Faster-X Adaptive Protein Evolution in House Mice

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ABSTRACT The causes of the large effect of the X chromosome in reproductive isolation and speciation have long been debated. The faster-X hypothesis predicts that X-linked loci are expected to have higher rates of adaptive evolution than autosomal loci if new beneficial mutations are on average recessive. Reproductive isolation should therefore evolve faster when contributing loci are located on the X chromosome. In this study, we have analyzed genome-wide nucleotide polymorphism data from the house mouse subspecies *Mus musculus castaneus* and nucleotide divergence from *Mus famulus* and *Rattus norvegicus* to compare rates of adaptive evolution for autosomal and X-linked protein-coding genes. We found significantly faster adaptive evolution for X-linked loci, particularly for genes with expression in male-specific tissues, but autosomal and X-linked genes with expression in female-specific tissues evolve at similar rates. We also estimated rates of adaptive evolution for genes expressed during spermatogenesis and found that X-linked genes that escape meiotic sex chromosome inactivation (MSCI) show rapid adaptive evolution. Our results suggest that faster-X adaptive evolution is either due to net recessivity of new advantageous mutations or due to a special gene content of the X chromosome, which regulates male function and spermatogenesis. We discuss how our results help to explain the large effect of the X chromosome in speciation.

THE X chromosome has a special role in speciation, harboring a disproportionate number of loci contributing to reproductive isolation. This phenomenon, also known as the “large-X” effect (or large-Z for species where the female is the heterogametic sex), has been documented in several species of *Drosophila*, *Lepidoptera*, birds, and mammals (Coyne and Orr 1989, 2004; Coyne 1992). Its causes are disputed, and several hypotheses have been proposed to explain it (Rice 1984; Charlesworth *et al.* 1987; Presgraves 2008). One hypothesis rests on the fact that the X chromosome is found in only one copy in males, and recessive mutations on the X are fully exposed to selection. If new advantageous mutations are partially or fully recessive, X-linked loci are therefore expected to have higher rates of adaptive evolution than autosomal loci (the faster-X hypothesis; Charlesworth *et al.* 1987). Faster-X adaptive evolution could partially or fully explain the large-X effect (Presgraves 2008).

The faster-X hypothesis has been highly influential, since it has generated testable predictions using genomic data. It also presented the intriguing possibility of estimating the dominance coefficient (h) of new advantageous mutations. Assuming an equal number of breeding females and males, that the fitness effects of new advantageous mutations do not differ between autosomes and the X, that the beneficial mutation rate is equal per X-linked and autosomal gene, and that most adaptive substitutions are from new mutations rather than from standing variation, then the ratio of the rates of adaptive evolution of X-linked loci over autosomal loci (R) is a function of h and the selective effects of new mutations in females (s_f) and males (s_m):

$$R \approx \frac{2hs_f + s_m}{2h(s_f + s_m)}. \quad (1)$$

When $s_f = s_m$, this reduces to a simple function of h :

$$R \approx \frac{2h + 1}{4h} \quad (2)$$

(Charlesworth *et al.* 1987; Vicoso and Charlesworth 2006).

Several researchers have set out to test the faster-X hypothesis, initially by comparing the rate of protein evolution (*i.e.*, the ratio of divergence at nonsynonymous sites to synonymous sites, d_N/d_S) between X-linked and

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autosomal genes (Betancourt *et al.* 2002; Counterman *et al.* 2004; Lu and Wu 2005; Musters *et al.* 2006; Mank *et al.* 2007, 2010). However, a higher d_N/d_S ratio for X-linked vs. autosomal loci could be caused by reduced efficiency of negative selection on the X due to its smaller effective population size (N_e) than the autosomes. A more powerful way of testing for positive selection is the McDonald–Kreitman test (McDonald and Kreitman 1991) and its derivatives, which contrast patterns of polymorphism and divergence at selected and putatively neutral classes of sites, and can enable inference of the proportion of substitutions that are adaptive (α). In *Drosophila*, some studies have found evidence for faster-X adaptive evolution (Begun *et al.* 2007; Baines *et al.* 2008; Mackay *et al.* 2012), whereas others have not (Thornton *et al.* 2006; Connallon 2007). Few studies have compared α between autosomal and X-linked genes in species other than *Drosophila*. A study that compared α for autosomes and X of two subspecies of the European rabbit found faster-X evolution for only one of the two species (Carneiro *et al.* 2012). Another recent study found strong evidence for faster-X adaptive evolution in central chimpanzees (Hvilsom *et al.* 2012).

Apart for a faster overall rate of X-linked adaptive evolution, additional predictions from the faster-X theory can be tested using genomic data. For example, Equation 1 can be simplified to show that for mutations with male-limited fitness effects ($s_m > 0$ and $s_f = 0$), R becomes an inverse function of h :

$$R \approx \frac{1}{2h}. \quad (3)$$

Apart for mutations with female-limited fitness effects ($s_m = 0$ and $s_f > 0$), $R \approx 1$ (Charlesworth *et al.* 1987; Vicoso and Charlesworth 2006). Therefore, a more pronounced faster-X effect is expected for recessive mutations with male-limited fitness effects, whereas no faster-X effect is expected for recessive mutations with female-limited fitness effects (Charlesworth *et al.* 1987; Vicoso and Charlesworth 2006). Baines *et al.* (2008) tested this prediction in *Drosophila* by investigating the evolutionary rate of genes with sex-biased expression, which they assumed had sex-limited fitness effects. Baines *et al.* (2008) found that genes that have male-biased expression show a stronger faster-X effect than unbiased or female-biased genes, as is expected from faster-X theory (Baines *et al.* 2008).

Exposure of recessive mutations in males is not the only process that can create conditions for faster- or slower-X evolution. A different gene content of the X chromosome and the autosomes could underlie differences in their evolutionary rate. For example, the X chromosome might be enriched for classes of genes that evolve rapidly, such as genes that are narrowly expressed (Meisel *et al.* 2012a,b). Moreover, genes on the X chromosome experience global inactivation during spermatogenesis (a process known as meiotic sex chromosome inactivation; MSCI (Lifschytz and Lindsley 1972). Evidence for MSCI in *Drosophila*, birds, and mammals has been documented (Turner 2007; Hense *et al.* 2007; Schoenmakers *et al.* 2009), and it has been

suggested that MSCI could be a universal feature of species with heteromorphic chromosomes (Namekawa and Lee 2009). A recent study showed that genes that escape MSCI and are expressed postmeiotically in spermatogenesis have a higher evolutionary rate than genes that remain silenced postmeiotically in mice (Sin *et al.* 2012). However, it is unknown whether the higher evolutionary rate of escapee than non-escapee genes of MSCI is due to stronger positive selection or weaker selective constraint on these.

