Germ Line Mutation Rates in Old World Monkeys

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Mutations that occur in the germ line are the ultimate source of genetic variation upon which natural selection can act. As such, an understanding of the germ line mutation rate is crucial for interpreting evolutionary processes and products, whether in response to emerging infectious disease or climate change. Even in closely related species such as primates, substantial variation exists in both mutation rates as well as the mutational spectra, partially owing to differences in life history traits. Though considerable attention has been given to humans and other great apes in these characterisations, Old World monkeys represent an excellent and underutilised model system to investigate the extent and time scale at which mutation rates have evolved across the primate clade.

Background

Mutation – one of the most fundamental processes in biology – alters the nucleotide sequence of an organism. Mutations can be caused by a variety of molecular processes, including errors in the function of polymerase during deoxyribonucleic acid (DNA) replication as well as spontaneous (i.e. nonreplicative) changes resulting from the intrinsic instability of the genome and its exposure to endogenous (e.g. oxygen-free radicals produced during aerobic respiration) or exogenous (e.g. ultraviolet radiation or chemical mutagens) mutagenic agents that cause DNA damage that is not repaired by the time of replication. Mutations can arise in both somatic and germ line cells, the latter of which give rise to gametes. In contrast to somatic mutations, those that occur in the germ line introduce heritable changes in the genetic information that is transmitted from parents to offspring, making them an important source of genetic variation upon which natural selection may subsequently act.

The fate of a mutation depends on its frequency as well as its effect on the fitness of an organism – with selective effects ranging from lethal to deleterious (i.e. decreasing fitness) to neutral (i.e. no change in fitness) to beneficial (i.e. increasing fitness). However, as newly introduced mutations are rare, their fate is largely determined by genetic drift, a random process that causes a variant to rise or fall in frequency, eventually leading to either fixation or loss from the population. Importantly, as the fitness effect of a mutation is determined by the product of the selection coefficient (s) and effective population size (N_e), the fraction of newly arising mutations that behave neutrally will fluctuate with population size. However, Kimura (1968) demonstrated that for strictly neutral mutations, the rate of fixation is simply given by the mutation rate (μ) – a so-called molecular clock. **See also: Mutation Rate**

Mutation is not a homogeneous process, and substantial variation exists in both mutation rates as well as mutation spectra among primate species (Elango *et al*., 2006; Kim *et al*., 2006; Amster and Sella, 2016; Gao *et al*., 2016; Moorjani *et al*., 2016a; Harris and Pritchard, 2017; Pfeifer, 2017a), resulting in a long-standing scientific interest in the topic of mutation rate evolution. In fact, many important questions in evolutionary and population genomics rely on accurate information about genomewide rates and patterns of mutations – from advancing evolutionary inference related to the genetic basis of disease, to improving our understanding of the chronology of primate evolution, to detecting signals of natural selection.

As mutations are a major cause of severe genetic disorders in humans ranging from aneuploidies (i.e. the duplication or deletion of an entire chromosome) to other developmental diseases, much previous work has focused on understanding mutation rates in our own species. In order to improve resolution in humans, this interest has also extended to our most closely related extant relative – the chimpanzee (Venn *et al*., 2014; Tatsumoto *et al*., 2017). Furthermore, though genomic resources exist in specific and more distantly related nonhuman primates owing to their importance in biomedical research, the connections between this data and the larger question of mutation rate evolution are only beginning to be explored. Most notably, Old World monkeys (OWMs) – including rhesus macaque (*Macaca mulatta*) and the African green monkey (*Chlorocebus sabaeus*) – have large-scale

