

EFFECTIVE POPULATION SIZE AND THE FASTER-X EFFECT: EMPIRICAL RESULTS AND THEIR INTERPRETATION

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The X or Z chromosome has several characteristics that distinguish it from the autosomes, namely hemizyosity in the heterogametic sex, and a potentially different effective population size, both of which may influence the rate and nature of evolution. In particular, there may be an accelerated rate of adaptive change for X-linked compared to autosomal coding sequences, often referred to as the Faster-X effect. Empirical studies have indicated that the strength of Faster-X evolution varies among different species, and theoretical treatments have shown that demography and mating system can substantially affect the degree of Faster-X evolution. Here we integrate genomic data on Faster-X evolution from a variety of animals with the demographic factors, mating system, and sex chromosome regulatory characteristics that may influence it. Our results suggest that differences in effective population size and mechanisms of dosage compensation may influence the perceived extent of Faster-X evolution, and help to explain several clade-specific patterns that we observe.

KEY WORDS: Faster-X, rates of evolution, sex chromosomes.

The X chromosome has several distinguishing characteristics (Vicoso and Charlesworth 2006), which may influence the rate of evolution of X-linked genes (Rice 1984; Charlesworth et al. 1987). First, the hemizygous sex possesses just one functional copy of the X chromosome, and this means that newly arisen recessive or partially recessive mutations (with dominance coefficient $h < 0.5$) are more often directly exposed to selection in the heterogametic sex when located on the X chromosome. In contrast, these mutations when present on autosomes will only be directly exposed in the homozygous state, a relatively rare event for new mutations at low frequencies in randomly mating populations. New, beneficial mutations on the X chromosome may therefore be detected more readily and fixed by positive selection whereas novel deleterious mutations will be more rapidly purged by purifying selection.

On the other hand, the effective population size (N_e) of the X chromosome, which controls the rate of genetic drift (Wright 1931), is three-quarters of that of an autosome when there are equal numbers of breeding males and females and random variation in offspring numbers for both sexes. Departures from these conditions, such as those caused by an increased variance in male reproductive success due to sexual competition among males (Andersson 1994), affect the X/autosome ratio of N_e values (Caballero 1995; Charlesworth 2001; Laporte and Charlesworth 2002). This ratio influences the relative rates of evolution of the X chromosome and autosomes, for both positively and negatively selected mutations (Vicoso and Charlesworth 2009b); other things being equal, a higher value increases the ratio of X/A rates of evolution for favorable mutations and decreases the ratio for slightly deleterious mutations.

The patterns predicted by these considerations have been extensively tested in a range of animals, and a complex signal has begun to emerge. Several studies of *Drosophila* species indicate a negligible or small Faster-X effect (Counterman et al. 2004; Thornton et al. 2006; Begun et al. 2007; Connallon 2007; Baines et al. 2008; Vicoso et al. 2008), whereas mammals (Torgerson and Singh 2003; CSAC 2005; Khaitovich et al. 2005; Lu and Wu 2005; Torgerson and Singh 2006; Baines and Harr 2007), and birds (Borge et al. 2005; Mank et al. 2007a) show evidence of more pronounced Faster-X evolution (for simplicity, we will refer to the Z chromosome of birds as an X chromosome throughout this article).

Although the origins of the sex chromosomes of therian mammals, *Drosophila*, and birds represent independent evolutionary events, the genetic characteristics that could produce a Faster-X effect are similar in all three systems. It is therefore somewhat puzzling that the signature of Faster-X evolution varies among these clades. It seems unlikely that the nature of new mutations differs sufficiently among these groups to cause the different Faster-X patterns observed, suggesting that some other variable, or set of variables, must be responsible.

The theoretical treatment of Vicoso and Charlesworth (2009b) indicates that demographic and mating system factors can strongly influence the strength of the Faster-X effect. The overall effective population size of the population (N_e) influences the extent of Faster-X evolution, because a larger proportion of new mutations are effectively neutral in smaller populations, and accumulate at similar rates on the X and on the autosomes. In populations with a large N_e , many more mutations under selection will experience the effects of differences in N_e between the X and autosomes. The ratio of the effective population sizes of the X and autosomes (N_{eX}/N_{eA}) is also a crucial parameter: when N_{eX}/N_{eA} approaches 1, there can be a Faster-X effect even for partially dominant favorable mutations, because of reduced genetic drift on the X.

Overall N_e values differ by orders of magnitude among *Drosophila* (Andolfatto 2001; Eyre-Walker et al. 2002), mammals (Won and Hey 2005; Garrigan et al. 2007; Geraldine et al. 2008), and birds (Primmer et al. 2002; Jennings and Edwards, 2005; Backström et al. 2008). If N_e is responsible, at least in part, for the differing levels of Faster-X evolution observed in these clades, then we would expect a relation between N_e and the Faster-X effect, and we examine this possibility in this article.

The ratio N_{eX}/N_{eA} differs among these clades as well, with potentially important effects on Faster-X evolution. With equal variances in male and female reproductive success, $N_{eX}/N_{eA} = 3/4$. A higher variance in male-mating success will increase N_{eX}/N_{eA} above 3/4 in male heterogametic species, but will reduce N_{eX}/N_{eA} below 3/4 for female heterogametic animals. If the effects of N_{eX}/N_{eA} on favorable mutations explain the observed differences

in the Faster-X effect, we might expect it to be most evident in *Drosophila*, least evident in birds, and intermediate in mammals, as we will show next.

The sex chromosome dosage compensation systems of mammals, *Drosophila*, and birds also differ (Mank 2009), and this can affect the signal and strength of the Faster-X effect (Charlesworth et al. 1987). Specifically, therian mammals equalize the difference in gene dose for the X chromosome in two steps. First, one X is transcriptionally inactivated in female embryos, resulting in functional female hemizygosity in marsupials where the paternal X is always inactivated (Sharman 1971), and patchy functional hemizygosity in placental females from the random X-inactivation that targets either X chromosome in a cell-specific manner (Payer and Lee 2008). Hyper-transcription of the active X in both sexes then achieves X:A transcriptional parity (Gupta et al. 2006). *Drosophila* also compensate for X chromosome gene dosage, but in a more straightforward manner: the male X chromosome is simply hyper-transcribed (Fagegaltier and Baker 2004). Birds do not have wholesale dosage compensation (Ellegren et al. 2007; Itoh et al. 2007), but rather compensate some critical genes locally (Mank and Ellegren 2009a), leaving the majority with higher expression in males.

