Morphometric characterization of the asymmetry in the mandible of the ferret (*Mustela putorius furo* Linnaeus, 1758)

Parés-Casanova, P.M.
pmpares@gencat.cat

Abstract

In order to analyse asymmetries between hemimandibles, a sample of 24 mandibles from ferrets was studied by means of geometric morphometric methods, using a set of 3 landmarks and 14 semilandmarks, on the lateral aspect. Results showed that both size and shape played a significative role in mandibular asymmetry. For shape, there appeared significative fluctuating and directional asymmetries, with an especially high level for this latter. Landmarks corresponding to muscular attachments showed greater landmark asymmetry. This it is supported the hypothesis of a chewing side preference, e.g., a mastication-related driver for mandibular shape asymmetry.

Keywords: craniofacial laboratory animal; mental foramen; mental nerve; polecat

Introduction

The ferret (*Mustela putorius furo*) is a small carnivore which represents the domesticated form of the European polecat (European fitch), in the family Mustelidae. The history of the ferret's domestication is uncertain, but it seems it has been domesticated for about 2000 years and kept for rabbit trapping in the old days (He et al., 2002).

The mandible consists of a pair of dentary bones that articulate anteriorly at the mandibular symphysis, a fibrocartilaginous joint. From the symphysis, the two dentary bones diverge from each other, forming the V-shaped mandibular space. The body of the mandible holds lower teeth. Rostrally, the body of the mandible presents several mental foramina, the rostral openings of the mandibular canal, the largest and most caudal located ventral to the septum between the third and fourth premolars (He et al., 2002). The ramus of the mandible resembles a triangle and contains the prominent coronoid process, which occupies most of it, and the condylar process (He et al., 2002). This latter forms the temporo-mandibular joint (He et al., 2002). The angle of the mandible, which is ventral to the condylar process, serves as the insertion of the pterygoid muscle medially and the masseter muscle laterally. Finally, the masseteric fossa accommodates the insertion of part of the masseter muscle (He et al., 2002). So, in addition to housing the dentition, the mandible is a major attachment for the jaw-opening and jaw-closing muscles, being dominated by its role in feeding.

The mandible can be considered to be divided into zones (Captier et al., 2006), each dependent on local functional activity. The mandibular body extends from the joint at the condyle to the symphysis that joins the two sides anteriorly; the alveolar process contains the teeth; and the ascending ramus is comprised of muscle attachment areas briefly described above. The mandible is generally assumed to be bilaterally symmetrical, its left-right asymmetry corresponding to matching symmetry, which is referred to in situations where two separate objects exist as mirror images of each.

Only brief descriptions exist of skull of the ferret (He et al., 2002). In the present paper we use geometric morphometrics to quantify the matched (a)symmetry in a sample of mandibles from ferrets.

Materials and methods

Source of material

A sample of 24 corpses of ferrets naturally dead from a pet farm in Térmens (Catalonia, Spain) was obtained. At laboratory, corpses were beheaded and soft tissues were removed by drying, detaching and rinsing. Clean skulls were then left to dry at room temperature before further photographs. All animals were totally dentated.

Each mandible was disarticulated and both parts levelled on a horizontal plan. 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 so that the focal axis of the camera was parallel to the horizontal plane and centred on the lateral aspects. A scale was put over each specimen.

Landmark Selection

Pictures were transported to TPSUtil v. 1.70 (Rohlf, 2015) to convert the files. The digitation process was followed utilizing TPSDig2 v. 1.40 (Rohlf, 2015). Three discrete landmark points and 14 semi-landmarks were located on each hemimandible. Landmarks defined the most rostral part of the hemimandible, the principal mental foramen and the most caudal part of the hemimandible on the tip of angular process, while semilandmarks defined ventral ridge of the mandibular body (Figure 1). Accessory mental foramina were not considered, as their numbers were variable between specimens. Ramus was not considered as some were broken during the preparation of skulls. The file to determine the sliding direction of the semilandmarks was created in TPSUtil v. 1.70 (Rohlf, 2015). Digitalization was bi-replicated to stablish the measurement error (Fruciano, 2016).

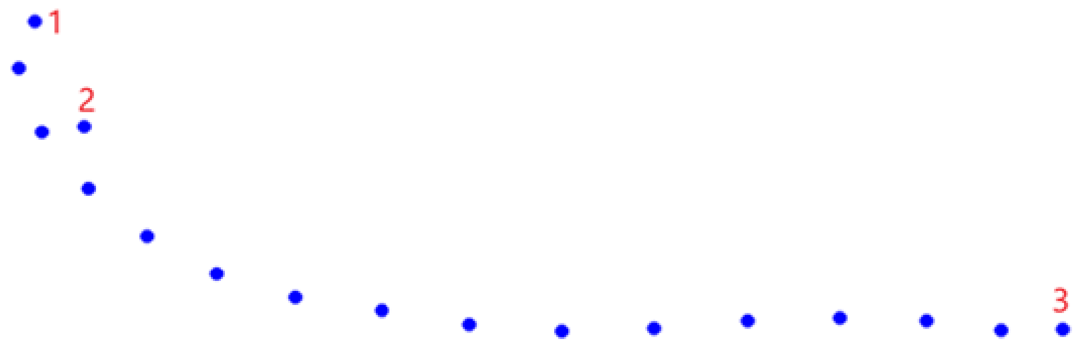

Figure 1. Set of 17 points (3 landmarks and 14 semilandmarks) on hemimandible (lateral aspect). Points 1, 2 and 3 correspond to landmarks on most rostral part of the hemimandible, principal mental foramen and most caudal part of the hemimandible, respectively (circles on the picture). Accessory mental foramina were not considered, as their numbers were variable between specimens.

