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Skull Asymmetry in Sheep is Dominated by Right Side

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Abstract

Random deviations from the perfect symmetry of normally bilaterally symmetrical characters for an individual with a given genotype occur during individual development due to the influence of multiple environmental factors. Directional asymmetry (DA) indicates that one side is consistently and significantly different than the other. DA is a characteristic of most vertebrates, most strikingly exhibited by the placement of various organs (heart, lungs, liver, etc.) but also noted in small differences in skeletal structures.

In the research presented here, we study the presence and level of skull DA in a sample of domestic sheep. For this purpose, a global sample of 40 skulls belonging to adult animals was studied by means of geometric morphometric methods.

The results of this study raise future questions about the influence of skull biomechanics on its asymmetrical development, but also about how management, ingesta-specific properties (such as abrasiveness) and domestication can influence this response.

Keywords: Bilateral asymmetry; Cranium; Directional asymmetry; Fluctuating asymmetry

Introduction

There are three types of asymmetry: fluctuating asymmetry, antisymmetry and directional asymmetry (DA). Fluctuating asymmetry is a pattern of bilateral variation where the mean difference between sides for a population is zero, and the variation is normally distributed around zero. Antisymmetry is present when the side which is bigger varies among individuals, creating a bimodal distribution for the differences. DA is the consistent difference between a pair of skeletal structures, such that the larger metric consistently occurs on one side (the smaller on the other). Although most mammals have bilaterally symmetrical skulls, a common departure from this ideal asymmetry is DA, which has been observed in a large number of taxa [1,2]. Many cases of DA have been found on wild animals (2010 for bibliographical citations [3]), but also in domestic mammals like pig [4] and sheep [5].

Geometric morphometrics extends the traditional approach of measuring left and right side traits to quantify individual variation and asymmetry in geometric shape of paired structures. This approach consists of landmarking photographic images of each specimen and creating mirror images of the right and left sides to form a consensus figure. Differences between landmarked points and consensus points are used to calculate Procrustes residuals as a measure of asymmetry for all landmarks, allowing shape variation to be partitioned into symmetric shape and asymmetry [6,7].

Objectives

The overall objective of this research was to determine, by means of geometric morphometric methods, whether DA appears in domestic adult sheep and, if so, to analyze this kind of asymmetry. Answers to these questions should provide a basis for establishing DA in domestic mammals, a topic which began to be studied only during the last few years.

Methods

Population studied

Spanish law allows carcasses of farm animals to be left in the field in some protected areas or to be taken to “muladares” (vulture feeding stations) to provide food for wild-life. A sample of skulls of domestic sheep was collected from three different vulture feeding stations located in Catalonia (NE Spain) during 2013. Herds to which animals belonged were managed under similar semi-extensive conditions and were composed of different pure meat breeds and their crosses. Skulls were from adult males and females who died from natural causes, but there was no information on the sex of each specimen. All skulls were generally well preserved and some had pathological lesions (as assessed on the basis of macroscopic examination) and the sole exclusion criterion was the inability to determine the precise anatomical points of reference. Therefore, only the individuals for whom both sides could be well measured were included in the final analysis. A final sample of 40 specimens was collected for this study. They are now held in the collection of the Dept. of Animal Science of Lleida University.

Morphometrics

In total, 21 two-dimensional homologous landmarks (anatomical points) were used on the dorsal side of skull (Table 1 and Figure 1). Sixteen of them were bilateral and five (3, 4, 7, 8 and 9) were midline landmarks. All these landmarks are considered to encompass elements of both vicerocranium and splanchnocranium.

Data acquisition

Each skull was placed in a support, always in the same position, levelled in accordance with a horizontal plan. Image capture was performed with a Nikon® D70 digital camera (image resolution of 2,240 × 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

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1. Left nuchal tubercle	12. Left contact point of zygomatic-lacrimal, lacrimo-maxillary and maxillo-zygomatic sutures
2. Right nuchal tubercle	13. Left facial tubercle
3. Occipito-temporal suture	14. Most ventral point of the maxillary tuberosity (left)
4. Fronto-parietal suture	15. Space between pM^3 and M^1 (left)
5. Left supraorbital foramen	16. Space between pM^3 and M^1 (right)
6. Right supraorbital foramen	17. Most ventral point of the maxillary tuberosity (right)
7. Medial point on the line between landmarks 5 and 6	18. Right facial tubercle
8. Left fronto-nasal suture	19. Right contact point of zygomatic-lacrimal, lacrimo-maxillary and maxillo-zygomatic sutures
9. Right fronto-nasal suture	20. Right zygomatic-lacrimal suture
10. Left temporo-zygomatic suture	21. Right temporo-zygomatic suture
11. Left zygomatic-lacrimal suture	

Table 1: Landmarks used for the study of asymmetries in sheep skull (dorsal aspect). In total, 21 two-dimensional landmarks were used on the dorsal side of skull. Sixteen of them were bilateral and five (3, 4, 7, 8 and 9) were midline landmarks. All these landmarks are considered to encompass elements of both vicerocranium and splanchnocranium.

