Canid Biology & Conservation

Research report

Accuracy of aging kit foxes using cementum annuli analysis

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Abstract

Determining the age of individual wild canids that are older than one year is challenging. Cementum annuli analysis is considered the most accurate technique for determining age in mammals. We compared age estimated by cementum annuli analysis to actual age for 62 kit foxes (*Vulpes macrotis*) of known age collected at the Naval Petroleum Reserves in California. The cementum annuli analysis was conducted by Matson's Laboratory. The estimated age matched the actual age for 82.3% of the samples. No estimate deviated from the actual age by more than one year. The proportion of foxes aged correctly was significantly higher for foxes ≤ 1 year old (89.4%) compared to foxes ≥ 2 years old (53.8%). A higher proportion of foxes aged incorrectly died within 0.1 year of their birth date (63.6%) compared to foxes dying > 0.1 year of their birth date (3.4%). Nine of 11 foxes aged incorrectly were females indicating a potential sex bias in aging accuracy. Cementum annuli analysis provided reasonably accurate estimates of age (in years) for kit foxes. Errors did occur and were most common among foxes ≥ 2 years old, foxes dying around the time of their birth date, and possibly females. Researchers should be cognisant of and account for these potential sources of error when interpreting results of cementum annuli analysis for kit foxes and possibly other fox species as well.

Introduction

Determining the age of individual wild canids that are older than 1 year is challenging. Tooth eruption or wear patterns have been used for some species such as grey wolves (*Canis lupus*; Fuller and Keith 1980, Landon et al. 1998, Gipson et al. 2000), coyotes (*Canis latrans*; Gier 1957, Linhart and Knowlton 1967, Nellis et al. 1978, Bowen 1982), black-backed jackals (*Canis mesomelas*; Lombaard 1971), African wild dogs (*Lycaon pictus*; Mbizah et al. 2016), crab-eating foxes (*Cerdocyon thous*; Olifiers et al. 2010), red foxes (*Vulpes vulpes*; Churcher 1960), grey foxes (*Urocyon cinereoargenteus*; Wood 1958, Root and Payne 1984), and island foxes (*Urocyon littoralis*; Laughrin 1980, Moore and Collins 1995). Eye lens weights have been used for some species such as coyotes (Scrivner et al. 2014), grey foxes (Lord 1961), Pampas grey foxes (*Dusicyon* [=*Lycalopex*] *gymnocercus*; Lord 1966), and the Patagonian grey fox (*Dusicyon* [=*Lycalopex*] *griseus*; Lord 1966). Cementum annuli analysis has been used for some species such as grey wolves (Goodwin and Ballard 1985, Ballard et al. 1995, Landon et al. 1998), coyotes (Linhart and Knowlton 1967, Allen and Kohn 1976, Roberts 1978, Bowen 1982, Scrivner et al. 2014), black-backed jackals (Lombaard 1971), African wild dogs (Mbizah et al. 2016), arctic foxes (*Alopex lagopus*; Grue and Jensen 1976 cited in Harris 1978), red foxes (Grue and Jensen 1973, Monson et al. 1973, Allen 1974, Harris 1978, Simon and Frydendall 1981, Cavallini and Santini 1995), and grey foxes (Root and Payne 1984). Other aging techniques tried on canids have included baculum attributes for black-backed jackals (Lombaard 1971); cranial suture fusion for island foxes (Moore and Collins 1995) and grey wolves (Landon et al. 1998); canine pulp cavity closure for grey wolves (Landon et al. 1998), coyotes (Knowlton and Whittemore 2001), African wild dogs (Mbizah et al. 2016), red foxes (Simon and Frydendall 1981), and grey foxes (Root and Payne 1984, Tumlison and McDaniel 1984); skull attributes and teeth weights for African wild dogs (Mbizah et al. 2016); and epiphyseal closure for red foxes (Sullivan and Haugen 1956, Reilly and Curren 1961).

