Integrative Zoology 2021; 0: 1–13 doi: 10.1111/1749-4877.12564

ORIGINAL ARTICLE

Functional correlates of skull shape in Chiroptera: feeding and echolocation adaptations

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Abstract

Morphological, functional, and behavioral adaptations of bats are among the most diverse within mammals. A strong association between bat skull morphology and feeding behavior has been suggested previously. However, morphological variation related to other drivers of adaptation, in particular echolocation, remains understudied. We assessed variation in skull morphology with respect to ecology (diet and emission type) and function (bite force, masticatory muscles and echolocation characteristics) using geometric morphometrics and comparative methods. Our study suggests that variation in skull shape of 10 bat families is the result of adaptations to broad dietary categories and sound emission types (oral or nasal). Skull shape correlates with echolocation parameters only in a subsample of insectivorous species, possibly because they (almost) entirely rely on this sensory system for locating and capturing prey. Insectivores emitting low frequencies are characterized by a ventrally tilted rostrum, a trait not associated with feeding parameters. This result questions the validity of a trade-off between feeding and echolocation function. Our study advances understanding of the relationship between skull morphology and specific features of echolocation and suggests that evolutionary constraints due to echolocation may differ between different groups within the Chiroptera.

Key words: diet, echolocation, functional morphology, geometric morphometrics, macroevolution

INTRODUCTION

Morphological changes in the mammalian skull are driven by a variety of functional demands including feeding ecology (Janis 1990), environmental context (e.g. habitat productivity: Cardini *et al.* 2007), and broad morphological drivers (e.g. allometric rule: Cardini 2019). Flying mammals of the order Chiroptera face the

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additional challenge of effective echolocation, and so their skulls also have to behave as acoustic horns for efficient sound emission (Pedersen 1998).

Previous studies have documented a strong association between bat skull morphology and feeding function. In particular, diet preferences, bite force, and masticatory muscles have been widely associated with skull size and shape variation in bats (Freeman 1998; Aguirre *et al.* 2002; Santana *et al.* 2012). Nevertheless, the majority of these studies have focused on one family only, the Phyllostomidae (Dumont 2007; Dumont *et al.* 2012; Hedrick *et al.* 2020, but see Senawi *et al.* 2015; Hedrick & Dumont 2018). Although this family is the most diverse in terms of diet and skull morphology (Wilson & Reeder 2005), comparisons within a broader taxonomic context are required to detect more general patterns.

Laryngeal echolocating bats use acoustic emissions not only to locate prey and navigate the environment but also to communicate (Jones & Siemers 2011). Different degrees of head rotation are associated with emission type in bats: in nasal emitters the head is folded towards the chest, while in oral emitters it rotates dorsally during ontogenesis (Pedersen 1998). Besides this well-described dichotomy between oral and nasal emitters, our understanding of the influence of echolocation adaptation on size and shape of bat skulls remains limited. Adaptations for echolocation are generally thought to be associated with soft tissue rather than bony structures (Elemans *et al.* 2011). Evidence that bat skull size is associated with echolocation parameters (in particular peak frequency, FP) has been detected in some bat families (Jacobs *et al.* 2014; Thiagavel *et al.* 2017), but there is still a significant gap in our understanding of how echolocation relates to morphology (particularly to skull shape) and whether or not a general pattern is present across families (but see Jacobs *et al.* 2014 for Rhinolophidae). Different selective pressures can drive related taxa towards different evolutionary optima, especially when they influence trade-offs between traits (Dumont *et al.* 2014; Arbour *et al.* 2019). Insectivorous bats are known to rely mainly on echolocation to detect and pursue their prey, in contrast with other bats (e.g. carnivorous species) that also rely on vision and olfaction (Bahlman & Kelt 2007; Surlykke *et al.* 2013; Ripperger *et al.* 2019). Thus, we set out to test the hypothesis that insectivorous species display an association between skull shape and echolocation characteristics due to a less flexible (but more specialized) sensory system. More specifically, we used geometric morphometrics and phylogenetic comparative methods to test the following main predictions:

(i) The association between feeding descriptors (i.e. diet, bite force, masticatory muscle) and skull morphology follows a general pattern within the Chiroptera. Hedrick *et al.* (2020) recently proposed the relative rostrum length as a key trait allowing Phyllostomidae to occupy the broad range of their dietary niches. Because this trait correlates strongly with bite force in bats and other mammals (Wroe *et al.* 2005; Dumont 2007; Santana *et al.* 2010), we predict that skull shape changes generally associated with face shortening and more powerful masticatory muscles (Herrel *et al.* 2008) should occur in Chiroptera that are able to generate relatively high bite force.