In this study, we analyze genome-wide polymorphism data from *Mus musculus castaneus*, a subspecies of the *M. musculus* species complex. A large volume of evidence has been accumulated showing a large effect of the X chromosome on hybrid incompatibilities in *M. musculus* (Tucker *et al.* 1992, p. 92; Oka *et al.* 2004, 2007; Storchová *et al.* 2004; Payseur *et al.* 2004; Teeter *et al.* 2008; Good *et al.* 2008). For example, the X chromosome displays reduced gene flow as compared to the autosomes in the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus* in Europe (Tucker *et al.* 1992; Payseur *et al.* 2004; Teeter *et al.* 2008). Additionally, laboratory crosses between different *M. musculus* strains have revealed a special role for the X in hybrid male sterility (Oka *et al.* 2004, 2007; Storchová *et al.* 2004; Good *et al.* 2008). However, it is unclear whether faster-X sequence evolution can account for the large-X effect that clearly underlies the evolution of reproductive incompatibilities in mouse speciation. Here, we contrast within-species polymorphism and between-species divergence for ~19,000 protein-coding genes and quantify the relative rates of adaptive protein evolution between the autosomes and the X chromosome. To test faster-X theory predictions, we investigate the evolution of genes that have biased expression in sex-specific tissues and of genes that are expressed at various stages of spermatogenesis.

Materials and Methods

Sampling of mice

We generated genomic sequences for 10 *M. musculus castaneus* individuals collected in northwest India (Baines and Harr 2007), seven females and three males. The sampling strategy is detailed in a previous study (Halligan *et al.* 2010) and was aimed at sampling nonrelated individuals from a single population. Tests for population structure and admixture (using the program *STRUCTURE*; Pritchard *et al.* 2000), which were conducted in a previous study, had shown no evidence for hidden population substructure or admixture between differentiated subspecies in our population sample (Halligan *et al.* 2010). We also sequenced the genome of an individual *Mus famulus* obtained from the Montpellier wild mice genetic repository to use as an outgroup.

Genome sequencing and Illumina read mapping

Illumina paired-end sequencing libraries were generated for each individual with fragment sizes 300–550 bp. Mapped

sequence coverage was 21–42× (average 29×) per sampled animal. The libraries were run at a mixture of 76-, 100-, and 108-bp read lengths on the Illumina GAIIx and HiSeq platforms. The program *SMALT* (<http://www.sanger.ac.uk/resources/software/smalt/>) using the parameters: -k 13 -s 6 was used to align the *M. m. castaneus* Illumina sequencing reads to the NCBI37/mm9 unmasked reference genome. We also generated genomic sequence for *M. famulus* to be used as an outgroup. Since *M. famulus* sequence is diverged from the reference (NCBI37/mm9), we used an iterative mapping procedure to improve alignment to the reference. More details on the iterative mapping procedure are given in Halligan *et al.* (2013).

SNP calling

We used the *SAMtools* package to call genotypes at each site (Li *et al.* 2009). This involves creating genotype-likelihood files using *mpileup* and obtaining SNP calls for every site in the genome using an iterative Bayesian approach with *bcftools*. More details on the procedure to call SNPs are given in Halligan *et al.* (2013). We excluded genotype calls that had no mapped reads or where there was significant evidence for departure from Hardy–Weinberg proportions (a cutoff of <0.0002 was used on the *P*-value of a χ^2 -based test obtained using *SAMtools*). For the X chromosome, SNP calls were made using females only, because *SAMtools* assumes diploids. Therefore, we had an allelic coverage of 20 for the autosomes and 14 for the X chromosome.

Obtaining the sequences for protein-coding genes

We obtained gene coordinates from the Ensembl database v. 62 (<http://apr2011.archive.ensembl.org/index.html>) for a total of 18,110 autosomal and 700 X-linked protein-coding genes with orthologs in both mouse and rat. For each gene, we obtained the coordinates for the canonical splice-form as annotated in the Ensembl database. We used these to obtain gene sequences for rat and to construct sequences for *M. m. castaneus* and *M. famulus* individuals based on their genotype calls. We then created separate alignments for each gene using MAFFT (Katoh *et al.* 2002) based on the translated amino-acid sequences and back-translated them to the DNA sequence to preserve the coding frame. We considered only zero-fold and four-fold degenerate sites as nonsynonymous and synonymous, respectively.

The site-frequency spectrum and summary statistics

We obtained the frequencies of the segregating alleles for each polymorphic site in our population sample by assuming that all sites are biallelic, while excluding sites where more than two alleles were present in our sample. We obtained the folded SFS by summing the sites over all possible minor allele frequencies. We did not use the unfolded site-frequency spectrum (SFS) in our analysis because the small number of genes on the X chromosome

meant that the high-frequency bins of the unfolded SFS were excessively noisy.

To summarize diversity, we calculated the average per-site heterozygosity π (Tajima 1983). We quantified the relative skew of the SFS compared to what is expected at Wright–Fisher equilibrium and an infinite-sites mutation model by calculating Tajima's *D* (Tajima 1989). Note that we bootstrapped by gene with replacement 1000 times to perform statistical comparisons of *D* between different classes of sites or with zero. We used *M. famulus* and the rat as outgroups to calculate between-species nucleotide divergence. For the polymorphic sites in *M. m. castaneus*, we calculated the average divergence between the *M. m. castaneus* alleles at a site with the outgroup base, accounting for their frequencies. We applied a Jukes–Cantor multiple hits correction to the divergence estimates (Jukes and Cantor 1969). CpG dinucleotides have higher mutation rates in mammals, and their frequency is higher close to and within genes than noncoding DNA that is far away from genes (Arndt *et al.* 2003). For analyses, we excluded sites that were preceded by C or followed by a G, as suggested by a previous study (Gaffney and Keightley 2008), unless specifically noted.

Assumption of neutral evolution for synonymous sites

We used synonymous sites of protein-coding genes as the neutral class for our analyses. Current evidence suggests the presence of very small selective constraints at synonymous sites of murids (Eory *et al.* 2010); therefore, we do not expect substantial underestimation of the strength of selection at nonsynonymous sites of autosomal and X-linked genes. However, if the selection pressure on synonymous sites is different between autosomal and X-linked genes, it is possible that we will obtain artificial evidence for faster- or slower-X evolution, as has been suggested previously for *Drosophila melanogaster* (Campos *et al.* 2013). A previous study that examined patterns of codon-usage bias in autosomal and X-linked genes of rodents found no evidence that codon-usage bias is due to selection for either autosomal or X-linked genes (Smith and Hurst 1999). Therefore, we do not expect to misinfer the relative strength of selection on nonsynonymous sites of autosomal and X-linked genes due to a different strength of selection on synonymous sites of autosomal and X-linked genes.

Estimating the distribution of fitness effects of new deleterious mutations

To infer the distribution of fitness effects (DFE), we used a maximum-likelihood (ML) method (*DFE-alpha*) that fits a selection and a demographic model to the SFSs of assumed selected and neutral classes of sites, respectively (Keightley and Eyre-Walker 2007). We used synonymous sites of protein-coding genes as the neutral class for our analyses and nonsynonymous sites as the selected class of sites.