For canids, most of the techniques above are primarily effective in distinguishing individuals < 1 year old from individuals ≥ 1 year old. For individuals ≥ 1 year old, cementum annuli analysis is the technique considered to be the most accurate and reliable (Johnston et al. 1987, Lyons et al. 2020). In mammals, cementum is continually deposited on the roots of teeth during the life of an animal. In the northern hemisphere, the deposition rate generally is more rapid in summer and then slows in winter culminating with the formation of a denser acellular band (Matson et al. 1993). The annual layers are termed annuli. Thin microhistological cross sections of a tooth can be stained to reveal these annuli, and counting the annuli provides an estimate of the age of the animal in years (Johnston et al. 1987).

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We assessed the accuracy of cementum annuli analysis for determining age for kit foxes (*Vulpes macrotis*; IUCN ranking LC). A 16-year (1980-95) investigation was conducted on the demography and ecology of endangered San Joaquin kit foxes (*V. m. mutica*) at the Naval Petroleum Reserves (NPR) Numbers 1 and 2, located in Kern County, California (Cypher et al. 2000). A primary objective of this investigation was to determine survival rates and causes of mortality. Thus, 525 adult and juvenile kit foxes were radio-collared and monitored, and 367 foxes were recovered dead. Various biological samples were collected from the dead foxes including a lower canine tooth for age determination. For canids, lower canines are considered to be the optimal teeth for determining age using cementum annuli analysis (Lyons et al. 2020). Many of the foxes in the NPR study were first collared as young of the year, and therefore, their exact age was known. Teeth from these foxes provided an opportunity to compare age estimated by cementum annuli analysis to actual age.

Methods

A lower canine tooth was extracted from the mandible of foxes found dead at the NPR, California. Each tooth was allowed to air-dry, and then was stored in a labelled coin envelope. The tooth samples were submitted to Matson's Laboratory (Manhattan, Montana) for cementum annuli analysis. In brief, the teeth were cleaned in a hot water bath to remove any dirt and soft tissue, decalcified in a weak acid solution, embedded in paraffin, and thinly sectioned using a rotary microtome. The sections were then mounted on microscope slides and stained to enhance visibility of the annuli. The annuli were counted by a certified technician and the results recorded. A certainty level also was recorded for each age estimate with "A" indicating high confidence for the estimate and "B" indicating that the evidence was less strong with possible error. Additional details on the processing and analysis of tooth samples are available from Matson's Laboratory (https://matsonslab.com).

For each sample, we compared the estimated ages provided by Matson's Laboratory to the actual known ages of the foxes. We determined the proportion of samples where the estimated and known ages agreed for the A and B confidence categories separately and for all samples combined. We assessed the strength of the relationship between estimated and known ages for all samples and for the A and B confidence categories using correlation analysis. We also compared the proportion of foxes aged correctly among age classes (0, 1, 2, ≥ 3) using Fisher exact tests. The proportions aged correctly also were compared between foxes of known age ≤ 1 year and foxes ≥ 2 years using a chi-square test with a Yate's correction for continuity. An examination of the dates of death for the foxes from which tooth samples were obtained revealed that a number of the foxes died within 0.1 year (ca. 36 days) of their putative birth date, which for kit foxes at the NPR was estimated to be 15th February (Zoellick et al. 1987). For all samples and for those in the A and B confidence categories separately, we compared the proportion of samples aged correctly between foxes dying within 0.1 year of 15th February and all other foxes using Fisher exact tests. For all statistical tests, we used an alpha level of 0.05.

Results

Tooth samples were available from 62 kit foxes of known age. Matson's Laboratory estimated fox age with a confidence level of A for 51 samples and a confidence level of B for 11 samples. The estimated age agreed with the known age for 88.2% of the level A samples, 54.4% of the level B samples, and 82.3% of all samples combined. The correlation between the estimated and known ages was significant for the level A samples ($r = 0.90$, $p < 0.01$), level B samples ($r = 0.88$, $p < 0.01$), and for all samples combined ($r = 0.92$, $p < 0.01$). The proportion of foxes aged correctly (Figure 1) did not differ between age classes 0 and 1 (Fisher $p = 0.62$) or between age classes 2 and ≥ 3 (Fisher $p = 0.59$), and the difference was near significant between age classes 1 and 2 (Fisher $p = 0.05$). The proportion of foxes aged correctly was significantly higher ($\chi^2_{61} = 6.37$, $p = 0.01$) for foxes ≤ 1 year old (89.4%) compared to foxes ≥ 2 years old (53.8%).

