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Assessment of skull asymmetries in *Camelus* species

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Abstract

Fluctuating asymmetry (FA) is defined as subtle differences between the left and right sides in bilaterally symmetrical organisms or their parts. FA represents a change in developmental homeostasis of adult morphology due to a loss of developmental stability, so environmental changes may increase it. In this study, we assessed cranial asymmetries in camels using geometric morphometric techniques. For this, 27 adult skulls of *Camelus* sp. (*C. dromedarius*, *C. bactrianus* and their hybrids) were used. A set of 16 anatomical landmarks on the ventral aspect of the skulls was selected and studied with standard multivariate techniques. The skulls exhibited high levels of FA, suggesting developmental instability. As developmental stability depends upon a balance between heterozygosity and coadaptation, possibly FA is due to gene complexes, rather than a decrease in functional traits. The novelty of this study resides in that the geometric morphometric approach for the asymmetric assessment has not previously been applied to camel skulls. We believe that these application goals will largely benefit more studies on camels' developmental instability.

Keywords: camel; directional asymmetry; fluctuating asymmetry; skull

Introduction

The external metazoan body plan is assumed to be symmetric. Asymmetry is defined as a deviation of an organism (or a part of it) from perfect symmetry (Klingenberg, 2015). In bilateral asymmetries, three types at the population level are commonly recognized. One type is fluctuating asymmetry (FA), which is defined as the random developmental variation of a trait (or characteristic) (Kwiatkowska et al., 2015). FA is a population-level measure of developmental instability, *i.e.*, the ability of an organism to consistently produce a perfect symmetrical shape in a given environment (Angelopoulou et al., 2009; Kwiatkowska et al., 2015; Urošević et al., 2015). In fact, many studies established, that FA can overall be considered a useful tool for assessing a population's average fitness, as a decrease of genetic diversity can potentially imply a decrease of fitness. This is the reason FA has been often used to detect populations under stress (Carter et al., 2009; Albarrán-Lara et al., 2010; Demontis et al., 2010).

Another type of asymmetry is directional asymmetry (DA), which occurs when one of the body sides is marked more strongly than the other or, in other words, when symmetry differs from zero (Kwiatkowska et al., 2015). DA indicates the presence of a genetic asymmetry influence, but it is not an indication of developmental instability. DA generally results from normal development (asymmetry being the norm); whereas FA occurs when symmetry is the norm, but normal development is perturbed (Van Valen, 1962). The third type of asymmetry is antisymmetry (AS), which occurs when there are deviations from symmetry towards either the right or left sides (Ludoški et al., 2012).

Geometric morphometric techniques have represented an improvement of the morphometrics as they have the ability for measuring displacements, deformations and rotations of objects, and as well to illustrate variations in shape (Adams et al., 2013). These methods enable a better representation of shape than traditional linear measurements, allowing a quantification of traits otherwise only described qualitatively (Alibert et al., 1996; Adams et al., 2013). In recent years, there appeared some studies applying geometric morphometric analyses of skulls of domestic mammals (Parés-Casanova, 2013; Parés-Casanova, 2014; Parés-Casanova & Bravi, 2014; Parés-Casanova & Medina, 2019; Parés-Casanova et al., 2020; Parés-Casanova & Domènech-Domènech, 2021).

Extant Old World domestic camelids have been divided into three species: the dromedary or Arabian camel (*Camelus dromedarius*, named by Linnaeus, 1758), known as "one-humped" camels, the Bactrian or Asian camel (*Camelus bactrianus*, named by Linnaeus, 1758), known as "two-humped" camels (Legesse et al., 2018; Martini et al., 2018; Dioli, 2020) and the wild Bactrian camel (*Camelus ferus*) (Wu et al., 2021). They interbreed easily (Martini et al., 2018) and such hybrids can be morphologically identical to pure-bred dromedaries (Dioli, 2020).

In this study, applying the tools of geometric morphometric techniques, we compared the symmetry of camel skulls. The results obtained will shed some light on these techniques and will be useful for future studies of camels' skulls.

Material and methods

A set of 27 adult camels' skulls (*C. dromedarius*, *C. bactrianus* and their hybrids) was used in this study. Specimens consisted of specimens housed at the Zoological Museum at Barcelona. All of them corresponded to fully grown specimens (with M^3 partly or totally erupted) and were from human-kept animals. As identification of hybrids is based on DNA or on the morphometric analysis of bones (Silbermayr et al., 2010; Lado et al., 2019) not available for all specimens, species were impossible to be considered and we worked at genus level.

Imaging

Each skull was photographed in high resolution in a standardized ventral view with a digital camera. Images were captured with a Nikon® D70 digital camera (image resolution of 2,240 x 1,488 pixels) equipped with a Nikon AF Nikkor® 28-200 mm telephoto lens. Scale was given for each photograph by placing a ruler next to the specimen.

