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MicroCT imaging of canid diaphyses: bone ontogeny from a zooarchaeological and digital perspective

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ABSTRACT
X-ray microCT offers the possibility of studying the internal structure of animal remains by detecting age-related changes in bone microstructure. In the present work, we analyse developmental patterns of the diaphyseal structure in canids. In particular, the first metacarpal of current and archaeological individuals of red fox (*Vulpes vulpes*) and wolf (*Canis lupus*) was analysed. Variables describing bone structure were measured by inferring bone development through the observation of cross-sections. The results show how bone structure changes over the course of a lifetime and how this approach makes it possible to separate young and older individuals. This is important from a zooarchaeological point of view to estimate the age at death of fragmentary animal remains and to discriminate taxa characterised by similar morphology but different adult body size using a non-destructive approach.

Section: RESEARCH PAPER

Keywords: Micro CT; Zooarchaeology; Wolf; Red fox; Bone ontogeny

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1. INTRODUCTION

Computed microtomography (microCT) has been proposed and successfully used in several zooarchaeological studies [1], [2], [3], [4], [5] during the last decade. An interesting, but as yet insufficiently investigated application in prehistoric studies, is the estimation of the age-at-death of individuals through structural analysis of bone tissue. This is of great importance when skeletal elements from archaeological contexts lack diagnostic features, such as metaphyses, that are useful for estimating the age-at-death. Ossification centres may be naturally absent (as, for example, in most of basipodial elements) or may be missing due to fragmentation. Correctly estimating the age-at-death of individuals is crucial for understanding the strategies adopted by past human communities in the exploitation of animal resources [3]; moreover, age-at-death can be useful in discriminating taxa or domestic breeds characterized by a close morphology but different adult body size [4]. Furthermore, bone microstructure may be helpful for taxonomic identification of bone remains [6]. As we have already tested the usefulness of analysing age-related parameters of spongy bone tissues in zooarchaeology [1], [4], this paper will focus on the diaphyseal structure. A sample of red fox and wolf metacarpals will be analysed, and porosity of the diaphyseal portions will be quantified to identify age-related changes in compact bone tissues. Our research is part of a project aimed at providing new tools for the identification of domestic animal bones from prehistoric sites.

2. MATERIALS AND METHODS

In this work, we investigate the first metacarpal of 10 modern red foxes (*Vulpes vulpes* Linnaeus, 1758), from a few months old cubs to adults, to define the microstructural parameters related to their age-at-death. The samples are part of the comparative osteological collections of the Department of Physical Sciences, Earth and Environment, Research Unit of Prehistory and Anthropology, of the University of Siena (Italy). As they belong to complete skeletons, age-at-death of individuals was estimated considering tooth eruption and epiphyseal fusion [7]. The results are shown in Table 1.

In addition, we analysed the first metacarpal of nine wolves (*Canis lupus* Linnaeus, 1758). Three of them are modern wild wolves from Italy (n° 362, 353, 361); three are modern zoo-wolves (n°180, 52, 214); among the latter, there are no data on their living conditions and whether they were born in captivity or not. Finally, three specimens (n° 1971, R23 R24) are Late Pleistocene wolves from Grotta Paglicci. This is an important prehistoric cave-site located in Apulia on the Gargano promontory (FG, southern Italy) [4], [8], [9]. The chronology of all three specimens indicates cold paleoenvironmental conditions referable to a time span between the late MIS 3/beginning of MIS 2 and the Late Glacial (i.e., between approximately 30,000 and 13,000 years before present) [10], [11], [12]. Stratigraphy of Grotta Paglicci is characterized by evidence of several phases of Upper Palaeolithic human frequentation; the accumulation of animal bones is mostly related to human hunting activities [9], [12]. Late Pleistocene wolves from Grotta Paglicci are characterised by a large body size, comparable to that of present-day wolves from northern Europe [4].

The modern specimens are part of the aforementioned osteological reference collection held at the University of Siena. Specimens 362 and 353 belong to not fully developed young individuals. Specimens 196, 361, and the zoo-wolves belong to individuals whose skeletal growth and development is complete. For this reason, they can all be considered adults [7], [13]. All archaeological specimens show a fully fused distal epiphysis. As these are isolated and scattered bones, complete data on the skeletal ontogeny are not available. For this reason, only a general age for wolves is given in Table 2.

