Size asymmetries in equine upper molar series

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Received March 1, 2014; Accepted November 25, 2014

Size morphological symmetry and asymmetry of complete horse molar series (n=16) belonging to 'Cavall Pirinenc Català' (Pyrenean horse) were decomposed using geometric morphometric methods. Fluctuating asymmetry (FA) was used as an indicator of environmental stress, and directional asymmetry (DA) as biomechanical constraints. Two-dimensional coordinates of 18 occlusal landmarks were digitized and analyzed using multivariate analyses which showed the presence of significant DA as well as FA. Stressors such as extreme temperatures, lack of dental care and parasites could explain the significative FA whereas DA could be explained by a lateralization in masticatory process, a preference for one side of the dentition during chewing, as has been described in humans.

'Cavall Pirinenc Català', cheek teeth, molar, morphological variation, *Equus*, Pyrenean horse, symmetry

Citation: Parés- P, Morros C. Size asymmetries in equine upper molar series. ECORFAN Journal-Mexico 2014, 5-13: 1064-1071

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December 2014 Vol.5 No.13 1064-1071

Introduction

Bilateral symmetry, which amongst others includes object and matched symmetries, is rarely perfect, and asymmetries can be found in normal growth and development as a typical adaptation of organisms to their environment. When deviations from expected perfect symmetry occur, organisms develop several kinds of asymmetries, among others fluctuating asymmetry (FA) and directional asymmetry (DA).

The level of FA often increases if individuals suffer developmental perturbations (Zakharov, 1989; Parsons, 1990). FA sometimes responds to genetic stressors such as inbreeding and selection, as inbreeding promotes homozygosity and this renders organisms less able to cope with changes in the environment and makes them less fit (Lerner, 1954) and is one of the best measures of developmental instability in populations (Leamy et al., 2001).

DA happens whenever one side on the plane of symmetry develops more than the other side, and has a proportion of genetic component (Van Valen, 1962; Palmer and Strobeck, 1986). Teeth are the hardest tissues and are constantly worn away by prolonged use (Getty, 1975).

From an evolutionary perspective dental wear is one of the prime selective forces that shaped not only the anatomy of teeth but the properties of the dental tissues themselves (Kaidonis, 2008) being the anatomical relationship and different wear characteristics of dentine and enamel essential for masticatory efficiency (Kaidonis, 2008).

There seems to be subtle variations in how the wear mechanisms act and they can be separated into abrasion, demastication, attrition, abfraction, resorption and erosion (Imfeld, 1996). Abrasion is the loss of tooth substance (Pindborg, 1970) due to tooth wear by exogenous material (Butler, 1972); although a multitude of foreign bodies can cause abrasion, the most common yet most overlooked is food (Kaidonis, 2008). The action of food on a tooth surface is 'non-anatomically specific'; that is, the action generally occurs over the whole occlusal surface producing a wear area as opposed to a facet (Kaidonis, 2008).

Demastication describes the wearing away of tooth substance during the mastication of food with the bolus intervening between opposing teeth. Attrition is defined as the loss of enamel, dentine, or restoration by tooth-to-tooth contact without the presence of food (Pindborg, 1970; Butler, 1972). The term abfraction is used to describe a special form of wedge-shaped defect at the cement-enamel junction of a tooth (Imfeld, 1996). Resorption describes the process of biological degradation and assimilation of substances or structures previously produced by the body due to cementoclastic, dentinoclastic and ameloclastic activity (Imfeld, 1996). And erosion is the loss of dental hard tissues by chemical action not involving bacteria (Eccles, 1982).

These mechanisms probably act together, each with different intensity and duration to produce a multitude of different wear patterns which expose modifications representing areas of differential wear resistance. For instance dentine and even cementum scoop soon after exposure. The interplay between wear and continual eruption determines the occlusal vertical dimension.

ISSN-Print: 2007-1582- ISSN-On line: 2007-3682

In general, by abrasion the buccal cusps of the lower molars and the palatal cusps of the upper molars wear faster (Kaidonis, 2008). If any of these processes affect right and left teeth sides differently, one could expect differences between series. It must be emphasized that no research studies have been conducted on these documented different clinical erosive patterns, and few studies on teeth right-left symmetries.

The horse, like other domestic mammals, has a heterogeneous dentition that consists of incisors, canines, premolars and molars of which the two last-named are similar, and because of this they are referred to simply as 'cheek teeth'. The cheek teeth dentition is characterized by most cheek teeth being hypsodont, possessing long crowns that are completely covered by coronal cement at eruption (Sahara, 2014) and continuing to erupt in length after apparition (König and Lieblich, 2009), such teeth having to last the horse until death (Budras et al., 2009). Matched symmetry between the left and right sides of the dentition due to wear is a form of matching symmetry and is expected since their genetic determination is theoretically the same, at least it is in humans.