(ii) Insectivorous bats display an association between skull morphology (both size and shape) and echolocation call parameters because they almost exclusively rely on sound emission to detect and pursue their prey. This hypothesis has been already proposed by Bogdanowicz *et al.* (1999) who identified that echolocation calls are dominated by low frequencies in bigger insectivores unlike smaller species which use higher frequencies (Jones 1999).

(iii) Insectivorous bats show a trade-off in skull shape between feeding and sensory function due to dual skull functions: processing hard food and optimizing sound emission. Hard food processing requires a relatively short rostrum for maximizing bite force (Herring 1993). This trait equally correlates with the ability to produce high frequency sounds that limit prey detection range for bats. Jacobs *et al.* (2014) proposed that these 2 functions have opposing effects on the evolution of skull shape variation. This hypothesis was validated in Rhinolophidae showing significant association between skull shape, bite force, and peak frequency.

MATERIALS AND METHODS

Sample

We examined 185 bat skulls, belonging to 67 species, from 10 different bat families. This is representative of 5% of the total diversity of extant Chiroptera that are currently divided into 21 families (Wilson & Reader 2005). The number of species was constrained by the need to consistently collect data on skull morphology, diet, emission type, echolocation parameters, masticatory muscle mass, and bite force using the same established protocols. Masticatory muscle data were available for a subsample of just over half of the species (96 specimens,

Table 1 Functional traits used as covariates in the present study

Feeding parameters	Sensory parameters	Diet category	Emission type
Bite force (BF)	Peak frequency (FP)	Insectivorous (I)	Non-laryngeal echolocation (NLE)
Digastric muscle (DIG)	Start frequency (SF)	Frugivorous (F)	Nasal (R)
Masseter muscle (MAS)	End frequency (EF)	Hematophagous (H)	Mouth (M)
Temporalis muscle (TEM)	Bandwidth (BW)	Vertebrate eater (V)	Both mouth and nasal (B)
Pterygoid muscle (PTE)	Duration (D)	Nectarivorous (N)	
	Sweep rate (SR)	Omnivorous (O)	
		Frugi/insectivorous (F,I)	
		Necta/fruigivorous (N,F)	
		Insect–vertebrate eater (I,V)	

34 species, 5 bat families) and were included in the analyses. Details on origins of specimens (museum collections) are reported in Table S1, Supporting Information.

Functional, ecological, and morphological data

Sensory (i.e. echolocation parameters) and feeding (i.e. bite force and muscles mass) data were acquired from the literature or collected in the field. Details on collection techniques and criteria for data selection are provided in Supporting Information together with the selected literature and raw data used in this study (Tables S2 and S3, Supporting Information, for sensory and feeding parameters, respectively). All sensory and feeding estimates were $\log_{10}$ transformed prior to the analyses.

Morphological data were collected by geometric morphometric methods (GMM) applied to 3D digital models of bat crania (landmark configuration described in Fig. 1 and Table S5, Supporting Information). An established photogrammetric protocol (Giacomini *et al*. 2019) and micro CT scans were employed to digitally reconstruct the models (Table S1, Supporting Information). The combination of 3D reconstruction techniques (i.e. photogrammetry and micro CT scan) has been demonstrated to provide robust biological results in macroevolutionary analyses when appropriate preliminary tests are performed on a subsample of the data (Shearer *et al*. 2017; Giacomini *et al*. 2019). Details on the GMM are reported in Supporting Information.

To assess the relationship between morphology and ecological groups, we classified species by broad diet categories, ability for laryngeal echolocation, and emission type. The full list of traits studied and parameter abbreviations used hereafter are reported in Table 1. Diet was

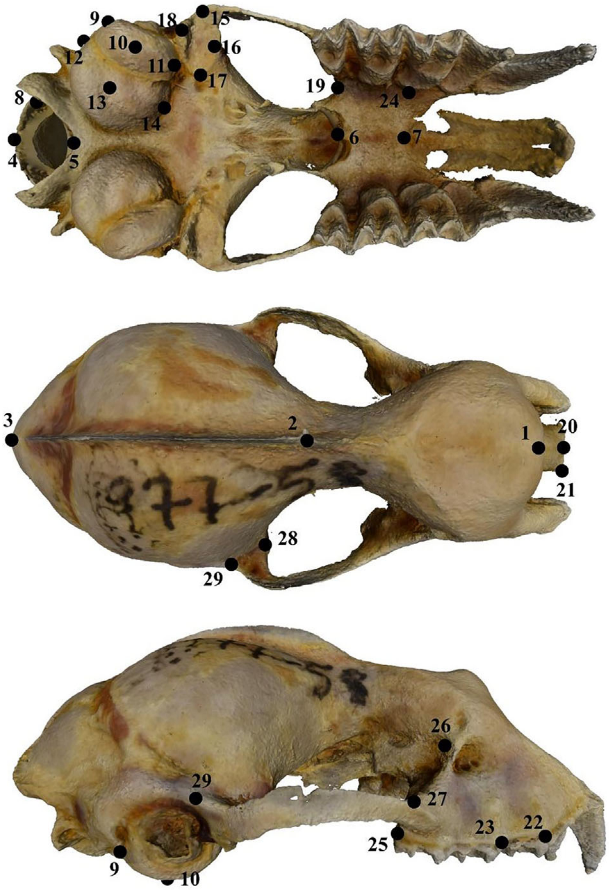

Figure 1 Landmark configuration on the skull of *Rhinolophus ferrumequinum*. Anatomical descriptions of landmarks are in Table S4, Supporting Information.

