Molecular Distance and Divergence Time in Carnivores and Primates[1]

R. K. Wayne, B. Van Valkenburgh,* and S. J. O'Brien†*

*Department of Biology, University of California, Los Angeles; and
†Laboratory of Viral Carcinogenesis, National Cancer Institute

Numerous studies have used indices of genetic distance between species to reconstruct evolutionary relationships and to estimate divergence time. However, the empirical relationship between molecular-based indices of genetic divergence and divergence time based on the fossil record is poorly known. To date, the results of empirical studies conflict and are difficult to compare because they differ widely in their choice of taxa, genetic techniques, or methods for calibrating rates of molecular evolution. We use a single methodology to analyze the relationship of molecular distance and divergence time in 86 taxa (72 carnivores and 14 primates). These taxa have divergence times of 0.01–55 Myr and provide a graded series of phylogenetic divergences such that the shape of the curve relating genetic distance and divergence time is often well defined. The techniques used to obtain genetic distance estimates include one- and two-dimensional protein electrophoresis, DNA hybridization, and microcomplement fixation. Our results suggest that estimates of molecular distance and divergence time are highly correlated. However, rates of molecular evolution are not constant; rather, in general they decline with increasing divergence time in a linear fashion. The rate of decline may differ according to technique and taxa. Moreover, in some cases the variability in evolutionary rates changes with increasing divergence time such that the accuracy of nodes in a phylogenetic tree varies predictably with time.

Introduction

Genetic distance data have been used to date divergence times and to reconstruct phylogenetic relationships. While the latter do not necessarily require the assumption of a molecular clock if genetic distance is appropriately measured (Nei 1987; Saitou and Nei 1987), an implicit assumption of studies that use genetic distance to estimate divergence times is an approximate constancy of molecular evolutionary rate over time. Given certain assumptions, such rate constancy is a specific prediction of the neutral or nearly neutral theory of molecular evolution (Kimura 1969, 1983). Early work did in fact show a linear trend with time in the evolution of various indices of genetic divergence (Zuckerkandl and Pauling 1962, 1965; King and Jukes 1969; Dickerson 1971; Fitch 1976; Sarich 1977; Wilson et al. 1977). Moreover, these indices appeared to show a linear relationship with one another, thus reinforcing the hypothesis of a constant molecular clock. More recently, however, studies comparing genetic measures with each other and with time have demonstrated varying degrees of association (e.g., see Brown et al. 1979; Maxson and Maxson 1979; Brownell 1983; Beverley

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Address for correspondence and reprints: R. K. Wayne, Department of Biology, University of California, Los Angeles, Los Angeles, California 90024.

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and Wilson 1984; O'Brien et al. 1985; Britten 1986; Gingerich 1986; Vawter and Brown 1986; Ochman and Wilson 1987; Sheldon 1987; DeSalle and Templeton 1988; Wayne et al. 1989). As evidenced by these recent reports, a firm consensus has not emerged; some authors advocate constancy of genetic change over long time spans while others maintain that the molecular clock is valid only when one is comparing very closely related species.

In the present study, we examine the empirical relationship between genetic distance and paleontological estimates of divergence time for a large number of taxa examined with several molecular techniques. The taxa include 72 carnivore and 14 primate species or subspecies. The techniques include one-dimensional protein electrophoresis (PE-1D), two-dimensional protein electrophoresis (PE-2D), DNA hybridization, and microcomplement fixation. These techniques are commonly used for phylogenetic reconstruction and for estimating divergence time. Most of the data have been published and were obtained in a single laboratory using the same protocols (table 1). The majority of divergence times are estimated from the relatively rich fossil record of mammalian carnivores. We are able to date phylogenetic branching points that span a wide range of divergence times, i.e., 0.01–55 Myr (Appendix, table A2). Hence, we evaluate changes in molecular evolutionary rate (as inferred from the rate of change of genetic distance with time) at progressively greater levels of evolutionary divergence.

Material and Methods

Genetic Distance Data

Pairwise genetic distance data were obtained from carnivore and primate taxa by using four different genetic techniques (table 1) that indirectly assess sequence variation in nuclear genes by differences in charge and molecular weight of proteins (protein electrophoresis), by hybridization of single-copy DNA, and by complement fixation assay (see Collier and O'Brien 1985; Wayne et al. 1989). The distance measures are (1) unbiased standard genetic distance (Nei 1972, 1987) based on one-dimensional and two-dimensional allozyme electrophoresis, (2) delta T_mR (Kohne 1970; Benveniste and Todaro 1976) based on DNA hybridization, and (3) albumin immunologic distance (Champion et al. 1974; Collier and O'Brien 1985) based on microcomplement fixation. These techniques were applied to nine families of carnivores and to six families

Table 1
Techniques, Number of Nodes, Taxonomic Groups, and Sources for Data Used in Present Study

Technique	No. of Nodes	Taxonomic Groups	Sources[a]
PE-1D	56	Felidae, Canidae, Ursidae Carnivora, Primates	1, 2, 3, 4, 5
PE-2D	13	Ursidae, Primates	1, 3, 6
DNA hybridization	47	Carnivora, Ursidae Primates, Canidae, Felidae	7, 8
Microcomplement fixation	12	Felidae, Primates	9, 10

[a] 1 = O'Brien et al. 1987; 2 = Wayne and O'Brien 1987; 3 = Goldman et al. 1989; 4 = S. J. O'Brien, unpublished data; 5 = Janczewski et al., 1990; 6 = Goldman et al. 1987; 7 = Wayne et al. 1989; 8 = O'Brien et al. 1985; 9 = Collier and O'Brien 1985; and 10 = Sarich and Wilson 1967.

of primates (Appendix, table A1). For dating of phylogenetic nodes and the evaluation of molecular evolutionary rates (see below), pairwise distance estimates derived from each technique were used to generate UPGMA trees (e.g., see fig. 1) with the NTSYS-pc program (Rohlf 1988).

Dating of Phylogenetic Nodes

No clear systematic approach has been advanced for dating the divergence of vertebrate taxa. Statistical approaches to estimate the confidence of fossil dates (Springer and Lilje 1988) can rarely be applied to terrestrial vertebrates because they are less abundant and less continuously distributed in the fossil record. Often, the earliest time of first appearance of taxa on either side of a phylogenetic node is used to date divergence time. However, because speciation is necessarily a branching process, and because ancestors may coexist with descendants, the older taxon is likely to be the ancestral species whose time of first occurrence bears no predictable relationship to the divergence time of the two taxa. For example, the brown bear is the probable ancestor of the polar bear (Kurten 1968); thus the time of first occurrence of the polar bear, rather than that of the brown bear, should be used to date node 1 in figure 1 because, given a perfect fossil record, this date equals the divergence time of the two taxa. Similarly, approaches that utilize an average of first occurrence dates of both descendant and ancestral species necessarily confound estimates of actual divergence time of the two taxa because only the former date directly reflects divergence time. Therefore, the time of first occurrence of the descendant species is a more desirable estimate of divergence time because, if the fossil record is good, it will more closely approach the actual divergence time of two taxa.

