A NEW METHOD OF AGING GRAY SQUIRRELS (*Sciurus carolinensis*) BY USE OF CEMENTUM ANNULI

by

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INTRODUCTION

A major problem in the management of wildlife populations is obtaining an understanding of the population dynamics of a species. Essential to this understanding is a technique for determining the age of individuals within a population. Many techniques (Taber, 1963) have been devised for aging mammals including maturation of long bones, horn growth, baculum length, eye lens weight, tooth replacement and wear, and presence of annual layers in tooth cementum. These techniques are not applicable to all species and some can be applied only to dead animals. Since most mammals have teeth throughout their life and can surrender a tooth for scientific research with few adverse side effects, the use of tooth-aging techniques has distinct advantages and has received increased attention in recent years.

Aging by tooth replacement can be very accurate for younger individuals, but aging older individuals by tooth wear requires observer judgment and can be misleading since tooth wear may be influenced by many factors other than age. Tooth cementum annuli, on the other hand, is of value in aging adult individuals and is based on the presence of distinct annuli.

There are two general ways to prepare a tooth for a study of the cementum annuli: the tedious task of sectioning, staining, and mounting tooth sections on slides for microscopic examination or sawing or grinding away part of the tooth with a carborundum disk and then polishing the exposed surface for examination with a dissecting microscope or magnifying glass.

While teeth from larger mammals are most often ground and polished for aging, the sectioning method is used on teeth of smaller mammals. These stained sections are prepared by complex, lengthy and expensive histological processes. These processes require two or three days for the preparation of each section and use equipment which would be impractical, if not impossible, to use in the field.

The purpose of this study was to develop a fast, easy method for preparing and aging squirrel teeth which would enable a large number of squirrels to be handled in a short time and with minimum equipment involved. Once the technique was developed, it was tested for accuracy against serial sections, eye lens weights and teeth from known-age and minimum-age squirrels.

LITERATURE REVIEW

Since the discovery by Scheffer (1950) that the dentine of pinnipeds forms annular ridges on the roots of teeth, the teeth of mammals have received much attention as a means of estimating age. Laws' (1952) method of determining the age of elephant seals by examining the dentine layers of thin sections of canine teeth has been applied to a number of species of pinnipeds by many other researchers. The deposition of cementum on the teeth of marine mammals is most often very slight, but where found in thick layers, they have been used to provide a check on counts of dentine layers.

The first attempt to age land mammals by cementum annuli was made by Sergeant and Pimlott (1959) using the incisor teeth of moose. After cutting the root of the incisor longitudinally with a jeweler's saw or band saw and grinding the exposed faces with a fine carborundum or aluminum oxide stone, the polished faces were then examined under a binocular microscope using reflected light. This method of cutting and polishing a tooth has since been used for aging grizzly bear (Mundy and Fuller, 1964), white-tailed deer (Ransom, 1966) and moose (Wolfe, 1969).

Low and Cowan (1963) found that a slide of a decalcified tooth prepared histologically and viewed with a microscope using transmitted light was the best method for aging black-tail deer by cementum annuli. This method has been used to age black bear (Stoneberg and Jonkel, 1966), coyotes (Linhart and Knowlton, 1967), mule deer (Erickson and Seliger, 1969), and white-tail deer (Gilbert, 1966).

For aging grizzly bears by tooth cementum annuli, Craighead et al. (1970) first sawed thin longitudinal sections from the teeth and then ground them to about 16 microns with carborundum paper. These sections were then decalcified, mounted on a slide and stained with hematoxylin and eosin.

The first study using cementum annuli for aging a rodent was made by van Nostrand and Stephenson (1964) on the beaver. By grinding away of about half the side of a molar tooth they produced longitudinal section. Adams and Watkins (1967) used the histological preparation of longitudinal sections of the lower M-3 tooth for the aging of California ground squirrels. Hefner (1971) used the histological preparation of longitudinal sections of the left lower M-1 tooth in his study on age determination of gray squirrels.

PROCEDURES AND TECHNIQUES

Choice of Tooth for Study

An examination of a squirrel's teeth showed that the M-3 tooth had one large root at the posterior end and two thinner roots at the anterior end (Fig. 1A), while the M-2 and M-1 teeth had four roots of equal size. Since the tooth cementum was found to be thickest near the base of the roots and it was much easier to bisect the large M-3 root than any of the thinner roots of the other teeth, the M-3 was chosen as the tooth to be used in this study.

It was also noted that, in extracting teeth from live squirrels, the loss of an M-3 tooth would most likely create fewer difficulties in mastication for the squirrel.

For this study, teeth were obtained only from dead specimens, both lower M-3 teeth being extracted with long-nosed pliers and stored in 5 ml vials containing 10% formalin.

Decalcification

Brief trials using Nitric Acid, Formic Acid-Sodium Citrate, and Hartman-Leddon decalcifying fluids indicated that teeth in a modified Formic Acid-Sodium Citrate fluid decalcified, sectioned, and stained best. Formic Acid-Sodium Citrate decalcifying fluid was prepared as follows:

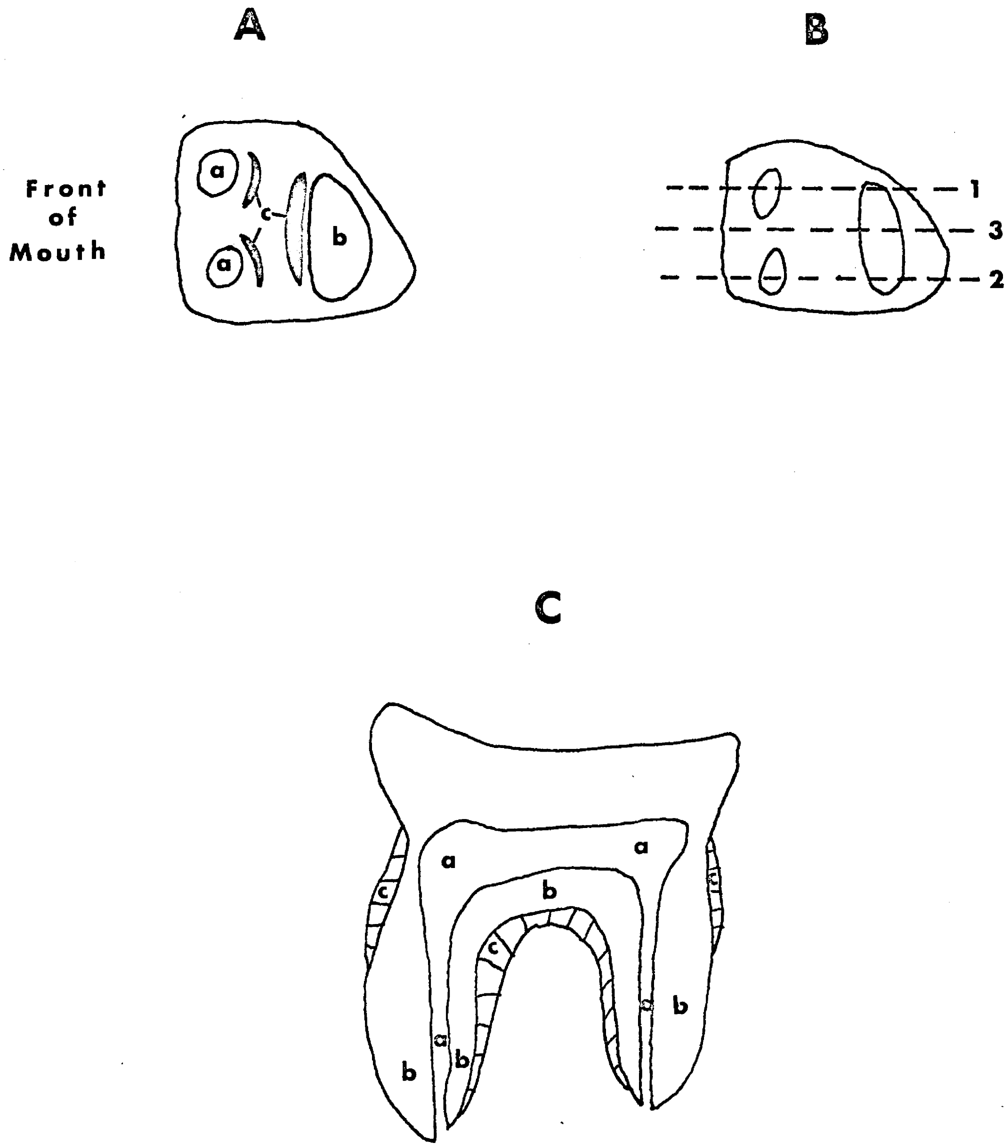

Fig. 1. Sketches of M-3 squirrel tooth

A. Basal view of M-3 squirrel tooth showing a) anterior roots, b) large posterior root and c) areas of thickest cementum.

B. Basal view of M-3 squirrel tooth showing first, second and third longitudinal cuts made with a single edge razor blade. Each cut bisected a root.

C. Lateral view of a longitudinal section of an M-3 squirrel tooth showing the location of a) pulp, b) dentine and c) cementum.

Solution A	Solution B
Sodium Citrate ... 50 gm	Formic Acid 90%...125 ml
Distilled Water...250 ml	Distilled Water...125 ml

Mix solution A and solution B in equal volumes. (Keep in separate containers and do not mix until ready to use.)

When 2 ml of solution A and 2 ml of solution B were placed in a 5 ml vial, a squirrel tooth was completely decalcified in 12-14 hours. Since a 24 hour decalcification period was desired, various dilutions of the solutions were tested. It was found that by diluting solution B with distilled water on a 1:1 ratio, complete decalcification was obtained in 24 hours.

Sectioning and Staining

After decalcification, the tooth was washed in tap water, then placed upside down (roots pointing upward) on a large glass slide. Held securely with forceps, the tooth was cut longitudinally with a single edge razor blade so that the roots were bisected (Fig. 1B). Depending on the exact shape of the tooth, three or four sections were produced.

These sections were moved from container to container through the staining sequence with the use of fine pointed forceps. The staining sequence was as follows:

Harris' hematoxylin - 10 seconds

tap water wash

lithium carbonate bluing

tap water wash

Eosin Y (1% in water) - 10 seconds

tap water wash

The sections were then placed on a slide, immersed in a drop of water, and examined with a compound microscope using reflected light. Fig. 1C is a lateral view of a longitudinal section showing the location of the pulp, dentine and cementum.

Storage and Restaining

After being examined and aged, the sections were placed in labeled 5 ml vials containing 10% formalin. Individual sections were sometimes rechecked, but they had to be restained since the formalin caused a decolorizing of the first staining. Decolorizing in formalin was used when overstaining occurred. The overstained sections were placed in 10% formalin for a few days and rechecked and restained if necessary.

Serial Sections

One of the questions generated by the razor blade method of preparing tooth sections was whether these sections would distort or fail to completely show the layers of cementum present on a tooth. It was decided to compare some sets of known-age squirrel teeth, of which serial sections of one M-3 tooth were prepared using the standard histological methods, while the other M-3 tooth was sectioned by the razor blade method and stained. Four sets of teeth of different ages were chosen for this purpose: T-112 (260 days), T-242 (418 days), T-204 (821 days) and T-3 (1743 days).

Aging Guide

There were two other questions to be answered. When did deposition of summer and winter cementum begin? Could juveniles be separated into spring and summer litters on the basis of tooth development?

The teeth of 12 immature known-age squirrels were chosen for the investigation of the deposition of summer and winter cementum. Of these 12 squirrels, one was born in April and died 11 months later, 6 were born in July and died from 6 to 9 months later, 3 were born in August and died from 5 to 11 months later, and of the 2 born in September, one died 5 months later and the other specimen died 7 months later.

As a further check on the time of deposition of summer and winter cementum, and on the annuli-aging technique, an examination of razor sections of selected teeth from a sample of 100 January-collected specimens was made. The specimens examined had eye lens weights of 23, 29, 32, 32, 35, 38, 41 and 56 mg.

The objective of these investigations was to develop a chart which could be used in determining the spring and summer born individual and to age adult squirrels to year classes.

Comparison Between Aging by Eye Lens Weight and Tooth Sections

A special hunt was held in York County, Virginia, during January, 1972, to obtain 100 squirrels for research at VPI & SU. With the data normally taken, the head of each specimen was removed, tagged and stored in formalin. The eye lenses were removed, dried, weighed and aged by another investigator.

The teeth from 50 of these specimens were chosen randomly and aged by this author. An attempt was made not only to age the adults by cementum, but also to separate the spring and summer litters on the basis of tooth development.

Testing With Known-Age Teeth

Teeth from 57 known-age and 19 minimum-age squirrels were obtained from Dr. Frederick Barkalow of the Department of Zoology of North Carolina State University in Raleigh. VPI & SU supplied teeth from two known-age squirrels. The teeth were obtained in two groups: those in the first group were North Carolina museum specimens which had been stored dry, and those in the second group were from North Carolina and VPI & SU and had been stored in formalin.

