

## Age determination of the raccoon dog in Finland

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We compared the age determination using the relative width of the pulp cavity in the lower canines and the one using the ossification stage of the epiphyseal cartilage of the radius and ulna with the most reliable method, namely, that based on the incremental lines in the tooth cementum in order to find a quick and reliable method for identifying young raccoon dogs *Nyctereutes procyonoides* (Gray, 1834), in Finland. Most juveniles could be identified by means of the pulp cavity/ tooth diameter ratio in early autumn, and a remarkable proportion of them could still be separated from adults in late autumn and early winter. When using a separation point of 50%, only 2% of adults were determined as young. In spring, by contrast, the method is of no use, because the pulp cavity/tooth diameter ratio rarely exceeds 50% even in the young. All individuals killed before December were correctly aged from the ossification stage of the long bones. In December-January the proportion of incorrect determinations was 2–3%, but in spring it rose rapidly, being 17% in April–May. Until the end of February this method is very safe; no adults are determined as young. In conclusion, the best method for identifying juveniles in the present study is that using the ossification stage of the long bones until the end of February. The method using the relative width of the pulp cavity is less practicable especially in late autumn. Neither of these methods works well in spring, so, at that time all animals should be aged from the incremental lines.

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### Introduction

The raccoon dog *Nyctereutes procyonoides* (Gray, 1834) which originates in eastern Asia, was introduced to the north-west Soviet Union in 1935–55. Soon afterwards it appeared in Finland, and during the past few decades it has rapidly colonized southern and central parts of the country (Helle and Kauhala 1987). Because the raccoon dog is the main vector of rabies in Finland (Westerling 1988), and it may have effects on other small game animals, it is important to understand the population dynamics of the species. In order to study the population dynamics it is essential to know the age structure of the population and, thus, to determine the age of a large number of animals.

Many different methods have been used to determine the ages of mammals. Age determination on the basis of annual incremental lines in the dentine and the tooth cementum is usually considered the most reliable and accurate of the methods (*e.g.* Low and Cowan 1963, Hewer 1964, Klevezal and Kleinenberg 1967, Conley 1968, Crowe 1972, Thomas 1977, Larson and Taber 1980).

Because preparing the teeth for age determination by the method of incremental lines is laborious and time consuming, other methods have also been used. Dolgov and Rossolimo (1966) used the relative width of the pulp cavity of canines for determining the age of arctic foxes *Alopex lagopus*. Knudsen (1976) and Jean *et al.* (1986) used the same method for determining the age of coyotes *Canis latrans*, especially for distinguishing between juveniles and older specimens, Johnson *et al.* (1981) used the method to age bobcats *Felix rufus*, Dix and Strickland (1986) and Nagorsen *et al.* (1988) to age martens *Martes americana*, Kuehn and Berg (1981) and Jenks *et al.* (1984) to age fishers *Martes pennanti*, Coman (1988) to age foxes *Vulpes vulpes* and Lüpms *et al.* (1987) to age badgers *Meles meles*. In many mammals, *e.g.* hares (Stroh 1931, Walhovd 1966, Broekhuizen and Maaskamp 1979, Soveri *et al.* 1986), the juveniles can also be identified by means of the ossification stage of the epiphyseal cartilage of long bones. Englund (1970) has also used this method to age red foxes, Fiero and Verts (1986) to age raccoons *Procyon lotor* and Lüpms *et al.* (1987) to age badgers.

Because most raccoon dogs to be aged in population studies are juveniles, a quick method for separating 'young' specimens (less than one year) from 'old' ones (at least one year) is needed. In that purpose we studied the reliability of the method using the relative width of the pulp cavity in the lower canines and the one using the ossification stage of the epiphyseal cartilage of the radius and ulna by comparing them with the method based on the incremental lines in the tooth cementum.

### Material and methods

Raccoon dog carcasses were collected from hunters in 1986-89 from six provinces in Finland: the provinces of Turku and Pori, Uusimaa, Häme and Kymi in southern Finland, and the provinces of North Karelia and Oulu, which lie further north. Most specimens were sampled using wire cage traps, hunting dogs or burrowing dogs during ordinary hunts between August and May. The pulp cavity was measured from the lower canines of 1140 animals from Häme and Kymi in 1986-89. The bones were examined from 988 individuals, the majority also from Häme and Kymi, in 1987-89.

### Incremental lines

The annual incremental lines are caused by periodic changes in the rate and nature of the calcification of the dentine and the tooth cementum as a result of seasonal differences in feeding, vitamin D intake and hormonal activity (Morris, 1972). In summer, growth is fast and a wide layer is formed, but in winter growth becomes slower and a narrow band (annual incremental line) is laid down (Kleinenberg and Klevezal 1966). In carnivores the first line is usually formed when the animals are 6 to 18 months old (Grue and Jensen 1979). In the raccoon dog the first line is formed during the first winter as we judged from 19 farmed raccoon dogs of known age. Annual growth lines are most pronounced in hibernators, *e.g.* the raccoon dog, and in seals, which fast for a long period every year (Mayer and Bernick 1963, Morris 1972).