In the present study we analyzed lateral asymmetries in the molar series of adult horse skulls using geometric morphometric (GM) techniques, with the aim to quantify asymmetries and to assess and describe differences between right and left series. These GM methods combined with tools of multivariate statistics make it possible to study the morphological variation with direct reference to the anatomical context. We have quantified FA and DA as components of the total asymmetry of the complete landmark configuration under consideration applying the Procrustes asymmetry assessment method.

Digitizing the landmarks as two-dimensional (2D) coordinates enabled us to test symmetry by interchanging pairs of landmarks and comparing the original configurations with their relabelled reflections. The within-samples sum of squares around the average of original and mirrored data, which expresses the extent to which the sample fluctuates about its own average asymmetry, correspond to FA, whereas the sum of squares for average asymmetry, i.e. the squared shape distance between the original and mirrored average, corresponds to DA.

The interest of this study is focused not only on morphological study per se, but on its implication for functional and clinical equine dental studies.

2. Materials and methods

Specimen collection

Sixteen skulls were obtained from corpses following natural death on grassland. The origin of these skulls represents a wide range of geographical origins, but all specimens belonged to the Pyrenean horse ('Cavall Pirinenc Català', CPC). No inbreeding was supposed. Specimens corresponded to adult animals > 40 months (assessed by total eruption of M^3). Classification into more closely defined age groups based on molar occlusal design was considered inaccurate as the use of surface roughness parameters (such as those proposed by Grant, 1982) to establish age is, in practice, very unclear in many of the animals sampled. No cases of cheek tooth diseases (peg-shaped, dental agenesis, asymmetrical wear, chronic abscesses...) nor osseous abnormalities (enthesopathies, osteomyelitis, periodontitis...), which could cause gross bony deformations intra vitam, were detected.

ISSN-Print: 2007-1582- ISSN-On line: 2007-3682

CPC is a local horse bred for meat production in the northeastern part of the Pyrenees along the Spanish-French border (Fernández et al., 2009), being compact and broad-built (Parés and Parés, 1997).

Genetic analysis suggests that this small population (<4,600 individuals) (Infante, 2011) is closely related to the French breeds, Breton and Comtois (Infante et al., 2010).

The horses are reared outdoors throughout the year and they are fed on grass, sometimes hay during winter, with no concentrate food except when they are being prepared for abattoir, normally around 1 year of age.

Farmers do not provide systematic clinical care to animals, so studies focused on their dentition, like this one, are representative of wearing from 'natural' grazing.

Data collection and geometric morphometric analyses

Clean and dry skulls were labelled and levelled on a horizontal plane on their dorsal plane, and then photographed once. Image capture was performed with a Nikon® D70 digital camera (image resolution of 2,240 x 1,488 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 skull ventral aspect. In total, 18-2D occlusal landmarks (homologous anatomical points) were used on each series (Figure 1).

The software TpsUtil v. 1.50 (Rohlf, 2012) was used to prepare and organize the images.

Landmarks were digitized twice using TPSDig v. 2.16 (Rohlf, 2010) by the same person (Morros) on two different days, in the same order, for assessing measurement error. In order to compare Procrustes to tangent space distances between individuals, a Generalized Procrustes Analysis superimposition (equivalent to generalized least squares) procedure of Rohlf and Slice (1990) was performed on each data set using TPSSmall v. 1.29 (Rohlf, 2014).

The high degree (r=0.999) of approximation of shapes in the sample (i.e. shape space) by the reference shape (i.e. tangent space) allowed accurate capture of the nature and extent of shape deformations in subsequent statistical analyses. Size was obtained as centroid size (CS), which is the square root of the summed square distance from each landmark to the centre of the form. An ANOVA was then used to quantify the amount of size FA and DA; results are reported as sum of squares (SS) and means squares (MS).

Finally, a Kruskal-Wallis H test was performed to know if size was different between corresponding counterpart individual dental pieces (M^1, M^2 and M^3), and a one-way ANOVA to know if mean squares differences were different for each of them too.

SAGE v. 1. 05, available at http://www-personal.umich.edu/~emarquez/morph/, was used to determine symmetries. The CoordGen6f software, available at http://www.canisius.edu/sheets, was used to obtain CS. PAST v. 2.17c, available at http://palaeo-electronica.org/2001_1/past/issue1_01.html, was used for the rest of the statistical analysis. Significance level was established at 5%.