For all 11 of the samples (6 level A, 5 level B) where the estimated and known ages did not agree, the estimated ages differed by 1 year; the estimated age was 1 year less than the actual age for seven samples (all adults) and 1 year more than the actual age for four samples (one adult and three young of the year). Of the 11 foxes where the estimated and known ages did not agree, nine were females and two were males. Among the nine females, estimated age was 1 year less than actual age for the six adults and estimated age was 1 year more than actual age for the three young of the year. For the two males where the estimated and known ages did not agree, both were adults and estimated age was 1 year more than actual age for one and 1 year less than actual age for the other.

The proportion of foxes dying ≤ 0.1 year of their putative birth date (15th February) was significantly higher for foxes in which the estimated age did not match the known age (Table 1) for level A samples and all samples combined. For level B samples, the proportions seemed quite different (16.7% versus 60.0%), but the Fisher's exact test did not yield a significant *p*-value, probably due to small sample sizes.

Discussion

Cementum annuli analysis provided a reasonably accurate estimate of age for kit foxes with over 80% of the known-age samples we submitted being aged correctly. Not unexpectedly, the error rate was higher for samples aged with a confidence level of B. Regardless of confidence level, the errors never were greater than 1 year. Allen (1974) claimed he correctly aged 95 known-aged adult red foxes using cementum annuli analysis after applying correction factors that he had devised based on when individuals were harvested. Grue and Jensen (1973) correctly aged 125 of 135 (92.6%) teeth from red foxes of known age. Scrivner et al. (2014) reported that Matson's Laboratory had correctly aged teeth from 28 known-aged coyotes.

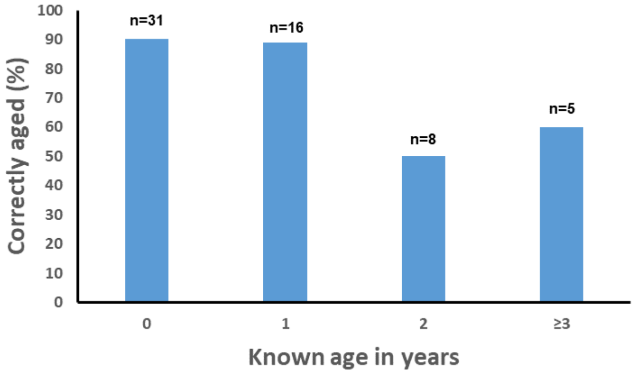

Figure 1. Proportion of tooth samples from known-age kit foxes that were correctly aged by Matson's Laboratory using cementum annuli analysis.

Table 1. Comparison of the proportions (%) of kit foxes dying ≤ 0.1 year of their putative birth day (15th February) between foxes in which age estimated by cementum annuli analysis was correct (i.e., matched known age) and foxes in which the estimated age was incorrect. The proportions were compared using a Fisher's exact test.

	Estimated age correct			Estimated age incorrect			
Samples	n	Foxes dying ≤ 0.1 year of birth day	%	n	Foxes dying ≤ 0.1 year of birth day	%	Fisher's exact test
Level A	45	1	2.2	6	4	66.7	p < 0.01
Level B	6	1	16.7	5	3	60.0	p = 0.24
All samples	51	2	3.4	11	7	63.6	p < 0.01

The source of the errors for the kit fox samples that were aged incorrectly is not known. Some estimated ages were 1 year more than the actual age and some were 1 year less. Double or uneven cementum layers can cause aging errors (Johnston et al. 1987), particularly the overestimation of age in juveniles and the underestimation of age in adults, as was documented with grey wolves (Landon et al. 1998, Gipson et al. 2000) and also black bears (*Ursus americanus*; Harshyne et al. 1998) and polar bears (*Ursus maritimus*; Hensel and Sorensen 1980). It is unknown whether these problems were encountered in the kit fox samples.

