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LINEAGE-SPECIFIC VARIATION IN SLOW- AND FAST-X EVOLUTION IN PRIMATES

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Theories predict that the evolutionary rates of X-linked regions can differ from those of autosomal regions. The male-biased mutation theory predicts a slower rate of neutral substitution on the X chromosome (slow-X evolution), as the X spends less time in male germlines, where more mutations originate per generation than in female germlines. The fast-X theory, however, predicts a faster rate of adaptive substitution on the X chromosome when newly arising beneficial mutations are, on average, partially recessive (fast-X evolution), as the X enjoys a greater efficacy of positive selection. The slow- and fast-X processes are expected to interact as the degree of male-biased mutation can in turn influence the relative rate of adaptive evolution on the X. Here, we investigate lineage-specific variation in, and the interaction of, slow- and fast-X processes using genomic data from four primates. We find consistent evidence for slow-X evolution in all lineages. In contrast, evidence for fast-X evolution exists in only a subset of lineages. In particular, the marmoset lineage, which shows the strongest evidence of fast-X, exhibits the lowest male mutation bias. We discuss the possible interaction between slow- and fast-X evolution and other factors that influence the degrees of slow- and fast-X evolution.

KEY WORDS: **Male mutation bias, effective population size, mating system, life history traits, adaptive evolution.**

Evolutionary theories provide conflicting predictions on the relative evolutionary rates of X-linked loci to those of autosomal loci. A theory based on male mutational bias, or "male-driven evolution," predicts that the X chromosome will accumulate neutral substitutions at a slower rate than autosomes (Miyata et al. 1987; Li et al. 2002; Ellegren 2007). Mutation rates in the male germline are generally higher than those in the female germline, owing to the greater number of replicative germ cell divisions involved in the production of sperm versus eggs per generation (Haldane 1947; Penrose 1955; Drost and Lee 1995). As X chromosomes spend less time in males than autosomes, the X experiences fewer mutations and hence fewer neutral substitutions. Male-biased mutation therefore predicts slow-X evolution.

In contrast, a theory based on hemizygous selection in the XY sex predicts that the X chromosome will accumulate adaptive substitutions faster than the autosomes (Charlesworth et al. 1987). In particular, provided that newly arising beneficial mutations are, on average, partially recessive ($\bar{h} < 0.5$), their probabilities of fixation are higher for X-linked loci than autosomal ones (Avery 1984; Charlesworth et al. 1987). Thus, assuming new beneficial mutations are generally partially recessive, theory predicts fast-X evolution.

Therefore, sites on the X-chromosome may evolve slower or faster than those on the autosomes, depending upon the main underlying evolutionary forces acting on them. Studies found that the strength of slow-X evolution varies among taxa (Shimmin et al. 1993; Chang et al. 1994; Bauer and Aquadro 1997; Ellegren and Fridolfsson 1997; Bohossian et al. 2000; Betancourt et al. 2002; Li et al. 2002; Makova and Li 2002; Ellegren and Fridolfsson 2003). Because slow-X evolution is a mutation-driven process, lineage effects likely reflect factors that affect lineage differences in mutation rate such as generation time, metabolic

rates, and, potentially, mating system (Bartosch-Härlid et al. 2003; Blumenstiel 2007; Presgraves and Yi 2009). Likewise, empirical studies of fast-X evolution have also yielded mixed results (Betancourt et al. 2002; Thornton et al. 2006; Presgraves 2008; Singh et al. 2008; Mank et al. 2010). In particular, in mammals, evidence of fast-X is largely restricted to genes expressed in testis (Torgerson and Singh 2003; The Chimpanzee Sequencing and Analysis Consortium 2005; Khaitovich et al. 2005; Baines et al. 2008).

Although these studies mostly focused on testing the prediction of either of the two (slow- vs. fast-X) theories, it is proposed that the mutation-based slow-X process and the selection-based fast-X process may interact. The $\bar{h} < 0.5$ condition of the fast-X theory assumed equal mutation rates in males and females ($\alpha = u_m/u_f = 1$) and assumed the effective population size of the X is three-fourths that of the autosomes ($N_X/N_A = 0.75$; Charlesworth et al. 1987). The conditions for fast-X evolution depend on both. In the presence of male mutation bias, the dominance condition for fast-X evolution becomes more restrictive (Kirkpatrick and Hall 2004). If, for instance, the male-to-female mutation rate ratio (α) = 5, then fast-X occurs only when $\bar{h} < 0.3$. Male-biased mutation can thus impede fast-X evolution. When $N_X/N_A > 0.75$, the dominance condition for fast-X evolution is more permissive (Vicoso and Charlesworth 2009). If, for instance, there is greater-than-Poisson variance in male reproductive success, N_X/N_A will exceed 0.75, and fast-X evolution can occur even when new beneficial mutations are partially dominant. In XY systems, then, sexual selection on males can facilitate fast-X evolution.