Given these considerations, the phylogenetic nodes defined by the UPGMA trees based on the genetic distance data were dated by the following strategy: First, for terminal nodes such as node 1 in figure 1, in which one of the two taxa that define the node is a probable ancestor, the divergence time was assumed to be the more

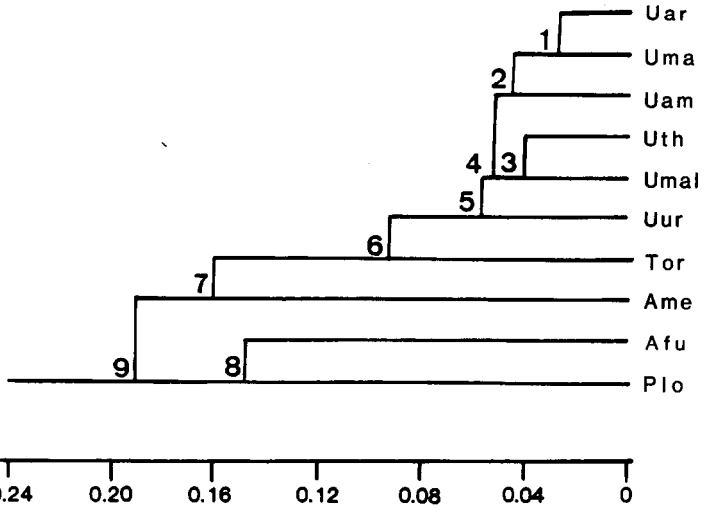

FIG. 1.—UPGMA tree of bear family—Ursidae—and of two taxa from raccoon family—Procyonidae—based on Nei's genetic distance values in table 2. Uar = *Ursus arctos;* Uma = *U. maritimus;* Uam = *U. americanus;* Uth = *U. thibetanus;* Umal = *U. malayanus;* Uur = *U. ursinus;* Tor = *Tremarctos ornatus;* Ame = *Ailuropoda melanoleuca;* Afu = *Ailurus fulgens;* and Plo = *Procyon lotor.*

recent first occurrence of the two taxa. In a group with a good fossil record, this date should most often be that of the descendant species, rather than that of the ancestral species. For example, the first brown bear appeared ~500,000 years ago, while its descendant, the first polar bear, appeared only 85,000 years ago (Appendix, table A1). Thus the divergence time of the two species would be taken as 85,000 years ago. For earlier nodes, such as node 6 in figure 1, some phylogenetic reasoning is required to estimate the time of first appearance of progenitors that gave rise to the modern taxa. For instance, we date this node by assuming that *Plionarctos* is the progenitor of *Tremarctos* (the spectacled bear) and that *Ursus minimus* is the progenitor of the ursine (genus *Ursus*) bears (Kurten and Anderson 1980; Hunt, accepted). The time of first appearance of *Plionarctos* is ~6 Mya and that of *U. minimus* is ~5 Mya. If we had no additional data on the relationship of the two taxa, we would use the later date, 5 Mya, as the divergence time. However, in this instance, we use the earlier date (6 Mya) because phylogenetic analysis suggests that *Plionarctos* is too derived to have given rise to any ursine bear (Hunt, accepted).

Molecular rate calculations based on this approach will be biased if the relationship of minimum divergence time to the true divergence time changes as a function of time or taxonomic group. The use of the same taxonomic groups for each of the techniques evaluated in the present paper minimizes taxonomic biases. Nevertheless, the minimum dating of recent nodes may be a more accurate reflection of the true divergence time than is the minimum dating of earlier nodes, because of compounded mistakes in phylogenetic reasoning and because of the increasing incompleteness of the fossil record as one goes backward in time (Raup and Stanley 1978). However, this trend may be somewhat offset by the increasing number of taxa used to date more distant phylogenetic nodes.

Correlation of Molecular Distance and Divergence Time

Because distance measures and divergence time are intercorrelated, we use a permutation test to assess their association (Dietz 1983). Permutations of distance matrices and permutations of divergence matrices were performed separately for each family because missing cells that result if data from all carnivores were pooled were not permitted. Pearson product moment and two nonparametric measures—Spearman rank order and Kendall tau statistics—were calculated as measures of association (Sokal and Rohlf 1969). These analyses were done with a program provided by E. J. Dietz (Department of Statistics, North Carolina State University, Raleigh).

Evaluation of Rate Constancy

To assess the absolute rate of molecular evolution, most studies compare measures of pairwise divergence between two taxa with an estimate of their divergence time based on data from the fossil record (e.g., see Sarich and Wilson 1967; Brown et al. 1979; Britten 1986). Because the taxa being compared are united by a common phylogenetic framework, this approach presents a problem of redundancy and intercorrelation. For example, in figure 1 many of the pairwise divergences among taxa in the UPGMA tree stem from the same phylogenetic node (e.g., *U. arctos* vs. *T. ornatus, U. maritimus* vs. *T. ornatus* fig. 1 node 6) and thus are not independent estimates of the relationship between genetic distance and divergence time. Moreover, in any phylogenetic tree the number of taxa that branch from nodes representing progressively greater genetic distance always increases; thus estimates across more distant nodes will necessarily be overrepresented relative to more proximal ones (table 2 and fig. 1). As

Table 2
Time and Distance between Pairs of Carnivores

	Species									
Species	*Ursus arctos*	*U. maritimus*	*U. americanus*	*U. ursinus*	*U. thibetanus*	*U. malayanus*	*Tremarctos ornatus*	*Ailuropoda melanoleuca*	*Ailurus fulgens*	*Procyon lotor*
U. arctos		0.028	0.046	0.050	0.048	0.041	0.086	0.158	0.200	0.181
U. maritimus	0.09 (1)		0.038	0.061	0.056	0.050	0.092	0.162	0.211	0.197
U. americanus	2.00 (2)	2.00 (2)		0.052	0.044	0.058	0.093	0.154	0.208	0.193
U. ursinus	? (5)	? (5)	? (5)		0.052	0.061	0.092	0.150	0.196	0.184
U. thibetanus	3.00 (4)	3.00 (4)	3.00 (4)	? (5)		0.039	0.091	0.154	0.190	0.177
U. malayanus	3.00 (4)	3.00 (4)	3.00 (4)	? (5)	? (3)		0.089	0.166	0.199	0.186
T. ornatus	6.00 (6)	6.00 (6)	6.00 (6)	6.00 (6)	6.00 (6)	6.00 (6)		0.150	0.184	0.191
A. melanoleuca	12.00 (7)	12.00 (7)	12.00 (7)	12.00 (7)	12.00 (7)	12.00 (7)	12.00 (7)		0.176	0.164
A. fulgens	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)		0.147
P. lotor	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	25.00 (9)	17.00 (8)	

NOTE.—Data are Nei's genetic distance values (above diagonal) based on PE-2D of 289 proteins (Goldman et al. 1989), fossil divergence times in millions of years (below diagonal), and (in parentheses) the node in fig. 1 to which each divergence time refers.

a result, in plots of genetic divergence against time, regression procedures will overemphasize more ancient nodes and attach less weight to more recent ones. Furthermore, if a distant node is incorrectly dated (which is more likely because of the incompleteness of the fossil record), then the regression line may be severely biased because of the great number of pairwise divergences stemming from that node. Therefore, we used (1) a single datum for each node of a tree, (2) an average genetic distance among taxa at each node, and (3) a minimum divergence time.

As recently discussed by Gingerich (1986), rate constancy can be effectively evaluated by use of the power function $d = at^b$, where in the present study, d is a measure of average genetic distance, t is an estimate of minimum divergence time at each node, and a and b are coefficients. This expression is equivalent to $\log(d) = \log(a)+b[\log(t)]$, and thus $\log(a)$ and b may be regarded as the intercept and slope, respectively, of the line defined by this relationship. A slope of 1 indicates that the rate of molecular evolution is constant with time, or *isochronic,* whereas slopes <1 or >1 indicate that molecular evolutionary rates change with time, or are *allochronic* (sensu Gingerich 1986).

Our approach was to evaluate graphically the relationship of genetic distance and divergence time and then fit the relation $\log(d) = \log(a)+b[\log(t)]$ by least-squares regression to the part of the curve which by inspection appears most linear. Other regression techniques, such as reduced major axis, can also be used, but the results are similar so long as the correlation among variables is high (Seim and Saether 1983; Smith 1984). We chose least-squares regression because correlation coefficients were high and because statistical analyses of slope and intercept differences are well defined (Zar 1983). However, it is important to note that the node-specific average genetic distances used in the present study are not independent, because many of the same taxa are used in computation of these averages.

