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Patterning of Variation Morphology in Equine Navicular Bone

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ABSTRACT

Size and shape variation in the equine navicular bone is explored through the decomposition of coordinate data into elliptic Fourier coefficients. For this purpose, 10 bony pieces belonging to “Cavall Pirinenc Català” breed were studied. This is a local equine breed for meat purposes whose range is in northeast Spain. The Fourier procedure used appeared well repeatable. Elliptic Fourier descriptors of one harmonic successfully captured most of the navicular morphology, whereas a larger number of harmonics would not have increased the information, but would have produced noise. The method described in the present study allows a sensible analysis of the morphology characteristics of the equine navicular bone. It could be extended to other breeds, as well as to horses affected by foot problems; and also it could be proposed for longitudinal evaluations if obtaining images *in vivo* (i.e. ecography) were standardised.

Keywords: Cavall Pirinenc Català”; elliptic Fourier analysis; elliptic Fourier descriptors

INTRODUCTION

Horses have a sesamoid bone called the navicular bone, located within the hoof, that lies between the second phalanx and third phalanx, on the palmar aspect of the coffin joint. The navicular region is an important structure in relation to equine lameness, responsible for as much as one-third of all cases of lameness in horses. It is involved with a significant disease process called “navicular syndrome”, which affects the front feet in particular (Wintzer, 1989). Recently, much of the original literature concerning navicular disease has been called into question, particularly the significance of radiographic changes as a sole diagnostic criterion. Newer imaging techniques have shown that damage to the soft tissues in the region may be significant contributors to lameness.

The clinical analysis of navicular must often be considered a complete quantification of the form. This quantification of shape variation has proven to be particularly problematic in some cases; it has long been recognised that conventional metric measurements (such as distances, angles, and ratios, and the indices derived from them), while replicable, summarise aspects of shape very poorly because of the vast amount of contour information that is lost in the process. The outline of a bone is a complex shape that cannot be reduced into Euclidean geometry and correctly considered by conventional measurements. Moreover, it might be difficult to select the best and most informative ones. One of the mathematical methods than can be used for the morphological characterisation is the Fourier analysis, in which the outline can be mathematically analysed and thus quantified.

Fourier analysis allows a quantitative analysis of shape and of its changes independent from size. Fourier theory is pretty complicated mathematically as it decomposes into series of cosine and sine functions. Cosine and sine coefficients can be standardised for size, thus giving a description of pure shape. The global shape characteristics of the object are thus quantified independently from its size, 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).

The use of both conventional and Elliptic Fourier analysis (EFA) has few precedents in domestic animal anatomy and remains a relatively uncommon method. Therefore, some discussion of the technique is merited here. Elliptic Fourier analysis is an extension of conventional Fourier analysis (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 the conventional Fourier analysis, the use of EFA does not require that points be equidistant (Lestrel, 1989). Unlike more commonly used morphometric techniques such as Euclidean distance matrix analysis (EDMA), which quantifies morphology based on a distance matrix, EFA is based on a trigonometric function. It uses sine/cosine measurements to quantify curvature.

Originally derived to measure sound waves, this method is applied here to capture curvilinear aspects of the external morphology of the navicular bone, on their palmar aspect. Each harmonic is described by four Fourier coefficients, two each for the *x*-and *y*-axes, generating a total of four *n* coefficients labelled a_n, b_n, c_n an d_n, where *n* is the number of harmonics. The first, largest harmonic describes the overall length of the specimen, and the following harmonics provide increasingly detailed information about its complexity. The terms together in the series combine to describe the repeated elements in a sinusoidal waveform. As the number of terms (harmonics) in the series increases (in other words, as *n* gets bigger), 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, Fourier analysis is able to converge upon and describe complex two-dimensional bounded outlines.

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 is applied to cross-sectional contours of equine navicular bone in order to define a method to quantitatively study the shape of the shape component of this bone independent of size, and provide a mathematical accounting of shape variation in a "global" sense; that is, by applying the "totality" of contour information in a comparative analysis. Shape variation is explored, but size is as well, by separating their shape and size components.

MATERIALS AND METHODS

Specimens

Ten equine front feet (six rights and four left side) from the “Cavall Pirinenc Català” breed were used. This is a local breed for meat purposes, ranged in an absolutely outdoor manner in the northern part of Catalunya (northeast Spain), in the Pyrenees region. Feet were obtained from a commercial abattoir.

