CEMENTUM ANNULI ARE UNRELIABLE REPRODUCTIVE INDICATORS IN FEMALE BROWN BEARS

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Abstract: The accuracy of using cementum layers in teeth to reconstruct reproductive histories has been evaluated for black bears (*Ursus americanus*) but not for brown bears (*Ursus arctos*). We tested the hypothesis that years when brown bears successfully reared cubs could be identified in teeth by a cementum layer that was thinner than layers in either the preceding or the following year. Using teeth from 29 brown bears with known reproductive histories, we identified potential cub-rearing years ("cub years") based on measurements of cementum layer thickness and compared results to known years of cub rearing. Of 62 known years when females reared cubs, only 13 were correctly identified. We failed to identify 49 known cub years, and we incorrectly identified as cub years 30 years when females did not rear cubs. We concluded that this method, though successful for black bears, was unreliable for brown bear populations.

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A knowledge of the reproductive histories of female bears is essential for understanding bear population dynamics. Reproductive histories provide the means to estimate mean age of first reproduction and the frequency with which cubs are reared successfully through their first full year of life. Reproductive histories have been successfully reconstructed by examining cementum layering patterns in histologically stained tooth sections from female North American black bears (Rogers 1975, 1978; Coy and Garshelis 1992; Carrel 1994). In many black bear populations, a marked thinning of the abundant, light-colored cementum occurs in females during years when they successfully raise one or more cubs. Our objective was to test whether this method would prove useful for brown bears as well.

METHODS

We examined tooth sections from 29 female brown bears with known histories for some or all of their reproductive life. We acquired known histories by monitoring individual radiocollared females for 2–13 years. Because monitoring was continuous, each history included not only years when a female was observed with cubs, but also years when she was accompanied by older offspring or when no cubs were observed. We defined cub as an offspring in its first year of life.

We confirmed presence or absence of cubs by visual observation or evidence of lactation when handling adult females. We considered cubs successfully reared if they were observed as yearlings with the adult female during spring after emergence from the den. At least one first premolar tooth (upper or lower) from each female was decalcified, sectioned longitudinally, and stained, using standard protocols for preparing tooth sections for cementum analysis (Matson et al. 1993). Our sample included 9 teeth from 7 Alaskan grizzly bears (*Ursus arctos horribilis*), 14 teeth from 14 Swedish brown bears (*Ursus arctos arctos*), and 8 teeth from 8 Kodiak (Alaska) bears (*Ursus arctos middendorffi*). Teeth were extracted from bears of various ages, upon occasions of capture. Two teeth extracted at different times from each of 2 Alaskan bears were analyzed independently. Only 1 of the bears in our study was of known age. We used the same tooth section from each bear to estimate its age and to look for indicators of reproduction.

We took photomicrographs at magnifications of 60X or 160X at 2 different points on one section from each tooth. We selected the tooth section and the points photographed based on where the full number of annuli were most plainly visible and where the deposition pattern of annual cementum layers was most regular. We avoided points where some of the annual cementum layers differed dramatically from their average thickness through-

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out the tooth section. Typically, but not always, points chosen for measuring annuli were near the gumline. Higher magnification for more accurate measurement was used for 2 tooth sections that showed cementum annuli very close together. When we converted negatives to 10 by 15 cm glossy prints, the resulting magnification was 70X or 180X.

We measured the thickness (mm) of each cementum layer directly from the photographic prints and entered measurements on a spreadsheet. Our measurement for a single layer included both the darkly staining, thin, acellular cementum annulus (formed during winter) and the lightly staining, abundant cellular cementum (formed during spring, summer, and fall). Based on the observations of thinned cementum during cub-rearing years of black bears (Rogers 1975, 1978; Coy and Garshelis 1992; Carrel 1994), we used 2 criteria for identifying a cub year in female brown bears: (1) the cementum layer was any measurable amount thinner than in the previous year; and (2) the cementum layer was any measurable amount thinner than in the following year.

We back-dated from the last-formed cementum layer to date cub years. We recorded a cub year whenever measurements in the 2 photographs from each tooth section were compatible. Measurements were compatible when cub years were identified in either of the 2 photographs, but not when a cub year was identified in one photograph and in a successive year in the other photograph.

