Reconstructing the history of variation in effective population size along phylogenies.

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Abstract

The nearly-neutral theory predicts specific relations between effective population size (N_e), and patterns of divergence and polymorphism, which depend on the shape of the distribution of fitness effects (DFE) of new mutations. However, testing these relations is not straightforward since N_e is difficult to estimate in practice. For that reason, indirect proxies for N_e have often been used to test the nearly-neutral theory. Here, we introduce an integrative comparative framework allowing for an explicit reconstruction of the phylogenetic history of N_e and u, thus leading to a quantitative test of the nearly-neutral theory and an estimation of the allometric scaling of π_N/π_S and dN/dS with respect to N_e. As an illustration, we applied our method to primates, for which the nearly-neutral predictions were mostly verified. The variation of π_N/π_S and dN/dS as a function of N_e appears to be too strong, however, to be compatible with current estimates of the DFE based on site frequency spectra. The reconstructed history of N_e in primates seems consistent with current knowledge, shows a ten-fold variation across primates and shows a clear phylogenetic structure at the super-family level. Altogether, our integrative framework provides a quantitative assessment of the role of N_e in modulating patterns of genetic variation, while giving a synthetic picture of the long-term trends in N_e variation across a group of species.

Introduction

Effective population size (N_e) is a central parameter in population genetics and in molecular evolution, impacting both genetic diversity and the strength of selection (Charlesworth, 2009; Leffler *et al.*, 2012). The influence of N_e on diversity reflects the fact that larger populations can store more genetic variation, while the second aspect, efficacy of selection, is driven by the link between N_e and genetic drift: the lower the N_e, the more genetic evolution is influenced by the random sampling of individuals over generations. As a result, long-term trends in N_e are expected to have an important impact on genome evolution (Lynch *et al.*, 2011) and, more generally, on the relative contribution of adaptive and non-adaptive forces in shaping macro-evolutionary patterns.

The nearly-neutral theory proposes a simple conceptual framework for formalizing the role of selection and drift on genetic sequences. According to this theory, genetic sequences are mostly under purifying selection; deleterious mutation are eliminated by selection, whereas neutral and nearly-neutral mutations are subject to genetic drift and can therefore segregate and reach fixation. The inverse of N_e defines the selection threshold under which genetic drift dominates. This results in specific quantitative relations between N_e and key molecular parameters (Ohta, 1995). In particular, species with small N_e are expected to have a higher ratio of nonsynonymous (d_N) to synonymous (d_S) substitution rates and a higher ratio of nonsynonymous (π_N) to synonymous (π_S) nucleotide diversity. Under certain assumptions, these two ratios are linked to N_e through allometric functions in which the scaling coefficient is directly related to the shape of the distribution of fitness effects (DFE) (Kimura, 1979; Welch *et al.*, 2008).

The empirical test of these predictions raises the problem that N_e is difficult to measure directly in practice. In principle, N_e could be estimated through demographic and census data. However, the relation between census and effective population size is far from straightforward. Consequently, many studies which have tried to test nearly-neutral theory have used proxies indirectly linked to N_e. In particular, life history traits (LHT, essentially body mass or maximum longevity) are expected to correlate negatively with N_e (Waples *et al.*, 2013). As a result, d_N/d_S or π_N/π_S are predicted to correlate positively with LHT. This has been tested, leading to various outcomes, with both positive and negative results (Eyre-Walker *et al.*, 2002; Popadin *et al.*, 2007; Nikolaev *et al.*, 2007; Lartillot, 2013; Nabholz *et al.*, 2013; Romiguier *et al.*, 2014; Figuet *et al.*, 2016).

More direct estimations of N_e can be obtained from π_S since, in accordance with coalescent theory, $\pi_S = 4N_e u$ (with u referring to the mutation rate per site per generation). Thus, one

would predict a negative correlation of d_N/d_S or π_N/π_S with π_S and a positive correlation between LHT and π_S. Such predictions have been tested, and generally verified, in several previous studies (Piganeau & Eyre-Walker, 2009; Romiguier *et al.*, 2014; Figuet *et al.*, 2016; Galtier, 2016; Chen *et al.*, 2017; James *et al.*, 2017). However, these more specific tests of the nearly-neutral theory are only qualitative, at least in their current form, in which N_e is indirectly accessed through π_S without any attempt to correct for the confounding effect of the mutation rate u and its variation across species.

New Approaches

In this study, we aim to solve this problem by using a Bayesian integrative approach, in which the joint evolutionary history of a set of molecular and phenotypic traits is explicitely reconstructed along a phylogeny. This method has previously been used to test the predictions of the nearly-neutral theory via indirect proxies of N_e (Lartillot, 2013; Nabholz *et al.*, 2013). Here, we propose an elaboration on this approach, in which the variation in the mutation rate per generation u is globally reconstructed over the phylogeny by combining the relaxed molecular clock of the model with data about generation times. This in turns allows us to tease out N_e and u from the π_S estimates obtained in extant species, thus leading to a complete reconstruction of the phylogenetic history of N_e and of its scaling relations with others traits such as d_N/d_S or π_N/π_S. Using this reconstruction, we can conduct a proper quantitative test of some of the predictions of the nearly-neutral theory and then compare our findings with independent knowledge previously derived from the analysis of site frequency spectra. The approach requires a multiple sequence alignment across a group of species, together with polymorphism data, ideally averaged over many loci to stabilize the estimates, as well as data about life-history traits in extant species and fossil calibrations. Here, we apply it to previously published phylogenetic and transcriptome data (Perelman *et al.*, 2011; Perry *et al.*, 2012), focussing the analysis on primates, a group for which coding-sequence evolution has been suggested to be globally compatible with a nearly-neutral regime (Eyre-Walker & Keightley, 2009; Galtier, 2016).

Results

Empirical phylogenetic correlation analysis

Using an integrative comparative approach, based on a multivariate log-Brownian covariant model for rates and traits (Coevol, Lartillot & Poujol, 2011), we first tested the predictions of the nearly-neutral theory in primates, taking life history traits (age of sexual maturity, body mass and longevity, hereafter abbreviated as LHT) as tentative proxies for effective population size N_e.

Neither π_N/π_S nor dN/dS appear to correlate with LHTs (Table 1), except for a positive correlation between d_N/d_S and longevity ($r = 0.49$). On the other hand, the correlations among molecular quantities are globally in agreement with the nearly-neutral predictions, although with rather unequal statistical support. Most notably, π_N/π_S shows a clear negative correlation with π_S ($r = -0.73$). As for dN/dS, it also shows a negative correlation with π_S ($r = -0.50$), although with very marginal support ($pp < 0.95$). The two variables, dN/dS and π_N/π_S are also positively correlated with each other ($r = 0.42$), but again, with marginal support. The weaker correlations observed for dN/dS, compared to the more robust correlation between π_N/π_S and π_S, could be due either to the presence of a minor fraction of adaptive substitutions or, alternatively, to a discrepancy between the short-term demographic effects reflected in both π_S and π_N/π_S and long-term trends captured by dN/dS.

Although not conclusive, the correlation patterns between π_S, π_N/π_S and dN/dS are compatible with the hypothesis that the nearly-neutral model is essentially valid for primates. The overall lack of correlation with LHT, on the other hand, suggest that there is no clear correlation between effective population size and body size or other related life-history traits in this group. Possibly, the phylogenetic scale might be too small to show sufficient variation in LHT that would be interpretable in terms of variation in N_e. Alternatively, N_e might be driven by other life-history characters (in particular, the mating systems), which may not directly correlate with body size. Of note, in those cases where the estimated correlation of dN/dS or π_N/π_S with LHTs were in agreement with the predictions of the nearly-neutral theory (Eyre-Walker *et al.*, 2002; Popadin *et al.*, 2007; Nikolaev *et al.*, 2007; Lartillot, 2013; Nabholz *et al.*, 2013; Romiguier *et al.*, 2014; Figuet *et al.*, 2016), the reported correlation strengths were often weak, weaker than the correlations found directly between π_S and π_N/π_S and dN/dS (Piganeau & Eyre-Walker, 2009; Romiguier *et al.*, 2014; Figuet *et al.*, 2016; Galtier, 2016; Chen *et al.*, 2017; James *et al.*, 2017).

Teasing apart substitution rates, divergence times and effective population size

The correlation patterns shown by the three molecular quantities π_S, d_N/d_S and π_N/π_S are suggestive of a role of N_e as the main driver of their interspecific variation. However, in its current form, this correlation analysis does not give any quantitative insight about the scaling of d_N/d_S and π_N/π_S as a function of N_e and, more generally, about the quantitative impact of N_e on the evolution of coding sequences. In order to achieve this, an explicit estimate of the key parameter N_e, and of its variation across species, is first necessary. In this direction, a first simple but fundamental equation relates π_S with N_e:

$$\pi_S \;=\; 4\,N_e\,u. \tag{1}$$

In order to estimate N_e from equation 1, an estimation of u is also required. Here, it can be obtained by noting that:

$$u \;=\; r\,\tau, \tag{2}$$

where r is the mutation rate per site and per year and τ the generation time. Assuming that synonymous mutations are neutral, we can identify the mutation rate with the synonymous substitution rate dS, thus leading to:

$$u \;=\; dS\,\tau. \tag{3}$$

Finally, combining equations 1 and 3 and taking the logarithm gives:

$$\ln N_e \;=\; \ln \pi_S - \ln d_S - \ln \tau - \ln 4. \tag{4}$$

This expression suggests to apply the linear transformation given by equation 4 to the three variables $\ln \pi_S$, $\ln d_S$ and $\ln \tau$, all of which are jointly reconstructed across the tree, which then gives a global phylogenetic reconstruction of $\ln N_e$. In addition, since the transformation is linear, the correlation patterns between $\ln N_e$ and the other variables included in the analysis can be recovered by applying elementary matrix algebra to the covariance matrix estimated under the initial parameterization (see Materials and Methods).