Landmark selection

After digitization, sixteen points (4 midsagittal and 12 bilateral) were chosen to analyze on the basicranium (Figure 1). The *x* and *y* coordinates of these landmarks were digitized using TpsDig 2.04 v. 1.40 (Rohlf, 2015). Since levels of FA are typically quite small in comparison with trait size and error can account for a large fraction of between-side variance and alter the results, digitalization was made twice to determine error level.

Symmetric and asymmetric variation

In geometric morphometrics, the nuisance parameters from the dataset, such as differences in size, orientation, and position, are removed through procrustean reorientation (Rohlf, 2005). Therefore, only the variation in shape of the landmarks' configurations is considered. This technique also scales configurations to have a centroid size (CS), "the square root of the summed squared distances from all landmarks to the configuration centroid" (Rohlf, 2005). The asymmetry was quantified through the landmark deviations of the original configuration from the symmetric consensus of the original and mirror image (Mardia et al., 2000). These Procrustes distances were analyzed with an ANOVA test, where the "individual" factor represents the random variation due to differences among individuals, the "side" factor represents variation due to DA, and the "individual-by-side" interaction indicates FA. *P*-values were obtained from permutation tests (10,000 iterations). This analysis also allowed identification of the measurement error (ME). To measure the amount of asymmetry, we calculated variance of both size (expressed as CS) and shape. Differences in the amount of asymmetry in both were tested with a Levene's test for homoskedasticity, that is, whether variances were equal between groups (averaged values). The asymmetry of CS was quantified using left and right values in hemi-skull.

Prior to calculation of ME and asymmetry, data were checked for AS. We used the Kolmogorov-Smirnov *D* test to analyze overall equal distribution of right and left hemi-skull size values with a permutation *p*.

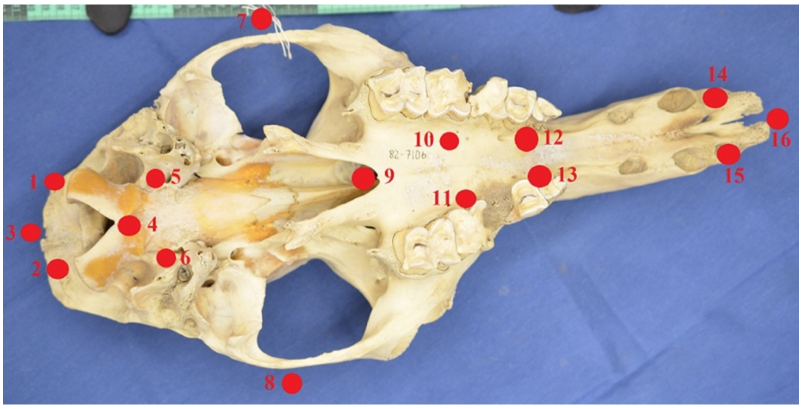

Figure 1. Characteristics used for morphological analysis of *Camelus* skulls, on their ventral aspect. Sixteen anatomical landmarks (4 midsagittal and 12 bilateral) were chosen.

To determine whether size had an effect on shape asymmetry (*i.e.*, allometry) we used a multivariate regression, treating shape completely in the multivariate context. Shape asymmetry values were regressed on to individual log-transformed CS values. The statistical significance of regression was estimated using the permutation test with 10,000 iterations against the null hypothesis of independence between size and shape.

To reveal the characteristics responsible for the observed patterns of morphological variation, a Principal Component Analysis (PCA) for allometric coefficients was made. The consensus configuration was determined.

Morphometric and statistical analyses were conducted using the Morpho J v. 1.06c (Klingenberg, 2011) and PAST v. 2.17c (Hammer et al., 2001) packages.

Results

Measurement error and antisymmetry

Mean squares of FA, DA and individual variation were found to exceed the error component, indicating that the contribution of ME to overall form variation was small. The Kolmogorov-Smirnov test demonstrated that the size difference between the right and left hemi-skulls did not depart significantly (D=0.203, p=0.197), reflecting an absence of AS in the data. Thus, we focused on the study of skull FA and DA.

Amounts of asymmetry

Procrustes ANOVA showed highly significant FA ("Individual x Side interaction") and DA ("Side effect") (p<0.05). FA represents the maximal significative contribution compared to DA (Table 1).

Table 1. Procrustes ANOVA for 27 *Camelus* skulls based on 16 anatomical points on ventral skull. “Side” effect represents directional asymmetry, while interaction “Individual*side” represents fluctuating asymmetry. Both were statistically significative, although this latter represents the maximal significative contribution compared to the former.

