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A Comparative Analysis of Brain Size in Relation to Foraging Ecology and Phylogeny in the Chiroptera

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Key Words
Mammals · Chiroptera · Comparative method · Brain evolution · Brain allometry · Foraging · Phylogeny

Abstract
Variations in total brain mass and in the mass of three brain regions (main olfactory bulb, hippocampus, auditory nuclei) were examined using a data set for 63 species of bats (Chiroptera). Using both conventional and phylogenetically based analysis of covariance (log body mass as covariate), we tested several hypotheses that relate total brain mass or the size of the components to variation in foraging ecology, categorized as phytophagous, gleaner, and aerial insectivore. In some analyses, the category phytophagous was split into phytophagous pteropodid and phytophagous phyllostomid to examine differences between two distinct clades of bats. Because the Megachiroptera orient primarily by vision and olfaction, whereas all other bats rely on laryngeal echolocation to locate their prey, we hypothesized that the former would differ in size of the main olfactory bulb, as compared with all other bats. This hypothesis was supported by our analyses. Our more general prediction was that insectivorous bats, which rely heavily on echolocation for the pursuit and capture of their prey, would have larger auditory nuclei than do phytophagous species. This, too, was supported. We also compared phytophagous (fruit or nectar consuming) bats in two families, the Pteropodidae and the Phyllostomidae. We hypothesized that the phyllostomids, which use echolocation while foraging, would have larger auditory nuclei. Although statistical power is low in phylogenetically informed comparisons of the two clades, we did find weak evidence in support of this hypothesis. We conclude that bat brains show evidence of adaptation to foraging ecology.

Introduction

Among the many tasks that brains perform are the filtering and preparation of sensory information. Different foraging ecologies necessarily entail different sensory requirements. For example, the senses that are most useful for an insectivorous bat (well-developed echolocation) may not be as useful for a nectar-feeding bat. If studies of ecology indicate that a particular organism is highly dependent on the sense of smell as a means of prey location, then several expectations might follow. In particular, one might expect that this hypothetical organism would have a large nasal area and a large olfactory bulb, because

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the former allows more space for olfactory epithelium and the latter effectively provides more processing power for incoming information. Such hypothetical examples are intrinsically comparative, as the questions of large nasal area and olfactory bulb necessarily imply reference to some norm.

Bats present an excellent opportunity to examine several facets of sensory specialization as it relates to foraging ecology. Although they are probably a monophyletic group [Hutcheon et al., 1998; Simmons and Geisler, 1998], bats have adopted a variety of approaches to acquiring prey, emphasizing different sensory modalities. The taxonomy of bats reflects some of these differences with the two suborders Megachiroptera and Microchiroptera. All bats of the putative suborder Microchiroptera [Simmons and Geisler, 1998] are capable of laryngeal echolocation [Fenton, 1984], although the manner in which it is employed can vary greatly [Arita and Fenton, 1997] and some bats do employ echolocation while foraging [Fenton, 1984]. Megachiropteran bats, on the other hand, do not employ laryngeal sonar at all. This observation alone has been the basis for much speculation about the likely phylogenetic affinities of the megabats. Many authors [cf. Pettigrew et al., 1989; Altringham, 1996; Speakman, 1999] have expressed reservations about the likelihood of a bat losing such a useful asset, which was presumably already present in the common ancestor of all bats [Novacek, 1985; Fenton et al., 1995]. This, in turn, has served as fodder for theories advocating a separate origin of megabats.

For this study, we elected to use phylogenetic trees based on molecular data. Several different molecular techniques have led to similar answers with respect to the position of the megabats [Hutcheon et al., 1998; Kirsch et al., 1998; Teeling et al., 2000; Hutcheon, 2001]. Moreover, the molecular data sets should be relatively independent of neurobiological data, thus reducing the probability of inappropriately biasing analyses of the latter [e.g., see de Queiroz, 1996].

Several comparative studies of bat brains have been published [Jolicoeur and Baron, 1980; Baron and Jolicoeur, 1980; Barton et al., 1995; Legendre and Lapointe, 1995; Barton 1999]. Not surprisingly, megachiropteran bats have usually been characterized as having 'olfactory' or 'visual' brains, whereas microchiropterans have been described as having 'auditory' brains. However, olfaction alone is unlikely to constitute a sufficient sensory arsenal for the detection of food items. Spatial memory, although not a sense per se, is nonetheless likely to be of great importance to a foraging frugivore. For example, a study by Fleming et al. [1977] involved erecting poles with ripe fruit on them and observing the fidelity of bats to these artificial feeding sites. The authors would also occasionally move the poles, and test the time it took for the bats to respond to this change in the distribution of their food supply. They concluded that frugivorous bats are 'highly responsive in the spatiotemporal distributional pattern of their food resources.' Fleming et al. [1977] did not perform any neurobiological analysis, but a number of papers have demonstrated a correlation between hippocampal volume and spatial memory in birds [Devoogd et al., 1993; Jarrard, 1995; Krebs et al., 1996; Szekely et al., 1996; Szekely, 1999]. Moreover, although hippocampal volume has not been specifically studied in this context in bats, several other brain characteristics do appear to follow foraging ecology among species of bats [Jolicoeur and Baron, 1980].

The relationship of ecology to brain size has important implications for models of mammalian neural evolution. One recent debate is that over the importance of developmental constraint [Finlay and Darlington, 1995; Clancy et al., 1999] versus adaptation [i.e., the 'mosaic theory' of Barton and Harvey, 2000]. On one hand, developmental constraint points to conserved features common to the brains of various orders and suggests that these features are the result of the order of neurogenesis. One implication of this hypothesis is that all brain features should follow predictable growth patterns and the size of any brain component might be predicted from the application of an allometric scaling equation. On the other hand, the adaptationist argument points to the various modules (i.e., nuclei) which together constitute a whole brain, and suggests that variation in selective regimes should influence the absolute or relative prominence of these nuclei. Based on the available molecular-phylogenetic information [Hutcheon et al., 1998; Teeling et al., 2000], frugivory has evolved twice in the chiropteran lineage, allowing for the comparison of brain traits in two distinct clades of bats and, arguably, one approach to evaluating the adaptation versus development argument.

A phylogenetic context is necessary to evaluate the correlations of relative sizes of brain areas with foraging ecology. Conventional statistical methods assume that data points are independent and identically distributed. Because bats (or any other species) did not originate independently, but rather are part of a branching, hierarchical phylogeny, species can only be assumed to be independent of one another as far back in time as their last common ancestor (and maybe not even then if phenomena such as character displacement occur). Hence, phyloge-

netically based statistical methods are required [e.g., see Felsenstein, 1985; Garland et al., 1993, 1999; Barton, 1999; Vanhooydonck and Van Damme, 1999; Deaner et al., 2000; Cruz-Neto et al., 2001; Iwaniuk and Nelson, 2001].

Hypotheses

A recent study of total brain mass in relation to diet and foraging mode in waterfowl did not find significant relationships [Iwaniuk and Nelson, 2001]. However, as those authors noted, even if total brain masses do not differ significantly among ecologically or phylogenetically defined groups, components of the brains might. Therefore, we examined both total brain mass and the volumes of three regions.

