


## 33

## AGE DETERMINATION



**B**eing able to ascertain or estimate age is important in studies of mammals. Knowledge of age structures of populations is necessary for their management (Morris 1972; Taber 1969) and for understanding the life history strategies of mammals (Caughley 1966; Cole 1954; Deevey 1947; Wilson 1975). Methods of determining the ages of individuals in a population are reviewed by Gandal (1954), Morris (1972), Spinage (1973), Pucek and Lowe (1975), and Harris (1978); also see the bibliography by Madson (1967) and articles in journals such as *Acta Theriologica*, the *Journal of Mammalogy*, and the *Journal of Wildlife Management*.

## TYPES OF AGING CRITERIA

When samples of mammals are taken from a population, it is usually impossible to assign a **known age** to any specimen unless birth was observed and the individual was uniquely marked for later identification. An **absolute age** can often be determined by counting incremental growth lines in various structures of wild-caught individuals. More commonly, a **relative age** may be assigned to an individual based on comparison with other individuals in the sample. The procedures for establishing absolute and relative ages should be standardized by study of individuals of known age (Morris 1972). However, if the individuals of known age were captive animals, the morphological changes that occurred during development may have been different than the changes experienced by individuals living in the wild, and thus the results obtained from captives may not be accurate for purposes of aging.

A rough estimate of relative age is used by many taxonomists to segregate adult from younger individuals of a sample. In mammalian population biology, the category **adult** generally refers to the larger and potentially reproductive members of a population. A **subadult** individual is generally a young of the year that may or may not be in reproductive condition. This individual is typically smaller than an adult but is otherwise similar. A **juvenile** individual is smaller than a subadult and often (but not always) has a pelage coloration that is different from that of subadults and adults. A **nestling** is a recently born individual that is still confined to a nest. In precocial species (e.g., artiodactyls, hares), the nestling stage is virtually nonexistent, and only a juvenile stage is present.

- 33-A Assemble a sample of skulls of one species from a single locality and, preferably, representing a small interval of dates of collection. Arrange the skulls in the order of their presumed age by examining tooth wear, tooth eruption, degree of ossification of bones, etc. (see Chapter 2). Can you separate these skulls into adult, subadult, and juvenile categories?
- 33-B Examine skins from the same sample of specimens assembled in Exercise 33-A. Does information from pelage morphology and coloration aid in assigning these specimens to age groups? Do these procedures tell you the absolute ages of these specimens?

## USE OF STATISTICS AND KNOWN-AGE SAMPLES

It is important to have a reference sample of known-age individuals so that the efficacy of an aging procedure can be evaluated. Some of the aging techniques discussed will be more accurate than others, although they may involve more time and care in processing the samples. The nature of the investigation will determine what level of accuracy you should strive for and whether you can accept a relatively larger error rate to save time for other procedures.

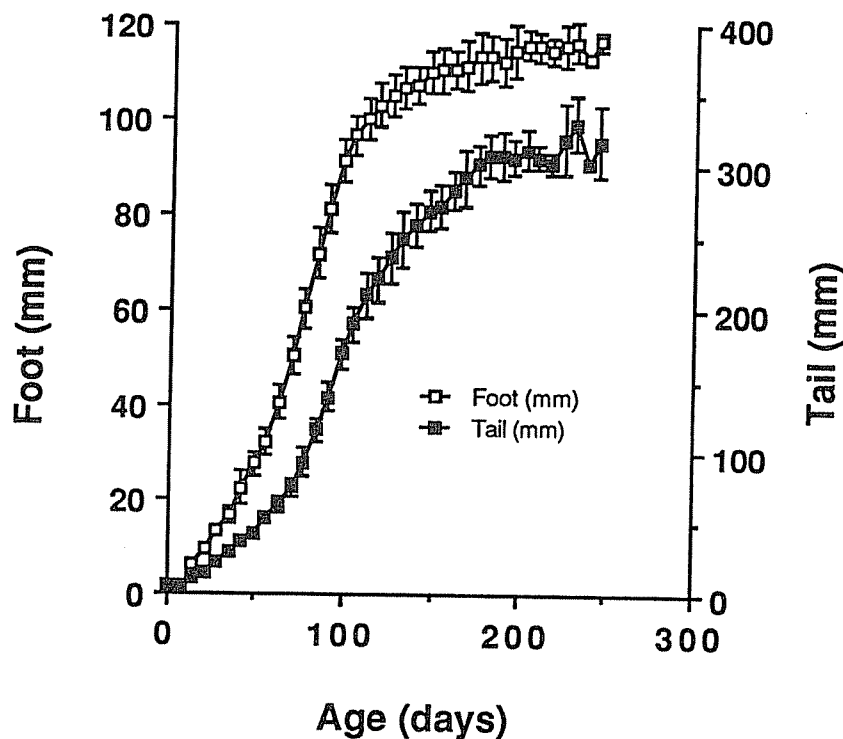
In studies of relative age, the technique of regression analysis is often used (Sokal and Rohlf 1969). With known-age individuals, age is a nonrandom variable (i.e., it is measured without error) and thus the quantity measured ( $Y$ , dependent variable) must be regressed on age ( $X$ , the independent variable). With samples of unknown age, age can be estimated by the method of inverse prediction (Dapson 1973; Dapson and Irland 1972; Sokal and Rohlf 1969:446–448) using values obtained from the measured variable (e.g., weight). In such cases, the computation of confidence limits for these regression lines is different from that of a Model I regression (Sokal and Rohlf 1969).

