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## **Molar asymmetry shows a chewing-side preference in horses**

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### **ABSTRACT**

*Deviations from expected perfect symmetry can occur, and organisms can develop several kinds of asymmetry. Among others are fluctuating asymmetry (FA) and directional asymmetry (DA). FA represents small random differences between corresponding parts on the left and right sides of an individual in bilaterally paired structures; it is thought to reflect an organism's ability to cope with genetic and environmental stress during growth. DA occurs whenever one side on the plane of symmetry develops more than the other side, and has a genetic component. In this research, we tested the presence of dental FA and DA by examining the expression of asymmetry in the upper first molar of 25 individuals belonging to the "CavallPirinencCatalà" horse breed. Size was not different between sides, but there was significant FA. We suggest that the predominant source of detected occlusal variation is only behavioural, being a chewing-side preference, a preference for one side of the dentition during chewing, as has been described in humans.*

**Key words:** "CavallPirinencCatalà", directional asymmetry, fluctuating asymmetry, molar size/shape, Pyrenean horse

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### **INTRODUCTION**

Teeth are the hardest of tissues and, unlike other tissues, are constantly worn away by prolonged use [1]. If this wearing is not uniform (whatever the cause), one could expect different occlusal appearances between sides [2]. Environmental factors could influence dental growth and can lead to bilateral tooth size asymmetries, despite the fact that both sides of equine dentition have the same basic genetic determination. Deviations from expected perfect symmetry can occur, and organisms can develop several kinds of asymmetry, including fluctuating asymmetry (FA) and directional asymmetry (DA). FA represents small random differences between corresponding parts on the left and right sides of an individual, variation in nondirectional differences between left and right sides of bilateral characters. Because of its characteristics, FA has been usually considered to be a measure of developmental noise [3, 4, 5]. DA occurs whenever one side on the plane of symmetry develops more than the other side, and has a genetic component [6, 7]. It has been suggested that DA may be related to developmental timing differences between antimeres, which may in turn result from developmental stress in a population [8]. The aim of the present research was to investigate the potential effect of developmental stability and environmental stress on molars in "CavallPirinencCatalà" (Catalan Pyrenean horse, CPC), assessed by means of FA and DA.

CPC is an inexpensive horse bred for meat production in the harsh environment of the northeastern part of the Pyrenees along the Spanish-French border [9], being compact, broad-built, and predominantly chestnut, with rather short limbs [10]. Genetic analysis suggests that this small population (<4600 individuals) [11] is closely related to the Breton and Comtois breeds [12]. The horses are reared outdoors throughout the year and do not receive any additional food besides some low-quality straw in winter. They do not receive any systematic clinical care and receive little dental care. The results of this study will contribute to new morphological information about "natural" tooth wear. Another added benefit is that teeth tend to be preserved very well in archaeological settings so the results can also act as an aid for zooarchaeologists evaluating results from studies on buried animals in comparison with living equine populations. To the authors' knowledge, no similar work has been done on this species to date.

### **MATERIALS AND METHODS**

#### *1.1. Sampling*

Thirty-five edentate skull specimens were obtained from horses belonging to the CPC breed, aged more than 8–12 months, and assessed by eruption of upper first molar ( $M^1$ ). No cases of tooth diseases (peg-shaped, dental agenesis,

asymmetrical wear, chronic abscesses, etc.) were detected in a previous ocular inspection of specimens, which are now held at the collection of the Animal Production Department in the University of Lleida.

