AGE DETERMINATION IN THE BUSH SQUIRREL, *PARAXERUS CEPAPI CEPAPI*

SAN VILJOEN
Mammal Research Institute, University of Pretoria

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ABSTRACT

Tooth eruption and wear, measurements of skulls, eye-lens mass determination and different aspects of phallic morphology were used in an attempt at age classification in the bush squirrel, *Paraxerus c. cepapi*. The first method proved the most practical and allowed for three immature and three adult age classes. Three known-age immature animals which fitted into the three immature tooth-wear classes, together with comparison of mass of known-age field young with that of collected young, enabled an estimate to be made of ages of the different immature classes. Beyond sexual maturity age could not be ascribed to animals.

INTRODUCTION

Morris (1972), commenting on mammalian age-determination methods, suggested tooth replacement in younger animals and tooth wear in older individuals as a guide to age, but he cautioned that these methods were not suitable for species which developed their permanent dentition early in life, and that inaccuracies due to diet and individual growth rates were unavoidable. He regarded mass of the dried eye-lens as a useful measure of rapid growth prior to attainment of adult size, which permits the separation of juveniles and adults and may be a useful age indicator in mature animals. In the present study age classification from tooth eruption, replacement and wear was the initial method applied, and it subsequently proved to be the most practical. In combination with data from three known-age slaughtered specimens and mass comparison of collected animals with that of known-age field animals, the tooth eruption/wear method enabled identification of adults, subadults and juveniles and the allocation of approximated ages to them. Skull measurements, eye-lens mass determination, bacular measurements and description of phallic morphology were completed later and then grouped according to the toothwear age classes for comparative purposes.

MATERIALS AND METHODS

Eighty-seven specimens of the bush squirrel, *Paraxerus cepapi cepapi* (A. Smith, 1836) were collected by live-trapping or by shooting, from various localities in the Transvaal (Table 1). Collection was aimed at adult animals for determination of seasonal reproduction, and therefore the juvenile/subadult cohort of the collection is abnormally small, and the relative

*Dr C. K. Brain, Transvaal Museum, Pretoria.

numbers of animals in these two age classes could not be used to estimate ages from birth dates as Fisher & Perry (1970) did on the grey squirrel, *Sciurus carolinensis.*

TABLE 1

Sample size of bush squirrels, *Paraxerus c. cepapi*, collected from different localities.

	Sample Size			
Locality	*Juveniles*	*Subadults*	*Adults*	*Total*
Naboomspruit 28°47′E/24°35′S	4	11	36	51
Limpopo River 30°35′E/22°20′S	—	6	10	16
Thabazimbi 27°25′E/25°0′S	—	—	8	8
Rustenburg 27°10′E/25°40′S	—	1	5	6
Potgietersrus 28°40′E/24°20′S	1	1	3	5
TOTAL	5	19	62	86

Skull measurements and tooth wear

All skulls were cleaned by dermestid beetles and then rinsed in commercial NaOCl-solution. The measurements taken on 76 of the skulls were based on Cockrum (1955): maximum skull length, zygomatic breadth, cranial breadth, minimal interorbital constriction, depth of the cranium, nasal length and breadth, and length of the maxillary and mandibular tooth-rows; anterior lengths of the incisors were measured; age classes were established according to tooth eruption and molar wear patterns. Consistency in ageing was only obtained after some practice and consequently, ageing on all skulls was repeated to ensure correct classification.

Eye-lens mass

Eyes of 76 animals were removed intact immediately after death and fixed in 10 per cent formalin. After 15 weeks, the lenses were removed, washed in 95 per cent alcohol for three to five minutes for partial dehydration and to prevent them from sticking to the vial (Fisher & Perry 1970), and stored dry until mass was determined. Just prior to mass determination eye lenses were dried in a force-draught oven at 80°C for two weeks until a constant mass had been attained. Average mass of the pair of lenses for each animal was determined except in a few cases where one lens had been too badly damaged by gunshot.

Bacular description and measurements

Twenty-six phalli, which had been stored dry, were processed in a two percent KOH-solution and stained with 0,003 per cent Alizarin red S (Lidicker 1968). Measurements were taken through a stereo microscope.

