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Does the application of geometric morphometric methods on skull allow a differentiation of domestic sheep breeds?

Parés-Casanova, Pere M.

Dept. of Animal Production; University of Lleida, Av. Alcalde Rovira Roure 191
25198 Lleida (Catalunya, Spain)
Correspondence: peremiquelp@prodan.udl.cat

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ABSTRACT

Geometric morphometric methods (GMM) make it possible to study shape and size independently. The skulls of 58 adult specimens of different domestic breeds of Ovis were analysed by means of GMM in order to know if shape and size would allow their racial grouping. For this purpose, breeds were grouped a priori in four groups according to the general area of the origin of each breed: "West Mediterranean" (Fardasca breed, n=21), "Central Europe" (German breeds, n=12, Friesland, n=3 and Ile de France, n=2), "East Mediterranean" (Karaguniko, n=5, Kephalonia, n=1, Khios, n=2, and one unknown breed but of Greek origin) and "Hebrides Islands" (Hebridean breed, n=7). A picture on the lateral aspect of each skull was taken and fourteen landmarks were placed on each. Skull variation was decomposed in both size (centroid size: CS) and shape components. No allometry appeared, and size differences between groups were significant only for Eastern group (Greek breeds), probably due to the influence of Asiatic breeds. Central Europe group presented the highest variation, probably because it encloses different morphological breeds, such Friesland and Ile de France. According to shape, significant differences appeared between groups except between West and East Mediterranean breeds for the discriminant function. Shape differences were mainly focused on basilar aspect of the skull. 63.8% of the specimens were properly classified a posteriori, increasing only to 70.6% of proper classification when size was used. The results suggest that a rather low discrimination among breeds appears and that size does not add much more information.

Keywords: ewe, *Ovis*, skull morphometrics, skull shape, skull size

INTRODUCTION

It has been said traditionally that the morphological and morphometric study of the skull does not only reflect contributions of genetic and environmental components to individual development but can also describe genetic and ecophenotypic variation. Traditional morphometrics use distances and ratios to characterize morphology of the skull from an ethnological point of view. However, such an approach has some well-known deficiencies. First, selection of variables is very arbitrary and can importantly affect the results obtained. The lack of an internationally accepted methodology, such as those existing for zooarchaeologists (the most commonly used being the monography by Von den Driesch, [2]), would partially explain this. Sometimes, authors give different importance to different parts, depending on their hypothesis, or whether or not breed is horned. Second, direct distances are typically more correlated with the overall size of an organism, making it impossible in practice to study size and shape separately. Finally, even if ratios (between informative distances, the so-called "ethnological indexes") were used as an attempt to correct the effect of size, such an approach has its own statistical problems. Therefore, it was evident that classical methods were imperfect to single out size and shape for classifying breeds.

It was Sir D'Arcy Wentworth Thompson who first proposed depicting changes in shape by using reference deformations of an organism superimposed on a grid [1]. However, a general approach to solve this measuring quantitatively did not appear until shape could be described by the coordinates of a set of well-defined points or landmarks [1]. The geometric morphometric (GM) techniques, which have been shown to be objective and efficient compared to traditional methods, can be used to analyse the size and shape variation in the skulls of the specimens.

The geometry of shape is captured by a configuration of topographically corresponding landmarks [3] which finally allow the comparison of geometrical forms of almost any structure. Size and shape are separated, and shape differences can be visualized with deformation grids in the style of Thompson [4]. Then, differences in shape among individuals will be directly characterized by differences in these coordinates. See [1, 5 and 6] for more details. GM links the geometry of the structure, the mathematics of deformation and biological inference.

The main aim of this paper is to introduce the use of GM techniques to some European groups of sheep breeds in order to know their "power" to characterize differences in skull shape and size.

MATERIALS AND METHODS

Samples

The skulls of 58 specimens of different domestic breeds of *Ovis* were analyzed for the purpose of this study. The material is housed in different private and public collections. Breeds (Table 1) were grouped *a priori* in four groups according to the general area of their origin (name for each group is just an author's decision): "West Mediterranean" (Fardasca breed, n=21), "Central Europe" (German breeds, n=12, Friesland, n=3 and Ile de France, n=2), "East Mediterranean" (Karaguniko, n=5, Kephalonia, n=1, Khios, n=2, and one unknown breed but of Greek origin) and "Hebrides Islands" (Hebridean breed, n=7). An effort was made to include only mature 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 *Ovis* genre.