Calculation of Standard Error of Phylogenetic Nodes

The variance in genetic distance estimates may also change as a function of divergence time (Nei 1987). The independence of molecular rate variation and divergence time was assessed by computing the standard error of pairwise genetic divergences of taxa on either side of a phylogenetic node. For example, in figure 1 the standard error of pairwise divergence values between the giant panda (Ame) and all other ursids would be the value of the standard error at the bear-panda node (node 7). The relationship between standard error and divergence time was estimated by least-squares regression as described above.

Results

Correlation of Molecular Distance and Divergence Time

Genetic distance and divergence time are highly associated, as indicated by several correlation statistics (table 3). Correlation values appear largest for PE-1D, PE-2D, and DNA hybridization and lowest for microcomplement fixation (AID). No group-specific differences in correlation are apparent, as the carnivore and primate families used in this analysis generally all have high values of correlation. However, the carnivore family correlation for PE-1D is relatively low. The permutation analyses indicate that the association between genetic distance and divergence time is significantly different from random, for each genetic technique and taxonomic group. Thus, our results strongly suggest that genetic distance data correlate well with divergence-time estimates from the fossil record.

Table 3
Correlation and Significance Statistics for Genetic Distance versus Divergence Time, Based on UPGMA Trees of Carnivores and Primates

Technique and Group	Tree[a]	No. of Species	Correlation[b]			Significance[b]		
			S	P	K	S	P	K
PE-1D:								
Canidae	1	12	0.84	0.98	0.61	0.000	0.000	0.000
Felidae	2	7	0.89	0.93	0.62	0.002	0.001	0.003
Ursidae	3	8	0.85	0.82	0.63	0.001	0.001	0.001
Carnivora	4	11	0.67	0.70	0.43	0.000	0.000	0.000
Primates	5	7	0.92	0.93	0.73	0.002	0.002	0.002
PE-2D:								
Ursidae	6	7	0.95	0.95	0.83	0.000	0.000	0.000
Primates	7	7	0.91	0.94	0.70	0.009	0.003	0.009
DNA hybridization:								
Carnivora	8	21	0.88	0.83	0.57	0.000	0.000	0.000
Primates	9	7	0.98	0.97	0.82	0.001	0.001	0.001
Microcomplement fixation:								
Felidae	10	9	0.69	0.95	0.47	0.026	0.019	0.046

[a] 1 = Wayne and O'Brien 1987, fig. 1a; 2 = O'Brien et al. 1987, fig. 4; 3 = Goldman et al. 1988; 4 = S. J. O'Brien, unpublished data for 11 species from seven carnivore families; 5 = Janczewski et al., 1990, fig. 4a; 6 = Goldman et al. 1989; 7 = Janczewski et al., 1990, fig. 2a; 8 = Wayne et al. 1989, fig. 17.5; 9 = O'Brien et al. 1985; and 10 = Collier and O'Brien 1985, fig. 1.

[b] S = Spearman rank order; P = Pearson product moment; and K = Kendall tau.

Plots of Genetic Distance Measures against Divergence Time

1. *PE-1D*

For nonursid carnivores, a curvilinear, rather than linear, relationship is suggested by the arithmetic plot of Nei's genetic distance against divergence time (fig. 2A, dots). Thus, genetic distance is not a constant proportion of time among nonursid carnivores and appears to decrease with increasing values of divergence time. In other words, the amount of genetic divergence between taxa of more ancient divergence times is proportionately less than that among those of more recent divergence times.

Protein evolution in ursids and primates seems to decrease at a rate similar to that of nonursid carnivores, but these rates may not be linear over the same time span in both groups (fig. 2B, circles and triangles respectively). The log/log plot suggests that the rate of decrease is linear among nonursid carnivores only for the interval between 3 and 55 Mya (fig. 2B, dots). Moreover, the ursid regression line, while similar in slope, is clearly below that of the nonursid carnivores (fig. 2B). This suggests that the rate of decrease of genetic distance per unit interval of time is similar in both groups but that the extent of genetic divergence is less among ursids than among other carnivores of equivalent divergence time. The giant panda appears as a distinct outlier from the ursid regression line, with a divergence time of only 12 Mya and genetic distance of ~0.3 (fig. 2A).

2. *PE-2D*

Data on PE-2D were available for ursids and primates. As is true for the PE-1D data, genetic distance does not appear to be a constant fraction of time; rather, it decreases as divergence time increases [fig. 2C, circles (ursids) and triangles (pri-

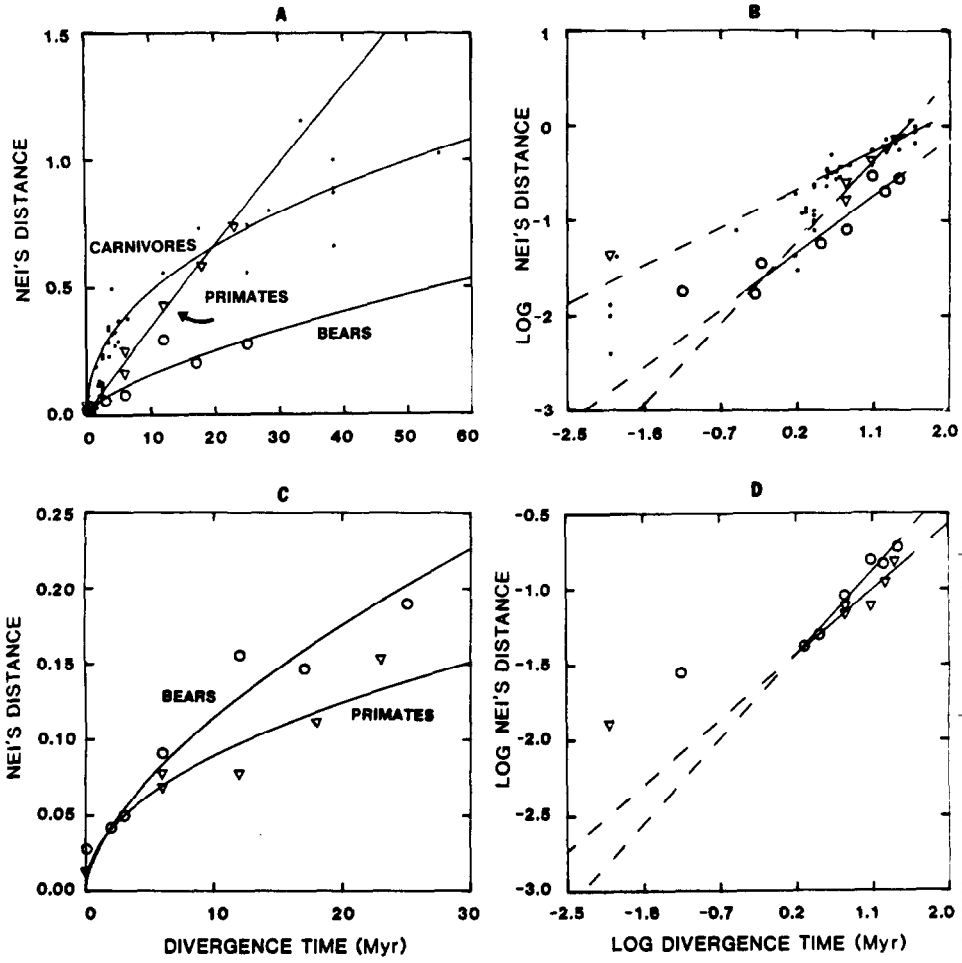

FIG. 2.—Arithmetic (*left*) and log/log (*right*) plots of genetic distance estimates against divergence time in millions of years. Techniques used are (A and B) PE-1D, (C and D) PE-2D, (E and F) DNA hybridization, and (G and H) microcomplement fixation (A.I.D.). Lines in arithmetic and log plots are derived from coefficients in table 4. Separate regressions were performed for primate and carnivore data—except in A and B, where the ursid data are analyzed separately from those for other carnivores. In A–D, dots (⊙) denote nonursid carnivores, circles (○) denote ursids, and triangles (▽) denote primates; in E–H, dots (⊙) denote carnivores, and triangles (▽) denote primates.

mates)]. The log/log plot suggests that the rate of decline is approximately linear over the time period of 2–~25 Mya (fig. 2D). The rate of decrease is similar in both groups. In both ursids and primates, nodes representing more recent branching events (brown bear vs. polar bear, 0.085 Mya; and Sumatran vs. Bornean orangutan, 0.01 Mya) appear as outliers, falling above the regression line (fig. 2D). Their inclusion in the regression line would decrease the apparent rate of molecular evolution, suggesting that rates of evolution have declined in more recently evolved taxa. Alternatively, the molecular data or fossil dates may be inaccurate.

