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## **Discrete Sexual Dimorphism in Minorcan Horse**

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## **Research Article**

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#### **ABSTRACT**

In this research, the authors studied sexual dimorphism in the Minorcan horse, an autochthonous breed from Minorca Island in the Balearic archipelago (NW Mediterranean Sea). For this purpose, a two-dimensional geometric morphometric approach was applied to 52 pictures of adult animals (24 males and 28 females) in their left lateral view. Fourteen landmarks were chosen to provide an adequate coverage of the body. Certain differences between sexes appeared, mainly on dorsal neck conformation and distal part of extremities, males being much more uniform. Surprisingly, withers height was not discriminatory between genders.

#### INTRODUCTION

The Minorcan horse breed is autochthonous from Minorca Island, Balearic archipelago (NW Mediterranean Sea). Although a breeding programme for the breed started in 1989, when it was officially recognized, the FAO lists it as "Endangered". In 2011, its total population was reported to be about 3,212 animals, of which 644 and 780 breeding males and females, respectively <sup>[1]</sup>. Official standard (published in 2008) described it as eumetrical (normal range in the species, which is 400 kg), uniform jet-black (tending to be browner during summer), with a sub-convex to straight profile. The height at wither were 161 and 157 cm for males and females, respectively <sup>[1]</sup>.

The term 'sexual dimorphism' refers to differences in dimensions and proportions of the body between males and females <sup>[2]</sup>. When compared with strong dimorphic species, such as bovids, gender dimorphism in horses is not pronounced, similar to that in species such as the cat, the rabbit or the Guinea pig <sup>[3,4]</sup>. Sexual conformation assessment for the breed is described in its racial standard, but this judgment is basically subjective. Thus, dimorphism is based on analogical and merely comparative appreciations, with expressions such as "females are slimmer than males, with longer head and body the neck finer and longer, and a more squared croup" (official standard). Masculinity and femininity are therefore characteristics that depend on the attitudes of trained technicians, with no information on whether or not they are linked to functional traits. Moreover, some animals can be registered even without having an adequate racial standard for their gender. Therefore, the implementation of objective methodologies (that can also assess functional morphology) is of major importance.

Geometric Morphometrics (GM) is a method to describe the form (size+shape) of an organism, normally using a set of landmarks. In horses, few studies have used GM analysis. Cervantes et al. [5] had applied this methodology for the Spanish and Arabian horses. Their study described Morphofunctional traits of that breed. In present investigation, full images of morphological sexual pattern in the Minorcan horses were examined to study shape variation through the application of GM.

## **MATERIALS AND METHODS**

#### **Material**

Sample consisted of 52 officially registered adult Minorcan horses (24 males and 28 females) aged more than 2.5 years (ranged from 3 to 16 years, averaged 8 years). The animals belonged to 48 different owners, and were kept in different local farms and riding centres, with uniform management and feeding conditions in all locations.

#### **Data gathering**

Images for each animal were collected by taking a digital high-resolution picture in left lateral view. Fourteen landmarks were situated by white sticks placed on each animal before taking its photograph. These landmarks were chosen to provide an adequate coverage of the body functionality (**Table 1 and Figure 1**), as they were located mostly at osseous articulations. A scale was placed on each image. Withers height was also registered. While measuring, the horses stood on a firm surface, assuming a natural position. Sony DSC-HX1 apparatus at high resolution (3,456 × 2,304 pixels) was used to collect the photographs. In order to avoid peripheral image distortion, sufficient distance was maintained to ensure that the animal occupied the central portion of focal space. All images were subsequently downloaded to a PC, and a file in tps format was created for each of the groups studied using tps Util software version 1.60 <sup>[6]</sup>. The entire set of images was then digitized using tps Dig software version 2.16 <sup>[7]</sup> to obtain the x, y coordinates of each landmark. To ensure that the localization of the selected points was accurate and ensure repeatability, specimen land marking was performed twice. The Discriminant Function Analysis (DFA) for two replicas gave p=0.999, so the error for digitizing landmarks was considered negligible. To test whether the shape variation was 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 tps Small software <sup>[8]</sup>. The correlation was very high (r=0.999). It was confirmed that shape variation between the specimens was sufficiently small, and therefore the distribution of points in the shape space could be represented satisfactorily by their distribution in the tangent space.

