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Are Megabats Big?

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Traditionally, bats (Order Chiroptera) are divided into two suborders, Megachiroptera ("megabats") and Microchiroptera, and this nomenclature suggests a consistent difference in body size. To test whether megabats are, in fact, significantly larger than other bats, we compared them with respect to average body mass (log transformed), using both conventional and phylogenetic statistics. Because bat phylogeny is controversial, including the position of megabats, we employed several analyses. First, we derived two generic-level topologies for 101 genera, one with megabats as the sister of all other bats ("morphological" tree), the other with megabats as the sister of one specific group of microbats, the Rhinolophoidea ("molecular" tree). Second, we used a recently published "supertree" that allowed us to analyze body mass data for 656 species. In addition, because the way body mass has evolved is generally unknown, we employed several sets of arbitrary branch lengths on both topologies, as well as transformations of the branches intended to mimic particular models of character evolution. Irrespective of the topology or branch lengths used, log body mass showed highly significant phylogenetic signal for both generic and species-level analyses (all $P \leq 0.001$). Conventional statistics indicated that megabats were indeed larger than other bats ($P \ll 0.001$). Phylogenetic analyses supported this difference only when performed with certain branch lengths, thus demonstrating that careful consideration of the branch lengths used in a comparative analysis can enhance statistical power. A conventional Levene's test indicated that log body mass was more variable in megabats as compared with other bats ($P = 0.075$ for generic-level data set, $P \ll 0.001$ for species-level). A phylogenetic equivalent, which gauges the amount of morphospace occupied (or average minimum rate of evolution) relative to topology and branch lengths specified, indicated no significant difference for the generic analyses, but did indicate a difference for some of the species-level analyses. The ancestral bat is estimated to have been approximately 20–23 g in body mass (95% confidence interval approximately 9–51 g).

KEY WORDS: Body size, comparative method, Chiroptera, disparity, echolocation, independent contrast, Megachiroptera, Microchiroptera, phylogenetic signal, supertree, systematics.

INTRODUCTION

Dobson's subordinal division of the Chiroptera into Megachiroptera (Pteropodidae) and Microchiroptera (16 other families) represents one of the most fundamental taxonomic distinctions in any order of mammals (Dobson, 1875). The morphological differences between the two suborders have been well documented, and are listed exhaustively in Pettigrew *et al.* (1989). Although body size per se has not been used as a character in phylogenetic analyses,

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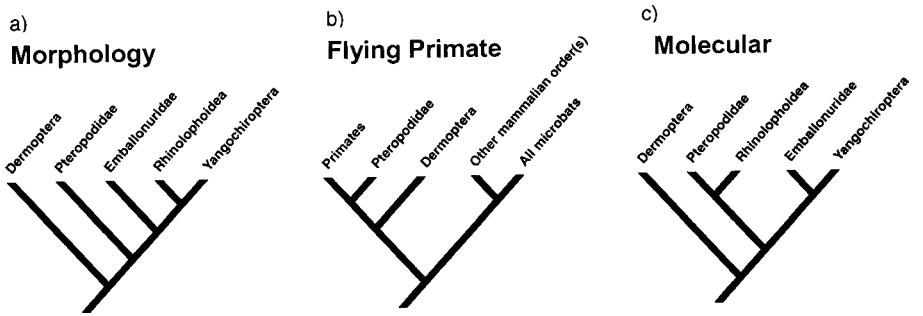

Fig. 1. Three alternative phylogenetic hypotheses for the Chiroptera illustrating differences in opinion with regard to the relative position of Pteropodidae (megabats). From (a) Simmons and Geisler (1998) based primarily on morphological data, (b) Pettigrew (1989) based primarily on neuroanatomical data, and (c) Hutcheon *et al*. (1998) based on single-copy DNA hybridization.

many "megabats" are large, and large body size is generally considered a characteristic of the group (e.g., see Hill and Smith, 1980; Altringham, 1996). Nonetheless, to our knowledge, a formal statistical comparison of the body sizes of megabats with other bats has never been presented.

Moreover, during the last decade, a lively debate has centered on the phylogenetic position of the megachiropteran bats (Pettigrew, 1991a,b; Simmons, 1994; Hutcheon *et al*., 1998). Three basic positions have arisen, which we will characterize as the "morphological" hypothesis, the "flying-primate" hypothesis and the "molecular" hypothesis (Fig. 1). The "morphological" hypothesis (Fig. 1(a)) is characterized by the traditional and primarily anatomically based studies of bats and designates the Megachiroptera (Pteropodidae) as the sister group to the Microchiroptera (all other bat families). This placement of Pteropodidae was also derived in the "supertree" analysis of Jones *et al*. (2002). The "flying primate" hypothesis (Fig. 1(b)), based primarily on characters of the optic tract, suggests that the megabats are not truly bats and are in fact a basal lineage of the primate radiation. The "molecular" hypothesis (Fig. 1(c)) is based on several recent molecular studies (Hutcheon *et al*., 1998; Kirsch *et al*., 1998; Teeling *et al*., 2000, 2002, 2003; Springer *et al*., 2001; Hutcheon and Kirsch, 2004; Van Den Bussche and Hoofer, 2004), all of which arrive at the conclusion that the megabats are most closely related to one specific group of microbats, the Rhinolophoidea.

The "flying primate" hypothesis advanced by Pettigrew *et al*. (1989) is concerned with bat systematics and, thus, might arguably warrant inclusion in this analysis. However, because Pettigrew *et al*. (1989) advocate the polyphyly of bats, an analysis based on their hypothesized phylogenetic relationships is impeded by the problem of choosing and representing outgroups. Thus, although we recognize the contributions of Pettigrew and colleagues as an important alternative hypothesis of chiropteran phylogeny, we have opted not to include it in this analysis, and have concentrated instead on phylogenies that assume chiropteran monophyly.

Perhaps one of the greatest objections to the "molecular" hypothesis of bat relationships is a perceptual-nomenclatural one, that the megabats are so much larger than the microbats that they most likely form a separate evolutionary lineage. Another objection to the pairing of pteropodids and rhinolophoids is the implied loss or multiple gain of

echolocation in the Chiroptera. Because echolocation is, to some extent, functionally constrained by body size (Barclay and Brigham, 1991; Jones, 1994), these arguments really constitute, to that extent, two sides of the same coin. The following analysis is a first step towards examining the distribution of body sizes among bats as a whole, ultimately relating the evolution of bat body size to echolocation ability.

The simplest way to compare the body sizes of megachiropteran and other bats is by conventional analysis of variance (ANOVA). However, one assumption of such a statistical analysis is that the data points (generic averages, in the present case: see Methods) in each group are independent and identically distributed. Given that all existing phylogenetic information indicates hierarchical structure within the various subclades of bats, including the Pteropodidae, the assumption of independence is probably unwarranted. This sort of realization has led to the adoption of phylogenetically based statistical methods for most comparative studies (reviews in Harvey and Pagel, 1991; Garland *et al*., 1999).

A conventional statistical analysis can be viewed as a phylogenetic analysis that assumes a particular phylogeny, i.e., a "star" or "comb" with no hierarchical structure and equal-length branches (Garland *et al*., 1999). The alternative of performing an analysis with a specified hierarchical topology requires knowledge of, or an assumption about, the length of each branch on the tree. Moreover, these branch lengths need to be in units proportional to expected variance of evolution for the character of interest, and this corresponds directly to divergence times only under particular models of character evolution, such as simple Brownian motion (Felsenstein, 1985; Garland *et al*., 1992, 1993). Although for most clades existing information may make it very clear that a star is not a good model for their phylogenetic history, that same information may not provide estimates of branch lengths that meet the foregoing criteria. Such is the case for bats.

