Forefeet are more asymmetrical than hindfeet in unshod horses

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Abstract

The size and shape of paired structures usually differ between the left and right sides. To study it among unshod horses, sample of 59 unpaired feet (14 right hindfeet, 14 right forefeet, 15 left forefeet and 16 left hindfeet) of clinically non lame yearlings were included in this study. Animals belonged to Catalan Pyrenean breed (*Cavall Pirinenc Català*). Radiographic images in dorso-palmar/plantar projections were individually obtained and analyzed by means of geometric morphometrics. A total of 20 paired landmarks occurring on both sides of the bones (distal metapodium [2], proximal phalanx [8], middle [4] and distal phalanx [6]) were located on each image. Results showed that shape variation between feet was highly significant both for fluctuating asymmetry -the deviation of the symmetry of an individual from perfect bilateral symmetry- and for directional asymmetry -a greater development on one side than on the other-. I found smaller asymmetries between hindfoot pair that between forefoot pairs, with a tendency to prone (to be rotated towards in) for right feet, and to supine (to be rotated towards out) for left feet. The influence of natural biomechanics status -hindimbs having more importance on animal motion and a natural asymmetry for other functional functions, such as mastication- can be the considered cause for these detected asymmetries.

Key words: Catalan Pyrenean horse; directional asymmetry; fluctuating asymmetry; foot balance; laterality

Introduction

In bilaterally symmetric organisms, the size and shape can differ between the left and right sides (Sforza et al. 1998). In fact, it is very hard to find a real organism perfectly symmetric, and small asymmetries can reflect phenotypic adaptation to the environment (Parés-Casanova 2013). Among bilateral asymmetries we find, between others, Fluctuating Asymmetry (FA) and Directional Asymmetry (DA) (Graham, Freeman, and Emlen 1993). FA is defined as the random deviation of the symmetry of an individual from perfect bilateral symmetry (Graham, Freeman, and Emlen 1993). These developmental accidents are generally the result of genetic or environmental stress (Alados et al. 2001) (Angelopoulou, Vlachou, and Halazonetis 2009) (Blackburn 2011) and are considered a negative indicator of the ability to resist small developmental accidents (Graham, Freeman, and Emlen 1993). On the other hand, DA occurs whenever there is a greater development of a character on one side of the studied bilateral structure than the other (Kharlamova et al. 2010). It has been traditionally stated that a proportion of DA has a genetic basis (Carter, Osborne, and Houle 2009).

Because skeletal elements of feet undergo remodeling during development, asymmetrical loading can cause morphological asymmetries (Breno, Bots, and Van Dongen 2013). DA in extremities have been suggested to be due to behavioral lateralization (White, Panjabi, and Hardy 1974) (Illmore, W, and Klingenberg 2005) (Wilson et al. 2009) (Parés-Casanova 2014a). In humans, DA appears to increase with age, possibly due to sustained mechanical loading (Blackburn 2011). Handedness is the cause of their morphological asymmetries (Lotto and Béguelin 2014) (Farmer, Krueger, and Byrne 2010), favoring the right side upper foot (Auerbach and Ruff 2006). But correlations between DA and lateralization in other vertebrates have been less studied (Breno, Bots, and Van Dongen 2013).

In horses, there are detected high frequencies of skeletal asymmetries (Wilson et al. 2009) (Nicolai et al. 2017) (Leśniak 2018), but their quantification has been rarely applied. Probably the oldest and most used method is to measure metric distances, areas, angles, and ratios in the left and right side separately and to calculate the difference between paired measurements (Sforza et al. 1998) (Roland et al. 2003). This method provides good information about the differences in size, but it does not reflect differences in global shape (Sforza et al. 1998), as geometric morphometrics allows. Researches on foot asymmetries have also shown that loadings are different within bilateral pairs (Wilson et al. 2009).

The aim of this study was to determine asymmetries in phalanges in an equine breed based on radiographs and assessed by geometric morphometric methods. As the breed is not subjected to work/ridden management, foot-trimming nor intensive husbandry, results can be considered to reflect natural balance of unshod horses. Moreover, from this study there are raised implications on health and management of horses maintained under extensive conditions, as it assesses natural conformational traits. This is the first time this research is done in a hypermetrical horse, as the Catalan Pyrenean breed is.