The presence or absence of dosage compensation can influence the rate of Faster-X evolution, with the Faster-X effect due to beneficial mutations potentially being less marked in the absence of dosage compensation (Charlesworth et al. 1987). In contrast, the patchy functional hemizygosity resulting from eutherian random X-chromosome inactivation may accelerate Faster-X for nondominant mutations, as we show below.

In the light of the major differences in overall N_e , as well as N_{eX}/N_{eA} , among *Drosophila*, mammals, and birds, we have analyzed the pattern of Faster-X evolution in these three clades, and then integrated our observations with demographic data to test the predictions of Vicoso and Charlesworth (2009b) concerning the effects of demography on the Faster-X effect. We also explored the effects on Faster-X evolution of differences in the mechanisms of dosage compensation.

Materials and Methods

COMPARATIVE GENOMIC DATA

For mammals, we used Ensembl-annotated genomes (www.emsembl.org) to estimate average divergence for X and autosomal genes, using only those genomes with fully annotated and mapped assemblies. For each nonoverlapping pairwise species comparison (human–chimpanzee, macaque–orangutan, mouse–rat, horse–cow) we extracted all one-to-one orthologs via BioMart, and estimated the number of synonymous and nonsynonymous substitutions with the Ensembl homology pipeline, which incorporates the codeml application of the PAML

package (Yang 1997), and includes a saturation filter where all orthologs with $d_S > 2$ are removed to avoid problems due to mutation saturation and multiple hits (d_S is the codon-based, maximum likelihood estimate of the divergence per synonymous site, derived from codeml). This filter is based on previous work showing that the transition-transversion ratio at fourfold degenerate sites shows a linear relationship with d_S for d_S values up to two, and a nonlinear relationship for $d_S > 2$ (Axelsson et al. 2008). We then further filtered the data by determining which orthologs mapped to autosomes in both species and which consistently mapped to the X in both species, discarding from further analysis all orthologs that have evolved under an amalgam of autosomal and sex-linked forces. For the remaining data, we computed the average d_N (the codon-based, maximum likelihood estimate of the divergence per nonsynonymous site) for both X and autosomal genes. We estimated d_S in a similar fashion. We then computed the normalized rate of adaptive evolution for each species pair, by computing the ratio of autosomal to X mean d_N divided by mean d_S .

For *Drosophila*, we searched the literature for studies comparing divergence estimates for autosomes and the X chromosome. From the studies using overlapping taxon samples, we included the one with the largest and least biased dataset, choosing whole-genome analysis over partial genome scans, yielding three point estimates of autosomal and X-linked divergence (Begun et al. 2007) for over 70% of the annotated transcriptome.

For birds, because full genomic analysis involving the zebra finch genome is still embargoed until publication of the draft genome, we used the data for the only Faster-X study to date (Mank et al. 2007a), which is a partial-genome comparison between chicken and zebra finch that encompasses roughly a quarter of the annotated avian transcriptome.

NUMERICAL ANALYSES OF EVOLUTIONARY RATES

We used the program described in Vicoso and Charlesworth (2009b) to compute the fixation rates of weakly beneficial mutations at X-linked and autosomal sites, using different values of N_{eA} and N_{eX} to compare the expected rates of evolution at X-linked and autosomal sites in birds, mammals, and flies. This uses standard diffusion equation formulae to compute the expected rates of substitution for mutations with a specified selection coefficient s , here assumed to be the same for both hemizygotes and homozygotes. The results were expressed as ratios of the rates of substitution for X and autosomes, each normalized relative to the putatively neutral rate to remove the effect of differences in mutation rate between the sexes (X/A ratios), as described by Vicoso and Charlesworth (2009b). We studied both the case in which the dominance coefficient, h (where hs is the fitness effect of a mutation in heterozygotes) was the same for autosomal loci and X-linked loci, and the case when the dominance coefficient (h)

for X-linked loci was always one-half, regardless of the value of h for autosomal mutations with the same selection coefficient. The purpose of this is to see how intermediate dominance that may be associated with X-inactivation in eutherian mammals affects the X/A ratio of the rate of evolution.

EFFECTIVE POPULATION SIZE ESTIMATES

N_e values were obtained from estimates of molecular sequence diversity (listed in Table 2), and corrected for mutational bias and differences in generation time. The effective population sizes used were $N_{eA} = 500,000$ (Primmer et al. 2002; Jennings and Edwards 2005; Backström et al. 2008) for birds. For mammals, we set $N_{eA} = 100,000$, which is intermediate between the low primate (Chen and Li 2001; Won and Hey 2005; Garrigan et al. 2007) and high rodent (Eyre-Walker et al. 2002) values. Previous studies have indicated a reduction in X or Z chromosome diversity consistent with $N_{eX}/N_{eA} < 1$ for both mammals and birds. For *Drosophila*, we used an estimate of $N_{eA} = 2,000,000$ (Andolfatto 2001; Eyre-Walker et al. 2002), with $N_{eX}/N_{eA} = 1$ (Andolfatto 2001; Connallon 2007; Singh et al. 2007; Vicoso and Charlesworth 2009a). We assumed that the ancestral population sizes were equal to N_{eA} and had equal numbers of breeding males and females.

