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by
HELEN GRUE & BIRGER JENSEN

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Introduction

The periodic growth of cementum around the roots of teeth is a well-known phenomenon, and the seasonal development of incremental lines in the cementum today provides the most accurate means available of establishing absolute age in mammals (MORRIS 1972). As part of a study of the nature of incremental cementum lines and the season for their development, canine teeth of arctic foxes *(Alopex lagopus)* from Greenland and from Denmark were investigated.

The distribution of the arctic fox is circumpolar, and it is mainly found along coasts and in the arctic tundra region. Since the beginning of this century it has been reared in fur-farms in many countries, being introduced as a farm animal to Denmark in the 1920's, when foxes from Alaska formed the breeding strain. Two colour phases (blue and white) occur both in the wild and on fur-farms.

The material of arctic foxes (blue phase) from Greenland comprised 44 skulls. The foxes were collected in western Greenland during 1952–54 in 4 different areas (24 animals from Thule, 10 from Sukkertoppen, 7 from Godthåb and 3 from Frederikshåb). This material was kindly made available by Dr. CHR. VIBE, Zoological Museum, Copenhagen.

The material from Denmark comprised skinned heads of 20 arctic foxes (blue phase), obtained during pelting-season in November 1974. The foxes had been reared at the »GRECA-MINK« farm and were of known age ($^1/_2$ to $5^1/_2$ years). The material was kindly placed at our disposal by Cand. agro. G. ENGEL, »GRECA-MINK«, Sorø, Denmark.

The specific aim of this work was to establish whether annual incremental lines develop in the cementum of wild arctic foxes from Greenland and in farm-reared animals in Denmark, and to evaluate the distinctness of the lines in regard to climatic conditions in these two areas. In addition closure of the pulp cavity in relation to age was studied.

The work was supported by a grant from The Danish Natural Science Research Council.

Method

The material from Greenland consisted of cleaned skulls with teeth glued into the alveoli. In order to soften the glue before extracting the canines the tip of the jaw was dipped into boiling water for about five minutes. Fresh jaws from the Danish animals required boiling for 20–30 minutes in order to extract teeth without damage to the cementum. After extraction the 4 canines from each specimen were x-rayed, and by projecting the picture onto a screen, the maximum width of the tooth and of the pulp cavity could be measured. The pulp cavity width was then calculated as a percentage of maximum tooth width (cf. JENSEN 1976). A canine from each specimen was then decalcified in 5% nitric acid (HNO_3), and sagittal histological sections were prepared as described by GRUE & JENSEN (1973). The thickness of the sections was approximately 12 µ.

Results

SIZE OF PULP CAVITY

JENSEN (1976) demonstrated the possibility of using size of the canine pulp cavity of the red fox *(Vulpes vulpes)* to separate young foxes from older ones. This method was applied to teeth of the arctic fox and for 44 arctic foxes from Greenland, the width of the pulp cavity of each animal was expressed as a percentage of the maximum width of the tooth, and plotted against time of death of the animal (Fig. 1).

In Greenland fox cubs are born during late May – early June (CHR. VIBE, pers. com.), permanent canines in this species erupting at an age of approximately 3 months (MACPHERSON 1969). Judging from Fig. 1, the width of the pulp cavity forms about 80% of the total width of the canine on 1 October. During the next six months the percentage decreases, and is only half of the original percentage by 1 April. Two specimens from Godthåb in March with a percentage pulp cavity width of 64 and 66 fall outside the general picture. However according to CHR. VIBE, it is possible that a labelling error has been made, especially as the skulls are part of a large collection made through trading posts in Greenland from local trappers.

The material represents two seasons (1952/53 and 1953/54) and four localities on the west coast of Greenland, from Thule (about 77° N) to Frederikshåb (about 62° N). There is a tendency for young foxes from more southern localities to have bigger pulp cavities than foxes from Thule, but the scanty data do not allow any detailed discussion of this point.