We tested the effect of foraging ecology on total brain mass and on the volume of three brain regions, after controlling statistically for correlations with body mass. Because it is expected that frugivorous and nectarivorous bats (here lumped together as 'phytophagous') necessarily rely upon the sense of smell and upon spatial memory to a greater extent than do insectivorous bats, we hypothesized that the former would have larger olfactory bulbs and hippocampi. Likewise, because insectivorous bats rely heavily on echolocation for the pursuit and capture of their prey, it is expected that these bats will have larger auditory nuclei [defined here as the cochlear nuclei complex and the superior olivary complex, following the convention of Baron et al., 1996] than do phytophages.

A subtler question deals with the evolution of plant use in bats. Two families of bats have phytophagous members: the Pteropodidae and the Phyllostomidae. These families are both monophyletic and separated by several nodes; therefore, phytophagy is presumptively convergent [Hutcheon et al., 1998; Simmons and Geisler, 1998]. The pteropodids are restricted to the Old World and include the flying foxes. These bats comprise the suborder Megachiroptera in most classifications [Simmons and Geisler, 1998] and, unlike all other bat families, do not employ laryngeal echolocation. The phyllostomid bats are restricted to the New World and are one of 17 families that comprise the other suborder of bats, the Microchiroptera.

Although serious controversies surround the phylogenetic position of pteropodid bats with respect to other bats [Hutcheon et al., 1998; Springer et al., 2001], no evidence suggests that they are particularly close to the phyllostomid bats. Plant use, whether frugivory or nectarivory, is thus convergently evolved and an explicit comparison just between these two families provides an opportunity to test for effects of phylogeny (clade differences) even when ecology is similar. Because the phyllostomid bats are able to employ echolocation, it is expected that they will differ from the pteropodid bats on the basis of auditory nuclei size, but will not significantly differ from them on the basis of olfactory bulb or hippocampus size (unless, of course, the phyllostomids make less use of these regions).

Materials and Methods

Brain volume and body mass data were obtained from Baron et al. [1996] for 63 species of bats and are presented with dietary category in table 1. Bat species were scored for diet using a classification similar to of Barclay and Brigham [1991], recognizing five categories:

Table 1. Diet, body masses, brain masses and volumes of three brain regions for 63 species of bats

Species	Diet	BOW	BRW	AUD	MOB	HIP
Rousettus aegyptiacus	1	136.30	2,070.00	9.88	105.77	125.97
Epomops franqueti	1	120.00	2,210.00	10.44	107.80	159.80
Eonycteris spelaea	1	58.70	1,310.00	5.48	67.00	97.70
Cynopterus sphinx	1	48.30	1,184.33	4.77	65.27	95.40
Dobsonia praedatrix	1	184.00	3,028.00	7.09	213.43	233.30
Eidolon helvum	1	262.00	4,290.00	12.77	208.70	258.10
Pteropus vampyrus	1	1,014.00	9,121.00	16.93	243.54	331.29
Macroglossus miniumus	1	14.60	561.00	2.40	30.05	52.95
Syconycteris australis	1	14.70	570.00	2.13	31.40	53.10
Nyctimene albiventer	1	29.70	825.00	4.56	68.93	81.40
Rhinolophus landeri	2	6.30	208.00	4.88	4.83	20.89
Hipposideros commersoni	2	101.90	750.00	8.79	9.50	27.68
Aselliscus stoiczkanus	2	4.90	150.00	2.72	1.88	11.60

➤

Table 1 (continued)

Species	Diet	BOW	BRW	AUD	MOB	HIP
Triaenops persicus	2	13.70	271.00	4.07	5.22	17.40
Megaderma spasma	3	22.60	644.00	10.56	14.25	35.85
Macroderma gigas	3	119.80	1,704.00	22.36	21.60	68.90
Cardioderma cor	3	26.00	670.00	10.81	8.07	32.30
Lavia frons	3	23.40	644.00	10.92	4.35	25.80
Nycteris thebaica	3	8.90	323.00	5.79	3.65	21.65
Rhinopoma hardwickei	3	12.90	275.00	5.20	7.48	20.95
Craseonycteris thonglongyai	3	2.56	87.00	1.84	0.66	4.80
Desmodus rotundus	4	36.30	999.00	9.77	33.00	42.40
Diphylla ecaudata	4	30.90	798.00	8.30	36.20	41.00
Brachyphylla cavernarum	1	44.50	1,196.00	8.63	42.20	78.80
Lionycteris spurrelli	1	9.90	393.00	3.71	10.30	29.50
Glossophaga soricina	1	10.60	414.00	3.74	12.20	35.00
Leptonycteris curasoae	1	24.50	610.00	5.57	18.60	44.95
Anoura geofroyi	1	16.00	586.00	5.20	14.15	41.40
Phylloderma stenops	1	46.10	1,338.00	10.20	87.40	91.70
Phyllostomus haustatus	1	90.10	1,517.00	12.74	34.33	65.60
Mimon crenulatum	1	11.80	326.00	5.92	7.30	18.20
Trachops cirrhosus	1	36.90	1,003.00	16.34	23.50	50.60
Tonatia bidens	1	27.67	684.67	13.37	17.96	28.30
Vampyrum spectrum	1	173.00	2,587.00	27.60	92.00	110.40
Micronycteris brachyotis	1	8.98	319.00	4.19	13.85	17.10
Carollia perspicillata	1	17.80	546.00	5.27	23.55	40.75
Rhinophlylla pumilio	1	8.90	356.00	4.57	18.80	30.30
Sturnira lilium	1	20.20	618.00	4.77	30.77	49.73
Artibeus lituratus	1	41.00	1,016.00	7.21	34.38	54.90
Uroderma bilobatum	1	16.20	612.00	5.98	28.70	42.70
Vampyrops vittatus	1	22.60	791.00	11.56	29.22	52.46
Chiroderma villosum	1	26.10	814.00	7.95	28.75	47.58
Pteronotus parnelli	2	20.20	543.00	5.52	7.98	22.23
Mormoops megalophylla	3	15.70	386.00	4.19	6.66	27.50
Noctilio albiventris	3	32.70	597.00	11.59	13.54	36.00
Natalus tumidirostris	3	6.90	245.00	3.28	3.58	27.10
Furipterus horrens	3	3.43	127.00	2.78	1.37	9.46
Molossus ater	3	33.60	526.00	7.07	10.26	20.13
Tadarida condylura	3	20.27	367.78	4.94	9.75	18.60
Molossops abrasus	3	19.35	301.00	5.38	6.93	15.87
Otomops martiensseni	3	41.50	756.00	11.41	14.30	30.60
Cheiromeles torquatus	3	167.00	1,362.00	13.20	40.50	61.20
Saccopteryx leptura	3	7.80	228.00	3.48	1.49	9.21
Cyttarops alecto	3	5.30	175.00	3.51	0.74	7.96
Cormura brevirostris	3	8.20	219.00	4.37	2.28	11.20
Peropteryx trinitatus	3	5.00	149.50	2.81	1.47	7.54
Rhynchonycteris naso	3	3.80	118.00	2.34	0.91	5.48
Emballonura monticola	3	5.30	166.00	3.16	1.30	8.93
Coleura afra	3	11.50	257.00	4.08	3.98	12.40
Taphozous saccolaimus	3	43.00	671.00	9.65	10.92	26.38
Kerivoula papilosa	3	5.73	209.67	4.47	2.52	19.02
Myotis myotis	3	7.00	190.00	7.50	5.23	16.24
Miniopterus medius	3	11.71	271.22	4.77	5.31	17.28

Data taken from Baron et al. [1996]. Diet categories: 1 = phytophage; 2 = gleaner; 3 = aerial insectivore; 4 = vampire. Abbreviations: BOW = body mass; BRW = brain mass; AUD = auditory nuclei volume; MOB = main olfactory bulb volume; HIP = hippocampus volume.

phytophagous pteropodid (a category that includes both frugivores and nectarivores), phytophagous phyllostomid, gleaner, aerial insectivore, and vampire. In the final analysis, we excluded the two vampires because of the small sample size in this category; thus, all tests included only 61 species of bats. In addition, to test differences between specific clades, phytophagous pteropodids and phyllostomids were treated as different categories in additional analyses of these groups alone. All data were $\log_{10}$ transformed prior to analysis.