In this study, we simultaneously investigate slow- and fast-X evolution as well as their interactions. To do so, we compare lineage-specific rates of substitution on the X and autosomes in four primates: human, orangutan, rhesus macaque, and marmoset. We contrast substitution data from intron and protein-coding sequences. As the evolutionary rates of introns are largely governed by the input of neutral mutations, whereas those of nonsynonymous sites are largely governed by selection, slow- and fast-X evolution can be inferred using introns and exons, respectively. We find that all four primates show strong evidence of slow-X evolution. However, the strength of slow-X evolution varies significantly among lineages, potentially due to the variation in life history traits affecting the strength of male mutation bias. Among the primates investigated here, the marmoset lineage exhibits the lowest male mutation bias. Interestingly, we find that evidence for fast-X evolution at nonsynonymous sites is mostly limited to marmoset.

We discuss potential interaction between the slow- and fast-X processes and other factors that influence the variation of these processes. We also demonstrate that lineage-specific variation of slow- and fast-X processes has implications for population genetic inferences about human demography.

Methods

ORTHOLOGOUS GENE ASSEMBLY

The human genome assembly is considered highly accurate or "finished," representing approximately eightfold coverage of euchromatic regions (International Human Genome Sequencing Consortium 2004). The genome sequences of orangutan, rhesus macaque, and marmoset are of similar coverages (6× for orangutan and marmoset, and 5× for rhesus macaque, UCSC Genome Browser). Non-human primate data are all obtained from females. Because females harbor 2× chromosomes, the X and autosomes are sequenced to similar depths. We retrieved and assembled orthologous gene sets from four primates—human, orangutan, rhesus macaque, and marmoset—with mouse as an outgroup from the Ensembl BioMart (version: Ensembl Genes 57). For any pair of species, we chose genes with orthology type marked as "ortholog_one2one." For genes with multiple transcripts, the longest transcript was selected. To estimate substitution rate differences between the X chromosome and autosomes, we chose genes that have remained X-linked throughout mammalian evolution, that is, those that are X-linked in human, orangutan, rhesus macaque, marmoset, and mouse. There are 303 such genes. For autosomal genes, we chose genes that are homologous to those on human chromosomes 5 and 6, which have sizes and G+C contents similar to those of the X chromosome. We further limited the analysis to genes that have remained on homologous chromosomes in orangutan and rhesus macaque; no synteny information for chromosomes homologous to human chromosomes 5 and 6 was available for marmoset at the time of the analysis. There are 977 such genes.

MALE MUTATION BIAS AND PROTEIN EVOLUTIONARY RATES

We used intron sequences to estimate male mutation bias. We aligned the repeat masked, concatenated intron sequences of each gene using MLAGAN v2.0 (Brudno et al. 2003). To minimize the influence of sites that may be under natural selection, we removed first introns as well as 100 bps adjacent to splice sites of the remaining introns. Introns shorter than 300 bps after this procedure were also removed. In addition, we masked hyper-mutable CpG dinucleotides (Kim et al. 2006). Lineage-specific numbers of nucleotide substitutions were estimated using PAML baseml 4.2, assuming the HKY model of substitution (Yang 2007). Male-to-female mutation rate ratios (α) were then estimated following Miyata et al. (1987). Confidence intervals were obtained using the bootstrapping resampling method (Makova and Li 2002; Lu and Wu 2005; Elango et al. 2009). Estimates of the male-to-female mutation rate obtained using the Kimura's 2-parameter model are similar, demonstrating that our results are robust against different substitution models used (Table S1).

Coding sequences were translated to amino acid sequences first and then aligned using ProbCons 1.08 (Do et al. 2005). Genes with fewer than 100 aligned nucleotides were removed from subsequent analyses. Lineage-specific dN (number of nonsynonymous substitutions per site), dS (number of synonymous substitutions per site), and dN/dS values were estimated using PAML codeml 4.2 (Yang 2007). Again, hyper-mutable CpG dinucleotides were removed. To avoid overestimation, we removed 18 X-linked and 54 autosomal genes with dN/dS values greater than 15 in any lineage (among these genes, all but two genes had dN/dS $>$ 100). The final data set comprised 216 X-linked genes and 702 autosomal genes. Confidence intervals were calculated by bootstrapping 1000 times. We also calculated dN/dI for genes with both intron and exon data available following the above filtering steps. There are 151 X-linked genes and 490 autosomal genes for which dN/dI data are calculated.

STATISTICAL TESTS

We tested whether evolutionary rates of protein-coding sequences exhibit lineage-specific patterns of slow- and fast-X using linear models. The analysis of covariance (ANCOVA) model for nonsynonymous rates is, for example, defined as

$$\mathrm{dN} \sim \mathrm{lineage} + \mathrm{chromosome} + \mathrm{dI} + \mathrm{lineage} : \mathrm{chromosome\ interaction}.$$

The lineage term accounts for the lineage effects, the chromosome term accounts for the effect of X versus autosomal linkage, and the dI term accounts for locus-specific variation in mutation rate. The lineage:chromosome interaction term tests the null hypothesis that the effect of X versus autosome is independent of lineage; rejection of the null therefore indicates lineage-specific difference in the X versus autosomal effects on dN. We repeated these analyses for dS and dN/dS. Because the continuous variables are not normally distributed, we used a permutation method to test for significance, with each term's significance examined by permutating the samples 100,000 times.

