

Directional Solar Asymmetries in Sound Pyrenean Catalan Horses

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Abstract

Most published attempts to quantify equine hoof form are based on lineal and angular measurements. Here we applied geometric morphometric methods to study shape variation of hoof outlines in a sample of 25 distal forelimbs (13 right and 12 left). Limbs belonged to sound "Cavall Pirinenc Català" horse, an equine meat local breed from Pyrenees, slaughtered in an abattoir. The outline of each hoof was represented by a set of two landmarks and 86 semi-landmarks. Results reflected directional asymmetric, e.g. consistent differences between medial and lateral contours for all four limbs. Right limbs tended to supinate (rotation of medial wall towards out), while left limbs tended to pronate (rotation of lateral wall towards in). Such morphological adjustments may be an important consideration for hoof practitioners and may imply an important reconsideration of "normal" feet evaluation.

Keywords: Equine • Hoof conformation • Forelimb • Laterality • Sidedness

Introduction

Bilateral symmetry among animals is rarely perfect, i.e., when measurements of left and right structural parts do not always are perfectly equal [1,2]. In a population, bilateral asymmetry can be observed to occur in three general patterns, *viz.* fluctuating asymmetry (FA), directional asymmetry (DA) and anti-symmetry (AS). FA represents a random variation on the right and left side; DA involves left and right sides differences but *in the same direction*; in AS can be considered a macroscopic form of FA [3-6].

Geometric morphometrics (GM) is based on the Cartesian coordinates of landmarks (measurement points) that are homologous across all measured individuals [7]. The set of all landmarks preserves the geometry of the studied configurations, so GM is of superior statistical power than most traditional morphometric approaches and is particularly effective for exploratory studies [7].

Landmark configurations need to be registered (superimposed) prior to any statistical analysis because the coordinates not only contain information on the shape of the measured objects, but also on their position, scale, and orientation [8]. The most common superimposition technique in GM is Generalized Procrustes Analysis, consisting of three steps: (i) translation to have the same centroid (average landmark position), (ii) scaling to have the same size, and (iii) iteratively rotation to minimize the summed squared distances between the landmarks and the corresponding sample average [7]. Overall size is measured as centroid size, the square root of the summed squared distances between the landmarks and their centroid [8]. The coordinates of the superimposed landmark configurations are called Procrustes shape coordinates as they contain information about the shape of the landmark configurations only [8].

Curvatures express the rate of change of the angle between the tangent to the curve and the x-axis [9]. Curvatures cannot be well described using visual or traditional metric methods [9]. Semi-landmarks are points along

curvatures. They are initially placed at approximately corresponding positions and their exact locations are ulteriorly estimated statistically in order to create geometrically homologous points that can be used in the subsequent analysis as if they were anatomical landmarks [9].

Many biological structures, such as hoof outlines, consist of relatively smooth curves and lack homologous landmark points that can be identified in all individuals. Object symmetry for hooves can be considered if the axis of symmetry is included within the structure itself as the axial plan and separates lateral and medial sides as "halves" [10,11]. The convenient statistical properties of GM together with its effective visualization allow for a potentially very powerful exploratory of the hoof solar structure [12]. Separation of DA and FA is of great importance to the shape analysis of symmetric structures, as it is a key step that allows for distinction between systematic asymmetry, which is genetically fixed and heritable for DA, and a sign of developmental instability for DA [6].

Handedness (or laterality, as it is referred to when applied to horses) means they prefer either the right or left side. Recent researchers have found that horses have a preferred side of the body, just like humans [13,14]. In the present paper we apply GM methods to study object shape asymmetry of the entire equine hoof outline. We extracted the hoof shape from surface scans of the sole (see the methods section below) using landmarks and semi-landmarks. To the best of our knowledge, symmetries of equine hoof outline shapes using GM have scarcely been studied [15].