Effect	Pillai trace	*P*
Side	0.77	0.0256
Individual*side	8.15	0.0002

Allometric effects

A significant effect of size on the shape asymmetry was observed. Asymmetry of size accounted for 9.9% in asymmetry of shape (p=0.026).

Patterns of asymmetric shape variation

In the PCA of the asymmetric component of the shape (regression scores), the PC1 summarized 40.38% of the variation and PC2 24.08% (PC1+PC2=64.64%). Loadings of some characteristics on PC1 were negatives so they cannot be interpreted as corresponding to general size. These results indicated anisotropy: cranial asymmetry was not homogeneous throughout its entire structure, being characteristics of splanchnocranium (muzzle) which contributed most to variance. Characteristics on muzzle (x14 and x15), zygomatic arches (y7 and y8) and lateral points of foramen magnum (y1 and y2) showed the highest tendency towards asymmetry (Table 2 and Figure 2).

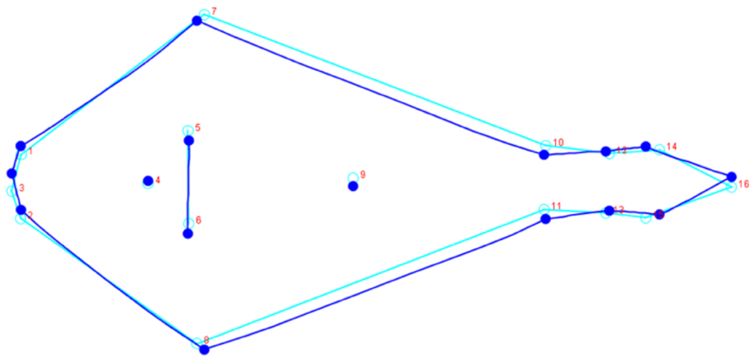

Figure 2. Shape deformation for the skull on its ventral aspect. Grey represents the starting shape, black represents the target shape.

Discussion

Asymmetry is defined as a deviation of an organism (or a part of it) from perfect symmetry and is composed by three different categories. Among them, the fluctuating asymmetry (FA) occurs when there are random deviations between the right and left sides (Klingenberg, 2015), and the directional asymmetry (DA) occurs when one side of a bilateral trait develops more than the other side (Klingenberg, 2015). The level of developmental instability is reflected by the level of FA, i.e., when the symmetry deviates from the developmental "plan" of the genome and cannot be corrected by the regulatory mechanisms of the organism (Auffray et al., 1999; Ludoški et al., 2012; Costa et al., 2015). The procedure for estimating asymmetries in this study used automatically provided information for the presence of FA and DA. Our results showed significant FA and DA, although FA represents the maximal significative contribution compared to DA.

Table 2. Principal component analysis for 27 *Camelus* skulls. PC1 summarized 40.38% of the variation and PC2 24.08% (PC1+PC2=64.64%). The most significative values (>0.3) appear in bold. Loadings of some characteristics on PC1 were negatives so they cannot be interpreted as corresponding to general size. Characteristics on muzzle (x14 and x15), zygomatic arches (y7 and y8) and lateral points of foramen magnum (y1 and y2) showed the highest tendency towards asymmetry.

	PC1	PC2
x1	0.028972	-0.01859
y1	0.301275	-0.04797
x2	-0.02897	0.018587
y2	0.301275	-0.04797
x3	0	0
y3	0.172518	-0.26254
x4	0	0
y4	-0.23155	-0.13072
x5	0.032056	-0.0569
y5	-0.16751	0.006346
x6	-0.03206	0.056899
y6	-0.16751	0.006346
x7	0.139027	-0.08141
y7	-0.12884	0.301033
x8	-0.13903	0.081405
y8	-0.12884	0.301033
x9	0	0
y9	-0.17839	0.118634
x10	0.000922	-0.02471
y10	-0.22248	0.248656
x11	-0.00092	0.024711
y11	-0.22248	0.248656
x12	-0.14129	-0.06251
y12	0.053812	-0.10265
x13	0.141287	0.062506
y13	0.053812	-0.10265
x14	-0.39413	-0.46438
y14	0.147908	-0.22469
x15	0.394134	0.464376
y15	0.147908	-0.22469
x16	0	0
y16	0.26909	-0.08685

Conclusion

Camel skulls exhibit high levels of developmental instability, expressed as fluctuating asymmetry. As developmental stability depends upon a balance between heterozygosity and coadaptation (Alibert et al., 1996), possibly fluctuating asymmetry is due to gene complexes, rather than a decrease in functional traits.

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Supporting information

Queries or any other issues regarding errors are requested to be addressed to author.

Conflicts of interest

The author declares no conflicts of interest to disclose related to this research.

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