Results

SLOW-X EVOLUTION IN PRIMATES

From the four primate species, including two apes (human and orangutan), one Old World monkey (rhesus macaque), and one New World monkey (marmoset), we extracted more than 15 million intron sites that are likely to be evolving under little selective constraint, including more than 4 million from the X-chromosome (see Materials and Methods). The phylogenetic tree and lineage-specific branch lengths obtained from the autosomal intron data are consistent with previous findings (Fig. 1). Notably, the human lineage has a shorter branch length than other apes (Elango et al. 2006) and Old World monkeys (Yi et al. 2002), whereas marmosets have a longer branch length than the catarrhines (Steiper and Young 2006).

For all four primate lineages, the estimated intron substitution rates from X-linked genes are significantly lower than those from autosomal genes (Wilcoxon test, $P < 10^{-10}$, Table S2), indicating slow-X evolution. We then estimated lineage-specific male-to-female mutation rate ratios (α) from the X and autosomal intron substitution rates (Miyata et al. 1987). Note that the species in this analysis are sufficiently diverged from one other that the effects of ancestral polymorphism, which can affect estimates of α, should be negligible (Makova and Li 2002). The degree of male mutation bias, measured by α, varies between different lineages, generally in accord with the previous findings. Our estimate of α for the human lineages is 4.36 that falls within the range of previously published estimates (Li et al. 2002). We report that the α from rhesus monkey is 3.07, again in accord with previous results (Rhesus Macaque Genome Sequencing and Analysis Consortium 2007; Elango et al. 2009). We report that the male mutation bias in the orangutan lineage is 3.40. Interestingly, the marmoset lineage exhibits an extremely reduced male mutation bias, $\alpha = 1.63$. This value is significantly lower than the male mutation bias from the other three primate lineages investigated in this study (Fig. 1). In fact, it is the lowest among the published values from primates and similar to the estimated values from rodents. Indeed, the male mutation bias from the outgroup branch, a composite of the rodent lineage leading to the mouse and the ancestral primate lineage leading to anthropoids, is 1.77, similar to that from the marmoset.

LINEAGE-RESTRICTED FAST-X EVOLUTION

To test for evidence of fast-X evolution, we investigated the relative evolutionary rates of protein-coding sequences (Table 1). In human, orangutan, and rhesus macaque lineages, the X/A ratios of mean and median dN are all less than 1 (Table 1). Interestingly, in the marmoset lineage, the X/A ratio of mean dN is 1.25, suggesting that the nonsynonymous sites of the X chromosome evolve faster than those of autosomes. The generally lower rates of nonsynonymous substitution on the X could arise for three reasons: a greater efficacy of purifying selection on the X; a sample of X-linked genes with greater average functional constraints than the sample from chromosomes 5 and 6; or a lower mutation rate at X-linked versus autosomal loci arising from male-biased mutation (see above). To begin to distinguish among these possibilities, we controlled for X-autosome mutation rate differences by studying nonsynonymous rates of substitution standardized by synonymous (dN/dS) and intronic rates of substitution (dN/dI). Although most branches showed X/A ratios of mean and median dN/dS and dN/dI greater than 1, the 95% confidence intervals tended to include 1. For those lineages showing some

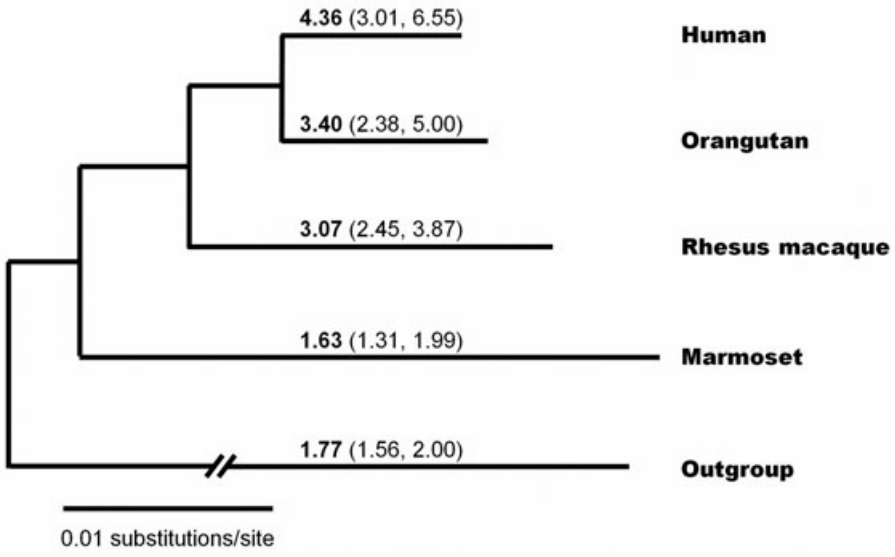

Figure 1. Phylogenetic tree of the four primates and the outgroup. The branch lengths estimated using the PAML follows the following tree: ([[human: 0.010402, orangutan: 0.011597]: 0.008192, rhesus: 0.026041]: 0.010851, marmoset: 0.056405, outgroup: 0.415723). The estimates of male-to-female mutation rate ratio α are marked in bold above each branch along with the 95% confidence interval in parentheses.

