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Diet, phylogeny, and basal metabolic rate in phyllostomid bats

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Summary

Aside from the pervasive effects of body mass, much controversy exists as to what factors account for interspecific variation in basal metabolic rates (BMR) of mammals; however, both diet and phylogeny have been strongly implicated. We examined variation in BMR within the New World bat family Phyllostomidae, which shows the largest diversity of food habits among mammalian families, including frugivorous, nectarivorous, insectivorous, carnivorous and blood-eating species. For 27 species, diet was taken from the literature and BMR was either measured on animals captured in Brazil or extracted from the literature. Conventional (nonphylogenetic) analysis of covariance (ANCOVA), with body mass as the covariate, was first used to test the effects of diet on BMR. In this analysis, which assumes that all species evolved simultaneously from a single ancestor (i.e., a "star" phylogeny), diet exerted a strong effect on mass-independent BMR: nectarivorous bats showed higher mass-independent BMR than other bats feeding on fruits, insects or blood. In phylogenetic ANCOVAs via Monte Carlo computer simulation, which assume that species are part of a branching hierarchical phylogeny, no statistically significant effect of diet on BMR was observed. Hence, results of the nonphylogenetic analysis were misleading because the critical values for testing the effect of diet were underestimated. However, in this sample of bats, diet is perfectly confounded with phylogeny, because the four dietary categories represent four separate subclades, which greatly reduces statistical power to detect a diet (= subclade) effect. But even if diet did appear to exert an influence on BMR in this sample of bats, it would not be logically possible to separate this effect from the possibility that the dietary categories differ for some other reason (i.e., another synapomorphy of one or more of the subclades). Examples such as this highlight the importance of considering phylogenetic relationships when designing new comparative studies, as well as when analyzing existing data sets. We also discuss some possible reasons why BMR may not coadapt with diet.

Key words: Basal metabolic rate, bats, comparative method, computer-simulations, diet

Introduction

The measurement of basal metabolic rate (BMR) was originally defined as a way to index the minimum rate of energy necessary to maintain homeostasis. Operationally it is defined as metabolic rate in the absence of physical activity in post-absorptive animals, within the zone of thermal neutrality, and during the inactive phase of the normal circadian cycle (McNab, 1997). BMR is a major component of the total energy expenditure of mammals, both under laboratory conditions and in the wild. For free-living mammals, BMR can represent up to 50% of the total daily energy budget (Nagy et al., 1999; Speakman, 2000), which gives this energetic measure an overt ecological significance. Thus, it is not surprising that studies seeking to understand the structural basis, functional significance, and evolutionary trends associated with BMR are still flourishing. Studies of the proximate and ultimate causes of variation in BMR have adopted a range of perspectives, with BMR being correlated (or used to predict) variation at the genomic and cellular level all the way up to zoogeographical patterns (e.g., Vinagrodov, 1995; Rolfe and Brown, 1997; Lovegrove, 2000). This optimistic view

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of BMR as a unifying measurement in mammalian energetics has been questioned (e.g., Speakman, 2000). Nevertheless, BMR remains the energetic trait most often measured in mammals (McNab, 1992a), and it provides a standardized measure of energy expenditure that can be used for intra- and interspecific comparisons.

Among the many factors that determine the variability and magnitude of BMR, body mass is the most pervasive, although functional explanations for this relationship are still controversial (e.g., West et al., 1997; Riisgard, 1998). When the effects of body mass are eliminated statistically, a residual variation still exists which demands an explanation in terms of adaptive significance (McNab, 1988). Several factors have been implied as responsible for this variation, and diet is chief among them (McNab, 1986, 1988, 1992b). The effect of diet on mass-corrected mammalian BMR is thought to be causative in the sense that certain properties of the diet (availability and quality) have brought about differences in the way natural selection has modified the rate by which energy is acquired and used.

Although the "food-habit hypothesis" (Thompson, 1992) might be correct, traditional procedures used to test it can be criticized. Although the food-habit hypothesis was framed in an explicitly evolutionary context, the statistical procedures used to test it usually have not taken phylogenetic history into full account. Traditionally, the relationship of BMR to body mass and diet has been analyzed by conventional analysis of covariance (e.g., McNab, 1992b) or even by informal analyses that did not present statistical tests (e.g., McNab, 1982, 1986). Species are part of a hierarchically structured phylogeny and therefore generally cannot be considered, for statistical purposes, as being independent (e.g., Felsenstein, 1985; Martins and Garland, 1991; Garland and Adolph, 1994; Ackerly, 1999; Garland et al., 1999; Purvis and Webster, 1999; Lapointe and Garland, 2001). Based on this consideration, some authors re-analysed the relationship between mass-independent BMR and diet in mammals within a partial phylogenetic context and found that many of the observed associations reflect taxonomic affiliation (e.g., Hayssen and Lacy, 1985; Elgar and Harvey, 1987). However, analyses that support this "taxonomic-affiliation hypothesis" can also be criticized because higher taxonomic categories (e.g., genera, families) are arbitrary in terms of phylogenetic inclusiveness, may or may not reflect actual cladistic structure, may not constitute statistically independent observations themselves, and do not account for hierarchical relationships within taxonomic levels (e.g., see Miles and Dunham, 1993).