The data in Fig. 1 clearly fall into two groups, canines with a pulp cavity width exceeding 40%, and those with a lesser value. Especially in comparison with results obtained for the red fox (JENSEN 1976) it is reasonable to assume all 33 animals in the first group to be less than one year old, and the 11 comprising the second group to be more than one year. By sectioning the teeth and investigating layers in the cementum (cf. below) it was possible to confirm the above assumption, and in Fig. 1 the number of layers present in the teeth sectioned are shown. For the whole winter period, it is possible to separate young of the previous year from older foxes on the basis of pulp cavity size. Furthermore the percentage pulp cavity size also decreases with age in foxes more than one year old, but within this latter group it is not possible to subdivide into age-classes on the basis of pulp cavity size.

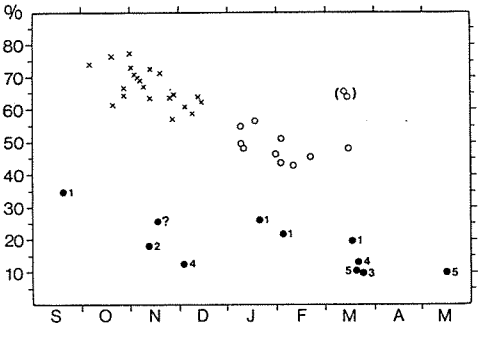

Fig. 1. Wild arctic fox, Greenland. Pulp cavity width as percentage of total canine tooth width, in relation to month in which each individual was killed. – x: Young foxes (< 1 year), canines not sectioned. – ○: Young foxes (< 1 year), canines sectioned. – ●: Older foxes, canines sectioned, numerals indicate number of incremental lines visible in each specimen.

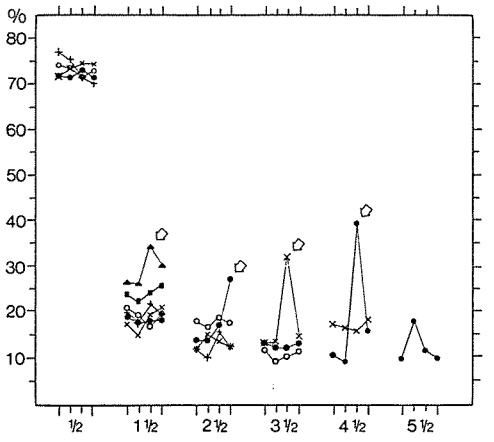

Fig. 2. Arctic fox, fur-farm reared, Denmark. Pulp cavity width as percentage of a total canine tooth width for each of 4 canines per individual, in relation to its known age in years. From left to right, values for upper right, upper left, lower left and lower right canine per individual are shown. Values for four broken canines are arrowed.

The canines of 20 arctic foxes of known age from the Danish fur-farm were collected in late November and x-rayed. The maximum pulp cavity width as a percentage of maximum tooth width for the 4 canines of each individual are shown in Fig. 2.

Farm foxes from Denmark are born about 1 June, almost the same time as cubs in Greenland, but by the end of November the four Danish farm foxes of less than one year have a somewhat greater pulp cavity size than young foxes from Greenland. Whether this difference is consistent and could possibly be explained by minor differences in time of birth or in growth rate ought to be investigated when more data are available. For foxes more than one year old, there is a general decrease in pulp cavity size with increasing age (Fig. 2). 18-month old foxes in particular have larger pulp cavities on average than older foxes.

Fig. 2 also illustrates the existence of differences in the percentage pulp cavity sizes of canines from the same individual. In four individuals, percentage pulp cavity size in one of the four canines deviated markedly from the remainder. However, these teeth all showed signs of having been broken to such an extent that the cavity was exposed, and tooth growth therefore probably ceased. This phenomenon must be taken into consideration if age determination of animals is based on size of pulp cavities.

INCREMENTAL LINES

21 of the foxes from Greenland killed before 1 January were judged from pulp cavity size to be juvenile, and their canines were not sectioned. Histological sections of canines of the remaining 23 animals all showed a distinct layer of cementum. A previous investigation of incremental lines in cementum of wild Danish red foxes (Grue & Jensen 1973) revealed that the first line in this species does not develop until the animal is at least 10 months old. In the arctic foxes, however, canines from all animals killed after 1 January when they are half a year old or more were sectioned regardless of the pulp cavity size. In the 12 specimens considered to be less than 1 year old and killed during the period January–March, no incremental lines were observed, except for 2 animals killed in February, where a developing first incremental line could be distinguished at the edge of the cementum. The other 11 animals, judged from Fig. 1 to be more than 1 year old, possessed from 1 to 5 incremental lines, although in 1 animal the lines merged