Materials and methods

A sample of 59 unpaired feet of clinically non lame colts belonging to Catalan Pyrenean breed (*Cavall Pirinenc Català,* CPC) below of 12 months of age (yearlings) was collected at the abattoir. The distribution of the sample was as follows: 14 right hindfeet, 14 right forefeet, 15 left forefeet and 16 left hindfeet. All animals were clinically sound and showed no lameness during the *ante mortem* abattoir official veterinary inspection previous to their sacrifice. Individual information was not possible for all samples, so exact age, sex and body weight could not be considered.

CPC is a breed raised for meat production under extensive management along NE Pyrenees. It is a hypermetrical (live body weight for adults more than 500 kg), compact, broad-built horse with rather short feet (Parés-Casanova 2011) (Parés-Casanova and Oosterlinck 2012). Genetic analysis suggests that this small population (<4,600 individuals) is closely related to the Breton and Comtois breeds (Infante González 2011) (Parés-Casanova 2011), and sometimes is ill known as *Hispano-Bretón*. The breed is never trimmed and is managed under semi-extensive conditions (natural grazing all year round), receiving a minimal preventive care (normally a vermifugation once per year).

Before radiographs were taken, feathers, sole and frog were cleaned with water. Images were then obtained using a computed radiography system. The exposure factors for dorso-palmar/plantar view was 60 kV and 3.2 mAs with each foot placed on a block *ca*. 5 cm high and the x-ray beam centered approximately to the fetlock. The cassette was vertically positioned as close as possible to the foot, without touching it. Each image was marked with a rigid radiodense marker for calibrating images.

A total of 20 paired landmarks occurring on both sides of the bones (distal metapodium [2], proximal phalanx [8], middle [4] and distal phalanx [6]) were located on each image (Figure 1). The landmarks were chosen to have a good representation of the overall autopodial shape and in a way that allow us to see sides asymmetry. The captured radiographic images were ulteriorly transformed using TpsUtil software v. 1.40 (Rohlf 2015a) and landmarks recorded using TpsDig software v. 2.26 (Rohlf 2010). TpsSmall software v. 1.33 (Rohlf 2015b) was used to test whether the distribution of points in the tangent space could be used as a good approximation of their distribution in shape space.

Scale was eliminated by setting the centroid size, the square root of sum of squared distances between the centroid and each landmark (Zelditch, Swiderski, and Sheets 2004). By eliminating size information, only the shape is extracted and the landmark coordinates can be used as quantified shape information in the subsequent mathematical analysis (Zelditch, Swiderski, and Sheets 2004).

Statistical analyses

Measurement error (ME) is of critical importance when analyzing FA (Palmer 1994). To assess the significance of FA relative to ME, all individuals were digitized twice. Then I applied a Procrustes ANOVA for shape taking into account all the values of mean squares of the ANOVA (Klingenberg, McIntyre, and Zaklan 1998) (Klingenberg and Monteiro 2005). Analyses were done for asymmetry component (measured from the differences between configurations from the left and right sides of each individual) (Klingenberg, Barluenga, and Meyer 2002). Allometry was studied by a regression of shape coordinates against centroid size (values log-transformed, 10,000 permutations round) (Klingenberg 2016). FA was interpreted as the effect interaction “individual * side”, while DA as the effect “side” (Klingenberg, Barluenga, and Meyer 2002). Finally, differences between feet were assessed performing a Canonical Variate Analysis (CVA) using

Mahalanobis distances and 10,000 permutation rounds. Analysis were done with MorphoJ software v. 1.06c (Klingenberg 2011) and PAST software v. 2.17c (Hammer, Harper, and Ryan 2001). For all tests, α=0.05.

Results

First analyses indicated an excellent correlation between the tangent and the shape space as the correlation (uncentered) between the tangent space *Y*, regressed onto Procrustes distance was 0.99994. The general estimate of shape was enough accurate as the measurement error was smaller than the true FA (mean squares values for individual x side for shape: 0.0000876291 in comparison with ME: 0.0000078441) (table 1). Feet presented no allometry trend (p=0.099), with only a 0.794% of shape variation explained by size. CVA indicated asymmetric variation between feet was highly significant between feet (p<0.0001), with the asymmetric component tending to group fore and hindfeet separately and being hindfeet less asymmetrical between sides (Figure 2). In forefeet, DA explained about 52.34% of the total variance (*versus* 8.80% of FA) and in the hindfeet it decreased to 45.37% of the total variance (*versus* 17.22% of FA). Right feet (both fore and hind) tended to present a proximal pronation (towards in), while left feet (both fore and hind) showed a more general change. Left forefoot also showed a proximal supination (towards out), a median pronation and a distal rotation, while left hindfoot supinated also distally. In general, directional changes were more extended in left feet (Figure 3).

