

Morphological correlates of bite force and diet in the skull and mandible of phyllostomid bats

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Summary

1. Bite force is an important performance measure for vertebrate and is related to a variety of ecological challenges. Phyllostomid bats present highly diversified feeding habits associated with extensive cranial shape divergence. Biomechanical models predict that the cranial shape changes are linked to dietary variation through bite force. However, the relationship of cranial shape, diet and bite force had not been explicitly tested before.

2. Here we use a combination of geometric morphometric techniques and comparative methods to test for morphological correlates of bite force and diet in 14 phyllostomid bat species. Skull and mandible shape variation were assessed by vectors, derived from a two-block partial least squares analysis of geometric shape and size-independent bite forces. The relationship between bite force, skull shape and diet was assessed by phylogenetic generalized least square regressions.

3. Most variation in the bite force data examined here (approximately 74%) was explained by size variation alone, but the shape vectors for both skull and mandible explained a significant part of the residual variation in bite force (83% and 56%, respectively), as did the dietary differences (56%). Although the first principal component of diet variation is associated with a negative correlation between insectivory and frugivory, residual bite force and cranial shape are not associated with this diet contrast. Shape and residual bite force variation in the sample were strongly associated with the second diet component, depicting an increase in nectarivory.

4. Species with stronger bites (insectivorous and frugivorous) than expected for their sizes presented a shorter rostrum and mandible, higher skulls, and more developed muscle attachment areas. On the other hand, the characteristic cranial elongation of nectarivorous species (supporting the long tongue) is a trade-off with bite force. These morphological correlates of bite force are similar to those observed in carnivores and non-herbivorous marsupials, and can be related to morphological characters used in biomechanical models for bite force prediction.

5. Our results reinforce the effectiveness of statistically integrating geometric shape variables to bite force and diet information in the investigation of patterns of cranial shape change and trophic radiation in ecologically diverse vertebrate groups.

Key-words: Chiroptera, comparative methods, feeding specialization, geometric shape, Phyllostomidae

Introduction

Earlier studies on ecological morphology appeared in the 1950s and 1960s following work by researchers interested in a variety of ecological questions such as resource partition and community structure (Bock 1994). The methodological approach used in these studies, namely correlational analyses

between morphological traits and relevant ecological features, is still widely used, and when conducted under an explicit phylogenetic scenario has the potential to shed light on the process of adaptation (Hutcheon *et al.* 2002; Westneat *et al.* 2005; Clabaut *et al.* 2007). For a deeper understanding of the adaptive value of a morphological trait, however, it is also necessary to investigate how an organism performs an ecologically relevant task using this trait, how (and if) this trait influences the observed performance, if the variation in

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Species	<i>N</i>	Insectivory	Frugivory	Nectarivory	Carnivory
<i>Micronycteris minuta</i> (<i>Mmi</i>)	12	6	1	0	0
<i>Lophostoma silvicolum</i> (<i>Lsi</i>)	7	5	1	1	1
<i>Phyllostomus hastatus</i> (<i>Pha</i>)	9	4	3	2	1
<i>Phyllostomus elongatus</i> (<i>Pel</i>)	3	6	1	1	1
<i>Phyllostomus discolor</i> (<i>Pdi</i>)	4	4	3	2	1
<i>Mimon crenulatum</i> (<i>Mcr</i>)	11	6	0	1	1
<i>Erophylla sezekorni</i> (<i>Ese</i>)	10	3	3	3	0
<i>Monophyllus redmani</i> (<i>Mre</i>)	10	3	1	3	0
<i>Glossophaga soricina</i> (<i>Gso</i>)	10	2	3	3	0
<i>Carollia perspicillata</i> (<i>Cpe</i>)	14	2	5	1	0
<i>Sturnira lilium</i> (<i>Stli</i>)	18	1	5	1	0
<i>Uroderma bilobatum</i> (<i>Ubi</i>)	7	1	6	1	0
<i>Platyrrhinus lineatus</i> (<i>Plli</i>)	20	1	6	1	0
<i>Artibeus obscurus</i> (<i>Aob</i>)	20	1	6	1	0

Table 1. Species, sample size (*N*), and diet of phyllostomid bats used to investigate bite force and jaw-skull shape relationships. Diet scores correspond to ranks of estimated percentage contribution of items (see text)

this trait has a genetic basis, and if the performance in question does really have an impact on the reproductive success of the organism (Aerts *et al.* 2002). A performance trait that has been largely investigated among vertebrates, and is linked to a cranial shape divergence and a variety of ecological challenges (e.g. food acquisition, mating, and predator avoidance) is bite force (Christiansen & Wroe 2007; Wroe & Milne 2007; Anderson *et al.* 2008).

