

Encephalization, Adaptation and Evolution of Chiroptera: A Statistical Analysis with Further Evidence for Bat Monophyly

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Key Words

Brain evolution · Phylogeny · Bat monophyly · Megabats · Microbats · Primates

Abstract

As part of a large-scale study on brain morphometrics and adaptations in mammals, we addressed the problem of chiropteran evolution. A specific statistical framework was designed to test which of two competing hypotheses (bat monophyly vs. diphyly) is more strongly supported by quantitative brain data. Our analyses, based on 120 species, revealed that megabats and microbats were more closely related to each other than to primates, and illustrated the convergent adaptations of the brain of bats to similar trophic (i.e. feeding related) niches. Ecologically-corrected characters were then used to derive a new phylogeny which also supports the chiropteran clade. The monophyletic origin of bats is the preferred hypothesis to explain brain quantitative evolution in chiropterans and primates.

Introduction

Until Pettigrew's [1986] challenging paper on chiropteran evolution, the monophyletic origin of bats has seldom been seriously challenged. Flying mammals were thought to be derived from a common insectivorous ancestor that might have possessed gliding membranes [Smith, 1976,

1977, 1980]. But brain studies have revived an old and different hypothesis [see Linnaeus, 1758]; microbats and megabats could have originated independently, with megabats related to primates. The strongest piece of evidence supporting this 'flying primate hypothesis' relies on the possession by megabats of advanced retinotectal pathways, a condition also found in primates [Pettigrew, 1986; Pettigrew and Cooper, 1986]. However, several studies based on morphological [Wible and Novacek, 1988; Thewissen and Babcock, 1991; Simmons, 1994] and molecular data [Bennett et al., 1988; Adkins and Honeycutt, 1991; Mindell et al., 1991; Ammerman and Hillis, 1992; Bailey et al., 1992; Stanhope et al., 1992; Kirsch et al., 1995] have supported the chiropteran clade. Thus, two hypotheses of bat evolution are competing: the monophyletic vs. diphyletic scenario [for a detailed account of the debate, see Baker et al., 1991; Pettigrew, 1991a, b; Simmons et al., 1991]. If flight has arisen only once during mammalian evolution, bats must constitute a monophyletic assemblage: megabats and microbats should form a clade to which primates would represent a sister group (fig. 1A). On the other hand, if the diphyletic hypothesis of Pettigrew [1986] is correct, primates and megabats should form a monophyletic clade, and microbats would represent its sister group (fig. 1B).

On molecular grounds alone, the problem of bat phylogeny seems to have been solved to indicate that bats are monophyletic, yet there still exists the impression that the megabat nervous system is more like the primate's than the microbat's. A quantitative investigation is thus undertaken in this paper to see if this is really true when the brain as a whole is included in the analysis. Interestingly, the different

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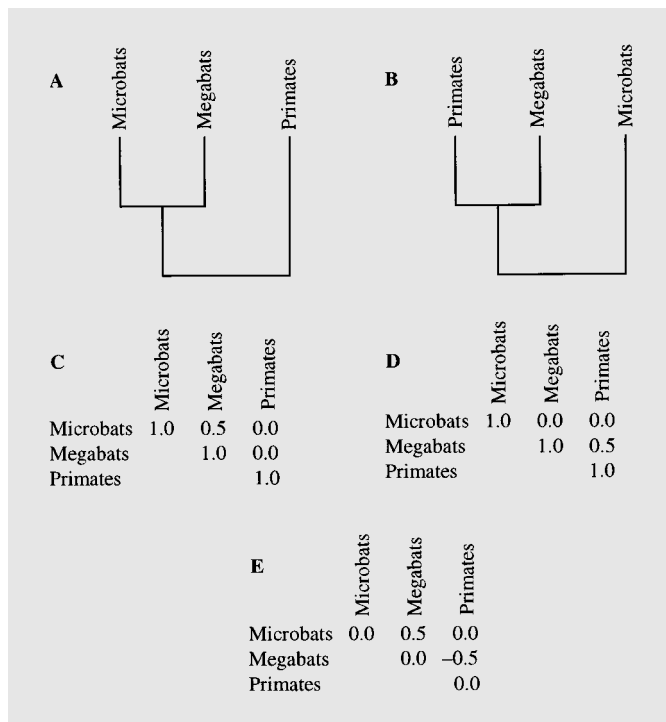