Discussion

In the very first stages of the vertebrates development, a midline divides them into two symmetric left and right parts (Sforza et al. 1998). The organism develops then a left-right axis early on that will be the base for the lateralization (Sforza et al. 1998). Most of the body will maintain an apparent macroscopic symmetric pattern, although detailed analysis will reveal more or less evident morphologic asymmetries.

The analyzed autopodes of CPC showed directional asymmetry (DA) between pairs, which was higher in forefeet. But for right and left feet, it was showed a different pattern in opposite directions: right feet tended to pronate, while left feet tended to supinate. The conformation of the equine foot can be viewed as a dynamic platform for its locomotor function (Pollitt and Collins

2011), so this DA is thought to be a reflection of a compensatory action (Auerbach and Ruff 2006). This would be congruent with frequent asymmetries in foot spread of animals, especially on forefeet, which suggest unequal loading of the feet (Wilson et al. 2009).

I am not able to assess the influence of asymmetrical strain exerted during the movement of the animals, but it may hypothesize that forces induced by physical exercise might play an important role in explaining our results. This asymmetric pattern can be attributed either to the mechanical consequences of handedness bias or to genetic factors. For some authors (Leśniak 2018) handedness would be likely a species trait, and specifically masticatory laterality in horses have been described function (Parés-Casanova 2014b). The varying patterns of asymmetry detected here may thus indicate that physical forces place differing stresses on each phalanx, yielding these a general right displaced asymmetry. Moreover, the detected breed has no performance (is only for meat production), and it has been noted by other authors (van Heel et al. 2006) (Wilson et al. 2009) (Hobbs et al. 2018).

Detected asymmetries would be a putative signal of lateralized motor activity (van Heel et al. 2006) (Hobbs et al. 2018), emerging from different mechanical loadings across sides due to handedness. If this observed directional asymmetry have a genetic/developmental basis cannot be stated, having de need for similar studies among other breeds, so it is strongly recommended to conduct a similar study in other breeds of horses, as well as in horses with lameness. Moreover, because lateralization can have genetic basis, and consequently it could develop prenatally as a pre-adaptation to adult life, similar studies but during early development would be very interesting to perform.

Ethics Statement

This study was carried out in material from slaughtered animals for commercial purposes other than those of the research, and in any case they were euthanized, so no Ethics Committee agreement was considered to be necessary.

Conflicts of interest

The author declares no conflicts of interest.

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Table 1. Result of Procrustes ANOVA. Both DA (effect “side”) as FA (effect interaction “Individual * Side”) appeared significative (in bold), being the mean square (MS) of the former clearly higher. Also, MS of Measurement Error was smaller than the true FA.

Effect	SS	MS	Df	F	P
Individual	0.27768252	0.0002706457	1026	3.09	<0.001
Side (DA)	0.00808788	0.0004493264	18	5.13	**<0.001**
Individual * Side (FA)	0.08990748	0.0000876291	1026	11.17	**<0.001**
Measurement Error	0.01637845	0.0000078441	2088	-0.05	NaN
Residual	-0.01154162	-0.0001603003	72		

