Age determination of Cape porcupines, *Hystrix africaeaustralis*

R.J. van Aarde
Mammal Research Institute, University of Pretoria, Pretoria

Attempts to determine the absolute age of free-ranging porcupines based on counts of cementum and periosteal lines, age-related changes in eye lens weight, sequential pattern of tooth eruption and replacement, and tooth attrition are described. Variation due to ramification and absorption of cementum and periosteal lines resulted in counts of these lines being unreliable indicators of age. Variation in counts of cementum lines apparently results from continual growth of the hypsodontic open-rooted premolars and molars. Heteroscadasticity resulted in the relationship between age and lens weight being unreliable for predicting age. Consistency in the age at which maxillary molars erupt and premolars are replaced, as well as the wear pattern of the occlusal surfaces, provide a method for distinguishing nine dental age classes. Chronological age, based on observations on captive porcupines could be accurately assigned to six of these.
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Pogings om die absolute ouderdomme van vrylewende ystervarke, gebaseer op tellings van sement- en periosteale lyne, ouderdomsverwante veranderinge in ooglensgewig, die opeenvolgende snyding en wisseling van kies- en voorkiestande en tandslytasie, te bepaal, word beskryf. Variasies as gevolg van vertakking en resorbsie van sementlyne en periosteale lyne gee daartoe aanleiding dat tellings daarvan nie geskik is vir ouderdomsbepaling nie. Heteroskedastisiteit dra daartoe by dat die verwantskap tussen ouderdom en ooglensgewig nie gebruik kan word om ouderdom akkuraat te bepaal nie. Die konstante ouderdom waarop maksillêre kiestande sny en die voorkiestande wissel, sowel as die verweringspatroon van die maaloppervlak van kiestande, bied 'n metode om nege ouderdomsklasse te onderskei. Gebaseer op waarnemings op ystervarke in gevangeskap kon werklike ouderdom akkuraat aan ses van hierdie klasse toegewys word.
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R.J. van Aarde
Mammal Research Institute, University of Pretoria,
Pretoria, 0002 Republic of South Africa

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The need to determine the absolute and/or relative ages of porcupines *Hystrix africaeaustralis* arose from attempts to define age at sexual maturity and senescence, age-specific fecundity schedules and age-specific reproductive values for free-ranging porcupines (van Aarde 1984). Porcupines apparently have a wide ecological tolerance and their natural habitats include tropical forests, woodlands, grassland savannas, and semi-arid and arid environments throughout the southern African subregion. Captive porcupines may attain an age of 20 years in captivity (van Aarde 1984).

Published accounts of techniques employed to determine the absolute ages of hystricomorph rodents are limited and include those of Earle & Kramm (1980) for the Canadian porcupine *Erethizon dorsatum*; Willner, Gale, Dixon, Chapman & Stauffer (1980) for the coypu *Myocastor coypus* and Collet (1981) for the paca *Agouti paca*.

All age determination criteria applied during the present study are well established (reviewed by Morris 1972) and include the sequential pattern of tooth eruption, tooth wear, counts of cementum annuli, counts of periosteal lines and the determination of age-related changes in dry eye lens weights. The present paper evaluates the applicability of these techniques to material collected from porcupines killed in the Tussen-die-Riviere Game Farm (30°25′S/26°12′E) as part of a culling programme.

Materials and Methods

Tooth eruption and replacement

The sequence of tooth eruption and replacement in the maxillary tooth row of known-age porcupines born in captivity at the Experimental Farm of the University of Pretoria were determined during bi-weekly inspections of restrained or immobilized animals. A tooth was considered erupted when its occlusal surface was visible above the gum line.

Based on the sequential eruption and replacement of teeth in the maxillary tooth row and the extent of occlusal surface wear, skulls of porcupines obtained from the Tussen-die-Riviere Game Farm (TdR) were placed into nine 'dental age classes'.