### 1.2. Morphometrics

All molars were photographed in the occlusal view with a Nikon®D70 digital camera (image resolution of 2240 × 1488 pixels) equipped with a Nikon AF Nikkor®28–200 mm telephoto lens. The camera was placed on a tripod parallel to the ground plane so the focal axis of the camera was parallel to the horizontal plane of reference and centred on the ventral aspect of each skull and at a distance of more than 1 m in order to minimise lens distortion consistently. Both right and left molar series inside the alveoli were included (a picture was taken for each skull, so the same picture included right and left molars). A scale was included in the images to standardise each specimen size (in mm). Tooth pictures were digitised using tpsDig version 2.16 [13]. A total of 12 landmarks symmetrically distributed on the left and right first upper molars ( $M^1$ ) were assessed (Figure 1). These points were chosen to ensure an adequate representation of the  $M^1$  that comprised the molar row. Enamel pattern (protocone, hypocone, etc.) was excluded from the analysis because of the poor precision of digitisation, especially in older animals. As FA is very small compared with the size of the trait being measured, typically around 1% of the trait size, measurement error is of particular concern [7]. Moreover, Smith *et al.* [14] noted that measurement error itself could mimic FA and that sampling effects could obscure true FA if the average amount of FA was small [14]. Therefore, landmarks were digitised twice (using the same image) in two separate measurement sessions and digitisation was tested to discern true asymmetry from measurement error [7].

Variations in the form of landmark configurations were examined using geometric morphometric methods rather than a distance-based approach, because they estimate the mean more precisely, have lower type I error rates, higher statistical power, and lower bias in simulations based on the assumption of independent isotropic distributions of landmarks [15]. The Procrustes analysis was performed to eliminate differences in the size, location, and orientation of the digitised images. This analysis uses the centroid of the landmark configuration as the starting point from which rotation and scaling occur in order to eliminate differences due to discrepancies in size. Procrustes distances among landmark configurations relate to Kendall's shape space [15], which is non-Euclidean. Subsequent multivariate analyses used a projection of the scatters of individuals from Kendall's space into a Euclidean space tangent to the consensus. The software tpsSmall version 1.20 [16] was used to verify that distortion introduced by this projection was insignificant. The correlation between tangent-projected and Procrustes distances was large and significant ( $N=100$ ,  $r^2=0.999$ ), indicating that their variations were sufficiently small that the tangent space approximated well the shape space in analyses. This is also reassuring with regard to the robusticity of the findings in relation to the choice of morphometric approach.

### 1.3. Allometry

Occlusal centroid size (CS) was computed for each specimen as the square root of the mean squared distance from each landmark to the centroid of the landmark configuration [17]. Natural log transformation was applied in order to scale centroid size relative to the mean properly [18].

### 1.4. Size and shape asymmetry

To analyse size asymmetry, we used a Mann-Whitney U test. To analyse shape asymmetry, we used a two-way, mixed-model analysis of variance (ANOVA) with repeated measures, where sides were fixed and individuals were random [7]. Measurement error was assessed by this [19]. In these analyses, the main factors were individuals and sides, with the side × individual ( $S \times I$ ) interaction assessing the extent of FA, which was evaluated by the F statistic using the remainder mean square ( $MS_{SJ}$ ) over the mean square due to measurement error ( $MS_M$ ). If the  $I \times S$  interaction mean square is significantly greater than the error mean square, it suggests FA [4]. If significant, the variance component not due to DA is  $\sigma_i^2 = (MS_{SJ} - MS_M)/2$ , where 2 is the number of repeated measures per tooth. DA was evaluated using the mean square for sides ( $MS_S$ ) over the remainder mean square ( $MS_{SJ}$ ). Measurement error was  $(MS_M)^{1/2}$ , where  $MS_M$  is the error mean square of this individual interaction [4]. Table 1 contains the ANOVA model with the expected mean squares, in which S is the number of sides, J is the number of individuals, and M is the number of measures per side. Principal component analysis (PCA) was carried out on a covariance matrix of  $I \times S$  interactions to estimate shape variation among individuals. It is also useful to visualise FA-related deformation.

MorphoJ v. 1.05 by Klingenberg [20] was used to obtain CS and study multivariate allometry. Asymmetry values were obtained using the 'Symmetry and Asymmetry in Geometric Data' (SAGE) program, version 1.21, available at <http://www-personal.umich.edu/~emarquez/morph/>. The rest of the data were analysed using PAST v. 3.01 Paleontological Statistics Software Package for Education and Data Analysis [21] available at <http://www.nhm.uio.no/norlex/past/download.html>. Analyses were conducted with the full dataset.

## RESULTS

The Mann-Whitney test indicated that there were no differences in median size among sides ( $U=1125$ ,  $p=0.396$ ). Shape was not related to  $\log CS$  ( $r=0.006$ ,  $p=0.947$ ).

