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Primate molecular divergence dates

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Abstract

With genomic data, alignments can be assembled that greatly increase the number of informative sites for analysis of molecular divergence dates. Here, we present an estimate of the molecular divergence dates for all of the major primate groups. These date estimates are based on a Bayesian analysis of ~59.8 kbp of genomic data from 13 primates and 6 mammalian outgroups, using a range of paleontologically supported calibration estimates. Results support a Cretaceous last common ancestor of extant primates (~77 mya), an Eocene divergence between platyrrhine and catarrhine primates (~43 mya), an Oligocene origin of apes and Old World monkeys (~31 mya), and an early Miocene (~18 mya) divergence of Asian and African great apes. These dates are examined in the context of other molecular clock studies.

Keywords: Primates; Molecular clocks; Divergence dates; Crown primates; Hominoid slowdown

1. Introduction

Zuckerkandl and Pauling (1965) forwarded the idea that molecular changes accrue in a clock-like fashion. The record of these changes, known as the 'molecular clock,' allows scientists to estimate the antiquity of a lineage and divergence dates using molecular data. Before the molecular clock, paleontology provided the only timeframe for species evolution. Since then, the molecular clock approach has been used across many taxa to help estimate the divergence dates among species. Within biological anthropology, the application of the molecular clock brought about a fundamental shift in our understanding of the age of the human lineage (e.g., Sarich and Wilson, 1967), but studies of primate divergence dates are a source of continuing debate. Specifically, genetic and paleontological studies sometimes conflict, with the estimated ages of molecular divergences of living primate taxa usually predating those derived from the fossil record (e.g., Seiffert et al., 2005b; Steiper et al., 2004).

Here, we present an analysis of the divergence times of all major primate groups based on a ~59.8 kbp[1] alignment of genomic data, based on a 1.9 Mbp alignment (Cooper et al., 2005). The primates studied include apes, Old World monkeys, New World monkeys, and strepsirrhines. The genomic region analyzed here is drawn from a region homologous to human chromosome 7 (Cooper et al., 2005; Thomas et al., 2003) and provides a long, unbiased genomic sample. The primate divergence dates are estimated here using a Bayesian MCMC molecular clock methodology (Thorne and Kishino, 2002; Thorne et al., 1998), which has been applied to a range of taxa (e.g., Crawford and Smith, 2005; Knapp et al., 2005; Raaum et al., 2005; San Mauro et al., 2005; Steiner et al., 2005). This method calculates lineage-specific rates and branch lengths and incorporates this information into divergence date estimates. This is particu-

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[1] *Abbreviations used:* mya, millions of years ago; kbp, kilobase pairs (1000 bases); Mbp, megabase pairs; LCA, last common ancestor.

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larly important for primates, where rate variation is known to occur (Bailey et al., 1991; Elango et al., 2006; Ellsworth et al., 1993; Goodman, 1961; Goodman et al., 1971; Koop et al., 1986; Li and Tanimura, 1987; Li et al., 1987, 1996; Seino et al., 1992; Steiper et al., 2004; Yi et al., 2002). This method also enables the incorporation of multiple fossil calibrations to the analysis. This analysis provides an estimate of the divergences among species of apes, Old World monkeys, New World monkeys, and strepsirrhines, as well as the dates for last common ancestors of catarrhines, platyrrhines, strepsirrhines, anthropoids, and primates.

2. Materials and methods

2.1. Dataset

The data analyzed here are from a 1.9 Mbp alignment of mammals examined and described by Cooper et al. (2005) (available at http://mendel.stanford.edu/supplementarydata/). This is a taxonomically expanded version of earlier studies of this region (Hwang and Green, 2004; Thomas et al., 2003). This region maps to the *CFTR* region of human chromosome 7. For the purposes of this study, the original alignment was reduced from 29 to 19 taxa. All of the major groups of primates were represented by at least three species. Included were the apes or hominoids (human [*Homo sapiens*], common chimpanzee [*Pan troglodytes*], gorilla [*Gorilla gorilla*], orangutan [*Pongo pygmaeus*]), Old World monkeys or cercopithecoids (rhesus macaque [*Macaca mulatta*], anubis baboon [*Papio anubis*], vervet [*Chlorocebus aethiops*]), New World monkeys or platyrrhines (common marmoset [*Callithrix jacchus*], dusky titi monkey [*Callicebus moloch*], Bolivian squirrel monkey [*Saimiri boliviensis*]), strepsirrhines (gray mouse lemur [*Microcebus murinus*], small-eared galago [*Otolemur garnettii*], and ringtailed lemur [*Lemur catta*]). Non-primate mammals included were rodents (house mouse [*Mus musculus*] and Norway rat [*Rattus norvegicus*]), carnivores (domesticated dog [*Canis familiaris*] and domestic cat [*Felis catus*]), African hedgehog [*Atelerix albiventris*], and nine-banded armadillo [*Dasypus novemcinctus*]. Rodents and carnivores were included as outgroups because they have been used as calibration points in other studies of eutherians (e.g., Springer et al., 2003; Yang and Yoder, 2003; Yoder and Yang, 2004) (further discussed below). Taxa were excluded from the original alignment either because they were not a primary focus of this analysis and/or because they had many gaps that significantly shortened the length of the final alignment. Finally, all positions with a gap in one or more species were removed, leaving only positions with a basepair present for all taxa. This process yielded an alignment consisting of 59,764 bp for 19 species (available upon request). This alignment was examined for the presence of repeat elements (e.g., SINEs) using REPEATMASKER (Smit et al., 1996-2004). It was determined that <1% of the sequences were repeat elements. This small amount was likely because relatively few of these elements were common to all taxa, and only basepairs that were common to all species were included in the final alignment.