For each bear, we compared the known reproductive history with the history suggested from cementum measurement. We deleted from our data set all known cub years that were either in the same year or in the year just prior to a tooth extraction in the spring season, because the cementum layer for the current year was not fully developed and therefore its thickness relative to the previous year could not be evaluated.

We also tested whether subjective examination of tooth sections was comparable to analysis by measuring cementum layers in photographs. Three experienced technicians microscopically examined each tooth section and identified possible cub years by sight, unaided by measurements, but using the same criteria as above. One of the technicians was experienced in cementum analysis, having aged approximately 19,000 brown bears and 129,000 black bears (G.M. Matson, unpublished data). The other 2 technicians were being trained in the application of Matson's standardized cementum aging model for brown bears (Matson et al. 1993) and each had already aged several hundred black bear teeth under supervision. We recorded a possible cub year whenever all 3 technicians agreed that both criteria were met.

RESULTS

Using cementum measurements from photographs, we correctly identified only 13 of 62 known cub years. We failed to identify 49 known cub years, and incorrectly identified 30 years that were not cub years (Table 1). Accuracy was similarly low for all 3 populations (Sweden, interior Alaska, and Kodiak Island). Cementum layer thinning was not evident during years when females were known to have successfully reared cubs (Fig. 1).

Our ability to identify cub-rearing years was no better using visual examination than by using measurements on photographs. We failed to identify all 62 known cub years and, in fact, agreed on only 3 potential cub years, all of which were incorrect. All 3 technicians agreed that there was no tooth section with annuli that unequivocally demonstrated the cementum criteria. In contrast, cub years are plainly evident in teeth in some populations of black bears (Fig. 2). Accuracy was better in teeth extracted from brown bears $\leq$10 years old than for older bears; we correctly identified 7 of 14 known cub years in the former group, but only 6 of 48 in the latter group ($\chi^2 = 1.09$; $P = 0.006$).

DISCUSSION

We found no evidence that the method developed for black bears, using cementum annuli to identify cub-rearing years, can be successfully applied to brown bears. The low accuracy of our results in bears from 3 populations in 2 continents suggests that the method has low potential as an indicator of cub rearing in *Ursus arctos*.

Our criteria for identifying possible cub years in teeth differed somewhat from the criteria used by other researchers. We used 2 criteria for identifying a cub year: (1) the cementum layer was any measurable amount thinner than in the previous year; and (2) the cementum layer was any measurable amount thinner than in the following year. Coy and Garshelis (1992) found that marked thinning in a cementum layer, typically to less than half the thickness of the previous cementum layer, accurately indicated cub rearing. Carrel (1994) computed a relative width index (RWI) of each annual cementum layer that compensated for (1) thickness variation in different parts of the tooth section; and (2) decreasing thickness with increasing age. We chose to apply the method of Coy and Garshelis (1992) because it was simple and could be used during routine cementum aging. We wanted to know whether either visual examination of tooth sections or simple measurement of layer thickness (after visually selecting sample points) could successfully identify cub rearing years in brown bears. We felt that our first criterion was more likely to detect all of the cub years than Coy and Garshelis's criterion of marked thinning, and thus would indicate any possible correspondence between layer thinning and cub rearing. We expected it to result in some false identifications of cub years (as happened); to decrease the chance of this occurring, we added the second criterion, "rebound" thickening the following year. This is consistent with the characteristics of cementum layering in black bears described by Coy and Garshelis (1992) and observed consistently in our laboratory. Re-

Table 1. Accuracy of cementum thickness as an indicator of cub rearing in female brown bears from 3 populations. By our criteria, the cementum layer indicated a cub year when it was thinner than the layers before and after.