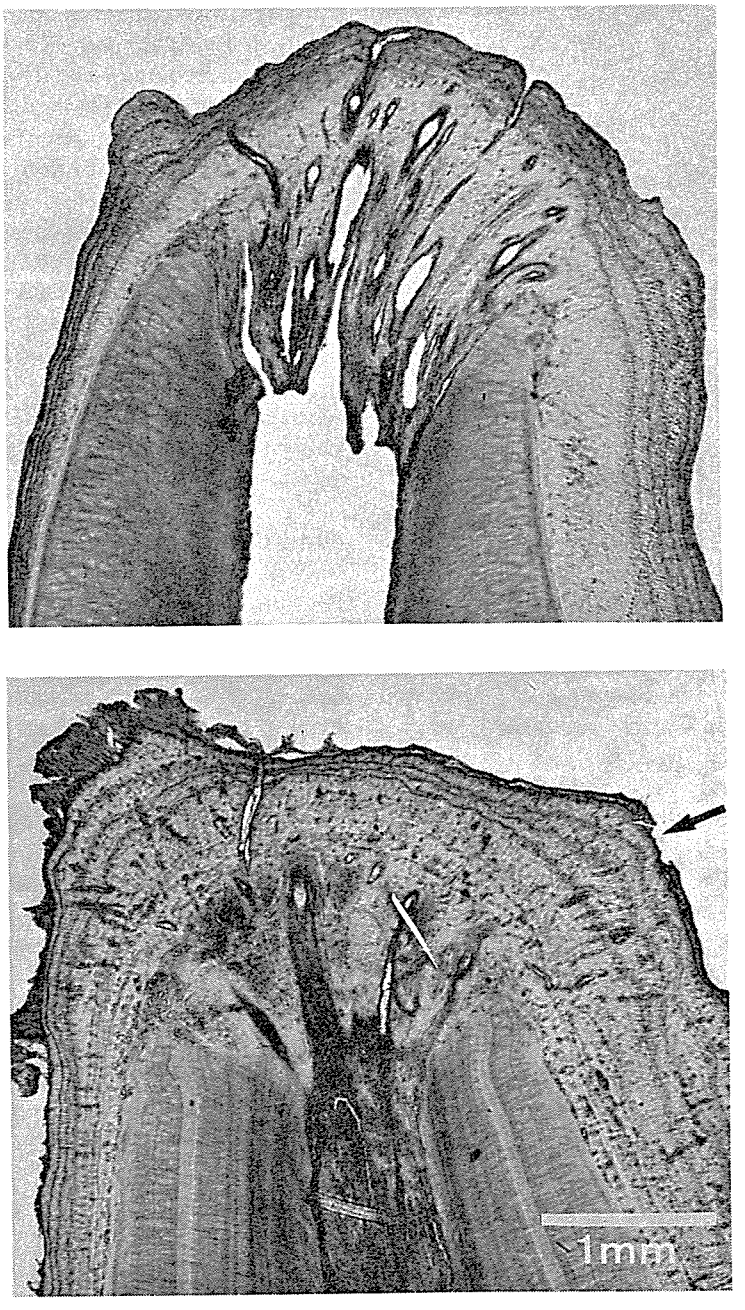

Fig. 3. Sections of canine teeth of arctic fox. Top, farm-reared fox from Denmark, killed late Nov., age 5½ years. 5 incremental lines visible in cementum, but no distinct stratification in dentine. – Bottom, wild fox from Greenland, killed late March. 3 incremental lines visible in cementum, and arrow points to a fourth being formed. Distinct stratification in dentine is also evident.

and the precise number could not be established.

In the older foxes, deposition of incremental lines was observed in animals killed between early February and May; the period for deposition of lines possibly extends into the summer months but material from the months June–August is required to verify this. Older foxes killed between 19 September and 20 January showed lightly stained homogeneous cementum deposited outside the last formed incremental line (Fig. 3, top).

The 2 young foxes from March, probably wrongly labelled as mentioned showed no signs of developing incremental lines; the absence of a line forming in these 2 animals substantiates the possibility of a labelling error.

