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Age determination in the Red fox in a Mediterranean habitat

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Abstract

The age determination of red foxes *Vulpes vulpes* was studied in Pisa Province, Central Italy, from January to May 1992. The presence of incremental lines in the cementum of canine teeth after decalcification, sectioning and staining has been checked for the first time in a Mediterranean species. We compared the weight of the eye lens and the width of pulpar cavity of canine teeth as methods of discriminating yearlings from adults with the incremental line count (a more reliable, but more time-consuming technique). 330 foxes (125 F; 205 M) were collected, but sample size was variously reduced for different methods. Incremental lines (up to 8) were clearly visible. Neither the eye lens nor the pulpar cavity methods discriminated unequivocally the two age classes, but a discriminant function using the two variables achieved 100% success. Pulpar cavity of females closed during their first oestrus. Geographical variability in the development rates of different organs suggests that no method (except incremental lines) can be used without prior verification.

Introduction

A variety of methods has been used to determine the age of various Carnivora: external measurements (Sargeant et al. 1981); weight or shape of baculum (Harris 1978; Nelson and Chapman 1982; Ogle et al. 1990); weight of eye lens (Harris 1978; Nelson and Chapman 1982); fusion of cranial or epiphyseal sutures (Harris 1978; Nelson and Chapman 1982; Yoneda and Maekawa 1982; Hancox 1988; Ogle et al. 1990; Rosas et al. 1993); width of pulpar cavity of teeth, as measured by either sectioning (Root and Payne 1984; Goszczynski 1989) or radiographing (Tumlison and McDaniel 1984; Ogle et al. 1990) one of the teeth; distance of the enamel line from the edge of the alveolus (Allen 1974; Root and Payne 1984); tooth wear (van Bree et al. 1974; Harris 1978; Root and Payne 1984; Hancox 1988); presence of the apical foramen of canine teeth (Root and Payne 1984); enumeration of incremental lines ot either teeth cementum (Sauer et al. 1966; Jensen and Nielsen 1968; Grue and Jensen 1973; Monson et al. 1973; Allen 1974; van Bree et al. 1974; Harris 1978; Artois and Salmon 1981; Nelson and Chapman 1982; Yoneda and Maekawa 1982; Goszczynski 1989; Ogle et al. 1990; Rosas et al. 1993), or teeth dentine (Harris 1978; Driscoll and Jones 1985) or bones (Harris 1978). Whereas a variety of techniques appears suitable to separate young from adults, the most reliable method to estimate age is enumeration of cementum lines (e.g. Harris 1978). Lines are formed during winter in temperate areas, and during dry season(s) in the tropics. Dark annuli may therefore be related to nutritional stress (Coy and Garshelis 1992). None of the above studies, however, was conducted in Mediterranean areas, where winters are mild and aridity during summers is not extreme. Under these conditions, food limitation is probably not highly seasonal, especially for an animal with a catholic diet such as the red fox (e. g. CAVALLINI and LOVARI 1991; CAVALLINI 1994). Red foxes, having only one oestrus cycle per year (in spring: LLOYD and ENGLUND 1973; CAVALLINI 1994), have a discrete age structure. Determination of age is therefore facilitated when date of sampling is known. Determination of age is most difficult in late winter and spring, when yearlings approach the size of adults (HARRIS 1978).

The aim of this study is to verify the suitability of three ageing techniques (eye lens weight, pulpar width and incremental lines) in a sample of red foxes *Vulpes vulpes* collected in late winter and spring from a Mediterranean area.

Material and methods

Foxes were collected by hunters in the Pisa province (43° N, 10–11° E), Central Italy, from January to May 1992, during the main fox hunting season. The area (52 km E-W by 75 km N-S; 2 448 km^2) is mostly flat and intensively cultivated (mainly cereals) in the north, becoming increasingly hilly (up to 800 m a. s. l.) and wooded towards the south. The climate is Mediterranean, with mild winters and dry, hot summers. In 1992, minimum temperatures (monthly average) ranged from 3.4 °C to 19 °C, and maximum temperatures from 12 °C to 31 °C. Monthly means were below 10 °C for 3 months, and above 20 °C for 4 months. Rainfall is heavier in autumn (35.9% ot total rainfall), in winter (28.9%) and in spring (23.7%), whereas only 11.5% of total rain occurs during summer. Interannual variation is large: in 1992, the least rainy months (less than 20 mm of rain per month) were January, February, March, August, and May (in increasing order; CAVALLINI 1994).

