



Original Article

Differences in the Morphology of the Distal Phalanx Are Not Reflected on the Distal Sesamoid Bone in Young Equines

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ABSTRACT

Correlations in distal phalanx and distal sesamoid area and shape were explored through the decomposition of coordinate data into elliptic Fourier coefficients. For this purpose, 10 equine distal sesamoid bones and distal phalanges on their solar aspect, belonging to young animals (< 24 months) of “Cavall Pirinenc Català” breed, were studied. This is a local equine breed for meat purposes ranged in extensive management in NE Spain. Distal sesamoid and distal phalanx did not appear interrelated, either in area or in shape, so the differences in the morphology of the distal phalanx were not reflected in the morphology of the distal sesamoid bone. Lack of correlation could possibly be due to youth of animals. This study should be extended to older animals, as well as to horses affected by foot problems and also it could be proposed for longitudinal evaluations if obtention of images *in vivo* (i.e. echography) were standardized.

Keywords: Cavall Pirinenc Català, elliptic Fourier analysis, hoof, navicular.

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INTRODUCTION

Radiographic assessment of the distal bones is the backbone of the veterinary evaluation of the equine digit and, recently, quantitative and objective radiographic measurements have reported new insights into its form and function (Burd *et al.*, 2014). The quantification of shape, and sometimes of size, can be particularly problematic in some cases as conventional metric measurements summarize these aspects very poorly. While the lunar form of the solar surface of the distal phalanx has been widely described, little information exists about the form of distal sesamoid bone, and less about the relationships of both bones. But sometimes the outlines are complex; they cannot be reduced into Euclidean geometry, as happens when only conventional measurements are considered.

One of the mathematical methods than can be used for the morphological characterization of complex shapes is the Fourier analysis (FA), in which the outline can be mathematically

analysed and thus quantified. FA allows a quantitative analysis of shape but also of its changes. Fourier theory is fairly complicated mathematically as it decomposes into series of cosine and sine functions. Unlike more commonly used morphometric techniques, which quantify morphology based on a distance matrix, FA is based on a trigonometric function, using sine/cosine measurements to quantify curvature using terms (harmonics).

Each harmonic is described by four Fourier coefficients, two each for the x - and y -axes, generating a total of $4n$ coefficients labelled a_n , b_n , c_n and d_n , where n is the number of harmonics. The harmonics together in the series combine to describe the repeated elements in a sinusoidal waveform. As the number of harmonics in the series increases (in other words, as n gets bigger), the Fourier series increasingly converges onto the form being analyzed. The first, largest harmonic describes the overall length of the specimen, and the following harmonics provide increasingly detailed information about its complexity, that is, each harmonic adds to and numerically describes the distortion of the form from the original circle described by the Fourier series when no harmonics are added. In this manner, FA is able to converge upon and describe complex two-dimensional bounded outlines.

Sine and cosine coefficients can be standardized for size; thus, description of pure shape independently from size can be obtained, but also from spatial orientation and relation to reference planes. This independence can be very important, as size could be a confounding factor in the analysis of changes in shape because the modifications in size are often of greater magnitude than the corresponding modifications in shape (Ferrario *et al.*, 1996).

Elliptic Fourier analysis (EFA) is an extension of conventional FA (Kuhl and Giardina, 1982), but has certain appealing properties not available in the conventional method (Lestrel, 1989). In brief, elliptic series allow an internal orientation of structures, performed by rotating the forms until the major axes of the first harmonic coincide. Such an orientation cannot be performed by conventional Fourier analysis and external, often arbitrary, references should be used. Moreover, unlike conventional Fourier analysis, the use of EFA does not require that points be equidistant (Lestrel, 1989).

This quantitative analysis can characterize more detailed variation in form than conventional FA, and in the case of foot bones provides researchers with an independent characterization that does not rely on simple linear measurements. Thus it has the potential of being a valuable tool in bone characterization.

