EVALUATION OF AGE DETERMINATION TECHNIQUES FOR GRAY WOLVES

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Abstract: We evaluated tooth wear, cranial suture fusion, closure of the canine pulp cavity, and cementum annuli as methods of age determination for known- and unknown-age gray wolves (*Canis lupus*) from Alaska, Minnesota, Ontario, and Isle Royale, Michigan. We developed age classes for cranial suture closure and tooth wear. We used measurement data obtained from known-age captive and wild wolves to generate a regression equation to predict age based on the degree of closure of the canine pulp cavity. Cementum annuli were studied in known- and unknown-age animals, and calcified, unstained thin sections were found to provide clear annulus patterns under polarized transmitted light. Annuli counts varied among observers, partly because of variation in the pattern of annuli in different regions of the cementum. This variation emphasizes the need for standardized models of cementum analysis. Cranial suture fusion is of limited utility in age determination, while tooth wear can be used to estimate age of adult wolves within 4 years. Wolves <7 years old could be aged to within 1–3 years with the regression equation for closure of the canine pulp cavity. Although inaccuracy remains a problem, cementum-annulus counts were the most promising means of estimating age for gray wolves.

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An accurate method of determining the age of gray wolves is necessary to describe age-specific mortality, an important component of wolf population dynamics. Most methods of gray wolf age determination can only distinguish juvenile and adult animals. Pups, yearlings, or adults have been separated via the epiphyseal fusion stage of the radius and ulna (Rausch 1967), the size and appearance of the posterior-most inguinal nipples (Mech et al. 1993), tooth eruption, replacement, and wear (Van Ballenberghe et al. 1975, Fuller and Keith 1980, Fritts and Mech 1981), the extent of closure of the apical foramen, and thickness of the dentin and cementum in canine teeth (Parker and Maxwell 1986).

Wolf age has been estimated to the nearest year only by cementum-annulus counts (Goodwin and Ballard 1985) and by marking and monitoring individuals (Mech 1988). Maximum longevity of wild wolves was estimated in Minnesota via radiotracking (Mech 1988), but this approach is both expensive and labor intensive. Cementum-annulus counts are a reliable technique for estimating age in many herbivorous species (Klevezal and Kleinenberg 1967, Fancy 1980), but interpretation of cementum patterns in carnivores has been problematic because cementum differs not only among individuals of the same species but also among tooth types from the same individual (Roberts 1978). The accuracy of age determinations has been complicated by the presence of multiple components within a single cementum annulus (Nellis et al. 1978) and an insufficient number of known-age samples for testing methods of age determination (Ballard et al. 1995).

There is currently no scientific evidence linking cyclic cementum production in wildlife with any causative factor (Matson et al. 1993). A laboratory experiment using captive animals has shown that both occlusal strain and growth rates are related to annulus formation in goats (Lieberman 1993). That study increased understanding of cementogenesis by identifying, via a controlled experiment, factors that are causally linked to annulus formation. However, it is unknown if the same mechanism applies broadly

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to wildlife species, especially those without a primarily herbivorous diet. Additional research aimed at understanding cementogenesis and annulus formation will strengthen cementum-annulus analysis.

This study evolved from a need to estimate age structure for the unexploited wolf population of Isle Royale National Park, Michigan (Waite 1994). Skulls and teeth from wolves of known age, known minimum age, and unknown age from Alaska, Minnesota, Ontario, and Isle Royale, Michigan, were used to evaluate age determination by tooth wear, cranial suture fusion, closure of the canine pulp cavity, and cementum annuli. The cementum analyses included an evaluation of calcified, unstained sections and an assessment of aging precision among experienced and inexperienced observers using decalcified, stained sections.

METHODS

Cementum Annuli

We prepared calcified, unstained thin-sections for polarized transmitted light analysis of cementum from the teeth of 5 Ontario and 29 Isle Royale adult wolves of unknown age. We prepared the sections by embedding the teeth in low-viscosity Epofix epoxy (Struers, Westlake, Ohio, USA), using vacuum impregnation to fill all voids in the dental tissues. We made a midsagittal cut through the tooth and epoxy with a VC-50 Vari/Cut low-speed saw (Leco, St. Joseph, Michigan, USA) with a 12.7-cm-diameter diamond tipped blade (0.038 cm thick). The cut face was polished on a strip-grinder and glued onto a microscope slide. A second cut removed the extra epoxy and dental tissue, leaving a section approximately 75–100 μm thick attached to the slide. We ground the section on a bench-grinder until nearly transparent (approx 30–35 μm thick), polished it on the strip-grinder, and topped it with a cover slip affixed with Permount (Fisher Scientific, Pittsburgh, Pennsylvania, USA). We examined these unstained sections microscopically (10–100×) with polarized transmitted light.

