Chapter 13

RECONSTRUCTING DIVERGENCE TIMES FOR SUPERTREES

A molecular approach

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Abstract: Here, we present a formal approach to estimating divergence dates derived from aligned DNA sequence data on MRP supertrees, using a new supertree for the Primates as a case study. We selected 40 sequence data sets that conform under various models of sequence evolution to the molecular clock. Each of these data sets covers only a subset of the taxa on the supertree, and so composite date estimates were obtained by calibrating the data sets on common nodes and subsequently combining the estimates from different genes for the same node. The internal consistency of our estimates is high. The estimates presented here also fit well with those from Purvis' 1995 primate supertree, although estimates for deeper splits are progressively older.

Keywords: divergence times; fossils; maximum likelihood; molecular clock; Primates; supertree techniques

1. Introduction

Supertrees can be applied usefully to research beyond that of descriptive systematics (Bininda-Emonds *et al*., 2002; Gittleman *et al*., 2004), including comparative studies of character evolution (Gittleman *et al*., 2004); studies of speciation, extinction, and diversification rates (Purvis *et al*., 1995; Moore *et al*., 2004); or establishing conservation priorities (e.g., based on the "evolutionary heritage" concept, the amount of independent evolutionary history embodied within a taxon; Mooers *et al*., in press). These applications require phylogenies for which divergence dates, relative or absolute, are established. Although estimates of relative branch lengths from consensus

Bininda-Emonds, O. R. P. (ed.) Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life, pp. 281–299. Computational Biology, volume 3 (Dress, A., series ed.).

techniques are possible (see Bryant *et al*., 2004), the most widely used technique for the amalgamation of source trees, matrix representation with parsimony analysis (MRP; Baum, 1992; Ragan 1992), does not result in branch lengths that can be interpreted as a temporal dimension. Instead, divergence dates on such supertrees are added afterwards, if at all. In some of the currently published supertrees, divergence dates were obtained through a combination of fossil dates, indirect estimates of sequence divergence by measuring branch lengths from published sources, and models for the expected age of clades given the number of taxa of that clade relative to its dated parent clade (Purvis, 1995; Bininda-Emonds *et al*., 1999). In other studies (e.g., Wojciechowski *et al*., 2000; Liu *et al*., 2001; Jones *et al*., 2002; Pisani *et al*., 2002), no effort was made to establish divergence dates. In any case, objective and robust methods to reconstruct divergence dates for MRP supertrees directly from molecular data sets have yet to be established. Here, we will comment on the advantages and pitfalls of different techniques and data sources, and then discuss a molecular approach as applied to a new supertree of the order Primates.

1.1 Fossils as tools for calibration

If a fossil can be ascribed clearly to a clade, it can offer a minimum estimate of the age of that clade. The application of fossils in estimating divergence dates is twofold: a fossil date can not only be used to define the minimal age of a single node or clade (and its sister group) in a tree, but also to calibrate the absolute depths of other nodes in the same tree if the relative depths of these nodes have been inferred (e.g., from gene sequence data). This distinction is worth mentioning in the context of supertrees: relative node ages are unknown for MRP supertrees and fossils can supply information only in the former manner (i.e., as an indicator of the minimum age of clades and their sister groups) without recourse to added data. The paucity of fossil data is therefore an especially big problem in this type of supertree construction and subsequent dating.

The data on the ages of taxa provided by the fossil record has conflicted with molecular phylogenetic data on several occasions. A textbook example of such conflict is the initial identification of *Ramapithecus* as a 9–12 million year old hominid, constraining the split between humans and the (non-hominid) chimpanzees to be older than that. The subsequent reclassification of *Ramapithecus* as being more closely related to orangutans reconciled the fossil-constrained age of the hominids with the mounting molecular evidence of a more recent origin (Ridley, 1996). Clearly, a misidentified fossil leads to correlated errors for all the node depth calibrations based on it. The reliability and independence of fossil dates

should therefore be evaluated critically, as stressed by, for example Lee (1999), who showed that recent molecular evidence for the earliest metazoan split (Xun, 1998) was calibrated on only two "fossil" dates — one of which was actually obtained from the other "with an additional (molecular) layer of uncertainty introduced" (Lee, 1999:387).

The carnivore supertree (Bininda-Emonds *et al*., 1999) is an example where fossils were used to derive the minimum age of sister groups: the time of first occurrence of either descendant lineage was used to date nodes. It is agreed generally that because fossils can be classified only once clade-defining morphological synapomorphies have arisen (Archibald, 1999), it is likely that the fossils of the earliest members of a clade are often overlooked as members of the clade (if these fossils have formed and were discovered at all). Thus, fossil dates will be too-young estimates of the age of clades. A famous example of this is the "Cambrian explosion" scenario, a hypothesized evolutionary burst (e.g., Gould, 1989; Lipps and Signor, 1992) that hinges on the assumption that sudden cladogenesis and trait evolution followed from the sudden appearance of most animal phyla in the Cambrian fossil record. Molecular studies, however, consistently support an extended period of Precambrian metazoan diversification (Bromham *et al*., 1998; Bromham and Hendy 2000) along "ghost lineages" (Novacek and Wheeler, 1992; Fortey *et al*., 1996), giving further evidence that fossils should not be considered as fixed ages of nodes, but rather as constraints on the minimum ages of nodes. However, despite the difficulties in working with fossils in terms of their rarity and their interpretation, the key attraction to fossils is that they are the only way, ultimately, that absolute ages of clades can be determined.