Analyses

Comparative analyses were undertaken in two ways. First, sets of conventional statistical tests were performed on each of the data sets. The implicit assumption of these tests was that all bat species originated from a single common ancestor (i.e., a 'star' phylogeny). Analysis of covariance (ANCOVA) was performed, with log body mass as the covariate and each species associated with a particular diet and/or clade, which allowed for testing group effects. [An alternative would be to use total brain mass as the covariate in analyses of brain regions, e.g., see Deaner et al., 2000.]

The second set of analyses assumed that bat species are part of a hierarchical, branching phylogeny. Because no phylogeny of all Chiroptera is available at the generic level, a composite tree was constructed for purposes of phylogenetically based statistical analyses. Tree topology was determined using the interfamilial relationships implied by Hutcheon et al. [1998]. Robbins and Sarich [1988], Griffiths et al. [1992], Griffiths [1994], Wetterer et al. [2000], Hand and Kirsch [1998], Kirsch et al. [1995], and Freeman [1981] were used to inform the within-family and intergeneric relationships of the tree (fig. 1). Because branch-length data were not available in all cases, arbitrary values were used, following Pagel's [1992] method. A check of the diagnostic for phylogenetically independent contrasts [Garland et al., 1992; Diaz-Uriarte and Garland, 1998] suggested that these arbitrarily set branch lengths adequately standardized the contrasts. Thus, these branch lengths are likely to be reasonable for simulations (see next paragraph).

Monte Carlo simulations were used to generate phylogenically based and empirically scaled null distributions of F statistics for ANCOVAs. The data set was simulated 1,000 times, using the PDSIMUL module of the Phenotypic Diversity Analysis Programs version 5 [PDAP; Garland et al., 1993, 1999]. The simulated data were analyzed with the PDANOVA module. From this distribution of F values for the simulated data, the 95th percentile was computed and used as the phylogenetically informed critical value (for $\alpha = 0.05$). This effectively recalibrates the critical value, often raising the criterion for significance.

Simulations were performed using a simple Brownian motion model of character evolution, and using the default values in PDSIMUL, except that limits to character evolution were set by the 'Replace' option [following Garland et al., 1993; Cruz-Neto et al., 2001]. Under the 'Replace' algorithm, if the program notices during a simulation that a change will cause a trait to exceed a specified limit, then a new random change is tried. If it would also cause a trait to go out of bounds, then another is tried until an acceptable change is drawn. In this way, the traits are never allowed to go out of bounds. For body mass, the lower and upper limits were set at 0 and 3 (on a $\log_{10}$ scale, corresponding to a range of 1 g to 1,000 g). For brain volumes, lower and upper limits were set on the $\log_{10}$ scale at 0 and 4. The auditory nuclei lower and upper limits were 0 and 1.6 ($\log_{10}$), and the olfactory bulb and the hippocampus limits were set at 0 and 3 ($\log_{10}$). All simulations were performed on the log-transformed scale. For all brain traits, the correlation with body mass was set to zero, because the intention was to test for effects of body mass as well as group effects.

A second set of analyses involved looking at correlations of masses of the different brain regions. Four sets of conventional correlations were carried out: a simple correlation between total brain mass of each and three different brain regions (i.e., auditory nucleus and olfactory bulb, auditory nucleus and hippocampus, olfactory bulb and hippocampus). However, simple correlations treat all the bats as a single group and three different grouping schemes had been used for the ANCOVAs; thus, correlations were also carried out using residuals from the ANCOVA model. Furthermore, a simple correlation does not correct for any potential effect of overall body size, a covariate known to be highly significant. Therefore, residuals were calculated from each of the ANCOVA models (four groups, three groups, and frugivores), and these were correlated with one another.

None of the foregoing correlations is informed by phylogeny, however. Therefore, as a final, phylogenetically correct correlation, standardized independent contrasts [Felsenstein, 1985; Garland et al., 1992; see also Barton and Harvey, 2000] of log body mass and each of the three log-transformed brain regions were computed in PDTREE. Regressions (through the origin) of contrasts of brain region on contrasts in body mass were computed, and residuals were saved. Finally, these independent-contrast residuals were correlated with each other.

