Human and Ape Molecular Clocks and Constraints on Paleontological Hypotheses

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Although the relationships of the living hominoid primates (humans and apes) are well known, the relationships of the fossil species, times of divergence of both living and fossil species, and the biogeographic history of hominoids are not well established. Divergence times of living species, estimated from molecular clocks, have the potential to constrain hypotheses of the relationships of fossil species. In this study, new DNA sequences from nine protein-coding nuclear genes in great apes are added to existing datasets to increase the precision of molecular time estimates bearing on the evolutionary history of apes and humans. The divergence of Old World monkeys and hominoids at the Oligocene-Miocene boundary (approximately 23 million years ago) provides the best primate calibration point and yields a time and 95% confidence interval of 5.4 ± 1.1 million years ago (36 nuclear genes) for the human-chimpanzee divergence. Older splitting events are estimated as 6.4 ± 1.5 million years ago (gorilla, 31 genes), 11.3 ± 1.3 million years ago (orangutan, 33 genes), and 14.9 ± 2.0 million years ago (gibbon, 27 genes). Based on these molecular constraints, we find that several proposed phylogenies of fossil hominoid taxa are unlikely to be correct.

Fossils of the earliest hominoids (21 million years ago) and the cercopithecoids (Old World monkeys; 19 million years ago) are known from the early Miocene (Gebo et al. 1997; Lewin 1999; Miller 1999; Pilbeam 1996). Between then and the end of the Miocene (approximately 5 million years ago), hominoids decreased and cercopithecoids increased in diversity in the fossil record (Fleagle 1999). Relating the Miocene apes to living species has proven to be problematic (Pilbeam 1996). There is no fossil species that is clearly a close relative of the gorilla, chimpanzee, or gibbon. It has been debated whether *Sivapithecus* (8–13 million years ago) or other Eurasian fossil apes are close relatives of the orangutan lineage (Pilbeam 1996; Ward 1997). Although the skull of one particular *Sivapithecus* species from 8 million years ago is orangutan-like, postcranial features and the morphology of the cheek teeth have suggested affinities with archaic hominoids (Pilbeam 1996). With this uncertainty, the orangutan divergence is of limited value as a calibration point for molecular time estimates. The absence of Plio-Pleistocene fossil apes from Africa contrasts strongly with the rich hominid fossil record during that same period and is most likely explained by ecological and preservation biases (Fleagle 1999). All of these factors make it difficult to impose time constraints on the origin of living species of hominoids.

With such uncertainty in the hominoid fossil record, considerable attention has been focused on molecular clocks during the last three decades. During the first half of the 20th century, anthropologists assumed that the great apes formed a single evolutionary group distinct from the human lineage, with a divergence time of approximately 30 million years ago (Lewin 1999). However, the first applications of molecular techniques to this problem showed that humans are closer to African apes than to Asian apes (Goodman 1962) and the human-African ape divergence occurred only 5 million years ago (Sarich and Wilson 1967). Many molecular studies have been published since then (Easteal et al. 1995) and have clarified the branching order ((((human, chimpanzee) gorilla) orangutan) gibbon). However, divergence time estimates have varied considerably (Figure 1). If the ratios of the distances or time estimates are considered, the results are more consistent among studies. This suggests that variation in time estimates is largely attributable to the calibration used in each study.

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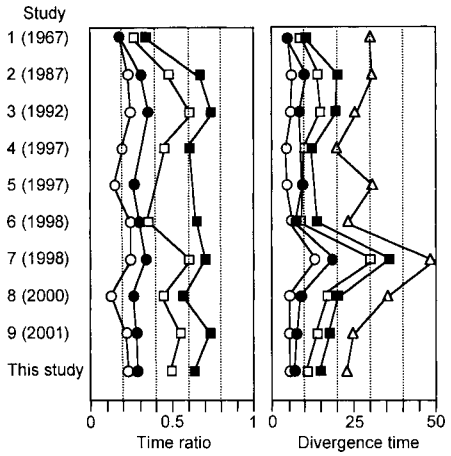