1.2 Relative divergence dates inferred from molecular phylogenies

DNA sequence data can provide information on when species have diverged, not only on the branching order that can be inferred from the phylogenetic signal they provide, but also on the relative timing of these branching events. For the latter to work, the locus under study must conform to the "molecular clock" (Zuckercandl and Pauling, 1965), which in practice means that substitution rates must be constant along all lineages, resulting in an ultrametric tree (i.e., a tree with the same root-to-tip path length for all lineages). Whether or not a particular locus conforms to the molecular clock can be tested by comparing the likelihood (Felsenstein, 1981) of the optimal topology under unconstrained rates to the likelihood of the same tree constrained to be ultrametric. The ultrametric tree will have a worse score,

but, if it is not significantly worse, the locus is considered to conform to the molecular clock hypothesis.

Clocklike loci are a useful source of information from which divergence dates for supertrees can be obtained. However, the MRP supertree technique does not allow for branch length information to be encoded in such a way that the resulting supertree reproduces meaningful divergence date estimates. Therefore, in earlier MRP supertree studies, molecular data on divergence times was used indirectly (Purvis, 1995; Bininda-Emonds *et al*., 1999) by rescaling previously published molecular phylogenies, calibrating them subsequently using fossil data, and then sticking the divergence dates so obtained on the supertree. This approach has two drawbacks. First, the rescaling process (as described in Purvis, 1995) is essentially a method by which source phylogenies are "ultrametricized" without recourse to the underlying sequence data. It is therefore not certain whether or not the particular locus actually conforms to the molecular clock. Second, the source trees sometimes do not match the topology of the supertree, rendering the source tree in whole or in part unusable. Given these drawbacks, we argue that using sequence data directly is an approach that warrants further research, a case study of which is discussed in this chapter.

1.3 Obtaining composite estimates of divergence dates from sequence data

Relative branch lengths from a set of congruent phylogenies that each cover a subset of taxa usually cannot be combined to derive the branch lengths for the phylogeny that covers the bigger set from which the subsets were drawn. However, the depths of nodes in the set of congruent phylogenies can be combined. For instance, Figure 1a shows a topology for which the divergence dates are unknown. The four trees in Figure 1b each cover a subset of the taxa of the tree in Figure 1a, and are congruent with the topology of that tree. The branch lengths for these ultrametric trees might have been derived from disparate data sources, such as different genes that conform to the molecular clock hypothesis. By calibrating these trees on a shared node — such as node 2 for trees II–IV in this example — the node depths of these trees can be combined to obtain the branch lengths for the topology of Figure 1a (as shown to the right in Figure 1c). From the example in Figure 1, it is evident that this method can be used only to combine divergent dates from multiple sources that share at least one node. However, this is not the only consideration that needs to be taken into account in choosing calibration points.

The location of the calibration point relative to the other nodes in the source trees has an effect on how variation in the estimates is distributed

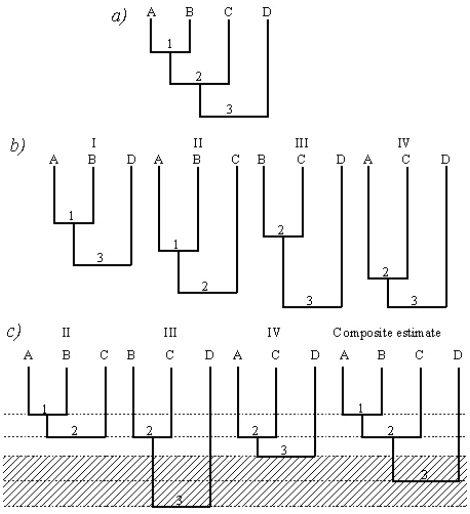

Figure 1. Combining and calibrating divergence dates. a) Hypothetical MRP supertree topology, for which relative branch lengths and labeled node depths are undefined. b) Aligned sequence data sets that conform to the molecular clock when fitted to the topology of the supertree. Node labels correspond with those in (a). c) Sequence data sets II, III and IV are calibrated on their shared node 2. Based on these combined data sets, the depths for all three nodes can be reconstructed in the composite estimate. Because there are two data points for node 3, there is a range (hatched area) from which the median is selected for the composite estimate.

over the tree. Figure 2 illustrates this via a simulation. Branch lengths on 1000 ultrametric and fully-unbalanced (i.e., comb-like) 32-taxon trees were simulated based on a pure birth model for clade growth (Harding, 1971). This is a common process for generating divergence times on trees, with the useful property that the waiting times between successive branching events are drawn from a negative exponential distribution with parameter n, where n is the number of extant taxa at any time (Nee *et al*., 1992; Nee, 2001). Relative waiting times (and so relative branch lengths) can therefore be simulated simply as $t = -\ln(p) / n$, where p is a uniformly distributed random number between 0 and 1 that represents the uniform distribution of probabilities. Although the trees so generated are all the same size and shape, they differ in their total depth as a result of the stochastic nature of the birth process.

