

No modularity at ventral level in the horse skull

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Abstract

Morphological integration and modularity are concepts that refer to the covariation level between the components of a structure. Morphological modules are independent subsets of highly correlated traits. The horse skull has been studied as a whole functional structure for decades, but the integrative approach towards quantitative examination of modules is scarce. We report here the first evaluation of cranial modularity in the horse at basal level. For this, we studied the modularity hypothesis for splanchnocranium and basicranium modules in the horse, two phenotypic regions under local influence by soft-tissue–hard-tissue interfaces. Using geometric morphometrics to capture the shape and location, we examined both modules in a sample of 23 dry skulls belonging to Pyrenean Horse Breed using 57 two-dimensional cranial landmarks. Modules were compared through partial least squares analyses and Escoufier (*RV*) coefficient. We tested whether the integration (measured by Escoufier *RV* coefficient) of splanchnocranium and basicranium strength modules and their covariation pattern (as analysed by partial least squares analysis) subordinate and express similar integration results. A clear modularity was observed. The lack of disproportions in the skulls of domestic horse breeds (compared to dog and cat breeds, for instance) might be an expression of the lack of single modules to evolve. On the other side, integration might have a positive impact on survival as long as the selection pressure is along the trajectory of integrated variation.

KEYWORDS

allometry, geometric morphometrics, morphological integration, 'Cavall Pirinenc Català'

1 | INTRODUCTION

Skull is integrated functionally as a whole, but its morphological integration is not uniform throughout. It is composed of multiple parts that are more or less distinct from each other on the basis of genetics, development or function (Curth et al., 2017). This coordination into subunits has long been known as morphological integration (Püschel, 2014). Integration and modularity concern the degree of covariation between parts of a structure (Klingenberg, 2009). Modules are developmentally distinct regions (Klingenberg, 2009). For example, some authors have suggested the rostrum and the braincase as two different modules of the dog cranium, as they are

units whose parts are strongly integrated internally but are weakly integrated between them (Drake & Klingenberg, 2010) (Curth et al., 2017), although the modules of the skull can never be fully independent from one another (Curth et al., 2017). On the other hand, skull modules can constrain or promote the potential of the skull to evolve into new shapes probably in the course of horse evolution in general.

The skull base represents a central and complex bone structure of the skull and forms the floor of the cranial cavity on which the brain lies (Barone, 1999). The skull base undergoes an elaborate sequence of development stages and represents a key player in

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skull development. Geometric morphometrics (GM) offers the possibility to study morphological integration and modularity (Curth et al., 2017). GM can determine whether a structure is a single integrated unit or consists of several distinct modules and evaluate hypotheses about their boundaries. The goal of this study was to assess whether basal splanchnocranium and basicranium, two spatially contiguous skull parts under local influence by soft-tissue–hard-tissue interfaces, conform to a single integrated unit or consist of two distinct modules, by means of GM techniques. A modular structure like this could allow those single modules to vary more independently without any negative consequences for other parts of the skull (Curth et al., 2017).

This exploratory study used GM to assess if two complex bony structures of the equine ventral skull—splanchnocranium and basicranium—grow homogeneously or at different rates. Our hypothesis was that there would be an ontogenic difference between those bones, with palate having more skull variability than the sphenoid, as this latter contains important neurocranial structures, such as cranial nerves.

2 | MATERIALS AND METHODS

2.1 | Sample

A sample of 23 complete dry skulls belonging to 'Cavall Pirinenc Català' (Pyrenean Horse Breed) were sampled from the osteologic collection held in the Department of Animal Science at the University of Lleida. Animals had at least eruption of their first upper molar. We selected only specimens from different localities (both males and females) with at least M¹ fully erupted dentition. The heads were aged by clinical molar examination using standard guidelines.

2.2 | Imaging

Image captures were performed with a Nikon® D70 digital camera (image resolution of 2,240 × 1,488 pixels) equipped with a Nikon DX® 18–105 mm telephoto lens and JPG file format was used. The camera was levelled horizontally. In order to reduce distortion artefacts due to parallax, the specimens were positioned at the centre of the field of view, and the horizontal position of skulls was checked visually prior to the taking of the photographs. The frontal bones touched the horizontal supporting surface. Scale was given for each photograph by placing a 10 mm scaled ruler.