Results

There are three point estimates for X and autosomal divergence in *Drosophila*, four in mammals, and one in birds (Table 1). This collection of genome sequences includes estimates of d_N and d_S for over 95,000 one-to-one orthologs after filtering; to our knowledge, it is the largest and most comprehensive test of Faster-X theory to date. Overall, these data suggest a pattern of Faster-X evolution in the three clades. For both mammals and *Drosophila*, the presence of multiple $(d_{NX}/d_{SX})/(d_{NA}/d_{SA})$ estimates in the clades make it possible to test for overall Faster-X effects. The overall $(d_{NX}/d_{SX})/(d_{NA}/d_{SA})$ ratio for both groups was significantly greater than one (Z-test, $P < 0.00001$ with standard deviation = 0.094 for mammals, and $P = 0.04$ with a standard deviation of 0.106 for flies), where the standard deviation is parametrically estimated from within-clade estimates. The single Faster-X test in birds precludes a similar test; however, there was a significant Faster-X effect with the single comparison available (Mank et al. 2007a). Our data show clear differences among clades in the strength of the Faster-X effect, with *Drosophila* showing the least difference between X and autosomal loci, and mammals and birds having a more pronounced Faster-X effect.

THE EFFECT OF N_{eX}/N_{eA}

N_{eX}/N_{eA} ranged widely over the taxa we assessed (Table 2). The ratio was lowest in birds (0.42), as might be expected from the

Table 1. Relevant studies of Faster-X evolution and point estimates of divergence for X versus autosomal coding sequences.

| Clade | Citation | Species | Dataset | X d_N/d_S | Autosomal d_N/d_S |
|-------------------|---------------------|--|--------------|-------------|---------------------|
| <i>Drosophila</i> | Begun et al. (2007) | <i>D. melanogaster</i> (with <i>D. simulans</i> and <i>D. yakuba</i>) | 10,403 genes | 0.0874 | 0.0878 |
| | | <i>D. simulans</i> (with <i>D. melanogaster</i> and <i>D. yakuba</i>) | 11,439 genes | 0.135 | 0.109 |
| | | <i>D. yakuba</i> (with <i>D. melanogaster</i> and <i>D. simulans</i>) | 10,150 genes | 0.109 | 0.102 |
| Birds | Mank et al. (2007a) | chicken and zebrafinch | 5020 genes | 0.110 | 0.0849 |
| Mammals | Ensembl | human and chimpanzee | 13,693 genes | 0.593 | 0.460 |
| | | macaque and orangutan | 15,396 genes | 0.401 | 0.350 |
| | | mouse and rat | 16,048 genes | 0.310 | 0.228 |
| | | horse and cow | 13,617 genes | 0.209 | 0.228 |

effects of sexual selection on the female-heterogametic sex chromosomes (Vicoso and Charlesworth 2009b). Mammals show a wide range of values of N_{eX}/N_{eA} , being largest in horses (approaching 1), possibly due to the pronounced harem mating system and its resulting influence on male reproductive success (Nowak 1999), and the relatively high rate of recombination on the ungulate X (Ihara et al. 2004). The ratio was smallest in rodents, which may be due in part to sampling error due to the small number of genes assessed (Salcedo et al. 2007; Geraldles et al. 2008), as the range of values for the genus is wide (0.15–0.84). Additionally, recent work suggests that *Mus musculus* has experienced a significant bottleneck during its expansion into Europe (Baines and Harr 2007); because of this, we employed the N_{eX}/N_{eA} estimate for *M.*

Table 2. Estimates of relative chromosomal effective population sizes. Where multiple estimates exist within a clade, the average N_{eX}/N_{eA} is given.

| Clade | Species | N_{eX}/N_{eA} |
|-----------------------|--|---|
| Birds ¹ | Chicken (<i>Gallus gallus</i>) | 0.30 (Sundström et al. 2004) |
| | Collared flycatcher (<i>Ficedula albicollis</i>) | 0.51 (Borge et al. 2005) |
| | Pied flycatcher (<i>Ficedula hypoleuca</i>) | 0.45 (Borge et al. 2005) |
| | median | 0.42 |
| Primates ² | Human (<i>Homo sapiens</i>) | 0.81 (ISMWG 2001) |
| Rodents ³ | <i>Mus castaneus</i> | 0.84 (Geraldles et al. 2008) |
| Ungulates | <i>Equus</i> | ≈1 (Lau et al. 2009) |
| <i>Drosophila</i> | <i>D. melanogaster</i> | ≈1 (Andolfatto 2001; Connallon 2007; Singh et al. 2007) |

¹Mutation and divergence rate estimates from Axelsson et al. (2004), Dimcheff et al. (2002), van Tuinen and Dyke (2004).

²Mutation and divergence rate estimates from CSAC (2005).

³Mutation and divergence rate estimates from MGSC (2002). We used the estimate from *M. castaneus*, as there is evidence of a severe bottleneck in European *Mus* lineages (Baines and Harr 2007).

castaneus as the rodent value for our analysis, because this is likely to reflect the ancestral state. The estimates for humans (0.81) are also similar to what would be expected with some variance in male reproductive success in a male-heterogametic system. N_{eX}/N_{eA} approaches 1 for the putatively ancestral *Drosophila melanogaster* population (Andolfatto 2001; Connallon 2007; Singh et al. 2007; Vicoso and Charlesworth 2009a). The divergence and N_{eX}/N_{eA} data taken together, as shown in Figure 1, do not show strong support for a relationship between N_{eX}/N_{eA} and the ratio of X/A divergence ($r^2 = 0.2526$, $P > 0.2$). In fact, the trend is in the opposite direction to what might be expected with positive selection of favorable nonsynonymous mutations (Vicoso and Charlesworth 2009b).