A representative sample of relative and absolute age-determination methods will be described below under the type of structure involved.

## GROWTH OF SKULL, SKELETON, AND BODY

### Dimensions and Weight

In early life, growth in most kinds of mammals is continuous and thus provides a means for separating the youngest individuals from adult members of a population. Some structures cease growth sooner than others. Not all morphological features are useful for aging individuals in a population. Increases in linear dimensions and weight may be useful indicators of age during the earliest portions of a mammal's life (Fig. 33.1) but rapidly lose their usefulness once adult dimensions are reached (Hoffmeister and Zimmerman 1967; Kirtpatrick and Hoffman 1960). These measurements are frequently utilized in live-trapping studies because of their simplicity and because of the frequent lack of other suitable criteria obtainable from live mammals.



**Figure 33.1** Increase in mean foot and tail lengths ( $\pm$  SD) from birth to maturity in a marsupial, the Tasmanian bettong, *Bettongia gaimardi*, Diprotodontia, Potoroidae. (Rose 1989: 255)

### Degree of Fusion of Epiphyseal Cartilage

The degree of fusion (determined by X-ray analysis or analysis of skeletons) of the distal epiphyseal cartilages of the radius and humerus has been used in many management studies to age specimens of bats, rabbits, and foxes (Sullivan and Haugen 1956). The technique is less accurate than most other methods (Wight and Conaway 1962).

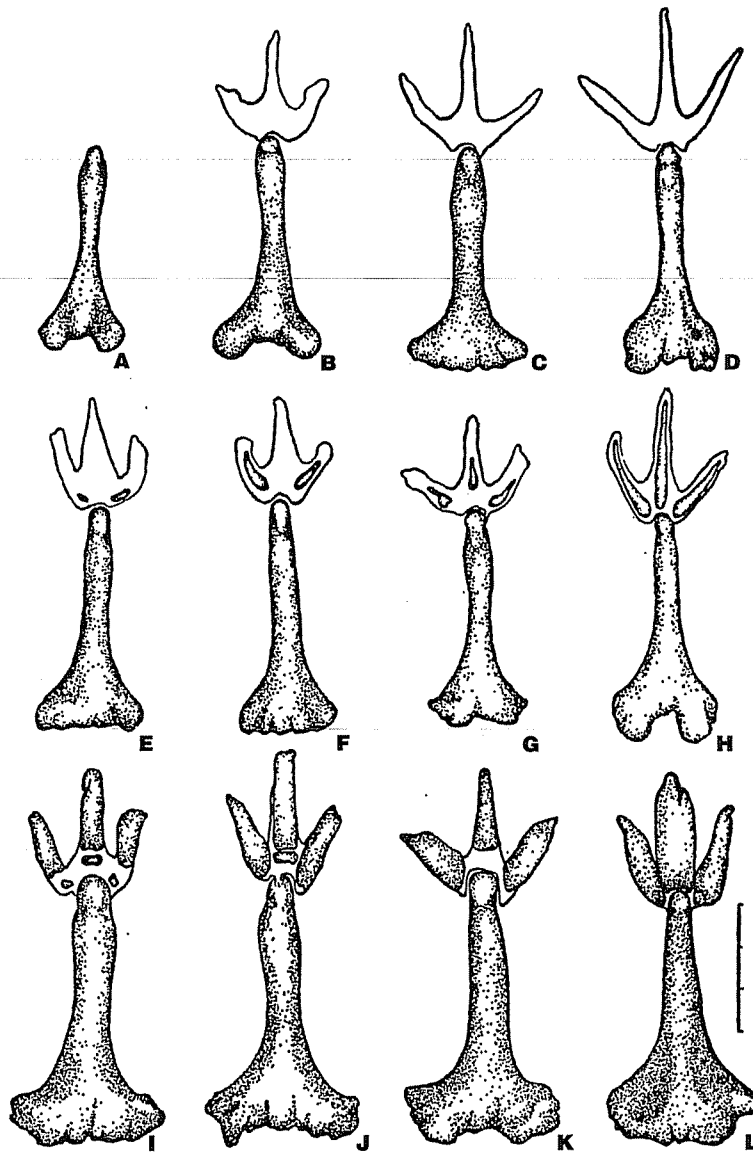
33-C Assemble a sample of known-age skulls (perhaps obtainable from laboratory colonies, a mink ranch, or a research project). Select several cranial measurements (see Chapter 2) and measure the skulls. Using age as the independent variable ( $X$ ) and a measurement variable as the dependent variable

