Comparison of Two Methods to Age Gray Wolf Teeth

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We compared the Harris' Modified Hematoxylin stain (HMH) with the Giemsa Stain (GS) for aging wolf canine and first premolar teeth. Ages derived from canine teeth for the two stains were not significantly different (P = 1.00). Premolar teeth stained with the HMH could not be accurately aged, but those stained with the GS provided ages that were not significantly different (P = 0.43) from those provided by canines with the HMH. Based on our results, wolf premolars stained by the GS method and canine teeth stained with the HMH method appear to provide similar ages, suggesting that premolar teeth extracted from live wolves can be used to estimate age by the less expensive GS method. However, further evaluation with known-age specimens is needed.

Introduction

Obtaining accurate and precise age estimates of gray wolves (*Canis lupus*) has been difficult. Gray wolves have been aged by tooth wear and replacement (Van Ballenberghe et al. 1975, Fuller and Keith 1980, Fritts and Mech 1981, Ballard et al. 1987), examination of epiphyseal cartilage of long bones (Rausch 1967), counts of cementum annuli (Goodwin and Ballard 1985), and dentine width and root closure (Parker and Maxwell 1986). Tooth wear and replacement can be unreliable, particularly beyond the pup and yearling age classes, because factors other than age may cause wear variation. Also, aging by tooth wear is subjective and depends on the experience of the estimator. Examination of long bones allows wolves to be placed into pup or adult age classes, but with unknown accuracy and precision. Similarly, root closure and dentine width only allows identification of pup and yearling age classes.

Counts of cementum annuli from teeth that have been sectioned and stained provide the most accurate and precise estimates of ages for most wildlife species (Grue and Jensen 1979, Fancy 1980). This method was reported useful with wolves, based on known-age specimens, by Goodwin and Ballard (1985). They used the Harris' modified hematoxylin stain (Cable 1958) with hot bath (HMH). Although the method provided relatively accurate age estimates, it was only useful on canine teeth and thus was not practical for live wolves. E.A. Goodwin (Alaska Dept. Fish and Game, Anchorage) and W.B. Ballard (unpubl. data) attempted to stain first premolar teeth using the same method but could not consistently identify cementum annuli. They concluded that further research was needed on the suitability of using incisors and premolars for aging wolves.

Since the late 1970's, biologists from Alaska, Northwest Territories, and Yukon Territory (G. Matson, Matson's Laboratory, Milltown, Mont., pers. commun.) have extracted premolar teeth from live wolves for determining age. The Giemsa stain (GS) (Schneider 1973, Stone et al. 1975) has been widely used (n = 2,481 wolves from Alaska and Canada [unpubl. data]) because teeth can be commercially processed at relatively low cost. However, the GS aging method has not been adequately evaluated with known-age specimens, nor has it been compared with other methods. Ideally the method should be tested with known-age specimens, but these are difficult to obtain. Goodwin and Ballard (1985) used known-age specimens for validation of the HMH method. We wanted to examine the GS staining method but lacked known-age specimens for adequate evaluation. Consequently, we compared the two staining and aging methods using many of the specimens aged by Goodwin and Ballard (1985). Because their method was developed based on known-age material, we assumed that the HMH ages using canine teeth were accurate and used them as baseline values.

Our objectives in this study were to: 1) compare estimated ages obtained from counting cementum annuli in wolf canine teeth using the HMH with hot bath to those obtained by staining with GS (Schneider 1973, Stone et al. 1975) using a standardized aging model (G.M. Matson, unpubl.

Table 1. Deviation of ages of wolf canine teeth using the Giemsa Stain method from those using the Harris' Modified Hematoxylin with hot bath (HMH).

	Deviation from HMH canine ages (years)		
HMH Ag	*-1*	*0*	*+1*
1		2	
2	1	2	1
3	2	4	1
4		1	3
5		1	
6	2		
7			
8	1		
9			
10			1
Totals	**6**	**10**	**6**

data); 2) test GS on premolar teeth from wolves aged by Goodwin and Ballard (1985) using HMH; 3) compare ages obtained between canine and premolar teeth using the GS method; and 4) provide a model for aging wolf canine and premolar teeth to standardize methods for aging gray wolves.

Methods

Procedures for cutting, staining, and estimating the age of wolf canine and premolar teeth using the HMH method were described by Goodwin and Ballard (1985); procedures used for the GS method were described by Schneider (1973), Stone et al. (1975), and Matson (1981). All specimens were obtained from wolves harvested in south-central Alaska. A wolf birthdate of 1 May (Ballard et al. 1991b) was used for aging purposes. Goodwin and Ballard (1985) determined canine cementum age by identifying the first well-developed dark annulus as the age indicator for two-year-olds. Their model was similar to that described by Linhart and Knowlton (1967) for aging coyote canines (*Canis latrans*). Matson (1981) determined both canine and premolar cementum ages based on a poorly defined but often present first annulus in one-year-old wolves. The latter model was similar to that described for coyote canines by Allen and Kohn (1976). Evidence from both wolf canine and first premolar tooth sections indicates the dark cementum annulus first becomes visible at the extreme periphery of canine and premolar teeth collected in March and April. The addition of one year to the dark annulus count in teeth collected during late winter provides the age to the nearest full year.