The method just described is typically used for archaeological specimens (Landon 1993, Lieberman 1994). An advantage of this method is use on fragile or partially decomposed material without damaging the cementum. The outermost annulus and the adjacent peripheral cementum are also well preserved, which facilitates estimation of the season of death (Lieberman and Meadow 1992). A disadvantage of this method is limitation on the number of sections potentially produced from a single tooth. Midsagittal cuts are made with a saw, and the section must be prepared so the plane of the cut passes at right angles through the cementum layers. The saw destroys part of the tooth as it cuts, and the tooth remnant usually has a limited area that can be recut and still produce a section with the cementum layer uniformly perpendicular to the plane of the slide.

We ranked skulls of 26 wolves of unknown age from Ontario from youngest to oldest via closure of the cranial suture and tooth wear (see below). We selected teeth from 5 of the oldest individuals and prepared calcified, unstained sections for cementum-annulus analysis. One sample of the upper first incisor, upper second incisor, and upper third premolar was sectioned, each from a different animal. In addition, we took the lower first premolar from 2 animals, the upper second premolar from 4 animals, and the upper third incisor, upper canine, and upper first premolar from each of the 5 wolves. We made sections of different tooth types to qualitatively evaluate whether a particular tooth type yielded the best annulus readings based on clarity and definition of the annuli. Two different observers independently counted cementum annuli in 32 sections from 24 teeth of 5 wolves. Annulus patterns were recorded, a subjective assessment of clarity and definition was made, and the location of the reading was noted on a sketch of the section.

For the 29 adult Isle Royale wolves of unknown age, we prepared calcified sections for cementum-annuli counts. To preserve the tooth-wear pattern of each animal for future study, we extracted a tooth for sectioning only if the corresponding tooth on the opposite side of the mouth was present and had a similar wear pattern. The canine was the first choice for sectioning because coyote cementum annuli are more distinct in this tooth type (Roberts 1978). If a canine was not available, preference was then given to a second or third premolar (double rooted), and lastly a first premolar (single rooted). We did not section teeth from 2 pups. We took 1 tooth from 23 animals and 2 from the other 6 animals. The sample comprised upper canines from 10 animals, upper second premolars from 9 animals, lower canines from 6 animals, upper first premolars from 4 animals, and upper third premolars, lower third premolars, and lower second premolars from 2 animals. Three different observers independently read and recorded the cementum annuli in 69 calcified, unstained sections from the 29 adult wolves from Isle Royale.

To evaluate several age-determination methods, we used skulls of 12 known-age wolves from Minnesota that covered the entire lifespan of the species. These wolves were live-captured and radiocollared as pups, and their skulls were collected after death. We examined first premolar midsagittal sections from each wolf that had been previously prepared by decalcification and staining via standard techniques. We chose these available sections, rather than sectioning other teeth, because of the scarcity and potential future uses of teeth from known-age animals. Three observers independently counted cementum annuli in each tooth section before knowing the true age.

Also included in the study were decalcified, stained sections of first premolar teeth extracted from 12 Minnesota wolves of known minimum age. These wolves were live-captured and radiocollared when they were >1 year old, and their skulls were collected after death. The sections from the Minnesota wolves were supplemented by decalcified, stained canine sections from 7 known-age Alaska wolves (Goodwin and Ballard 1985). All known-age wolves were taken from the wild, except 1 from Minnesota and 2 from Alaska.

We used sections from known-age wolves to test observer variation and to try to standardize an analysis model by describing the thickness of the first translucent cementum band, the location of the first opaque band (annulus), and annulus patterning relative to actual age. We supplemented known-age material by including calcified sections from teeth of 7 Isle Royale wolves for which date-of-birth had been estimated from bomb ^{14}C activity in molar dentin, as determined by accelerator mass spectrometry (J. L. Bada, Scripps Institute of Oceanography, unpublished data). We matched wolf bomb ^{14}C levels to a bomb ^{14}C extinction curve for Isle Royale moose (*Alces alces*; Bada et al. 1990). We determined age estimates for the 7 animals by subtracting the derived date of birth from the known date of death, and used these age estimates to check the accuracy of the cementum-annulus analysis.