( $Y$ ), perform a regression analysis on these data (see Sokal and Rohlf 1969 or similar reference).

33-D Examine skeletal elements (pelves, distal elements of limbs) of a sample of known-age material (see Exercise 33-C). Is the degree of fusion of the epiphyses a useful indicator of age? At what age (in the species studied) do the epiphyses completely fuse with the diaphysis (i.e., shaft of longbone)?

### Baculum

For male mammals, the weight, length, and volume of the baculum often provide a means for separating a juvenile from an adult (Fig. 33.2) individual (Elder 1951; Friley



**Figure 33.2** Age changes in bacula of muskrats, *Ondatra zibethicus*, Rodentia, Muridae, Arvicolinae. The top (A–D) and middle (E–H) rows are from juvenile individuals five to eight months of age. The bottom row (I–L) shows patterns in adults 15 months or more of age. The scale is in millimeters.

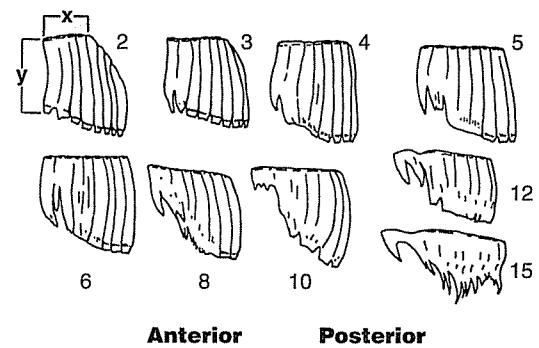
(Elder and Shanks 1962:146)

1949). More precise age determinations are generally not possible using this bone (Harris 1978; Morris 1972).

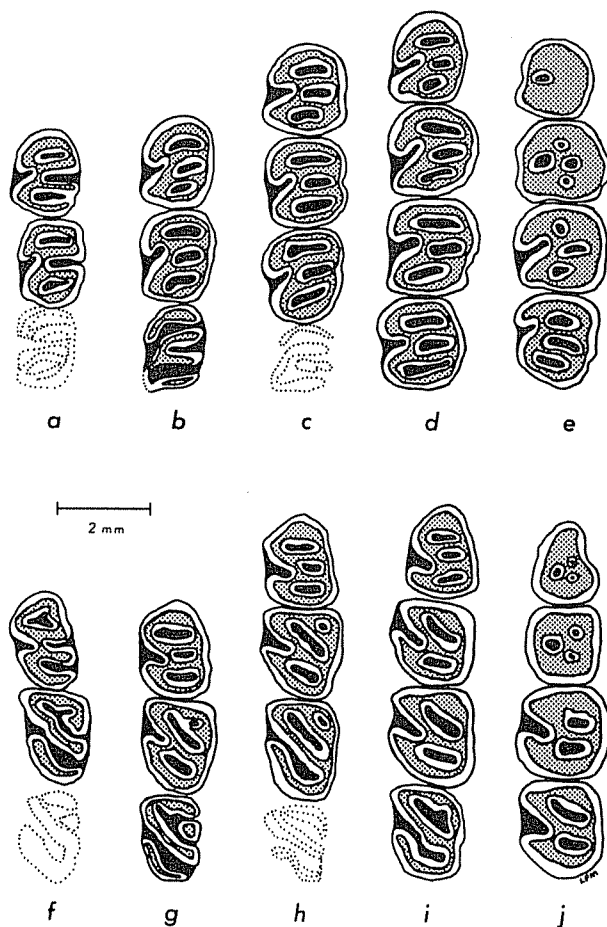
## RELATIVE GROWTH AND MORPHOLOGY OF TEETH

### Tooth Wear

Relative wear on teeth has been widely utilized to separate mammals into age groups for taxonomic studies (Fig. 33.3) and for population and management studies (Figs. 33.4 and 33.5). Spinage (1973) noted that the pattern of wear on teeth generally follows a negative exponential curve. Thus, younger animals tend to be classified as older than their true age. Harris (1978) found that 65.5% of the red foxes (*Vulpes vulpes*) he examined could be aged correctly with an error of 1 year or less. Aging was accurate in 93.3% of the cases with foxes up to 4 years of age. The accuracy of this technique also