The nature of incremental lines in the 10 adult specimens in which the lines present could be counted was very similar to the pattern previously observed in the wild red fox of Denmark (GRUE & JENSEN 1973). The existence of synchrony between line deposition and season of the year which results in one line per year seems probable in the wild arctic fox of Greenland, but in order to make a definite statement, material of known age is required.

In the Danish arctic foxes reared in farm, the lower left canines were sectioned and a distinct cementum layer was seen in all. No incremental lines could be distinguished in the four animals known to be less than one year old. In 16 animals, ranging in age from $1^1/_2$ to $5^1/_2$ years of age, incremental lines were observed (Fig. 3). For 13 of these the number of lines present corresponded precisely to the age of the animal in years.

Of the remaining 3 foxes, 1 animal listed as being $3^1/_2$ years of age showed 6 distinct lines in the cementum, indicating an age of $6^1/_2$ years. According to information from the fur farm, it is possible that an error was made in labelling the fox cages when breeding stocks were moved to clean cages after the pelting season.

In the last 2 animals, the cementum showed many thin lines and it was not possible to count the number present. The cementum growth had obviously been disturbed during development; these two very canines were broken, and from the size of the pulp cavity the event probably happened when the animals were approximately 1 year old. Sectioning of another canine from each of these two animals showed the number of incremental lines to be in accordance with the known age of the animal. In one of the other animals for which the number of lines corresponded to the age in years the canine sectioned was also known to be broken. In this specimen however, the single incremental line had obviously been developed before the tooth was damaged and the accessory lines formed as a consequence of the injury could easily be distinguished and did not interfere with the annual line once laid down.

The canine tooth of farm foxes often get caught in the wire netting of the cages, resulting in broken teeth. This possibility must be taken into consideration if farm-material of known age is used as reference material for the age determination of animals in the wild.

As all the farm foxes were killed during the pelting season in November, the time of year in which incremental lines are developed could not be precisely established. However in all animals the last-deposited line lay far from the edge of the cementum, indicating deposition in spring or summer (Fig. 3).

Incremental lines in the cementum of arctic foxes in the wild were generally

more clearly defined than in the fur-farm reared animals in Denmark. However, line distinctness varies in individuals, and it proved impossible to distinguish specimens from the two localities on the basis of line clarity alone.

It is noteworthy that the animals from Greenland showed distinct dentine stratification, whereas the dentine of the Danish fur-farm animals was more homogeneous. Lines in the dentine could not be related to animal age, based on lines in cementum. Though dentine may be deposited periodically as is cementum, due to rapid filling of the pulp cavity dentine is not normally suitable for age determination in terrestrial mammals beyond the first year or so of life (MORRIS 1972).

Fig. 4. X-ray of upper right canine tooth from fur-farm reared arctic foxes of known age. From left to right 5½, 3½, 1½ and ½ years old. The canine to the far right is from an animal 1½ years old in which filling up of the pulp cavity stopped after the tooth was broken.

Discussion

Measurements of the canine pulp cavity show that foxes less than one year old can be separated from older ones on a size basis, while foxes more than one year old cannot be further divided into age classes. This method might be especially useful in population studies where it is sufficient to distinguish between potentially reproductive animals and the yearly offspring. However, initial elimination of young animals beforehand could save much time even when a more detailed age classification is needed, for instance based on the incremental lines present in cementum.

This investigation has shown, that well-separated incremental lines are present in the canine cementum of arctic foxes living as wild animals in Greenland, or reared on fur-farms in Denmark. As the age of the Danish fur-farm animals was known, it was also possible to establish that incremental lines develop according to a seasonal cycle, and that the number of lines present corresponds to the age of an animal in years.

For the foxes from Greenland no animals of known age were available, but the nature of the incremental lines being the same as that of foxes reared on Danish fur-farms provides strong indication that in the wild arctic fox from Greenland they also develop according to a seasonal cycle, and that the number of lines present in a tooth coincides with the age of the animal in years. However, considering climatic differences between the two countries, age determination of wild arctic foxes from Greenland based on reference material from fur-farm animals in Denmark has

to be performed with caution until material from animals of known age from Greenland can be obtained.