Counts of cementum annuli

The left maxillary permanent premolars and/or first molars of all skulls and all teeth in the tooth row of nine skulls were decalcified in a 5% nitric acid solution after being extracted from the alveolus, often by placing the cleaned skull into boiling water for 5 to 10 min. The nitric acid solution was changed every 24 h and complete decalcification required 48 to 140 h, depending on the size of the tooth. The lack of a white precipitate forming withing 5 min after a 5% solution of ammonium oxalate was added to the nitric acid solution was used as a criterion for complete decalcification (Earle & Kramm 1980).

Decalcified teeth were stored in water and their roots serially cross-sectioned at 20 μm, either in a cryostat with a cooled blade or by using a microtome following routine embedding in paraffin wax. Fifteen to 20 cross-sections were mounted on glass microscope slides and stained for 20 to 25 min with Carazzi's haematoxylin. Stained sections were routinely dehydrated, and following clearing in two xylol baths, mounted with D.P.X. mountant (BDH Chemicals Ltd, Poole, UK). Sections were examined through a light microscope and the number of incremental lines in cementum were counted at 10 separate locations on each section.

Counts of periosteal lines

Cross-sections of a part of the shaft of the left humerus of each specimen were also prepared for microscopic examination. Decalcification in a nitric acid solution was usually complete within 48 h and 20-μm sections, prepared on a slice microtome, were stained in Carazzi's haematoxylin after routine embedding in paraffin wax. Ten to 15 sections were examined and periosteal lines were counted at 10 separate locations.

Eye lens weight

Eye lenses collected from 118 porcupines killed in the Game Farm were removed from the eyeballs after a 14 to 21 day fixation period in 10% formalin (40% formaldehyde solution diluted 1:9 in water), cleaned, weighed and oven-dried at 80 °C to constant weight for eight weeks. Lenses were weighed at 14-day intervals and care was taken not to allow the hygroscopic dried lenses to absorb moisture by putting groups of dried lenses into a desiccator over anhydrous calcium chloride when taken out of the oven before weighing.

Tooth measurements

The length of permanent maxillary premolars of each culled porcupine (n = 118) was measured with a steel vernier caliper to the nearest 0,1 mm. The area of the occlusal surfaces of the same teeth were measured using an electronic planimeter (AAC-400, Hayashi Denkoh Co. Ltd, Tokyo) after a 120 × magnification drawing on cardboard with the aid of a drawing tube fitted to a stereoscopic dissecting microscope. The area (mm^2) was calculated as the mean of four consecutive readings corrected for magnification.

Results

Sequence of tooth eruption and replacement

Incisors of captive born porcupines were fully erupted at birth (n = 24) and the deciduous premolars started erupting at an age of approximately 14 days. Eruption of the first maxillary molars commenced 2,0 to 2,5 months (n = 8) after birth and the second molars were visible above the gumline at an age of five to six months (n = 21). The third maxillary molars erupted at an age of eight to 11 months (n = 12), with eruption being complete at an age of 12 months. Eruption of the permanent premolars was complete at the age of 23 to 25 months (n = 12) and the occlusal surfaces of the premolars were slightly worn 24 to 30 months (n = 8) after birth. All the flexi of the premolars were still distinct at this age. Descriptions and illustrations of the maxillary tooth row of these age classes are provided in Table 1 and Figure 1.

Skulls of specimens collected on the TdR Game Farm could thus be grouped into one of nine 'dental age classes' (Figure 1), with age class I to III representing porcupines less than eight months of age, age class IV animals eight to 18 months of age, age class V and VI animals older than 18 months but less than 30 months of age, and age class VII to IX porcupines older than 30 months of age.

