

This study estimated the magnitude of sexual dimorphism in a relict sheep from Catalunya (NE Spain), called *Fardasca*, using geometric morphometric techniques. For these analyses, a total of 18 skull specimens (2 adult males and 16 females) were studied. Our results suggest that the breed is not cranially dimorphic, so sex determination using landmarks described here as criteria is likely to yield poor results.

KEY WORDS craniometric points, *Fardasca* breed, sexual dimorphism, shape, size.

### INTRODUCTION

Comparison of anatomical characters between organisms has been a core element in comparative biology for centuries. In the early twentieth century, comparative biology entered a transition from the description field and quantitative science, where morphological analysis had a similar revolution of quantification (Bookstein, 1998). Based on this quantitative mathematical revolution, the study of morphology has had an important role in developing statistical shape analysis. This made possible the combination of multivariate statistical methods and new ways to visualise a structure (Adams and Funk, 1997; Dryden and Mardia, 1998).

In geometric morphometrics (GM), the shape is defined as "any geometric information that remains when the effects of translation, scaling and rotation are removed from an object" (Kendall, 1977). Landmark GM is currently the most widely used tool in sexual dimorphism studies, where equivalent and homologous specific points are fixed in the biological structure being studied. These tools allow the study of an organism's shape and size, providing sound graphic analyses to quantify and visualise morphometric variation within and between organism samples. One of the most interesting sources of phenotypic variation in animals and plants has been sexual dimorphism. Sexual differences in morphological characters are a common phenomenon in many animal taxa, and their most conspicuous aspect is body size (Gannon and Rácz, 2006).

Sexual dimorphism is of interest in zooethnological studies since the differences between sexes are frequently not obvious or the individuals are very small; thus, finding discriminating characters allows easy determination of sexes. Sexual size dimorphism (SSD) is a widespread phenomenon in different animal taxa, including the subfamily of goats and sheep (Caprinae), which belong to the most dimorphic mammalian groups (Polák and Frynta, 2009). Domestication has led to a remarkable decline in SSD of domestic sheep (Polák and Frynta, 2009).

Morphometrics is both the study of size and shape variation and its co-variation with other variables. Shape is mathematically defined as all the geometric features of an object except its size, position and orientation (Dryden and Mardia, 1998).

The following is a study of sexual dimorphism of a relict sheep breed and its evaluation using GM tools. This GM approach can be considered a complete methodology to study size and shape differences (Marcus *et al.* 1993).

## MATERIALS AND METHODS

For the morphometric analyses, a total of 18 dry skulls belonging to 2 males and 16 females of *Fardasca* population were used. This population is restricted to SE Catalunya (Spain), and have been only recently discovered, to date it has not been recognised as a breed, although it appears to be clearly differentiated from neighbouring breeds, such as *Maellana*, *Ojalada* and *Ripollesa*. It has a straight frontal profile, sublongilinial proportions, eumetrism, and a conspicuous black splashed coat. Highly rustic, the sheep is well adapted to the harsh environment of the area and has a meat purpose.

To minimise potential ontogenetic effects, only adults (with all of the permanent teeth erupted) were used. The geometric analysis was performed using a photograph in the lateral right view of skulls with an NIKO D90 digital camera. The focal axis of the camera was parallel to the right lateral aspect of each skull. A ruler was used in this process. Fourteen anatomically equivalent and homologous landmarks were digitised (Figure 1) on each picture, by tps Dig, version 2.04.

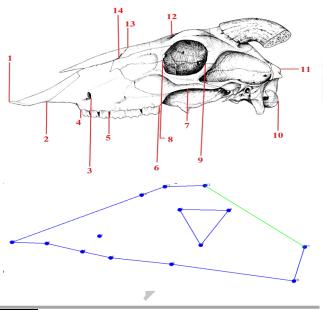


Figure 1 Lateral aspect of the *Ovis* skull in the established position for obtaining the shape from the 14 landmarks used

Once the Cartesian x-y coordinates were obtained for all landmarks, the shape information was extracted with a full Procrustes fit and centroid size (CS, the square root of the sum of the squared distances among the landmarks in a configuration and their centre of mass extracted), which were also obtained using CoordGen6f (H. D. Sheets, www.canisius.edu/sheets). Procrustes superimposition is a procedure that removes the information of size, position and orientation to standardise each specimen according to the centroid size. To correct the size, ln of CS was used.

As a normal distribution appeared for size (W=0.957, p=0.467), univariate analysis (Student test) was employed to verify the existence of significant differences between sexes. Sexual shape differences were assessed by a nonparametric multivariate analysis of variance (NPMANOVA). Under MANOVA, a canonical variates analysis (CVA) produces a scatter plot of specimens along the two first canonical axes, producing maximal and second to maximal separation between all groups (multigroup discriminant analysis). Finally, a Principal Component Analysis (PCA) over the variance-covariance matrix of the procrustes was carried out to explore multivariate differences between sexes. The relationship between size and shape was also examined by plotting centroid size versus the procrustes configuration.