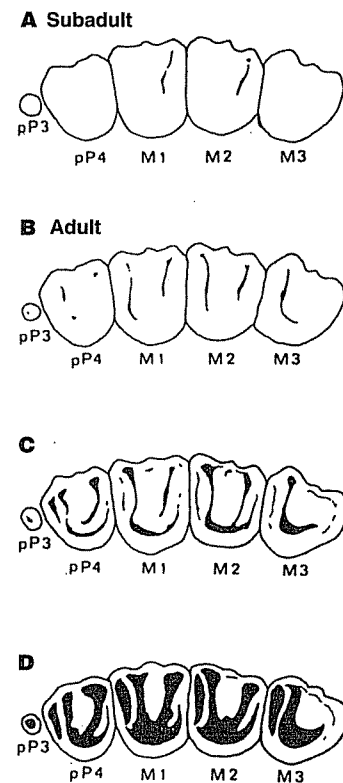


**Figure 33.4** Lateral views of upper molars ( $M^3$ ) in warthogs *Phacochoerus* sp., Artiodactyla, Suidae, of 2 to 15 years of estimated age. The X (occlusal surface) and Y refer to measurements used to derive ratios (X/Y) useful for aging this species. (Spinage, C.A., and G.M. Jolly. 1974. Age estimation of warthog. *J. Wildlife Manag.* 38(2):229-233, fig. 1. Copyright 1974 The Wildlife Society. Reprinted with permission.)



**Figure 33.3** Relative age classes based on tooth wear in museum specimens of spiny rats, *Proechimys* sp., Rodentia, Echimyidae. Tooth rows (a-e, left upper; f-j, left lower) arranged from left to right in order of increasing tooth wear and presumed age. Dotted lines indicate teeth that are not at occlusal level. True ages unknown.

(Martin 1970:4)



**Figure 33.5** Occlusal views of the left upper molariform teeth of four known-age gray squirrels, *Sciurus carolinensis*, Rodentia, Sciuridae, from Wake County, North Carolina, to show the sequende of dental wear through two age classes. (A) Subadult, 338 days old; (B) adult, 494 days; (C) adult, 1,122 days; (D) adult, 2,413 days. Dark areas represent exposed dentine on the occlusal surface. Dental notation: pP3 and pP4 = permanent third and fourth premolars; M1, M2, and M3 = molars.

(Hench et al. 1984. Age classification for the gray squirrel based on eruption, replacement, and wear on molariform teeth. *J. Wildlife Manag.* 48(4):1409-1414. Copyright 1984 The Wildlife Society. Reprinted with permission.)

depends on the type of tooth examined. The incisors are useful for aging many species of canids (Harris 1978), whereas molars are useful for aging cervids (Taber 1969). Generally, this technique is less accurate for aging than methods based on discrete data (e.g., tooth eruption, annulations in tooth or cementum; Morris 1972).

### Tooth Eruption

In many rodents, the pattern of tooth eruption is a useful indicator of age during the first few months following birth. In artiodactyls, relative degrees of tooth eruption may be used to group individuals into year or seasonal classes during the first 2 to 3 years of life (Dimmick and Pelton 1996; Larson and Taber 1980; Taber 1969).

### Pulp Cavities

Radiographs are used to measure the extent of pulp cavities in selected teeth in order to group animals into age categories. Tumilson and McDaniel (1984) found that the pulp cavities of juvenile gray foxes, *Urocyon cinereoargenteus*, were significantly more open than those in adult foxes.

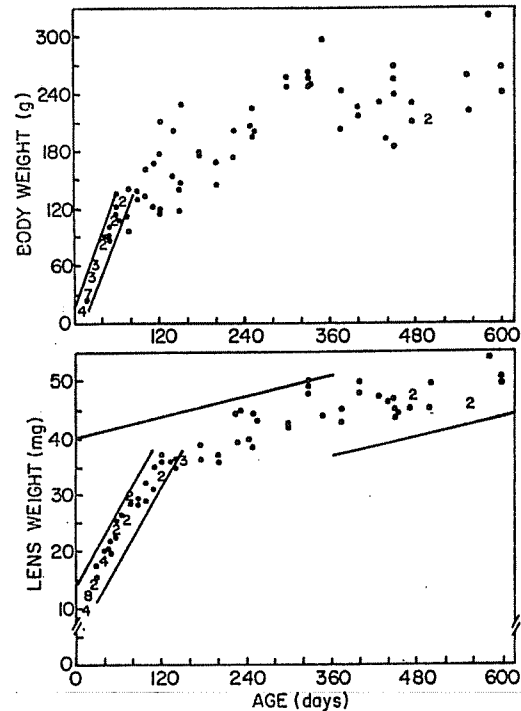
- 33-E Examine teeth in the sample of skulls assembled for Exercise 33-C. Can you group these specimens into age categories (or an age sequence) based upon tooth eruption and tooth wear without relying upon the known ages? How do your tooth wear age categories compare with the true ages of the specimens?

