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A MOLECULAR TEST OF BAT RELATIONSHIPS: MONOPHYLY OR DIPHYLY?

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Abstract.—Two conflicting hypotheses concern the origin of flying mammals. The traditional hypothesis states that the two major groups of bats, the microchiropterans and the megachiropterans, are sister groups that constitute the taxon Chiroptera. In contrast, the diphyly hypothesis suggests that megachiropterans are more closely related to primates than to microchiropterans. Different suites of morphological characters provide support for each of these hypotheses, and previous molecular studies have not provided a clear resolution of the problem. We analyzed a region of the mitochondrial 12S ribosomal RNA gene from 11 species of mammals, including 2 species of megachiropterans, 2 species of microchiropterans, a primate, a colugo (Dermoptera), a tree shrew (Scandentia), and 4 outgroups, to test the diphyly hypothesis. A phylogenetic analysis of 257 base pairs resulted in two shortest unrooted trees that significantly support the monophyly of the bats and also suggest that the colugo is more closely related to primates than to the bats: (((Primates, Dermoptera) Scandentia) (Microchiroptera, Megachiroptera)). The topology supporting the diphyly hypothesis is 10 steps longer than the most-parsimonious unrooted tree. Although the traditional hypothesis is supported with respect to bat monophyly, the rDNA data support the nontraditional grouping of colugo and primates (a hypothesis also supported by neurological data). [Bat monophyly, phylogenetic confidence, Chiroptera, Archonta.]

All mammals capable of true flight traditionally have been recognized as a monophyletic group, Chiroptera. The two major lineages of bats have been united based on several unique musculoskeletal specializations associated with the presence of wings (Baker et al., 1991b). Recently, doubt has been raised over the support for a bat clade. Are the large fruit-eating bats of the Old World (Megachiroptera) the closest living relatives of the smaller predominantly insectivorous cosmopolitan bats known as microchiropterans? The monophyly of Chiroptera was questioned first by Smith (1977) and Smith and Madkour (1980). They concluded that derived features of the penis, neurosensory system (size of neocortex), and limb joints suggest a diphyletic origin for bats, or that mammals evolved flight twice. The diphyly hypothesis also has been supported by Pettigrew (1986) based upon several features related to the patterns of connection between the retina and midbrain (superior colliculus) that are shared in primates and megachiropterans. Predictably, these findings have sparked a controversy over bat relationships. The evidence supporting opposing sides of the argument was recently summarized by Pettigrew (1991a, 1991b), Baker et al. (1991b), and Simmons et al. (1991).

The disagreement among existing morphological studies (Simpson, 1945; Smith, 1977; Smith and Madkour, 1980; Novacek, 1982; Pettigrew, 1986; Shoshani, 1986; Wible and Novacek, 1988; Pettigrew et al., 1989; Thewissen and Babcock, 1991) makes this particular problem an excellent candidate for molecular analysis. Which suite of characters are truly the result of common ancestry and which are convergent? The differences between the megabats and microbats are widely known (Pettigrew et al., 1989), but synapomorphies for Chiroptera have been much more elusive.

The bats are generally regarded as part of the superorder Archonta (Novacek, 1989), along with Primates, Dermoptera (colugos or flying lemurs), and Scandentia (tree shrews). Bats and colugos traditionally have been recognized as sister taxa (Fig. 1a), whereas Pettigrew et al. (1989) hypothesized a sister-group relationship between one of the groups of bats (megachiropterans) and primates (Fig. 1b). There is very little consensus regarding the relation-

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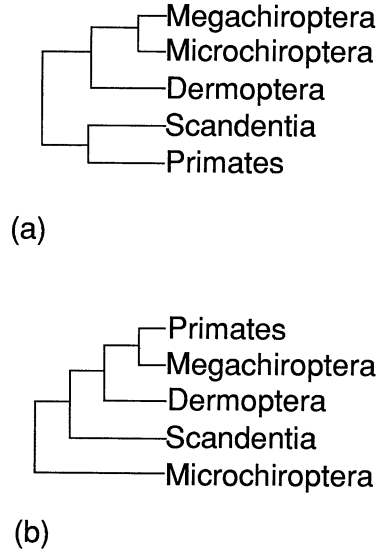

FIGURE 1. (a) The traditional relationships of the archontan mammals (from Novacek, 1989). (b) The relationships proposed by Pettigrew et al. (1989).

ships among many orders of eutherian mammals (Benton, 1988; Novacek et al., 1988). Therefore, it was necessary to use several individual outgroups to address the possibility that a potential outgroup might actually be part of the ingroup.