At least one lower M-3 tooth, and in many cases both teeth, from each specimen was aged by the razor section method. The age determined from razor sectioning was then compared with the known age as a check on the tooth sectioning accuracy.

RESULTS

Serial Sections

Photomicrographs were taken of both the serial section and razor section of the four known-age teeth, but none of the pictures were of satisfactory quality and detail.

Aging Guide

Examination of the teeth from known-age squirrels showed that winter cementum usually started forming in December, but sometimes not until January. The summer cementum began appearing in April.

Two photomicrographs were taken of juvenile tooth sections: Fig. 2 is of a 3-1/2 month old squirrel tooth and Fig. 3 is a sketch of the same section showing the location of the pulp, dentine, crown, root tip opening and peridontal tissue; Fig. 4 is of a tooth from a squirrel approximately 6 months old (eye lens weight of 30 mg.) and Fig. 5 is a sketch of the same section showing the location of the pulp, dentine, crown, root tip opening and cementum.

From the study of all these sections, the following guide was developed for aging squirrel teeth:

1) Three months of age: pulp occupies 50-80% of the root; root tip wide

Fig. 2. Razor section of a 3-1/2 month old squirrel tooth. (40X)

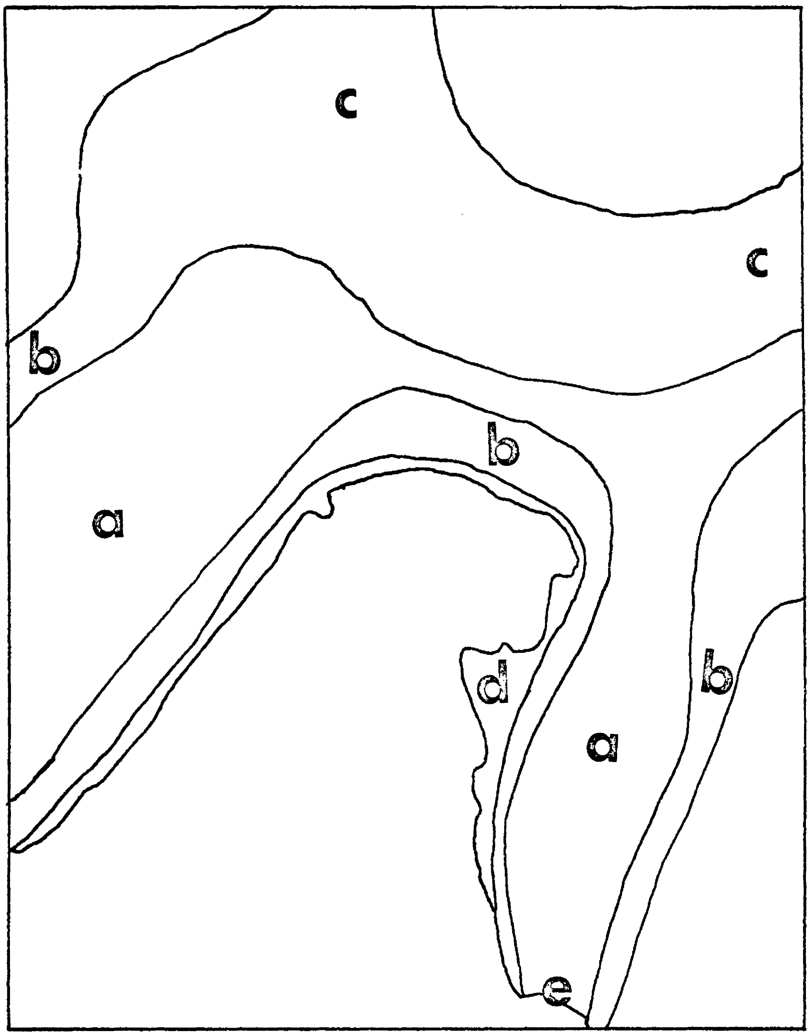

Fig. 3. Sketch of photomicrograph in Fig. 2 showing a) pulp, b) dentine, c) crown, d) peridontal tissue and e) root tip opening. No cementum is present on this 3-1/2 month old squirrel tooth.

Fig. 4. Razor section of M-3 tooth from a squirrel approximately 6 months old (eye lens weight of 30 mg.). (70X)

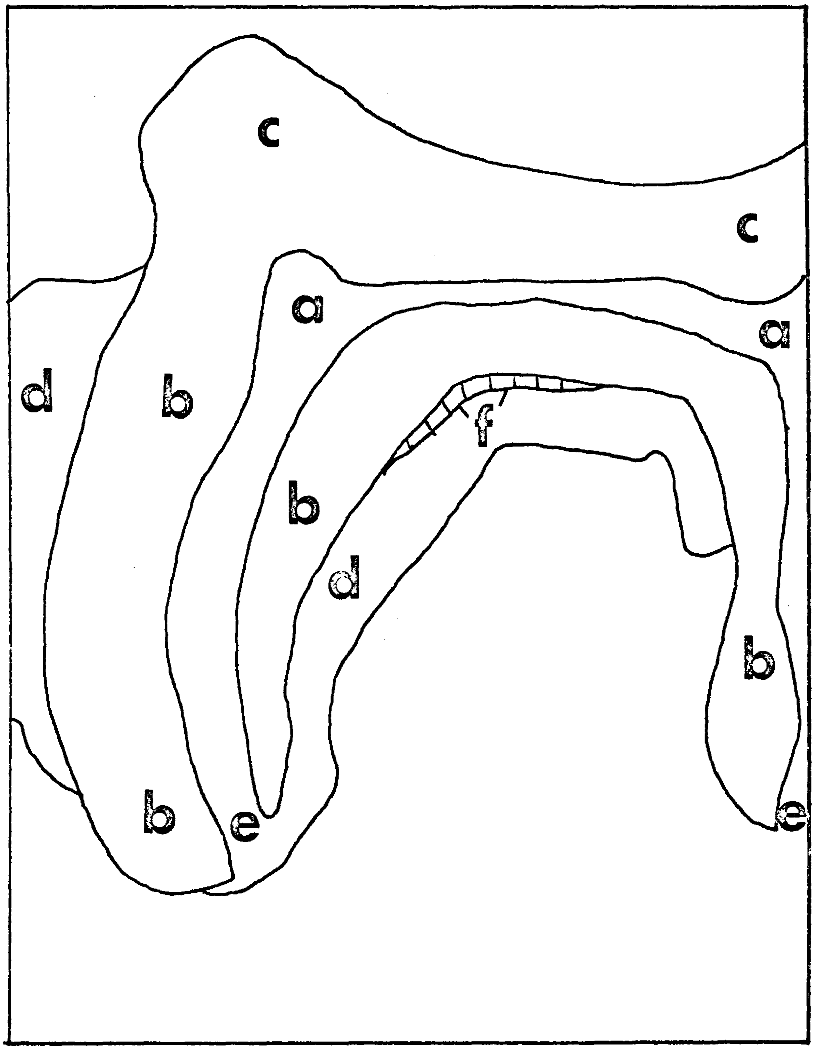

Fig. 5. Sketch of photomicrograph in Fig. 4 showing a) pulp, b) dentine, c) crown, d) peridontal tissue, e) root tip opening and f) cementum.