Maximal bite force varies both within and across species, which in several taxa is mainly a result of differences in muscle mass and/or leverage systems associated to changes in body size (Anderson *et al.* 2008). Additional traits, however, may also contribute to this variation, such as skull shape (Herrel *et al.* 2007) and the architecture of bite-related muscles (Van der Meij & Bout 2004; Herrel *et al.* 2008). Presenting unusually high levels of morphological and ecological diversity, bats have long been subject to ecomorphological studies (Findley 1993; Swartz *et al.* 2003). Recurrent in many of these studies is the search for correlations between cranial shape and diet (Van Cakenberghe *et al.* 2002; Dumont 2004; Nogueira *et al.* 2005), an issue of particular interest in phyllostomids, whose dietary diversification has no parallel among mammalian families (Freeman 2000; Wetterer *et al.* 2000; Nogueira & Peracchi 2003). In the last decade, bite force has been incorporated into some of these studies, allowing for a more refined analysis of the link between skull morphology and feeding strategies (Aguirre *et al.* 2002; Dumont 2007). However, while a clear association between residual (size-corrected) bite force and masticatory muscle characteristics has been demonstrated (Herrel *et al.* 2008), an expected correlation with shape variables has not been observed (Dumont 2007).

Here we employ geometric morphometric techniques to quantify skull and mandible shape, and investigate the hypothesis that evolutionary variation in size-corrected bite force is associated with shape changes related to muscle insertion areas (temporalis and masseter) and dietary differences. Based on previous analyses exploring relationships among these traits using linear measurements (Aguirre *et al.* 2002), we predict that nectarivory will be negatively correlated with shape changes linked to increased bite force. These changes

(e.g. higher and shorter mandibles, increased muscle insertion areas) are expected to be maximally expressed in bats engaged in carnivory, but we have no *a priori* hypothesis regarding their expression in the specialized fruit or insect-eating bats studied here, because food items explored by each group can be highly variable in hardness (Aguirre *et al.* 2002, 2003). We have focused our analysis on phyllostomid bats, for which a large data base on skull and mandible images has been obtained for ongoing studies of shape variation and morphological integration.

Materials and methods

SAMPLE

A total of 155 specimens representative of 14 species and 12 genera of phyllostomid bats were analysed (Table 1, Fig. 1). This set of species was selected based on the availability of bite force data on phyllostomids in the published literature (Aguirre *et al.* 2002; Dumont & Herrel 2003).

All specimens were considered adults based on the ossification of the basisphenoid region. We examined the lateral view of both skull and mandible of all these specimens, except in the case of *Carollia perspicillata*, which was excluded from the skull analysis because of the incompleteness of the zygomatic arch in this taxon. Skull and mandible images were obtained with a digital camera Nikon Coolpix 8700. Particular care was taken so that all specimens were photographed under the same plane regarding the camera lens (reference points in the specimens were used to align the sagittal plane with the focal plane). To capture as much shape variation as possible about the lateral view of both skull and mandible, 14 and 11 landmarks, and 20 and 25 semi-landmarks, respectively, were selected (Fig. 2). All landmarks and semi-landmarks were digitalized in the tpsDig software (Rohlf 2004). The skull and mandible configurations of points were treated as separate data sets in all analyses described below.

BITE FORCE MEASUREMENTS AND DIET

Bite force data used here were obtained from Aguirre *et al.* (2002), and complemented with information from Dumont & Herrel (2003) for *Erophylla sezekorni* and *Monophyllus redmani*. These measurements



Fig. 1. *Lophostoma silvicolum*, one of the phyllostomid bats studied here.

were obtained by a force transducer and correspond to *in vivo* measurements of the maximal force (at least five trials were performed for each animal) during bilateral canine biting (see original articles for more details). Because no shape data was available for *Artibeus jamaicensis*, we associated bite force for this species with cranial shape from the similar-sized, and closely related phylogenetically, *Artibeus obscurus* (Larsen *et al.* 2007).