categorized by traditional groups inferred from Wilson and Reeder (2005) and is reported in Table 1. We followed Thiagavel *et al.* (2018) to categorize species according to whether they are capable of laryngeal echolocation (LE) or not (NLE). LE bats were further categorized according to emission type, as species that use mouth emission (M), nasal emission (R), or emission from both nose and mouth (B), following references in Table S2, Supporting Information, and additional references (Pedersen 1998; Goudy-Trainor & Freeman 2002; Surlykke *et al.* 2013; Seibert *et al.* 2015; Jakobsen *et al.* 2018).

Comparative analyses

All the analyses in this study were performed above the species level (=interspecific scale) using a phylogenetic comparative approach (phylogenetic generalized least squares, PGLS; phylogenetic Partial Least Squares, phylo-PLS). The raw data (both functional traits and skull morphological data) were averaged by species. Phylogenetic histories were represented by a series of pruned trees extracted from the calibrated and ultrametric phylogenetic tree built by Shi & Rabosky (2015), with tips corresponding to the species of our dataset (and sub datasets). The tree was used to compute the phylogenetic variance-covariance matrices employed in PGLS and phylogenetic PLS (Rohlf 2006, 2007; Adams & Felice 2014). The analyses were performed using the R packages "geomorph" (Adams & Otárola-Castillo 2013), "caper" (Orme *et al.* 2013), "mvMorph" (Clavel *et al.* 2015), and "phytools" (Revell 2012).

Size variation

Skull size was here represented by $\log_{10}$ centroid size (defined as the square root of the squared distances from each landmark to the barycenter of the landmark configuration). This variable was used as independent in order to explore allometric variation in feeding and sensory parameters (Table 1). PGLS was applied to account for phylogenetic non-independence of interspecific data. We employed the method proposed by Revell (2010) that allows simultaneous estimation of regression parameters and lambda (λ: a measure of the strength of phylogenetic signal in the data with $0 =$ no phylogenetic structure, and $1 =$ data fully explained by the phylogeny) using maximum likelihood. The allometric equations were tested based on sample availability ($n = 67$ for bite force; $n = 61$ for sensory parameters, including all the species except Pteropodidae; $n = 34$ for muscle parameters) and residuals were subsequently used for the analyses of association between functional parameters and skull shape.

Skull shape variation, phylogenetic signal, and ecological grouping

A Principal Component Analysis (PCA) was performed on Procrustes shape coordinates in order to visualize the skull shape variation in the sample. The 3D model of *Artibeus jamaicensis* was warped on the consensus (i.e. mean shape of the dataset), and the result was subsequently warped on the maximum and minimum shape of the first 2 PC axes to indicate major morphological variation in the dataset (Klingenberg 2013). The warped model on the consensus was used as the reference mesh in all the subsequent shape visualizations to facilitate comparisons between the different analyses. Wireframe visualizations were equally presented in the Supporting Information figures to provide a more detailed representation of shape changes strictly associated to changes in the landmark spatial positioning.

The K statistic of Blomberg *et al.* (2003) was used to test for the presence of a phylogenetic signal in the shape data. The K statistic reflects the degree of congruence between the trait and the phylogeny (Blomberg *et al.* 2003) and has been extended to multivariate data (K_{multiv}) by Adams (2014). Statistical significance of K_{multiv} was assessed using randomization (Adams 2014).

Associations between skull shape and ecological categories (diet and emission type) were tested using phylogenetic multivariate analysis of variance (pMANOVA; Clavel & Morlon (2020). This is a novel approach that allows incorporation of several evolutionary models. Specifically, skull shape data represented the dependent variable (Y, multivariate) and ecological categories the independent (X). Phylogeny was accounted for using the following models: Brownian Motion (BM) where the amount of evolutionary change in a trait is proportional to branch lengths; Early Burst (EB) that assumes exponentially reductions in diversification rates through time as found in adaptive radiations (Harmon *et al.* 2010; Ingram *et al.* 2012); Ornstein–Uhlenbeck (OU), a model that takes into account stabilizing/divergent selection and stasis so that traits can evolve towards a single or multiple optima (Hansen 1997; Butler & King 2004; Beaulieu *et al.* 2012); lambda branch length transformations that stretch basal or terminal nodes approximating different levels of phylogenetic signal in the data (Pagel 1999). Models were compared using penalized likelihood and best model implemented in the final pMANOVA.