3. *DNA Hybridization*

The arithmetic plot of the DNA hybridization data suggests that the change in delta T_mR per interval of time decreases in carnivores but increases with time in

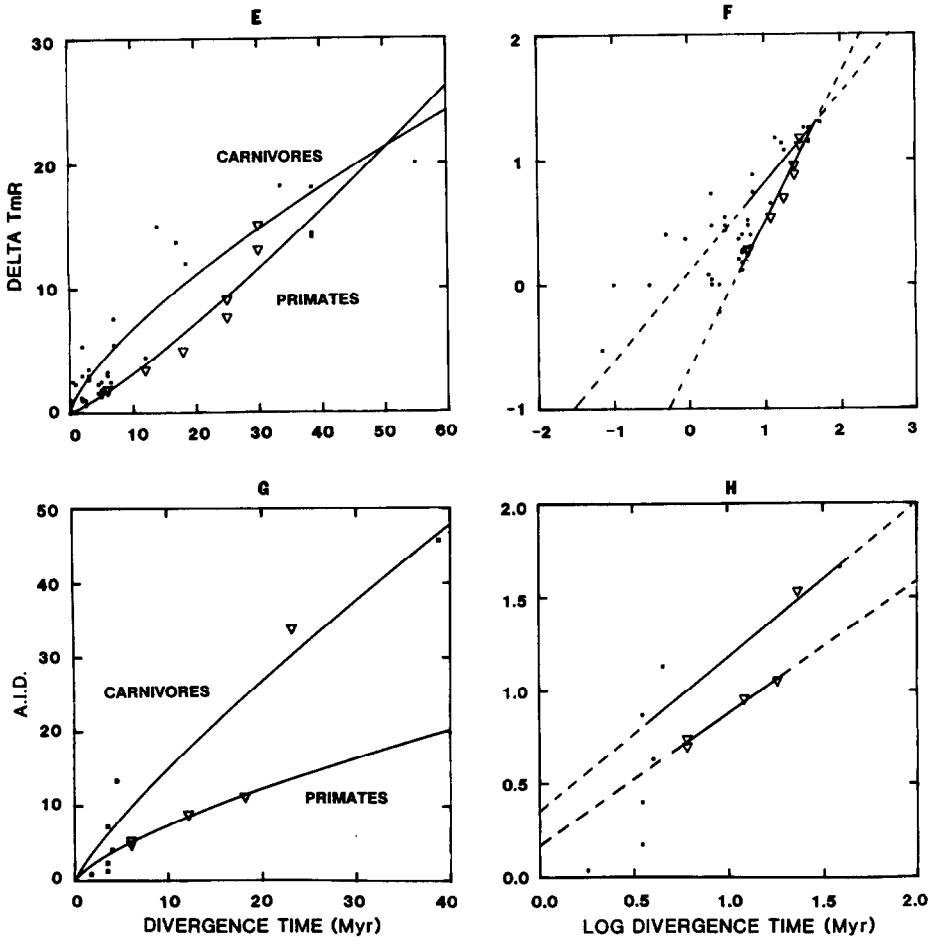

FIG. 2. (*Continued*)

primates (fig. 2E, dots and triangles, respectively). Because the closely related primates used in the present study are humans and apes, rates of evolution appear slower among the higher primates. The carnivore curves are characterized by much more scatter, especially among carnivores of <6 Mya of divergence time. As suggested elsewhere (Wayne et al. 1989), DNA hybridization poorly reflects phylogenetic relatedness among closely related carnivores. If species with divergence times <6 Mya are excluded, there is an apparent difference between primate and carnivore slope values that indicates that, for the time range 6–31 Mya, the rate of molecular evolution, as determined by DNA hybridization, may be lower in carnivores than in primates.

4. *Microcomplement Fixation*

The microcomplement fixation data were available only for recently diverged species of felids and primates. Thus the relationship of AID to time is not as well defined as with other techniques. The AID data for felids appear to correspond poorly with divergence time (figs. 2G and H, dots). In fact, the regression analysis for nodes that have a divergence time <4 Mya is not significant ($P > 0.05$). This may reflect the poor fossil record of small fields, rather than variability in the metric. In the

primates the relationship between AID and time seems linear for divergence times <20 Mya (figs. 2G and H, triangles). The primate rate declines initially for recent divergence times and then increases (if one assumes that the dating of the final node is correct). This result suggests a slower rate of molecular evolution among the higher primates.

Time Span of Linearity and Rates of Evolution

By visual inspection of the log/log plots we defined a time span over which the rate of change in genetic distance appeared to be a linear function of divergence time (table 4). Only points from the linear part of the curve were used in the calculation of regression statistics (table 4). In general, linearity is most apparent among nodes of intermediate and ancient divergence times (e.g., 3–30 Mya; fig. 2). Many of the log/log plots suggest that very recent nodes (<3 Mya) may either define a different regression line than do nodes of intermediate divergence times or not be significantly associated. In fact, for PE-1D data and for felid microcomplement fixation data, regressions of all nodes <5 Mya are not significant ($P > 0.05$).

If the constraints of the linear model are assumed, nearly all the slopes of the regression line of log genetic distance against log divergence time are significantly less than 1, except for the primate DNA hybridization analyses (table 4, col. b). This indicates that rates of evolution are actually decreasing with increasing divergence times. For carnivores the rate of decrease is greatest for PE-1D ($b = 0.45$), whereas for primates the rate of decrease is greatest for PE-2D ($b = 0.48$) (table 4).

Differences in both slope and intercept are seen among taxonomic groups. For PE-1D the regression slopes of ursids and nonursid carnivores are not significantly different (table 4 and fig. 2B). However, the intercept value is considerably less for ursids, suggesting that the rate of molecular evolution is decreasing at the same rate

Table 4
Regression Coefficients of Log Genetic Distance on Log Divergence Time, by Technique and Group

Technique and Group	No. of Nodes	Time Span[a] (Myr)	Regression Coefficient[b]			
			a	b (SE)	r	P
PE-1D:						
Carnivora	22	3.0–55.0	−0.76	0.45* (0.04)	0.93	0.000
Ursidae	7	0.5–25.0	−1.47	0.67* (0.09)	0.96	0.001
Primates	5	6.0–23.0	−1.41	0.95 (0.14)	0.97	0.007
PE-2D:						
Ursidae	6	2.0–25.0	−1.56	0.62* (0.06)	0.98	0.000
Primates	5	6.0–23.0	−1.53	0.48* (0.14)	0.90	0.039
DNA hybridization:						
Carnivora	13	6.0–55.0	0.17	0.68* (0.13)	0.85	0.000
Primates	8	6.0–31.0	−0.98	1.19 (0.09)	0.98	0.000
Microcomplement fixation:						
Felidae	3	4.0–38.5	0.35	0.83 (0.41)	0.90	0.291
Primates	4	6.0–18.0	0.17	0.71* (0.06)	0.99	0.007

[a] Time span of linearity is approximate and based on visual examination of plots in fig. 2.

[b] a = Intercept; b = slope; SE = standard error of slope; r = Pearson product-moment correlation; and P = significance of regression.