open; cementum not present.

2) Six months of age: pulp occupies one-third or less of the root; root tip constricting; thin cementum layer present.

3) Nine months of age: pulp occupies less than one-third of root; constriction of root tip and part of root canal obvious; thick cementum layer present.

4) Adults: each winter band counted as one full year, a summer band counted as one-half year in age.

Comparison Between Aging by Eye Lens Weight and Tooth Sections

Fig. 6 shows the distribution of eye lens weight of the 100 squirrels collected in January, 1972.

Table 1 compares the results of aging the 50 squirrels by eye lens weight and by tooth development and cementum annuli.

The results of aging each individual squirrel by eye lens weight and by tooth development are in Appendix Table I.

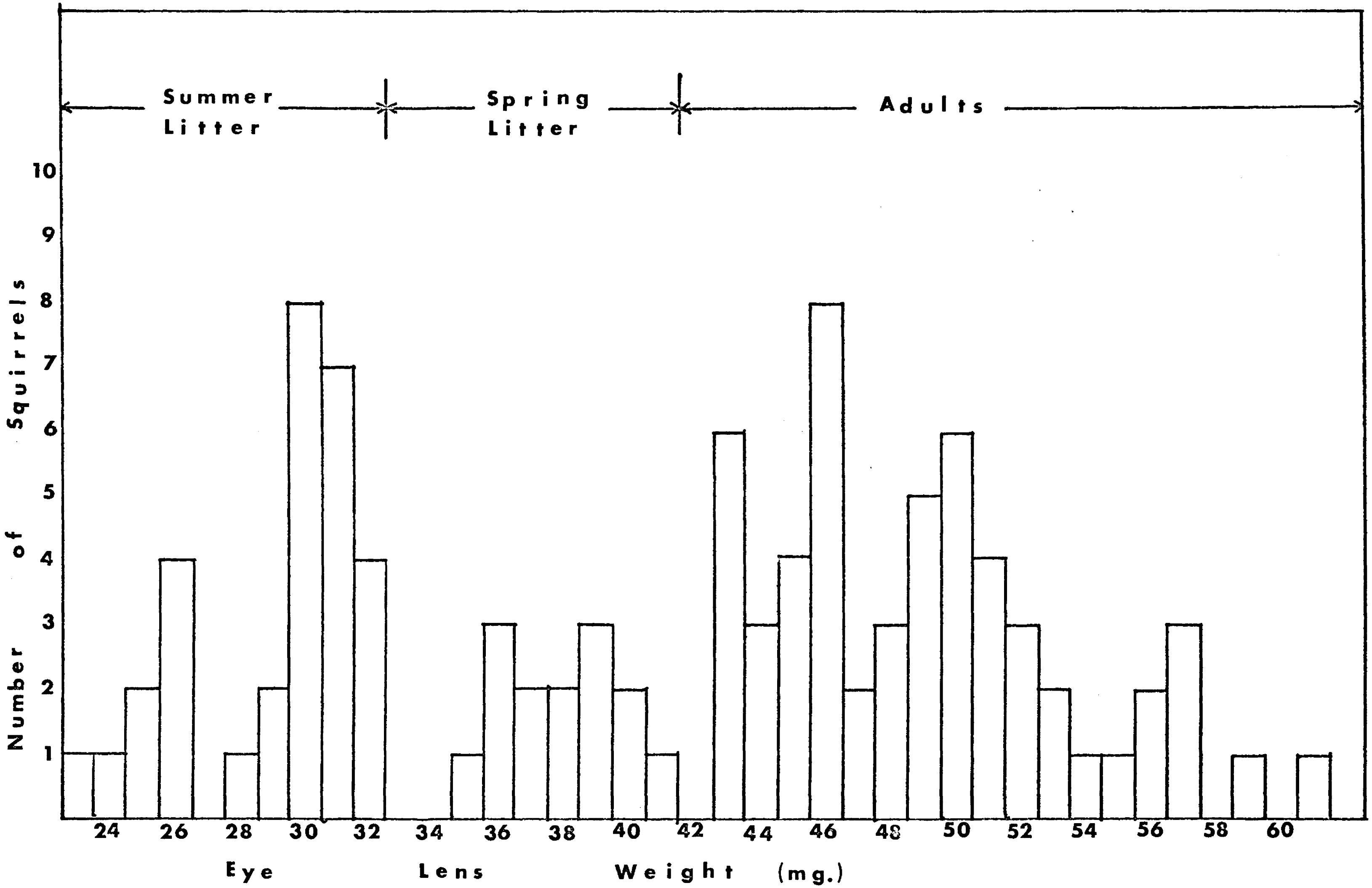

Fig . 6. Distribution of eye lens weights of 100 squirrels collected in January, 1972 and their separation into age groups based on Hefner's lens weight criteria.

Table 1. Comparison of aging by eye lens weight and by tooth development and cementum annuli of 50 squirrels collected January, 1972, in York County, Virginia

Age group	Aging Technique: Eye lens weight (Number)	Aging Technique: Tooth development and cementum annuli (Number)
Summer litter (3-6 months)	15	13
Late spring-early summer (7-8 months)	0	2
Spring litter (9-11 months)	10	8
Adults (Total)	25	27
2 yrs.	--	12
3 yrs.	--	6
4 yrs.	--	5
5 yrs.	--	3
6 yrs.	--	0
7 yrs.	--	1

Testing With Known-Age Teeth

Table 2 summarizes the results of aging the known-age teeth on the basis of tooth development and cementum annuli.

The results of aging each individual known-age squirrel are in Appendix Table II.