This suggests that mating system effects cannot in themselves explain the differences in the apparent strength of the Faster-X effect. We used approximate estimates of N_{eX} and N_{eA} from mammals, birds, and *Drosophila* from estimates of DNA sequence diversity (see Methods and Table 2) to compute the expected rates of evolution on the X and the autosomes for these organisms for some chosen selection coefficients, for both beneficial and deleterious mutations (see Methods). Figure 2 shows the expected

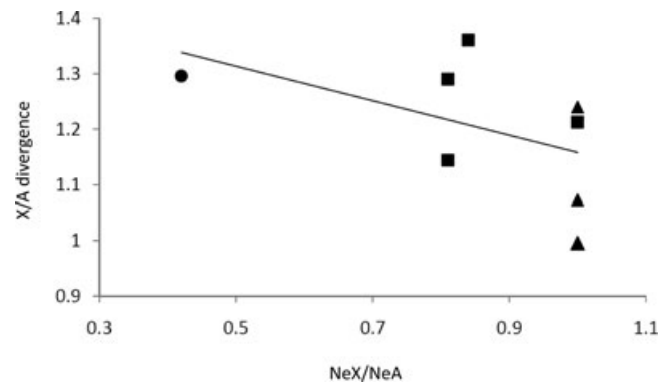


Figure 1. Relative strength of Faster-X effect in birds (circle), mammals (squares), and *Drosophila* (triangles). Average point estimates of normalized divergence (d_N/d_S estimates) of X/autosomal coding sequence are plotted against the relative N_{eX}/N_{eA} (taken from Table 2).

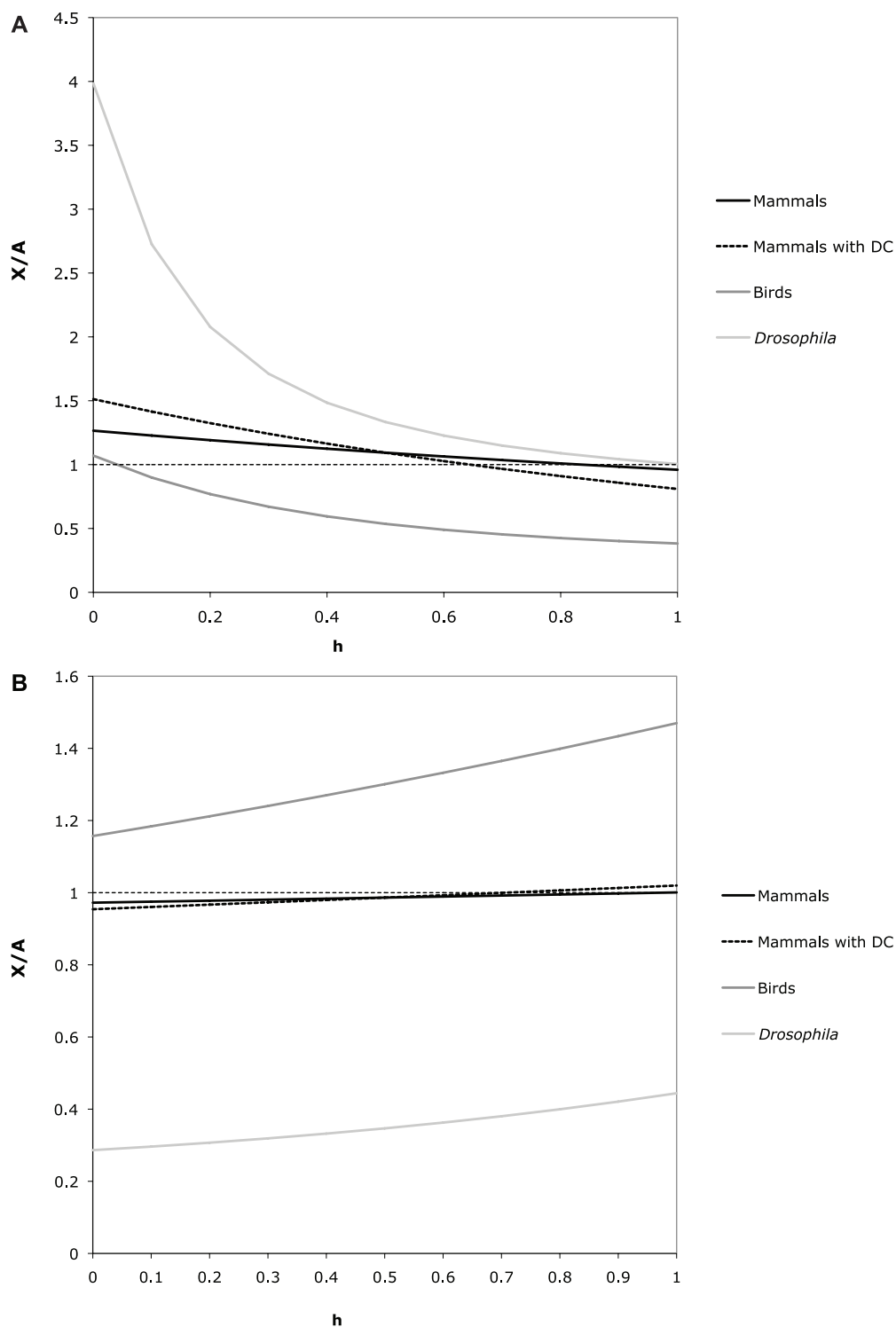


Figure 2. Normalized rates of evolution of the X compared with the autosomes, $X/A = (d_{NX}/d_{sX})/(d_{NA}/d_{sA})$, for beneficial mutations ($s = 10^{-5}$, panel A) and mildly deleterious mutations ($s = 10^{-6}$, panel B). The values for mammals with dosage compensation (DC) assume that h is always 0.5 for X-linked loci (see text). N_{eX}/N_{eA} estimates are from Table 2.

normalized rates of evolution of the X compared with the autosomes, the equivalent of the ratio of d_N/d_S for the X versus that for the autosomes, for beneficial mutations ($s = 1 \times 10^{-5}$, upper panel) and mildly deleterious ones ($s = -1 \times 10^{-6}$, lower panel),

using the assumption that the dominance coefficient h is the same for both autosomal and X-linked mutations, as in most previous work (Charlesworth et al. 1987; Vicoso and Charlesworth 2009b). Changing the magnitudes of the selection coefficients only affects

the curves quantitatively (see Vicoso and Charlesworth 2009b and Figs. S1 and S2), so that the qualitative nature of these patterns is expected to be seen for other values. There is a clear conflict between the effects of N_{eX}/N_{eA} on the rates of fixation of favorable versus deleterious mutations, which may well confuse the overall pattern observed for d_N/d_S .

