Variable Autosomal and X Divergence Near and Far from Genes Affects Estimates of Male Mutation Bias in Great Apes

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Abstract

Male mutation bias, when more mutations are passed on via the male germline than via the female germline, is observed across mammals. One common way to infer the magnitude of male mutation bias, α, is to compare levels of neutral sequence divergence between genomic regions that spend different amounts of time in the male and female germline. For great apes, including human, we show that estimates of divergence are reduced in putatively unconstrained regions near genes relative to unconstrained regions far from genes. Divergence increases with increasing distance from genes on both the X chromosome and autosomes, but increases faster on the X chromosome than autosomes. As a result, ratios of X/A divergence increase with increasing distance from genes and corresponding estimates of male mutation bias are significantly higher in intergenic regions near genes versus far from genes. Future studies in other species will need to carefully consider the effect that genomic location will have on estimates of male mutation bias.

Key words: male mutation bias, natural selection, X chromosome, X-inactivation, divergence.

Introduction

In mammals, mutations accumulate faster in the male germline than the female germline primarily due to more germline cell divisions in males versus females (Vogel and Motulsky 1997; Drake et al. 1998), resulting in a male mutation bias. If errors in DNA replication during germline cell divisions are the major source of mutations, and the analyzed sequences are unconstrained (neutral), male mutation bias can be estimated from substitution rates (Miyata et al. 1987). Substitution rates in unconstrained regions (a proxy for mutation rates) on the X and autosomes are routinely compared with estimate the ratio of the mutation rate in males to the mutation rate in females (α), because these genomic regions spend different amounts of time in the male and female germline (Miyata et al. 1987; Ellegren 2007).

Male mutation bias is observed across mammals (Li et al. 2002; Taylor et al. 2006; Wilson Sayres et al. 2011), birds (Axelsson et al. 2004; Smeds et al. 2016), fish (Ellegren and Fridolfsson 2003), and flies (Bachtrog 2008). In mammals, the magnitude of α varies tremendously, and is predominantly explained by variation in generation time across species (Wilson Sayres et al. 2011). Whereas previous studies focused primarily on pairwise estimates of α (Taylor et al. 2006; Elango et al. 2009), additional genome sequences make it possible to infer branch-specific (i.e., species-specific) α values (Makova and Li 2002; Berlin et al. 2006; Wilson Sayres et al. 2011). Branch-specific α values have been reported for the great apes, but vary (Taylor et al. 2006; Presgraves and Yi 2009; Wilson Sayres and Makova 2011). Direct estimates of *de novo* mutation rates have also indicated a strong male mutation bias in humans (Kong et al. 2012) and chimpanzees (Venn et al. 2014). Estimates of α between recently diverged species can be biased by not accounting for variations in the amount of ancestral polymorphism between genomic regions (Li et al. 2002; Makova and Li 2002). Genome-wide substitution rate comparisons are less prone to locus-specific variation, and more likely to reflect the true male mutation bias (Makova et al. 2004; Taylor et al. 2006; Wilson Sayres et al. 2011). Sex specific differences in life histories can also affect genome-wide neutral substitution rate accumulation and estimates of male mutation bias (Amster and Sella 2016). However, if substitutions accumulate differently with distance from genes on the X chromosome versus autosomes, it will have a tremendous impact on estimates of male mutation bias, even when genome-wide comparisons are utilized.

Natural selection can affect the evolution of nearby unconstrained region across the genome that is less likely to be separated from the selected allele by recombination. For example, levels of genetic diversity are reduced in both coding genes and the regions around genes, either because purifying selection removes harmful alleles from coding regions and nearby neutral sites are influenced by background selection (Charlesworth 2012), or because positive selection increases the frequency of beneficial alleles and nearby neutral sites are influenced by genetic hitchhiking (Smith and Haigh 1974). There has been substantial effort to understand how natural selection has shaped patterns of genetic diversity within populations at putatively neutral sites (Nielsen et al. 2007; Akey 2009; Lohmueller et al. 2011; Wilson Sayres et al. 2014). Genetic diversity within human populations is reduced near genes and other conserved sequences (Hammer et al. 2004, 2008, 2010; Gottipati et al. 2011; Arbiza et al. 2014), consistent with background selection or genetic hitchhiking. The X chromosome, in particular, has significantly increased levels of purifying and positive selection versus autosomes in humans (Veeramah et al. 2014), and it has been shown to be affected by independent strong selective sweeps in great apes (Nam et al. 2015). As a consequence, linked selection affects diversity differently on the X chromosome and the autosomes within populations: genetic diversity on chromosome X increases faster with increasing distance from genes than it does on the autosomes across both human and great ape populations (Hammer et al. 2010; Gottipati et al. 2011; Prado-Martinez et al. 2013; Arbiza et al. 2014).

Whereas it is known that patterns of sex-linked and autosomal genetic diversity in unconstrained regions of the genome are shaped by natural selection, it is debated whether genetic divergence across the genome is affected by linked selection over long evolutionary times. It has been suggested that linked selection does not affect selectively neutral sites, as recombination over long evolutionary time is expected to unlink selected sites from linked neutral loci (Birky and Walsh 1988). A few studies have suggested that selection may affect the accumulation of substitutions and estimates of species divergence in neutral regions (Hellmann et al. 2003; Reed et al. 2005; Begun et al. 2007; McVicker et al. 2009; Lohmueller et al. 2011), in particular if background selection acts on ancestral polymorphism, especially in species with large ancestral population sizes (Phung et al. 2016). However, differences in estimates of divergence near and far from genes between the sex chromosomes and the autosomes have not been investigated, nor the effect on estimates of male mutation bias.

Here, we investigate patterns of divergence with increasing distance from genes on the X chromosome and the autosomes in great apes, including human. We analyze patterns of divergence along the genome over the long evolutionary time measured both from the present to the MRCA of great apes, as well branch-specific estimates of divergence, and make an attempt to correct for differences in ancestral polymorphism between genomic regions. We find that genetic divergence typically increases with increasing distance from genes, and notably that this increase in divergence with distance from genes is faster on the X chromosome than the autosomes. This leads to an increasing ratio of X/A divergence with distance from genes. Consequently, measurements of male mutation bias vary with distance from genes, increasing with increasing distance from genes. Our results suggest that evolutionary forces shape patterns of divergence differently on the X and autosomes in ways that significantly impact estimates of male mutation bias.