Figures 2a–c depict different calibration scenarios for these simulated trees. In Figure 2a, all 1000 trees were calibrated on the root, forcing them all to have the same total depth. The graph plots the median depth over the sets of equivalent estimated nodes (i.e., the most recent split, the second-most, the third-most, through to the root) as the x-axis and the coefficient of

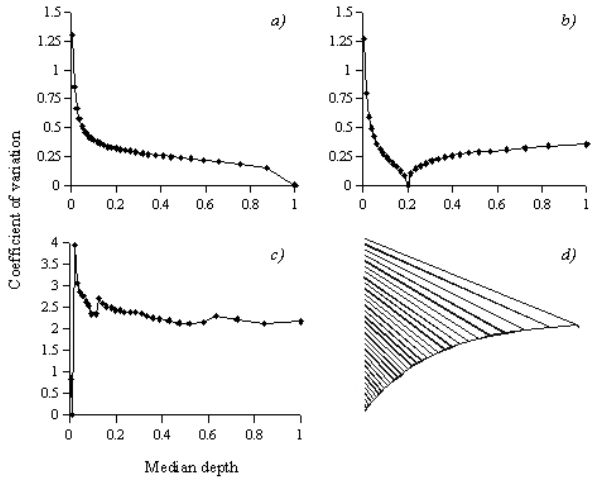

Figure 2. Simulated calibration scenarios: a) calibration on the root, b) calibration on an intermediate node, and c) calibration on a recent node. Each data point represents a set of equivalent nodes over 1000 comb-like, ultrametric trees. For instance, the rightmost point represents a set of a thousand roots, whereas the leftmost point represents the set of nodes that splits the most recent pair of sister species. Median depth over each set is plotted on the horizontal axis such that values of 0 and 1 correspond with the tips and the root, respectively. On the vertical axis, the coefficient of variation over each set is given, give the following calibration scenarios An example of a 32-taxon ultrametric tree with branch lengths simulated under a Yule model, such as the trees used in these calibration scenarios, is given in (d). Its orientation is identical to the data points in (a)–(c) (i.e., with the oldest nodes on the right and the newest on the left).

variation over each of these sets as the *y*-axis. The data point with the largest depth (i.e., the root) had a coefficient of variation of zero because it was used as the calibration point. As we moved away from the calibration point (i.e., leftward), the coefficient of variation increased because of the cumulative effect of the randomness propagating through the tree. Figure 2b shows how the coefficient of variation behaves when a node of intermediate depth was chosen as calibration point. Once again, the coefficient of variation increased as we moved away from the calibration point, both when we moved nearer to the tips or to the root. However, the mean coefficient of variation over all nodes was lower (here, 0.309 versus 0.368 when calibrated on the root). Figure 2c shows the behaviour when a recent node was used as calibration point: the mean coefficient of variation over all nodes was the highest of all scenarios (2.324). This is probably because of the Central Limit Theorem: the depth of the first split, unlike all that follow, is not the result of the sum of a series of draws from the exponential distribution, but rather of a single draw, and so the variation over a set of such nodes is accordingly higher than that over any set of deeper nodes. Thus, constraining a set of these first,

more variable, splits to the same depth will increase the variation over each set of deeper nodes.

In comb-like trees, all nodes are ordered consistently and linearly, and so the trees in our simulation provide a highly simplified and somewhat extreme example of the effect of choosing a single calibration point on the overall variation over all other nodes. Nevertheless, we expect that the same effect will hold for real data sets, albeit to a lesser extent because most real trees are not fully unbalanced.

Because it is desirable to choose a calibration point that minimizes the total variation over node depth estimates, the best choice would be to choose an intermediate node for calibration. However, even if the variation over different estimates is so minimized, it is still likely to be high as a result of outliers caused by, for instance, 1) saturated genes reducing the estimated depth for deeper nodes or 2) genes that give highly discrepant estimates for other reasons such as different strengths and modes of selection along different lineages. In earlier studies where divergence dates were combined in supertrees (e.g., Purvis, 1995), the influence of such outliers was minimized by taking the median instead of the mean over the set of estimates. We do the same here.

From the simulations, it is evident that overall variation can be reduced by choosing an optimal calibration point. However, even if one were to choose the node that is located optimally within the topology of the tree, stochasticity will still propagate through the tree such that nodes located away from the calibration point will be highly variable. By using multiple calibration points located in disparate regions of the tree, we can minimize this effect. This approach has the added merit of including more previously known information on divergence dates.

Consider Figure 1 again. In this example, the trees were calibrated on the shared node 2. All prior information on the other divergence dates is thus disregarded when obviously we should strive to incorporate all available, robust, information in the estimates. We will do this by averaging all divergence date estimates for a given node across all different calibration points for which prior information is available. For instance, if node 1 in Figure 1 would also be used as a calibration point, we would get two data points for node 2: one where it was used as a calibration point as shown in Figure 1c, and one from tree II calibrated on node 1. Similarly, we would get two estimates for node 3 (one from the median of the estimates obtained by calibrating trees III and IV on node 2, and one from tree I calibrated on node 1) as well as for node 1 (one obtained be calibrating tree II on node 2 and one where it is used as a calibration point for trees I and II). We then average over the data points for each the respective nodes and incorporate the results into the supertree. We apply this method below.

2. Methods

2.1 Phylogeny construction

The primate phylogeny we used in this study will be presented in full in a companion article (Vos and Mooers, in prep.), and so we offer only the briefest outline here. We collected 217 source trees from 126 articles published after 1993 and combined these with the data from the primate supertree of Purvis (1995). We then combined all these data sets into one large MRP matrix using RadCon (Thorley and Page, 2000) and used the parsimony ratchet (Nixon, 1999) strategy as implemented in the program PAUPRat (http://viceroy.eeb.uconn.edu/paupratweb/pauprat.htm) to search tree space under various models of character state change. Finally, we constructed majority-rule and strict consensus trees over each of the resulting sets of unique optimal trees.

2.2 Molecular data collection

To collect suitable candidate genes for the inference of relative divergence dates we downloaded the Primates section of the NCBI-GenBank Flat File Release 132.0 from ftp.ncbi.nlm.nih.gov. We indexed this data set using the standalone BLAST tool formatdb and performed keyword frequency ("grep") searches to collect genes that were sequenced over a broad taxonomic range. We refined these results using BLAST (Altschul *et al.*, 1990) searches. This yielded 55 candidate genes. We aligned these sequence data sets using Clustal W's default settings and method (Thompson *et al.*, 1994) and subsequently by hand. We then ran ModelTEST (Posada and Crandall, 1998) on each data set using the likelihood-ratio test statistic $d = -2 \log L$ to identify the appropriate nucleotide substitution model from a nested set.