2.3 | Geometric morphometrics

Pictures were transported to TPSUtil v. 1.70 (Rohlf, 2015). The digitation process was followed utilizing TPSDig2 v. 1.40 (Rohlf, 2015). On each skull photograph, we identified and digitized a total of 57 points (subset of 3 mid-sagittal, 7 paired landmarks (discrete homologous points), a dense set of semilandmarks points

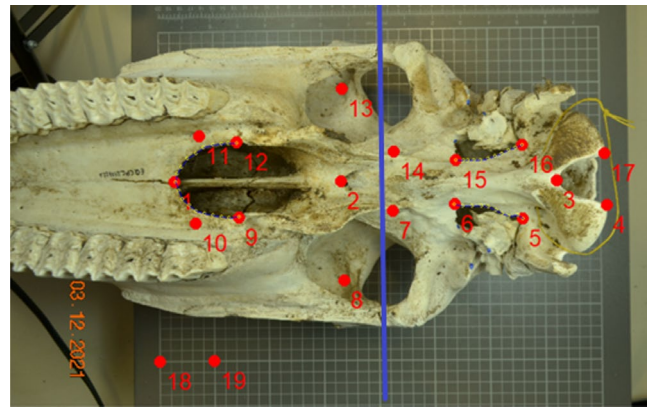


FIGURE 1 Landmark configuration used in the study, composed of a total of 57 points (subset of 3 sagittal and 7 paired landmarks (discrete homologous points) and 10 semilandmarks (points on an outline determined by extrinsic criteria) per side on the choanae and on the *foramen lacerum*). Inference about the boundaries of modules from the patterns of covariation was made by partitioning the traits into two subsets (divided by the blue line). Landmarks 18 to 19 signal the ruler (50 mm). Ventral view

on an outline determined by extrinsic criteria), 10 per side on the choanae and 10 semilandmarks per side on the *foramen lacerum* (Figure 1). The semilandmarks were important for quantifying shape in those areas that lack clear definable points. Digitalization was bi-replicated to reduce the measurement error. The semilandmarks were anteriorly slid using bending energy with TPSUtil v. 1.70 (Rohlf, 2015). A generalized full Procrustes fit was performed on two-dimensional landmark coordinates to extract shape information. Size was computed as centroid size (CS), 'the square root of the sum of squared distances from each landmark to the specimen's centroid' (Adams et al., 2013). A consensus (mean) configuration was obtained. No information contained in the original landmark configurations is lost at this step except the one about CS, which can be analysed independently, and translation and rotation, which have no biological mean.

The hypotheses of landmark partitioning of the basal skull in two modules—the splanchnocranium and the basicranium—were considered. These two modules will be handled as a 'black box', since no functional nor developmental or genetic factors, but merely an anatomical differentiation, have been considered.

2.4 | Allometry

The effect of allometry was verified using the multivariate regression of shape (Procrustes coordinates) on size (\log_{10} -transformed CS), which was treated here as a proxy for general size.

2.5 | Study of modules

Inferences about the boundaries of modules from the patterns of covariation were made by partitioning the symmetric components into

two subsets (splanchnocranium and basicranium) and comparing the degree of covariation between them (250 rounds) (Figure 1). Two-block partial least squares analysis of regression residuals allowed to explore patterns of covariation between two sets of variables (Rohlf & Corti, 2000). Figure 2 shows hypothesized partitions. For both data sets, the landmark configurations from both sides were included in a generalized Procrustes fit (with appropriate reflections). The averages of the configurations of each individual were used to compute the among-individual covariance matrices.

The Escoufier (*RV*) coefficient was calculated as a measure of integration strength (Klingenberg, 2009). It represents a multivariate generalization of the squared Pearson correlation coefficient (Adams, 2016). Hypotheses concerning the boundaries of cranial modules were tested by comparing these *RV* coefficients. The proportion of partitions for which the *RV* coefficient is less than or equal to the *RV* value for the partition of interest was interpreted as the analogue of a *p*-value.

Because the strength of covariation between different regions of a structure is the criterion for assessing integration and modularity in morphometric data, a measure for quantifying covariation between sets of landmarks is of critical importance. *RV* coefficient is a scalar measure of the strength of association between the coordinates of two sets of landmarks and presents a new generalization of this measure for multiple sets of landmarks (Klingenberg, 2009). When the *RV* coefficient values are higher, the covariance of two blocks is stronger (Romaniuk, 2018). Boundaries between modules were evaluated by partitioning the configuration in different ways and comparing the *RV* coefficients between subsets of landmarks. If the division of the traits into subsets coincides with the boundary between modules, the covariation between the subsets results from the few or weak interactions between traits belonging to different modules and accordingly, the degree of correlation between the subsets will be relatively low (Romaniuk, 2018). Finally, a partial least squares analyses (PLS) was performed which ascertain the main trajectories of covariation between two sets of landmarks and order them according to the amount of total covariation they explain (Bookstein, 1991).

For all statistical analyses, we used MorphoJ software v. 1.07a (Klingenberg 2011), available on web site www.morphometrics.org, and PAST software v. 2.17c (Hammer et al., 2001), with $\alpha = 0.05$.