Given a lack "real" branch lengths (e.g., estimates of divergence times) that might be used for a phylogenetic analysis of character evolution, one can employ arbitrary branch lengths, and several different types are in common usage (see Methods). Recent studies have emphasized the approach of comparing the fit of trees with alternate sets of branch lengths (which constitute alternate models of character evolution), and preferring the results of analyses based on the best-fitting trees. As an additional refinement, arbitrary "starter" branch lengths can be transformed, with the best-fitting transform preferred for analyses. Finally, the fit of the best-fitting tree can be compared with the fit of a star phylogeny as a strong guide to which analysis is more reliable (Freckleton *et al*., 2002; Blomberg *et al*., 2003). Such is the approach adopted herein.

METHODS

We used two different compilations of body masses, the first based on generic averages and the second based on species-level data. For the first, male and female body masses were obtained from Silva and Downing (1995). In cases with multiple data points per species, values were averaged (not weighted by sample size), separately by sex. Multiple species within genera were then averaged (not weighted by sample size) to obtain generic mean values. This is equivalent to assuming a star phylogeny within each genus, and also that each genus is monophyletic. All genera used ($N = 101$) were assigned a unique two-character code for use in later analyses (see Table I). The issue of bat taxonomy itself is contentious, however, we elected to use the generic names from Wilson and Reeder (1993).

Table I. Averaged Body Masses for 101 Genera of Bats (kg)

Genus	Abbreviation	Male body mass	Female body mass	*n* Males	*n* Females	*n* Species
Eidolon	Ei	0.241	0.205	3	4	1
Pteropus	Pt	1.080	0.844	20	20	15
Acerodon	Ac	0.759	0.759	4	4	2
Rousettus	Ro	0.102	0.094	18	16	5
Megaloglossus	Mg	0.014	0.013	7	6	1
Epomophorus	Ep	0.087	0.071	28	27	4
Eonycteris	Eo	0.065	0.062	6	7	2
Dobsonia	Do	0.329	0.233	7	6	4
Cynopterus	Cy	0.096	0.102	14	14	4
Sphaerias	Sp	0.026	0.030	1	1	1
Macroglossus	Mc	0.016	0.017	8	7	2
Syconycteris	Sy	0.016	0.016	2	3	1
Myonycteris	My	0.071	0.041	5	5	1
Paranyctimene	Pn	0.025	0.026	2	2	1
Nyctimene	Ny	0.050	0.051	5	4	6
Rhinopoma	Rp	0.015	0.016	6	5	3
Craseonycteris	Cs	0.002	0.002	1	1	1
Nycteris	Nt	0.013	0.013	22	20	8
Megaderma	Md	0.042	0.043	7	8	2
Macroderma	Mr	0.150	0.150	3	3	1
Cardioderma	Cd	0.026	0.023	2	2	1
Lavia	Lv	0.020	0.025	1	1	1
Rhinolophus	Rh	0.011	0.011	86	86	43
Hipposideros	Hp	0.030	0.024	47	48	28
Asellia	As	0.020	0.019	4	4	1
Aseliscus	Al	0.007	0.007	2	3	2
Cloeotis	Cl	0.004	0.004	2	2	1
Triaenops	Tr	0.012	0.012	1	1	1
Coelops	Cp	0.007	0.008	2	1	1
Noctilio	Nc	0.051	0.039	15	14	2
Pteronotus	Po	0.032	0.030	10	10	4
Mormopterus	Mm	0.017	0.016	3	3	1
Mystacina	Ms	0.012	0.012	20	16	7
Macrotus	Mt	0.011	0.011	2	2	1
Lonchorhina	Lo	0.029	0.024	3	5	3
Macrophyllum	Mh	0.008	0.007	2	1	1
Lionycteris	Ly	0.007	0.006	2	2	1
Lonchophylla	Ln	0.025	0.022	3	2	1
Anoura	Ao	0.011	0.011	10	11	4
Lichonycteris	Lc	0.007	0.006	1	2	1
Hylonycteris	Hy	0.007	0.007	2	3	1
Scleronycteris	Sc	0.049	0.049	1	1	1
Glossophaga	Gl	0.013	0.013	14	14	4
Monophyllus	Mu	0.015	0.013	1	1	1
Leptonycteris	Le	0.022	0.022	5	5	3
Brachyphylla	Br	0.040	0.040	1	1	1
Carollia	Ci	0.016	0.018	19	14	4
Rhinophylla	Rl	0.009	0.010	2	2	1
Diphylla	Dp	0.026	0.026	1	2	1
Diaemus	Da	0.038	0.036	4	4	1
Desmodus	Ds	0.031	0.033	12	12	1
Tonatia	To	0.043	0.046	15	12	8
Mimon	Mi	0.032	0.042	6	4	2
Phyllostomus	Ph	0.079	0.086	13	13	4
Phylloderma	Pd	0.055	0.058	3	1	1
Trachops	Tc	0.036	0.036	4	4	1
Chrotopterus	Ch	0.052	0.054	6	6	1

Table I. Continued

Genus	Abbreviation	Male body mass	Female body mass	*n* Males	*n* Females	*n* Species
Vampyrum	Va	0.176	0.169	4	4	1
Choeroniscus	Ce	0.007	0.008	6	5	4
Choeronycteris	Cc	0.019	0.017	2	5	1
Sturnira	Su	0.039	0.041	23	20	9
Uroderma	Ur	0.033	0.034	7	7	2
Vampyrops	Vm	0.047	0.040	18	14	8
Vampyrodes	Vp	0.034	0.035	3	3	1
Vampyressa	Vy	0.037	0.041	7	6	5
Chiroderma	Ck	0.021	0.022	11	11	4
Ecotphylla	Ec	0.016	0.016	2	2	1
Mesophylla	Mf	0.006	0.007	2	3	1
Artibeus	Ab	0.038	0.041	49	39	14
Pygoderma	Py	0.019	0.019	3	3	1
Ametrida	Am	0.008	0.011	2	3	1
Centurio	Cu	0.022	0.018	4	1	1
Sphaeronycteris	Sn	0.015	0.015	2	2	1
Natalus	Na	0.005	0.006	5	3	2
Furipterus	Fu	0.003	0.003	3	2	1
Thyroptera	Ty	0.004	0.004	3	3	1
Kerivoula	Ke	0.004	0.005	10	10	9
Myotis	Mx	0.011	0.009	132	149	49
Miniopterus	Mq	0.016	0.013	25	23	9
Mormopterus	Mj	0.011	0.012	5	5	4
Molossus	Ml	0.039	0.041	25	26	8
Myopterus	Mw	0.011	0.011	1	1	1
Tadarida	Td	0.020	0.030	47	52	22
Chaerephon	Cn	0.020	0.030	1	1	1
Molossops	Mp	0.020	0.030	12	13	5
Otomops	Ot	0.033	0.025	1	1	1
Eumops	Eu	0.055	0.044	16	18	7
Promops	Pr	0.014	0.015	5	5	2
Mops	Mv	0.045	0.042	1	1	1
Cheiromeles	Cm	0.181	0.181	2	2	1
Taphozous	Tz	0.028	0.028	23	24	13
Emballonura	Em	0.005	0.005	4	4	3
Mosia	Mo	0.003	0.003	1	1	1
Coleura	Co	0.008	0.009	2	2	2
Rhynchonycteris	Rn	0.004	0.004	4	4	1
Centronycteris	Ct	0.023	0.023	1	1	1
Saccopteryx	Sx	0.006	0.007	11	10	3
Balantiopteryx	Bx	0.008	0.009	3	4	2
Diclidurus	Di	0.066	0.066	5	5	3
Cormura	Cr	0.017	0.018	3	3	1
Peropteryx	Pe	0.010	0.011	7	7	2

Note. First 15 genera (*Eidolon* through *Nyctimene*) are megabats (Pteropodidae). *n* males and *n* females refer to the number of values taken from Silva and Downing (1995) and may have included multiple individual bats.

