Side differences in the skull of sheep: an assessment by geometric morphometrics

Short running title: Skull asymmetries in sheep

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Abstract

Effects of perturbations during development can be due to environmental and/or genetic factors, resulting in increased developmental instability which in turn can be expressed as fluctuating asymmetry (FA), defined as the non-directional deviation (right-left differences) from bilateral symmetry. However, other asymmetry types can appear, such as and directional asymmetry (DA), characterized by a distribution skewed to one side (right or left) at the, which is originated as a response to external stimuli that affect differentially on both sides of the organism. In order to describe asymmetric patterns in the ovine skull, we studied 165 specimens from animals belonging to the sheep breed "Navarra" from North Spain, using geometric morphometric methods. On digital pictures we analyzed two midline and 8 bilateral two-dimensional landmarks on skull dorsal aspect. Results showed that FA accounted for a reduced amount of total variation, while DA explained most of it. We suggest that presence of side differences due to lateralizated muscular function (mastication) is the most important factor in skull asymmetry. Obtained results should provide a basis for relating asymmetries to the mechanics of cranial skeletum in sheep.

Key words: cranium; directional asymmetry; morphological variation; Navarra sheep breed; *Ovis*

Introduction

In structures that present bilateral symmetry, random disturbances can alter the observable symmetry at macroscopic level (Graham et al. 2010). Due to its random nature, such disturbances affect both sides indistinctly, leading to an increase in fluctuating asymmetry (FA) (Cocilovo, Varela, and Quevedo 2006), defined as the non-directional deviation (right-left differences) from bilateral symmetry. However, other bilateral asymmetry types can appear, such as and directional asymmetry (DA), which is characterized by a distribution skewed to one side (right or left) at the population level. DA originates as a response to external stimuli that affect differentially on both sides of the organism (Graham, Freeman, and Emlen 1993) (Klingenberg, Barluenga, and Meyer 2002). Finally, antisymmetry (AS) occurs when there are deviations from symmetry towards either the right or left sides (Ludoški et al. 2012). Although the bases of FA are far from fully known, it is usually considered as a measure of genetic or environmental noise (Angelopoulou, Vlachou, and Halazonetis 2009), while DA has a proportion of genetic component (Cocilovo et al. 2006).

Here we investigate asymmetries in skull of a sheep breed managed under extensive conditions, analyzing a robust data base and using geometric morphometric techniques. Obtained results should provide a basis for relating asymmetries to the mechanics of cranial skeletum in sheep.

Materials and methods

Specimen collection

A sample of 165 skulls from “Navarra” sheep breed were randomly obtained from four different vulture feeding points in the Spanish slope of Central Pyrenees. Specimens belonged to different herds, but exact origin for each individual was impossible to be known. Breed’s geographical distribution is limited to the western half and south of the province of Navarra and to bordering provinces (Álava, Soria, La Rioja, Huesca and Zaragoza) in Spain (Jordana and Ribó 1991) (MAPAMA 2014). This breed is notable for its ability to adapt to adverse environments with heavy rain and snow, its resistance to sudden changes in temperature and the practice of transhumance (MAPAMA 2014). At present, it is used mainly in meat production (the production of young lambs), having lost its prior classification as a triple-purpose breed. Specimens corresponded to adult and subadult animals (assessed by at least a total eruption of M^2). Some cases of advanced cheek tooth diseases (peg-shaped, dental agenesis, asymmetrical wear, chronic abscesses...) were detected as well as osseous abnormalities (enthesopathies, osteomyielitis, periodontitis...), which caused osseous deformations *intra vitam*. These skulls were excluded from the analysis. Skulls that presented clear evidence of deformation by the action of postdepositational factors were equally excluded. Gender was not known for most of specimens, so it was not considered in our statistical analysis. Specimens are currently deposited in the bone collection of the Department of Animal Science at the University of Lleida (for consults: first author).

Data collection and photographing specimens

Skulls were labelled and levelled on a horizontal plane, and then photographed in their dorsal view. Image capture was performed 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. The camera was placed on a tripod parallel to the ground plane so the focal axis of the camera was parallel to the horizontal plane of reference and centered on the dorsal aspect of each skull. A scale was included in the images (mm unit).