Materials and Methods

Sequence Data for Estimating Divergence from MRCA of Great Apes Using Great Ape Population Data

We analyzed data from whole genome sequences of 77 individuals from 11 great ape populations [*Homo sapiens* (African), *Homo sapiens* (Non-African), *Pan paniscus, Pan troglodytes ellioti, Pan troglodytes schweinfurthii, Pan troglodytes troglodytes, Pan troglodytes verus, Gorilla beringei graueri, Gorilla gorilla gorilla, Pongo pygmaeus*, *and Pongo abelii*] (Prado-Martinez et al. 2013). Genomes were mapped to the human reference assembly NCBI build 36 (UCSC hg18), and variant calling and filtering were performed as previously described (Prado-Martinez et al. 2013). Loci in the pseudoautosomal regions of the X chromosome were excluded from analyses (Prado-Martinez et al. 2013). We obtained nucleotide diversity and divergence values, calculated for callable bases in 20 kbp windows (Prado-Martinez et al. 2013). We divided regions on autosomes and X chromosome into six nonoverlapping bins of increasing genetic distances from the nearest genes (in centiMorgans), based on the fine-scale recombination map of Hinch et al. (2011): ([0–0.05], [0.05–0.1], [0.1–0.2], [0.2–0.4], [0.4–0.8], [0.8–2.0]). We computed divergence for each great ape subspecies along the branch to the MRCA of all great apes (fig. 2A), meaning all branches cover the same amount of evolutionary time (Prado-Martinez et al. 2013).

We computed 95% confidence intervals (CI) for each bin using the nonparametric bootstrap method, where 1000 replicates with replacement are generated from the observed data for each bin (https://github.com/WilsonSayresLab/MMB_apes; last accessed September 24, 2016).

Sequence Data for Estimating Branch-Specific Divergence across Reference Genomes

We used the Neutral Region Explorer webserver (Arbiza et al. 2012) to extract putatively neutral regions for the hg19 human reference genome, masking the genome for genic regions (known genes, gene bounds, and spliced ESTs), duplicated regions (segmental duplications, copy number variants,

and self-chain regions), repetitive regions (simple repeats and repetitive elements) and phastCons (44wayPlacental) (Pollard et al. 2010). We required filtered regions to have at least 500 contiguous bases to be included in further analysis. Intergenic regions were divided into non-overlapping bins on the basis of genetic distance (in cM) from the nearest gene given by [0–0.05], [0.05–0.1], [0.1–0.2], [0.2–0.4], [0.4–0.8], [0.8–2.0], and physical distance (in kbp) from the nearest gene given by [0–50], [50–100], [100–200], [200–400], [400–800], [800–2000]. Genetic distances were defined based on the HapMap recombination map (International HapMap Consortium 2005). We used 100-way multiZ new (hg19) (Blankenberg et al. 2011) multiple alignment files (MAFs) for extracting alignments for the filtered regions. For each filtered region we extracted a five-way multiple sequence alignment including the reference genomes of human (hg19), chimpanzee (panTro4), gorilla (gorGor3), orangutan (ponAbe2), and rhesus macaque (rheMac3) using the Galaxy interface (Goecks et al. 2010). In the autosomal regions, number of bases in each bin ranged from 15 Mb (closest to the genes) and 700 kb (farthest from the genes) when genetic distance was considered, and 6 Mb (closest to the genes) and 658 kb (farthest from the genes) when physical distance was considered. On the other hand, X chromosome had fewer bases in each bin with base counts ranging from 155 kb (closest to the genes) to 3 kb (farthest from the genes) when genetic distance was considered, and 39 kb (closest to the genes) to 5 kb (farthest from the genes) when physical distance was considered.

Substitution Rates Calculation

Alignments in each bin are divided into 5 kb windows and substitution rate is calculated for each window using PhyML software (Guindon et al. 2010). We used the HKY85 (Hasegawa et al. 1985) nucleotide substitution model with a transition/transversion ratio in the maximum likelihood framework for calculating the substitution rates. We kept the tree topology constant, and optimized the branch lengths and model parameters. The 95% confidence intervals (CI) for autosomes and X were computed using bootstrap method by randomly selecting windows 1000 times with replacement and computing the mean and 95% confidence intervals from each pseudo-sample.

Computing Male Mutation Bias

We used Miyata's framework to estimate male mutation bias (Miyata et al. 1987). If most mutations are due to errors during replication then, we can infer that substitutions on the autosomes represent equal contributions from mutations accumulated in genetic males and females because the autosomes spend half of their time in male germline and half in female germline, whereas substitutions on the X chromosome represent unequal contributions from the male and female germline, because the X chromosome spends only one-third of its time in male germline:

$$A = \frac{1}{2}\mu_{male} + \frac{1}{2}\mu_{female}, \quad (1)$$

and

$$X = \frac{1}{3}\mu_{male} + \frac{2}{3}\mu_{female}, \quad (2)$$

where μ_{male} is the mutation rate in genetic males, and μ_{female} is the mutation rate in genetic females, and X and A represent neutral divergence rates calculated for the X chromosome and autosomes (Miyata et al. 1987). Solving the two equations above for the ratio of the mutation rate in males to the mutation rate in females yields, α, the estimate of the magnitude of male mutation bias:

$$\frac{\mu_{male}}{\mu_{female}} = \alpha_{X/A} = \frac{3\left(\frac{X}{A}\right) - 4}{2 - 3\left(\frac{X}{A}\right)} \quad (3)$$

We computed X/A divergence ratios along with 95% confidence intervals (CI) for each bin of increasing genetic and physical distance from genes and used the ratios to compute male mutation bias (α) for each bin along with 95% confidence intervals (CI) as explained in equations (1)–(3).

Divergence Estimates in Exons, Introns, and Intergenic Near and Far from Genes

We extracted positions for exonic and intronic regions for both autosomes and X chromosome for human hg19 reference using RefSeq dataset from the UCSC table browser (Karolchik et al. 2004). We used 100-way multiZ new (hg19) (Blankenberg et al. 2011) multiple alignment files (MAFs) for extracting alignments for these regions. For both autosome and X chromosome, we extracted a five-way multiple sequence alignment including the reference genomes of human (hg19), chimp (panTro4), gorilla (gorGor3), orangutan (ponAbe2), and rhesus macaque (rheMac3) using the Galaxy interface (Goecks et al. 2010). Mean and 95% confidence intervals for substitution rates were calculated using PhyML (Guindon et al. 2010), as described above.