## GROWTH OF EYE LENSES

### Weight of Eye Lens

The weight of the eye lens increases with age (Fig. 33.6) and thus many studies of age structures of populations have relied on weighing the lenses removed from mammal specimens (see Lord 1959 and review in Friend 1968; Morris 1972). Several investigators (Adamczewska-Andrzejewska 1973; Myers et al. 1977) found eye lens weight to be accurate for estimating ages of several species of myomorph rodents. Morris (1972) reported that the technique has been most successfully applied with animals of medium size (e.g., rabbits, hares) during the period of rapid growth prior to the attainment of adult size. Harris (1978), studying red foxes (*Vulpes vulpes*), did not find lens weights to be useful for separating year classes of animals that had attained adult size.

Refer to Friend (1968), Morris (1972), and Harris (1978) for precautions that must be followed to insure accuracy and precision in the measurement of lens weights.



**Figure 33.6** Changes with age in body weight and eye lens weight in cotton rats, *Sigmodon hispidus*, Rodentia, Muridae, Sigmodontinae. Heavy lines indicate 95% confidence intervals.

(Birney, E.C., R. Jenness, and D.D. Baird. 1975. Eye lens proteins as criteria of age in cotton rats. *J. Wildlife Manag.* 39(4):718-728, fig. 1. Copyright 1975 The Wildlife Society. Reprinted with permission.)

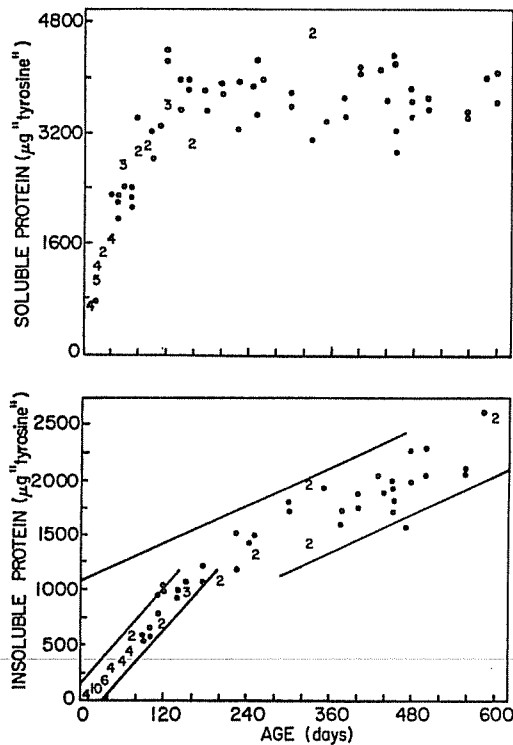
### Measurements of Lens Protein

Dapson and Irland's (1972) technique found that the amount of the soluble tyrosine (lens protein) increased linearly during approximately the first year of life in old-field mice (*Peromyscus polionotus*). They also found that the increase in the insoluble tyrosine fraction was a curvilinear function of age during the first 750 days of life in these rodents. Birney et al. (1975) demonstrated the accuracy of this technique (Fig. 33.7) for aging cotton rats (*Sigmodon hispidus*) but cautioned that the most accurate technique is not necessarily the one that should be used if time is limited or if the needed specialized equipment is lacking.

- 33-F Compare the changes in body weight and lens weight in Figure 33.6 with the changes in soluble and insoluble lens protein in Figure 33.7. How do the results differ? At what ages do all of the techniques produce the best results?

## GROWTH LINES

The growth of teeth and bones in mammals is not uniform throughout the year. Thus, narrow and wide layers



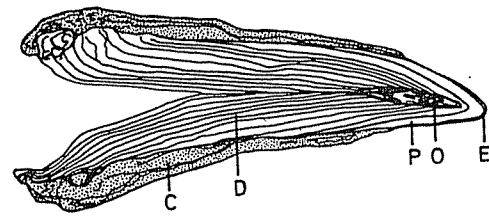
**Figure 33.7** Changes with age in soluble and insoluble lens protein in cotton rats, *Sigmodon hispidus*, Rodentia, Muridae, Sigmodontinae. Heavy lines indicate 95% confidence intervals.

