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RESEARCH ARTICLE

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Variation in morphological assessment of the equine distal phalanx solar surface

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ABSTRACT

Area and shape variations on the solar surface of the equine distal phalange were explored through the decomposition of coordinate data into elliptic Fourier coefficients. For this purpose, 10 equine distal phalanges belonging to the "Cavall Pirinenc Català" breed were studied. This is a local equine breed raised for meat whose range is NE Spain. Elliptic Fourier descriptors of the first principal component successfully captured most of the distal phalange morphology, whereas a larger number of harmonics did not increase the information but did produce noise. As repeatability was an unimportant factor contributing to the variation, this method seems to be sufficiently sensitive to detect morphologic background (area and shape). It could be extended to other breeds as well as to horses affected by foot problems and could also be proposed for longitudinal evaluations if obtention of images in vivo (i.e. ecography) were standardized.

Key words: "Cavall Pirinenc Català", elliptic Fourier analysis, elliptic Fourier descriptors, phalanx

INTRODUCTION

Radiographic assessment of the distal phalanx is the backbone of veterinary evaluation of the equine digit. Recently, quantitative and objective radiographic measurements have been reported that give new insight into the form and function of the equine digit [1]. While the lunar form of the solar surface (*margo solearis*) has been widely described, little information exists regarding the manner in which this form may be quantified.

The quantification of shape variation has proven to be particularly problematic in some cases, as conventional metric measurements summarize aspects of shape very poorly. In fact, distances, angles, and ratios and the indices derived from them, while replicable, lose a vast amount of contour information. Moreover, the outline of a bone is a complex shape that cannot be reduced to Euclidean geometry and correctly considered by conventional measurements. Moreover, it might be difficult to select the best and most informative examples.

One mathematical method that can be used for the morphological characterization of complex shapes is Fourier analysis (FA), in which the outline can be mathematically analysed and thus quantified. FA allows quantitative analysis of a shape and changes it undergoes. Fourier theory is pretty complicated mathematically, as it decomposes into a 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 $4\,n$ coefficients labelled a_n, b_n, c_n and d_n, where n is the number of harmonics. The harmonics in a series combine together to describe the repeated elements in a sinusoidal waveform. As the number of harmonics in the series increases (in other words, as n gets larger), the Fourier series increasingly converges onto the form being analysed. 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 giving descriptions of pure shape independent of size, but also from spatial orientation and relation to reference planes. This independence can be very important, as size can be a confounding factor in the analysis of changes in shape, because modifications in size are often of greater magnitude than the corresponding modifications in shape [2].

Elliptic Fourier analysis (EFA) is an extension of conventional Fourier analysis [3] that has certain appealing properties not available in the conventional method [4]. 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 [4].

Although EFA is a powerful tool for analysing biological shapes and has been applied to many organisms, to the authors' knowledge, nothing has been done with equine bones. In this study, EFA was applied to cross-sectional contours of the equine distal phalange on its plantar surface in order to quantitatively define the shape of this bone independent of size (understood as area) and also the shape in a "global" sense; that is, by applying the "totality" of contour information (shape and area).

MATERIALS AND METHODS

Specimens

Ten equine front feet (6 right side and 4 left side) from the "Cavall Pirinenc Català" breed were used. This is a local outdoor breed raised for meat, whose range is the Northern part of Catalonia (NE Spain), in the Pyrenees area. The feet were obtained from a commercial abattoir from animals < 24 months. All feet were free of macroscopic pathologic changes. Individual sex and coat colour were recorded but they were did not taken into account for our analysis. The distal phalange bone was obtained by maceration and manual removal of soft tissues. Lozano was responsible for this operation.

Data collection

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

The validity of the procedure was tested using a duplicate recording by the same author (Lozano), and data from the two replicates were analysed with a Wilcoxon paired test for area and with a Mantel test with 5,000 permutations for shape. A NPMANOVA for morphological data (for both area and shape) was also applied to determine the variance in the replicates. Repeatability measures the proportion of variance due to true variation among individuals and is defined as the percentage of variation not due to measurement error, ranging from 0 to 100. Repeatability was calculated using the formula, $100-[S^2_{within} / (S^2_{within} + S^2_{among}) \times 100]$ as indicated in Muñoz-Muñoz and Perpiñán [5]. The actual values analysed statistically were based on the 4 coefficients of each harmonic and done with the PAST package [6].