Table 2 Summary statistics of PGLS models using log centroid size as independent variable and feeding or sensory parameters (all log transformed) as dependent

Dependent variable		N	Adj. R^2	Intercept	P	β	P	*lambda*	95% CI
Feeding	Bite force	67	0.649	−4.073	<0.001	3.108	<0.001	0.719	(NA, 0.965)
	Digastric	36	0.671	−3.134	<0.001	2.772	<0.001	0.000	(NA, 0.495)
	Masseter	34	0.693	−3.015	<0.001	2.879	<0.001	0.305	(NA, 0.797)
	Temporalis	34	0.675	−2.100	<0.001	2.752	<0.001	0.000	(NA, 0.845)
	Pterygoid	34	0.686	−2.803	<0.001	2.576	<0.001	0.348	(NA, 0.827)
Sensory	Peak frequency	61	0.194	2.742	<0.001	−0.666	<0.001	1.000	(0.928, NA)
	Start frequency	61	0.129	2.737	<0.001	−0.616	0.003	0.996	(0.779, NA)
	End frequency	61	0.194	2.698	<0.001	−0.701	<0.001	1.000	(0.851, NA)
	Bandwidth	61	0.000	1.972	0.008	−0.468	**0.319**	0.977	(0.700, NA)
	Duration	61	0.076	−0.542	**0.374**	0.948	0.018	1.000	(0.880, NA)
	Sweep rate	61	0.050	2.556	0.023	−1.443	0.046	1.000	(0.901, NA)

P values are reported for both intercept and slope ($=\beta$). In bold are the non-significant P values. 95% confidence interval is reported for the lambda parameter estimated using maximum likelihood.

Evolutionary trade-off between feeding and sensory function

Phylogenetic Partial Least Squares (phylo-PLS) was employed to test for association between skull shape data and functional parameters in the sub-sample of laryngeal echolocating bats ($n = 61$) and insectivore species ($n = 19$). This method was preferred because it allows phylogenetic relatedness (using the function "phylo.integration," Adams & Felice 2014) and the relative association within and between each block of multivariate variables (in our case, skull shape data and feeding/echolocation parameters, Table 1) to be simultaneously taken into account without assuming any directionality (there is no dependent or independent block of variables). Phylo-PLS model should be significant if bats are able to optimize skull shape for feeding function and frequency emission (see Jacobs *et al*. 2014).

For the subsample of laryngeal echolocating bats ($n = 61$), bite force was the only representative feeding variable while in the insectivores ($n = 19$) relative masticatory muscle masses were also analyzed. These variables were represented by residuals from PGLS allometric models (see Nogueira *et al*. (2009) that equally identified covariation between Phyllostomidae skull shape and residual bite force). Finally, the block of functional parameters was divided into feeding and echolocation for further phylo-PLS models. Strength of association between shape data and functional blocks of variables was quantified using the RV coefficient (Escoufier 1973) and compared between models using z-scores (Adams & Collyer 2016). The reference skull mesh was warped on the maximum and minimum shapes for the phylo-PLS models to visualize shape covariation with feeding and echolocation. The comparison of shape changes that were related to echolocation and feeding provided insights into possible functional trade-offs.

RESULTS

Size variation in bat skulls

The PGLS models identified a significant positive association between skull size and feeding parameters in all cases, while negative relationships were found for all echolocation parameters except "Duration" (Table 2). Lambda varied between 0.00, indicating no phylogenetic signal (e.g. the digastric and temporalis muscle mass) and 1.00, indicative of traits following Brownian Motion model (as found for the majority of sensory parameters). This supports the presence of a significant phylogenetic and allometric signal in both feeding and echolocation parameters.

Skull shape variation and ecological grouping

Most of the morphological variation between the 67 bat species was described by principal components 1 (PC1) and 2 (PC2) (33.35% and 27.02% of variance,