Landmark selection and digitization of sample images

The captured images were transformed to TpsUtil software v. 1.40 (Rohlf 2015) and landmarks recorded using TpsDig software v. 2.26 (Rohlf 2010). The craniofacial morphology was relieved by registering 10 two-dimensional (2D) Cartesian coordinates of midline (2) and bilateral (8) landmarks (on both sides of the skull) on the dorsal side of cranium (Figure 1). All these LMs are considered to encompass elements of both viscerocranium -which supports the functions of feeding and breathing, and forms the face in mammals- as neurocranium -which surrounds and protects the brain-. Landmarks were digitized twice by the same person (RC) on two different days for assessing measurement error (ME).

Cartesian *x-y* coordinates were then extracted with a full Procrustes fit, a procedure that removes information about position, orientation and rotation and standardize each specimen. The size of each specimen was accessed through the centroid size (CS): the square root of the summed squared Euclidean distances from each landmark to the specimen centroid (Webster and Sheets 2010). Then we analyzed both symmetric and asymmetry components of variation; the first one is the average of left and right sides and represents the shape variation component, whereas the asymmetry component represents the individual left-right differences. The measure of asymmetries was computed for each individual by a procedure that involves the following: (1) a reflection of each of the original configurations of landmarks (each individual) to its mirror image (a reflected copy of each configuration); (2) a Procrustes fit, which generated an average of the original and mirrored configurations for each specimen; and (3) a computation for each individual as the deviation of the original configuration of landmarks from the symmetric consensus. The test for the error term was made by a Procrustes ANOVA procedure, which adds up sums of squares and mean squares over the coordinates of the landmarks and can quantify the amount of shape variation as a measure of the magnitude of the effects. The model allows to simultaneously assess the effect of side (DA) and interaction individual*side (FA) whereas the first factor, such as a fixed effect and the second, as a fixed and the second, as a random effect. To detect AS we used the Kolmogorov-Smirnov *D* test to analyze overall equal distribution of right and left hemiskull size values with a permutation *p*.

From the superposition were extracted a matrix containing the asymmetrical component, that is estimated from the bilateral landmarks and is obtained as the difference between the coordinates on both sides of the axis of symmetry. Finally, a linear regression of the asymmetric component of the shape *versus* log CS was done in order to study the possible allometry.

All analyses were then performed using MorphoJ version 1.05 (Klingenberg 2011) except the MANOVA which was performed with the package base PAST version 2.17c (Hammer, Harper, and Ryan 2001).

Results

Preliminary analysis

The control of digitizing error in studies with FA is fundamental as FA is the result of a subtle biological effect (Fruciano 2016). The Procrustes ANOVA indicated that the ME (mean squares for error term: 0.0000161971) was 4.5 times smaller than FA (i.e. individual-by-side interaction; mean squares for individual*side: 0.0000729348) (Table 1) and therefore the amount of ME was negligibly small compared to the source of variation dealt in the analysis. The variation explained by FA only reached to 1.4% of the total, while AD represented a 91.1% of the total. MANOVA test confirmed these asymmetries (Pillai trace 0.58 and 6.21 for DA and FA respectively, $p<0.0001$). The reduction in the number of variables using a Principal Component Analysis was not necessary since we disposed more cases than variables (e.g., Procrustes coordinates). Kolmogorov-Smirnov test demonstrated that size difference between right and left hemiskulls did not depart significantly ($D=0.039$, $p=0.956$), reflecting an absence of AS in the data. The spatial configuration showed asymmetry mainly in viscerocranium: facial tubercles ("thick face") and the dorsal ridges of the orbita (Figure 2). It should be noted that the DA vectors were oriented towards right.

Although the regression of asymmetric component against the log-transformed CS revealed that asymmetry had a significant increase during development ($p=0.0374$), this ontogenetic shape change through the asymmetric component was markedly low (1.9%). The shape changes observed in the skull during the development included relative changes on the muzzle length in smaller specimens towards relative width changes in bigger specimens.

Discussion

In this study, we have applied a geometric morphometric analysis to the study of symmetrical shape variation in skulls from a local sheep breed maintained under extensive conditions. The method used allowed the decomposition of the total shape variation into components of symmetric variation (i.e. differences among individuals) asymmetric variation. The results obtained in our analyses indicated firstly that the magnitude of fluctuating asymmetry was low compared to directional asymmetry, which constituted the relevant factor in the estimation of the asymmetric component of the variation.

We suggest that presence of fluctuating asymmetry in sheep skulls may be purely related to subtle stress factors, as no skull deformities were observed and similar results have been obtained for other domestic species (Parés-Casanova 2014a) (Parés-Casanova 2014b). This fluctuating asymmetry would be below the ‘threshold phenomenon’, that is, not due to stress and a low genetic buffering capacity. In other words, the skull fluctuating asymmetry would not be exceeded due to pathologic reasons.