2.2. Molecular model

Divergence times were estimated using a Bayesian method that allows for rate variation among lineages (Thorne and Kishino, 2002; Thorne et al., 1998). To employ this method, we used the programs ESTBRANCHES and MULTIDIVTIME (available at http://statgen.ncsu.edu/thorne/multidivtime.html), following an established protocol (Rutschmann, 2004). The method estimates rates for each lineage in conjunction with date constraints (calibrations) on certain nodes and a user-input phylogenetic tree. The user-input tree was based on the current best-estimate of mammalian phylogenetic relationships of Murphy et al. (2004) and primate phylogenetic relationships of Goodman et al. (1998): ((African hedgehog, (domesticated dog, domesticated cat)), ((((gray mouse lemur, ringtailed lemur),small-eared galago), (((common marmoset, Bolivian squirrel monkey), dusky titi monkey), (((((human, common chimpanzee), gorilla), orangutan), ((anubis baboon, rhesus macaque), vervet)))), (house mouse, Norway rat))), nine-banded armadillo). This topology was also recovered from the entire dataset (Cooper et al., 2005), except within the New World monkeys. However, reanalysis of the 59.8 kbp alignment of primates recovered the accepted platyrrhine phylogeny used here. Calibration points are discussed below.

The analysis included two steps. First, maximum likelihood branch lengths and their variance–covariance matrix were estimated using the ESTBRANCHES program. The ML parameters used by ESTBRANCHES were estimated with the F84 + γ model (Yang, 1994) by BASEML (Yang, 1997). Second, the rates of molecular evolution and the ordinal date estimates with their 95% credibility intervals were calculated by MULTIDIVTIME employing MCMC sampling. A burn-in of 100,000 generations was followed by 10,000 generations sampled every 100 cycles. A prior γ-distribution of 100 mya was chosen for the root (the node connecting all the species except armadillo), with a standard deviation of 50 mya, and an absolute oldest estimate of 200 mya. Adjustment of this prior for the root node by 20 mya in either direction (80 and 120 mya) resulted in date estimates that were <0.3% different from those estimated from a root of 100 mya. Adjustment of the standard deviation of this node by 25 mya in either direction (25 and 75 mya) resulted in date estimates that were <0.4% different from those estimated using a 50 mya standard deviation. The rate of autocorrelation (brownmean) was chosen as 0.01 (Rutschmann, 2004). Adjustment of this parameter by an order of magnitude in either direction (0.1 and 0.001) resulted in date estimates that were <0.9% different from a 0.01 estimate for brownmean. The rate of molecular change at the root node was estimated from the dataset to be approximately 0.0014 substitutions per site per million years. This is based on the mean and median time from the root to the tips of the trees.

Assuming the slowest rate from root to tip (0.0013) changed the node estimates by 0.07%, while assuming the fastest rate from root to tip (0.0018) changed the estimates by 0.17%. Overall, these analyses showed that the prior estimates for the parameters had minor impact on the resulting estimates of the node ages. Each analysis was performed twice, with each run arriving at similar date estimates for all nodes, implying convergence. The averages of these two runs are presented as the results.

2.3. Date estimates

There are many potential pitfalls in the calibration of molecular clocks, including a poor fossil record, use of single calibration points, assumption of error-free calibrations, use of secondary (non-paleontological) calibrations, and over-reliance on interpolated or extrapolated dates (Graur and Martin, 2004; Lee, 1999; Raaum et al., 2005; Reisz and Muller, 2004; Shaul and Graur, 2002; Smith and Peterson, 2002; Yoder and Yang, 2000).

In this study, all calibrations are based on well-supported paleontological evidence and are treated as intervals, and multiple calibration points were used both within primates and the outgroups. Dates were first estimated using both the youngest point of the calibration range and the oldest point. Second, all calibration points were estimated as intervals. This approach yielded a range of date estimates that allowed an assessment of the effect of the calibrations on our estimates.