* Significantly different from a slope of 1 ($P < 0.05$; Zar 1984).

in both groups but that overall differences among taxa at various degrees of phylogenetic divergence are lower among ursids than among nonursids. Ursids also differ from primates, but in this case intercepts are similar and slopes differ (table 4). The steeper slope for primates suggests that genetic distance does not decrease as rapidly with increasing divergence times among primates as among carnivores. For the DNA hybridization data, primates appear to have steeper slopes as well. The primate slope value is >1, indicating that evolutionary rates may increase with increasing divergence time.

Standard Error of Phylogenetic Nodes

The standard error of pairwise genetic distances among taxa at each phylogenetic node often shows a linear trend with divergence time (fig. 3 and table 5). For data from PE-1D and PE-2D and microcomplement fixation, the slopes are <1 for regressions of the standard error against time (table 5). Thus, the standard error about phylogenetic nodes is decreasing with increasing divergence times for these metrics. The rate of decrease as indicated by the slope values is similar to that of genetic distance measures against divergence time (tables 4 and 5). Therefore, the standard error is a similar fraction of genetic distance irrespective of divergence time. According to theoretical studies (Li and Nei 1975), the proportion of standard error to genetic distance should decline as genetic distance increases, although the magnitude of the variance increases.

In contrast, the standard error of genetic distance for DNA hybridization data appears to increase sharply with average delta T_mR and divergence time (slopes >1; table 5). However, the standard error for the slope value is large. If the slope values are taken as representative of the real trend, then they suggest that distant nodes will have proportionately more error than do more proximal nodes, because the change in DNA hybridization distance per unit time in carnivores decreases with increasing divergence time (table 4) as the standard deviation increases.

Some indication of the relative magnitudes of the standard error as a proportion

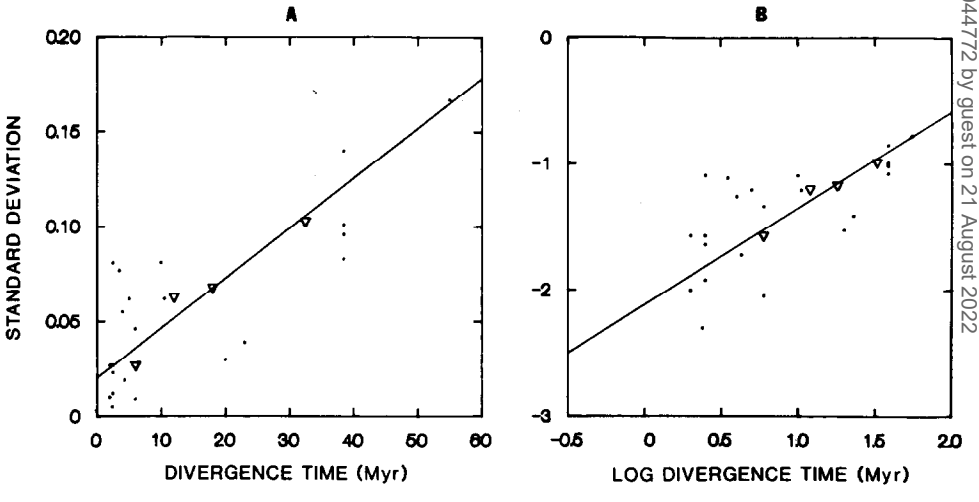

FIG. 3.—Arithmetic (A) and log/log (B) plots of SD of phylogenetic nodes vs. divergence time in millions of years, for PE-1D data. Dots (⊙) denote carnivores, and triangles (▽) denote primates. Lines in arithmetic and log plots are derived from coefficients in table 5.

Table 5
Regression Coefficients of Log SD on Log Divergence Time, by Technique and Group

Technique	Group	No. of Nodes	Regression Coefficient[a]			
			a	*b* (SE)	*r*	*P*
PE-1D	All taxa	26	−1.88	0.55* (0.12)	0.68	0.000
PE-2D	Ursidae, Primates	10	−2.50	0.24 (0.24)	0.34	NS
DNA hybridization	Carnivora	11	−3.76	2.12 (0.62)	0.75	0.008
Microcomplement fixation	Felidae	8	−0.10	0.42* (0.21)	0.64	0.089

[a] As defined in table 4. NS = not significant.
* Significantly different from a slope of 1 ($P < 0.05$; Zar 1984).

of the mean genetic distance can be gained by comparing predicted standard errors with predicted genetic distances at a given divergence time (table 6). Apparently, DNA hybridization has the smallest proportional error (0.005–0.029; table 6). However, as mentioned above, the standard error increases with greater divergence times for this metric, and thus ancient nodes are more uncertain. By contrast, the standard error as a proportion of the mean genetic distance for PE-1D data is considerably larger (0.10–0.12) but increases much less with greater divergence time. Thus, with protein-electrophoresis data we have equal confidence for phylogenetic nodes having large and small divergence times.

For PE-2D the ratio of the standard error to the mean may be estimated as the mean standard error over the mean genetic distance, since the regression of these two variables is not significant. The average value for PE-2D, 0.028, is considerably less than the value for PE-1D. This lower value may reflect the larger number of loci sampled in PE-2D (289) as opposed to PE-1D (44). In sum, the PE-1D data exhibit the most overall variability about phylogenetic nodes, followed by microcomplement fixation, PE-2D, and, last, with the smallest standard error, DNA hybridization data.

Discussion

Our results demonstrate that molecular distance data are highly correlated with estimates of divergence time. Moreover, although genetic measures of distance increase with divergence time, the rate of increase is rarely constant. Most frequently, less genetic change appears to have occurred per unit time with increasing values of divergence time. This result supports the prediction that the amount of genetic distance per unit time, as measured by Nei's genetic distance, should appear to decrease with time as parallel mutations at the same locus appear in long-separated taxa (Nei 1987). Moreover, especially for protein loci, the frequency of back and parallel mutations may be higher because of purifying selection (Peetz et al. 1986). Our results also indicate that the tempo of genetic change is smoothly decreasing over only a limited time span. Departures from the linear trend on log/log plots are most apparent among phylogenetic nodes of recent divergence time.

Rates of change in genetic measures of divergence vary according to technique and taxonomic group. In the present study, rates of change in genetic measures are highest with DNA hybridization data. In primates the DNA hybridization data suggest that the rate of genetic evolution increases slightly with divergence time, whereas in carnivores the opposite is found (figs. 2E and F). Gingerich (1986) also found that,

Table 6
Predicted SD as Fraction of Predicted Mean Genetic Divergence at Several Divergence Times

Technique	Predicted SD as Fraction of Predicted Mean Genetic Divergence				
	Divergence time (Mya)				
	10	20	30	40	Average
PE-1D	0.100	0.110	0.110	0.120	0.110
PE-2D	. . .	. . .	. . .	. . .	0.028[a]
DNA hybridization	0.005	0.012	0.020	0.029	0.017
Microcomplement fixation	0.120	0.098	0.087	0.080	0.096

NOTE.—Predicted values are based on regression coefficients given in table 4 for Carnivora (PE-1D and DNA hybridization) and Felidae (microcomplement fixation) and given in table 5 for all taxa (PE-1D), Carnivora (DNA hybridization), and Felidae (microcomplement fixation).

[a] Reported because regression in table 5 is not significant.

for primates, DNA hybridization data indicated a molecular evolutionary rate increasing with divergence time. His slope values of 1.08 and 1.14 from two studies of primates are similar to our value of 1.19. He also found that the rate of change in immunological distance increased with time, having a slope coefficient of 1.43 for primates, as opposed to our values of 0.83 for felids and 0.71 for primates. This apparent discrepancy may reflect the difference in time scale between our study and his; our primate divergence dates fall within the past 20 Myr (fig. 2H), whereas the four dates in his study span 20–65 Mya. In fact, the most ancient data point in figure 2H (which was not included in the regression calculations) does suggest that the rate of molecular change increases with greater divergence times. However, our results from PE-1D and PE-2D based on many divergence dates over a time span of 1–55 Mya do not support his general claim (based on many fewer divergence dates) that for Cenozoic mammals, molecular evolutionary rates increase with divergence time.