Table 1 Description of dental age classes based on the sequential eruption, replacement and wear of the occlusal surfaces of teeth in the maxillary tooth row of captive porcupines examined at bi-weekly intervals

Dental age class	Absolute age (months)	Description of maxillary tooth row
I	Birth – 0,5	Deciduous premolar newly erupted
II	2,0 – 2,5	Deciduous premolar slightly worn with first molar newly erupted
III	5,0 – 6,0	Deciduous premolar and first molar slightly worn with second molar newly erupted
IV	8,0 – 11,0	Third molar erupted and deciduous premolar heavily worn
V	18,0 – 23,0	Permanent premolar erupting. Flexi of second and third molar still distinct and occlusal surface of first molar comprises of a number of 'dentine islands', defined as fosettes (see Maguire 1976)
VI	24,0 – 30,0	Permanent premolar fully erupted and slightly worn with paraflexus, mesoflexus, metaflexus, entoflexus and hypoflexus still distinct. Fosettes forming on occlusal surface of second molar and flexi still visible on third molar
VII	30,0	Fosettes distinct on all molars
VIII	30,0	Fosettes worn away on first molar
IX	30,0	Fosettes worn away on first and second molar

Counts of cementum lines

Distinct annuli were visible in the cementum of all erupted teeth and no annulations were present in the cementum of newly erupted premolars and molars. Most cementum was deposited between the roots with cementum lines being most discrete in the thick cementum pad forming at the proximall end of the roots. The degree of definition of the cementum lines and the distances between these lines varied considerably.

Ramification of the lines at lacunae in the cementum and the uneven deposition of cementum around roots resulted in considerable variation in the number of lines counted. The coefficient of variation for 10 counts of cementum annuli conducted on four to eight sections of each tooth, varied from 0,0 to 117,0% with the greatest variation recorded for the older dental age classes (VII – IX). Most coefficients of variation were larger than 20% but less than 50%.

Assuming that cementum lines may be formed on an annual basis and considering the eruption interval of the permanent molars and the premolars (Table 1), it is expected that the mean number of lines in the permanent premolar of a specific individual would be two less than in the first molar. The number of lines in the first molar should thus be one less than in the third molar. The mean number of cementum lines counted in cross-sections of all teeth in the maxillary tooth row of nine porcupines, presented in Table 2, support this hypothesis, suggesting that at least some of the lines are formed on an annual basis. The numbers of cementum lines in the premolars were consistently less than those in the first molar and more lines were deposited in the