Divergence estimates were calibrated both within primates and the outgroups. Within primates, the split between humans and common chimpanzees was calibrated using *Sahelanthropus tchadensis*, the earliest known hominin, which has been dated between 6 and 7 mya using faunal comparisons (Brunet et al., 2002; Vignaud et al., 2002), with earlier portions of this range more likely (Brunet et al., 2005). This date is supported by two other fossil taxa: *Orrorin tugenensis* (~5.8 mya), a potential hominin from the Tugen Hills (Senut et al., 2001), and *Ardipithecus kadabba* (5.2–5.8 mya), a hominin from Ethiopia (Haile-Selassie et al., 2004). Although there are no known fossil chimpanzees to date (with the notable exception of a very recent fossil (McBrearty and Jablonski, 2005)), the mix of both primitive features and similarities to later hominins in *Sahelanthropus* strongly suggests that the ancestors of chimpanzees and humans had diverged by 7 mya (Brunet et al., 2005; Zollikofer et al., 2005). To accommodate the uncertainty in dating of these specimens, ages of 6 and 7 mya were used to calibrate the human–chimpanzee node.

The primate portion of the tree was also calibrated using an estimate of the divergence of rhesus macaque and anubis baboon lineages. Macaques are known from North Africa and Europe by 5.5 mya (Delson et al., 2000), and in East Asia slightly later (Delson, 1980), although the ago of this latter fossil is questionable (Delson, pers. comm.). Papionins other than macaques (e.g., *Theropithecus*) are well-sampled from the middle to late Pliocene (~2–4 mya) (Jablonski, 2002). Delson (1992) and Delson et al. (2000) estimate these lineages split by ~7–8 mya. We use a conservative estimate of 6–8 mya.

Two non-primate calibrations were also incorporated: *Mus-Rattus* (rodents), and *Felis-Canis* (carnivores). Based on evidence presented by Jacobs and Downs (1994), many molecular clock studies have utilized a 12–14 mya divergence for *Mus* and *Rattus* (e.g., Blair et al., 2005; Chevret and Dobigny, 2005; Chevret et al., 2003; Douzery et al., 2003; Dubois et al., 1999; Ducroz et al., 1998; Huchon et al., 2002; Liu et al., 2004; Salazar-Bravo et al., 2001; Springer et al., 2003). Steppan et al.'s (2004) recent analysis and review of both morphological and molecular evidence was critical of this divergence time and instead estimated an younger date (8.8–10.3 mya) for this node. Conservatively, a *Mus-Rattus* divergence range from 8.8 to 14.0 mya was chosen. The *Felis-Canis* divergence has also been used as a calibration point in molecular clock studies (e.g., Springer et al., 2003; Yang and Yoder, 2003; Yoder et al., 2003; Yoder and Yang, 2004) with conservative estimates between about 45 and 65 mya (Benton, 1993; Flynn, 1996; McKenna and Bell, 1997). This range (45–65 mya) is used in the present analysis.

2.4. Rate testing

Differences between the rate of molecular evolution in hominoids and cercopithecoids were examined using a quartet method implemented by the *Qdate* program (v 1.1) (Rambaut and Bromham, 1998). This method examines a quartet of species, using a calibration point within each species pair. This method tests for differences in rates of molecular evolution, enabling a test of the hominoid slowdown hypothesis. Here, we use the dates estimated using a Bayesian model as calibration points within each pair. Likelihoods were calculated assuming the most likely model of molecular evolution, as chosen by MODELTEST (Posada and Crandall, 1998) under three different conditions of lineage-specific rate heterogeneity. First, the likelihoods of the 'One-Rate' condition were calculated, with a single rate of molecular evolution assumed for all of the branches of the tree. Second, the likelihoods of the 'Two-Rate' condition were calculated, assuming one rate of molecular evolution for the hominoid branches and a second rate for the cercopithecoid branches, allowing an estimation of the hominoid slowdown. A third 'Free-Rate' condition was examined, where each branch was allowed to evolve at its own rate and no molecular clock was assumed. Comparing the likelihoods under these different conditions, rate constancy can be examined with a likelihood ratio test (Felsenstein, 1981) using a χ^2 approximation to test the significance of the difference in log likelihood values between the given conditions (tests had 1 d.f.), as implemented in *Qdate*. First, the 'One-Rate' Condition is tested against the 'Free-Rate' Condition, to test whether one rate of molecular evolution characterizes the data. Second, the 'Two-Rate' condition is tested against the 'Free-Rate' condition, to test if a model

where cercopithecoids and hominoids are evolving at two different rates is a statistically better fit than a model with each branch allowed to evolve at a different rate which enables a test of the 'hominoid slowdown' hypothesis.

3. Results

3.1. Substitution rates

Substitution rates were calculated for all branches (Table 1a). For this dataset, there was between a 3 and 15% difference in the substitution rates between hominoids and cercopithecoids (Table 1b). Using the estimate based on the calibration intervals, the average hominoid rate was 0.57×10^{-3} and the average cercopithecoid rate was 0.65×10^{-3} substitutions per site, per million years. The substitution rates estimated here were slightly slower than those calculated for the human and chimpanzee lineages by Yi et al. (2002) (0.79×10^{-9} substitutions per site per year), which used a divergence of 7.5 mya and Ebersberger et al. (2002) (0.89×10^{-9}) using a divergence of 7 mya. Both the hominoid and cercopithecoid substitutions rates were about twice as fast as those observed in platyrrhines and strepsirrhines. Strepsirrhines and platyrrhines were evolving at approximately the same rate. Within strepsirrhines, galagos were evolving about 3 times more rapidly than the lemurs, which supports previous findings (Bonner et al., 1980; Koop et al., 1986; Porter et al., 1995).