The results of aging the minimum-age squirrels are in Appendix Table III.

Fig. 7 shows the cementum bands of a 6-1/2 year old squirrel.

Table 2. Accuracy of age determination by razor section of teeth based on comparison with known-age specimens

Age group (Year)	Known-age (Number)	Age by cementum (Number)	Correctly aged (Number)	Correctly aged (%)
		DRY MUSEUM SPECIMENS		
< 1 yr.	6	7	6	100
1 yr.	7	8	6	86
1-1/2 yrs.	2	2	2	100
2 yrs.	4	5	3	75
3 yrs.	6	5	4	67
4 yrs.	5	6	3	60
4-1/2 yrs.	2	0	0	0
5 yrs.	2	3	0	0
5-1/2 yrs.	1	0	0	0
7 yrs.	1	0	0	0
		SPECIMENS STORED IN FORMALIN		
< 1 yr.	18	18	18	100
1-1/2 yrs.	1	1	1	100
3 yrs.	2	2	2	100
6-1/2 yrs.	1	1	1	100
8 yrs.	1	1	1	100

Fig. 7. Razor section of tooth from a minimum-age squirrel, aged as 6-1/2 years old.

DISCUSSION

Serial Sections

Tooth T-112 (260 days) was from a squirrel born Feb. 8, 1958, and died Nov. 30, 1958. Scattered amounts of thin cementum were observed through the microscope on both serial section and razor section and the size of the pulp and the micro anatomy of both sections were nearly identical.

Tooth T-242 (418 days) was from a squirrel born Feb. 2, 1963 and died Mar. 6, 1964. The cementum on the serial section was very thin, but a summer and a winter band could be discerned. The cementum on the razor section was much thicker and the summer and winter bands were easily noticed.

Tooth T-204 (821 days) was from a squirrel born Aug. 13, 1959, and died Nov. 11, 1961. Both serial and razor sections showed thick cementum with two winter bands and a third summer band. The third winter band had not begun forming.

Tooth T-3 (1743 days) was from a squirrel born Mar. 3, 1957, and died Dec. 8, 1961, and should have had cementum displaying five winter bands. The cementum on the serial section was very thin and showed only two winter bands. Cementum on the razor section was also very thin and showed two dark winter bands with a faint third winter band between. At 400X, two small areas of cementum showed what appeared to be three faint winter bands between two darker bands, but these were disregarded due to uncertainties involved when using high magnifications. The tooth, therefore, was aged as three years old. T-3 was one of the North Carolina museum specimens. These dry-stored teeth continually displayed very thin cementum or no cementum at all, leading this author to believe that the cementum either shrank from dehydration or peeled off. Fresh teeth and those stored in formalin did not exhibit this problem.

The comparison between serial sections and razor sections showed that the technique of razor sectioning did not lose or distort any of the detail of the cementum annuli.

Aging Guide

Figs. 1 and 2 are of a razor section of a 3-1/2 month old squirrel tooth which just recently erupted. The root is composed mostly of pulp and the extremely large opening of the root tip is obvious. No cementum is visible yet.

Figs. 3 and 4 are of a razor section of a tooth from a squirrel approximately 6 months old, based on an eye lens weight of 30 mg. The size of the root pulp cavity has noticably decreased to the point where it occupies only about one-third of the root. The root tip has narrowed and some deposits of cementum are present but not apparent in the picture.

The tooth development of a 9 month old squirrel is like that of an adult, the pulp occupying less than one-third of the root, the root tip constricted and a thick cementum layer present. Spring litter squirrels (born Feb.-April) will reach 9 months of age before winter cementum begins appearing in January and will, therefore, have a thick layer of summer cementum. Squirrels born from May-Sept. will possibly begin producing winter cementum before reaching the age of 9 months. Since the appearance of the dark winter cementum band in the major criterion for assigning an individual to the one-year-old class, some individuals 8-10 months of age, which are collected during the winter months, will be included in this class.

For adult squirrels, each winter band is counted as one full year and an additional summer band as one-half year in age.

Comparison Between Aging by Eye Lens Weight and Tooth Section

The majority of squirrels in the spring litter are born from Jan.-April and would be 9-12 months old when collected in January. Squirrels in the summer litter are born from June-Sept. and would be 5-7 months old when collected in January. According to Hefner (1972), the heaviest eye lens weight within the 95% confidence interval for seven month old squirrels would be 31.5 mg, and the heaviest weight for twelve month old squirrels would be 40.5 mg. Therefore, the 100 squirrels collected for this study were divided by eye lens weight into summer litter, spring litter and adults, as shown in Fig. 6.

Fifty of these 100 squirrels were aged by tooth development and cementum and 15 were placed in the summer litter by eye lens weight; 13 of these 15 were also placed there on the basis of tooth development. However, squirrel number 46 (30 mg.) was placed in the late spring-early summer group because it had cementum thicker than that found in a summer litter tooth, but not as thick as in a spring litter tooth. It also had a larger pulp but less root tip constriction than in a spring litter tooth. Number 92 (31 mg.) was aged as a spring litter squirrel because the pulp occupied less than one-third of the root and the cementum showed a thick summer band and the beginning of a winter band.

Of the 10 squirrels placed in the spring litter by eye lens weight, 7 were also placed there on the basis of tooth development. Number 54 (39 mg.) was classified as late spring-early summer, and two individuals [number 17

(37 mg.) and number 29 (40 mg.)] were found to be two-year-old adults.

All 25 adults by eye lens weight were also found to be adults by tooth cementum and were placed in their appropriate year classes. The results of each individual aging will be found in Appendix Table I.

This writer believes that the results show that tooth development and cementum annuli can be used as accurately as eye lens weight for separating spring and summer litters. Aging by tooth sections has the added advantage of being able to separate the adults into year classes.

Testing With Known-Age Teeth

Table 2 shows that all 23 teeth which had been stored in formalin were correctly aged, including an 8 year-old individual. Twelve of the 36 dry museum specimens were incorrectly aged, 9 of the 12 having very thin cementum. Nearly all the museum specimens had thinner cementum than those which were freshly extracted or stored in formalin.

The one-year-old individual which was incorrectly aged was T2-315 (217 days). It was born July 26, 1967, and died Feb. 28, 1968, and should have had both a summer and winter band. However, no cementum was visible and it was aged as 3-6 months old.

The incorrectly aged two-year-old individual was T-2-259 (665 days), born Feb. 10, 1966, and died Dec. 4, 1967. The second winter band should have started forming when it died, but the section showed only one thin dark band and the tooth was, therefore, aged as one year old.