Region	Bear identification (age at tooth extraction, years)	Age span monitored (yr)[a]	Ages of known cub rearing (yr)[b]	Ages of correctly (incorrectly) identified cub rearing (yr)	Accuracy, by region (%)[c]
Sweden	W9308 (7)	3–7	3, 5	3, 5	
	BD18 (14)	6–14	7, 10	none (6)	
	BD51 (7)	4–7	5	5	
	BD62 (16)	15–16	14	none (13)	
	W8808 (13)	6–13	3, 6, 8, 10	none (7, 11)	
	W8905 (9)	2–9	4, 7	none	
	W9008 (10)	4–10	5, 7	7	
	BD01 (13)	1–13	5, 9	none (8, 10)	
	W8702 (8)	7–8	7	none	
	W8802 (7)	4–7	3, 5	3	
	W8909 (12)	7–8	6, 8	8 (5)	
	W9001 (8)	5–8	4, 6	none (5)	
	BD10 (9)	9	7	7 (4)	
	BD27 (13)	13	11	none	28%
Interior Alaska					
	1308-94A (18)	5–18	6, 8, 11, 14	None (7, 12)	
	AKR91-1308 (15)	5–15	6, 8, 11	none (13)	
	1607-95A (15)	8–11, 15	9	9	
	AKR90-1608 (23)	20–23	21	none (20)	
	1311-95 (27)	13–27	14, 16, 19, 22	none (13, 15, 18, 25)	
	1362-95 (15)	4–10, 14–15	6, 14	14	
	1391-95HK[d] (8)	0–8	5	5	
	1398-94 (16)	8–16	9, 13	none (12)	
	96-1608 (29)	20–29	21, 24, 27	none	14%
Kodiak Island	055 (23)	12–23	14, 17, 20	none (15, 19)	
	431 (17)	10–17	13, 16	13 (15)	
	443 (13)	8–13	10	none (11)	
	727 (19)	11–19	13, 17	17	
	741 (19)	7–19	9, 13, 16	none (8, 15)	
	776 (13)	6–13	8	none	
	426 (20)	12–20	14, 16	14 (17)	
	086 (21)	6–21	8, 13	none (12, 15, 17, 19)	19%

[a] Each bear was radiocollared at the beginning of the monitoring period and followed annually throughout the indicated age span, except for 2 Alaska bears that weren't monitored for periods of 3 years.

[b] The age of known cub rearing was back-dated from the year of capture. Actual age of cub rearing may be incorrect because of cementum aging error. Cub rearing in years prior to the year of capture was determined by the ages of offspring with the captured female. Does not include cub-rearing year at age of tooth extraction or 1 year younger when extraction was in the spring season, because cementum criteria for identifying cub-rearing years would not yet be present for that year.

[c] Percent accuracy = Number of correctly identified cub-rearing years/number of known cub-rearing years x 100.

[d] The only known-age bear in our study.

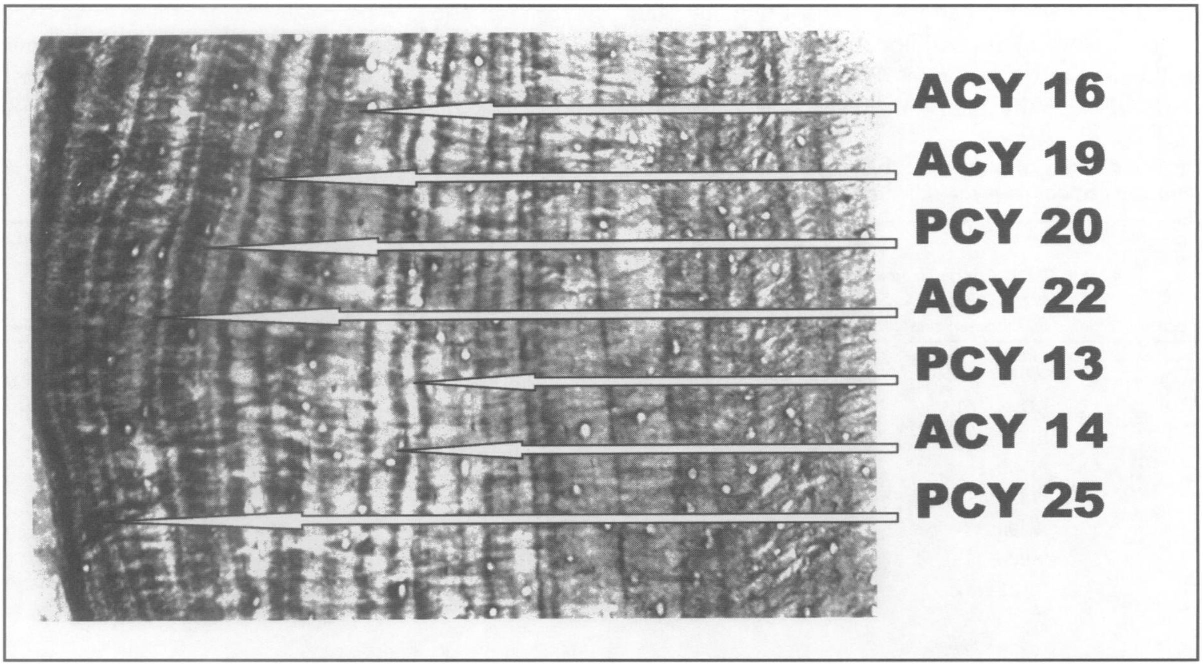

Fig. 1. Tooth section from a female interior-Alaska brown bear (cementum age = 27 yrs; date of tooth extraction = 11 June). Cub-rearing years predicted by our cementum measurement criteria (pcy) match none of the actual cub-rearing years (acy). Numerals indicate age (yr).