To examine the phylogenetic relationships of the Megachiroptera and the Microchiroptera to each other and to other orders of mammals, we examined the DNA sequence of the mitochondrial ribosomal RNA small subunit gene (12S rDNA). By taking a molecular approach, we assume our data to be independent of the possible adaptive convergences of visual pathways and flight found in the existing morphological studies. The conservative nature of ribosomal RNA genes make them potentially valuable for investigating divergences among mammalian orders (Miyamoto and Boyle, 1989). The 12S ribosomal RNA gene sequence has been analyzed phylogenetically in numerous studies of intraordinal mammalian relationships (Brown et al., 1982; Hixson and Brown, 1986; Miyamoto and Boyle, 1989; Miyamoto et al., 1989, 1990; Thomas et al., 1989; Kraus and Miyamoto, 1991) as well as for relationships with much older divergence times (reviewed in Hillis and Dixon, 1991). In this study, the divergence time is at least 50 million years ago (MYA), which is the age of the oldest fossil bat (Jepsen, 1970). The actual divergence time between the archontan orders is probably much older (around 68 MYA; Benton, 1990).

Two methods have been proposed recently for ascertaining whether a specific data set contains useful phylogenetic signal or if the data are no more structured than would be expected at random: the g_1 statistic (Hillis, 1991; Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992) and permutation tail probabilities (Faith and Cranston, 1991). With these procedures, one can test for the presence of hierarchical structure in the data and assess confidence in the resulting phylogenetic hypotheses. In our test of bat monophyly, we employed the new topology-dependent cladistic permutation test proposed by Faith (1991). The results of our tests of Faith (1991) are compared with those of other methods for estimating significance of particular nodes or topologies (g_1 statistic [Hillis, 1991], bootstrapping [Felsenstein, 1985], and Templeton's test [Templeton, 1983]).

METHODS

Whole genomic DNA preparations of two megachiropterans (*Pteropus scapulatus* [flying fox], *Penthetor lucasi* [dusky fruit bat]), two microchiropterans (*Tadarida brasiliensis* [mexican free-tailed bat], *Glossophaga soricina* [long-tongued bat]), a dermopteran (*Cynocephalus volans* [colugo or flying lemur]), a scandentian (*Tupaia glis* [tree shrew]), an edentate (*Dasypus novemcinctus* [nine-banded armadillo]), and a marsupial (*Phascolarctos cinereus* [koala]) were used as templates in polymerase chain reactions (PCR) (Innis et al., 1990). Primers 28–33 base pairs long (5′-AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT-3′ and 5′-TGACTGCAGAGGGTGACGGGCGGTGTGT-3′) were used to amplify a region of the mitochondrial 12S ribosomal RNA gene of approximately 400 nucleotides. This region corresponds to base positions 701–955 in the published *Mus* mtDNA sequence (Van Etten et al., 1980). A modified

Taq polymerase (US Biochemical) was used to produce a double-stranded DNA product (Mullis and Faloona, 1987). Each sample was subjected to 30 cycles of denaturation (94°C), annealing (55°C), and extension (72°C) in the presence of 2 mM $MgCl_2$.

Double-stranded PCR product was chloroform extracted, ethanol precipitated in the presence of ammonium acetate, and resuspended in 50–100 μl dH_2O. Further purification of the DNA solution was by one of two methods: Sepharose CL6B columns or Millipore Ultrafree-MC 30,000 NMWL filter units. About 1 μg of purified double-stranded DNA product was denatured in 0.2 M NaOH for 5 min, added to 4 μl of 1% acrylamide and 16 μl of 3 M ammonium acetate, and precipitated with 150 μl of absolute ethanol. The samples were frozen 20–60 min at −20°C, washed with 70% ethanol, and allowed to dry. The dry template was annealed to 20 ng of primer by either heating to 65°C and gradually cooling to 37°C or by boiling for 5 min and cooling immediately on ice for 10 min. We then followed standard dideoxy sequencing and electrophoresis protocols as described in Hillis et al. (1990).