Subsequently, we tested whether the molecular clock could be rejected using the same statistical approach, but with a liberal alpha for rejection of 0.001. We chose this alpha level for two reasons. First, given that the likelihood-ratio test for rate constancy is a test of significance, the usual alpha level of 0.05 will reject the clock by chance alone once in every twenty tests on average, even if all loci behave in a clocklike manner (i.e., a Type I error). Lowering the alpha level reduced this risk and so served as a correction for multiple comparisons. Second, lowering the alpha level to 0.001 allowed us to include data sets that evidently deviate somewhat from rate constancy such that they would have been rejected under the more commonly used level of 0.05.

Because this approach by itself yielded too few data sets, we developed a program that iteratively prunes from the non-clocklike data sets those taxa that are the most divergent from the mean root-to-tip path length, and subsequently tests whether the data set then conforms to the molecular clock. The routine stops once $p > 0.001$. Essentially, this program removes those lineages from a data set within which substitution rates have increased or decreased significantly relative to the average of that data set. Data sets where the program stopped when three taxa remained were discarded because conforming to the molecular clock with so few taxa is essentially meaningless.

Using this approach, which could be described as "gene shopping" followed by "taxon shopping", 40 loci conformed to the molecular clock. The loci analyzed in this study are listed in Table 1; those that conform to the molecular clock and that we used to obtain divergence dates are indicated by an asterisk.

1.3 Inferring and calibrating divergence dates

We labeled each node in the topology of the supertree by appending a serial number — and, to remain compliant with the NEXUS format (Maddison *et al*., 1997), the word "node" — to each closing bracket of the tree description. The result is similar to the labeling on the tree in Figure 1a. For each aligned clock-like sequence data sets, we then pruned all taxa that were absent in that data set from the supertree so as to obtain constraint trees congruent with the consensus supertree, while keeping track of the initial node-labeling scheme. This resulted in a set of trees with labeled nodes like those shown in Figure 1b. The labeling and pruning was done using Perl scripts, which are available from the authors upon request. We then estimated the branch lengths on these constraint trees under the appropriate models using PAUP* (Swofford, 2002). We calculated relative node depths from these branch lengths using the ape package (http://stat.ethz.ch/R-CRAN/doc/packages/ape.pdf) for the R program. The routine that calculates these depths visits all labeled nodes and, for each, calculates the path length from that focal node to the tips and writes it to a table. Because the routine does not take all possible paths into consideration, it gives meaningful results only for ultrametric (i.e., clocklike) trees. We then combined the results from the individual genes into a larger table to calibrate these multiple loci on shared nodes. We surveyed the recent literature for estimates of the timing of major, uncontested splits in the evolutionary history of the primates that could function as calibration points (e.g., Gingerich and Uhen, 1994; Adachi and Hasegawa, 1995; Adachi and Hasegawa, 1996; Arnason *et al*., 1996a, b,

Table 1. Loci used in this study. The abbreviated models are the following: HKY85: Hasegawa, Kishino, Yano (Hasegawa *et al*., 1985); K80: Kimura two-parameter (Kimura, 1980); GTR: General Time Reversible (Rodríguez *et al*., 1990; Yang *et al*., 1994); +Γ: variation in rates among sites modeled using a gamma distribution (Yang, 1996); +I; a proportion of sites modeled as invariant (Hasegawa *et al*., 1985). The number of taxa after pruning (see text) is given.

Gene	Model	Clock test *p*-value	No. of taxa
alpha-1,3-Galactosyltransferase	GTR+I	1.09752×10^{-23}	19
ATP6	GTR+Γ+I	3.72376×10^{-10}	17
ATP7A	HKY85+Γ	0.13700341*	7
ATP8	GTR+Γ+I	3.06905×10^{-10}	17
BRCA1	HKY85+Γ	0.01145129*	7
Calmodulin	HKY85	0.815719539*	6
CCR5	K80+Γ+I	0.00046635	67
CD4	GTR+Γ	0.00189824*	22
COII	GTR+Γ+I	4.56×10^{-8}	57
CXCR4	HKY85+Γ+I	8.91×10^{-5}	42
DRD4	HKY85+Γ	0.07035867*	14
FUT1	HKY85+Γ	7.3035×10^{-149}	32
Gamma1 globin	HKY85+Γ	0.0093968*	13
G6PD	HKY85+Γ	0.00121083*	23
IL-2	HKY85	0.92550696*	11
IL-3	GTR+I	0.020268243*	4
IL-4	GTR	0.13912934*	8
IL-6	HKY85	9.88×10^{-14}	8
IL-10	HKY85	0.16400763*	9
IL-16	GTR+I	0.08135042*	7
Interferon gamma	HKY85+Γ	0.19943861*	13
IRBP (intron 1)	K80+Γ	0.00010886	37
IRBP (partial cds)	HKY85+Γ	0.01551987*	23
LZM	HKY85+Γ	0.000427075	17
nd1	GTR+Γ+I	0.36658552*	12
ND2	GTR+Γ+I	0.00033689	13
ND3	GTR+Γ+I	0.13023191*	36
ND4L	GTR+Γ+I	0.25098333*	45
ND5	GTR+Γ+I	0.00024931	27
ND6	GTR+Γ+I	0.26791855*	12
NRAMP1	HKY85	0.13333399*	14
PLCB4	GTR	0.85023707*	7
PNOC	GTR+Γ	0.28885825*	7
SRY	HKY85+Γ	0.01145427*	59
TSPY	HKY85+Γ	0.00116896*	41
tRNA-ala	GTR+Γ	0.101676857*	10
tRNA-arg	HKY85+Γ+I	0.1082015*	36
tRNA-asn	HKY85+Γ	0.000246114	10
tRNA-asp	HKY85+Γ	0.054571469*	10
tRNA-cys	GTR+Γ	0.597145544*	10

Table 1. Continued.