Using *DFE-alpha*, we first fitted a two-epoch demographic model of a step change in population size in the past to the neutral SFS. It has previously been shown that bottlenecks

or population subdivision do not greatly affect the accuracy of inference of selection by *DFE-alpha* if a two-epoch model is fitted to the neutral SFS (Keightley and Eyre-Walker 2010; Kousathanas and Keightley 2013). Nevertheless, we also fitted a three-epoch demographic model to the neutral SFS to investigate whether our results are robust to a more complex demographic history and to investigate the possibility of a bottleneck in the studied population.

Using *DFE-alpha* we then fitted a gamma distribution to the selected SFS to infer the DFE of deleterious mutations. We assumed that new mutations in the selected class are unconditionally deleterious. In natural populations some fraction of new mutations might be advantageous; however, it has previously been shown that these will not affect the estimates of the parameters of the DFE for deleterious mutations (Keightley and Eyre-Walker 2010). We also fitted multispike distributions to the nonsynonymous data to investigate whether our results are robust to a multimodal DFE. Moreover, *DFE-alpha* assumes that sites are unlinked, which could affect its inferences. However, it has previously been shown that the effect of linkage can be taken substantially into account by fitting a two-epoch demographic model to a neutral reference that is interdigitated with the selected sites (Kousathanas and Keightley 2013; Messer and Petrov 2013).

Measuring the rate of molecular adaptation

To infer the rate of adaptive divergence between two species, we use an extension of the McDonald–Kreitman (MK) test (McDonald and Kreitman 1991). The standard MK test compares the ratio of nonsynonymous to synonymous divergence (d_N/d_S) between two species with the ratio of nonsynonymous to synonymous polymorphism (p_N/p_S) within a species. Because positively selected mutations are not expected to contribute substantially to polymorphism, an excess of d_N/d_S relative to p_N/p_S is interpreted to be the result of adaptive substitutions. The rate of molecular adaptation is usually quantified by calculating the proportion of substitutions that have been fixed by positive selection (α) as

$$\alpha = \frac{d_N - d_S(p_N/p_S)}{d_N} \quad (4)$$

(Fay *et al.* 2001; Smith and Eyre-Walker 2002), where d_N is the observed nonsynonymous divergence between two species and $d_S(p_N/p_S)$ is the expected divergence explained by neutral and slightly deleterious mutations.

When comparing estimates of α between different classes of genes or between different species, differences in α can be due to a difference in the contribution of slightly deleterious mutations to d_N rather than a different rate of adaptive substitution. This can be controlled for by calculating the rate of adaptive relative to neutral substitution (ω_a):

$$\omega_a = \frac{d_N - d_S(p_N/p_S)}{d_S} \quad (5)$$

(Gossmann *et al.* 2010).

Given the inferred DFE from the polymorphism data, we can calculate the average fixation probability of new deleterious and neutral nonsynonymous mutations relative to the fixation probability of neutral synonymous mutations ($\bar{u}$) by integrating over the DFE. We modified Equations 4 and 5 and calculated α and ω_a as

$$\alpha = \frac{d_N - d_S \bar{u}}{d_N} \quad (6)$$

$$\omega_a = \frac{d_N - d_S \bar{u}}{d_S} \quad (7)$$

(Eyre-Walker and Keightley 2009). There are two advantages of using fixation probabilities to infer α and ω_a . First, slightly deleterious mutations that can contribute disproportionately to polymorphism in the selected class, leading to underestimation of α and ω_a , are explicitly modeled. Second, by using the framework to estimate the DFE as detailed above, the recent demographic history of the population can be taken into account. Demographic changes can produce a signal in the polymorphism data that can bias estimates of α and ω_a (Eyre-Walker 2002).

Keightley and Eyre-Walker (2012) had previously shown that the estimates of α and ω_a can be biased if the divergence between the species compared is low relative to within-species polymorphism (Keightley and Eyre-Walker 2012). We corrected the divergence estimates for the contribution of polymorphism by using their suggested approach (Keightley and Eyre-Walker 2012). Unless otherwise stated, our estimates of the d_N/d_S ratio, α and ω_a , are all corrected using that method.

We also used nonparametric estimators to calculate α , because they can be potentially more powerful when analyzing small numbers of loci. We used the program *MKtest* (Welch 2006) to calculate α_{FWW} and α_{SEW} developed by Fay *et al.* (2001) and Smith and Eyre-Walker (2002), respectively. The first estimator (α_{FWW}) is calculated by summing the counts of divergent and polymorphic nonsynonymous and synonymous sites (D_N , P_N , D_S , P_S , respectively) across genes and using the following equation:

$$\alpha_{\text{FWW}} = 1 - \frac{D_S P_N}{D_N P_S} \quad (8)$$

The α_{FWW} estimator has been shown to be biased if there is a correlation between selective constraint and diversity. The α_{SEW} estimator has been introduced by Smith and Eyre-Walker (2002) to control for this bias by averaging D_N , P_N , D_S , P_S across genes and using the following equation:

$$\alpha_{\text{SEW}} = 1 - \frac{\overline{D_S} \overline{P_N}}{\overline{D_N} \overline{P_S + 1}} \quad (9)$$

Estimates for α_{FWW} and α_{SEW} were not corrected for the contribution of polymorphism to divergence.

Table 1 Number of sites and summary statistics for nonsynonymous and synonymous sites of autosomal and X-linked loci

Cpg-prone status	Site class	Chr.	No. sites (Mbp)	% π	% d (<i>M. famulus</i>)	% d (rat)	Tajima's D
All sites	Nonsyn	A	17.7	0.136 [0.133, 0.139]	0.590 [0.579, 0.601]	3.64 [3.58, 3.70]	−0.906 [−0.928, −0.884]
	Nonsyn	X	0.634	0.057 [0.05, 0.063]	0.594 [0.537, 0.658]	3.91 [3.53, 4.39]	−0.829 [−0.954, −0.704]
	Syn	A	4.33	0.849 [0.839, 0.857]	3.53 [3.51, 3.56]	18.2 [18.1, 18.3]	−0.609 [−0.623, −0.595]
	Syn	X	0.147	0.372 [0.348, 0.397]	2.70 [2.60, 2.81]	15.0 [14.3, 15.7]	−0.636 [−0.731, −0.542]
Non-CpG-prone	Nonsyn	A	9.59	0.125 [0.122, 0.128]	0.575 [0.565, 0.586]	3.45 [3.39, 3.51]	−0.817 [−0.843, −0.790]
	Nonsyn	X	0.370	0.0536 [0.0470, 0.0607]	0.592 [0.533, 0.656]	3.72 [3.36, 4.16]	−0.833 [−0.983, −0.674]
	Syn	A	1.49	0.631 [0.622, 0.641]	2.73 [2.71, 2.76]	14.8 [14.8, 14.9]	−0.643 [−0.665, −0.619]
	Syn	X	0.0495	0.306 [0.276, 0.335]	2.34 [2.21, 2.49]	13.7 [13.2, 14.3]	−0.726 [−0.898, −0.562]

Statistics are given for all sites and non-CpG-prone sites. Nucleotide divergence (d) is not corrected for the contribution of polymorphism. 95% confidence intervals are given in brackets.