Two three-year-olds were incorrectly aged. T2-257 (994 days) was born July 21, 1964, and died April 11, 1967, and should have had three complete winter bands. The tooth section had one thick layer of cementum with no bands and was aged as one year old. T-182 (112 days) was born Feb. 28, 1959, and died Mar. 28, 1962, and also should have had three complete winter bands. The tooth section was aged as two years old when only two winter bands were seen in the very thin cementum.

Two of the four-year-olds were incorrectly aged. T-16 (1352 days) was born July 20, 1960, and died April 2, 1964, and should have had four complete winter bands. Only two winter bands were visible in the very thin cementum, so the tooth was aged as two years old. T2-191 (1426 days) was born Feb. 28, 1959, and died Jan. 24, 1963, and should have been producing its winter band. The tooth section was aged as five years old, most likely because a deep staining dentino-cementum interface was mistaken as a fifth winter band.

Both four-and-a-half-year-olds were incorrectly aged. E-8 (1571 days) was born July 30, 1957, and died

Nov. 17, 1961. There should have been four winter bands and a fifth summer band. It is unlikely a fifth winter band would be forming in mid-November. The tooth section was, however, aged as five years old. I think it is more likely that what was taken as the fifth winter band was really the deep purple stained nuclei of a dense layer of gum tissue next to the summer band. T-3 (1743 days) was aged as a three-year-old and has been discussed in the serial section part of this study.

Both five-year-olds (T2-82♂ and T2-49) were incorrectly aged as four-year-olds for probably the same reason - one of the winter bands was missed in the thin cementum.

T2-189 (2105 days) was born Feb. 28, 1959, and died Dec. 3, 1964. While it was put in the five-and-a-half-year-old group, the sixth winter band could possibly have begun forming when it died, thus putting it in the six-year-old group. This could explain why the tooth section was aged as five years old: again, one winter band was missed in the thin cementum.

Seven-year-old T-140 (2413 days) was aged as four years old, also because of thin cementum.

Appendix Table III shows the results of aging the minimum-age squirrel teeth. These squirrels were captured as adults, tagged and later recovered dead. Since the date of birth of an individual squirrel was not known, only its minimum age could be estimated. For testing with minimum-age teeth, an age by tooth section less than the minimum-age was considered incorrect and an age greater than the minimum-age was considered correct. Of the 19 minimum-age teeth aged by tooth section, 7 were incorrect and 12 were considered correct.

The minimum-age teeth were stored as dry museum specimens and most had thin cementum. One notable exception was a 3 year 4-2/3+ month minimum-age squirrel (E2-157) which was aged as 6-1/2 years (Fig. 7). Magnifications of 100X to 200X were normally required for distinguishing winter bands, but the cementum on this individual was thick enough to age at only 70X.

While the aging results of the dry museum specimens are not ideal, the results of aging the specimens stored in formalin show that the razor section method is accurate for aging squirrels. The results also show that the teeth should be stored in formalin and aged as soon after extraction as possible.

SUMMARY AND CONCLUSIONS

The purpose of this study was to develop a fast, easy method for preparing and aging squirrel teeth which would enable a large number of squirrels to be handled in a short time and with minimum equipment involved.

A comparison of serial sections and razor sections of sets of teeth from four selected known-age squirrels showed that the razor sectioning technique did not lose or distort any of the detail of the cementum annuli.

An examination of the teeth from known-age squirrels showed that winter cementum usually started forming in December, but sometimes not until January, and summer cementum usually began appearing in April.

Based on these facts and on the examination of many juvenile and adult tooth sections, the following aging guide was developed for aging squirrel teeth:

1) Three months of age: pulp occupies 50-80% of the root; root tip wide open; cementum not present.
2) Six months of age: pulp occupies one-third or less of the root; root tip constricting; thin cementum layer present.
3) Nine months of age: pulp occupies less than one-third of root;

constriction of root tip and part of root canal obvious; thick cementum layer present.

4) Adults: each winter band counted as one full year, a summer band counted as one-half year in age.

A comparison between aging squirrels by eye lens weight and by tooth development and cementum annuli showed that tooth sections can be used as accurately as eye lens weight for separating spring and summer litters and also had the added advantage of being able to separate the adults into year classes.

Testing with known-age teeth showed that the razor section method is accurate for aging adult squirrels. It also showed that the teeth should be stored in formalin rather than being stored as dry museum specimens.

LITERATURE CITED

Adams, L., and S. G. Watkins. 1967. Annuli in tooth cementum indicate age in California ground squirrels. J. Wildl. Mgt. 31(4): 836-839.

Craighead, J. J., F. C. Craighead, and H. E. McCutchen. 1970. Age determination of grizzly bears from fourth premolar tooth sections. J. Wildl. Mgt. 34(2): 353-363.

Erickson, J. A., and W. G. Seliger. 1969. Efficient sectioning of incisors for estimating ages of mule deer. J. Wildl. Mgt. 33(2): 384-388.

Gilbert, F. F. 1966. Aging white-tailed deer by annuli in the cementum of the first incisor. J. Wildl. Mgt. 30(1): 200-202.

Hefner, J. M. 1971. Age determination of the gray squirrel. M. S. Thesis, The Ohio State University, Columbus, Ohio 51 pp.

Laws, R. M. 1952. A new method for age determination for mammals. Nature 169(4310): 972-973.

Linhart, S. B., and F. F. Knowlton. 1967. Determining age of coyotes by tooth cementum layers. J. Wildl. Mgt. 31(2): 362-365.

Low, W. A., and I. M. Cowan. 1963. Age determination of deer by annular structure of dental cementum. J. Wildl. Mgt. 27(3): 466-471.

Mundy, K. R. D., and W. A. Fuller. 1964. Age determination in the grizzly bear. J. Wildl. Mgt. 28(4): 863-866.

Ransom, A. B. 1966. Determining age of white-tailed deer from layers in cementum of molars. J. Wildl. Mgt. 30(1): 197-199.

Scheffer, V. B. 1950. Growth layers on the teeth of Pinnipedia as an indication of age. Science 112(2907): 309-311.

Sergeant, D. E., and D. H. Pimlott. 1959. Age determination in moose from sectioned incisored teeth. J. Wildl. Mgt. 23(3): 315-321.

Stoneberg, R. P., and C. J. Jonkel. 1966. Age determination of black bears by cementum layers. J. Wildl. Mgt. 30(2): 411-414.

