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ORIGINAL ARTICLE

Lower palatine developmental instability in hybrid Old World camelids

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ABSTRACT

Objective: In this research study, we explore the fluctuating asymmetry (FA) of palate *Camelus* hybrids and their parental species (dromedary and Bactrian).

Materials and Methods: We studied a sample of pictures from 27 adult skulls of pure *Camelus dromedarius* (n = 13), *Camelus bactrianus* (n = 7), and their crosses (n = 7), from two different collections. A set of 11 semilandmarks was located on the palatal region and was studied by means of geometric morphometric methods. The asymmetric variation was analyzed and evaluated for allometric effects, and variation among these three groups was studied using a canonical variates analysis.

Results: Among hybrids, there appeared a significantly lower amount of FA in comparison to the parental species, which may reflect the lower levels of genetic stress and higher levels of directional asymmetry, which may suggest the presence of strongly transgressive mastication compared to pure species.

Conclusion: Camel hybrids would present increased developmental stability and better adaptation over those of parenteral lines.

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Introduction

In general, there are three types of bilateral asymmetry: fluctuating asymmetry (FA), directional asymmetry (DA), and anti-symmetry (AS). FA is characterized by random differences between the right and left sides [1,2]. FA is generally influenced by developmental instability (DI), which refers to an individual's inability to produce a specific phenotype under a given set of environmental conditions [3,4]. The randomness of FA is expressed as a bell-shaped distribution of deviations from the target phenotype [5]. Developmental accidents are generally the result of genetic or environmental stress [1,6]. Sometimes, deviations are distributed preferentially in one direction, thus generating DA. In DA, there is a tendency for the development of one specific side in relation to the other, leading to a distribution with the average deviation being significantly different from zero [7,8]. AS is found whenever the position of the larger side varies randomly in the population, leading to a bimodal distribution of the differences between the left and right sides, with symmetric individuals being less frequent [9,10].

The skull is a composite structure made up of the neurocranium and the splanchnocranium. The neurocranium (the "cranial vault" or "calvarium") surrounds and protects the brain, while the splanchnocranium forms mainly the skeleton of the face [11,12]. Multiple skeletal components of the skull originate asynchronously [12], with those two cranial parts presenting different growth processes. The region of the neurocranium reaches maturation first and is followed by the splanchnocranium [11,13]. The bilateral asymmetry of the skull, which describes the left–right morphological differences, is an inherent characteristic of its morphology. In this context, the pattern of skull asymmetry has been widely used as a marker for DI [14,15].

Domestic Old World camelids are divided into two species: the one-humped dromedary or Arabian camel

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(*Camelus dromedarius* Linnaeus, 1758) and the two-humped Bactrian or Asian camel (*Camelus bactrianus* Linnaeus, 1758) [16–18]. Dromedaries and Bactrian camels are morphologically close and present a large intraspecific variation [18]. They interbreed easily, and it is said that hybrids were regularly produced in the past, mainly because of their good laboring abilities [16,18,19].

Hybrids (interspecific crossed) are expected to be phenotypically intermediate between parental lines, with their fitness decreasing dramatically [3,20,21]. If we accept this hypothesis, it would be expected to find a higher level of FA, as it is an expression of DI, in hybrids compared to parental species.

In this circumstance, the primary goal of this study was to compare splanchnocranial asymmetries between pure and hybrid Camelus. We wished to determine whether the amount of FA and DA differ significantly among hybrids and parenteral species. The hard palate is the most crucial region of the splanchnocranium, and this is why we have focused this study on this region.

Because of their unique ability to measure displacements, deformations, and rotations of objects, as well as to illustrate multivariate variations in shape [21,22], geometric morphometrics (GM) techniques have been used in this research. With GM, it is possible to obtain a better representation of shape than the traditional linear and angle measurements, allowing to quantify traits and also allowing visualization of shape differences [23,24,25]. Viewing scientific research studies, few researchers have worked on hybrids phenotypic asymmetries using GM techniques [20], and none reported on *Camelus* species. Hence, our research study fills this non-explored topic for the first time.