To compare the divergence in the exonic and intronic regions to intergenic regions, we used the previously aligned intergenic regions obtained for the branch specific estimates using reference genomes above, and merged the regions from bin1 to bin6, from bin2 to bin6, from bin3 to bin6, bin4 to bin6, bin5 to bin6, and calculated the mean divergence and 95% CI for both X and autosome using PhyML (Guindon et al. 2010), and calculated mean X/A ratio and α along with 95% CI (supplementary table S3, Supplementary Material online).

GC Content and Divergence Estimates

We used neutral regions on chromosome 8 to examine the relationship between GC content and divergence estimates (independent of distance from genes). We divided the

chromosome into 5 kb windows, and selected the windows where number of filtered loci was greater than 2 kb. We performed multiple sequence alignment in Galaxy (Goecks et al. 2010) using the reference genomes as described above and calculated mean and 95% confidence intervals for substitution rates using PhyML program (Guindon et al. 2010) described above.

Results

Divergence from the MRCA of Great Apes Differs with Distance from Genes by Chromosome Type

To examine how divergence varies with distance from genes on both the autosomes and the X chromosome, we evaluated divergence in windows of increasing genetic distance from the nearest gene for ten great ape populations (fig. 1). We found that divergence in putatively neutral regions increased as the distance from genes increased on both the X and the autosomes (fig. 1). We observed that divergence estimates exhibit a similar pattern along the genome for all great ape subspecies (fig. 1). This was expected because divergence estimates were computed from the MRCA of great apes (approximately 10.5 million years ago, MYA) (Prado-Martinez et al. 2013) to each modern species (fig. 2A), therefore much of the evolutionary history is shared along the branches. Divergence increases monotonically on the autosomes, and increases across most bins on the X chromosome (fig. 1). Interestingly, we also observed that divergence on the X chromosome increases faster than the autosomes with increasing distance from genes (fig. 1). As a consequence of different patterns of divergence on the X chromosome and autosomes, we observed that the ratio of X/A divergence increases with increasing distance from genes, driven by a faster increase in the rate of divergence on the X chromosome relative to the autosomes (supplementary fig. S1, Supplementary Material online). However, the observed divergence in the recently diverged populations could be inflated by diversity present in the ancestral population. To attempt to correct these divergence estimates for ancestral polymorphism, we used diversity estimates (and multipliers of diversity estimates) of autosomes and X chromosomes from the modern populations as a proxy for diversity in ancestral populations (supplementary text S1 and fig. S1, Supplementary Material online). Because modern and ancestral populations likely differ in effective population size (Pool and Nielsen 2007; Prado-Martinez et al. 2013), we acknowledge that this correction may not be sufficient to get the exact estimate of divergence.

Estimates of Male Mutation Bias Are Higher Near Genes than Far from Genes When Measured to the MRCA of Great Apes

Male mutation bias, the ratio of the mutation rate in males to females (α), is estimated from genetic divergence data on the X and autosomes, assuming they spend different amounts of time in the male and female germline (Miyata et al. 1987; Li et al. 2002; Makova and Li 2002; Makova et al. 2004; Taylor et al. 2006; Wilson Sayres and Makova 2011). Our finding that patterns of divergence on the X and autosomes are different near and far from genes suggests that genomic regions analyzed will greatly affect estimates of α from substitution rates. To investigate this further, we computed α with increasing distance from genes. For all great apes we observed higher α values in genomic regions near genes, and lower estimates far from genes (fig. 2; supplementary fig. S2 and table S1, Supplementary Material online). Using both the corrected and uncorrected X/A divergence ratios, we computed α with increasing distance from genes. For all great apes, α values are higher in genomic regions near genes, and lower far from genes (supplementary fig. S2, Supplementary Material online). Estimates of male mutation bias near genes are approximately twice as high as estimates in regions farthest from the genes (fig. 2B).

Ape Branch-Specific Substitution Rates Vary with Distance from Genes When Measuring in Both Genetic and Physical Distance

We computed genome-wide, branch-specific divergence estimates in bins corresponding to varying distances from genes measured using both genetic distance (in centimorgans, cM) and physical distance (in base pairs, bp) to investigate species-specific estimates of α. Across the genome, divergence increases as either genetic distance (fig. 3A) or physical distance (fig. 3B) from genes increases. Paralleling observations of substitutions to the great ape common ancestor, divergence on X chromosome increases faster than autosomes with increasing distance from genes (fig. 3). Consequently, the branch-specific ratio of X/A divergence also increases with increasing distance from the genes (fig. 3). However, the X/A ratio increases monotonically with increasing physical distance (fig. 3B) but not genetic distance (fig. 3A), due to a reduction in X-chromosome divergence in the 0.1–0.2 cM bin in most species.

Very Similar X/a Ratios Can Give Dramatically Different Estimates of Male Mutation Bias Using the Current Framework

Using the X/A ratios calculated from branch-specific divergence in great apes species, we computed the magnitude of male mutation bias (α) with increasing distance from the genes, using both genetic and physical distance (fig. 4 and supplementary table S2, Supplementary Material online). Branch-specific estimates of the X/A ratio focus on recent time. The observed branch-specific X/A ratios are lower than X/A ratios measured to the MRCA of great apes, and corresponding branch-specific α values are much higher than

