



## Shape, Not Only Size, Differentiate Wild And Domestic Ovis

### KEYWORDS

morphometrics; ovinae; "phyletic" allometry; skull allometry

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**ABSTRACT** *The skulls of 74 specimens of different species and domestic breeds of Ovis were analysed by means of geometric morphometric methods in a two-dimensional view of their lateral aspect. Specimens were grouped a priori into three groups: domestic, feral and wild. Wild specimens were not differentiated for subspecies. With respect to size, there only appeared to be differences between the domestic and wild specimens, and feral specimens showed no differences with either wild or domestic specimens. Shape differences were significant for all groups. No specific landmarks differentiated the groups, thus the general shape seems to differentiate the groups. As a result it is quite easy to distinguish the wild and domestic specimens, although the morphological differences are not limited to a concrete part of the skull. This fact could be related to different feeding strategies, but we believe that there are other possible interpretations related to the phenotypic features linked to the domestication process.*

### INTRODUCTION

The skeleton, more than any other phenotypic feature, provides phylogenetic links between vertebrates, revealing the course of their evolution. Because of the basic supportive and protective roles of the skeleton, we can understand in broad terms how different bone morphologies might be adaptive in different environments and for different life history traits and, hence, how natural selection might influence the evolution of these different morphologies.

The morphological and morphometrical studies of the skull reflect the contributions of genetic and environmental components to the individual's development and describe genetic and ecophenotypic variations (Wehausen and Ramey, 2000). Most of these studies have been done from a "classical" point of view that is by using linear measurements (Kairimi et al., 2001; Özcan et al., 2010). Linear measurements, capturing total lengths, widths and perimeters, are used as variables. In Geometric Morphometrics (GM), the role that shape variables take in an experimental design depends on the biological questions being investigated; one of the most powerful components of geometric morphometric analysis being the visualisation of shape variability. The measurement of shape has been classically derived from linear distance measurements, by sampling dimensions of length, height and width (Marcus, 1990).

The GM approach allowed us for the first time to describe some of the size of skull variabilities in *Ovis*, in order to study size and shape changes between the wild and domestic forms of this genre.

### MATERIAL AND METHODS

#### Samples

The skulls of 74 specimens of different species and domestic breeds of *Ovis* were analysed for the purpose of this study. The material is housed in different mammal collections. Specimens (Table 1) were grouped *a priori* into three groups: domestic, feral and wild. Wild species were not differentiated for subspecies. All were adult specimens and no distinction was made between male and female specimens, as the skulls included were chosen primarily for dental completeness and ontogenetic stage, although only one specimen did not include data on sex. Not distinguishing between sexes could, however, introduce a slight bias in some of the data, since previous studies have demonstrated sexual skull dimorphism in the *Ovis* genre, but no sexual bias should be suspected

since males and females can both be inferred to be represented in the data sample.

#### Data gathering

Images were taken by photographing each skull in direct lateral view. Fourteen landmarks were placed on the lateral aspect. Landmarks were chosen to provide an adequate coverage of the skull shape (Figure 1). A ruler (20 mm) was placed on each image. The second author was responsible for landmarking all specimens. The images were then digitised using TpsDig software version 2.16 (Rohlf, 2010) to obtain the x, y coordinates of the landmarks. To ensure that the localisation of the selected points was accurate, Fardasca specimens were replicated twice. The Mantel test, with 5,000 permutations, reflected  $R=0.904$ ,  $p=0$ . So the error in digitising landmarks was considered negligible. To test whether shape variation is small enough to permit the use of approximations in tangent space, a correlation between specimen distances in tangent space and Procrustes space was performed in tpsSmall (Rohlf, 2003). The correlation was very high ( $r>0.996$ ), indicating that no significant distortion was introduced by the tangent space approximations.

#### Morphometrics: size

To quantify the size of a specimen, centroid size (CS) was computed from the raw coordinates of the landmarks (Dryden and Mardia, 1998) using the CoordGen6f software (Sheets, 2003). CoordGen6f was also used to translate and rotate the images. CS is a measure of geometric scale, calculated as the square root of the summed squared distances of each landmark from the centroid of the landmark configuration. In the present study, CS was not correlated to skull length (measured as the distance between landmarks 1 and 10) of the specimens ( $r^2=0.011$ ,  $p=0.363$ ).

#### Shape

Body shape was analysed using landmark-based geometric morphometric methods (Rohlf, 1990; Bookstein, 1991). Once the specimens were aligned, the mean configuration of the landmarks was computed, and the specimens were projected to a linear shape tangent space. The mean configuration is usually called the consensus or reference shape because it is the configuration of landmarks that corresponds to the point of tangency between the exact curved shape space and the approximating tangent space in which the linear multivariate statistical analyses are performed (Rohlf and Slice, 1990; Rohlf, 1999; Slice, 2001) (Figure 2). Thin-plate spline defor-

mations were used to study shape deformations as a function of position along a discriminant axis.

