

Evolutionary rate variation in Old World monkeys

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We analysed over 8 million base pairs of bacterial artificial chromosome-based sequence alignments of four Old World monkeys and the human genome. Our findings are as follows. (i) Genomic divergences among several Old World monkeys mirror those between well-studied hominoids. (ii) The X-chromosome evolves slower than autosomes, in accord with ‘male-driven evolution’. However, the degree of male mutation bias is lower in Old World monkeys than in hominoids. (iii) Evolutionary rates vary significantly between lineages. The baboon branch shows a particularly slow molecular evolution. Thus, lineage-specific evolutionary rate variation is a common theme of primate genome evolution. (iv) In contrast to the overall pattern, mutations originating from DNA methylation exhibit little variation between lineages. Our study illustrates the potential of primates as a model system to investigate genome evolution, in particular to elucidate molecular mechanisms of substitution rate variation.

Keywords: Old World monkeys; substitution rates; male mutation bias; comparative genomics

1. INTRODUCTION

The genomics of humans and apes (hominoids), particularly of humans and chimpanzees, is an active field of research. Here, we propose that Old World monkeys offer an excellent genomic model system, complementary to hominoids for several reasons. Old World monkeys are the closest outgroup to hominoids. Divergence times among some Old World monkeys are comparable to those among well-known hominoids. For example, the rhesus macaque and baboon share a similar divergence time (6–8 Myr ago) to that of humans and chimpanzees (Delson *et al.* 2000; Steiper *et al.* 2004, although see Raauum *et al.* (2005) for an alternative, older estimate). A large amount of genomic resources are available from Old World monkeys. Furthermore, since some Old World monkeys, especially the rhesus macaque and baboon, served as non-human primate model systems for biomedical studies (e.g. Higley *et al.* 1991),

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comparative genomic analyses will complement functional data.

As the first step to developing a genomic model system, we present here the comparative genomic analyses of several Old World monkeys. We generated a large amount of sequence alignments between four Old World monkeys (macaque, baboon, grivet and colobus monkey) and the human. From these, we obtained estimates of genomic divergence among these species. We also compared substitution rates (also referred to as ‘evolutionary rates’) of the X-chromosome and autosomes, and between different Old World monkey lineages. Finally, we investigated whether mutations that mainly originate from DNA methylation exhibit different patterns of evolutionary rate variation compared with other mutations, as suggested recently (Kim *et al.* 2006).

2. MATERIAL AND METHODS

We downloaded bacterial artificial chromosome (BAC) sequences of the following species from GenBank in April 2008: 443 baboon (*Papio anubis* and *Papio hamadryas*) BACs; 301 black-and-white colobus monkey (*Colobus guereza*, referred to as ‘colobus monkey’ here) BACs; and 305 grivet (*Chlorocebus aethiops*) BACs. Pairwise alignments of human and rhesus macaque (*rheMac2*), chimpanzee (*panTro1*) and orangutan (*ponAbe2*) were downloaded from the UCSC genome browser. Alignment of these sequences and annotation followed methods in Elango *et al.* (2008) (see the electronic supplementary material).

To analyse substitution rates at CpG and non-CpG sites, we assembled alignments of human–chimpanzee–orangutan–baboon–macaque–colobus monkey. The orangutan and colobus monkey are used as outgroups to the human–chimpanzee and baboon–macaque pairs, respectively. CpG sites (sites that were likely to have been a part of CpG dinucleotide) and non-CpG sites are identified using the parsimony method as in Meunier & Duret (2004) and Elango *et al.* (2008). C to T (or G to A) transitions are then counted for CpG and non-CpG sites separately. Only non-coding, non-repetitive sites are used for this analysis.