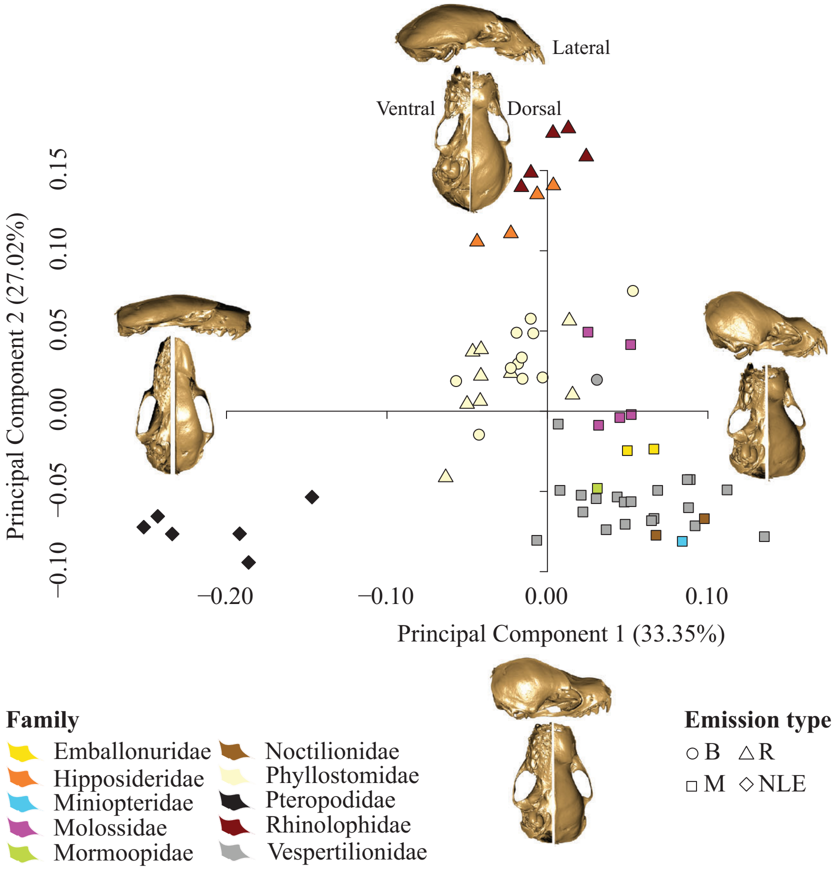

Figure 2 Plot of principal component scores (vector 1 vs 2) for all the species of the dataset ($n = 67$) displayed by family and emission type (laryngeal echolocation: both mouth and nasal [B], nasal [R], mouth [M]; non-laryngeal echolocation, [NLE]). Shape variation was reported on dorsal (D), ventral (V), and lateral (L) views by warping maximum and minimum PC variation of each axis on the reference mesh.

respectively) (Fig. 2; Fig. S1, Supporting Information). PC1 displayed shape variation related to rostrum length, zygomatic arch length, and braincase height, and separated NLE species (i.e. Pteropodidae family, frugivores) from LE species (generally insectivores). PC2 showed variation mainly related to palatal length (i.e. maxillary and palatine bones) and braincase length with mouth emitting species displaying a longer palatal length but a shorter braincase respect to nasal and nasal/mouth emitting species (Fig. 2; Fig. S1, Supporting Information).

A significant phylogenetic signal was identified in skull shape ($P < 0.001$) with $K_{\text{multiv}} = 1.255$, a value greater than expected under the BM model of evolution.

EB model was found to be the best mode of evolution when testing for association between skull shape and diet (loglikelihood = 13950.11, compared to 13865.6 found for BM, OU, and lambda models did not differ at all from BM in loglikelihood). Using the EB model, pMANOVA identified a significant impact of diet on skull shape (Pillai's trace = 5.286, $P = 0.001$) explaining 13.93% of total variance. EB model was also favored for the emission type categories (loglikelihood = 15080.28 compared to 15043.91 found for BM, OU, and lambda), which were found to explain 52.34% of skull shape variance (Pillai's trace = 2.276, $P = 0.001$).

Analyses of trade-offs between feeding and sensory function

Phylo-PLS identified 7 pairs of covariation vectors for skull shape and functional variables in 61 LE bats of which the first was significant (r-PLS = 0.662, $P = 0.0014$, Effect Size = 3.244). The PLS1 vector was strongly loaded on residual bite force and much less so on echolocation parameters (Table 3). When phylo-PLS was repeated using only echolocation parameters as a functional block, no significant association could be detected with the shape vector (r-PLS = 0.541, $P = 0.080$, Effect Size: 1.474), while correlation between skull shape and residual bite force remained significant when in isolation (r-PLS = 0.646, $P = 8\text{e-}04$, Effect Size = 3.366, Fig. 3a). This relationship is mainly described by the relative length of the rostrum: species with relatively greatest