Statistical testing

Confidence intervals for parameter estimates were obtained by bootstrapping by gene 200 times, unless otherwise stated. To compare different classes of genes, we performed a nonparametric bootstrap test and unless otherwise stated, the two-tailed P -value is reported. Mann–Whitney U -tests were performed using R (<http://www.r-project.org/>).

Analysis of gene expression

To define functional categories of genes, we analyzed several gene expression data sets from microarray experiments. We used the GNF gene expression atlas (Su *et al.* 2004) to define male- and female-specific genes. This data set contains measurements of gene expression for several thousand mouse and human genes in a large number of tissues (61 in mice). For this data set, we defined a gene as expressed in a tissue when its expression value was higher than the median (=140.5) for the whole microarray experiment, following the authors' suggestions (Su *et al.* 2004). We defined a gene as specifically expressed in a tissue when the expression of that gene in the focal tissue was twofold higher than the median expression of the gene over all tissues, excluding the focal tissue. Male-specific genes were defined as those that specifically expressed in testis or prostate, whereas female-specific ones were defined as those with expression specifically in ovary or uterus. To calculate the expression breadth (τ) of each gene in our data set, we used the formula

$$\tau = \sum_{i=1}^N \frac{1 - \log T_i}{\log T_{\max}} / (N - 1), \quad (10)$$

where N is the number of tissues examined, T_i is the expression value in each tissue, and $T_{\max}$ is the maximum expression over all tissues (Liao *et al.* 2006).

To define genes expressed at different stages of spermatogenesis, we used the data set of Namekawa *et al.* (2006). This contains gene expression measured in four types of germ cells, corresponding to different stages of spermatogenesis. These are A and B spermatogonia, pachytene spermatocytes, and round spermatids. A and B spermatogonia correspond to the

early premeiotic stage of spermatogenesis (stage 1), pachytene spermatocytes represent the stage where meiotic sex inactivation of the X chromosome (MSCI) occurs (stage 2), and round spermatids are mature postmeiotic cells (stage 3). The data set contains gene expression levels for each cell type computed from microarray signal intensities. Expression values had been scaled to a trimmed mean signal intensity of 125 for each microarray chip. There were two replicates per cell type and we averaged the expression levels of the replicates. Genes that had a signal intensity <100 at all stages of spermatogenesis were considered as not expressed during spermatogenesis (following suggestions of Namekawa *et al.* 2006). A gene was considered as expressed during a stage if its expression value was >125. We defined three groups of genes based on their expression during stage 1 and stage 3: group A for genes that are expressed in stage 1, and not in stage 3, group B for genes that are expressed during both stages 1 and 3, and group C for genes that are nonexpressed in stage 1 and expressed in stage 3. These groups of genes correspond roughly to the groups defined by Namekawa *et al.* (2006).

Results

Diversity and divergence for autosomal and X-linked loci

We analyzed polymorphism within *M. m. castaneus* and divergence from *M. famulus* and the rat at nonsynonymous and synonymous sites in a total of 18,110 autosomal and 700 X-linked protein-coding loci. We examined results for all sites and non-CpG-prone sites separately. The pairwise nucleotide diversity at synonymous sites (π_s) is substantially lower for X-linked than for autosomal loci ($P < 0.01$; Table 1). X-linked synonymous site divergence (d_s) from *M. famulus* and rat is also significantly lower than that of the autosomes ($P < 0.01$ for all comparisons; Table 1). If we assume that synonymous sites evolve neutrally, then π_s is proportional to the product of the effective population size (N_e) and the mutation rate (μ). Therefore, the lower π_s for X-linked loci relative to the autosomes could be attributed to a lower N_e or μ . After controlling for a difference in μ between the chromosomes by dividing π_s with d_s from

Figure 1 The site-frequency spectra (SFS) for nonsynonymous and synonymous site classes of autosomal and X-linked genes. Shading indicates the expected SFS for an equilibrium population under a neutral Wright–Fisher model of evolution. The SFSs are for non-CpG prone sites.

M. famulus, we obtained a diversity ratio (X/A) equal to 0.58, which should reflect the ratio of effective population sizes between the X and the autosomes (*i.e.*, N_{eX}/N_{eA}). The X chromosome is found in two copies in females and one copy in males, and as a result, this ratio is expected to be 0.75 under neutrality and if variance in reproductive success is equal between males and females. The observed N_{eX}/N_{eA} is significantly lower than this expectation ($P < 0.01$ for all sites and non-CpG-prone sites comparisons, using either *M. famulus* or rat to calculate divergence). The observed reduction in X-linked diversity could be explained by a bottleneck (Wall *et al.* 2002; Pool and Nielsen 2007), by unequal variance in reproductive success between males and females (Charlesworth 2001) or by a larger effect of selective sweeps or background selection in eliminating X-linked synonymous diversity (Betancourt *et al.* 2004; Charlesworth 2012).

We then obtained the folded or minor allele SFS for each site class by summing the minor allele frequency over all sites per class (Figure 1). We also generated the expected SFS for a population at equilibrium under a neutral Wright–Fisher

model for comparison (Figure 1). We observed a deviation from the equilibrium expectation for autosomal and X-linked genes for both synonymous and nonsynonymous sites (Figure 1), consistent with the observed Tajima’s D values, which are negative for all site classes (Table 1). If we consider that synonymous sites are selectively neutral, then their negative D -values support either that the population has experienced a population expansion or, alternatively, that there is an effect of Hill–Robertson interference from nearby sites under selection (Hill and Robertson 1966).

Model fitting to infer demography and selection

We estimated the distribution of fitness effects of new deleterious mutations (DFE) for autosomal and X-linked loci by applying a maximum-likelihood approach (*DFE-alpha*) that fits a demographic and a selection model to the SFSs from neutral and selected classes of sites (Keightley and Eyre-Walker 2007). We used synonymous sites to infer effects of population size changes and nonsynonymous sites to infer selection. A two-epoch demographic model gave a good fit to autosomal and X-linked synonymous data (Supporting Information, Figure S1), and a three-epoch model produced only a marginally better fit (Table S1 and Figure S1).

We then fitted several types of models to the non-synonymous data to infer the DFE (Table S1 and Figure S2). A model with three discrete selection coefficients had a better fit to the autosomal data than the gamma distribution (Table S1). However, the three-spike model did not fit substantially better than the gamma distribution to the X-linked data (Table S1). For consistency of the analysis of autosomal and X-linked loci we used the gamma model to infer the DFE, while controlling for the population history inferred from the two-epoch model. Note that we investigate below the effect of fitting different models of the DFE and different demographic models on our inferences.