Materials and Methods

The sample

We collected 27 adult skulls currently housed at the collection of the Osteoarchaeological Laboratory of the Faculty of Veterinary Medicine at Istanbul University (*n* = 20: nine *C. dromedarius*, four *C. bactrianus*, and seven hybrids), and at the Zoological Museum at Barcelona (*n* = 7: four *C. dromedarius* and three *C. bactrianus*). The sample comprised only fully grown specimens (with M^3 partly or totally erupted) and without any apparent skeletal disorders.

Imaging

Image capturing was accomplished with the implementation of a Nikon® D70 digital camera with an image resolution of 2,240 × 1,488 pixels, and the images were stored as digital files. The camera was equipped with a Nikon AF Nikkor® 28–200 mm telephoto lens. Each skull was levelled dorsally, following a horizontal plan with the camera placed with its focal axis parallel to the horizontal plane and centered on the ventral aspect of each skull. The scale was given for each photo by placing a ruler.

Landmark selection

Anatomical points should be marked by landmarks, while distinct curves on surfaces, such as ridges, should be treated as discrete points called semilandmarks. As very few landmarks were available on palate sides, outlines were digitized as a series of semilandmarks [25], and the bilateral shape of the palatal region was finally described by a set of 11 semilandmarks per side (Fig. 1). These semilandmarks were ulteriorly slid along each curve until they were in positions that matched the reference configuration [23], and these new discrete coordinates were used for further analysis [23].

The software TPSUtil v. 1.50 [26] was used for preparing and organizing the images. To establish digitizing error, landmarks were digitized twice by one of the authors (C.M.), using TPSDig v. 1.40 [26], in two different sessions. To ensure that the results of the Procrustes analysis were amenable to classic multivariate statistics, which require Euclidian (flat) space [23], we carried out an analysis using TPSSmall v. 1.33 [26]. This analysis reflected a high degree of approximation of shape space to tangent space (r = 0.999), which allowed accurate capturing of the nature and extent of palatine shape deformations in subsequent statistical analyses.

Morphometric methods

Through the generalized Procrustes superimposition approach, the nuisance parameters, such as differences in size, orientation, and position, were removed from the data set [23]. These superimposition steps were taken to minimize the sum of squared distances between corresponding

Figure 1. Set of semilandmarks. Eleven points per side were outlined and were chosen and used for morphometric analysis of the palatal region in the ventral skull.

landmarks [27]. The scale was eliminated by setting the centroid size (CS), the square root of the sum of squared distances between the centroid and each landmark, the same in all specimens [23]. Since the size was removed, the analysis was more sensitive to subtle shape differences, and consequently, only the variation in shape (not in size) of the landmarks configurations was compared.

Multivariate analysis

Shape asymmetry was analyzed using configurations superimposed as dependent variables in a Procrustes analysis of variance (ANOVA) [5]. In this model, the effect of the side corresponded to DA, the interaction between the side of the palate and the specimen corresponded to FA, and the residual term corresponded to the measurement error [27]. AS was analyzed using scatter plots of the differences between the left and right side for each point, following Klingenberg and McIntyre's method [28]. The formation of clusters of points in this distribution would correspond to a bimodal distribution in the differences between the left and right sides, and therefore to a presence of AS [29].

The effect of allometry (the influence of size on the shape, expected to contribute to transgressive effects on the shape possibly) was analyzed using the multivariate regression of shape on size. In this study, the size was treated as the $\log_{10}$-transformed CS, while the information about the shape variation was extracted from the Procrustes coordinates [23]. An ANOVA test was used to examine the differences between CS (log-transformed values).

Subsequent shape analysis of the asymmetric component was carried out by a canonical variates analysis (CVA). CVA was applied for statistically testing the differentiation of the three groups. It was based on Mahalanobis distance (Md, the distance between a point and a distribution) using 10,000 permutation rounds. The residuals from the multivariate regression (corrected size values for removing the influence of allometry) were employed for this analysis.

All morphometric analyses were carried out with MorphoJ software v. 1.06c [27]. The confidence level was established at 95%.

Results

The results in all Procrustes ANOVAs, regardless of species, showed that error contributed the least to total variation (Table 1) so it was unlikely to have significantly influenced the results. The scatter plots of left–right differences for each landmark evidenced no clustering of points (Fig. 2); thus, we also discarded AS and focused on the study of net asymmetry (FA and DA). The overall Procrustes ANOVA results indicated that both FA ("individual*side" effect) as DA ("side" effect) showed significant effects for all three groups except DA for *C. bactrianus* (Table 1).