Table 1. X/A ratios of mean or median dN, dS, and dN/dS in four primates.

		dN	dS	dN/dS	dN/dI
Human	Mean	0.80 (0.58–1.15)	0.76 (0.68–0.86)	1.03 (0.80–1.30)	1.04 (0.70–1.50)
	Median	0.75 (0.61–1.09)	0.84 (0.72–0.94)	1.19 (0.81–1.75)	1.06 (0.76–1.45)
Orangutan	Mean	0.80 (0.57–1.12)	0.83 (0.69–0.99)	0.85 (0.67–1.06)	0.96 (0.68–1.33)
	Median	0.85 (0.62–1.12)	0.83 (0.69–0.98)	0.86 (0.57–1.15)	1.04 (0.75–1.37)
Rhesus	Mean	0.89 (0.65–1.19)	0.77 (0.64–0.91)	1.30 (1.03–1.60)	1.04 (0.76–1.39)
	Median	0.77 (0.58–1.06)	0.71 (0.62–0.83)	1.13 (0.80–1.41)	0.94 (0.75–1.38)
Marmoset	Mean	1.25 (0.94–1.62)	0.94 (0.83–1.05)	1.21 (0.96–1.50)	1.40 (1.04–1.86)
	Median	0.95 (0.76–1.27)	0.93 (0.88–1.05)	1.07 (0.80–1.44)	1.04 (0.88–1.44)

Note: 95% confidence intervals are given in parenthesis.

significant evidence for fast-X evolution, the signals were not consistent across measures. For instance, the rhesus macaque lineage shows suggestive evidence for fast-X evolution using mean dN/dS but not using median dN/dS, mean dN/dI, or median dN/dI. Similarly, the marmoset lineage shows suggestive evidence for fast-X evolution using mean dN/dI but not using median dN/dI, mean dN/dS, or median dN/dS. The fact that the X/A ratios of means, but not medians, tend to exceed 1 suggests that the weak signal of fast-X evolution in this analysis comes from the upper tails (high dN/dS or dN/dI) of the distributions (see below).

The X/A ratios of evolutionary rates in Table 1 vary considerably among lineages. We therefore used ANCOVA models with evolutionary rates (dN, dS, or dN/dS) as response variables and lineage (to account for lineage-specific evolutionary rates), chromosome (to account for X or autosomal linkage), and intron substitution rate (dI, to account for gene-specific mutation rates) as explanatory variables. We also included a lineage:chromosome interaction term to test whether relative evolutionary rates of X-linked and autosomal genes vary significantly among the four lineages. Because evolutionary rate data are not normally distributed, we assessed significance using permutation tests. As Table 2 shows, lineage has a highly significant effect on dN and dS. Notably, dN/dS, which is corrected for lineage-specific divergence, and thus supposedly measures only selective constraints, also shows a highly significant lineage effect. The effective level of functional constraints thus appears to vary among primate lineages. Neither dN nor dS show significant effects of chromosome, whereas dN/dS does. Thus, controlling for lineage and locus-specific mutation rate, dN/dS shows a significant fast-X effect. Across loci, intronic substitution rate (dI) is highly correlated with dN and dS but not with dN/dS, suggesting that the dN/dS ratio adequately standardizes for locus-specific mutation rate.

Table 2. Significance of lineage, chromosome, locus-specific intron divergence, and lineage-chromosome interaction on evolutionary rates.

	Four species (external branches only)		
	F-value	Pr(>*F*)	*P*-value (permutation)
Response variable: dN			
Lineage	18.01	$<10^{-10}$	$<10^{-5}$
Chromosome	2.24	NS	NS
dI	34.54	$<10^{-8}$	$<10^{-5}$
Lineage:chromosome interaction	2.52	0.056	0.057
Response variable: dS			
Lineage	14.70	$<10^{-8}$	$<10^{-5}$
Chromosome	1.61	NS	NS
dI	76.40	$<10^{-17}$	$<10^{-5}$
Lineage:chromosome interaction	1.14	NS	NS
Response variable: dN/dS			
Lineage	7.74	$<10^{-4}$	10^{-4}
Chromosome	5.12	0.02	0.02
dI	12.39	$<10^{-3}$	10^{-3}
Lineage:chromosome interaction	3.74	0.01	0.01

NS: Not significant, that is, $P > 0.1$.

Finally, the lineage:chromosome interaction term is marginally significant for dN and significant for dN/dS but not for dS. The lineage:chromosome interaction effect implies that the magnitude of the X-autosome difference in dN/dS varies significantly among lineages.

A LINEAGE-SPECIFIC FAST-X SIGNAL FOR GENES WITH HISTORIES OF RAPID EVOLUTION

The fast-X theory is concerned with differences in the rate of adaptive evolution between the X and autosomes. We therefore tested for a signal of fast-X evolution by estimating the proportion of X-linked versus autosomal genes for which dN/dI $>$ 1, as expected given histories of recurrent positive selection (Table 3). In the marmoset lineage, the X chromosome shows a significant 2.6-fold excess of genes with dN/dI $>$ 1 relative to autosomes. In the composite outgroup lineage, the X shows a similar significant excess of genes with dN/dI $>$ 1. None of the other three primate lineages show an excess of positive selection on the X.