The images were processed and analysed using SHAPE, ver. 1.3 [7], a package of programs that identifies outlines and generates an elliptic Fourier description. Twenty harmonics were used to describe the shape. The accuracy of the overall process was qualitatively assessed by reconstructing the distal phalange and comparing the shape of the actual distal phalange bone image and the principal component analysis reconstruction using the NEFview program from the same package. The size normalization procedure consisted of recalculating the phalange outline using the same value of the enclosed area for all specimens. Principal Component Analysis (PCA) was performed from a covariance matrix with the SHAPE, ver. 1.3 package.

RESULTS

The procedure used to image and quantify the outlines of the distal phalange bone was well repeatable: replicates did not appear to differ significantly either in size (W=30, p=0.845) or shape (R=0.812, p=0.0002). The repeatability reached 99.8% and 89.9% for area and shape, respectively. The NPMANOVA showed that morphology variance accounted for only 1.2% of the total variance. For posterior calculations we proceeded with averaged values. Area ratios ranged between 16.1 and 24.5 and presented a coefficient of variation of 14.1%. The outline shape variations described by the 1st PC accounted for 96% of the total variance (Table 1). Shape variations along the first 5 PCs are presented in Figure 1.

DISCUSSION

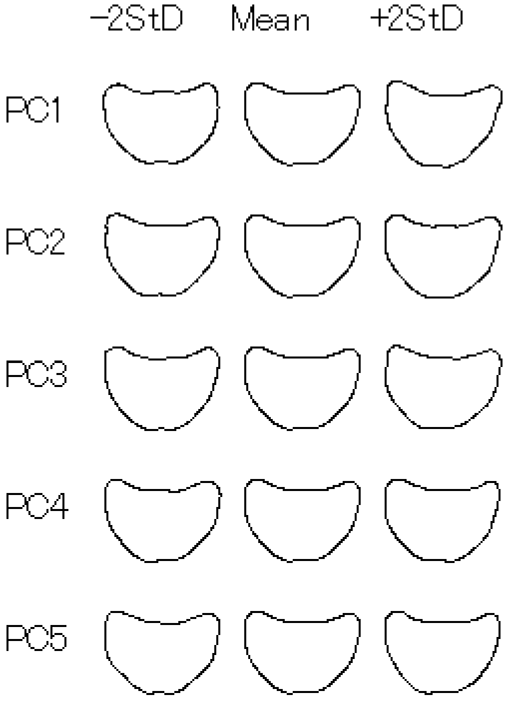
In the present investigation, a method based on elliptic Fourier analysis for quantitative analysis of the equine distal phalange is proposed. The method appears to be fast, easy to perform, and inexpensive (it requires neither special pictures nor needs highly specialized personnel and the software is downloadable for free). This method supplies a very close approximation of the analysed trace, the goodness of fit (and thus the detail in the analysis) being a function of the number of harmonics used in the reconstruction. The first–harmonic truncation allowed very good definition of all the details of the distal phalange outline. Repeatability (understood as taken by the same observer with the same method) was not an important factor contributing to the variation, so this method would seem to be sufficiently sensitive to detect the morphological background (size and shape) of minimal functional variations in the distal phalange, at least on its solar aspect.

One of the limitations of Fourier analysis is that it provides a great deal of data (in the present study, each distal phalange outline was described by four coefficients for each of the 20 harmonics, i.e. 80 coefficients were available for each outline).

Table 1 Values for the first 10 principal components (PC)

PC1	3.83×10^{-4}	38.327	38.327
PC2	1.66×10^{-4}	16.611	54.939
PC3	1.33×10^{-4}	13.282	68.221
PC4	7.59×10^{-5}	7.602	75.823
PC5	5.69×10^{-5}	5.696	81.520
PC6	5.33×10^{-5}	5.338	86.858
PC7	3.46×10^{-5}	3.462	90.321
PC8	2.24×10^{-5}	2.238	92.559
PC9	1.76×10^{-5}	1.760	94.319
PC10	1.59×10^{-5}	1.595	95.915

Figure 1. Recreation (mean ± standard deviation) showing the characterization of distal phalange shape using Elliptical Fourier descriptors generating from 1 to 5 principal components (PC). The distal phalange was successfully delineated in PC1



Out of methodological considerations, one important potential application of this analysis is for studying the individual symmetry of distal phalange bones and solar surface changes with age. Morphological values from other equine breeds would be interesting, too, in order to compare aptitudes (saddle, meat...).

In conclusion, the method described in the present study allows a sensible analysis of the morphological characteristics of the equine distal phalange bone and is full of applications. Obviously we have worked with dissected bony pieces, so this was just a *post dissectional* study. But it could be proposed for longitudinal evaluations if obtention of images *in vivo* (i.e. ecography) were standardized. Nevertheless, the authors hope that a better understanding of this bone will yield improvements in hoof care and the treatment of foot related disease.

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