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Estimating Ages of Cottontails by Periosteal Zonations

Abstract

Mandibles from 78 eastern cottontails (*Sylvilagus floridanus*) and 58 Nuttall's cottontails (*S. nuttallii*) were decalcified, cross-sectioned, stained, and examined under the microscope. Densely stained lines in the periosteal zone were absent in 64 (98.5%) of 65 mandibles from cottontails whose lens-weights, unossified epiphyseal cartilages, or known ages indicated that they were young-of-the-year. One or more densely stained lines were present in mandibles from 68 (95.8%) of 71 cottontails for which there was corroborating evidence that they were 1 year old or older. The periosteal zonation technique appears to be useful for separation of age classes among the adult cohort of cottontail populations, but provides no particular advantage over the lens-weight technique for separating adults from young-of-the-year. Use of the two techniques in combination provides a means by which age structures of cottontail populations can be established more precisely than heretofore possible.

Introduction

This is a report on the determination of ages of eastern and Nuttall's cottontails (*Sylvilagus floridanus* and *S. nuttallii*) from densely stained lines ("adhesion lines") in the mandibular periosteum.

Ages of cottontails are commonly estimated by the degree of ossification between the epiphysis and diaphysis of long bones (Thomsen and Mortensen, 1946; Hale, 1949; Petrides, 1951) and from the weight of the dried lens of the eye (Lord, 1959). Lens-weight reportedly is more reliable than ossification of the epiphyseal cartilage for separating adults from young-of-the-year (Wight and Conaway, 1962; Edwards, 1962; Rongstad, 1966). However, Edwards (1962), Rongstad (1966), and Friend (1968) considered the lens technique to be unreliable for separating year classes of cottontails older than 1 year. Klevezal' and Kleinenberg (1967) reported the occurrence of adhesion lines between annual layers of bone deposited in the periosteal zone of mandibles of Asiatic pikas (*Ochotonidae*) and other mammals. Millar and Zwickel (1972) used these adhesion lines to estimate ages of North American pikas (*Ochotona princeps*). We wished to determine if the technique were applicable to other lagomorphs.

Methods and Materials

Seventy-eight eastern cottontails were collected on the E. E. Wilson Game Management Area about 11.5 km north of Corvallis, Benton County, Oregon, between 23 August and 30 September 1973. These dates were chosen because the oldest eastern cotton-

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tail born in 1973 was expected to be less than 7 months old at this time (Trethewey and Verts, 1971); consequently, this group could be distinguished from adults on the basis of ossification of the epiphyseal cartilages of their radii and ulnae (Hale, 1949). Twenty-five Nuttall's cottontails were collected between 11 September and 11 October 1973, about 4.9 km west of Terrebonne, Deschutes County, Oregon; the oldest individual born in 1973 in this collection was expected to be less than 6 months old (Powers and Verts, 1971). Presumably, these juveniles also could be distinguished from adults by unossified epiphyseal cartilages in the bones of their forelegs. An additional 15 Nuttall's cottontails were collected in the same area between 1 April and 14 June 1972 (a period during which adults were distinguished from juveniles by size and reproductive status), and 18 more were collected at irregular times during 1972 and 1973. Of the 58 Nuttall's cottontails, 27 were recoveries of individuals marked as juveniles and 5 marked as adults, providing a sample for which a maximum or minimum age was known.

Cottontails were returned to the laboratory where both eyes, one radius and ulna, one humerus, and the mandibles were collected from each individual. Eyes were preserved in 10 percent Formalin for at least 30 days (Friend, 1968). Lenses were removed, dried for 7 days at 80° C, and weighed to the nearest 1.0 mg. Radii, ulnae, and humeri were cleaned in a 25 percent solution of laundry bleach (Clorox), and were examined to determine the degree of ossification of the epiphyseal cartilages. Mandibles were decalcified in a 5 percent solution of 67 percent nitric acid for about 12 hours (until bone and teeth were flexible), and rinsed in running water for 12 hours. The diastema portion was removed, dehydrated in a graded series of alcohol solutions, infiltrated with paraffin, and embedded in paraffin (Humason, 1967). Embedded tissues were cross-sectioned at 11 μ , mounted on slides with Mayer's albumen, and stained with Papanicolaou (Harris) hematoxylin. Slides were examined under the microscope for the presence or absence of dark stained lines in the periosteum (Fig. 1).

Counting the densely stained lines and assigning individuals to age classes was usually straightforward. However, branching of adhesion lines in mandibles of some individuals required that several sections be examined and that each line be traced to insure that it did not terminate as a side branch rather than continue the full length of the section. We commonly found adhesion lines to be more distinct and more easily counted on the labial and ventral sides of the mandibles. "Lines of resorption" between the periosteum and mesosteum (Klevezal' and Kleinenberg, 1967) were visible in some sections, but usually could be separated easily from adhesion lines because they were relatively deeper in the bone, and because they tended to be irregular rather than parallel to the periphery of the mandible (Fig. 1).