The results of this linearly-transformed correlation analysis are gathered in Table 1 (last column). In accordance with the predictions of the nearly-neutral theory, π_N/π_S and d_N/d_S show a negative correlation with N_e ($r = -0.73$ for π_N/π_S and -0.58 for dN/dS).

Quantitative scaling of π_N/π_S and d_N/d_S as a function of N_e

Once an explicit estimate of N_e and of its variation is available, the scaling behavior of π_N/π_S and d_N/d_S as a function of N_e can also be quantified. Here, the variables are log-transformed, such that the correlation analysis conducted here entails the following log-linear relations:

$$\ln \pi_N/\pi_S = -\beta_1 \ln N_e + \alpha_1, \quad (5)$$

$$\ln dN/dS = -\beta_2 \ln N_e + \alpha_2, \quad (6)$$

or equivalently:

$$\pi_N/\pi_S = \kappa_1 N_e^{-\beta_1}, \quad (7)$$

$$dN/dS = \kappa_2 N_e^{-\beta_2}, \quad (8)$$

where $\kappa_i = e^{\alpha_i}$, for $i = 1, 2$. In other words, the slopes of the log-linear relations, β_1 and β_2, are just the scaling coefficients of π_N/π_S and d_N/d_S as a function of N_e. These two scaling coefficients can be directly obtained based on the covariance matrix estimated above. In the present case, the estimates of β_1 and β_2 are of similar magnitude, with point estimates of 0.17 and 0.10, respectively (Table 2).

It is worth comparing these estimates with those that would obtain if we were using π_S directly as a proxy of N_e (i.e. without correcting for u). The slopes of the log-linear scaling of π_N/π_S and dN/dS as a function of π_S are steeper than those obtained as a function of N_e, with point estimates of 0.29 and 0.13 (Table 2), suggesting that the confounding effects of u are not negligible on the evolutionary scale of a mammalian order such as primates and, as such, can substantially distort the scaling relations if not properly taken into account.

Relation with the shape of the distribution of fitness effects

Mechanistically, the slope of $\ln \pi_N/\pi_S$ and $\ln d_N/d_S$ as a function of $\ln N_e$ can be interpreted in the light of an explicit mathematical model of the nearly-neutral regime. Such mathematical models, which are routinely used in modern Mac-Donald Kreitman tests (Charlesworth & Eyre-Walker, 2008; Eyre-Walker & Keightley, 2009; Halligan *et al.*, 2010; Galtier, 2016), formalize how demography modulates the detailed patterns of polymorphism and divergence. In turn, these modulations depend on the structure of the distribution of fitness effects (DFE) over non-synonymous mutations (Eyre-Walker & Keightley, 2007). Mathematically, the DFE is often modelled as a gamma distribution. The shape parameter of this distribution (usually denoted as β) is classically estimated

based on empirical synonymous and non-synonymous site frequency spectra. Typical estimates of the shape parameter are of the order of 0.15 to 0.20 in humans (Boyko *et al.*, 2008; Eyre-Walker *et al.*, 2006), thus suggesting a strongly leptokurtic distribution, with the majority of mutations having either very small or very large fitness effects.

When the shape parameter β is small, both π_N/π_S and d_N/d_S are theoretically predicted to scale as a function of N_e as a power-law, with a scaling exponent equal to β (Kimura, 1979; Welch *et al.*, 2008). This relation was used previously for analyzing the impact of the variation in N_e along the genome in *Drosophila* (Castellano *et al.*, 2018). Assuming that the DFE is constant across primate species, it also predicts the interspecific allometric scaling coefficients β_1 and β_2 estimated above should both be equal to the shape parameter β of the DFE. In the present case, the two estimates β_1 and β_2 are indeed congruent with each other, with overlapping credible intervals (Table 2). They are also compatible with previously reported independent estimates of the shape parameter of the DFE, such as obtained from site frequency spectra in Humans and in other great apes (also reported in Table 2).

A mechanistic nearly-neutral phylogenetic codon model

Since all of the results presented thus far are compatible with a nearly-neutral regime, we decided to construct a mechanistic version of the model directly from first principles. Thus far, a phenomenological approach was adopted, in which the whole set of variables of interest (dS, dN/dS, π_S, π_N/π_S and generation-time τ) were jointly reconstructed along the phylogeny, as a multivariate log-normal Brownian process with 5 degrees of freedom. Only in a second step were N_e and u extracted from this multivariate process, using log-linear relations.

Here instead, only three degrees of freedom are considered (which could be taken as u, N_e and τ), and then the empirically measurable molecular quantities dS, dN/dS, π_S and π_N/π_S are obtained as deterministic functions of these three fundamental variables, according to the equations introduced above (3, 4, 6 and 5). In practice, the model is more conveniently expressed, via a log-linear change of variables, in terms of π_S, π_N/π_S and τ as the three free variables, since these are directly observed in extant primates – from which dS, dN/dS and N_e are then derived by inverting the functional relations given by equations 3, 4, 6 and 5 (see Materials and Methods). Finally, the shape parameter β, along with κ_1 and κ_2 in equations 6 and 5, are structural parameters of the model, representing the DFE, itself assumed to be constant across species.

This mechanistic model was implemented in two alternative versions. A first naive version

assumes that genetic divergence takes place exactly at the splitting times defined by the underlying species phylogeny. Identifying genetic divergence with species divergence, however, amounts to ignoring the time it takes for coalescence to occur in the ancestral populations. For that reason, an alternative model was considered, based on the argument that the mean coalescence time in the ancestral population at a given ancestral node of the phylogeny is equal to $\delta t = 2N_e\tau$, where N_e is the effective population size and τ the generation time prevailing at or around that node of the species tree. Since N_e and τ are both reconstructed along the entire species phylogeny, it is therefore possible to use the local information about the current value of N_e and τ to account for the extra amount of divergence δt induced by coalescence in the ancestral population when calculating the sequence likelihood (see methods). In the following, these two alternative versions of the mechanistic model are called the 'naive-phylogenetic' and 'mean-coalescent' versions.

Fitting the model on the data returns an estimate of the structural parameters of the DFE as well as a dated tree, annotated with the complete history of the variation in mutation rate and in effective population size along its branches. These different aspects of the output of the analysis are now considered in turn.

Mechanistic estimate of the shape parameter of the DFE

The mechanistic model yields an estimate of β (Table 2) that is congruent with, although somewhat higher than, the slopes β_1 and β_2 estimated previously with the phenomenological approach, with a posterior median at 0.27 and a credible interval equal to $(0.22, 0.33)$. The credible interval is smaller than those for β_1 and β_2, reflecting the fact that the mechanistic model is more constrained. Our estimate of β also appears to be higher than independent estimates obtained from site frequency spectra. Of note, there is some discrepancy among those DFE-based estimates, whose credible intervals do not overlap. However, our estimate is significantly higher than the more recent estimate obtained by jointly analyzing the site frequency spectra across great apes (Castellano *et al.*, 2019), which is probably the most relevant one in the present context. This observation suggests a potential violation of the mechanistic model (see Discussion).

Estimated mutation rates

The dated phylogeny, together with the reconstructed history of the mutation rate per year r, is shown in Figure 1. Here, the mechanistic model works like a standard molecular dating method, using fossil calibration and a relaxed clock model to tease out times and rates from raw synonymous

sequence divergence. Generation time, also reconstructed along the tree by the model, is then used to convert the mutation rate per year r into a mutation rate per generation u (Figure 2). Point estimates (medians) and 95% credible intervals of both r and u for some extant species of interest and a few key ancestors are shown in Table 3.

The naive-phylogenetic model tends to return higher mutation rates, compared to the mean-coalescent approach, in particular for extant taxa and, more generally, for recent ancestors. For instance, in humans, the mutation rate per year is estimated at $0.80.10^{-9}$ when coalescence in the ancestral population is ignored, versus $0.67.10^{-9}$ when it is accounted for in the model. This reflects a bias induced by ignoring ancestral coalescence. For instance, the Human-Chimpanzee split is constrained at around 5 to 7 My, but coalescence in the ancestral population can easily go back to 9 My, which, if dated at 7 My, automatically induces an overestimation of the mutation rate along the branch leading to Humans.

The estimates obtained here under the mean-coalescent model are intermediate, lower than previously reported phylogenetic estimates but still higher than pedigree-based estimates. For instance, typical phylogenetic estimates for the mutation rate in humans are typically of the order of 10^{-9} per year, or 3.10^{-8} per generation, whereas pedigree-based estimates are generally about half these values. Concerning other primates, our estimates are also higher than pedigree based estimates previously reported for macaques (Wang *et al.*, 2020) and baboons (Wu *et al.*, 2019). On the other hand, they are congruent for Gorilla, Pongo (Besenbacher *et al.*, 2019) and Aotus (Thomas *et al.*, 2018). In the following, only coalescent-aware estimates are further considered.