Table 1. Explanation of landmarks used to derive the 14 landmarks used in the morphometric analyses of Minorcan horse.

1	Forehead (above the eyes)	8	Fetlock
2	Forelock	9	Hip (anterior)
3	Poll	10	Hip (posterior)
4	Withers	11	Buttock
5	Point of shoulder	12	Stifle
6	Elbow	13	Hock
7	Knee	14	Ankle



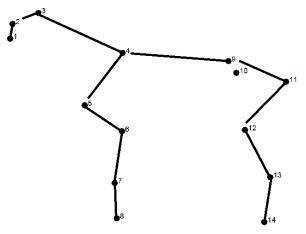


Figure 1. Landmarks digitised on the surface of the body.

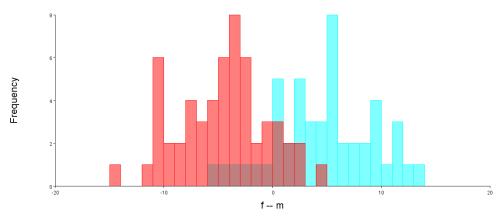
After that, the configurations were rotated to minimize the squared differences between landmarks, and the aligned coordinates (consensus configuration) were obtained with tps Relw software [9]. The final "shape variables" averaged between replicas were used for further statistical analysis.

#### Statistical analysis

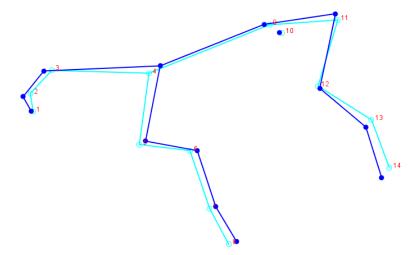
To examine the amount of differences in sexual shape dimorphism, the p-values were obtained from a permutation test (10,000 permutation rounds) to get Mahalanobis distances between sexes. Mann-Whitney test was used to perform the comparison between withers heights, which were obtained from official individual register. Correlation with withers height (log transformed) was carried out with a linear model. Statistical analyses were undertaken with the MorphoJ [10] and PAST [11] packages.

#### **RESULTS**

DFA based on the shape variables revealed 11.5% misclassification among individuals and, in the alternative, more stringent cross-validation classification test; it resulted in 26.9% misclassification between the two gender groups. Despite this, permutation test (10,000 permutation rounds) for Mahalanobis distances showed differences between males and females (p<0.0001) (Figure 2). First three principal components explained 74.0% of the variance. Landmarks 2 (forelock), 4 (withers), 8 (fetlock), 13 (hock) and 14 (ankle) were those which contributed most to sexual differences. The vector plot depicted in Figure 3 illustrates these landmark displacements. Displacements were much less for males, probably suggesting a greater selective pressure. According to wither height, no differences appeared between males and females (U=249, p=0.658); therefore, scaling of characters were examined. All of them were isometric (p<0.05). Isometry also appeared when both genders considered separately.



**Figure 2.** Discriminant function analysis for 24 male adults and 28 female adults belong to Minorcan horse breed. Permutation test (10,000 permutation rounds) for Mahalanobis distances showed differences between males and females (p<0.0001). Males graph indicated with blue colour on right, females graph indicated with red colour on left.



**Figure 3.** Landmark displacements for females and males. Landmarks 2 (forelock), 4 (withers), 8 (fetlock), 11 (buttock), 13 (hock) and 14 (ankle) were those which contributed most to sexual differences.

The results obtained showed that the morphostructure is not sufficient to differentiate sexes, as the correct classification of individuals according to sex is about 70%. Sexual differences were accumulated mainly on dorsal neck conformation and distal part of extremities. Surprisingly, withers height was not gender discriminatory.

In conclusion, the breed exhibits a discrete sexual dimorphism, GM detecting these morphological differences between

"males" and "females". Obviously, perimetral traits (such as thoracic girth) and widths cannot be studied by this 2-D method. However, the conclusions were congruent with the low sexual dimorphism usually described in modern horse [12], and detected sexual differences probably explained by a functional plane: only males are used for saddle, so breeders tend to select males more accurately (head and neck, and extremities also being of highest importance) than females, which would be subject to a mere "reproductive inertia".

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