Directional and fluctuating asymmetries in domestic sheep skulls

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ABSTRACT

Morphological symmetry and asymmetry of three different sheep geographical populations (n=39), managed under semi-extensive conditions, were decomposed using geometric morphometric methods, on dorsal aspect of skulls. Fluctuating asymmetry (FA) was used as an indicator of environmental stress, and directional asymmetry (DA) as biomechanical constraints. The two-dimensional coordinates of 21 landmarks were digitized and analyzed using geometric morphometrics. Multivariate analyses showed the presence of subtle but significant directional asymmetry and fluctuating asymmetry in the entire sample, but no distinctive differences were detected between populations. These results are on the whole indicative that environmental stress, if present was not sufficient to infer on symmetry of the skull, and no alterations could be noted on functional loadings. On the whole morphometric studies should open up promising areas of research in this almost unexplored field, in domestic ungulates.

Key words: geometric morphometric, morphological variation, *Ovis*, symmetry

INTRODUCTION

Bilateral symmetry, a key feature of vertebrate body plans, is rarely perfect, and mild asymmetries can be found in normal growth and development, as a typical adaptation of the organisms to their environment. Deviations from expected perfect symmetry can occur, and organisms can develop several kinds of asymmetries, among others are fluctuating asymmetry (FA) and directional asymmetry (DA). The first is defined as non-directional deviation from bilateral symmetry, expressed as individual differences between left and right sides (*r - l*), and because of its characteristic is usually considered as a measure of developmental noise [1, 2, 3]. DA happens whenever one side on the plane of symmetry develops more than the other side, and has a proportion of genetic component [4, 5].

In the present study we analyzed left right asymmetries in the sheep skulls with geometric morphometric techniques, with the aim to quantify asymmetries and to assess and describe differences between three different geographical populations. The Geometric morphometrics was used to determine the overall differences and/or similarities of the differences in skull between groups. These methods combined with powerful and flexible tools of multivariate statistics make it possible to study morphological variation with direct reference to the anatomical context of the structure.

MATERIALS AND METHODS

2.1 *Specimen collection*

Thirty-nine skulls were obtained from three different vultures feeding points located in Catalunya (NE Spain). The origin of these skulls represents a wide range of breeds of European origin. Samples were pooled according into those three sampling origins, codified as AU (n=11), PAL (n=10) and SOL (n=18), which will be considered as operational groups. All animals were from semi-extensive local farms, and were all raised for meat purposes. No inbreeding was supposed for herds. Specimens corresponded to adult animals (assess by total eruption of M^3). Some cases of advanced cheek tooth diseases (peg-shaped, dental agenesis, asymmetrical wear, chronic abscesses...) were

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detected as well as osseous abnormalities (enthesopathies, osteomyielitis, periodontitis...), which caused gross bony deformations *intra vitam*. These individuals, although the more stressed ones, were retained for the analyses, as detection of asymmetries was one of the purposes of this study.

2.2 Data collection and geometric morphometric analyses

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 mandible. A scale was included in the images to standardize each specimen size (mm unit). Skulls were digitized using tpsDig version 2.04 [6]. In total, 21 two-dimensional (2D) landmarks (LMs, homologous anatomical points) were used on the dorsal side of cranium (Figure 1). Sixteen of them were bilateral and five (3, 4, 7, 8 and 9) were midline landmarks. All these LMs are considered to encompass elements of both vicerocranium as esplachnocranium. Landmarks were digitized twice by the same person (PMPC) on two different days in the same order, for assessing measurement error. All analyses were then performed using MorphoJ version 1.05 [7].