Cranial Suture Closure and Tooth Wear

We developed 5 tooth-wear classes based on the degree of enamel wear and rounding of the points of the teeth. We then developed 5 classes of cranial suture closure based on the degree of fusion of the basioccipital–basisphenoid and basisphenoid–presphenoid sutures of the skull. We recorded cranial suture and tooth-wear class for wolf skulls of known age and known minimum age from Minnesota, and for selected wolves from Ontario and Isle Royale aged by cementum-annulus counts. The known ages, known minimum ages, and cementum-annulus ages were all used to derive estimates of age range for classes of tooth wear and cranial suture closure.

Pulp-Cavity Measurements

We calculated percentage closure of the canine pulp cavity for 16 Minnesota and 7 Alaska wolves with ages known to within 1 year. We measured canine tooth and pulp-cavity widths with a calibrated ocular micrometer (20×, for thin-sections) or a dial caliper with a least count of 0.05 mm (for radiographs of whole teeth; Tumlison and McDaniel 1984). Widths were measured on a midsagittal plane near the enamel–cementum junction, where the tooth is typically widest and composed mainly of dentine whereby

$$\text{Percent closure} = \frac{(\text{Total width} - \text{Pulp-cavity width})}{\text{Total width}} \times 100.$$

We used these percent closure measurements to develop a regression equation to predict age based on the percent closure of the canine pulp cavity at the enamel–cementum interface. We used the curve-fitting program "CURFIT" (Keen and Spain 1992) to determine the best-fit curve through the data points ($n = 23$). We performed a "Woolf" linear transformation on the data to determine the coefficients for the nonlinear regression equation (Keen and Spain 1992) and determined 95% confidence limits for the regression line.

RESULTS

Cementum Annuli

Decalcified, Stained Sections.—The annulus counts recorded for 12 known-age Minnesota wolves show the extent of variation among observers (Table 1). Observer 1 made the most

Table 1. Annuli readings from 3 independent observers of decalcified, first premolar thin sections of 12 known-age Minnesota gray wolves.

Animal	Age (yr)	Expected count	Observer[a] 1	Observer[a] 2	Observer[a] 3
2[b]	4.6	4	4–9[c]	4–7	6–7
3	6.8	6	3	3	5
7	4	3	3	2	6
50	3.6	3	5	7–9	6
66	5	4	6	7	10
87	1.5	1	NA[d]	NA	6
93	2	1	3	2	5
2449	4.5	4	4	6	4–9
5079	5.8	5	11	7	6
5781	4.5	4	3	7–8	7
5954	0.5	0	0	0	0
Lady	14	13	13	11–14	10

[a] Observers 1 and 3 used brightfield illumination, while observer 2 used a combination of brightfield and polarized illumination.
[b] Animal 2 exhibited a pattern of 4 darkly stained annuli with lighter staining, discontinuous lines in between.
[c] When a range is stated, the lower value corresponds to the number of obvious dark annuli. The higher value is a combination of the obvious dark annuli plus possible false or not as darkly stained annuli.
[d] No observable annuli pattern for this animal.

accurate annulus counts, followed by observers 2 and 3. One source of variation is the different experience and training of the observers. Observers 2 and 3 had counted cementum annuli in other noncarnivorous taxa, but none of the 3 observers had experience with wolf tooth sections. Relative experience levels counting cementum annuli in other taxa did not translate to success in counting annuli in wolf teeth. Observer 3, the most experienced analyst, was typically off by the widest margin, while observer 1, the least experienced analyst, was most correct. Patterns of cementum annuli vary among taxa, and experience in taxa-specific analysis is an important source of potential error.