The New World bat family Phyllostomidae exhibits the greatest diversity of food habits of any mammalian

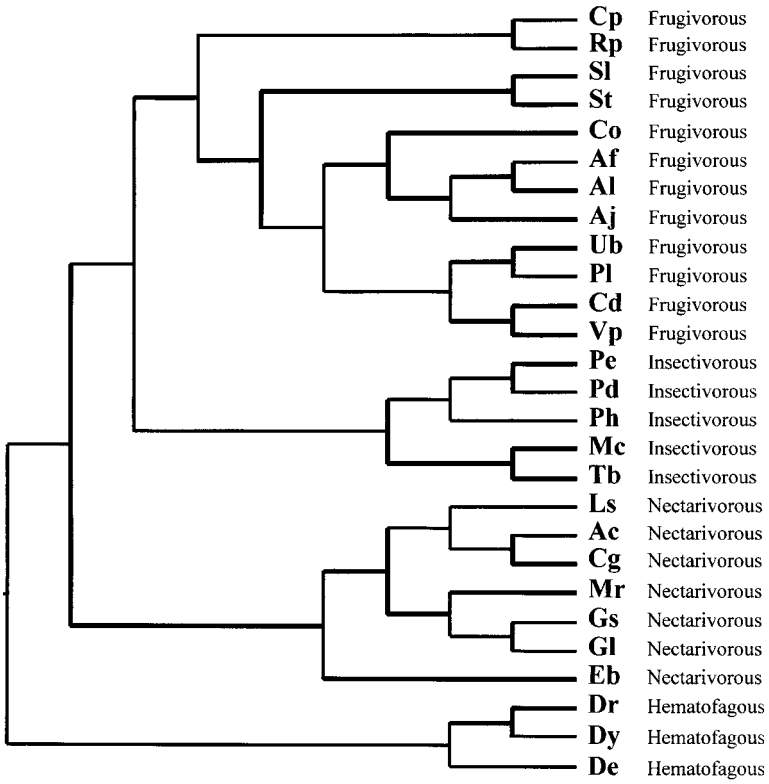

Fig. 1. Cladogram showing hypothesized phylogenetic relationships of the 27 species of phyllostomid bats used in this study, based on Owen (1987) and Wetterer et al. (2000). Branch lengths are arbitrary, using the method of Pagel (1992), and are the same as used in computer simulations. See Table 1 for species coding.

family, with frugivorous, nectarivorous, insectivorous, carnivorous and hematofagous species (Freeman, 2000; Wetterer et al., 2000). McNab (1969, 1982, 1992b) has suggested that the association between BMR and diet within the family Phyllostomidae is as pervasive, and independent of phylogeny, as it is for other mammals. For a wide range of mammalian taxa, McNab (1992b) used conventional ANCOVA models to test for the relationship of BMR to body mass, diet, taxonomic affiliation, and various other factors, depending on the set of species analyzed. In his analysis of 17 species of phyllostomids, with body mass as a covariate ($P = 0.0001$), McNab (1992b) found that diet had a highly significant effect on BMR ($P = 0.0010$), whereas analyses including taxonomic affiliation (genus) and/or climate never showed a significant effect for either of these factors ($P > 0.05$). However, as noted above, the use of generic affiliation alone to account for phylogenetic associations in a statistical analysis is inadequate.

In this article, we re-analysed the relationship of BMR to body mass and diet, while taking into consideration the phylogenetic history of the phyllostomid bats, using an available cladogram and phylogenetically based statistical models (Garland et al., 1993, 1999). We expanded the data-base used for this analysis by including data on BMR for 27 species of phyllostomids, including some measured here for the first time. A preliminary inspection of the cladogram (Fig. 1) showed that diet is perfectly confounded with phylogeny in this sample of species. The confounding of diet and phylogeny in Phyllostomidae was not apparent at the time of McNab's (1992b) previous analysis because he did not attempt to construct phylogenetic trees for the species analyzed and, in any case, not as much phylogenetic information was then available.

Material and methods

Data set

Data on basal metabolic rate (BMR) and body mass of 27 species of bats of the family Phyllostomidae were extracted from the literature (Table 1) or measured

Table 1. Basal Metabolic Rate (BMR – ml $O_2 \cdot h^{-1}$) and body mass (BM – grams) of 27 species of bats of the family Phyllostomidae used in the present study.

Code	Species	Diet	BM	BMR	Source
Cp	*Carollia perspicillata*	Frugivorous	13,9	29,1	1,7
Rp	*Rhinophylla pumilio*	Frugivorous	9,5	16,2	1
Sl	*Sturnira lilium*	Frugivorous	21,9	39,2	1
St	*Sturnira tildae*	Frugivorous	20,5	39,9	7
Co	*Artibeus concolor*	Frugivorous	19,7	32,9	1
Af	*Artibeus fimbriatus*	Frugivorous	63,9	77,96	7
Al	*Artibeus lituratus*	Frugivorous	69,7	86,4	1,7
Aj	*Artibeus jamaiscensis*	Frugivorous	45,2	56,5	1
Ub	*Uroderma bilobatum*	Frugivorous	16,2	26,6	1
Pl	*Platyrrhinus lineatus*	Frugivorous	22,3	30,9	1,7
Cd	*Chiroderma doriae*	Frugivorous	19,9	31,1	7
Vp	*Vampyressa pussila*	Frugivorous	8,8	18,6	7
Pe	*Phyllostomus enlogatum*	Insectivorous	35,6	38,5	1
Pd	*Phyllostomus discolor*	Insectivorous	33,5	34,5	1
Ph	*Phyllostomus hastatus*	Insectivorous	84,2	70,7	1
Mc	*Macrotus californicus*	Insectivorous	11,7	14,6	2
Tb	*Tonatia bidens*	Insectivorous	27,4	39,2	1
Ls	*Leptonycteris curasoae*	Nectarivorous	23	39,3	4,5
Ac	*Anoura caudifer*	Nectarivorous	11,3	28,1	1,7
Cg	*Choeroniscus godmani*	Nectarivorous	10,1	19,9	4
Mr	*Monophyllus redmani*	Nectarivorous	8,7	11,1	6
Gs	*Glossophaga soricina*	Nectarivorous	8,8	21,4	1,3
Gl	*Glossophaga longirostris*	Nectarivorous	13,5	26,5	4
Eb	*Erophylla bombifrons*	Nectarivorous	16,1	17,7	6
Dr	*Desmodus rotundus*	Hematofagous	34,5	32,8	1
Dy	*Diaemus youngi*	Hematofagous	36,6	34,1	1
De	*Diphylla ecaudata*	Hematofagous	27,8	33,9	1