Table 1a
Substitution rates $\times 10^{-3}$ per million years

Branches	Young[A]	Old[B]	Intervals[C]
Strepsirrhines			
Mouse lemur	0.85	0.59	0.73
Lemur	0.58	0.40	0.50
Internode	0.95	0.66	0.82
Galago	2.41	1.67	2.08
Strepsirrhine stem	1.37	0.95	1.20
Average 5 strepsirrhine branches	1.23	0.85	1.07
Platyrrhines			
Squirrel Monkey	1.27	0.92	1.11
Marmoset	1.44	1.05	1.26
Internode	1.34	0.99	1.18
Dusky Titi	1.01	0.74	0.89
Platyrrhine stem	1.25	0.91	1.10
Average 5 platyrrhine branches	1.26	0.92	1.11
Hominoids			
Human	0.65	0.57	0.59
Chimpanzee	0.58	0.50	0.53
Internode 1	0.61	0.51	0.55
Gorilla	0.65	0.56	0.59
Internode 2	0.60	0.48	0.54
Orangutan	0.63	0.54	0.57
Hominoid stem	0.65	0.49	0.60
Average 7 hominoid branches	0.62	0.52	0.57
Cercopithecoids			
Baboon	0.58	0.41	0.51
Macaque	0.64	0.48	0.59
Internode	0.71	0.54	0.65
Vervet	0.83	0.62	0.77
Cercopithecoid stem	0.83	0.64	0.74
Average 5 cercopithecoid branches	0.72	0.54	0.65

A = Used calibrations as youngest points.
B = Used calibrations as oldest points.
C = Used calibrations as intervals from youngest to oldest estimate.

Table 1b
Rate ratios

Branches	Young	Old	Intervals
Cercopithecoid/Hominoid	1.15	1.03	1.15
Cercopithecoid/Strepsirrhine	0.58	0.63	0.61
Hominoid/Strepsirrhine	0.51	0.61	0.53
Platyrrhine/Strepsirrhine	1.02	1.08	1.04

Quartet analysis (Rambaut and Bromham, 1998) was used to further explore the rate differences between the hominoids and cercopithecoids. Quartet analysis tests for differences between a model where no molecular clock is assumed from models with a single molecular clock or two molecular clocks hold. In this test, the two-clock model approximates the hominoid slowdown hypothesis. When the whole dataset was tested, a non-clock model fit the data better than both the one- and two-clock models in a majority of cases (Table 2) which supports the idea that there are slight differences in rates even among hominoids (Elango et al., 2006). When the dataset is broken down into 6 individual sets of approximately 10,000 bp the one-rate model is a poor fit to the data, while the two-rate model is a much better fit in most cases. This supports the hominoid slowdown hypothesis, but underscores the idea that rates may differ between different regions of the genome and there may be additional rate variation among different lineages.

Support for the 'hominoid slowdown' has been offered by many studies (Bailey et al., 1991; Elango et al., 2006; Ellsworth et al., 1993; Goodman, 1961; Goodman et al., 1971; Koop et al., 1986; Li and Tanimura, 1987; Li et al., 1987, 1996; Seino et al., 1992; Steiper et al., 2004; Yi et al., 2002). One of these analyses (Steiper et al., 2004) examined a dataset similar to the one used here, but only for a taxonomic subset of the species analyzed (two hominoids and two cercopithecoids). Steiper et al. (2004) found that cercopithecoids were evolving at a 45% faster rate than the hominoids, while the present analysis recovered a rate between 9 and 21% faster. The present analysis potentially recovered a less pronounced slowdown because the analysis of Steiper et al. (2004) removed dinucleotide substitutions from their alignment, omitting GpG mutations, which are known to evolve in the most 'clock-like' manner (Hwang and Green, 2004). It also may simply be the case that regions show different levels of slowdown, as shown in Table 2. Similarly, Yi et al. (2002), which overwhelmingly supported the hominoid slowdown, found different levels of slowdown in the 20 of 27 genes that showed a slower rate in hominoids.