For example, in the case of *Drosophila*, with very large N_{eA} and high N_{eX}/N_{eA} , both of which facilitate Faster-X evolution for beneficial mutations, we expect the X chromosome to have a higher rate of adaptive evolution than the autosomes, as seen in Figure 2A. This is, however, counteracted by the strongly reduced rate of fixation of mildly deleterious mutations on the X chromosome, compared to the autosomes (shown in Fig. 2B). These results imply that Faster-X evolution may only be detected in *Drosophila* if a very large fraction of the divergence were caused by positive selection, and only a small fraction by drift. Because most estimates for this clade do not suggest that more than 50% of amino-acid substitutions in *Drosophila* have been fixed by positive selection (Smith and Eyre-Walker 2002; Bierne and Eyre-Walker 2004; Welch 2006; Andolfatto 2007), it is thus not surprising that Faster-X evolution is difficult to detect overall in *Drosophila*.

THE TOTAL EFFECTIVE SIZE OF THE POPULATION

The above results suggest that an additional factor that may be important is the total effective size of the population (we will use the autosomal value N_{eA} as a measure of this), which differs greatly among clades (see Methods). The contrast between mammals, especially primates, and *Drosophila* is of special interest, because estimates of the distribution of selection coefficients against newly arisen nonsynonymous mutations are available for large datasets on both human and *D. melanogaster* populations, based on the same method of estimation (Keightley and Eyre-Walker 2007). The parameter $N_{eA}s$, which determines the effectiveness of purifying selection against deleterious mutations with selection coefficient s against homozygous carriers, has a very wide distribution in both cases. However, in humans 34% of new nonsynonymous mutations are estimated to fall into the region with $N_{eA}|s| < 1$, compared with only 5% in *D. melanogaster* (these estimates are both for the putatively ancestral African populations of these species). The proportions of mutations in the category $1 \leq N_{eA}|s| < 10$ are similar in the two species (9% and 6%, respectively). Mutations with bigger effects than this, which constitute the bulk of the distribution, have no chance of fixation (Kimura 1983, p. 43), so that only mutations in these two classes can contribute much to nonsynonymous evolution.

This argument can be made more quantitative by using the estimated distributions of fitness effects of nonsynonymous mutations, which Keightley and Eyre-Walker (2007) assumed to follow gamma distributions (see their Table 5 for estimates of the

parameters of the distributions for human and *D. melanogaster* populations). An analytic formula for the rate of substitution for a given selection coefficient relative to the neutral value is available for this case (Kimura 1983, their eq. 3.14); this depends only on $N_{eA}|s|$. When mutations are semidominant ($h = 1/2$), with the same selection coefficient in males and female, the ratio of the rates of change of allele frequency for X versus autosomes for a given allele frequency is simply 4/3 (see eqs. 3 and 5b of Vicoso and Charlesworth 2009b). The corresponding product of N_e and selection coefficient for an X-linked mutation that appears in the expression for fixation probability is thus $4N_{eX}|s|/3$. Knowing the ratio N_{eX}/N_{eA} , we can obtain predicted rates of substitution for deleterious mutations at both autosomal and X-linked loci by integrating Kimura's formula over the estimated distribution of $N_{eA}s$ values.

There are three important differences between humans and flies; a smaller shape parameter of the gamma distribution (approximately 0.1–0.15 for Africans and 0.3 for Zimbabwe *D. melanogaster*), a smaller mean value of $N_{eA}|s|$ (2500–5300 for Africans, and 9800 for Zimbabwe *D. melanogaster*), and a lower N_{eX}/N_{eA} for humans than *D. melanogaster* (0.81 vs. 1). Although the exact value of the mean value of $N_{eA}|s|$ is only poorly estimated from the polymorphism data, the shape parameter estimates are reasonably accurate (Keightley and Eyre-Walker 2007). For a given shape parameter, we can obtain estimates of $N_{eA}|s|$ from the observed d_N/d_S values for species differences. For human–chimpanzee divergence and the *D. melanogaster* lineage, our analysis suggests autosomal d_N/d_S values of 0.46 and 0.088, respectively. About 10–20% of nonsynonymous substitutions in the case of human–chimpanzee and about 50% in the *D. melanogaster* lineage are estimated to have been fixed by positive selection (Keightley and Eyre-Walker 2007; Boyko et al. 2008), so that the deleterious d_N/d_S values are likely to be at most about 0.37 and 0.044, respectively. Using shape parameters of 0.10 and 0.30 for humans and *D. melanogaster*, these d_N/d_S values are consistent with $N_{eA}|s|$ values of 150 and 6600, respectively (see Supporting information). Using the above values for N_{eX}/N_{eA} , the predicted d_N/d_S values for X-linked mutations are then 0.369 and 0.040, respectively. These are approximately 99% and 91% of the corresponding values for autosomes.

It thus seems possible that a low ratio of X/A evolution for deleterious mutations in *Drosophila* may swamp any Faster-X effect of selectively favorable mutations, which is expected to be relatively modest unless they have highly recessive fitness effects (Fig. 2A). This swamping effect can be expressed algebraically as follows. Let D_{Xa} and D_{Aa} be the expected numbers of fixed nonsynonymous differences per gene that are driven by positive selection for the X chromosome and autosomes, respectively. Similarly, let D_{Xd} and D_{Ad} be the corresponding numbers of differences driven by drift acting on neutral or nearly

neutral, slightly deleterious mutations. The total expected numbers of fixed differences per gene for the X and autosomes are $D_X = D_{Xa} + D_{Xd}$ and $D_A = D_{Aa} + D_{Ad}$, respectively. The expected X/A ratio is $D_X/D_A = (D_{Xa}/D_{Ad})(1 + D_{Xd}/D_{Ad})(1 + D_{Aa}/D_{Ad})$. In the case of *Drosophila*, the relatively low value of D_{Xd}/D_{Ad} (0.91) makes it nearly impossible for a higher value of D_{Xa}/D_{Ad} relative to D_{Aa}/D_{Ad} to cause the overall value of D_X/D_A to exceed one. For example, if D_{Aa}/D_{Ad} is approximately 1 (see above), even if D_{Xa}/D_{Ad} were 1.2, the overall value of D_X/D_A is close to 1. If, on the other hand, (D_{Xd}/D_{Ad}) is approximately 1, as seems likely for mammals, a sufficiently high value of D_{Xa}/D_{Ad} compared with D_{Aa}/D_{Ad} can result in $D_X/D_A > 1$.