RESULTS

Tooth eruption and wear

According to Walker (1964) the accepted squirrel tooth formula is $\frac{1}{1}\frac{0}{0}\frac{1\text{–}2}{1}\frac{3}{3}$. In the bush squirrel it is $\frac{1}{1}\frac{0}{0}\frac{2}{1}\frac{3}{3}$. However, in a few squirrels collected in the more southern locality of Rustenburg the premolars of the maxilla were sometimes absent in older animals. This situation can be considered an abnormal regional difference. From Table 2 it is clear that the lower incisors (which erupted before birth) were consistently longer than the upper ones (which erupted on day seven). The mean incisor lengths of squirrels of the youngest tooth wear age class were 4,9 mm (upper) and 8,4 mm (lower). Captive squirrels with similar incisor lengths were two to three months old. However, the gums of live animals made comparison with processed skulls difficult. Incisor lengths of adults measured from 7,0 mm (upper) and 10,8 mm (lower) in processed skulls. The original premolars in the processed skull were of slightly darker hue than the white permanent molars and premolars. Tooth wear on permanent teeth was noticeable earlier in the mandibular than in the maxillary tooth-rows. In the mandibular tooth-row wear was slowest on the antero-lateral aspect of $PM\overline{1}$ and reasonably slow on the postero-medial aspect of $M\overline{3}$, whereas in the maxillary tooth-row wear was slowest on the antero-medial aspect of $PM\underline{2}$ and reasonably slow on the postero-lateral aspect of $M\underline{3}$. These regions of lesser wear graded to a maximum of wear towards the middle of the tooth-rows on M1 and M2.

Toothwear classes

Skulls were placed in the following classes according to eruption and wear of teeth (Figures 1 and 2):

Juveniles

Class x — Young squirrels, all molars not yet fully erupted

Subadults

Class xx — All molars present, premolars worn and still to be replaced

Class xx+ — Premolars replaced, M1, 2 and 3 already showing signs of wear but dentine not yet visible;

Adults

Class xxxx− — Dentine visible on all molars but not yet on the premolars which show no wear. The enamel pattern still clearly visible on M1, 2 and 3;

Class xxxx — Still little wear on the premolars. The enamel pattern on the other molars fading, sometimes absent;

Class xxxx+ No pattern left on the molars which are worn smooth with M3 sometimes broken in two. The original pattern still detectable on the premolars, which are worn as in the molars of Class xxxx.

The distinction between age-classes xxxx— and xxxx was the most difficult to make. Juvenile, subadult and adult separation was substantiated by a histological study of the reproductive organs (Viljoen 1975).

Skull measurements

The mean measurements for the different age classes are presented in Table 2 and show an increase with increasing age. Only in two instances did the mean measurements in young animals slightly exceed those of the older age classes: the cranial breadth and the length of the mandibular tooth-row of Class xx exceeded the respective measurements for Class xx+ and were the same as for the adult Class xxxx. The measurements for the depth of cranium and the length of the maxillary tooth-row also attained adult dimensions in subadult animals. Of all the measurements the maximum length of skull showed the most noteworthy increase (from 38,8 mm to 43,7 mm) and seemed the character which took the longest to attain adult dimensions. This would therefore probably be the best age indicator of the skull measurements. Juvenile and subadult animals could still be recognized by their squat faces, when all other external body dimensions appeared adult.

Eye-lens mass

Both lens masses in a squirrel were always similar, and decreased substantially during the first 48 hours of drying. Thereafter, a slight fluctuation was found and decrease was negligible. From Table 3 it is clear that there is an overlap in the ranges of eye-lens mass when compared to the different toothwear age classes, especially in the three adult age classes. However, the means of eye-lens mass increased with increasing toothwear age classes, and eye lenses of mass 5–8 mg could be considered to belong to juveniles, 8–12 mg to subadults and those of mass 12–18 mg, which were the most abundant in the sample, represented adult animals.

