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RESEARCH ARTICLE

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No bilateral variation accompanies the skull size change in domestic pig

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ABSTRACT

Morphological symmetry and asymmetry in a sample of 41 domestic pig skulls was decomposed into a component of symmetric and asymmetric variation using geometric morphometric methods. The left–right symmetric variation was similar to the asymmetric variation, and both mainly centred on the caudal part of skull.

Key words: allometry, developmental instability, directional asymmetry, fluctuant asymmetry, heterochrony

INTRODUCTION

In biology, deviations of body plans from expected perfect symmetry normally occur, and organisms can develop several kinds of asymmetry. In geometry, symmetry is defined as invariance of an object to a particular transformation that can be applied to it, such that the object is the same before and after transformation. The object remains unchanged after the transformation precisely if it is symmetric with respect to that transformation. Because bilateral symmetry is the most widespread and simplest type of symmetry, it has been the most studied in various contexts. The symmetry and asymmetry of shape have been studied with the methods of geometric morphometrics (i.e. of the Procrustes tangent coordinates) in that there is a morphometric framework for decomposing symmetry and asymmetric components of morphological variation [1]. The great advantage is that the use of geometric morphometrics plus multivariate statistical techniques provides a graphic visualisation of the morphological variation.

These methods were used in our study. We used the entire organism (the entire skull) in order to consider the variation in form holistically. Theoretically, the skull is bilaterally symmetric as a whole because it is invariant to reflection about its sagittal plane. This reflection brings the left side of the face onto the right side and vice versa, but because the two sides are mirror images of each other, the skull as a whole is left unchanged.

In this study, authors have studied bilateral asymmetries on the skulls of domestic pig (on their ventral aspect) in order to assess the magnitude of this symmetry, evaluated both as symmetric and asymmetric components, according to size. The first one is the average of left and right sides and represents the shape variation component, whereas the asymmetric component represents the individual left-right difference [2].

MATERIALS AND METHODS

A sample of 41 dentulous dry complete skulls from domestic pig, chosen at random from a vulture feeding point in Catalonia (northeast Spain), was studied. The sex of these samples was unknown. Complete, undamaged, and clearly non-pathological skulls (as assessed on the basis of macroscopic examination) were chosen. Only skulls with at least the M¹ erupted were studied.

Data acquisition

Image capture was performed with a Nikon® D70 digital camera (image resolution of $2,240 \times 1,488$ pixels) equipped with a Nikon AF Nikkor® 28–200 mm telephoto lens. The focal axis of the camera was parallel to the horizontal plane of the skull and centred on its ventral aspect. Images always included a scale (interval 50 mm). The data consist of 12 pairs of bilateral landmarks and four midline landmarks recorded in two dimensions. In addition to being highly repeatable, these landmarks encompass elements of the entire cranium. Specimens were positioned so that all of the landmarks could be digitised without repositioning them during measurement.

Sixteen landmarks were plotted in order to describe the size and shape variations, producing a set of 32 raw coordinates for each specimen. Twelve of these landmarks were topologically equivalent, being four landmarks on the mid-sagittal plain. Figure 1 shows the location of the anatomical landmarks. Data were analysed using geometric morphometric methods [3, 4].

Shape analysis

All images were saved in JPEG format and stored on a personal computer. The software TpsUtility v. 1.50[5] was used to prepare and organise the images. Measurements were directly recorded as x- and y-coordinates using TpsDig v. 2.04 software[6]. To determine measurement error, each individual was measured two times by the same observer (Esteve-Puig) on separate days. TpsSmall v. 1.29 software [7] was used to assess the correlation between Procrustes and the Kendall tangent space distances to ensure that the amount of shape variation in a data set was small enough to allow subsequent statistical analyses. As the correlation between Procrustes and the Kendall shape spaces was very high ($r=0.999$, root MS error: 0.00002), we proceeded with the subsequent morphometric analyses.