Allometries, meaning size-shape covariances, are at the heart of morphological change, and lend prominently to its complexity. Skeletal allometries accompany both evolutionary change ("phyletic" allometries) and developmental change ("developmental" or "growth" allometries). The change in proportions related to variations in size can be termed "phyletic" allometry, but it has not been studied in sheep. Thus a regression of size on the size was needed to characterise the allometry. The amount of shape variation for which each regression accounted was expressed as a percentage of the total variation around the sample's mean. To remove non-shape variations a permutation test using 1,000 runs was used to test the null hypothesis of independence between shape and size. Analysis was done with tps Repr version 1.36 software (Rohlf, 2009).

### Statistical analysis

A Canonical Variates Analysis (CVA) was initially performed. The squared Mahalanobis distance (the Mahalanobis distance is calculated from the pooled within-group covariance matrix, giving a linear discriminant classifier). In addition, group assignment was cross-validated by a leave-one-out cross-validation (jackknifing) procedure. A confusion matrix which assigned each specimen to the different groups by the classifier was ultimately performed. The NPMANOVA was used to test differences between sizes (according to log CS). Statistical analyses were done with the PAST package (Hammer et al., 2001) and MorphoJ (Klingenberg, 2011).

### RESULTS

According to size there only appeared to be a difference between domestic and wild specimens ( $p=0.024$ ), and feral specimens showed no differences with either the wild or domestic specimens ( $p<0.05$ ). The regressions of shape on size were not significant (Wilks'  $\lambda=0.190$ ,  $F_{28,45}=6.837$ ,  $p<<<0.0001$ ) (Figure 3). Only a 1.354% of shape is explained by size, so no allometry could be suspected. Differences between groups based on procrustes distances were significant for all groups (Wilks'  $\lambda=0.062$ ,  $F_{56,88}=4.732$ ,  $p<<<0.0001$ ). The average procrustes distance between the domestic and the feral groups was 24.7, whereas the procrustes distance between the domestic and wild group was 40.4. Discriminant function analysis based on the shape variables only revealed two misclassifications (2.7%) among individuals. The plot of Axis1 against Axis2 produced a good separation of groups (Figure 4). In an alternative, more stringent jackknifing classification test, fourteen specimens were misclassified (this test resulted in 19.0% of misclassifications between the three groups), the confusion matrix indicated that all misclassified specimens belonged to the domestic group. No specific

landmarks differentiated the groups, thus the general shape seems to differentiate them (Table 2).

Procrustes distances, giving single measures of the shape disparities, support the interpretation that the shape deformations from consensus between domestic and feral groups are less prominent than the deformation between the wild group and domestic and feral specimens. Domestic specimens present a CV of 79.4% along the axis 1, whereas the wild and feral specimens present 20.8 and 40.1% respectively. This fact could be related to different feeding strategies, but as they are not clearly related to areas in which masticatory muscles are attached, we believe that there are other possible interpretations related to phenotypic features, linked to the domestication process. In fact, some morphologic changes occurred in domesticated and wild sheep's bones during the Neolithic (Ryder, 1981; Zohary, 1998; Marinis and Asprea, 2006). In comparison to wild forms, domestic animals finally presented different morphological features due to migration, changes in climate, differences in nutrition, and artificial selection (Yağcı et al., 2010). The dispersion of domestic shape specimens would corroborate this. So we believe that the study of skull shape, not only size, can be extremely useful in understanding in biological terms how animals are responding to human selection. In this study the morphologic differences are defined at both the neurocranium and splanchnocranium level, and both for size and shape.

The researcher should check in practice the real statistical importance of the geometric variables as well as check experimentally possible errors in the technique, before attempting to try a more detailed and more interesting biological interpretation of shape differences. Evidently, our results could be influenced by the small sample used, and perhaps by changes occurring in the skeletal morphology of wild animals when kept in captivity (some of the specimens were provided from zoo specimens), so a larger sampling and a review of samples of well-known origin would be desirable for further study. A sliding semilandmarks (non-discrete anatomical loci that represent a homologous curve) approach to outline the analysis of profiles would be another interesting approach for this kind of study, in order to examine the profile differences between groups.

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**Table 1. Ovis species, subspecies and breeds studied.**

Species	Breed or subspecies	Fe-males	Males	Unknown Sex	Area of origin	Assigned Group	Collection of precedence
Domestic sheep ( <i>Ovis aries</i> )	Fardasca	19	2	0	NE Spain	Domestic	1
	German breeds	1	15	0	Germany	Domestic	3
	Karaguniko	4	1	1	Greece	Domestic	2
	Kephalonia	0	1	0	Greece	Domestic	2
	Khios	1	1	0	Greece	Domestic	2
	Friesland	1	2	0	Britain	Domestic	2
	Ile de France	1	1	0	France	Domestic	2
	Hebridean	0	7	0	Scotland	Feral	3
	Croatian	0	1	0	Croatia	Domestic	3