In the arctic foxes from Greenland, developing incremental lines were visible at the edge of the cementum in animals killed during February-May. In all the Danish animals killed in November, the last-formed line lay well within the cementum layer indicating in this case also deposition in spring or summer. Corresponding results were observed in the wild red fox in Denmark (GRUE & JENSEN 1973). In this species, development of the first incremental line is not visible until the animal is approximately one year old in March. The deposition of incremental lines was mostly observed between March and September, with a maximum in May-June.

Farm-reared arctic foxes from the Saltykov state farm, USSR, have previously been investigated for age-related structures in the tooth-cementum (KLEINENBERG & KLEVEZAL 1966). The age of the animals was known, and the number of incremental lines in the cementum corresponded to the age of the animals in years. The first-formed line in these animals was noted to be just slightly distinguishable from the surrounding homogeneous cementum, and seemed to increase in clarity with advancing age. Quite the opposite was observed in 5 species of Danish carnivores investigated by one author here (H. G.), as in Danish animals the first-formed line is clear in younger animals but often becomes diffuse with advancing age.

The farm foxes from the USSR were killed in November, and all showed homogeneous cementum deposited outside the last-formed incremental line. The period of deposition of incremental lines in arctic foxes in the USSR as well as for 10 other mammalian species was stated to be December to March (KLEVEZAL & KLEINENBERG 1967) but no further details were given.

It is generally believed that the natural environmental fluctuations to which animals are exposed somehow affect the physiological processes leading to the development of annual incremental lines. A slight difference in the cementum zones and a more marked in the dentine was observed between arctic foxes from Greenland and Denmark. Danish farm foxes are usually kept in outdoor cages and are exposed to climatic fluctuations to almost the same extend as wild animals, and it is improbable that captivity in this respect should affect the natural periodicity of dentine development.

The observed difference in distinctness of incremental lines both in cementum and dentine between animals from Denmark and Greenland could have a climatic explanation. The yearly mean temperature range at 70° N on the westcoast of Greenland is 25° C while in Denmark it is 17° C (a difference of 8° C) but there are marked differences in the absolute temperature levels. In Greenland and Denmark respectively July means are 6,°4 C and 17° C, and for January –19,°5 C and 0° C. It is therefore possible that climatic differences rather than conditions of captivity are the cause of differences in distinctness of incremental lines in animals from the two countries observed in this work.

Climatic influence on the distinctness of incremental cementum lines has been observed in beaver *(Castor fiber)*, in moose *(Alces alces)* and in two species of *Apodemus* by KLEVEZAL (1973). In these species incremental cementum lines were less distinct in animals from regions with a weak continental climate than in those from regions of typical continental climate.

Variation in the distinctness of incremental lines with the geographical location of animals has also previously been demonstrated in red deer *(Cervus elaphus)* by LOWE (1967) and SMITH (1974).

The influence of seasonal changes in environment on the development of annual incremental cementum lines is furthermore substantiated by results from an investigation of the cementum in teeth of dogs *(Canis familiaris)* (GRUE 1976). The annual periodicity in deposition of incremental lines previously observed in five species of wild Danish carnivores was not found in the dog, indicating that the development of annual lines requires a more direct exposure to seasonal climatic fluctuations than Danish domestic dogs normally receive in their mainly indoor environment. In sledge-dogs from Greenland however, incremental lines similar to those observed in the arctic fox were found, indicating the influence of climatic fluctuations on their deposition.

Dansk resumé

Årlige lagdannelser i hjørnetændernes rodcement hos polarræve (*Alopex lagopus* (L.)) fra Grønland og Danmark

Aldersbestemmelse af pattedyr på grundlag af lagdeling i tændernes rodcement finder i dag udstrakt anvendelse. Som led i studier over lagdannelsen er undersøgt histologiske præparater af hjørnetænder fra 20 polarræve af kendt alder, født og opvokset på en dansk pelsfarm. Disse præparater er sammenlignet med tilsvarende af hjørnetænder fra 23 vildtlevende polarræve fra forskellige lokaliteter på Grønland. De danske farmræve viste en klar årscyklus i dannelsen af rodcement, og antallet af linier i cementen svarede til dyrenes alder i år. De fleste grønlandske polarræve viste noget tydeligere linier i cementen end de danske, og det må antages, at antallet af linier i rodcementen også for de grønlandske ræve svarer til dyrenes alder i år. Hos de grønlandske ræve, men ikke hos de danske, iagttoges også en lagdeling i hjørnetændernes dentin, men disse lag kunne ikke sættes i relation til lagene i cementen. Årscyklus i lagdannelsen i tænderne hos grønlandske vildtlevende polarræve synes således at være mere udpræget end hos polarræve, der lever i farm i Danmark under et mildere klima og med en stabil fødetilgang. Årscyklus er dog fuldt bevaret hos danske farmræve, men det er usikkert, hvilke faktorer, der regulerer den.