The high degree of variation among the 3 observers also shows the need for clearly defined models of cementum-age analysis that describe the taxa-specific patterns of cementum annuli and define criteria for distinguishing and counting annuli. Part of the variation among the 3 observers is the result of where in the cementum band they chose to count the annuli, and what they defined as an annulus. The annulus counts of observer 3 generally were greater than the number expected. Observer 3 counted in an area of the cementum where the greatest number of annuli occurred, typically near the apex of the root where the cementum band was wide. Observer 1 searched for an area with a clear pattern further up the root away from the apex, where the cementum layer was thinner, and considered multiple closely spaced lines near the root apex not to be true annual lines. These types of observer differences can be minimized by starting with a standardized model of cementum-age analysis, as has been done for Alaska brown bear (*Ursus arctos*; Matson et al. 1993; see below).

One of the known-age Minnesota wolves exhibited 3 closely spaced paired annuli in the cementum band. This pattern indicated this animal had been a breeding female, which was confirmed by the animal's history. The formation of paired annuli in breeding females is possibly due to lactation stress and calcium depletion (Coy and Garshelis 1992). A similar paired annulus pattern was not observed in any of the other known- or unknown-age wolves examined. Possibly, no other breeding females were included in the samples studied. Females sometimes do not breed until they are 4–5 years old (Mech and Seal 1987), and breeding females represent a small proportion of a wolf population (typically only 1/pack).

Calcified, Unstained Sections.—Calcified, unstained sections showed distinct annuli under polarized transmitted light (Fig. 1). Our assessment of which tooth type produced the best annuli pattern was inconclusive, although our qualitative impression was that the canine and the double-rooted premolars provided the clearest and most definite patterns in calcified, unstained sections. From this study, it appears the opaque annulus (darkly staining in histological sections) forms in mid to late winter (Jan–Mar) in gray wolves, as in many other mammalian species (Klevezal and Kleinenberg 1967, Klevezal 1996). The abundant and more highly cellular cementum layer produced during spring–fall appears translucent in the calcified, unstained sections viewed with polarized transmitted light microscopy. The same layer appears only lightly stained in the decalcified, stained sections viewed with ordinary brightfield microscopy. Viewed with the same equipment, the cementum annulus, formed during the winter, appears opaque in the calcified, unstained sections, and darkly stained in the decalcified, stained sections.

Gray wolves are typically born in April or May. By 5.5 months of age, all adult premolars are present and adult canines have erupted about halfway (Mech 1970). Goodwin and Ballard (1985) noted that no dark annulus was

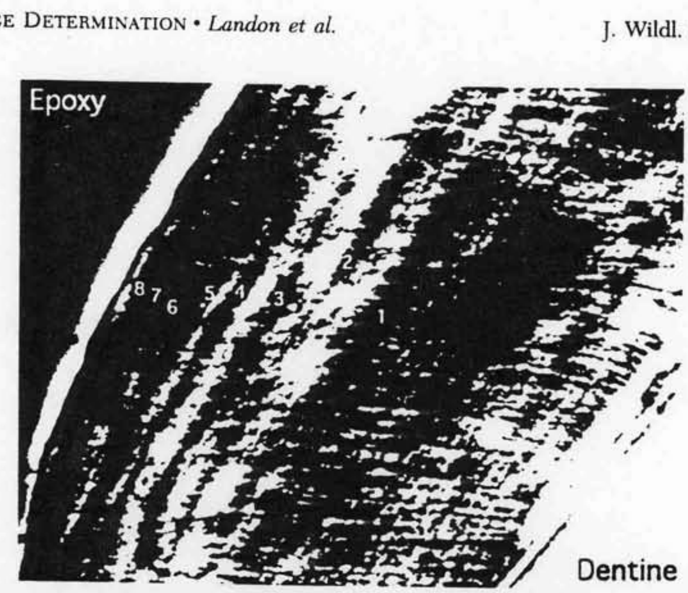

Fig. 1. Digitized micrograph of a calcified, unstained section showing the cementum annuli below the enamel–cementum junction. Isle Royale wolf 1516 (male) estimated at 9.7 years old, upper second premolar. The annulus labeled 1 is likely the 2-year annulus. The 1-year annulus is difficult to identify in the diffuse and mottled initial cementum band. Note the closer spacing of annuli as the animal ages.

present in thin sections from 49 known-age pups ranging from 6 to 10 months old (Nov–Mar of their first year). In coyote canines, the first annulus was identified as a 2-year age indicator by Linhart and Knowlton (1967) and as a 1-year indicator by Allen and Kohn (1976). In wolf canines, the first annulus was identified as a 2-year age indicator by Goodwin and Ballard (1985), and as a 1-year indicator by Ballard et al. (1995). Different annulus identification criteria may have resulted in these contrasting interpretations.