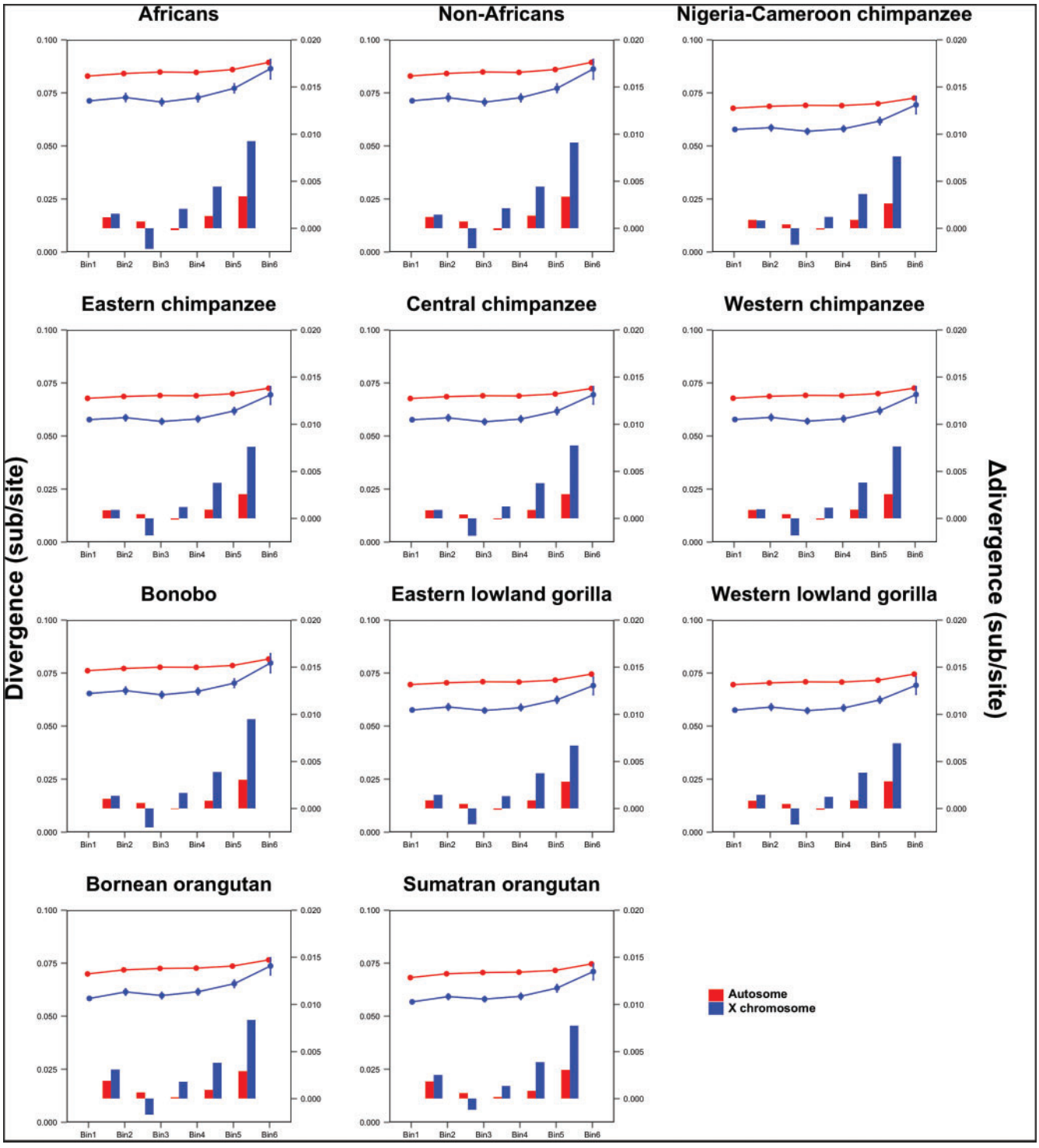

Fig. 1.—Divergence from the MRCA across great apes using population data. Divergence increases with distance from genes faster on the X chromosome (blue) than on the autosomes (red) for all great ape subspecies, plotted as lines with bars representing 95% confidence intervals calculated using 1000 bootstrap replicates. The difference between the divergences of two consecutive bins is plotted as bars between each pair of bins (Bin 1 = 0–0.05 cM, Bin 2 = 0.05–0.1 cM, Bin 3 = 0.1–0.2 cM, Bin 4 = 0.2–0.4 cM, Bin 5 = 0.4–0.8 cM, Bin 6 = 0.8–2.0 cM).

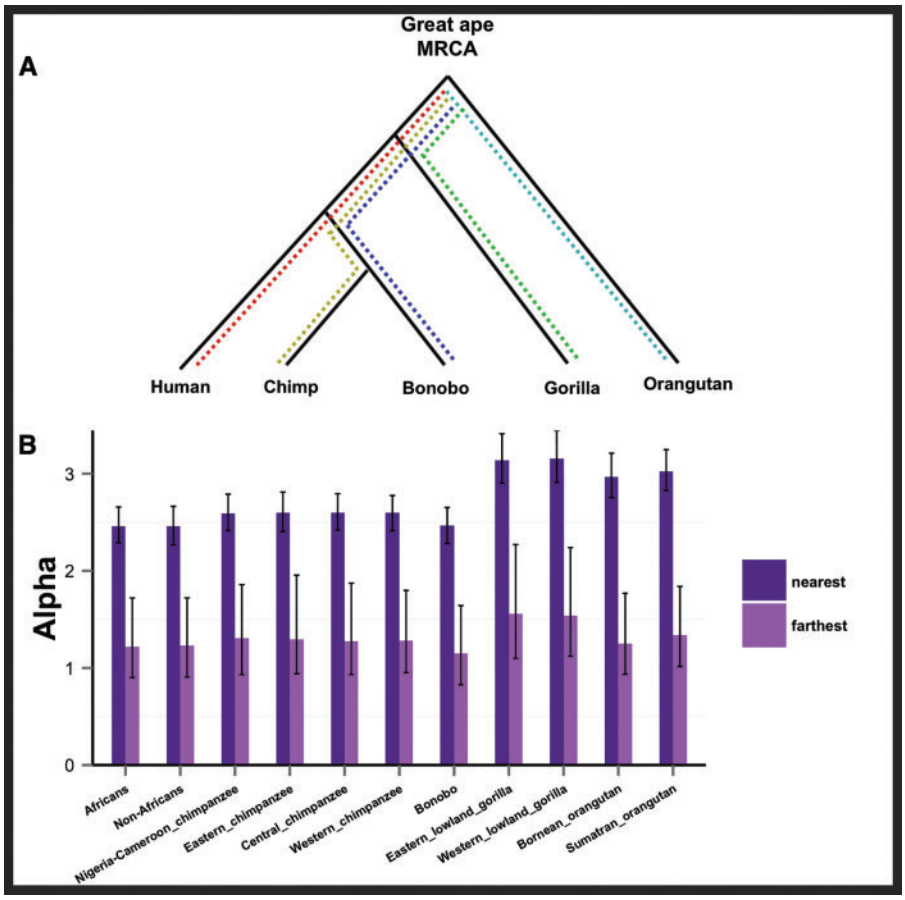

Fig. 2.—Male mutation bias computed along each branch of great ape to the MRCA. (*A*) Phylogeny of great apes species to estimate divergence from the great ape MRCA. (*B*) Male mutation bias estimates (α), corrected for ancestral diversity (pi), near (Bin 1 = 0–0.05 cM) and far (Bin 6 = 0.8–2.0 cM) from the genes.