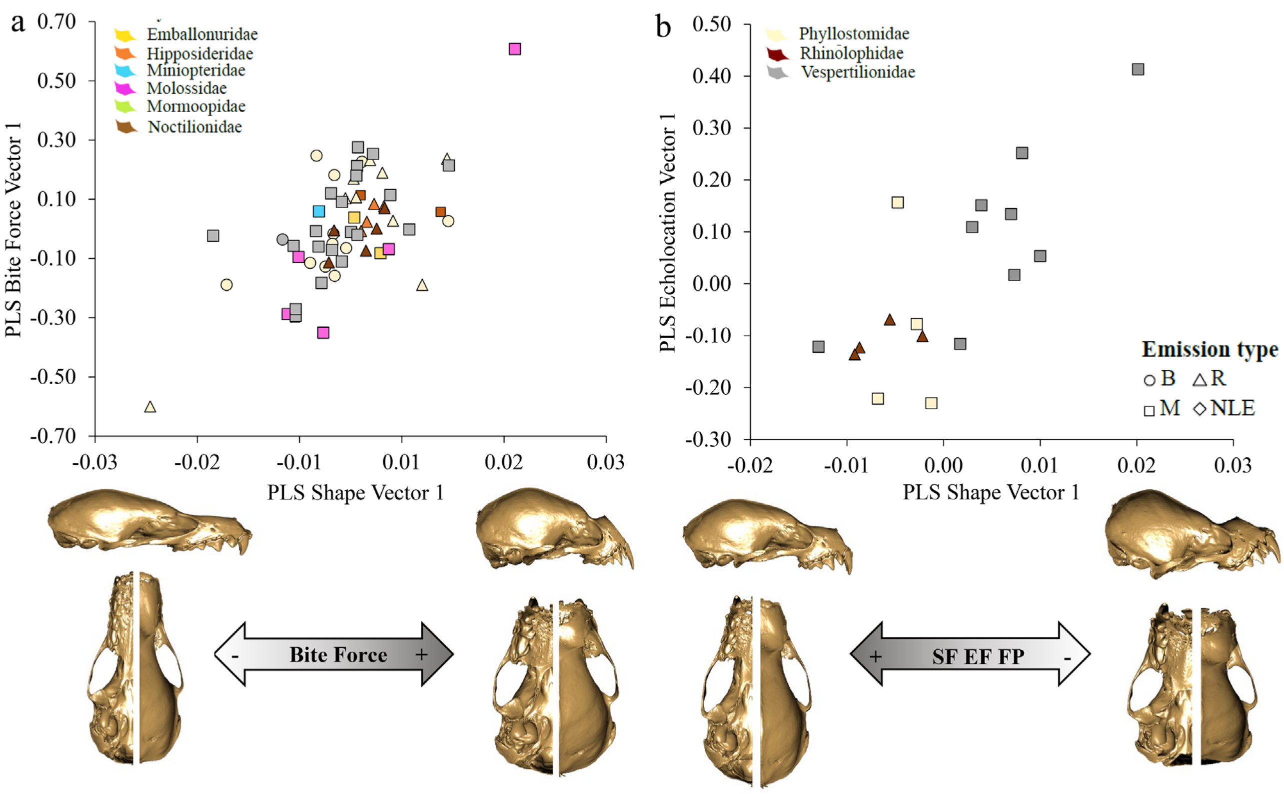

Figure 3 Plot of phylo-PLS showing association between skull shape and bite force in a subsample of LE bats (n = 61) (a), and correlation between skull shape versus echolocation parameters in 17 insectivore species (b). Skull warpings show the shape variation related to the minimum (left) and maximum (right) values PLS vector scores.

bite force for their skull size (e.g. *Molossus molossus*) had a rostrum length that was much shorter than the braincase (Fig. 3a; Fig. S2, Supporting Information). Within a subsample of 19 insectivores, a significant association between skull shape and all functional variables (including relative muscle mass) still holds (r-PLS = 0.867, P = 0.006, Effect Size = 2.6504). PLS vector loading again revealed a predominant impact of feeding rather than sensory parameters (Table 3), so separate phylo-PLSs were conducted. Significant correlation occurs between skull shape and feeding variables in isolation (r-PLS = 0.865, P = 0.007, Effect Size = 2.6716) but not between skull shape and sensory variables (r-PLS = 0.6518, P = 0.2761, Effect Size = 0.61251). A closer inspection of the phylo-PLS plot (Fig. S3, Supporting Information) showed 2 significant outliers of the Molossidae family. When both species were removed (n = 17) phylo-PLS identified an opposite trend (Fig. 3b). The skull shape vector was not significantly correlated with the feeding vector (r-PLS = 0.698, P = 0.063, Effect Size = 1.543), but was correlated with the sensory vector (r-PLS = 0.7716, P = 0.0127, Effect Size = 2.31483). This was negatively loaded on start, end, and peak frequency (Table 3) so that species at positive scores are characterized by a diagonally tilted and shorter rostrum, wider braincase, and larger bullae (Fig. 3b).

DISCUSSION

The chiropteran skull is optimally designed for multiple functions. By using a representative taxonomic sample of 10 out of 21 families, we identified a predominant impact of feeding adaptations on skull shape above species level. This pattern was abundantly validated in the diverse Phyllostomidae family (Dumont 2007; Nogueira *et al.* 2009) and our results extend it to many more bat families. Relative rostrum length plays a significant role in optimizing bite force (Fig. 3a) thus supporting the recent proposal by Hedrick *et al.* (2020) that this trait allows occupation of multiple feeding niches along a line of least evolutionary resistance.