Table 1. Area of origin, assigned group and collection of origin for the breeds studied (n=58).

Breed	N	Area of origin of the breed	Assigned group	Collection of procedence
Fardasca	21	Spain	West Mediterranean	1
German breeds	16	Germany	Central Europe	3
Karaguniko	5	Greece	East Mediterranean	2
Kephalonia	1	Greece	East Mediterranean	2
Khios	2	Greece	East Mediterranean	2
Unknown	1	Greece	East Mediterranean	2
Friesland	3	Britain	Central Europe	2
Ile de France	2	France	Central Europe	2
Hebridean	7	Scotland	Hebridas Islands	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"

Data gathering

Images involved taking a photograph of each skull in direct lateral view. Fourteen landmarks were placed on lateral aspect. Landmarks were chosen to provide an adequate coverage of the skull shape (Figure 1). A scale bar (20 mm) was placed on each image for rescaling purposes. The images were then digitized using TpsDig software version 2.16 [7] to obtain the x, y coordinates of the landmarks. The author was responsible for landmarking all specimens. To ensure that the localization of the points selected was accurate, Fardasca specimens (n=21) were replicated twice. Mantel test, with 5,000 permutations, reflected R=0.904, p=0. The error for digitizing landmarks was therefore considered to be negligible. To test if 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 software version 1.20 [8]. The correlation was very high (r>0.996), indicating that no significant distortion was introduced by tangent space approximations. We thus proceeded with subsequent analysis.

Morphometrics: size

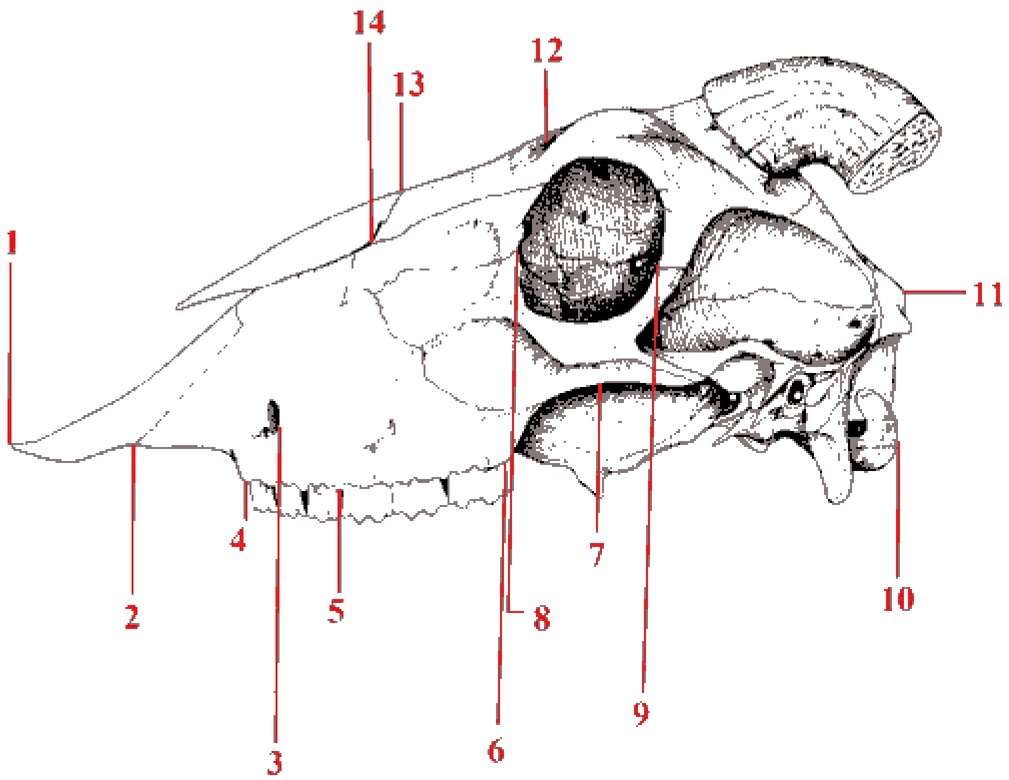
Skull variation was decomposed in both size and shape components. To quantify the size of a specimen, centroid size (CS) was computed from the raw coordinates of the landmarks [9] using the CoordGen6f software [10]. CS is a measure of geometric scale, calculated as the square root of the summed squared distances of each landmark from the centroid of the landmark configuration. Differences in sizes were studied according to Mahalanobis distances of ln CS.

