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Age-dependent mandibular asymmetries in domestic pigs

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Abstract

A sample of 41 dentulous dry mandibles from adult domestic pigs were studied in order to compare both the right and left sides according to age. Samples were grouped according to dental status: "subadults" (erupting third molar, n=19) and "adults" (fully erupted third molar, n=22). Individual levels of asymmetry were analysed from the x- and y-coordinates of 16 landmarks on the dorsal aspect of the mandible. The analyses separated directional asymmetry (one side of the body with a larger character value than the other) and fluctuating asymmetry (small random deviations from perfect symmetry), which were both found to be significant. The condylar ramus was the most asymmetric structure for both age groups.

Introduction

Developmental instability arises from genetic or environmental stressors that disturb the normal developmental pathways of different continuous characteristics, producing developmental noise. This is commonly measured as fluctuating asymmetry (FA) (Van Valen, 1962; Palmer and Strobeck, 1986; Leamy and Klingenberg, 2005). FA is the variance in subtle differences between the left and right sides in bilaterally symmetrical organisms or parts of organisms, and provides a measure of how well an individual can buffer its development against internal genetic and external environmental stress during ontogeny (Van Valen, 1962).

Conversely, directional asymmetry (DA) 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 for developing structures on either body side (Klingenberg et al., 1998). Unlike FA, which concerns the dispersion of individual left-right differences, DA pertains to the mean left-right difference in a sample, and is thus statistically less difficult to estimate (Klingenberg et al., 1998). Because DA is a mean, the variance of estimates due to random measurement errors is inversely proportional to the 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).

The statistical properties of geometric morphometrics (GM) are superior to those of distance-based or angle-based methods (Rohlf, 2006 and 2007), with the supply graphics being far more legible and interpretable to the biologist. The method of GM, which is based on the study of landmarks, has made it easier to parameterise shape in this way, visualise changes in shape, and test hypotheses statistically.

Analyses of symmetry consider the left and right sides separately (Solon et al., 2012). Variation among individuals is analysed using the averages of the left and right configurations. Asymmetry is then measured by the differences in configurations on the left and right sides of each individual (Klingenberg and Savriama, 2002).

The aim of this research was to determine the degree of asymmetry between the hemimandibles in the domestic pig, whether this was due to fluctuating morphological asymmetry, and if so, whether the asymmetry was functional or mechanical.

Material and method

Samples

We studied a sample of 41 dentulous dry mandibles (os dentale) from domestic pigs, fully preserved and collected from a vulture feeding point. The sex of the samples was unknown. The mandibles were disarticulated and the skulls were not studied. The samples were cleaned and initially subdivided into three age groups according to the second and third molar (M) eruption: second M only ("young", n=3), erupting third M ("immature", n=16) and fully erupted third M (complete dentition, "adult", n=22).

Digitisation and formatting

Mandibles were labelled and levelled on a horizontal plane, and then photographed in their dorsal aspect. 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 focal axis of the camera was parallel to the horizontal plane of reference and centred on the dorsal aspect of each mandible. A ruler was used in this process (interval 50 mm). Landmarks were digitised using tpsDig version 2.04 (Rohlf, 2006). Sixteen landmarks were plotted on the mandible in

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order to describe the variations in size and shape, producing a set of 32 raw coordinates for each specimen. Fourteen of these landmarks were right-left equivalent. Figure 1 shows the location of the landmarks. "Nomina Anatomica Veterinaria" (2005) and von Driesch (1976) were used to determine the spelling of the anatomical and zoological terms in this investigation.

Procrustes fitting/superimposition

The individual landmark configurations were superimposed by generalised Procrustes analysis (GPA) implemented in the CoordGen6f software (H. D. Sheets, www.canisius.edu/sheets), standardising the size of the bones and optimising their rotation and translation 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. The TpsSmall version 1.20 software (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 between Procrustes and the Kendall shape spaces was very high ($r=0.9999$), we proceeded with the morphometric analyses. To estimate the amount of measurement error due to digitising, duplicate measurements were taken for all samples by the same person, and a Procrustes analysis of variance (NPMANOVA) was carried out. No differences appeared between replicas ($p=0.653$ and $p=0.839$ for the right and left hemimandibles respectively) so we proceeded with the average values across replicas. Size was computed as the centroid size (CS), which corresponds to the sum of the squared distances from the landmarks to the centroid of configuration (Bookstein, 1991). The CS was extracted using CoordGen6f (H. D. Sheets, www.canisius.edu/sheets).

Size and shape comparisons of the three age groups were performed initially by means of a one-way NPMANOVA using Gower distance and Bonferroni-corrected values, with 9,999 permutations. However, as the hemimandible size for each side appeared to be different between "adults" and the remaining samples ($p<0.001$), we opted to group "immatures" and "youngs" into a new category named "subadults" for all analyses. Comparisons between these two new groups were made using the two-sample Hotelling's T2 test for shape data and the Mann-Whitney U test for size data. The statistical significance of the regressions was tested with permutation tests against the null hypothesis of independence (Good, 2000).

Investigation of symmetry

A Procrustes NPMANOVA approach was used to quantify the different components of variation (Anderson, 2001). The ANOVA approach was a two-factor, mixed-model ANOVA design containing individuals and sides as the factors (Leamy, 1984; Palmer and Strobeck, 1986). DA ("sides", one side is systematically different from the other), FA ("individual x side interaction", small random deviations from perfect symmetry), and their respective errors were included as effects. Linear size dependence for FA was removed by natural log-transforming all data to obtain a size-scaled measure of FA. Finally, the patterns of shape variation related to size were compared with a multivariate regression of shape variables onto CS, using the CS and configuration of each specimen (TpsRegr version 1.28) (Rohlf, 1998).