Gene	Model	Clock test *p*-value	No. of taxa
tRNA-gln	HKY85+Γ	0.406863018*	9
tRNA-glu	GTR+Γ	0.006563358*	10
tRNA-gly	HKY85+Γ	0.005765017*	30
tRNA-ile	GTR+I	0.003574332*	10
tRNA-lys	HKY85+Γ	0.007495502*	10
tRNA-met	GTR+I	0.552294012*	10
tRNA-phe	HKY85	4.28761×10^{-9}	12
tRNA-pro	GTR+Γ	0.22147066*	11
tRNA-thr	GTR+I	0.010557853*	12
tRNA-trp	HKY85+Γ	7.52579×10^{-6}	10
tRNA-tyr	GTR+Γ	0.012303697*	10
tRNA-val	HKY85+Γ	0.048727903*	26
ZFX	HKY85+Γ+I	0.0130834*	18
ZFY	GTR+Γ	0.00138935*	13
vWF	HKY85+Γ	0.031415638*	17

1998, 2000; Easteal and Herbert, 1997; Porter *et al*., 1997; Yoder, 1997; Goodman *et al*., 1998; Kumar and Hedges, 1998; Stauffer *et al*., 2001; Nei and Glazko, 2002).

3. Results

The majority-rule consensus tree that we dated was based on a search using irreversible character-state changes, and had a resolution of 0.917 (over 15 242 unique optimal trees), and a consistency index of 0.82.

Figure 3 presents the relationship between the median depth of a set of equivalent estimated nodes and coefficient of variation over that set under three calibration scenarios. Figure 3a depicts the variation over the divergence dates if the depths were calibrated on the split between *Homo* and *Pan*, which is a recent split in the context of primate phylogeny. The total variation was highest under this scenario (mean coefficient of variation, CV = 0.622). Variation was lowered when all node depths were calibrated on the root (mean CV = 0.513; Figure 3c). The coefficient of variation was lowest when the split between the Colobinae and Cercopithecinae was used for calibration (mean CV = 0.345; Figure 3b). The results shown in Figure 3 demonstrate that the actual data behaved as we assumed from the results of our simulations: the lowest overall variation was obtained by calibrating on a node of intermediate depth, whereas recent nodes used as calibration points led to the highest variation. Note that the comparison is not exact for several reasons: first, different numbers of genes were common to each calibration;

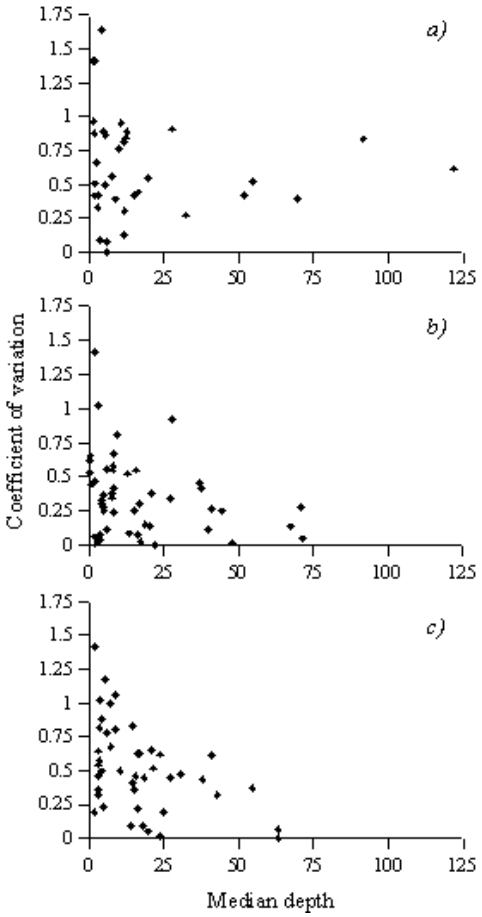

Figure 3. Three calibration scenarios: a) calibration on the split between *Homo sapiens* and *Pan* (the calibration point lies at a depth of 6 MYA); b) calibration on the split between the Cercopithecinae and the Colobinae (22.2 MYA); and c) calibration on the root (63 MYA).

second, the topology of the supertree is not comb-like; and finally, the model used in our simulations was a simplified approximation of the actual process of clade growth (of which a molecular phylogeny is again an approximation).

The depths of the calibration points used in Figure 3 were obtained by taking the median over the estimates we found in a search through the recent literature (Table 2). These previously published dates were obtained through a variety of methods and data sources: from fossils (Goodman *et al*., 1998); from a coalescence model for species diversity (Gingerich and Uhen, 1994); from maximum likelihood estimates using mtDNA calibrated on divergences

Table 2. Recently published estimates of dates for major primate splits. 1 = apes-Old World monkeys; 2 = *Homo*-*Pan*; 3 = (*Homo*, *Pan*)-*Gorilla*; 4 = ((*Homo*, *Pan*),*Gorilla*)-*Pongo*; 5 = great apes-gibbons; 6 = Old World monkeys-New World monkeys; 7 = root; 8 = lemurs-lorisiforms; 9= Colobinae-Cercopithecinae. All ages are in millions of years ago.