Mitochondrial 12S sequences from 10 taxa were each aligned to the mouse 12S ribosomal DNA sequence (Van Etten et al., 1980) using the MacVector program (IBI, Pustell and Kafatos, 1986; see Fig. 2). The ingroup (superorder Archonta) was assumed to be monophyletic in the initial analyses (Novacek, 1989). The data from the ingroup taxa were analyzed with equal weighting of transitions and transversions using the exhaustive search option of PAUP version 3.0q (Swofford, 1991). The tree was rooted four times using four separate outgroups (mouse, armadillo, cow, and koala). These sequences also were used as a composite outgroup and analyzed using the branch-and-bound option of PAUP with the following constraint: (koala (armadillo (mouse, cow, ingroup))). A separate phylogenetic analysis of transversions alone was also carried out (for both the ingroup taxa and for all taxa) for comparison.

We estimated the reliability of branches on the shortest trees using 1,000 bootstrap replicates (each of which was analyzed using the branch-and-bound option of PAUP). An estimate of skewness of the tree-length distribution (g_1) also was obtained using PAUP. Alternative hypotheses were located in the distribution of all trees using the topological constraints option of PAUP. After initial parsimony analyses, the data were subjected to maximum-likelihood analysis (Kimura, 1980) and a modified Templeton's (1983) test using the Macintosh version of PHYLIP 3.3 (J. Felsenstein, Department of Genetics, Univ. Washington, Seattle).

We conducted the topology-dependent cladistic permutation test proposed by Faith (1991) for an a priori test of bat monophyly. The a priori test is appropriate in this study because bats were hypothesized to be monophyletic before analysis of this particular data set. This method compares the lengths of the shortest tree(s) exhibiting monophyly and nonmonophyly from the observed analysis to the same lengths resulting from analyses of randomized data. The proportion of the randomizations where this difference value is equal to or greater than the observed difference value is referred to as the topology-dependent cladistic permutation tail probability (T-PTP) (Faith, 1991).

T-PTPs were generated by analyzing 1,000 randomized data sets with PAUP. The character states for each character were randomized among taxa while maintaining the identity of each taxon (Faith, 1991). For each PAUP analysis, the length of the shortest tree supporting monophyly was subtracted from the length of the shortest tree not supporting monophyly to come up with difference values. The proportion of the randomizations in which the difference value matched or bettered that of the original nonrandomized data set was recorded. This proportion equals the T-PTP (Faith, 1991).

RESULTS

Unrooted Analyses

We were able to unambiguously align 257 base pairs from the 12S rRNA gene

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Mus           TTAAAACTCA AAGGACTTGG CGGTACTTTA TAT-CCATCT AGAGGAGCCT
Phascolarctos N......... .........A ....G-.CCT A.GNNN.... ..........
Dasypus       .......... .......... ....G..... C.....T... ..........
Bos           .......... .......... ....G..... ......T... ..........
Pongo         .......... ......C... ....G...C. ..C...CC.. ..........
Tupaia        CC........ .......... ....G..... .......... ..........
Cynocephalus  CA........ .........A .N..G..CC. C.....CC.. .......T..
Pteropus      NNN....... .......... ....G..... C..G..C... ..........
Penthetor     C......... .......... ....G..... C......... ..........
Glossophaga   NNN....... .......... ....G.N.C. ......C... ..........
Tadarida      N.T....... .N........ ....G..... .......... ..........