Figure 1. Molecular divergence time estimates for apes and human. The results of selected studies published during the last four decades are shown, where an Old World monkey (cercopithecoid) also was included. Left panel shows the ratio of the human-ape divergence time divided by the hominoid-cercopithecoid divergence time. Right panel shows the actual divergence times. Symbols represent the following divergences: human-chimpanzee (open circles), human-gorilla (closed circles), human-orangutan (open squares), human-gibbon (closed squares), and human-cercopithecoid (open triangles). The data are from the following studies: 1 (Sarich and Wilson 1967), 2 (Sibley and Ahlquist 1987), 3 (Bailey et al. 1992), 4 (Easteal and Herbert 1997), 5 (Takahata and Satta 1997), 6 (Kumar and Hedges 1998), 7 (Arnason et al. 1998), 8 (Yoder and Yang 2000), and 9 (Page and Goodman 2001).

To gain better and more precise estimates of hominoid splitting we have collected new sequence data from nine nuclear protein-coding genes in selected apes. Analyses of these data, along with all other available sequence data, have helped to constrain hypotheses concerning the phylogenetic placement of important fossil hominoids. One major element of uncertainty is the time of the human-chimpanzee divergence. Although the hominid fossil record is relatively good, there are no undisputed Pliocene fossils of African apes (chimpanzees and gorillas) and no Miocene ape fossils that clearly constrain a lower limit to that divergence. An advantage of molecular time estimates is that they measure the mean time of separation rather than the minimum, and the amount of molecular data available has increased in recent years. However, even the most recent molecular studies (Arnason et al. 1998, 2001; Easteal and Herbert 1997) have resulted in widely spaced estimates (3.6–14 million years ago) for the human-chimpanzee split. Because several major oscillations in global climate occurred over intervals of a few million years in the late Miocene and Pliocene (Crowley and North 1991; Pagini et al. 1999), much greater precision in time estimation is necessary to establish postulated relationships between the origin of hominids themselves and bipedal locomotion (the first major hominid adaptation) and environmental change. It also is possible that extinction rather than speciation events are correlated with climate change (Foley 1994).

Materials and Methods

Portions of complementary DNAs (cDNAs) from the following nine nuclear genes were amplified and sequenced for *Gorilla gorilla* and *Pongo pygmaeus*: acyl-coA: cholesterol acyltransferase I, alcohol dehydrogenase 1, beta-glucuronidase, Cd 46, CMP-N-acetylneuraminic acid hydroxylase, interleukin-α_1, prostaglandin D_2 synthase, chemokine receptor 2, and muscarinic acetylcholine receptor 5. A cDNA pool for each species was created by reverse transcription polymerase chain reaction (RT-PCR) (Perkin-Elmer RNA Core kit). RNA was extracted using the RNAqueous kit (Ambion, Inc., Austin, TX) from fibroblast cell cultures established and characterized at the Zoological Society of San Diego (www.sandiegozoo.org/cres/frozen.html). Primers were designed from conserved regions of the cercopithecoid and human sequences in the public databases. Gene fragments were amplified (PCR) and complimentary strands were sequenced. Gene fragments for each gene were combined and aligned using CLUSTAL W (Thompson et al. 1994). All primer sequences, alignments, and sequence accession numbers for this project are available at http://www.evogenomics.org/publications/data/primate/.