Table 3 Loadings on phylo-PLS vector 1 for different functional parameters, based on analyses of association between skull shape and functional traits, in a subsample of 61 laryngeal eholocating bats, 19 insectivores, and the same sample after the exclusion of 2 Molossidae outliers ($n = 17$)

	PLS Vector 1 loadings			
	LE bats	Insectivores		
	$N = 61$	$N = 19$		$N = 17$
Bite force	0.959	0.474	0.237	—
Digastric mass	—	0.531	0.747	—
Masseter mass	—	0.381	0.351	—
Temporalis mass	—	0.435	0.347	—
Pterygoid mass	—	0.388	0.376	—
StartF (KHz)	−0.035	−0.053	—	−0.673
EndF (KHz)	0.155	−0.049	—	−0.493
BW (KHz)	−0.165	0.009	—	−0.107
Fpeak (KHz)	0.124	−0.050	—	−0.537
Duration (ms)	0.033	−0.018	—	0.033
Sweep.rate (KHz ms)	−0.105	0.026	—	−0.056

Diet explains a substantial proportion of shape variation in our sample, and the implementation of more complex evolutionary models suggested that Brownian motion is not an appropriate model fit to investigate skull phenotypic adaptations in bats. For our sample, Early Burst provided a better implementation of the comparative methods in line with the rapid radiation of bat morphologies conjunctly with echolocation adaptations (Arbour *et al.* 2019). It is thus expected that the categorical variable "emission type" provided in our case even stronger exploratory power for skull shape variation (≈50%) than diet (≈13%). Such a profound impact is well supported by a recent embryonic study that identified common developmental origin in the hearing apparatus of NLE bats (pteropodids) and all other of non-chiropteran mammals (Noijri *et al.* 2021).

The deep divergence of Pteropodidae from all other LE bats is depicted by our PCA (Fig. 2). Bigger cochlea and tympanic bulla are common morphological traits to all LE bats, supporting the idea that cochlea hypertrophy is linked to laryngeal echolocation ability (Simmons *et al.* 2008). In fact, cochlea size is known to scale with the vestibular system and to correlate with canal morphologies which differentiate LE from NLE bats (Davies *et al.* 2013). We also found that LE bats have taller braincases, which might represent the need to accommodate a brain with different spatial constraints from NLE bats. For example, LE bats display larger auditory nuclei than NLE (Hutcheon *et al.* 2002), even though their relative brain size is smaller (Jones & MacLarnon 2004; Thiagavel *et al.* 2018).

Within LE bats, mouth emitters significantly differed in shape from nasal and nasal/mouth emitters. Nasal emission is an innovation in bat skull morphology and implies deep cranial rearrangements (Pedersen 2000). The shorter and narrower palate, together with the increased length and decreased height of the braincase seems to be connected to shape rearrangements due to the nasal emission (and nasal/mouth emission). Cochlear features (i.e. basilar membrane length and number of cochlea turns) correlate with echolocation frequencies (Davies *et al.* 2013); therefore, differences in cochlea and tympanic bulla relative size between the 2 groups can relate to variation in hearing limits or echolocation characteristics.

In terms of skull size, allometric patterns for all feeding and sensory parameters have been here established implementing phylogenetic regression (Revell 2010). The scaling of bite force and masticatory muscle mass with skull size was explored by Herrel *et al.* (2008) on 16 species providing slopes unsurprisingly similar to those presented here on a larger sample (Table 2). Muscle mass scales with skull size following a geometric expectation (slopes between 2.58–2.88) with temporalis not showing significant departure from this pattern.

The allometry of sensory parameters supported a pattern established for peak frequency in some families of insectivorous bats (Jones 1999; Thiagavel *et al.* 2017; Jacobs & Bastian 2018). Species with bigger body size and, hence, longer vocal folds produce lower frequencies confirming the negative allometry also for start frequency, end frequency, and sweep rate. Interestingly, we found no significant association between these echolocation parameters and skull shape at broad taxonomic scale.

Thiagavel *et al.* (2018) hypothesized the retention of a trade-off between vision and echolocation in extant LE bat species. Nectar, fruit, blood, and vertebrate eating species use vision and smell in combination with echolocation to detect and locate food items (Bahlman & Kelt 2007; Surlykke *et al.* 2013; Ripperger *et al.* 2019). These species share a similar hunting ecology: they hunt static food items in cluttered environments through a passive or active gleaning mode (Denzinger & Schnitzler 2013). In contrast, insectivorous bats have evolved the use of echolocation as their main sensory system for prey detection and pursuit of rapidly-moving prey. This might explain why we only identified a significant association between skull shape and echolocation for some insectivorous bats.