The digital skeleton of the horse is reduced to the third digit. It consists of three phalanges and the sesamoid bones (König and Liebich, 2009). The distal sesamoid bone (*os sesamoideum distale*, also known as “navicular bone”) is an element of the distal interphalangeal joint (*art. interphalangea distalis*) between the middle and distal phalanx (*phalanx media* and *phalanx distalis*) within the hoof (*ungula*) (Komosa *et al.*, 2013). It is boat-shaped with a straight proximal border (*margo proximalis*) and a convex distal border (*margo distalis*), which is attached to the distal phalanx by a strong ligament (König and Liebich, 2009).

It also stabilizes the distal interphalangeal joint. Despite its small size, sesamoid bone plays an important role in the movement of the limb (Komosa *et al.*, 2013). Recently, distal sesamoid problems have frequently been diagnosed, especially in sport horses (Komosa *et al.*, 2013). FA provides a description of form without reference to landmarks.

To the authors' knowledge, nothing has been done with equine bones. Elliptical Fourier decomposition was chosen for this study because it does not require that points on the outline of the specimen be equally spaced (Crampton, 1995), thus allowing greater sampling from sections of complex shape or high variability of curvature. In this study, EFA is applied to cross-sectional contours of equine distal sesamoid bone and distal phalanx on its solar surface in order to define whether or not their size (interpreted as area in this research) and shape are correlated.

MATERIALS AND METHODS

Specimens

10 equine front feet (6 right and 4 left sides) from “Cavall Pirinenc Català” (CPC) breed were used. CPC is a local breed for meat purposes, ranged in absolutely outdoor management in the N part of Catalonia (NE Spain), in the Pyrenees area. Feet were obtained from a commercial abattoir from animals < 24 months which were destined for slaughter for meat purposes (thus a reason unrelated to this research). Feet were free of macroscopical pathologic changes. Individual sex and coat were recorded but we did not take them into account for our analysis. Bones were obtained by maceration and manual removing of soft tissues. Lozano was responsible for this operation.

Data Collection

Data collection from clean bones followed a three-step process: 1) manually obtaining outline tracings using a pen on normal paper. The solar outlines were obtained, 2) scanning the outlines using a HP Photosmart at 200 ppi. A reference of 20 × 30 mm accompanied each specimen, and 3) quantifying the outlines using an EFA program.

The images were processed and analyzed using SHAPE, ver. 1.3 (Iwata and Ukai, 2002, downloadable at <http://lbm.ab.a.u-tokyo.ac.jp/~iwata/shape/>), a program that identifies the outlines and generates an elliptic Fourier description. Twenty harmonics were used to describe the shape. The size normalization procedure consisted of a recalculation of the outlines using the same value of the enclosed area for all specimens.

Quantifying the outlines was tested by a duplicate recording by the same author (Lozano). Total percentage of measurement error was calculated using the formula $[S^2_{\text{within}} / (S^2_{\text{within}} + S^2_{\text{among}}) \times 100]$ as indicated in Muñoz-Muñoz and Perpiñán (2010). Correlation between distal sesamoid and phalange bones was studied with a Mantel test (Mantel and Valand, 1970). This is a permutation test for correlation in which the *R* value is simply the Pearson’s correlation coefficient between all the entries in the two matrices. The permutation test compared the original *R* to *R* computed in 5,000 random permutations using the Euclidean distance for both matrices. The actual shape values analyzed statistically were based on the 4 coefficients of each harmonic. The non-parametric correlation coefficient Kendall’s τ was also used to re-investigate area data between both bones; values were log-transformed. In this test all possible $N(N-1)/2$ pairs of bivariate data points are considered. The two pairs can have the same direction in *x* as in *y* (*x* and *y* either decrease, or both increase) or not. All statistical analyses were performed with the PAST package (Hammer *et al.*, 2001, downloadable at <http://folk.uio.no/ohammer/past/>). Significance level was established at 5%.

RESULTS

Total percentages of measurement error appear in Table 1. Results of Mantel test for area, shape and both appear in Table 2. There was no significant correlation between any variable, although p-value for area data is close to 5%. Kendall’s τ was -0.022 ($p=0.928$), thus reinforcing that area was discordant for both bones (Figure 1). In other words, there was no morphological correlation between distal sesamoid bone and distal phalanx either for area or for shape.