Mus           GTTCTATAAT CGATAAACCC CGCTCTACCT CACCATCTCT TGCTAATTCA
Phascolarctos ..C......C ....G.N... ..A.AC...C ....TCT... ...C...A..
Dasypus       .......... .......... ..A.A..... .....C.C.. .......A..
Bos           .......... .......... ..A.AA.... .....AT... .......A..
Pongo         .....G.... .......... ..A..A.... .....C.C.. ...----...
Tupaia        ..C..G.... .......... ..A.AC.... .......C.. .....T.C..
Cynocephalus  .........C .......... ..A.A....C T.-..C.... -..C-.CC..
Pteropus      .....G.... .......... ..A.AA.... .....A.... .......A..
Penthetor     .......... .......... ..A.AA.... .....A.C.. .......C..
Glossophaga   .......... .......... ..A.AA.... .....A.C.. ...C..A...
Tadarida      .......... .......... ..A.AA.... .....A.C.. ..........

Mus           GCCTATATAC CGCCATCTTC AGCAAACCCT AA-AAAGGTA TTAAAGTAAG
Phascolarctos .......... .......G.. ...CT....C ...T.G..AC CA........
Dasypus       .......... .......... ....G..... .G.T....C. CC.C...G..
Bos           .T........ .......... .......... ........A. AA........
Pongo         .......... .......... .......... .T.G....CC ACG.......
Tupaia        .......... .......... .........A .-.......AC GAC.......
Cynocephalus  ....G..... .......... .........C CC..GG.CAT GA........
Pteropus      .......... .......... ..NNN..... ..A..G.AA. CA........
Penthetor     .......... .......... .......... ........A. CC.T......
Glossophaga   .......... .......... .......... G....G..NN .A.T......
Tadarida      .......... .......... .......... .......CA. ...T......

Mus           CAAAAGAATC AAACATAAAA ACGTTAGGTC AAGGTGTAGC CAATGAAATG
Phascolarctos GC....GN.. .CCT..... -......... .......... AT.....GA.
Dasypus       ..C..T..CA TN.......G .......... .......... TT...GGG..
Bos           .GT..TT..G .T........ .......... ........A. .T........
Pongo         .GC..AC.-. CC..G....G .......... .......... .C...G.G..
Tupaia        .TT..TC... CT........ .A........ .......... .C...GG...
Cynocephalus  .CC..T.... C-........ .......... ......C... .C....GG..
Pteropus      .....AC..A .......... .......... .......... .T....GT..
Penthetor     .....CC..G G......... .......... .......... .C...GGT..
Glossophaga   .TC..CT.CA .C.TG..... .......... .......... TT...GGT..
Tadarida      ..C..AC..T GC........ .......... .......... ......GT..

Mus           GGAAGAAATG GGCTACATTT TCTTATAAAA GAACATTACT ATACCCTTTA TGAAACT
Phascolarctos .A...TG... ......T... ...A.C.T-. ...A.....G GACTATC... ......C
Dasypus       ..N....... .......... ...A....-. ..G..AA..A .A.AA...A. ......A
Bos           .......... .........C ..ACTACC.. ..GATCA..G .A.GTTA... ......C
Pongo         .C........ .......... ...AC.TC-. ...A.C...G ...A..C.C. .....TC
Tupaia        .....TC... .......... ...AG.CC-. ...T.CA..G CC.A.TCA.. .......
Cynocephalus  .-........ .A........ ...A..CT-. .....AC..G .C.G....C. .....TC
Pteropus      .A........ .......... ...AGC.T-. .....CC..G .A.ATT..CG .....TC
Penthetor     .......... .......... ...AGC..T. ......C..G .A.ATT..CG ......C
Glossophaga   .A........ .......... C..A..T.T. .G...CA..G .A.A.TCC.G .....TC
Tadarida      .......... .......... C....CC.T. .G...A...G .ATAT..C.G .....TC
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FIGURE 2. A portion of the mitochondrial ribosomal small subunit (12S) sequence collected from two species of megachiropterans (*Penthetor, Pteropus*), two species of microchiropterans (*Tadarida, Glossophaga*), a colugo (*Cynocephalus*), and a tree shrew (*Tupaia*) using PCR amplification procedures. The primate sequence (*Pongo*) was obtained from Hixson and Brown (1986). Sequence from an armadillo (*Dasypus*), a mouse (*Mus*) (Van Etten et al., 1980), a cow (*Bos*) (Anderson et al., 1982), and a koala (*Phascolarctos*) were used as outgroups. Each sequence was aligned to the published *Mus* sequence for parsimony analysis. Periods indicate nucleotides that are the same as that in mouse; dashes indicate gaps to maintain alignment. The gene fragment analyzed corresponds to base positions 701–955 of *Mus*.

