ABSTRACT
Deviations from expected perfect symmetry in biological forms can occur, and organisms develop several kinds of asymmetries. Among others, there are fluctuating asymmetry (FA) and directional asymmetry (DA). Thirty-two complete skulls were obtained from horses belonging to the “Cavall Pirinenc Català” breed and the mesiodistal and the palatinovestibular lengths of the right and left upper molar series were analyzed in order to study FA and DA. Neither FA nor DA were detected in the studied sample. The results of this study will provide researchers with new morphological information about natural teeth wearing (based on grazing) and may also be used as a reference for designing experimental studies, especially on mandibular catch-up growth, and as an aid to zooarchaeologists in evaluating results from buried animal studies for comparison with living equine populations.

Keywords: Cheek teeth, morphometry, Pyrenean horses.

INTRODUCTION
Bilateral symmetry, a key feature of vertebrate body plans, is rarely perfect and mild asymmetries can be found in normal growth and development as a typical adaptation of the organisms to their environment. Deviations from expected perfect symmetry can occur and organisms can develop several kinds of asymmetries, which include fluctuating asymmetry (FA) and directional asymmetry (DA) among others. FA represents small random differences between corresponding parts on the left and right side in bilaterally paired structures; it is thought to reflect an organism’s ability to cope with genetic and environmental stresses during development, and it is usually considered as a measure of developmental noise (Palmer, 1994).
This deviation from perfect bilateral symmetry can be caused by environmental stresses, developmental instability or genetic problems during development. It is thought that the more perfectly symmetrical an organism is, the better it has been able to handle developmental stress and the more developmental stability it has.

DA happens whenever one side on the plane of symmetry develops more than the other side, and this has a genetic component (Van Valen, 1962; Palmer & Strobeck, 1986). Deviations from perfect symmetry can be measured as the variance (or related measures of dispersion) of linear dimensions, shape variation involving landmarks, or continuous symmetry measures (Graham et al., 1993). The classic measure of FA is the variance (or standard deviation) of the difference between the values of a trait on the left and right sides. If $d_i = r_i - l_i$, where $d_i$ is the signed (with plus or minus) left-right asymmetry of individual $i$, $l_i$ is the value of the trait on the left side, and $r_i$ is the value of the same trait on the right side. Thus, a measure of FA is $\text{Var}(d_i)$ or its square root (standard deviation) (Graham et al., 1993). In perfectly symmetrical objects, these points will be mirror images of one another, and $d_i$ will be symmetrically distributed around a mean of zero. DA is characterized by a symmetry distribution that is not centred on zero, but is significantly biased towards larger traits on either the left or the right side. FA is not likely to be adaptive as symmetry is expected to be the ideal state. On the other hand, DA is developmentally controlled and is therefore likely to have adaptive significance (Van Valen, 1962; Palmer, 1994).

The teeth are the hardest of all the tissues and, unlike other tissues, are constantly worn away by prolonged use. If this wear is not uniform (whatever the cause), different occlusal appearances between sides can be expected. Asymmetry is of particular interest as an indicator of environmental stress in populations. Environmental factors can influence dental growth and can lead to bilateral tooth size asymmetries, despite the fact that both sides of the equine dentition have the same basic genetic determination.

The aim of this work was to investigate the potential effect of developmental stability and environmental stress on
Cavall Pirinenc Català horse breed (Catalan Pyrenean Horse, CPC) molars. To the authors’ knowledge, no similar studies have been reported on domestic Equus to date.

MATERIALS AND METHODS

Specimen collection
Thirty-two complete skulls were obtained from CPC horses, older than 8–12 months. CPC is a horse breed for meat production quite well adapted to the harsh environment of the north-eastern part of the Pyrenees along the Spanish–French border (Fernández et al., 2009). The breed has been described as compact, broad-built, with a predominantly chestnut coat with rather short limbs (Parés&Parés, 1997). Genetic analysis suggests that this small population (<4,600 individuals) (Infante, 2008) is closely related to the Breton and Comtois breeds (Infante et al., 2010). Horses are reared outdoors throughout the year and do not receive additional food except for some low-quality straw in winter. Animals do not receive any systematic clinical care, and dental care is almost inexistent.

Skulls were collected opportunistically from deceased animals and none had been prepared for the abattoir. Dead animals were of unknown sex. Specimens corresponded to different age groups according to molar teeth eruption: only $M^1$ fully erupted (n=3, 8–12 months), $M^2$ fully erupted (n=11, 20–26 months) and $M^3$ fully erupted (complete molar series, n=18, more than 40 months). Estimated ages according to molar eruption were established according to Barone (2009). No cases of tooth diseases (peg-shaped, dental agenesis, asymmetrical wear, chronic abscesses, etc.) were detected in a previous ocular inspection of the specimens. After the experiment, skulls were held at the collection of the Animal Production Department in the University of Lleida.