Because no estimate of the distribution of $N_{eA}|s|$ values is available for deleterious nonsynonymous mutations in birds, it is not possible to predict how they would behave. But in this case, another factor influences the Faster-X effect: the greatly reduced N_{eX} compared with N_{eA} ($N_{eX}/N_{eA} = 0.4$ in our computations). This results in increased drift on the X chromosome compared with the autosomes, leading to $D_X/D_A < 1$ even for fairly recessive favorable mutations (Fig. 2A). This suggests that, for birds, the Faster-X evolution detected in the data is caused primarily by a corresponding increase in the rate of fixation of mildly deleterious mutations on the X chromosome (Fig. 2B), and not by more efficient positive selection on this chromosome.

DOSAGE COMPENSATION

Birds, mammals, and *Drosophila* also show major differences in the mechanisms by which they compensate for sex chromosome dosage in the heterogametic sex (Mank 2009), and this can affect Faster-X evolution (Charlesworth et al. 1987). Here, we investigated the role of eutherian X chromosome inactivation, which for cell-autonomous proteins results in functional hemizyosity in females, on the evolution of X-linked genes (Fig. 2). With random X-chromosomal inactivation as in eutherian mammals, mutations that are cell autonomous would be expected to cause one-half of the cells in the body to express the wild-type allele and one-half the mutant allele. This seems likely to cause approximate semidominance for the fitness effects of the mutations concerned, at least for the Kacser-Burns model of dominance (Kacser and Burns 1981), because the amount of the total end product of the pathway in which the gene is involved will be approximately 50% from each of the two types of cell. In contrast, for autosomal loci with comparable effects, heterozygotes will have amounts of end product that reflect the influence of a single mutant allele on the throughput of the pathway. For beneficial mutations (Fig. 2A), the Faster-X effect expected with this model of the effect of X-inactivation with low h is somewhat greater than that expected under other models, slowly decreasing with h in such a way that dominant beneficial mutations should show a slower rate of fixation when X-linked in a lineage with X-inactivation

compared with other cases. For deleterious mutations (Fig. 2B), there are only small effects of X-inactivation for partially recessive mutations, and the X/A ratio for mammals is always close to 1. Our conclusions for the case of semidominance are, of course, unchanged.

Discussion

Here, we present the first meta-analysis of the Faster-X effect, encompassing whole-genome data from animals in three distinct clades, and including data from over 95,000 one-to-one orthologs. This analysis clarifies the differences among taxa in Faster-X evolution, and indicates that the mechanism of sex chromosome dosage compensation, overall N_e , and the X-autosome ratio of effective population sizes (N_{eX}/N_{eA}) all affect the likelihood of detecting a Faster-X effect from comparisons of d_N/d_S ratios between X and autosomes. We now discuss the effects of various genetic and demographic factors on these comparisons.

OVERALL EFFECTIVE POPULATION SIZE

Point estimates of N_{eA} vary greatly over the animals assessed here, as described in the Methods section. For example, analyses of nuclear gene polymorphism data suggest a larger N_{eA} in birds than in mammals. N_{eA} for the chicken is estimated to be approximately 600,000 based on diversity data (ICPMC 2004) and mutation rate estimates (Axelsson et al. 2004). Wild passerine populations show molecular diversity patterns indicating slightly lower N_{eA} estimates of 200,000–500,000 (Primmer et al. 2002; Jennings and Edwards 2005; Backström et al. 2008). Mammalian species show large variation in N_{eA} , as expected from the extreme variation in body size seen between different mammals. This is evident when comparing N_{eA} in primates, which ranges from 10,000 in humans (Chen and Li 2001; Garrigan et al. 2007), 21,000 in chimpanzee to 25,000 in gorillas (Chen and Li 2001; Won and Hey 2005), with N_{eA} in mice, which is estimated to be 161,000–280,000 (Eyre-Walker et al. 2002), or even higher (Baines and Harr 2007). In contrast, N_{eA} in African populations of *D. melanogaster* (1,450,000) and *D. simulans* (2,580,000) (Andolfatto 2001; Eyre-Walker et al. 2002) are much higher than for both vertebrate clades.

The Faster-X pattern is clearly affected by N_{eA} . Larger populations are expected to have higher rates of adaptive evolution on the X compared with the autosomes (Fig. 2 and Vicoso and Charlesworth 2009b), so that we might expect species with the highest N_{eA} values to exhibit the strongest pattern of Faster-X evolution. Due to their small body size and correspondingly large carrying capacity, N_{eA} for *Drosophila* is by far the highest of our three analyzed clades, but this is, paradoxically, the group with the weakest Faster-X effect. As shown in the Results section, more effective selection against the accumulation of deleterious mutations on the X with a high N_{eA} , even with a higher rate of adaptive evolution on this chromosome, can result in a similar overall rate

of evolution on the X and the autosomes. This may well account for the weak Faster-X effect in *Drosophila*.

Mammals and birds have lower N_{eA} values compared with *Drosophila* and are thus likely to be accumulating a relatively large fraction of mildly deleterious mutations at similar rates on the X and the autosomes (as many mildly deleterious mutations behave neutrally in smaller populations). This may allow a Faster-X effect due to the fixation of favorable mutations to be detected more easily than in *Drosophila*, because the high effective population size of *Drosophila* causes a low ratio of X/A substitutions of deleterious mutations, which is likely to overcome any Faster-X effect due to favorable mutations (see Results).