3.2. Date estimates

Date estimates for all primate nodes are found in Table 3. Each date estimate utilized the calibration information differently in order to model the stochastic nature of both the molecular clock and the uncertainty of the fossil

Table 2
Rate testing within catarrhines

Taxa	Included basepair regions													
	1–10,000[A]		10,001–20,000[B]		20,001–30,000[C]		30,001–40,000[D]		40,001–50,000[E]		50,001–59,764[F]		All Included[G]	
((Cercopithecoids), (Hominoids))	One rate	Two rate	One rate	Two rate	One rate	Two rate	One rate	Two rate	One rate	Two rate	One rate	Two rate	One rate	Two rate
((baboon,macaque), (human,chimp))														
((baboon,vervet), (human,chimp))	*		**				*				**		**	**
((macaque,vervet), (human,chimp))	*						*	*			*		*	*
((baboon,macaque), (human,gorilla))														
((baboon,vervet), (human,gorilla))	**						*				**		**	**
((macaque,vervet), (human,gorilla))	**						*	*			**		**	*
((baboon,macaque), (chimp,gorilla))														
((baboon,vervet), (chimp,gorilla))	**		*				**	*			**		**	**
((macaque,vervet), (chimp,gorilla))	**						**	*			**		**	*
((baboon,macaque), (human,orangutan))					*								*	
((baboon,vervet), (human,orangutan))	**		*		*		*				**		**	**
((macaque,vervet), (human,orangutan))	**		*		*		*	*			**		**	*
((baboon,macaque), (chimp,orangutan))													*	
((baboon,vervet), (chimp,orangutan))	**		*				*				**		**	**
((macaque,vervet), (chimp,orangutan))	**		*				**	*			**		**	*
((baboon,macaque), (gorilla,orangutan))					*									
((baboon,vervet), (gorilla,orangutan))	**				*						**		**	**
((macaque,vervet), (gorilla,orangutan))	**				*		*	*			**		**	*
Percent significant	67%	0%	33%	0%	33%	0%	69%	28%	0%	0%	67%	0%	88%	75%

A = model = HKY, transition ratio = 2.55, γ-parameter = 0.5164.
B = model = general-time reversible approximation of Tamura/Nei (A–C = 1, A–G = 6.51, A–T = 1, C–G = 1, C–T = 4.19, G–T = 1), γ-parameter = 0.269.
C = model = HKY, transition ratio = 2.54.
D = model = HKY, transition ratio = 2.63, γ-parameter = 0.8307.
E = model = HKY, transition ratio = 2.77, γ-parameter = 0.3124.
F = model = general-time reversible approximation of Tamura/Nei (A–C = 1, A–G = 3.94, A–T = 1, C–G = 1, C–T = 5.41, G–T = 1), γ-parameter = 0.9687.
G = model = general-time reversible approximation of TVM (A–C = 1.145, A–G = 5.3306, A–T = 0.6268, C–G = 1.754, C–T = 5.3306, G–T = 1), γ-parameter = 0.269 (A = 31.21%, C = 17.81%, G = 18.68%, T = 32.31%).
* $p < 0.05$.
** $p < 0.01$.

information. In Estimates A and B, all calibrations were the youngest or oldest point in the calibration range, respectively. As expected, estimates from A yielded the youngest and estimates from B yielded the oldest divergence dates for all of the nodes. For Estimate C all calibrations were set as ranges. The confidence intervals of Estimate C approximate the oldest and youngest estimates in A and B. Here, we attempt to accommodate uncertainty in the calibration points by reporting the mean estimates from A and B as the date range for a node and the estimate from C as the best estimate of the result. Fig. 1 illustrates these primate divergence dates in the context of a phylogeny.

Table 3
Primate divergence date estimates

Taxa	A: Young calibration points		B: Old calibration points		C: Calibration intervals	
	Estimate[b]	Credibility interval[c]	Estimate	Credibility interval	Estimate	Credibility interval
Human/Chimpanzee[a]	**6.0**	**6.0–6.0**	**7.0**	**7.0–7.0**	**6.6**	**6.1–7.0**
(Human, Chimpanzee)/Gorilla	7.7	7.2–8.3	9.2	8.6–9.9	8.6	7.7–9.4
(((Human, Chimpanzee), Gorilla)/Orangutan)	16.3	14.6–18.2	20.8	18.6–23.3	18.3	16.1–20.8
Macaque/Baboon[a]	**6.0**	**6.0–6.0**	**8.0**	**8.0–8.0**	**6.6**	**6.0–7.7**
(Macaque, Baboon)/Vervet	8.9	8.3–9.6	11.8	11.0–12.7	9.9	8.8–11.4
Hominoidea/Cercopithecoidea [Catarrhine LCA]	26.9	23.1–30.7	36.4	31.1–41.9	30.5	25.9–35.8
Squirrel Monkey/Marmoset	15.0	11.7–18.7	20.5	15.7–25.9	17.1	13.3–21.8
(Squirrel Monkey, Marmoset)/Titi Monkey [Platyr. LCA]	18.2	14.5–22.3	24.9	19.5–30.8	20.8	16.5–26.0
Catarrhine/Platyrrhine [Anthropoid LCA]	37.3	31.8–42.8	52.4	44.2–60.5	42.9	36.1–51.1
Ringtailed Lemur/Mouse Lemur	35.3	28.0–42.8	51.0	39.4–62.4	40.9	31.7–51.4
Lemur/Galago [Strepsirrhine LCA]	49.4	42.8–56.2	71.4	61.5–81.4	57.1	48.0–68.7
Anthropoidea/Strepsirrhini [Primate LCA]	67.1	60.8–75.0	97.7	88.2–110.2	77.5	65.9 –93.2

[a] Divergences used as calibrations are in bold.
[b] All dates are in mya.
[c] 95% credibility intervals determined by *multidivtime*.