Diet data were also obtained from the literature, and complemented with unpublished information from us. For each species we selected studies that potentially covered seasonal variation in diet, and that evaluated the possible use of all the main food resources explored by

phyllostomid bats (insects, vertebrates, fruits and nectar/pollen) (e.g. Reis & Peracchi 1987; Ascorra *et al.* 1996; Pedro & Taddei 1997; Giannini & Kalko 2004; Soto-Centeno & Kurta 2006). Data from each study were organized as percentage of use of each food category (those cited above) for each species. Afterwards, an average value was calculated for each of these categories, minimizing possible regional trends in diet. Because our knowledge on the bats' diet is still scarce for several species, we transformed the percentage contribution of food items to an ordinal scale ranging from zero to six. A 'zero' score was attributed when a particular food category was absent from the species' diet, 'one' was used when those categories represented up to 10% of the species' diet, 'two' was associated with values between 11% and 25%, 'three' between 26% and 50%, 'four' between 51% and 75%, 'five' between 76% and 90%, and 'six' was used when the percentage contribution was higher than 90%. The data base used to calculate the scores (Table 1) is available from the authors upon request. In a few cases, when no data were available on the use of a particular food category, but information on closely related species suggests the contrary, we arbitrarily assigned a score 'one' (e.g. insectivory in *A. obscurus*). The same score was attributed when a record was available, but came from anecdotal observations (e.g. carnivory in *Phyllostomus discolor*).

Because there was a high correlation between some of the diet variables (particularly a strong negative association between frugivory and insectivory), we summarized the correlated variation patterns observed among the four diet variables with the first two principal components (PCs) of the diet variables (based on the correlation matrix). By doing this, we reduced the problems associated with multicollinearity of independent variables when performing the regressions described below (Johnson & Wichern 1988).

SHAPE VARIABLES

Geometric shape descriptors were obtained after a least squares Procrustes superimposition (generalized procrustes analysis) of the configuration coordinates and their projection to the tangent space

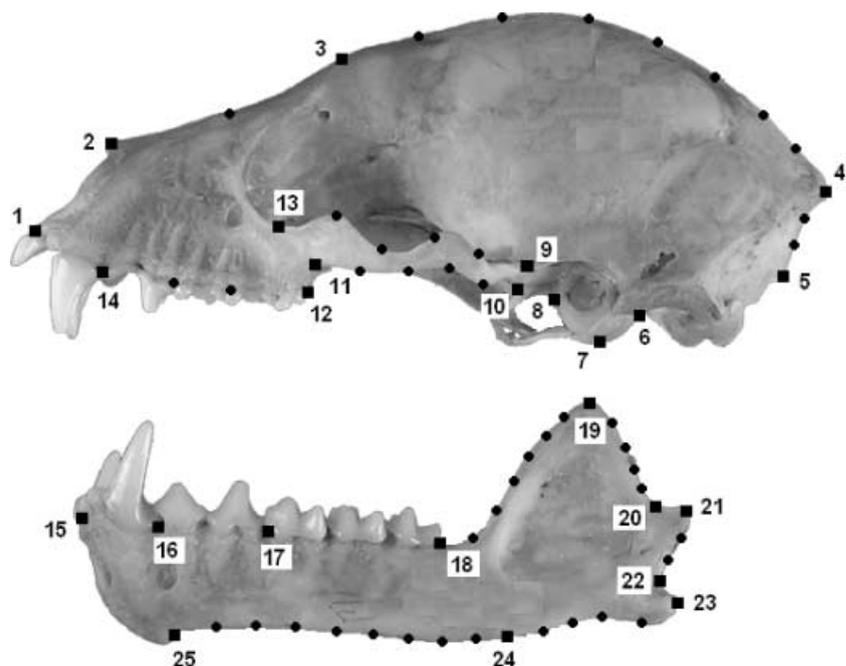


Fig. 2. Landmarks (squares) and semi-landmarks (circles) used to capture shape from the lateral view of the skull and mandible of phyllostomid bats. See Appendix S1 in Supporting Information for landmark definitions.

