

Research Article

Sexual differences of skull asymmetry in *Carollia brevicauda* (Phyllostomidae: Carollinae) from Colombia

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Abstract

The analysing of asymmetry enables evaluation of morphological adaptation as well responses to environmental or genetic stress. Research presented here is a study of the three types of common bilateral asymmetries, fluctuating asymmetry (FA), directional asymmetry (DA) and antisymmetry (AS), by means of 15 skull landmarks on dorsal aspect of 45 specimens of the Silky short-tailed bat *C. brevicauda* (24 males and 21 females) from different localities in Colombia. DA appeared to be significant, possibly related to echolocation function, and with differences between sexes. The neurocranium exhibited a DA pattern of higher asymmetry than splanchnocranial components. FA was similar between sexes. AS was not detected. These results are congruent with the fact that sexual dimorphism in *Carollia* is not related to size but to different skull shape.

Keywords: Chiroptera; echolocation; neurocranium; shape sexual dimorphism; silky short-tailed bat.

Introduction

There are three main types of bilateral asymmetry: directional asymmetry (DA), fluctuating asymmetry (FA) and antisymmetry (AS). DA is a pattern where left and right sides consistently differ from each other [1]. FA is present when there are subtle non-directional differences between sides [1]. And AS represents a directional deviation from symmetry towards either the right or left side [2]. Within populations, large values of FA are interpreted to reflect lower individual quality in terms of fitness [3,4] whereas DA and AS are presumed to be heritable [3,4]. *Carollia* Gray, 1838 is a bat included in the Phyllostomidae family, which is widespread on the Neotropical region [5,6]. They are of robust and medium size, having a pronounced lancet extending dorsally [5]. *Carollia* uses short-wavelength echolocation to forage and

navigate [7,8]. Eight species in the genus are currently recognized [5]: *C. benkeithi*, *C. brevicauda*, *C. castanea*, *C. manu*, *C. monoherndezii*, *C. perspicillata*, *C. sowelli* and *C. subrufa* [9]. Colombia holds the highest variety of phyllostomid species, where until now four species have been documented: *C. brevicauda*, *C. castanea*, *C. monoherndezii* and *C. perspicillata* [10]. Silky short-tailed bats (*Carollia brevicauda*) inhabits a wider variety of ecosystems [5, 11, 12] from forests to altered areas, cultivated areas, grasslands, gardens and even places close to urban centres. *Carollia* species are known as frugivorous bat species, with fruits being the main food resource in their diet. Insects are a complementary food only. Its cranio-dental morphology is characteristic of frugivorous bat species [5]. Variation in skull and body morphology has been used broadly to establish ontogenetic processes in bats [12,13,14,15], but much less work has been done on asymmetries [9]. The objective of this study was to identify possible skull asymmetries in *Carollia brevicauda* which allow comparisons between both sexes and sexual allometric trends. Moreover, I wanted to determine if the neurocranial region (“cranium”) has an independent shape change in relation to splanchnocranial region (“face”).

Materials and Methods

Sample collection

Studied material is housed at the *Universidad del Cauca*, in Popayán (Colombia). Pathological and damaged skulls were previously excluded. Skull images were captured on the dorsal aspect using a digital camera (Nikon D1500) equipped with a lens (Nikon DX de 18-105 mm), with skull levelled ventrally on the horizontal plane and each image including a 10 mm ruler for calibration. The images were later stored in JPEG format. Finally, a total of 45 adult specimens (24 males and 21 females) was studied.

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Geometric morphometrics

I employed landmark-based geometric morphometric techniques for the study. A set of 15 landmarks, expressed as x, y-coordinates in Cartesian space (Figure 1), was positioned on the dorsal aspect of each skull using the TpsDig v. 2.26 software [16]. These landmarks were considered to be homologous and topographic equivalents. Differences in size, position, translation, and rotation were deleted using Generalised Procrustes Analysis (GPA) [17]. Shape variation was separated into a symmetric component (among-individual variation) and an asymmetric component (within-individual variation), but only the asymmetric component was included in this study. To quantify and minimize measurement error, all skulls were digitized two times.

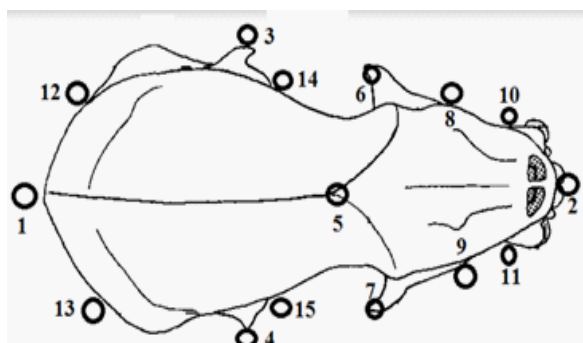


Figure 1: Location of the 15 landmarks on the dorsal view of *Carollia brevicauda* skull.

