Paired left-right asymmetries of the hoof surface in the Pyrenean Catalan yearlings are less marked among hindlimbs

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Abstract. *Most published researches that describe an equine hoof form are based on lineal and angular measurements. Here we apply geometric morphometric methods to study symmetries between fore and hind pairs in a pure horse breed. For this purpose, we studied a sample of 27 right-left pairs of distal limbs (12 forelimb and 15 hindlimb pairs) from young Pyrenean Catalan Horses ("Cavall Pirinenc Català"), a meat local breed from Catalonia (Spain), managed under semi-extensive conditions at NE Pyrenees. The outline of the hoof was represented by a set of 2 landmarks and 88 semi-landmarks, which were studied by means of geometric morphometric methods. Surfaces were similar for all limbs but significant shape variations appeared in fluctuating asymmetry, with less contra-paired differences in hindlimbs than between forelimbs. The detected asymmetry of the solar surface in this study may provide, in our opinion, and indicator of mechanical, not environmental, stress, as hooves, acting as robust but malleable pieces, can change shape according to pressure forces. So, if the hoof surface in the Pyrenean Catalan Horse exhibits asymmetry, it may be just a mere plasticity due to pressure forces. This asymmetry would be expressed less markedly in the hind pair, which represents the "motor" part of the equine body and so, much linked to a perfect symmetrical functional oerforming.*

Introduction

Bilateral symmetry among animals is rarely perfect, i.e., left and right parts, areas, lengths or widths do not measure perfectly equal (Adams et al., 2013). In a population, bilateral asymmetry can occur in three general patterns (Auffray et al., 1999), *viz*: fluctuating asymmetry (FA), directional asymmetry (DA) and antisymmetry (AS). FA is a random deviation from bilateral symmetry, DA involves repeatable deviations from symmetry towards the same side, and AS is bimodal asymmetry that is random with respect to side (Auffray et al., 1999) (Pither & Taylor, 2000) (Mancini et al., 2005).

Geometric morphometrics (GM) is of superior statistical power than traditional morphometric approaches (Reyment, 2010) (Adams et al., 2013). As GM is based on sets of Cartesian coordinates of landmarks (measurement points that are homologous), it preserves the geometry and, thus, can represent shape deformation studies better than linear morphometrics (Reyment, 2010) (Adams et al., 2013). Semi-landmarks are points along such smooth outlines that are initially placed at approximately corresponding positions (Gower, 1975) (Webster & Sheets, 2010)shape variation, and covariation of shape with other biotic or abiotic variables or factors. The resulting graphical representations of shape differences are visually appealing and intuitive. This paper serves as an introduction to common exploratory and confirmatory techniques in landmark-based geometric morphometrics. The issues most frequently faced by (paleo. Their exact locations are ultimately estimated statistically in order to create geometrically homologous landmarks that can be used as if they were anatomical landmarks (Gower, 1975) (Webster & Sheets, 2010).

In the present paper, we apply GM methods to study paired size and shape asymmetries of the hoof outline in a sample of a horse breed.

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Materials and methods

Study population

The Pyrenean Catalan Horse ("*Cavall Pirinenc Català*") is a compact, broad-built, predominantly chestnut horse with rather short limbs with a small population (< 4,600) located in the North Eastern part of the Pyrenees, along the Catalan-French border (Infante Gil, 2011). Genetic analysis suggests that it is closely related to the Breton and Comtois breeds (Infante Gil, 2011). Today mainly managed for meat production, it is reared outdoors throughout the year in a free all year-round grazing lifestyle, normally without receiving additional food beside some low-quality straw in winter (Infante Gil, 2011). When selected for sacrifice, yearlings are gathered in paddocks and receive additional feeding with hay and concentrates during the last 2–3 months before slaughter, at 10–12 months of age ("poltres", average body weight 350 kg) (Parés-Casanova, 2011). Animals of this breed do not receive any hoof care, trimming, or shoeing; therefore, their hooves must be considered "normally" shaped (Parés-Casanova, 2011). In the breed, hoof wall problems are rarely found, being the "splay foot", i.e., the hoof wall flaring outwards, the most frequently found non-clinics abnormality (*pers. obs.*).