The dN/dI $>$ 1 criterion for inferring positive selection is stringent. We therefore considered an arbitrary range of dN/dI values, assuming that there is greater enrichment for positive selection as our criteria become increasingly restrictive, from dN/dI $> 0.2 \rightarrow >$ 1 (Table 3). In the marmoset lineage, the signal of fast-X evolution gets stronger as we enrich for positive selection: for dN/dI > 0.8 and $>$ 1, the X shows a significant approximately 2.6-fold excess of rapidly evolving genes; for dN/dI $>$ 0.4 and $>$0.6, the signal diminishes to approximately twofold excess of rapidly evolving genes on the X; and for dN/dI $>$ 0.2, the signal of fast-X evolution in marmoset disappears entirely. In the composite outgroup lineage, the X shows a similar significant (or marginally significant) qualitative enrichment for rapidly evolving genes as the dN/dI criterion increases from $> 0.6 \rightarrow >$ 1. None of the other three primate lineages show similar enrichment of rapid evolution on the X. Finally, the distributions of dN/dI and dN/dS both differ significantly between the X and autosomes in the marmoset lineage (Wilcoxon test, $P =$ 0.016 and 0.036, respectively) but not in the other three lineages.

FAST-X EVOLUTION CONFIRMED AT TESTIS-EXPRESSED GENES IN HUMANS

Fast-X evolution is expected to be especially strong for mutations having male-beneficial fitness effects (although the condition that $\overline{h} < 0.5$ for new favorable mutations holds; (Charlesworth et al. 1987; Vicoso and Charlesworth 2006). We therefore studied evolutionary rates at genes with testis- or sperm-specific functions in humans (Torgerson and Singh 2003; Khaitovich et al. 2005). In our data set, five of 151 X-linked genes and 12 of 490 autosomal genes show testis-specific expression. The X-linked testis-specific genes have significantly higher dN, dN/dS, and dN/dI than X-linked genes not expressed in testes (Figure S1). X-linked testis-specific genes also show greater dN, dN/dS, and dN/dI than autosomal testis-specific genes, although the significance was marginal due to the small sample size (Figure S1). These findings are consistent with previous ones showing that human testis-specific genes show elevated rates of substitution on the X (Torgerson and Singh 2003; Khaitovich et al. 2005).

Discussion

In this article, we provide a simultaneous look at predictions of slow- and fast-X evolution by studying sites enriched for neutral molecular evolution in introns and sites enriched for purifying and positive natural selection in exons from four primate lineages. We find evidence for significant slow-X evolution among all four primate lineages, consistent with male mutation bias. The degree of slow-X evolution, however, varies between lineages, largely in accord with previous observations. We also find evidence consistent with fast-X evolution in marmosets and, at least for genes with male-biased expression, in humans. Below we discuss the possible causes of lineage effects on the strength of male-biased mutation and, hence, slow-X evolution. We also consider the consequences of lineage differences in slow-X evolution for fast-X

Table 3. Lineage-specific signals of fast-X for genes under different dN/dI thresholds.

	X			A				
	+[1]	−[2]	%pos_sel[3]	+	−	%pos_sel	*P*[4]	X/A[5]
dN/dI>1								
Human	2	149	0.013	10	480	0.020	0.741	0.6
Orangutan	17	134	0.113	41	449	0.084	0.330	1.3
Rhesus	24	127	0.159	68	422	0.139	0.595	1.1
Marmoset	8	143	0.053	10	480	0.020	**0.047**	2.6
Outgroup	3	148	0.020	0	490	0.000	**0.013**	Inf
dN/dI>0.8								
Human	10	141	0.066	15	475	0.031	0.056	2.2
Orangutan	21	130	0.139	57	433	0.116	0.477	1.2
Rhesus	29	122	0.192	82	408	0.167	0.539	1.1
Marmoset	11	140	0.073	13	477	0.027	**0.014**	2.7
Outgroup	3	148	0.020	2	488	0.004	0.088	4.9
dN/dI>0.6								
Human	11	140	0.073	29	461	0.059	0.565	1.2
Orangutan	23	128	0.152	79	411	0.161	0.899	0.9
Rhesus	38	113	0.252	104	386	0.212	0.315	1.2
Marmoset	16	135	0.106	26	464	0.053	**0.036**	2.0
Outgroup	5	146	0.033	3	487	0.006	**0.021**	5.4
dN/dI>0.4								
Human	20	131	0.132	73	417	0.149	0.693	0.9
Orangutan	39	112	0.258	122	368	0.249	0.830	1.0
Rhesus	49	102	0.325	142	348	0.290	0.417	1.1
Marmoset	35	116	0.232	61	429	0.124	**0.003**	1.9
Outgroup	11	140	0.073	29	461	0.059	0.565	1.2
dN/dI>0.2								
Human	56	95	0.371	164	326	0.335	0.434	1.1
Orangutan	70	81	0.464	234	256	0.478	0.780	1.0
Rhesus	70	81	0.464	236	254	0.482	0.710	1.0
Marmoset	60	91	0.397	167	323	0.341	0.207	1.2
Outgroup	38	113	0.252	114	376	0.233	0.662	1.1

Significant signals of fast-X evolution are shown in bold fonts.

[1]Number of genes having dN/dI > threshold.

[2]Number of genes having dN/dI < threshold.

[3]Frequency of genes having dN/dI > threshold.

[4]*P*-value of Fisher's exact test.

[5]X/A ratio of number of the percentage of genes having dN/dI > threshold.

evolution and, more practically, for population genetic inferences of human sex-specific demography.