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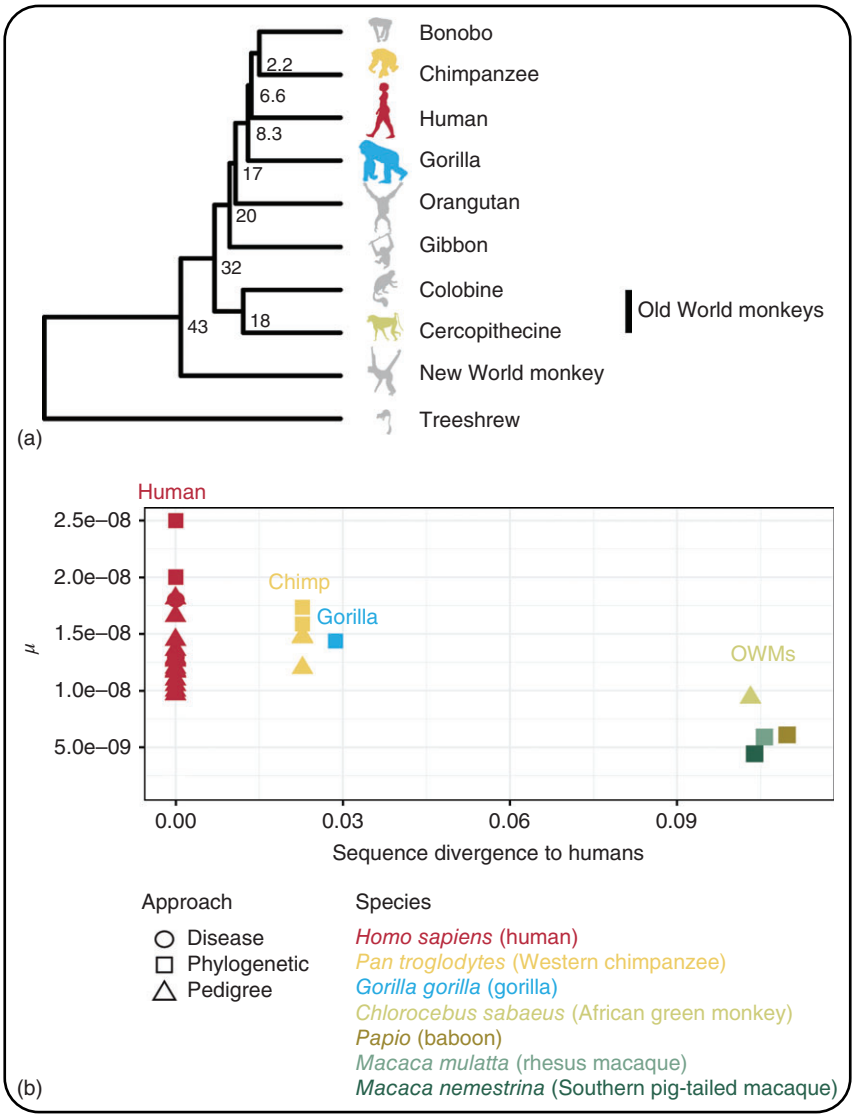

Figure 1 **Mutation rate variation in primates**. (a) Phylogeny of selected primates, including Hominoidae (bonobos, chimpanzees, humans, gorillas, orangutans and gibbons), Old World monkeys (family Cercopithecidae) and New World monkeys (superfamily Ceboidea). Species for which mutation rate information is available are colour coded, the remainder are represented in grey. Numbers indicate the time of divergence provided in millions of years. Topology and divergence times were taken from Perelman *et al.* (2011) (Supplementary Information, Tree #5). (b) Estimates of mutation rate (μ) per base pair per generation for several primate species plotted as function of their sequence divergence from humans. Colours denote taxon; shapes denote the approach used to estimate mutation rate. Mutation rate estimates were taken from Fischer *et al.* (2004); Thalmann *et al.* (2007); Hernandez *et al.* (2007); Evans *et al.* (2010); Wegmann and Excoffier (2010); Boissinot *et al.* (2014); Ségurel *et al.*, (2014); Moorjani *et al.*, (2016b); Jónsson *et al.* (2017); Narasimhan *et al.* (2017); Pfeifer (2017a); and Tatsumoto *et al.* (2017). The Boissinot *et al.* (2014) mutation rate estimate for baboons of 7.6×10^{-10} per base pair per year was converted to 6.08×10^{-9} per base pair per generation using a generation time of 8 years (Charpentier *et al.*, 2012). Sequence divergence was calculated as pairwise distance between tips using branch lengths of the phylogeny from Perelman *et al.* (2011).

and publicly available population-level data; yet they are simply two species of the highly diverse Cercopithecidae family, containing hundreds of others. This breadth, combined with the proximity of their relationship to the *Hominoidea*, makes them an excellent model system to investigate the extent and time scale on which mutation rates have evolved across primates (**Figure 1**).

This article first introduces several methods utilised to estimate mutation rates in primates, discusses known and potential determinants of mutation rates and finally summarises the current state of knowledge pertaining to germ line mutation rate in primates, with a particular focus on Old World monkeys.