For directional asymmetry we must look the explanation on the masticatory apparatus, as it would suggest a direct association to chewing mechanical factors. In vertebrates, these directionalities in left±right dimensions have been found (Bartosiewicz, Van Neer, and Lentacker 1993) (Laia et al. 2015) (Bishop et al. 2016) (Del Castillo et al. 2016) (Gourso et al. 2018). A genetic background for the phenomenon has been suggested (Hackert et al. 2008), although until recent years no specific genes have been found to cause the lateralized behavior (Carter, Osborne, and Houle 2009). Mastication is dominated by the masseter muscle and has its origin on the skull, where it is attached from the zygomatic bone till the facial tubercle (Sisson, Grossman, and Getty 1982). Individuals with this asymmetrical muscular development as a result of chewing side preference, a right side in our studied case, were expected to have increased level of directional asymmetry. Thus, a normal directional asymmetry may well be of functional origin in sheep, in the same way as there is a definite right-side preference in chewing in primates, including humans (Kwiatkowska et al. 2015) (Singleton 2015), and in other vertebrates (Zamanlu et al. 2012) (Parés-Casanova 2014a) (Leśniak 2018).

Moreover, the fact that asymmetric component of shape fitted to the size suggests an imperceptible increase of asymmetry with age. Thus, if directional asymmetry might continue to change with the size increase, this would reinforce the hypothesis that is the mechanical loading the main explanatory factor, as animals must increase their feeding requirement if their mass is bigger, as facial structures have been shown to be strongly dependent on the muscular balance. Moreover, being skull morphogenesis a complex phenomenon, the face in the last region to mature, so environmental factors may modify this region more markedly.

In summary, our data suggest that for the sheep skull, right and left sides are differentially biased, giving rise to directional asymmetry which results in fixed differences between the two sides mainly on viscerocranium. Random effects around these fixed differences (i.e., environemntal noise, expressed as fluctuating asymmetry) perturb slightly the magnitude of the effects.

A potential impact of these results may be on the study of ovine models in which intracranial asymmetries might have an impact (Boltze et al. 2008) (Hoffmann et al. 2014) (Nitzsche et al. 2015). Future studies that incorporate a greater number of populations and to broaden the range of ecological variation analyzed will help deepen our understanding of the processes of morphological variation in domestic sheep.

Authors' contributions

PMPC designed the experiments, analyzed the data and wrote the paper. AT and RC performed the field study. Refer to first author for supplementary material as the posted materials are not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the first author.

Competing interests.

Authors have no competing interests.

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Table 1. Procrustes ANOVA test performed for both centroid size (CS) and shape (SH). DA=directional asymmetry; FA=fluctuating asymmetry. Mean squares (MS) are the amount of variation from the one higher level in the hierarchy. The *F* value represents the comparison of each MS to the one lower level of MS which could be the source of error. Sums of squares (SS) and MS are in units of Procrustes distances (i.e. dimensionless).

		SS	MS	*df*	*F*	P
CS	Individual	61959.371213	377.801044	164	26.68	<.0001
	Error	2279.897654	14.160855	16	0.04	1.000
	Residual	1058.448875	352.816292	3		
SH	Individual	0.48964293	0.0003732035	1312	5.12	<.0001
	DA	0.03693527	0.0046169091	8	63.30	<.0001
	FA	0.04172378	0.0000161971	1312	4.50	<.0001
	Error	0.04712376	0.0000181805	2576	-0.01	