Fig. 1. Phylogenetic hypotheses discussed in the text. **A** The monophyletic hypothesis; **B** the diphyletic hypothesis. Under each model is its unique matrix representation (**C** and **D**) in which evolutionary relationships among species are coded numerically: two species in the same taxonomic group (i.e. primates, megabats, microbats) are given maximum similarity (i.e. 1), species within the same clade are coded by an intermediate value (i.e. 0.5), whereas species in sister groups are given a null similarity. These matrices can be used instead of phylogenetic trees to assess competing hypotheses by comparing each model to a path-length matrix representing the derived brain phylogeny. Alternatively, a simpler way to test these models is to build a single matrix **E** that differentiates the competing hypotheses; it is obtained by subtracting matrix **D** from **C**, two blocks of values are of interest: the similarities between megabats and microbats (0.5), and the similarities between megabats and primates (-0.5) (the relationship between microbats and primate is not relevant here as it always occurs in sister-groups, that is, the relationships always exhibits a null similarity). Using these values as linear contrasts, one can test whether megabats and microbats are more closely related to each other than megabats to primates, or vice versa. The direction of the correlation value provides evidence supporting either the monophyletic (positive correlation) or the diphyletic (negative correlation) hypothesis.

hypotheses have seldom been assessed statistically with neural characters. In particular, too few species were used for the diphyletic model to reach phylogenetic significance [e.g. Pettigrew, 1986]. Indeed, most of the earlier studies have dealt with only one representative species of megabats, microbats and primates to evaluate the competing hypothe-

ses, thus considering a very narrow spectrum of taxonomic diversity and overlooking the most important feature of any phylogenetic study: variation. This drawback becomes even more important when dealing with neural traits which are highly adaptive; bat species sharing similar behaviors or occupying the same ecological niche are very likely to exhibit similar brain organization [Pirlot and Stephan, 1970; Stephan and Pirlot, 1970; Baron, 1974, 1977; Stephan et al., 1974, 1981, 1987a, b, 1991; Pirlot and Pottier, 1977; Eisenberg and Wilson, 1978; Pirlot and Nelson, 1980; Stephan and Nelson, 1981], and this could lead to biased estimations of the real phylogeny unless a correction is made for convergent adaptations. Our main objective is to compare the two phylogenetic scenarios using a relevant statistical framework [Lapointe and Legendre, 1990, 1992a] capable of considering adaptation and variation of brain quantitative characters. Assuming that the brain provides good phylogenetic descriptors, we should be able to assess the two competing hypotheses and determine which one is better. If bats actually are monophyletic, one would expect to find a significant correlation between the monophyletic model and a phylogeny based on brain characters. A significant correlation between the diphyletic model and the brain tree would provide new evidence for the independent origin of megabats and microbats.