The raw coordinates matrix was then transformed with the generalised Procrustes analysis. The algorithm consists of a series of iterative superimpositions of homologous landmarks to adjust them by scale, translation and rotation. Then, the resulting variables are shape variables, which can be analysed with multivariate statistical methods. A NPMANOVA (nonparametric multivariate analysis of variance) with 9,999 permutation rounds was used to assess differences between replicas, which did not appear to be significant ($F=0.909$, $p=0.909$). The R coefficient of the Mantel test with 5,000 permutation rounds in two sets showed also a high correlation between replicas ($R=0.894$, $p=0.0002$). To study skull size variation, we used an isometric estimator, the centroid-size (CS), which was calculated as the square root of the sum of the squared distances between the centre of the configuration of landmarks and each landmark [8]. It was obtained with CoordGen6f (H. D. Sheets, www.canisius.edu/sheets), and their log transformed values were used as a measurement independent of shape [3].

Static allometry denotes size-related shape changes measured in different individuals at the same developmental stage within a population or species [9]. To assess the effect of size on shape, we explored the size-related shape changes by regressing the shape variables on log CS.

Shape variation was analysed in the averaged dataset with principal component analysis (PCA) based on the covariance matrix of symmetric and asymmetric components of shape variation. Levene's test for homogeneity of variance (homoskedasticity), that is, whether variances were equal in PC1 for both symmetric and asymmetric components, was assumed by ANOVA. As it was significant, meaning that we had unequal variances, the unequal variance (Welch) test was used. Finally, regressions of shape (PC1 coordinates for each specimen) on size (log-transformed) were done both for symmetric and asymmetric components.

Statistical analyses were carried out using MorphoJ v. 1.06b [10] and PAST v. 3.01 [11].

RESULTS

The first two PCs for the symmetric component accounted for 77.41% ($PC1 + PC2 = 58.42\% + 18.99\%$) of the total shape variation, and provided a good approximation of the total amount of variation, with the other PC components that accounted for no more than 7% of the variation each. For the asymmetric component, the first three PCs accounted for 74.73% ($PC1 + PC2 + PC3 = 54.76\% + 10.24\% + 9.72\%$) of the total shape variation, with the other PC components accounting for no more than 7% of the variation each. The amounts of variation described by symmetric and asymmetric components did not differ between them ($F_{52,76} \ll 0.0001$, $p=1$). The permutation tests for homogeneity of multivariate dispersions based on symmetric and asymmetric PC axes resulted in insignificant differentiation of component dispersions. Most of the symmetric and asymmetric variation was contained in the caudal landmarks (7 to 12). The regression analysis of shape *versus* size showed a linear relationship between shape changes and increase in size ($R^2=0.154$, Wilk's $\lambda=0.020$, $F_{32,8}=11.990$, $p \ll 0.0001$), although for components, no significant regression appeared for the symmetric component *versus* size ($R^2=0.085$, Wilk's $\lambda=0.914$, $F_{1,39}=3.662$,

$p=0.063$) (Figure 2), nor for the asymmetric component ($R^2=0.069$, Wilk's $\lambda=0.930$, $F_{1,39}=2.933$, $p=0.094$) (Figure 3).

DISCUSSION

Directional asymmetry occurs throughout the animal kingdom and a left–right axis has now been demonstrated for most major phyla, including all the principal model organisms in developmental biology. The skull could seem symmetric in structure, the development of which involves several processes including the induction of the cranial neural crest and the specification, migration and differentiation of neural crest cells. Using the entire skull in accordance with the methodology proposed by Alibert *et al.* [12], which considers the variation in form holistically (and this approach allows a more realistic view of the variation in skull shape), it can be demonstrated that pig skull is not perfectly symmetric and small asymmetries can reflect phenotypic bilateral variations.

Over half of the symmetric component of shape variation is associated with the first two PCs, with important variation between them, indicating that there can be external pressure determining shape change, but not due to allometry. The non-prominent role of phenotypic plasticity may also explain the similarities in the patterns of covariation between the symmetric and asymmetric shape variation.