Our first analysis employed generic averages because at the time we began it no comprehensive phylogenetic hypotheses for all species of bats are presently available. Since then, Jones *et al*. (2002) have presented a "supertree" derived by combining 105 estimates of bat phylogenetic relationships published since 1970. It includes a large number of soft polytomies and its overall topology differs from both the "morphological" and "molecular"

trees, an outcome predicted by Lapointe *et al*. (1999). Much recent debate has concerned the potential merits (Bininda-Edmonds *et al*., 2002) and disadvantages (Gatesy *et al*., 2002; Lavasseur *et al*., 2003; Weins, 2003; Gatesy and Springer, in press) of supertrees. In any case, we also used the supertree and the "combined" (male and female) body masses for 656 species (including three recently extinct species) presented by Smith *et al*. (2003). Although the topology of the Jones *et al*. (2002) supertree is like neither our "morphological" or "molecular" tree, it is important to note that it places Pteropodidae as the sister of all other bats, like our "morphological" tree.

For the generic analysis, we constructed two composite topologies for purposes of phylogenetically based statistical analyses. For the "morphological" tree (Fig. 2), in which the Pteropodidae ($N = 15$ genera) are depicted as the sister of all other bats ($N = 86$ genera), topology was determined using the interfamilial relationships implied by Simmons and Geisler (1998). Freeman (1981), Robbins and Sarich (1986), Griffiths (1992, 1994), Kirsch *et al*. (1995), Hand and Kirsch (1998), and Wetterer *et al*. (2000) were used to inform the within-family and intergeneric relationships. For the "molecular" tree (Fig. 3), the interfamilial relationships implied by Hutcheon *et al*. (1998) were used, along with the same intergeneric relationships. Because the interfamilial topology was based on an older study, the placement of Nycteridae does not reflect the result of recent molecular studies (Teeling *et al*., 2002, 2003; Hutcheon and Kirsch, 2004). However, given both the placement of this family (within a cluster of families) and the fact it is monogeneric (and thus represented by one tip), we consider it unlikely that the results obtained from this topology are misleading. Thus, the within-family topologies were identical in the morphological and molecular trees. In the context of the molecular tree, it is also of interest to compare the Pteropodidae with only their sister lineage, the Rhinolophoidea ($N = 14$ genera), so we created a "reduced molecular" tree with only 29 tips. In principle, this more focused comparison might yield increased statistical power to detect differences because it avoids using bats that are more distantly related (and might vary in body size for numerous reasons), but it may have less power because such extreme phylogenetic clumping of groups to be compared tends to reduce statistical power (see Garland *et al*., 1993; Vanhooydonck and Van Damme, 1999).

A *t*-test or conventional one-way analysis of variance (ANOVA) comparing two groups is statistically equivalent to a least-squares linear regression of the dependent variable on a 0–1 dummy variable coding for group membership, and the regression approach is perhaps the simplest to implement with phylogenetically independent contrasts (Felsenstein, 1985; Garland *et al*., 1992; examples in Garland *et al*., 1993). Therefore, we used the PDTREE module of the Phenotypic Diversity Analysis Program (PDAP) package (Garland *et al*., 1993, 1999; Garland and Ives, 2000) to implement the regression approach with phylogenetically independent contrasts, and thus to compare $\log_{10}$ male or female body masses between the megachiropteran ($N = 15$) genera and all other bat ($N = 86$) genera. Conventional regression statistics are also reported by PDTREE, and they can also be obtained from the contrasts calculations after first collapsing the phylogeny to a star with equal-length branches (Purvis and Garland, 1993). All P values for comparisons of mean body masses are one-tailed because the working hypothesis is that megabats are significantly larger than other bats.

As explained in Garland *et al*., (1993), for a two-group comparison of means, a simple nonparametric phylogenetic test is to ask whether the contrast between pteropodids and

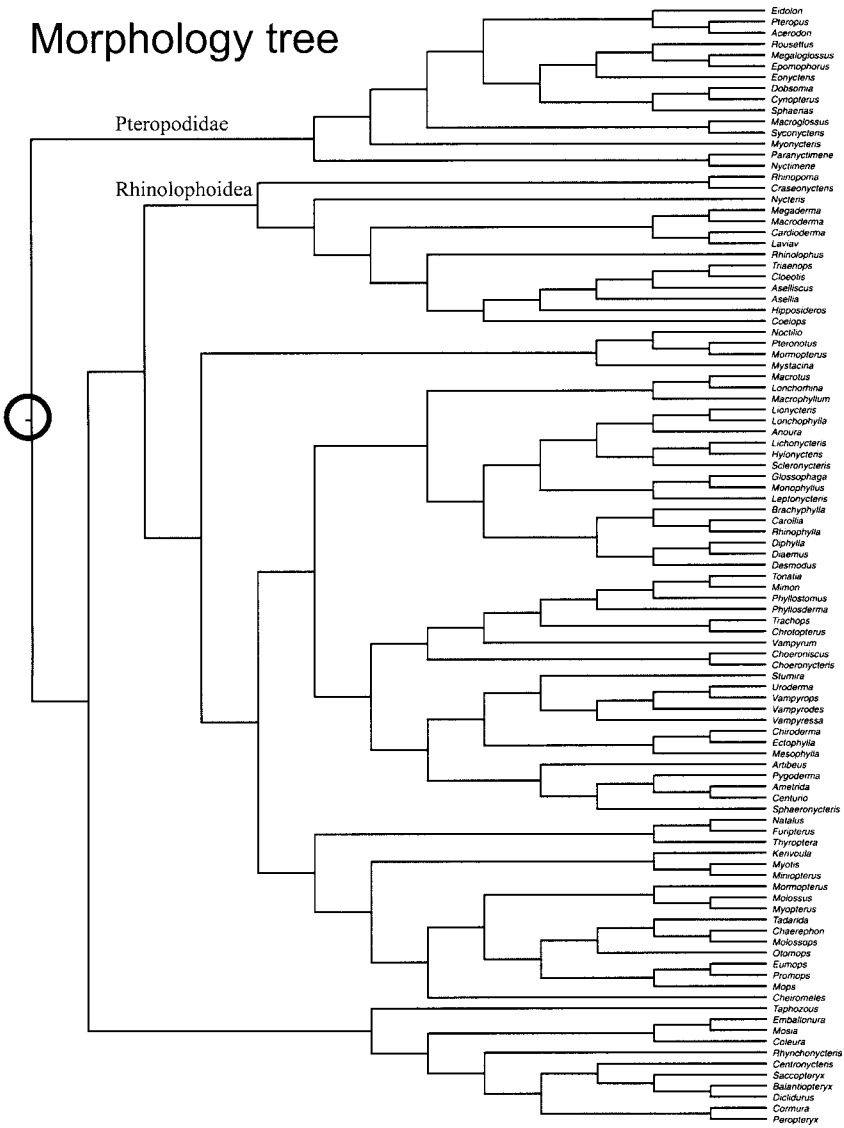

Fig. 2. The "morphological" tree, a composite phylogeny of 101 genera of bats. Interfamilial relationships are based on the phylogeny presented by Simmons and Geisler (1998). The 15 recognized genera of Pteropodidae ("megabats"), *Eidolon* through *Nytcimene*, are the sister clade to all other bats (86 genera). Circle indicates base node of the independent contrast that is used to test whether Pteropodidae are larger than other bats.