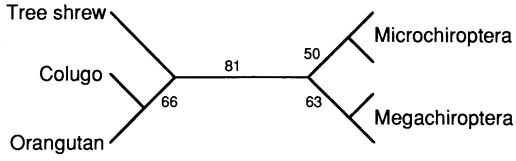

FIGURE 3. One of the two most-parsimonious unrooted trees (167 steps) resulting from the exhaustive search option of PAUP using all characters. This result supports the bat monophyly hypothesis. The values on the branches indicate the result of a bootstrap analysis on 1,000 pseudoreplicates. The highest bootstrap value is on the branch separating the bats from the other taxa.

(Fig. 2). An unrooted parsimony analysis of 44 informative sites yielded two shortest unrooted trees of 167 steps and with consistency indices (CI) of 0.66. The topology of one of the shortest unrooted trees is presented in Figure 3. The alternative topology differed with respect to the relationships within the bats (one microchiropteran species, *Tadarida,* united with the megachiropterans). The relationships in Figure 3 are consistent with the traditional hypothesis of relationships except for the position of the colugo (see Fig. 1a). Two additional steps are required to reconstruct the traditional relationships of bats with the colugo (169 steps). The diphyly hypothesis of Pettigrew et al. (1989) requires 10 additional steps (177 steps) (Fig. 4). The shortest tree to support nonmonophyly of the bat clade is four steps longer than the shortest tree. The shortest nonmonophyletic hypothesis requires 171 steps and groups the tree shrew with the microchiropterans, to the exclusion of the megachiropterans.

Maximum-likelihood analysis evaluates the likelihood that a given phylogeny will yield the observed DNA sequences under a specified model of evolution (Swofford and Olsen, 1990). Maximum-likelihood analysis of the shortest unrooted tree showed each branch to be significantly supported except for the branch between the orangutan/colugo clade and the tree shrew (see Fig. 3). This branch collapsed under the assumptions of the Kimura (1980) two-parameter model of maximum likelihood, which indicates that the particular resolution of relationships among these three taxa is not well supported.

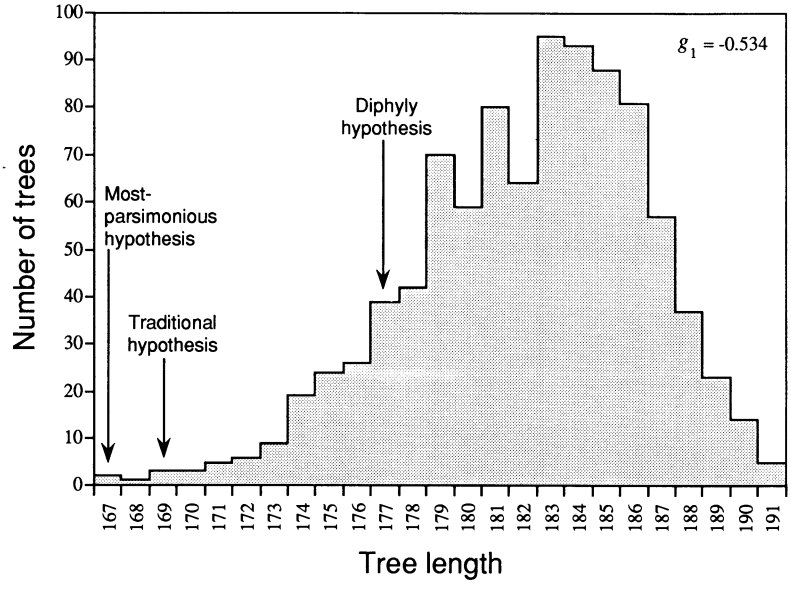

FIGURE 4. The distribution of all possible trees from the analysis of all data for seven taxa. The locations in the distribution of the most-parsimonious hypothesis, the traditional hypothesis, and the diphyly hypothesis are indicated by arrows. The most-parsimonious hypothesis is shown in Figure 3.