We collected foxes (N = 330; 125 females and 205 males) from hunters within 6 hours of death and stored them in plastic bags in a refrigerator cell (≤ 48 hours, −2 °C) until dissection (within 2 days from refrigeration). The protocol of HARRIS (1978) for measuring eye lens weight (± 0.1 mg) was followed without modifications. The average between the two lenses was taken, and pairs of lenses that differed by more than 1% were discarded. We extracted both right (or, in their absence, left) canine teeth from the skull after maceration (6 months) in water. They were radiographed using standard techniques (courtesy of Hospital "Le Scotte", Siena). After tracing a perpendicular to the major axis of the tooth in the point of its maximum width, we measured external diameter of the tooth and diameter of the cavity (± 0.1 mm) with a calliper. The ratio of the two measures was used for subsequent analyses. In the case of teeth damaged during the life of the animal, dentine deposition is inhibited, and this technique cannot give unequivocal results.

The same teeth were then decalcified in aqueous solution of HNO_3 (10%) for 36–48 h (according to tooth dimension). The tooth was then rinsed for 24 h under running tap water and cut sagittally (20 μm) with a freezing microtome (–20 °C). After air-drying (24 h), sections were stained with ematossilyne and mounted with standard histological techniques. This protocol is slightly modified from that of GRUE and JENSEN (1973). Incremental lines were counted by use of an optical microscope (40×).

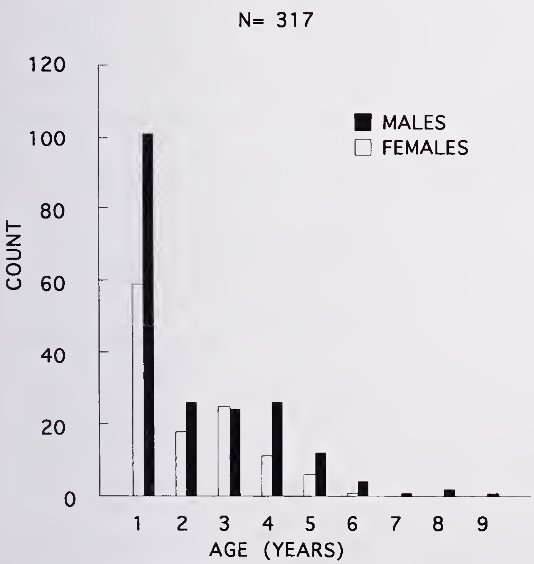

Fig. 1. Age composition (as determined by incremental annuli) of 317 foxes sampled in Pisa Province, Central Italy, January–May 1992.

Results

Microscopic analysis of decalcified sections showed that approximately one half of the samples did not show any incremental lines; these foxes were therefore considered to be in their first year. In the other foxes, lines (up to 8, corresponding to an age of 9 years) were clearly visible (Fig. 1).

Weights of eye lens showed two peaks, one around 200 mg (yearlings) and the other around 240 mg (adults). However, the overlap between the two distributions was very high (Fig. 2 a). Both the effect of age (pooling samples ≥4 years) and that of sex on eye lens weight were significant (age: $F = 167.3$, $p < 0.0001$; sex: $F = 19.6$, $p < 0.0001$, $N = 179$). Their interaction did not have an effect ($F = 1.2$, $p = 0.3$).