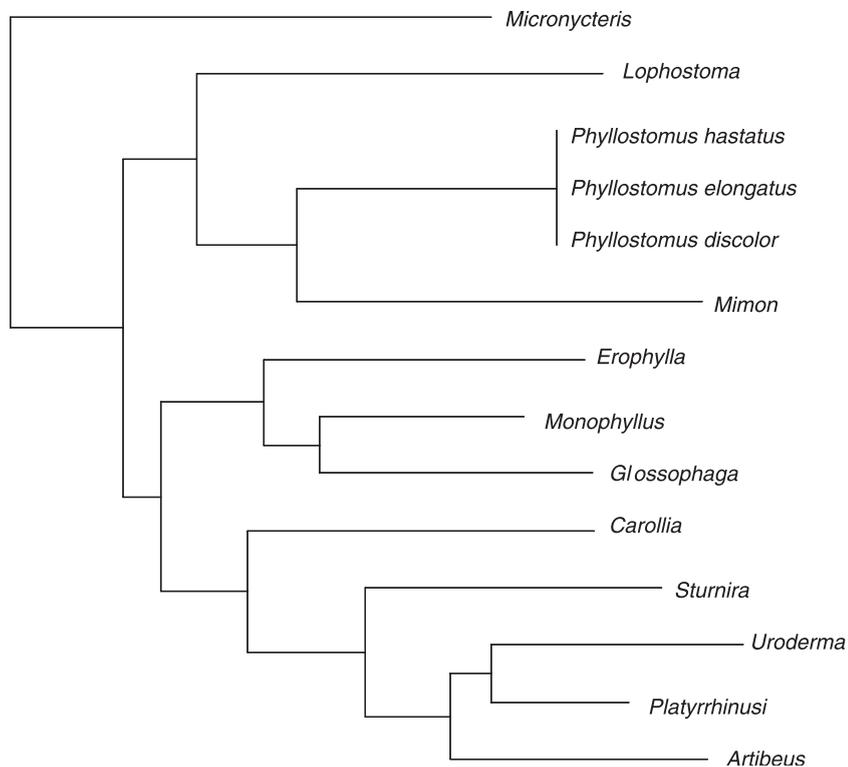


Fig. 3. Phylogenetic relationships among phyllostomid bats based on molecular data (Baker *et al.* 2003). The original tree was pruned to exclude species not investigated here.

of partial warps and uniform components (Rohlf 1996; Monteiro & Reis 1999). The superimposition removes differences related to scaling, position, and orientation from the coordinate data. The semi-landmarks were also allowed to slide along tangent vectors to the object contours to reduce the bending energy of the transformation (Bookstein 1997; Monteiro *et al.* 2004). Through this superimposition we obtained mean configurations (consensus) for each species and, then, a general grand mean shape, which was used as reference for the calculation of partial warps and uniform components. The partial warps and uniform components were used as shape variables in the multivariate statistical analyses.

COMPARATIVE STATISTICAL ANALYSES

In comparative analyses, species closely related phylogenetically do not constitute independent observations (Felsenstein 1985), which might artificially raise the type I error rates in both parametric and nonparametric conventional statistical models, where the residuals are expected to be independent and normally distributed (Rohlf 2001). To correct the bite force data for the influence of size, we calculated a phylogenetic regression of log-transformed bite force against log-transformed mean head length for each species (Dumont & Herrel 2003). Head length data were obtained in the same studies from which bite force data were available (Aguirre *et al.* 2002; Dumont & Herrel 2003). The phylogenetic generalized least squares (PGLS) regression incorporates the expected pattern of covariation among species due to phylogenetic relatedness into its error structure (Rohlf 2001, 2006a). In our study, this information was obtained from a pruned molecular tree (Fig. 3) derived from the phylogeny provided by Baker *et al.* (2003), and the phylogenetic covariance structure proposed by Martins & Hansen (1997), which incorporates several possible evolutionary models (e.g. Brownian motion, varying degrees of stabilizing selection), depending on parameters estimated from data by maximum likelihood (Paradis *et al.* 2004). The regression of

bite force on head length accounts for the fact that species with larger mean sizes are expected to have stronger bites. The deviation of bite force residuals from the size predictions was tested for significance using 95% confidence limits for predicted bite forces based on a *t*-distribution and the generalized least squares model parameters (Garland & Ives 2000).

In order to find a linear combination of the shape variables showing maximum covariance with bite force, we employed a two block partial least squares (PLS) analysis (Rohlf & Corti 2000). This method explores the patterns of covariance between two sets of variables, finding linear combinations of variables within blocks that account for as much covariance between sets as possible. The PLS shape vector represents a linear vector in shape space explaining maximum covariation between shape variables (partial warps and uniform components) and residual bite force. Because one of the variable sets was composed of a single variable (bite force), the PLS analysis yields a single pair of vectors (whereas the one corresponding to bite force is the variable itself). The correlation between the PLS shape vector scores and residual bite force was calculated, and its statistical significance was evaluated through a randomization test with 9999 permutations of the original data (where PLS vectors were recalculated at each resampling stage). To account for phylogenetic non-independence in the statistical tests relating bite force with skull and mandible shape, the PLS shape vectors were used as independent variables in PGLS models with residual bite force as a dependent variable.

The association between bite force, cranial shape and diet was assessed through PGLS multiple regression models relating residual bite force and PLS shape vectors (each separately as dependent variables) and PCs of diet as independent variables.