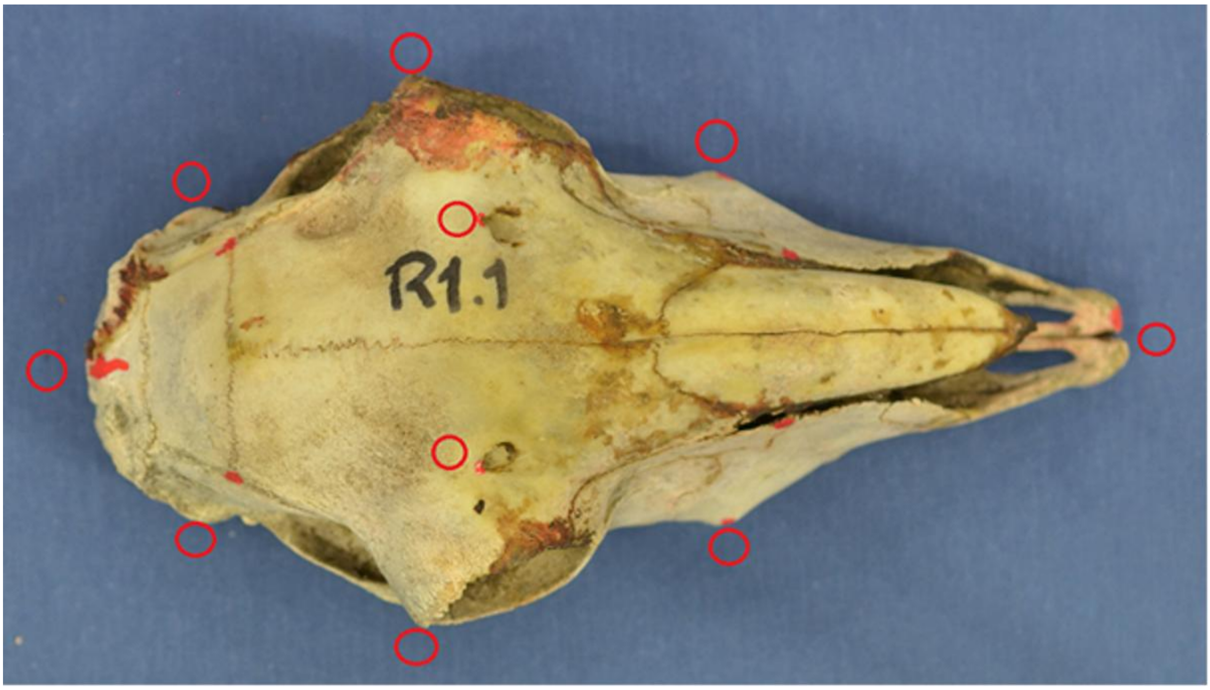

Figure 1. Landmarks (LMs) digitized on the surface of the skull (dorsal aspect). Skulls were labelled and levelled on a horizontal plane, and then photographed in their dorsal view. Eight of them were bilateral and two were midline LMs. All of them were considered to encompass elements of both viscerocranium as neurocranium.

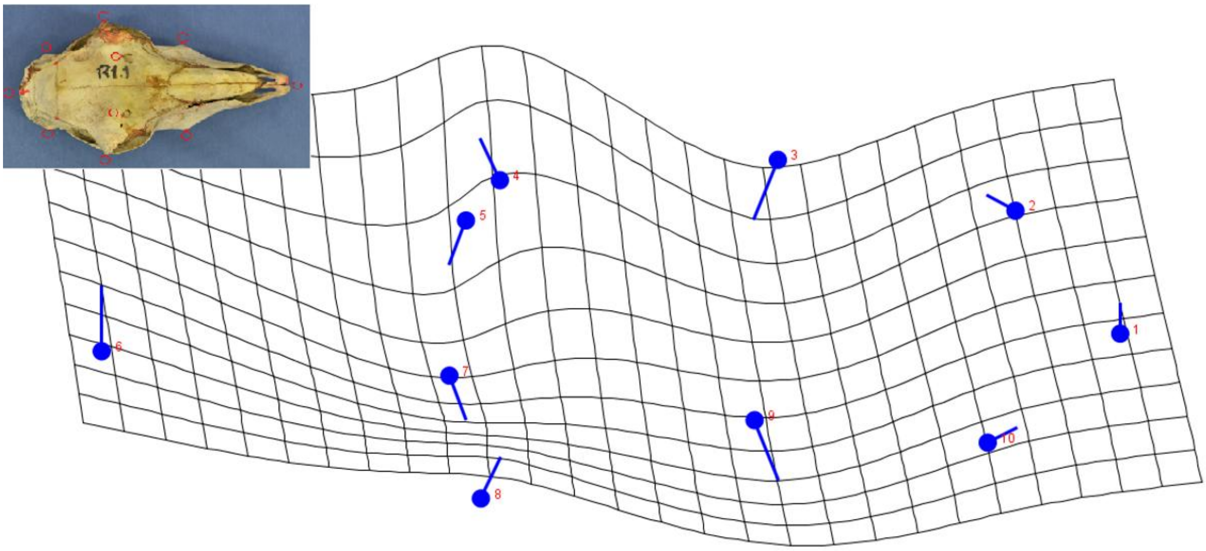

Figure 2. Deformation grid to capture the morphological shape differences and changes. This spatial configuration showed asymmetry mainly in viscerocranium: facial tubercles ("thick face", landmarks 4 and 8) and the dorsal ridges of the orbita (landmarks 3 and 9).