An important implication of our results is that *rates of molecular evolution cannot be accurately calibrated with a single or a few widely spaced divergence times.* Because rates of molecular change generally decrease with increasing divergence time, the use of a younger, better-established divergence time to date more difficult ancient divergences will provide an underestimation of that date. For example, suppose we assume, incorrectly, that rate constancy applies when PE-1D data are used. If the brown bear–Asiatic black bear divergence (Nei's distance 0.017, divergence time 0.5 Mya) is used to predict the ursid-procyonid divergence date, a divergence time of ~8.2 Mya is obtained, on the basis of their genetic distance of 0.280. This conflicts with the 25-Mya date from the fossil record. If instead we use genetic distance as the independent variable in a regression of log genetic distance and log divergence time for the ursid data (slope 1.37, intercept 2.05), we obtain, when given the same genetic distance of 0.280, a date of 19.6 Mya, which is a much closer fit. By using empirically based regression equations based on several phylogenetic nodes, we reduce the influence of a single node that, because of a poor fossil record, may be inaccurately estimated.

Caution should also be used in assuming that similar rates hold for different taxonomic groups. In PE-1D the bear regression deviates from the carnivore regression

in intercept but not in slope. Thus, in both groups, different equations should be used to estimate divergence time on the basis of genetic distance data. Similarly, primate and carnivore regression equations often differ in slope or intercept (e.g., see figs. 2B and F). Also, because genetic measures differ in their relationship to divergence time (fig. 2 and table 4), differences in their rates of evolution may vary according to the time span over which they are compared. For example, recent comparisons of nuclear and mtDNA evolution in vertebrates, sea urchins, and bacteria analyze taxa that differ widely in divergence time; consequently, analogous parts of the molecular evolutionary curve might not have been compared (Brown et al. 1979; Britten 1986; Gingerich 1986; Vawter and Brown 1986; Ochman and Wilson 1987). Indeed, the timing of peak evolutionary rates in mtDNA or nuclear genes might differ among these groups more than does the overall rate.

The present study suggests that a good evolutionary metric should have two important characteristics. First, it should change in a linear fashion with divergence time, such that the rate of change is constant (slope = 1; table 4). If this condition holds, then a unit change in genetic distance between phylogenetic nodes will correspond to the same unit change in time throughout the entire phylogenetic tree. A second important characteristic is that the standard error of pairwise distance values about a phylogenetic node should be small and should be a constant fraction of the mean genetic divergence at a given node. If this is true, then the error about phylogenetic nodes is proportionately the same over the entire phylogenetic tree.

The metrics used in the present study deviate from these criteria, to various degrees. For PE-1D, regression slopes are significantly <1, indicating that rates of genetic change are decreasing with increasing divergence time. Therefore, ancient nodes in the phylogenetic tree are more compressed (i.e., have more similar genetic distance values than expected) and appear closer in time than they really are. However, the standard deviation about these nodes decreases at a similar rate with respect to divergence time. Consequently, although more distant nodes are closer together, the deviation about them is proportionately reduced. Nevertheless, the phylogenetic nodes defined by PE-1D have the greatest overall variability of any of the techniques (table 6).

For PE-2D data, the genetic distance per unit increment of time also decreases with increasing divergence time, but the standard error at each phylogenetic node does not show a significant relationship with divergence time. As a result, we cannot predict the degree of certainty of phylogenetic nodes according to divergence time. However, the overall level of variability is less than that of PE-1D, suggesting that the standard error is reduced as more loci are sampled.

For the time spans examined in the present study, DNA hybridization has several desirable characteristics. Because the rate of decrease for primates is near 1, evolutionary rates do not change greatly with time, and thus the genetic distance between phylogenetic nodes is nearly independent of divergence time. Moreover, the standard deviation of phylogenetic nodes is lowest overall (table 6) but does increase with divergence time. Thus, the uncertainty of phylogenetic nodes is greater for more ancient divergences. However, very recently evolved carnivore taxa are not well discriminated by this technique, suggesting that variability among very recent phylogenetic nodes is also high (Wayne et al. 1989). This technique is best used for taxa with divergence times >6 Mya.

The last technique is difficult to evaluate, because of insufficient data. The rate of change in AID decreases with divergence time, and thus more distantly related

nodes are compressed with respect to elapsed time. The inaccuracy due to compression is somewhat offset by a higher rate of decrease in the standard error about phylogenetic nodes. Past studies of the variance of AID provide variance/mean distance estimates of 0.3–9.1 (Nei 1977; Beverley and Wilson 1984). If we assume that the mean distance is approximately half the average pairwise divergence value, then, our variance/mean distance ratio, when averaged over the four time points in table 6, is 0.57.

In conclusion, the above discussion suggests that phylogenetic trees need to be corrected for secular trends in the rate of molecular evolution. When the regression of log divergence time against log genetic distance is used for as many taxa as possible, phylogenetic trees relating allied taxa can be adjusted such that the differences among nodes reflect the same difference in divergence time, throughout the tree. Moreover, the confidence of phylogenetic nodes as a function of divergence time can be assessed by utilizing the empirically based trends in variability of phylogenetic nodes over time. Finally, for estimating divergence time our approach provides an empirically based method that is more specific to a particular taxonomic group and genetic distance technique than are more general theoretical models.

APPENDIX

Table A1

Estimated First Dates of Occurrence of Carnivore and Primate Taxa—or of Lineages That Led to Them—Used to Date Nodes of UPGMA Trees Used for Present Paper.

Taxon	Estimated First Occurrence (presumed earliest taxon) in Fossil Record (Mya)	Source(s)
Carnivora:		
Canidae:	32–35 (*Hesperocyon*)	Savage and Russell 1983
Alopex lagopus	0.1–0.3	Kurten 1968
Alopex sp.	1–3 (*Vulpes alopecoides*)	Kurten 1968
Canis sp.	6–7 (*C. davisii*)	Savage and Russell 1983
C. adustus	2–3 (*C. terblanchi*)	Ewer 1956; Hendey 1974
C. aureus	2–3 (*C. lupaster*)	Kurten 1965
C. familiaris	0.012	Clutton-Brock 1987
C. latrans	3–4 (*C. lepophagus*)	Kurten 1974
C. lupus	1–2	Kurten 1968
C. mesomelas	2–3	Hendey 1974; Turner 1985
Cerdocyon sp.	4–5	Berta 1987, 1988
Chrysocyon sp.	3–4	Berta 1987
Dusicyon sp.	2–3 (*Pseudalopex*)	Berta 1987, 1988
Lycaon sp.	2–3	Turner 1985
Nyctereutes sp.	4 (*N. donnezani*)	Kurten 1968
Speothos sp.	2–3 (*Protocyon* sp.)	Berta 1987
Vulpes sp.	9–12 (*Leptocyon*)	Savage and Russell 1983
V. chama	1–2	Savage 1978
V. macrotis	0.5–1	Kurten and Anderson 1980
V. vulpes	0.5–1	Kurten 1968
Urocyon sp.	4–6	Kurten and Anderson 1980
Felidae:	37–40 (*Proailurus*)	Hunt 1989
Acinonyx sp.	3–5	Ficcarelli 1984; Turner 1987
Caracal sp.	3–5	Savage and Russell 1983; Turner 1987
Felis catus	0.004–0.01	Clutton-Brock 1987
F. chaus	0.1	Kurten 1968
F. libyca	0.3[a]	Kurten 1968
F. sylvestris	1–3 (*F. lunensis*)	Kurten 1968
Leopardus sp.	1.5–2.5	Berta 1983
Leptailurus serval	3–5	Turner 1985

Table A1 (*Continued*)