Sources: 1 – McNab (1969, 1989); 2 – Bell et al. (1986); 3 – Cruz-Neto and Abe (1997); 4 – Arends et al. (1995); 5 – Carpenter & Graham (1967); 6 – Rodriguez-Duran (1997); 7 – this study.

in our laboratory using the methods described in Cruz-Neto and Abe (1997; see below). Data were only used if they were obtained via flow-through respirometry and under the standard conditions necessary to measure BMR (McNab, 1997). Most of the data came from McNab or from our own laboratory, which should minimize "noise" introduced by variation in experimental protocols. Whenever two values were available for the same species, and provided that they did not differ by more than 5%, we took a simple average and used it as representative of BMR for this species. When two values were available for a given species and they differed by more than 5%, we used the lower value, in accordance with the definition of BMR. This was only the case for *Carollia perspicillata* and *Anoura caudifer*, and hence we did not use the values reported by McNab (1989) for these species.

According to information in the original sources (see Table 1), only recently collected individuals were measured, thus minimizing possible effects of captivity on BMR (Studier and Wilson, 1979). The second approach to guide the choice of species was associated with the comparative nature of the analysis used in this study, which demands the use of a well-resolved phylogeny. Although data were available for the carnivorous *Chrotopetrus auritus* (mean BMR = 101.9 $mlO_2 \cdot h^{-1}$; mean body mass = 96.1 grams; McNab, 1989) whose phylogenetic position within Phyllostomidae is well resolved (Wetterer et al., 2000), we omitted this species because it is the only carnivorous species for which data are presently available.

We measured BMR in eight species, four of which where not previously studied (*Chiroderma doriae* n = 2, *Vampyressa pusilla* n = 3, *Artibeus fimbriatus* n = 4, and *Sturnira tildae* n = 4). The other four species (*Artibeus lituratus* n = 25, *Platyrrhinus lineatus* n = 23, *Carollia perspicillata* n = 8, and *Anoura caudifer* n = 7) had their BMR previously quantified by McNab (1969, 1989). All species were captured with mist nets at several localities in São Paulo State, Southeast Brazil, and immediately transported to our laboratory, where they were kept in captivity for no longer than five days. During captivity, bats were fed a mixed supply of fruits. Usually, BMR was quantified within two days of capture. Once the experiments were done we returned the animals to the places where we originally collected them.

BMR was quantified by measuring rates of oxygen uptake at temperatures ranging from 5 to 35 °C, between 8:00 and 18:00 h, with an open-flow respirometric system. Briefly, animals were weighed and placed in a respirometer chamber (80–800 ml), which was then placed inside a temperature-controlled cabinet. Dry, CO_2-free room air was pushed through the chamber at rates varying from 100–1,000 ml/min, depending on body mass and experimental temperature. Downstream to the chamber, air was dried and scrubbed of CO_2 and an aliquot of 20–120 ml was diverted to the sensor of an Applied Electrochemistry model S-3A O_2 analyzer. Readings from the analyzer was continuously monitored and stored in a computer for later analysis by a data acquisition system (Sable Systems). Temperature of the chamber was also constantly monitored by a thermocouple linked to the data acquisition system. Bats were tested at a given temperature until oxygen uptake rates had been stable and minimal for at least 10 min. At the end of the experiments, bats were removed from the chamber and body temperature measured by inserting a thermocouple in the rectum. Plots of oxygen uptake rates against ambient temperature were obtained for each bat. From this plot we determined the thermoneutral zone according to Cruz-Neto and Abe (1997), and used the average values of oxygen uptake within this zone as indicative of BMR.

Data analysis

Values of BMR and body mass presented in Table 1 were log transformed prior to analysis. To avoid statistical problems associated with the comparison of a variable (body mass) with a mathematical function of itself (mass-specific rates of oxygen consumption), several of the values of BMR that had been reported in the literature were returned to absolute (whole-animal) rates before analysis (Packard and Boardman, 1999). Comparative analyses were carried out in two ways. First, we performed a conventional, nonphylogenetic analysis, which implicitly assumed that all species evolved from a single common ancestor (star phylogeny) and, in effect, that character evolution had been similar to Brownian motion (e.g., no evolutionary interactions among species). Thus, we tested for the effects of diet on BMR with a conventional Analysis of Covariance (ANCOVA), with log body mass as the covariate, while testing for the assumptions of normality (Shapiro-Wilkins test) and homoscedasticity (Hartley F-max test) of the residuals. Each species was associated to a particular type of diet, according to McNab (1986). For species not included in that paper, we used the original classification reported by the authors. An α level of 0.05 was used to judge statistical significance.