MicroCT scanning was performed at the Multidisciplinary Laboratory of the Abdus Salam International Centre for Theoretical Physics of Trieste, in Italy [14]. The instrumentation is designed for the analysis of archaeological and palaeontological specimens [3], [15]. The obtained µCT slices were reconstructed with the commercial software DigiXCT (DIGISENS) in 32-bit format. A semi-automatic threshold-based segmentation was performed using AVIZO software (Thermo Fisher Scientific Inc.) to separate the bone component from the interstitial air [16], [17], [18].

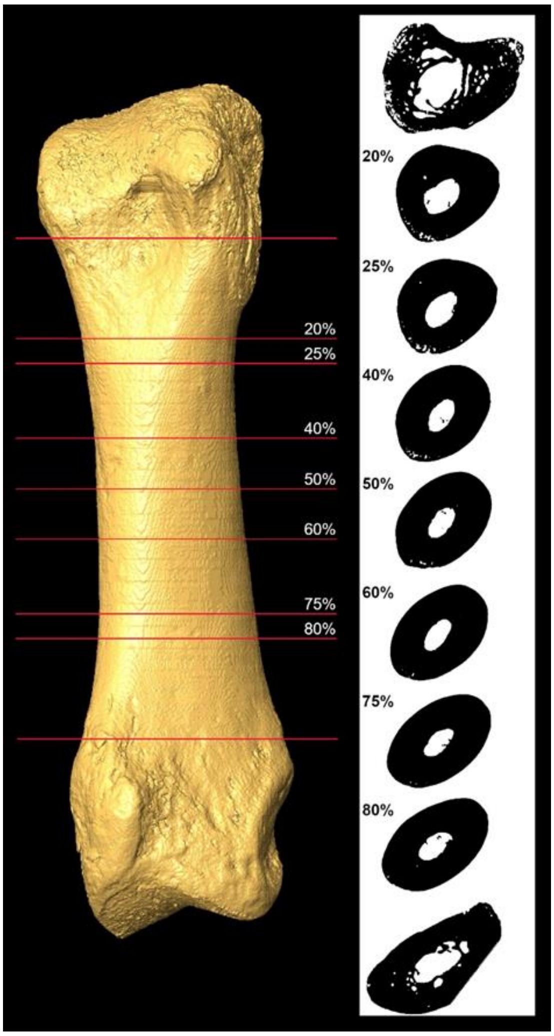

Figure 1. Cross-sections on Zoo-Wolf 52

After segmentation, all specimens were aligned to their longitudinal axis. The diaphysis was isolated by separating the two epiphyses. The proximal one was separated from the rest of the bone using a transverse plane tangent to the distal ridge of the articular facet on the palmar side. The distal epiphysis was separated using a transverse plane tangent to the most proximal ridge of the distal articular facet on the palmar side (Figure 1). Once the diaphysis was isolated, seven cross-sections were digitally extracted, starting from the proximal end, at 20 %, 25 %, 40 %, 50 %, 60 %, 75 % and 80 % of the length of the diaphysis respectively. When extracting cross-sections, we did not consider the total biomechanical length of the bone as proposed by other authors [19], to extend the method even to fragmentary archaeological remains. Using ImageJ software [20], the Porosity Surface to Bone Surface (PS/BS) ratio was calculated on the seven cross-sections for each specimen. Marrow cavity was not considered as part of the “Porosity surface”.

Table 1. Red fox specimens analysed in this paper.

Specimen	Age (months)
Fox 253	2
Fox 254	2
Fox 329	5 - 6
Fox 458	6
Fox 73	6 - 8
Fox 313	6 - 8
Fox 160	8 - 12
Fox 47	> 12
Fox 299	> 12
Fox 338	> 12

Table 2. Wolf specimens analysed in this paper.