Cartesian *x-y* coordinates were then extracted with a full Procrustes fit [8, 9], a procedure that removes information about position, orientation and rotation and standardize each specimen to unit centroid size (CS – a measure computed as the square root of the summed squared Euclidean distances from each landmark to the specimen centroid, and provides an estimation of the size of the structure under study). Due to the symmetry of the structure, reflection was removed including the original and the mirror images of all configurations in the analysis and superimposing all of them simultaneously [10]; all information on the asymmetry of the studied structure were used to observe eventual phenomena of FA and DA. Within a symmetrical structure, DA happens whenever one character developed more in one side of the plane or planes of symmetry than in the other, while FA is defined as the non-directional deviation from bilateral symmetry (right-left differences, r-l). We used Procrustes ANOVA, as assessed for study on symmetry [10, 11, 12], to quantify the amount of symmetric variation and asymmetry; results are reported as sum of squares (SS) and means squares (MS) that are dimensionless. Additionally, to avoid the assumption of having isotropic (equal and independent) variation on all LMs, we performed a MANOVA test for both symmetric and asymmetry components [10].

We then assessed shape variation in the whole dataset performing a PCA (Principal Component Analysis), taking into account 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. Differences between the three sampled populations were assessed with a CVA (Canonical Variate Analysis), a multivariate statistical test that allow finding shape characteristics that best distinguish among several groups of specimens. Results are reported as Mahalanobis distance, a multivariate measure of distance relative to the within sample variation. All analyses were computed with 10,000 permutation runs.

RESULTS

Measurement error resulted smaller than FA (MS value for FA, compared to MS value for individuals, Table 1), so we proceed with all subsequent analyses. Procrustes ANOVA indicated that variation between populations was not significant concerning size, whereas it was significant concerning shape, latter result confirmed by MANOVA test for symmetric component of variation (Pillai trace = 1.41, P = 0.004). Additionally, both DA and FA emerged as highly significant and were confirmed by MANOVA test (for DA Pillai trace = 0.81, P = 0.0008; for FA Pillai trace = 13.65, P<0.0001).

We then used PCA to assess and describe this pattern of individual variation and asymmetry. PCA for the symmetric component of variation (DA) showed that the firsts three PCs explained 65.3% of the total shape variation, with the all other PC which account for no more than 9.5% of variation (see Figure 2 and Table 2 for details); all LMs contributed quite equally to the whole shape variation. On the reverse PCA for the asymmetric component of shape variation (FA) showed that the first PC contributed alone for 47.6% of the total variation and each other PCs contributed no more than 10% to the total shape variation (refer to Table 2 for details).

Morphological variability between populations was assessed and displayed with CVA, which showed highly significative differences (in each case P<0.0001) between the three populations analyzed, and quite similar one another in both symmetric component (Mahalanobis distances: AU vs PA = 2.27; AU vs SO = 2.61; PA vs SO = 3.84) and asymmetry component (Mahalanobis distances: AU vs PA = 3.65; AU vs SO = 2.20; PA vs SO = 2.19) of variation (Figure 3).

DISCUSSION

In this study, authors have applied the general method for geometric morphometric analysis of symmetric shape variation and asymmetry in sheep skulls. The method used allowed the decomposition of the total shape variation into components of symmetric variation (i.e. differences among individuals) from components of asymmetry (multiple components might occur according to the symmetry of the object). Using this approach, purely symmetric variation spanned only about 33% of the total variation. Conversely, majority of the variation was described by individual patterns of asymmetry as it has been illustrated that asymmetric variation did only account for more than 47% of the total morphological variation in all 3 studied groups
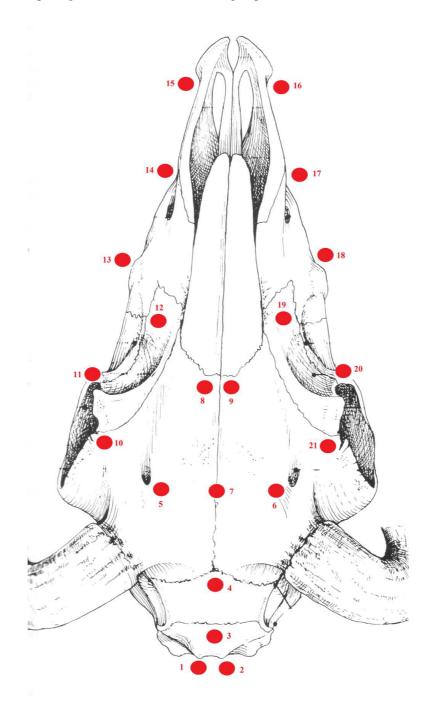