Approaches to Estimate Mutation Rates

Accurately estimating mutation rates is challenging owing to the rarity of spontaneous mutations in primates. In humans,

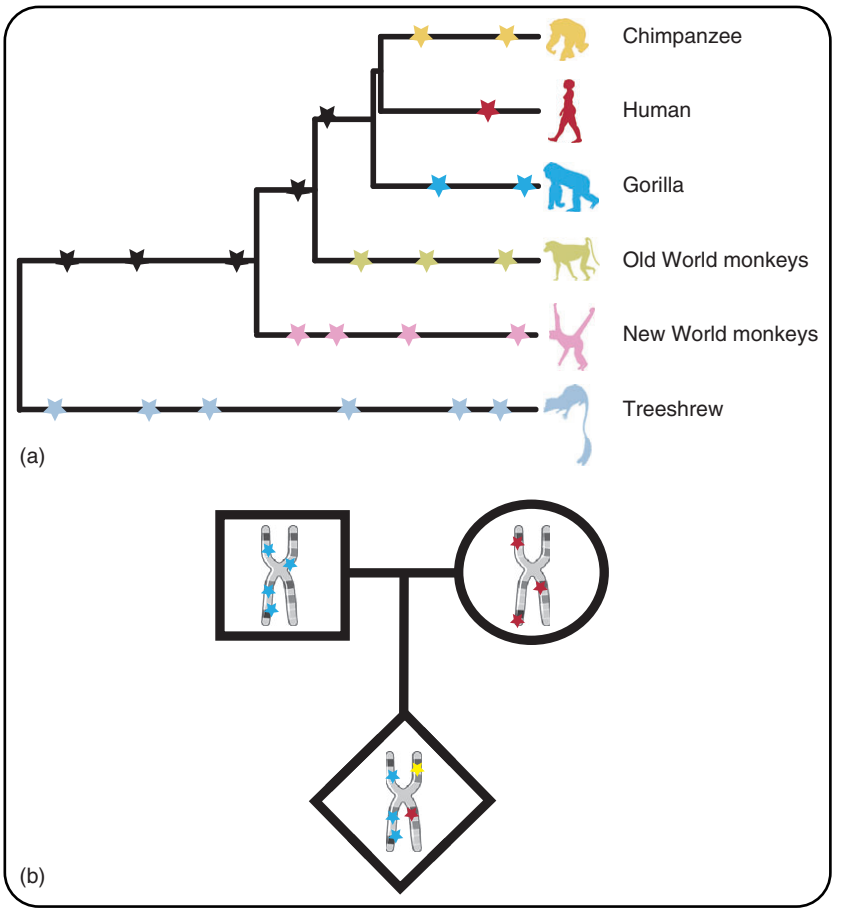

Figure 2 **Approaches to estimate mutation rates**. (a) Indirect approach: The phylogenetic approach measures phylogenetic distances by calculating the number of substitutions at putatively neutral sites (indicated as stars) that have accumulated in independent lineages between pairs of taxa. Coloured stars indicate species-specific substitutions; black stars indicate substitutions in the ancestral linage. Given an independent estimate of the split time from securely dated fossils, mutation rates are then estimated from the number of substitutions that accumulated between species. The topology was taken from Perelman *et al.* (2011) (Supplementary Information, Tree #5). (b) Direct approach: Mutation rates are estimated by sequencing the genome of a pedigree (father: square, mother: circle, and child: diamond) to identify mutations present in offspring but not their parents (i.e. *de novo* mutations, shown as yellow stars).

these estimates have historically been obtained through classical genetic approaches, illustrated by Haldane's (1935) work utilising information from incidents of dominant monogenic Mendelian disorders appearing in a population. However, these disease incident-based approaches require large family surveys and are intrinsically limited to certain types of diseases and mutations, such as those with large fitness effects which produce observable phenotypic changes in all carriers (i.e. are fully penetrant), making it impossible to survey the occurrence and distribution of mutations on a genomewide scale. As a result, many mutation rate estimates in primates have been obtained indirectly using phylogenetic approaches, based on Kimura's observation noted earlier. The application of these methods has led to a better understanding of the effects of genomic factors as well as sex, age and life history traits on mutation rates (see 'Determinants of mutation rates'). Today, technological and methodological advances permit the direct investigation of mutation rates by studying the genome of multigeneration pedigrees. Although this direct approach offers more detailed insights into the genomic distribution of mutations as well as the underlying molecular mechanisms, few such estimates yet exist in nonhuman primates (**Figure 1b**). **See also:** Mutation Rates: Data