Across primates, the mutation rate per year r shows a 5-fold variation from $0.6.10^{-9}$ to $3.0.10^{-9}$ point mutations per year and per nucleotide site (Figure 1). The rate of mutation is relatively high in the ancestor of primates ($\sim 2.10^{-9}$). On the side of Strepsirrhini, it remains high in Lorisiformes ($\sim 2.10^{-9}$) but is lower in Lemuriformes ($\sim 10^{-9}$). Concerning Haplorrhini, the rate undergoes a net slowdown in Catarrhini ($\sim 10^{-9}$), further accentuated in apes, which are among the slowest evolving primates ($\sim 0.6.10^{-9}$). Compared to apes, the other catarrhine group, Cercopithecidae (old world monkeys) shows a globally higher rate of evolution (around 10^{-9}). As for Platyrrhini (new world monkeys), they are globally fast evolving, ranging from $1.5.10^{-9}$ to above 2.10^{-9} more specifically in Cebidae. These observations are globally in accordance with previous observations, in particular, emphasizing that the slowdown occurring in apes (Steiper *et al.*, 2004) is in fact in the continuity of a broader process of deceleration more generally across catarrhine primates (Perelman *et al.*, 2011). Of note, a higher contrast is observed here between old-world monkeys and apes, or

between the fast-evolving Lorisiformes and the more slowly-evolving Lemuriformes, compared to previous reports (Perelman *et al.*, 2011).

Compared to the mutation rate per year, the mutation rate per generation u varies over a more moderate range, showing a 3-fold variation across primates. Moderately high ancestrally ($1.5.10^{-8}$), it shows a convergent decrease in Lorisiformes, Lemuriformes, and Cercopithecidae (reaching below 10^{-8} in several species in these three clades), but otherwise, remains more in the range of 1.5 to 2.10^{-8}, although with many local deviations from this global trend. The mutation rates per year r and per generation u tend to show opposite patterns: slow-evolving lineages, with a low mutation rate per year, tend to have a higher mutation rate per generation (high u). This is particularly apparent for apes (low r, high u) or for Lorisiformes (high r, low u). However, there are some exceptions, of lineages that have both a high r and a high u, most notably Platyrrhini (new world monkeys).

Phylogenetic Reconstruction of N_e

The marginal reconstruction of the history of N_e along the phylogeny of primates returned by the mechanistic model is shown in Figure 3. More detailed information, with credible intervals, is given in Table 4 for several species of interest and key ancestors along the phylogeny.

The N_e estimates for the four extant hominids (*Homo, Pan, Gorilla and Pongo*) are globally congruent with independent estimates based on other coalescent-based approaches (Prado-Martinez *et al.*, 2013). In particular, for Humans, N_e is estimated to be between 13 000 and 24 000. For other hominids, they tend to be somewhat higher than coalescent-based estimates. Concerning the successive last common ancestors along the hominid subtree, our estimation is also consistent with the independent-locus multi-species coalescent (Rannala & Yang, 2003).

The history of N_e shows a clear large-scale structure over the primate phylogeny. Starting with a point estimate at around 100 000 in the last common ancestor of primates, N_e then goes down in Haplorrhini, stabilizing at around 65 000 in Cercopithecidae (old-world monkeys), 50 000 in Hominoidea (going further down more specifically in Humans), and 40 000 in Platyrrhini (new-world monkeys). Conversely, in Strepsirrhini, N_e tends to show higher values, staying at 100 000 in Lemuriformes and going up to 160 000 to 200 000 in Lorisiformes. Finally, rather large effective sizes are estimated for the two isolated species *Tarsius* and *Daubentonia* – although the credible intervals are very large (Table 4). These estimates may not be so reliable, owing to the very long branches leading to these two species.

The reconstruction of N_e shown in Figure 3 mirrors the patterns of dN/dS estimated over the tree (Supplementary Figure 1). Thus, dN/dS starts in the range of 0.25 in the early primate lineages. On the side of Haplorrhini, it goes up to 0.30 in Simiiformes, stabilizes around this value in Catarrhini (old-world monkeys), goes further up to 0.32 in Platyrrhini (new-world monkeys). On the other branch (Strepsirrhini), the dN/dS is around 0.25 in Lemuriformes, versus 0.22 in Lorisidae. The two species *Daubentonia* and *Tarsius* show the lowest dN/dS values (around 0.20).

In order to get some insight about how much dN/dS and π_N/π_S inform the reconstructed history of N_e, an alternative version of the model was also explored, in which all time-dependent variables are assumed to evolve independently from each other. Under this control, N_e is only informed at the tips by $\pi_S = 4N_e u$ and by the estimates of u implied by the relaxed clock and data about generation times. Compared to the mechanistic version just presented, this uncoupled model gives lower N_e estimates most notably for the last common ancestor of primates (30 000, versus 100 000 under the mechanistic model), but also for the two species *Daubentonia* and *Tarsius*. Conversely, it returns higher N_e estimates in Lemuriformes. The reconstruction under the phenomenological version of the model gives an intermediate picture (Supplementary Figure 2), although its estimate for the crown primate ancestor is closer to that of the uncoupled model.

Interestingly, under the uncoupled model, there is a substantial uncertainty about the estimation of N_e across the tree: the 95% credible intervals span one order of magnitude on average. This uncertainty is reduced under the reconstructions relying on the additional information contributed by dN/dS and π_N/π_S, quantitatively, by 30% under the phenomenological, and by 50% under the mechanistic covariant models. In the end, there is thus on average a factor 5 between the lower and the upper bound of the 95% credible intervals on N_e estimates under the most constrained (mechanistic) model. Concerning the deep branches of the tree, most of this reduction in uncertainty is primarily contributed by dN/dS – which thus gives an idea of how much information can be extracted from multiple sequence alignments about very ancient population genetic regimes.

Of note, the N_e estimates are lower under the naive-phylogenetic than under the mean-coalescent approach. This difference between the two models is due to the fact that, given π_S, any bias in the estimation of u has to be compensated for by an opposite bias of the same magnitude in the estimation of N_e. These discrepancies are relatively minor, however, and the global patterns of the history of N_e along the tree are very similar in both cases.

Finally, the phylogenetic history of u, the mutation rate per generation, mirrors that of N_e, such that species with smaller N_e tend to have a higher value for u (compare Figures 1 and 2).

These opposite patterns of variation, combined with the fact that N_e shows a greater amplitude in its variation across primates, compared to u, results in π_S being mostly driven by N_e, although with a partial dampening of its overall variation. This joint pattern for N_e and u explains why the regression slopes of $\ln \pi_N/\pi_S$ and $\ln dN/dS$ against π_S are steeper than those against N_e (Table 2).

Discussion

A Bayesian integrative framework for comparative population genomics

The question of the role of N_e in the evolution of coding sequences has motivated much work over the years. One main problem that has attracted particular attention is to understand to what extent N_e modulates the ratio of non-synonymous over synonymous polymorphism (π_N/π_S) or divergence (d_N/d_S). Often, π_S has been used as a proxy for N_e. However, π_S also depends on u, the mutation rate per generation, which differs between species.

In this context, the main contribution of the present work, which is primarily methodological, is to propose a Bayesian integrative phylogenetic framework for conducting such comparative analyses in a way that allows for direct quantitative estimation of N_e and of its impact on molecular evolution across a clade of interest. Relying on an integrated relaxed clock model to calibrate mutation rates, the program leverages an estimate of N_e based on π_S, correcting for u. Simultaneously, it conducts a regression analysis, returning an estimate of the scaling exponent of molecular quantities such as dN/dS and π_N/π_S, but also potentially other variables or quantitative traits, directly as a function of N_e. As a byproduct, the approach also returns a global reconstruction of the history of effective population size and mutation rate across the phylogeny.

As can be seen from Table 4, correcting for variation in mutation rate between species (for u), as opposed to regressing directly against π_S, does have an impact on the estimated scaling relations. In the present case, the slopes as a function of π_S tend to be steeper than as a function of N_e, a pattern that is more generally expected if species with large N_e also tend to have lower mutation rates per generation (Lynch *et al.*, 2011). The approach introduced here should therefore represent a useful methodological contribution in the context of the current discussions on the role played by those scaling coefficients in molecular evolution (James *et al.*, 2017; Castellano *et al.*, 2018, 2019; Galtier & Rousselle, 2020).

Estimating mutation rates

Conceptually, our approach for extracting N_e is merely a reformulation, in a Bayesian integrative framework, of the classical idea of estimating N_e from π_S by factoring out the mutation rate u, itself estimated based on a molecular clock argument. The integrative approach presents several advantages, however. First, it imposes the same assumptions about the molecular clock, relying on the same sequence data and the same global set of fossil constraints, uniformly for all species included in the analysis. Second, it automatically propagates the uncertainty about estimates of u, which themselves incorporate the uncertainty about divergence times, onto the credible intervals eventually reported for N_e or for the slopes of the regressions. Third, these slopes are also automatically corrected for phylogenetic non-independence. Finally, on purely practical grounds, the application of the method is straightforward, just requiring as its input a multiple sequence alignment for the clade of interest, estimates of π_S and π_N/π_S for some or all of the extant species and fossil calibrations.

An alternative to the phylogenetic estimation of u is to rely on high-throughput sequencing of pedigrees. As of yet, such estimates are available only for 7 primates (Chintalapati & Moorjani, 2020), but this is likely to change in the future. For these 7 species, the phylogenetic estimates obtained here are higher than pedigree-based estimates, thus in line with previous observations. The reasons for this discrepancy are not yet well-understood (Scally & Durbin, 2012; Ségurel *et al.*, 2014; Chintalapati & Moorjani, 2020). Interestingly, accounting for coalescence in the ancestral populations contributes a lot to making phylogenetic estimates closer to those obtained in the same species by sequencing pedigrees, although not entirely.