3 Results

Measurement error resulted in FA being smaller than MS values for individuals, DA and FA (Table 1), so we proceeded with all subsequent analyses. Size of molar series differed significantly between sides, being mean CS of the left series 11.76 (SD=0.422) bigger than those of the right series 11.70 (SD=0.445) (these numbers are dimensionless) (Table 1 and Figure 2). ANOVA indicated that variation between size sides was significant concerning both FA and DA (see Table 1 for details). No differences appeared for size asymmetries between corresponding counterpart individual dental pieces (H=0.233, p=0.850), and although mean squares were highest for M^3 (0.0008, 0.0006 and 0.0021 for M^1, M^2 and M^3 respectively) no differences appeared in their variances (F=0.012, p=0.988) (Figure 3).

4 Discussion

Tooth wear is a normal physiological phenomenon where teeth, although worn, remain functional throughout life. In this study, authors have applied the general method for GM analysis of bilateral size variation and asymmetry in equine molar series in the CPC breed, which due to an extensive management can be considered as representative of a ‘natural’ grazing wearing.

It was noted that there was considerable inter-individual variation, probably due to the wide range of ages (not being possible to be adjusted merely on wearing). Increased FA may occur for various genetic reasons: homozygosity for deleterious recessive alleles, presence of certain dominant mutant alleles, deleterious gene combinations, aneuploidy, or chromosome aberrations.

Disruption of the genetic composition of coadapted gene complexes by inbreeding or selection for traits, so that the buffering potential is diminished, may increase the magnitude of developmental instability, resulting in increased FA (Schaefer et al., 2006).

Many studies show overall FA to be higher in homozygotes than in heterozygotes (e.g. Palmer and Strobeck, 1986), and some reports in the literature support the hypothesis that developmental instability, resulting in increased FA, is associated with inbreeding and homozygosity. But as it has been demonstrated the low rates of consanguinity in CPC horses (Infante, 2011), and stressors in the environment may be the reasons for such asymmetries.

Stressors such as pollution, malnutrition, toxins, extreme temperatures, or parasites have been described (Clarke, 1992; Markow, 1994, 1995; Woolf and Markow, 2003). Pollution and malnutrition can be excluded, as being bred for meat purposes, animals are rarely seen in poor corporal condition; moreover, they thrive in mountainous areas ‘a priori’ far from polluted areas.

Extreme temperatures for a horse that thrives all year round outdoors in Pyrenean areas (where minus zero temperatures and long snow and especially cold periods are not rare during winter), the lack of dental care, and generally poor anthelmintic programmes (Infante, 2011) could explain the significant detected levels of FA, uniformly distributed on the occlusal molar series. For DA, it could be assumed not to be an indicator of developmental (in)stability but a mere preference for performing an action with one side of the body or to one side.

The tooth form functions when the mandible moves with a wide, 'lateral' masticatory cycle to produce a shearing action made possible by a shallow glenoid fossa. That is, the opposing enamel blades move past one another to produce what is called 'scissorial point cutting', which is a very efficient masticatory action (Every, 1972) and this may induce the appearance of bilateral asymmetry, which for our study would consist of enhanced development of left compared to right series. Over time, mastication causes tooth wear, which in turn would affect tooth size and hence the occlusion and again in time the masticatory pattern.

Our study revealed the presence of significant size FA and DA in equine molar series. The results of FA on the whole would indicate the presence of some stressors (extreme temperatures, lack of dental care, and a poor anthelmintic programme), apparently sufficient to infer developmental stability and minimize fitness and DA would be explained by a lateralization in masticatory function. But the interpretation remains just that: an interpretation, not an inference.

We know too little about how wearing is physically and chemically related to components of the diet or the habitat, and to what extent they are the result of tooth morphology and function, foraging and chewing behaviour, digestive physiology, thegosis, salivary and food chemistry, in horses.

A clear shortcoming of this study is the fairly low sample size, which probably would mask the different levels of asymmetry between molar pieces.

Moreover, occlusal surface must be described as a complex landscape with more or less elevated alterations, only possible working with 3-D techniques which have not been applied here (the level of 'texture' is not possible to be evaluated with the picture-based method used in this study, which takes into account only horizontal features – spacing parameters).

The detected phenomena must be explored further through future studies, as well as with experimental models. What is clear is that these morphometric studies should open up promising areas of research in many anatomical parts of domestic ungulates, taking into account the 'breed' and 'management' factor. The study of counterpart mandibular cheek teeth (upper and lower series) would no doubt give more clues about the mechanical forces which are involved in these asymmetries.

Sources of funding

None to declare.

Conflict of interests

None to declare.

5 References

Budras K-D, Sack WO, Rock S. Anatomy of the Horse. Schlütersche Verlagsgesellschaft mbH & Co. KG., Hans-Böckler. Hannover 2009.

Butler PM. Some functional aspects of molar evolution. Evolution 1972, 26(3), 474-483.

Clarke G M. Fluctuating asymmetry: a technique for measuring developmental stress of genetic and environmental origin. Acta Zool. Fenn. 1992, 191, 31–35.