Materials and Methods

Studied population

The Catalan Pyrenean horse ("Cavall Pirinenc Català") is a compact, broad-built horse with a small population (<4,600) which is located in the NE part of the Pyrenees, along the Catalan-French border [16]. Mainly bred for

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meat production, it is reared outdoors throughout the year, normally not receiving additional food beside some low-quality straw in winter except when foals are selected for slaughter [17]. Then, they are gathered in paddocks and receive additional feeding with hay and concentrates during the last months before slaughter, at about 10-12 months of age ("poltres", body mass about 350 kg) [17]. Animals of this breed do not receive any hoof care, trimming, or shoeing; therefore, their hooves must be considered naturally shaped. Anyway, hoof problems are rather rarely encountered, being the forelimb "splay foot" -the hoof wall flaring outwards- the most frequently found non-functional abnormality (pers. obs.).

Sampled limbs

At a commercial abattoir, 25 distal forelimbs (13 right and 12 left) were obtained from Catalan Pyrenean yearlings (<12 months) after slaughter. Sex and exact age were not registered. Animals were apparently healthy and sound. At the abattoir, limbs were disarticulated at the level of the carpus and were rinsed with water to clarify hoof outline. Ground surface was taken outlining the round edges with a pen on a paper. The original drawings were subsequently photocopied.

Extraction of shape

The outline of the hoof was digitized by 86 semi-landmarks and two true landmarks at the two axial-most positions (Figure 1). We used the sliding landmark algorithm to estimate the position of the semi-landmarks in all individuals, enabling the joint analysis of anatomical landmarks and curves (represented by semi-landmarks) [18]. All landmark configurations were superimposed by a Generalized Procrustes Analysis, standardizing for position, size, and orientation of the configurations. The resulting Procrustes shape coordinates were used for further statistical analysis [7]. The most common algorithm for this purpose is the sliding landmark algorithm, which iteratively slides the semi-landmarks along their curves in order to minimize local shape differences (the bending energy of the thin-plate spline interpolation) between each individual and the sample average [9].

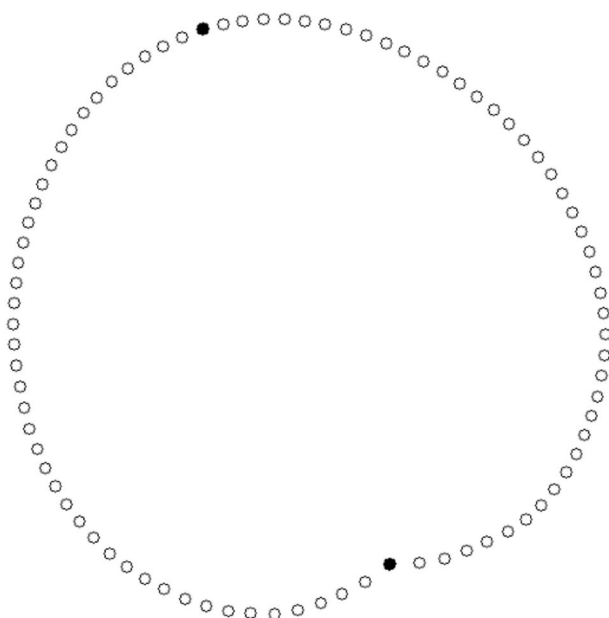


Figure 1: Position of landmarks to describe the outline of the hoof. Two landmarks were in fixed positions (filled dots: axial-most positions), with the remaining 86 landmarks allowed to slide along the outline between the fixed landmarks (empty dots).

The software TpsUtil v. 1.50 [19] was used to prepare and organize the images. Landmarks were digitized twice, using TpsDig v. 1.40 [19], by one of the authors (Noelia) in two different sessions. In order to compare Procrustes to tangent space distances between individuals, the procedure performed using TpsSmall v. 1.33 [19] reflected a high degree of approximation of

shapes in the sample (i.e., shape space) in relation to the reference shape (i.e., tangent space) ($r=0.999$), which allows accurate capture of the nature and extent of skull shape deformations in subsequent statistical analyses.

A regression of centroid size versus shape (regression scores) was done to verify if allometry existed. Size was computed as centroid size (or, the square root of the sum of squared distances from the landmarks to their centroid) [7]. In the absence of allometry, it is the only size measure uncorrelated with all the shape variables [7].

For shape, a Procrustes ANOVA indicated the degrees of freedom, means of squares, F and P values for the effects from individuals, sides, Individual \times sides effect and measurement error. The individual's effect denoted the individual variations of shape and size of hooves individual animals. The main effect of sides indicated the variation between sides and considered as the measure of DA. The Individual \times sides is the mixed effect, which indicates there is failure of hoof shape differences to be on the same side in every case and therefore indicates there FA in the data. Lastly, measurement error is a gauge of the possible effect of measurement error on the estimates of FA [20].

