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Age determination of European otters (*Lutra lutra* - Linnaeus 1758) by pulp cavity volume reduction of canine and carnassial teeth.

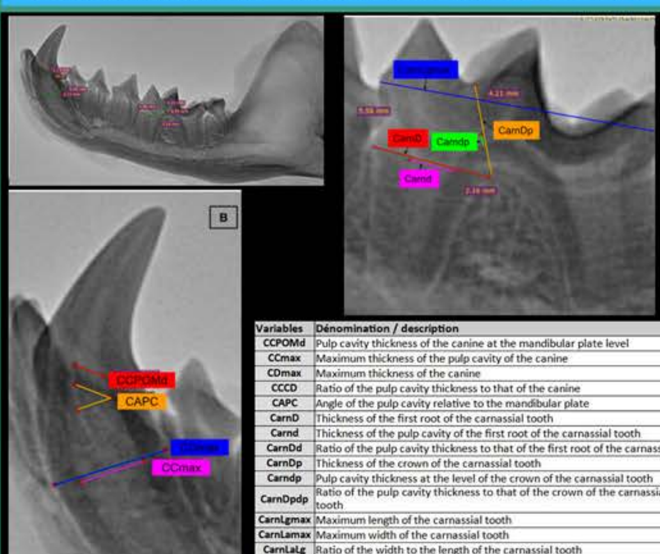
SAINT-MARTIN J.[1], MARQUET M.[2], SIMONNET F.[3], BETTI E.[4], BOUE F.[5], CAILLOT C.[6], MONTFORT D.[7], FUSELLIER M.[8], RAMOS M.[9], RENARD M.[10], LARRAT S.[11], THORIN C.[12], GUINTARD C.[13]

Introduction

- The European otter (*Lutra lutra*) is a semi-aquatic mustelid widespread in several countries. In France, the European otter is a protected species subject to a national action plan (KUHN 2009 et 2019) for the protection of indigenous populations.
- Determining the age of individuals is essential in monitoring populations and for collecting data on the species for further studies.
- Several data in the literature allow the age of otters to be determined based on morphological, biometric, and cranial criteria, including counting incremental lines of cementum at the canine root level.
- A study in sables (*Martes zibellina*) (MONAKHOV 2004) and in dogs (LECOMPTE 1986) yielded rather satisfactory results. However, no data on the pulp cavity volume reduction of teeth in European otters are currently available.

Material and method

- **Biological materials** : 59 European otters (26 females and 33 males).
- The mandibles were extracted and then conditioned during the dissections.
- **X-ray equipment** : An Primax Opera Evolution X-ray machine was used.
- The equipment features the following specifications : on a flat panel detector with constants of 44 kV and 5 mAs.
- **Location** : ONIRIS VetAgroBio Nantes, (FRANCE).
- The radiographic images were processed with the RadiAnt DICOM Viewers (64-bit) for Windows 10 reading software, on which the dental measurements were performed.
- Descriptive, bivariate, multivariate analysis and multivariate logistic regression were performed using R software version 4.4.3.
- A description of five age categories was proposed based on the state of epiphysation of the long bones of the thoracic and pelvic limbs : Baby (Birth to 6 months), juvenile (6 months - 1 year), Sub-adult 1 (1-2 years), Sub-adult 2 (2-3 years), Adult (more than 3 years). The "Baby" age category was eliminated due to the small number of individuals (2 individuals) and because the permanent teeth have not yet come through.



Variables	Dénomination / description
CCPOMd	Pulp cavity thickness of the canine at the mandibular plate level
CCmax	Maximum thickness of the pulp cavity of the canine
CDmax	Maximum thickness of the canine
CCCD	Ratio of the pulp cavity thickness to that of the canine
CAPC	Angle of the pulp cavity relative to the mandibular plate
CarnD	Thickness of the first root of the carnassial tooth
Carnd	Thickness of the pulp cavity of the first root of the carnassial tooth
CarnDd	Ratio of the pulp cavity thickness to that of the first root of the carnassial
CarnDp	Thickness of the crown of the carnassial tooth
Carndp	Pulp cavity thickness at the level of the crown of the carnassial tooth
CarnDpdp	Ratio of the pulp cavity thickness to that of the crown of the carnassial tooth
CarnLgmax	Maximum length of the carnassial tooth
CarnLamax	Maximum width of the carnassial tooth
CarnLaLg	Ratio of the width to the length of the carnassial tooth

A: Entire mandible; B: Canine measurements; C: Carnassial measurements.

