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Evaluation of bovine hemimandible morphology by means of elliptic Fourier descriptors

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Abstract
The aim of this research was to compare size (area) and shape variations of bovine hemimandibles according to age. Digital photographs were obtained for 34 hemimandibles belonging to different European breeds of cattle. The specimens were classified according to age, as determined by molar eruption: 6 months (“young”, M$_1$ erupting, n=8), 10 months (“immature”, M$_2$ erupting, n=9) and over 24 months (“adult”, M$_3$ fully erupted, n=17). Captured images were then digitally analysed based on elliptic Fourier descriptors, which mathematically characterise the area and shape. Hemimandibular areas only showed significant differences between the adults (2752.3 cm$^2$ ± 250.4) and young subjects (2373.8 cm$^2$ ± 300.2). The areas for each age group were not linked to linear shape modifications, which was the same for all age groups. So, bovine hemimandibular form change is mainly related to size changes. Shape variability is centred on the condylar ramus.

Key words
Cattle, image analysis, jaw, mandible, outline fitting functions.

Key to abbreviations:
H = harmonic
PC = principal component
PCA = principal component analysis

Introduction
Digital image analysis is one way to compensate for the weakness in the qualitative evaluation of continuous variation. The advantages of using image analysis are numerous: (i) errors made by experts can be overcome (typing/writing errors); (ii) measurements are totally independent of the expert and time (this is not crucial for easy parameters but it is for scores etc.); (iii) less straightforward parameters can be measured such as projected area, etc. – which is not possible manually; (iv) all data are available on a continuous scale which simplifies statistical processing; and (v) many samples can be analysed in a short amount of time (Lootens et al., 2007).
morphology of objects is studied, two approaches can be followed: classical parameters and shape-describing methods (Rohlf and Archie, 1984; White et al., 1988; Furuta et al., 1995). The quantification of shape variation by means of conventional linear measurements (and the indices derived from them), while replicable, very poorly summarise aspects of the shape due to the vast amount of contour information that is lost in the process. If the mandible is described by morphometric methods based on landmarks, its curved shape mainly provides type-2 landmarks - which are points of maximum curvature (Bookstein, 1991). Such landmarks are likely to be sensitive to measurement error. Elliptic Fourier descriptors allow an outline analyses with a low loss of contour information. Most multivariate morphologic analyses are based on landmark analysis, which requires the presence of homologous landmarks or outlines for superimposition of specimens (Crampton, 1995); mandibles do not have many identifiable homologous features. Fourier analysis provides a description of form without reference to landmarks. A number of different methods can be used in Fourier shape analysis (Rohlf and Archie, 1984). Elliptical Fourier decomposition was chosen for this study because it does not necessitate points on the outline of the specimen to be equally spaced (Crampton, 1995), thus allowing greater sampling from sections of complex shape or with high variability of curvature. The method also does not require the prior definition of a biologically homologous centroid, or geometric centre (Crampton, 1995). This is why the author has chosen to use outline analysis based on Fourier methods, which allows the overall shape to be mathematically described by transforming coordinate information concerning its contour. For an extended introduction of these techniques, refer to any of the several survey papers (Loncaric, 1998; van Otterloo 1991; Veltkamp, 2001).

This study aims to compare the bovine hemimandible overall shape and size (area) variations according to age, by utilising the above mentioned digital tool; the hemimandible outline has been defined as a two-dimensional projection of the mandible bone viewed from its buccal side (Renaud et al., 1996). The mathematical characterisation of elliptical Fourier analysis has been accompanied by multivariate analysis.

**Materials and methods**

**Osteological material**

The study sample consisted of 34 dentulous hemimandibles. As all individuals presented complete cheek dentition it was possible to establish their individual ages. So specimens were classified according to age determined by molar eruption (Dyce et al., 1996): 6 months (“young”, M1 erupting, n=8), 10 months (“immature”, M2 erupting, n=9), over 24 months (“adult”, M3 fully erupted, n=17). The animals belonged to different European breeds: Pyrenean Brown, Charollais, Limousine, Salers, Friesian and Aubrac. The selected material came from a skeletal collection available at the Department of Animal Production at the University of Lleida.

**Extraction of the mandibular outlines**

Image capture was performed 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 of reference and centred on the left lateral aspect of each hemimandible. Images always included a scale (interval 1 mm). The image was imported as a GIF file. Subsequently, image noise and teeth were manually removed from each image using a graphics software package (GIMP v. 2.6.11®), and thus the image of the outline of each hemimandible was obtained.