Several factors appeared to be associated with higher error rates in aging kit fox teeth using cementum annuli analysis. More errors were observed among foxes of known age ≥ 2 years. Grue and Jensen (1973) were able to accurately age foxes < 1 year or > 3 years old but were less accurate determining the age of foxes in their second or third year. Harshyne et al. (1998) also reported that error rates increased with age for black bears.

Higher error rates were observed for samples from kit foxes that died within 0.1 years (approximately 36 days) of their putative date of birth. Wide (light staining) layers form in spring and summer, narrow (dark staining) layers form in autumn and winter (Lombaard 1971, Matson et al. 1993). The dark layers define the boundary between years. These layers can be difficult to distinguish when they are forming or have just formed (Johnston et al. 1987, Matson et al. 1993). Exactly when this layer forms in kit fox teeth is unknown, but apparently there can be considerable variation in the timing. Grue and Jensen (1973) found that this layer can form any time from March to September in northern European red foxes. Similarly, Harris (1978) found that it formed any time from April to August in British red foxes. The layer had already formed for some kit foxes dying before the birth date, and the layer had not yet formed for some foxes dying after the birth date. Consequently, accuracy and reliability of age estimates may be lower for kit foxes dying around the time of year of their birth. Harshyne et al. (1998) also reported higher aging error rates depending upon the time of year black bears died and attributed the errors to annual variation in cementum deposition patterns.

Interestingly, most of the errors in the age estimates were among female kit foxes. This result may have simply been random. However, formation of cementum annuli also may have been affected by reproduction, as has been reported for female black bears (Carrel 1992 cited in Matson et al. 1993, Coy and Garshelis 1992). For all six adult female kit foxes that were aged incorrectly, the estimated age was 1 year less than actual age indicating that the formation of the dark line marking the end of an annulus had been delayed. At least two of these six adult females were known to have reproduced during the winter/spring in which they died. The roles of hormone and nutrient cycles in cementum layer deposition are unknown and warrant further investigation (Johnston et al. 1987). Lombaard (1971) reported the presence of a second dark line within one annulus in a male black-backed jackal that he referred to as a "rut line", presumably resulting from changes in hormone levels or nutrition related to breeding. Such rut lines apparently are common in male red deer (*Cervus elaphus*; Mitchell 1967) and could alter age estimates if not accounted for.

Although cementum annuli analysis is a relatively robust method for estimating age among mammals, the technique does have some significant limitations. For a number of species including kit foxes, the optimal tooth to analyse is a canine. Removing a canine tooth from a carnivore, particularly in the field, is a highly invasive procedure, and the removal of a canine would place the individual at significant disadvantage in acquiring food or defending itself. Thus, removing a canine tooth from a live wild carnivore is considered unethical (Lyons et al. 2020). Consequently, samples generally are only available from dead animals (e.g., telemetered individuals dying during studies, individuals collected in conjunction with harvest or control programs, museum specimens). Removal of teeth such as incisors or premolars, which work well for some species, may have less of an impact on a live animal, but still may be controversial (Festa-Bianchet et al. 2002, Lyons et al. 2020).

Also, specialized equipment and expertise are required to appropriately process samples and accurately interpret results (Johnston et al. 1987). Few facilities and individuals have the necessary equipment and expertise. Services such as Matson's Laboratory are available to conduct cementum annuli analysis, but of course a cost is involved. However, when post-juvenile age estimates are needed and the appropriate equipment and expertise is available or funding is available to purchase analytical services, then cementum annuli analysis may be the optimal option for securing age estimates for individual carnivores.

In summary, cementum annuli analysis provided reasonably accurate estimates of age (in years) for kit foxes. However, errors did occur and were most common among foxes ≥ 2 years old, foxes dying around the time of their birth date, and possibly females. Researchers should be cognisant of these potential sources of error when interpreting results of cementum annuli analysis for kit foxes and possibly other fox species as well. For example, when kit foxes that died within 0.1 year of their birth date were removed from the level A samples, the agreement between estimated and actual age improved to 97.9%. Thus, accounting for known sources of error can significantly improve accuracy of cementum annuli analysis results.

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Biographical sketch

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