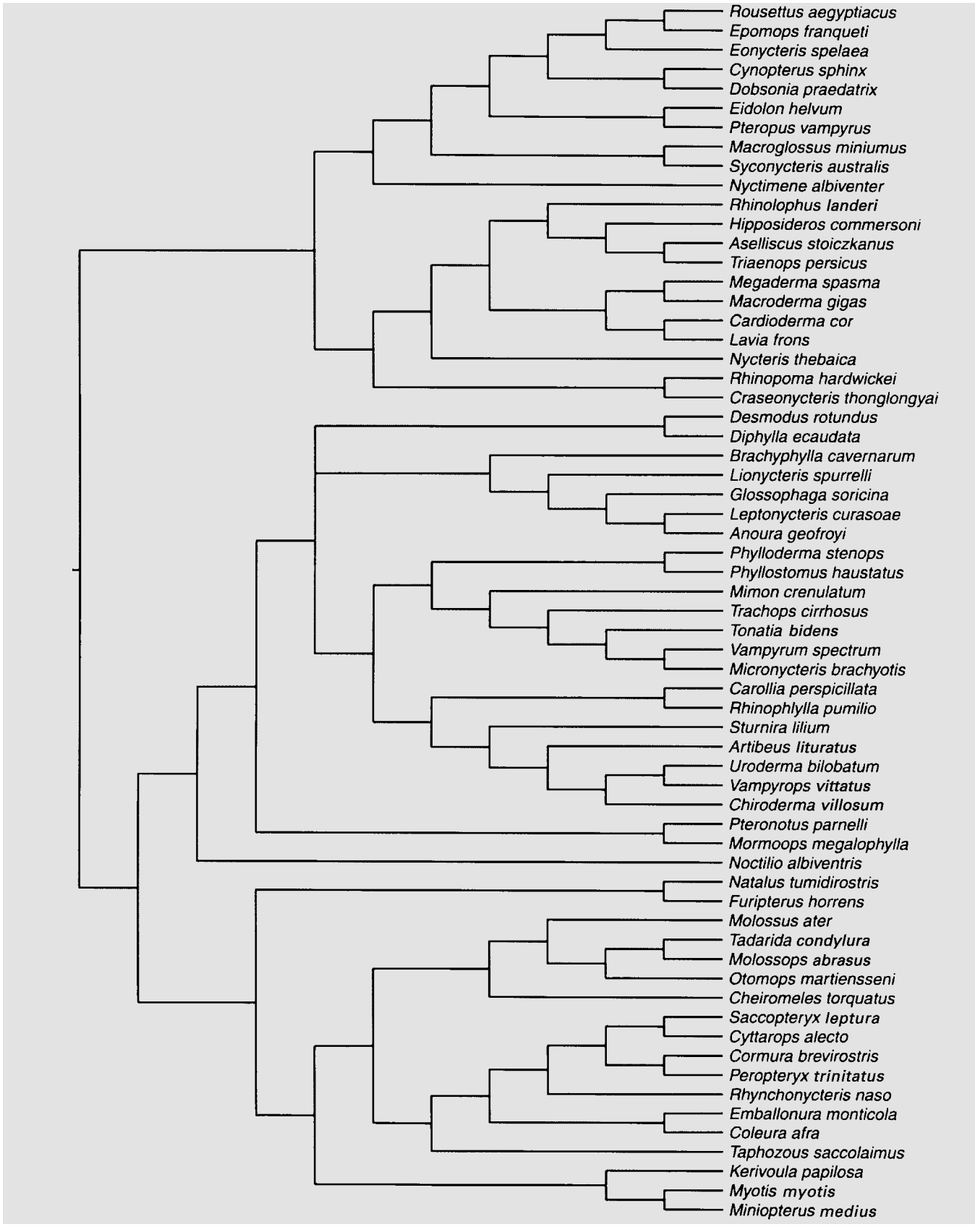
Results

Total Brain Mass

Plotting log total brain mass against log body mass reveals clustering of all phytophagous bats relative to all other bats (fig. 2). Results of the conventional ANCOVA indicate that bats differ significantly in brain mass when body mass is taken into account ($p < 0.0005$; tables 2 and 3). In the phylogenetically based analyses of brain mass, the covariate (body mass) remained highly significant ($p < 0.001$; tables 2 and 3). The main effect (diet) was significant when considering all bats, irrespective of whether the bats were grouped ecologically (table 2) or ecologically plus phylogenetically (table 3).

Auditory Nuclei

When log auditory nucleus volume is plotted against log body mass, the pteropodid bats form a distinct cluster (fig. 3). Results of the conventional ANCOVA indicated differences among categories when body mass is taken into account ($F = 288.38$; d.f. = 1,3; $p < 0.001$). In all analyses, both conventional and phylogenetic, body mass (the covariate) had a significant effect, but the significance of the main effect (diet) varied. In conventional analyses, it gave a consistently significant effect (tables 2, 4). The phylogenetically based ANCOVAs yielded different results, depending upon the grouping scheme used. When 61 spe-


Rousettus aegyptiacus
Epomops franqueti
Eonycteris spelaea
Cynopterus sphinx
Dobsonia praedatrix
Eidolon helvum
Pteropus vampyrus
Macroglossus miniumus
Syconycteris australis
Nyctimene albiventer
Rhinolophus landeri
Hipposideros commersoni
Aselliscus stoiczkanus
Triaenops persicus
Megaderma spasma
Macroderma gigas
Cardioderma cor
Lavia frons
Nycteris thebaica
Rhinopoma hardwickei
Craseonycteris thonglongyai
Desmodus rotundus
Diphylla ecaudata
Brachyphylla cavernarum
Lionycteris spurrelli
Glossophaga soricina
Leptonycteris curasoae
Anoura geofroyi
Phylloderma stenops
Phyllostomus haustatus
Mimon crenulatum
Trachops cirrhosus
Tonatia bidens
Vampyrum spectrum
Micronycteris brachyotis
Carollia perspicillata
Rhinophylla pumilio
Sturnira lilium
Artibeus lituratus
Uroderma bilobatum
Vampyrops vittatus
Chiroderma villosum
Pteronotus parnelli
Mormoops megalophylla
Noctilio albiventris
Natalus tumidirostris
Furipterus horrens
Molossus ater
Tadarida condylura
Molossops abrasus
Otomops martiensseni
Cheiromeles torquatus
Saccopteryx leptura
Cyttarops alecto
Cormura brevirostris
Peropteryx trinitatus
Rhynchonycteris naso
Emballonura monticola
Coleura afra
Taphozous saccolaimus
Kerivoula papilosa
Myotis myotis
Miniopterus medius

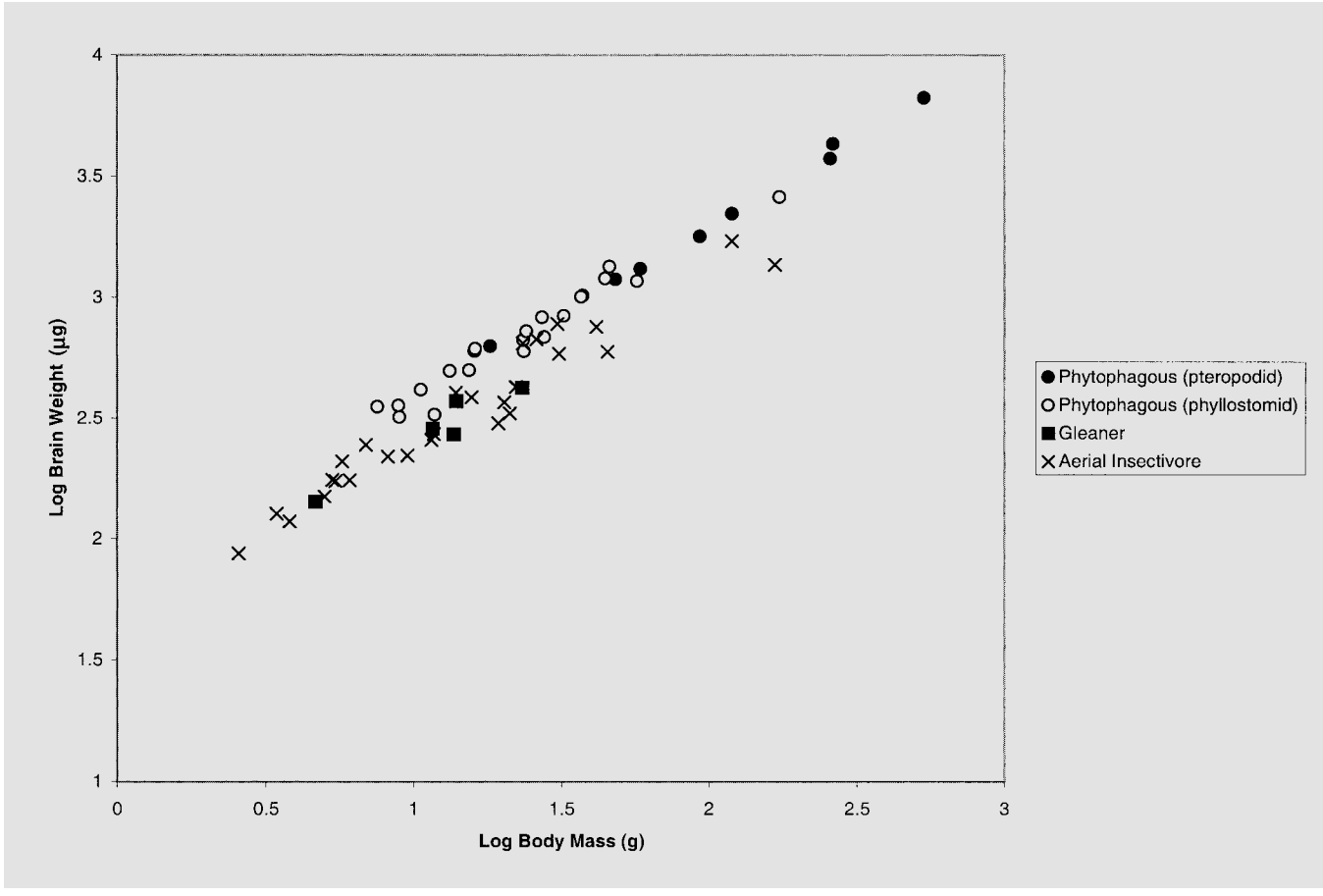


Fig. 2. Log total brain mass plotted against log body mass for 63 species of bats. For statistical analyses, the two vampire species were omitted.

cies were considered and the phytophagous bats were differentiated as belonging to either pteropodid or phyllostomid groups, a significant result of $p < 0.033$ was obtained (table 2). However, when only three foraging ecologies were recognized, the difference became non-significant, $p \leq 0.812$ (tables 2–4).