The other nuclear genes analyzed were 5-hydroxytriptamine receptor 1a, alpha 1,3 galactosyltrasferase, alanine: glyoxylate aminotransferase, atrophin, beta-nerve growth factor, blue opsin, carbonic anhydrase, c-myc oncogne, cytochrome oxidase subunit 4, DDX5 (p68 RNA helicase), decay accelerating factor, dopamine 4 receptor, dystrophin, eosinophil-derived neurotoxin, fusin, glycophorin A, hemoglobin α1, hemoglobin β, hemoglobin ϵ, hemoglobin γ-α, histamine receptors H_1 and H_2, homeodomain proteins OTX1 and OPTX2, intracellular adhesion molecule 1, interleukin (IL)-3, IL-β8 receptor, IL-16, involucrin, L-selectin, leptin, lysozyme C, muscarinic acetylcholine receptors 2 and 3, myelin basic protein, myoglobin, olfactory receptor, preproinsulin, protamine p2, relaxin, rhesus-like factor, RNase k6, Sp100-HMG, testis-specific protein Y, and zinc finger Y. All genes included in the analyses satisfied two criteria: (1) a sequence was available for *Homo* and at least one other ape genus (*Pan, Gorilla, Pongo, Hylobates*), and (2) at least one calibration species (from Cercopithecidae, Artiodactyla, or Rodentia) and a mammalian or avian outgroup species sequence was available for relative rate testing. Furthermore, all *Pan* and *Gorilla* sequences that were identical to the corresponding *Homo* sequence were deemed uninformative and were therefore eliminated. All analyses were performed on both the group of rate-constant genes only and on the entire dataset.

The relatively low pairwise distances for most protein coding genes in these comparisons of closely related species, combined with limited sequence lengths, favors the more variable nucleotide data (all three codon positions) instead of amino acid data. For time estimation, the Kimura (1980) two-parameter with gamma model was used, which accounts for rate variation among sites. The gamma parameter was estimated by maximum likelihood estimation (Yang 1997) for each gene. Between-group distance estimation was made using PHYLTEST (Kumar 1996), and two methods of time estimation were used. The multigene method uses the mean (or mode) of single-gene time estimates (Hedges et al. 1996; Kumar and Hedges 1998). The average distance method is similar, but averages the concatenated distances, each weighted by sequence length (Lynch 1999; Nei et al. 2001). Rate tests (Takezaki et al. 1995) were made for all comparisons using PHYLTEST.

We used the hominoid-cercopithecoid divergence, set at 23.3 million years ago, as the primate calibration point. It is a fossil calibration point, because the earliest fossils of each lineage are known from 19–21 million years ago (see above). The specific date used (23.3 million years ago) is the geologic boundary between the Oligocene and Miocene epochs (Harland et al. 1990). Most boundaries between geologic periods are times of major or catastrophic change in Earth history or climate, resulting in a greater than average number of extinctions followed by adaptive radiation. The resulting faunal change provides a sharp delineation or time marker in the fossil record. Thus, not considering other factors, it is more likely that the speciation event leading to these two major groups occurred at the boundary rather than slightly earlier or later. Also, the same time of 23.3 million years ago for the hominoid-cercopithecoid divergence was obtained by analysis of protein se-

Table 1. Divergence time estimates (million years ago) between the four major lineages of hominoid primates and the human lineage based on analyses of nuclear and mitochondrial DNA

	Chimpanzee			Gorilla			Orangutan			Gibbon		
	Time	SE	Genes	Time	SE	Genes	Time	SE	Genes	Time	SE	Genes
Nuclear												
Primate calibration, MG	**5.41**	0.55	36	**6.41**	0.74	31	**11.29**	0.68	33	**14.94**	1.01	27
	(4.87)	(0.52)	(25)	(5.49)	(0.65)	(22)	(10.64)	(0.70)	(23)	(14.56)	(1.07)	(21)
Nonprimate calibration, MG	4.65	0.68	22	6.35	1.43	18	10.54	1.29	16	10.73	2.12	6
	(4.54)	(1.07)	(12)	(4.48)	(0.76)	(10)	(8.07)	(1.01)	(10)	(9.28)	(2.99)	(4)
Primate calibration, AD	4.31			6.21			10.03			12.99		
Mitochondrial												
Unadjusted, MG	5.9	0.49	11	7.8	0.59	11	13.2	0.76	11	15.4	0.63	11
Adjusted, MG	4.8	0.59	11	6.4	0.71	11	12.3	0.83	11	14.6	0.70	11