Eccles JD. Tooth surface loss from abrasion, attrition and erosion. Dent Update 1982, 9, 373-381.

Every. A new terminology for mammalian teeth: founded on the phenomenon of thegosis. Parts 1 & 2. Pegasus, Christchurch, New Zealand 1972.

Fernández M, Gómez M Delgado JV, Adán S, Jiménez M. Guía de Campo de las Razas Autóctonas Españolas. SERGA. Madrid 2009.

Getty R. Sisson and Grossman's The Anatomy of the Domestic Animals, 5th ed., vol. 1. W.B. Saunders Co. Philadelphia 1975.

Grant A. The use of tooth wear as a guide to the age of domestic animals. In: Wilson N, Grigson C, Payne S, eds. Ageing and sexing animal bones from Archaeological sites. BAR British Series 109, 1982, 91-108.

Imfeld T. Dental erosion. Definition, classification and links**.** Eur J Oral Sci 1996, 104, 151-155.

Infante JN. Caracterización y gestión de los recursos genéticos de la población equina de carne del Pirineo Catalán (Cavall Pirinenc Català): interrelación con otras razas cárnicas españolas. Tesis Doctoral. Univ. Autónoma de Barcelona. Spain 2011.

Infante JN, Ferrando A, Parés PM, Jordana J. Estructura genética poblacional en la raza equina Cavall Pirinenc Català (CPC). Su relación con otras razas cárnicas españolas y la influencia de razas pesadas francesas. Gijón, Asturias, España: Libro de Comunicaciones VII Congreso Ibérico sobre los Recursos Genéticos Animales 2010, 66.

Kaidonis JA. Tooth wear: the view of the anthropologist. Clin Oral Invest 2008, 12, Suppl 1, 21–26.

Khalaf K, Elcock C, Smith RN, Brook AH. Fluctuating dental asymmetry of multiple crown variables measured by an image analysis system Archives of Oral Biology 2005, 50, 249—253.

König HE, Lieblich H-G. Veterinary Anatomy of Domestic Mammals. Schattauer. Stuttgart 2009.

Leamy L.J., Meagher S., Taylor S., Carroll L., Potts W.K. 2001. Size and fluctuating asymmetry of morphometric characters in mice: their associations with inbreeding and t-haplotype. Evolution 2011, 55 (11), 2333–2341.

Lerner IM. Genetic homeostasis. Oliver and Boyd, Edinburgh, U.K. 1954.

Markow TA. Developmental instability: its origins and evolutionary implications. Kluwer. Dordrech 1994.

Markow TA. Evolutionary ecology and developmental instability. Annu Rev Entomol 1995, 40, 105-120.

Palmer AR, Strobeck C. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 1986, 17, 391-421.

Palmer AR, Strobeck C. Fluctuating asymmetry analyses revisited. In: Polak M, ed. Developmental instability: cuases and consequences. Oxford Univ. Press. Oxford. 2003.

ISSN-Print: 2007-1582- ISSN-On line: 2007-3682

Parés PM, Parés R. Algunas características fanerópticas del caballo Bretón Ceretano. Av Alim Mejora Anim. 1997, 37, 53-57.

Parsons PA. Fluctuating asymmetry: an epigenetic measure of stress. Biol. Rev. 1990, 65, 57–72.

Pindborg JJ. Pathology of the dental hard tissues. 1st ed. Copenhagen: Munksgaard 1970.

Rohlf FJ. TPSDig version 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook. New York 2010.

Rohlf, FJ. TPSUtility version 1.50. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook. New York 2012.

Rohlf FJ. TPSSmall version 1.29. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook. New York 2014.

Rohlf FJ, Slice D. Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Zoology 1990, 39, 40e59.

Sahara N. Development of Coronal Cementum in Hypsodont Horse Cheek Teeth. Anat. Rec. 2014, 297, 716-730.

Schaefer K, Lauc T, Mitteroecker P,Gunz P, Bookstein FL. Dental Arch Asymmetry in an Isolated Adriatic Community. American Journal of Physical 2006, 129, 132-142.

Van Valen L. A study of fluctuating asymmetry. Evolution 1962, 16, 125-142.

Vishalakshi C, Singh BN. Effect of developmental temperature stress on fluctuating asymmetry in certain morphological traits in Drosophila ananassae. Journal of Thermal Biology 2008, 33, 201-208.

Woolf CM, Markow TA. Genetic models for developmental homeostasis: historical perspectives. In: Polak M, editor. Developmental instability: causes and consequences. Oxford University Press. New York 2003.

Zakharov VM. Future prospects for population phenogenetics. Sov. Sci. Rev. F. Physiol. Gen. Biol. 1989, 4, 1–79.