Phallic measurements and morphology

The bony elements of the penis are divided into a crest, baculum and cartilage (Viljoen 1975; see also this volume p. 229). Age classes were established principally according to the bone development of the crest and to a lesser degree by the form of the baculum:

Subadults

Class I

No development of bone is present on the crest although the tissue surrounding the baculum already shows distinct folds along the line where the bony crest will develop. The form of the baculum is somewhat variable. It is narrower than the typical adult form when viewed from the ventral surface and does not yet have the characteristic pear shape. Laterally, the distal portion is of adult shape but the proximal portion is still narrower than the middle section and will still enlarge. Age-class I includes an artificially large proportion of the population and can be divided into two sub-classes:

Ia: phalli with no crestal bone development, bacula of irregular form;

Ib: a slightly older class with phalli which have bacula of more or less adult proportion and yet no crestal bone development.

Class II

The form of the baculum remains constant throughout further classes. Bones have started to develop on the crest but are still more or less circular and definitely separate. A wide gap remains at the dorsal proximal juncture of the two crest halves.

TABLE 2

Mean skull measurements in the different toothwear age classes of 76 bush squirrels.

	Tooth-wear age class					
	x	xx	xx+	xxxx—	xxxx	xxxx+
Sample size	5	6	13	18	20	14
Measurements (mm):						
Maximum length of skull	38,8	41,8* (5)	42,6 (10)	43,2 (17)	43,4 (19)	43,7
Zygomatic breadth	23,0	25,2 (5)	25,5	25,8 (17)	26,2 (19)	26,2
Cranial breadth	19,3	20,1 (5)	19,9	20,0 (17)	20,2 (19)	20,2
Minimum inter-orbital constriction	10,5	11,8	12,2	12,8	12,9 (19)	13,2
Depth of cranium	18,0	18,4 (4)	19,0	19,1 (17)	19,2 (19)	19,4
Nasal: length	10,7	12,2	12,6 (9)	12,5	12,7 (18)	12,8
breadth	5,7	6,4	6,6 (9)	6,7	7,0 (18)	7,0
Length of tooth-rows: maxillary		8,5	8,6	8,5 (17)	8,6	8,3
mandibular		8,9	8,7	8,9	8,9	8,7
Length of incisors: upper	4,9	6,2	6,3	6,9 (11)	7,2 (18)	7,6 (8)
lower	8,4	9,1	9,9	10,8 (11)	11,1 (18)	11,8 (8)

*Figures in brackets denote divergent sample size.

Adults

Class IIIa

Bones on the crest start to join giving a zig-zag appearance to the crest. However, gaps are still in evidence between groups of joined bones. The first part to join completely and firmly, is the wing of the crest where it encircles the baculum to proceed antero-ventrally. The postero-dorsal gap is sometimes already closed by a few loose bones.

Class IIIb

Bone development on the crest is at a maximum having very few if any gaps. The crest is also thicker than in Class IIIa. The postero-dorsal curvature of the two crest halves is closed by the bony extension of each half. In some squirrels cartilage can clearly be seen in the tip anterior to the baculum and in the portion enclosed by the crest.

Both the squirrels in the juvenile Toothwear-class x were of the youngest age according to the bacular development (Class Ia). Subadult Toothwear-classes xx and xx+ were represented in Bacular-class Ib except for one squirrel in Class xx+ which had bacular development of Class II. Therefore the two subadult toothwear-classes are mainly represented by the one subadult Bacular-class Ib. Bacular-class II was transitional between immature and mature animals and some of these animals already had fully active testes (Viljoen 1975). A consistent toothwear/bacular development relationship was found in the oldest Toothwear-classes xxxx+ where all bacula were of Class IIIa or IIIb. Relative age can thus be estimated by this method. Measurements of bacular elements appear in Table 4. Measurements in Class Ia which exceeded those of Class Ib were those of the posterior breadth (g) of the baculum as seen from the ventral surface, the tip anterior to the baculum (h) as viewed laterally and the total lateral

TABLE 3

Eye-lens mass and estimation of subadult age of the different toothwear age classes of 76 bush squirrels.