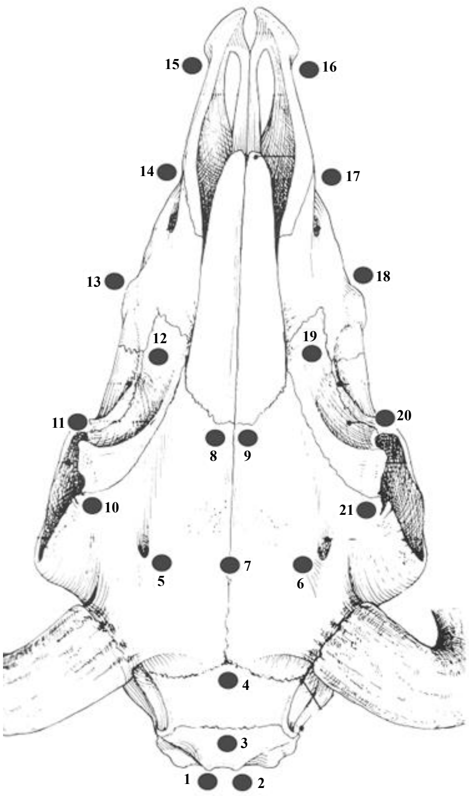

Figure 1: Position on landmarks used for the study of asymmetries in sheep skull (dorsal aspect). In total, 21 two-dimensional landmarks were used on the dorsal side of skull. Sixteen of them were bilateral and five (3, 4, 7, 8 and 9) were midline landmarks. All these landmarks were considered to encompass elements of both viceroy cranium and splanchno cranium.

plane so that the focal axis of the camera was parallel to the horizontal plane of reference and centred on the skull dorsal aspect. A scale was put over each specimen. Twenty-one landmarks were used, of which 18 were bilateral. The software TPSUtil v. 1.50 [8] was used to prepare and organize the images. Landmarks were digitized twice, using TPSDig v. 2.16 [9], by the same author on two different days, in the same order, to assess measurement error. In order to compare Procrustes to tangent space distances between individuals, a Generalized Procrustes Analysis superimposition (equivalent to generalized least squares) procedure was performed on each data set using TPSSmall v. 1.29 [10]. The high degree (r=0.999) of approximation of shapes in the sample (i.e. shape space) in relation to the reference shape (i.e. tangent space) allowed accurate capture of the nature and extent of shape deformations in subsequent statistical analyses.

Shape asymmetry

Shape asymmetry of skulls was studied by superimposing the configurations of landmarks from each side of the skull using a Procrustes superimposition [11]. First, landmark configurations of the left sides of the skulls were reflected to their mirror images by subtracting the x-values from a constant to align corresponding landmarks of right and left sides. The centroid size (CS) is a measurement of the dispersion of landmarks around their centroid, and was computed as the square root of the sum of squared distances of all landmarks from the centroid. After configurations were scaled to unit centroid size, configurations were rotated around their centroid (the point with average coordinates). Finally, asymmetry was measured as the deviations between the pairs of the corresponding superimposed landmarks.

Intra-observer error

To establish the degree of error in the acquisition of this landmark series, we repeated the results twice on different days for all specimens. We tested measurement error to observe whether our asymmetry estimates were significantly larger than predicted due to error alone.

Statistical analysis

We used a one-factor mixed-model ANOVA. Degrees of freedom for the shape ANOVA were the degrees of freedom for each of the effects multiplied by the number of landmark coordinates minus four. To compare distances to sagittal line, a paired test was applied. In order to linearize data a previous log transformation for all distances was applied (Bookstein, 1991).

All analyses were performed using MorphoJ version 1.05 [6] and PAST software [7]. “Nomina Anatomica Veterinaria” (2005) was used as guide book in the spelling of anatomic terms in this research.