CAUSES OF LINEAGE-SPECIFIC VARIATION OF MALE MUTATION BIAS

Male mutation bias arises from the asymmetry in the numbers of cell divisions between male and female germlines (Haldane 1947; Miyata et al. 1987; Crow 1997; Hurst and Ellegren 1998). The magnitude of the asymmetry likely varies with life history traits across species. For example, it has been proposed that as generation time increases, the cumulative difference between the number of cell divisions in the male and female germlines will also increase, thereby increasing α (Chang et al. 1994; Li et al. 2002; Bartosch-Härlid et al. 2003; Goetting-Minesky and Makova 2006; Sayres et al. 2011). Consistent with a generation time effect on male mutation bias, estimates of α from humans, which have relatively long generation times, converge on large values, 4–6, whereas those from mouse, which have relatively short generation times tend to be smaller, approximately 2 (Li et al. 2002).

Our primate data provide further support for the generation time effect on male mutation bias. In particular, the ranks of male mutation bias observed between the primate lineages included in

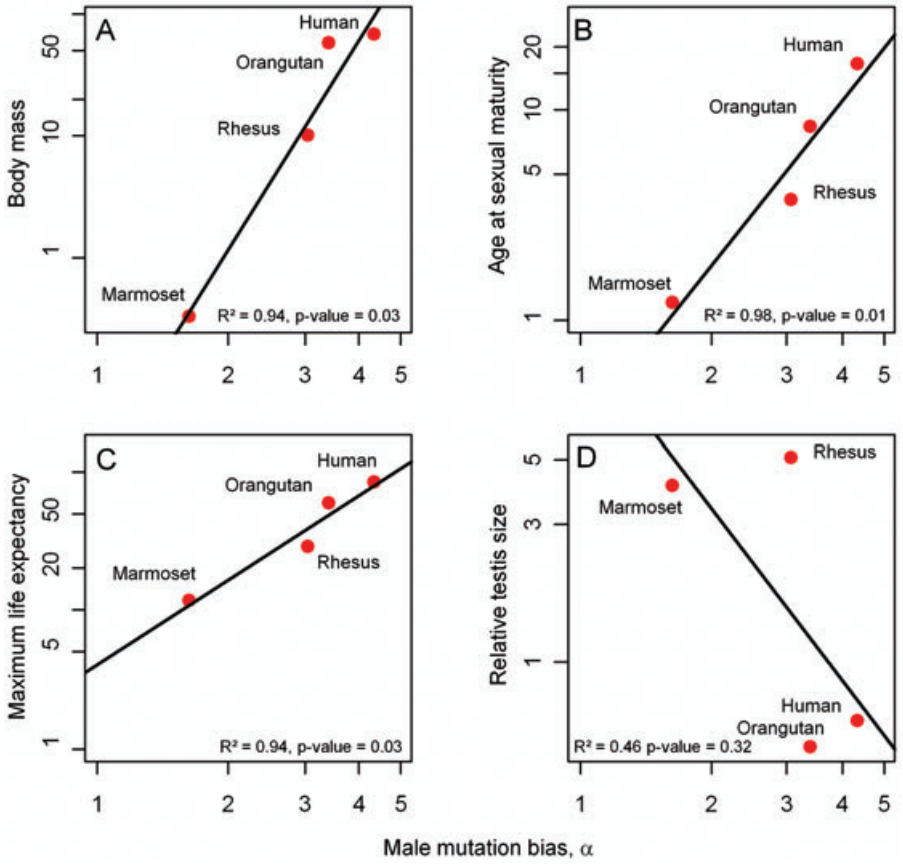

Figure 2. **Correlations between the male mutation bias (α) and several life history traits. Male mutation bias is positively correlated with (A) body mass, (B) age at sexual maturity, (C) maximum life expectancy. However, it is not correlated with (D) relative testis mass. Values are log-transformed to improve normality.**

the current study (human > orangutan > rhesus monkey > marmoset) correspond well to the ranks of several life history traits known to co-vary with generation times. For instance, body mass, age at sexual maturity, and maximum life expectancy are significantly correlated with the estimated male mutation bias (Pearson's $\rho = 0.97$, 0.99, and 0.97, $P = 0.03$, 0.01, and 0.03, respectively; Fig. 2). While suggestive, these correlations are not corrected for phylogeny. Doing so, using Felsenstein's (1985) independent contrasts method, leaves just three phylogenetically independent points. Even though the correlations between the above life history traits and male mutation bias remain highly positive (e.g., the correlation between age at sexual maturity and male mutation bias, after correcting for phylogenetic independence, is 0.96), no meaningful test of significance can be obtained from three data points. Details are presented in Table S3.