Table 1: Total percentage of measurement error for distal sesamoid bone and distal phalange (size and shape) (n=10)

Parameter		S^2_{within}	S^2_{among}	Measurement error (%)
Distal sesamoid bone	Area	1.288	585.804	0.21
	Shape	2.715	9.775	21.74
Distal phalanx	Area	3.569	2771.690	0.12
	Shape	2.354	21.020	10.07

Table 2: Results of Mantel test between distal sesamoid bone and distal phalanx (n=10) for area, shape and both. The reported *p* value is one-tailed (the test being at 5% significance level)

	R	<i>p</i>
Area	0.733	0.090
Shape	0.041	0.364
Area and shape	0.041	0.373

DISCUSSION

The use of EFA on distal phalanx and distal sesamoid bone has allowed a comprehensive and complete depiction and quantification of their area and shape. Distal sesamoid and distal phalanx did not appear interrelated, either in area or in shape, so the differences in the morphology of the distal phalanx are not reflected in the morphology of the distal sesamoid bone. This could be due to the fact that samples used in this research were from young animals, aged below 2 yr and thus not yet fully grown. Then, a lack of absolute integration (integration is concerned in some way with covariation among the parts or traits) (Klingenberg, 2013) could be expected between bones for growing horses. The closure time of selected growth plates of the limbs has been determined for some horse breeds (Pezzoli and Del Bue, 1975; Yoshida *et al.*, 1982; Uhlhorn *et al.*, 2000; Strand *et al.*, 2007), showing that sometimes it is not achieved before the age of six years. The appendicular skeleton of CPC horses has probably not completed its bone growth at least before 2 years of age.

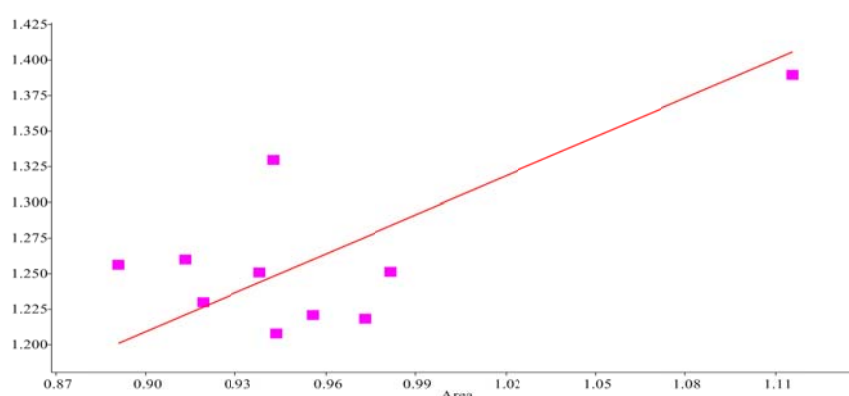


Figure 1: Correlation between areas of distal sesamoid and distal phalanx bones (log transformed values). Kendall's τ was -0.022 ($p=0.928$), so area was discordant for both bones

This was just a post-dissectional study. A limitation for inferences from this study is that we have focused on a small sampling and from very young animals. This study should be extended to older animals, as well as to horses affected by foot problems and also it could be proposed for longitudinal evaluations if obtention of images *in vivo* (i.e. echography) were standardized. Nevertheless, we are sure that a better understanding of this bone will yield improvement in hoof care and treatment of foot-related disease.

CONCLUSIONS

Distal sesamoid and distal phalanx did not appear interrelated, either in area or in shape, so the differences in the morphology of the distal phalanx are not reflected in the morphology of the distal sesamoid bone. This could be due to the fact that samples used in this research were

from young animals, aged below 2 yr and thus not yet fully grown. The appendicular skeleton of CPC horses has probably not completed its bone growth at least before 2 years of age.

Ethics Statement

No protected animals or live animal manipulations were involved in this study.

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None to declare.

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