estimates using divergence to the ape MRCA (supplementary table S1 and fig. S2, Supplementary Material online). Further, branch-specific estimates of α vary widely with increasing distance from genes (supplementary table S2, Supplementary Material online). Estimates of α in the three bins nearest to genes are 3–10 times greater than α estimates in the three bins farthest from genes, which were much more similar to each other (fig. 4; supplementary table S2, Supplementary Material online). Therefore, to reduce the stochasticity in estimates of male mutation bias due to unequal divergence with distance from genes on the X versus the autosomes, we measured the X/A ratio in the intergenic regions far from genes, which corresponds to removing the first 0.2 cM (if using genetic distance) or 200 kbp (if using physical distance) of intergenic regions near genes (supplementary fig. S3, Supplementary Material online; X/A ratios, α values, and 95% CI for all species in intergenic regions far from genes are in supplementary table S3, Supplementary Material online).

Different Divergence Estimates for Genic and Intergenic Regions on Autosomes and X-Chromosomes

To further investigate differential patterns of divergence on the X chromosome and autosomes in humans, we calculated and compared the ratio of X/A divergence and α for exons, introns, intergenic regions near genes (Bin 1–Bin3), intergenic regions far from genes (Bin 4–Bin 6), and all intergenic regions (Bin 1–Bin 6), and (supplementary fig. S3 and table S3, Supplementary Material online). For both the X chromosome and autosomes, branch-specific divergence was significantly lower within exons than introns or any of the intergenic regions (supplementary fig. S3, Supplementary Material online). Whereas divergence in introns was significantly lower than divergence in any of the intergenic intervals on the autosomes, divergence on the X chromosomes is very similar between introns and intergenic regions near genes (supplementary fig. S3, Supplementary Material online). Consequently, we do not observe a monotonic increase in the X/A divergence ratio across exons, introns, intergenic regions near genes, and

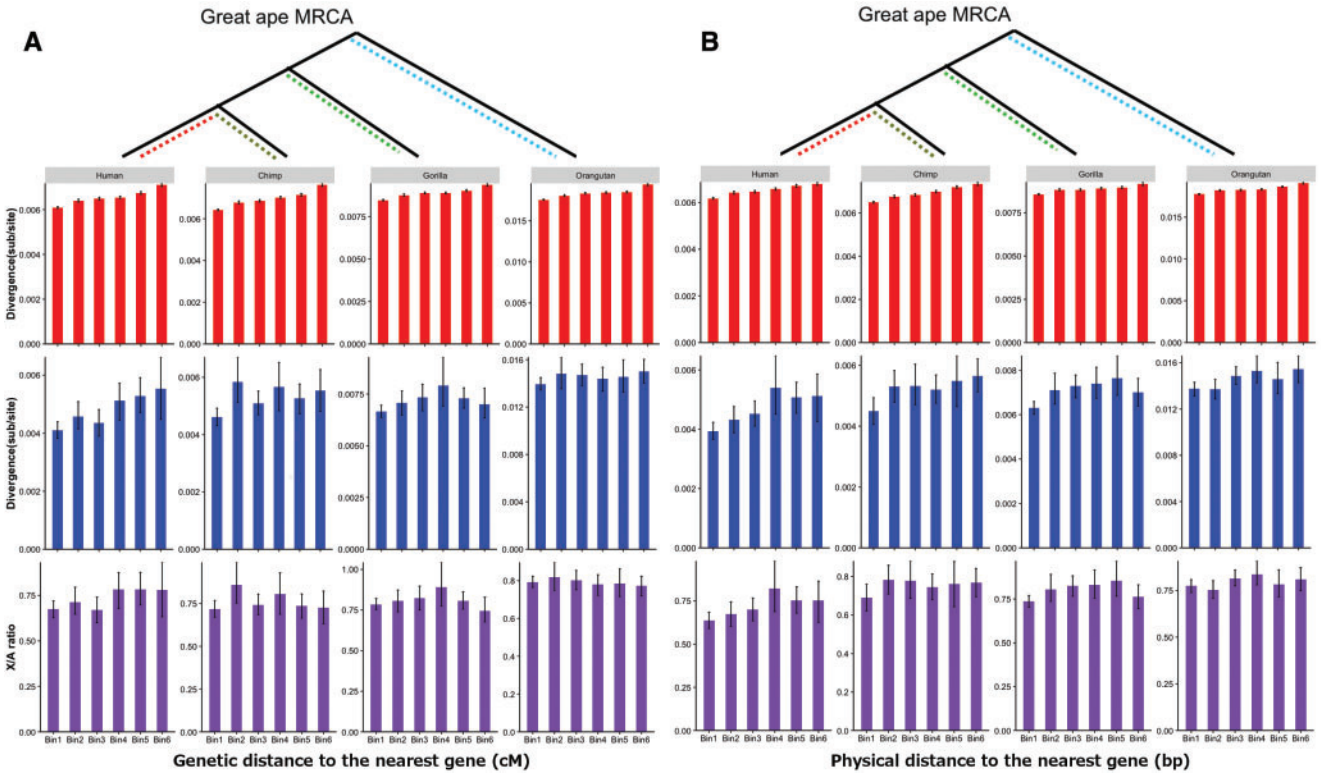

Fig. 3.—Branch specific divergence estimates across great apes using reference genome. (*A*) Divergence on autosomes, X chromosome and X/A ratio plotted in bins with genetic distance from genes (Bin 1 = 0–0.05 cM, Bin 2 = 0.05—0.1 cM, Bin 3 = 0.1–0.2 cM, Bin 4 = 0.2–0.4 cM, Bin 5 = 0.4–0.8 cM, Bin 6 = 0.8–2.0 cM). (*B*) Divergence on autosomes, X chromosome and X/A ratio plotted in bins with physical distance from genes (Bin 1 = 0–50 kbp, Bin 2 = 50–100 kbp, Bin 3 = 100–200 kbp, Bin 4 = 200–400 kbp, Bin 5 = 400–800 kbp, Bin 6 = 800–2000 kbp).

far from genes (supplementary fig. S3, Supplementary Material online).