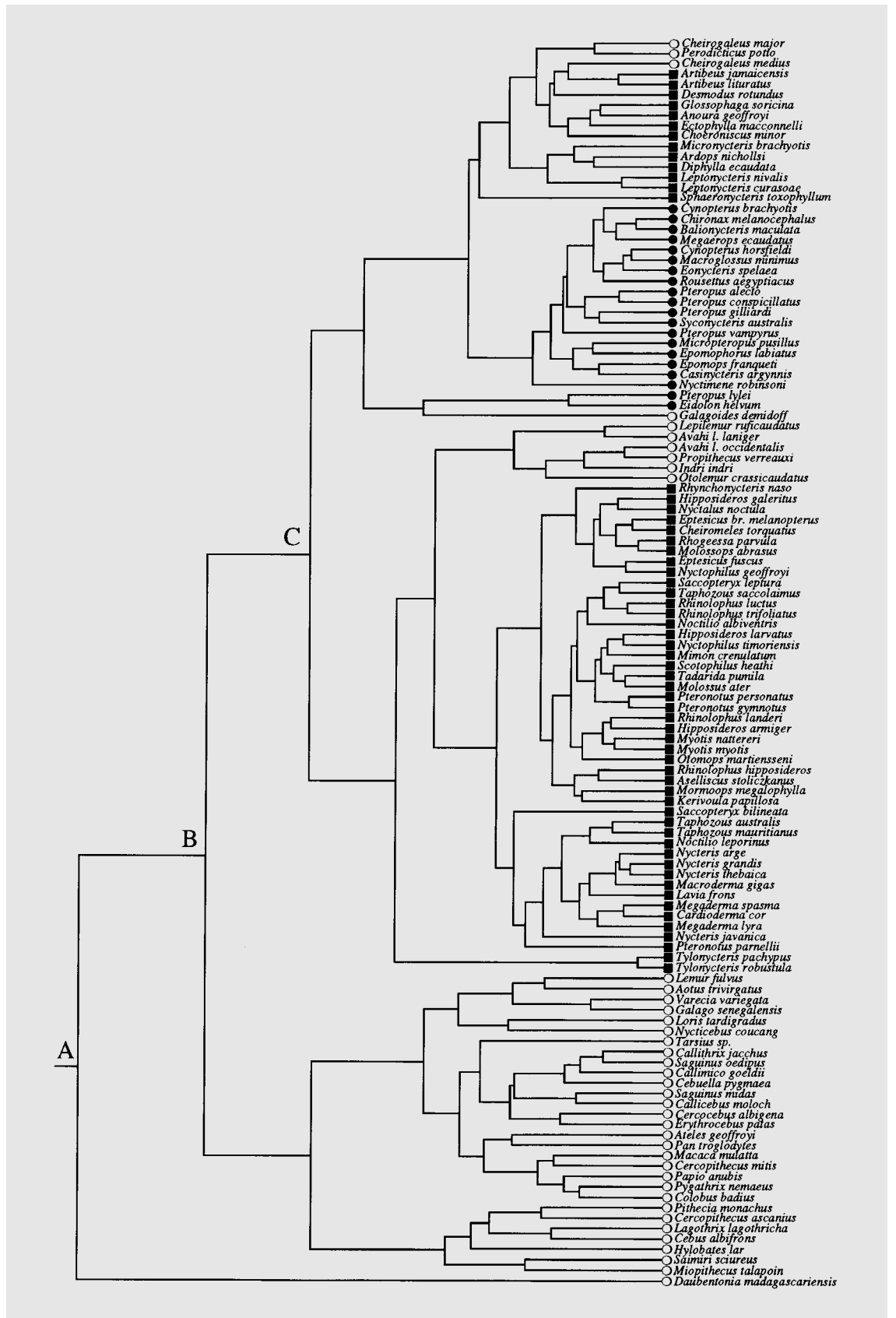
Materials and Methods

Forty species of primates (twelve families), twenty megabats (one family) and sixty microbats (nine families) were included in our analyses [data from Stephan et al., 1988; Baron et al., 1996]. These species represent all the major families and cover the entire range of ecological adaptations of bats and primates.

Neural Characters

Twelve variables representing brain component volumes were measured in each species. These include the classic subdivisions of the brain: medulla oblongata, cerebellum, mesencephalon, diencephalon and telencephalon. The telencephalic component was further subdivided into septum, striatum, hippocampus, amygdala, paleocortex, schizocortex, neocortex and bulbus olfactorius. Character polarity was defined with respect to a basal group of Insectivora (Tenrecinae) which possess the most primitive brain among extant mammals [Stephan et al., 1991]. The ratios of the actual components in any species to the expected size in the corresponding basal group were treated as size indices [Stephan, 1967], readily showing whether a given brain component is more or less developed than in a hypothetical average primitive stock of species of the same body mass.

Fig. 2. Phylogenetic tree based on size-corrected brain characters. The capital letters A, B and C refer to specific dichotomies discussed in the text. ○ = Primates; ● = megabats; ■ = microbats.



2

Correction for ecological differences among species was applied using an approach similar to that used to correct for allometric differences. To remove the effect of a species diet [Wilson, 1973; Norberg and Rayner, 1987; Stephan et al., 1988] on neural characters, an average size component was computed for each variable and for all different feeding habits (omnivores, folivores, frugivores, nectarivores, aerial insectivores, gleaning insectivores, carnivores, and sanguinivores). The ratios of brain components in any given species to the average size of that component in each feeding category formed ecologically-corrected variables that were used in further analyses.