The DFE for autosomal and X-linked loci

The inferred parameters for the best-fitting two-epoch and gamma distribution models are given in Table 2. The two-epoch model gave evidence of a population expansion for both autosomal and X-linked loci (Table 2). Even though our analysis included several thousand genes, the mean strength of selection for deleterious mutations ($N_e E(s)$) was very imprecisely estimated for both autosomal and

Table 2 Estimates and 95% confidence intervals for parameters of the two-epoch demographic model and the gamma DFE for autosomal and X-linked loci

Chr.	Demography (two-epoch)		Selection (gamma DFE)	
	N_2/N_1	t_2/N_1	$N_e E(s)$	b
A	2.79 [2.79, 3.07]	1.47 [1.3, 2.09]	9.47×10^5 [2.74×10^5 , 3.36×10^7]	0.11 [0.088, 0.13]
X	4.09 [3.07, 7.26]	2.19 [0.85, 6.14]	3.73×10^8 [1.23×10^4 , ∞]	0.085 [$\rightarrow 0$, 0.21]

The two-epoch model parameters are the magnitude of a population size change (N_2/N_1) and the time in generations since the size change (t_2/N_1), and are scaled by N_1 , which is the initial size of the population. The parameter estimates for the gamma DFE are the mean strength of selection ($N_e E(s)$) and the shape (b) of the distribution.

Figure 2 The distribution of fitness effects of new nonsynonymous mutations binned into four classes of effects for autosomal and X-linked genes. The estimates are for non-CpG prone sites; 95% confidence intervals were generated by bootstrapping by gene.

X-linked loci (indicated by the very wide confidence intervals; Table 2). The point estimate for $N_e E(s)$ for the X chromosome is very high (3.73×10^8). Even if we assume an N_e of 10^6 for *M. m. castaneus*, the $E(s)$ value would still be >100 . This high value for $E(s)$ should not be considered realistic, but rather an artifact of the method to estimating the DFE. When the inferred gamma distribution is highly leptokurtic there is a disproportionately large contribution of mutations with strong effects to the mean, and since *DFE-alpha* allows for $s > 1$, $E(s)$ could also be inferred to be much higher than 1. The shape parameter (b) was estimated with more precision than $N_e E(s)$ and indicated a strongly leptokurtic DFE for both autosomal and X-linked loci (Table 2). Overall, we did not observe significant differences in the parameters of the DFE between autosomal and X-linked loci (Table 2).

We also compared the proportion of mutations assigned to four $N_e s$ ranges and found that they did not differ between X-linked and autosomal loci (Figure 2). These results suggest that the efficacy of purifying selection acting on nonsynonymous mutations is similar in X-linked and autosomal genes on average. This is unexpected, given that we infer that the X chromosome N_e is smaller than three-

quarters that of the autosomal genes and therefore experiences a stronger effect of drift. One possible explanation is that new deleterious mutations are on average recessive and therefore are removed more efficiently from the X than from the autosomes. It is possible that these two processes (smaller N_e of the X chromosome and recessivity of new deleterious mutations) cancel each other to some extent.

Evolution of autosomal and X-linked loci

The inferred parameters of the DFE can be used together with the divergence between two species to infer the proportion of adaptive substitutions and the rate of adaptive relative to the rate of neutral substitution (α and ω_a respectively, calculated using Equations 4 and 5). We considered significantly different ω_a values between two compared classes of genes as indicating different rates of adaptive evolution, but we also computed and compared d_N/d_S and α because these are more widely used than ω_a and can thus be compared with other studies. We compared estimates of d_N/d_S , α and ω_a for nonsynonymous sites of autosomal and X-linked genes (Table 3). X-linked loci have significantly higher d_N/d_S , α , and ω_a than autosomal loci ($P < 0.05$ for all parameter comparisons between autosomes and the X, using either *M. famulus* or rat as the outgroup; Table 3). These results provide strong support for faster X adaptive protein evolution. Faster-X evolution was also inferred when using a three-epoch model to infer the demographic history or with a DFE model consisting of three point masses (Table S2) or when using nonparametric estimators of α ("All" in Figure S3).

Evolution of male- and female-specific genes

The faster-X effect is expected to be more pronounced if selection acts on males only, whereas equal rates of adaptive evolution are expected if selection acts on females only (Charlesworth *et al.* 1987; Vicoso and Charlesworth 2006). To investigate this prediction, we compared rates of adaptive evolution for genes that are narrowly expressed in sex-specific tissues, which we assumed had sex-limited fitness effects. We used gene expression data for several tissues of mice from the atlas of gene expression (Su *et al.* 2004) to define those categories of genes. We defined male-specific genes as those with specific expression in testis or prostate and female-specific genes as those with specific expression in ovary or uterus. Genes that were not narrowly expressed in either male- or female-specific tissues were defined as non-sex-specific.

Table 3 Estimates of % d_N , % d_S , d_N/d_S , α , and ω_a for autosomal and X-linked genes using *M. famulus* and rat as outgroups

Outgroup	Chr.	% d_N	% d_S	d_N/d_S	α	ω_a
<i>M. famulus</i>	A	0.367 [0.358, 0.378]	1.54 [1.46, 1.55]	0.238 [0.235, 0.255]	0.316 [0.289, 0.365]	0.0753 [0.0695, 0.0923]
	X	0.506 [0.451, 0.564]	1.76 [1.61, 1.91]	0.287 [0.257, 0.330]	0.480 [0.363, 0.646]	0.138 [0.0983, 0.201]
Rat	A	3.23 [3.18, 3.30]	13.4 [13.3, 13.5]	0.241 [0.238, 0.246]	0.322 [0.284, 0.347]	0.0774 [0.0687, 0.0843]
	X	3.63 [3.26, 3.97]	13.1 [12.6, 13.5]	0.278 [0.252, 0.300]	0.463 [0.356, 0.617]	0.129 [0.0956, 0.172]

The estimates are for non-CpG prone sites and are corrected for the contribution of polymorphism to divergence. 95% confidence intervals are given in brackets.

Figure 3 Molecular evolution (A) and breadth of expression (B) of genes that have male- or female-specific expression and non-sex-specific expression. (A) Estimates for d_N/d_S , α , and ω_a were calculated using *M. famulus* as the outgroup and were corrected for the contribution of polymorphism to divergence. Error bars are 95% confidence intervals (CIs) obtained by bootstrapping by gene. Two-tailed bootstrap tests were performed to compare d_N/d_S , α , and ω_a estimates with the autosomal average (indicated by the dashed line) and between autosomal and X-linked genes of each class. Asterisks indicate significance for the comparison to the autosomal average (*, $P < 0.05$; **, $P < 0.01$). (B) Boxes indicate 25th and 75th percentiles of the distribution of τ and whiskers are ~95% CIs. Solid line within boxes indicates the median τ and notches are ~95% CIs for the median. Dashed line indicates the genomic average τ . A Mann–Whitney *U*-test was performed to compare median τ between autosomal and X-linked genes of each class. (A and B) Signs indicate significance for the comparisons between autosomal and X-linked genes (one sign, $P < 0.05$; two signs, $P < 0.01$).

We found significantly faster-X adaptive evolution for male- but not for female-specific genes (Figure 3A). Estimates for d_N/d_S , α , and ω_a were not significantly different between the autosomes and the X chromosome for non-sex-specific genes (Figure 3A). This could be due to lack of

power to detect a significant difference in ω_a between the autosomes and the X chromosome or due to a substantial contribution of male-specific genes to the faster-X effect observed for all genes. We obtained similar results for male- and female-specific genes using nonparametric estimators of α , although genes lacking sex-specific expression showed significantly faster-X evolution (“Sex-specific” in Figure S3). Defining sex bias as a twofold difference in expression between testis and ovary (following Zhang *et al.* 2010) produced similar results. Male-biased and -unbiased genes had significantly higher ω_a for the X chromosome than the autosomes, whereas female-biased genes did not have significantly different ω_a for autosomal and X-linked genes (Figure S4).