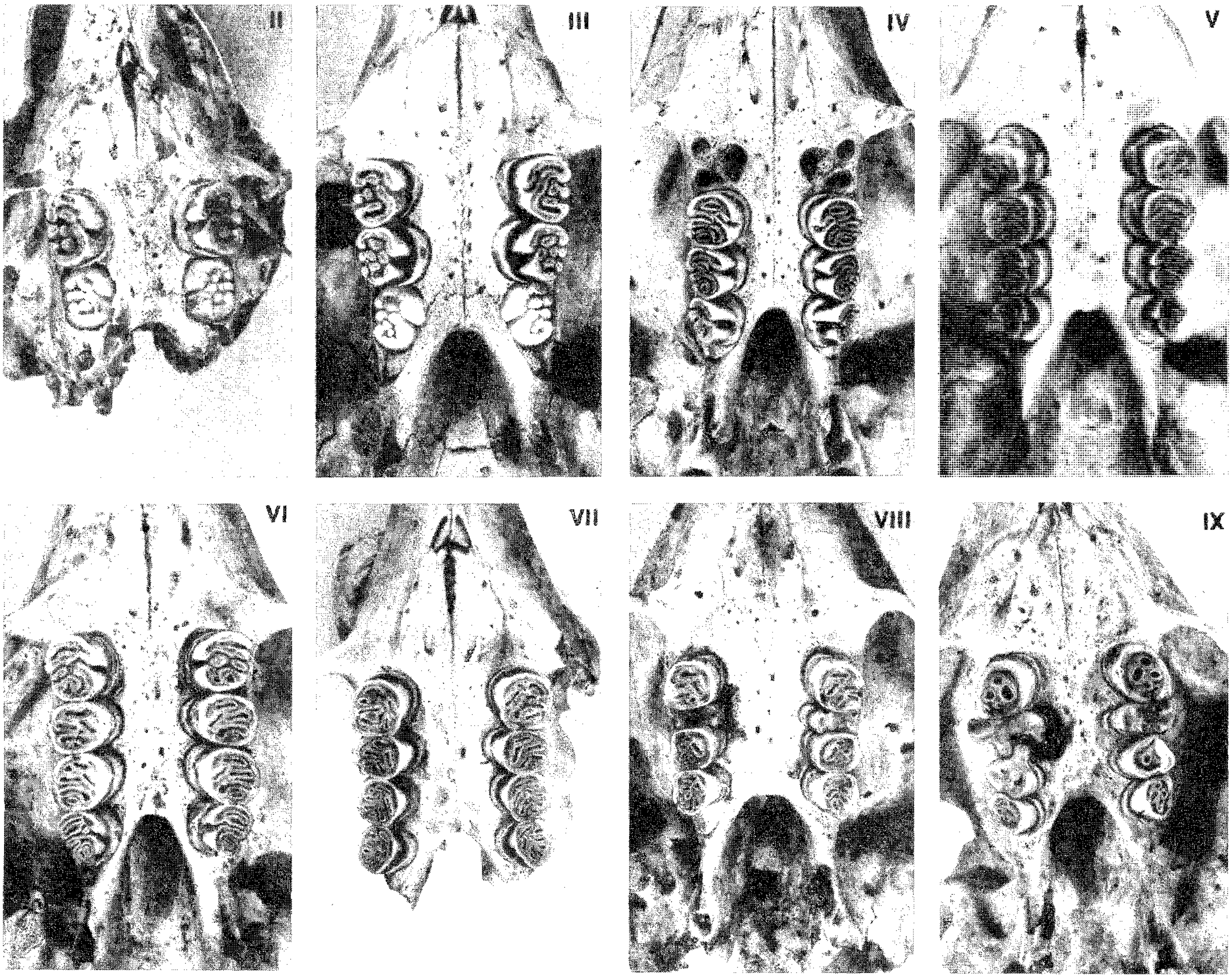

Figure 1 The maxillary tooth rows of eight of the nine dental age classes distinguished on the basis of the sequence of tooth eruption and replacement. A description of the tooth row of each of the nine age classes is presented in Table 1.

Table 2 Mean (± *S.D.*) number of cementum lines counted at ten different locations on four to eight decalcified cross-sections of the maxillary premolar, first, second and third molars of nine porcupines culled on the Tussen die Riviere Game Farm

Dental age class	Mean number of cementum lines			
	Premolar	First molar	Second molar	Third molar
IV	0,0[a]	1,8 ± 0,41	1,6 ± 0,53	0,0
V	0,0	1,4 ± 0,53	0,0	0,4 ± 0,55
VI	1,0 ± 0,00	3,8 ± 0,75	4,2 ± 0,41	3,0 ± 0,00
VI	1,4 ± 0,55	3,0 ± 0,00	2,0 ± 0,82	1,8 ± 0,45
VII	1,6 ± 0,53	3,8 ± 0,94	1,8 ± 0,44	2,2 ± 0,41
VII	1,7 ± 0,52	4,7 ± 0,49	3,6 ± 0,74	3,2 ± 0,41
VII	2,3 ± 0,52	4,0 ± 0,89	5,0 ± 1,00	2,4 ± 0,55
VII	2,4 ± 0,55	–	3,8 ± 0,41	2,5 ± 0,55
VIII	5,0 ± 0,00	6,5 ± 1,05	7,3 ± 0,52	5,0 ± 0,00

[a]Deciduous tooth.

Table 3 The difference between the mean number of cementum lines counted in the permanent premolar and the third molar and between the first and third molar of nine porcupine culled on the Tussen die Riviere Game Farm

Dental age class	Difference between means	
	Premolar/First molar	First molar/Third molar
IV	1,83	1,83
V	2,00	1,03
VI	1,60	1,20
VI	2,82	0,82
VII	2,26	1,66
VII	3,04	1,64
VII	1,67	1,60
VII	1,60	1,50
VIII	1,50	1,50
Average difference	2,04	1,41

cementum of the first molar than the third molar (Table 2). These differences were not affected by age and were also not within the expected limits for all animals (Table 3).