Tooth-wear age class	*Sample size*	*Eye-lens mass (mg)*		*Known age of 3 pups (months)*	*Estimated age by mass comparison of collected and known-age field pups (months)*
		mean	*range*		
x	5	6,14	5,8–8,2	1,5	0–4
xx	6	8,78	7,6–11,0	3,5	4–7
xx+	13	10,35	9,3–12,0	9,0	7–12
xxxx—	18	13,90	12,3–16,6	—	—
xxxx	20	14,78	13,2–16,2	—	—
xxxx+	14	15,99	14,5–18,0	—	—

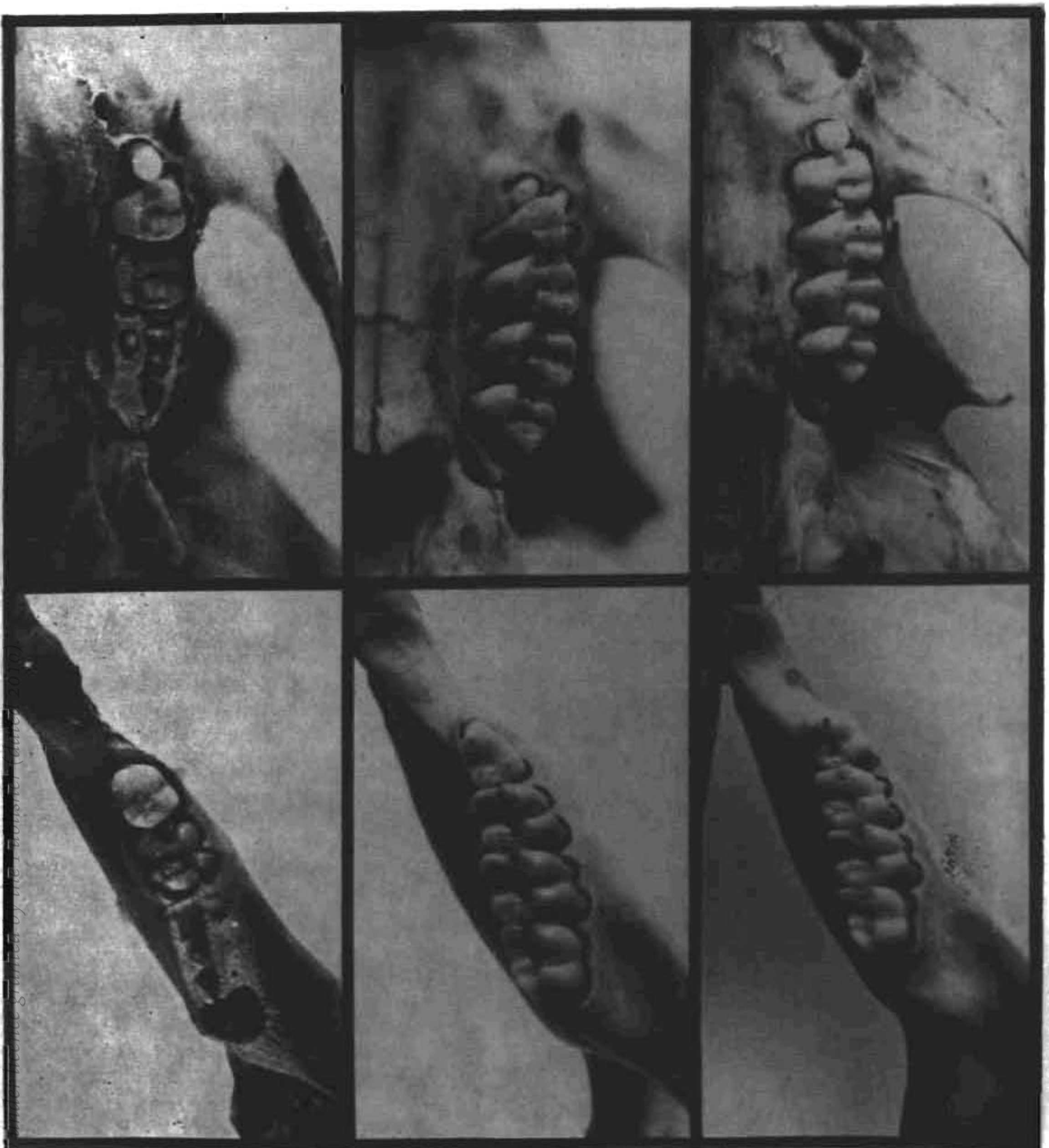

FIGURE 1
Left maxilla (top row) and right mandible (bottom row) of the three subadult age classes based on tooth eruption and wear in *Paraxerus c. cepapi*. Age class x: all molars not yet erupted; xx: premolars not yet replaced; xx+: premolars replaced.