Statistical analyses were then run using PAST- "Paleontological Statistics Software Package for Education and Data Analysis" (Hammer *et al.* 2001) and MorphoJ (Klingenberg, 2011). All of the programs used in this study are available over the Internet by FTP from the "morphmet" directory at life.bio.sunysb.edu or via the WWW at http://life.bio.sunysb.edu/morph/. All probability values are with  $\alpha$ = 0.05.

## **RESULTS AND DISCUSSION**

#### Skull size and shape

No significant differences in size appeared between sexes (t=-1.021, p=0.330) (Figure 2).

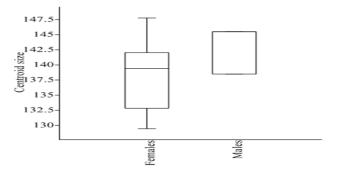


Figure 2 Box plot of *Fardasca* values for size (expressed as centroid size). For each sex, the 25-75 per cent quartiles are drawn using a box. The median is shown with a horizontal line inside the box. The minimal and maximal values are shown with short horizontal lines ("whiskers")

No differences in shape were found (p=0.058). The dimorphism indexes yielded no skull shape differences between the sexes. The following procrustes presented individual differences (P<0.05) between sexes for few characters: X5, Y6, Y9 and Y10, with only the last two related to the neurocranium being larger for males. If a CVA is performed, then sexes appeared separated (Wilk's  $\lambda$ =0.365, p=0.007). Therefore, the absence of sexual shape differences seems to be related to the landmarks chosen (Figure 3).

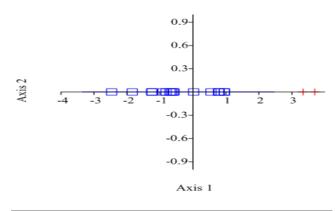


Figure 3 Canonical Variates Analysis scatter plot showing the distribution of male and female populations based on the outline analysis of the shape of the skulls. Sexes appear separated (Wilk's  $\lambda$ =0.365, p=0.007) Legend:

Squares= female and Crosses= male

#### Principal component analysis

The PCA plot for the procrustes data showed that the first three principal components (PCs) accounted for 63.68% (PC1+PC2+PC3=30.43%+18.77%+14.47%) of the total shape variation and provided a reasonable approximation of the total amount of variation, with the other PCs accounting for no more than 9.3% of the variation. PCA showed a slight differentiation of sexual shape dimorphism, with males exhibiting a slight bias towards positive values of PC1 and females towards negative values (Figure 4).

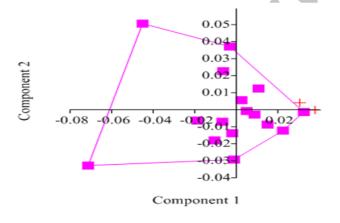
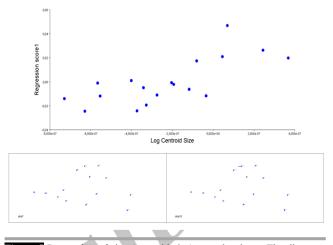


Figure 4 Principal component analysis for shape data. Filled squares are females (n=16) and crosses are males (n=2). The first two principal component s accounted for 49.21% (PC1+ PC2=30.43%+18.77%)

Regression of size (centroid size) over the shape is shown in Figure 5. The diagrams represent the extremes of skull shape associated with larger (upper right) and smaller (down left) skulls. The relationship between the shape and centroid size indicates that dimorphism is not related to size ( $R^2$ =0.071, p=0.153). Shape differences are dispersed above all the skull surface. Sexual size dimorphism, a difference in body size between sexually mature males and females, is a fundamental morphological characteristic of many animals, including most ungulates (for a review see Andersson, 1994).



**Figure S** Regression of size (centroid size) over the shape. The diagrams represent the extremes of skull shape associated with larger (upper right) and smaller (down left) skulls. The relationship between the shape and centroid size indicates that dimorphism is not related to size ( $R^2$ =0.071, p=0.153). Shape differences are dispersed over the entire skull surface

Sexual size dimorphism has important consequences for ecology, behaviour, population dynamics, and evolution (LeBlanc *et al.* 2001). The adoption of new techniques to determine variation in the shape of both animals and plants is currently a widely discussed issue (Lawing and Polly, 2010). Geometric morphometrics can unify methodologies to quantify and visualise shapes in all of the possible ways.

Although it has been said that sheep display a relatively high level of sexual dimorphism (Michelena *et al.* 2006), in terms of GM in skulls and for the *Fardasca* animals studied, these differences are not evident, at least with the landmarks studied. According to form (size+shape), males and females are similar, although males tend to have a more elevated neurocranium. A criticism of this study is that landmarks have been subjectively chosen, and do not necessarily represent the "true form" of the skull. Moreover, the number of landmarks has been limited because of the small sample. Further studies with larger samples would permit more landmarks to be investigated, which would help to adjust the results obtained here.

### CONCLUSION

In conclusion, we argue that GM methods are excellent yet rarely used resources for testing hypotheses of skull sexual dimorphism in domestic animals.

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