Figure 1. Landmarks digitized on the surface of the skull.

a) Symmetric

PC1

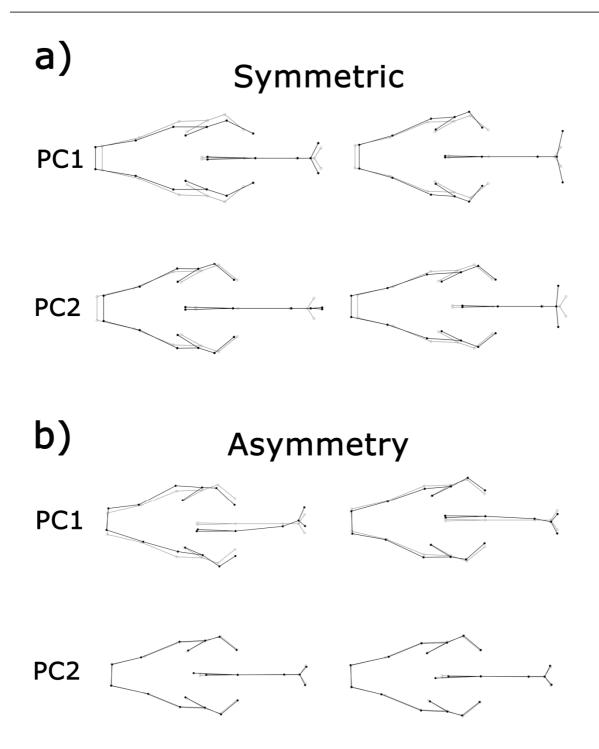PC2

b) Asymmetry

PC1

PC2

Figure 2: PCA shape deformations: a) for symmetric and b) for asymmetry components of variation. Grey represents the starting shape, black represents the target shape

We suggest that the large proportion of morphological asymmetry in sheep skulls may be related with different environmental factors for each geographical group. Although some skull deformities were observed, on the whole DA displayed no clear shape variation patterns, indicating that no side was consistently different from the other side. Perfectly bilateral symmetric organisms are hardly to find, and the asymmetries emerged from the present study might therefore be considered as deviations from the underlying ontogenetic trajectories.

Some authors [13, 14, 15] have reported that muscle contraction in juvenile pigs increases strain in the braincase. If muscle action had an effect on symmetry, symmetry differences would appear both in splachnocranium (where are inserted the muscles *dilatator naris apicalis*, *depressor labii superioris* and *buccinator –pars buccalis-*, for instance) as in neurocranium (insertion of *orbicularis oculi* and *malaris*, for instance). An increased strain can be supposed to

be localized to specific portions of the skull that correspond directly to the masticatory action. In sheep, the masticatory apparatus is dominated by the masseter muscle [16], which has its origin on the skull, where it is attached from the zygomatic bone till the facial tubercle (LMs 13-18). Necessarily, fibers that attach to different surfaces of an aponeurosis must have different orientations, raising the possibility that differential contraction of fibres could change the direction of muscle pull, a possibility that has been confirmed in a variety of masticatory muscles [15, 17]. But given the relatively small size of the muscles of mastication in sheep it is reasonable to assume that these muscles will play a minor role in the resulting variation found in ovine skulls. Individuals with asymmetrical muscular development as a result of either chewing side preference or simply a product of pathologies, are expected to have increased level of DA, but this is not the case, either, as some specimens presented clearly asymmetrical oclussal wearings and they did not appear separated. Thus, although action line could vary dynamically throughout a masticatory cycle, its action must be globally constant. Pathological skulls did no appear separated, either.
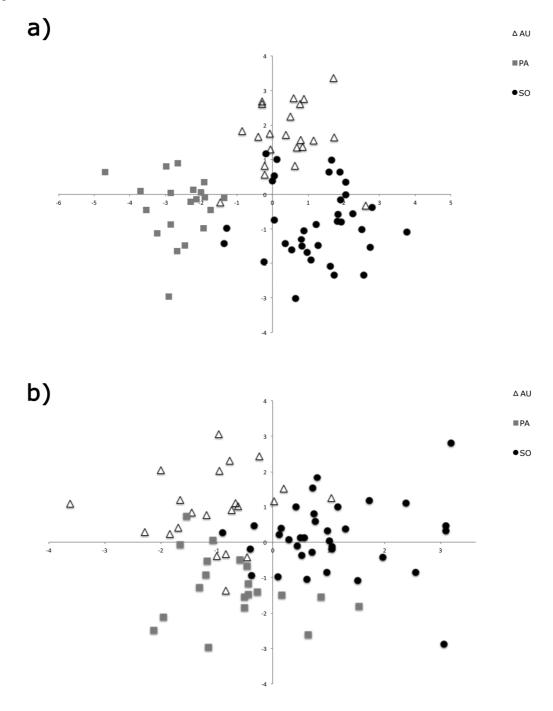