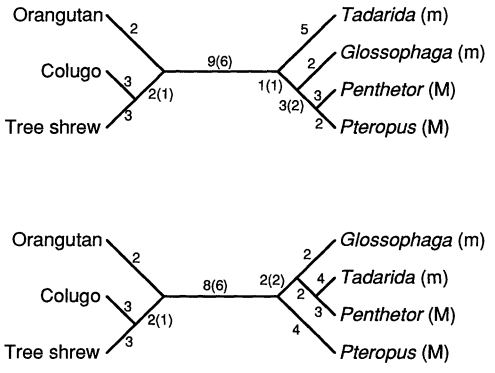

FIGURE 5. The two shortest unrooted trees resulting from the analysis of transversions. The values on the branches indicate the number of character changes supporting each clade. The numbers in parentheses are the number of transversions that do not change elsewhere on the tree. m = microchiropterans; M = megachiropterans.

The transversion : transition ratio of this data set was approximately 1:1 when calculated for the seven ingroup taxa (21 transversions out of 44 informative sites). One method often used with data sets that have a high transition bias is to analyze transversions alone (Hixson and Brown, 1986; Miyamoto and Boyle, 1989). This approach theoretically limits the amount of homoplasy expected from the frequent back mutations of transitions in divergent taxa. When the parsimony analysis of the ingroup was limited to transversions (sequence data coded as purines and pyrimidines), there were two shortest unrooted trees (Fig. 5). In this analysis, the tree shrew and colugo are sister groups. There is no possible rooting of either tree that would support a primate/megachiropteran clade. This result is consistent with the result from the data set containing both transitions and transversions in that it supports a bat clade (megachiropterans plus microchiropterans) and a primate clade (orangutan/tree shrew/colugo). A bootstrap analysis of transversions alone supported a bat branch in 97% of 1,000 trees. There are six transversions supporting the bat branch that do not change elsewhere on the tree (see Fig. 5; one site of the six is an unknown base in *Glossophaga*).

Statistical Confidence in Unrooted Analyses

Several statistical methods have been proposed for determining the confidence that can be placed on a particular data set or the confidence one can have in a particular hypothesis of phylogenetic relationships. These statistical methods can be useful for determining if a data set contains phylogenetic information that is significantly different from results that would be obtained from random data. The significance of the results in Figure 3 were tested by the criteria of four separate statistical methods (g_1 statistic, bootstrapping, Templeton's test, and T-PTP).

The g_1 statistic measures the skewness of a tree-length distribution. Results of computer simulation studies suggest that this statistic provides a good indicator of phylogenetic information (Huelsenbeck, 1991). By comparing the g_1 statistic of the observed data set with the g_1 of randomized data, we tested the hypothesis that this data set has significantly more phylogenetic information or structure than expected from random data. The 12S rDNA sequence data yield a significantly skewed tree distribution (Fig. 4; $g_1 = -0.534$; $P < 0.01$) when compared with the g_1 of 1,000 randomized data sets.

Felsenstein's (1985) bootstrapping test is a resampling method designed to establish the support for particular clades. The bat clade (megachiropterans plus microchiropterans) was supported more often than any other branch (81% of the time) in the analysis of both transversions and transitions and was supported 97% of the time in the transversions-only analysis. Recent simulation studies designed to test the reliability of bootstrap values provide evidence that the bootstrap method usually is a very conservative test (Hillis and Bull, submitted).

Templeton's (1983) test is designed to compare two competing hypotheses of relationships and determine whether one hypothesis is significantly better supported than the other. This method is basically a nonparametric signed-ranks test that compares the distribution of characters on

the two trees. The shortest tree was significantly better supported ($P < 0.01$) than the diphyly hypothesis (Fig. 1b) but was not significantly better than the traditional hypothesis (Fig. 1a).

Topology-dependent cladistic permutation tail probabilities (T-PTP) measure the degree of support for a specific hypothesis of monophyly in comparison to the shortest corresponding nonmonophyly hypothesis (Faith, 1991). Is there support for bat monophyly in this cladistic analysis that represents a significant departure from randomness? The shortest trees that exhibited monophyly and nonmonophyly were four steps apart. The difference value resulting from our analysis of 1,000 randomized data sets was ≥ 4 only twice. The topology-dependent cladistic permutation test therefore significantly supports the monophyly of the bat clade (T-PTP = 0.002).