The four comparisons are chimpanzees (*Pan*) versus humans, gorillas (*Gorilla*) versus humans + chimpanzees, orangutans (*Pongo*) versus humans + chimpanzees + gorillas, and gibbons (*Hylobates*) versus humans + other apes. In all cases, a gamma model was used. Results using only genes passing rate constancy tests are shown in parentheses. AD = average distance method; MG = multigene method. For the mitochondrial DNA estimates, results are based on a primate calibration; rate-adjusted times involve a correction for the long branch in orangutan. Time estimates based on the optimal combination of data and methods are indicated in bold.

quences from 56 nuclear genes calibrated with nonprimate divergences (Kumar and Hedges 1998).

We compared the results obtained using the primate calibration with application of a nonprimate calibration. Two nonprimate calibration points were selected: one was the divergence between ferungulates (carnivores and artiodactyls) and primates (92 million years ago) and the other was the divergence between rodents and primates (110 million years ago). These two calibration points are themselves molecular time estimates from an analysis of 333 and 108 nuclear proteins, respectively (Kumar and Hedges 1998). In turn, they derive from a fossil calibration of 310 million years ago for the separation of reptiles and mammals. The advantage of these particular calibrations is the availability of sequences of cattle (*Bos taurus*) and mouse (*Mus musculus*) for most of the genes used. For the nonprimate calibration, we obtained an average rate by linear regression, with the regression line fixed through the origin.

For comparison of our results with previous studies, we also analyzed the complete mitochondrial genomes of *Homo sapiens, Pan troglodytes, Pan paniscus, Gorilla gorilla, Pongo pygmaeus, Hylobates lar,* and *Papio hamadryas,* using the same methods described above. As in previous studies by other authors, we excluded NADH6 from the analysis due to its unusual location on the opposite strand, and COXII because of its accelerated rate of evolution (in primates) compared with other mitochondrial genes. Because of the long branch length of *Pongo* in trees of mtDNA, possibly causing a bias, the divergence time of *Pongo* was also calculated using a lineage-specific method described elsewhere (Schubart et al. 1998). Essentially the time was estimated using only the *Homo* + *Pan* + *Gorilla* lineage. Furthermore, *Pongo* mtDNA was excluded from pairwise length calculations of *Pan, Gorilla, Hylobates,* and *Papio* to prevent possible skewing of results caused by extended branch length.

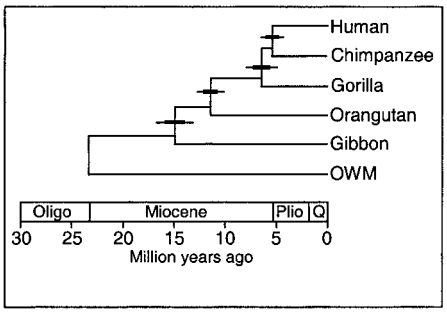

Figure 2. Time tree of catarrhine primates based on divergence time estimates from this study (nuclear genes, Table 1). Time estimates are shown with ± 1 SE (heavy bar) and 95% confidence interval (narrow bar). Abbreviations are Oligo (Oligocene), OWM (Old World monkey), Plio (Pliocene), and Q (Quaternary).

Results

Of the genes newly sequenced, only betaglucuronidase, Cd46, chemokine receptor 2, IL-α1, and prostaglandin d2 synthase demonstrated nucleotide substitution rate constancy. In addition, IL-α1 and alcohol dehydrogenase 1 could not be amplified for *Pongo,* and so the new sequences contributed 7082 base pairs of *Gorilla* sequence and 5556 base pairs of *Pongo* sequence to the analyses for these two species. The new sequences in this article have been deposited in the GenBank database (accession nos. AF354622–AF354638).

Total aligned nucleotide sites and the number of genes (in parentheses) examined for each species divergence (compared with human lineage) are *Pan* 40,668 sites (47 genes), *Gorilla* 29,999 sites (39 genes), *Pongo* 32,966 sites (41 genes), and *Hylobates* 19,307 sites (28 genes). The effects of eliminating the earliest and latest date from the arithmetic and weighted averages to account for possible paralogy problems (Kumar and Hedges 1998) were examined and found to have little effect on divergence estimates (not shown).