Statistical analysis

Prior to calculation of measurement error and asymmetries, the data were analysed for AS with Kolmogorov-Smirnov D test for overall equal distribution of signed right-left skull size. Size was expressed as centroid size (CS, e.g. the square root of the sum of squared distance between each landmark and the skull centroid) [17]. For shape, a Procrustes ANOVA on Procrustes coordinates were used to assess the contribution of individual variation, DA (“Sides” as the fixed factor), FA (“Individuals*Sides” interaction), and measurement error [1].

Results

Permutation tests revealed significant differences in asymmetry of mean dorsal skull shapes between sexes (Mahalanobis distances 1.377, $p=0.038$), thus in subsequent analyses two sexes were analysed separately. Skull sizes were similar for both sexes (Levene’s value $F_{1,43}=0.023$, $p=0.879$).

Measurement error and antisymmetry

Kolmogorov-Smirnov test demonstrated that signed difference between right and left hemiskull sizes did not differ significantly for both sexes ($D=0.125$, $p=0.992$; $D=0.142$, $p=0.939$ for males and females respectively), thus, rejecting the presence of AS. For shape, Procrustes ANOVA showed that FA, DA, and individual variation exceed the error component, indicating that the contribution of measurement error to overall shape variation was small.

Size and shape asymmetries

Levene’s test revealed no significant difference in the amount of skull

size asymmetry between sexes (Levene’s value 0.650, $F_{1,43}=0.099$, $p=0.754$). FA and DA were significantly different for both sexes (Mahalanobis distance 1.377, $p=0.038$), and most variation was concentrated in DA (89.0% and 85.2% for males and females respectively). It contrasted with shape FA (3.3% and 3.5% to total shape variation for males and females respectively), which showed no significant differences between sexes ($p>0.05$). Shape differences detected by canonical variates were illustrated on a deformation grid (Figure 2). In the PCA, skull asymmetry varied primarily along the two first PCs which explained a 70.1% of the total observed variance ($PC1+PC2=57.5\%+12.5\%$). Differences were located mainly on neurocranium length (coordinates x3, 4, 12 and 13) and fronto-nasal area (landmarks x, y14 and 15) (Table 1). Both PCA and CVA showed that the neurocranium has greater variance than the splanchnocranium.

Table 1: Loadings for Principal Components 1 and 2, which explained together a 70.1% of the total observed variance ($PC1+PC2=57.5\%+12.5\%$). Differences were centred on neurocranium length (coordinates x3, x4, xy12 and xy13) and fronto-nasal length and width (landmarks xy14 and xy15) (in bold).

	PC1	PC2
x1	0	0
y1	0.059822	-0.229617
x2	0	0
y2	-0.194529	0.326105
x3	-0.378424	-0.038454
y3	0.169316	0.250314
x4	0.378424	0.038454
y4	0.169316	0.250314
x5	0	0
y5	-0.008829	-0.704726
x6	-0.161428	0.000387
y6	-0.046408	-0.251980
x7	0.161428	-0.000387
y7	-0.046408	-0.251980
x8	-0.134784	-0.049856
y8	-0.077296	0.043284
x9	0.134784	0.049856
y9	-0.077296	0.043284
x10	-0.035126	-0.032419
y10	-0.184503	0.138913
x11	0.035126	0.032419
y11	-0.184503	0.138913
x12	-0.246647	-0.046886
y12	-0.079152	0.118223
x13	0.246647	0.046886
y13	-0.079152	0.118223
x14	-0.262029	0.060051
y14	0.289811	0.005365
x15	0.262029	-0.060051
y15	0.289811	0.005365

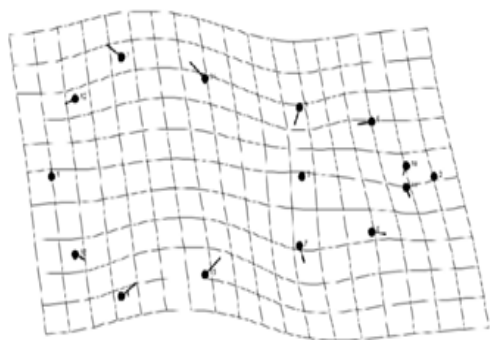


Figure 2: Deformation grid of shape differences detected by canonical variates for skulls of *Carollia brevicauda* (n=45). Landmarks are represented by black filled circles. This grid produces a geometric description of shape to detect deformations relative to a consensus to explain the shape change between males and females.

Landmarks involving the neurocranium (1, 3, 4, 12, 13, 14 and 15) were selected as a block. All of the other landmarks composed a second block involving splanchnocranium. PLS1 accounted for 91.03% of the total covariance, indicating that PLS1 represented the main covariance of two blocks. For PLS1, the pairwise correlation between blocks was up to 0.62 ($p < 0.001$), as noted in the plots distributed around the diagonal line of the PLS1 scores coordinate (Figure 3). Although the RV coefficient was only 0.271, it indicated that the overall strength of association between blocks was relatively weak ($p < 0.001$). A correlation was noted between two blocks but as it was not very strong, and the null hypothesis was rejected.