We identified a diffuse 1-year annulus in decalcified, stained sections. In these same sections, the 2-year annulus appeared more darkly stained and regular. In calcified, unstained sections, the identification of the 1-year annulus was not definite. The cementum peripheral to the dentine was often very mottled and irregular in appearance, and the determination of what to count as the first annulus was unclear. The first layer peripheral to the dentine was generally translucent (lightly staining in histological sections) and typically got wider and more diffuse from the enamel–cementum interface down the cementum band to the apex of the root.

In calcified, unstained sections, the annuli pattern got much more regular beginning with what we interpret as the 2-year annulus. From the 2-year annulus toward the peripheral edge of the tooth, the annuli typically were more regularly spaced and uniform in appearance, with the space between annuli often decreasing toward the periphery. Irregularities in the shape of the peripheral edge of the tooth often made it difficult to identify the outermost annulus. In some samples, the clarity of the outer annuli was less distinct than annuli closer to the center of the cementum band.

Many of the annuli were complex, with more than 1 component. In known-age samples, the annulus counts were most accurate toward the coronal end of the cementum band. In both young and older animals, the cementum was typically thinnest near the enamel–cementum junction and progressively thickened until it flared out considerably near the apex of the root (Fig. 2). At the root tip, where the cementum is abundant, annulus counts in known-age animals were typically greater than the determined age. Annuli that appeared united as a single structure at the coronal end of the cementum band appeared to split into multiple structures toward the apex of the root. This characteristic of the cementum band was identified in both calcified, unstained sections, and decalcified, stained sections.

Cranial Suture Closure and Tooth Wear

We used known-age Minnesota wolves and age estimates developed for Isle Royale wolves to determine the age of fusion of the basioccipital–basisphenoid and basisphenoid–