An explanation can be found in that most internal organs show consistent laterality, which is very stable within species [13]. The function of the brain has long been known to be asymmetric, and recent advances in soft tissue analyses and diagnostics have made it possible to show that highly asymmetric brain function correlates with anatomic and behavioural expression [14]. A genetic background for the phenomenon has been suggested, although until recent years no specific gene has been found to cause the lateralisation.

These results should be interpreted with caution, however, and need to be re-evaluated with a larger dataset. Here, the small sample size limits the statistical power, and the small number of landmarks -which in turn are conditioned by sample size- have limited the spatial information available for the analyses.

Figure 1. Designated landmarks for geometric morphometric analysis of the domestic pig skull (ventral aspect). Landmarks 1 to 4 were on the mid-sagittal plane. The rest of them were bilaterally equivalent

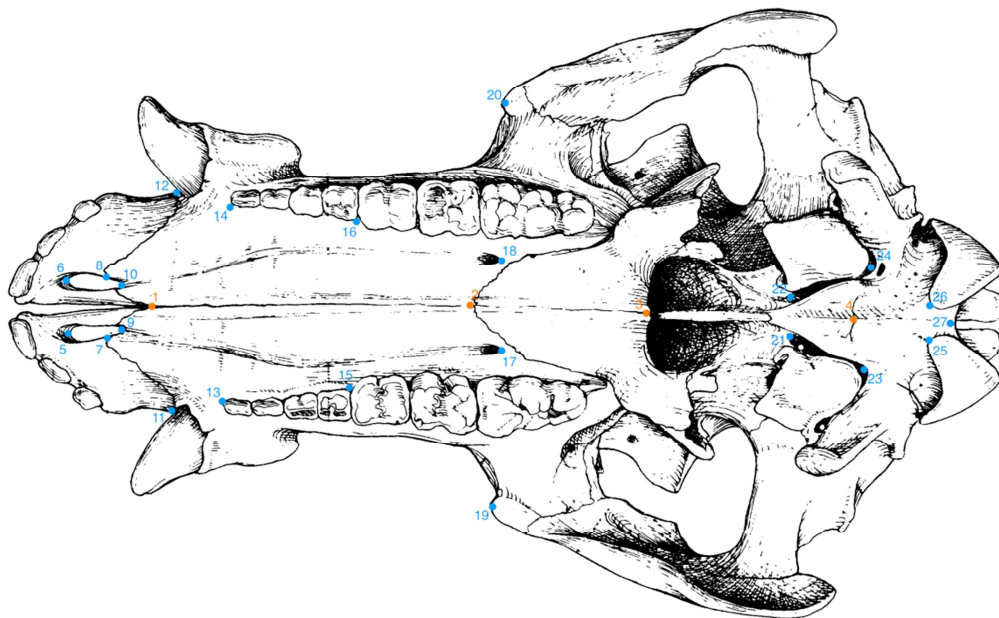


Figure 2. Regressions of shape (PC1 coordinates for each specimen) on size (log-transformed) for symmetric component. No significant regression appeared ($R^2=0.0858$, Wilk's $\lambda=0.914$, $F_{1,39}=3.662$, $p=0.063$)