Main Olfactory Bulb

Figure 4 shows a plot of the log volume of the main olfactory bulb against log body mass, and shows that the phytophagous bats all appear to form one large cluster. Conventional ANCOVA indicated that the most significant difference was to be found in the variation of the olfactory bulb (with log body mass as the covariate). This was true when pteropodid and phyllostomids were both split (4 group, table 2) and lumped (3 group, table 3). In fact, the F value increased in the three-group conventional ANCOVA (table 3). ANCOVA using the phylogenetically based simulations likewise yielded a significant result. As with the analysis of the auditory nuclei above, the critical value was raised; however, the distribution of the null F statistics was such that the diet effect was still statistically significant in both cases.

Hippocampus

In many respects, plotting log hippocampus volume against log body mass gives a result similar in appearance to the plot involving log volume of the olfactory bulb (fig. 5). The similarity of these plots is consistent with the strong positive correlation between these two regions (see

Fig. 1. Composite phylogeny of 63 species of bats. Interfamilial relationships are based on the phylogeny presented by Hutcheon et al. [1998]. Branch lengths are arbitrary [Pagel's 1992 method]. For statistical analyses, the two vampire species were omitted.

Table 2. Analysis of covariance (ANCOVA, with $\log_{10}$ body mass as the covariate) testing the effects of foraging ecology on $\log_{10}$ total brain mass and on three brain regions in 61 species of bats

Source of variation	d.f.	F	Conventional		Phylogenetic	
			critical value	p	critical value	p
Log total brain mass						
Covariate	1	1,235.5	4.02	<0.0005	13.73	<0.001
Main effect	3	26.08	2.78	<0.0005	23.19	≤0.036
Error	56					
Log auditory nuclei						
Covariate	1	288.38	4.02	<0.0005	14.02	<0.001
Main effect	3	27.32	2.78	<0.0005	23.57	≤0.033
Error	56					
Log main olfactory bulb						
Covariate	1	300.48	4.02	<0.0005	13.97	<0.001
Main effect	3	57.22	2.78	<0.0005	23.96	≤0.002
Error	56					
Log hippocampus						
Covariate	1	272.57	4.02	<0.0005	14.94	<0.001
Main effect	3	34.07	2.78	<0.0005	24.55	≤0.016
Error	56					

Categories used were gleaner, aerial insectivore, phytophagous phyllostomid, and phytophagous pteropodid. Critical values for F statistics and significance levels (p) are presented for conventional tabular values (which assume no hierarchical phylogenetic relationships among species) and based on Monte Carlo simulations (N = 1,000; see text) using the phylogeny shown in figure 2.

Table 3. As in table 2, except that phytophagous phyllostomids and pteropodids were lumped so that only three categories were used in the ANCOVA

Source of variation	d.f.	F	Conventional		Phylogenetic	
			critical value	p	critical value	p
Log brain mass						
Covariate	1	1,501.09	4.02	<0.0005	19.19	<0.001
Main effect	2	49.60	2.78	<0.0005	17.84	<0.001
Error	57					
Log auditory nuclei						
Covariate	1	92.92	4.02	<0.0005	17.94	<0.001
Main effect	2	1.21	3.17	0.304	17.70	≤0.812
Error	57					
Log main olfactory bulb						
Covariate	1	378.97	4.02	<0.0005	18.11	<0.001
Main effect	2	82.30	3.17	<0.0005	19.13	<0.001
Error	57					
Log hippocampus						
Covariate	1	332.44	4.02	<0.0005	14.94	<0.001
Main effect	2	41.05	3.17	<0.0005	24.55	≤0.002
Error	57					

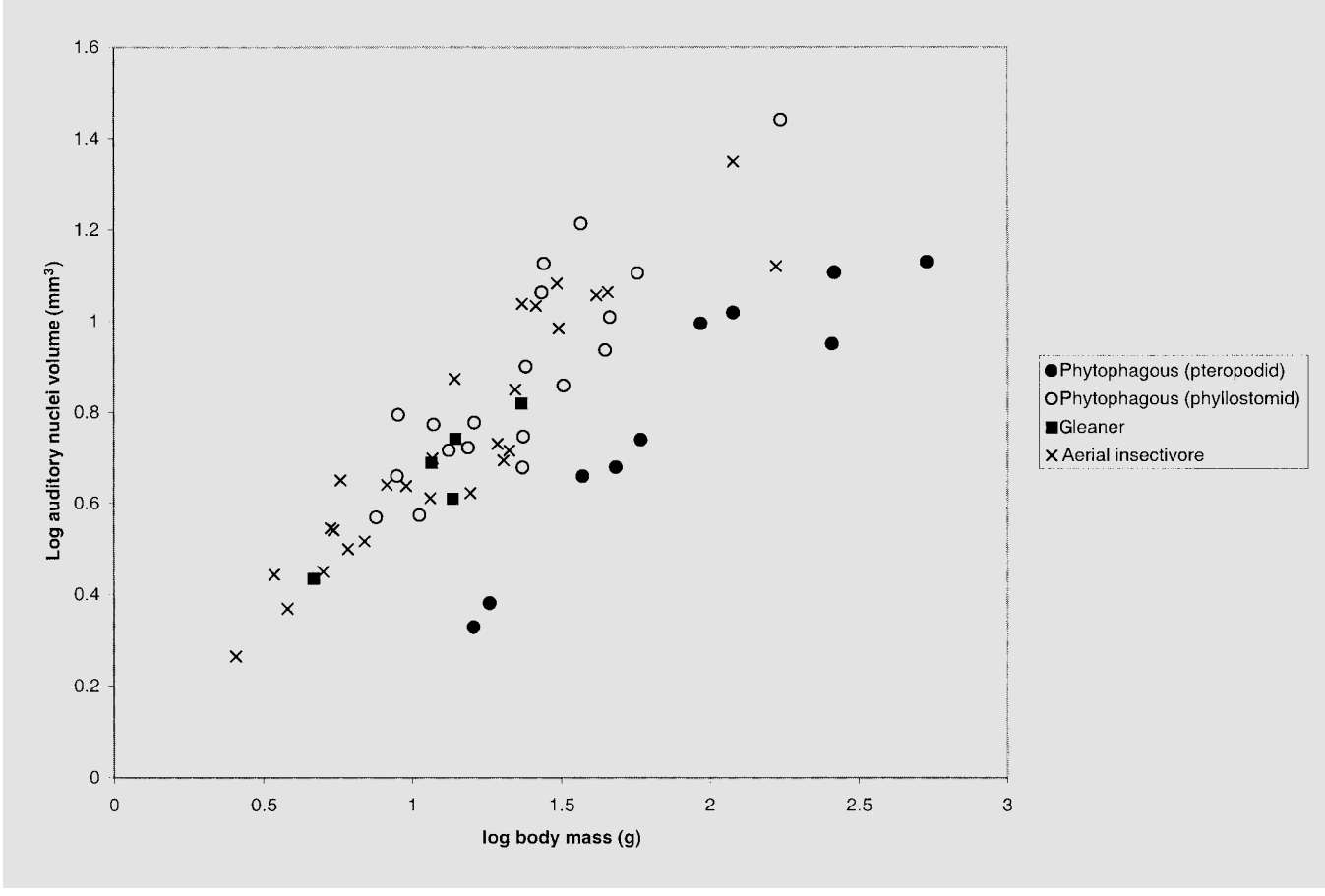


Fig. 3. Log auditory nuclei volume plotted against log body mass for 63 species of bats. For statistical analyses, the two vampire species were omitted.