Statistical and Phylogenetic Analyses

A pairwise resemblance matrix [Gower, 1971] was computed from the size-corrected characters (and/or ecologically-corrected variables) to obtain estimates of brain similarity among bats and primates. The least-squares method of De Soete [1983] was then used to derive a phylogenetic tree from the similarity matrix. The tree obtained could then be tested against the competing hypotheses (fig. 1) to assess the statistical significance of both the monophyletic and diphyletic hypotheses. The comparison test evaluates whether the actual correlation between the brain tree and each phylogenetic hypothesis is significantly different (i.e. larger or smaller) than that between a random phylogeny [Lapointe and Legendre, 1991] and the hypothetical models (fig. 1C, D) [Legendre et al., 1994; Legendre and Lapointe, 1995]. The null hypothesis of no correlation is rejected when the actual value of the statistic is as extreme or more extreme than most (e.g. 95%) of the correlation values obtained through randomization [Lapointe and Legendre, 1992b]. In our case, a contrast matrix (fig. 1E) was built to consider both hypotheses simultaneously instead of computing two tests; a positive correlation between the contrast model and the brain phylogeny would corroborate the monophyletic hypothesis, whereas a negative correlation would support the diphyletic scenario. The tests were one-tailed.

Results

The phylogenetic tree obtained from the size-corrected brain data is presented in figure 2. At first sight, no clear separation of primates, megabats and microbats is obvious from this tree. The first dichotomy (fig. 2A) isolates the aye-aye (*Daubentonia madagascariensis*) from all other species considered in the analysis, confirming its unique position among primates [Oxnard, 1981]. The second furcation (fig. 2B) separates a set of 29 primates from a second group of species encompassing all bats and some strepsirrhine primates. This latter group can be further divided into two well-defined subsets (fig. 2C), the first represented by 47 insectivorous microbats and 6 folivorous primates, the second composed of all 20 megabats, 4 primates, and 13 microbats that all share a primarily frugivorous and/or nectarivorous diet.

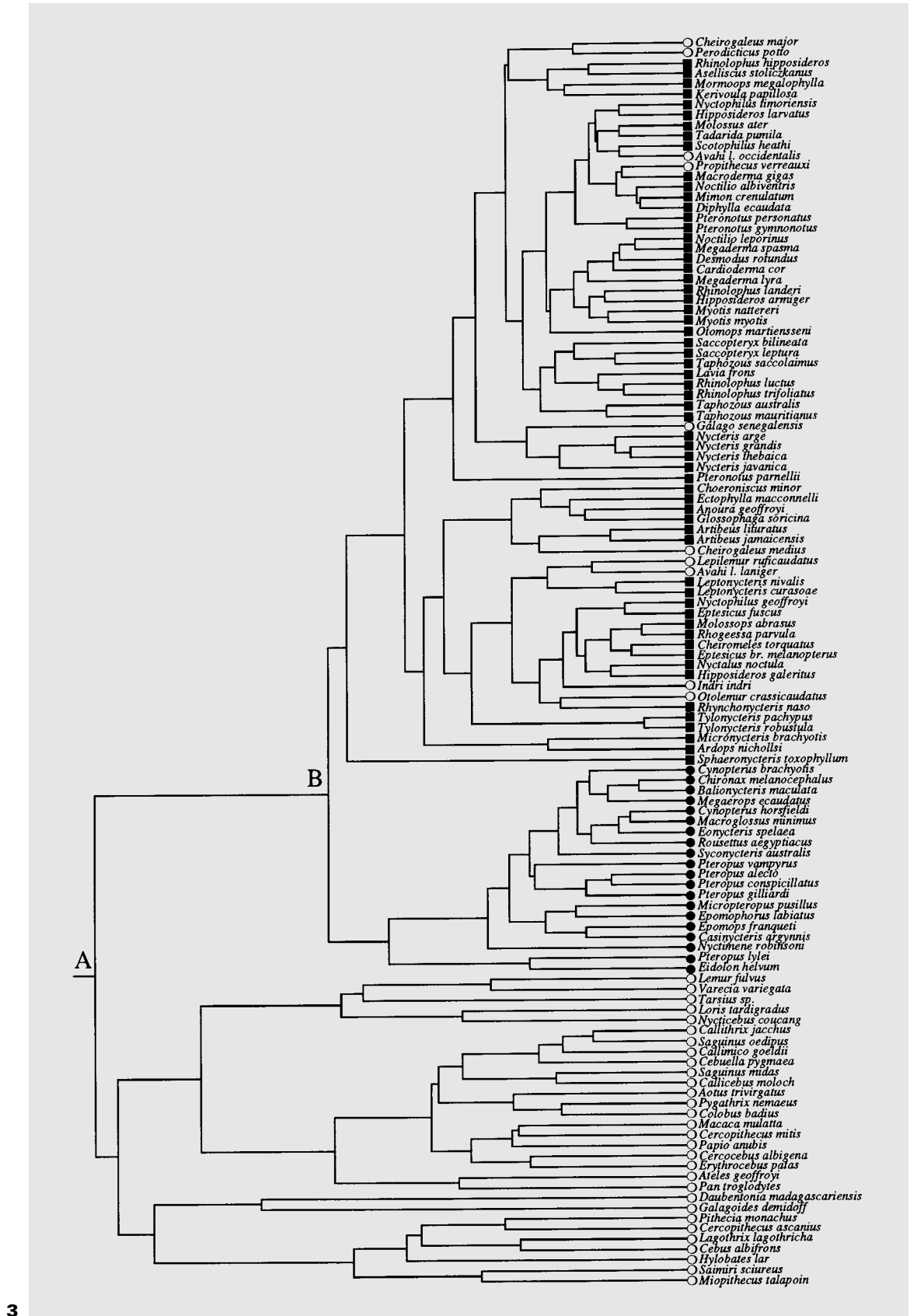
These associations reflect obvious adaptive convergences of the brain to trophic niches. Instead of a taxonomic characterization, one could clearly distinguish a folivorous,

