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Proximo-distal gain of asymmetry in lamb metacarpals

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Abstract

The ability of an organism to produce an ideal phenotype, despite the disturbances encountered during its development, is the causal mechanism of developmental stability. This ability is used to evaluate a variety of stress types and the genotypic ability to correct them. Geometric morphometric techniques were used to study the matched symmetry in form (size and shape) of right and left metacarpal bones in a sample of 48 lambs collected from abattoir facilities, on which 10 landmarks were located. Left and right metacarpal form showed statistically significant directional asymmetry, i.e. left and right body sides differed consistently from each other. The main shape differences were on the condylar reliefs of the distal part of the bone. These findings provide a reliable reference data set for future investigation on whether the morphology of the metacarpals is influenced by age and other factors such as the productive use of the animal (e.g. for meat, milk or wool).

Key words

Developmental instability, directional asymmetry, fluctuating asymmetry, laterality, left-right axis, morphometry.

Introduction

Developmental instability is the tendency for the phenotypic value of a trait to deviate from the value expected for an individual of a given genotype in a given environment (Palmer, 1996). Random errors, which occur during development, can lead to small deviations from perfect symmetry between body sides for bilateral characteristics. Developmental instability has been argued to be controlled genetically through such mechanisms such as levels of whole-genome heterozygosity or genomic coadaptation (Clarke, 1993) but much of the work described in the literature fails to provide a general biological mechanism that would explain patterns and mechanisms of stability in natural populations (Clarke, 1997). Developmental instability is believed to increase as a response to outside stress when buffering mechanisms that are supposed to maintain symmetrical development fail to counteract an increased number of small random errors. These errors are difficult to observe directly on the trait, but can be estimated from the increased variance in the asymmetry of bilateral characteristics across a population (Palmer, 1994; Klingenberg and McIntyre, 1998).

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The measurement tool mostly used to estimate the developmental instability is fluctuating asymmetry, this being defined as random deviations from ideal, perfect symmetry (Van Valen, 1962; Palmer, 1996). Statistically, fluctuating asymmetry can be estimated as the variance of a distribution of the differences between the left and right sides among individuals. While fluctuating asymmetry remains a controversial measure of developmental instability (Van Dongen and Lens, 2002), it has attracted increasing attention for its seemingly straightforward prediction of stress. On the other hand, directional asymmetry appears when the left and right body sides differ consistently from each other (Klingenberg et al., 1998). Its expression is mediated by a left-right axis conveying distinct positional identities to developing structures on either body side (Klingenberg et al., 1998). Unlike fluctuating asymmetry, which concerns the dispersion of individual left-right differences, directional asymmetry pertains to the mean left-right difference in a sample, and is thus statistically less difficult to estimate (Klingenberg et al., 1998). Because directional asymmetry is a mean, the variance of estimates due to random measurement errors is inversely proportional to sample size multiplied by the number of replicate measurements (Klingenberg et al., 1998). Therefore, even with a moderate sample size and two replicates, random measurement error becomes negligible (Klingenberg et al., 1998).

A large number of studies have shown examples of developmental instability in many wild species, but few studies have been done on domestic species, such as horses. According to author's knowledge, no developmental instability studies have been done on s A large number of studies have shown examples of developmental instability in many wild species, but few studies have been done on domestic species, such as horses sheep, and this is the first study to apply geometric morphometric techniques to *Ovis* species.

Material and methods

Samples

Ninety-six metacarpals were collected from 48 lambs aged 2-3 months. The animals were from different farms and were slaughtered in a commercial abattoir. No specimen presented anomalies in locomotion or deformities in limbs (assessed visually before slaughter). Metapodes were cut from the carcass and cleaned in air during ca. 5 months and then dissected in order to obtain the metacarpal. Sex and breed of animals were individually unregistered, although they belonged to typical Catalan meat breeds: Xisqueta, Ripollesa, Aranesa and "Roja del Rosselló". No specific permits were required for this study as it did not involve either *ex-professo* slaughter of animals or collection of endangered or protected organic parts.