Feet were free of macroscopically pathologic changes. Animals were < 24 months. Sex was recorded but did not take into account for analysis. Left and right feet were sampled indistinctly. Navicular bone and distal phalange was obtained by maceration and manual removing of soft tissues. Lozano was responsible of this operation.

Data collection

Data collection followed a three-step process: 1) obtaining manually outlined tracings of the navicular bone using a pen on normal paper. Once the palmar outlines were obtained, 2) the outlines were entered into the EFA program using a HP Photosmart at 200 ppi. A reference of 20 × 30 mm was used for each specimen, and 3) the outlines were quantified using the Elliptic Fourier program.

The validity of the procedure was tested by a duplicate recording, digitisation, and Fourier analysis of the size and shape of all subjects. The subjects were repeated by the same author (Lozano) and data from two replicas analysed with a Wilcoxon paired test for size, and with a Mantel test with 5,000 permutations for shape. A NPMANOVA for morphology data (for both size and shape) was also applied to know the variance due to replicas.

The actual values analysed statistically in this study are based on the amplitudes of each harmonic. These values represent calculations of the maximum height of the waveform. They are orthogonal and non-cumulative in nature. That is, the value of subsequent harmonic amplitudes is independent of and does not influence the value of previous harmonic amplitudes. The amplitudes are equal to the value of the Fourier coefficient that proceeds each sine or cosine term (a_i and b_i). The amplitude of each harmonic measures an aspect of the total deformation of the form from a circle, which is what the Fourier series describes when no terms are added to the constant (a_0). The harmonic amplitudes, therefore, represent a measure of the influence that each harmonic has on the form in distorting it from the original circle.

The images were processed and analysed using SHAPE, ver. 1.3 (Hammer *et al.*, 2001) a package of programs that identifies the outlines and generates an elliptic Fourier description (Figure 1).

Twenty harmonics were used to describe the navicular bone shape. The accuracy of the overall process was qualitatively assessed by reconstructing the navicular bone from the EFDs and comparing the form of the actual navicular bone image and the principal component analysis reconstruction using programme NEF view from the SHAPE package. The size normalisation procedure consisted of a recalculation of the navicular outline using the same value of the enclosed area for all specimens. The measurement was calculated using the formula $S^2_{within} / (S^2_{within} + S^2_{among}) \times 100$ as indicated in Muñoz and Perpiñán (2010) and Iwata and Ukail (2002). Principal component analysis (PCA) was performed from covariance matrix. Regression was performed using log area as an independent variable. The rest of the multivariate statistical analyses were performed with PAST software (Muñoz and Perpiñán, 2010).

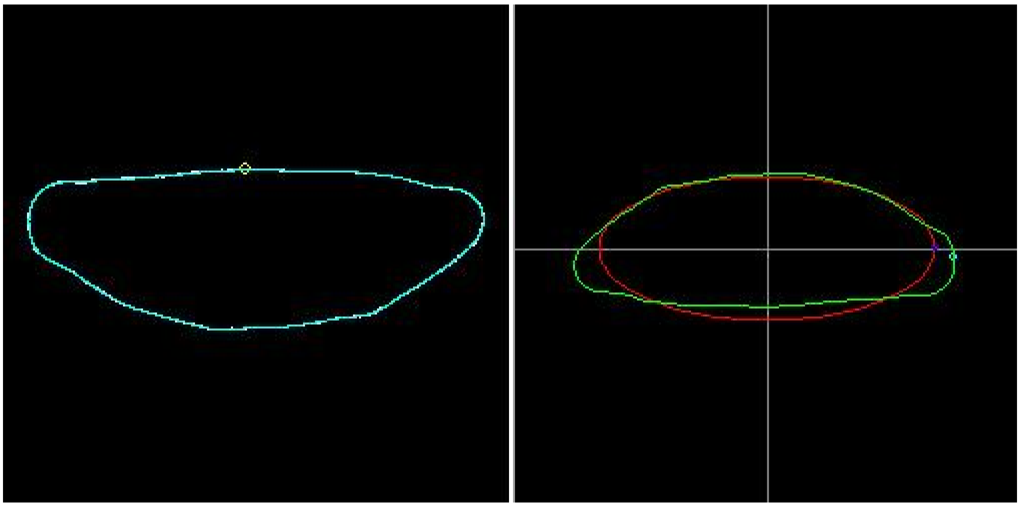

Figure 1. Contour reconstructed by the normalized elliptic Fourier descriptors. In blue (left), contour recorded by chaincode. In green (right), contour reconstructed by the normalized elliptic Fourier descriptors

RESULTS

The procedure used to image and quantify the outlines of the navicular bone was well repeatable; replicas appeared not to be significantly different for size (W=35, p=0.492) or shape (R=0.724, p=0.0002). The size estimation error percentage was 8.92%, and the NPMANOVA showed that morphology variance in the EFDs between replicas accounted only for 3.4% of the variance.