Periosteal lines

Definite lines were present in the periosteum of most humeruses cross-sectioned. The calculated coefficients of variation for ten counts conducted on four to six sections of each humerus varied, however, from 0,0 to 36,0% and precluded the reliable estimation of age based on counts of periosteal lines alone.

The minimum and maximum means calculated for individuals in each dental age class furthermore varied considerably and did not increase consistently with an increase in age, thereby reducing confidence in counts of periosteal lines as a parameter to estimate ages of adult porcupines (Table 4).

Variation in the number of lines counted also resulted in a poor relationship between the number of periosteal and cementum lines with the number of periosteal lines tending to decrease with an increase in the number of cementum lines, possibly due to periosteal lines being absorbed faster than cementum lines.

Table 4 The minimum and maximum mean (± *S.D.*) number of periosteal lines calculated for individuals in each of the adult age classes

Dental age class	Minimum and maximum mean (± *S.D.*) values calculated for individuals in age class	
	Minimum	Maximum
V	6,3 ± 1,37	–
VI	3,0 ± 0,00	3,7 ± 0,59
VII	4,0 ± 0,88	7,9 ± 2,09
VIII	3,2 ± 1,08	9,8 ± 2,59
IX	2,8 ± 0,50	6,0 ± 1,96

Eye lens weight

Mean combined dry eye lens weight increased linearly (y = 0,04 x + 0,019; n = 55) and significantly (r = 0,83; p < 0,001) over the first 24 months of life (dental age classes II – V). The relationship between these variables for the adult classes (VI – IX) was, however, not significant (r = 0,41; n = 57). The discontinuity in the relationship between dry eye lens weight and dental age class (Figure 2) can be ascribed to unequal age intervals being represented by each age class (see Table 1).

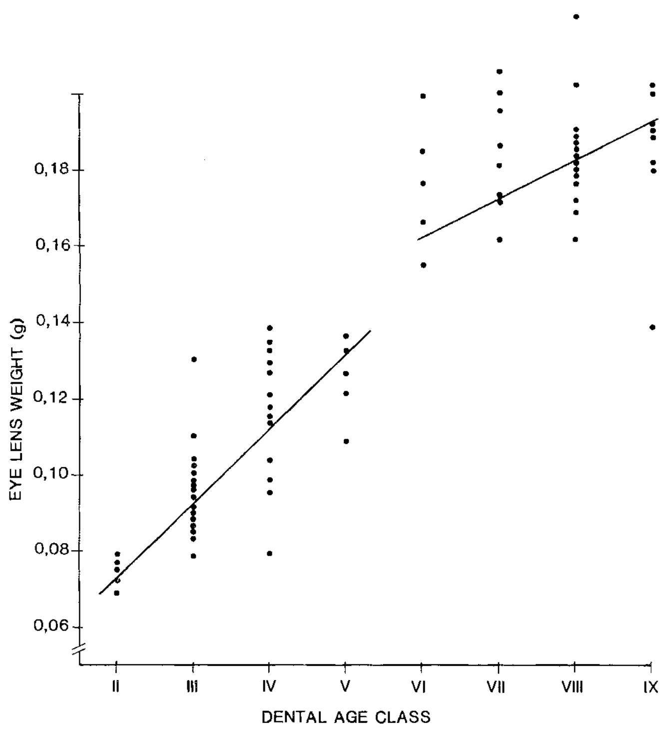

Figure 2 The relationship between combined eye lens weight (g) and dental age class. The lines were fitted through least square regression analyses.