Potential Confounders Cannot Explain Differences in X-Linked and Autosomal Divergence with Increasing Distance from Genes

To investigate the potential role of GC content with substitution rate variation we accessed how GC content correlates with divergence in bins of increasing distance from genes (supplementary fig. S4, Supplementary Material online). GC content decreases with increasing distance from genes, whereas divergence increases with distance from genes. This either suggests that there is a negative correlation between GC content and substitution rate (contrary to some expectations and observations) (Piganeau et al. 2002; Smith et al. 2002), or that both GC content and divergence are correlated with distance from genes. To distinguish between these two alternatives, we computed divergence and GC content in windows independent of distance from genes and found no relationship between GC content and substitution rate (supplementary fig. S4, Supplementary Material online).

To investigate if reduced X-linked divergence could be due to differences in gene density of autosomes and X chromosomes, we calculated relative gene density for all the chromosomes in human (supplementary fig. S5, Supplementary Material online). Although not the lowest, we observe that the density of genes on the X chromosome is relatively low, compared with most autosomes (supplementary fig. S5, Supplementary Material online). However, a lower gene density would be expected to correlate with more unconstrained regions, and higher estimates of divergence between species, thus, it is unlikely that gene density explains lower X-linked versus autosomal divergence near genes. We also compared sizes of introns on both autosomes and X-chromosome (supplementary fig. S6, Supplementary Material online). The distribution of intron sizes for autosomes and X chromosomes are not significantly different (Wilcoxon rank-sum test *P*-value: 0.511), suggesting that differential patterns of divergence on X and autosomes are not due to differences in intron sizes (supplementary fig. S6, Supplementary Material online).

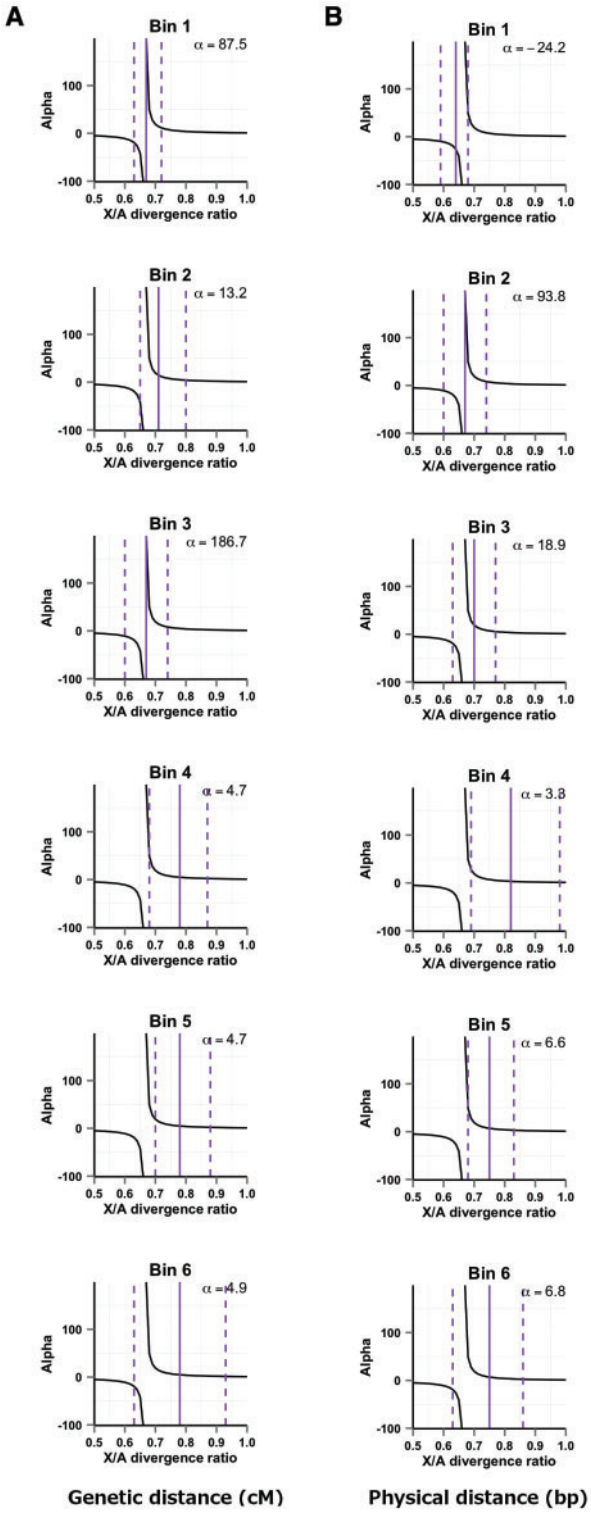

Fig. 4.—Male mutation bias estimates for humans using branch specific divergence estimates. (*A*) α values and 95% CIs represented on plots of variation of alpha as a function of X/A ratio (eq. 3), where X/A divergence ratio is calculated using genetic distance from genes. (*B*) α values and 95% CIs represented on plots of variation of alpha as a function of X/A ratio (eq. 3), where X/A divergence ratio is calculated using physical distance from genes.

Discussion

Factors Affecting Intergenic X-Linked and Autosomal Divergence

Across great ape species, and on different time scales, we observe that divergence increases with distance from genes across the genome, and that divergence increases faster with distance from genes on the X chromosome than on the autosomes. This parallels observations of similar patterns of differential population diversity between X and autosomes with distance from genes within humans (Hammer et al. 2010; Gottipati et al. 2011; Prado-Martinez et al. 2013; Arbiza et al. 2014; Veeramah et al. 2014; Nam et al. 2015). Variable patterns of divergence with distance from genes results in increasing X/A divergence ratios and corresponds to decreasing estimates of the magnitude of male mutation bias with increasing distance from genes. There are several possible explanations for the patterns we observe, but a likely explanation is that selection is acting to reduce divergence differently on the X chromosome and autosomes. Increasing divergence with distance from genes across the autosomes and X chromosomes could occur if selection is acting to affect fixation of polymorphic alleles in unconstrained regions near selected regions in the ancestral population (Hellmann et al. 2003; Reed et al. 2005; Begun et al. 2007; McVicker et al. 2009; Lohmueller et al. 2011). An alternative explanation for the pattern we observe is that regions near genes are not truly unconstrained, and the filters for neutral regions that we used are missing regions near genes that are also directly affected by selection.