	1	2	3	4	5	6	7	8	9
Nei and Glazko (2002)	23	6	7			33			
Stauffer *et al*. (2001)	23	5.4	6.4	11	15				
Gingerich and Uhen (1994)							63		
Yoder (1997)								54	
Arnason *et al*. (1998)	50					60	80		30
Porter *et al*. (1997)	25								
Goodman *et al*. (1998)						38			
Adachi and Hasegawa (1995)		4		16					
Easteal and Herbert (1997)				8.5					
Kumar and Hedges (1998)	23.3	5.5	6.7	8.2	14.6	47.6			
Arnason *et al*. (1996b)		6.1							
Adachi and Hasegawa (1996)		4.3							
Arnason *et al*. (2000)		13	16	30	35	70			
Arnason *et al*. (1996a)		10.4	14.2	19.2	32.4				
Purvis (1995)	27.5	7.0	8.3	14.5	18.2	40.5	57.5	45.1	14.4
Median of published studies	24.1	6	7.6	14.5	18.2	44.0	63	49.5	22.2
Present estimates	32.8	5.9	6.3	15.2	18.8	49.8	77.5	51.6	16.8

outside the order (Arnason *et al*., 1996a; Arnason *et al*., 1998), inside the order (Adachi and Hasegawa, 1995, 1996; Yoder, 1997), or calibrated on geological data (Arnason *et al*., 1996b; Stauffer *et al*., 2001) or using the method of Li *et al*. (1987; Arnason *et al*., 2000); from nuclear sequences calibrated on nodes outside (Easteal and Herbert, 1997; Kumar and Hedges, 1998) or inside the order (Porter *et al*., 1997); from amino acid sequences calibrated inside and outside the order (Nei and Glazko, 2002); and using the mixed fossil and rescaled phylogenies technique outlined earlier (Purvis, 1995). The estimates, all in millions of years ago (MYA), are listed in Table 2. We calibrated our data on the median values over these estimates and averaged over the nine resulting sets of estimates (i.e., one for each calibration point), some of the results of which are listed in the bottom row of Table 2. Figure 4 presents date estimates for the same nine splits we found using our method by calibrating trees first on each of these published estimates in turn and then averaging the results.

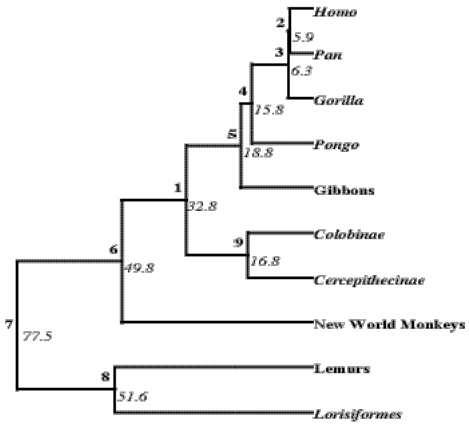

Figure 4. Selected dates of primate divergences using the methods outlined in the text. Numbers above nodes are from Table 2; numbers in front of nodes are divergence dates in MYA.

4. Discussion

The divergence dates estimated using the method described here generally fit well with previously published estimates from different sources (see the examples in Table 2). The correlation between dates estimated here and those for the equivalent nodes in the only other large-scale study (that of Purvis, 1995) is strong (Figure 5). Note that the topology of our supertree is different from that of Purvis in this comparison, and so we compared only those nodes that were unambiguously equivalent (the subtrees descending from these nodes could be different, however). The comparison is therefore not exact, and any differences observed could still be a result of different methods, different topologies, or both.

Compared with the date estimates in Purvis (1995), the estimates presented in this paper were increasingly older with their depth in the tree. We suspect that this is a result of a trend in primate phylogenetics that can be ascribed to both newly discovered, older fossil finds as well as the use of more sophisticated models of sequence evolution in more recent studies.

One potential weakness of our approach is that we have not been able to cover every node in the supertree with the currently available data. On the most resolved topology, 55% of the nodes had date estimates, with all the missing data concentrated around recent nodes in rarely studied clades. Although the amount of sequence data in public databases is growing rapidly, some way of incorporating more non-clocklike loci would seem desirable, perhaps using methods akin to those pioneered by Sanderson (1997, 2002). Even so, missing data points will probably remain in our tree

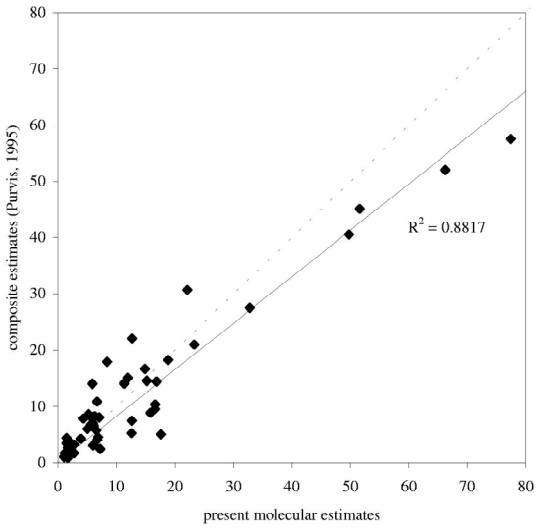

Figure 5. Comparison of previously published composite estimates of divergence dates (from Purvis, 1995) with present estimates. Dotted line indicates 1:1 relationship. See text for further details.

that would have to be interpolated based on models for clade growth such as those used in previous supertree studies (Purvis, 1995; Bininda-Emonds *et al*., 1999).