Our second approach assumed that species are part of a hierarchical, branching phylogeny. To analyse our data set within this framework, a cladogram that described the hypothesized phylogenetic relationships of the 27 species used in this study was necessary. Recently, Wetterer et al. (2000) used diverse types of data to construct a phylogenetic tree at the generic level for phyllostomid bats. Phylogenetic relationships within genera were taken from Owen (1987). Because information on

branch lengths proportional to expected variance of character evolution (see Felsenstein, 1985, Garland et al., 1999) was not available, we used arbitrary values for branch lengths, according to the method of Pagel (1992), as shown in Figure 1. We checked the diagnostic for phylogenetically independent contrasts as suggested by Garland et al. (1992; see also Díaz-Uriarte and Garland, 1998; Harvey and Rambaut, 2000) and found that these arbitrary branch lengths adequately standardized the contrasts. Hence, these branch lengths seem reasonable for simulations as well.

We used Monte-Carlo simulations to generate phylogenetically correct ("PC"), empirically scaled null distributions of F statistics for ANCOVA (Garland et al., 1993; Vanhooydonck and Van Damme, 1999), using the cladogram shown in Figure 1. As discussed above, conventional F-statistics for ANCOVAs cannot properly be judged for significance against the usual tabular values, owing to phylogenetic non-independence of species values. An approach to overcome this problem is to simulate the data set many times, and generate a null-distribution of F values that allows critical values to be set for hypothesis testing. From this distribution, the critical value, at $\alpha = 0.05$, is obtained by computing the 95th percentile. If this critical value exceeds the F ratio for the real data set, then we cannot reject the null hypothesis of no effect of diet on BMR. Actual P values were calculated as the number of F values derived from simulations that were larger than the F values for the real data, divided by the total number of simulations (1,000 in the present study).

Because the actual way in which BMR evolves is unknown, computer simulations were performed under five different evolutionary models, using the PDSIMUL module of the Phenotypic Diversity Analysis Programs version 5 (PDAP – Garland et al., 1993, 1999). For all models we set the correlation between the two traits (log body mass and log BMR) equal to zero because we also aimed to test for effects of body mass. All simulations were performed on the log-transformed scale. Expected variances at the tips of the phylogeny were always set equal to the actual variances of the data (the default in PDSIMUL). Also for all models, initial and final means were set as being equal to the estimated root node values obtained by an independent contrast analysis of the log-transformed real data set (see Garland et al., 1993, 1999). Back-transformed, these values were 26.45 grams for body mass and 30.9 ml $O_2 \cdot h^{-1}$ for BMR.

We used Gradual and Speciational Brownian motion models without limits or trends for evolution. We further simulated data under gradual Brownian motion with limits to evolution as 2 – 1,500 grams for body mass and 6.34 – 630 ml $O_2.h^{-1}$ for BMR. For body mass, this represents the range for extant bats (lower value for bumblebee bats, *Craseonycteris thonglongyai*; upper value for flying foxes, genus *Pteropus* – Nowak, 1994). For BMR, the range was calculated using the allometric equation derived by McNab (1988) for Chiroptera. For simulations using the gradual Ornstein-Uhlenbeck model (O-U model – Felsenstein ,1988), we set adaptive peaks as being equal to final means (estimated root node values). Finally, we simulated data under punctuated equilibrium (Gould and Eldredge, 1977; see Garland et al., 1993).

Results

The results of conventional, nonphylogenetic analysis indicated that both log body mass and diet significantly affected log BMR in phyllostomid bats (Fig. 2; Table 2). Phylogenetic ANCOVA also showed that body mass had a significant effect on BMR, irrespective of the evolutionary model considered ($P < 0.001$ in all cases; Table 2). However, when F-values for the main effect of diet (while controlling for body mass) were compared with the phylogenetically correct critical values, P values varied between 0.43 and 0.65, depending on the evolutionary model used for simulation. Thus, under the assumption that species are part of branching hierarchical phylogeny, we could not detect any effect of the diet on mass-independent BMR for any of the five evolutionary models employed in this analysis.

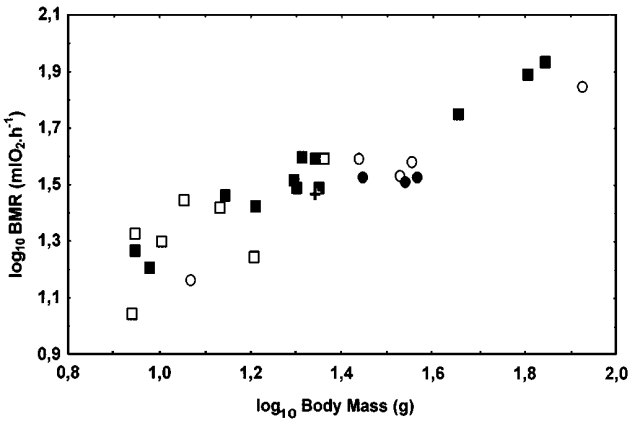

Fig. 2. Relationship between $\log_{10}$ basal metabolic rate (BMR – ml $O_2 \cdot h^{-1}$) and $\log_{10}$ body mass (grams) for the 27 species of phyllostomid bats used in this study, as a function of diet. Open circles = insectivorous; Closed circle = hematofagous; Closed square = frugivorous; Open square = nectarivorous. Cross indicates estimated values at the root of the phylogenetic tree (Fig. 1), using the independent contrasts method presented in Garland et al. (1993, 1999).

Table 2. Analysis of Covariance (ANCOVA) testing the effects of diet on $\log_{10}$ basal metabolic rate of 27 species of phyllostomid bats, with $\log_{10}$ body mass as the covariate. Critical values for F statisitcs and sigificance levels are presented for conventional ANCOVA and for ANCOVAs based on Monte Carlo simulations upon the phylogeny showed in Figure 1 (using Pagel's [1992] arbitrary branch lengths). These latter analyses were done under five different models of evolution implemented with the PDSIMUL program (Garland et al. 1993).