Figure 3: Scatter plot of CVA analysis showing differences between geographical populations, a) for symmetric component of variation; b) for asymmetry component of variation

Table 1. Procrustes ANOVA test performed for both centroid size (CS) and shape (SH).
SP = sampled populations; ID = individual; DA =directional asymmetry; FA = fluctuating asymmetry; ER = error.

		SS	MS	*df*	F	P (param.)
CS	SP	29979.52	14989.76	2	0.36	0.7022
	ID	1511614.13	41989.28	36	132.63	<0.0001
	ER	12347.30	316.60	39		
SH	SP	0.026	0.00069	38	2.14	0.0001
	ID	0.22	0.00032	684	4.35	<0.0001
	DA	0.02	0.0009	19	11.80	<0.0001
	FA	0.05	0.000074	722	1.58	<0.0001
	ER	0.07	0.000047	1482		

Table 2. Principal component analysis of shape variation, for both symmetric and asymmetry components.
Values reported are the eigenvalues and percentage which each PC (Principal Component) accounts for.

Symmetric component			Asymmetry component	
PC	Eigenvalues	% Variance	Eigenvalues	% Variance
1.	0.00122819	33.06	0.00037517	47.58
2.	0.00076447	20.74	0.00008245	10.46
3.	0.00042325	11.49	0.00006245	7.20
4.	0.00035331	9.59	0.00005412	6.63
5.	0.00024634	6.68	0.00005069	6.43
6.	0.00014294	3.88	0.00003297	4.18
7.	0.00011093	3.01	0.00002657	3.37
8.	0.00009909	2.69	0.00002055	2.61
9.	0.00007300	1.98	0.00001593	2.02
10.	0.00006676	1.81	0.00001448	1.84
11.	0.00004530	1.23	0.00001125	1.43
12.	0.00003778	1.03	0.00000899	1.14
13.	0.00003158	0.86	0.00000810	1.03
14.	0.00002506	0.68	0.00000696	0.88
15.	0.00001445	0.39	0.00000605	0.77
16.	0.00001335	0.36	0.00000523	0.66
17.	0.00001012	0.28	0.00000312	0.34
18.	0.00000509	0.14	0.00000185	0.24
19.	0.00000440	0.12	0.00000162	0.21

It has been suggested that different traits have different tolerances to levels of stress, making some better at buffering external stressors [18]. Another possible explanation of this little, but significant DA presence could be that the neural crest, which gives rise to diverse derivatives, including the peripheral nervous system and the craniofacial skeleton [19], involves a large number of processes, which would offer a mechanism of buffering any stress. Moreover, from the present study size has no significant influence on shape variation between groups. This finding would be consistent with the phenotypic architecture of the morphometric traits studied.

To conclude, from our study emerged the presence of both DA and FA in sheep skull, but their values, although statistically significant, appeared as subtle. These results on the whole indicate the presence of some asymmetries in the structure, apparently not sufficient to infer developmental stability, but instead they should act to counteract stressors and maximize fitness.

Dental disease can be a serious problem for sheep, as it has been described in other countries [20] but it seems no to be an important source of stress, at least for the sample studied. This hypothesis must be explored further through future studies, as well as experimental models.

From our study emerged that such these morphometric studies should open up promising areas of research in this almost unexplored field, in domestic ungulates.

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