discussion of correlations below). As with the olfactory bulb, conventional ANCOVA indicated a significant effect in the mass of the hippocampus between groups (tables 2 and 3). Grouping scheme had an effect on significance. Although both the 4-group and 3-group analyses indicated a statistically significant effect, the 3-group effect was more so ($p < 0.002$ vs. $p < 0.016$; tables 2 and 3). Again, phylogenetic ANCOVA yielded significant results although the critical value was slightly raised.

The interaction between body mass and group (i.e., covariate and main effect) was not significant for overall brain mass or for the volumes of the four brain regions. Statistical non-significance for this interaction was the case both in the conventional ANCOVA and in the phylogenetic ANCOVA. This indicates that the slopes of the groups do not differ significantly from one another.

Pteropodids and Phyllostomids

As a further test of phylogenetic effect, ANCOVAs were performed on a reduced set of real and simulated data that included only phytophagous bats, members of the Pteropodidae or Phyllostomidae. This provides a chance to compare brain traits in ecologically similar, but phylogenetically distinct, groups. Whereas conventional ANCOVA indicates significant variation between pteropodids and phyllostomids, ANCOVAs performed on the simulated data did not indicate a significant variation in any of the brain regions. The auditory nucleus, however, came the closest of any of the regions to showing a significant trend with a phylogenetic p value of 0.068 (table 4).

Correlations

Under simple, conventional statistical analysis, with no accounting for dietary or phylogenetic groupings, all volumes of all brain regions appear to be correlated with

Table 4. Analysis of covariance (ANCOVA, with $\log_{10}$ body mass as the covariate) testing the effects of phylogeny on $\log_{10}$ total brain mass and three brain regions for 29 species of phytophagous bats

Source of variation	d.f.	F	Conventional		Phylogenetic	
			critical value	p	critical value	p
Log brain mass						
Covariate	1	1,124.1	4.02	<0.0005	10.22	<0.001
Main effect	1	1.713	2.78	<0.202	60.02	≤0.678
Error	26					
Log auditory nuclei						
Covariate	1	109.15	4.22	<0.0001	10.89	<0.001
Main effect	1	53.73	4.22	<0.0001	65.61	≤0.068
Error	26					
Log main olfactory bulb						
Covariate	1	111.19	4.22	<0.0001	11.06	<0.001
Main effect	1	10.71	4.22	0.003	59.27	≤0.389
Error	26					
Log hippocampus						
Covariate	1	141.90	4.22	<0.0001	9.95	<0.001
Main effect	1	11.78	4.22	0.002	57.93	≤0.245
Error	26					

one another at a statistically significant level (table 5a). Such a result is unsurprising, because all brain volumes are highly correlated with body mass (see fig. 2–5). When an ANCOVA model is applied to these analyses, a much different result occurs. The correlation between the auditory nuclei and the hippocampus is negative (table 5b, c, d). Between the auditory nuclei and the main olfactory bulb the correlation is negative, but not significantly so in two cases (table 5b, c, d). In all cases, a significantly positive correlation between the volume of the hippocampus and the main olfactory bulb is obtained.

Correlation of the residuals of phylogenetically independent contrasts yields a negative correlation between the auditory nuclei and the hippocampus and main olfactory bulb, and a positive correlation between the hippocampus and the main olfactory bulb. All three of these correlations are statistically significant (table 5e).

Discussion

When phylogeny and body mass are taken into account, some of the primary brain structures that are hypothesized to be associated with foraging ecology do indeed differ significantly in size among groups of bats that differ also in foraging ecology. Additionally, overall brain size appears to be significantly affected by foraging ecology. Iwaniuk and Nelson [2001] recently published a study of total brain size of 55 species of waterfowl that varied in foraging mode or diet. They did not find differences in mass-independent brain size among their categories. Hence, either adaptation of the chiropteran brain to foraging ecology is more extensive than in waterfowl or, as suggested by Iwaniuk and Nelson [2001], perhaps evidence for adaptation in the avian brain is still to be found in the relative size of specific brain regions, which they did not study.

Although much attention is paid to the echolocation abilities of the microbats, the differences between groups of bats involving the auditory nuclei are not as extreme as those involving either the main olfactory bulb or the hippocampus, at least when comparing all bats (e.g., compare fig. 4 and 5). However, when the data set is reduced to include only pteropodids and phyllostomids, then auditory nucleus becomes the only area that approaches a statistically significant difference in size (table 3). Given the ecological similarities between these two clades, the significance of the auditory nuclei likely rests on the fact that phyllostomids use echolocation while foraging [Thies et al., 1998; von Helverson and von Helverson, 1999], whereas the pteropodids do not.