Shape

Body shape was analysed using landmark-based geometric morphometric methods [1, 11]. Once the specimens were aligned, the mean configuration of 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 [12, 13, 14].

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). Thus, a regression of skull shape on skull size was considered necessary to determine the amount of shape variation due to size changes.

Figure 1. Landmarks (14) used to capture cranial size and shape in *Ovis* skull. All landmarks were located on left lateral aspect in each specimen. Two additional landmarks (not appearing here) were used on a scale bar for rescaling purposes. See text for the exact anatomical description of each landmark.



Statistical analysis
Canonical Variates Analysis (CVA) was applied to obtain a scatter plot of specimens along the first two canonical axes, producing maximal and second to maximal separation between all groups (multigroup discriminant analysis). CVA classifies the data, assigning each point to the group that gives minimal Mahalanobis distance (calculated from the pooled within-group covariance matrix, giving a linear discriminant classifier). In addition, a cross validation statistical analysis was also accomplished by a leave-one-out cross-validation (jackknifing) procedure. This gives us information about the chance of confusing two (or more) groups.

All statistical calculations were performed PAST package [15].

RESULTS AND DISCUSSION

Size did not explain a significant amount of variation in shape using multivariate regression (Wilks' λ=0.212; $F_{28,29}$=3.837, p<0.001). Size differences between groups were significant only for Eastern group (p<0.05) (Table 2 and Figure 2). Central Europe group presented the highest variation (2.4%), probably because it encloses different morphological breeds, such as Friesland and Ile de France. According to shape, significant differences appeared between groups except between West and East Mediterranean breeds for the discriminant function (Wilks' λ=0.0067; $F_{84,81.65}$=4.185, P<0.005) (Figure 3). Only 63.8% of the specimens were properly classified *a posteriori*, increasing to 70.6% of proper classification when size was used. Shape differences were mainly focused on basilar aspect of the skull.

Table 2. Results of size (expressed as ln Centroid Size) between breed groups studied. Significant differences (p<0.05, in bold) only appeared between East Mediterranean breeds and the others.

	Central Europe	East Mediterranean	West Mediterranean
Central Europe			
East Mediterranean	**0.0443**		
West Mediterranean	0.2678	**0.0001**	
Hebridas Islands	0.6024	**0.0198**	0.8607

Figure 2. Box plot for ln Centroid Size for the groups according to the general area of origin: "West Mediterranean" (W), "Central Europe" (C), "East Mediterranean" (E) and "Hebrides Islands" (H).

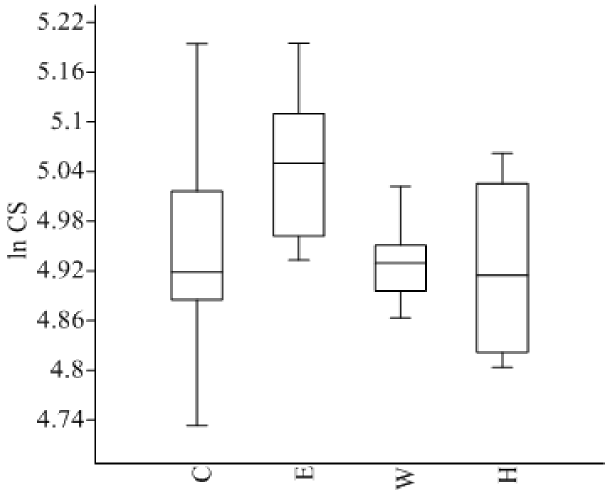
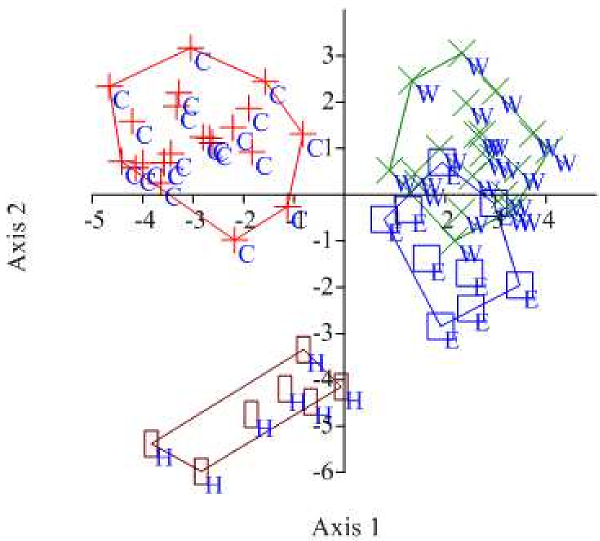