an insectivorous/carnivorous, and a frugivorous/nectarivorous clade based on the size-corrected brain data. We have thus applied an 'ecological correction' to the data, in order to test for the significance of trophic adaptations on the derived phylogeny. If brain characters are good phylogenetic descriptors, one should find no difference between the trees derived from the original and corrected data sets based on species' diets. A difference between these solutions would imply a significant adaptation effect. If this is the case, ecologically-corrected data should be used, bearing less confounding ecological noise and possibly more phylogenetic information.

A new phylogeny (fig. 3) was derived from the ecologically-corrected characters to improve the first solution. That second tree is very different from the initial one. The first dichotomies separate most primates from the bats (fig. 3A), and all megabats from microbats (fig. 3B). This phylogeny is in agreement with the monophyletic model, with the exception of ten strepsirrhine primates that still occur within the microbat clade reflecting ecological affinities other than simply diet. Different corrections based on other eco-ethological characters such as activity period or locomotion type [Stephan et al., 1988] did not succeed in improving this solution. However, further analyses based on independent observations [Frahm et al., 1984; Stephan et al., 1984] revealed a consistent pattern in the brain development of these primates species. Indeed, the ten strepsirrhines falling within the microbat clade were found to share a significantly reduced area striata (primary visual cortex) in comparison with all other primates of the same taxonomic group ($t = -5.688$, $p < 0.0001$). The same ten species were also shown to be statistically different from other strepsirrhine primates in terms of neocortical volume ($t = -5.288$, $p < 0.0001$) and development of the corpus geniculatum laterale ($t = -3.863$, $p < 0.0007$).

Statistical evaluation provided significance levels for the two phylogenetics with respect to the competing hypotheses. The phylogenetic trees (fig. 2, 3) were thus tested against the contrast model (fig. 1E) to assess whether the monophyletic or the diphyletic model is better supported by our brain data. A significant positive correlation was obtained with both size-corrected ($r = 0.148$, $p = 0.001$) and diet-corrected variables ($r = 0.219$, $p = 0.001$), in support of the monophyletic origin of bats (all tests were based on 999 permutations). The fit to the monophyletic model increases

Fig. 3. Phylogenetic tree based on ecologically-corrected brain characters. The capital letters A and B refer to specific dichotomies discussed in the text. ○ = Primates; ● = megabats; ■ = microbats.



when correcting for species diet, but the opposite effect is observed for the alternative hypothesis. Compared to only 10 mismatches between the brain phylogeny and the monophyletic scenario, 30 species rearrangements would have been required to perfectly match the diphyletic model (moving 10 primates and all megabats to the primate clade). The monophyletic hypothesis should be preferred to explain bat evolution as it is the most parsimonious (fewer rearrangements necessary) and most likely model given our brain data. On the other hand, the diphyletic model is not correlated to the brain phylogeny to a greater extent than random trees.

Discussion

Major forces in chiropteran evolution have been those associated with flight and feeding habits [Gillette, 1977; Findley and Wilson, 1982; Norberg and Rayner, 1987]. For this reason, the classification of bats and their separation from other mammalian orders has relied mostly upon flight-related characters, whereas species relationships can often be correlated solely with diets and feeding strategies [Freeman, 1981]. Most earlier studies on bat phylogeny have been based on adaptive traits associated with flight [e.g. Thewissen and Babcock, 1991]. Therefore, the conclusions of these studies might be biased by a conflation of anatomical, physiological, ecological and behavioral constraints on evolution. It is possible for similar convergent adaptations to have occurred independently in two different lineages of mammals sharing the same lifestyle. Resolution of the bat phylogeny thus needs to be addressed with characters not associated directly with flight or diet.