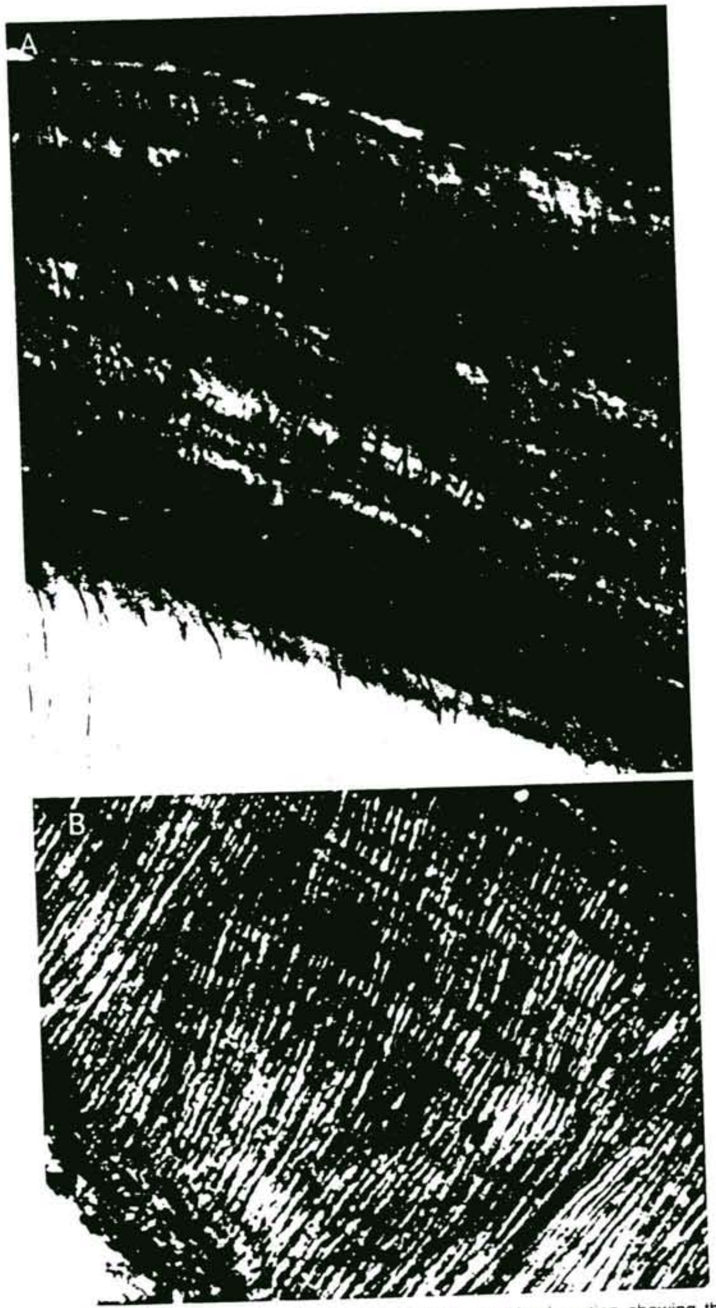

Fig. 2. Digitized micrographs of different areas of the same calcified, unstained section showing the extent of variation in cementum patterning in a single tooth. (A) Section halfway down root showing a pattern of 7 opaque annuli. (B) Section of the cementum at the apical end of the root showing a complex pattern of numerous thin bands. The tooth edge is toward the top and right of each image, and the cementum–dentin junction is toward the bottom and left. Isle Royale wolf 2201, upper second premolar, sex unknown.

presphenoid cranial sutures (Table 2). Of the 2 cranial sutures examined, the basioccipital–basisphenoid fuses first, with complete fusion occurring no later than 4 years of age and possibly as early as 3 years of age. The basisphenoid–presphenoid suture fuses second, with complete fusion occurring as early as 3.8 years or not occurring at all during the life of the animal. While the closure of these sutures might help place animals in relative age order,

Table 2. Basioccipital–basisphenoid (BB) and basisphenoid–presphenoid (BP) age ranks of cranial suture and fusion ages (yr).

Class	BB suture status[a]	BP suture status	Minnesota wolves[b]		Isle Royale wolves[c]	
			Age	n	Age	n
1	Open	Open			0.2–0.5	2
2	Partially closed	Open			0.7–2	3
3	Closed with or without line visible	Open			3.8–4.8	3
4	Closed with or without line visible	Partially closed	1.5–>4	9	3.8–10	9
5	Closed with or without line visible	Closed with or without line visible	3.6–14	10	5.0–12.7	14

[a] Open = fusion not yet begun; Partially closed = part of suture fused, but not completely fused; Line visible = fused, but suture line still visible.
[b] Includes 12 known-age wolves (yr) and 7 known minimum-age wolves with ages known to within 1 year.
[c] Estimated ages (yr) based on cementum annuli counts and tooth wear; includes 2 pups whose teeth were not sectioned.

it appears highly variable and of limited use for age determination.

We used the ages from cementum-annuli counts for the 5 Ontario and 29 Isle Royale wolves to estimate age ranges for adult tooth-wear classes (Table 3). We used the mean age from cementum-annulus counts for each age class with 1 standard deviation on each side to delimit the age range. The age ranges for the tooth-wear classes are broad and some overlap slightly, but classification of tooth wear seems to provide closer estimates of age than cranial suture closure.

Pulp-Cavity Measurements

We explored the quantitative relation between the degree of closure of the canine pulp cavity (measured as a percentage of total width) and age in a regression analysis comparing age and percent closure in known-age animals. A hyperbolic equation yielded the best fit based on the calculated F-value from the analysis of variance and the standard error of the residuals (Fig. 3). Based on our regression analysis, a formula was derived for estimating age from the percent closure of the pulp cavity in canine teeth ($r^2_{22} = 0.97$, $P < 0.001$):

$$\text{Age(yr)} = \frac{(1.181 \times \text{Percent closure})}{(94.359 - \text{Percent closure})}.$$

This equation adequately predicted age for animals up to 7 years old.

DISCUSSION

This study evaluated 4 methods of age determination. The timing of cranial suture fusion is variable, and therefore does not appear to be a reliable indicator of age. Tooth-wear classification provided a relatively easy method of accurately determining the ages of pups and determining the ages of older animals to within 4 years. Tooth wear also has the advantage of being potentially applicable to live animals. However, none of the cementum ages used to develop adult tooth-wear age classes were from known-age animals, and further evaluation of this method is needed..