Pictures

For each animal, paired metacarpals were leveled on a millimetrical sheet base with the palmar surface (*facies palmaris*) down and then pictures were taken from their dorsal surface (*facies dorsalis*). In order to reduce systematic measurement errors (from optical distortion) between right and left bones, pictures were taken simultane-

ously of each pair with a Nikon (Tokyo, Japan) D70 digital camera (image resolution of 2,240 x 1,488 pixels) equipped with a Nikon AF Nikkor® 28-200 mm telephoto lens. The focal axis of the camera was parallel to the horizontal plane. Ten right-left homologous landmarks were chosen for each bone: one of type 1 on the foramen, and nine of type 2 (three landmarks located on the proximal part *-basis-* and six landmarks located on the distal part *-caput-*) (Figure 1). These landmarks, producing a set of 40 raw coordinates for each pair of metacarpals, were considered to describe the size and shape variations on the entire articulation surfaces. Landmarks were independently placed on each of the pictures twice to assess the effects of digitizing on the measurement error. A test for significance of differences between two replicate groups yielded highly non-significant results ($p = 0.9028$), thus suggesting that digitizing error can be considered negligible. Landmark positions were digitized using the TpsDig software v. 2.04 (Rohlf, 2005).

Procrustes Fitting/Superimposition

The individual landmark configurations were superimposed by generalised Procrustes analysis, optimising the rotation and translation of bones so that the distances between corresponding landmarks were minimised. This step effectively scales, rotates, and translates the XY coordinate data bringing all specimens to a standardised size, orientation, and position before subsequent analysis. Generalised Procrustes analysis was implemented with the CoordGen6f software (from Sheets H.D., at www3.canisius.edu/~sheets). The TpsSmall software v. 1.20 (Rohlf, 2003) was used to assess the correlation between Procrustes and the Kendall tangent space distances to ensure that the amount of shape variation in a data set was small enough to allow subsequent statistical analyses. As the correlation of Procrustes and the Kendall shape spaces was very high ($r = 0.999$), we proceeded with the morphometric analyses.

Antisymmetry, fluctuating asymmetry and directional asymmetry

The ability of an organism to produce an ideal phenotype, despite the disturbances encountered during its development is the causal mechanism of

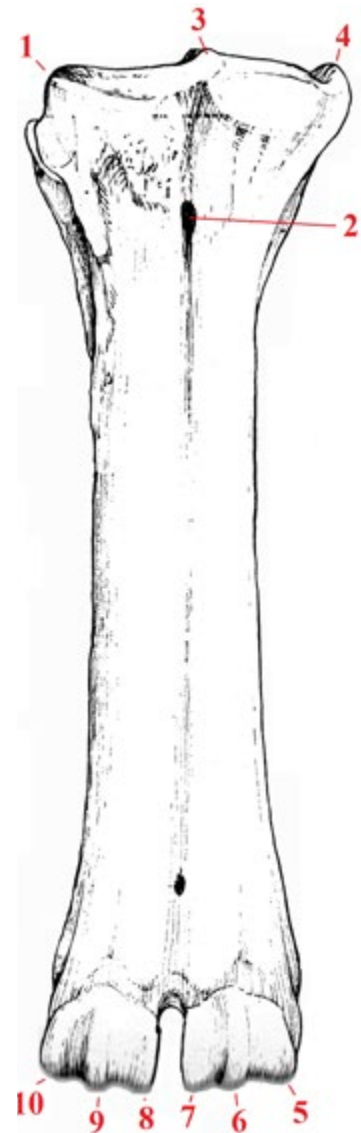


Figure 1 – Designated landmarks for geometric morphometric analysis of each metacarpal (dorsal aspect).

developmental stability (Benitez and Parra, 2001). This ability is used to evaluate a variety of stress types and the genotypic ability to correct them (Benitez and Parra, 2001). Antisymmetry, the pattern of bilateral variation where the difference between sides is consistent, but non-directional, was examined using Kolmogorov-Smirnov tests of the frequency distribution of the centroid sizes compared to an expected normal distribution (if present, antisymmetry would artificially inflate the levels of fluctuating asymmetry).

Asymmetry in overall size was estimated using the unit centroid size. The centroid size for each side of each metacarpal was calculated as the square root of the sum of squared distances from all of the landmarks on each side to their centroid (Slice et al., 1996). Each metacarpal was scaled to the unit centroid size in the analysis to eliminate the effect of individual size using CoordGen6f. To assess the fluctuating asymmetry and directional asymmetry of the metacarpal size and shape, the Procrustes ANOVA following Marquez (2008) was used, in which *p*-values were computed using the *F*-distribution (parametric test). In addition, the main effect of sides accounts for the directional asymmetry and the main effect of individuals accounts for individual variation in size. The individual×side interaction was used as the mean square for error to test the significance of the main effects. The measurement error, which was the sum of the placement and digitizing errors, was used to test for the significance of the individual×side interaction effect. The variance component of the interaction term provides an unbiased estimate of fluctuating asymmetry. For shape, directional asymmetry in each side is described as a vector of left-right differences in landmark positions. To test whether the directional asymmetry differences differ among samples, we conducted pairwise T^2 tests. Statistical analyses were conducted using SAGE v. 1.05 (Marquez, 2008). For this study a level of significance of 0.05 was used, however levels of $p < 0.001$ and $p < 0.0001$ were recorded separately.