Taber, R. D. 1963. Criteria of sex and age. pp. 119-189. In H. S. Mosby (Ed.), Wildlife Investigational Techniques. The Wildlife Society, Washington, D. C., 419 pp.

van Nostrand, F. C., and A. B. Stephenson. 1964. Age determination for beavers by tooth development. J. Wildl. Mgt. 28(3): 430-434.

Wolfe, M. L. 1969. Age determination in moose from cemental layers of molar teeth. J. Wildl. Mgt. 33(2): 428-431.

Appendix Table I. Comparison between aging by eye lens weight and by tooth development and cementum annuli of 50 squirrels collected January, 1972, in York County, Virginia

I.D. Number	Eye lens weight(mg)	Age by eye lens weight	Age by tooth section
64	23	Summer litter	Summer litter
28	28	Summer litter	Summer litter
74	29	Summer litter	Summer litter
24	30	Summer litter	Summer litter
46	30	Summer litter	Late spring-early summer
59	30	Summer litter	Summer litter
67	30	Summer litter	Summer litter
93	30	Summer litter	Summer litter
14	31	Summer litter	Summer litter
47	31	Summer litter	Summer litter
71	31	Summer litter	Summer litter
92	31	Summer litter	Spring litter
12	32	Summer litter	Summer litter
80	32	Summer litter	Summer litter
84	32	Summer litter	Summer litter
5	35	Spring litter	Spring litter
85	36	Spring litter	Spring litter
17	37	Spring litter	Adult - 2 yrs.
100	37	Spring litter	Spring litter

Appendix Table I. (cont.)

I.D. Number	Eye lens weight(mg)	Age by eye lens weight	Age by tooth section
11	39	Spring litter	Spring litter
38	39	Spring litter	Spring litter
54	39	Spring litter	Late spring-early summer
29	40	Spring litter	Adult - 2 yrs.
66	40	Spring litter	Spring litter
98	41	Spring litter	Spring litter
8	43	Adult	Adult - 2 yrs.
15	43	Adult	Adult - 2 yrs.
72	43	Adult	Adult - 2 yrs.
20	44	Adult	Adult - 2 yrs.
91	44	Adult	Adult - 2 yrs.
6	45	Adult	Adult - 2 yrs.
53	45	Adult	Adult - 2 yrs.
87	45	Adult	Adult - 3 yrs.
56	46	Adult	Adult - 3 yrs.
65	46	Adult	Adult - 5 yrs.
78	46	Adult	Adult - 2 yrs.
10	47	Adult	Adult - 4 yrs.
58	48	Adult	Adult - 3 yrs.
83	48	Adult	Adult - 2 yrs.
60	49	Adult	Adult - 4 yrs.

Appendix Table I. (cont.)

I.D. Number	Eye lens weight(mg)	Age by eye lens weight	Age by tooth section
61	50	Adult	Adult - 3 yrs.
69	50	Adult	Adult - 4 yrs.
75	50	Adult	Adult - 5 yrs.
33	51	Adult	Adult - 2 yrs.
41	51	Adult	Adult - 2 yrs.
97	52	Adult	Adult - 4 yrs.
43	53	Adult	Adult - 3 yrs.
62	57	Adult	Adult - 5 yrs.
95	57	Adult	Adult - 3 yrs.
13	61	Adult	Adult - 7 yrs.

Appendix Table II. Results of aging known-age teeth by razor section

I.D. Number	Known age	Expected banding and age	Age by razor section	Comments
**2011FSB	48 days	No cementum (3 mo.)	3 mo.	Tooth almost all pulp
**2005FSB	63 days	No cementum (3 mo.)	3 mo.	
**3533	69 days	No cementum (3 mo.)	3 mo.	
**2007FSB	72 days	No cementum (3 mo.)	3 mo.	
**4077-4078	83 days	No cementum (3 mo.)	3 mo.	
**3819-4076	84 days	No cementum (3 mo.)	3 mo.	
**4081	93 days	No cementum (3 mo.)	3 mo.	
**3820-3821	96 days	No cementum (3-6 mo.)	3-6 mo.	Layer of new dentine visible
T-80	96 days	No cementum (3-6 mo.)	3-6 mo.	
**B3C1D4	100 days	No cementum (3-6 mo.)	3-6 mo.	
**2006FSB	108 days	No cementum (3-6 mo.)	3-6 mo.	
E-270	3-1/2 mo.	No cementum (3-6 mo.)	3-6 mo.	
**4082	115 days	No cementum (3-6 mo.)	3-6 mo.	
**2003FSB	144 days	No cementum (3-6 mo.)	3-6 mo.	

Appendix Table II. (cont.)

I.D. Number	Known age	Expected banding and age	Age by razor section	Comments
**B3C2D1	148 days	No cementum (3-6 mo.)	3-6 mo.	
**B3C5D5	165 days	[SB]* (6-9 mo.)	6-9 mo.	Very thin cementum visible
**B1C1D5	191 Days	SB (6-9 mo.)	6-9 mo.	Very thin cementum visible
**3786	228 days	SB (6-9 mo.)	6-9 mo.	Very thin cementum visible
**4080	244 days	SB (6-9 mo.)	6-9 mo.	Very thin cementum visible
T-112	260 days	SB (6-9 mo.)	6-9 mo.	Very thin cementum visible
**2001FSB	--	--	6-9 mo.	SB thin - pulp 1/3-1/2
T-220	139 days	SB[WB] (6-9 mo.)	6-9 mo.	SB very thin-no [WB]
T2-117	163 days	SB[WB] (6-9 mo.)	6-9 mo.	SB - no [WB]
T2-89	173 days	SB[WB] (6-9 mo.)	6-9 mo.	SB - no [WB]
T2-82♀	143 days	SB-WB (1 yr.)	1 yr.	SB-WB - cementum very thin
T2-315	217 days	SB-WB (1 yr.)	3-6 mo.	No cementum visible
T-218	245 days	SB-WB (1 yr.)	1 yr.	Thin SB - thick WB
E2-259	257 days	SB-WB (1 yr.)	1 yr.	SB-WB cementum thin

Appendix Table II. (cont.)