THE N_{eX}/N_{eA} RATIO

Sexual competition among males increases the variance in reproductive success among males (Andersson 1994), reducing the effective population size of males. This can influence the Faster-X effect, by altering the difference in N_e between the X chromosome and the autosomes (see Results and Vicoso and Charlesworth 2009b). In many mammals, sexual selection acts through male–male competition, enabling high-ranked, dominant males to sire more offspring than low-ranked males [e.g., gorillas (Bradley et al. 2005), rhesus macaques (Widdig et al. 2004), and chimpanzees (Inoue et al. 2008)], reducing the number of males that are actually involved in reproduction. Sexual competition through female mate choice can also cause a male mating skew, as is the case in many bird species (Andersson 1994 and references therein), even in many socially monogamous species, where females seek extra-pair copulations (Kempnaers et al. 1992; Sheldon and Ellegren 1999; Rutstein et al. 2004). Sexual selection can also be more subtle, acting after actual mating, through postcopulatory female choice of sperm or sperm competition (Harshman and Clark 1998; Pizzari and Birkhead 2000; Dean et al. 2006).

Molecular diversity studies are not entirely consistent with the predicted effects of these differences. N_{eX} is roughly equal to N_{eA} in *D. melanogaster* (Andolfatto 2001; Connallon 2007; Singh et al. 2007) whereas some populations of vertebrates show reduced variation on the major sex chromosome compared to the autosomes, suggesting a reduction in N_{eX} compared to N_{eA} (Table 2). However, a high variance in male reproductive success should affect female- and male-heterogametic systems in opposite directions, increasing N_{eX}/N_{eA} to more than 3/4 in male-heterogametic lineages, and decreasing it below 3/4 in female-heterogametic lineages (Laporte and Charlesworth 2002). As we showed in the Results section, the Faster-X effect in birds may well simply reflect the faster accumulation of slightly deleterious mutations on the X chromosome due to its relatively low effective size.

The effect of sex differences in variance in reproductive success between the sexes, however, cause only relatively small de-

viations from the null value of N_{eX}/N_{eA} of 3/4, when biologically plausible values are considered (Vicoso and Charlesworth 2009b). This suggests that the strong deviations found in *Drosophila* ($N_{eX}/N_{eA} \approx 1$) and birds ($N_{eX}/N_{eA} \approx 0.42$) are probably influenced by other factors as well, and this may, in part, explain why we failed to find a statistical relationship between N_{eX}/N_{eA} and Faster-X evolution.

Recombination rate differences between the X and autosomes may play a role in some of the observed patterns, as this parameter can affect the extent of Hill–Robertson effects on the X and the autosomes, affecting N_{eX}/N_{eA} (Vicoso and Charlesworth 2009a). Specifically, the global suppression of crossing over in *Drosophila* males means that the X chromosome experiences a higher recombination rate than autosomes (Langley et al. 2008; Connallon 2007), which may partially explain the higher diversity levels for genes on the *Drosophila* X chromosome (Vicoso and Charlesworth 2009a). This is not the case in the other vertebrates assayed here, where recombination occurs in both sexes except for the part of the X that fails to cross over with the Y in males, so that the mammalian and avian X chromosomes would experience lower recombination rates than autosomes for all but the small pseudoautosomal regions. This reduced recombination may contribute to the reduced variation observed for the avian X chromosome (Table 2 and references therein). However, it is currently not clear to what extent a reduction in N_{eX}/N_{eA} caused by Hill–Robertson effects will be reflected in a corresponding difference between X and autosomes in the rate of fixation of either favorable or deleterious mutations, except for weakly selected mutations of the type involved in codon usage (e.g., Singh et al. 2005; Vicoso et al. 2008).

Whatever the causes of these biased N_{eX}/N_{eA} values, can the observed values contribute to the Faster-X patterns seen here? As in the case of the overall effective population size, the accumulation of deleterious mutations on the X is predicted to be much more sensitive to the amount of drift it experiences than the accumulation of beneficial mutations. Because increasing N_{eX}/N_{eA} decreases the amount of drift on the X versus the autosomes, it counteracts the (enhanced) Faster-X effect by strongly reducing the rate of accumulation of mildly deleterious mutations on this chromosome. We did not observe a significant relationship between N_{eX}/N_{eA} and the Faster-X effect (Fig. 1). This suggests that other factors that differ among our taxa, including the overall value of the effective population size (discussed above), sex chromosome dosage compensation, male-biased mutation, and the nonrandom distribution of genes between the X chromosome and autosomes, may affect the strength of the Faster-X Effect.

SEX CHROMOSOME DOSAGE COMPENSATION

Mammals, *Drosophila*, and birds have very different methods of dealing with the gene dosage effects of sex chromosomes.

Whereas birds lack dosage compensation entirely (Ellegren et al. 2007), *Drosophila* simply hyper-transcribe the genes on the X chromosome in males, (Fagegaltier and Baker 2004). Eutherian mammals have the most complex mechanism, based on random X chromosome inactivation. Either X can be inactivated, with the selection process randomized in each cell of the embryo and maintained in daughter cells (Payer and Lee 2008). This results in a patchy chimeric distribution of X chromosome transcription, making female tissues locally hemizygous.

Although there are not enough independent origins of the different types of sex chromosome dosage compensation to test theoretical predictions with empirical data at this time, dosage compensation can affect the pattern of Faster-X evolution. Charlesworth et al. (1987) assumed that an absence of dosage compensation would cause the selection coefficients in the heterogametic sex to be smaller in magnitude than in the presence of dosage compensation, due to the smaller amounts of gene products in the heterogametic sex. It is not at all clear whether this is biologically plausible, so we have not included this in our models. Overall, the results of Charlesworth et al. (1987) for their model with no dosage compensation suggested that the Faster-X effect is reduced for favorable mutations and strengthened for deleterious mutations; this may be another factor in the Faster-X effect in birds, which we have suggested is primarily caused by deleterious mutations (see Results).