3. RESULTS

(a) Genomic divergence among Old World monkeys and humans

We assembled alignments of 8.28 million base pairs over 20 autosomes (excluding chromosomes 3 and 17) and the X-chromosome of four Old World monkeys (macaque–baboon–colobus–grivet) and the human reference sequence (electronic supplementary material, table 1). Genomic divergence estimates from whole data are given in table 1. Results from non-coding sequences (with or without repetitive sequences) are shown in the electronic supplementary material. Overall, repetitive sequences accumulate more mutations than non-repetitive sequences and divergence estimates from data including repetitive sequences are usually greater than estimates from those without repetitive sequences. For instance, the divergence between humans and rhesus macaques from the whole data (including autosomes and the X-chromosome) is 6.19 per cent. Non-coding sequences including repetitive sequences show greater divergence (6.36%). Divergence estimated from non-coding, non-repetitive portions is 5.73 per cent for the same species pair. Other pairwise comparisons showed similar trends.

The genetic divergences among the four Old World monkey species mirror those between well-studied hominoids: the rhesus macaque and baboon are approximately 1.35 per cent divergent in the non-coding,

Table 1. Divergence among the Old World monkeys and humans estimated from whole alignments. Below and above diagonals are autosomal and X-chromosome divergence, respectively.

	baboon	macaque	grivet	colobus	human
baboon	—	0.0106 ($\pm 1.23 \times 10^{-4}$)	0.0153 ($\pm 1.48 \times 10^{-4}$)	0.0270 ($\pm 1.98 \times 10^{-4}$)	0.0538 ($\pm 2.85 \times 10^{-4}$)
macaque	0.0156 ($\pm 0.95 \times 10^{-4}$)	—	0.0164 ($\pm 1.54 \times 10^{-4}$)	0.0281 ($\pm 2.02 \times 10^{-4}$)	0.0543 ($\pm 2.86 \times 10^{-4}$)
grivet	0.0223 ($\pm 0.95 \times 10^{-4}$)	0.0233 ($\pm 0.56 \times 10^{-4}$)	—	0.0280 ($\pm 2.02 \times 10^{-4}$)	0.0547 ($\pm 2.88 \times 10^{-4}$)
colobus	0.0330 ($\pm 0.96 \times 10^{-4}$)	0.0340 ($\pm 0.69 \times 10^{-4}$)	0.0340 ($\pm 0.69 \times 10^{-4}$)	—	0.0564 ($\pm 2.92 \times 10^{-4}$)
human	0.0617 ($\pm 0.94 \times 10^{-4}$)	0.0627 ($\pm 0.95 \times 10^{-4}$)	0.0631 ($\pm 0.95 \times 10^{-4}$)	0.0640 ($\pm 0.96 \times 10^{-4}$)	—

Table 2. Male-to-female mutation ratio (α_m) estimated by comparing the X-chromosome and autosomal divergence. The 95% CIs using bootstrapping are shown.

	baboon	macaque	grivet	colobus
macaque	-32.6 [-872.8, 728.49]	—		
grivet	17.0 [-228.2, 310.9]	17.3 [-5242, 5462]	—	
colobus	3.13 [2.96, 3.88]	3.35 [2.95, 3.87]	3.16 [3.06, 4.07]	—
human	2.04 [1.91, 2.25]	2.14 [1.96, 2.32]	2.13 [1.97, 2.33]	2.03 [1.82, 2.15]

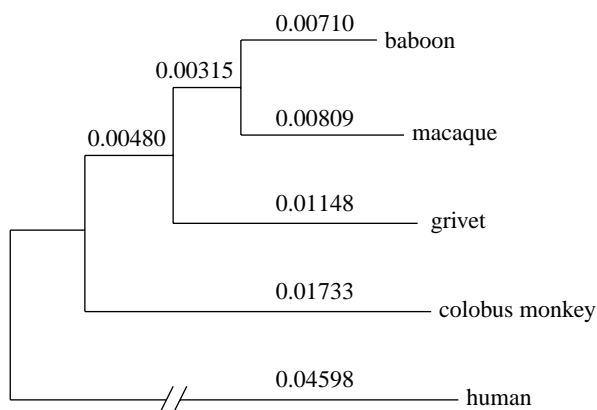


Figure 1. Phylogenetic relationship and branch lengths (roughly drawn to scale) among the five species analysed using the neighbour-joining method (Saitou & Nei 1987). The estimated numbers of substitutions per site are shown above each branch.