(Birney, E.C., R. Jenness, and D.D. Baird. 1975. Eye lens proteins as criteria of age in cotton rats. *J. Wildlife Manag.* 39(4):718-728, fig. 2. Copyright 1975 The Wildlife Society. Reprinted with permission.)

of dentin, cementum, and bone may be laid down in different seasons of the year or in annual increments (Klevezal and Kleinenberg 1967; Morris 1972). Generally, these **growth lines** or **incremental lines** are the only useful criteria for distinguishing year classes of adult individuals in or from a population, other than marking groups of the same age (i.e., cohorts) when they are young or recently born. These growth lines also provide an absolute age for an individual because the units are discrete and not subject to the continuous variation inherent in the criteria for relative growth described previously (but see cautions in Harris 1978). Phillips et al. (1982) stated that considerable confusion exists in the literature in the use of these terms, along with confusion about the types of dentin layers added to a tooth. They defined *primary dentin* as that formed in the initial phase of tooth development and *secondary dentin* as that formed after the tooth erupts. They considered incremental lines to be those appearing in the secondary dentin.

### Teeth: Dentin and Cementum

Growth lines in teeth (Fig. 33.8) have been utilized widely for the aging teeth of marine mammals since publication of the paper by Laws (1952). Subsequently, the



**Figure 33.8** Outline drawing of tooth section from a bottlenose whale, *Hyperoodon ampullatus*, Cetacea, Ziphiidae showing growth lines in the dentin. C, cementum; D, dentin; E, enamel; O, osteodentin; P, prenatal dentin.

(Christensen 1973:333)

technique has been applied successfully to other long-lived species such as moose, bears (Stoneberg and Jonkel 1966), caribou (Miller 1974), and others. Harris (1978), in studies of red foxes, found that sections of only the tooth cementum gave good resolution of growth lines that could be demonstrated consistently. Growth lines of dentin were less reliable for aging, and he also cautioned that the determined age should be based on sections of at least two teeth from the same individual.

Erickson and Seliger (1969) and Morris (1972) summarized the various methods used to prepare sections of teeth for study. For larger teeth (whales, pinnipeds, artiodactyls), a section is made of a tooth and it is then ground down to a thin layer using carborundum powder or a grindstone. Smaller teeth can be treated similarly if the teeth are mounted on a cork block (or slide) for easier handling. A second method for making sections involves decalcification and then sectioning of the teeth using a microtome. Formic acid is the most commonly used decalcifying agent although, with caution, a dilute solution of nitric acid is also permissible (Morris 1972). Tumilson and McDaniel (1983) used a decalcifying solution prepared as follows:

0.7 g. ethylenediaminetetraacetic acid (EDTA)

0.2 sodium potassium tartrate

1.0 liter 10% HCl,

which decalcified canines of bobcats (*Lynx rufus*) and river otters (*Lontra canadensis*) within two to three days. Hematoxylin stain generally gives good resolution of the growth lines (Harris 1978), but Gridley's silver impregnation, Masson's trichrome, or the periodic acid-Schiff reaction stains have also been used (Phillips et al. 1982).

33-G Select a carnivore tooth or artiodactyl tooth (incisors are frequently utilized because the permanent set of these teeth appears early in development) with which you can practice the preparation of thin sections. Cut thin slices of the tooth and mount in resin on a slide. Grind the tooth to a thin section using various abrasive carborundum powders. Examine the section microscopically under

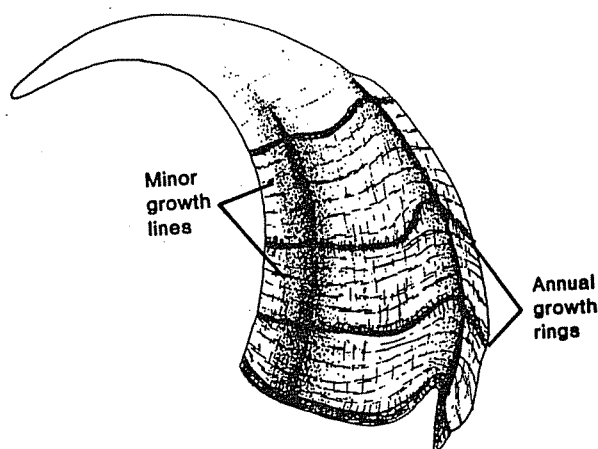
transmitted light (students should select teeth of different ages for later comparison). Can growth lines be detected in the sections? (It may be necessary to stain the teeth lightly with hematoxylin; Morris 1972.) If known age material is available, how well do the numbers of growth lines correspond with the true ages? Is there always only one growth line per year?

