### DANISH REVIEW OF GAME BIOLOGY Vol. 10 no. 2

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by Helen Grue

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#### Introduction

Investigation of the periodic development of tooth cementum is to-day a commonly-used method for determining the age of mammals (MORRIS 1972). Even though the method is used as a routine tool, practically nothing is known about the physiological processes leading to development of the annual incremental lines. It is, however, generally believed that natural fluctuations in the environment to which an animal is exposed somehow affect these processes.

Most of the species investigated for the presence of annual cementum structures come from the northern temperate zone, where environmental conditions show marked seasonal variation. However it has been shown that not only wild animals exposed to natural conditions, but also farm-raised specimens, leading an existence far from natural, develop incremental lines that can be correlated with an annual periodicity (Klevezal & Kleinenberg 1967, Grue & Jensen 1976).

As part of a study of the nature of incremental lines, especially the season for their development and their distinctness in relation to climate, it was of interest to establish whether annual increments would develop in any chosen specimen, no matter how artificial the nature of its environment might be. Of the Danish species of carnivores, the dog

(Canis familiaris), kept as a domestic animal, leads the least natural existence both with regard to diet and exposure to climate. Furthermore as material of known age is easily obtained, the domestic dog seemed the most suitable animal for study. The specific aim of this investigation was to establish whether distinct incremental lines are present in the tooth cementum of domestic dogs, and to verify whether the visible pattern of lines can be related to a regular time scale. The investigation was supplied by a grant from the Danish National Science Research Council.

The material studied includes teeth from 16 domestic dogs from Denmark, ranging in age from 8 months to 13 years, plus 2 specimens of unknown age. 10 of the dogs of known age were obtained as skinned heads from the Department of Pathology, Royal Veterinary and Agricultural University, Copenhagen, and comprised 2 Alsatians, 2 poodles, 1 Newfoundland, 2 Labrador retrievers, 1 Irish wolfhound, 1 Airedale terrier and 1 Pekingese. 4 dogs of known age (2 wirehaired dachshunds, 1 Alsatian and 1 wirehaired Fox terrier) were obtained from the Institute of Comparative Anatomy, University of Copenhagen. From 2 poodles kept as pet dogs by the author, I1 and I2 were extracted in vivo for hygienic reasons. For one of these dogs, 14 months elapsed between extraction of the two teeth. Lastly, heads of 2 large dogs, age and race unknown, were obtained from the Game Biology Station, Kalø.

In order to compare the nature of the incremental lines in domestic dogs from two different climates, teeth from 8 sledge-dogs (Canis familiaris) of unknown age and 1 of known age from Greenland were sectioned. The skulls of unknown age were collected at Scoresbysund by Mr. ALWIN PEDERSEN during the years 1927–29, and that of known age (20 months) by Mr. HARRY MADSEN at

Godhavn, Disko in 1948. A canine and an incisor ( $I_1$  or  $I_2$ ) were kindly placed at my disposal by cand. mag. P. Valentin-Jensen, the Zoological Museum of Copenhagen.

Sledge-dogs in Greenland lead an existence which with regard to exposure to environmental fluctuations is almost the same as that of a wild carnivore. The dogs are kept in the open all year round and are most often only fed during winter, when they are used as work-animals; the rest of the year they are almost wild and must seek their own food.

#### Method

Fresh jaws were boiled in water for 10 minutes in order to allow teeth to be extracted without damage to the cementum.

Teeth were then decalcified in 5% nitric acid (HNO $_3$ ) and sagittal histological sections were prepared as described by Grue & Jensen (1973). The thickness of sections was approximately 12  $\mu$ .

Various teeth from the Danish dogs were sectioned in order to establish which tooth would be the most suitable for further sectioning. As the pattern of incremental lines did not vary noticeably from one tooth to another in the same specimen, it was assumed that under normal conditions development of the lines would be identical in a whole set of teeth, this being in accordance with observations

in a number of wild carnivore species (Klevezal 1970 and own unpublished results). However, it has been stated that injuries to the tooth may cause an abnormal thickening of the cementum (Sicher & Bhaskar 1972). Furthermore, in 5 species of Danish carnivores previously studied, it was observed that hypercementosis affects the development of incremental lines, usually resulting in a high production of lines. Consequently, in this study teeth showing signs of abnormal cementum development were omitted. Generally two teeth, a canine and an incisor or premolar, were sectioned from each specimen. In the two live domestic dogs only incisors were sectioned.