We determined the age from longitudinal thin (0.15 mm) ground sections of the canines under a transilluminating microscope. For checking the ground section method, a histological (decalcified and stained) section was made from the teeth of 295 specimens in Matson's laboratory, Milltown, Montana, the ages of which were determined by us. Both techniques gave the same results in distinguishing between juveniles and adults. The growth lines were counted from the cementum, and the age was assessed in full years, assuming that the raccoon dogs were born at the end of May. In this study the age determined from the

incremental lines in the tooth cementum is considered the true age, with which ages determined by other methods are compared.

#### Relative width of the pulp cavity

Because the dentine grows inwards, gradually filling the pulp cavity, the cavity is large in young animals but becomes smaller with age (Laws 1953). Thus, the ratio between the pulp cavity and the outside diameter of the whole tooth, as seen in the cross sections of canines, can be used for age determination (e.g. Morris 1972). In most mammals dentine growth practically ceases after the animal reaches sexual maturity, and so this method can only be used to distinguish between juveniles and older specimens.

We made a cross section of the lower canine at the gum line on the anterior edge of the tooth. The gum line was easily identified on all teeth. The maximum diameter of the pulp cavity and that of the whole canine were measured to 0.1 mm under a microscope in order to calculate the pulp cavity/diameter ratios. The separation point between juveniles and adults was chosen in such a way, that none or a minimum number of adults are determined as young rather than *vice versa*, because the teeth of those determined as adults are later sectioned and young specimens among them thus revealed. We also calculated the dividing point between young and adults using the formula of Dix and Strickland (1986):

$$D = \bar{x}_a + (\bar{x}_b - \bar{x}_a)(SD_a)/(SD_b + SD_a), \text{ where } \bar{x}_a < \bar{x}_b.$$

#### Ossification stage of the epiphyseal cartilage

Growth takes place in a cartilaginous region near the end of the long bones (Weinmann and Sicher 1955). When growth ceases, the epiphyseal cartilage ossifies, and the terminal part of the bone (the epiphysis) becomes solidly fused to the rest of the bone. An epiphyseal line is often seen at the site of the recently ossified epiphyseal cartilage. Thus, the ossification stage of the epiphyseal cartilage can be used as an indicator of age. Epiphyses close at different ages in different bones (Morris 1972). The radius and ulna were used in this study, because they ossify rather late (Washburn 1943).

The bones were boiled for three hours, after which the flesh was separated from the bones. The distal ends of the bones were examined with the unaided eye, but some of the bones were also radiographed.

## Results and discussion

### Pulp cavity

The cavity/diameter ratio becomes smaller in both males and females in successive age groups until the age of 2.5 years (Mann-Whitney U-test, Sokal and Rohlf 1981), but considerable overlap exists between these age groups (Fig. 1). After the age of 2.5 years there is very little change in the cavity/diameter ratio. A nonlinear relationship with age in the pulp ratios was found also in coyotes (Jean *et al.* 1986) and martens (Nagorsen *et al.* 1988).

The cavity/diameter ratio is more than 50% in only 2% of the old specimens. In contrast, in juveniles, the ratio is more than 50% in 98% of specimens in September, in 89% in October, in 69% in November and in 37% in December – January. Thus, most juveniles can be identified by means of this method in early autumn, and a considerable proportion of them can still be distinguished from old animals in late autumn and winter, when using the separation point of 50%. When using the separation point of 54%, no old specimens are determined as young, but the proportion of juveniles that can be identified is smaller. Jean *et al.* (1986) used a separation point of 48% for

coyotes and they did not find any overlap between young killed in November – January and adults.

According to the formula of Dix and Strickland (1986), the dividing point between raccon dog juveniles and adults would be 47% for females and 49% for males in September, 45% and 47% in October, 40% and 43% in November and 40% and 41% in December and January, respectively. Hence, a higher proportion of juveniles could be identified by using the formula, but, as a consequence, a higher proportion of adults would be incorrectly determined as young, especially in late autumn. Because we want to minimize the number of adults that are incorrectly determined as young, we prefer our separation point of 50% (or 54%).

In February – May the relative width of the pulp cavity is more than 50% in only 2% of young individuals, so this method is of no use in spring. Because of the large overlap between successive age groups among older specimens, the method cannot be used to determine the exact age of old animals either. Johnson *et al.* (1981), Jean *et al.* (1986), Dix and Strickland (1986) and Nagorsen *et al.* (1988), among others, came to the same conclusion in their studies on other carnivores.