non-repetitive portions of the data. The divergence between the grivet and the baboon-macaque pair is approximately 2 per cent. All three Old World monkeys are approximately 3 per cent different from the colobus monkey. Thus, divergences between the baboon-macaque-grivet-colobus monkey closely resemble those between the human-chimpanzee-gorilla-orangutan (e.g. Chen & Li 2001), even though the actual divergence times between these four species may differ from those between the hominoids (e.g. Raaum *et al.* 2005).

(b) Low male mutation bias in Old World monkeys compared with hominoids

In primates, in particular between humans and chimpanzees, the X-chromosome exhibits lower divergence than autosomes (Nachman & Crowell 2000).

While a commonly cited explanation for such phenomena is the 'male-driven evolution' hypothesis (Li *et al.* 2002), some studies propose that lineage-specific evolutionary forces such as hybridization may be responsible (Patterson *et al.* 2006). Here, we examined the divergence of the X-chromosomes and autosomes separately (table 1). In all comparisons, regardless of the type of sites used, the X-chromosome shows lower divergence than the autosomes.

We estimated the male-to-female mutation ratio, denoted as α_m , from the comparison of the divergence on the X-chromosome (X) to autosomal divergence (A), following Miyata *et al.* (1987), as $(X/A) = (2(2 + \alpha_m)/3(1 + \alpha_m))$ (table 2).

Between closely related Old World monkey species, α_m estimates fluctuated greatly and the confidence intervals included zero (e.g. between the macaque and baboon). It is likely that ancestral polymorphism between closely related Old World monkeys hinders accurate estimations of α_m (e.g. Makova & Li 2002). Still, it is interesting to note that the mean α_m from all pairwise comparisons is approximately 2, much less than the earlier estimates of 5–6 in primates (Shimmin *et al.* 1993). Even when we restrict our comparisons to the comparisons between humans and Old World monkeys (instead of between different Old World monkeys) to avoid confounding effects of ancestral polymorphism, the mean α_m is approximately 2. Thus, the male mutation bias in Old World monkeys appears to be lower than that in hominoids.

(c) Evolutionary rate variation between Old World monkey lineages

We analysed evolutionary rate variation among Old World monkeys using the relative rate test (Wu & Li 1985), with the human as an outgroup. Figure 1

Table 3. Transitions at CpG and non-CpG sites exhibit qualitatively different patterns of molecular evolution. Divergences between human–chimpanzee (K_{HC}) and between macaque–baboon (K_{MB}) are shown separately. The 95% CIs of $K_{\text{MB}}/K_{\text{HC}}$ are obtained from bootstrapping 1000 times.

	repetitive		non-repetitive		total	
	CpG	non-CpG	CpG	non-CpG	CpG	non-CpG
K_{HC} ($\times 10^{-2}$)	10.1	0.75	7.31	0.63	8.39	0.67
K_{MB} ($\times 10^{-2}$)	9.2	0.98	8.11	0.82	8.50	0.87
$K_{\text{MB}}/K_{\text{HC}}$ [95% CI]	0.915 [0.80, 1.03]	1.31 [1.20, 1.43]	1.109 [0.99, 1.22]	1.29 [1.20, 1.38]	1.01 [0.93, 1.09]	1.29 [1.22, 1.36]

depicts the branch lengths estimated from the total data. It is known that substitution rates vary significantly among hominoids (Elango *et al.* 2006). Here, we find that evolutionary rate variation is even more conspicuous in Old World monkeys than in hominoids. Interestingly, the baboon lineage appears to be evolving more slowly than the other three Old World monkey branches, regardless of the nature of the dataset used ($p < 10^{-5}$ in all comparisons using the relative rate test). For example, the rate difference between the macaque and baboon lineages ranges between 5 (non-coding, non-repetitive dataset) and 14 per cent (total data).