We attempted to expedite the process by sectioning the decalcified mandibles with a freezing microtome, but cottontail mandibles were so cancellous that sections fragmented badly. Also, we sectioned portions of the mandible beneath the row of cheek teeth and from the angle of the jaw of some individuals in an attempt to locate areas in which branching of adhesion lines was somewhat less than in the diastema. No particular advantage in using these portions was noted, and the diastema was easier to embed and section. Adhesion lines were visible in cross sections of humeri, but were not separated sufficiently to permit counting them easily.

Results and Discussion

One or more adhesion lines were present in mandibles from 68 (95.8%) of 71 cottontails for which there was corroborating evidence that they were 1 year old or older. Among Nuttall's cottontails for which approximate ages were known and in the sample of eastern cottontails there was complete agreement between ages determined by lens-

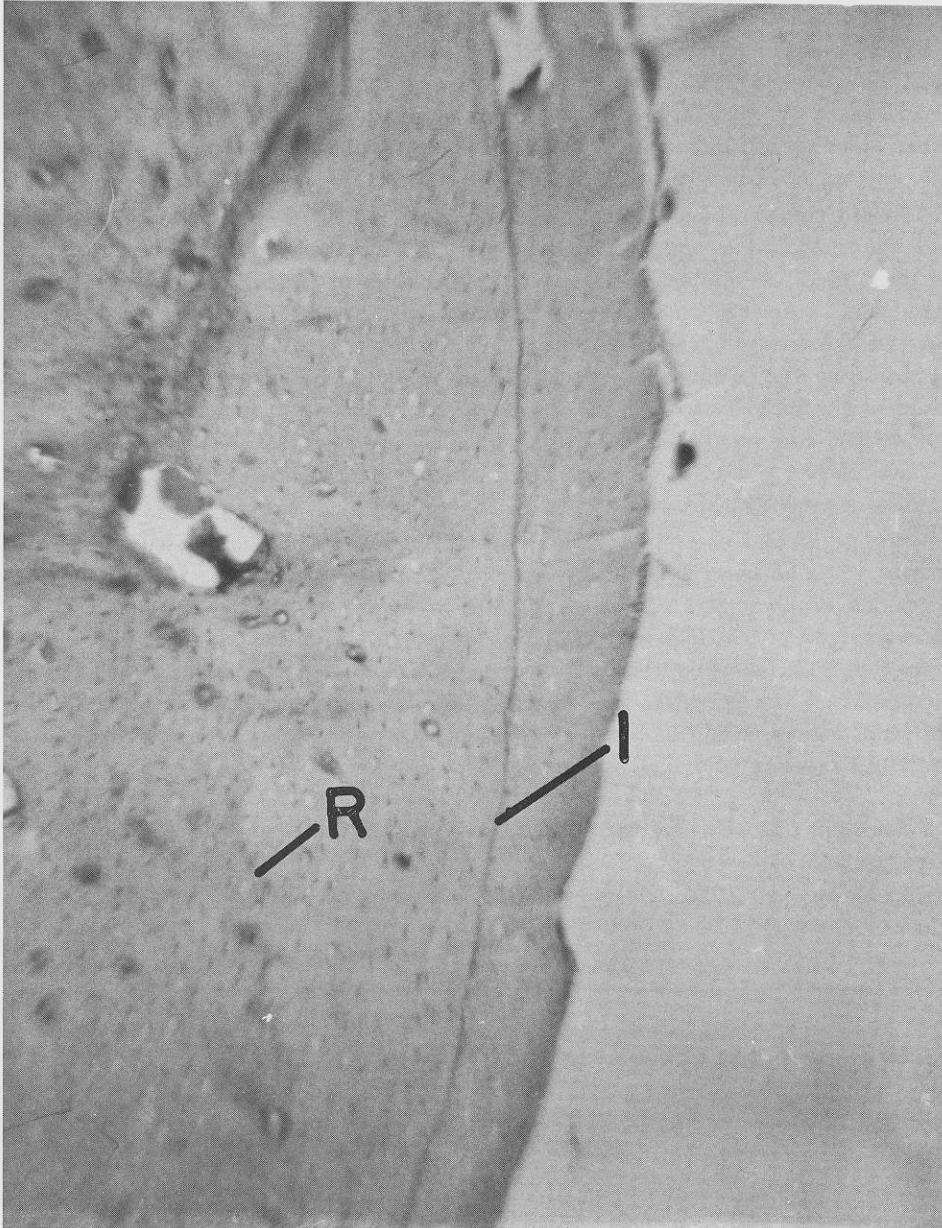


Figure 1. Photomicrograph of a cross section through the diastema of the mandible of an eastern cottontail showing one adhesion line in the periosteal zone (1). Note difference between adhesion line and resorption line (R).