The difference between the X and autosomes is consistent with observations that selection on linked neutral sites has a stronger effect on the X chromosome than on the autosomes across the great apes (Hudson and Kaplan 1995; Charlesworth 1996; Orr and Betancourt 2001; Vicoso and Charlesworth 2006; Ávila et al. 2015; Coolon et al. 2015). Natural selection is expected to be more efficient on the X chromosome because it is hemizygous in males, and recessive alleles will be routinely exposed to selection (Vicoso and Charlesworth 2006, 2009; Coolon et al. 2015). Although divergence in unconstrained regions has been predicted to be unaffected by linked selection (Birky and Walsh 1988), other work suggests that selection in the ancestral population may affect estimates of species divergence in linked neutral regions (Hellmann et al. 2003; Reed et al. 2005; Begun et al. 2007; McVicker et al. 2009; Lohmueller et al. 2011; Phung et al. 2016).

Another possible explanation for the difference in substitution rates in intergenic regions of the X chromosome versus the autosomes across great apes is the evolution of X-inactivation (Carrel and Willard 2005). Gene expression on the X chromosome evolved in response to gene loss on the Y chromosome (Wilson Sayres and Makova 2013), with the X chromosome accumulating motifs predicted to be associated with inhibiting or allowing gene-specific silencing on one X chromosome (Carrel et al. 2006). Long interspersed repeats (L1s) hypothesized to be involved in X inactivation (Lyon 1998), are found abundantly in the regions close to inactive genes (Carrel et al. 2006). Motifs related to X-inactivation are overrepresented within repetitive elements, and primarily occur in intergenic regions near affected genes (Carrel et al. 2006). Although most motifs are expected to occur in repetitive elements, which are filtered out of our analysis, some unidentified motifs may also accumulate near genes on the X chromosome (Horvath et al. 2013) and may be included in putatively neutral regions. Selection acting to maintain these motifs would affect estimates of substitution rates on the X chromosome, but not the autosomes, near genes. Consistent with this explanation, we observed that the divergence is reduced more on the X than the autosomes (yielding a lower X/A ratio) in intergenic regions near genes, where these motifs are predicted, than in intronic regions, where a difference between the X and autosomes due to X-inactivation is not expected (supplementary fig. S3, Supplementary Material online).

We also investigated if factors affecting mutation rate variation along the genome, like local GC content, can explain differences in X-linked and autosomal divergence with increasing distance from genes. GC content is a potential confounder of substitution rate variation, but the expected correlation is unclear (Piganeau et al. 2002; Smith et al. 2002; Arndt et al. 2005; Mugal and Ellegren 2011). GC content decreases with increasing distance from genes, whereas divergence increases with distance from genes (supplementary fig. S4, Supplementary Material online), and there is no correlation between GC content and divergence in a chromosome (supplementary fig. S4, Supplementary Material online). Thus, our analysis suggests that both GC content and substitution rate vary with distance from genes, but that it is not GC content itself that is driving changes in substitution rate variation with distance from genes. Also, we show that both the differences between the gene density (supplementary fig. S5, Supplementary Material online), as well as intron sizes (supplementary fig. S6, Supplementary Material online), between X and autosomes, is unlikely to be responsible for the higher X-linked divergence. Other factors like context dependent effects, especially the accumulation of substitutions at methylated CpG dinucleotides, are known to affect the mutation rate in mammals (Hwang and Green 2004), and could potentially differ between the X chromosome and autosomes. Biological mechanisms like variations in transcription coupled repair can expose the DNA to mutagens (Hanawalt and Spivak 2008), thus affecting mutation rate variation in the region. Additional processes, like differential replication timing of X chromosome and autosomes (Lubelsky et al. 2014), or different recombination rates in males and females (Coop and Przeworski 2007), could impact mutation rate variation differently on autosomes and X chromosome. Though, given our

estimates of gene density and intron size on the X and autosomes, neither of these mechanisms would be expected to result in the observed pattern.

Male Mutation Bias Decreases with Distance from Genes Regardless of Measuring Using Genetic or Physical Distance

We used genetic distances to estimate divergence from the MRCA for great apes. Our results show an increase in divergence as the distance from genes increases on both autosomes and X chromosomes, but the increase on the X chromosome is non-monotonic (fig. 1). Estimates on the X chromosome show a reduction in divergence at a distance of 0.1–0.2 cM from the genes (fig. 1). The low X/A divergence ratio and high male mutation bias estimates around the same genetic distance (0.1–0.2 cM) appear to be driven by the low divergence on the X relative to the autosomes (supplementary figs. S1 and S2, Supplementary Material online). This could be an artifact of the genetic map used to map the genetic distances, or due to additional constrained regions on the X chromosome. Interestingly, X-linked nucleotide diversity in human populations showed a similar trend around 0.11–0.19 cM in two previous studies (Gottipati et al. 2011; Arbiza et al. 2014). We also observed lower divergence on the X chromosome at this distance using branch-specific estimates for the human branch, using the human recombination map (International HapMap Consortium 2005), when distance from genes was measured using cM but not bp (fig. 3). Genetic maps used to estimate genetic distance rely on obtaining accurate estimates of genome-wide recombination rates, and are influenced by multiple factors, therefore accurate determination of genetic distance is very challenging. Recombination hotspots vary significantly across species and even between populations of the same species (Auton et al. 2012). Further, the correlations among broad-scale recombination rates are found to decline more rapidly than nucleotide divergence between species across great apes (Stevison et al. 2016), questioning the reliability of using the genetic map of a single species for comparative genomics purposes. Divergence estimates within the 0.1–0.2 cM from genes on the X chromosome could be reduced due to additional constrained regions on the X chromosome not filtered out, but then such a reduction would also be expected—but was not observed—in similar windows of physical distance (fig. 3). We used both the genetic and physical distances from genes to calculate male mutation bias for the branch specific estimates, and both methods show an overall decrease in α as distance from genes increases (fig. 4, supplementary table S2, Supplementary Material online), and provide similar α estimates in intergenic regions far from genes (supplementary fig. S3 and table S3, Supplementary Material online). However, given the high turnover of recombination hotspots, we propose that for the purposes of examining the patterns of divergence, physical distance from genes is a more reliable metric than genetic distance, as it is free from any skews stemming from interspecies recombination rate variation.