Specimen	Development
Wolf 362	Not fully developed
Wolf 353	Not fully developed
Wolf 361	Adult
Zoo-Wolf 180	Adult
Zoo-Wolf 52	Adult
Zoo-Wolf 214	Adult
Pleistocene Wolf 1971	Adult
Pleistocene Wolf R23	Adult
Pleistocene Wolf R24	Adult

3. RESULTS

The data collected on foxes can be seen in Table 3 and Figure 2, while those on wolves can be seen in Table 4 and Figure 4. Red fox cubs show a very porous diaphysis: PS/BS

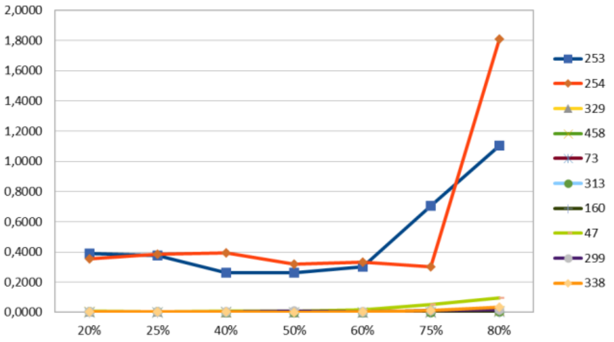

Figure 2. Red fox: PS/BS across the diaphysis.

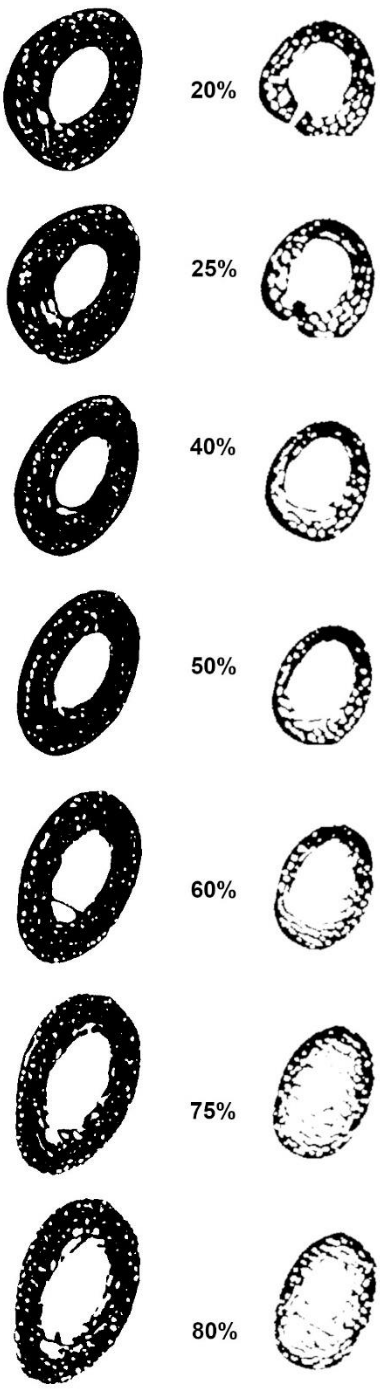

Figure 3. Cross-sections on young wolf 353 (left) and on fox cub 254.

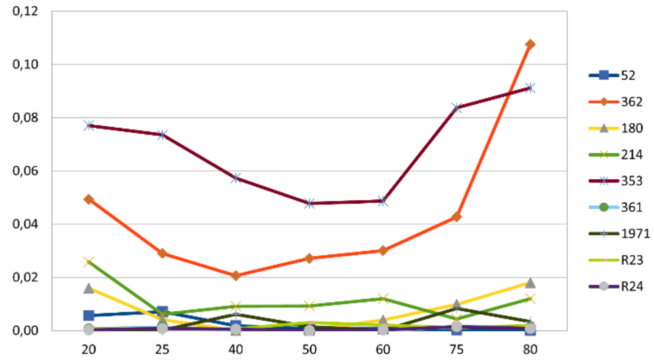

Figure 4. Wolves: PS/BS across the diaphysis.

ratio is always above 0.2 and reaches values above 1 in the distal part of the element. When individuals reach full body size, although still young (5-6 months), the porosity decreases (values are always below 0.1) and is comparable to that of adults. Individuals aged between 5 and 12 months show the most compact diaphysis. Older individuals (>12 months) show a very slight increase in porosity. This may be due to age- and life conditions-related elaboration of the compact bone tissue. Indeed, as observed in cancellous tissue, the architectural adaptation phase of growing bones to external loads is preceded by an increase in bone volume [21].

