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Use of canine pulp cavity size in separating juvenile and adult wolverines (*Gulo gulo*)

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Using 360 harvested wolverines, we examined techniques to separate juvenile and adult age classes using canine pulp cavity size or ossification of cranial sutures, and assessed which of 3 tooth types provides the most reliable counts of cementum annuli. Greatest confidence in age estimates from counts of cementum annuli were obtained using lower canine teeth rather than first premolars or third incisors. Percent closure of internasal or zygomatic sutures separated 71–100% of sex and age classes into juveniles and adults. While percent pulp cavity (pulp width/tooth width × 100) of canines decreased with age, overlap in tolerance limits among all adult year classes meant that percent pulp was only useful in separating juveniles (≤ 16 months) from adults (> 18 months). Calculated percent pulp dividing points between juveniles and adults (juveniles > dividing point) were 51.7% and 45.6% for males and females, respectively. These dividing points correctly aged > 97% of wolverines as juveniles or adults. Examination of percent pulp may provide a relatively rapid, inexpensive and reliable method for identifying juvenile and adult age classes of wolverines in the harvest.

1. Introduction

In management of wolverines (*Gulo gulo*) we need to both assign ages by year and by age class. For example, in evaluating population status by the age of first reproduction and age-specific pregnancy rates, we need to age females by year. However, as a preliminary evaluation of the effects of harvesting we only need to be able to differentiate juvenile and adult age classes.

Rausch & Pearson (1972) evaluated a number of aging methods for wolverines and concluded that only cementum analysis provided a relatively reliable estimate of age beyond 1 year. Layers of tooth cementum in canines agreed with age in 7 wolverines of known age (Myhre 1968, A. Magoun, Alaska Fish and Game, Fairbanks, pers. commun., 1991), however, annulations are not as distinct nor easy to read as for other animals (Rausch & Pearson 1972, Banci 1982, G. Matson, Matson's Lab., Milltown, MT, pers. commun., 1991). Cementum analysis also is expensive (US$3.30–4.80/tooth at commercial facilities) and time-consuming (2–3-month turnaround at com-

mercial facilities). In addition, its level of discrimination down to year class occasionally is more than what is required.

Other than cementum analysis, most techniques to distinguish juveniles from adult wolverines are unreliable. Bacula measurements appear unreliable as criteria of age (Banci & Harestad 1988) and are not useful for females. Ossification of cranial sutures has shown promise to identify animals up to 11 months of age (Rausch & Pearson 1972, Banci 1982). However, wolverines may be born from December to late March (Banci & Harestad 1988), thus in harvests from the Northwest Territories (NWT), Canada, where the harvest season covers 1 November to 30 April in most regions, the "juvenile" age class (animals in their first complete winter) may include animals up to 16 months of age.

The ratio of pulp cavity width to tooth width (percent pulp) from canine teeth measured using radiographs has proven effective at separating juvenile from older age classes in several species of mustelids, including marten (*Martes americana*), fisher (*M. pennanti*), otter (*Lutra canadensis*), badger (*Taxidea taxus*), and striped skunk (*Mephitis mephitis*) (Kuehn and Berg 1981, 1983, Fredrickson 1983, Jenks et al. 1984, Dix & Strickland 1986a, Poole et al. 1994). D. Palmisciano (pers. commun., cited in Hash 1987) reported that percent pulp gives a valid year-class in wolverines up to 3 years of age, but provided no supporting evidence.

Previous researchers have counted annulations in canines (Rausch & Pearson 1972, Liskop et al. 1981, Gardner 1985) and first upper premolars (PM^1) (Banci & Harestad 1988) to age wolverines, but no study has examined which tooth provides the most reliable results. Our objectives were to determine if percent pulp of lower canines or estimates of cranial suture closure are reliable predictors of age class (juveniles ≤ 16 months old, adults > 18 months old) of wolverines in the NWT, and to determine which of 3 tooth types provides the most reliable counts of cementum annuli.

2. Methods

Wolverine carcasses were collected from hunters and trappers from 3 areas in the NWT during the 1985–1986 to 1991–1992 harvest seasons (1 Nov to 30 Apr). Trapping areas sampled were ≤ 1200 km apart; the Kitikmeot area (central NWT, centred on 67°N, 110°W), the Inuvik area (northwestern NWT, 69°N, 130°W), and the Ft. Reliance area (southcentral NWT, 63°N, 108°W), east of Great Slave Lake. Lower canines, first lower premolars (PM_1), and lower outer incisors (I_3) were extracted by simmering the mandible in hot water (90–95°C) for ≤ 2 hr. A canine from each specimen was glued buccal side up on 190 × 270 mm file folders, and radiographed using a Senograph 600T Mammo Unit and Kodak Mammography film exposed at 36 Kv and 7 Mas. Following procedures outlined in Dix & Strickland (1986a), maximum pulp cavity width and tooth width at the same point were measured on the radiographs using a Canon microfiche reader. The radiographs were magnified 17.5× and the images were measured to the nearest 1 mm. Percent pulp [(pulp cavity width/tooth width) × 100] was calculated.