Alle de danske farmræve var aflivet i november og havde en bred lys yderzone i rodcementen. De grønlandske ræve var nedlagt i perioden september–maj, og dannelse af en smal mørk linie yderst i rodcementen sås hos dyr nedlagt i perioden feb.–maj.

Inden der fremstilledes præparater af hjørnetænderne, blev de røntgenfotograferet, og pulpahulens bredde i forhold til tandens bredde blev udmålt på fotografiet. Hos de danske farmræve af kendt alder havde de ca. 1/2 år gamle dyr en meget stor pulpahule og lod sig let adskille fra de ældre på denne karakter. Hos de ældre ræve aftog pulpahulens størrelse med alderen, men en yderligere aldersklassificering af dyrene på grundlag heraf var ikke mulig (Fig. 2). Enkelte hjørnetænder hos ældre dyr havde en påfaldende stor pulpahule, men i alle tilfælde skyldtes det, at tanden var brækket og udfyldningen af pulpahulen med dentin dermed ophørt. De grønlandske polarræve kunne på grundlag af pulpahulestørrelse opdeles i to grupper, og undersøgelsen af rodcementen bekræftede, at gruppen med en stor pulpahule bestod af unge dyr (< 1 år).

Резюме на русском языке

Образование годовых слоев в цементе клыка песцов (*Alopex lagopus* (L.)) из Гренландии и Дании

В настоящее время широко применяется способ определения возраста млекопитающих по слоистой структуре корневого цемента зубов. В порядке изучения слоистой структуры были исследованы гистологические препараты клыков 20 песцов известного возраста, рожденных и выросших на датской звероферме. Эти препараты сравнялись с соответветствующими препаратами клыков 23 живших на воле песцов из разных местностей Гренландии. У песцов с датской зверофермы обнаружен четкий годовой цикл образования корневого цемента, и число линий в цементе соответствовало числу годов возраста животных. У большинства гренландских песцов линии в цементе были несколько более четки, чем у датских, и можно предполагать, что число линий в корневом цементе гренландских песцов также соответствует числу годов возраста этих животных. У гренландских песцов, но не у датских, наблюдалась слоистая структура также и в дентине клыков, но не было возможно найти соотношения между этими слоями и слоями цемента.

Все песцы с датской фермы были убиты в ноябре и имели широкую светлую зону у наружного края корневого цемента. Гренландские песцы были убиты в течение периода с сентября по май, и у животных, убитых с февраля по май, у наружного края корневого цемента наблюдалась узкая темная линия. Таким образом кажется, что годовой цикл образования слоистой структуры зубов у живущих на воле гренландских песцов выражен более четко, чем у песцов, живущих на фермах в Дании при более мягком климате и постоянном снабжении пищей. Однако, годовой цикл у песцов с датских ферм полностью сохранился, хотя не выяснено, какие факторы регулируют его.

Перед приготовлением из них препаратов были сделаны рентгеновские снимки клыков, и на фотографии измерялась ширина пульповой полости по отношению к ширине зуба.

Из песцов известного возраста с датской зверофермы, у животных возрастом в полгода была очень большая пульповая полость, и их по этому признаку было легко отличить от более старых особей. У животных старшего возраста величина пульповой полости уменьшалась по мере их старости, но добавочная классификация животных по возрасту на этой основе была невозможна (Фиг. 2). Некоторые клыки сравнительно старых животных имели замечательно большую пульповую полость, но это во всех случаях объяснялось тем, что зуб был сломан, и заполнение пульповой полости дентином поэтому прекратилось. Гренландских песцов на основании величины пульповой полости можно было разделить на две группы, и исследование корневого цемента подтвердило, что группа с большой пульповой полостью составлялась из молодых животных (< 1 лет).

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