DA: Directional Asymmetry

FA: Fluctuating Asymmetry

SS: Sum of Squares

MS: Mean Squares

Df: degrees of freedom

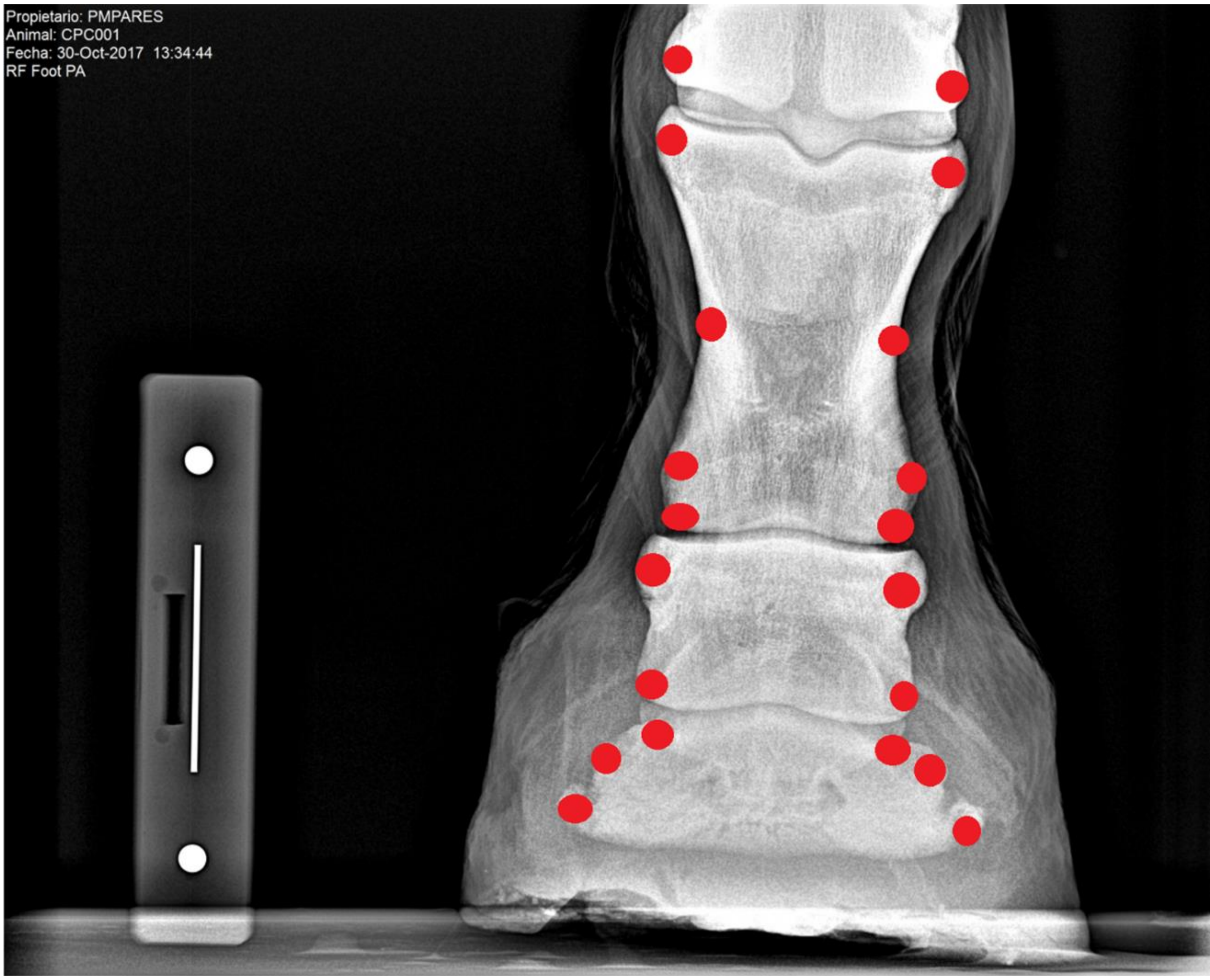

Figure 1. Dorso-palmar/plantar view of autopodium on which a total of 20 paired landmarks occurring on both sides of the bones (distal metapodium [2], proximal phalanx [8], middle [4] and distal phalanx [6]) were located on each image.