The percentage closure (to the nearest 10%) of the internasal and zygomatic sutures was visually estimated by 1 observer (AG) from a sample of cleaned, but not air-dried, skulls from Kitikmeot wolverines. The skulls were cleaned by simmering only long enough to remove the muscle mass.

Wolverine teeth were sectioned and aged by cementum analysis at a commercial laboratory by G. M. Matson (Matson's Laboratory, Milltown, MT). A subjective confidence code was assigned to each age assessment by the laboratory based primarily on the distinctness of the cementum annuli: "A" = reliable, "B" = some doubt, and "C" = unreliable (G. Matson, pers. commun., 1991). Up to 3 teeth from the same animal were aged in blind tests. Pairs of lower canine teeth ($n = 21$), canine and PM_1 ($n = 91$), and canine and I_3 ($n = 13$) were examined. We had no wolverine teeth of known age for comparison, thus we are unable to comment on the "accuracy" of the techniques (agreement between the true and the estimated age). We considered ages determined from percent pulp and suture closure as "reliable" if they agreed with the age derived from lower canine cementum analysis.

We compared group means using 1-way analysis of variance. We used 95% tolerance

limits (Remington & Schork 1970:188) to assess overlap among means (Dix & Strickland 1986b). Dividing points to separate juveniles from adults using percent pulp were calculated for males and females using the formula of Dix & Strickland (1986a):

$$D = \bar{x}_a + ((\bar{x}_b - \bar{x}_a)(SD_a)/(SD_a + SD_b))$$

where SD = standard deviation of each group, $\bar{x}$ = mean of each group, and $\bar{x}_a < \bar{x}_b$.

3. Results

Three hundred and sixty wolverines were examined (Kitikmeot, $n = 211$; Inuvik, $n = 97$; Ft. Reliance, $n = 52$). The commercial laboratory had greater confidence aging canine teeth than I_3 or PM_1 teeth (Table 1). PM_1 had the lowest proportion of "A" codes and the highest proportion of "C" codes, a result of resorption of cementum frequently removing or remodelling much of the root (G. Matson, pers. commun., 1991). In all tooth types there was a noticeable trend of decreased aging confidence as estimated age increased.

In cementum aging tests using multiple teeth from the same animal, 85% of canine–I^3, 81% of canine–canine, and 57% of canine–PM_1 comparisons agreed (Table 2). Agreement in estimated ages in all paired comparisons was highest in the younger age categories. All 6 juvenile canine–canine comparisons agreed, whereas 4/15 canine–canine age estimates from 1–3-year old wolverines were in error and none of the disagreements overlapped into the juvenile age category. Age estimates from cementum analysis of lower canines probably allow separation of juvenile from adult age classes.

In juveniles and adults, percent pulp in canines from males was larger than that of females (t-tests on arc-sin transformed data, $t > 2.6$, $P \leq 0.01$). There were no differences among the collection areas in percent pulp for male and female juvenile and adult age classes (as determined by canine cementum analysis; all $P > 0.3$), therefore we pooled wolverines from all areas for subsequent analyses.

Percent pulp declined rapidly during the harvest season for juveniles (males: $F_{1,52} = 80.1$, $P < 0.001$; females: $F_{1,21} = 23.1$, $P < 0.001$), with an average decline in pulp of 19.4% for males and 24.8% for females over the length of the season. Percent pulp continued to decrease to about 4 years of age, however substantial overlap in tolerance limits among all adult year classes meant that percent pulp was only useful in separating juveniles (≤ 16 months) from adults (> 18 months). Calculated dividing points between juveniles and adults (juveniles > dividing points) were 51.7% for males and 45.6% for females (Table 3). Although there

Table 1. Distribution (%) of confidence codes assigned for aging wolverine teeth using cementum analysis.

Tooth type	Cementum age	n	Confidence code[a] A	B	C
Canine	0	122	95.9	4.1	0
	1	140	90.0	10.0	0
	2	40	50.0	50.0	0
	≥ 3	58	37.9	51.7	10.3
	All	360	79.2	19.2	1.7
I_3	0	7	100.0	0	0
	1	4	0	75.0	25.0
	2	2	0	0	100.0
	All	13	53.8	23.1	23.1
PM_1	0	23	13.0	73.9	13.0
	1	49	12.2	67.4	20.4
	2	12	0	75.0	25.0
	≥ 3	7	14.3	71.4	14.3
	All	91	11.0	70.3	18.7

[a] "A" = reliable, "B" = some doubt, and "C" = unreliable.

Table 2. Degree of agreement between estimated age by cementum analysis of paired wolverine teeth by age category.

Paired comparison	Age category[a]	Difference in estimated age of paired teeth 0	±1 year	±≥2 years
Canine–canine	0	6/6	–	–
	1–3	11/15	4/15	–
Canine–I_3	0	6/6	–	–
	1–3	5/7	2/7	–
Canine–PM_1	0	13/15	2/15	–
	1–3	37/62	21/62	4/62
	4–10	2/14	1/14	11/14

[a] Age assigned from canine age in canine–I_3 and canine–PM_1 comparisons. Both members of each paired canine–canine comparison were in either the 0 or the 1–3 year age category.

were some overlap in tolerance limits (Table 3), > 97% of wolverines were aged correctly as juveniles or adults using these dividing points (Table 4).