In principle, pedigree-based estimates of u could be included in the framework presented here, as additional constraints at the tips of the phylogeny to inform the reconstruction of N_e. However, given the still unexplained mismatch between pedigrees and phylogenies, it is perhaps more meaningful to compare them after the fact, as was done here (Table 3), and then further investigate the various entry points in the model, at the level of the relaxed clock, the prior on divergence times, the fossil constraints, that could be responsible for this discrepancy.

Mechanistic models of coding sequence evolution

Our phylogenetic approach was implemented in two alternative versions, using either a phenomenological or a mechanistic modeling strategy. The phenomenological model implements the idea of

conducting comparative regression analyses directly against N_e, such as discussed above. In itself, this approach is agnostic about the underlying selective regimes over proteins and, in particular, is not inherently committed to a nearly-neutral interpretation.

The mechanistic model, on the other hand, makes more aggressive assumptions about the underlying selective regime. It is fundamentally a Bayesian phylogenetic implementation of the nearly-neutral theory. Accordingly, it assumes that protein-coding sequences are exclusively under purifying selection. Another key assumption of the model, not necessarily implied by the nearly-neutral theory, is that the DFE is constant across species. These assumptions give more constraint to the analysis and return more focussed estimates. However, the estimate of the shape parameter of the DFE obtained under this model turns out to be significantly higher than some of the estimates based on SFS obtained in Humans or in great apes (Table 2), suggesting that one of these two assumptions might not be strictly valid.

The question of whether the DFE is constant across species has recently motivated both methodological work for jointly analyzing the site frequency spectra of multiple species (Tataru & Bataillon, 2019) and empirical investigations (Castellano *et al.*, 2019; Galtier & Rousselle, 2020). These empirical analyses suggest that the shape parameter is rather stable across great apes (Castellano *et al.*, 2019) and more broadly across primates (Galtier & Rousselle, 2020). The mean of the distribution, on the other hand (usually denoted $\bar{s}$), was found to be potentially variable across great apes (Castellano *et al.*, 2019), such that species with larger N_e values also tend to have more strongly deleterious non-synonymous mutations. The rather steep variation of the population scaled mean $\bar{S} = 4N_e\bar{s}$ as a function of π_S observed across metazoans (Galtier & Rousselle, 2020) might also be interpreted as reflecting an underlying positive covariation of $\bar{s}$ with N_e. Importantly, since dN/dS and π_N/π_S scale as $(N_e\bar{s})^{-\beta}$, this specific pattern of covariation between the unscaled mean of the DFE and N_e predicts that the regression slopes should be steeper than β. This could explain the high estimate of β obtained here under the mechanistic model. Of note, the scaling of π_N/π_S and dN/dS with respect to N_e returned by the phenomenological model are compatible with SFS-based estimates, but this might just be a consequence of the rather large credible intervals obtained in their case.

To further investigate this question, conducting a broader analysis with polymorphism data obtained for a larger number of primate species would certainly be an important direction to pursue, as it would consolidate the results presented here and, in particular, would yield more precise estimates of the scaling coefficients under the phenomenological model. If this confirms

the discrepancy between the interspecific scaling coefficients and SFS-based estimates, then, the assumption of a constant DFE under the mechanistic model could be relaxed, although this would then require incorporating information, not just about mean diversity (π_S and π_N/π_S), but also about site frequency spectra in extant species, in order to constrain the estimation.

Concerning the other assumption of the mechanistic model, of an exclusively purifying selection regime, the dN/dS ratio may in fact contain a fraction of adaptive substitutions, susceptible to distort the relation between dN/dS and N_e. Although a relatively minor problem in the case of primates (Eyre-Walker & Keightley, 2009; Galtier, 2016), adaptive substitutions might be a far more important issue when applying the method to other phylogenetic groups. Here also, the model could be further elaborated, by explicitly including an adaptive component to the total dN/dS. Quite interestingly, the resulting model could then be seen as an integrative multi-species version of the Mac-Donald Kreitman test, returning an estimate of the history of the adaptive substitution rate over the phylogeny – which could then be compared with independent estimates based on pairs of sister species (Charlesworth & Eyre-Walker, 2008; Eyre-Walker & Keightley, 2009; Halligan *et al.*, 2010; Galtier, 2016).

Finally, another potential issue, which concerns both the mechanistic and the phenomenological model, is that short-term N_e (such as reflected by π_S) may be strongly dependent on recent demographic events (Charlesworth, 2009) and may thus not be identical with long-term N_e (such as reflected by d_N/d_S). This might be one of the reasons why d_N/d_S shows a weaker correlation with π_S than π_N/π_S. A possible improvement of our model in this direction would consist in allowing for an additional level of variability at the leaves, representing the mismatch between long- and short-term N_e. Other sources of variance in extant diversity estimates could also be modeled, in particular, the additional stochasticity contributed by the random genealogy or by the low counts of SNPs. These last two points are probably a minor issue for nuclear exome-wide polymorphism data, such as explored here. In contrast, they could be quite relevant in the case of the small and non-recombining mitochondrial genome, for which the question of the inter-specific scaling behavior of dN/dS and π_N/π_S as a function of N_e is also of interest (James *et al.*, 2017).

Evolution of mutation rates, N_e and life history across primates

The global phylogenetic history of N_e (Figure 3) and mutation rates (Figures 1 and 2) obtained here offers interesting insights into the macro-evolutionary trends in primates, making connections between life-history and molecular evolution. Previous analyses have repeatedly pointed out a

slowdown of the molecular clock in apes (Steiper *et al.*, 2004), more broadly in catarrhine primates (Perelman *et al.*, 2011), or even more globally throughout the evolutionary history of the entire order (Steiper & Seiffert, 2012), suggesting a trend towards increasing body size and longer generation times in this group. Our reconstruction confirms this global picture, adding another feature, in the form of a global decrease in effective population size, although more specifically in simians (Figure 3). A global picture only in terms of evolutionary trends along a small-versus-large body size axis, however, would be an oversimplification. In particular, dS and dN/dS appear to respond differently to LHT, dS being negatively correlated with body size (Table 1) as previously reported (Steiper & Seiffert, 2012), whereas dN/dS correlates only with longevity but not with body size, a pattern also observed across mammals (Nikolaev *et al.*, 2007; Lartillot, 2013).

The trend in decreasing N_e observed here is primarily driven by the underlying variation in dN/dS. As such, it provides another illustration of the more general result that molecular evolutionary patterns inferred from genetic sequences using phylogenetic methods can be informative about life-history evolution (Lartillot & Delsuc, 2012; Romiguier *et al.*, 2013; Figuet *et al.*, 2014; Wu *et al.*, 2017). Compared to previous work, however, an important new contribution of the present work is a quantitative reconstruction, over the phylogeny, directly in terms of the canonical parameters of population genetics, the mutation rate u and the effective population size N_e. Such broad-scale reconstructions, as opposed to focussed estimates in isolated extant species, are potentially useful in several respects. First, they provide a basis for further testing some of the key ideas about the role of mutation rate or genetic drift in genome evolution (Lynch *et al.*, 2011; Lefébure *et al.*, 2017). Second, the integrative framework could be augmented with trait-dependent diversification models (Fitzjohn, 2010), so as to examine the role of N_e or u in speciation and extinction patterns.

Materials & Methods

Coding sequence data, phylogenetic tree and fossil calibration

The coding sequences were taken from Perelman *et al.* (2011) and modified. It consists in a modified subset, codon compliant, based on 54 nuclear autosomal genes in 61 species of primates, and of a total length 15.9 kb. We used the tree topology published by Perelman et al. (itself based on a maximum likelihood analysis), as well as the eight fossil calibrations that were used in this previous study to estimate divergence times. These calibrations were encoded as hard constraints on the

molecular dating analysis.

Life History Traits

We used four life history traits (LHT) in this study. Adult body mass (as a proxy for body mass, 16 missing values), maximum recorded lifespan (ML, as a proxy for longevity, 19 missing values) and female age of sexual maturity (ASM, 26 missing values) were obtained from the AnAge database (de Magalhaes & Costa, 2009). Estimates about generation time for great apes were taken from Besenbacher *et al.* (2019). For the other primate species, they were calculated from maximum longevity and age at maturity following a method detailed by UICN (Pacifici *et al.*, 2013):

$$\tau = ML \times 0.29 + ASM.$$

Estimation of Polymorphism (π_S and π_N/π_S)

The estimates of the synonymous nucleotide diversity π_S and the ratio of non-synonymous over synonymous diversity π_N/π_S and π_S of 9 primate species were obtained from Romiguier *et al.* (2014); Figuet *et al.* (2016), themselves recalculated based on sequence data produced by Perry *et al.* (2012). For each species, estimates were based on 4 individuals. We matched these polymorphism data for the three species *Pan troglodytes*, *Propithecus vereauxi coquereli* and *Eulemur mongoz*, to *Pan paniscus*, *Propithecus verreauxi* and *Eulemur rufus*, respectively, from the Perelman et al multiple sequence alignment. Of note, π_S and π_N/π_S were estimated on different subset of sites, using the hypergeometric method, such as used in James *et al.* (2017), so as to avoid the artifactual correlations that would be induced between these two parameters by shared data sampling error.