#### Results and discussion

A distinct layer of cementum was present in teeth from all 18 Danish dogs, and incremental lines were visible in 17 of them. The single animal lacking lines was the only specimen known to be less than one year old, namely an Alsatian of 8 months age. In the domestic dog permanent incisors, canines and premolars erupt before the animal reaches an age of approximately 8 months (Sisson & Gross-

MAN 1938). As cementum formation is usually initiated before eruption (Sicher & Bhaskar 1972), the number of incremental lines could be expected to equal the age in years, if the domestic dog follows the pattern observed in other members of the family Canidae. The question is whether the observed number of incremental lines can be correlated with a time scale (not necessarily annual), as in wild animals.

Examination of the incremental lines made it clear that assessment of the exact number was difficult in most cases and impossible in some, due to the nature of the lines. In general, these appeared faint and poorly-defined compared with lines seen in the Danish red fox (Vulpes vulpes) (Grue & Jensen 1973). Since the preparation of sections was performed exactly as for the red fox, and as sections were satisfactory from a histological point of view, it was assumed that the cause for the diffuseness of the lines lay in the material itself and not in the preparation technique. 2 of the 18 specimens (a 16-months old wire-haired Fox terrier and a 10-year old Pekingese) possessed lines as clear as those found in the red fox. Nevertheless, for the 15 specimens of known age older than one year, it was very evident that the number of lines present by far exceeded the number of years the animal had existed (Fig. 1). Thus, the above mentioned 16-months old Fox terrier and the 10-year old Pekingese showed 3 and 14 lines respectively, while a 6-year old Labrador retriever showed 11 lines, and one of the live poodles, 13 years old, showed at least 27 lines.

It proved to be impossible to establish any correlation between the age of a dog and the number of incremental lines in its teeth, and the pattern of lines was much more irregular than that observed in 5 species of wild Danish carnivores previously studied. It was therefore concluded that the development of incremental lines is not correlated with seasonal cycles in dogs kept as domestic animals in Denmark.

In the 9 sledge-dogs from Greenland canine cementum showed a pattern of incremental lines that did not correspond in appearance to the general picture observed in the Danish dogs, but was much more like that previously observed in the arctic fox (Alopex lagopus) from Greenland (GRUE & JENSEN 1976) and in the red fox (Vulpes vulpes) from Denmark (GRUE & JENSEN 1973). Furthermore, as the 20-month old sledge-dog, killed in October showed 1 well-defined incremental line in the cementum, it seems reasonable to assume that in dogs living unsheltered in the arctic, incremental cementum lines develop according to a seasonal cycle, and that the number of lines present corresponds to the age of the animal in years as is the case in wild carnivores.

Other species of the genus Canis investigated for age-related structures in teeth include the coyote (Canis latrans) (LINHART & KNOWLTON 1967) and the black-backed jackal (Canis mesomelas) (LOMBAARD 1971). Distinct incremental lines were found in both species, and through studies on animals of known age, it was established that the number of lines present corresponded to the age of the animal in years. Material for these two studies was collected in the temperate zone, (northern and the southern parts respectively), as the coyotes were from Wyoming and Montana, North America, and the black-backed jackal from Transvaal, South Africa.