Tumlinson and McDaniel (1984) used radiographs to subjectively assess relative width of the canine pulp cavity and classify gray foxes (*Urocyon cinereoargenteus*) as either juveniles or adults. We extended this work by determining a quantitative relation between closure of the canine pulp cavity and age, which appears a useful tool for estimating the age of wolves. The regression equation developed in this study appears to estimate age fairly well for animals <7 years of age. For wolves with canine pulp cavities >80% closed, the range of possible ages

Table 3. Tooth-wear classes with age ranges based on the age estimates of gray wolves from Ontario and Isle Royale, Michigan.

Class	Description of relative wear	Age (yr)[a]
1	Pup or yearling, no wear evident.	<1
2	Slight signs of wear.	1–4
3	Points and edges of enamel generally rounded.	3–7
4	Significant wear, uniform rounding of enamel.	7–11
5	Severe wear, >50% of the crown worn down.	>9

[a] Age estimates from counts of cementum annuli.

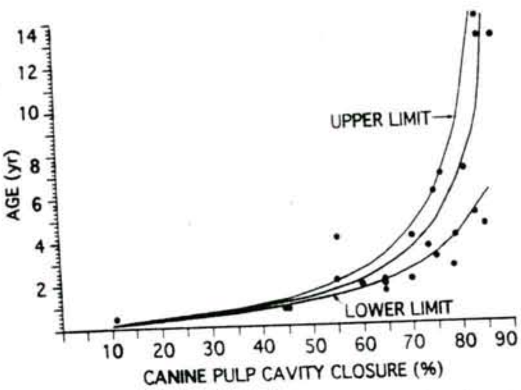

Fig. 3. Hyperbolic curve fit through 23 data pairs from known-age gray wolves showing the relation between age and percent closure of the canine pulp cavity. Upper and lower limits show the 95% confidence interval for age estimates based on the regression.

within the confidence limits is quite large (Fig. 3). However, the wide confidence limits may be due to the lack of available data from known-age wolves between 7 and 13 years of age. Despite this problem, this method of age determination has value. Percent closure of the canine pulp cavity can be measured from radiographs, and thus is potentially applicable to live animals.

Calcified, unstained sections observed under polarized transmitted light are a valid alternative for cementum-annulus analysis. When specimens are fragile or weathered, this method of preparation might be superior to decalcification and staining. We found that both canine and premolar teeth had sufficiently distinct annuli for age determination. Incisors proved difficult to section by the methods we used, and we did not attempt to section any molars.

Accuracy of cementum ages was evaluated with the tooth sections from known-age wolves. For the decalcified, stained, first premolar sections of the 12 Minnesota wolves, errors of up to 6 years occurred. For the calcified, unstained sections of the 7 Isle Royale wolves with ages estimated from bomb radiocarbon levels, estimates of cementum-annulus age for 5 animals were within 1 year, while the other 2 estimates were within 3 and 5 years. Part of this error is likely the result of observer inexperience and lack of training with a standardized analysis model. Observer variation in the interpretation of annuli patterns remains an important limitation in cementum-annulus analysis of wolf teeth. Taxa-specific experience in the analysis of cementum annuli is important in age determination.

The further development of a standardized model for interpretation of cementum annuli in wolf teeth will improve analysis accuracy. Many of the wolf teeth observed in this study had a great deal of variation in the regularity and patterning of the annuli in different areas of the cementum. Counts in areas with the highest number of annuli, often at the apical end of the root, did not necessarily result in the best age estimate. The cementum at the apical end of the root of the tooth was often irregularly formed and wavy, which made interpretation difficult. In calcified, unstained sections, the identification of the 1-year annulus, the determination of the annulus pattern at the peripheral edge of the tooth, and the differentiation of annual lines from nonannual components were all especially problematic. Additional improvements in accuracy of cementum-annulus analysis are needed, but short of intensive monitoring of individuals, this technique remains the most accurate way to estimate the age of adult wolves.

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LITERATURE CITED

ALLEN, S. H., AND S. C. KOHN. 1976. Assignment of age classes in coyotes from canine cementum annuli. Journal of Wildlife Management 40:796–797.

BADA, J. L., R. O. PETERSON, A. SCHIMMELMANN, AND R. E. M. HEDGES. 1990. Moose teeth as monitors of environmental isotopic parameters. Oecologia 82:102–106.

BALLARD, W. B., G. M. MATSON, AND P. R. KRAUSMAN. 1995. Comparison of two methods to age gray wolf teeth. Pages 455–460 *in* L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute, Occasional Publication 35.