					Brownian Motion									
			Conventional		Gradual		Speciational		Gradual – Limits		Ornstein – Uhlenbeck		Punctuated equilibrium	
Source of Variation	df	F	Critical Value	P	Critical Value	P	Critical Value	P	Critical Value	P	Critical Value	P	Critical Value	P
Main Effect	3	4.31	2.99	<0.02	29.8	0.65	21.2	0.55	29.2	0.64	14.7	0.43	19.8	0.53
Covariate	1	128.0	4.24	<0.001	8.09	<0.001	7.71	<0.001	7.1	<0.001	6.44	<0.001	8.5	<0.001
Explained	4	40.7	2.76	<0.001	25.8	<0.001	17.8	<0.001	25.7	<0.001	13.1	<0.001	17.8	<0.001
Error	22													
Total	26													

Discussion

Effects of body mass and diet

Our results for the effects of body mass and diet on BMR showed that, under the assumption of a star phylogeny, both factors strongly affect BMR of phyllostomid bats. Also working under the assumption of a star phylogeny, McNab (1992b) found that body mass had a strong effect on BMR for 17 species of phyllostomid bats; larger-bodied species had higher absolute BMR. When our data were analysed under the assumption of a branching, hierarchical phylogeny, body mass still showed a significant effect on BMR, and this effect was strong under all five different evolutionary models used in the computer simulations (Table 2). Thus, the dependence of BMR on body mass is clear, irrespective of whether we assume a star or a branching hierarchical phylogeny (for a similar example, see Garland et al., 1993).

McNab (1982, 1992b) has suggested that in phyllostomid bats the residual variation in BMR is strongly correlated with dietary guild. Using conventional ANCOVA, he showed that diet had a strong effect on mass-corrected BMR of phyllostomid bats (McNab, 1992b). He further suggested that high absolute BMR would be associated with easily digestible diets, food that is free from chemical agents, and/or food that is available throughout the year (McNab, 1992b; see also McNab, 1986). Fruits and nectar seem to fit this category while the converse applies for an insectivorous or hematofagous diet (McNab, 1986). However, when our data were analysed under the more plausible assumption of hierarchical, branching phylogenetic relationships, we could not detect a statistically significant effect of diet on mass-independent BMR, irrespective of the evolutionary model (Table 2). This disagreement between the two types of analysis may suggest that mass-independent BMR is not affected by diet, with similarities of species within clades being attributable to recency of common ancestry. Alternatively, we may not have been able to demonstrate an effect of diet on mass-independent BMR because of methodological limitations.

Caveats of the method: diet categorization, phylogeny, and evolutionary models

One caveat of our analysis is the way we classified diet. The categories we adopted in the present study were conservative, but in accordance with the hypothesis we aimed to test (McNab, 1986). However, with this categorization diet and phylogeny are strongly confounded (see Table 1 and Fig. 1). Nevertheless, assignment of diet categories was done at an early stage in our study and the clear relationship with phylogenetic position was not apparent until we assembled the phylogenetic relationships. The problems associated with such colinearity have been discussed elsewhere (Garland et al., 1993; Vanhooydonck and Van Damme, 1999); chief among them is a reduction in statistical power. When an independent variable is strongly associated with phylogenetic position, the power to detect an effect of that independent variable is reduced (e.g., see figure 5 in Vanhooydonck and Van Damme, 1999). Nonetheless, despite potentially low statistical power, differences among clades can be shown to exist with fully phylogenetic statistical methods, at least when such effects are strong (e.g., see Garland et al., 1997; Binninda-Emonds and Gittleman, 2000).

Statistical power to detect differences among groups, in a phylogenetic context, can be estimated by computer

simulations (e.g., see Martins and Garland, 1991; Garland and Adolph, 1994). For example, the current version of PDANOVA (available on request from T.G.) allows the user to add a constant to the values for one or more of the groups (e.g., dietary categories) before performing the ANCOVA (or ANOVAs). Hence, for any phylogeny and set of tip data, it is possible to see how large group differences would need to be in order for most (e.g., 80–95%) of the F values for the modified simulated data to exceed the critical values determined by computer simulation and analysis of the unmodified data (e.g., as reported in Table 2). In other words, one would first perform simulations to determine phylogenetically correct critical values for F statistics under one or more models of character evolution, as we report in Table 2. Then one would reanalyze the same data with PDANOVA, but adding (or subtracting) a constant to the log BMR of all species within one or more of the diet categories. This mimics the evolution of a higher (or lower) mass-adjusted BMR in one or more of the groups. The F statistics for these data would tend to be larger than for the unmodified data. By trying different values, one could determine how large group differences would need to be in order for them to be considered statistically significant (i.e., exceed the critical values) frequently enough to reach the desired statistical power. We have not done such simulations here because we did not have clear a priori expectations for the pattern and magnitude of diet-related differences in BMR. However, for someone in the process of designing a comparative study (i.e., deciding which species to measure), such simulations could be very useful (see also Vanhooydonck and Van Damme, 1999; Ackerly, 2000).