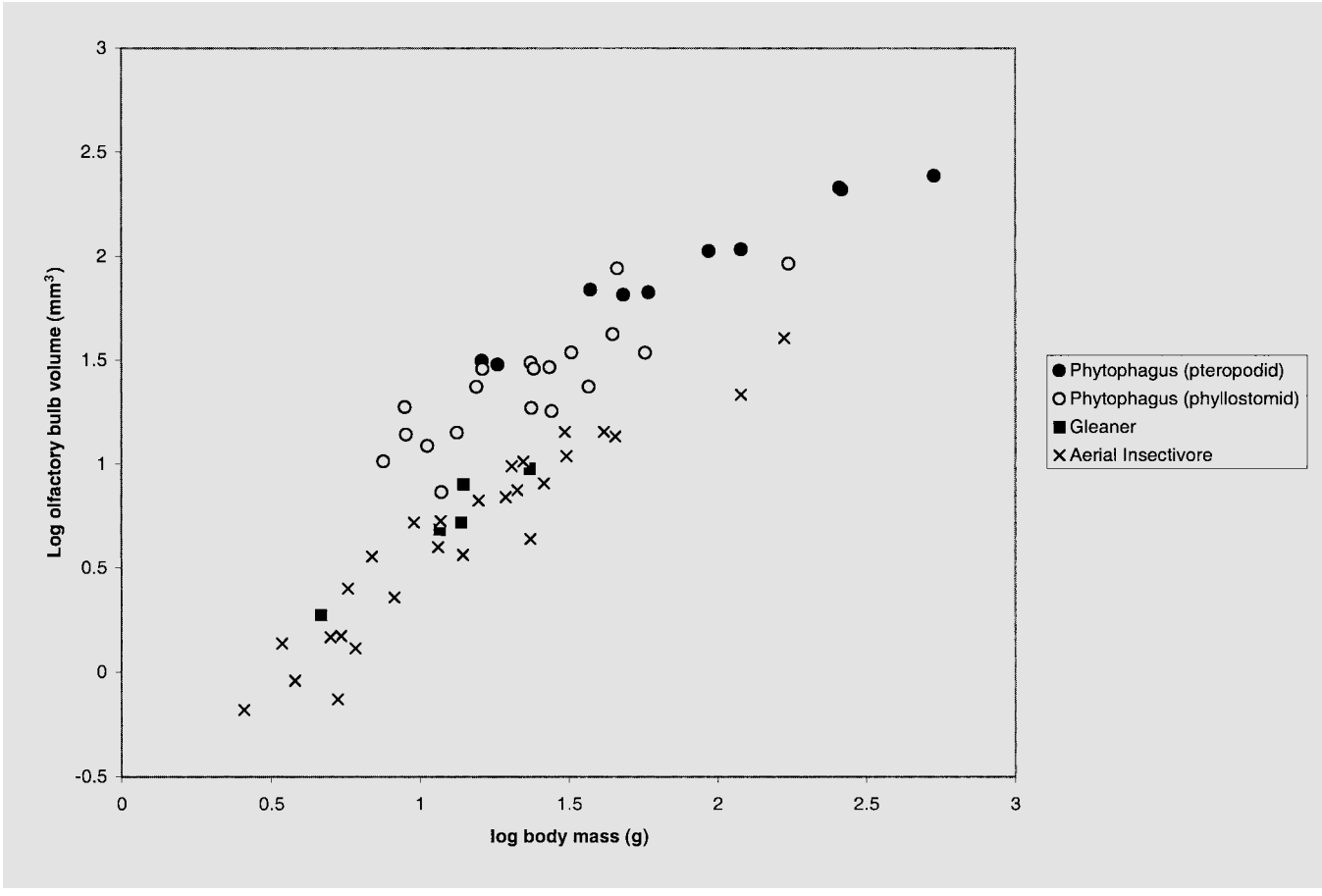


Fig. 4. Log main olfactory bulb volume plotted against log body mass for 63 species of bats. For statistical analyses, the two vampire species were omitted.

At the same time, neither olfactory bulb nor hippocampus size differed between these two groups with body mass as a covariate. The phytophagous-only analysis, however, is one that must be considered carefully. Although the difference is not statistically significant, it appears to be large in some of the graphs (see fig. 4 and 5), so this might just reflect a lack of statistical power because of reduced sample size (n = 29 as opposed to 61 in the other analyses) and because comparisons of clades seem to entail inherently low statistical power [see also Garland et al., 1993; Vanhooydonck and Van Damme, 1999; Brashares et al., 2000; Cruz-Neto et al., 2001]. Taking the results at face value, it could be that phytophagous bats, whether they are pteropodid or phyllostomid, employ at least some similar strategies while foraging – that is, reliance on the sense of smell and spatial memory. If this is the case, then it might well explain why trees which have evolved 'bat flowers' in the New World (i.e., in the range of the Phyllostomidae) continue to prosper when transplanted to the Old World (the range of the Pteropodidae) [Cox et al., 1991].

Healy and Guilford [1990] observed that the olfactory structures of nocturnal birds were larger than in diurnal birds. This observation has been taken as some evidence of tradeoffs in sensory specialization, given the constraints that low light levels place on vision [Barton et al., 1995]. In bats, however, nocturnality is the norm [Speakman, 1999] and if any bats might be described as partially diurnal it would be the pteropodids, the very bats with the greatest reliance on olfaction.

Although it seems intuitively attractive to posit tradeoffs among various sensory systems, it should be remembered that each sensory system also has its own limitations. A negative correlation between the size of the auditory nuclei and the olfactory bulbs has been reported in other papers [Jolicoeur and Baron, 1980; Baron and Joli-

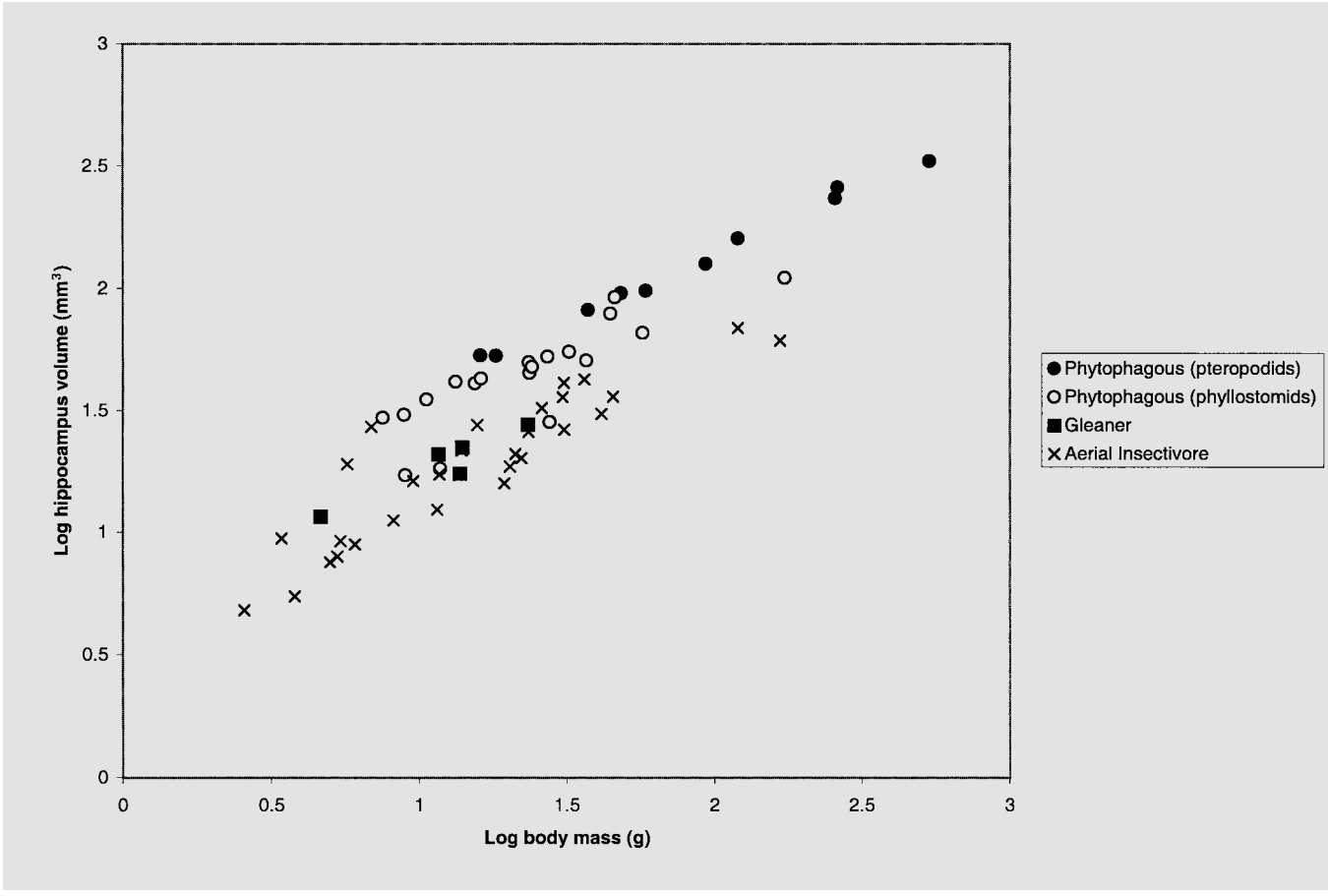


Fig. 5. Log hippocampus volume plotted against log body mass for 63 species of bats. For statistical analyses, the two vampire species were omitted.