their sister lineage is unusually large in magnitude as compared with the distribution of contrasts within pteropodids and within other lineages. In the morphological tree (Fig. 2), the contrast of interest is across the root node of the entire tree. In the molecular tree, the contrast of interest is between the pteropodid bats and the rhinolophoid clade (Fig. 3). Both

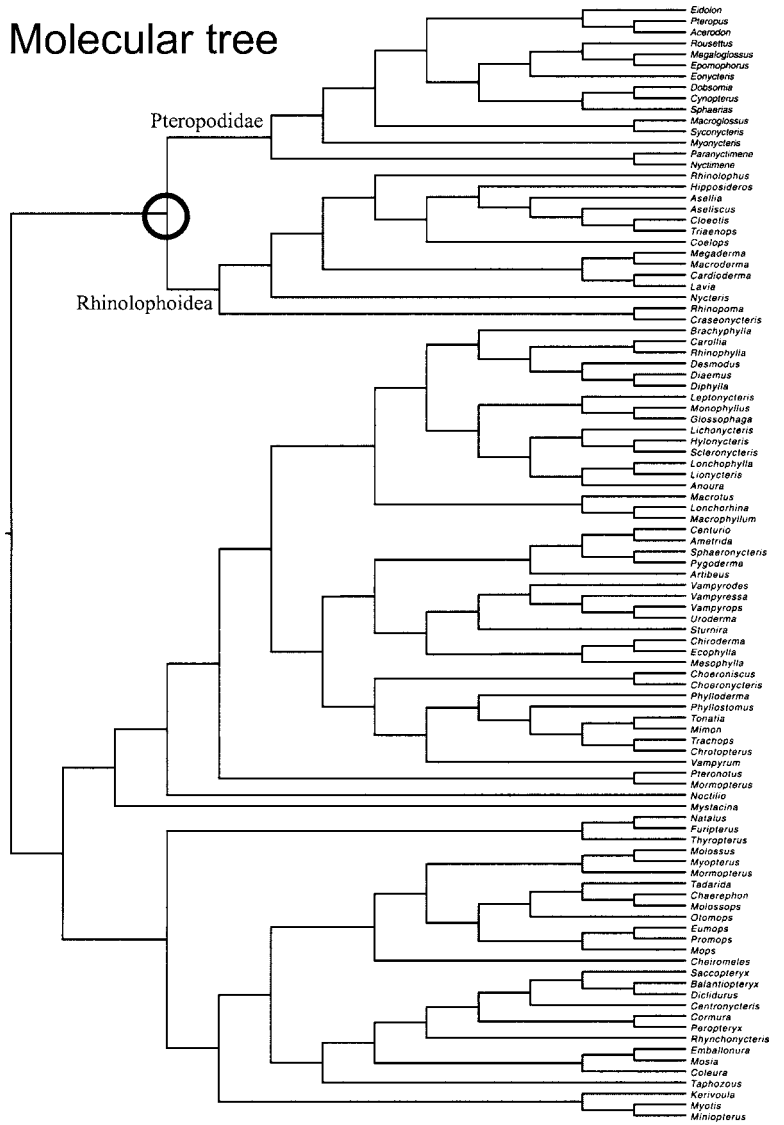

Fig. 3. The "molecular" tree, a composite phylogeny of 101 genera of bats. Interfamilial relationships are based on the phylogeny presented by Hutcheon *et al.* (1998). Megabats (Pteropodidae) are the sister clade to Rhinolophoidea, a lineage that includes 14 recognized genera (*Rhinolophus* through *Craseonycteris*). Circle indicates base node of the independent contrast that is used to test whether Pteropodidae are larger than other bats.

trees include 101 tip data points (generic averages), which yield 100 contrasts; hence, if the contrast between the megabats and its sister node was one of the five most extreme, then that was taken as evidence for megabats having a statistically larger average body mass at $P \leq 0.05$. For the reduced molecular tree with 29 tips, the contrast would have to be *the* most extreme ($1/28 = 0.0357$) to be considered statistically significant. For the supertree

analysis, which involves 655 contrasts, the megabat-sister contrast needed to be one of the 32 most extreme to be considered significant at $P < 0.05$.

Irrespective of any difference in mean body mass, megabats might occupy more or less "morphospace" as compared with other bats, which we addressed in a conventional statistical fashion with Levene's test (ONEWAY procedure in SPSS for Windows, Release 11.5.0). In a phylogenetic context, comparisons of amount of morphospace occupied are equivalent to comparisons of the rate of phenotypic evolution. All else being equal (e.g., no difference in speciation or extinction rates), a higher rate of evolution within a given clade will lead to a greater amount of morphospace being occupied, as compared with the expectation of a null model that specifies a particular topology, set of branch lengths, and model of character evolution. The model of character evolution appropriate for a given trait in a given clade is difficult to know (e.g., see Garland *et al*., 1993; Harvey and Rambaut, 2000; Blomberg *et al*., 2003), which makes it particularly difficult to test how morphologically diverse a given clade is in and of itself. However, it is easier to compare rates of evolution (amounts of morphospace occupied) across clades (Garland *et al*., 1992) because the test is then relative rather than absolute (although one must still assume that the topology, branch lengths, and model of character evolution are appropriate for generating a null expectation, and that nonrandom sampling (Ackerly, 2000) or differential speciation/extinction rates have not confounded comparisons (e.g., see Harvey and Rambaut, 1998)).

Following Garland *et al*. (1992), we compared rates of evolution between megabats and other bats (two-tailed) by comparing measures of central tendency for the distributions of the absolute values of the standardized phylogenetically independent contrasts. We first used non-parametric Mann–Whitney *U* tests (SPSS), as recommended by Garland *et al*. (1992), but, in an attempt to increase statistical power, we also employed conventional one-way ANOVAs after transformation of the values to improve normality. In the case of the generic morphological tree (Fig. 2) and the species-level supertree, the basal contrast between the Pteropodidae and all other bats was excluded, whereas for the generic molecular tree (Fig. 3) the contrast between Pteropodidae and their putative sister the Rhinolophoidea was excluded, as well as the contrast between that lineage and all other bats (i.e., again the basal contrast of the tree). As discussed in Garland *et al*. (1992), branch lengths in units of time have the greatest intuitive appeal for yielding "rate" comparisons, but we are really just dealing with ratios estimating (minimum) standardized amounts of divergence in log body mass, and the scaling metric to be used depends on the biological question one wishes to address. Nonetheless, because arbitrary branch lengths were used (see below), these analyses should be viewed with caution because the branch lengths could be differentially "wrong" within megabats vs. other bats (see Clobert *et al*., 1998; Garland and Ives, 2000). Another way to compare the amount of morphospace occupied is to use Levene's test in conjunction with computer simulations to create phylogenetically correct null distributions (Garland *et al*., 1993, e.g., the PDSIMUL and PDANOVA programs of their PDAP package; for a related example, see Harmon *et al*., 2003).