The results of Procrustes ANOVA are shown in Table 2. As measurement error did not exceed mean square FA values, the results are considered to be valid. Both individual (I), which estimates shape variation among individuals, and individual  $\times$  side interaction (I  $\times$  S), which estimates FA, appeared to be significantly different ( $p<0.0001$ ). The factor 'individual' explained the largest proportion of the total variation around the symmetric consensus. The second factor that contributed to the highest variation was I  $\times$  S interaction, denoting an effect of increased developmental instability. Side (S), which estimates the effects related to DA, appeared to be non-significant ( $p=0.606$ ). The first principal component explained 49.3% of the total observed variation, and showed a deformation pattern mainly located at the medio-palatine aspect of the tooth (landmark 2).

## DISCUSSION

The results obtained in this research provide meaningful insights into occlusal  $M^1$  symmetry in domestic horse, and more specifically in the CPC horse, a breed that is managed under extensive conditions, with minimal care. Thus, it can be supposed that their 'dental behaviour' is practically natural.

In CPC,  $M^1$  FA appeared to be significant, but not DA. Most studies have successfully linked FA and increased environmental stress, leading to the generally accepted study of FA as an indicator of developmental instability [22], although specific environmental disturbances that may cause bilateral asymmetry in the dentition and skeleton of humans are not well understood [7]. FA and stress are related. However, as some authors [23] have proposed, DA might result from environmental or genetic stressors that cause unilateral acceleration of mitotic rates in enamel organs. The lack of DA in the studied sample allows us to affirm that stress is not the cause of FA in CPC. Environmental circumstances such as poor medical care likely increase asymmetry, at least in humans [24], but the lack of evidence of dental pathology in the studied sample would indicate no pathological dental stress during growth and development.

In ruminants, the size of the occlusal surface area also plays an important role in the chewing efficiency, since it is assumed to be directly correlated with the quantity of food caught between the upper and lower tooth rows, increasing the number of food fragments cut or ground per chew [25]. If there is no size difference between the sides in CPC, and if that observation can be extrapolated to horses in general, the lack of size asymmetry would have no effect on chewing efficiency. The tooth wear would be explained by just motor performance (e.g., the non-allometric scaling of shape would be explained by similar physical-mechanical demands, independently of molar size).

Laterality means preference for a particular side. Hemispheric laterality relates to the portion of the brain, i.e., the cerebral hemisphere, which determines laterality in the function of peripheral organs [26]. Lateralisation was once thought to be a unique feature of the human brain. However, it is now recognised that lateralisation can be observed across a variety of animal species [27]. It is also largely unclear at what level lateral asymmetry exists in animals. Hemispheric laterality or lateral dominance in horses could be determined by chewing sidedness, as has been described in humans [28]. According to Christensen and Radue [28], chewing side preference occurs when mastication is performed exclusively/consistently or predominantly on the same side. Thus, in horses, for which a previous study has reported that they present a preferential chewing side [29], chewing would be a functional activity with right and left symmetrical components. The natural lateralised movement of the jaw during chewing would create dental wear that would be compensated for by continuous eruption of hypsodont teeth (so the occlusal area would be similar between sides), but resulting in differential wear due to uneven forces (so the occlusal shape would be different between sides). This would promote what we will define as 'kinematic fluctuating asymmetry'. We suggest that the predominant source of occlusal  $M^1$  variation in CPC is only behavioural, being due to individuals' tendency to chew more with the right or left mirror series.

Symmetry and asymmetry in antimeric tooth eruption have been shown to be sex- and race-specific in humans [30], although other authors have not found significant sexual differences in tooth FA [31, 32, 33]. Smith *et al.* [14] argued that differences in dental asymmetry could be explained by sampling effects on the small sample sizes typically used. The aspect of differences between sexes could not be evaluated as we have no information about the sex of our studied specimens. Therefore, although our final conclusion seems plausible, it should be interpreted with some caution, as not only the sample size was small, but individual sex was not known. More research with a bigger sample size of sexed specimens would be interesting. Moreover, testing could be done with more age groups



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