Indirect estimates

In the phylogenetic approach, substitution rates are inferred from genetic distances, which are estimated by comparing putatively neutral, orthologous sites among species. Specifically, given an independent estimate of the split time from securely dated fossils, mutation rates can be estimated from the number of substitutions that have accumulated between species (Kimura, 1968) (**Figure 2a**). Although this approach is easily applicable to a variety of species, there are several well-documented uncertainties. First, inference of mutation rates from phylogenetic data is often clouded by uncertainties in estimates of divergence and

generation times. Uncertainty in these parameter estimates is not only influenced by the data itself (e.g. the number and types of molecular markers and the species available for comparison) but also by the statistical methods used to identify and align orthologous sites and to estimate branch lengths, as well as ambiguities in the dating of available fossils (or a complete absence of a fossil record), making it difficult to calibrate the molecular clock. Second, the unintentional inclusion of sites that fail the assumptions of neutral evolution or rate homogeneity through time can contribute to uncertainties, generally leading to an underestimation of mutation rates due to the pervasive effects of purifying selection (Ségurel *et al*., 2014). Contributing to this underestimation are substitution saturations (i.e. the occurrence of multiple mutations at a single site), reducing estimates of sequence divergence between species. In addition, evolutionary processes including biased gene conversion can impact substitution rates even at neutral sites (Ségurel *et al*., 2014). Third, the phylogenetic approach relies on the assumption that the molecular clock is ticking at a constant speed throughout evolutionary time – an assumption that, at least in primates, appears to be violated, making it difficult to translate substitution rates into yearly mutation rates (Yi *et al*., 2002; Elango *et al*., 2006, 2009; Kim *et al*., 2006; Gao *et al*., 2016; Scally, 2016; Moorjani *et al*., 2016a). Lastly, as mutation rates are based on information gathered from several individuals across many generations, only historical, sex-averaged estimates can be obtained from this method.

Direct estimates

In contrast to indirect approaches providing information about historically averaged mutation rates in a species, high-throughput sequencing enables the direct and comprehensive study of genomewide mutation rates in single individuals by sequencing the genome of parent–offspring trios or large multigeneration pedigrees to identify mutations present in the offspring but not their parents (**Figure 2b**). Although this approach is applicable for a variety of organisms, only a handful of direct estimates exist for primates at present as the method is still tempered by a few limitations. First, the rare occurrence of genuine *de novo* mutations can make it difficult to detect them, particularly in low- or medium-coverage sequencing data (Tatsumoto *et al*., 2017). Second, direct mutation rate estimates are highly sensitive to errors introduced during sequencing, as errors occur much more frequently than spontaneous mutations in many primate species (Pfeifer, 2017b). Although false positives arising from spurious sequencing can often be mitigated computationally using a number of distinct characteristics, the application of stringent filtering criteria comes at the cost of potentially missing genuine mutations (Ségurel *et al*., 2014). This makes it necessary to estimate both false negative and false discovery rates, which often is not trivial (Pfeifer, 2017a). Furthermore, the calculation of a mutation rate per site requires an accurate unbiased estimate of the number of loci at which genuine mutations could have been detected if they had occurred. This correct calculation of this denominator is often overlooked, though it is crucial for obtaining accurate rate estimates. A third potential problem arises from the frequent usage of somatic, rather than germ line, samples for sequencing (Scally, 2016). However, these errors can be ameliorated by sequencing samples obtained from multiple generations and comparing mutations among more than two generations (Ségurel *et al*., 2014; Scally, 2016; Pfeifer, 2017a). Lastly, uncertainty is of course increased by sampling error in studies based on small pedigrees.

Discrepancies in estimates obtained from indirect and direct approaches

There is a known discordance of indirect mutation rate estimates based on phylogenetic divergence data calibrated against a fossil record, and those estimated directly from pedigree data. For example, the indirect average mutation rate in humans estimated from rates of human–chimpanzee divergence in pseudogenes (i.e. a gene that has been inactivated and is thus likely not subject to strong selective pressures) is approximately 2.5×10^{-8} per base pair per generation (Nachman and Crowell, 2000) – more than a twofold increase compared to the direct per-generation mutation rate of $\sim 10^{-8}$ estimated from complete genome sequences of parents and their offspring (Moorjani *et al*., 2016b). These discrepant estimates of human mutation rate from indirect and direct approaches may reflect evidence for a gradual hominoid-specific evolutionary slowdown in germ line mutation rate along the human ancestral lineage, and hence a change of the molecular clock through time (Goodman, 1961; Li and Tanimura, 1987; Yi *et al*., 2002; Elango *et al*., 2006; Kim *et al*., 2006; Scally, 2016). However, it is hard to discern the effects of such a hominoid-specific slowdown in mutation rate from lineage-specific evolutionary changes in life history traits, which results in differences in branch lengths (Ségurel *et al*., 2014; Amster and Sella, 2016; Scally, 2016).