More comparisons of our approach with that of Bininda-Emonds *et al*. (1999) will be necessary, as will further exploration of the relative power of this hybrid MRP + model-based method and traditional tree-building algorithms that consider the genetic data directly, incorporate multiple genes and multiple models, and, most dauntingly, mixed clock and nonclock scenarios for different data partitions. This, however, is for the future.

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References

ADACHI, J. AND HASEGAWA, M. 1995. Improved dating of the human/chimpanzee separation in the mitochondrial DNA tree: heterogeneity among amino acid sites. *Journal of Molecular Evolution* 40:622–628.

ADACHI, J. AND HASEGAWA, M. 1996. Tempo and mode of synonymous substitutions in mitochondrial DNA of Primates. *Molecular Biology and Evolution* 13:200–208.

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W., AND LIPMAN, D. J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403–410.

ARCHIBALD, J. D. 1999. Molecular dates and the mammalian radiation. *Trends in Ecology and Evolution* 14:278–278.

ARNASON, U., GULLBERG, A., BURGUETE, A. S., AND JANKE, A. 2000. Molecular estimates of primate divergences and new hypotheses for primate dispersal and the origin of modern humans. *Hereditas* 133:217–228.

ARNASON, U., GULLBERG, A., AND JANKE, A. 1998. Molecular timing of primate divergences as estimated by two nonprimate calibration points. *Journal of Molecular Evolution* 47:718–727.

ARNASON, U., GULLBERG, A., JANKE, A., AND XU, X. 1996a. Pattern and timing of evolutionary divergences among hominoids based on analyses of complete mtDNAs. *Journal of Molecular Evolution* 43:650–661.

ARNASON, U., XU, X. F., GULLBERG, A., AND GRAUR, D. 1996b. The "*Phoca* standard": An external molecular reference for calibrating recent evolutionary divergences. *Journal of Molecular Evolution* 43:41–45.

BAUM, B. R. 1992. Combining trees as a way of combining data sets for phylogenetic inference, and the desirability of combining gene trees. *Taxon* 41:3–10.

BININDA-EMONDS, O. R. P., GITTLEMAN, J. L. AND PURVIS, A. 1999. Building large trees by combining phylogenetic information: a complete phylogeny of the extant Carnivora (Mammalia). *Biological Reviews* 74:143–175.

BININDA-EMONDS, O. R. P., GITTLEMAN, J. L., AND STEEL, M. A. 2002. The (super)tree of life: procedures, problems and prospects. *Annual Review of Ecology and Systematics* 33:265–289.

BROMHAM, L. D., RAMBAUT, A., FORTEY, R., COOPER, A. AND PENNY, D. 1998. Testing the Cambrian explosion hypothesis by using a molecular dating technique. *Proceedings of the National Academy of Sciences of the United States of America* 95:12386–12389

BROMHAM, L. D. AND HENDY, M. D. 2000. Can fast early rates reconcile molecular dates with the Cambrian explosions? *Proceedings of the Royal Society of London B* 267:1041–1047

BRYANT, D., SEMPLE, C., AND STEEL, M. 2004. Supertree methods for ancestral divergence dates and other applications. In O. R. P. Bininda-Emonds (ed). *Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life*, pp. 129–150. Kluwer Academic, Dordrecht, the Netherlands.

COLLESS, D. H. 1980. Congruence between morphometric and allozyme data for *Menidia* species: a reappraisal. *Systematic Zoology* 29:288–299.

EASTEAL, S. AND HERBERT, G. 1997. Molecular evidence from the nuclear genome for the time frame of human evolution. *Journal of Molecular Evolution* 44(Suppl. 1):S121–S132.

FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* 17:368–376.

FORTEY, R. A., BRIGGS, D. E. G., AND WILLS, M. A. 1996. The Cambrian evolutionary "explosion": decoupling cladogenesis from morphological disparity. *Biological Journal of the Linnean Society* 57:13–33.

GINGERICH, P. D. AND UHEN, M. D. 1994. Time of origin of primates. *Journal of Human Evolution* 27:443–445.

GITTLEMAN, J. L., JONES, K. E., AND PRICE, S. A. 2004. Supertrees: using complete phylogenies in comparative biology. In O. R. P. Bininda-Emonds (ed.), *Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life*, pp. 439–460. Kluwer Academic, Dordrecht, the Netherlands.

GOODMAN, M., PORTER, C. A., CZELUSNIAK, J., PAGE, S. L., SCHNEIDER, H., SHOSHANI, J., GUNNELL, G. F., AND GROVES, C. 1998. Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence. *Molecular Phylogenetics and Evolution* 9:585–598.

GOULD, S. J. 1989. *Wonderful Life*. Norton, New York.

HARDING, E. F. 1971. The probabilities of rooted tree shapes generated by random bifurcation. *Advanced Applied Probability* 3:44–77.

HASEGAWA, M., KISHINO, H., AND YANO, T.-A. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22:160–174.

JONES, K. E., PURVIS, A., MACLARNON, A., BININDA-EMONDS, O. R. P., AND SIMMONS, N. B. 2002. A phylogenetic supertree of the bats (Mammalia: Chiroptera). *Biological Reviews* 77:223–259.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.

KLUGE, A. AND FARRIS, S. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18:1–32.

KUMAR, S. AND HEDGES, S. B. 1998. A molecular timescale for vertebrate evolution. *Nature* 392:917–920.

LEE, M. S. Y. 1999. Molecular clock calibrations and metazoan divergence dates. *Journal of Molecular Evolution* 49:385–391.

LI W.-H., WOLFE, K. H., SOUDIS, J., AND SHARP, P. 1987. Reconstruction of phylogenetic trees and estimation of divergence times under nonconstant rates of evolution. *Cold Spring Harbor Symposia on Quantitative Biology* 52:847–856.