### Periosteal Lines in Bone

Millar and Zwickel (1972) and Franson et al. (1975) successfully utilized sections of mandibles to age, respectively, pikas (*Ochotona princeps*) and mink (*Mustela vison*). Usually, sections of mandibles are prepared by decalcification and are sectioned using histological procedures. Morris (1972) recommended that formalin-fixed material be utilized because the process of cleaning the bones may result in the loss of some structural detail. Minor accessory growth lines may be seen among the true annual growth lines in bones (Morris 1972). Periosteal growth lines are generally thicker than growth lines in cementum and are thought by some workers to be easier to interpret for this reason.

### Growth Lines in Horns

Seasonal changes in forage quality cause the deposition of the keratinized epithelial layers in horns to be unequal (Taber 1969). Thus, these growth lines (Fig. 33.9), with adjustments, can be used to estimate year classes of members of the family Bovidae (Artiodactyla) that possess horns (Morris 1972). Murie (1944) successfully utilized this technique in a classic study of Dall sheep (*Ovis dalli*) mortality. Caughley (1965, 1966) summarized the use of this technique in the preparation of a life table for the Himalayan tahr (*Hemitragus jemlahicus*) introduced



**Figure 33.9** Horn of Himalayan tahr (*Hemitragus jemlahicus*, Artiodactyla, Bovidae) showing minor growth lines and true annual growth lines. (After Caughley 1965)

into New Zealand in 1904. Geist (1966), working with known-age bighorn sheep (*Ovis canadensis*), found that counts of growth lines were satisfactory for aging males but gave erratic results with females.

33-H Examine a number of bovid horns (preferably from animals of known age) of a species. Can growth lines be detected on the horns? Can you differentiate between annual and minor growth lines? If possible, compare horns of species living in temperate climates with those of species living in tropical climates. What differences, if any, are apparent in the growth lines?

### Growth Ridges in Baleen

Growth ridges are formed in the baleen plates of mysticete whales as new material is added at the base of these keratin structures. Seasonal variation in growth rate results in ridges that can be related to annual increments of age, although Morris (1972) stated that it is difficult to obtain precise results. Further details and a review of the literature can be found in Jonsgård (1969).

### Epithelial Earplugs of Whales

Each auditory meatus or canal of a mysticete whale is closed. Consequently, the epithelial lining that sloughs off the walls of this canal cannot exit from the body of the animal and instead forms a layered earplug (Morris 1972). Alternate light (higher fat content) and dark layers apparently represent seasonal feeding patterns. Roe (1967) provided a review of the literature in this field, along with data concerning the fin whale (*Balaenoptera physalus*).

### Counts of Antler Tines Inaccurate for Aging Individuals

There is a common misconception among hunters and laymen in general that the number of tines or "points" possessed by a deer is an accurate index of the age of the animal. Cahalane (1932) reviewed the evidence for this claim and found no convincing evidence for it.

## AGE DETERMINATION IN LIVE MAMMALS

Certain population and behavior studies and those involving critically endangered species require that animals not be killed when one is attempting to get age estimates under field conditions. Several methods have been developed to place animals into age classes.

### External and Pelage Characteristics

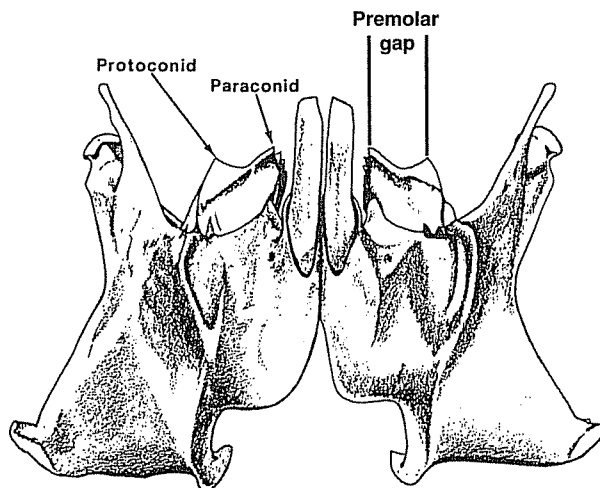
Most mammals exhibit very rapid growth following birth, after which body size (e.g., mass and dimensions)

is not a good indicator of age (see Fig. 33.1). Thus, for species maturing within the first year of life, adult body size may be reached in a few months after birth. This pattern is especially pronounced in rodents in which estimation of age by body mass becomes problematic in as little as three to 12 months after they are born.