Determinants of Mutation Rates

Genomic factors

One of the most abundant (and best understood) types of mutation are point mutations (i.e. a substitution of a single nucleotide), which are affected by different genomic factors such as replication timing, transcription and repair mechanisms, as well as nucleotide composition, influencing mutation rates on both fine and broad scales. (**See also: Mutation Rates: Evolution**; **Single-base Mutation**)

Mutations caused by replication errors are influenced by local nucleotide composition. Specifically, higher mutation rates are observed in GC-rich regions, likely due to stabilisation of double-stranded DNA (i.e. three hydrogen bonds connect cytosine (C) with guanine (G) compared to two hydrogen bonds connecting adenine (A) with thymine (T)) that makes it more difficult for DNA polymerase to separate strands for error repair, and thus lowers replication fidelity (Petruska and Goodman, 1985). Mutations that arise due to spontaneous or induced changes are largely influenced by sequence context. Specifically, an elevated mutability of CpG sites (the 'p' denotes the phosphodiester bond between the neighbouring C and G nucleotides; Hwang and Green, 2004; Hodgkinson and Eyre-Walker, 2011) results from the tendency of

cytosine residues to be methylated Methylation of cytosine destabilises the dinucleotide, and promotes spontaneous deamination from C to T (or G to A on the complementary strand), leading to mismatches that are less efficiently repaired (Ehrlich and Wang, 1981). As a result, CpG transitions are the most common type of mutation in humans, with a 30-fold higher-than-average rate (Nachman and Crowell, 2000; Hwang and Green, 2004; Hodgkinson and Eyre-Walker, 2011). CpG transversion rates are also elevated, but the underlying reasons remain largely unknown. **See also: Mutation Rate of Non-CpG DNA**

In general, transitions occur twice as often as transversions in primates (i.e. a 'transition–transversion bias') likely due to varying frequencies of DNA mispairing during replication, suggesting that mutability differs between nucleotides (with a twofold higher mutability from G or C to A or T (Hodgkinson and Eyre-Walker, 2011)).

While methylation and transition–transversion bias exhibit some of the strongest genomic effects, other factors (such as recombination, nucleosome occupancy, DNaseI hypersensitivity and repeat content) might also play an important role in mutation rate variability, but their effects remain less well understood (Hodgkinson and Eyre-Walker, 2011; Pfeifer and Jensen, 2016) **See also: Mutational Biases**

Life history traits

As most mutations are deleterious (e.g. see review of Bank *et al*., 2014), purifying selection may act to reduce the mutation rate until it reaches the limits set by genetic drift (Lynch, 2010). Therefore, most variation in mutation rates observed between species might reflect differences in the efficiency of natural selection to improve replication fidelity. The efficiency of selection directly depends on the effective population size (N_e) of a species, which is inversely proportional to the power of genetic drift. Concordantly, an inverse correlation between mutation rates and N_e has been observed in many species due to the fact that genetic drift overpowers selection when the fitness advantage of further reducing the deleterious mutation rate is lower than $1/2N_e$ (i.e. the drift barrier; Lynch, 2010). In other words, species with smaller N_e generally have higher per-generation mutation rates.

In addition, yearly mutation rates are expected to roughly scale inversely with generation times – a hypothesis commonly referred to as the 'generation time effect' (Goodman, 1961; Ohta, 1993; Li *et al*., 1996). This notion rests on the common assumption that most mutations occur by errors in DNA replication and are thus dependent on the number of germ line cell divisions per generation (Drake *et al*., 1998) (often measured indirectly as the average age of reproduction) which, per unit time, is higher in species with shorter generation times (Ohta, 1993; Li *et al*., 1996). Accordingly, late-replicating regions in the hominid (i.e. great apes) germ line exhibit 20–30% higher mutation rates than early-replicating ones (Stamatoyannopoulos *et al*., 2009). As a consequence, mutations that result from replication errors are expected to vary among species, following a generation-time-dependent molecular clock (Elango *et al*., 2009). In contrast, mutations that are independent of replication (such as transitions at CpG dinucleotides that primarily arise due to methylation) accrue in proportion to absolute time – in other words, the molecular clock for replication-independent mutations is relatively constant over time (Hwang and Green, 2004; Kim *et al*., 2006; Elango *et al*., 2009; Moorjani *et al*., 2016a).