Digitation and Shape Analysis

A Generalized Procrustes Analysis (GPA) approach eliminated the scale, and the translational, and rotational differences of the coordinate data among subjects (Webster & Sheets, 2010). The shape of each specimen was calculated as the centroid size (CS, the square root of the sum of squared distance between each landmark and the hemimandible centroid) (Bookstein, 1991). Thus, the CS and GPA-scaled coordinates represent surrogates of size and shape, respectively (Webster & Sheets, 2010).

To detect the components of variances and deviations both for size and shape, a Procrustes ANOVA was used. In this analysis, the *individuals* effect denoted the individual variations of each specimen; the main effect of *sides* indicated the variation between sides and was considered as the measure of directional asymmetry (DA, consistent left *versus* right differences); the *individuals x sides* was the mixed effect, this indicating fluctuating asymmetry (FA) (Klingenberg & McIntyre, 1998); and the *measurement error* (factor “double land-marking”) represented the variation due to measurement error in taking landmarks in separate sessions (Klingenberg & McIntyre, 1998). In Procrustes ANOVA there are more degrees of freedom than in conventional ANOVA because the squared deviations are summed over all the landmark coordinates (instead of a single sum of squares in conventional ANOVA). Therefore, the number of degrees of freedom is that for ordinary ANOVA multiplied by the shape dimension, which is, for our two-dimensional coordinate data, twice the number of landmarks minus four (the number of coordinates minus two dimensions for translation and one each for scaling and rotation) (Klingenberg & McIntyre, 1998).

Allometry

Allometry -e.g., change of shape asymmetry to size- was tested with a regression of shape coordinates against CS (log transformed).

Principal Component Analysis

A Principal Component Analysis (PCA) was done to detect most informative anatomical points. We used regression residuals.

Canonical Variate Analysis

Finally, a Canonical Variate Analysis (CVA) using Mahalanobis distances of regression residuals was applied to detect differences between side shapes.

For all statistical analyses, we used MorphoJ software v. 1.07a (Klingenberg 2011) with a = 0.05.

Results

There was a marked effect of DA (i.e., factor 'side') both for size and shape (Table 1). For size, right hemimandibles tended to be bigger. The effect of intra-observer error was near negligible. In addition to directional asymmetry, hemimandible exhibited a less noticeable extent of fluctuating asymmetry, which as significative in Pillai's trace (11.4, *p*=0.0052). A significant allometric effect appeared (p<0.05), with a 13.6% and a 5.2% of shape change explained by size change for right and left hemimandibles respectively. For right hemimandible, three first principal components explained a 74.9% of total observed variance (PCA+PC2+PC3 = 52.1%+15.6%+7.1%). For left hemimandible, results were similar, with three first principal components explaining a 71.2% of total observed variance (PCA+PC2+PC3 = 49.1%+14.1%+7.9%). CVA detected statistical shape differences between sides (p<0.0001), which were mainly focused on caudal landmark on the tip of angular process (*Y*3), with a vertical displacement, and to less degree, on mental foramen (*Y*2) and the rest of most caudal points close to angle of mandible.

Table 1. Procrustes ANOVA results for 24 ferrets' mandibles expressed in shape and size variables, with a significant effect of "side*individual" effect (FA) for both groups. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). The individuals MS can be interpreted as the measure of total phenotypic variation. For shape there was a marked effect of directional asymmetry and a much less noticeable extent of fluctuating asymmetry, which was significative in Pillai's trace (11.4, *p*=0.0052).

A/ Shape

	SS	MS	df	F	P
Individuals	0.135561	0.000184	736	2.81	<.0001
DA	0.019491	0.000609	32	9.30	<.0001
FA	0.048209	6.55E-05	736	0.79	0.999
Error	0.127424	8.30E-05	1536		

B/ Size

	SS	MS	df	F	P
Individuals	1540.049	66.95865	23	36.73	<.0001
DA	1.488989	1.488989	1	0.82	0.375
FA	41.93196	1.823129	23	1.42	0.150
Error	61.57716	1.282857	48		

Discussion

The aim of this work was to assess how and to which extent shape asymmetry occurs in mandibles in the ferret (*Mustela putorius furo*). Mandibles had not paired shapes. Shape directional asymmetry (DA) for both hemimandibles appeared statistically significative. DA means that most of the individuals in the population exhibit a side lateralization. Fluctuating asymmetry (FA) was also significative, although presenting much lower contributions. This would add an important caveat to the co-interpretation of within species shape FA because it may have an independent source not linked to stress. Generally, DA is adaptive, while presence of FA can be because of minor developmental constraints during mandible development.

The fact that directionality is focused mainly on ventro-caudal part of the mandible, area of attachment of the masseter muscle, would support the hypothesis of a chewing side preference, as muscle size and thus attachment area requirements for individual muscles are likely drivers of mandibular shape (Brassard et al., 2020). The angular process presents the highest masticatory stress. For asymmetry of the mental foramen, it is not very marked. Among humans, bilaterally asymmetric mental foramen has been reported (McKay et al., 2018) and possibly it has no functional importance.

The fact that allometry plays a significative role in shaping mandibular asymmetry, corroborates that developmental plasticity of the mandible is impacted by high levels of biomechanical stress and thus would explain more asymmetry on bigger mandibles, as a bone's plastic response to mastication force.

Although we focused on the asymmetries of the ventral part of the body, differences in other morphological features related with feeding habitats (such those on ramus or on the alveolar process) might be studied in further analyses. Evidently, kinematics events associated with chewing must be also analysed in future researches. Last conclusion is that in biometrical studies of mustelid mandibles, bilateral characters should not be examined on only one side.

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Competing Interests

The author declares there were no competing interests.

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