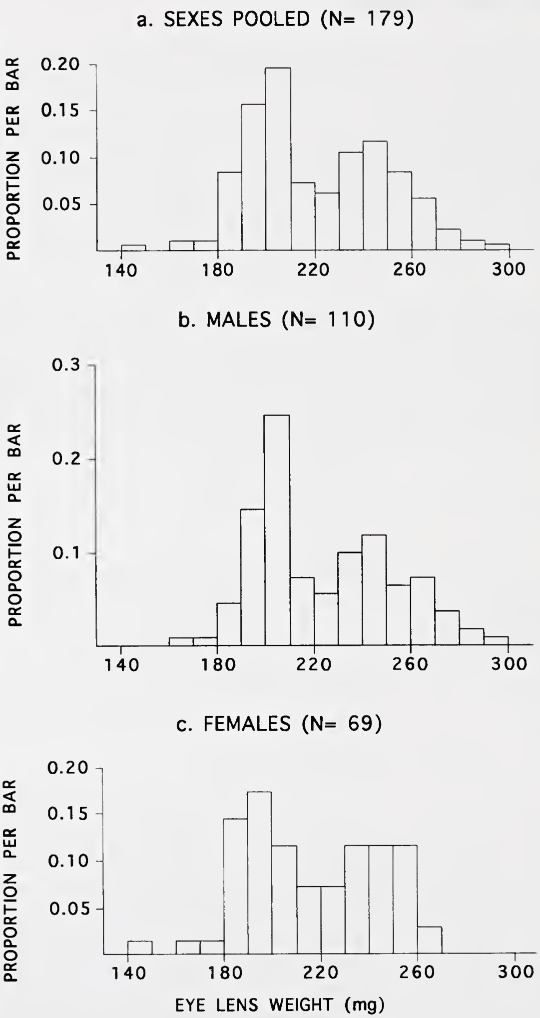

Fig. 2. Frequency distribution of lens weight of foxes from Pisa Province. The distributions for sexes pooled (a), for males (b) and for females (c) are shown. N is the number of individuals.

However, separation of male and female samples did not improve the discriminant power of this technique (Fig. 2 b, c). Weights of eye lens of juvenile foxes averaged 198.0 ± 13.4 mg ($N = 90$), whereas lenses of adults were heavier (244.4 ± 17.8; $N = 88$; Mann-Whitney $U = 181.5$, $P < 0.0001$). Eye lens weight continued to increase with age, although slowly (Fig. 3 a). Most foxes with eye lenses heavier than 210 mg were adults (82 of 95, or 86%), whereas those with lenses lighter than 210 mg were mostly yearlings (77 of 81, or 95%).

The effects of age (pooling samples ≥4 years) and sex on pulpar cavity ratio were significant (age: $F = 309.0$, $p < 0.0001$; sex: $F = 10.4$, $p = 0.001$, $N = 304$). Their interaction did not have an effect ($F = 0.3$, $p = 0.8$). When ages are pooled, pulpar cavity ratio was higher for males than for females, although they were not statistically different (upper: 0.332 ± 0.174; $N = 194$ vs. 0.301 ± 0.165; $N = 110$; Mann-Whitney $U = 11911.5$, $p = 0.092$; lower: 0.285 ± 0.155; $N = 192$ vs. 0.253 ± 0.139; $N = 117$; Mann-Whitney $U = 12703.5$, $p = 0.053$). The frequency distribution of pulpar cavity ratios showed two peaks (wide cavity, i.e. yearlings, and narrow cavity, i.e. adults). The discrimination based on the upper canine tooth was more reliable than that on the lower canine (Fig. 4). For females,

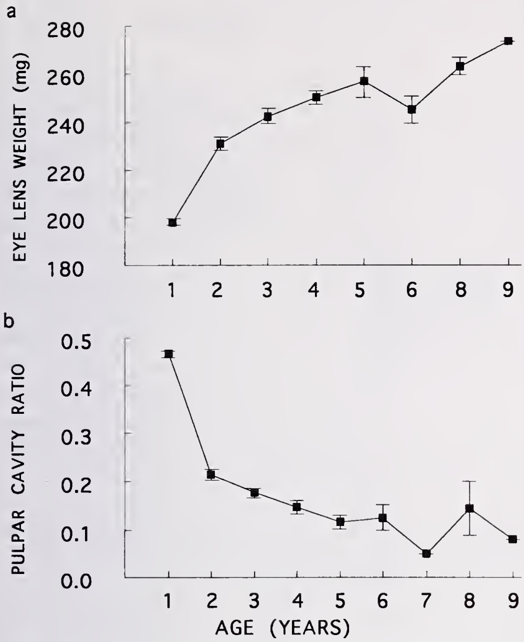

Fig. 3. Variation with age of: (a) lens weight; (b) ratio of pulpar cavity diameter to external diameter of the upper canine tooth. Averages and standard errors are shown. Sample sizes are the same as in Fig. 2 a and 4 a.

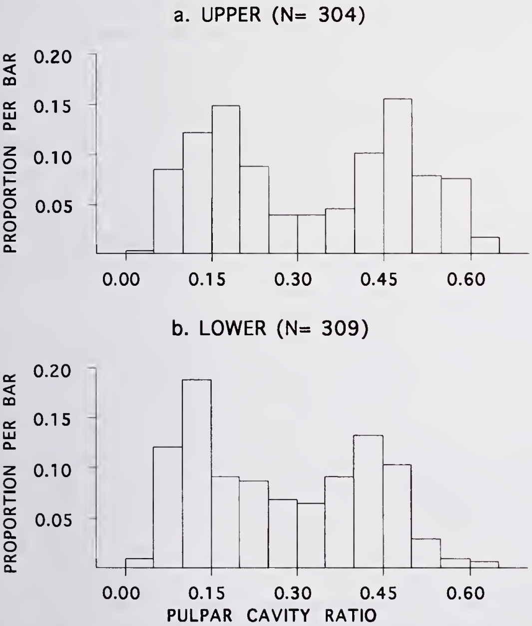

Fig. 4. Frequency distribution of the ratio of pulpar cavity diameter to external diameter ot the canine tooth of foxes from Pisa Province. The distributions for upper (a) and lower (b) canine teeth are shown. N is the number of individuals.