As a final step, we compared the shape variation associated with bite force (the PLS shape vector) with unconstrained interspecific patterns of major variation in shape space (which are not necessarily correlated with bite force or diet). These latter patterns were assessed

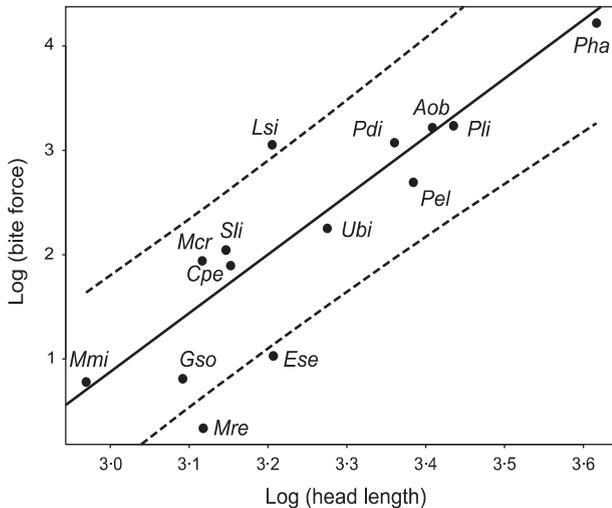


Fig. 4. Regression of log bite force against log head length of phyllostomid bats. Dashed lines represent 95% confidence intervals for predictions of the phylogenetic generalized linear model.

by a PCs analysis of partial warps and uniform components (Relative Warps Analysis – Rohlf 1996) obtained from the same species means used for the PLS analysis. The association between unconstrained (Principal Component Analysis – PCA) and constrained (PLS) patterns of shape variation will indicate whether the variation associated with bite force is aligned with the major interspecific shape differences.

Superimposition, sliding of semi-landmarks, and the relative warp analysis were performed in tpsRelw (Rohlf 2007). The PLS analysis, plus related correlation and permutation tests, were conducted in tpsPLS (Rohlf 2006b). The phylogenetic regressions and the diet PCA were performed in the R-system software (R Development Core Team 2007), supplemented by the packages ‘ape’ (Paradis *et al.* 2004) and ‘nlme’ (Pinheiro *et al.* 2008).

Results

Most variation in the bite force data was explained by size variation alone, as revealed by the regression ($r^2 = 0.74$, $P < 0.0001$). Nectar-feeding bats, such as *E. sezekorni* and *M. redmani*, bite significantly weaker than expected for their size, deviating from the allometric prediction, whereas the opposite was remarkably noted in *Lophostoma silvicolium*, a predominantly insectivorous bat (Fig. 4). PLS shape vectors derived for both skull and mandible presented, each, a higher correlation with residual bite force than it could be expected based on chance alone (skull: $r = 0.91$, $P = 0.0023$; mandible: $r = 0.70$, $P = 0.0275$). The PGLS analyses also revealed a significant association between the shape vectors and residual bite force (skull: $r^2 = 0.83$, $P < 0.0001$; mandible: $r^2 = 0.57$, $P = 0.0012$).

The visualization of shape changes described by the PLS shape vector derived from skull data showed that lower residual bite force is associated with an elongation of the rostrum, a narrowing of the zygomatic arc, and no appreciable dorsal or posterior expansion in the braincase, which in the interparietal region is suggested by the concave lateral profile of the

supraoccipital bone (Fig. 5a). In the opposite extreme of the same shape vector, high values of residual bite force are associated with a shortening of the rostrum, relative increase in skull height (via development of the sagittal crest at the braincase level), a more anterior insertion of the zygomatic arc, which is also more robust, particularly on its anterior portion, and a posterior extension of the interparietal region, as revealed by the convex lateral profile of the supraoccipital bone (Fig. 5b). Regarding the mandible, low values of residual bite force are associated with a low dentary, an expanded premolar tooth row, a contraction of the molar toothrow, an expansion of the gap between the last molar and the ascending ramus, a low coronoid process, and a reduced angular process (Fig. 6a). Features of the mandible associated with high residual bite force include a tall dentary, a contracted premolar toothrow, and an expanded molar toothrow, with the last molar being closer to the fulcrum, a high coronoid process, and an expanded angular process (Fig. 6b).

The first PC of diet variables explains 61.4% of total variation (Fig. 7) and depicts a contrast between the highly associated insectivorous and carnivorous vs. the frugivorous diet. The second PC explains 26.2% and is negatively associated with the nectarivorous diet. The PGLS models for the multiple regressions of residual bite force and PLS shape vectors on the PCs of diet were significant and explained a considerable proportion of variance in the dependent variables (Table 2). The first diet PC was not significantly associated with any of the dependent variables, but the second diet PC was significantly and positively associated with residual bite force and the shape vectors. Therefore, although the contrast between insectivory + carnivory vs. frugivory is the major pattern of diet variation in this sample, it is associated with neither the bite force nor the associated pattern of shape differences. The second diet PC, on the other hand, is positively associated with bite force and shape vectors, and negatively associated with nectarivory, linking this diet specialization with a weaker bite force and the associated shape differences.

