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RELIABILITY OF TECHNIQUES FOR DETERMINING AGE IN SOUTHERN WHITE-TAILED DEER

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ABSTRACT

Mandibles from known-age animals were used to compare reliability of determining age in white-tailed deer *(Odocoileus virginianus)* by tooth wear and replacement and enumeration of cementum annuli in the second premolar. Error ranged 0-7 and 0-8 for cementum annuli and tooth-wear-and-replacement estimates, respectively. Median error and consistency were similar for both techniques, but were dependent of age; an interaction of age and technique influenced median error. There was no consistent bias associated with either technique. Ages determined by use of the two methods agreed in 64% of the comparisons; ages of only 33% were determined correctly by both techniques. Agreement varied among age classes with the greatest disparity occurring among 6.5 year-olds.

Ages of white-tailed deer (*Odocoileus virginianus* typically are determined according to a schedule of tooth wear and replacement (Severinghaus, 1949), or enumeration of annuli within dental cementum (Laws, 1952; Scheffer, 1950). The tooth-wear-and-replacement technique is reliable for age classes 3.5 years, but precision diminishes rapidly for older age classes (Ryel et al., 1961). Because wear patterns vary among deer in different habitats, reliability of tooth-wear-and-replacement estimates depend on local variations in diet and training and experience of biologists (Ludwig, 1967). Numerous investigators attempted to determine the reliability of the cementum-annuli technique by use of known-age samples (DeYoung, 1989; Erickson and Selinger, 1969; Low and Cowan, 1963; Sauer, 1971, Thomas and Bandy, 1973, 1975). Most investigators concluded that enumerating cementum annuli provided an unbiased and fairly precise estimate of age. The reliability of such estimates, however, is clearly dependent on training and experience (Roseberry, 1980).

Enumerating annuli was considered a better estimator of age than tooth wear and replacement in northern USA and Canada (Erickson et al., 1970; Gilbert, 1966; Gilbert and Stolt, 1970; Sauer, 1971; Thomas and Bandy, 1975), but many of these investigators assumed that derived ages were correct. Use of cementum annuli counts as reliable estimates of age for white-tailed deer in southern regions recently was questioned (Cook and Hart, 1979; Hackett et al., 1979; Roseberry, 1980). Ages of white-tailed deer consistently were underestimated in Texas; only four (16%) of 25 were estimated accurately (Cook and Hart, 1979). In Mississippi, ages of white-tailed deer consistently were overestimated by counts of cementum annuli (Hackett et al., 1979). More recently, DeYoung (1989) reported that ages of young deer in south Texas determined by counts of cementum annuli were underestimated, whereas the ages of older deer were overestimated with this technique.

Both incisors and molariform teeth have been used to prepare histological sections of dental cementum for enumerating annuli. However, significant variation in definition and clarity of annuli occurs between sections prepared from different white-tailed deer dentition (McCullough and Beier, 1986). In the South, annuli counts have been obtained exclusively from the cementum of incisors (Cook and Hart, 1979; DeYoung, 1989; Hackett et al., 1979). Some investigators have reported success using molariform teeth to estimate age of northern cervids, including white-tailed deer (Mitchell, 1963; Ransom, 1966; Smith, 1982; Wolfe, 1969). In southwestern Oregon, clearly defined annuli were obtained in preparations of second premolars from white-tailed deer (Smith, 1982). The feasibility of using molariform teeth to reliably age southern white-tailed deer has yet to be investigated.

The purpose of our paper is to assess the reliability of cementum annuli counts from second premolars and the tooth-wear-and-replacement in estimating the age of southern white-tailed deer. Specific objectives include testing the hypotheses that the error that occurs in estimating the ages of deer is similar for cementum annuli counts and the tooth-wear-and-replacement technique; both techniques provide accurate estimates with similar consistency; and error, consistency, and agreement among estimates are independent of age.

METHODS

We obtained mandibles of known-age white-tailed deer collected from southern and central Mississippi (94 previously marked deer), the Radford Army Ammunition Plant, Virginia (eight tagged as fawns), and Mississippi State University (48

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captives). The mandibles and a corresponding list of months within which each deer died were forwarded to an experienced deer biologist who used the tooth-wear-and-replacement technique (Severinghaus, 1949) to estimate their respective ages. Following their examination, deer mandibles were returned to us and the second permanent premolar was extracted from all lower jaws still bearing this tooth (n=69).

Each permanent second premolar was placed in a decalcifying solution (0.7 g ethylenediaminetetraacetic acid and 0.2 g sodium tartrate in one liter of 10% hydrochloric acid) for 2-3 days (Tumilson and McDaniel, 1983). Following decalcification (indicated by flexible root tip), teeth were placed in tap water for four hours to allow dissolution of excess acid. Each tooth was labeled with a tag attached by an insect pin and placed into a 1:1 anhydrous ether-ethanol mixture for 24 h. Teeth then were placed in a thin celloidin solution of 1:1 ether-ethanol and 5% celloidin for a week, then transferred to 5% stock (thick) celloidin for an additional two weeks. Teeth were embedded within 100-ml polypropylene beakers in 50 ml of stock celloidin. Beakers were placed in a desiccator containing ether; after one hour, the ether was replaced with chloroform (Tumilson and McDaniel, 1983).