Data analysis

All morphometric analysis were analysed in MorphoJ v. 1.06c [21] and PAST v. 2.17c softwares [22]. Confidence level was established at 95%.

Results

Allometry

No significant regression of centroid size versus shape appeared, with only a 1.32% of shape variation explained by size variation ($p=0.200$).

Hoof shape symmetries

For hoof shape, Procrustes ANOVA highly significant variations in symmetry within individuals and sides (DA), but not for side*individual interaction (FA) (Table 1). FA presented mean squares about 1.6 to 7.2 times lower than for DA, which accounted for around a 25% of total asymmetric variation. FA was significant for left forelimb. Right limbs tended to supinate (rotation of medial wall towards out), while left limbs tended to pronate (rotation of lateral wall towards in).

Table 1: Procrustes ANOVA of hooves shape in object symmetry for 25 distal forelimbs (13 right and 12 left) belonging to sound "Cavall Pirinenc Català" horse. Mean squares (MS) are the amount of variation from the one higher level in the hierarchy. The F value represents the comparison of each MS to the one lower level of MS which could be the source of error. Shape differences among individual hooves ("Individual" effect) were strongly significant ($p<.0001$) and were approximately about 0.4 to 1.2 times larger than the asymmetry between both sides of the hooves. DA ("Sides" effect) proved to be highly significant and considerably larger than the FA ("Individual \times sides" interaction effect), which was very subtle for left forelimb. Error is a gauge of the possible effect of measurement error on the estimates of FA.

1/ Right forelimb (n=13)					
Effect	SS	MS	df	F	P
Individual	0.09501	0.0000920673	1032	3.41	<.0001
Sides	0.00428	0.0000497111	86	1.84	<.0001
Individual \times sides	0.02787	0.0000270028	1032	1.19	0.0006
Error	0.05093	0.0000227758	2236		

2/ Left forelimb (n=12)

Effect	SS	MS	Df	F	P
Individual	0.08183	0.0000865028	946	3.19	<.0001
Sides	0.00387	0.0000449869	86	1.66	0.0003
Individual × sides	0.02563	0.0000270950	946	1.07	0.0998
Error	0.05212	0.0000252497	2064		

Discussion

This study aim was to assess hoof asymmetries on solar surface in a local equine breed maintained under extensive management and consequently, which has no care of feet, so conclusions may express horse natural wearing. We applied geometric morphometric methods to study shape asymmetries for each forelimb separately with an outline represented by a set of landmarks and semi-landmarks.

Hooves are not pieces of rigid tissue that forever hold the shape. They are malleable enough to change shape when put under pressure, and such forces would not act uniformly [23]. The medial side of the hoof bears most of the descending weight of the horse [24]. In response to this, the 'plastic' hoof develops a medial side that is steeper and a lateral side that is more oblique, so it changes shape. Hoof DA in the studied sample is largely present, and attributable to differential mechanical loading, e.g. laterality. Laterality represents a dominance. Limb dominance is a common occurrence in many species [25], including horse [14,26]. Like humans [27] most horses are right forelimb dominant [14,28,29] and this would explain the detected subtle FA only in left forelimb, which would represent a very low disturbing effect. As asymmetric shape variation of hoof surface was not determined by allometric changes relating to size, we suppose that FA is due to a function process rather than to a developmental (e.g. wall growth) effect.

Conclusion

"Normal hooves" in horses are therefore directionally asymmetrical but, notably, with a clear right supination and a left pronation. These results would be considered by both farriers and veterinarians as an important feature of hoof conformation. Now it would be interesting to study if this unevenness coincides with both kinetic and kinematic asymmetrical pressure differences.

Conflicts of Interest

The authors declare no conflicts of interest. The post-mortem use of this kind of non-edible parts did not require an approval from the Ethics Committee.

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Authors' Contributions

PMPC designed the study and directed implementation and data collection. NLN collected data. Both authors analyzed the data and drafted the manuscript.

Supporting Information

The contents of all supporting data are the sole responsibility of the authors.

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