weight, ossification of epiphyseal cartilages, and periosteal zonation methods (Tables 1 and 2). In these samples, no adhesion lines were visible in sections of mandibles of individuals in which ossification of epiphyseal cartilages was complete, which had lens-weights indicating that they were young-of-the-year, or which a minimum known age indicated that they were young-of-the-year. Conversely, one or more adhesion lines was present in sections of mandibles of all individuals in these samples for which there was corroborating evidence that they were 1 year old or older. One female marked as an adult on 13 July 1972, and killed on 19 September 1973 (thus, at least 780 days old), had three adhesion lines indicating that it was born in 1970, and was 2 years old when marked. Four other adults known to be at least 407 days old when killed all had more than one adhesion line: two had one line, one had two lines, and one had three lines (Table 1).

Among 26 unmarked Nuttall's cottontails for which no maximum or minimum age was known, three adults collected in April had no visible adhesion lines in sections of their mandibles (Table 2). Because the first litter of Nuttall's cottontails is usually born about 1 April in central Oregon (Powers and Verts, 1971), there is no possibility that these individuals were misclassified. Formation of densely staining lines may be occurring at this season among young-of-the-previous-year, perhaps in response to the onset of the reproductive season. A female collected on 12 September 1973, whose lens-weight and unossified epiphyseal cartilages indicated that it was born in 1973 (Table 2) had a distinct adhesion line in sections of its mandible. We are not able to offer a plausible explanation for this exception.

Because we did not collect cottontails between mid-October and April, we are not certain when adhesion lines were formed in cottontail mandibles. It appeared that formation of lines occurred sufficiently late to permit use of the technique at least through mid-October, but whether the technique could be used to determine ages of cottontails collected during hunting seasons that extend through December and beyond is unknown. We speculate that formation of the lines occurs at different times in different regions, requiring that the time of formation be established locally if the periosteal zonation technique is to be used with specimens collected in late autumn and winter.

The high rate of agreement between the different methods of estimating age, the

TABLE 1. Numbers of marked Nuttall's cottontails with ossified and unossified epiphyseal cartilages and numbers with 0, 1, 2, and 3 adhesion lines in sections of their mandibles, grouped by maximum and/or minimum lens-weight and age. Specimens were collected in 1972-73 in Deschutes County, Oregon.

Age when marked ¹	Year marked	Weight of lens (mg)	Age when killed (days)	Epiphyseal cartilage				Number of adhesion lines			
				Radius & ulna		Humerus		0	1	2	3
				Open	Closed	Open	Closed				
Juvenile	1972-73	<150	<177	21	3	24	0	24	0	0	0
Juvenile	1972	201-225 ²	383-537	0	3	0	2 ³	0	3	0	0
Adult	1973	220-250	>407	0	4	0	4	0	2	1	1
Adult	1972	219	>780	0	1	0	1	0	0	0	1

¹ Based on size and reproductive status.

² No lens available for one specimen.

³ One sample not suitable for determination.

TABLE 2. Numbers of each of two species of cottontails with ossified and unossified epiphyseal cartilages, and numbers with 0, 1, 2, and 3 adhesion lines grouped by age based on the dry weight of the lens.

Species	Weight of lens (mg)	Age (days) ¹	Epiphyseal cartilage				Number of adhesion lines			
			Radius & ulna		Humerus		0	1	2	3
			Open	Closed	Open	Closed				
<i>Sylvilagus nuttallii</i>	<150	<177	4 ²	0	5	0	4	1	0	0
<i>Sylvilagus nuttallii</i>	>177 ³	>291	0	20 ²	0	19 ⁴	3	13	4	1
<i>Sylvilagus floridanus</i>	<168	<220	36	0	36	0	36	0	0	0
<i>Sylvilagus floridanus</i>	>185	>269	0	42	0	42	0	23	16	3

¹ Estimated on the basis of lens-weight.

² One sample not suitable for determination.

³ No lenses available for two specimens.

⁴ Two samples not suitable for determination.

gradual decline in numbers of individuals in older age classes, and the absence of cottontail rabbits with more than three adhesion lines in the periosteum tends to indicate that the periosteal zonation technique will be useful for separating year classes among cottontail populations. Because the lens-weight technique appears to be reliable, and considerably more expeditious to use, we do not advocate use of the periosteal zonation technique for separating adult cottontails from young-of-the-year. However, use of the two techniques in combination appears to provide a means by which age structures of cottontail populations can be established more precisely than heretofore possible.

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