Sampled limbs

At a commercial abattoir, 27 pairs of distal limbs (12 from forelimbs and 15 from hindlimbs) were obtained from 15 different young unshod Pyrenean Catalan yearlings (< 12 months) immediately after normal slaughter for commercial purposes. The healthy and sound sampled yearlings were unshod; no hooves had received trimming or other podal interventions. At the abattoir, the limbs were disarticulated at the level of the basipodium, being rinsed with water before measurements were performed. Sex and exact days age were not registered. The lack of three forelimb pairs was due to a sampling loss in the abattoir for reasons other than authors' procedures.

Extraction of size and shape

The outline of each hoof was drawn on a sheet and the contour was digitized by means of 88 semi-landmarks and two anatomical landmarks located at the two axial-most positions (most dorsal on the wall and most posterior on the bulb of heel) (Fig. 1). This procedure was repeated for two replicas of each image. Finally, all landmark configurations were superimposed by a Generalized Procrustes Analysis to standardize for position, size, and orientation of the configurations (Adams et al., 2013). The resulting Procrustes shape coordinates were used for further statistical analysis. The reader could directly consult primer references, as (Klingenberg, 2015) and (Savriama & Klingenberg, 2011).

The software TPSUtil v. 1.70 (Rohlf, 2015) was used to prepare and organize the images. Landmarks were digitized twice, using TPSDig v. 1.40 (Rohlf, 2015), by one of the authors (Noelia) in two different blind sessions. In order to compare Procrustes to tangent space distances between individuals, we applied a previous analysis with TPSSmall v. 1.33 (Rohlf, 2015), which 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) ($r = 0.999$). For each limb, we examined size and shape variation separately. Size (interpreted as hoof surface) was computed as centroid size (CS, the square root of the sum of squared distances from the landmarks to their centroid) (Webster & Sheets, 2010). A regression of CS (log-transformed) *versus* shape (regression scores) was done to verify if allometry (size-related shape changes) existed. Differences in CS between right-left pairs and between limbs were analysed by a two-way NPMANOVA (Non-Parametric Multivariate Analysis of Variance) using Euclidean distances with 9,999 permutations, and "side" (right/left) and limb (fore and hind) as factors. This Procrustes ANOVA indicated the degrees of freedom, means of squares, F and p values for the effects from individuals, sides (expressing DA), individuals* sides (expressing FA) and measurement error (expressing differences between replicas).

Finally, a Canonical Variate Analysis (CVA) was done to compare shapes among four limbs, expressing their differences as Mahalanobis distances (Md). The major purpose of CVA is to maximize differences between groups by producing weighted variables, referred to canonical variates. Typically, the first canonical variates account for most of the variation present. All morphometric analyses were done with MorphoJ v. 1.06c (Klingenberg, 2011) and PAST v. 2.17c software (Hammer et al., 2001). The confidence level was established at 95%.

Results

Allometry

Significative regression of centroid size *versus* shape appeared with a 2.7% and 3.4% of shape variation explained by size variation for fore and hindlimbs, respectively ($p < 0.001$).

Hoof size for each limb

Sizes were similar among all limbs ($p > 0.05$) (Table 1 and Fig. 2).

Hoof size symmetry for fore and hindlimbs

For hoof size, Procrustes ANOVA showed highly significant variations in symmetry within individuals and sides*individual interaction (FA), but no for sides (DA) (Table 2).

Hoof shape symmetry for fore and hindlimbs

For hoof shape, Procrustes ANOVA showed highly significant variations in symmetry within individuals and sides*individual interaction (FA), but not for sides (DA) (Table 3), with a 2.15% and 23.8% of the variance for FA for fore and hindlimbs, respectively. CVA reflected statistical differences between all

Table 1. Results of the two-way NPMANOVA (Non-Parametric Multivariate Analysis of Variance) using Euclidean distances for shape coordinates, and "side" and fore or hindlimb as factors. There appeared no differences among limbs.