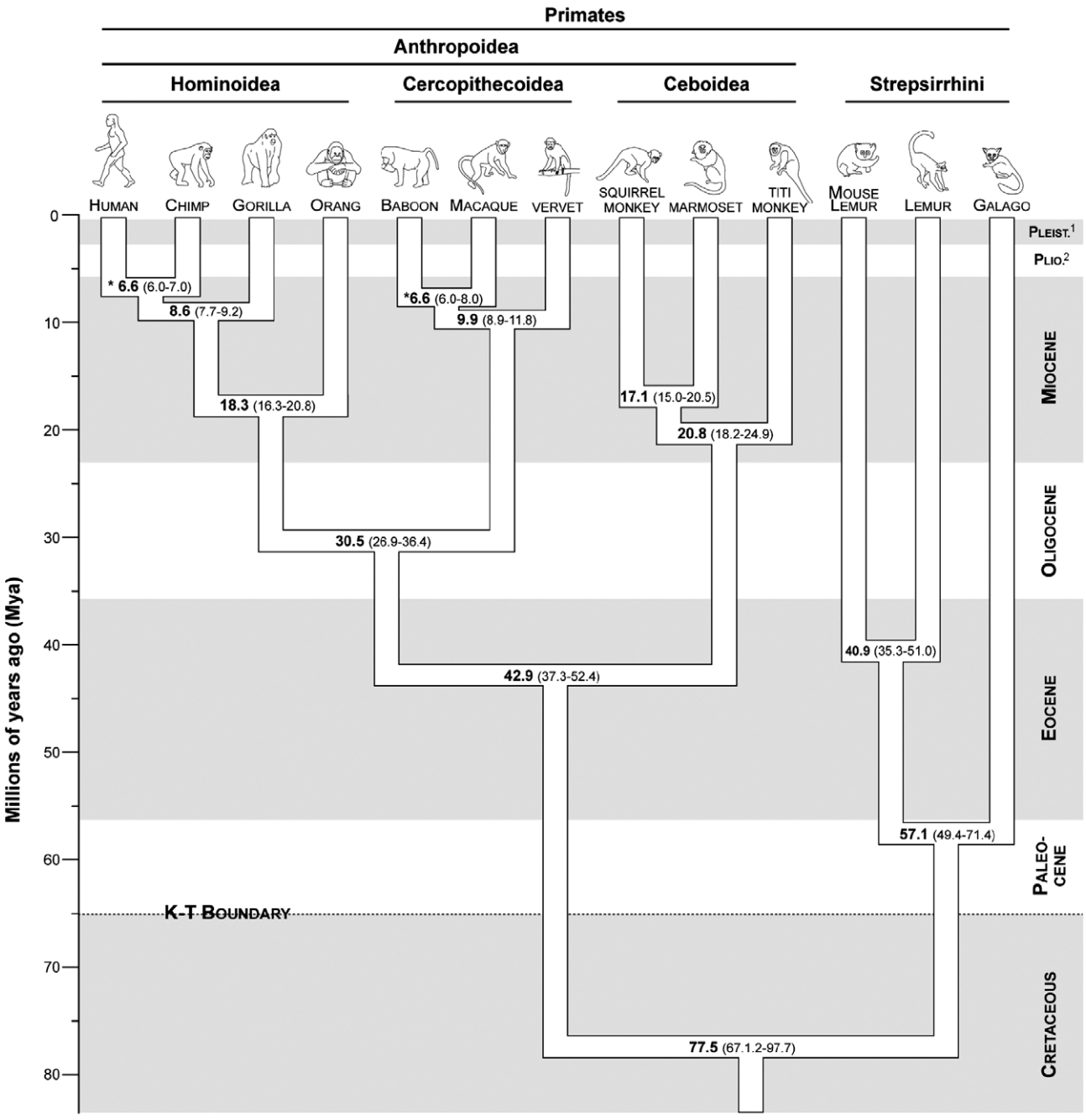

Fig. 1. Phylogeny of the taxa analyzed in this paper. Dates on nodes are based on the interval-estimated dates, with the high and low estimates derived from the dates based on the young and old calibration estimates. Asterisks denote calibrated nodes. [1]Pleist = Pleistocene, [2]Plio = Pliocene.

4. Discussion

4.1. Intra-hominoid dates

Gorilla was estimated to have diverged from human and chimpanzee between 7.7 and 9.2 mya, with a preferred estimate at 8.6 mya. *Pongo* was estimated to have diverged between 16.3 and 20.8 mya, with a preferred estimate of 18.3 mya. The dates estimated here are older than most previous studies that employed a number of different analytical methods and molecular datasets (Table 4). This difference is partially explained by the use of human/chimpanzee as a calibration point. Most studies used either the divergence of Asian and African apes (based on *Sivapithecus* at 9.5–12.5 mya) or the hominoid/cercopithecoid divergence (often at 23–25 mya), recovering relatively young human/chimpanzee dates.