The PLS vectors were highly correlated with unconstrained major axes of shape variation (first relative warps) for the skull ($r = 0.96$) and mandible ($r = 0.99$), indicating that the ordination of species based on maximum variance in shape space is largely associated with the interaction of shape and bite force.

Discussion

Interspecific size differences are a dominant factor, but do not explain all interspecific variation in bite force found among bats (Aguirre *et al.* 2002; Dumont 2007). Here, we demonstrate that an additional part of this variation can be attributed to shape differences in the skull and mandible, both associated with large scale (cranial elongation or shortening) and small scale changes (relative areas for muscle insertion). This pattern is particularly significant in phyllostomid bats because of the extensive shape and bite force changes associated to the specialization on nectar. A similar pattern, however, can evolve even in the absence of specialized nectarivory, as is the

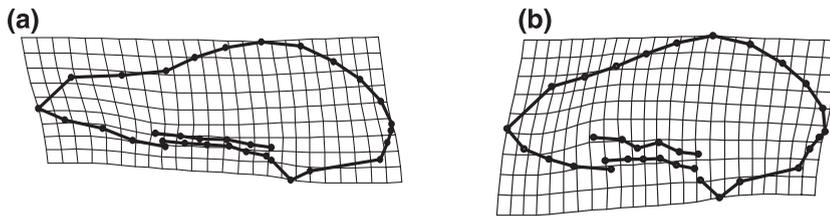
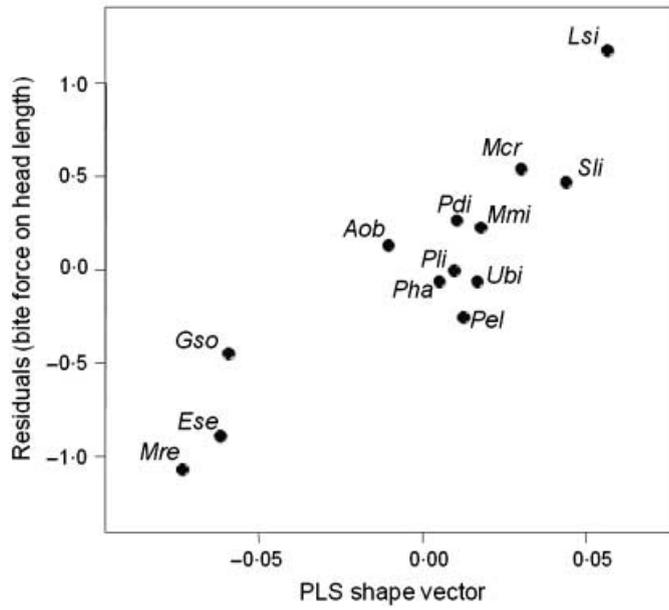


Fig. 5. Regression of size-adjusted bite force against the PLS shape vector derived from a geometric analysis the lateral view of the skull of phyllostomid bats. Grids depict shape changes associated with negative (a) and positive (b) scores of the PLS vector.

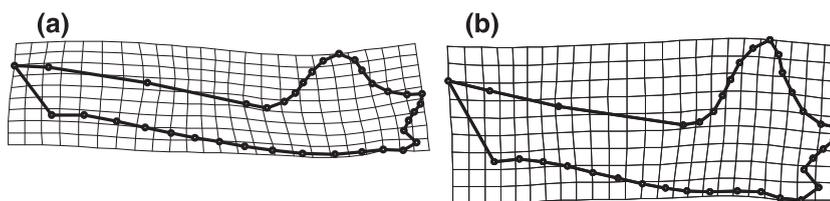
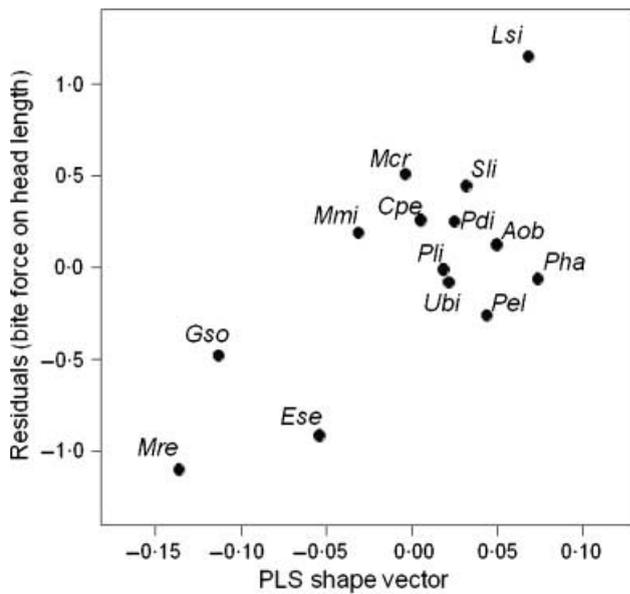


Fig. 6. Regression of size-adjusted bite force against the PLS shape vector derived from a geometric analysis the lateral view of the mandible of phyllostomid bats. Grids depict shape changes associated with negative (a) and positive (b) scores of the PLS vector.