Figure 3. Representation of the first two principal components in all specimens studied with no size component (Wilks' λ=0.0067; $F_{84,81.65}$=4.185, p<0.005). Breeds were grouped *a priori* in four groups according to the general area of origin: "West Mediterranean" (W), "Central Europe" (C), "East Mediterranean" (E) and "Hebrides Islands" (H).



Differences between domestic sheep breeds are shown in different studies, using conventional analyses of linear distances measured on each specimen [16]. By employing GMM, it is clear that sheep skulls cannot properly classify breeds. The evolution of morphological structures by selection depends on the availability of genetic variation for the traits in question. Particularly for multidimensional features such as shape, the response to selection depends critically on the patterns of genetic and phenotypic variation. The differences in skull size and shape between groups have probably been driven both by extrinsic factors (for instance adaptation for feeding on different kinds of grasslands) and by intrinsic ones (a shared developmental pathway of the sheep breed differentiation, which posed some biomechanical constraints on the direction of the evolution of sheep). It is clear that the choice of landmarks for characterizing morphological form can evidently not reflect the "biologically important" skull shape, or be a mere choice of researcher. Consequently, an effort must be made to unify methodologies between researchers in order to obtain results with total comparative possibilities.

CONCLUSION

- Size did not explain a significant amount of variation in shape
- Size differences between groups were significant only for Eastern group.
- According to shape, significant differences appeared between groups except between West and East Mediterranean breeds
- Shape differences were mainly focused on basilar aspect of the skull.

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REFERENCES

[1] Bookstein, F.L. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, Cambridge, **1991**
[2] Driesch, Von den A. *Peabody Museum Bulletin,* **1976**, 1, 1-135.
[3] Marcus, L.F. Traditional morphometrics. In: Rohlf, F. J. and F. L. Bookstein (Eds.): P*roceedings of the Michigan morphometrics workshop Special Publication*, Ann Arbor, University of Michigan Museum of Zoology, **1990**, 2, 77-122.
[4] Wehausen, J.D., Ramey, R.R. *Journal of Mammalogy,* **2000**, 81, 145-161.
[5] Rohlf, F.J., Marcus, L.F. A revolution in morphometrics. *Tree,* **1993**, 8, 129-132.
[6] Monteiro, L.R., Reis, S.F. *Principios de morfometria geométrica*. Ribeirão Preto, Brazil: Holos Editora, **1999**.
[7] Rohlf, F.J. *TpsDig version 2.16*. Department of Ecology and Evolution, State University of New York, Stony Brook, NY, **2010**.
[8] Rohlf, F.J. *TpsSmall version 1.20*. Department of Ecology and Evolution, State University of New York, Stony Brook, NY, **2003**.
[9] Dryden, I.L., Mardia, K.V. *Statistical Shape Analysis*. John Wiley & Sons, New York, **1998**.
[10] Sheets, H.D. *IMP-integrated morphometrics package*. Department of Physics, Canisius College, Buffalo, New York, **2003**.
[11] Rohlf, F.J. *Annual Review of Ecology and Systematics*, **1990**, 21, 299-316.
[12] Rohlf, F.J., Slice, D. *Systematic Zoology,* **1990**, 39, 40-59.
[13] Rohlf, F.J. *Journal of Classification,* **1999**, 16, 197-223.
[14] Slice, D.E. *Systematic Biology,* **2001**, 50, 141-149.
[15] Hammer, Ø., Harper, D.A.T., Ryan, P.D. *Paleontologia Electronica,* **2001**, 4, 1. [on line]. URL: http://palaeo-electronica.org/2001_1/past/issue1_01.html (accessed on April 23rd 2013).
[16] Guintard, C., Fouché, S. Etude ostéométrique de têtes osseuses de mutons (*Ovis aries*, L.). *Revue de Médicine Véterinarie*, **2008**, 159, 12, 603-617.