Phylogenetic methods for continuous-valued traits can also be used to estimate the values of hypothetical ancestors at various points on a phylogenetic tree (e.g., Schluter *et al*., 1997), including the root, interior nodes, and even points along a branch (Garland *et al*., 1999; Garland and Ives, 2000). We used PDTREE to estimate the values and 95% confidence intervals at the roots of the 101-tip morphological and molecular trees, and at the root of the 656-tip supertree. For the trees with Nee's branch lengths transformed under

the ACDC and OU models (see next paragraph), we subtracted 1 *df* for estimating the confidence intervals, although this may be only an approximate solution (cf. Grafen, 1989, p. 142).

Initially, four different sets of arbitrary branch lengths were employed on all three tree topologies for the generic data set (morphological, molecular, reduced molecular): Pagel's (1992, as shown in Figs. 2 and 3); all segments equal to one ("constant"); Grafen's (1989; each node is set at a depth equal to the number of descendant tips minus one); Nee's (cited in Purvis, 1995, p. 416; each node is set at a depth equal to the log of the number of descendant tips, as shown in Fig. 4) (for discussions of setting branch lengths, see Garland *et al*., 1992; Díaz-Uriarte and Garland, 1996, 1998). Of these, the Nee branch lengths performed best (see below), so we also used these as a starting point for implementation of the Ornstein-Uhlenbeck (OU) and Accelerating-Decelerating (ACDC) branch length transformations, as described in Blomberg *et al*. (2003; MatLab programs PHYSIGOU.M and PHYSIGACDC.M, respectively). These transformations are intended to mimic particular models of character evolution, although they can be applied to any types of "starter" branch, including the arbitrary ones of Nee, as done here. We did not perform all analyses with the OU or ACDC transforms on the supertree because of software limitations. However, we did estimate the ACDC transformation parameter and obtained a value (0.684) that makes very little change to the branch lengths. Hence, results reported for Nee's branch lengths should be similar to those that would result under transformation by the ACDC model of character evolution.

To gauge which branch lengths were the best justified for phylogenetic statistical analyses, we used two criteria. First, we computed the widely used diagnostic of Garland *et al*. (1992), which is the Pearson correlation (not through the origin) between the absolute values of the standardized contrasts and their standard deviations (square roots of sums of corrected branch lengths). A significant correlation indicates lack of fit of the tree to the tip data, e.g., because a Brownian motion model of character evolution along those branches is not an adequate descriptor of character evolution. For the full trees (101 tips, 100 contrasts), the 2-tailed critical value for 98 *df* is 0.197. For the reduced molecular tree (29 tips, 28 contrasts), the two-tailed critical value for 26 *df* is 0.374. For the supertree (656 tips, 655 contrasts), the two-tailed critical value for 653 *df* is 0.077; thus, very small correlations can be considered statistically significant.

Second, we computed the Mean Squared Error (MSE), which is equivalent to the variance of standardized independent contrasts once the trees have all been scaled such that the determinant of the variance-covariance matrix is a constant (Blomberg *et al*., 2003). Thus, a lower MSE indicates that a tree better fits the tip data. For a given topology, the relationship between these two alternate criteria for choosing branch lengths is not a direct one, although they often point in the same direction (A. R. Ives and T. Garland Jr., unpublished data). Moreover, both can be sensitive to influential points or outliers. Simulations studies have demonstrated the utility of the Garland *et al*. (1992) diagnostic (Díaz-Uriarte and Garland, 1996, 1998; Harvey and Rambaut, 2000), but have not yet compared its effectiveness with that of the criterion of minimum MSE.

For each combination of topology and branch lengths, we tested whether $\log_{10}$ (female, or combined for the supertree) body mass exhibited statistically significant phylogenetic signal (a tendency for related species to resemble each other: Blomberg and Garland, 2002) using the randomization test and associated MatLab program (PHYSIG.M) described in

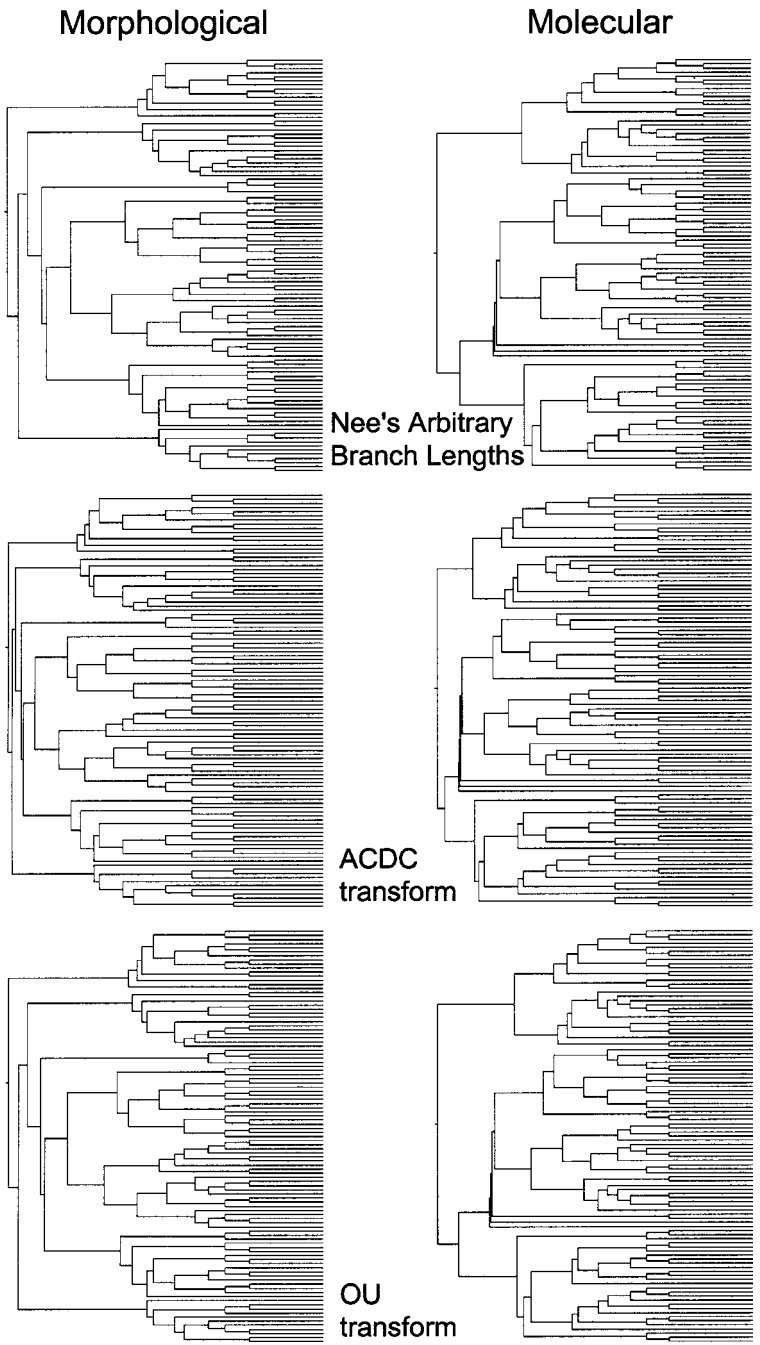

Fig. 4. Nee's arbitrary branch lengths (top) as transformed to optimize fit to the tip data ($\log_{10}$ generic average female body mass) under the Ornstein-Uhlenbeck (OU) and Accelerating-Decelerating (ACDC) models of character evolution (see text and Blomberg *et al*., 2003).