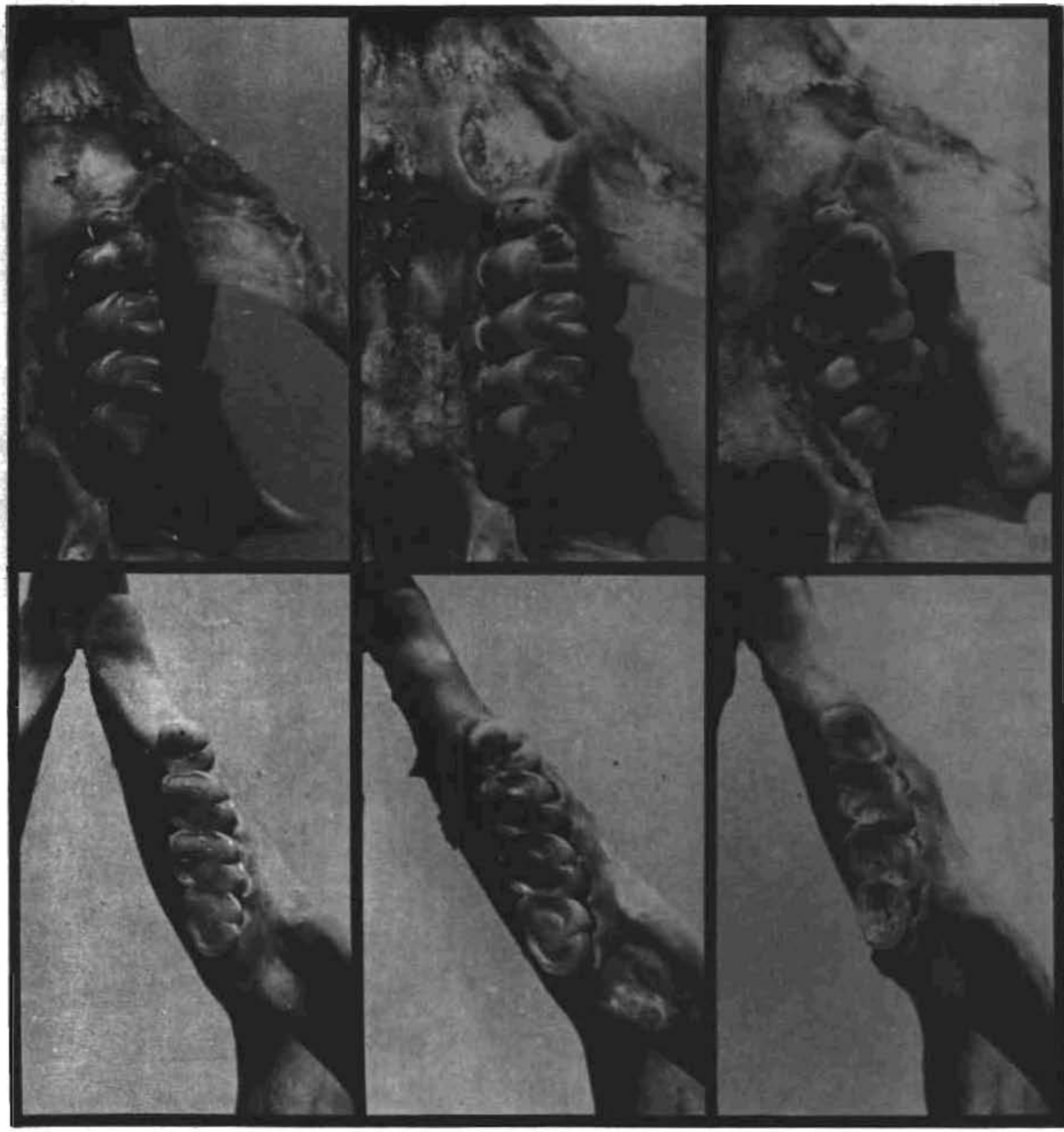

FIGURE 2
Left maxilla (top row) and right mandible (bottom row) of the three adult age classes based on tooth eruption and wear. See text for definition of classes.

TABLE 4

Bacular measurements (mm) for the dorsal, ventral and lateral aspects in the different age classes of 25 male bush squirrels.