COY, P. L., AND D. L. GARSHELIS. 1992. Reconstructing reproductive histories of black bears from the incremental layering in dental cementum. Canadian Journal of Zoology 70:2150–2160.

FANCY, S. G. 1980. Preparation of mammalian teeth for age determination by cementum layers: a review. Wildlife Society Bulletin 8:242–248.

FRITTS, S. H., AND L. D. MECH. 1981. Dynamics, movements, and feeding ecology of a newly protected wolf population in northeastern Minnesota. Wildlife Monographs 80.

FULLER, T. K., AND L. B. KEITH. 1980. Wolf population dynamics and prey relationships in northeastern Alberta. Journal of Wildlife Management 44:583–602.

GOODWIN, E. A., AND W. B. BALLARD. 1985. Use of tooth cementum for age determination of gray wolves. Journal of Wildlife Management 49:313–316.

KEEN, R. E., AND J. D. SPAIN. 1992. Computer simulation in biology: a BASIC introduction. Wiley-Liss, New York, New York, USA.

KLEVEZAL, G. A. 1996. Recording structures of mammals: determination of age and reconstruction of life history. Translated from Russian by M. V. Mina and A. V. Oreshkin. A. A. Balkema, Rotterdam, Netherlands, and Brookfield, Vermont, USA.

———, AND S. E. KLEINENBERG. 1967. Age determination of mammals from annual layers in teeth and bones. Translated from Russian by the Israel Program for Scientific Translations, Jerusalem, Israel.

LANDON, D. B. 1993. Testing a seasonal slaughter model for colonial New England using tooth cementum increment analysis. Journal of Archaeological Science 20:439–455.

LIEBERMAN, D. E. 1993. Life history variables preserved in dental cementum microstructure. Science 261:1162–1164.

———. 1994. The biological basis for seasonal increments in dental cementum and their application to archaeological research. Journal of Archaeological Science 21:525–539.

———, AND R. H. MEADOW. 1992. The biology of cementum increments (with an archaeological application). Mammal Review 22:57–77.

LINHART, S. B., AND F. F. KNOWLTON. 1967. Determining the age of coyotes by tooth cementum layers. Journal of Wildlife Management 31:362–365.

MATSON, G., L. VAN DAELE, E. GOODWIN, L. AUMILLER, H. REYNOLDS, AND H. HRISTIENKO. 1993. A laboratory manual for cementum-age determination of Alaska brown bear first premolar teeth. Alaska Division of Wildlife Conservation, Anchorage, Alaska, USA, and Matson's Laboratory, Milltown, Montana, USA.

MECH, L. D. 1970. The wolf: the ecology and behavior of an endangered species. Natural History Press, Garden City, New York, USA.

———. 1988. Longevity in wild wolves. Journal of Mammalogy 69:197–198.

———, T. J. MEIER, AND U. S. SEAL. 1993. Wolf nipple measurements as indices of age and breeding status. American Midland Naturalist 129:266–271.

———, AND U. S. SEAL. 1987. Premature reproductive activity in wild wolves. Journal of Mammalogy 68:871–873.

NELLIS, C. H., S. P. WETMORE, AND L. B. KEITH. 1978. Age-related characteristics of coyote canines. Journal of Wildlife Management 42:680–683.

PARKER, G. R., AND J. W. MAXWELL. 1986. Identification of pups and yearling wolves by dentine width in the canine. Arctic 39:180–181.

RAUSCH, R. A. 1967. Some aspects of the population ecology of wolves, Alaska. American Zoologist 7:253–265.

ROBERTS, J. D. 1978. Variations in coyote age determination from annuli in different teeth. Journal of Wildlife Management 42:454–456.

TUMLINSON, R., AND V. R. MCDANIEL. 1984. Gray fox age classification by canine tooth pulp cavity radiographs. Journal of Wildlife Management 48:228–230.

VAN BALLENBERGHE V., A. W. ERICKSON, AND D. BYMAN. 1975. Ecology of the timber wolf in northeastern Minnesota. Wildlife Monographs 43.

WAITE, C. A. 1994. Age determination of gray wolves from Isle Royale National Park. Thesis, Michigan Technological University, Houghton, Michigan, USA.

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