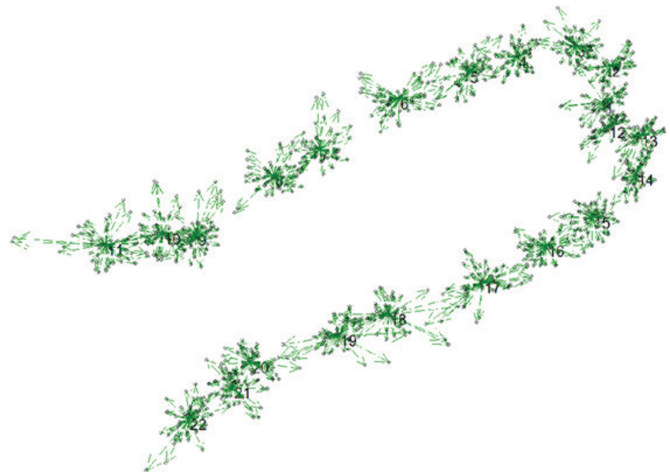

Figure 2. Scatter plots for each semilandmark after samples superimposition by the Procrustes algorithm. The morphological landmark is represented by dots, while the fluctuation is denoted by pointed vectors. There was no evidence for clustering of these points that would have suggested AS.

Table 1. Procrustes ANOVA for 13 C. dromedarius, six *C. bactrianus*, and eight hybrids based on 11 paired anatomical points on the palatal region.

Effect	SS	MS	Df	*f*	*p*	% MS
Individual	0.192186	0.000801	240	4.430	<0.0001	60.39
Side	0.005999	0.000300	20	1.660	0.041	22.62
Ind*side	0.043356	0.000181	240	4.050	<0.0001	13.62
Error	0.023167	0.000045	520			3.36
Bactrian						
Effect	**SS**	**MS**	**Df**	***f***	***p***	**% MS**
Individual	0.109455	0.000912	120	4.890	<0.0001	64.16
Side	0.005914	0.000296	20	1.580	0.067	20.80
Ind*side	0.022397	0.000187	120	6.890	<0.0001	13.13
Error	0.007584	0.000027	280			1.91
Hybrids						
Effect	**SS**	**MS**	**Df**	***f***	***p***	**% MS**
Individual	0.115694	0.000964	120	5.000	<0.0001	44.26
Side	0.019084	0.000954	20	4.950	<0.0001	43.81
Ind*side	0.023122	0.000193	120	2.870	<0.0001	8.85
Error	0.018820	0.000067	280			3.09

Results indicate that both directional ("side" effect) and fluctuating asymmetries (interaction "ind*side") showed significant effects for hybrids. Error represented a small amount of the total variance. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). Df = degrees of freedom. % MS of the total variation is computed by dividing the sum of squares for each term by the total sum of squares.

The multivariate regression showed that allometry was statistically significant ($p = 0.0001$; 10,000 random permutations); so the relationship between palate shape and size was quite clear. However, the percentage of shape variance related to size was low, as it accounted only for 11.44% of the total shape variance. Palate CS was higher in hybrids; the ANOVA showed statistical differences between them and Bactrians ($p = 0.039$). The small number of specimens does not enable us to test potential difference between groups statistically.

In the CVA for corrected size values, the asymmetric component allowed the differentiation of all three groups (CV1 + CV2 = 85.50% + 14.49%; $p < 0.001$). CVA also demonstrated that hybrids were separated in morphospace with no overlapping with pure individuals (Fig. 3), with a higher Md from hybrids to dromedaries (Md = 4.945) and Bactrians (Md = 4.600) than from Bactrians to dromedaries (Md = 2.155).

Discussion

Hybridization results in a new genetic combination, with the introduction of semicompatible genes into another genome [3]. Human-mediated hybridizations can lead to the production of more competitive phenotypes, which is generally considered as the result of this heterozygosis, known as "hybrid vigour" [19]. Among the most familiar examples of hybrids are mules and hinnies, the crosses between a donkey and a horse [19].

Hybridization has been historically practiced in camel breeding as a means to enhance the quality and fitness of offspring. In these crosses, heterosis takes the form of improved physical and physiological characteristics, notably for strength or endurance. Although this hybrid vigour is widely recognized, the phenotypic expression of developmental stability among camels remains unclear, particularly from an osteological point of view, but a large part of this vigour must be related to physical performance which is targeted to fit human needs and uses.