Age class determination from suture lines for 193 skulls was examined (Table 5). For males, a visually fitted 40% dividing point of internasal suture closure correctly aged 93% of the juveniles and 98% of the adults, whereas 90% zygomatic closure correctly identified 88% of the juveniles and 94% of the adults. Similarly, for females a dividing point of 40% internasal suture closure correctly identified 89% of the juveniles and 100% of the adults, and 90% zygomatic suture closure correctly identified 71% of the juveniles and 100% of the adults.

Table 3. Percent pulp [(pulp cavity width/tooth width) × 100] for lower canines in juvenile and adult wolverines taken in the Northwest Territories, 1985–1992.

	Cementum age class	*n*	$\bar{x}$	SE	Range	95% tolerance limits
♂	Juvenile	72	64.5	0.84	48.5–82.7	48.2–80.7
	Adults	153	33.4	0.83	7.7–58.6	11.2–55.6
♀	Juvenile	36	60.4	1.34	44.8–80.5	40.5–80.2
	Adult	78	27.4	1.11	10.3–46.5	5.0–49.8

Table 4. Percent pulp [(pulp cavity width/tooth width) × 100] of lower canines for separating juvenile and adult wolverines taken in the Northwest Territories, 1985–1992. Age as determined by cementum analysis is assumed to be correct.

	Dividing point (% pulp)	% juveniles aged correctly (*n*)	%adults aged correctly (*n*)
♂	51.7	97.2 (72)	97.4 (153)
♀	45.6	97.2 (36)	98.7 (78)

Table 5. Percentage of wolverines within estimated suture closure classes.

	Cementum age class	*n*	Zygomatic suture closure 0–70	80–90	100	Internasal suture closure 0–40	50–90	100
♂	Juvenile	24	75	13	13	93	7	0
	Adult	85	0	6	94	1	63	36
♀	Juvenile	17	71	0	29	89	6	6
	Adult	62	0	0	100	0	53	47

4. Discussion

Use of percent pulp cavity of lower canine teeth provides a relatively inexpensive, rapid and reliable technique to separate juveniles and adults, either solely, or as collaboration of ages derived from cementum analysis. Percent pulp as a technique to determine age class met at least 2 of Larson & Taber's (1980:143) 4 criteria for an ideal age determination technique. Separation into classes is clear and objective and the technique is relatively quick and reliable. However, the technique is not suitable for living animals (unless a portable radiograph machine is available), and it is unclear whether dentin deposition in the pulp cavity is affected by irregular nutritional and physiological variations. We found that teeth from up to 80 wolverines could be extracted, mounted on file folders, and their radiographs measured in 1 day. Radiography costs at commercial facilities were <$20 per file folder ($0.25 per tooth). The technique may be particularly attractive to persons inexperienced at cementum analysis, because an objective measurement of the percent pulp may yield more reliable results than inexperienced decisions on what constitutes an annulus.

The dividing points for percent pulp derived in this study effectively aged wolverines from large areas of the NWT, and may well be applicable to other regions of the north. However, regional differences may exist, and dividing points should be calculated from sufficient specimens in each study area. Because of the rapid decline in percent pulp in juvenile wolverines in their first full winter of life, the techniques may provide better results in animals taken earlier in the winter. The few cases of overlap of percent pulp between juveniles and adults in our data set took place in males harvested after 1 February and females taken after 1 March.

Wolverine teeth are among the most difficult to age by cementum analysis techniques (Rausch & Pearson 1972, Banci 1982, G. Matson, pers. commun., 1991). Human error, poor slides and abnormal tooth histologies can contribute to incorrect aging. Many of the discrepancies between percent pulp and cementum age might be attributed to incorrect aging by cementum analysis. A second canine could be cementum aged from all "B" or "C" code ages if increased precision is

desired. Larger samples would reduce the variability of the tolerance limits and refine the dividing point of the percent pulp cavity technique. Known-age individuals would enhance the evaluation of all aging techniques (Dapson 1980).

Our limited examination of cementum analysis by tooth type suggests that the canine should be the preferred tooth type when a carcass is available. For live animals the I_3 cannot realistically be removed, and the PM_1 may provide less than reliable data if the exact age of the animal is required. However, because agreement was good between cementum ages derived from paired canines and PM_1's in the juvenile age class (Table 2), cementum analysis of PM_1's may provide a good indication of juvenile vs. adult status in live animals. If greater precision is required, the researcher may wish to pull and section both PM_1's, making multiple sections (> 5) from each tooth in an attempt to get a section showing distinct annuli.

Rausch & Pearson (1972) used both ossification of long bones and skull sutures to minimize errors in estimating age classes, but they still found considerable variability. Measurement of percent pulp may be the preferred technique to separate juvenile and adult age classes, because it provides greater reliability than estimates of suture closures, and it is easier to conduct than other techniques when many samples are involved.

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