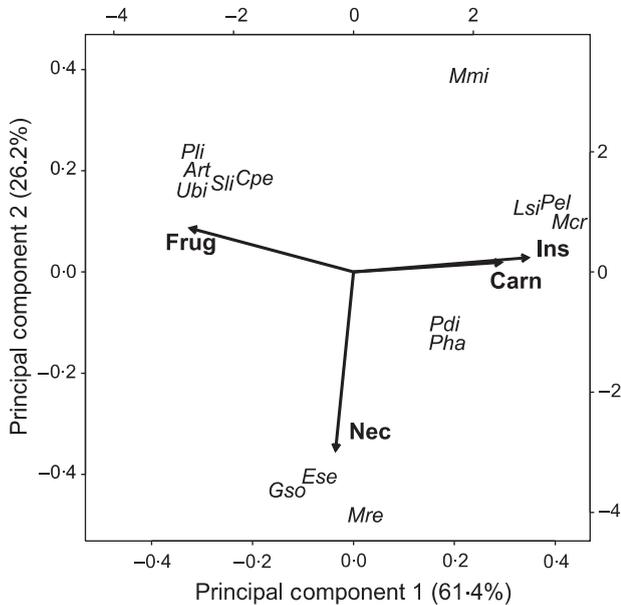


Fig. 7. Principal component biplot for diet scores of phyllostomid bats. Species scores are depicted by species acronyms in italics. Diet variables are depicted as vectors identified by variable names in bold. Abbreviations as in Table 1.

Table 2. Phylogenetic generalized least squares multiple regressions of residual bite force and shape on the principal components of diet

	Partial <i>b</i>	<i>P</i>	<i>R</i> ²
Residual bite force			0.56
dietPC1	0.0901	0.2288	
dietPC2	0.3835	0.0046	
Mandible PLS shape vector			0.43
dietPC1	-0.0007	0.9389	
dietPC2	0.0379	0.0121	
Lateral skull PLS shape vector			0.76
dietPC1	0.0076	0.0749	
dietPC2	0.0313	0.0003	

case in the order Carnivora and non-herbivorous marsupials (Wroe & Milne 2007).

Contrary to the condition described for nectarivory, our results provide no evidence that the shift in the core of the diet of phyllostomids from insects (probably primitive in the family; Wetterer *et al.* 2000) to fruits has been accompanied by selection for changes in size-independent bite force. The same result was found by Aguirre *et al.* (2002), who argued that it could be a consequence of the low resolution in their adopted diet categories. Our study presents the same problem, since we have not distinguished between soft and hard object frugivorous and insectivorous (Van Cakenberghe *et al.* 2002). Another shortcoming is the limited representation of some trophic morphs and the absence of extreme forms (e.g. short-faced frugivorous and specialized carnivorous).

As emphasized by Aguirre *et al.* (2002), most studies linking evolutionary changes in diet and skull shape in bats have based their conclusions on biomechanical models explaining

bite force, but without properly testing for the assumed correlation of this latter variable with skull shape. Our study shows, for the first time, strong quantitative evidence of such a correlation, at least in the context of the evolution of specialized nectarivory. Our results are, nevertheless, limited by the number of trophic morphs included in the analysis, which was a consequence of the scarcity of *in vivo* bite force data available for phyllostomid bats in the literature. A possible solution for this problem is the use of the dry skull biomechanical model of Thomason (1991), broadly applied to investigations on the trophic radiation in Carnivora (Christiansen & Wroe 2007; Wroe & Milne 2007). This model is based on linear and area measurements in skulls, and allows prediction of bite force even for extinct taxa (Wroe & Milne 2007). The use of experimental measurements of bite force, however, is advantageous because it provides bite forces that are independent from morphology. Shape variables correlated with these data can, therefore, be confronted with variables in Thomason's model, at least in an exploratory fashion. In the dry skull model, stronger bite forces are associated with an increase in cross-sectional areas of the temporalis and the masseter, along with an increase of the temporalis moment arm (relative increase of coronoid process) and a decrease of the outlever moment arm (relative shortening of rostrum and mandible). Therefore, the cranial morphological correlates of bite force observed for phyllostomid bats largely corroborate the functional importance of the morphological variables used by Thomason (1991) to predict bite force (but see Ellis *et al.* (2008) for a more direct comparison and calibration of postmortem with *in vivo* bite forces for canids).