there was no overlap between the upper canine pulpar width of young and adults in the period before oestrus (until 23 February; Cavallini 1994). The females with a ratio of less than 0.3 were adults, whereas those with a ratio >0.4 were yearlings, i.e. before their first oestrus (Fig. 5 a). During the period of oestrus (9 to 31 March), the overlap increased (Fig. 5 b), until after oestrus (from 12 to 30 April), when the two peaks were not noticeable (Fig. 5 c). For males, no gap was evident in any period. The cavity ratio of yearlings was significantly higher than that of adults (0.466 ± 0.090; $N = 155$ vs. 0.170 ± 0.079; $N = 149$; Mann-Whitney $U = 22\,609$, $P < 0.0001$). The ratio was 0.521 ± 0.074 ($N = 43$) at the beginning of the sampling period (until 20 February), and dropped to 0.403 ± 0.087 ($N = 38$) at the end (after 31 March; sexes pooled). Closure of the cavity is rapid in the first year, thereafter it is continuous, but very slow (Fig. 3 b).

Better results could be obtained by combining the results of the above analyses (Fig. 6): all points above the line indicated adults, whereas all the points below the line indicated yearlings. A Fisher Linear Discriminant Function (following Wilkinson 1990) was calculated. Complete discrimination of the two age classes was achieved without misclassifications (Wilks' $L = 0.106$, $F = 702.45$, $P < 0.0001$, $N = 169$). The classification functions were: $136.5x + 0.909y - 124.9$ for yearlings and $61.6x + 1.073y - 136.8$ for adults (where x = pulpar cavity ratio and y = eye lens weight). These functions can be applied to new data and each case may be assigned to the group with the largest function for that case.

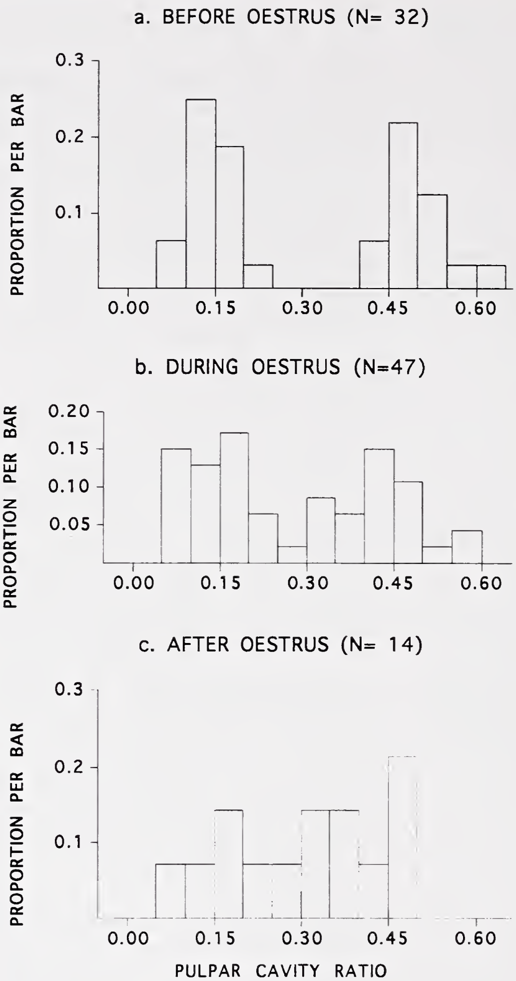

Fig. 5. Frequency distribution of pulpar cavity ratio of the upper canine tooth of female foxes from Pisa Province, divided in to three sampling periods (before oestrus (a): until 23 February; during oestrus (b): 9 to 31 March; after oestrus (c): from 12 to 30 April). N is the number of individuals.