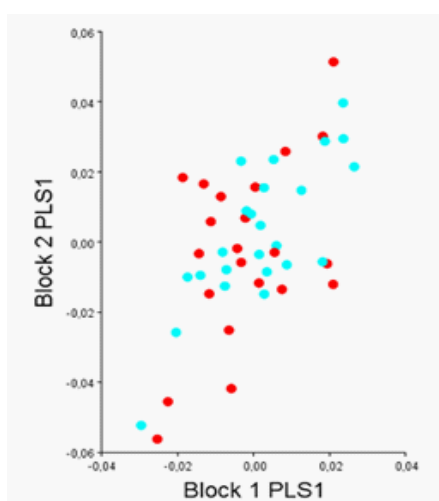


Figure 3: Scatter plot of the Partial Least Squares1 (PLS1 and PLS2) of two blocks (neurocranium and splanchnocranium) for skulls of *Carollia brevicauda* (n=45). The pairwise correlation between blocks was up to 0.62 ($p < .0001$).

Based on the PLS analysis results, a modularity analysis was performed to evaluate whether both parts were separate modules (Figure 4). The RV coefficient of the a priori hypothesis partition was 0.601 which was higher than that of the PLS analysis. Thus, null hypothesis partition was rejected, and we considered that the neurocranial and splanchnocranial parts of the skull covary. In conclusion, both parts of the *Carollia* skull are not separated modules, being integrated.

Associations of size and shape variation

Correlations between the amount of shape asymmetry and CS were non-significant for both sexes (8.40% and 4.91% of shape variation explained by size variation for males and females respectively, $p > 0.05$).

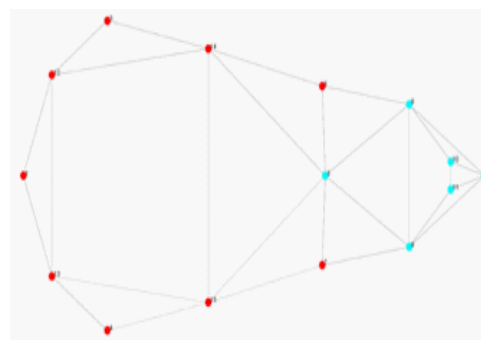


Figure 4: Modularity test results for skulls of *Carollia brevicauda* (n=45). The hypothesized partition: neurocranial part involving landmarks 1, 3, 4, 12, 13, 14 and 15, and the remaining landmarks as the visceral part; different colour presents different modules. Modules are units exhibiting a high degree of integration from many or strong interactions but relative independence from other such units.

Discussion

The use of geometric morphometrics in this study gave rise to the identification and quantification of previously unknown asymmetric features of *Carollia brevicauda* skulls. My results showed no size sexual dimorphism but shape asymmetric dimorphism was highly significant between sexes. These results are congruent with the fact that sexual dimorphism in *Carollia* is not given by skull size but by distinct asymmetric shape [11]. Is there a functional explanation for these significant asymmetric differences?

Developmental instability arises from processes that cannot buffer development against extrinsic or intrinsic accidents that occur under environmental and/or genetic conditions [20, 21]. FA has been used as an indicator of developmental stability in populations and there is evidence of a correlation between FA and a variety of intrinsic (genetic, e.g. inbreeding, increased homozygosity, hybridization....) and extrinsic (environmental, e.g. pollution, food level, parasites, developmental temperature....) stressors [22,23,24]. But the contribution of shape FA to the total shape variation was clearly lower than DA. DA must be interpreted as a reflection of adaptative traits and with more significance than the subtle FA.

Echolocation has a great anatomical effect on the bat cranium [8] and structural asymmetries allow detection of sound fields, thus reducing left-right ambiguity [25]. Thus, it is considered that DA -according to my results located mainly in braincase and the fronto-nasal line- may be the expression of the asymmetrical support plate for an asymmetric echolocation apparatus.

In this sense, DA sexual dimorphism possible appeared in *C. brevicauda* cranium because to that both sexes would have slight differences in their diet, as different conformation of echolocation traits and thus DA imply different ultrasound frequencies [7]. Moreover, perhaps the contralateral space would be devoted to pheromonal perception, which is very important among the Phyllostomidae [26] and thus it would be different between sexes, too.

The modularity test demonstrated that two skull modules, neurocranium and splanchnocranium, are units exhibiting a high degree of integration and relatively low independence between them. Conse-

quently, neurocranium and splanchnocranium are not distinct modules reflecting phenotypic and genetic variation. But which is the nature of the modularity interactions of these skull parts? Generally, it can be developmental, functional, or genetic [27]. Some studies have suggested that in families like Phyllostomidae echolocation may experience trade-offs with other sensory modalities, such as olfaction (on splanchnocranium) and vision (on neurocranium) [8]. Herein, it is argued that it is the echolocation function to which both units ultimately contribute.

Finally, when allometry is tested it is found that shape asymmetry and CS are not correlated in any of the sexes, meaning that bigger skulls are not accompanied by higher asymmetry. This lack of significant correlation of size and shape asymmetry suggests a minimal developmental connection between size and shape, which means that both are under different developmental constraints.

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Supplementary materials

The supplementary materials for this article can be sent upon request to author, the contents of all supporting data being his sole responsibility. The posted materials have not been copyedited.

Conflict of interests

Author has no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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