Source	Sum of squares	Degrees of freedom	Mean square	F	p
Fore/hind	3841	1	3841.0	1.1335	0.2949
Right/left	5124.8	1	5124.8	1.5123	0.2262
Interaction	–13727	1	–13727	–4.0508	0.8371
Residual	3.52E + 05	104	3388.8		
Total	3.48E + 05	107			

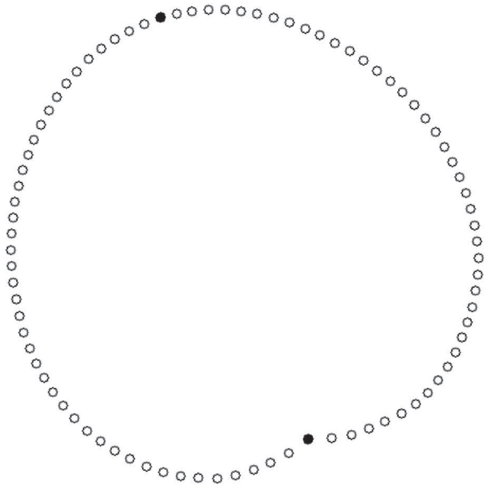

Fig. 1. Hoof outline, digitized by a set of 88 semi-landmarks and two anatomical landmarks (black dots) located at the two axial-most positions (most dorsal and most posterior).

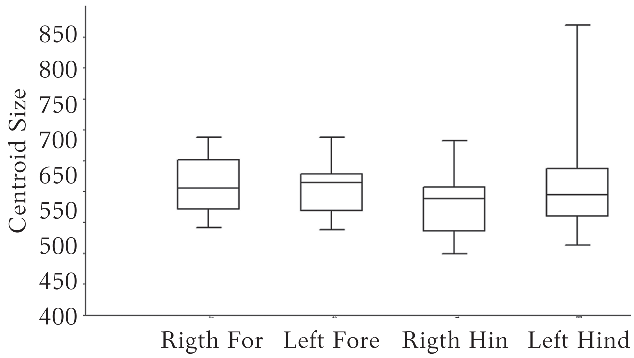

Fig. 2. A box plot for centroid sizes (interpreted as hoof surface) for 12 right forelimbs, 12 left forelimbs, 15 right hindlimbs and 15 left hindlimbs in Pyrenean Catalan Horse ("*Cavall Pirinenc Català*").

For each sample, the 25–75% quartiles are drawn using a box. The median is shown with a horizontal line inside the box. The minimal and maximal values are shown with short horizontal lines ("whiskers"). Centroid sizes were statistically similar among all limbs.

Table 2. Procrustes ANOVA of hooves size and shape in matching symmetry for 12 forelimb pairs for Pyrenean Catalan Horse ("Cavall Pirinenc Català"). Sums of squares and mean squares are in units of Procrustes distances (i.e. dimensionless).

1/Size

Effect	Sum of squares	Degrees of freedom	Mean square	F	*p*
Individual	73818.127911	6710.738901	11	17.34	< .0001
Sides	36.894910	36.894910	1	0.10	0.7633
Individual*sides	4256.736665	386.976060	11	2.69	0.0208
Error	3456.387249	144.016135	24		

2/Shape

Effect	Sum of squares	Degrees of freedom	Mean square	F	*p*
Individual	0.15618727	0.0000806752	1936	2.12	< .0001
Sides	0.00692511	0.0000393472	176	1.04	0.3627
Individual*sides	0.07351831	0.0000379743	1936	1.54	< .0001
Error	0.10406824	0.0000246374	4224		

Note: sides = directional asymmetry (DA); individual*sides = fluctuating asymmetry (FA).

Table 3. Procrustes ANOVA of hooves size and shape in matching symmetry for 15 hindlimb pairs from Pyrenean Catalan Horse ("*Cavall Pirinenc Català*"). Sums of squares and mean squares are in units of Procrustes distances (i.e., dimensionless).

1/Size

Effect	Sum of squares	Degrees of freedom	Mean square	F	*p*
Individual	182141.272015	13010.090858	14	2.86	0.0293
Sides	10297.793839	10297.793839	1	2.27	0.1545
Individual*sides	63649.296804	4546.378343	14	22.10	< .0001
Error	6172.944814	205.764827	30		

2/Shape

Effect	SS	MS	df	F	*p*
Individual	0.16326655	0.0000662608	2464	1.55	< .0001
Sides	0.00761430	0.0000432631	176	1.01	0.4411
Individual*sides	0.10524435	0.0000427128	2464	1.36	< .0001
Error	0.16521947	0.0000312916	5280		

Note: sides = directional asymmetry (DA); individual*sides = fluctuating asymmetry (FA).