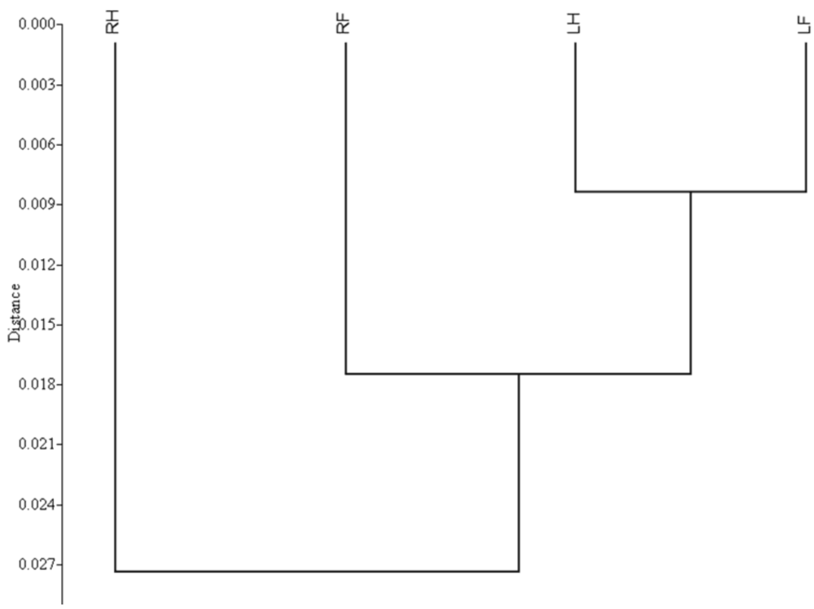

Figure 2. Clustering of asymmetric components per foot (averaged values) for the sample of sample of 59 feet of clinically non lame horses belonging to Catalan Pyrenean breed (14 right hindfeet RH; 14 right forefeet RF; 15 left forefeet LF; and 16 left hindfeet LH). Cluster showed a tendency to group fore and hindfeet separately, being hindfeet less asymmetrical than forefeet.

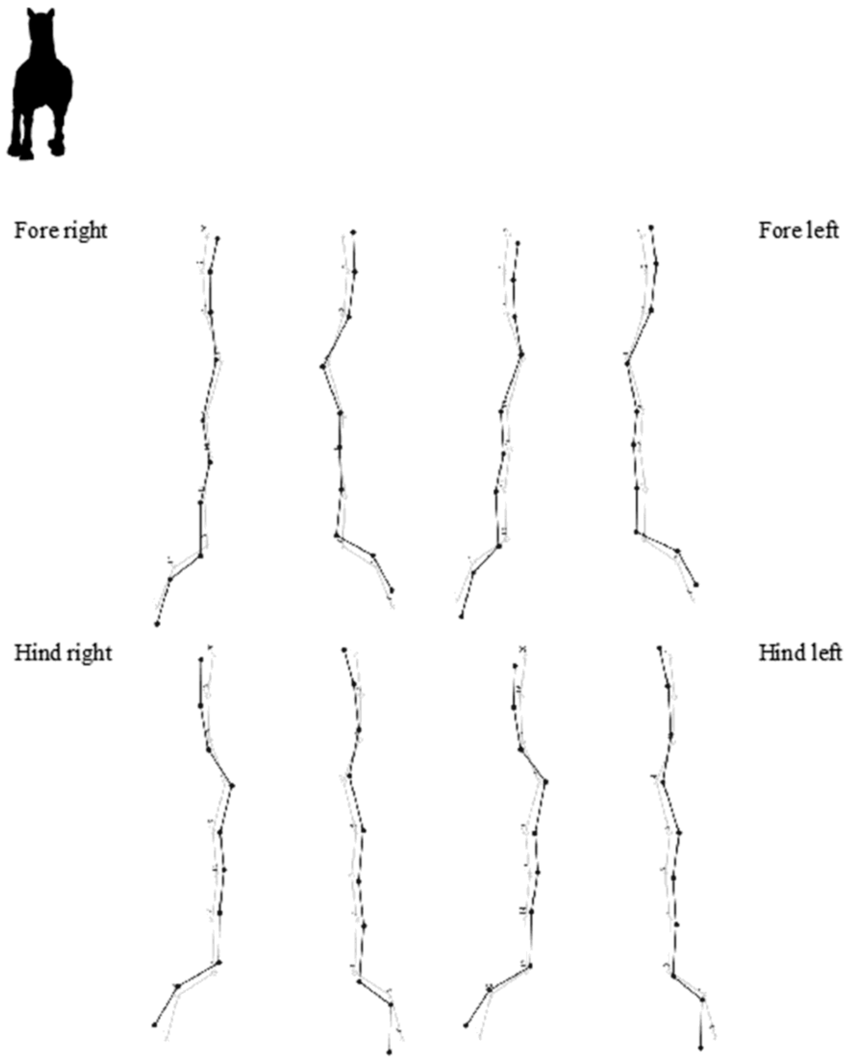

Figure 3. Shape changes for directional asymmetry (dorsal view). Clearer line represents the starting shape, darker represents the target shape. Forefeet tended to displace towards left, while hindfeet towards right. Note that the component distances are not side-specific measurements but concern the relation between the separate sides and their joint midline.