For similar reasons, characteristics of the pelage may be sufficient to separate juveniles from subadults or adults. In murid rodents, individuals of the juvenile age class often have a distinctly gray color in contrast to the the nongray pelages of subadults and adults. In gray squirrels, *Sciurus carolinensis*, characteristics of the ventral surface of the tail can be used to separate individuals into juvenile and subadult categories (Sharp 1958). Methods for determining age in many game and furbearing species can be found in Taber (1969), Larson and Taber (1980), and Dimmick and Pelton (1996).

### Molar Wear (Attrition)

Wear on molariform teeth has often been used to age dead mammals but only rarely to age live mammals. Hoogland and Hutter (1987) were able to measure molar wear on live, restrained black-tailed prairie dogs, *Cynomys ludovicianus*. From the degree of wear, they were able to establish four age categories corresponding to 0.5, 1.5, 2.5, and  $\geq 3.5$  years. Cox and Franklin (1990) found that premoar gap width (Fig. 33.10), the distance between the protoconid and paraconid, increased with age in black-tailed prairie dogs, enabling them to establish five age categories (Table 33.1) verified by known-age animals.



**Figure 33.10** Anterior view of mandible of black-tailed prairie dog, *Cynomys ludovicianus*, Rodentia, Sciuridae, showing location of premoar gap. The width of this gap (see Table 33.1) was used to estimate age in live, restrained individuals.

(Cox, M.K., and W.L. Franklin. 1990. Premolar gap technique for aging live black-tailed prairie dogs. *J. Wildlife Manag.* 54(1):143-146, fig. 1. Copyright 1990 The Wildlife Society. Reprinted with permission.)

**TABLE 33.1** Ages of black-tailed prairie dogs, *Cynomys ludovicianus*, Rodentia, Sciuridae, estimated by the premoar gap technique on live, restrained individuals<sup>1</sup>

Age Class (year)	Age (weeks)	Premoar Gap Mean (mm)
0 <sup>2</sup>	20	1.80
1	72	2.47
2	124	3.14
3	176	3.82
4	228	4.49

Table adapted from Cox and Franklin 1990:145.

<sup>1</sup>Refer to Cox and Franklin (1990) for details on the procedure and methods for calculating confidence limits for the age estimates.

<sup>2</sup>Age class 0 = juvenile

### Strength of Collagen

The strength of collagen fibers in the tails of Belding's ground squirrels, *Spermophilus beldingi*, was used by Sherman et al. (1985) to estimate the ages of individuals in a wild population. The collagen fiber was extracted from the dorsal side of the tail after a small area was shaved and a transverse cut was made through the skin to expose the fibers. The incision was treated with an anti-septic, and the animals did not experience any long-term adverse effects from the procedure. The equation describing the strength of the collagen in relation to age was the following (Sherman et al. 1985:876):

$$\log_{10}(\text{collagen breaking time}) = 0.42 \ln(\text{age in years}) + 5.20$$

$$(r^2 = 0.79, N = 143)$$

### Size of Footprints and Shoulder Height

Because of the size and logistical difficulties associated with capture of very large mammals, some investigators have developed ways to estimate ages of individuals indirectly. An interesting technique was developed by Western et al. (1983) to estimate the ages of African elephants, *Loxodonta africana*, Proboscidea, by measuring the lengths of footprints. With this technique, 12 age categories could be recognized, corresponding to chronological ages 0-1 years up to > 15.1 years. The footprint length method was correlated strongly ( $r^2 = 0.983$ ,  $N = 13$ ,  $P < 0.001$ ) with shoulder-height data that had been used previously (Laws 1966) to estimate the ages of elephants.

## LIMITATIONS OF AGE-DETERMINATION METHODS

Few studies of age determination of mammals produce essentially equal age estimates when different methods

are compared (Morris 1972). Body weights, body dimensions, degrees of epiphyseal fusion, and tooth-wear indices generally produce very different results. For aging mammals prior to adulthood, the weight of the eye lens and the lens protein content generally produce the most accurate results (Dapson and Irland 1972). For animals that live in strongly seasonal environments and for more than 1 year, the development of annuli in teeth (dentin or cementum) or in bones has proved to be a very successful and accurate method for aging mammals (Harris 1978; Morris 1972). Phillips et al. (1982) found

that the use of incremental lines in secondary dentin and cementum, to ascertain age in bats, was of questionable accuracy. Additional comparative studies of age determination can be found in the *Journal of Mammalogy* and the *Journal of Wildlife Management*, among others (see, e.g., Fiero and Verts 1986; Root and Payne 1984).

For any species studied, tests should be made with known-age animals, using a variety of methods, so as to evaluate the accuracy of the various procedures and to determine whether measurements can be made precisely so as to minimize measuring error.