Asymptotic Estimates of Alpha from the Current Framework

In the current framework for estimating α from the X/A substitution rate ratio (eq. 3; "Materials and Methods" scetion), an asymptote occurs at an X/A ratio of 2/3. As the X/A approaches 2/3, the denominator approaches 0, yielding α values of infinity, and negative α values if the ratio of X/A divergence is lower than 2/3. A consequence of using this equation, and relying on the X/A divergence estimates computed between species to infer male mutation bias is that small changes in the X/A ratio can yield drastic variations in α, and the possibility of α values that are not meaningful (e.g., negative α values; fig. 4). The ratio of X/A divergence in bins from Bin1 to Bin3 is relatively low (<0.75) mainly driven by low divergence on X chromosome, which leads to larger estimates of α in these regions (fig. 3; supplementary fig. S3 and supplementary table S2, Supplementary Material online). Therefore, we removed the first 0.2 cM (if using genetic distance) or 200 kbp (if using physical distance) of intergenic regions near genes, thus only including genetic regions from Bin4 to Bin6 (supplementary fig. S3 and table S3, Supplementary Material online). We propose that future studies of the magnitude of male mutation bias report the X/A ratio and 95% confidence interval as well as the corresponding α value to allow for better interpretation of variation in α.

Male Mutation Bias across the Great Ape Lineage

Generation time is the strongest predictor of variation in male mutation bias (Wilson Sayres and Makova 2011), likely because in species with shorter generation times, there will be fewer differences in the number of male germline cell divisions relative to female germline cell divisions. Estimates of generation time are highest in modern humans (29.1 years), and decrease with increasing evolutionary divergence, with chimpanzees (24.63 years), and gorillas (19.28 years) (Langergraber et al. 2012). Orangutans have a similar average generation time to chimpanzees of 24.4 years (Wich 2009). Branch-specific α estimates also vary across the great apes. For humans, chimpanzees, and gorillas (which have roughly similar branch lengths in this comparison), using genetic and physical distance, respectively, we estimate that male mutation bias is highest for chimpanzees 5.33 (95% CI: 2.88–17.73) and 7.96 (95% CI: 4.34–23.48), lowest for gorilla 3.32 (95% CI: 2.08–5.66) and 3.10 (95% CI: 2.13–4.79), and intermediate for humans at 4.31 (95% CI: 2.57–9.26) and 6.45 (95% CI: 3.60–21.94) (table 1). These branch-specific estimates are largely consistent with previous estimates for chimpanzee (Presgraves and Yi 2009; Wilson Sayres and Makova 2011), slightly higher than previous estimates for

Table 1
Branch-specific estimates of α from the present study, in comparision with previous estimates

Species	Present study (genetic distance, physical distance)	Wilson Sayres et al. 2011	Presgraves and Yi 2009
Human	4.31 (2.57–9.26) 6.45 (3.60–21.94)	20.09 (8.34–inf)	4.91(2.97–9.16)
Chimpanzee	5.33 (2.88–17.73) 7.96 (4.34–23.48)	3.61 (2.54–5.28)	4.39(2.80–8.21)
Gorilla	3.32 (2.08–5.66) 3.10 (2.13–4.79)	2.53 (1.44–4.54)	2.08(1.53–2.93)
Orangutan	4.89 (3.42–7.21) 4.11 (3.14–5.69)	3.53 (2.62–5.04)	1.96(1.59–2.46)

gorilla (Presgraves and Yi 2009; Wilson Sayres and Makova 2011), and similar to estimates for human (Taylor et al. 2006; Presgraves and Yi 2009; Wilson Sayres and Makova 2011; Xu et al. 2012). In contrast, we estimate male mutation bias to be higher in orangutan than previous reports (Presgraves and Yi 2009; Xu et al. 2012): 4.89 (95% CI: 3.42–7.21) and 4.11 (95% CI: 3.14–5.69), using genetic and physical distance, respectively (table 1).

Generation time in modern chimpanzees is not as long as in modern humans (Langergraber et al. 2012), so the largest α estimate in chimpanzees cannot be attributed to differences in generation time between these species. Alternatively, larger α in chimpanzees may be due to differences in other life history traits, differences in natural selection acting on the X in chimpanzees relative to humans (Nam et al. 2015) as well as technical artifacts (e.g., poorer sequence quality, especially on the X chromosome in chimpanzee, for which a male was sequenced (Chimpanzee Sequencing and Analysis Consortium 2005). Variation in male to female generation times may also explain some differences in estimates of male mutation bias (Amster and Sella, 2016). Theory suggests that sperm competition could drive a male-biased mutation rate, if sperm competition results in a larger quantity of sperm being produced (Blumenstiel, 2007). Interestingly, the estimated measures of α among great apes corresponds to the rank order of the intensity of sperm competition in great apes: chimpanzee > human > orangutan > gorilla (Harcourt et al. 1981; Austin and Short 1985; Dixon 2009). Sperm competition was not found to be a significant predictor of variation in male mutation bias across the mammalian phylogeny (Wilson Sayres and Makova 2011). However, it is possible that predictors of sperm competition, and the competition itself, evolve quickly, and so any signal of the effect of sperm competition was washed out when considering patterns of male mutation bias over long evolutionary time (such as across all mammals), but may be apparent over shorter evolutionary time (such as in these primates).

Conclusions

We studied X chromosome and autosomal divergence across the great ape showing that over both long (from the MRCA of great apes) and short (branch-specific) evolutionary time divergence increases with increasing distance from genes. Further, divergence increases faster on the X chromosome than the autosomes, differentially shaping patterns of divergence on the X chromosome and autosomes, and affecting estimates of male mutation bias. The increasing divergence in the intergenic regions with increasing distance from genes may be explained by constrained regions that are not filtered out across the genome, or potentially by linked selection acting to reduce variation near constrained regions. Divergence estimates from the MRCA of great apes result in estimates of male mutation bias close to genes that are twice as high as estimates farthest from genes. Similarly, branch-specific divergence estimates results in estimates of α that are 3–10 times greater near genes than far from genes. However, the overall pattern is that male mutation bias has increased along the great ape lineage in modern apes relative to ancestral populations. Curiously, when taking a conservative estimate of male mutation bias in regions far from genes, we find that estimates of male mutation bias across great apes positively scale with levels of sperm competition. Our results shed light on differential patterns of divergence across genomic regions, across autosomes and X-chromosome, across species, and advance our understanding of male mutation bias in apes.

Supplementary Material

Supplementary figures S1–S6, text S1, and tables S1–S3 is available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

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