coeur, 1980; Barton et al., 1995; Barton 1999] and was obtained in this analysis as well (table 1). Further, the assumption that echolocation might not be useful to frugivorous bats makes some intuitive sense, given the apparent difficulties of distinguishing the target fruit from a cluttered background. However, behavioral observations have shown that echolocation can be a valuable component of the sensory repertoire even of frugivores. Bats have been shown to use olfaction to detect fruit and echolocation for localization [Thies et al., 1998], and to use echolocation to assess the nectar content of flowers in the genus *Mucuna* [von Helverson and von Helverson, 1999]. The phyllostomids, at least, seem to be able to ride a sort of middle ground as sensory generalists. They are able to echolocate, and, on the basis of their olfactory bulbs, probably have an acute sense of smell, and indeed some members of the family are in fact apparently strictly insectivorous.

The only *positive* association to show up in this analysis is the correlation between hippocampus volume and main olfactory bulb volume when corrected for body mass. Fleming et al. [1977] observed that frugivorous bats are extremely sensitive to changes in the spatiotemporal distribution of fruit resources. Although Fleming et al. [1977] studied a phyllostomid bat, it seems likely that this observation would generally be true of frugivorous bats.

Interestingly, the correlation between the auditory nucleus and both the olfactory bulbs and the hippocampus was negative and statistically significant in the phylogenetic analysis (table 2e). Although this might be expected generally, it is interesting that this appears to hold true for the echolocating frugivores as well. That is, the phyllostomids, in retaining the ability to echolocate, have not developed their olfactory bulbs or hippocampi to the same extent as the pteropodids.

Table 5. Correlations of chiropteran brain regions across 61 species of bats under different grouping schemes

	a No groups			b Residuals under 4-group scheme		
	LOGMOB	LOGHIP	LOGBRW	LOGMOB	LOGHIP	LOGBRW
LOGAUD						
Correlation	0.615	0.623	0.761	−0.195	−0.224	0.395
Significance	<0.0005	<0.0005	<0.0005	0.126	0.078	0.002
LOGMOB						
Correlation	0.962	0.938		0.509	0.156	
Significance		<0.0005	<0.0005		<0.0005	0.229
LOGHIP						
Correlation			0.963			0.662
Significance			<0.0005			<0.0005
	c Residuals under 3-group scheme			d Residuals of phytophages only		
	LOGMOB	LOGHIP	LOGBRW	LOGMOB	LOGHIP	LOGBRW
LOGAUD						
Correlation	−0.265	−0.303	0.151	−0.221	−0.530	−0.183
Significance	0.036	0.016	0.245	0.250	0.003	0.343
LOGMOB						
Correlation		0.542	0.180		0.636	0.611
Significance		<0.0005	0.165		<0.0005	<0.0005
LOGHIP						
Correlation			0.660			0.782
Significance			<0.0005			<0.0005
	e Residuals of phylogenetically independent contrasts					
	LOGMOB	LOGHIP	LOGBRW			
LOGAUD						
Correlation	−0.282	−0.274	0.124			
Significance	0.026	0.031	0.336			
LOGMOB						
Correlation		0.603	0.443			
Significance		<0.0005	<0.0005			
LOGHIP						
Correlation			0.702			
Significance			<0.0005			

a Correlations of regions for all bats, no groups used. b Correlations of residuals computed from conventional ANCOVA models with $\log_{10}$ of body mass as covariate and four groups of bats identified (aerial insectivores, gleaners, phytophagous pteropodids, phytophagous phyllostomids). c Correlations of residuals from ANCOVAs separating bats into only three groups (aerial insectivores, gleaners, phytophagous). d Correlations of residuals from an ANCOVA including only phytophagous bats (pteropodids versus phyllostomids; n = 29). e Correlations of residuals based on phylogenetically independent contrasts of all bats (no groups separated). Two-tailed significance levels are shown. Abbreviations: LOGAUD = Log_{10} of auditory nuclei; LOGBRW = Log_{10} of brain mass; LOGMOB = Log_{10} of main olfactory bulb; LOGHIP = Log_{10} of hippocampus.

The foregoing results would seem to support the idea of mosaic evolution of the brain [Barton and Harvey, 2000; de Winter and Oxnard, 2001]. That is, the various functional subsystems of the brain can respond to fairly specific natural (or sexual) selection. The argument has been advanced that such mosaic changes can be limited by developmental constraint, which induces coordinated changes in the sizes of the components of the brain [Finlay and Darlington, 1995; Clancy et al., 1999] and, therefore, renders the sizes of any brain components predictable from overall brain size.

However, our results indicate significant difference in some brain structures of members of the same order. This is made especially evident by the fact that the Pteropodidae and Phyllostomidae, two phylogenetically distinct families, are so similar in the relative sizes of their olfactory bulbs and hippocampi. At the same time, both the pteropodid and phyllostomid bats are significantly different from their insectivorous sister groups in the volumes of their olfactory bulbs, hippocampi, and auditory nuclei. This point is further underscored by the fact that the Phyllostomidae is not a wholly frugivorous family. In addition to the vampires, some of the phyllostomids are almost entirely insectivorous. The olfactory regions of both types of bat phytophages are much larger than those of their insectivorous sister taxa, as are their hippocampi.

In an effort to use brain characteristics as phylogenetically informative characters, Lapointe et al. [1999] studied 120 species of bats, plus several outgroup taxa, using 12 neural characters and concluded that bats are a monophyletic group. Furthermore, Lapointe et al. [1999] emphasized the convergent nature of the megachiropteran and primate brains. One of the goals of their paper was to remove the confounding effects of ecology. When this was accomplished, support for a relationship between megabats and primates, using brain characters, was eliminated. Therefore, they concluded that the only meaningful convergence demonstrated by megabats and primates was with respect to their ecology, rather than neurobiological characters.

A remaining question with regard to bat foraging ecology is: why do the megachiropterans not echolocate? The answer must surely lie in the body size of their last common ancestor with the microchiropterans [Arita and Fenton, 1997; Speakman, 1999]. Given that echolocation is not incompatible with being a frugivore – witness the success of the phyllostomid bats – it is likely that the major tradeoff with which the megachiropteran had to deal was that of body size vs. echolocation. Given the reported differences in fruit sizes between the New and Old Worlds, those of the old world being on average larger [Mack, 1993], it might be that there are other differences between the flora of the Neotropics and Paleotropics that could drive the apparent emphasis on olfaction seen in the pteropodids [Springer et al., 2001].

Seen in this light, the analysis of head-space, the chemical composition of volatiles, becomes particularly relevant to questions of bat evolution. A few studies of the scent composition of bat-pollinated flowers have been conducted [Knudsen and Tollsten, 1995; von Helverson et al., 2000]. Some scent chemists [R. Raguso, pers. comm.] suspect differences between the odors of New and Old World flowers and fruits. If this conjecture is correct, then geographic differences in fruit sizes and smells might be the key to answering the lingering question of why megachiropterans do not echolocate.

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