Rooted Analyses

The trees generated from the analysis of both transitions and transversions were unrooted. The relationships expressed in Figure 3 do not necessarily indicate whether bats are monophyletic because the root of the tree could fall along one of the bat lineages. The shortest tree was rooted using a composite outgroup that was constrained as follows: (koala (armadillo (mouse, cow, ingroup))). This constraint is reasonable given that marsupials are widely accepted as the sister group of eutherian mammals and most studies agree that edentates are an early lineage of eutherian mammals (Novacek, 1989). This analysis yielded one shortest tree of 293 steps with a CI of 0.52 (Fig. 6). The same relationships among the ingroup taxa were achieved without assuming that the armadillo is outside of mouse and cow. In addition, each of the above taxa, when used individually as outgroups, rooted the tree identically and also resulted in shortest trees that supported bat monophyly.

The above analyses assume the monophyly of Archonta. When the entire data set (Fig. 2) is coded as purines and pyrimidines and analyzed using the branch-and-bound option of PAUP, the monophyly of Archonta is not supported. Koala was used as the outgroup for this analysis. There were 28 informative transversions. A strict consensus of the 24 shortest trees supports a primate/dermopteran/tree shrew clade. The cow clustered with this clade in 58% of the shortest trees. There was not support for bat monophyly in the strict consensus tree because one of the megachiropterans (*Pteropus*) clustered with the other bats in less than 50% of the shortest trees.

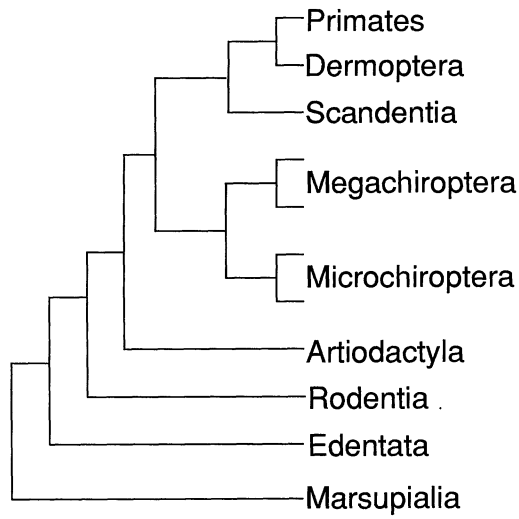

FIGURE 6. The most-parsimonious rooted tree (293 steps) from analysis of all data using a composite outgroup (see text). The same result is achieved without assuming that Edentata is outside of Rodentia and Artiodactyla. An analysis using each individual outgroup also results in shortest trees that support bat monophyly.

DISCUSSION

Bat Monophyly

Analyses of the 12S rDNA gene sequences strongly support the monophyly of bats. This result is congruent with numerous analyses of cranioskeletal (Wible and Novacek, 1988; Novacek, 1989), muscular (Thewissen and Babcock, 1991), and vascular (Wible and Novacek, 1988) characters. Our results are incongruent with the hypothesis that the two groups of bats evolved separately. The monophyly result requires that the neurological features described by Pettigrew et al. (1989) and features of penial morphology (Smith, 1977)

either be interpreted as convergent characteristics of primates and megachiropterans or as symplesiomorphies of Archonta. With exception of the analysis using transversions, our results also support the validity of the two chiropteran suborders.

The results of this study are largely in agreement with those of other molecular studies that have addressed the question of bat monophyly. Baker et al. (1991a) examined many taxa with a restriction enzyme analysis of the nuclear ribosomal gene repeat. With this technique they were unable to find support for one hypothesis over the other. The tree supporting monophyly was one step shorter than the tree supporting diphyly, but both trees had high levels of homoplasy. Adkins and Honeycutt (1991) found support for bat monophyly in a phylogenetic analysis using transversions of the cytochrome oxidase II gene. They were unable to demonstrate statistical significance for this result. Mindell et al. (1991) examined sequences from two genes (mitochondrial 12S ribosomal RNA gene and cytochrome oxidase I gene) for a microchiropteran and a megachiropteran. They compared their sequences to human and mouse. Their results supported bat monophyly but suggested nothing about the relationships of the other closely allied orders, such as the dermopterans.