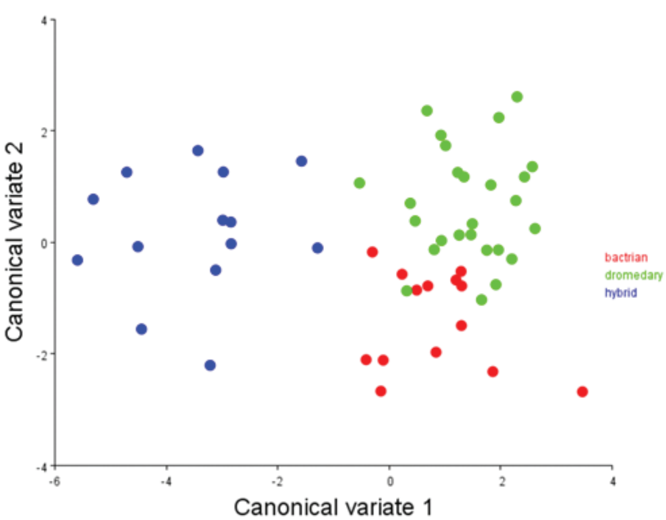

Figure 3. CVA for corrected size values of asymmetric component values allowed the differentiation of three groups (13 *C. dromedarius*, seven *C. bactrianus*, and seven hybrids). CV1 + CV2 = 85.50% + 14.49%. Although the asymmetric component allows the differentiation of all three groups ($p < 0.001$), hybrids appear more separated with no overlapping with pure individuals (Dromedaries and Bactrians) in morphospace.

DI is generally considered to increase with genetic and environmental stresses [3,10,30]. In the absence of stressors, selection can maintain DI acting directly on a character or indirectly through the selection on a correlated character favoring high phenotypic variance [31]. Hybridization has been considered to be one of the biological factors underlying the changes in levels of FA [3,35], which is inversely correlated with developmental stability [3,5]. Accordingly, individuals with highly unstable and thus more variable traits will have a selective advantage in new environments, or even asymmetry could be insignificant to the survival of individuals [20,31]. Increased FA in interspecific hybrids is often attributed to genetic incompatibilities causing meiotic irregularities or physiological and developmental abnormalities [3]. Given that both sides of the skull, and so the palate, are under the control of the same genetic pathways during development, one might expect that any deviation from symmetry will affect the product of disturbances that would break its developmental homeostasis.

DA is generally considered to have a genetic basis and is thus usually not considered to be driven by environmental or developmental stress [10]. Mastication in many domestic mammals is accepted to be asymmetrical [32–34], with chewing happening more on one side. In this context, lateralization of mastication and biting (e.g., side preference) may influence palate asymmetry, expecting to be a positive effect of palate asymmetry on bite force. In other words, side preference would be linked to side performance. So high levels of palate DA among hybrids would suggest the presence of strongly transgressive mastication compared to pure species, although it is however more complicated because little is known about mastication and biting in camels.

In this research study, we conclude that most of the symmetric variations in palate among pure and hybrid *Camelus* are explained by DA – directionally consistent difference between sides – which is higher in hybrids than in pure specimens of *Camelus* spp.; so, functional or adaptative mechanisms are better among the former group. But in hybrids, the palate also exhibits comparatively a low degree of significative FA-non-directional random deviations from symmetry. There may be opposite effects of function on directional and fluctuating asymmetries in the palate. Although the function itself (mastication and biting) may be lateralized, which would increase DA, it may be more developmentally stable causing their FA to be lower and therefore generating a negative correlation between these two components of asymmetric variation.

Conclusion

The observed values of FA for hybrid camels stand to be the result of increased developmental stability and those of DA, as higher functional or adaptative mechanisms.

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Conflict of interest

The authors declare no conflicts of interest.

Authors' contributions

The manuscript was conceived and designed by Pere M. Parés-Casanova; data were acquired by Cristina Morilla; analysis and interpretation of data were made by Pere M. Parés-Casanova and Cristina Morilla. Finally, Pere M. Parés-Casanova, Abu Bakar Siddiq, and Vedat Onar drafted the manuscript. All authors approved the completed article.

Supporting information

Supporting data can be requested from the first author.

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