Taxon	Estimated First Occurrence (presumed earliest taxon) in Fossil Record (Mya)	Source(s)
Lynx sp.	3–4	Werdelin 1985
Panthera leo	1–2	Neff 1982
P. onca	1.6	Kurten and Anderson 1980
P. pardus	3	Turner 1987
P. tigris	1.8	Neff 1982
Puma concolor	3–3.5 (*Miracinonyx* sp.)	Kurten 1976; Van Valkenburgh et al. 1990
Earliest ancestor of South American small cats	4–5 (*F. lacustris* or *F. rexroadenosis*)	Werdelin 1985
Ursidae:	37–40 (*Cephalogale*)	Hunt, accepted
Ailuropoda sp.	12 (*Agriarctos*)	Thenius 1979; R. H. Tedford, personal communication
Tremarctos sp.	5–7 (*Plionarctos*)	Kurten and Anderson 1980; Hunt, accepted
Ursus sp.	4–6 (*U. minimus*)	Kurten 1968
U. americanus	2.5–3.5	Kurten and Anderson 1980
U. arctos	0.5	Kurten 1968
U. maritimus	0.07–0.1	Kurten 1968
U. malayanus	0.2–1 (? or 5–10)	Savage and Russell 1983
U. thibetanus	1	Kurten 1968
U. arctos–U. americanus split	1–3 (*U. etruscus*)	Kurten and Anderson 1980
Ailuropodinae-Tremarctinae split	12	
Hyaenidae:	15–20 (*Herpestides*)	Hunt 1989
Crocuta sp.	4–5	Barry 1987
Crocuta crocuta	3–4	Turner 1985
Hyaena sp.	4–6	Turner 1987
Proteles cristatta	1–2	Savage and Russell 1983
Crocuta lineage	9–11 (*Adcrocuta*)	Werdelin and Solounias 1991
Mustelidae:	37–40 (*Mustelictis*)	Baskin, accepted-*b*
Subfamily (Mephitinae)	12–16	Baskin, accepted-*b*
Lutra sp.	6–8	Kurten 1968
Mephitis sp.	4–5 (*Promephitis*)	J. Baskin, personal communication
Mustela frenata	0.7–1	Kurten and Anderson 1980
M. putorius	1–3	Kurten 1968
M. vison	0.7–1	Kurten and Anderson 1980
Spilogale sp.	3	Kurten and Anderson 1980
Viverridae	37–40 (*Paleoprinodon*)	Hunt 1989
Herpestidae	18–19 (*Leptoplesictis*)	Schmidt-Kittler 1987; Hunt 1989
Procyonidae	25 (*Amphictis*)	Baskin, accepted-*a*
Phocidae	6–8 [?(20, *Potamotherium*)]	Savage and Russell 1983; Tedford et al. 1987
Otariidae	7–9	Savage and Russell 1983
Pinnipedia	27–30 (*Enaliarctos*)	Berta et al. 1989[b]
Procyonid-ailurid split	17	J. Baskin, personal communication
Aeleuroid-arctoid split	55	Flynn and Galiano 1982
Primates:		
Pongo pygmaeus pygmaeus	>0.010	Heaney 1986
P. p. abelli	0.010	Heaney 1986
Hominidae	6	Simons 1989
Pongidae	12 (*Sivapithecus*)	Andrews 1986
Hylobatidae	18	Andrews 1986
Cercopithecoidea	23 (*Prohylobates*)	Simons 1969; Said 1990

Table A1 (*Continued*)

Taxon	Estimated First Occurrence (presumed earliest taxon) in Fossil Record (Mya)	Source(s)
Hominoidea	20–22	Andrews 1986
New World-Old World Monkey split	31 (*Branisella*)	Fleagle et al. 1986

[a] Kurten (1968) considered *F. libyca* to be a subspecies of *F. sylvestris*.
[b] Berta et al. (1989) provide a date of ~23 Mya for *Enaliarctos*, but recent evidence suggests that 27–30 Mya is a better date for the deposits in which it was found (Pyramid Hill member of Jewett Sand Fm.; T. Demere, personal communication).

Table A2
Data Used in Figure 2

Node (species[a] on either side of node)	Mya	Distance
Fig. 2A and B (PE-1D; distances are Nei distances):		
Carnivores:		
1. (4) (6)	0.012	0.042
2. (4, 6) (15)	2.500	0.228
3. (10) (8)	2.500	0.101
4. (10, 8) (9)	3.500	0.235
5. (4, 6, 15) (10, 8, 9)	4.500	0.277
7. (16) (14)	5.000	0.293
8. (16, 14) (13)	4.000	0.323
9. (16, 14, 13) (19, 11)	5.000	0.375
10. (4, 6, 15, 10, 8, 9) (16, 14, 13, 19, 11)	6.500	0.383
11. (4, 6, 15, 10, 8, 9, 16, 14, 13, 19, 11) (48)	33.500	1.157
12. (4, 6) (5)	1.500	0.043
13. (4, 6, 5) (12)	2.500	0.115
14. (4, 6, 5, 12) (7)	2.500	0.241
15. (4, 6, 5, 12, 7) (9)	3.500	0.296
16. (1, 18)	0.300	0.079
17. (1,18) (11, 19)	2.000	0.137
18. (1, 18, 11, 19) (17)	1.500	0.194
19. (7) (3)	2.500	0.079
20. (7) (2)	2.500	0.096
21. (2) (3)	2.500	0.130
22. (20) (21)	0.010	0.004
23. (35) (36)	0.010	0.013
24. (39) (40)	0.010	0.010
25. (35) (40)	1.800	0.121
26. (40) (37, 38)	2.000	0.079
27. (35) (37, 38)	2.000	0.124
28. (37, 38)	1.600	0.030
29. (43, 21) (22)	3.500	0.339
30. (43, 21, 22) (44)	3.500	0.353
31. (40, 35, 37, 38, 41, 34) (43, 21, 22, 44)	4.000	0.497
32. (40, 35, 37, 38, 41, 34, 43, 21, 22, 44) (69)	38.500	1.000
33. (53, 55)	25.000	0.560
34. (46, 45)	12.000	0.560
35. (53, 55) (46, 45)	28.500	0.802
36. (4, 54)	38.500	0.661
37. (4, 54) (58)	25.000	0.747
38. (53, 55, 46, 45) (4, 54, 58)	38.500	0.871
39. (40) (31)	4.500	0.330
40. (67) (70, 71)	17.500	0.732
41. (40, 31) (65, 70, 71)	38.500	0.889
42. (53, 55, 46, 45, 4, 54, 58) (40, 31, 65, 70, 71)	55.000	1.024