Brain traits have been used extensively to study mammalian evolution [see Johnson et al., 1994, and references therein]. However, their use in chiropterans remains controversial. For example, Pettigrew [1986] compared neural traits among various mammal species to support his 'flying primate hypothesis'. According to his studies [Pettigrew and Jamieson, 1987; Pettigrew et al., 1989], primates and megachiropterans should be considered sister-groups because they share a similar retinotectal projection [see Pettigrew, 1986; but also Thiele et al., 1991]. However, these findings were falsified by every molecular study that has been published to date [for a review, see Honeycutt and Adkins, 1993]. Thus, whether the chiropteran brain can provide sound characters for phylogenetic analysis remains a question. If bats are really monophyletic, as indicated by molecular data, neural characters must be convergent in megabats and primates. On the other hand, if Pettigrew is

right, the implications are that DNA is homoplastic in bats and that flight has evolved twice in mammals.

It is now recognized that animals with a high metabolic rate (e.g. flying animals like bats) have GC-enriched DNA [Bernardi, 1993]. According to Pettigrew [1994], this condition is convergent in bats and does not reflect shared ancestry. Therefore, chiropterans are not monophyletic and the so-called 'flying DNA hypothesis' does not falsify the 'flying primate hypothesis'. Different studies have assessed this molecular issue. On the one hand, this problem was addressed with a new phylogenetic method [Steel et al., 1993] designed to correct for such base-compositional biases. The results were consistent with all other molecular studies and confirmed the monophyletic status of all chiropterans [Van den Bussche et al., 1998]. On the other hand, an experimental approach was used to fractionate the DNA into AT- and GC-enriched fractions, and the two components were independently analyzed to assess their effect on phylogenetic relationships [Pettigrew and Kirsch, 1995, 1998; Kirsch and Pettigrew, 1998]. Interestingly, the results were similar with both fractions of DNA and also confirmed bat monophyly. However, these analyses also revealed that microbats could be paraphyletic [Hutcheon et al., 1998], as some rhinolophoids seem to be more closely related to pteropodid megabats than they are to other microchiropterans. If this is true, flight would have evolved only once in mammals, but echolocation would have evolved twice in microbats [for a review of echolocation in bats, see Arita and Fenton, 1997].

In the light of these results, our study was designed to address the problem of bat evolution with a new perspective. We used quantitative neural characters (as opposed to qualitative traits used in earlier studies) to assess the phylogenetic position of megabats and microbats with respect to primates, but we were also interested in evaluating the general usefulness of brain component volumes for phylogenetic studies. Our results are convincing on both grounds.

For one, we have shown that a reasonable phylogeny can be derived from quantitative brain characters. Indeed, our tree is in agreement with the current classification of bats and primates, except for a few species which do not fall into place. We were able to clearly identify dietary groups when size-corrected data were used, whereas accepted taxonomic groups were obtained with ecologically-corrected characters. Interestingly, it appears that neocortex development in primates [see Barton, 1996] is mainly responsible for their separation from bats, and to a lesser extent for the separation of megabats and microbats. The importance of cortical and non-cortical visual areas of the brain represents a major factor that determines the neurological similarities among primate and bat species [Barton et al., 1995]. Further analyses

of the area striata and corpus geniculatum laterale revealed that these brain components are reduced in ten strepsirhine primates that fell into the microbat clade in the ecologically-corrected phylogeny. From a neurological standpoint, it thus seems that brain component volumes are adaptive characters that reflect phylogenetic relationships as well as ecological affinities among bat and primate species.

More importantly, we have established statistically that megabats are more closely related to microbats than either group is to the primates. This conclusion remains valid when size-corrected brain data are analyzed or when an ecological correction is used to account for different feeding habits. We have suggested that correcting for such homoplastic events could improve the accuracy of the phylogenetic analysis. In this particular case, the monophyletic

hypothesis was more strongly supported with the corrected data. These results corroborate all molecular studies to date, but contradict neurological evidence based on qualitative characters. On the basis of our quantitative brain characters, it is clear that megabats are not flying primates.

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