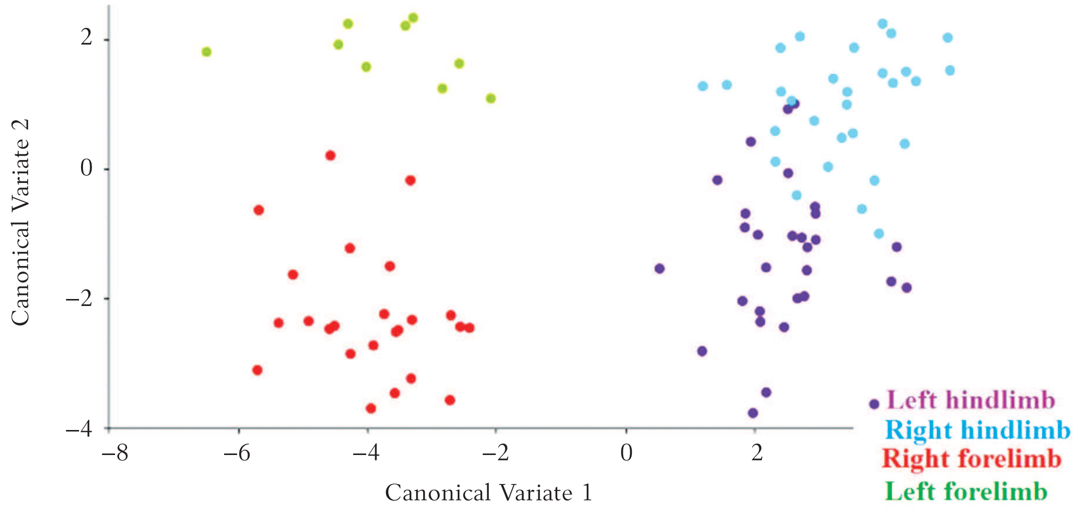

Fig. 3. Canonical Variate Analysis for 12 forelimb pairs and 15 hindlimb pairs shape in Pyrenean Catalan Horse ("*Cavall Pirinenc Català*"). It reflected statistical differences between pairs ($p < 0.05$), and left and right hindlimbs appeared less asymmetrical between them.

limbs, although hindlimb pair showed less asymmetry (Md = 2.735) than forelimb pair (Md = 3.651) (p values from 10,000 permutation rounds < 0.001) (Fig. 3).

Discussion

The study aim was to assess hoof size and shape asymmetries on the solar surface in a local yearling breed maintained under extensive management. Having not care of feet, conclusions of this research can express natural horse hoof wearing. We applied geometric morphometric methods to study asymmetries.

Few studies have evaluated asymmetries using geometric morphometric methods in horses. The more symmetrical certain characters are, the fitter the individual is said to be; thus, increased levels of stress supposedly disrupt developmental processes and correlate with increased levels of asymmetry (de Coster et al., 2013). Detected fluctuating asymmetry of the solar surface in our study may provide, in our opinion, and indicator of mechanical, not environmental, stress. In fact, hooves are rigid pieces that, as a robust but malleable tissue, can change shape according to pressure forces. So, if hoof surface in the Pyrenean Catalan Horse exhibits asymmetry, it may be just a mere plasticity due to pressure forces. This plasticity would be expressed more uniformly in the hind pair, the "motor" part of the horse body. Moreover, larger solar surfaces (measured by centroid size) had different shapes, something logical as horse mass influences hoof morphology (Leśniak et al., 2019).

In conclusion, horses' hooves are not perfectly symmetric and actually do not form mirrored pairs with their opposing hoof, at least in yearlings of Pyrenean Catalan Horse. Among other age group or elite performance breed, data could be totally different. It has to be studied in further studies.

Conflicts of interest

The authors declare no conflicts of interest.

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Supporting information

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

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