Differences in yearly mutation rates between primates can also result from important temporal and biological differences in the germ cell cycle between sexes. In females, mitotic cell divisions in oogenesis (i.e. egg production) are completed before birth. At birth, germ cell division is arrested and remains quiescent until after sexual maturity when selected oocytes are recruited at each oestrous cycle. In contrast, the proliferation of germ cells in males starts from a zygote in the growing testis before puberty. At puberty, spermatogenesis (i.e. sperm production) starts and continues throughout adult life, with one germ cell division per spermatogenic cycle. Assuming that most mutations occur during replication, higher mutation rates per generation are thus expected in males (i.e. a 'male mutation bias') due to the larger number of germ cell divisions during spermatogenesis relative to oogenesis (Haldane, 1947). In males, both an early onset of puberty relative to the reproduction age as well as an older average age of reproduction will increase the male mutation bias (Moorjani *et al*., 2016a). Consistently, most mutations in primates are paternal in origin, and the number of mutations inherited by a child increases with the father's age (i.e. a 'paternal age effect') (though note that recent evidence suggests that maternal age can, although to a lesser extent, also influence mutation rate (Moorjani *et al*., 2016b)). Support for male-driven evolution comes from the observation that there are fewer neutral substitutions on the X chromosome (that spends twice as many generations in females) than on the autosomes (that spend the same number of generations in males and females) or the Y chromosome (that spends all of the time in males) (Shimmin *et al*., 1993; Nachman and Crowell, 2000; Makova and Li, 2002). Although this pattern appears pervasive in many taxa, the degree of male mutation bias varies considerably (Elango *et al*., 2009; Wilson Sayres *et al*., 2011). Taken together, these observations suggest that maternal and paternal generation times might differently affect mutation rates in primates **See also: Mutation Rate: Sex Biases**. Beyond generation times, differences in species-specific life history traits that lead to differences in the number of cell divisions (such as the age of puberty and reproduction or germ line developmental processes) will also influence yearly mutation rates and thus contribute to the differences observed between primates (Ségurel *et al*., 2014; Amster and Sella, 2016; Gao *et al*., 2016).

In addition, correlated traits such as the degree of sperm competition in different mating systems may indirectly influence mutation rates. Thereby, the assumption that spermatogenetic cell divisions will not only vary with generation times but also with mating systems (and thus associated levels of sperm competition) is supported by the observation that promiscuous species produce sperm at a faster rate than monogamous species (**Table 1**; and see Ségurel *et al*., 2014). In species with high male–male (and thus presumably sperm) competition, males typically have larger relative testis sizes to accommodate a higher sperm production (Ramm and Stockley, 2010). Higher sperm production rates cause an increase in the number of mitotic cell divisions per unit time during spermatogenesis, which in turn might elevate the number of replication-driven mutations per generation, potentially yielding an accelerated male mutation rate in these species

Table 1 *Life history trait data for select primate species* including species-level body mass, basal metabolic rate (BMR), age of sexual maturity, mating system, rate of spermatogenesis and testis mass

Species	Body mass (kg)	BMR (ml O_2 h^{-1})	Age of sexual maturity (years)		Mating system	SECL (days)	Testis mass (g)
			♂	♀			
Western chimpanzee *Pan troglodytes*	26.0–70.0	1408	8–15	9–13	Polygynandrous	14	118.8–139.0
Human *Homo sapiens*	ø 67.0	13 377	14	13	Monogamous Polyandrous Polygynous Polygynandrous	16	40.5–50.2
Western gorilla *Gorilla gorilla*	ø 180.0	/	11–15	8–10	Polygynous	/	23.2–29.6
Bornean orangutan *Pongo pygmaeus*	ø 87.0	1514	8–15	5.8–11.1	Polygynous	/	34.2–35.3
White-handed gibbon *Hylobates lar*	4.4–7.6	1071	9	6–9	Monogamous Polyandrous	/	5.5
Anubis baboon *Papio anubis*	14.0–25.0	2958	7–10	7–8	Polygynandrous	11	93.5
Chacma baboon *Papio ursinus*	15.0–31.0	5067	5	3	Polygynous Polygynandrous	/	72.0
Guinea baboon *Papio papio*	13.0–26.0	2732	/	4.3	Polygynous Polygynandrous	/	88.9
Yellow baboon *Papio cynocephalus*	ø 12.0 ♀ ø 23.0 ♂	7929	4–7	5–6	Polygynandrous	10.2	52.0