Previous studies have shown that narrowly expressed genes have a higher d_N/d_S than widely expressed genes (Liao *et al.* 2006) and that the X chromosome is enriched for genes with narrow expression (Meisel *et al.* 2012b). To investigate whether a difference in tissue specificity between autosomal and X-linked genes could affect our results, we calculated the breadth of expression (τ) using Equation 10. Small values of τ correspond to broad expression, whereas large values correspond to narrow expression. We found that τ is not significantly different between autosomal and X-linked genes that have male- or female-specific expression (Mann–Whitney *U*-test $P > 0.05$; Figure 3B). X-linked genes that were not male- or female-specific had a significantly higher τ than autosomal genes (Mann–Whitney *U*-test $P < 0.01$; Figure 3B). Therefore, a narrower breadth of expression of X-linked than autosomal genes might partially account for faster-X evolution of genes that are non-male- or female-specific, but cannot account for faster-X evolution of the male-specific genes.

Evolution of genes expressed during spermatogenesis

During spermatogenesis, each diploid spermatogonium cell undergoes two rounds of meiosis to give four haploid spermatids. Only X-linked recessive mutations are exposed early in spermatogenesis (premeiotically), whereas both X-linked and autosomal recessive mutations are exposed late in spermatogenesis (postmeiotically). Moreover, during the first meiosis in spermatogenesis (meiosis I), X-linked genes experience global suppression of their expression (MSCI; Lifschytz and Lindsley 1972). However, a few X-linked genes escape MSCI and are expressed postmeiotically (Namekawa *et al.* 2006). Based on these considerations, faster-X evolution would be expected for genes expressed during early spermatogenesis and not for genes expressed exclusively late in spermatogenesis.

We obtained gene expression data for male germ cells at different spermatogenetic stages (data set of Namekawa *et al.* 2006) to investigate the evolutionary rate of genes that have different expression patterns during spermatogenesis. We defined three groups of genes: genes that are expressed premeiotically and suppressed postmeiotically (group A; Figure 4A), genes that are expressed both premeiotically and postmeiotically (group B; Figure 4A), and genes that

are suppressed premeiotically and expressed postmeiotically (group C; Figure 4A). We then calculated d_N/d_S , α and ω_a for autosomal and X-linked genes within each group. We found that group A genes had similar d_N/d_S , α , and ω_a for the X chromosome and the autosomes (Figure 4B). This is unexpected, because new advantageous X-linked recessive mutations are exposed in males if they occur in group A genes. However, genes with exclusively early expression have been reported to be female biased (Zhang *et al.* 2010). Female-biased genes are expected to evolve at similar rates for the autosomes and the X chromosome (Charlesworth *et al.* 1987; Vicoso and Charlesworth 2009). Therefore, the observation that group A X-linked and autosomal genes evolve at a similar rate does not necessarily contradict faster-X theory.

Group B genes had significantly higher d_N/d_S , α , and ω_a for the X chromosome than for the autosomes (Figure 4B). For group B, X-linked genes have a different expression profile than autosomal genes, because MSCI affects only the X chromosome (Figure 4A). Therefore, X-linked and autosomal genes within group B might not be comparable. However, X-linked genes within group B have significantly higher α and ω_a than the autosomal average (Figure 4B). The rapid adaptive evolution of X-linked group B genes might be related to their escape from MSCI.

Group C genes had similar d_N/d_S , α , and ω_a for the X chromosome and the autosomes (Figure 4B). This is expected, since cells are haploid late in spermatogenesis and recessive mutations are therefore exposed on both the autosomes and the X chromosome. Nonparametric estimators of α produced equivocal results (“Sperm” in Figure S3). The Fay *et al.* (2001) estimator (α_{FWW}) showed similar results to *DFE-alpha*, but the Smith and Eyre-Walker (2002) estimator (α_{SEW}) showed significantly higher α for X-linked than autosomal genes for all classes of genes examined (“Sperm” in Figure S3). However, both estimators showed α to be significantly higher than zero for X-linked genes of group B and autosomal genes of group C, which is consistent with our findings when using *DFE-alpha*. All methods consistently show rapid adaptive evolution for X-linked genes that escape MSCI and also autosomal genes that are postmeiotically expressed.

Discussion

The first main finding of this study is that the X chromosome has significantly lower synonymous diversity than expected for a population in Wright–Fisher equilibrium with equal variance in reproductive success between males and females. We discuss below the possible causes of the reduced diversity on the X chromosome. Our second main finding is that the X chromosome displays a higher rate of adaptive protein evolution than the autosomes. We discuss how violation of the assumption of neutrality for synonymous sites would affect this finding. We also found that X-linked genes with male-specific expression evolve particularly rapidly, whereas X-linked and autosomal genes with female-specific expression evolve at similar rates. We discuss

two possible causes of these observations: first that new advantageous mutations are on average recessive and second that genes expressed during spermatogenesis evolve faster on the X than the autosomes due to genetic conflict. We then proceed to discuss how faster-X adaptive evolution may contribute to the large effect of the X chromosome in speciation (large-X; Coyne and Orr 2004).

The causes of reduced diversity on the X chromosome

The X chromosome is expected to have 75% of the autosomal diversity if the population is at equilibrium and males and females have equal reproductive success. After controlling for a difference in mutation rate between chromosomes, we observed a X/A diversity ratio significantly lower than this expectation (58%). This could be explained by unequal variance in reproductive success between males and females (Charlesworth 2001), by population size reductions or bottlenecks (Wall *et al.* 2002; Pool and Nielsen 2007), or by a stronger effect of selective sweeps or background selection in eliminating X-linked synonymous diversity (Betancourt *et al.* 2004; Charlesworth 2012).

If females have a larger variance in reproductive success than males, then the female N_e is expected to be smaller than male N_e . This could produce a X/A diversity ratio smaller than 0.75. Comparisons of autosomal and mitochondrial diversity in populations of *M. musculus* have shown evidence for a larger female N_e (Baines and Harr 2007), which would imply a X/A diversity ratio >0.75 (i.e., contrary to our observation). Unequal variance in reproductive success between males and females is therefore an unlikely explanation for the observed X/A diversity ratio.

Population size reductions or bottlenecks have been shown to reduce the X/A diversity ratio (Wall *et al.* 2002; Pool and Nielsen 2007). A two-step demographic model gave a good fit to the autosomal and X-linked synonymous SFSs, providing evidence for a population expansion, which has been shown to increase the X/A diversity ratio (Pool and Nielsen 2007). However, given that synonymous polymorphism can also be affected by selection on linked sites, and this could bias demographic inference, we do not consider our inference of an expansion necessarily realistic. Therefore, we cannot definitively exclude the possibility of a bottleneck in the history of *M. m. castaneus*, which could at least partially explain the reduced diversity on the X chromosome.