Results

After adjusting the overall error rate to the 0.05 level, there were no significant deviations from normality as indicated by the Kolmogorov-Smirnov test ($D = 0.1167$, $p = 0.480$). Thus, we concluded that there was no evidence of antisymmetry in any of the studied population samples.

Measurement error was addressed during the *F*-tests and it was tested whether the fluctuating asymmetry estimates were significantly larger than predicted due to error alone. The measurements of directional asymmetry for size was statistically significant with $p = 0.001$ (Table 1) and the right metacarpal bigger than the left one ($p < 0.0001$). Pairwise comparisons showed that the directional asymmetry differences were also significantly different ($T^2 < 0.0001$, $F = 884.4$, $p < 0.0001$). Metacarpal shape displayed clear directional asymmetry in shape (Table 2) thus indicating that one side was consistently and significantly different in form (in shape and also in size) than the other side. Given this directional asymmetry in metacarpals form, we conducted further analyses to examine whether left-right displacements of landmarks were the same in all points but it appeared that directional asymmetries were not identical for all homologous ones. As shown in Table 3, landmarks 6, 8 and 9, which correspond to condylar reliefs, gave the major contribution to the variation in shape of the metacarpals.

Table 1 – Procrustes ANOVA of size between sides of lamb metacarpals.

Cause of variation	Sum of squares	Mean square	Degrees of freedom	F	P
Individual	21554.257	458.601	47	296.977	<<0.0001
Side	73.540	73.540	1	47.462	<<0.0001
Individual×side	72.824	1.549	47	0.110	NS
Measurement error	110.536	1.151	96	--	

The interaction term “individual×side” in this model represents the variation in left-right differences among individuals, which is a measure of fluctuating asymmetry.

Table 2 – Procrustes ANOVA of shape between sides of lamb metacarpals.

Cause of variation	Sum of squares	Mean square	Degrees of freedom	F	P
Individual	0.0511	<<0.0001	752	8.225	<<0.0001
Side	0.0075	0.00047	16	57.356	<<0.0001
Individual×side	0.0062	<<0.0001	752	0.215	NS
Measurement error	0.0589	<<0.0001	1536	--	

The interaction term “individual×side” in this model represents the variation in left-right differences among individuals, which is a measure of fluctuating asymmetry.

Table 3 – Relative contribution of each landmark point to variance, measured from the ‘Sum of Squares’ recorded from the generalised Procrustes analysis. The major contributors to variance are indicated in bold.

Landmark	Sum of Squares
1	0.00140
2	0.00000
3	0.00006
4	0.00090
5	0.00517
6	0.22642
7	0.14475
8	0.25313
9	0.36206
10	0.00610

Discussion

It has been shown that the size and the shape of metacarpal exhibit form directional asymmetry. For directional asymmetry, the more variable points that explain these systematic right-left differences are those located on the distal part of the bone (*caput*), and more specifically on the condylar reliefs of the distal part of the bone. Long bones of the limbs ossify by endochondral ossification. In sheep fetuses, the primary ossification centre in the metacarpal firstly appear in the diaphysis at 47 days (Ahmed, 2008), being metacarpal bones mono-epiphysarial. It might seem that as the sequence of appearance of the limb bones ossification centres occurs in a proximo-distal direction (Ahmed, 2008), the detected differences have an embryological explanation. However, as the most variable landmarks correspond to functional points of articulation with the 1st phalange, instead of this embryological explanation we propose a functional cause: articulation of the metacarpal with the 1st phalanges, being ossified later in time in lambs, is more prone to subtle (in a clinical sense) but detectable (statistically) asymmetry.

Directional asymmetry occurs throughout the animal kingdom (Palmer et al., 1996). The facts that, in this research, the detected left-right asymmetry appeared mostly located on the distal part of the bones and that sample animals were all juveniles raise the question if symmetric changes occur with age and whether the morphology of the metacarpals is primarily influenced by age or by other factors such as use of the animal (e.g. for meat, milk or wool).

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