I.D. Number	Known age	Expected banding and age	Age by razor section	Comments
T2-141	272 days	SB-WB (1 yr.)	1 yr.	SB-WB - very thin
T2-41	388 days	SB-WB (1 yr.)	1 yr.	SB-WB
T-242	418 days	SB-WB-[SB] (1-1-1/2 yrs.)	1 yr.	SB-WB - no [SB]
T2-318	244 days	SB-WB-SB (1-1-1/2 yrs.)	1-1/2 yrs.	SB-WB-SB (thin)
T2-275	297 days	SB-WB-SB (1-1/2 yrs.)	1-1/2 yrs.	SB-WB-SB (thin)
**VPI 2087-88	18 mo.	SB-WB-SB (1-1/2 yrs.)	1-1/2 yrs.	SB-WB-SB
T2-218	495 days	SB-WB-SB-[WB] (1-1/2-2 yrs.)	2 yrs.	Second WB present but thin
T2-259	665 days	SB-WB-SB-[WB] (1-1/2-2 yrs.)	1 yr.	Cementum very thin
T2-214	601 days	2 WB (2 yrs.)	2 yrs.	Cementum very thin
T-241	779 days	2WB-[SB] (2-2-1/2 yrs.)	2 yrs.	Cementum very thin
T-204	821 days	2WB-SB-[WB] (2-1/2-3 yrs.)	3 yrs.	Third WB just beginning to form
T2-37	938 days	3 WB (3 yrs.)	3 yrs.	Cementum very thin
T2-243	948 days	3 WB (3 yrs.)	3 yrs.	Cementum very thin

Appendix Table II. (cont.)

I.D. Number	Known age	Expected banding and age	Age by razor section	Comments
T2-225	963 days	3 WB (3 yrs.)	3 yrs.	Cementum very thin
T-182	1124 days	3 WB (3 yrs.)	2 yrs.	Cementum very thin
**3870-71	3 yrs. 80 days	3 WB (3 yrs.)	3 yrs.	Thick cementum third WB just beginning to form
**A0B1C4D4	3 yrs.	3 WB (3 yrs.)	3 yrs.	Thick cementum
T2-257	994 days	3 WB-[SB] (3-3-1/2 yrs.)	1 yr.	One thick layer of cementum-no bands
T2-140	1240 days	3 WB - SB-[WB] (3-1/2-4 yrs.)	4 yrs.	4 WB
T-171	1395 days	3 WB-SB-[WB] (3-1/2-4 yrs.)	4 yrs.	Fourth WB forming
T2-191	1426 days	4 WB (4 yrs.)	5 yrs.	5 WB
T-16	1352 days	4 WB-[SB] (4-4-1/2 yrs.)	2 yrs.	Cementum very thin
T2-177	1483 days	4 WB-[SB]	4 yrs.	[SB] not forming
E-8	1571 days	4 WB-SB (4-1/2 yrs.)	5 yrs.	
T-3	1743 days	4 WB-SB-[WB] (4-1/2-5 yrs.)	3 yrs.	One faint WB between two thick WB's

Appendix Table II. (cont.)

I.D. Number	Known age	Expected banding and age	Age by razor section	Comments
T2-82 ♂	1664 days	5 WB (5 yrs.)	4 yrs.	Fourth WB forming
T2-49	1697 days	5 WB (5 yrs.)	4 yrs.	4 WB
T2-189	2105 days	5 WB-SB-[WB] (5-1/2-6 yrs.)	5 yrs.	5 WB
**VPI 592-2255	6-1/2 yrs.	6 WB-SB (6-1/2 yrs.)	6-1/2 yrs.	Crown worn to pulp 6 WB's 7th SB obvious next to gum tissue
T-140	2413 days	7 WB (7 yrs.)	4 yrs.	Fourth WB just forming
**3805-3806	8 yrs. 132 days	8 WB-[SB]	7-8 yrs.	Too many bands to be sure if 7 or 8

*SB - Summer Band

**Specimens Preserved in Formalin

WB - Winter Band

[] - Band Possibly Present

Appendix Table III. Results of aging minimum-age squirrel teeth (all dry museum specimens)

I.D. Number	Minimum age (Years:Months)	Age by tooth section	Comments
E2-180	2:4-1/4	6-9 months	Root canal 33-50% Cementum very thin
E2-110	2:6	4 yrs.	Fourth WB* beginning
E2-194	2:6+	3-1/2 yrs.	3 WB; SB** beginning
E-98	3:0 1/3	3-1/2-4 yrs	Not certain if fourth WB forming
E2-96	3:0 1/3+	5 yrs.	5 WB present
E2-108	3:1 1/5+	2 yrs.	Cementum very thin Second WB forming
E-158	3:3 3/4+	3 yrs.	Third WB forming
E2-157	3:4 2/3+	6-1/2 yrs.	Crown worn to pulp 6 WB present - also 7th SB
E2-141	3:8-1/2+	4 yrs.	4 WB present
E-159	3:11	3 yrs.	3 WB present
E2-33	3:11-1/2	5 yrs.	5 WB present
E-76	4:0 1/2+	3 yrs.	Third WB forming
E-119	4:5 1/3+	5-1/2 yrs.	Sixth SB forming
E2-78	4:11+	5-1/2 yrs.	Sixth SB forming
E-95	5:2	5 yrs.	Cementum thin 5 WB present
E2-79	5:8 1/2	4 yrs.	Cementum thin 4 WB present

Appendix Table III. (cont.)

I.D. Number	Minimum age (Years:Months)	Age by tooth section	Comments
E2-89	5:8-1/2+	6-1/2- 7-1/2 yrs.	Thick cementum 6-7 WB SB
E-90	6:1 1/4+	4 yrs.	4 WB apparent Crown worn to pulp (must be older)
E2-228	Old Adult	3-1/2 yrs.	3 WB SB apparent Crown worn (older)

*WB - Winter Band

**SB - Summer Band

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A NEW METHOD OF AGING GRAY SQUIRRELS (*Sciurus carolinensis*) BY USE OF CEMENTUM ANNULI

by

John George Fogl

(ABSTRACT)

Squirrel teeth were decalcified in a modified formic acid-sodium citrate solution and sectioned longitudinally with a razor blade. The sections were stained with Harris' hematoxylin and eosin and viewed under a microscope using reflected light. Tooth development was used for separating spring and summer litters, while cementum annuli were used for placing the individuals into year classes. Magnifications of 100X - 200X were best for aging by cementum annuli.

It was found that spring and summer litters could be separated as accurately with tooth development and cementum annuli as with eye lens weights. Tooth sectioning had the added advantage of being able to separate the adults into year classes.

Testing with formalin preserved known-age teeth showed that the razor section method is accurate for aging adult squirrels. Dry stored teeth are more difficult to age with accuracy, apparently due to the shrinkage of the cementum.