Cementum ages of canine teeth previously aged by Goodwin and Ballard (1985) were compared with ages obtained with the GS method based on canines extracted from the same wolves. One or two first premolars were extracted from each wolf skull, stained and aged by the GS method, and then ages were compared with those obtained from the canines using the HMH and GS methods. Teeth that were severely altered in histology because of resorption, heat exposure, or breakage were excluded from analyses. Ages for different teeth from the same wolf were assigned without knowledge of prior results. Comparisons among tooth-stain methods were compared by Wilcox on matched pairs tests (Ott 1988).

Results and Discussion

Wolf ages estimated from canine teeth (n = 22) using the HMH and GS method were not significantly different ($\underline{Z} = 0.0$, $\underline{P} = 1.00$). All of the GS ages were within one year of the estimates provided by the HMH method (Table 1). Both methods of staining and annulus counting provided similar age estimates for canine teeth.

There were no significant differences ($\underline{Z} = 0.79$, $\underline{P} = 0.43$) between HMH canine ages and premolars stained by the GS method. Forty-eight percent of the GS ages (n = 67) were identical to the ages provided by the HMH method and 87% within ± 1 year of the HMH age (Table 2). There was a slight tendency for older animals (> 6 years) to

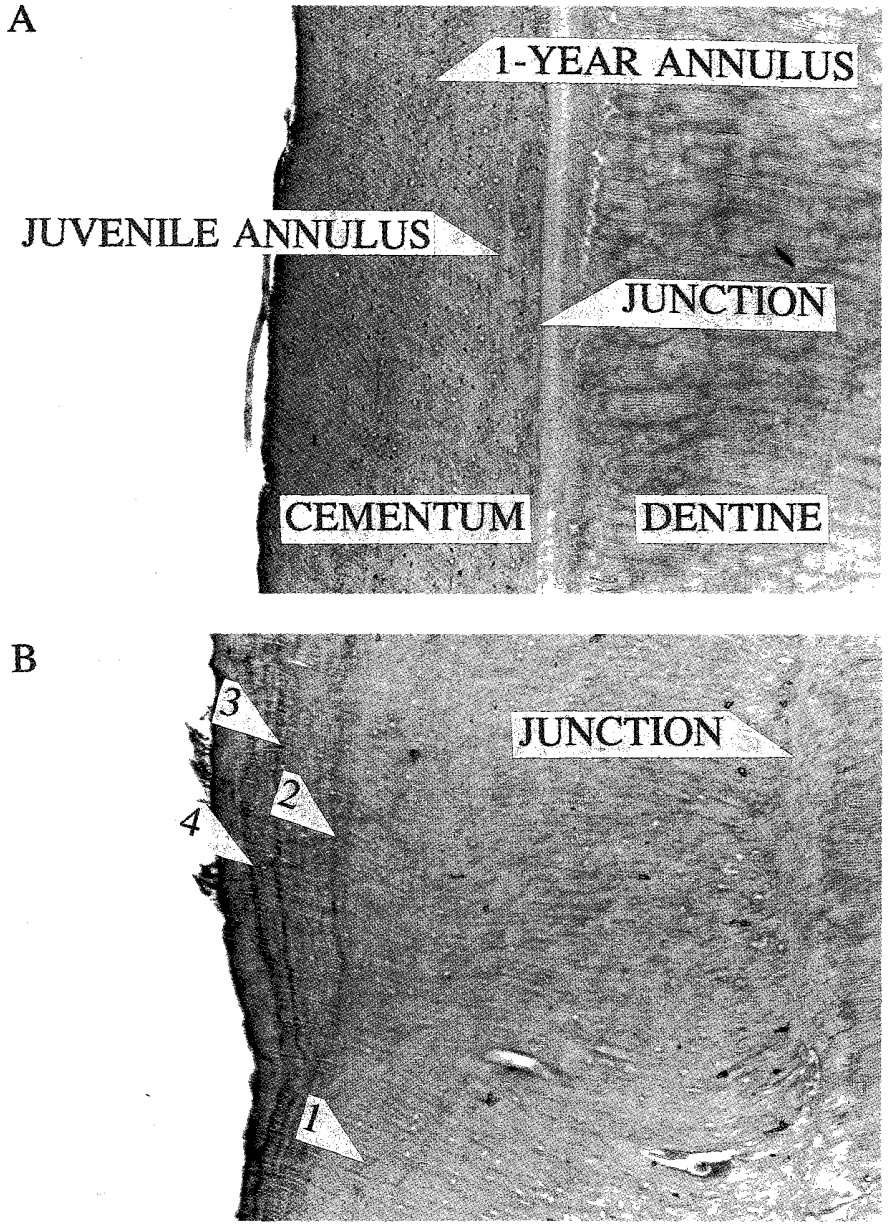

Fig. 1. *Wolf canine midsagittal sections. 60X. A. Wolf No. 122062. 5 mm above root tip. Known age = 22 months. Tooth collected on 21 March. B. Wolf No. 122179. 2 mm above root tip. 60X. Not known age. Cementum age = four years. Tooth collected on 31 December. A "juvenile annulus" (JA) may be formed before the age of one year. It is differentiated from the 1-year annulus by two characteristics: 1) it is present at the root tip, as the first identifiable annulus distal to the dentine, but is absent above the root tip; and 2) it is simple with only a single component and stains indistinctly. The one-year annulus is present both at the root tip and above it, and stains less distinctly than the two-year annulus. The first prominent annulus is the two-year annulus. Annuli formed during subsequent years are similar to the two-year annulus in staining intensity and characteristics. All annuli may be complex to varying degrees, having more than a single component. Light cementum is produced in successively narrower bands, with the greatest width occurring during the first summer and autumn of life. The dark annuli appears to be formed during winter, and first becomes visible in late March or April just before the assumed 1 May birthday. To determine the age of summer-autumn-winter-collected teeth each dark annulus is counted as one year. To determine the age of spring-collected teeth the last formed annulus is not counted as a year of age until after the assumed birthday of 1 May. Fractions of the year may be added to the annulus count according to the month of tooth collection.*