(d) *Contrasting patterns of molecular evolution of transitions at CpG and non-CpG sites*

Most mutations are considered to occur by errors in DNA replication. Consequently, species that undergo more DNA replication in a given amount of time may accumulate more mutations compared with those that undergo fewer replication events. This is the reasoning underlying the so-called ‘generation-time effect’ (Yi *et al.* 2002).

However, mutations at CpG dinucleotides often originate from DNA methylation, rather than replication error. Since DNA methylation is not restricted solely to the DNA replication process, it was proposed that mutations at CpG sites may accumulate relatively constantly over time (Kim *et al.* 2006). Here, we revisited this question, with a larger amount of data and closer outgroups.

We compared substitution rates between the human–chimpanzee pair with that between the baboon–macaque pair. The absolute divergence times within the two pairs are similar (Delson *et al.* 2000; Steiper *et al.* 2004), while evolutionary rates are generally greater in Old World monkey lineages, due to the generation–time effect (Yi *et al.* 2002; Steiper *et al.* 2004). Indeed, when only non-CpG sites are analysed, the baboon–macaque pair has accumulated approximately 30 per cent more substitutions than the human–chimpanzee pair (table 3), well in accord with the known degree of rate difference (Yi *et al.* 2002; Steiper *et al.* 2004). When only CpG sites are analysed, however, the two pairs have accumulated similar numbers of substitutions (table 3), for both repetitive and non-repetitive sites. Our results thus support the proposal that transitions at CpG sites exhibit relative time dependency (Kim *et al.* 2006).

4. DISCUSSION

This study illustrates that non-hominoid primate genomics are readily available to provide a useful genomic model system complementary to hominoids. By comparing evolutionary rate variation in Old World monkeys with those from hominoids, we can distinguish common patterns of primate genome evolution from those specific to certain lineages.

Male-driven evolution can reduce the divergence of the X-chromosomes compared with autosomes. The male-to-female mutation ratio (α_{m}) in primates is considered to be approximately 5, mostly based upon comparisons between humans and chimpanzees (Shimmin *et al.* 1993; Li *et al.* 2002). We showed that between humans and Old World monkeys, α_{m} is lower than earlier estimates. A previous study also noted that α_{m} between humans and macaques is lower than 5 (RMGSAC 2007). Therefore, the degree of male mutation bias appears to be variable, even within primates. Male mutation bias may be correlated with generation time, because the relative contributions of male germlines towards the mutational pool should increase with generation times (Bartosch-Harald *et al.* 2003). Our observation is in accord with this view. However, the extent of variation of male mutation bias and causative mechanisms underlying the observed variation should be investigated further.

Evolutionary rates vary greatly between different Old World monkeys. Human substitution rates are known to be lower than chimpanzee rates (Elango *et al.* 2006). We observe a comparable phenomenon between baboons and macaques. In fact, baboons appear to evolve more slowly than the other three branches in Old World monkeys. Such observations pose at least two immediate questions. First, what caused the reported substitution rate differences? Life-history traits may influence evolutionary rates, but it is not clear which are the main determinants of rate variation between the Old World monkeys. Detailed analyses of evolutionary rates between lineages, while controlling for specific life-history traits, will allow us to evaluate the role of different life-history variables on genome evolution. Second, the fact that substitution rates vary substantially and significantly between even closely related lineages make us ask whether we can confidently infer divergence times and other evolutionary events from molecular data. On the one hand, methods incorporating rate changes need to be developed urgently.

On the other hand, the observation that CpG sites exhibit relatively constant molecular evolution compared with non-CpG sites (Kim *et al.* 2006, this study) may provide an alternative means to infer past evolutionary events.

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