Blocks hardened within 2-7 days and were trimmed individually to accommodate microtome sectioning. Reblocked teeth were immersed in 100% chloroform to promote further hardening, placed in 70% ethanol for one hour before sectioning, and mounted on wooden blocks. Sections were cut at 20 microns on a sliding microtome, stained in toluidine blue, and prepared on glass slides with a cover slip and Permount (Fisher Scientific, Fair Lawn, NJ) mounting medium. Prepared slides were examined under a binocular microscope at 100x, and number of cementum annuli was recorded. Age in years was estimated as the number of annuli plus one because the permanent premolars are not fully erupted until 19 months (Sauer, 1984). Thus, individuals with permanent dentition but no cementum annulus were classified as 1.5 year olds. Both estimates were obtained without prior knowledge of ages.

We divided the sample into the following age classes according to difficulty and accuracy of the tooth-wear-and-replacement technique (Ryel et al., 1961): I, fawns-3.0-year olds; II, 3.5-6.0-year olds; III, 6.5 year olds.

Nonparametric two-factor analysis of variance (Zar, 1984:219) was used to test whether estimating technique, age class, or combined effects influenced our estimates of age. Comparison of proportions in independent samples (Snedecor and Cochran, 1980:124) were used to determine whether techniques were equally consistent (percent correct), and a chi-square test of independence was used to determine if age significantly influenced consistency. Tendencies of each technique to produce over- or underestimates of age of all deer and among age classes were examined with multiple contingency-table analysis and the G-statistic (Zar, 1984:71). A probability of <0.05 was accepted as statistical justification for rejecting the null hypothesis.

RESULTS AND DISCUSSION

Ages of 69 deer were estimated by both techniques (Table 1). A scatterplot (Figure 1) and Spearman rank correlation analyses (Zar, 1984) indicated that there was close agreement between estimates produced by use of each method and actual ages. A significant correlation was indicated for both tooth-wear-and-replacement estimates (r_s=0.84, P<0.001) and cementum-annuli estimates (r_s=0.77, P<0.001). Absolute error ranged 0-7 and 0-8 years for cementum-annuli and tooth-wear-and-replacement estimates, respectively. Overall agreement between techniques was 64%, but ages of only 33% of the deer were determined correctly by use of both methods. Median error of cementum-annuli (1.0) and tooth-wear-and-replacement estimates (1.0) were similar (H=0.396, $d.f.$=1, P>0.50), but error was not independent of age (H=53.550, $d.f.$=2, P<0.001). Larger absolute errors occurred in the oldest age class (Tables 1 and 2). Interaction of age and technique on the observed disparity of estimates from actual ages was significant (H=83.050, $d.f.$=2, P<0.001), and tooth-wear-and-replacement estimates were accurate more frequently in the younger age classes. Consistency (% correct) did

Table 1. Number and percentage (of total) of known-age white-tailed deer from the southeastern United States that were aged correctly, overestimated, and under-estimated by tooth wear and replacement.

Known-age class	n	Aged Correctly n	%	Over estimated n	%	Under estimated n	%
0.5	3	3	4.3				
1.5	6	4	5.8	2	2.9		
2.5	18	14	20.0	3	4.3	1	1.4
3.0	1			1	1.4		
3.5	14	6	8.7	4	5.8	4	5.8
4.0	2					2	2.9
4.5	13	4	5.8	4	5.8	5	7.2
5.5	1	1	1.4				
6.5+	11			3	4.3	8	11.6
Totals	69	32	46.0	17	24.0	20	30.0

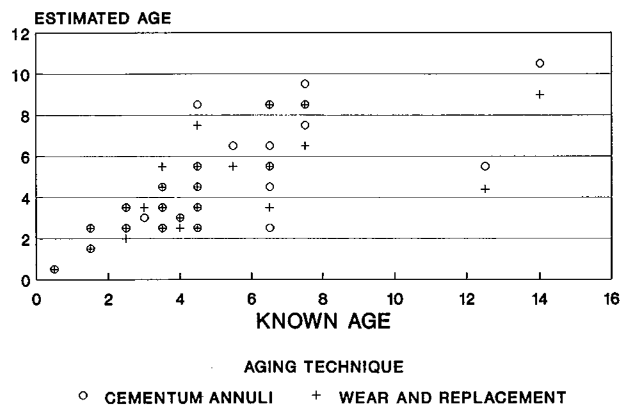

Figure 1. Scatterplot of cementum-annuli estimates and tooth-wear-and-replacement estimates against known ages of a sample of white-tailed deer from the southeasten United States.

Table 2. Number and percentage (of total) of known-age white-tailed deer from the southeastern United States that were aged correctly, overestimated, and under-estimated by enumerating cementum annuli.