be underaged by 1–2 years with premolars. Age estimates from premolars using the GS method were significantly lower ($\underline{Z} = 1.98$, $\underline{P} = 0.048$) than GS canine ages (Table 3).

These differences suggest there are unidentified sources of error in the aging models of one or both tooth types. Canine teeth sections have thicker cementum layers than premolars, and greater separation between annuli permits the complex repeated annual patterns to be more easily identified. The illustrated description of the aging method for canines (Fig. 1) and premolars (Fig. 2) provides reference that helps insure consistency of annulus interpretation. The complete reference standard or "model" defines the criteria for annulus identification and identifies the season during which the annulus was formed.

Goodwin and Ballard's (1985) known-aged sample was largely composed of pup and yearling age classes (86% of 63 samples). Consequently both the HMH and the GS methods are in need of validation with a larger sample size of older wolf age classes. Based on our results, wolf premolars stained by the GS method and canine teeth stained with the HMH method appear to provide similar ages. Perhaps more importantly, premolar teeth can be extracted from live wolves and aged by the GS method.

Use of the GS method for aging canine and premolar wolf teeth has two distinct advantages over the HMH method described by Goodwin and Ballard (1985): 1) the GS method has been successfully used on premolar teeth; and 2) the GS method is considerably cheaper to use. The HMH method

Table 2. Deviation of wolf premolar teeth ages using Giemsa stain method from ages obtained from canine teeth using Harris' modified hematoxylin stain with hotbath (HMH).

	Deviation from HMH canine ages (years)						
HMH Age	*-4*	*-3*	*-2*	*-1*	*0*	*+1*	*+2*
1					4	3	
2				3	7	3	2
3			3	5	8	2	
4				1	8	4	1
5					4	1	
6			1	4	1		
7							
8	1		1				
Totals	**1**	**0**	**5**	**13**	**32**	**13**	**3**

Table 3. Deviations of wolf premolar teeth ages from canine teeth ages using Giesma Stain (GS) method.

	Deviation from GS canine ages (years)						
GS Canine Age	*-3*	*-2*	*-1*	*0*	*+1*	*+2*	*+3*
1			1				
2		1	3	1	1		
3		2	7				
4	1		2				
5	1	2	6	1			
6							
7	2		2				
Totals	**2**	**7**	**19**	**2**	**1**	**0**	

was labor intensive (25 teeth processed per day) and currently cost approximately $10.55/tooth (E.A. Goodwin, unpubl. data). Because of the high costs and intensive labor, large numbers of teeth could not be processed on a routine basis. In contrast, the GS method currently costs about $2.85/tooth and commercial processing is available.