Molossidae within the insectivore bat families were clear outliers in skull shape morphology and this might be due to their extremely durophagous adaptation in hunting beetles (Freeman 1981). Indeed, they also exhibit proportionally higher bite forces when compared to sampled insectivores with similar skull size (e.g. *M. molossus* with 8.4 Newton compared to *Micronycteris megalotis* with 2.31 Newton, Table S1, Supporting Information). Aguirre *et al*. (2003) already proposed that within insectivorous species a degree of morphological distinctiveness occur in relation to prey size and food hardness, and by sampling a more homogenous skull shape bauplan of species within Verspertilionidae, Phyllostomidae, and Rhinolophidae, we were able to detect association with echolocation parameters. This relationship was clearly driven by the rostral tilting which is greater in species emitting higher frequencies. Interestingly, within this same subsample, no relationship between skull shape and feeding parameters could be detected thus questioning the idea of trade-off between feeding and echolocation function.

Higher bite forces and larger muscles are functionally advantageous as they allow for the possible consumption of a wider range of prey (Aguirre *et al*. 2003; Nogueira *et al*. 2009). On the other hand, whether high frequencies are disadvantageous is debatable. A known disadvantage of high frequencies is the range of effectiveness: atmospheric attenuation is severe, allowing detectability in the short-field only (Lawrence & Simmons 1982). Species emitting low frequencies have a long-field resolution but their bite force is weaker and their long rostrum is less resistant to torsion. Higher frequencies might promote niche specialization allowing for the detection of smaller prey: the wavelength of the sound emitted has to be shorter than the circumference of the object in order to produce strong echoes (Pye 1993; Jones 1999). Species emitting very low-frequency calls are potentially unable to detect small preys (Barclay 1986; Barclay & Brigham 1991; Safi & Siemers 2010). It is argued, however, that most of the bats use frequencies three or more times higher than is necessary to detect the smallest prey in their diet (Jakobsen *et al*. 2013). Furthermore, higher frequencies allow for higher beam directionality which maximizes the effectiveness of the echoes in the focal area and "isolates" echoes from the periphery (Surlykke *et al*. 2009). Thus, while beam directionality and detectability of smaller prey appear to be potential advantages in niche exploitation, the potential disadvantage is atmospheric attenuation.

In conclusion, skull diversification among bat families is mainly driven by sound emission type and broad diet preferences. Echolocation parameters were associated with skull shape in a subsample of insectivorous species only, suggesting that within similar feeding niches a stronger selection might occur due to the preferential use of echolocation as sensory system. The trade-off between feeding and echolocation function was questioned by our data analyses that instead support a predominant impact of dietary adaptations on skull shape variation in bats at a broad taxonomic scale.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of the following museums for providing access and allowing collection of morphological data: Royal Belgian Institute of Natural Science (Brussels), Magyar Természettudományi Múzeum (Budapest), Statens Naturhistoriske Museum (Copenhagen), World Museum (Liverpool), London Natural History Museum, Muséum National d'Histoire Naturelle (Paris) and Naturhistorisches Museum (Vienna). This research received support from the SYNTHESYS 3 Project http://synthesys3.myspecies.info/ which is financed by the European Community Research Infrastructure Action under the FP7 (BE-TAF-6601, HU-TAF-6926, DK-TAF-6870, FR-TAF-6924, AT-TAF-6820; awarded to the first author). The authors would like to thank Stefan Greif for helping with muscle dissections.

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SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Methods

Table S1 Specimens information and techniques used to reconstruct in 3D the bat skulls

Table S2 Estimates for sensory traits and categorical variables with data sources

Table S3 Estimates for feeding traits and categorical variables with data sources

Table S4 Anatomical definitions of 29 unilateral landmarks

Table S5 Main sources of variation of echolocation call parameters in bats that were controlled for within this study

Figure S1 Plot of principal component scores for all species of the dataset (n=67) displayed by diet. Shape variation is reported on lateral and ventral views using wireframe graphs as implemented in MorpoJ. Light blue represent the consensus (mean shape configuration) while in dark blue there is shape deformation related to the extreme PC scores.

Figure S2 Wire frame showing shape variation from the mean (light blue) along Partial Least Squares vector 1 from negative to positive scores in a) 61 bat species associated with Bite Force, b) 17 bat species associated with echolocation parameters.

Figure S3 Phylo-PLS scatter plot showing association between feeding parameters and skull shape in a subsample of 19 insectivorous bats. The Feeding vector 1 is heavily loaded on relative digastric mass. Skull wireframe show the shape variation related to the minimum (left) and maximum (right) values PLS vector scores.

Supplementary References

Cite this article as:

Giacomini G, Herrel A, Chaverri G *et al*. (2021). Functional correlates of skull shape in Chiroptera: feeding and echolocation adaptations. *Integrative Zoology* **00,** 1–13.