Models

General Principles of the integrative strategy (Coevol)

Coevol is a Bayesian inference software program based on a comparative approach applied to molecular data (Lartillot & Poujol, 2011). The principal aims of this program are to estimate ancestral continuous traits and to determine the correlations between the different molecular parameters along a phylogeny. Coevol follows a generative modeling strategy to describe the evolution of continuous traits along a phylogenetic tree. The joint evolutionary process followed by traits is modeled as a log-Brownian process. This process is parameterized by a variance-covariance matrix, which thus captures the correlations between traits corrected for phylogenetic inertia. Sequence

evolution is described by a codon model (with a separate evolution of d_S and d_N/d_S along the tree). The model is conditioned on data obtained in current species (multiple sequence alignments and quantitative traits), with fossil calibrations, and samples from the joint posterior distribution are obtained by Markov Chain Monte-Carlo (MCMC). The analysis returns an estimation of correlation patterns between traits (covariance matrix) and a reconstruction of the history of the traits along the phylogeny.

Ex-post log-linear transformation of the correlation analysis

As a first step, we ran the original model (such as defined by the current version of Coevol), which we call the *phenomenological* model in the following. We then used the outputs from these runs to estimate N_e and its correlation patterns with other traits. At any given time, the multivariate Brownian process is structured as follows:

$$\begin{cases} X_1 &= \ln dS \\ X_2 &= \ln dN/dS \\ X_3 &= \ln \tau \\ X_4 &= \ln \pi_S \\ X_5 &= \ln \pi_N/\pi_S \\ ... \end{cases}$$

Where τ is the generation time and entries X_i for $i > 5$ correspond to all other LHT. Using equation 4, which gives $\ln N_e$ as a linear combination of the components of the Brownian process, we can define the following linear change of variables:

$$X = \begin{pmatrix} \ln dS \\ \ln dN/dS \\ \ln \tau \\ \ln \pi_S \end{pmatrix} \quad \rightarrow \quad Y = \begin{pmatrix} \ln dS \\ \ln dN/dS \\ \ln \tau \\ \ln N_e \end{pmatrix}$$

where

$$Y_4 = X_4 - X_1 - X_3 + K,$$

where K is a numerical constant (depending on the absolute time scale). So, if we define the matrix A:

$$A = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ -1 & 0 & -1 & 1 \end{pmatrix}$$

then $Y = AX + K$. Finally, since X follows a Brownian process (parameterized by a variance-covariance matrix Σ_X), according to elementary multivariate normal theory, Y follows a Brownian process parameterized by a variance-covariance matrix $\Sigma_Y = A \times \Sigma_X \times A^{-1}$. In practice, we added a new method in Coevol to read the output and apply the linear transformation (from X and Σ_X to Y and Σ_Y) on each sample from the posterior distribution. This allows us to produce a reconstruction of N_e (posterior mean, credible intervals) and of the correlation matrix Σ_Y.

Mechanistic Nearly-Neutral Model

This alternative model uses the original Coevol framework but introduces additional constraints, such that some of the parameters are deduced through deterministic relations implying other Brownian dependent parameters. Specifically, the Brownian free variables are now:

$$\begin{cases} X_1 &= \ln \pi_S \\ X_2 &= \ln \pi_N/\pi_S \\ X_3 &= \ln \tau \end{cases}$$

Then, using equations 3, 4, 7 8, the other variables of interest can be expressed as deterministic functions:

$$\begin{cases} \ln N_e &= -1/\beta \left(\ln \pi_N/\pi_S + \ln \kappa_2 \right), \\ \ln dS &= \ln \pi_S - \ln 4N_e - \ln \tau, \\ \ln dN/dS &= -\beta \ln N_e + \ln \kappa_1. \end{cases}$$

This model has three structural free parameters, β, κ_1 and κ_2, which were each endowed with a normal prior, of mean 0 and variance 1.

The naive-phylogenetic version of the model uses the default approach used in Coevol, i.e. assumes a single dated tree T, with branch lengths measured in time. The Brownian multivariate process X runs along this tree, splitting into two independent processes whenever a speciation node is encountered along the phylogeny. Conditional on T and X, the sequences then evolve along that same tree T, using a codon-model in which dS and dN/dS are modulated across branches, such as

implied by X (Lartillot & Poujol, 2011). For the mean-coalescent version of the model, on the other hand, conditional on T and X, the sequence evolutionary process is assumed to run on a dated tree T' different from T. Compared to T, the node ages of T' are all shifted further back into the past by an amount $\delta t = Max(2N_e\tau, \delta t_0)$, where N_e and τ are the instant values of effective population size and generation time implied by the process X at the corresponding node in the original tree T, and δt_0 is the difference between the age of the focal node on T and the age of its oldest daughter node in T'. This additional constraint is meant to ensure that a node should always be older than its daughter nodes in T'. Owing to the variation in N_e and τ between successive nodes, this constraint may not be automatically realized, in particular in regions of the tree in which successive splitting times are within coalescence time from each other – precisely those splits that are potentially under a regime of incomplete lineage sorting. In practice, this problem is rarely encountered (less than one node of the whole tree on average under the posterior distribution).

Uncoupled Model

The *uncoupled* model, already implemented in Coevol, is similar to the phenomenological version of the model, except that the variables of interest (dS, dN/dS, π_S, π_N/π_S and τ) are modelled as independent Brownian processes along the tree. Equivalently, we use a multivariate Brownian model with a diagonal covariance matrix (see Lartillot and Poujol, 2011, for details).

Markov Chain Monte-Carlo (MCMC) and post-analysis

Two independent chains were run under each model configuration. Convergence of the chains was first checked visually (a burnin of approximately 1000 points, out of 6000, was taken for the phenomenological model, and of 100 out of 3100 for the mechanistic model) and quantified using Coevol's program Tracecomp (effective sample size greater than 500 and maximum discrepancy smaller than 0.10 across all pairs of runs and across all statistics, except for the age of the root of the tree, which typically has a lower effective sample size). We used the posterior median as the point estimate. The statistical support for correlations is assessed in terms of the posterior probability of a positive or a negative correlation. The slope is estimated for each covariance matrix sampled from the distribution, which then gives a sample from the marginal posterior distribution over the slope.

Software and data availability

Coevol (Lartillot & Poujol, 2011) is an open source program available on github: `https://github.com/bayesiancook/coevol`. All models and data used here, along with scripts to re-run the entire analysis, are accessible through the branch coevolNe.

Authors contribution

All modifications of Coevol and new models made in this study are attributable to MB, who also gathered and formatted the data and conducted all analyses, in the context of an internship (master Biosciences of École Normale Supérieure de Lyon). MB and NL both contributed to the writing of the manuscript.

Competing interests

The Authors have no competing interests.

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References

Besenbacher, S., Hvilsom, C., Marques-Bonet, T., Mailund, T. & Schierup, M. H. 2019 Direct estimation of mutations in great apes reconciles phylogenetic dating. *Nat Ecol Evol*, pp. 1–10.

Boyko, A. R., Williamson, S. H., Indap, A. R., Degenhardt, J. D., Hernandez, R. D., Lohmueller, K. E., Adams, M. D., Schmidt, S., Sninsky, J. J. *et al.* 2008 Assessing the evolutionary impact of amino acid mutations in the human genome. *PLoS Genet.*, **4**(5), e1000 083.

Castellano, D., James, J. & Eyre-Walker, A. 2018 Nearly Neutral Evolution across the Drosophila melanogaster Genome. *Mol. Biol. Evol.*, **35**(11), 2685–2694.

Castellano, D., Macià, M. C., Tataru, P., Bataillon, T. & Munch, K. 2019 Comparison of the Full Distribution of Fitness Effects of New Amino Acid Mutations Across Great Apes. *Genetics*, **213**(3), 953–966.

Charlesworth, B. 2009 Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation. *Nat. Rev. Genet.*, **10**(3), 195–205.

Charlesworth, J. & Eyre-Walker, A. 2008 The McDonald-Kreitman test and slightly deleterious mutations. *Mol. Biol. Evol.*, **25**(6), 1007–1015.

Chen, J., Glémin, S. & Lascoux, M. 2017 Genetic Diversity and the Efficacy of Purifying Selection across Plant and Animal Species. *Mol. Biol. Evol.*, **34**(6), 1417–1428.

Chintalapati, M. & Moorjani, P. 2020 ScienceDirectEvolution of the mutation rate across primates. *Curr. Opin. Genet. Dev.*, **62**, 58–64.

de Magalhaes, J. & Costa, J. 2009 A database of vertebrate longevity records and their relation to other life-history traits. *J. Evol. Biol.*, **22**, 1770–1774.

Eyre-Walker, A. & Keightley, P. D. 2007 The distribution of fitness effects of new mutations. *Nat. Rev. Genet.*, **8**(8), 610–618.

Eyre-Walker, A. & Keightley, P. D. 2009 Estimating the rate of adaptive molecular evolution in the presence of slightly deleterious mutations and population size change. *Mol. Biol. Evol.*, **26**(9), 2097–2108.

Eyre-Walker, A., Keightley, P. D., Smith, N. G. C. & Gaffney, D. 2002 Quantifying the slightly deleterious mutation model of molecular evolution. *Mol. Biol. Evol.*, **19**(12), 2142–2149.

Eyre-Walker, A., Woolfit, M. & Phelps, T. 2006 The distribution of fitness effects of new deleterious amino acid mutations in humans. *Genetics*, **173**(2), 891–900.

Figuet, E., Nabholz, B., Bonneau, M., Mas Carrio, E., Nadachowska-Brzyska, K., Ellegren, H. & Galtier, N. 2016 Life History Traits, Protein Evolution, and the Nearly Neutral Theory in Amniotes. *Mol. Biol. Evol.*

Figuet, E., Romiguier, J., Dutheil, J. Y. & Galtier, N. 2014 Mitochondrial DNA as a tool for reconstructing past life-history traits in mammals. *J. Evol. Biol.*, **27**(5), 899–910.