Age	*Sample*	*Dorsal*				*Ventral*			*Lateral*				
Class	*Size*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*
Ia	2	1,020	0,300	1,040	0,900	1,040	0,180	0,720	0,920	1,060	2,680	0,520	4,780
Ib	6	1,200	0,368	1,128	1,040	1,280	0,308	0,612	0,728	1,280	2,907	0,632	4,480
II	5	1,264	0,400	1,128	1,040	1,312	0,352	0,736	0,768	1,272	3,072	0,680	4,640
IIIa	8	1,250	0,376	1,112	1,036	1,272	0,360	0,712	0,784	1,272	2,896	0,684	4,560
IIIb	4	1,300	0,372	1,112	0,980	1,380	0,372	0,752	0,772	1,372	2,952	0,772	4,760

a — length of to baculum
b — length of tip anterior to baculum
c — distal width of crest
d — proximal width of crest
e — length of baculum
f — distal width of baculum
g — proximal width of baculum
h — length of tip anterior to baculum
i — length of baculum
j — length of crest
k — proximal width of baculum
l — total length of everted part

length of the everted portion (l). Measurements of Class I were consistently less than those of Class II. A decrease was evident with increasing age in certain measurements: the length of the tip anterior to the baculum (b), the distance between the bony crests at the anterior broadest part (c) and at the proximal broadest portion (d), and the length of the crest (j). The bony development of the crest therefore causes slight shrinkage of the crest with increasing age. All measurements of the baculum itself increased consistently with age.

DISCUSSION

From the ecological point of view of the present study it was important to know when subadults had been born, at what age they would reach sexual maturity, and which adults were yearlings. As a result of the small sample size, especially of subadult animals, the degree of overlap made it impossible to develop age classes for bush squirrels according to skull or bacular measurements, or eye-lens mass. However, it was possible to establish relative age classes by the tooth eruption/wear method. On comparing these toothwear age classes with the age classes derived by other methods, arbitrary classes could also be distinguished in the latter. Kirkpatrick & Barnett (in Morris 1972), working with grey squirrels, similarly found that no simple osteological measurements would permit proper separation of age classes, but that the degree of overlap was small. Age estimation of subadults is shown in Table 3. The mass of captive young was only comparable to that of field young in the youngest Tooth-class x; thereafter the captive young gained mass much more rapidly than their field counterparts. Histological work (Viljoen 1975) and observations in the field on known-age animals indicate that squirrels can reach sexual maturity at 10 months in the field and in captivity even sooner where the length of the scrotum can reach mature dimensions at six months of age and sperm can be detected at nine months of age. Judging by ovarian features, females in the field reproduced when in Toothwear-class xxxx—, and one-year-old females in the study area had young of approximately one month old. The estimated ages of subadult bush squirrels agree with those of other tree sciurids, namely the American red squirrel (*Tamiasciurus hudsonicus*), where juveniles were three to five months old and subadults six to nine months old (Farentinos 1972). Fox squirrels, *Sciurus niger* (Kirkpatrick 1955), and grey squirrels, *S. carolinensis* (Smith 1968), matured at the age of one year, a situation similar to that in bush squirrels. Longevity was not established, but Brain* (*pers. comm.*) had a captive bush squirrel which reached ten years of age.

On comparing the eye-lens mass with toothwear age classes, the overlap could possibly be accounted for by individual and regional variation. For instance, Morris (1972) stated that nutrition influences age/lens relationships. Type of food could also affect toothwear but not necessarily to the same extent as it would the eye-lens mass. An obvious disadvantage of age determination using the baculum was that only males could thus be classified. The shape of the baculum of a two-to-three-month-old squirrel resembles that of an adult and although it is smaller it soon reaches adult dimensions. Similarly, Zimmerman (1972) found it thirteen-lined ground squirrels, *Spermophilus tridecemlineatus*, and Adams & Sutton (1968) in species of

*Dr C. K. Brain, Transvaal Museum, Pretoria.

Eutamias only slight bacular variation between adults and immature specimens. Development of the bony crest is a slower process than bacular growth and thus presents a better indicator of relative age of the bush squirrel. Bony crestal development can moreover be detected without prior staining, therefore yielding an easier age determination method than measuring the baculum. Cartilage development was unfortunately not followed throughout the different age classes.

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