Data collection and error measurement
Occlusal lengths are the distances between the points of maximum curvature of occlusal surfaces of a tooth: width (maximal palatinovestibular distance) and length (maximal mesiodistal distance) on the occlusal face, measured perpendicularly to the
main axis (root-occlusal surface) (Figure 1).

Figure 1. Measurements taken on the occlusal face of horseteeth. Width: maximal palatinovestibular distance. Length: maximal mesiodistal distance. Upper part of the picture: mesial aspect; left: palatine aspect.

Lengths were collected from both sides for each molar into their alveoli. The tooth dimensions were measured using a digital calliper Ratio® 150 mm, (0.01mm precision). All measurements were performed directly on the skull by a single researcher. As measurement error could be a confounding factor when assessing FA (Palmer & Strobeck, 1986), teeth were measured twice on different days. An evaluation of the repeatability was provided by two different tests: a Non-Parametric Multivariate Analysis of Variance (NPMANOVA) with Bonferroni p-corrected values, and a Mantel test using 5,000 permutations. Euclidean distances were considered for both tests. To test for consistency between pieces (right and left sides), Spearman’s $r_s$ correlation was used.

Variation is expressed as the coefficient of variation. In general, values between 4% and 10% are good average points. Much lower values usually indicate that the number of specimens is not adequate to show the variability, and much higher values usually indicate that the sample is not pure (MacFadden, 1989). NPMANOVA was applied to discriminate the differences between age groups according to the correlation matrix for all linear measurements using 9,999 permutations. For $M_1^1$, differences between age groups were assessed by means of a multivariate analysis of variance (MANOVA) test. Finally, to analyse dental asymmetry, a two-way, mixed-model ANOVA with repeated measures was used, where the sides were fixed and the specimens were random.
To assess asymmetries, the upper molar teeth from thirty-two horses (from upper first to third molar: M¹, M² and M³) were studied, quantifying asymmetries and describing differences between the left and right sides. Data were analysed using the Paleontological Statistics Software Package for Education and Data Analysis (PAST) (Hammer et al., 2001), available at http://www.nhm.uio.no/norlex/past/download.html).

RESULTS AND DISCUSSION
Tooth functionality is of the highest importance throughout a horse’s life. If teeth are unable to process food efficiently, animals may face malnutrition, reduced fecundity and accelerated senescence (Veiberget al., 2007). Besides the cracking or chipping of the enamel due to mechanical forces, tooth functionality can be compromised by atrophy of the protective enamel layer due to repeated use. As a consequence of both tooth use and ingested materials, wear has frequently been championed as a useful comparative device for detecting dental adaptations to the diet (Veiberget al., 2007). Thus, the study of dental FA can be useful for understanding masticatory and ingestive behaviour, as well as life history, and constitutes an indicator of the influence of environmental factors on a population and a tool for the study of developmental stability and genetics. Although Parés & Morros (2014), using geometric morphometric methods, detected a significant FA in the upper first molar of this breed, they found no size differences between sides.

Image-capturing device precision
The NPMANOVA showed no difference in values between the two replicates (F=0.072, p=0.927), and the Mantel test was also highly significant (R=0.943, p=0.0002), showing that the obtained precision was unlikely to constrain the results of subsequent statistical analyses in the present study. Correspondence between both lengths was high (r>0.7). Descriptive statistics were generated for each tooth (per side): range, mean, standard deviation and coefficient of variation (Table 1). Although the skewness in the measurement results from some individuals exhibited extreme values (data not shown), these outliers were kept in the data set because they can be
considered biological extremes.

**Table 1. Descriptive statistics for Cavall Pirinenc Català teeth**

<table>
<thead>
<tr>
<th></th>
<th>M&lt;sup&gt;1&lt;/sup&gt;L</th>
<th>M&lt;sup&gt;2&lt;/sup&gt;L</th>
<th>M&lt;sup&gt;3&lt;/sup&gt;L</th>
<th>M&lt;sup&gt;1&lt;/sup&gt;W</th>
<th>M&lt;sup&gt;2&lt;/sup&gt;W</th>
<th>M&lt;sup&gt;3&lt;/sup&gt;W</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right side</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>24.7-37.8</td>
<td>25.1-37.5</td>
<td>28.7-38.8</td>
<td>25.5-33.8</td>
<td>24.9-33.8</td>
<td>24.5-29.9</td>
</tr>
<tr>
<td>Mean± s.d.</td>
<td>30.5±3.419</td>
<td>30.1±3.106</td>
<td>32.4±2.131</td>
<td>30.3±1.476</td>
<td>29.2±1.605</td>
<td>27.1±1.252</td>
</tr>
<tr>
<td>CV</td>
<td>11.19</td>
<td>10.33</td>
<td>6.57</td>
<td>4.88</td>
<td>5.49</td>
<td>4.62</td>
</tr>
<tr>
<td><strong>Left side</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>24.7-37.6</td>
<td>26-37</td>
<td>29-38</td>
<td>28-34</td>
<td>25-33</td>
<td>25-30</td>
</tr>
<tr>
<td>Mean± s.d.</td>
<td>30.5±3.412</td>
<td>30.0±3.035</td>
<td>32.2±2.075</td>
<td>30.1±1.390</td>
<td>29.1±1.619</td>
<td>27.0±1.147</td>
</tr>
<tr>
<td>CV</td>
<td>11.19</td>
<td>10.09</td>
<td>6.43</td>
<td>4.61</td>
<td>5.55</td>
<td>4.24</td>
</tr>
</tbody>
</table>