Node (species[a] on either side of node)	Mya	Distance
Bears:		
1. (48) (51)	0.500	0.017
2. (48, 51) (50)	0.085	0.018
3. (48, 51, 50) (49)	0.600	0.036
4. (47) (46)	3.000	0.058
5. (47, 46) (52, 48, 51, 50, 49)	6.000	0.081
6. (45) (47, 46, 52, 48, 51, 50, 49)	12.000	0.298
7. (53, 54)	17.000	0.205
8. (53, 54) (45, 47, 46, 52, 48, 51, 50, 49)	25.000	0.280
Primates:		
1. (84, 85)	0.010	0.044
2. (76, 77)	6.000	0.169
3. (81, 82) (76, 77)	6.000	0.257
4. (81, 82, 76, 77) (84, 85)	12.000	0.433
5. (81, 82, 76, 77, 84, 85) (86)	18.000	0.588
6. (81, 82, 76, 77, 84, 85, 86) (75)	23.000	0.743
Fig. 2C and D (PE-2D):		
Bears:		
1. (48, 50)	0.085	0.028
2. (48, 50) (47)	2.000	0.042
3. (48, 50, 47) (49, 51)	3.000	0.050
4. (48, 50, 47, 49, 51, 52) (46)	6.000	0.091
5. (48, 41, 47, 49, 51, 52, 46) (45)	12.000	0.156
6. (53, 54)	17.000	0.147
7. (53, 54) (48, 41, 47, 49, 51, 52, 46, 45)	25.000	0.196
Primates:		
1. (84, 85)	0.010	0.013
2. (77) (81, 82)	6.000	0.069
3. (77, 81, 82) (76)	6.000	0.078
4. (84, 85) (77, 81, 82, 76)	12.000	0.078
5. (84, 85, 77, 81, 82, 76) (86)	18.000	0.112
6. (84, 85, 77, 81, 82, 76, 86) (75)	23.000	0.154
Fig. 2E and F (DNA hybridization; distances are ΔT_m):		
Carnivores:		
1. (4) (7)	2.500	1.000
2. (4, 7) (1)	2.000	3.000
3. (4, 7, 1) (60, 64, 61, 62, 63, 59, 53, 54, 48, 49, 46, 45, 56, 57)	33.500	18.220
4. (60, 64)	3.000	3.500
5. (61, 63)	0.900	2.300
6. (61, 63) (62)	2.000	5.400
7. (61, 63, 62) (59)	7.000	7.600
8. (60, 64) (61, 62, 63, 59, 53, 54, 48, 49, 46, 45, 56, 57)	14.000	15.020
9. (53) (54)	17.000	13.700
10. (48, 49, 46, 45) (61, 62, 63, 59, 53, 54, 56, 57)	38.500	14.400
11. (48) (49)	0.500	2.500
12. (48, 49) (46)	6.000	3.300
13. (48, 49, 46) (45)	12.000	4.500
14. (48, 45, 49, 46) (53, 54)	25.000	14.300
15. (48, 45, 53, 54) (4)	38.500	18.100
16. (56) (57)	8.000	5.500
17. (4, 7, 1, 60, 64, 61, 62, 63, 59, 53, 54, 48, 49, 46, 45, 56, 57) (66, 69, 68, 67, 70, 71, 23, 24, 30, 29, 35, 38)	55.000	20.030
18. (66, 69, 68) (67)	18.500	12.000
19. (66, 69, 68, 67) (70, 71, 23, 24, 30, 29, 35, 38)	38.500	14.070
20. (70) (71)	5.000	3.000
21. (23, 24) (30, 29)	4.500	2.300
22. (23, 24) (35, 38)	3.000	3.000
23. (23, 28, 26, 24, 25, 27) (35)	3.000	2.700

Table A2 (*Continued*)

Node (species[a] on either side of node)	Mya	Distance
24. (23) (28)	0.007	0.300
25. (23, 28) (26)	0.100	1.000
26. (23, 28, 26) (24)	0.100	1.100
27. (23, 28, 26) (25)	0.300	1.100
28. (23, 28, 26, 24, 25) (21)	3.500	2.700
29. (35) (40)	1.800	1.200
30. (35, 40) (38)	2.000	1.100
31. (4) (10, 8, 9)	4.500	1.600
32. (4) (6)	0.012	0.000
33. (19) (16)	5.000	1.800
34. (19) (14)	5.000	2.500
35. (19) (13)	4.000	1.300
36. (19) (1)	2.000	1.000
37. (16) (14)	5.000	1.500
38. (16) (13, 1, 11, 19)	5.000	1.870
Primates:		
1. (76, 77)	6.000	1.850
2. (76, 77) (82)	6.000	1.900
3. (76, 77, 82) (85)	12.000	3.500
5. (76, 77, 82, 85) (78, 86)	18.000	5.022
6. (77, 82, 76, 78) (83)	23.000	7.700
7. (76, 77, 82, 85, 78, 86, 83) (73)	31.000	13.130
8. (73, 74, 79) (83)	31.000	15.100
9. (85, 78, 86) (83)	23.000	8.933
Fig. 2G and H (microcomplement fixation; distances are AID):		
Cats:		
1. (35) (40)	1.800	1.100
2. (35, 40) (33)	3.500	1.500
3. (35, 40, 33) (21)	3.500	2.530
4. (35, 40, 33, 21) (32)	4.000	4.280
5. (35, 40, 33, 21, 32, 34, 43) (44)	3.500	7.370
6. (35, 40, 33, 21, 32, 34, 43, 44) (23)	4.500	13.410
7. (35, 40, 33, 21, 32, 34, 43, 44, 23, 30) (69)	38.500	45.480
Primates:		
1. (77) (82)	6.000	5.000
2. (77, 82) (76)	6.000	5.500
3. (77, 82, 76) (85)	12.000	9.000
4. (77, 82, 76, 85) (78, 86)	18.000	11.300
5. (77, 82, 76, 85, 78, 86) (80)	23.000	33.800

[a] Species reference numbers are as follows: Carnivora, Canidae—1, *Alopex lagopus;* 2, *Canis adustus;* 3, *C. aureus;* 4, *C. familiaris;* 5, *C. latrans;* 6, *C. lupus;* 7, *C. mesomelas;* 8, *Cerdocyon thous;* 9, *Chrysocyon brachyurus;* 10, *Dusicyon vetulus;* 11, Fennecus zerda; 12, *Lycaon pictus;* 13, *Nyctereutes procyonoides;* 14, *Otocyon megalotis;* 15, *Speothos venaticus;* 16, *Urocyon cinereoargenteus;* 17, *Vulpes chama;* 18, *V. macrotis;* and 19, *V. vulpes.* Carnivora, Felidae—20, *Acinonyx jubatus* (east Africa); 21, *A. jubatus* (South Africa); 22, *Caracal caracal;* 23, *Felis catus;* 24, *F. chaus;* 25, *F. libyca;* 26, *F. margarita;* 27, *F. nigripes;* 28, *F. silvestris;* 29, *Leopardus geoffroyi;* 30, *L. pardalis;* 31, *L. wiedii;* 32, *Leptailurus serval;* 33, *Lynx canadensis;* 34, *Neofelis nebulosa;* 35, *Panthera leo* (African lion); 36, *P. leo* (Asiatic lion); 37, *P. onca;* 38, *P. pardus;* 39, *P. tigris* (Sumatra); 40, *P. tigris* (Bengal); 41, *P. uncia;* 42, *Prionailurus bengalensis;* 43, *Profelis temmincki;* and 44, *Puma concolor.* Carnivora, Ursidae—45, *Ailuropoda melanoleuca;* 46, *Tremarctos ornatus;* 47, *Ursus americanus;* 48, *U. arctos;* 49, *U. malayanus;* 50, *U. maritimus;* 51, *U. thibetanus;* and 52, *U. ursinus.* Carnivora, Procyonidae—53, *Ailurus fulgens;* and 54, *Procyon lotor.* Carnivora, Phocidae—55, *Mirounga angustirostris;* and 56, *Phoca vitulina.* Carnivora, Otariidae—57, *Eumetopias jubatus.* Carnivora, Mustelidae—58, *Ictonyx striatus;* 59, *Lutra canadensis;* 60, *Mephitis mephitis;* 61, *Mustela frenata;* 62, *M. putorius;* 63, *M. vison;* and 64, *Spilogale putorius.* Carnivora, Viverridae—65, *Galidia elegans;* 66, *Genetta genetta;* 67, *Herpestes* sp.; 68, *Paradoxurus hermaphroditus;* and 69, *Viverra tangalunga.* Carnivora, Hyaenidae—70, *Crocuta crocuta;* 71, *Hyaena hyaena;* and 72, *Proteles cristatus.* Primates, Hominidae—73, *Alouatta* sp.; 74, *Cebus* sp.; 76, *Colobus guereza;* 76, *Gorilla gorilla;* 77, *Homo sapiens;* 78, *Hylobates lar;* 79, *Lagothrix* sp.; 80, *Maccaca mulata;* 81, *Pan paniscus;* 82, *P. troglodytes;* 83, *Papio cynocephalus;* 84, *Pongo pygmaeus abelii;* 85, *P. p. pygmaeus;* and 86, *Symphalanges syndactylus.*

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