Remarkably, divergence times were relatively consistent across genomes (mitochondrial versus nuclear), calibrations (primate and non-primate), rate consistency of gene, and time estimation methods (Table 1). Across all of these variables the divergence time estimates for human versus chimpanzee ranged from 4.2 to 6.3 million years ago, although most estimates were between 5 and 6 million years ago. The optimal method of analysis involves nuclear genes, primate calibration, and multigene method. Divergence times (and 95% confidence intervals) between the human lineage and apes using that method (Table 1) are 5.4 ± 1.1 million years ago (chimpanzee), 6.4 ± 1.5 million years ago (gorilla), 11.3 ± 1.3 million years ago (orangutan), and 14.9 ± 2.0 million years ago (gibbon) (Figure 2).

The difference between time estimates from rate-constant genes versus all genes is relatively small, and therefore the use of all genes is preferred because it yields a lower variance. The multigene method yielded similar estimates to the average distance method except in the case of the human-chimpanzee divergence, where it was slightly low (4.3 million years ago). A variety of weighting schemes can be used with the average distance (or "concatenated distance") method, besides the one used here (sequence length), but the statistical properties of this method are not well known and deserve further study

(Nei et al. 2001). We include those estimates here for comparison, but emphasize the better-known multigene method.

The time estimates from mtDNA (Table 1) are similar to those from nuclear DNA. However, we present these times only for comparison with the nuclear results and with previous studies. The relatively large amount of rate variation in this molecule makes it less desirable for use in time estimation and may explain (in part) why previous time estimates and time ratios from mtDNA have varied (Arnason et al. 1996, 1998; Yoder and Yang 2000) (Figure 1).

Discussion

Hominoid Divergence Times

The divergence time estimates and time ratios from these new sequence data are robust to different methods and calibrations (Table 1). In general, calibrations that are closer to the time estimate are preferred because they require less extrapolation and therefore we advocate use of the primate (hominoid-cercopithecoid) calibration. Using this calibration as a reference point, and the nucleotide-gamma method (Table 1), the resulting time ratios (divergence with human lineage) are 0.23 (chimpanzee), 0.28 (gorilla), 0.48 (orangutan), and 0.64 (gibbon). Here we have assumed that the hominoid-cercopithecoid divergence was 23 million years ago, for the reasons described above. In the future, additional molecular evidence will give increased precision to these distance and time ratios, but the actual time estimate will continue to depend on the calibration. For example, if hominoid or cercopithecoid fossils are found at 30 million years ago, the molecular time estimates would be pushed back by 30%, yielding (for example) a human-chimpanzee split of 6.9 million years ago. However, a similar increase in the synapsid-diapsid (mammal-bird) divergence, to approximately 400 million years ago, would place it earlier than the fish-tetrapod transition in the fossil record (Benton 1997), which would be unlikely. The fact that the nonprimate and primate calibrations now yield similar time estimates suggests some stability to the calibrations used here.

In theory, the divergence times estimated here may be overestimates of the actual population divergences because of coalescence (earlier divergence) of alleles within ancestral populations (Edwards and Beerli 2000; Takahata and Satta 1997). The overestimation is likely to be greatest in recently diverged populations and negligible in ancient splitting events of species (Edwards and Beerli 2000). The amount of overestimation depends on knowledge of population parameters (e.g., population size and generation time) that are difficult to estimate for extinct species in the distant past. However, the closeness of our molecular time estimate of the human-chimpanzee divergence to the fossil record constraint (Haile-Selassie 2001) suggests that the overestimation due to coalescence may be small.