Long-tailed macaque *Macaca fascicularis*	3.0–7.0	3458	4–6	3.5–4	Polygynous Polygynandrous	10.16	35.2–35.7
Japanese macaque *Macaca fuscata*	ø 8.4 ♀ ø 11.3 ♂	4362–4524	4–4.5	3.5–4	Polygynandrous	/	/
Rhesus monkey *Macaca mulatta*	4.0–12.0	2222–2273	4.5–7	2.5–4	Polygynandrous	9.5–10.5	46.2–76.0
Stump-tailed macaque *Macaca arctoides*	7.5–10.02	/	4.5–5.5	3.5–4	Polygynandrous	11.6	48.2
Blue monkey *Cercopithecus mitis*	4.0–6.0	3392	3–4.5	3–4.5	Polygynous Polygynandrous	/	/
Red-capped mangabey *Cercocebus torquatus*	ø 9.5	1605	5–7	5–7	Polygynous	/	25.1
African green monkey *Chlorocebus aethiops*	3.0–5.0	/	5	3	Polygynous	10.2	13.0–20.6
Mantled guereza *Colobus guereza*	5.0–14.0	2978	6	4	Polygynous	/	3.0
Patas monkey *Erythrocebus patas*	7.0–13.0	640–1068	4–4.5	3	Polygynous Polygynandrous	/	7.2

Rate of spermatogenesis is measured as the length of the seminiferous epithelium cycle (SECL), which is the time period between two successive occurrences of the same seminiferous stage. Information on basal metabolic rate (BMR) and testis mass was taken from Harcourt *et al*. (1995); Wlasiuk and Nachman (2010); Wilson Sayres *et al*., (2011). Information on the rate of spermatogenesis was taken from Amster and Sella (2016). Information on body mass, age of sexual maturity and mating system was taken from 'Animal Diversity Web' (ADW), an online database of animal natural history, distribution, classification and conservation biology at the University of Michigan (http://animaldiversity.org; last accessed June 2018), and 'AnAge – The Animal Ageing and Longevity Database', part of Human Ageing Genomic Resources (http://genomics.senescence.info; last accessed June 2018). The presence of a 'ø' indicates the average, and '/' indicates that information is currently unavailable.

Source: Data from Animal Diversity Web and AnAge – The Animal Ageing and Longevity Database.

(Ségurel *et al*., 2014). However, strong sexual selection against male germ line mutations seems to partially counteract these elevated male mutation rates (Ségurel *et al*., 2014). Furthermore, it remains unclear whether relative testis size is a reliable proxy for the degree of sperm competition (Møller and Cuervo, 2003).

Other factors suggested to be associated with substitution rate are body mass and the basal metabolic rate. Specifically, smaller animals often have higher substitution rates (Martin and Palumbi, 1993). In addition, high levels of oxidative stress associated with fast metabolism might damage DNA, thus potentially resulting in higher rates of substitution (Martin and Palumbi, 1993). However, a phylogenetic analysis of mammals suggested that the observed correlation between metabolic rate and autosomal substitution rate is nonsignificant after controlling for the effect of generation time (Wilson Sayres *et al*., 2011).

Germ Line Mutation Rates in Old World Monkeys

In general, there is a paucity of mutation rate estimates for Old World monkeys, with a single direct estimate available for African green monkeys (also commonly referred to as vervet monkeys; Pfeifer, 2017a) and three indirect estimates for baboons and macaques (Hernandez *et al*., 2007; Evans *et al*., 2010; Boissinot *et al*., 2014) (**Figure 1b**).

For rhesus macaques, the mutation rate was estimated to be 5.9×10^{-9} per base pair per generation, assuming a generation time of 6.5 years and a divergence time with baboon of 6.6 million years (Hernandez *et al*., 2007). A similar mutation rate of 4.4×10^{-9} per base pair per generation has been reported for Southern pig-tailed macaques, assuming a generation time of 5 years and a divergence time with humans of 37 million years (Evans *et al*., 2010). Compared to the indirect mutation estimates of macaque, a higher germ line mutation rate of 9.4×10^{-9} per base pair per generation has been estimated for African green monkeys, using a three-generation pedigree (Pfeifer, 2017a). In contrast, a substantially lower mutation rate of 7.6×10^{-10} per site per year has been reported in baboons, assuming a divergence time of 30 million years between humans and baboons (Boissinot *et al*., 2014) – consistent with an earlier analysis using relative rate tests that suggested that baboons evolve at a slower rate than macaques and vervets (Elango *et al*., 2009).