Blomberg *et al*. (2003) and 1000 permutations. We used the K statistic of Blomberg *et al*. (2003) as a descriptor of the amount of phylogenetic signal present in log body mass. A value of unity indicates that a trait has exactly the amount of signal expected under Brownian motion evolution along the specified topology and branch lengths, whereas values less than unity indicate less signal than expected, and values of K greater than unity indicate more.

RESULTS

A simple scatter-plot (male, female) of the generic-average body masses of bats shows ample overlap between the between pteropodids ($N = 15$) and other ($N = 86$) bats (Fig. 5). The two most extreme data points, both somewhat separated from the rest, represent the pteropodid genera *Pteropus* and *Acerodon*, both of which have average body masses of approximately 1 kg. Conventional ANOVA indicated a highly significant difference in mean generic body mass for both males ($F = 28.7$, $df = 1, 99$, $P \ll 0.0001$) and females ($F = 25.9, df = 1, 99, P \ll 0.0001$). As also shown in Fig. 5, male and female body masses were very strongly correlated (conventional $r = 0.992$ for log-transformed values), and a conventional paired t-test of male-female body masses indicated no significant difference between the sexes ($t = 0.046, df = 100$, two-tailed $P = 0.964$). Therefore, in the following we present results only for female generic averages.

Considering all 101 genera, for both the morphological and the molecular tree, the diagnostic test of Garland *et al*. (1992) indicated significant lack of fit with both Grafen's and Pagel's arbitrary branch lengths (Table II). Consistent with this, these branches also

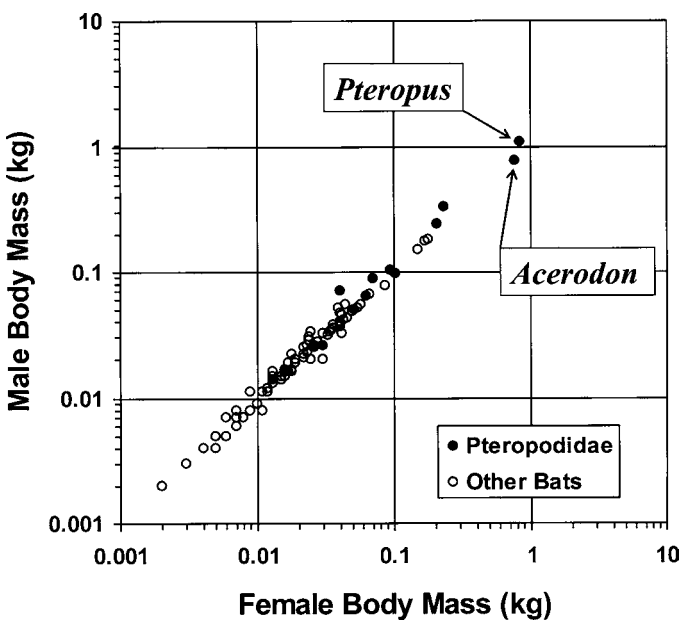

Fig. 5. Scatter-plot ($\log_{10}$ axes) of generic average male versus female body mass. Open circles are microbats, solid symbols are megabats.

Table II. Statistical Analyses of Generic-Average Body Masses and Tests for Adequacy of Various Sets of Branch Lengths

Topology	Branches	Diagnostic correlation	MSE	K	F for body mass comparison	One-tailed P for body mass comparison[a]	One-tailed P for rank contrast comparison	Lower CI 95%	Ancestral body mass (g)	Upper 95% CI
Star, $N = 101$			0.2231		25.92	1×10^{-6}	0.01			
Morphology $N = 101$	Constant	−0.006	0.1637	0.425	0.82	0.184	0.35	7.9	23.8	72.1
	Grafen	−0.312[b]	0.2107	0.080	0.06	0.402	0.67	0.2	21.4	2,010.0
	Pagel	−0.200[b]	0.1654	0.484	0.72	0.199	0.38	5.5	21.0	81.1
	Nee	−0.067	0.1530	0.698	1.28	0.130	0.26	8.3	21.4	55.5
	Nee ACDC[c]	−0.006	0.1487	1.026	3.25	0.037	0.10	12.0	21.5	38.6
	Nee OU[d]	0.042	0.1501	0.856	1.78	0.093	0.21	9.4	21.6	49.3
Molecular $N = 101$	Constant	−0.042	0.1663	0.484	2.00	0.080	0.19	4.8	15.0	46.4
	Grafen	−0.323[c]	0.2276	0.082	0.30	0.294	0.50	0.1	19.8	3,079.0
	Pagel	−0.234[c]	0.1755	0.460	1.27	0.132	0.22	5.1	18.6	68.2
	Nee	−0.122	0.1579	0.695	2.41	0.062	0.15	7.6	20.8	57.5
	Nee ACDC[e]	−0.139	0.1524	1.037	4.95	0.014	0.05	11.8	21.2	38.1
	Nee OU[f]	−0.031	0.1519	0.911	3.69	0.029	0.10	9.0	20.9	48.4
Star, $N = 29$			0.3795		13.12	0.0006	0.036			
Reduced	Constant	0.016	0.2193	0.943	1.36	0.127	0.286			
Molecular $N = 29$	Grafen	−0.328	0.3068	0.339	0.19	0.334	0.607			
	Pagel	−0.324	0.2663	0.733	0.82	0.187	0.429			
	Nee	−0.113	0.2310	1.102	1.47	0.118	0.286			
	Nee ACDC[g]	−0.291	0.2305	1.339	2.95	0.049	0.107			
	Nee OU[g]	−0.253	0.2296	1.330	2.19	0.076	0.214			

Note. Diagnostic correlation: values farther from zero are worse; Mean Squared Error: smaller values are better; K describes the amount of phylogenetic signal (larger indicates more; 1.00 is amount expected under Brownian motion character evolution); F and Ps are for comparing $\log_{10}$ generic average body masses of "megabats" (Pteropodidae) with all other bats (total $N = 101$ genera); and estimating the body mass of the ancestral bat at the root of the phylogenetic tree (backtransformed from logarithms).

[a] For the 101-tip trees, $df = 1, 99$, except for the ACDC- and OU-transformed trees, where df are reduced by one to account for estimation of the branch-length transformation parameter (see Díaz-Uriarte and Garland, 1996, 1998). For the reduced molecular tree with 29 tips, df are 1, 27 or 1, 26.

[b] Two-tailed $P < 0.05$, indicating significant lack of fit of branch lengths to tip data.

[c] Estimated ACDC transformation parameter was 0.168, which was significantly different from zero ($P < 0.001$) but not from unity ($P = 0.15$).

[d] Estimated OU transformation parameter was 0.945, which was significantly different from zero ($P < 0.001$) but not from unity ($P = 0.13$).

[e] Estimated ACDC transformation parameter was 0.141, which was significantly different from zero ($P < 0.001$) but not from unity ($P = 0.09$).

[f] Estimated OU transformation parameter was 0.925, which was significantly different from zero ($P < 0.001$) but not from unity ($P = 0.08$).