Results

DA of shape was significantly larger than the variance expected due to measurement error ($p<0.0001$; (Table 2) and was statistically significant. First two PCS explained 60.0% of the total variance observed (PC1 + PC2 = 48.6% + 11.4%) (Table 3). On PC1, landmarks located both on neurocranium (1, 2, 5, 6, 9, 11 and 20) and on splanchnocranium (13, 14, 15, 16, 17 and 18) presented the highest contribution to the explanation of the asymmetry observed (Table 4). Most discriminant landmarks on PC2 were 8, 9, 11, 14, 17 and 20 (Table 4). These most discriminant landmarks on PC1 presented a clear lateral displacement, mainly toward left (except for landmarks

Effect	Sums of Squares	Mean Square	Degrees of freedom	F	*P*
Individual	0.26883304	0.0003627976	741	4.77	<0.0001
DA	0.01717238	0.0009038095	19	11.89	<0.0001
Error	0.06976597	0.0000458987	1520		

Table 2: ANOVA results, Directional Asymmetry (DA) of shape was significantly larger than the variance expected due to measurement error, being statistically significant.

PC	Eigenvalues	% of variance	Cumulative variance %
1	0.000351	48.670	48.670
2	8.24E-05	11.411	60.081
3	5.75E-05	7.961	68.043
4	4.86E-05	6.735	74.777
5	4.03E-05	5.582	80.359
6	2.83E-05	3.915	84.274
7	2.55E-05	3.533	87.807
8	1.80E-05	2.497	90.304
9	1.60E-05	2.219	92.523
10	1.33E-05	1.846	94.369
11	9.61E-06	1.332	95.701
12	8.04E-06	1.114	96.815
13	6.82E-06	0.945	97.759
14	5.39E-06	0.747	98.507
15	3.87E-06	0.537	99.043
16	3.10E-06	0.429	99.472
17	2.28E-06	0.316	99.788
18	8.20E-07	0.113	99.901
19	7.10E-07	0.099	100

Table 3: Variance explained for each Principal Component (PC). First two PCs explained 60.0% of the total variance observed (PC1+PC2=48.6%+11.4%).

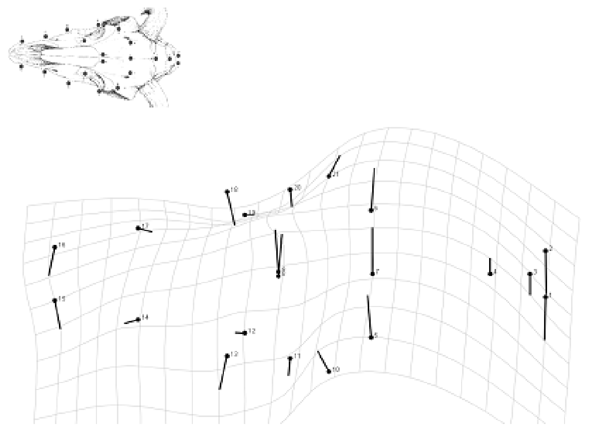

Figure 2: Distortion grids a leftward shift of cranial bones near the rostral and caudal vertexes, and a rightward shift in the frontal area were observed. Muscle insertions related to masticatory function are attached to the anatomical points: 14 to 17, for buccinator muscle (*buccinator, pars buccalis*), 13 and 18, for masseter (*masseter, pars superficialis*), and 1 and 2, for parieto-scutular (*parietoscutularis*) and cervico-scutular (*cerivocoscutularis*).

5, 6, 7 and 8, located on the most rostral part of the neurocranium). A leftward shift of cranial bones near the rostral and caudal vertexes and a rightward shift in the frontal area could be observed (Figure 2). Paired test showed bigger distances from lateral points to sagittal line on right side (t=-2.44, p=0.02), which moreover showed less average variance (51.5% and 48.0% for left and right side points, respectively). These deviations appear not to be a factor diminishing individual life expectations, as a wide age spectrum (assessed by occlusal molar wearing (data not presented here) was collected.