Distinct incremental lines have also been observed by the author in a single specimen of wolf (Canis lupus), Sudbury, Canada. The animal killed in June, showed a developing first incremental line border-

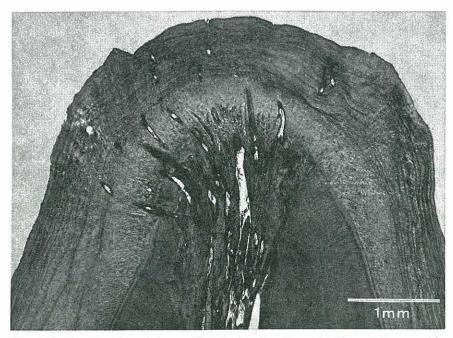


Fig. 1. Section of canine of 6-year old Labrador dog, Denmark. Close-set incremental lines are visible in the cementum. 11 lines were discerned in the section, and although they are less clear in the figure, the number visible exceeds the animal's age.

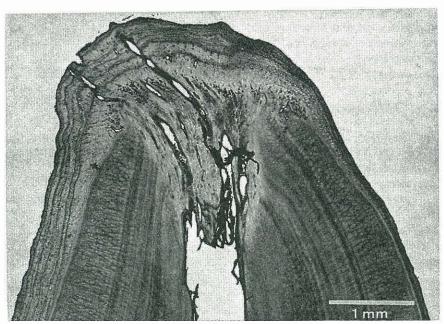


Fig. 2. Section of canine of sledge-dog (CN 3301), Greenland. 5 well-defined incremental lines are visible in the cementum.

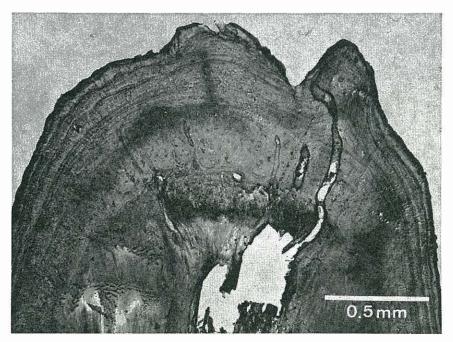


Fig. 3. Section of canine of cat (IK 27) from the early medieval period, Denmark. 4 incremental lines are seen in the cementum. The position of the outermost line, close to the periodontal membrane, indicates its deposition shortly before the animal died.

ing the exterior margin of the cementum.

From this study of the periodic growth of cementum in dogs, it can be concluded that endogenous cycles cannot alone maintain a strict annual periodicity in the deposition of incremental lines. Obviously, the development of annual lines requires a more direct exposure to seasonal fluctuations in external factors than domestic dogs are subjected to in their mainly indoor environment in Denmark. Captivity as such does not affect the annual deposition of incremental lines, for both arctic foxes and minks (Mustela vison), reared on fur-farms in Denmark develop annual lines similar to those of wild animals (GRUE & JENSEN 1976 and own unpublished results). Nor does the lack of seasonal variation in composition of the diet seem to influence the development of annual lines, for even living on

an unvaried diet, minks produce incremental lines on an annual basis (own unpublished results).

The domestic dog provides ideal material for further experiments in this field, for instance in attempts to define more precisely the type of external influence requiered to produce synchrony between deposition of incremental lines and seasons of the year.

It would be of interest if determination of age by means of incremental cementum lines were also applicable to archaeologically obtained carnivore teeth. Examination of the outermost cementum layer is furthermore of special interest to archaeologists, as the nature of this layer may determine in which season of the year the animal died. In this way a study of incremental cementum lines in excavated teeth not only gives information about

the age of animals but also contributes to the knowledge of activity and site occupation (cf. Saxon & Higham 1968, Kay 1974). So far this method has been tried only on teeth from herbivores where the presence of a thick cementum layer allows lines to be investigated in undecalcified ground sections. However in carnivores the cementum layer is thin, and stained sections of decalcified teeth are required both for a reliable assessment of the number of lines and especially for interpretation of the last-formed layer.

In connection with this study of incremental lines in domestic dogs, the opportunity to examine a few teeth of dogs from the Mesolithic age was taken. The teeth were excavated at Ertebølle, Denmark, during the period 1893-97 and since then had been kept untreated at the Zoological Museum of Copenhagen. 5 teeth (3 canines and 2 premolars) were kindly placed at the author's disposal by mag. scient. Tove Hatting. A major problem with teeth from such excavations is their poor condition and especially their porosity, resulting from a loss of organic material. Indeed the remains of organic material in the 2 premolars proved to be too little to allow the tissue to be sectioned after decalcification. Even though the 3 canines were more solid after decalcification and sections could be prepared, the cementum was completely destroyed and just a lattice-work of organic material was left. The dentine was also

affected, but regions of unaffected dentine found near the pulp cavity did show stratifications similar to those found in the dentine of sledge-dogs from Greenland.