Known-age class	*n*	Aged Correctly n	%	Over estimated n	%	Under estimated n	%
0.5	3	3	4.3				
1.5	6	4	5.8	2	2.9		
2.5	18	14	20.0	4	5.8		
3.0	1	1	1.4				
3.5	14	2	2.9	6	8.7	6	8.7
4.0	2					2	2.9
4.5	13	2	2.9	6	8.7	5	7.2
5.5	1			1	1.4		
6.5+	11	2	2.9	3	4.3	6	7.2
Totals	69	28	41.0	22	32.0	19	27.0

not differ between cementum-annuli (41%) and tooth-wear-and-replacement (46%) estimates (Z=0.59, P>0.50).

Age influenced the probability of estimates from either technique being correct (x^2=48.58, *d.f.*=2, P<0.001). None of the tooth-wear-and-replacement estimates for deer in the 6.5 age class were correct (Table 1), but tooth-wear-and-replacement estimates for deer in age class II were accurate more frequently than estimates derived from cementum annuli counts (x^2=6.22, *d.f.*=1, P<0.025). Neither technique provided estimates that classified deer as younger or older more or less often than expected from random chance (x^2=0.247, *d.f.*=1, P>0.50), nor was there a significant bias (G=1.945, *d.f.*=2, P>0.25) associated with any age class.

We correctly determined the age of 41% of our sample with cementum annuli counts, which was comparable to the 39% reported for cementum annuli counts of incisors extracted from live white-tailed deer in Texas (DeYoung, 1989). However, our tooth-wear-and-replacement estimates of age were correct more often (46%) than similar estimates obtained for white-tailed deer in Texas (35%). Then again, our study provided ideal conditions for using tooth-wear-and-replacement to estimate age—disarticulated mandibles were closely examined in hand. In contrast, DeYoung (1989) examined intact lower jaws of live white-tailed deer. Unlike Cook and Hart (1979) and Hackett et al. (1979), we did not observe a consistent age bias for either technique; nor did either technique tend to overestimate or underestimate age such as reported by DeYoung (1989).

Our analysis indicated that counts of cementum annuli in second premolars may provide a more reliable means of estimating age of southern white-tailed deer than previously suggested (Cook and Hart, 1979; Hackett et al., 1979). We determined ages of 80% of fawns and yearlings correctly. Admittedly, estimates of older deer were less reliable. Overall, we were only able to determine ages of about 50% of the sample correctly.

Inappropriate or unrefined technique during sectioning and staining of dental cementum contributes to error, especially by the less-experienced technician. For example, annuli often are torn during microtome sectioning; close examination of what appears as 2 annuli may be an annulus split during sectioning. Extent of previous training and experience among technicians accounts for much of the variability among cementum-annuli estimates (Lockard, 1972; Roseberry, 1980). But the expense of commercial laboratories often prohibits many agencies from obtaining cementum annuli estimates from professional technicians. Even then greater precision is not guaranteed, especially when protocol does not include means to account for variation that probably exists among regional populations (DeYoung, 1989).

The technician who prepared and examined dental cementum in our study had limited experience; still our cementum annuli estimates were as reliable as tooth-wear-and-replacement estimates. Moreover, cementum-annuli estimates appear to suffer less from bias and "provide a more realistic age distribution" (DeYoung 1989). In our study, blind re-examination of tooth sections from previously misclassified deer indicated that ability improved with experience. Reference slides prepared from local known-age deer would further improve the reliability of cementum-annuli estimates.

Additional research is needed to determine whether most of the error in cementum annuli estimates results from preparation and examination by technicians, or whether annulus formation does not occur as expected. If we understood the environmental circumstances and related physiological processes that produce annuli in teeth, then perhaps we could better interpret discrepancies in counts of annuli in comparison with known age.

Where distinct seasonality occurs, deer physiology appears to be interrupted (Seal et al., 1972). Annuli formation within dental cementum, consequently, is distinct and predictable, and counts of cementum annuli apparently yield reliable results (Low and Cowan, 1963; Sauer, 1971). Absence of a clearly defined period of interrupted metabolism in the Southeast may preclude systematic formation of cementum annuli. White-tailed deer in the Southeast reduce forage intake and probably experience some weight loss during winter, but the extent or nature of metabolic changes associated with this phenomenon is largely unknown (K.V. Miller, pers. comm.).

Also, late summer to early fall is typically a period of drought when forage availability and quality diminishes. Hackett et al. (1979) proposed that much of the unreliability of annuli counts was a result of false annuli that occur in years of extreme drought when southern white-tailed deer experience biannual (winter and summer) nutritional stress. If this were true, cementum annuli counts should be on the order of twice that of northern populations where deer are exposed to physiological stress during winter. Our results do not support this hypothesis.

Even with improved techniques and understanding, local known-age material needs to be assembled and used as reference (DeYoung, 1989; Roseberry, 1980). Otherwise, current methods are, at best, 50% reliable in classifying older deer correctly in the Southeast.

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