	PC1	PC2
x1	-0.00606	0.030952
y1	-0.30564	0.092829
x2	0.00606	-0.03095
y2	-0.30564	0.092829
x3	0	0
y3	-0.14672	-0.0367
x4	0	0
y4	0.10976	-0.09779
x5	-0.02567	-0.06083
y5	0.30004	0.01476
x6	0.02566	0.06083
y6	0.30004	0.01476
x7	0	0
y7	0.32797	0.04586
x8	0.02413	-0.51440
y8	0.29747	-0.07565
x9	-0.02413	0.51439
y9	0.29747	-0.07565
x10	-0.07708	-0.16910
y10	0.14451	0.09287
x11	-0.01077	0.28815
y11	-0.11914	-0.17008
x12	-0.06394	0.09727
y12	0.00173	-0.00302
x13	-0.05355	0.00895
y13	-0.23706	-0.01822
x14	-0.09229	-0.20641
y14	-0.02492	0.07572
x15	0.04212	0.03217
y15	-0.20252	0.03509
x16	-0.04213	-0.03218
y16	-0.20252	0.03509
x17	0.09228	0.20641
y17	-0.02492	0.07572
x18	0.05355	-0.00895
y18	-0.23706	-0.01822
x19	0.06393	-0.09728
y19	0.00173	-0.00302
x20	0.01076	-0.28816
y20	-0.11914	-0.17008
x21	0.07708	0.16910
y21	0.14451	0.09287

Table 4: Loadings for Principal Components (PC) 1 and 2 (PC1+PC2=48.6%+11.4%) for each landmark. Highest absolute loadings (>[0.2]) appear in bold. Most discriminant landmarks on PC1 were 1, 2, 5, 6, 8, 9, 13, 15, 16 and 18. Most discriminant landmarks on PC2 were 8, 9, 11, 14, 17 and 20.

Discussion

If the expression of bilateralism is determined by the same genome, then the asymmetry between the sides must be a consequence of modifications in the normal development programme, which may have genetic and/or environmental causes [12]. Now consider mechanical forces as a possible cause of development modification. The dominance of one side might be determined by a right or leftsidedness in chewing. In bone, some studies have found that the trabecular architecture maintains its shape but adapts according to mechanical stimuli [13-15], and in skull it has been stated that craniofacial morphology responds to changes in mechanical stimuli [16], so the morphology of the skull, or at least part of it, could change according to variations in mechanical stimuli during mastication to compensate for mechanical imbalances. This phenomenon has been described in mandibles, and many authors [17] report that the morphology of the mandible is affected by the masticatory function [18], in particular, report that patients with developmental mandibular asymmetry had more asymmetrical activity in the masseter. In humans, acquired asymmetries have been described because of chewing side preference [19,20], and, as previously mentioned, asymmetries have been described in horse [21], pig [4] and sheep [5,22]. Therefore, an oriented asymmetry of the ovine skull could be determined by a greater use of one side than the other, a lateralization due to the direction of jaw movement during rumination, and thus greater mechanical forces on one side than the other. Evidently, mechanical forces of different power during mastication would affect the morphology and internal structure of the bony structure differently, at least at those parts where masticatory muscles are attached, as the processes of bone formation and resorption are influenced by the mechanical environment, with bone morphology regulated to maintain strength. In our study, the right skull side is more uniform and bigger than the left one, probably because it is where the chewing function is more pronounced.

This seems plausible for the data we obtained, as most of the muscle insertions related to masticatory function are attached to the most variable anatomical points detected: 14 to 17, for buccinator muscle (*buccinator, pars buccalis*), 13 and 18, for masseter (*masseter, pars superficialis*), and 1 and 2, for parieto-scutular (*parietoscutularis*) and cervico-scutular (*cerivocoscutularis*), the latter probably as compensation for the lateralized mastication.

And why does this lateralization occur? Could it compensate for hemispheric laterality? It occurs in humans, where facial directionalities have been linked to compensatory adjustments for right hemispheric dominance [23-25]. A variety of left-right asymmetries in the behaviour of other vertebrates, including sheep, have also been discovered in recent years, which seem to reflect asymmetries in brain function [25]. The assumption that asymmetry in different mechanical lateral forces is due to asymmetries in brain function, and that the former result in clear anatomical skull reactions, would thus be logical [26,27].

However, the study of brain lateralization, and also of mechanical stimuli (such as grinding teeth and use of salt bite blocks) and ingesta-specific properties (such as abrasiveness), should be investigated in future research, as should those referring to how domestication could have influenced this response.

Conclusions

Directional Asymmetry of shape in a sampling of 40 sheep skulls appeared statistically significant using geometric morphometric methods. Landmarks located both on neurocranium and on splanchnocranium presented the highest contribution to the explanation of the asymmetry observed. It is suggested that this lateralization is due to the direction of jaw movement during rumination, and thus greater mechanical forces on one side than the other.

The results of this study raise future questions about the influence of skull biomechanics on its asymmetrical development, but also about how management, ingesta-specific properties (such as abrasiveness) and domestication can influence this response.

Sources of Funding

None to declare.

Conflict of Interests

None to declare.

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