Our numerical results suggest that mammalian X inactivation will increase the strength of Faster-X evolution for partially recessive ($h < 0.5$) favorable mutations, and causes X/A for deleterious mutations to be approximately 1 regardless of the dominance coefficient for autosomal mutations (Fig. 2). This strengthens the case that the Faster-X effect in mammals is caused by adaptive evolution.

MALE-BIASED MUTATION

Genomic regions that are more often transmitted through males than females may exhibit higher mutation rates than the genome-wide average (Crow 2000), and this could potentially affect X chromosome evolution. For male-heterogametic genomes, the X chromosome is present less often in males than the autosomes, so that the X will experience fewer de novo mutations than the autosomes when there is male-biased mutation. Male-biased mutation can influence the evolution of the major sex chromosome in important ways (Kirkpatrick and Hall 2004), and this is likely more so for mammals (Makova and Li 2002; Sandstedt and Tucker 2005) and birds (Axelsson et al. 2004) than *Drosophila* (Bauer and Aquadro 1997; Betancourt et al. 2002), although Bachtrog (2008) has proposed a higher male mutation rate in *D. miranda*. However, our use of the normalized d_N/d_S statistic removes this effect from our analysis by effectively controlling for the mutation rate, as shown by Vicoso and Charlesworth (2009b).

NONRANDOM DISTRIBUTION OF SEX-BIASED GENES

The *Drosophila* X chromosome is depauperate in genes that are highly expressed in males relative to females (Parisi et al. 2003; Ranz et al. 2003; Sturgill et al. 2007), as is also true of the mammalian X chromosomes (Potrzebowski et al. 2008). However, the X chromosome in mammals also appears to be enriched for genes expressed in males, other than those expressed at late stages of spermatogenesis (reviewed by Vicoso and Charlesworth 2006). This means that the composition of the sex chromosomes is not a truly randomized subset of autosomal gene functionalities, but rather the X contains a smaller proportion of male-biased genes that function in late spermatogenesis in both species (Vibrantovski et al. in press). Because this class of genes is among the fastest evolving (Zhang et al. 2004; Cutter and Ward 2005; Good and Nachman 2005; Khaitovich et al. 2005; Pröschel et al. 2006), there may be a concomitant bias in divergence estimates that produces the deflation of the X mean rate of adaptive evolution.

The demasculization of the *Drosophila* X chromosome is more pronounced than the demasculinization of the mammalian X. Comparisons of groups of genes with similar sex-biased expression patterns shows that the Faster-X effect in *Drosophila* is more pronounced for male-biased genes (Baines et al. 2008). This suggests that the lack of male-biased genes on the X may explain, at least in part, the low observed level of Faster-X in this clade.

The situation is reversed in birds, where the absence of wholesale dosage compensation produces an excess of genes that are expressed more strongly in males than in females (Ellegren et al. 2007; Itoh et al. 2007). It is important to point out that this male-bias is not due to a nonrandom distribution of gene functionalities, but rather to dosage effects alone. In addition to the dosage effects, birds show a nonrandom distribution of adult sex-biased genes on the X chromosome, due to a combination of defeminization due to unbalanced sex-specific selection (Mank and Ellegren 2009b) and ephemeral meiotic sex chromosome inactivation in females (Schoenmakers et al. 2009). However this excess of male-biased X-linked genes probably does not greatly influence Faster-X evolution in birds, as the limited evidence suggests that male-biased genes do not show the accelerated rates of divergence seen in flies and mammals (Mank et al. 2007b).

Conclusions

We have found evidence from comparative genomic data from diverse animal clades that the strength of Faster-X evolution is influenced by multiple evolutionary and demographic factors. When interpreting these results, it is worth considering the nature of the genomic data we employed. The data from mammals and birds are based on two-species comparisons, so that the rate of adaptive evolution represents the forces shaping the entire intervening lineage. The *Drosophila* data are single-branch estimates based

on three-species comparisons (Begun et al. 2007). The latter data represent the evolutionary forces that occurred in a single taxonomic lineage because it diverged from the other species considered. Although this is a methodological difference, it would not be expected to bias our results, because all the two-species comparisons included only those orthologs that were linked to the X or an autosome for whole of the phylogenetic time-span linking them. Additionally, although the branch lengths vary among the species considered here, especially for the ungulates that are more distant than the other species pairs, we focused on the ratio of X to autosomal divergence, and this corrects for differences in evolutionary time.

Employing ratios of divergence, although necessary for correcting in differences in underlying mutation rate among genes and for differences in divergence time among clades, carries associated concerns. Specifically, d_N/d_S ratios can be influenced by codon bias (Powell and Moriyama 1997), which violates the assumptions of neutrality for synonymous substitutions (Vicoso et al. 2008) and can act predominantly on X-linked genes under some scenarios (McVean and Charlesworth 1999). Codon bias may be a particular concern for estimates of the Faster-X Effect in *Drosophila* (D12GC 2007; Singh et al. 2008), although less for mammals (Urrutia and Hurst 2001) and birds (ICGSC 2004).

Overall, our results suggest that it may be possible to account for the differences between taxa in their Faster-X patterns that we have found largely in terms of differences in overall effective population size, together with differences in the N_{eX}/N_{eA} ratio; the low ratio of effective sizes of X and autosomes in birds may be the main cause of their Faster-X effect.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Normalized rates of evolution of the X (Z, in the case of birds) compared with the autosomes ($X/A = (K_{aX}/K_{sX})/(K_{aA}/K_{sA})$) for a beneficial mutation ($s = 1 \times 10^4$), for the three clades analyzed.

Figure S2. Normalized rates of evolution of the X (Z, in the case of birds) compared with the autosomes ($X/A = (K_{aX}/K_{sX})/(K_{aA}/K_{sA})$) for a deleterious mutation ($s = 1 \times 10^{-5}$, upper panel), for the three clades analyzed.

Supporting Information may be found in the online version of this article.

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