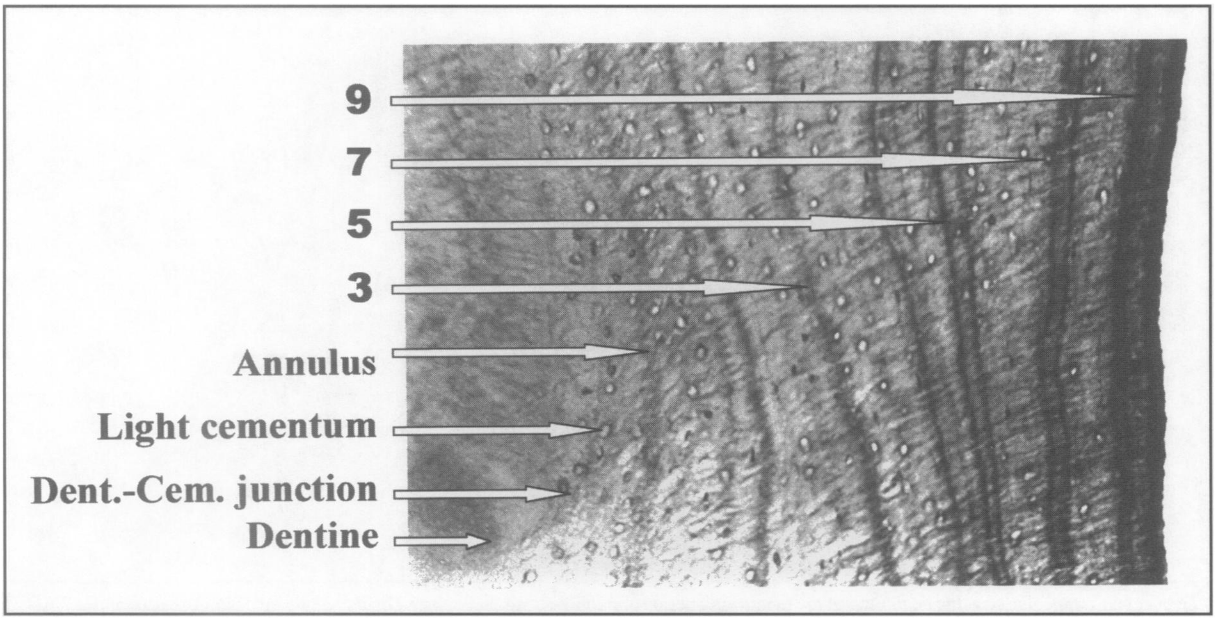

Fig. 2. Typical tooth section (first premolar) of a female black bear. The abundant, cellular light cementum is produced during spring, summer, and fall. The cementum annulus is produced during winter. The thinned light cementum layers indicate successful cub rearing at ages 5, 7, and 9. Numerals indicate age (yr). Dent. - cem. = Dentine-cementum.

bound thickening possibly reflects a release in growth inhibition after cub rearing.

However, the very low accuracy of our results suggests that annual cementum layer thickness may be too variable in brown bears to be useful as an indicator of cub rearing. There are several possible explanations for our inability to reconstruct brown bear reproductive histories. First, the irregular layering that is a frequent characteristic of cementum deposition can cause cementum aging error. Sometimes the last visible annulus, which is the indicator for the year of tooth extraction, is incorrectly identified. Assigning the wrong year to the last visible annulus would cause consistent errors in reconstructed cub years because we back-dated from the year of extraction to date the apparent cub year. For bear number 1311-95 (Table 1), known cub years and reconstructed cub years consistently differed by 1 year. Other evidence of aging error may be found in the cementum ages of 3 Swedish brown bears; our cementum criteria indicated that these 3 bears reared cubs as 3-year-olds (Table 1), yet researchers have not found cub rearing in known-age Swedish brown bears younger than 4 years (A. Söderberg, Research Unit, Swedish Hunters Association, Uppsala, Sweden, personal communication, 1998).