Noncoding DNA sequences also have been used to time human and ape divergences, although higher rates of sequence change limit comparisons to closely related species. Therefore the cercopithecoid calibration usually is not available. In a recent study (Chen and Li 2001) using approximately 24 kb of noncoding sequence, the time ratio (extrapolated from orangutan) for gorilla versus human (0.26) was similar to the coding DNA value reported here, but the human-chimpanzee ratio (0.20) was lower than the corresponding value here (0.23). Assuming the orangutan divergence time estimated here (11.3 million years ago), the resulting human-chimpanzee divergence time with those noncoding data (4.7 million years ago) still is within the 95% confidence limit of our estimate (5.4 $\pm$ 1.1 million years ago).

Mitochondrial DNA has figured prominently in the timing of human and ape divergences in recent years. In large part, this is because of the availability of complete mitochondrial genomes for the species. However, much of the variability in divergence time estimates and time ratios concerns different analyses of these same data (Figure 1). For example, in some studies (Arnason et al. 1996, 1998, 2001) time estimates were two to three times greater than in other studies, whereas the time ratios were not unusually large or skewed, suggesting that the difference was in the calibration. In another case (Yoder and Yang 2000), both the time estimates and time ratios were skewed (e.g., human-chimpanzee divergence was one-seventh of hominoid-cercopithecoid divergence) compared with other studies (Figure 1). Most or all of these problems with timing primate divergences using mtDNA probably stem from the well-known rate increase in the primate lineage in this molecule (Penny et al. 1998) and use of nonprimate calibrations. Although rate adjustments can and have been made, in cases like this where such major rate differences are known, it might be best to avoid using the molecule (especially with many nuclear genes available) or to use only a primate calibration. Taking the latter course in this study, we have obtained time estimates and time ratios for mtDNA more consistent with the fossil record and other molecular datasets.

In one recent study (Easteal and Herbert 1997) the time estimate for the human-chimpanzee divergence (3.6 million years ago) postdates the first appearance of hominid fossils. This raised the possibility that chimpanzees evolved from an upright hominid such as *Australopithecus* (Easteal and Herbert 1997) and that chimpanzees later lost the many morphological adaptations to bipedalism. Our dates using a larger dataset are more consistent with the hominid fossil record.

Temporal Constraints on Hominoid Evolution

Knowledge of an accurate timescale of primate evolution can help constrain interpretations of phylogeny and the relationships of fossil to living taxa. For example, the Early Miocene (21 million years ago) *Morotopithecus* from Uganda was suggested to be either a primitive great ape or the sister taxon of all living hominoids (Gebo et al. 1997). Under the timescale supported here, the first alternative can be rejected because the split between the great and lesser apes is estimated as 14.9 $\pm$ 2.0 million years ago and 21 million years ago is not included in the 95% confidence interval. The second hypothesis is not rejected by our data. Phylogenetic interpretations of some Eurasian fossil apes provide another example. The divergence of the orangutan lineage from the African ape and hominid lineage, 11.3 $\pm$ 1.3 million years ago, is only barely consistent with *Sivapithecus* (12.75–7.0 million years ago) (Ward 1997) being on the orangutan lineage; a smaller confidence interval would reject that hypothesis for at least the earlier specimens of *Sivapithecus.* New hominoid fossils named *Orrorin tugenensis* from the approximately 6.0-million-year-old deposits of the Lukeino Formation of Kenya (Pickford and Senut 2001; Senut et al. 2001) are said to be the earliest hominids, and this date is included in our 95% confidence interval for the chimpanzee-human split. However, the describers of these fossils postulate that African great apes and hominids split 8.5 million years ago, and this is not supported by our estimate. The recently described hominid fossils of *Ardipithecus* from Ethiopia (Haile-Selassie 2001), dated at between 5.2 and

Table 2. Comparisons of paleontological hypotheses of primate phylogeny with molecular time estimates