One general way to increase statistical power is simply to increase sample size, as more information on BMR, food habits, and the phylogenetic relationships of phyllostomid bats becomes available. This may have little effect, however, unless species are added that break the strong relationship between diet and phylogeny that is present in the existing data set (Fig. 1). In this regard, additional data on more species of the sub-family Phyllostominae, especially those with a carnivorous diet, would be profitable. Unfortunately, only one carnivorous phyllostomid has yet been studied (*Chrotopterus auritus*, McNab, 1989; see Materials and Methods) which precluded us from including it in analyses. A possibly more promising way to increase power would be to classify diet on a continuous scale (see also Garland et al., 1993) based on, for example, percent of fiber, energetic content or digestibility. Another possibility would be to classify diet on a different discrete scale (see also comments in McNab, 1992a). Not all of the bats present strict fidelity in relation to the categories we used. Diversity of diet, with geographical and seasonal variations, is more common in bats than hitherto believed (Martuscelli, 1995; Kunz and Diaz, 1995; Ferrarezi and Gimenez, 1996). Based on such information, Ferrarezi and Gimenez (1996) and Wetterer et al. (2000) recently refined dietary categorization for several species of bats, attributing to most of the phyllostomid bats polymorphic diet categories. Although we used monotypic diet categories, our classification is not meant to imply that bats are committed exclusively to a single diet category (except for hematofagous bats); it only suggests that a given diet category is the predominant food type taken by a given species of bat. In fact, this was the only difference between the way we categorized diet and the new categorization proposed by Wetterer et al. (2000). In this regard, the categorization of Wetterer et al. (2000) would be not particularly helpful and, more important, it also does not avoid the problem of colinearity. Thus, the caveat of the effects of diet categorization on the outcome of such analyses is still an open question.

A second methodological problem is the fact that phylogenies are hypotheses subject to modifications, and the cladogram we used in this study may not reflect truth. Third, we do not have information on divergence time between the species, so we used arbitrary branch lengths (see Fig. 1). Inaccurate branch-length information will reduce the accuracy of significance tests derived from computer simulations, but perhaps not to a fatal extent (Díaz-Uriarte and Garland, 1998). Branch lengths interact with evolutionary models in phylogenetic analyses, and the consistency of our results across models (Table 1) suggests that errors in branch lengths may not have had a strong effect on our final results.

A fourth point is that none of the five evolutionary models we used to simulate data may be biologically realistic, which may cause misleading significance tests (see also Price, 1997; Díaz-Uriarte and Garland, 1998; Harvey and Rambaut, 2000). Although the concordance of results irrespective of the model employed is reassuring, it should be noted, for example, that the absence of evolutionary trends we imposed in our simulations is in contrast with the trend for increasing body mass that has occurred in other mammals (Alroy, 1998).

Diet and BMR: evolutionary adaptation vs. phenotypic plasticity

Assuming that the above methodological limitations have not unduly biased our results, then it is possible that mass-independent BMR of phyllostomid bats does not, in fact, coadapt with diet. The core of the "food-habit hypothesis" is that certain properties of the food (e.g., quality and availability) limit the rate by which the energy is acquired and spent, which causes selec-

tion for a particular (high or low) level of BMR (McNab, 1986). Given the apparent positive relationship between BMR and rates of daily energy expenditure in the wild (Ricklefs et al., 1996), mammals faced with "low-quality" diets should have acquired, over evolutionary time, low rates of basal energy expenditure, thus allowing a reduction in their total daily rates of energy expenditure. However, Speakman (2000) was not able to show any effects of diet on rates of daily energy expenditure in mammals, after taking the effects of phylogeny into account, which suggests that mammals may respond differently to the effects of diet than predicted by the "food-habit hypothesis." Irrespective of the debate as to whether BMR is linked to daily rates of energy expenditure, our results seems to parallel Speakman's, further arguing against the "food-habitat hypothesis."

At the level of phenotypic plasticity rather than evolutionary adaptation, although some mammals downregulate BMR when faced with low-quality diet, the universality of this response has been contested (review in Speakman, 2000). Moreover, when faced with a low-quality diet, some mammals do not downregulate BMR but, instead, increase food intake (e.g., Thompson, 1992; Voltura and Wunder, 1998), in order to maintain a relatively constant intake of nutrients and energy. Increase in food intake leads to a decrease in digestive efficiency, and to compensate for such decrease, some mammals alter their gastrointestinal tract (Piersma and Lindström, 1997; Speakman, 2000). Alteration in gut morphology, via increase in some parts of the gastrointestinal tract, may increase the energetic cost of maintenance and, thus, BMR. Although we do not have data on the proximate effects of diet quality on BMR of bats, the diversity of food habits among phyllostomids is paralleled by an extreme plasticity of some components of the dietary apparatus, including the gastrointestinal tract (Forman et al., 1979; see also Freeman, 2000 on plasticity of other components of the dietary apparatus in bats). This plasticity, in turn, is associated with differences in absorption capacities in these bats (Kovtun and Zhukova, 1994; Delmore and Thomas, 1996) which may underscore their responses to a decrease in food quality and/or availability. Taken together, these factors may have contributed to mitigate the possible evolutionary effects of diet on mass-independent BMR and reduced the variation in BMR among clades to a level that prevented us from detecting statistically significant differences. Studies on the phenotypic plasticity of BMR in bats in response to manipulations of diet quality and availability will be pivotal to test this hypothesis.