Considering the age of the material and the duration of the storage period, the negative result obtained here does not exclude the possibility that useful histological sections could be prepared from excavated carnivore teeth especially from more recent periods. Not only the age of the material but also its conservation state may be of importance for a successful decalcification of archaeologically obtained teeth. In addition the duration and nature of storage before processing may affect the ability of the tissue to withstand decalcification. Indeed the organic parts of teeth of the domestic cat (Felis domestica) from early medieval layers excavated in Odense, Denmark during 1970-71 and processed while containing natural moisture, withstood treatment with a decalcifying agent (T. HAT-TING & K. ROSENLUND, pers. com.) and Fig. 3 shows a section prepared by K. Rosenlund.

Both the 5% nitric acid used in this investigation and the 3% hydrochloric acid (HCl) used for cat teeth are harsh decalcifying agents and it is possible that with very old teeth better results could be obtained by using a more gentle reagent, for instance EDTA.

## Dansk resumé

## Uperiodiske lagdannelser i tandcementen hos hunde (Canis familiaris L.)

I forbindelse med studier over årligt fremkomne linier i tændernes rodcement hos en række danske vildtlevende rovdyr er også undersøgt tænder af 18 danske hunde, hvoraf 16 af kendt alder. Histologiske præparater af hjørnetænderne viste uregelmæssige cementlinier, som i antal langt oversteg dyrenes alder i år, og korrelation mel-

lem individernes alder og liniernes antal kunne ikke påvises. Til sammenligning undersøgtes hjørnetænder fra 9 grønlandske slædehunde, hvoraf 1 var af kendt alder (20 mdr.). Histologiske præparater af disse tænder viste velafgrænsede cementlinier, der mindede om de årligt fremkomne linier hos bl. a. vildtlevende rødræv

og polarræv. Den 20 mdr. gamle slædehund havde dannet én cementlinie, og det antages, at liniernes antal hos de grønlandske slædehunde svarer til dyrenes alder i år. En klar årlig cyklus i rodcementdannelsen kunne således konstateres hos de grønlandske slædehunde, der er udsat for store sæsonmæssige ændringer i livsvilkårene, mens den ikke fandtes hos danske »stue«-hunde, der i mindre grad er udsat for sådanne variationer. Muligheden for fremstilling af histologiske præparater af rovdyrtænder fra arkæologiske fund er også omtalt.

#### Резюме на русском языке

## Непериодические слоистые структуры зубов собак (Canis familiaris L.)

В связи с исследованиями образовавшихся ежегодно линий в корневом цементе зубов некоторых живущих на воле датских хищных животных, также исследовались зубы 18 датских собак, из которых 16 были известного возраста. Гистологические препараты клыков показали нерегулярные линии цемента, число которых значитечьно превышало число годов возраста животных, и не было возможно найти какого нибудь соотношения между возрастом особей и числом линий. Для сравнения были исследованы клыки 9 гренландских упряжных собак, из которых был известен возраст одной (20 месяцев). Гистологические препараты этих зубов показывали четко ограниченные цементные линии, напоминающие о ежегодно образующихся линиях у, между прочим, живущих на воле красных лисиц и песцов. У 20-месячной упряжной собаки образовалась цементная линия, и предполагается, что число линий у гренландских упряжных собак соответствует числу годов возраста этих животных. Таким образом, четкий годовой цикл образования корневого цемента был обнаружен у гренландских упряжных собак, которые подвержены значительным сезонным изменениям условий их жизни, между тем как он отсутствовал у датских «комнатных» собак, в более низкой степени подверженных таким изменениям. Обсуждается также возможность приготовления гистологических препаратов зубов хищных животных из археологических нахо-

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