W: width (mm); L: length (mm); s.d.: standard deviation; CV: coefficient of variation (%).

**Asymmetries**

Twelve normality tests for the right–left distributions were performed. Differences in M<sup>3</sup> width did not present normal distributions (p<0.05), although the Wilcoxon test showed no significant differences. Length values for M<sup>1</sup> and M<sup>2</sup> were between 10% and 11%, and the others were between 4% and 7%. For M<sup>1</sup> lengths, statistical differences were observed both between age groups (Wilk’s λ=0.387, F<sub>4,116</sub>=17.59, p<<0.001), as well as for and M<sup>2</sup> lengths (Wilk’s λ=0.596, F<sub>2,53</sub>=17.69, p<<0.001), with a tendency to shorter (lower in length) M<sup>1</sup> and M<sup>2</sup> with age.

Table 2 shows the ANOVA results with expected mean squares for the mixed-model ANOVA. The effect called *sides* is the variation between the two sides and is a measure of DA. The *interaction* (interaction individual-sides) is the failure of the effect of individuals to be the same from side to side and is a measure of FA. ANOVA indicated that FA and DA did not exist and so DA was unlikely to confound interpretations about FA variation, *i.e.* deviations about the mean directional asymmetry are largely due to developmental instability. Most studies have successfully linked FA and increased environmental stress, leading
to the generally accepted use of FA as an indicator of developmental instability (Leamy & Klingenberg, 2005). Nevertheless, specific environmental disturbances that may cause bilateral asymmetry in the dentition and skeleton of humans are not well understood (Palmer & Strobeck, 2003).

Environmental circumstances such as poor medical care likely increase asymmetry, at least in humans (Schaefer et al., 2006), but the lack of evidence of dental pathology in the studied sample would indicate no pathological dental stress during growth and development.

Table 2. Two-way replicate NPMANOVA with expected mean squares for the mixed-model results testing for FA (fluctuating asymmetry) and DA (directional asymmetry)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side</td>
<td>2.8098</td>
<td>1</td>
<td>2.8098</td>
<td>0.00427</td>
<td>0.998</td>
</tr>
<tr>
<td>Replica</td>
<td>93.201</td>
<td>1</td>
<td>93.201</td>
<td>0.14172</td>
<td>0.861</td>
</tr>
<tr>
<td>Interaction</td>
<td>3.5341</td>
<td>1</td>
<td>3.5341</td>
<td>0.00537</td>
<td>0.998</td>
</tr>
<tr>
<td>Residual</td>
<td>81549</td>
<td>124</td>
<td>657.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>81649</td>
<td>127</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Side = DA (directional asymmetry); Individual x sides interaction = FA (fluctuating asymmetry).

Significance was tested with 9,999 permutations.

In this study, mesiodistal length variation appeared to be more than 10% for M¹ and M², which could be explained by the fact that the sample included animals of decidedly different ages. Results showed no asymmetries between right and left sides. This fact could be explained assuming that CPC breed has a good bilateral chewing, i.e. right and left teeth should wear similarly over the lifespan. However, when FA for both sides is assessed by testing for differences in variance, small differences are not likely to be detected unless sample sizes are large. Smith et al. (1982) found that sample sizes of several hundred are required for this variance ratio, while Kieser (1990) argued that sample sizes of 75 or more are adequate. Sample sizes smaller than this may have led to type II errors in some studies that have attempted to test
the relationship between FA and environmental stress (Guatelli-Steinberg et al., 2006). Therefore, further research focusing on the issue of developmental stability in the equine dentition must include a large number of specimens. As in Parés & Morros (2014), the results obtained in this research provide meaningful insights into occlusal symmetry in domestic horses, 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. Our preliminary data may be used as a reference for researchers designing experimental studies, especially on mandibular catch-up growth, and as an aid to zooarchaeologists in evaluating results from buried animal studies for comparison with living equine populations.

CONCLUSIONS
The results of this study provide new morphological information about natural teeth wearing in domestic horses. FA and DA did not exist in molar series of CPC horse breed. Deviations about the mean directional asymmetry are largely due to developmental instability. Further studies must include a large number of specimens.

REFERENCES