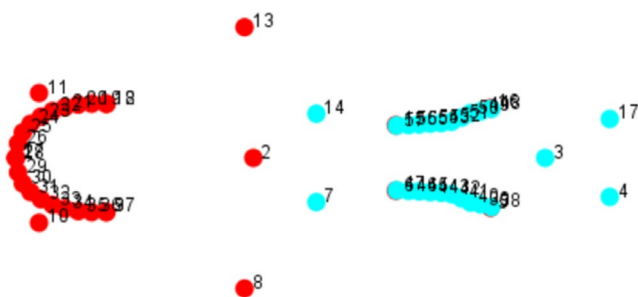


FIGURE 2 Modularity test results. The hypothesized partition: splanchnocranium and basicranium; different colour presents different modules

3 | RESULTS

Measurement error using Procrustes ANOVA showed that its effect on shape amounted to a mere 2.7%, clearly below fluctuating symmetry (9.7%), so being therefore negligible. The variation among individuals was higher (40.1%) than that induced by the digitizing procedure. These results suggest that the data are indicative of real biological differences.

The relationship between modules shape and size is quite clear. The multivariate regression of the Procrustes coordinates on log₁₀-transformed CS showed that allometry is statistically significant (10,000 random permutations). Log₁₀-transformed splanchnocranium CS accounted for 6.17% ($p = .0221$) of the total shape, and for basicranium, it accounted a 16.25% ($p < .0001$) of shape change explained by size. Since there was a significant allometric effect of size on shape, for PLS analysis we used the residual component of the regression of shape on CS, making it possible to compare shapes with minimum interference from differing size.

The analysis of symmetric regression scores between splanchnocranium and basicranium showed that the pairwise correlation of PLS scores between their symmetric components was statistically significant ($r = .611$; $p = .0195$). PLS analysis of symmetry component of covariation revealed a $RV = 0.180$ ($p = .0193$; 10,000 randomization rounds). Although the overall strength of association between blocks was weak, the correlation was high, and the hypothesis of no covariation was rejected, meaning that the specific shape in one module covaries with its specific shape in the other. PLS1 possessed 60.0% of total covariation score, indicating that it represented the main covariance of two blocks. Figure 3 presents plots distributed around the diagonal line of the PLS1 scores coordinates. The shape variance of basicranium appeared to be more conservative than splanchnocranium.

4 | DISCUSSION

The main objective of this research was to test whether splanchnocranium and basicranium are integrated or independent from each other, being the integration—the degree to which both are structurally and developmentally connected—to be significant.

It has been advocated that a high integration of modules leads to decreased diversity in the superordinate structure because non-integrated modules may vary more freely when variations have no negative impact on others, although some authors disagree (Curth et al., 2017). The observed integration of splanchnocranium and basicranium in the horse skull in this study would show a low skull plasticity and thus would hinder the formation of new shapes, at least in basal parts, so not allowing for ‘flexibility’. In fact, in horses, the morphological changes induced by the process of domestication are less pronounced than in other species, such as dogs or pigs (Heck et al., 2018). The lack of disproportions in the skulls of domestic

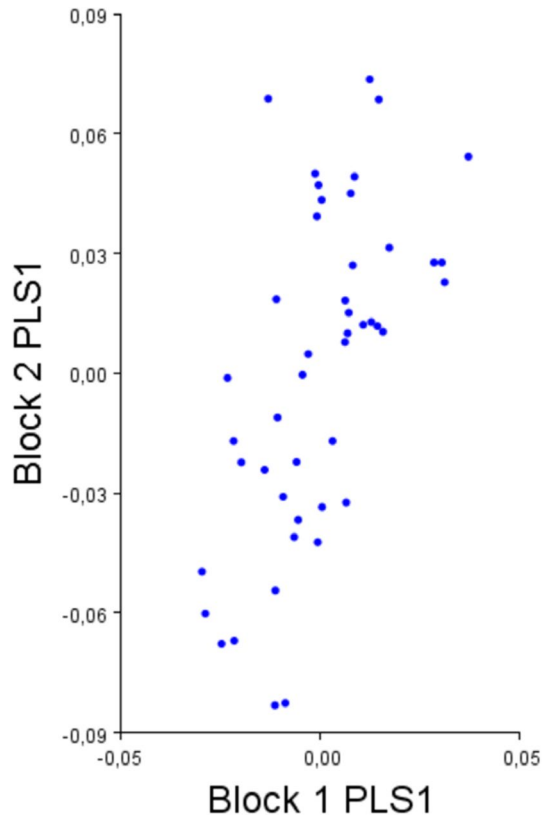


FIGURE 3 PLS analysis results: scatter plot of the PLS1 of two blocks (Block 1: basicranium; Block 2: splanchnocranium). The shape variance of basicranium is more conservative than splanchnocranium

horse breeds (compared to dog and cat breeds, for instance) might be this expression of independent modules to change.

CONFLICT OF INTEREST

All authors declare there are no potential competing interests.

DATA AVAILABILITY STATEMENT

The contents of all supporting data are the sole responsibility of the authors. The data sets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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