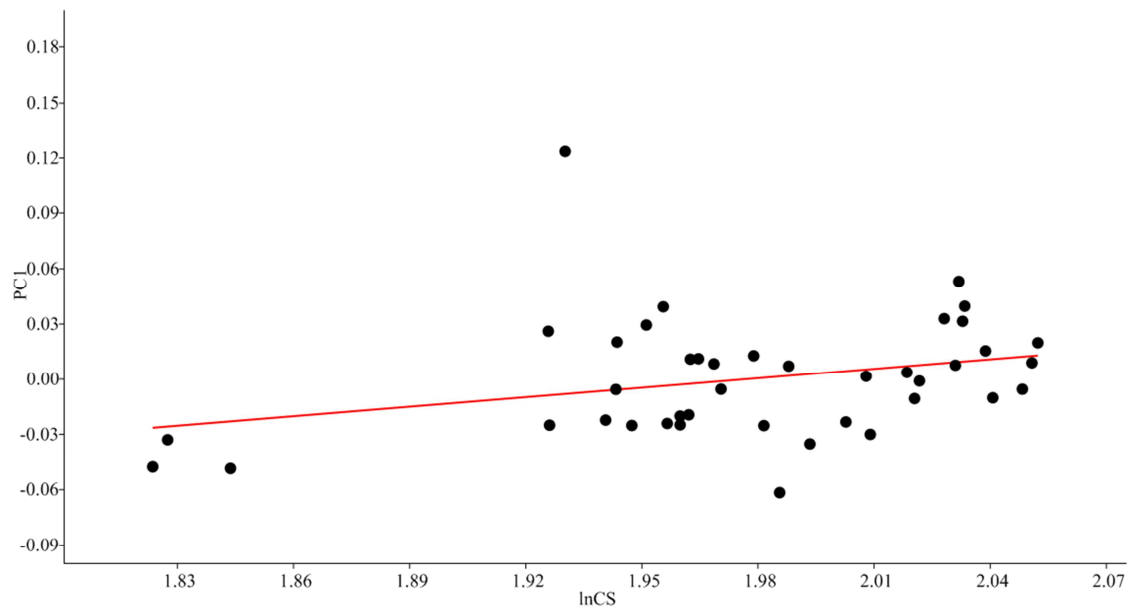
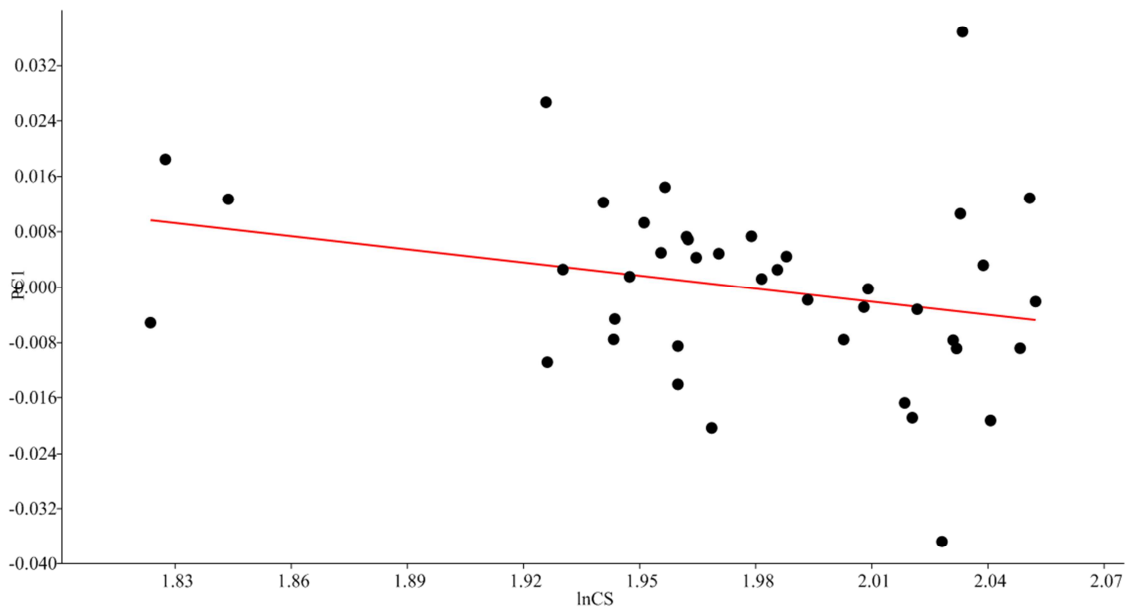


Figure 3. Regressions of shape (PC1 coordinates for each specimen) on size (log-transformed) for asymmetric component. No significant regression appeared ($R^2=0.069$, Wilk's $\lambda=0.930$, $F_{1,39}=2.933$, $p=0.094$)



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