Finally, the X chromosome may experience a stronger effect of selective sweeps or background selection than the autosomes, reducing neutral diversity linked to selected loci. A stronger effect of selective sweeps on neutral diversity is plausible, given that we find a higher rate of adaptive evolution on the X than the autosomes.

Assumption of neutrality for synonymous sites

In our analysis we assumed that synonymous sites evolve neutrally. Violation of this assumption could artificially produce signatures of faster-X substitution. For example, in

Figure 4 Expression pattern (A) and molecular evolution (B) of three groups of genes expressed during spermatogenesis. (A) Group A are genes that are expressed exclusively premeiotically, group B are genes that are expressed during premeiosis and postmeiosis, and group C are genes that are expressed exclusively postmeiotically. Boxes indicate 25th and 75th percentiles of log₂ expression intensity and whiskers are ~95% CIs. Solid line within boxes indicates median expression intensity and notches are ~95% CIs for the median. The dashed line indicates the expression intensity threshold that was used to define a gene as being expressed. (B) Estimates for d_N/d_S , α , and ω_a for group A, group B, and group C. d_N/d_S , α , and ω_a were calculated by using *M. famulus* as the outgroup and were corrected for the contribution of polymorphism to divergence. Error bars are 95% CIs obtained by bootstrapping by gene. Two-tailed bootstrap tests were performed to compare d_N/d_S , α , and ω_a estimates with the autosomal average (indicated by the dashed line) and between autosomal and X-linked genes of each class. Asterisks indicate significance for the comparison to the autosomal average (*, $P < 0.05$; **, $P < 0.01$). Signs indicate significance for the comparisons between autosomal and X-linked genes (one sign, $P < 0.05$; two signs, $P < 0.01$).

Drosophila, selection on codon usage is stronger on the X chromosome than on the autosomes (Singh *et al.* 2005). It has been suggested that the documented faster-X effect in *D. melanogaster* (Mackay *et al.* 2012) could partially be due to a lower d_S for X-linked genes than for autosomal genes (Campos *et al.* 2013).

In contrast to *Drosophila* where selection on codon usage is well established, Smith and Hurst (1999) showed that selection is unlikely to operate on codon usage in mice. Although we did observe a lower d_S for the X chromosome than for the autosomes in our data (Table 1), we believe that this is driven mainly by the lower mutation rate of the X chromosome than of the autosomes. This can be clearly seen when looking at d_S for all sites and non-CpG-prone sites: the ratio $d_S(X)/d_S(A)$ is much closer to 1 when including CpG-prone sites than when excluding CpG-prone sites (Table 1). This is because those sites have a much higher mutation rate in genic regions, therefore making the difference in d_S between X and autosomes much more pronounced when those sites are included.

Moreover, the X chromosome has much lower synonymous diversity than the autosomes even when controlling for their difference in mutation rates. Therefore, when calculating d_S , there is a larger contribution of polymorphism to divergence for autosomal synonymous diversity than for X-linked synonymous diversity. Therefore, the autosomal d_S will be inflated compared to the X-linked d_S if not corrected for the contribution of polymorphism to divergence. To see this effect one can compare d_S calculated in Table 1 without the correction and Table 2 where the correction was applied. The corrected d_S calculated between

M. m. castaneus and *M. famulus* is in fact higher for the X chromosome than the autosomes and d_S calculated between *M. m. castaneus* and rat is not significantly different. Therefore, even if there is selection on codon usage in mice (contrary to the conclusions of Smith and Hurst 1999) and if its strength differs between the autosomes and the X chromosome, it appears to have a minimal effect on our estimate of the ratio of adaptive substitution between X and autosomes and on our conclusion for faster-X evolution.

Dominance of new advantageous mutations

We found that X-linked genes evolve faster than autosomal genes on average and that genes with male-specific expression display faster-X evolution, while genes with female-specific expression do not. These observations are compatible with the predictions of the faster-X theory as outlined in the Introduction. We can use our estimate of the ratio of the rate of adaptive substitution of X-linked to autosomal loci ($R = \omega_{aX}/\omega_{aA}$) to predict the average dominance coefficient (h) for new, advantageous mutations that would explain our observed R . We used Equation 3 for all genes and genes with no sex-specific expression, and Equation 3 for genes with male-specific expression. The predicted value of h when using Equations 2 and 3 is ~0.2 (Table 4). However, these equations require the assumption that $N_{eX}/N_{eA} = 0.75$ and our results showed that this is unlikely to be true. Vicoso and Charlesworth (2009) who investigated the relationship between R and h for varying N_{eX}/N_{eA} showed that average recessivity ($h < 0.5$) of new advantageous mutations would be expected even for $R = 1$, if $N_{eX}/N_{eA} < 0.75$ (Vicoso and

Charlesworth 2009). Given that we estimated $N_{eX}/N_{eA} < 0.75$, we expect that h for new advantageous mutations should be even smaller than 0.2 to explain our observations. Moreover, Connallon *et al.* (2012) showed that genetic architecture underlying bouts of adaptive substitution can influence the assumptions of the theoretical predictions of Charlesworth *et al.* (1987) and that a contribution of standing variation to adaptive substitution can dampen the predicted relationship between R and h (Connallon *et al.* 2012). Therefore, we would need to assume that a large number of genes ($>>1$) contribute to individual bouts of adaptation and that most adaptive substitutions are from new mutations (Connallon *et al.* 2012) to support the case that the observed faster-X evolution is due to average recessivity of new advantageous mutations with $h < 0.2$. The validity of these assumptions remains to be seen since very little is known about how many genes are involved to individual bouts of adaptation and whether adaptation proceeds mainly through fixation of new mutations or standing variation (Orr 2010; Connallon *et al.* 2012).

Evolution of genes expressed during spermatogenesis

Previous studies have revealed evidence for rapid evolution of genes expressed during spermatogenesis (Torgerson *et al.* 2002; Torgerson and Singh 2006), particularly those expressed postmeiotically (Good and Nachman 2005). A recent study that compared genes expressed during different stages of spermatogenesis in mice and humans found an elevated d_N/d_S for X-linked genes that escape MSCI compared to X-linked genes that do not escape MSCI (Sin *et al.* 2012). Interestingly, another recent study in mice found that the majority of these escapee genes are young additions to the X chromosome (originating <50 MYA; Zhang *et al.* 2010). In our study, we showed that the high d_N/d_S of the escapee genes compared to the non-escapee genes is likely due to a faster rate of adaptive evolution and not due to more relaxed constraint.