Discussion

In our area, incremental lines were clearly shown even in the absence of snowy winters or dramatically dry summers. This technique appears therefore suitable (though time consuming) not only in cooler northern environments or dry-and-wet tropics, but also in warm Mediterranean areas. By contrast, eye lens weight was related to age, but it was not a reliable indicator of age in late winter and spring. The point (210 mg) that allows a partial separation between yearlings and adults among foxes from North America (FRIEND and LINHART 1964), from Switzerland (WANDELER 1976) and from surburban London (until March; HARRIS 1978) is also partially discriminating for our population. This coincidence is surprising, given the morphological differences among these fox populations (e.g. CAVALLINI 1994), and the different sampling periods (September and October in North America, October to March in London, January to May in Central Italy). In Australia, the 200 mg point seems a better separation point, but this method has limited applicability (until January or February, i.e. when juveniles are about 6 months old, judging from Fig. 4 of RYAN (1976), or until December, according to the author (RYAN 1976)). Reasons for this discrepancy are not clear.

The pulpar cavity method is not suitable for separating yearlings from adults in spring, especially for males. The cavity of females closes rather quickly during their first oestrus, thus yearling females can be identified only up until their first oestrus. This is in contrast to the findings for foxes in Poland, where clear results can be obtained at least until the end of March (GOSZCZYNSKI 1989) and in London where foxes in their second autumn can be separated from older ones (HARRIS 1978). Other differences between our results and those from Poland (GOSZCZYNSKI 1989) are evident: (1) within each age class, females have lower ratios than males in Italy, whereas no difference was found in Poland; (2) the ratio of upper canines was different from that of lower canines in Italy, but not in Poland. Absolute values of the ratio for yearlings (60% in autumn, 40% after the end of March), however, are similar in the two areas. Geographical variation in both indices of age classes is therefore important, and only incremental lines can be recommended. Other

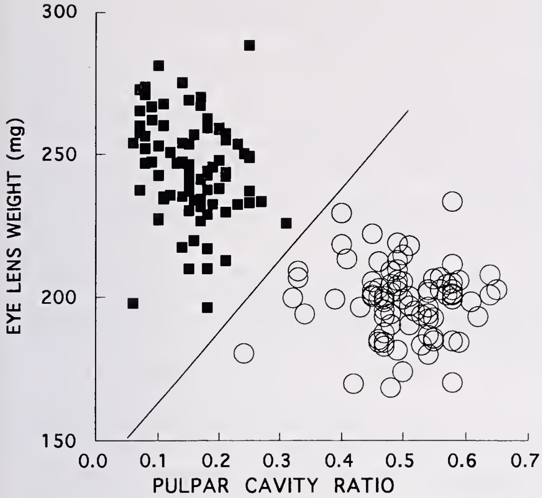

Fig. 6. Scatter plot diagram of pulpar cavity ratio vs. lens weight. Closed squares are adult foxes (> 1 year), open circles are yearlings (≤ 1 year).

methods should be validated for each population (as suggested by HARRIS 1978). The combination of the two less time-consuming techniques (eye lens and pulpar width; see Fig. 6) gave good results for the population studied, in spite of the late sampling period (until May), and may be useful for other areas. The numeric values of the discriminant functions should be verified on other populations.

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Zusammenfassung

Altersbestimmung beim Rotfuchs in einem mediterranen Habitat

Wir haben verschiedene Methoden zur Altersbestimmung des Rotfuchses (*Vulpes vulpes*) in Zentralitalien (Region um Pisa) von Januar bis Mai 1992 getestet. Erstmalig wurde in einem mediterranen Habitat die Anwesenheit von Wachstumslinien im Zement der Eckzähne nach deren Entkalkung, Sektion und Färbung untersucht. Messungen von Augenlinsengewichten und Pulpahöhlenweiten der Eckzähne als Methoden zur Unterscheidung einjähriger von erwachsenen Tieren wurden mit der Methode der Zählung von Wachstumslinien verglichen. Insgesamt 330 Füchse (125 W; 205 M) sind gesammelt worden, aber die Stichprobenzahl für die verschiedenen Methoden war unterschiedlich. Bis zu 8 Wachstumslinien waren deutlich erkennbar. Weder die Methode der Augenlinsengewichte noch die der Pulpahöhlenweite bestimmten die beiden Altersklassen eindeutig, aber eine Diskriminanzfunktion der zwei Variablen erreichte einen 100%igen Erfolg. Die Pulpahöhle weiblicher Tiere schließt sich während des ersten Oestrus. Die geographischen Unterschiede in der Wachstumsrate verschiedener Organe lassen vermuten, daß nur die Methode der Wachstumslinien zu gesicherten Altersangaben führt.

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