LIPPS, J. H. AND SIGNOR, P. W. 1992. *Origin and Early Evolution of Metazoa*. Plenum, New York.

LIU, F.-G. R., MIYAMOTO, M. M., FREIRE, N. P., ONG, P. Q., TENNANT, M. R., YOUNG, T. S., AND GUGEL, K. F. 2001. Molecular and morphological supertrees for eutherian (placental) mammals. *Science* 291:1786–1789.

MADDISON, D. R., SWOFFORD, D. L., AND MADDISON, W. P. 1997. NEXUS: an extensible file format for systematic information. *Systematic Biology* 46:590–621.

MOOERS, A.O., HEARD, S. B., AND E. CHROSTOWSKI, E. In press. Evolutionary heritage as a metric for conservation. In A. Purvis, T. L. Brooks, and J. L. Gittleman (eds), *Phylogeny and Conservation*. Oxford University Press, Oxford.

MOORE, B. R., CHAN, K. M. A., AND DONOGHUE, M. J. 2004. Detecting diversification rate variation in supertrees. In O. R. P. Bininda-Emonds (ed.), *Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life*, pp. 487–533. Kluwer Academic, Dordrecht, the Netherlands.

NEE, S. 2001. Inferring speciation rates from phylogenies. *Evolution* 55:661–668.

NEE, S., MOOERS, A. Ø., AND HARVEY, P. H. 1992. Tempo and mode of evolution revealed from molecular phylogenies. *Proceedings of the National Academy of Sciences of the United States of America* 89:8322–8326.

NEI, M. AND GLAZKO, G. V. 2002. Estimation of divergence times for a few mammalian and several primate species. *Journal of Heredity* 93:157–164.

NIXON, K. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15:407–414.

NOVACEK M. J. AND WHEELER, Q. D. 1992. Introduction: extinct taxa. In: Novacek M. J. and Q. D. Wheeler (eds), *Extinction and Phylogeny*: New York: Columbia University Press, 1–16.

PISANI, D., YATES, A. M., LANGER, M. C., AND BENTON, M. J. 2002. A genus-level supertree of the Dinosauria. *Proceedings of the Royal Society of London B* 269:915–921.

PORTER, C. A., PAGE, S. L., CZELUSNIAK, J., SCHNEIDER, H., SCHNEIDER, M. P. C., SAMPAIO, I., AND GOODMAN, M. 1997. Phylogeny and evolution of selected primates as determined by sequences of the ε-globin locus and 5' flanking regions. *International Journal of Primatology* 18:261–295.

POSADA, D. AND CRANDALL, K. A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14:817–818.

PURVIS, A. 1995. A composite estimate of primate phylogeny. *Philosophical Transactions of the Royal Society of London B* 348:405–421.

PURVIS, A., NEE, S. AND HARVEY, P. H. 1995. Macroevolutionary inferences from primate phylogeny. *Proceedings of the Royal Society of London B* 260:329–333.

RAGAN, M. A. 1992. Phylogenetic inference based on matrix representation of trees. *Molecular Phylogenetics and Evolution* 1:53–58.

RIDLEY, M. 1996. *Evolution*, 2nd edition. Blackwell Science, Inc., Cambridge, Massachusetts.

RODRÍGUEZ, F., OLIVER, J. L., MARÍN, A., AND MEDINA, J. R. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretic Biology* 142:485–501.

SALAMIN, N., HODKINSON, T. R., AND SAVOLAINEN, V. 2002. Building supertrees: an empirical assessment using the grass family (Poaceae). *Systematic Biology* 51:112–126.

SANDERSON, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* 14:1218–1231.

SANDERSON, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19:101–109

STAUFFER, R. L., WALKER, A., RYDER, O. A., LYONS-WEILER, M., AND HEDGES, S. B. 2001. Human and ape molecular clocks and constraints on paleontological hypotheses. *Journal of Heredity* 92:469–474.

SWOFFORD, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sinauer, Sunderland, Massachusetts.

THOMPSON, J. D., HIGGINS, D. G., AND GIBSON T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680.

THORLEY, J. L. AND PAGE, R. D. M. 2000. RadCon: phylogenetic tree comparison and consensus. *Bioinformatics* 16:486–487.

WILSON, D. E. AND REEDER, D. M. (eds). 1993. *Mammal Species of the World*. Smithsonian Institution Press, Washington DC.

WOJCIECHOWSKI, M. F., SANDERSON, M. J., STEEL, K. P., AND LISTON, A. 2000. Molecular phylogeny of the "temperate herbaceous tribes" of papilionoid legumes: a supertree approach. In P. Herendeen and A. Bruneau (eds), *Advances in Legume Systematics* 9:277–298. Royal Botanic Garden, Kew.

XUN, G. 1998. Early metazoan divergence was about 830 million years ago. *Journal of Molecular Evolution* 47:369–371.

YANG, Z., GOLDMAN, N., AND FRIDAY, A. 1994. Comparison of models for nucleotide substitution used in maximum likelihood phylogenetic estimation. *Molecular Biology and Evolution* 11:316–324.

YANG, Z. 1996. Among-site variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution* 11:367–371.

YODER, A. D. 1997. Back to the future: a synthesis of strepsirhine systematics. *Evolutionary Anthropology: Issues, News, and Reviews* 6:11–22.

ZUCKERCANDL, E. AND PAULING, L. 1965. Evolutionary divergence and convergence in proteins. In V. Bryson and H. J. Vogel (eds), pp. 97–165 *in Evolving Genes and Proteins*. Academic Press, New York.