The cavity is relatively larger in males than in females. However, the differences are

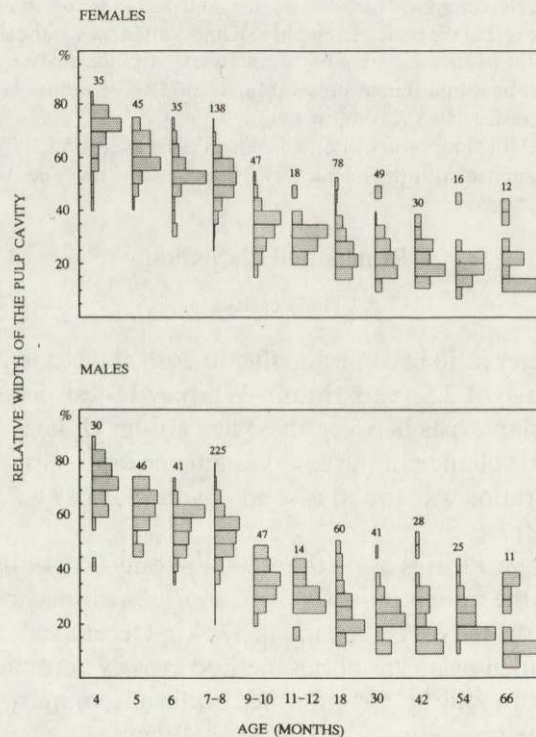


Fig. 1. The relative width of the pulp cavity (percentage frequency distribution) of male and female raccoon dogs at different ages in Finland. Figures give the sample sizes.

small, and in 15 cases out of 20 they are not significant (Mann-Whitney U-test) when the data at any month are compared. In 17 cases out of 20, though, the cavity/diameter ratio is higher in males than in females, which means that there is a small, but significant, difference between the sexes (binomial test,  $p=0.04$ ). A difference between the sexes in the relative width of the pulp cavity has been also found in the arctic fox (Dolgov and Rossolimo 1966) and in the marten (Dix and Strickland 1986, Nagorsen *et al.* 1988). However, the differences between the male and female raccoon dogs are so small that they do not interfere with the age determinations.

Small differences also exist in the cavity/diameter ratios between the years (Fig. 2). The cavity was relatively largest in early autumn in the exceptionally cold year of 1987, probably indicating a slower growth rate of juveniles or later time of birth, or both, in that year. The difference between 1987 and 1988 was significant in October for both males and females ( $p=0.018$  for males,  $p=0.007$  for females; Mann-Whitney U-test). Hence, after a very warm summer a slightly smaller proportion of juveniles can be aged by means of this method than after an exceptionally cold summer. However, because the differences between the years are small and not significant except the one mentioned above, they have, generally speaking, little effect on the age determination.

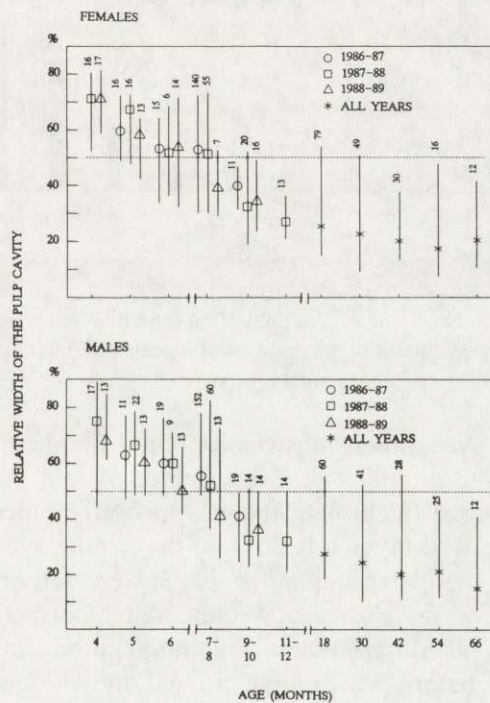


Fig. 2. The relative width of the pulp cavity (median and range) of female and male raccoon dogs in Finland in different years. The separation point between young and old (50%) is marked with a dashed line. Figures give the sample sizes.

### Ossification stage of the radius and ulna

Age determination succeeded better when the bones were examined with the unaided eye rather than from radiographs, because in the radiographs the epiphyseal line is sometimes also visible in adults, which can be confusing. Therefore we used here only the results from visual examinations, which succeeded equally well from both bones.

All animals killed before the end of November were correctly determined as 'young' or 'old' by means of the ossification stage of the epiphyseal cartilage of the radius and ulna (Fig. 3). In December, 2% of the age determinations using the long bones differed from those made using the incremental lines and were considered incorrect. The proportion of incorrect determinations made using the long bones increased in late winter and spring from 3% in January to 17% in April–May.