	Mongol	0	1	0	Mongolia	Domestic	3
	Persian	0	1	0	Somalia	Domestic	3
Argali ( <i>Ovis ammon</i> )		1	2	0	Central Asia	Wild	3
Bighorn ( <i>Ovis canadensis</i> )		0	1	0	N America	Wild	3
Dall sheep ( <i>Ovis dalli</i> )		0	1	0	N America	Wild	3
Mouflon ( <i>Ovis aries</i> )	Orientalis	2	2	0	Caucasus, N Iraq and NW Iran	Feral	3
Urial ( <i>Ovis orientalis</i> )	Vignei	2	1	0	W central Asia	Wild	3

- 1: Private collection (Sabaté Family of Rasquera)
- 2: Faculty of Animal Science and Aquaculture, Agricultural University of Athens
- 3: "Sektion Mammalogie und Osteologie Staatliches Museum für Naturkunde Stuttgart"

Figure 1. Lateral aspect of the *Ovis* skull in the established position for obtaining the shape from the 14 landmarks used.

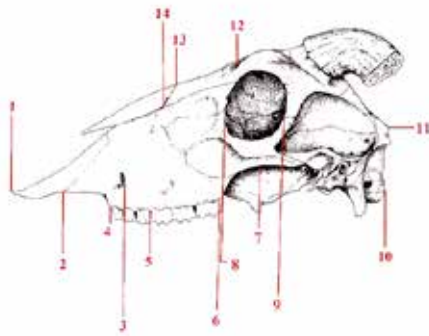


Figure 2. Consensus or reference shape.

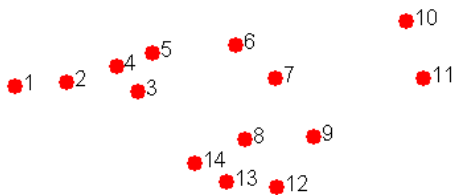


Figure 3. Relationship between log centroid size and size of all the specimens in this study, represented with shape scores as a function of log CS. Filled circles indicate specimens. Generalised Goodall F-test:  $F_{24, 1728} = 3.1297$ ,  $p = 0.0000$ . Only 1.354% of shape is explained by size.

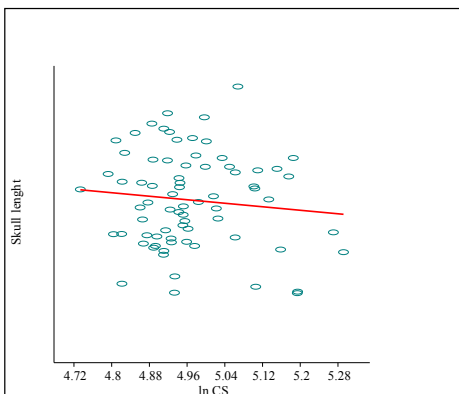


Figure 4. Discriminant function analysis of size distinguishes wild (crosses), feral (filled rectangles) and domestic (open

squares) *Ovis* specimens. The jackknifing classification test resulted in 19.0% misclassification between the three groups. The analyses are based on procrustes distances. The plot shows the convex hulls that include all the specimens for each group. Wilks'  $\lambda = 0.062$ ,  $F_{56, 88} = 4.732$ ,  $p < < < 0.0001$ .

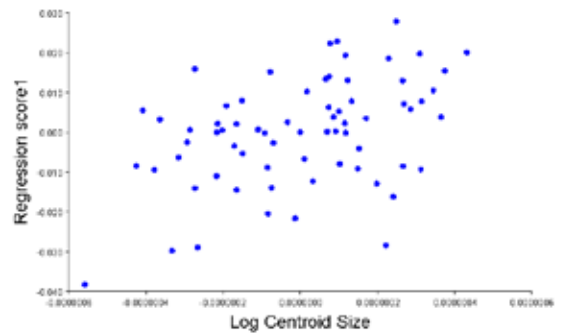


Table 2. Loadings of procrustes distances for PC1 and PC2. Highest values appear in bold.

	Axis 1	Axis 2
X1	0.079	-0.148
Y1	-0.202	-0.327
X2	0.024	-0.005
Y2	-0.137	-0.117
X3	-0.052	-0.054
Y3	-0.080	0.002
X4	0.003	-0.040
Y4	-0.085	-0.018
X5	0.103	-0.207
Y5	0.000	0.090
X6	0.279	-0.172
Y6	-0.151	0.206
X7	-0.246	0.017
Y7	0.153	0.085
X8	-0.280	0.082
Y8	0.226	0.106
X9	-0.399	0.101
Y9	0.153	0.266
X10	0.293	0.166
Y10	0.116	-0.430
X11	0.357	-0.079

Y11	-0.185	-0.447
X12	-0.343	0.258
Y12	0.037	0.173
X13	0.121	0.140
Y13	0.060	0.206
X14	0.060	-0.060
Y14	0.095	0.206

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