[g] ACDC and OU transformation parameters were not estimated for these trees; rather, they were produced by pruning directly from the 101-tip molecular tree.

exhibited the highest MSEs, indicating that they fit the data less well than did other types of branch lengths. Indeed, the MSEs with Grafen's branch lengths were as large as for a star phylogeny (Table II). As would be expected, the K statistic, which indicates the amount of phylogenetic signal relative to the expectation under Brownian motion evolution, varied inversely with the MSE. The trees with Grafen's arbitrary branch lengths were clear outliers, exhibiting very low K values. Nonetheless, in the randomization tests for significance of phylogenetic signal, all P values for the 101-tip trees were ≤ 0.001. Thus, even in the context of branch lengths that yield relatively poor fits to the tip data (relatively high MSE) and low estimated amount of phylogenetic signal (low K), a significant tendency for related species to resemble each other is still apparent. Thus, phylogenetically based statistical analyses are to be preferred. This conclusion is also indicated by the fact that the MSE on a star phylogeny is larger than for any of the hierarchical trees, except with Grafen's branch lengths, which clearly give poor fit to the data (Table II). Given the poor fit of both Grafen's and Pagel's branch lengths on both of the 101-tip trees, results with these branch lengths will not be considered further, but they are presented in Table II for completeness.

For the supertree, the diagnostic correlation indicated significant lack of fit for all branch lengths except constant (Table III). Nonetheless, MSEs were always much lower than on the star phylogeny, and the randomization test for phylogenetic signal was highly significant for all four types of arbitrary branch lengths. Therefore, as for the generic-level trees, phylogenetic statistical analyses should be more reliable than conventional ones. As with the 101-genus trees (Table II), Grafen's branch lengths yielded substantially worse diagnostic correlations, lower MSEs, and lower K values. Therefore, results with Grafen's branch lengths will not be mentioned further. (Note that the poor fit of Grafen's arbitrary branch lengths to the body mass data should not be taken as a criticism of his paper (Grafen, 1989). Rather, he intended them as one arbitrary way to begin if no "real" branch lengths were available, and he presumed they would be transformed to improve the fit to the tip data.)

For the genus-level morphological tree, which depicts megabats as the sister lineage to all other bats, the parametric comparison of log body mass was statistically significant only with Nee's arbitrary branch lengths transformed under the ACDC model of character evolution ($df = 1, 98$, one-tailed $P = 0.037$), while the rank comparison of the phylogenetically independent contrasts was never significant (smallest P was 0.10) (Table II). With the molecular tree, statistical significance in the parametric comparisons was attained with Nee's branch lengths as transformed under both the ACDC ($P = 0.014$) and OU ($P = 0.029$) models of character change. Figure 4 shows the ACDC and OU transformations of Nee's arbitrary branch lengths on both topologies. With the pruned molecular trees that included only Pteropodidae and their sister lineage ($N = 29$ total), the parametric comparison of body masses was still significant for the ACDC transform of Nee branches ($P = 0.049$), but not for the OU transform ($P = 0.076$), and none of the rank comparisons was significant (Table II).

For the body mass data associated with the supertree (Table III), analysis on a star phylogeny (equivalent to a conventional ANOVA) indicated a highly significant difference between megabats and other bats ($P = 4 \times 10^{-6}$). With constant branch lengths (the best-fitting ones), the megabat-other difference in $\log_{10}$ body mass was statistically significant ($P = 0.047$). The difference was also significant with Nee's arbitrary branch lengths ($P = 0.0004$), but not with Pagel's ($P = 0.112$).

Table III. Statistical Analyses of Species-Level Body Masses and Tests for Adequacy of Various Sets of Branch Lengths

Topology	Branches	Diagnostic correlation	MSE	K	F for body mass comparison	One-tailed P for body mass comparison[a]	One-tailed P for rank contrast comparison	Lower 95% CI	Ancestral body mass (g)	Upper 95% CI
Star, $N = 656$			0.3091		774.15	4×10^{-113}	0.0015			
Supertree $N = 656$	Constant	−0.056	0.0648	1.020	2.81	0.047	0.0977	9.9	22.5	51.4
	Grafen	−0.345[b]	0.1213	0.062	0.08	0.391	0.6336	0.03	21.0	12,723.2
	Pagel	−0.132[b]	0.0650	0.847	1.48	0.112	0.1893	6.7	19.2	54.7
	Nee	−0.122[b]	0.0688	1.913	11.43	0.0004	0.0076	11.4	20.1	35.2

Note. Diagnostic correlation: values farther from zero are worse; Mean Squared Error: smaller values are better; K describes the amount of phylogenetic signal (larger indicates more; 1.00 is amount expected under Brownian motion character evolution); F and Ps are for comparing $\log_{10}$ body masses of "megabats" (Pteropodidae) with all other bats; and estimating the body mass of the ancestral bat at the root of the phylogenetic tree (backtransformed from logarithms).

[a]For the 656-tip super trees, $df = 1, 654$.

[b]Two-tailed $P < 0.05$, indicating significant lack of fit of branch lengths to tip data.

As shown in Fig. 5, generic-average body mass on a logarithmic scale does not appear obviously more variable in pteropodids as compared with other bats, and a conventional Levene's test did not indicate a statistically significant difference in variance between the two groups (Levene statistic $= 3.23$, $df = 1, 99$, two-tailed $P = 0.075$). The phylogenetic comparisons also indicated no significant difference for either the morphological trees (absolute values of the log body mass contrasts were transformed by raising to the 0.4 power for the one-way ANOVAs; smallest two-tailed P was 0.082 with Nee's branch lengths OU-transformed) or the molecular trees (raised to the 0.5 power; smallest P was 0.120, also with Nee's branch lengths OU-transformed). The reduced molecular trees also indicated no difference (all parametric $P > 0.6$).

In the supertree analysis of 656 species, a conventional Levene's test indicated a highly significant difference in variance between the two groups (Levene statistic $= 74.73$, $df = 1$, 654, $P = 4 \times 10^{-17}$). For the phylogenetic comparison, we did not consider Grafen's arbitrary branch lengths because of their relatively poor fit (Table III). For the other branch lengths, results varied depending on the analysis, but generally suggested a significant difference in variance (constant: Mann–Whitney $U = 31{,}424$, $Z = 1.845$, asymptotic two-tailed $P = 0.065$; ANOVA of absolute values of log mass contrasts raised to the 0.4 power, $F = 2.55$, $df = 1$, 652, two-tailed $P = 0.111$; Pagel's: $U = 29{,}240$, $Z = 2.962$, $P = 0.003$; $F = 7.18$, $P = 0.008$; Nee's: $U = 31{,}015$, $Z = 2.054$, $P = 0.040$; $F = 3.561$, $P = 0.060$).

Based on generic averages and considering the best-fitting branch lengths (ACDC and OU transformations of Nee's), the estimated ancestral bat body mass was about 21 g with a 95% confidence interval of about 9–49 g (Table II). Based on the 656-species supertree analysis with constant branch lengths, ancestral mass was about 23 g with a 95% confidence interval of 10–51 g (Table III).

DISCUSSION

Conventional statistical analyses indicate that megabats (Pteropodidae) average significantly larger in log body mass, as compared with all other bats ($P = 1 \times 10^{-6}$ for the generic-level analysis, $P = 4 \times 10^{-113}$ for the species-level analysis). Significance levels for all phylogenetic analyses were much larger (Tables II and III). This general pattern of conventional analyses—which effectively assume a star phylogeny with no hierarchical relationships—yielding smaller P values is typical of comparative studies that compare mean values of clades or other groups that are strongly phylogenetically clumped (Garland *et al*., 1993; Vanhooydonck and Van Damme, 1999). Nevertheless, for the generic analyses with both the morphological and molecular topologies, one or more sets of branch lengths indicated significant ($P \leq 0.05$) differences, and these were the branches that provided the best fit to the tip data, based on the standard diagnostic test of Garland *et al*. (1992) and/or the criterion of lowest MSE (Blomberg *et al*., 2003). Significance levels were always lower for the 101-genus molecular tree as compared with the morphological tree, which presumably reflects the higher statistical power when the megabats occupy an interior position on the tree rather than being the outgroup to all other bats (Garland *et al*., 1993; Vanhooydonck and Van Damme, 1999). For the 656 species-level analysis, significant differences were also indicated with some branch lengths, especially the best-fitting ones (Table III). Thus, careful choice of branch lengths can improve power in phylogenetically based statistical analyses.