4.2. Intra-cercopithecoid dates

The estimated divergence of vervets and papionins was 9.9 mya (range = 8.9–11.8 mya). These estimates closely match those based on mtDNA (9.8 mya, Raaum et al., 2005), X-linked DNA (11.5 mya, Tosi et al., 2005), and nuclear

Table 4
Summary of hominoid divergence date estimates (adapted from Raaum et al. (2005))

Reference	Divergence estimates[a]			
	Human/Chimpanzee	Human/Gorilla	Human/Orangutan	Hominoid/Cercopithecoid
Mitochondrial DNA				
Arnason and Janke (2002)[A]	11	14	27	47
Hasegawa et al. (2003)[B]	7.4	10.5	17.1 **(13–18)**	34.6
Horai et al. (1995)[C]	4.9	6.6	**13**	—
Huelsenbeck et al. (2000)[D]	7	10	17.8	—
Nikaido et al. (2001)[E]	6.6	9.3	16.8 **(13–18)**	38.1
Raaum et al. (2005)[F]	6.3 (**>6**)	8.6	14.5 (**>14**)	26.5
Yoder and Yang (2000)[G]	*5*	*8*	—	*35*
Nuclear DNA, Immunological Distance, & DNA-DNA Hybridization				
Chen and Li (2001)[H]	*5.4*	*7.3*	***14***	—
Easteal and Herbert (1997)[I]	4	—	9	20
Eizirik et al. (2004)[J]	6.8 **(6–8)**	—	—	28.1(**>23**)
Glazko and Nei (2003)[K]	6	7	***13***	23
Goodman et al. (1998)[L]	6	7	14	**25**
Hasegawa et al. (1987)[M]	4.9	5.9	11.9	25.3
Kumar and Hedges (1998)[N]	5.5	6.7	8.2	23.3
Page and Goodman (2001)[O]	*5.8*	*7.4*	*14.5*	**25**
Sakoyama et al. (1987)[P]	6.4	—	17.3	30
Sarich and Wilson (1967)[Q]	5	5	8	***30***
Satta et al. (2004)[R]	6	7.2	18	34
Sibley and Ahlquist (1987)[S]	*6.6*	*9.4*	***14.6***	*29.5*
Stauffer et al. (2001)[T]	5.4	6.4	11.3	**23.3**
Steiper et al. (2004)[U]	***6.5***	—	—	*31.6*
Wildman et al. (2003)[V]	*5.5*	*6.3*	***13.9***	***25.3***
Median	6.0	7.3	14.0	28.1
This study	**6.6 (6–7)**	8.6	18.3	30.5

A = From their Figure 6.
B = Only mtDNA dates used from this analysis from their Table 2. Nuclear analysis did not include these hominoid taxa.
C = From their p. 534.
D = From their Figure 12.
E = From their Figure 2.
F = From Bayesian estimate in their Table 3.
G = From their p. 1087.
H = From their p. 452.
I = Table 7, column 3.
J = From their Figure 4.
K = From their p. 432.
L = From their Table 5.
M = From their p. 132.
N = From their Figure 4.
O = From their Table 4, column 1.
P = From their p. 1080.
Q = From their p. 1202.
R = From their p. 486.
S = From their p. 99.
T = From their p. 469.
U = From their Table 2, column 12.
V = From their p. 7185.

[a] All dates in mya. Point estimate given with two exceptions. Italics denote average when date was given as a range. Bold denotes use as a calibration point.

genes (10 mya, Goodman et al., 1998), but are older than the 7.8–8.1 mya estimate of Page and Goodman (2001). Overall, the present data are in accord with the previous studies.

4.3. Hominoid/cercopithecoid divergence

The hominoid/cercopithecoid divergence ranged from 26.9 to 36.4 mya, with an estimate of 30.5 mya. This date is in agreement with the median estimate based on other studies (28.1 mya) (Table 4). Recalibrating the other studies using a human/chimpanzee divergence at 6.6 mya, the median age of the hominoid/cercopithecoid node was 30.9 mya. This further supports the molecular and paleontological evidence that this node is probably older than has often been assumed as a calibration point (sensu Steiper et al., 2004). Instead, this node is closer in age to the original

primate molecular clock calibration estimate of 30 mya (Sarich and Wilson, 1967), although not as old as has been proposed elsewhere (~50 mya, Arnason et al., 1998).

4.4. Platyrrhine divergences

The node connecting squirrel monkeys and marmosets, one of the deepest nodes within the Cebidae, was estimated at 17.1 mya ago. Schneider et al. (2001) dated the Cebidae node to 23 mya assuming a 26 mya divergence for the crown platyrrhine node. Goodman et al. (1998) estimated the Cebidae node at 22 mya based on a 40 mya date for the last common ancestor of anthropoids. The node connecting squirrel monkeys and marmosets to the dusky titi monkey estimates the divergence of Cebidae to the Pithecidae. In many phylogenetic reconstructions of platyrrhines, this is the deepest node linking crown New World monkeys (e.g., Chaves et al., 1999; Schneider et al., 2001; Steiper and Ruvolo, 2003; von Dornum and Ruvolo, 1999). This node was estimated at 20.8 mya ago (range = 18.2–24.9 mya). Goodman et al. (1998) estimated the deepest crown platyrrhine node at 25 mya. Therefore, the dates estimated here are slightly younger than have been previously proposed.