Fitzjohn, R. G. 2010 Quantitative traits and diversification. *Syst. Biol.*, **59**(6), 619–633.

Galtier, N. 2016 Adaptive Protein Evolution in Animals and the Effective Population Size Hypothesis. *PLoS Genet.*, **12**(1), e1005 774.

Galtier, N. & Rousselle, M. 2020 How much does N_e vary among species? . **141**(4), 1619.

Halligan, D. L., Oliver, F., Eyre-Walker, A., Harr, B. & Keightley, P. D. 2010 Evidence for pervasive adaptive protein evolution in wild mice. *PLoS Genet.*, **6**(1), e1000 825.

James, J., Castellano, D. & Eyre-Walker, A. 2017 DNA sequence diversity and the efficiency of natural selection in animal mitochondrial DNA. *Heredity*, **118**(1), 88–95.

Kimura, M. 1979 Model of effectively neutral mutations in which selective constraint is incorporated. *Proc. Natl. Acad. Sci. USA*, **76**(7), 3440–3444.

Lartillot, N. 2013 Interaction between Selection and Biased Gene Conversion in Mammalian Protein-Coding Sequence Evolution Revealed by a Phylogenetic Covariance Analysis. *Mol. Biol. Evol.*, **30**(2), 356–368.

Lartillot, N. & Delsuc, F. 2012 Joint reconstruction of divergence times and life-history evolution in placental mammals using a phylogenetic covariance model. *Evolution*, **66**(6), 1773–1787.

Lartillot, N. & Poujol, R. 2011 A phylogenetic model for investigating correlated evolution of substitution rates and continuous phenotypic characters. *Mol. Biol. Evol.*, **28**(1), 729–744.

Lefébure, T., Morvan, C., Malard, F., François, C., Konecny-Dupré, L., Guéguen, L., Weiss-Gayet, M., Seguin-Orlando, A., Ermini, L. *et al.* 2017 Less effective selection leads to larger genomes. *Genome Res.*, **27**(6), 1016–1028.

Leffler, E. M., Bullaughey, K., Matute, D. R., Meyer, W. K., Ségurel, L., Venkat, A., Andolfatto, P. & Przeworski, M. 2012 Revisiting an old riddle: what determines genetic diversity levels within species? *PLoS Biol.*, **10**(9), e1001 388.

Lynch, M., Bobay, L.-M., Catania, F., Gout, J.-F. & Rho, M. 2011 The repatterning of eukaryotic genomes by random genetic drift. *Annu Rev Genomics Hum Genet*, **12**, 347–366.

Nabholz, B., Uwimana, N. & Lartillot, N. 2013 Reconstructing the phylogenetic history of long-term effective population size and life-history traits using patterns of amino acid replacement in mitochondrial genomes of mammals and birds. *Genome Biol. Evol.*, **5**(7), 1273–1290.

Nikolaev, S. I., Montoya-Burgos, J. I., Popadin, K., Parand, L., Margulies, E. H., National Institutes of Health Intramural Sequencing Center Comparative Sequencing Program & Antonarakis, S. E. 2007 Life-history traits drive the evolutionary rates of mammalian coding and noncoding genomic elements. *Proc. Natl. Acad. Sci. USA*, **104**(51), 20 443–20 448.

Ohta, T. 1995 Synonymous and nonsynonymous substitutions in mammalian genes and the nearly neutral theory. *J. Mol. Evol.*, **40**, 56–63.

Pacifici, M., Santini, L., Di Marco, M. & Baisero, D. 2013 Generation length for mammals. *Nature*.

Perelman, P., Johnson, W. E., Roos, C., Seuánez, H. N., Horvath, J. E., Moreira, M. A. M., Kessing, B., Pontius, J., Roelke, M. *et al.* 2011 A Molecular Phylogeny of Living Primates. *PLoS Genet.*, **7**(3), e1001 342.

Perry, G. H., Melsted, P., Marioni, J. C., Wang, Y., Bainer, R., Pickrell, J. K., Michelini, K., Zehr, S., Yoder, A. D. *et al.* 2012 Comparative RNA sequencing reveals substantial genetic variation in endangered primates. *Genome Res.*, **22**(4), 602–610.

Piganeau, G. & Eyre-Walker, A. 2009 Evidence for variation in the effective population size of animal mitochondrial DNA. *PLoS One*, **4**(2), e4396.

Popadin, K., Polishchuk, L., Mamirova, L., Knorre, D. & Gunbin, K. 2007 Accumulation of slightly deleterious mutations in mitochondrial protein-coding genes of large versus small mammals. *Proc. Natl. Acad. Sci. USA*, **104**(33), 13 390.

Prado-Martinez, J., Sudmant, P. H., Kidd, J. M., Li, H., Kelley, J. L., Lorente-Galdos, B., Veeramah, K. R., Woerner, A. E., O'Connor, T. D. *et al.* 2013 Great ape genetic diversity and population history. *Nature*, **499**(7459), 471–475.

Rannala, B. & Yang, Z. 2003 Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics*, **164**(4), 1645–1656.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., Chiari, Y., Dernat, R., Duret, L. *et al.* 2014 Comparative population genomics in animals uncovers the determinants of genetic diversity. *Nature*.

Romiguier, J., Ranwez, V., Douzery, E. J. P. & Galtier, N. 2013 Genomic evidence for large, long-lived ancestors to placental mammals. *Mol. Biol. Evol.*, **30**(1), 5–13.

Scally, A. & Durbin, R. 2012 Revising the human mutation rate: implications for understanding human evolution. *Nat. Rev. Genet.*, **13**(10), 745–753.

Ségurel, L., Wyman, M. J. & Przeworski, M. 2014 Determinants of Mutation Rate Variation in the Human Germline. *Annu Rev Genomics Hum Genet*, **15**(1), 47–70.

Steiper, M. E. & Seiffert, E. R. 2012 Evidence for a convergent slowdown in primate molecular rates and its implications for the timing of early primate evolution. *Proceedings of the National Academy of Sciences*, **109**(16), 6006–6011.

Steiper, M. E., Young, N. M. & Sukarna, T. Y. 2004 Genomic data support the hominoid slowdown and an Early Oligocene estimate for the hominoid-cercopithecoid divergence. *Proceedings of the National Academy of Sciences*, **101**(49), 17 021–17 026.

Tataru, P. & Bataillon, T. 2019 polyDFEv2.0: testing for invariance of the distribution of fitness effects within and across species. *Bioinformatics*, **35**(16), 2868–2869.

Thomas, G. W. C., Wang, R. J., Puri, A., Harris, R. A., Raveendran, M., Hughes, D. S. T., Murali, S. C., Williams, L. E., Doddapaneni, H. *et al.* 2018 Reproductive Longevity Predicts Mutation Rates in Primates. *Curr. Biol.*, **28**(19), 3193–3197.e5.

Wang, R. J., Thomas, G. W. C., Raveendran, M., Harris, R. A., Doddapaneni, H., Muzny, D. M., Capitanio, J. P., Radivojac, P., Rogers, J. *et al.* 2020 Paternal age in rhesus macaques is positively associated with germline mutation accumulation but not with measures of offspring sociability. *Genome Res.*

Waples, R. S., Luikart, G., Faulkner, J. R. & Tallmon, D. A. 2013 Simple life-history traits explain key effective population size ratios across diverse taxa. *Proc. Roy. Soc. London Ser. A*, **280**(1768), 20131 339.

Welch, J. J., Eyre-Walker, A. & Waxman, D. 2008 Divergence and polymorphism under the nearly neutral theory of molecular evolution. *J. Mol. Evol.*, **67**(4), 418–426.

Wu, F. L., Strand, A., Ober, C., Wall, J. D., Moorjani, P. & Przeworski, M. 2019 A comparison of humans and baboons suggests germline mutation rates do not track cell divisions. **10**(1), 4053.

Wu, J., Yonezawa, T. & Kishino, H. 2017 Rates of Molecular Evolution Suggest Natural History of Life History Traits and a Post-K-Pg Nocturnal Bottleneck of Placentals. *Curr. Biol.*, **27**(19), 3025–3033.e5.