It is important to remember that the subordinal names Microchiroptera and Megachiroptera are taxonomic ranks and not descriptive diagnoses. Although the rank names would suggest a clear grouping of "large" and "small" bats into their respective taxa, they actually show considerable overlap in body size (Fig. 5). The Megachiroptera are comprised of one family (Pteropodidae), restricted to the Old World, and all are primarily frugivorous. The Microchiroptera are comprised of 16 families and, as a group, are found worldwide. One family within the Microchiroptera, the Phyllostomidae, has also evolved plant usage. The phyllostomid bats are restricted to the New World, and while many members of the family are frugivorous, some are partial frugivores, supplementing with insects, and a few are wholly insectivorous, carnivorous or vampires (see McNab, 2003). In her analysis of evolution in the Phyllostomidae, Freeman (2000) suggests that the ancestral phyllostomid was a relatively unspecialized insectivore, which later diversified into four novel eco-morphological groups, including frugivores. What the data instead seem to show is a continuum of body sizes between the phytophagous bats. If the pteropodid nectarivores represent one end of a continuum, surely the large fruit-eating bats represent the other. One might argue that, all else being equal, the comparative body sizes between the fruit-eating bats of the New and Old Worlds should be relatively similar. This begs the question of what ecological differences might exist between what might otherwise be considered to be fundamentally the same sort of animal: volant, nocturnal, frugivorous mammals. We suggest that "prey" (i.e., fruit) size and echolocation are likely to be important factors.

Barclay and Brigham (1991) argue persuasively that the small body size of insectivorous bats increases their efficiency in handling small insects. The same may hold true with fruits. The zoochorous fruits of the Old World are, on average, larger in mass than those of the New World (Mack, 1993). Therefore, the handling requirements for Old World fruits will likely require a larger body mass. Mack (1993) suggested that the larger body sizes of Old World frugivores might have driven the observed difference, but the opposite could just as easily be true; perhaps larger fruit sizes has driven larger body sizes, the cases of *Pteropus* and *Acerodon* representing the extreme of this scenario in the fruit-eating pteropodid bats (Marshall, 1983). This hypothesis could be tested in the future with quantitative data on fruit size, which are not presently available for all of the genera (let alone species) in our study.

Another important consideration is the use of echolocation. The New World phyllostomid bats utilize echolocation even while foraging (Kalko and Condon, 1999; Thies *et al*., 1998; von Helverson and von Helverson, 1999), whereas the Old World pteropodid bats do not echolocate. Previous studies (Barclay and Brigham, 1991; Jones, 1994) have noted the relationship between small body size and echolocation ability, the former being scaled with wing beat pulse and the frequency of the echolocation call If, in fact, large body size necessarily decreases echolocation efficiency, then there may be a dual constraint (echolocation and small fruit size) limiting the size of New World fruit-eating bats (Phyllostomidae). It should be noted, however, that not echolocating is not inconsistent with a small body size; some megabats—particularly nectarivores—are small, as evidenced by such small exemplars of the family as *Eonycteris spelaea*, which has a body mass of 0.057–0.066 kg (Silva and Downing, 1995; Smith *et al*., 2003).

Extreme members of the family Pteropodidae notwithstanding, it is clear that the megachiropteran bats are not uniformly large-bodied: pteropodid body sizes in Smith *et al*. (2003) range from 14.2 g (*Balionycteris maculata*) to 1,075 g (*Acerodon jubatus*), with

38 of 136 species (28%) being below 50 g. We suggest that the perception otherwise is fostered by traditional nomenclature (Megachiroptera vs. Microchiroptera) and the fact that the most visible members of the family are species in the anomalously large *Pteropus* (119–1,039 g). The origin of flight in bats, particularly with respect to echolocation, has been a topic of considerable debate (Norberg, 1990; Fenton *et al*., 1995; Speakman, 2001). Norberg (1994) notes that the ancient bats had short, broad wings as well as low aspect ratio and high wing loading. This would suggest that the ancient bats were capable of foraging in cluttered environments (Norberg and Rayner, 1987; Habersetzer and Storch, 1989). Based on the data in Smith *et al*. (2003), as used in our supertree analysis, extant bats range in body size from 2 g (*Kerivoula minuta*) to 1,075 g (*Acerodon jubatus*). J. D. Smith (1976) speculated on the nature of the putative *ur* bat, suggesting that it might have originated in the late Cretaceous and concluded that it was "emballonuroid like" but gave no indication as to what he considered a likely body size. In the Smith *et al*. (2003) data set, Emballonuridae range in size from 3.3 g (*Mosia nigrescens*) to 100 g (*Saccolaimus peli*). Although many of the above-cited works suggest a small size, we are not aware of any specific hypotheses about the body size of the ancestral bat. Considering the best fitting branch lengths (Nee's with ACDC or OU transform) in the generic-level analyses, we estimate that the ancestral bat was approximately 21 g (Table II), whereas the constant branch lengths in the species-level analysis lead to an estimate closer to 23 g. The 95% confidence intervals associated with those estimates range from about 9–51 g. This range of values includes most—but not all—of the emballonurids in Smith *et al*. (2003). The upper value of 51 g would include the mean body mass of several genera within the megabats, although several other genera of megabats and some genera of non-megabats are much larger than this and exhibit what could be considered a "derived" large body size (e.g., see Fig. 5).

In the species-level phylogenetic analysis, we found some evidence for a statistically significant difference in amount of morphospace occupied (or, equivalently, average rate of evolution *sensu* Garland *et al*. (1992)) between megabats and other bats. This difference was not apparent in the generic-level analysis, probably because of lower statistical power when using 101 genera vs. 656 species. As noted in the Methods section, these analyses must be viewed cautiously because arbitrary branch lengths were employed, and reanalyses will be important when information on divergence times (or some other common metric, such as genetic distances (e.g., see avian example in Garland and Ives, 2000)) become available (see Garland *et al*., 1992; Clobert *et al*., 1998).

Caveats about branch lengths aside, what might account for a higher average rate of body mass evolution in the pteropodid lineage? Because body size is correlated with so many morphological, physiological, behavioral, and ecological traits, it is difficult to formulate cogent hypotheses. In addition, any synapomorphy of the Pteropodidae could, in principle, be the cause of a higher rate of body size diversification (constitute a "key innovation"). Indeed, the causes of clade differences in morphological variability have long been of interest (e.g., see James, 1982) and "disparity" is presently the subject of intense study, typically involving multivariate analyses of morphometric traits intended to indicate variation in overall body plans, rather than simple variation in body mass (e.g., Harmon *et al*., 2003; McClain *et al*., 2004). It would be of considerable interest to attempt multivariate phylogenetic analyses of clade differences in bat body size and shape, e.g., including measures of the skull (e.g., Van Cakenberghe *et al*., 2002), dentition

(e.g., Freeman, 2000), and wings (e.g., Norberg, 1994). Combined with ecological and behavioral information, this could allow elucidation of the causes of diversification within the megabats.

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