Figure 1 : Measurements taken on mandibular canine and carnassial teeth from dental radiographs (©Medical Imaging Service, ONIRIS VetAgroBio Nantes).

Results

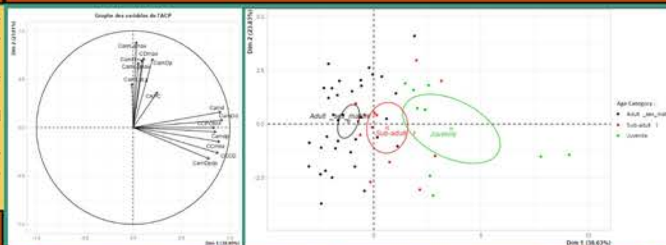


Figure 2 : Graph of variables (left figure) and individuals (right figure) of the principal component analysis (PCA) (59 individuals, 14 variables, factorial plan I-II) for the study of measurements from dental radiographs.

- The graph of variables (left graph) shows a grouping of the constituent teeth measurements of the thicknesses of the canines and carnassials (CDmax, CarnD, CarnDp, CarnLgmax, CarnLamax) with low variability around the axis of Dimension 2 and a grouping of variables whose measurement varies more strongly around the axis of Dimension 1.
- The individuals (right graph) are centered on axis 1 of the PCA, describing the age categories according to a gradient. Individuals can be separated into three categories : "Baby", "Sub-Adult 1" and "Sub-Adult 2" / "Adult". Indeed, the average weights of the "Sub-Adult 2" and "Adult" groups are included in their respective confidence ellipses. Furthermore, the confidence ellipses overlap, suggesting that there are no significant differences between these two groups.
- Individuals can be as old as two years based on dental radiographs. Beyond two years, the separation becomes more complex.
- A discriminant model grouping the three groups defined in the PCA made it possible to identify the CCCD and CarnDpdp variables as being the most discriminating with a good classification rate of individuals of 78%.

Table 1 : Discriminant values (in their respective unit of measurement in millimeter), error rate and performance (AUC : area under the curve) of the two-by-two logistic regression between age categories for the canine and the carnassial.

Age category	Juvenile – Sub-adult1			Sub-adult1 – Adult sex mature		
Canine teeth variables	Discriminant value	Error rate	AUC	Discriminant value	Error rate	AUC
CCPOMd	2.9	0.41	0.70	2.4	0.23	0.81
CCmax	**3.5**	**0.27**	**0.85**	**2.7**	**0.21**	**0.91**
CCCD	**54**	**0.27**	**0.87**	42	0.21	0.88
Age category	**Juvenile – Sub-adult1**			**Sub-adult1 – Adult sex mature**		
Carnassial teeth variables	Discriminant value	Error rate	AUC	Discriminant value	Error rate	AUC
Carnd	**1.7**	**0.27**	**0.83**	**1.6**	**0.27**	**0.75**
CarnDd	**36**	**0.32**	**0.83**	**31**	**0.23**	**0.76**
Carndp	0.5	0.32	0.74	0.5	0.23	0.64
CarnDpdp	16	0.27	0.73	14	0.23	0.65

- The variables with the best error rates and model performance are those of the canine pulp cavity thickness (CCmax) and the ratio of the canine pulp cavity thickness to that of the canine (CCCD), the first carnassial root pulp cavity thickness (Carnd) and the ratio of the first carnassial root pulp cavity thickness to that of the first carnassial root (CarnDd), allowing to best separate the age groups.
- The error rate remains acceptable between 20 and 30%, which suggests that less than a third of individuals can be misclassified with this model.
- The model performances are good to very good meaning that these discriminating values are relatively reliable.

Conclusion

- To conclude, the age determination of European otters by pulp cavity volume reduction of canine and carnassial teeth is possible up to two years. Then it is difficult to evaluate a precise age for adult otters.
- A comparative analysis with an histologic analysis of canine root cementum would perhaps allow us to refine the results of this work as in dogs and sables.

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