Tables

Table 1. Correlation coefficients between dS, dN/dS, π_S and π_N/π_S, life-history traits and N_e.

	dS	dN/dS	Maturity	Mass	Longevity	π_S	π_N/π_S	Gen. time	N_e
dS		0.24	-0.38	-0.64**	-0.27	-0.60	0.45	-0.42	-0.72**
dN/dS			0.15	0.08	0.49	-0.50	0.42	0.42	-0.58*
Maturity				0.61**	0.52**	0.05	0.10	0.64**	-0.01
Mass					0.53**	0.38	-0.19	0.64**	0.32
Longevity						-0.24	0.26	0.88**	-0.32
π_S							-0.78**	-0.04	0.92**
π_N/π_S								0.11	-0.73**
Gen. time									-0.17

Asterisks indicate strength of support (**: $pp > 0.975$, *: $pp > 0.95$)

Table 2. Scaling coefficient of dN/dS and π_N/π_S as functions of N_e and π_S, compared with estimates of the shape parameter β of the distribution of fitness effects.

method or source	point estimate	credible/confidence interval
$\pi_N/\pi_S \sim N_e$	0.17	(0.02, 0.3)
$dN/dS \sim N_e$	0.10	(-0.02, 0.22)
$\pi_N/\pi_S \sim \pi_S$	0.29	(0.12, 0.51)
$dN/dS \sim \pi_S$	0.13	(-0.12, 0.51)
β (mechanistic model)	0.27	(0.22, 0.33)
Eyre-Walker *et al.* (2006)	0.23	(0.19, 0.27)
Boyko *et al.* (2008)	0.18	(0.16, 0.21)
Castellano *et al.* (2019)	0.16	(0.13, 0.17)

Table 3. Estimates of mutation rate per year r and per generation u (posterior median and 95% credible interval), for several extant and ancestral species.

species	r (per 10^9 years)		u (per 10^8 generation)		
	without anc. pol.[a]	with anc. pol.[b]	without anc. pol.[a]	with anc. pol.[b]	pedigrees[c]
Homo	0.81 (0.61, 1.11)	0.67 (0.50, 0.91)	2.36 (1.76, 3.21)	1.95 (1.46, 2.65)	1.23 - 1.29
Pan	0.79 (0.67, 0.93)	0.67 (0.56, 0.80)	1.91 (1.61, 2.23)	1.62 (1.34, 1.92)	1.26 - 1.48
Gorilla	0.73 (0.44, 1.13)	0.55 (0.32, 0.85)	1.39 (0.84, 2.15)	1.05 (0.60, 1.62)	1.13
Pongo	0.84 (0.54, 1.20)	0.73 (0.46, 1.04)	2.11 (1.36, 3.00)	1.84 (1.16, 2.59)	1.66
Homo - Pan	0.72 (0.51, 1.00)	0.61 (0.43, 0.86)	1.72 (1.29, 2.26)	1.46 (1.08, 1.94)	
Homo - Gorilla	0.74 (0.52, 1.06)	0.66 (0.46, 0.94)	1.68 (1.26, 2.23)	1.47 (1.09, 1.97)	
Homo - Pongo	0.80 (0.51, 1.23)	0.73 (0.46, 1.14)	1.66 (1.19, 2.29)	1.49 (1.04, 2.11)	
Hominoidea	0.76 (0.46, 1.21)	0.70 (0.43, 1.16)	1.42 (0.95, 2.01)	1.27 (0.84, 1.87)	
Macaca	0.68 (0.54, 0.81)	0.58 (0.46, 0.71)	0.95 (0.75, 1.14)	0.82 (0.64, 1.00)	0.37
Papio	0.90 (0.50, 1.59)	0.71 (0.38, 1.30)	1.23 (0.77, 1.94)	0.98 (0.57, 1.59)	0.55
Cercopithecidae	1.08 (0.73, 1.58)	1.00 (0.65, 1.51)	1.26 (0.92, 1.70)	1.14 (0.81, 1.60)	
Catarrhini	0.87 (0.54, 1.45)	0.91 (0.55, 1.52)	1.24 (0.83, 1.79)	1.23 (0.82, 1.85)	
Aotus	1.09 (0.63, 1.77)	1.02 (0.56, 1.74)	1.16 (0.70, 1.78)	1.08 (0.61, 1.70)	0.81
Platyrrhini	1.48 (1.00, 2.15)	1.42 (0.96, 2.09)	1.57 (1.18, 2.15)	1.53 (1.12, 2.06)	
Simiiformes	1.37 (0.75, 2.48)	1.43 (0.80, 2.61)	1.52 (0.98, 2.37)	1.58 (1.00, 2.44)	
Haplorrhini	2.00 (1.01, 4.07)	2.09 (0.98, 4.42)	1.44 (0.88, 2.38)	1.60 (0.92, 2.84)	
Lorisiformes	2.02 (1.19, 3.43)	2.10 (1.15, 3.80)	1.16 (0.77, 1.80)	1.23 (0.77, 1.98)	
Lemuriformes	1.01 (0.58, 1.79)	1.04 (0.57, 1.83)	0.99 (0.64, 1.50)	1.00 (0.66, 1.58)	
Strepsirrhini	2.57 (1.34, 4.99)	2.77 (1.29, 5.94)	1.65 (1.02, 2.73)	1.88 (1.08, 3.38)	
Primates	2.07 (1.06, 4.24)	2.20 (1.05, 4.79)	1.45 (0.87, 2.47)	1.62 (0.94, 2.93)	

[a] naive-phylogenetic method (not accounting for ancestral polymorphism)

[b] mean-coalescent method (accounting for ancestral polymorphism)

[c] from Table 1 of Wu et al (2019)

Table 4. Estimates of effective population size ($\times 10^{-3}$, posterior median and 95% credible interval) for several extant taxa and ancestors.

species	mechanistic	mech. w/o anc. pol.	phenomenological	uncoupled	coal.[a]	hmmcoal[b]
Homo	23 (17, 30)	19 (14, 25)	19 (12, 36)	20 (13, 32)	(13, 16)	8
Pan	64 (54, 78)	54 (46, 64)	67 (45, 101)	60 (40, 94)	(31, 62)	30
Gorilla	64 (25, 170)	67 (27, 159)	110 (37, 354)	47 (20, 108)	(28, 57)	21
Pongo	42 (15, 109)	38 (15, 89)	52 (20, 131)	32 (10, 103)	(42, 85)	19
Homo-Pan	44 (28, 69)	43 (28, 66)	49 (29, 87)	39 (24, 64)	(10, 47)	50
Homo-Gorilla	45 (26, 73)	46 (28, 74)	54 (31, 101)	41 (24, 70)	(27, 61)	47
Hominidae	48 (24, 91)	44 (24, 78)	52 (26, 104)	45 (21, 97)		
Hominoidea	56 (28, 111)	52 (28, 98)	63 (31, 139)	53 (24, 121)		
Cercopithecidae	64 (34, 114)	70 (41, 118)	81 (43, 156)	72 (36, 147)		
Catarrhini	67 (33, 137)	68 (36, 128)	70 (34, 157)	57 (26, 128)		
Platyrrhini	42 (22, 79)	37 (20, 67)	32 (16, 60)	32 (14, 79)		
Simiiformes	56 (26, 130)	53 (26, 110)	43 (18, 97)	39 (15, 101)		
Tarsius	392 (80, 2220)	285 (74, 1477)	89 (18, 391)	49 (4, 568)		
Haplorrhini	96 (40, 240)	88 (39, 199)	46 (15, 131)	45 (14, 149)		
Lorisiformes	117 (55, 253)	115 (58, 244)	53 (16, 135)	53 (21, 135)		
Lemuriformes	102 (47, 218)	97 (48, 197)	118 (48, 285)	152 (66, 377)		
Daubentonia	863 (162, 6174)	893 (183, 5296)	739 (142, 5583)	335 (43, 2796)		
Strepsirrhini	93 (37, 253)	84 (36, 200)	39 (12, 116)	45 (16, 132)		
Primates	97 (40, 251)	88 (39, 200)	47 (14, 132)	46 (15, 151)		

[a] from Prado-Martinez et al, 2013, table 1, for extant hominids, and from Rannala and Yang, 2003 for ancestral species; [b] from Prado-Martinez et al, 2013, figure 2