Table 1: Values for first 5 principal components.

PC	Eigenvalue	% variance	Cumulative variance
1	2.53×10^{-3}	59.804	59.804
2	1.19×10^{-3}	28.218	88.023
3	1.84×10^{-4}	4.342	92.365
4	7.40×10^{-5}	1.750	94.115
5	5.69×10^{-5}	1.346	95.462

The measurement error reached 11.7% and 21.7% for area and shape, respectively. For ulterior calculations we proceeded with averaged values. Area ratios ranged between 7.77 and 13.04. Only five principal components had a proportion larger than 1/number of analysed coefficients. First two principal components explained 88.0% of the observed variance (Table 1 and Figure 2). The area presented a coefficient of variation of 16.0%, and the first two harmonics were not regressed on it (R^2=0.171, Wilk's λ=0.202, $F_{8,1}$=0.491, p=0.808).

DISCUSSION

In the present investigation, a method based on elliptic Fourier analysis for the quantitative analysis of equine navicular bone is proposed. The method appeared to be fast, easy to perform, and inexpensive (it neither requires special pictures nor needs highly specialised personnel, and software is a free download). This method supplies a very close approximation of the analysed trace, the goodness of fit (and thus the detail of the analysis) being a function of the number of harmonics used in the reconstruction. The first harmonic truncation allowed a very good definition of all the details of the navicular outline, whereas a larger number of harmonics would not have increased the information but would have produced noise. As the measurement error was low, too, this method would seem to be sufficiently sensitive to detect the morphologic background (size and shape) of minimal functional variations of the navicular bone.

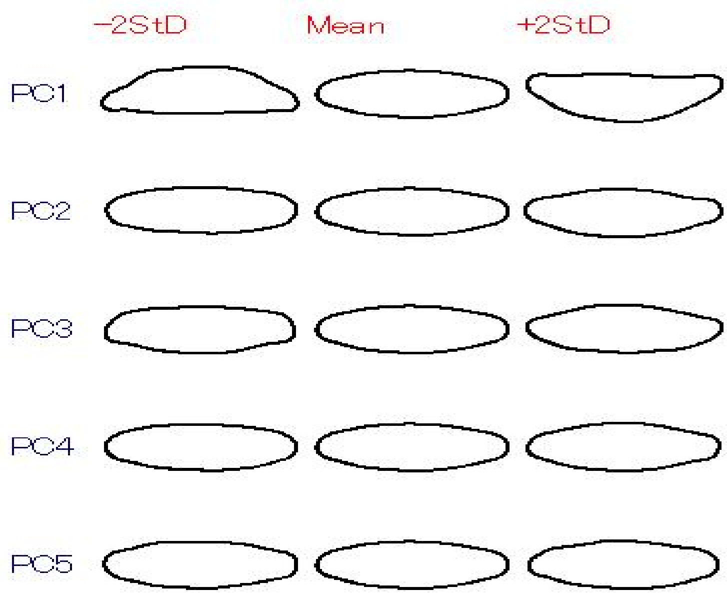

Figure 2. Progression showing characterisation of navicular shape using increasing number of harmonics. Recreations using elliptical Fourier descriptors generated from one to five harmonics (only these five principal components had a proportion larger than 1/number of analysed coefficients).

One of the limitations of Fourier analysis is that it provides a great deal of data. In the present study, each navicular outline was described by four coefficients for each of the 20 harmonics, i.e. 80 coefficients were available for each outline. But for navicular bone, information is not fragmented in the first harmonic.

The absolute clinical significance of the individual size and shape in navicular bone cannot be established as no lameness case has been studied, and no quantitative investigations comparable with the present one were found. Moreover, it is impossible to state if the values are different from other breeds (especially according to the aptitude). An important application of this analysis of shape could also be performed for the study of individual symmetry of the navicular bone; the method could provide normal right and left outlines that could be used as a reference for the quantitative diagnosis of horse patients.

CONCLUSION

The method described in the present study allows a sensible analysis of the morphology characteristics of the equine navicular bone. It could be extended to other breeds, as well as to horses affected by foot problems. Obviously we have worked with dissected bony pieces, so it is just a *post mortem* study. But it could be proposed for longitudinal evaluations if obtaining images *in vivo* (i.e. ecography) was standardised.

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