Although models particularly designed to predict bite force values have not been used with bats, the application of more general biomechanical models has been extensive and was pioneered by Freeman (1979, 1981a,b), whose findings have been used as hypotheses by several subsequent authors (Jacobs 1996; Barlow *et al.* 1997; Dumont 1997; Van Cakenberghe *et al.* 2002). According to Freeman (*op. cit.*), hard-object specialists (those expected to have evolved stronger bite force) present thicker dentaries, higher coronoid process, and greater development of cranial crests. These features are linked to the cross-sectional area and mechanical advantage of the temporalis (Maynard-Smith & Savage 1959; Thomason 1991), and are clearly supported by our results. In addition, we also observed a change in the profile of the supraoccipital, related to a posterior projection of the interparietal region, resulting in a relative increase of the area occupied by the origin of temporalis. In fact, an evolutionary correlation in bats has recently been shown between the bite force, temporalis mass and fibre length (Herrel *et al.* 2008). The deeper dentary correlated with stronger residual bite forces, is also a typical feature of durophagous bats (Freeman 2000). Robustness of the dentary seems to be functionally linked in carnivore mammals with an increased development of the temporalis (Maynard-Smith & Savage 1959) providing increased support against the stress generated by biting, particularly for species whose feeding behaviour includes cracking bones (Therrien 2005).

Bats that feed on hard items are expected to present the connection of the zygomatic arch with the rostrum more anteriorly positioned than soft-object specialists (Freeman 1979). This feature, along with a shortening of the rostrum, puts the last molars in a more vertical alignment with the masseter, which originates in this connection point (Freeman 1979). In our data, not only this anterior displacement and the shortening of the rostrum are present, but the anterior zygoma becomes consistently deeper as residual bite force increases. The importance of the masseter in increasing bite force among the bats studied here is also supported by the expansion we detected on the angular process, the site of insertion for this muscle. Such expansion may be even greater than described here, since our two-dimensional view of the mandible is probably underestimating the lateral flaring of this process. Contrary to the results found for the temporalis, however, Herrel *et al.* (2008) did not find a significant correlation between masseter mass and bite force when using phylogenetic independent contrasts.

A common morphological correlate with increasing bite force is shortening and widening the face, which brings the canines closer to the fulcrum and may allow an increase in the volume of both masseter and temporalis (Swartz *et al.* 2003). This pattern of shape differentiation is also repeatedly observed in placental and marsupial carnivores (Wroe & Milne 2007), but within a smaller range of dietary adaptations. A clear negative association between residual bite force and lengthening of the rostrum is represented in our data, which reveals its importance even in the absence of extreme long-nosed or short-faced bats in the analysis. The mandible follows the same morphological trend detected in the rostrum, but in this case it is interesting to note that higher residual bite forces are associated with a noticeable shortening of the space between canines and molars, but not of that between first and last molars (the mandibular elongation is more pronounced in its anterior region). The reason for this may rely on the importance of these latter teeth to process hard food (they are closer to the fulcrum than are the premolars), and their minor relevance to species whose diet is mainly liquid, like nectar-feeding bats (Freeman 1995). The elongated rostrum is a shared feature of nectarivorous bats in both phyllostomid and pteropodid families (Freeman 1995), and is clearly associated with the development of an enlarged, specialized tongue. The trade-off between bite force and cranial elongation imposes a constraint against harder items in the diet of more specialized nectar-feeding bats.

Diet differences are not the only factor that can be explored when interpreting evolutionary divergence in cranial shape and bite force. Bats of the genus *Lophostoma* are apparently unique among phyllostomids because of their habits of using teeth to excavate holes in active termite nests (Kalko *et al.* 2006). The hardness of termite nests may generate an additional functional demand influencing the evolution of the jaw–cranial system in this group. This extra demand may explain why the bite force in *L. silvicolum* is significantly larger than predicted for its size, since its diet is, in general, similar to that of *M. crenulatum*, also present in our sample (Reis & Peracchi 1987;

Giannini & Kalko 2005). Evidence based on bite force measurements of more extreme specialists (e.g. short-faced frugivores, long-faced nectarivores, and larger carnivorous bats), as well as behavioural studies and more detailed bio-mechanical models, will further improve our understanding about the relationship of diet, bite force, and skull shape, possibly allowing for more generalized and robust conclusions on the fitness consequences of postulated functional adaptations.

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