Though aging error may have occurred, we minimized its effect by back-dating from the last-formed cementum layer to date the cub year. As an example, consider a hypothetical 15-year-old bear captured in 1996 with a cub year identified in a thinned cementum layer of 1994. The chance of aging error in the 3 years between cub rearing and capture is less than for the entire 15 years of age. If there is no aging error in the last 3 years, the cub-rearing year would be correctly identified even though the age at cub rearing could be incorrect because of aging error in earlier years.

A second possible explanation for our low accuracy in reconstructing reproductive histories applies to teeth from old-age (>10 years) bears. Accurate interpretation of the entire complement of cementum annuli is difficult because annual cementum layers are typically thin at advanced ages. Due to the progressive growth reduction that occurs with increasing age (Pearson 1975), there may be a less-pronounced effect of cub rearing on annulus thickness in older ages. We suspected that if cementum thinning occurred as a result of cub rearing, it should be more apparent at younger ages when cementum layers were thickest overall (Pearson 1975). In fact, we found no significant difference in accuracy identifying cub years that occurred at ages <10 versus >10 years old (9 of 35 and 4 of 27, respectively, were correctly identified). We did, however, find that accuracy was significantly better in teeth extracted from bears at younger ages (50% correct in teeth extracted when bears were ≤10 years old versus 12.5% in older bears). At advanced ages, separation between adjacent cementum annuli is not always clearly visible. This can cause 2 annuli to appear as a single annulus, resulting in an unavoidable error in back-dating apparent cub years (e.g., an event that occurred 6 years prior to the tooth extraction would appear to have occurred only 5 years before). Even if annuli thicknesses accurately reflected cub rearing in young bears (<10), any "missed annuli" occurring at later ages in the same tooth would make it impossible to back-date those cub-rearing events to the correct year.

Although these types of errors likely explained some of our failure to accurately reconstruct reproductive histories, we do not believe they were the only problem. In a study of 75 known-age Alaska brown bear teeth, the most experienced technician was correct for 61.7% of the readings (Matson et al. 1993), which is higher than our accuracy identifying cub years. Moreover, despite similar problems interpreting the thinned annuli in teeth from older black bears, scientists routinely have been able to date known cub-rearing years from black bear cementum patterns. Only the study of known-age bears can determine the relative importance of aging error versus other factors in preventing this method from being useful for brown bears.

We do not know why black bears produce cementum layers that accurately reflect reproductive histories but brown bears do not. It could be argued that our criteria may not be appropriate for brown bears due to reproductive differences in the 2 species. Cubs are normally produced every other year in black bears but are often produced only every third year in brown bears. The alternating thick-thin cementum layering that correlates with cub production in black bears may not occur with the 3-year cycle of brown bears if the second year of maternal care is nutritionally costly and if nutrition is ultimately responsible for cub-related variation in cementum thickness. However, we were no more successful identifying cub years in brown bears that raised cubs every 2 years ($N = 11$ known 2-yr intervals; Table 1) versus every 3 years.

Even among different black bear populations, there are some that lack cub-rearing indicators (Coy and Garshelis 1992). Possible causative factors include genetically-based differences in growth and calcium metabolism. Environmental and dietary differences, marked between most North American black bears and the brown bears of our study, may play a role. Our understanding of cementum growth dynamics is handicapped by a lack of information, as there have been no controlled studies of the factors causing cyclic cementum growth in any mammal species commonly hunted or trapped in the northern hemisphere.

Because the accuracy of cub year identification was too low to be useful for any of the 3 populations we evaluated, we did not test for significant differences in accuracy among them. Recent genetic study has found 2 mitochondrial DNA clades in Swedish brown bears (Taberlet et al. 1995). Our study did not evaluate whether these or other genetic differences were reflected in our results.

Despite our higher success using teeth extracted from younger bears, accuracy was still not great enough (50%) to make this a reliable technique, even if restricted to bears <10 years old. We encourage future study, using teeth of known-age brown bears, to determine whether first successful cub rearing can be detected consistently from cementum patterns; if so, the method could still be useful for estimating the average age of first reproduction in a population, even if reconstruction of entire reproductive histories was unreliable.

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