The tooth cementum method, though useful, is in need of more evaluation and refinement. The development of an accurate and precise method of cutting, staining, counting cementum annuli, and estimating ages of wolf teeth has been inadequately evaluated because of lack of standardization and an insufficient number of known-age samples from several age classes. There is a strong need for wolf biologists to pool their known-age specimens so that aging methods can be appropriately tested and refined. A number of questions remain concerning the deposition of annuli and their interpretation. Goodwin and Ballard (1985) estimated that the first annulus was deposited between 18 and 22 months of age in canine teeth, but the assessment was based on only four wolves. Using different criteria a "juvenile" annulus, formed before one year of age, and a one-year annulus can be identified in known-age teeth (Figs. 1 and 2). We need to

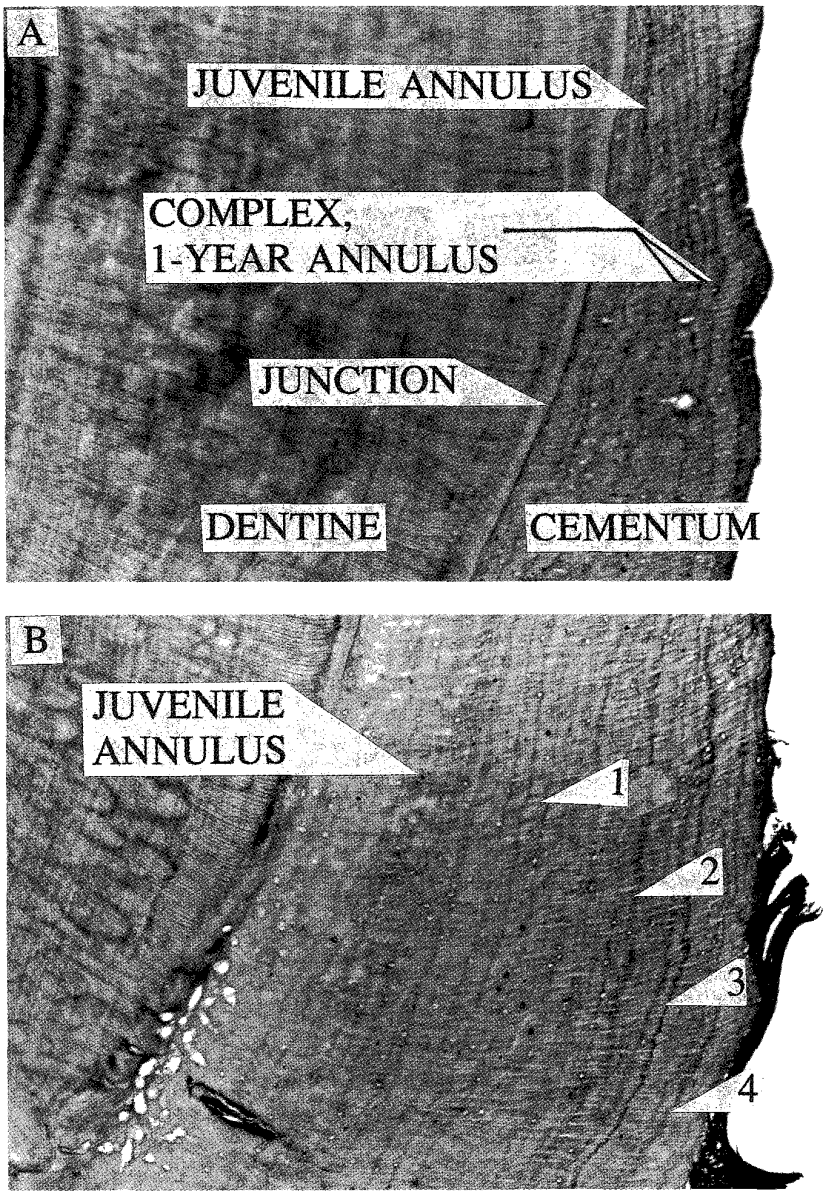

Fig. 2. *Wolf PM1 midsagittal sections. 60X. A. Wolf No. 122062. 2 mm above root tip. Known age = 22 months. Tooth collected on 21 March. B. Wolf No. 122179. 2 mm above root tip. 60X. Not known aged. Cementum age = 4 years. Tooth collected on 31 December. This aging model is similar to that for the canine tooth but the smaller size of the premolar compresses annuli closely together, making identification of annuli different. Complex annuli are sources of cementum aging error in canine and premolar teeth. There are two criteria for differentiating the complex annulus from its nonannual components: 1) the major annulus component is uniformly present at most points of the tooth section; and 2) complex annuli are uniformly spaced at regular but diminishing distances during successive years.*

determine if there are differences in the timing and extent of annulus deposition in different teeth and in wolves from different regions.

Use of the GS method for processing wolf premolar teeth has potential for estimating ages of free-ranging wolves but requires further testing with known-age specimens. Accuracy can be further improved by using only experienced personnel and a combination of root closure, examination of long bones, and tooth eruption and wear, along with the estimates provided by cementum annuli.

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