Although only a handful of mutation rate estimates are available for OWMs, they generally support the generation time effect hypothesis. Generation times for Old World monkeys are less than half that of hominoids and twice that of New World monkeys and, consequently, evolutionary rates of replication-dependent substitutions are slower in hominoids than in Old World monkeys than in New World monkeys (Goodman, 1961; Li *et al*., 1996; Yi *et al*., 2002; Kim *et al*., 2006; Elango *et al*., 2009; Moorjani *et al*., 2016a). In particular, Yi *et al*. (2002) reported an average substitution rate of 1.17×10^{-9} to 1.5×10^{-9} along the human and OWM lineages, depending on whether the human–OWM divergence was assumed to have occurred 30 or 25 million years ago, respectively. In concordance with this earlier result, a recent study by Moorjani *et al*. (2016a) reported an average autosomal substitution rate for OWMs that is 1.33× higher than the estimate for hominoids. In their study, estimates for substitution rates differed between replication-dependent and replication-independent sites, as expected from the different origins of mutation. At replication-dependent mutational targets, the average autosomal substitution rate for OWMs was 1.38× higher than that for the hominoid lineage, while CpG transitions exhibited only a 1.07× higher rate, consistent with the notion that replication-independent mutations accumulate in a more 'clock-like' manner (Moorjani *et al*., 2016a). Concordant with the notion that male mutation bias is correlated with generation time, OWMs also exhibit a lower male-to-female mutation ratio than hominoids (Elango *et al*., 2009).

Positively correlated with generation time is the rate of spermatogenesis (Moorjani *et al*., 2016a). Differences in spermatogenic cycle lengths between Old World monkeys (varying between 9.5 days in rhesus monkeys and 11.6 days in stump-tailed macaques) and hominoids (14 days in chimpanzees and 16 days in humans; **Table 1**) likely contribute to the differences in mutation rates as well as strengths of paternal age effects observed between primate species. Furthermore, evolutionary processes such as biased gene conversion may contribute to the variation in substitution rates observed among primates due to differences in effective population sizes and fine-scale recombination rates between the species (Moorjani *et al*., 2016a).

Concluding Remarks

Primates are known to differ in their life history traits such as body size, generation time, onset of puberty, rates of spermatogenesis and metabolic rates (**Table 1**). This article thus highlights several areas that would benefit from increased attention in future research efforts. Most importantly, there is a pressing need for further investigations in which mutation rate is directly estimated from multigeneration pedigree data from across a greater diversity of primates species. In particular, the rich diversity of OWMs, the abundance of population and species-level life history and trait information, and the extended evolutionary distance to humans relative to the well-studied apes promise many fruitful insights in the near future. Such avenues are expected to allow deeper and perhaps conclusive investigations pertaining to life history correlates of mutation rate, as well as the explanatory power of the hominoid slowdown and generation time effect hypotheses in general.

Glossary

Biased gene conversion A process that leads to substitution patterns that are enriched in guanine (G) and cytosine (C) nucleotides due to a higher rate of mutation from strong bases (G or C) to weak bases (adenine (A) or thymine (T)).

De novo *mutation* A mutation that is present in the child but not the parents.

Effective population size (N_e) The idealised number of individuals resulting in the same level of genetic drift as is observed in the true population.

Fitness Measures the capacity of an individual to reproduce (also accounts for survival to reproductive age).

Genetic drift The random change in allele frequencies through time due to stochastic fluctuations inherent to finite populations.

Molecular clock The notion that neutral nucleotide sequences evolve at a constant rate over time.

Monogamous Mating system in which a female mates with a single male.

Mutation rate (μ) The rate at which DNA sequence changes accumulate per base pair per unit time, which under neutrality is also equivalent to the expected rate of fixation.

Old World monkeys Part of the highly diverse *Cercopithecidae* family. They inhabit regions in Africa, Asia and Europe (today in Gibraltar but the fossil record suggests other potential native habitats in Europe) as opposed to New World monkeys whose habitats are located in the Americas.

Polyandrous Mating system in which a female mates with several males.

Polygynandrous Mating system in which a female mates with several males, each of which mates with several females.

Polygynous Mating system in which a male mates with multiple females.

Transition An exchange of one purine (A or G) with another purine or of one pyrimidine (C or T) with another pyrimidine.

Transversion An exchange of a purine (A or G) to a pyrimidine (C or T) or vice versa.

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