In summary, the conclusion as to whether diet affects mass-independent BMR in phyllostomid bats depends on the assumptions made about the phylogenetic relationship among the species. The assumption of a star-like phylogeny leads to corroboration of the hypothesis. However, this assumption is unrealistic based on presently available phylogenetic information (Wetterer et al., 2000), and the results of the analysis are misleading because critical values for hypothesis testing were underestimated (Table 2). Under the more trustworthy assumption that species are part of a branching, hierarchical phylogeny (as shown in Fig. 1), we were not able to detect a statistically significant effect of diet on the residual variation of BMR, perhaps because phyllostomid bats evolved different strategies to cope with variations in the quality and availability of their diets. However, owing to the colinearity between diet and phylogeny, positive results would only be obtained if BMR varied greatly among diet groups. Moreover, even if such variation occurred, we could not logically separate the effect of diet from the possibility that these 27 species of phyllostomid bats differ for some other reason besides diet (i.e., some synapomorphy of one or more subclades). Hence, phyllostomid bats, in spite of their great dietary diversity, do not necessarily represent a good model for testing associations between diet and other aspects of the phenotype. Examples such as the present one should serve as a cautionary tale for the design of comparative studies (see also Garland et al., 1993; Ackerly, 1999, 2000; Purvis and Webster, 1999; Vanhooydonck and Van Damme, 1999).

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References

Ackerly, D. D. 1999. Comparative plant ecology and the role of phylogenetic information. In: Physiological plant ecology (M.C. Press, J. D. Scholes and M. G. Braker, eds). Blackwell Science, Oxford, U.K., pp. 391–413.

Ackerly, D. D. 2000. Taxon sampling, correlated evolution, and independent contrasts. Evolution 54: 1480–1492.

Alroy, J. 1998. Cope's rule and the dynamics of body mass evolution in North American fossil mammals. Science 280: 731–734.

Arends, A., F.J. Bonaccorso, and M. Genoud.1995. Basal rates of metabolism of nectarivorous bats (Phyllostomidae) from a semi-arid thorn forest in Venezuela. J. Mammal. 76: 947–956.

Bell, G.P., G.A. Bartholomew and K.A. Nagy. 1986. The roles of energetics, water economy, foraging behaviour and geother-

mal refugia in the distribution of the bat *Macrotus californicus*. J. Comp. Physiol. B 156: 441–450.
Bininda-Emonds, O.R.P. and J.L. Gittleman. 2000. Are pinnipeds functionally different from fissiped carnivores? The importance of phylogenetic comparative analyses. Evolution 54: 1011–1023.
Carpenter, R. and J.B. Graham. 1967. Physiological responses to temperature in the long-nosed bat, *Leptonycteris sanborni*. Comp. Biochem. Physiol. 22: 709–722.
Cruz-Neto, A.P and A.S. Abe. 1997. Taxa metabólica e termoregulação no morcego nectarívoro *Glossophaga soricina* (Chiroptera, Phyllostomidae). Rev. Bras. Biol. 57: 203–209.
Delorme, M. and D.W. Thomas. 1996. Nitrogen and energy requirements of the short-tailed fruit bat (*Carollia perspicillata*): fruit bats are not nitrogen constrained. J. Comp. Physiol. B 166: 427–434.
Díaz-Uriarte, R. and T. Garland, Jr. 1998. Effects of branch length errors on the performance of phylogenetically independent contrasts. Systematic Biology 47: 654–672.
Elgar, M.A. and P.H. Harvey. 1987. Basal metabolic rates in mammals: allometry, phylogeny and ecology. Funct. Ecol. 1: 25–36.
Felsenstein, J. 1985. Phylogenies and the comparative method. Amer. Nat. 125: 1–15.
Felsenstein, J. 1988. Phylogenies and quantitaive characters. Ann. Rev. Ecol. Syst. 19: 445–471.
Ferrarezi, H. and E.A.Gimenez. 1996. Systematic patterns and the evolution of feeding habits in Chiroptera (Archonta: Mammalia). J. Comp. Biol. 1: 75–94.
Freeman, P.W. 2000. Macroevolution in microchiroptera: recoupling morphology and ecology with evolution. Evol. Ecol. Res. 2: 317–335.
Forman, G.L., C.J. Phillips and C.S. Rouck. 1979. Alimentary Tract. In: Biology of Bats of the New World Family Phyllostomidae (R.J. Baker, J. Knox Jones Jr. and D.C. Carter,eds). Spec. Publ. Mus. Texas Tech Univers. 16. Part III, pp. 205–227.
Garland, T., Jr., and S. C. Adolph. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. Physiol. Zool. 67: 797–828.
Garland, T., Jr., P.H. Harvey and A.R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Syst. Biol. 41: 18–32.
Garland, T., Jr., K.L.M. Martin, and R. Diaz-Uriarte. 1997. Reconstructing ancestral trait values using squared-change parsimony: plasma osmolarity at the origin of amniotes. In: Amniote origins: completing the transition to land (S.S. Sumida and K. L. M. Martin,eds). Academic Press, San Diego, pp. 425–501.
Garland, T., Jr., P.E. Midford and A.R. Ives. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. Amer. Zool. 39: 374–388.
Garland, T., Jr, A.W. Dickerman, C.M. Janis and J.A. Jones. 1993. Phylogentic analysis of covariance by computer simulation. Syst. Biol. 42: 265–292.
Gould, S.J. and N. Eldredge. 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3: 115–151.
Harvey, P.H. and A. Rambaut. 2000. Comparative analyses for adaptive radiations. Phil. Trans. Roy. Soc. Lond. B 355: 1599–1605.
Hayssen, V. and R.C. Lacy. 1985. Basal metabolic rates in mammals: taxonomic differences in the allometry of BMR and body mass. Comp. Biochem. Physiol. 81A: 741–754.
Kovtun, M.F. and N.F. Zhukova. 1994. Feeding and digestion intensity in chiropterans of different trophic groups. Folia Zool. 34: 377–386.
Kunz, T.H.and C.A. Diaz. 1995. Folivory in fruit-eating bats, with new evidence from *Artibeus jamaiscensis* (Chiroptera: Phyllostomidae). Biotropica 27: 106–120.
Lapointe, F.-J. and T. Garland, Jr. 2001. A generalized permutation model for the analysis of cross-species data. Journal of Classification. In press.
Lovegrove, D. 2000. Zoogeography of mammaliam BMR. Am. Nat. 156: 201–219.
Martins, E. P., and T. Garland, Jr. 1991. Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. Evolution 45: 534–557.
Martuscelli, P. 1995. Avian predation by the round-eared bat (*Tonatia bidens*, Phyllostomidae) in the Brazilian Atlantic forest. J. Trop. Ecol. 11: 461–464.
Miles, D.B. and A.E. Dunham. 1993. Historical perspectives in ecology and evolutionary biology: the use of phylogenetic comparative analysis. Ann. Rev. Ecol. Syst. 24: 587–619.
McNab, B.K. 1969. The economics of temperature regulation in neotropical bats. Comp. Biochem. Physiol. 31: 227–268.
McNab, B.K. 1982. Evolutionary alternatives in the physiological ecology of bats. In: Ecology of Bats (T.H. Kunz, ed.). Academic Press, New York, pp. 151–200.
McNab, B.K. 1986. The influence of food habits on the energetics of eutherian mammals. Ecol. Monographs 56: 1–19.
McNab, B.K. 1988. Complications inherent in scaling the basal rate of metabolism in mammals. Q. Rev. Biol. 63: 25–54.
McNab, B.K. 1989. Temperature regulation and rate of metabolism in three bornean bats. J. Mammal. 70: 153–161.
McNab, B.K. 1992a. Energy expenditure: a short history. In: Mammalian Energetics: Interdisciplinary Views of Metabolism and Reproduction (T.E. Tomasi and T. H. Horton eds.). Cornell University Press. Ithaca, pp. 1–15.
McNab, B.K. 1992b. A statistical analysis of mammalian rates of metabolism. Funct. Ecol. 6: 672–679.
McNab, B.K. 1997. On the utility of uniformity in the definition of basal metabolic rate. Physiol. Zool. 70: 718–720.
Nagy, K.A., I.A. Girard, and T.K. Brown. 1999. Energetics of free-ranging mammals, reptiles, and birds. Annu. Rev. Nutrition 19: 247–277.
Nowak, R.M. 1994. Walker's Bats of the World. John Hopkins University Press, Baltimore.
Owen, R.D. 1987. Phylogenetic analysis of the bat subfamily Stenodermatinae (Mammalia: Chiroptera). Texas Tech University Press, Lubbock.
Packard, G.C. and T.J. Boardman. 1999. The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? Comp. Biochem. Physiol. 122A: 37–44.
Pagel, M.D. 1992. A method for the analysis of comparative data. J. Theor. Biol. 156: 431–442.
Piersma, T. and A. Lindström. 1997. Rapid reversible changes in organ size as a component of adaptive behavior. Trends Ecol. Evol. 12: 134–138.
Price, T. 1997. Correlated evolution and independent contrasts. Phil. Trans. Royal Soc. Lond. B 352: 519–529.
Purvis, A. and A.J. Webster. 1999. Phylogenetically independent comparisons and primate phylogeny. In: Comparative Primate Socioecology (P.C. Lee ed.). Cambridge University Press, Cambridge, pp. 44–70.
Ricklefs, R.E., M. Konarzewski and S. Daan. 1996. The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. Am. Nat. 147: 1047–1071.
Rolfe, D.F.S. and G. C. Brown. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol. Rev. 77: 731–758.