Relationships of Dermoptera

The position of Dermoptera as the sister group to primates is an unexpected result of our study. However, it is consistent with recent findings by Beard (1990) and Kay et al. (1990) that the early primate fossil group, Plesiadapiformes, was allied with the dermopterans and not the primates. A primate/dermopteran clade is also supported by DNA sequence data from the cytochrome oxidase II gene (Adkins and Honeycutt, 1991). The most-parsimonious hypothesis based on the data in this study (Fig. 3) is not significantly better than the traditional hypothesis (Fig. 1a) supporting a dermopteran/bat clade. The primate/dermopteran/tree shrew clade was well supported in the analysis of transversions for all taxa, but these results do contradict traditional views and suggest that more work is needed to understand the phylogenetic position of this group.

Relationships of Archonta

We found no support for the monophyly of Archonta when only transversions were analyzed for all taxa, primarily because of the position of the cow. Because artiodactyls have never seriously been proposed as a close relative of the Archonta, we doubt this weakly supported result. However, more data may show that the Archonta is an artificial group. Numerous morphological and molecular studies have failed to locate synapomorphies of Archonta (Cartmill and MacPhee, 1980; Miyamoto and Goodman, 1986; Kay et al., 1990; Adkins and Honeycutt, 1991; and see Novacek et al. [1988] for review) and indicate that our understanding of the relationships of these orders is very poor. We expect that the relationships we attempted to reconstruct by including all taxa (including a marsupial) are simply too divergent for this part of the gene (Novacek, 1982; Hillis and Dixon, 1991). A more conservative sequence, such as a nuclear ribosomal RNA gene sequence, may be more useful in resolving older divergences such as these.

Phylogenetic Confidence

The fact that the complete 12S data set (both transitions and transversions) shows hierarchical structure significantly different from random (from g_1 test) lends credence to the conclusions drawn from these data. Critics may argue that the mitochondrial 12S gene is not suitable for answering phylogenetic questions concerning mammalian orders because of its rapid rate of evolution. If that were the case, we would not expect to find significant phylogenetic signal in the data. We would expect, however, that transversions would be less homoplastic and would provide a more conservative look at relationships among these taxa. However, all weighting schemes are in agreement and provide no reason to reject bat monophyly.

The g_1 statistic has been compared with

other indicators of phylogenetic confidence (such as the bootstrap) using simulation studies of known phylogenies and has been found to be a good measure of phylogenetic structure (Huelsenbeck, in prep.). Although skewness of tree-length distributions (as measured by g_1) may be useful for identifying phylogenetic structure in a data set, the Faith (1991) test may be useful for discerning the relative confidence that can be placed in a particular branch of a cladogram. Few applications of the test of Faith (1991) exist, and the usefulness of this relatively new method is largely unknown. However, the results of Faith's test are in general agreement with those of the other tests in supporting bat monophyly.

Problems in Mammal Phylogeny

Many unanswered questions remain in the field of mammalian systematics. Mammalogists today have less confidence in the branching order of the 18 orders of mammals than they did 100 years ago. Baker et al. (1991a) proposed that the rapid radiation of mammals in the Cretaceous makes it difficult to recover informative molecular synapomorphies. A pattern may not be recoverable using molecular data because of short internal branch lengths and long terminal branches. This problem is especially prominent with a group such as the bats that might have been under strong selective pressure for a short amount of time as they became adapted for flight. The molecular and morphological changes did not necessarily occur at the same rate, but the short period of common ancestry may limit the number of informative molecular characters that can be retrieved. There have not yet been enough molecular studies at this level of mammalian divergence to know if the relatively rapid radiation of mammals is going to be a complicating factor in our efforts to reconstruct mammal phylogeny using molecular characters. The results of this analysis are very encouraging. Despite the size of the data set, enough phylogenetic signal was evident to test and support the hypothesis of bat monophyly with a high degree of confidence.

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