Figures

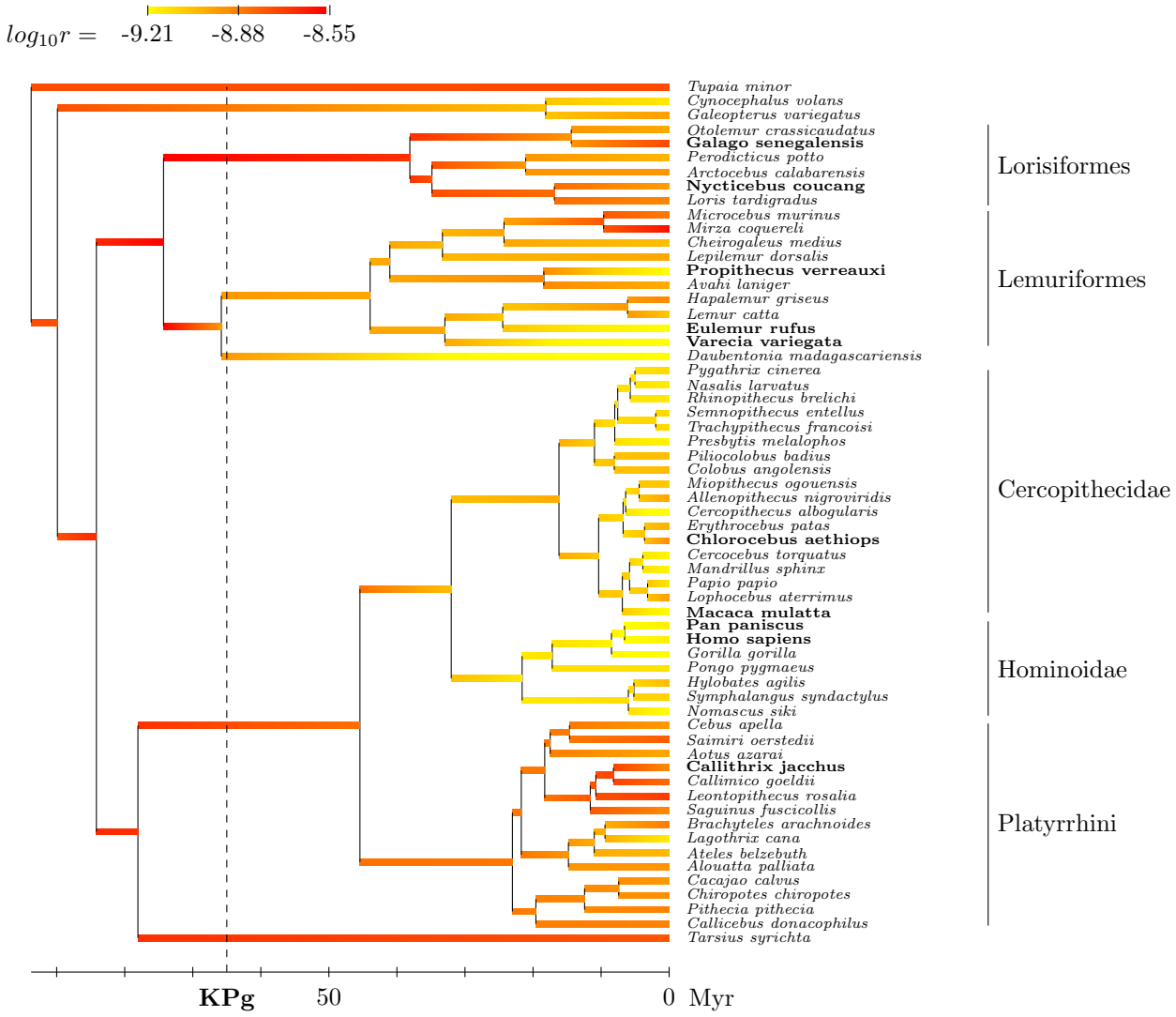

Figure 1. Reconstructed phylogenetic history of the mutation rate per year r (posterior median estimate) under the mechanistic model. Species for which polymorphism data are available are in bold face.

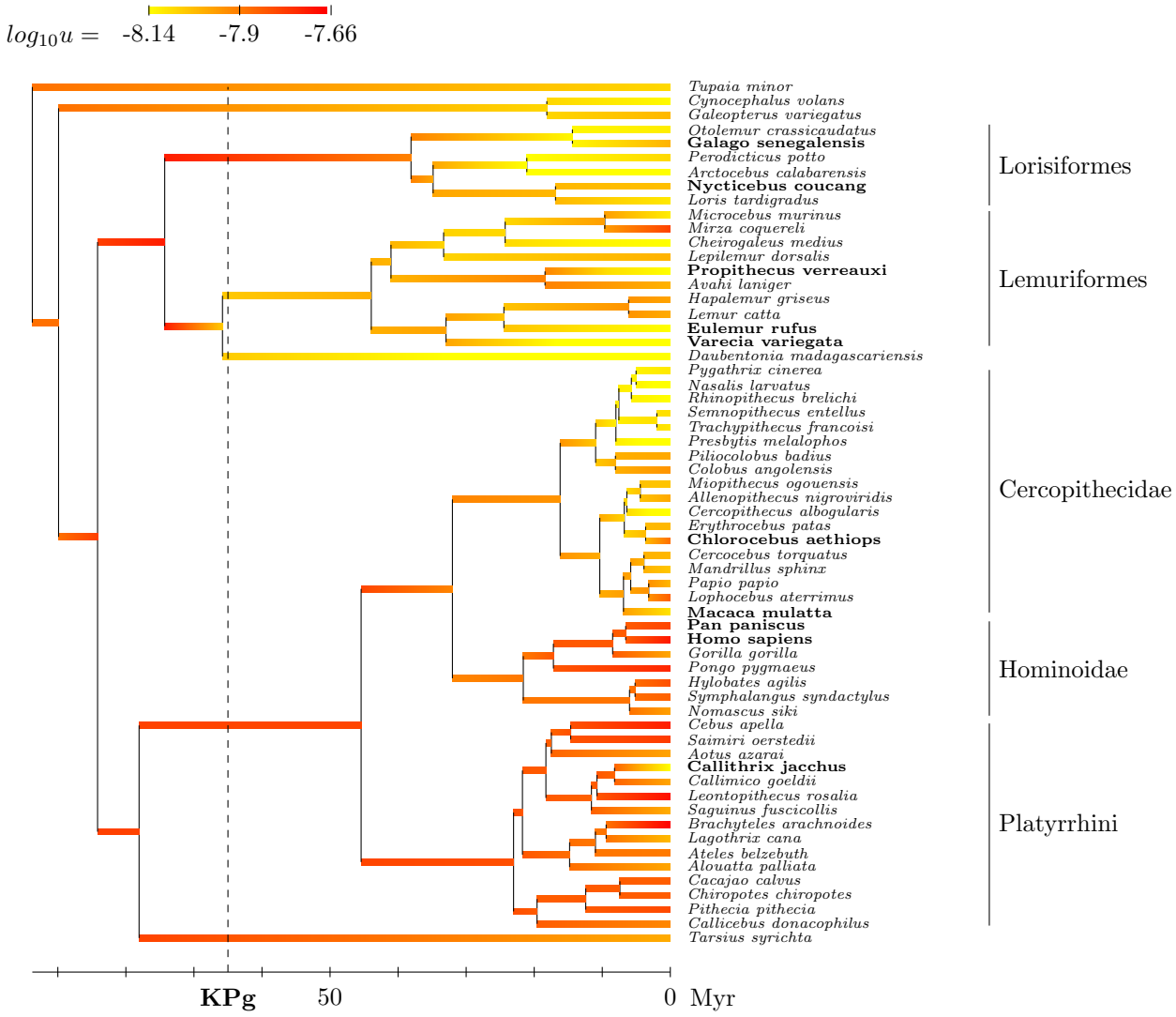

Figure 2. Reconstructed phylogenetic history of the mutation rate per generation u (posterior median estimate) under the mechanistic model. Species for which polymorphism data are available are in bold face.

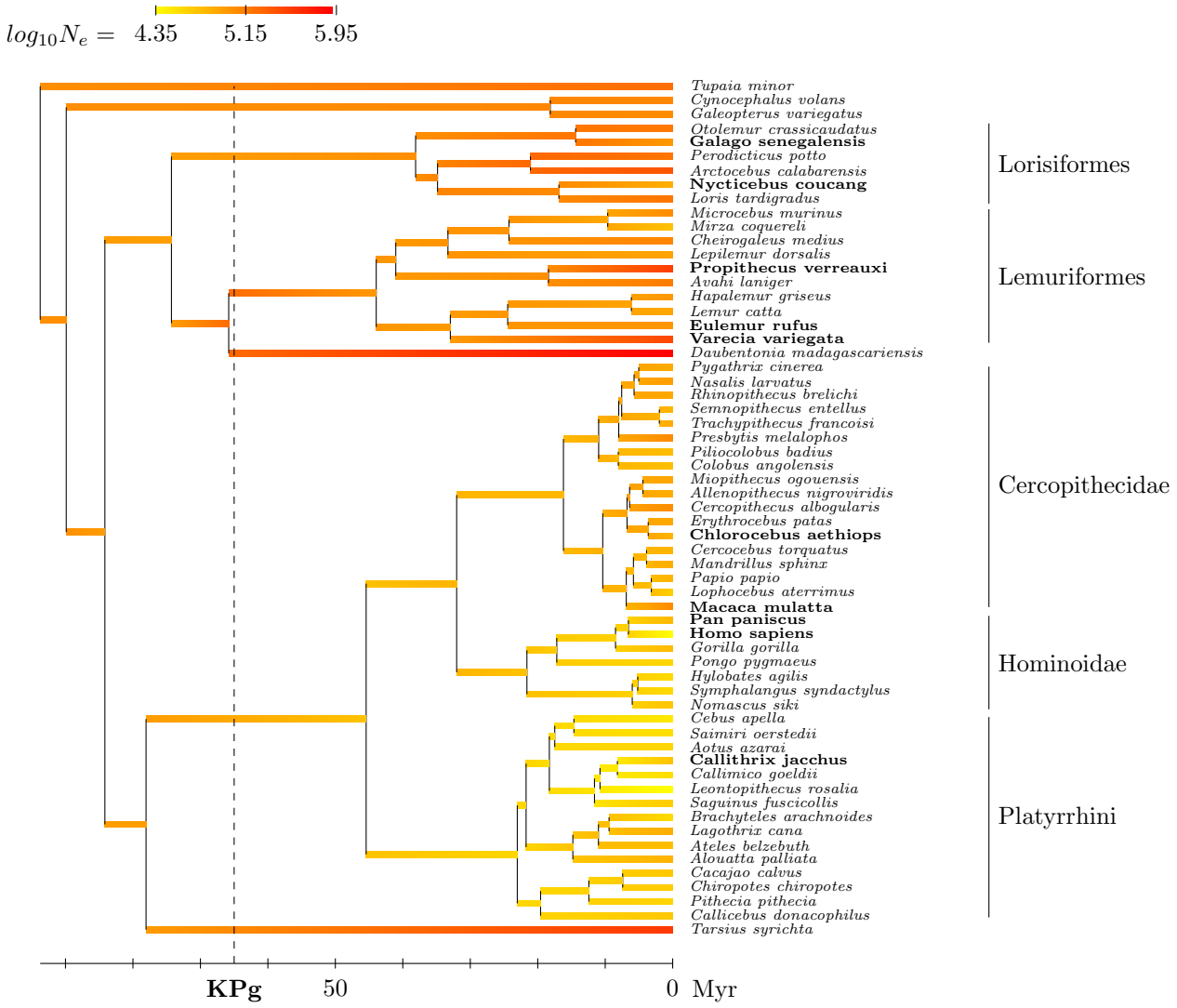

Figure 3. Reconstructed phylogenetic history of N_e (posterior median estimate) under the mechanistic model. Species for which polymorphism data are available are in bold face.