Other life history traits may also affect the strength of male mutation bias. The intensity of sperm competition, for instance, varies considerably with primate-mating systems (Harcourt et al. 1981, 1995; Dixson and Anderson 2001). In systems for which the risk of sperm competition is high, males have evolved greater investment in sperm production—larger relative testis mass and greater numbers of sperm per ejaculate (Harcourt et al. 1981; Smith 1984; Dixson and Anderson 2001). If adaptation to sperm competition involved the evolution of more male germline cell divisions, to produce more sperm faster, then elevated male mutation bias may evolve as an incidental byproduct (Blumenstiel 2007; Presgraves and Yi 2009). Previously, we reported evidence for a positive correlation between α and relative testis mass in hominids (Presgraves and Yi 2009). The present analysis spans a wider range of taxa to include hominids, Old World monkeys and New World Monkeys, but does not support a simple, general relationship between male mutation bias and indexes of sperm competition. Of the four primates studied, rhesus macaque experiences the greatest intensities of sperm competition, as evidenced by its mating system (multimale and multifemale) and the largest relative testis mass among the species investigated (Harcourt et al. 1995, Table S3). In comparison, humans and orangutans exhibit generally single-male–mating systems (Harcourt et al. 1995; Martin 2007). Relative testis sizes of human and orangutan are much lower than that of rhesus macaque (Table S2). However, male mutation bias is greater in humans and orangutans than in rhesus macaque (Fig. 1). Marmoset, whose relative testis size is

the second largest among the four primates, also exhibits little male mutation bias (Fig. 1, Table S3). These observations suggest either that sperm competition has little effect on male mutation bias or that other life history traits, like generation time, are much more important. A recent study of distantly related mammals also reached a similar conclusion (Sayres et al. 2011).

LINEAGE-SPECIFIC SLOW-X EVOLUTION CAN AFFECT INFERENCES ABOUT HUMAN DEMOGRAPHY

Because the X chromosome and autosomes are inherited differently with respect to sex, comparing DNA sequence polymorphism for the X and autosomes can be informative about human demographic history. In a population with equal numbers of effective males and females, the effective number of X chromosomes (N_X) should be three-fourths that of autosomes (N_A). However, if the effective sex ratio deviates from 1, then the N_X/N_A ratio can deviate from $^3/_4$ (Charlesworth 2001; Vicoso and Charlesworth 2009). For instance, in a polygynous population, males have higher variance in reproductive success. Consequently, the effective number of males could be considerably lower than that of females, causing the N_X/N_A ratio to be greater than $^3/_4$. In contrast, if a population is founded by a male-biased group, the N_X/N_A ratio would be less than $^3/_4$. Two recent studies using population genetic approaches to estimate the N_X/N_A ratio in humans reached different conclusions (Hammer et al. 2008; Keinan et al. 2009). Keinan et al. (2009) estimated that N_X/N_A is unusually low in non-African populations and proposed a male-biased dispersal model (Keinan and Reich 2010). Hammer et al. (2008), however, estimated that N_X/N_A is approximately 1 in all populations examined, including African and non-African populations.

We hypothesize that species differences in male mutation bias may partially explain the discrepancy (see also [Bustamante and Ramachandran 2009]). N_X/N_A is inferred from the ratios of X versus autosomal polymorphism, corrected for X versus autosomal mutation rate differences:

$$\left(\frac{N_X}{N_A}\right) = \left(\frac{4N_X\mu_X}{4N_A\mu_A}\right) \times \left(\frac{\mu_A}{\mu_X}\right) = \left(\frac{\pi_X}{\pi_A}\right) \Big/ \left(\frac{\mu_X}{\mu_A}\right).$$

Lacking an experimentally defined μ_X/μ_A from humans, the μ_X/μ_A parameter is often inferred from human X/A ratios of divergence from an outgroup species. For instance, Keinan et al. (2009) used human divergence from macaque, whereas Hammer et al. (2008) used human divergence from orangutan. As shown above, however, the degree of male mutation bias, and consequently μ_X/μ_A, differs among these lineages. Male mutation bias is weaker in rhesus macaque than in orangutan (Fig. 1). The μ_X/μ_A from rhesus macaque used in Keinan et al. (2009) is approximately 0.875, whereas the μ_X/μ_A from orangutan used in Hammer et al. (2008) is approximately 0.750, consistent with our estimates. We examined other studies that reported μ_X/μ_A ratios, including pairwise estimates and lineage-specific estimates (Table 4). As expected if α for human > orangutan > rhesus macaque (Fig. 1), the different estimates in Table 4 consistently show a trend that μ_X/μ_A are human < orangutan < rhesus macaque. Using divergence data from macaque or orangutan to correct for the higher male-biased mutation rate in humans will thus cause underestimates of N_X/N_A. As rhesus macaque has a higher μ_X/μ_A than orangutan, using divergence from macaque will more strongly underestimate the true N_X/N_A.

The difference in μ_X/μ_A estimates used by Keinan et al. (2009) and Hammer et al. (2008) cannot, however, fully account for the discrepancy in N_X/N_A between the two studies. For example, if we correct nucleotide polymorphism from Keinan et al. (2009) using human-specific μ_X/μ_A obtained from our current study, N_X/N_A of the West African population increases from 0.763 to 0.844. The estimates of N_X/N_A of Hammer et al. (2008) decrease slightly after being corrected using the human-specific μ_X/μ_A but still higher than those of Keinan et al. (2009). For example, the mean N_X/N_A of Hammer et al. (2008)'s data decreases from 1.036 to 0.989. Other factors, such as different sensitivities of analytical techniques used in the two studies (Emery et al. 2010) and the effect of natural selection on a subset of sites nearby genes (Gottipati et al. 2011) are likely to account for the remaining discrepancies.