Fossil genus of group	Age (million years ago)	Postulated fossil relationship or event	Reference	Consistent with molecular timescale
Orrorin	6	Stem hominid	Pickford and Senut (2001)	Yes
Orrorin	8.5	African ape-hominid split	Pickford and Senut (2001)	No
Ouranopithecus	9.5	Stem African ape	Andrews et al. (1997)	Yes
Ouranopithecus	9.5	Stem hominid	DeBonis and Koufos (1997)	No
Samburupithecus	9.5	Stem African ape	Ishida and Pickford (1997)	Yes
Dryopithecus	10	Stem African ape	Begun and Kordos (1997)	Yes
Dryopithecus	10	Orangutan clade	Moya-Sola and Kohler (1996)	Yes
Otavipithecus	13–12	Stem African ape	Pickford et al. (1997)	Yes
Sivapithecus	12.75	Orangutan clade	Ward (1997)	Yes
Proconsul	20	Stem great ape	Walker and Teaford (1989)	No
Proconsul	20	Stem hominoid	Walker (1997)	Yes
Gibbons	20	Pre-*Proconsul* divergence	Rae (1997)	No
Morotopithecus	21	Stem hominoid	Gebo et al. (1997)	Yes
Morotopithecus	21	Stem great ape	Gebo et al. (1997)	No
Small Miocene apes	23	Gibbon clade	Andrews et al. (1997)	No

Different hypotheses of relationships may be inferred from the morphological characters of a single fossil. Here a selection of fossil genera and groups is listed along with relationships (hypotheses) postulated by different authors. The age of the fossil places a temporal constraint on each hypothesis of relationship. Consistency between the paleontological hypothesis and the molecular time estimate (95% confidence interval) for the corresponding divergence among living hominoids is indicated.

5.8 million years ago (WoldeGabriel et al. 2001), show signs of being close to the split between humans and chimpanzees, which again is consistent with our time estimate. The author discounts claims by Senut et al. (2001) that *Ardipithecus* is on the lineage leading to chimpanzees, and that *Orrorin* possesses characters placing it on the hominid lineage (Haile-Selassie 2001). Other recent hypotheses concerning the relationships of extinct hominoids can be addressed by our divergence times (Table 2).

Molecular time estimates also can provide insight into the historical biogeography of hominoid primates. It is assumed that the living and fossil hominoids of Eurasia represent an early dispersal out of Africa. However, the origin of the African great apes and humans (AAH) has been debated. Either they arose from a preexisting lineage of African hominoids, or, as has been suggested (Sarich and Cronin 1976; Stewart and Disotell 1998), they represent dispersal back to Africa. In the recent revival of the "back to Africa" hypothesis (Stewart and Disotell 1998), that scenario was deemed more parsimonious when fossil taxa were considered because it required fewer dispersal events. However, the relationships of fossil hominoids are controversial (Pilbeam 1996) and two African taxa are from a critical period. *Samburupithecus,* an approximately 9.5-million-year-old large hominoid from Kenya (Ishida and Pickford 1997) is postulated on morphological and chronological grounds to be a close relative of the AAH clade. Our time estimates do not rule out this possibility. Similarly, the African *Otavipithecus* (13–12 million years ago) has been thought to be a close relative of the AAH clade (Pickford et al. 1997). Although our mean estimate of the splitting time between the orangutan clade and AAH is 11.3 million years ago, we are unable to reject this hypothesis based on overlap of the 95% confidence interval (12.6–10.0 million years ago) with the time of the fossils of *Otavipithecus.* Considering these fossils, the possibility that the ancestors of the gibbon lineage may have lived in Africa, and the general uncertainty of fossil hominoid relationships, an African origin for the AAH clade is perhaps more likely than a Eurasian origin. More evidence is needed before either hypothesis can be robustly supported.

The larger number of nuclear genes that will be available for analysis in the future will permit increased precision in time estimation and the opportunity to further test these and other hypotheses. This increased precision also will mean that calibration error will take on greater importance. Because fossil calibrations represent minimum (not average) time estimates for the divergence of two lineages, the use of many poorly constrained calibration points may yield a calibration that is a significant underestimate. A more accurate calibration (and resulting time estimate) may be obtained by using only the best-constrained calibration point or points.

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