**Elliptic Fourier descriptors and multivariate analysis**

Image capture was carried out using the SHAPE® software package developed by Iwata and Ukai (2002), which identifies the outline of the hemimandible and generates an elliptical Fourier description. Briefly, the procedure was as follows: images were made binary (i.e. transformed into white for the bone outline and black for the background, in pixels) so the outlines of each continuous contour (interface between the black and the white pixels) were automatically obtained and digitalised; the area enclosed in each outline was automatically calculated. An average of 3,290 points was positioned along the outline of each specimen. The coefficients of the elliptic Fourier descriptors, which describe each harmonic (H), were calculated by the discrete Fourier transformation of the chain-coded contour, the position of the first point being on the standardised outline for all hemimandibles. Shape was approximated by the first 20 harmonics. Each harmonic corresponded to the four coefficients defining the ellipse in the xy-plane. Coefficients were subsequently normalised for size and aligned by the major axis of the ellipse described by H1 (Kuhl and Giardina, 1982). As SHAPE® adjusted for size and orientation, the first harmonic does not contain morphologic information (Crampton, 1995) and so seventy-six [(4x20)-4] standardised Fourier descriptors were finally used for the outline analysis. One-way Anova was conducted to study the area differences. A non parametric multivariate Anova (MANOVA) was carried out in order to study differences in shape, using a correlation index as the distance measure. Wilk’s lambda criterion was used in this test. Principal component analysis (PCA) was used to summarise the coefficients for the studied sample. It was based on the variance-covariance matrix of the coefficients. Geometric interpretation for each principal component was assessed from the reconstructed contours using inverse Fourier transforms. To visualise the variation along a principal component axis, inverse Fourier transforms were calculated for the mean ± 2 standard deviation (SD) of the principal component (PC) scores, with the remaining components set to zero (Iwata and Ukai, 2002). This visualisation may be helpful for giving the morphological meaning of the variation evaluated by each PC. The descriptive statistics were determined with the PAST® package (Hammer et al., 2001).

**Results**

The results of one-way Anova for hemimandibular areas only showed significant differences between adults (2752.3 cm²±250.4) and the 6 month old group (2373.8 cm²±300.2) (p < 0.005; Figure 1), but the shape of mandibles was not different between age groups (p < 0.05). The cumulative contribution ratio of the first PC...
Figure 1 – Box plot for size (mean ± SD) for each age group (2373.8 cm² ± 300.2; 2550.1 cm² ± 161.3 and 2752.3 cm² ± 250.4 for young, immature and adult respectively. The results of one-way Anova for hemimandibular areas showed significant differences between adults and the 6 month old group (p < 0.005).

Figure 2 – Principal component analysis (PCA) for PC1 (45.94% of observed variance) and PC2 (16.51% of the observed variance) after removing the size factor, using elliptic Fourier coefficients of the first 20 harmonics from hemimandible outlines. Filled squares correspond to 6 month old animals (“young”, M₁ erupting), crosses correspond to 10 month old animals (“immature”, M₂ erupting), and stars correspond to 24 month old animals (“adult”, M₃ fully erupted).
for size and shape was 100%, but after removing the size factor the observed variance in the PC1 decreased to 45.94%, in which PC2 accounted for 16.51% of the total observed variance. In the PCA plot it must be acknowledged that the adult specimens are more widely distributed on both planes of the PCA than the rest of the specimens (Figure 2), probably because the sample included a wide range of adult ages. For both approaches (taking into account the size or not), the proportion of correctly classified specimens according to age was similar, about 45%. The morphological analysis of the step by step reconstruction (Figure 3) allowed for a demonstration of the geometrical contribution of the first three PC, with the major anatomical characteristics being fully described using PC1. PC1 was then a good measure of the general appearance of the mandible. After the exclusion of H1, used for size standardisation, H2 and H3 were considered for size and shape relationships. Shape was not correlated with area ($R^2 = 0.090$, Wilk’s $\lambda = 0.347$, $p < 0.0005$).
Discussion

These results indicate that the age change in the cattle mandible is mostly focused on area change, and subtle changes appear on the condylar ramus. Minor limitations of this study were the following: animals in the early stages of their development (below 6 months of age) were not studied, so possibly important macroscopic shape changes in that age range would have escaped detection; and age classification was reduced to three classes according to molar teeth eruption, so one cannot exclude that a more detailed classification, for instance based on dental attrition, might allow a better analysis at the price of markedly increasing the sample size.

Although a limitation of the Fourier analysis is the high number of coefficients computed which prevents its use for small samples, it has allowed an approach that represents interesting perspectives for the characterisation and outline comprehension of the mandibular outline morphology in cattle. It also appeared as a reliable tool in the analysis of shape changes during growth, enabling the visualisation of both the growth trajectory (how morphological variability is related to size change) and the growth pattern (how the arrangement of biological structures changes over time).

References