Since recessive advantageous mutations are exposed only on the X chromosome early in spermatogenesis, but on both the autosomes and the X chromosome late in spermatogenesis, we would expect faster-X evolution to manifest most strongly for genes that are expressed exclusively early in spermatogenesis. However, we found that autosomal and X-linked genes that are expressed exclusively early in spermatogenesis evolve at similar rates. Therefore, faster-X evolution is unlikely to be explained only by partial recessivity of new advantageous mutations. Our results suggest that the MSCI process, which is unique to the X chromosome, may create conditions for rapid adaptive evolution of certain classes of X-linked genes. For example, such conditions could manifest through genetic conflict between host genes and selfish genetic elements to control expression during spermatogenesis (Presgraves 2008). Recurrent bouts of invasion of selfish genetic elements could trigger an evolutionary arms race with the host to suppress their expression. Therefore, the faster-X evolution that we

Table 4 Quantitative prediction for the dominance coefficient (h) based on our estimated ratio of adaptive substitution for X-linked over autosomal loci ($R = \omega_{aX}/\omega_{aA}$)

Gene class	Expected R	Estimated R	Predicted h
All genes	$R \approx \frac{2h+1}{4h}$	1.8 [1.2, 2.6]	0.19 [0.12, 0.36]
Not sex specific		1.6 [0.78, 2.5]	0.24 [0.13, 0.89]
Male specific	$R \approx \frac{1}{2h}$	2.4 [1.3, 4.3]	0.21 [0.12, 0.38]

We assumed that $N_{eX}/N_{eA} = 0.75$ and that variance in reproductive success is equal between males and females. We calculated R using *M. famulus* as the outgroup. 95% confidence intervals are given in brackets.

observe could be a consequence of a high concentration of genes that evolve rapidly on the X chromosome due to genetic conflict. Male-specific genes and genes that are expressed postmeiotically in spermatogenesis might be more likely to be involved in these arms races, which could explain the more pronounced faster-X evolution of those genes.

Faster-X evolution and the large effect of the X chromosome in speciation

Faster-X evolution due to average recessivity of advantageous mutations could partially or fully explain the large effect of the X chromosome in speciation (large-X). This is because loci that contribute to hybrid incompatibilities will evolve faster when located on the X chromosome than on the autosomes (Presgraves 2008). Future studies should focus on documenting precisely the excess of X-linked relative to autosomal loci that cause hybrid incompatibility in mice and investigate to what extent a $1.8\times$ faster rate of adaptive substitution on the X can explain that excess.

Another explanation for the large-X that is compatible with our data is related to the regulation of genes expressed during spermatogenesis (Presgraves 2008). Spermatogenesis might be a process that is inherently sensitive to perturbation, which is likely to occur in hybrids. For example, a recent study in *M. musculus* found a strong association between X-linked hybrid male sterility and disruption of MSCI (Campbell *et al.* 2013). The phenomenon of MSCI may be universal to species with heteromorphic chromosomes (Namekawa and Lee 2009) and it has been suggested that MSCI evolved as a defense mechanism against selfish genetic elements such as sex-ratio distorters (Meiklejohn and Tao 2010). Therefore, the ultimate cause of the large-X phenomenon could be that genetic conflict manifests more often on the X chromosome than the autosomes. Theoretical studies have shown that sex-ratio distorters are indeed more likely to invade a population when located on the sex chromosomes than the autosomes (Frank 1991; Hurst and Pomiankowski 1991). However, very few empirical studies document selfish genetic elements such as sex-ratio distorters and identify their location in the genome (Meiklejohn and Tao 2010). Future studies should focus on extensive mapping of these elements and further dissection of their potential evolutionary link with MSCI and the large-X.

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Faster-X Adaptive Protein Evolution in House Mice

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Figure S1 The observed synonymous site frequency spectrum and the expectation generated by assuming a stationary population size and two demographic models.

Figure S2 The observed nonsynonymous site frequency spectrum and the expectation generated by assuming no selection, gamma distribution and a distribution consisting of 3 discrete selection coefficients.

Figure S3 Estimates for α using non-parametric estimators. We used the Fay et al. (2001) and Smith and Eyre Walker (2002) estimators (α_{FWW} and α_{SEW} , respectively [3,4]). Autosomal and X-linked genes are compared for all genes (All), genes with sex-specific expression (Sex-specific) and genes with a different expression pattern during spermatogenesis (Sperm.). Estimates were obtained by using *M. famulus* as the outgroup. Error bars are 95% confidence intervals obtained by bootstrapping by gene 10,000 times. Stars indicate significance for $\alpha > 0$ (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Figure S4 Molecular evolution of genes that have male- or female-biased expression and non-sex-biased expression. Estimates for d_N/d_S , α and ω_a were calculated using *M. famulus* as the outgroup. Error bars are 95% confidence intervals (CIs) obtained by bootstrapping by gene. Two-tailed bootstrap tests were performed to compare d_N/d_S , α and ω_a estimates with the autosomal average (indicated by the dashed line), and between autosomal and X-linked genes of each class. Stars indicate significance for the comparison to the autosomal average (* $P < 0.05$, ** $P < 0.01$). Signs indicate significance for the comparisons between autosomal and X-linked genes (one sign; $P < 0.05$, two signs, $P < 0.01$).

Table S1 Goodness of fit of demographic and selection models. The demographic models were fitted to the synonymous sites and the selection models to nonsynonymous sites for autosomal and X-linked genes separately. The log-likelihood difference ($\Delta\log L$) and the corrected akaike information criterion difference (ΔAIC) from the best fitted model is reported. The spike and step models consist of discrete selection coefficients that are fitted to the selected data. To infer the DFE with these models, we incremented the number of spikes/steps until the improvement of fitting additional spikes/steps is less than 2 AIC units. In parentheses we report the number of spikes/steps of the best fitted spike model.

Sites	Chr.	Model	$\Delta\log L$	ΔAIC
Synonymous	A	Stationary	-1,727	-3,446
		2-epoch	-7	-10.1
		3-epoch	0	0
	X	Stationary	-36.7	-68.8
		2-epoch	-0.2	0
		3-epoch	0	-3.5
Nonsynonymous	A	No selection	-24,133.4	-48,256.8
		Gamma	-21.3	-36.5
		Spike (3)	0	0
		Step (2)	-2.8	-1.6
	X	No selection	-407.4	-810.4
		Gamma	-0.2	0
		Spike (3)	0	-5.5
		Step (2)	0	-1.5

Table S2 Point estimates for $\frac{\overline{u_{del}}}{\overline{u_{neu}}}$, α and ω_a when fitting different combinations of demographic and selection models to autosomal and X-linked data. The models used in the present study are highlighted in yellow. We used the rat as an outgroup to calculate α and ω_a . The divergences from *M. m. castaneus* were not corrected for the contribution of polymorphism to divergence because this correction is not implemented yet for some combinations of models. This is not expected to affect our inferences for α and ω_a , because the rat is distantly related to *M. m. castaneus* (~18% synonymous divergence). The models used in the present study are highlighted in yellow.

Chr.	Demographic model	Selection model	$\frac{\overline{u_{del}}}{\overline{u_{neu}}}$	α (rat)	ω_a (rat)
A	2-epoch	Gamma	0.17	0.28	0.065
		Spike (3)	0.19	0.20	0.046
	3-epoch	Gamma	0.16	0.29	0.068
X	2-epoch	Gamma	0.15	0.45	0.12
		Spike (3)	0.17	0.38	0.10
	3-epoch	Gamma	0.15	0.45	0.12