All analyses were carried out in PAST: "Paleontological Statistics Software Package for Education and Data Analysis" (Hammer et al., 2001).

Results

Age dimorphism

Hemimandibles differed in both size and shape between "adults" and "subadults" ($p<0.01$). This is congruent with the multivariate regression of shape and CS, which was highly statistically significant ($p<0.0001$), and accounted for 25% of the total amount of shape variation. A generalised Goodall F-test (Rohlf, 1998) based on such distances suggested a significant effect for size or shape ($F=51.2239$, $df=28, 4312$, $p<<0.00001$). The hemimandible size increased from young to adult animals (Figure 2).

[enlarge]

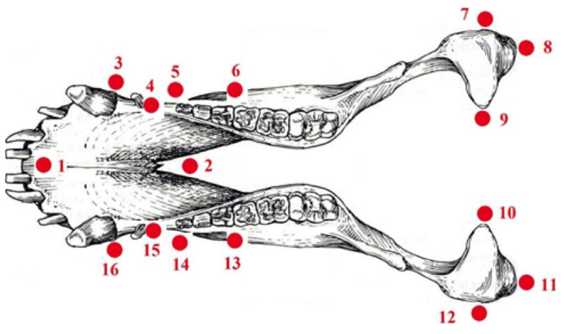

Figure 1. Designated landmarks for GM analysis of the mandible (dorsal aspect).

[enlarge]

However, between sides, size was similar in both "subadults" and "adults" ($U=168$ and $U=199$ respectively, $p>0.95$). Nevertheless, shape differed between sides for both ages ($T2<<0.0001$). This would indicate that the mandibles developed bilaterally in a similar way for size but not for

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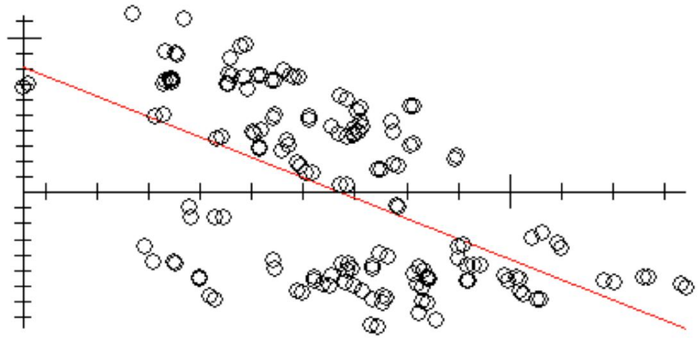

Figure 2. Scatter plot of shape against centroid size for all ages (all specimen configurations are included).

shape on each side for both age groups. According to discriminant loadings, for "subadults", the main differences were focused on C alveoli and on the lateral part of the condylar process, whereas the main differences in "adults" centred on the nuchal and lateral part of the condylar process.

Procrustes ANOVA and MANOVA

Results from the Procrustes two-way NPMANOVA for studying the left-right variation in both age groups are given in Tables 1 and 2. The consensus determined by Procrustes ANOVA indicated that DA accounted for the largest proportion of the total variation, causing the main variation in symmetric shape. Since the amount of DA greatly exceeded that of measurement error, the test for the sides yielded a highly significant result with both methods. DA was especially high for "subadults".

Source	Sum of squares	Degrees of freedom	Mean square x 10^6	F	P
Replicas	0.0085	1	0.0082	0.680	0.4578
Sides	5.1017	1	5.1017	420.440	0.0001
Individuals x side	-0.0051	1	-0.0051	-0.426	0.9952
Measurement error	0.8736	72	0.0121		

Table 1. Results of the Procrustes ANOVA conducted on the landmark sets for domestic pig "subadults" (n=19).

Discussion

Source	Sum of squares	Degrees of freedom	Mean square x 10^6	F	P
Replicas	0.0060	1	0.0060	0.57826	0.5065
Sides	5.2278	1	5.2278	501.84	0.0001
Individuals x side	-0.0029	1	-0.0029	-0.27899	0.9870
Measurement error	0.8750	84	0.01041		

Table 2. Results of the Procrustes ANOVA conducted on the landmark sets for domestic pig "adults" (n=22).

The mandible is an asymmetric bone, as many studies in humans have shown (Pierrakou, 1990; Ponyi et al., 1991; Westesson et al., 1994; Mattila et al., 1995; Gustina et al., 1997; Türp et al., 1998). Melnik et al. (1992) observed a strong trend of left-to-right dominance in the mean mandibular length, although Ponyi et al. (1991) noted that the right side of the mandible appeared to be larger than the left side slightly more frequently than vice versa. Pierrakou (1990) found asymmetry of the mandible in 82% of the study cases, with the right side being smaller than the left side in 47.5%, but this asymmetry was different between the ramus and the corpus. As shown in this study, the mandible is an asymmetric bone according to shape but not size, and is centred on the condylar ramus. This could be explained by functional and mechanical reasons, as the chewing forces from the mandible to the cranium during mastication seems to be related to condylar size in humans (Uthman and Al-Rawi, 2006) and so the same might be expected in pigs. There was also an effect of dentition (age) on condylar asymmetry. However, the shape differences between the two hemimandible sides seemed low enough not to be clinically relevant.

That mandible is strongly conserved is emphasised by the finding that differences in shape across species (similar results have appeared for wild boar, Parés and Caballero, unpublished data) are more commonly associated with changes in the magnitude rather than in the pattern of asymmetry.

The ramus, the mandibular notch and the condylar process are highly variable and probably play an adaptation role in relation to the cranial base or masticatory apparatus to maintain the symmetry of the occlusion. To support this idea, further studies are necessary, notably in foetuses, to compare the symmetry of the neural part of the mandible before tooth development and masticatory function. Our results also demonstrated that size did not change with age for the age period studied.

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