Riisgard, H.U. 1998. No foundation of a “3/4 power scaling law” for respiration in biology. Ecol. Letters 1: 71–73.

Rodriguez-Duran, A. 1995. Metabolic rates and thermal conductance in four species of neotropical bats roosting in hot caves. Comp. Biochem. Physiol. 110A: 347–355.

Speakman, J.R. 2000. The cost of living: field metabolic rates of small mammals. Adv. Ecol. Res. 30: 178–297.

Studier, E.H. and D.E. Wilson. 1979. Effects of captivity on thermoregulation and metabolism in *Artibeus jamaiscensis* (Chiroptera: Phyllostomatidae). Comp. Biochem. Physiol. 62A: 347–350.

Thompson, S.D. 1992. Gestation and lactation in small mammals: basal metabolic rate and the limits of energy use. In: Mammalian Energetics: Interdisciplinary Views of Metabolism and Reproduction (T.E. Tomasi and T. H. Horton, eds.). Cornell University Press, Ithaca, pp. 213–259.

Vanhooydonck, B. and R. Van Damme. 1999. Evolutionary relationships between body shape and habitat use in lacertid lizards. Evol. Ecol. Res. 1: 785–805.

Vinogradov, A. E. 1995. Nucleotypic effect in homeotherms: body-mass-corrected basal metabolic rate of mammals is related to genome size. Evolution 49: 1249–1259.

Voltura, M.B. and B.A. Wunder. 1998. Effects of ambient temperature, diet quality and food restriction on body composition dynamics of the praire vole, *Microtus ochrogaster*. Physiol. Zool. 71: 321–328.

West, G.B., J.H. Brown and B.J. Enquist. 1997. A general model for the origin of allometric scaling laws in biology. Science 276**:** 122–126.

Wetterer, A.L., M.V. Rockman and N.B. Simmons. 2000. Phylogeny of phyllostomid bats (Mammalia, Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. Bull. Am. Mus. Nat. Hist. 248: 1–200.