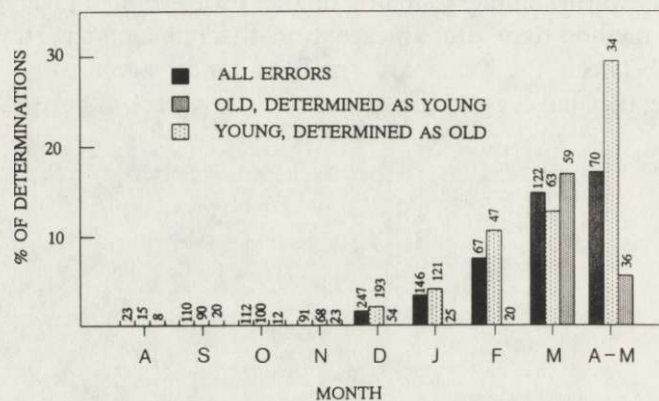


Fig. 3. The percentage of incorrect age determinations of raccoon dogs made using the ossification stage of the epiphyseal cartilage of the radius and ulna in different months in Finland. The true age is determined from the incremental lines. Figures give the sample sizes.

Before the end of November all juveniles could be identified by means of this method. Only 3% of the juveniles were determined as 'old' in December–January, but from the beginning of February the proportion of incorrect determinations rose rapidly, and in April–May nearly 30% of the 'young' were determined as 'old'.

Altogether 5% of the old specimens ( $n=257$ ) were determined as 'young' using the ossification stage of the radius and ulna. All incorrect determinations were made between March and May. Thus, this method can be considered very safe until the end of February, before which time no old individuals were determined as 'young'. But between March and May the age of every individual should be determined using other methods, because as many as 13% of adults were determined as 'young'. This method saves much time in case of material prior to the end of February, for instance, in the present study 98% of young individuals could be

identified using it between August and February.

In hares ossification of the long bones ends at the age of 7–9 months (*e.g.* Hale 1949, Petrides 1951). Thus, Soveri *et al.* (1986) could identify young mountain hares *Lepus timidus* from radiographs until the end of December. By means of palpation of the prominence of the ulna (the distal epiphyseal cartilage of the ulna) they could reliably distinguish between juveniles and older individuals until the latter half of November. Therefore we conclude that the method using visual examination enables the age of raccoon dogs to be determined at least as long as that of hares by palpation or radiography. Palpation can also be used to some extent in identifying live juvenile raccoon dogs in autumn (Helle and Kauhala, unpubl.).

Lüps *et al.* (1987) used the ossification stage of the epiphyseal cartilage in order to identify juvenile badgers; they could in most cases distinguish between juveniles (less than one year) and adults by using both the distal and proximal ends of the tibia.

### Conclusions

All young raccoon dogs killed before the end of November and most of those killed between December and March are identified as young by means of the ossification stage of the radius and ulna (Fig. 4). Thus, this method saves much time and should be used to age animals killed before the end of February. In spring, however, the method is not safe, because too many adults are determined as young. If their teeth are not sectioned, the erroneous results are not revealed but remain in the data.

The method using the relative width of the pulp cavity works well in early autumn,

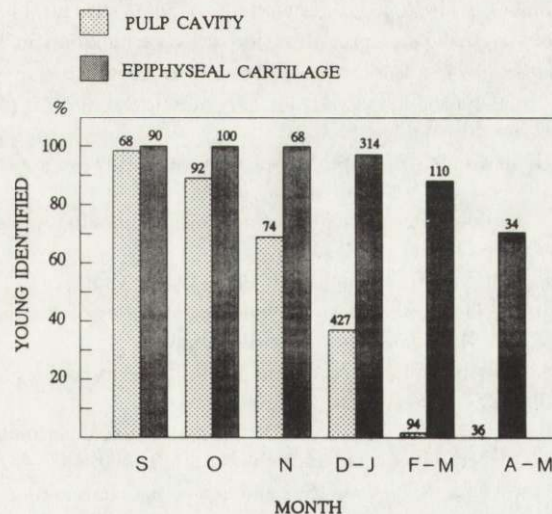


Fig. 4. The percentage of young raccoon dogs identified using the relative width of the pulp cavity and the ossification stage of the epiphyseal cartilage of the radius and ulna in different months in Finland. Figures give the sample sizes.

when the cavity/diameter ratio is more than 50% in most juveniles. However, it is less practicable in late autumn and is of no use in spring. If we use a separation point of 50%, some adults are still determined as juveniles in early autumn. Thus, the method using the ossification stage of the epiphyseal cartilage is more efficient and more reliable for identifying juveniles than that using the relative width of the pulp cavity. Between March and May the age should always be determined by means of the annual incremental lines of the tooth cementum.

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