Young wolves show a similar pattern to foxes: their porosity is very different from that of adult individuals. In this case, the porosity of specimens 353 and 362 is much lower than that of young foxes, but this can be explained by the fact, that young wolves analysed in this paper are not just a few months old cubs. These are growing wolves that have left the den and probably have already taken part in pack activities [22]. In contrast, the fox cubs analysed in this work still had a limited range of activities and did not leave the den. Therefore, the mechanical loads applied to their bones were very weak. The difference in porosity between a fox cub (n° 254) and a young wolf (n° 353) can be seen in Figure 3. Furthermore, by comparing Figures 1 and 3, the differences in porosity between an adult wolf, even a zoo wolf, and a growing wolf can be seen. However, among adults, the zoo-wolves are the individuals with the highest porosity at the extremities of the diaphysis. Adult wild wolves, both modern and fossils, form a homogeneous group (Table 4, Figure 4).

4. DISCUSSION AND CONCLUSIONS

Our data, albeit at a preliminary level due to the small sample size, show that the internal diaphyseal structure can provide information on the age-at-death of individuals. This cannot be used to construct accurate mortality profiles due to the low sensitivity of the method. In fact, only young and still growing individuals can be separated from the others. However, this type of data can be used to discriminate between different taxa characterized by similar morphology and different adult body size. From this point of view, it is interesting to point out that 5-6 months old foxes, which should have already reached adult body size [23], [24], show a bone porosity that is comparable to that of older individuals. As already stated in a previous methodological paper [1], the possibility of discriminating between populations characterized by different body size is of pivotal importance for the study of domestication. A similar method, based on the analysis of spongy bone tissue, has already been successfully applied on a Palaeolithic sample from Grotta

Paglicci, leading to the identification of the oldest domestic dog remains discovered in Italy so far [4]; here we add clues on a different type of bone tissue. Furthermore, the increased porosity of the diaphysis in zoo-wolves is of interest for the study of animal domestication: the external and internal structure of bones are influenced by external mechanical loads (i.e., living conditions) [25], [26], [27], [28] and can be used to identify, among zooarchaeological samples, bones of individuals who lived in captivity [29], [30]. MicroCT has already been applied on human remains to infer about locomotive behaviour [31], while some preliminary results already show the potential of these methods in identifying the impact of captivity on the skeleton of domestic animals [32]. This is of crucial importance when research focuses on the early stages of animal domestication, when domesticated animals were morphologically very close to their wild counterparts [24], [33], [34] but could live in very different conditions.

Table 3. Red fox: PS/BS ratio in the seven cross-sections.

Specimen	20 %	25 %	40 %	50 %	60 %	75 %	80 %
253	0.390	0.374	0.263	0.260	0.302	0.705	1.104
254	0.354	0.387	0.394	0.318	0.332	0.302	1.811
329	0.003	0.001	0.000	0.000	0.001	0.003	0.013
458	0.002	0.001	0.000	0.000	0.000	0.001	0.009
73	0.001	0.000	0.001	0.000	0.000	0.001	0.004
313	0.002	0.003	0.000	0.000	0.000	0.001	0.003
160	0.000	0.000	0.000	0.000	0.000	0.000	0.000
47	0.006	0.002	0.007	0.009	0.014	0.052	0.097
299	0.005	0.004	0.004	0.006	0.006	0.010	0.019
338	0.004	0.003	0.002	0.001	0.002	0.012	0.035

Table 4. Wolf: PS/BS ratio in the seven cross-sections.

Specimen	20 %	25 %	40 %	50 %	60 %	75 %	80 %
Wolf 362	0.106	0.097	0.074	0.063	0.067	0.130	0.148
Wolf 353	0.063	0.036	0.025	0.033	0.037	0.055	0.140
Wolf 361	0.001	0.002	0.000	0.000	0.000	0.002	0.001
Zoo-Wolf 180	0.020	0.005	0.000	0.000	0.004	0.011	0.021
Zoo-Wolf 52	0.006	0.008	0.002	0.001	0.001	0.000	0.000
Zoo-Wolf 214	0.034	0.008	0.011	0.011	0.014	0.005	0.015
Pleistocene Wolf 1971	0.000	0.000	0.007	0.002	0.000	0.009	0.004
Pleistocene Wolf R23	0.001	0.001	0.001	0.003	0.002	0.001	0.002
Pleistocene Wolf R24	0.000	0.001	0.001	0.000	0.000	0.002	0.001

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