4.5. Anthropoids

The divergence of platyrrhines and catarrhines was estimated at 42.9 mya (37.3–52.4 mya). This node, connecting the crown anthropoid primates, has been used as a calibration for earlier molecular clock studies (Goodman et al., 1998) with an estimate of 40 mya, which is in good agreement with the date proposed here. An analysis of mtDNA sequences placed this node at 35 mya, using a 25 mya date for the cercopithecoid/hominoid node as a calibration (Schrago and Russo, 2003). Hasegawa et al. (2003), in a Bayesian reanalysis of the data of Murphy et al. (2001a), (64 taxa, 7 primates, 15 nuclear, and 3 mtDNA genes, ~10 kbp), arrived at an estimate of 37.5 mya. An analysis of mtDNA amino acids (Arnason and Janke, 2002) estimated this node at 60 mya, but was based on a unique primate phylogenetic tree (see below). A Bayesian analysis by Eizirik et al. (2004) dated this divergence to 43.6 mya. The date proposed here is earlier than some of those previously proposed (perhaps due to the choice of calibration), but is very similar to the estimate of Eizirik et al. (2004).

4.6. Strepsirrhini

The estimated divergence of ringtailed lemur and mouse lemur, 40.9 mya, is close to a recent estimate by Yoder and Yang (2004), based on four loci (35.3–51.0 mya). The divergence time of galagos from these lemurs provides an estimate of the deepest node within strepsirrhines. This node was estimated as 57.1 mya, near to another estimates at 59.6 mya (2004), but slightly younger than the estimate of 68.5–74.9 mya (Yoder and Yang, 2004).

4.7. Crown primates

The divergence of crown primates (the last common ancestor of anthropoids and strepsirrhines) was estimated at 77.5 mya (67.1–97.7 mya). While the youngest parts of the credibility interval of Estimate A were Tertiary, most of this estimate falls in the Cretaceous. Three other studies have also dated this divergence using the Bayesian method. Hasegawa et al. (2003) estimated the crown primate node at 73.1 ± 2.7 mya. Springer et al. (2003) analyzed the data of Murphy et al. (2001b) (42 taxa, 2 primates, 19 nuclear, and 3 mtDNA genes, ~16 kbp) and estimated the human/strepsirrhine (crown primate) node at ~70–84 mya. Eizirik et al. (2004) expanded the taxonomic sampling of the Murphy et al. (2001b) dataset to 10 primates, analyzing an 8182 bp alignment. Interestingly, this phylogenetic analysis supported a Prosimii group (linking tarsiers and strepsirrhines), instead of Haplorhini (Anthropoidea and tarsiers). Based on this data set, Eizirik et al. (2004) dated the deepest primate divergence (Anthropoidea/Prosimii) at 77.2 mya. A non-Bayesian analysis of eutherian mitochondrial amino acid sequences estimated the last common ancestor of primates at 91 mya (Arnason and Janke, 2002), although this was based on an unusual phylogeny that included Dermoptera as the sister group of Anthropoidea (Dermosimii), with Prosimii as the sister group to this clade, and tarsiers as the most basal extant primate lineage. The Bayesian estimates fit within the most conservative window presented here for the crown primate divergence and are also all very close to the estimate of 77.5 mya. The mitochondrial estimate is slightly older, perhaps because the analytical methods employed accounted for rate variation in different ways. Estimates based from a mathematical model of primate fossil preservation rate suggest the crown primates diverged earlier, around 81.5 mya (95% CI = 72.0–89.6 mya) (Tavaré et al., 2002), which is closer to molecular estimates. Other paleontological interpretations have been presented which also posit early divergences of crown primates (Miller et al., 2005). These analyses provide support for Martin's (1993) argument that fossil preservational biases will necessarily lead to underestimations of nodes. Indeed, recent analyses of fossil strepsirrhines bolster the idea that the crown primate divergence may be early (Seiffert et al., 2005b). Our estimates are consistent with a Cretaceous origin of crown primates.

5. Concluding remarks

The primate divergence times estimated here are consistent with most other molecular studies which often predate current paleontological estimates. Incongruence of divergence dates based on molecular and paleontological data can occur for a number of reasons including sampling issues (Martin, 1993); i.e., gaps in the fossil record that overlap phyletic divergence times, and dissociations between phyletic and morphological divergences, which cause difficulty in recognizing early members of a lineage

(Cooper and Fortey, 1998). Recent work (Seiffert et al., 2005a; Seiffert et al., 2005b) offers hope that the excavation and reanalysis of fossil materials can also contribute to a reconciliation of the differences in divergence dates between molecular and paleontological studies.

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