LINEAGE-SPECIFIC SIGNAL OF FAST-X EVOLUTION

To date, strong evidence for fast-X evolution resulting from beneficial substitutions has been lacking. In birds, the faster evolution of Z-linked loci is consistent with the fixation of slightly deleterious mutations due to the much smaller effective size of the Z versus the autosomes (Mank et al. 2009). In mammals and flies, evidence for fast-X evolution is strongest for genes with male-biased or testis-specific expression (Torgerson and Singh 2003; Khaitovich et al. 2005; Torgerson and Singh 2006; Baines et al. 2008). Over the *Drosophila* phylogeny, the distributions of dN/dS and the incidence of positively selected genes are both elevated for some lineages but not others (Singh et al. 2008). We find similar lineage-restricted evidence for fast-X evolution over the primate phylogeny.

Marmoset is unique among the four primate lineages, showing several signals of fast-X evolution. First, the X/A ratio of mean dN/dI significantly exceeds 1, and the X/A ratio for mean dN/dS nearly does (Table 1). Second, the distributions of dN/dI and dN/dS are both significantly shifted toward higher values on the X relative to the autosomes. Third, the X shows a significant excess of rapidly evolving genes, an excess that gets stronger as the sample is progressively enriched for genes with histories of positively selected genes (Table 3). Among the subset of genes with dN/dI > 1, GO term analyses showed no excess of genes with sperm-specific functions on the X (1 on the X, 1 on the

Table 4. **X chromosome to autosome ratios of mutation rates estimated using different outgroups. Some studies used estimates obtained from pairwise comparisons. Although there exists considerable variation among studies, the rate differences between the X chromosome and autosomes are consistently larger in human–orangutan comparisons than in human-macaque comparisons. Several studies now provide lineage-specific X to autosomal ratios for human, orangutan, and macaque. Again, the pattern is obvious that male mutation bias is greater in apes than in available estimates of lineage-specific estimates are also available.**

μ_X/μ_A in pairwise comparisons	Human–Orangutan	Human–Macaque
Hammer et al. (2008)	0.755	
Keinan et al. (2009)		0.875
Patterson et al. (2006) Five species comparison	0.877	0.921
Elango et al. (2009)		0.866
Rhesus Macaque Genome Sequencing and Analysis Consortium (2007)		0.839
Ebersberger et al. (2007)	0.790	0.823
Current study	0.805	0.840

Lineage-specific estimation of μ_X/μ_A	Human	Orangutan	Macaque
Patterson et al. (2006) Five species comparison	0.785	0.892	0.941
Patterson et al. (2006) Four species comparison	0.797	N/A	0.913
Ebersberger et al. (2007)	0.746	0.790	0.851
Current study	0.791	0.818	0.830

autosomes), although these analyses are limited by the lack of experimental genome annotation data from marmoset. Nevertheless, the fact that we observe strong signals of fast-X in an unfiltered data set (with respect to male-biased expression) is highly unusual and marks marmoset as one of the few, if any, mammalian lineages exhibiting evidence of fast-X evolution.

Why might marmoset differ from the other three primate lineages? The possibility of faster adaptive evolution on the X depends on the strength of male mutation bias (Kirkpatrick and Hall 2004) and on the ratio of effective sizes, N_X/N_A (Vicoso and Charlesworth 2009). Lineage-specific variation in either parameter could therefore give rise to lineage-restricted fast-X evolution. Kirkpatrick and Hall (2004) showed strong male mutation bias—which reduces the mutation rate on the X—impedes fast-X evolution. It is therefore interesting that the marmoset lineage, which has the lowest male mutation bias among the four primates investigated, has the strongest signal of fast-X evolution. Long-term average N_X/N_A may vary among lineages as well, possibly with mating system. All else being equal, $N_X/N_A > {}^3/_4$ facilitates and $N_X/N_A < {}^3/_4$ impedes fast-X evolution (Vicoso and Charlesworth 2009). Unfortunately, we have little direct empirical knowledge of long-term N_X/N_A in primates. Nevertheless, among the four primates we analyze, the marmoset is unique in having a polyandry-like mating system (Sussman and Garber 1987). As polyandry entails an increased variance in female reproductive success, there is some reason to expect $N_X/N_A < {}^3/_4$ in marmosets. If true, then it is surprising that marmoset provides the best signal of fast-X evolution. Given the number of species in our analysis, our conclusions on the interaction of male mutation bias and N_X/N_A on fast-X evolution must be considered tentative. Direct estimates of N_X/N_A from a range of primates along with lineage-specific estimates of slow- and fast-X evolution are needed for a larger number of primate lineages.

Conclusion

We provide a first simultaneous look at slow- and fast-X evolution as well as their interactions within a primate phylogeny. We show that slow-X evolution is universal among the four primate lineages, although its magnitude varies significantly, mostly because of the generation time effect on male mutation bias. Unlike slow-X evolution, we demonstrate that marmoset is the only species exhibiting compelling general evidence of fast-X evolution, possibly due to its weak male mutation bias compared to the other primates. Finally, we consider the possibility that the variation in the strength of male mutation bias among lineages may influence the estimates of important parameters in human demography.

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Supporting Information

The following supplementary material is available for this article:

Table S1. Comparison of alpha values obtained from the HKY method and the Kimura's 2-parameter method.
Table S2. Mean and median of intron substitution rates of X-linked and autosomal genes.
Table S3. Variation of life history traits among the primate species examined.
Figure S1. Comparisons between X-linked and autosomal human genes according to their expression patterns.

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

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