Tooth measurements

The hypsodontic open-rooted premolars of porcupines are conical in shape with the diameter at the crown of the newly erupted tooth being less than at the neck, where it is wider than at the roots. The area of the occlusal surface of the permanent premolars decreased slightly (b = −0,10) with an increase in the length of the premolar (Figure 3) but the relationship between the two variables was not significant (r = 0,20; n = 50).

The relationship between the area of the occlusal surface and dental age class was best described by a third degree polynomial curve following the equation y = 73,07 − 39,76x + 7,24 x^2 − 0,41 x^3 (r^2 = 0,72; n = 48) with occlusal surface as the dependent variable (Figure 4). The area of the occlusal surface thus decreased from age classes VII to VIII after increasing exponentially between age classes IV and VII.

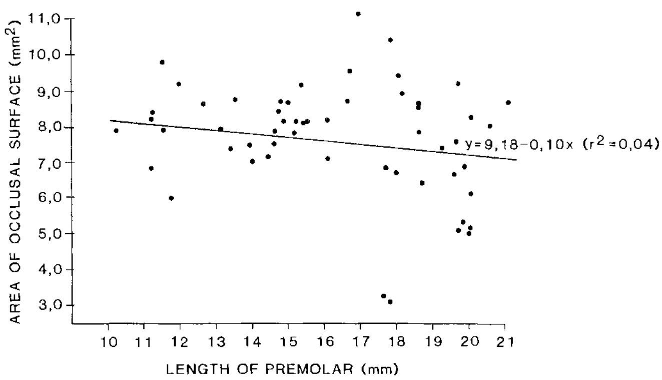

Figure 3 Relationship between the length (mm) and the area of the occlusal surface (mm) of the premolars of adult porcupines. The line was fitted through least square linear regression analysis.

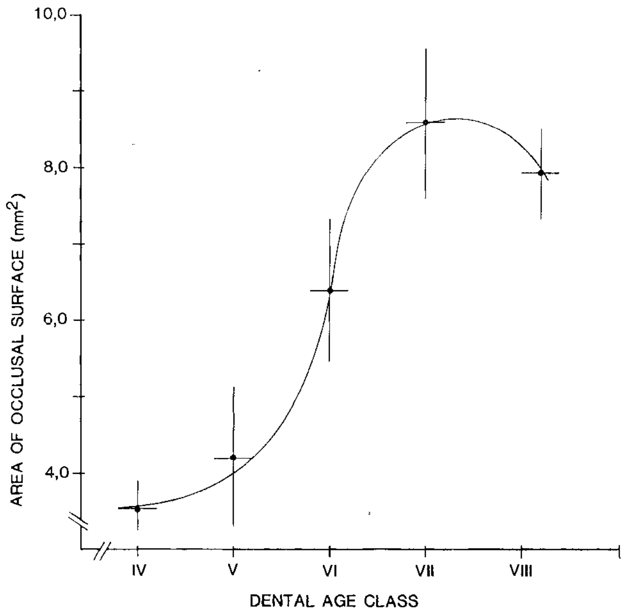

Figure 4 The relationship between the area of the occlusal surface (mm) and dental age class. The third degree polynomial curve is described by the equation y = 73,07 − 39,76x + 7,24x^2 − 0,41x^3. Vertical lines present one standard deviation of the mean.

Discussion

Factors affecting the annular pattern of cementum growth have been the subject of much speculation but consideration of these is beyond the scope of the present paper. However, it is generally accepted that the calcification rhythm in teeth may be disturbed by changes in the level of nutrition or periods of stress associated with reproduction (Klevezal' & Kleinenberg 1969; Morris 1972).

Information available on the eruption interval of molars and permanent premolars and the differences between the number of lines in the cementum of these teeth (Tables 2 & 3) suggests that primary cementum lines are formed on an annual basis in the teeth of porcupines during the first few years of life.

Ramification of cementum lines owing to the uneven deposition of cementum as a result of continuous longitudinal growth of the open-rooted hypsodontic teeth, however, introduced variability in the counts of these lines beyond the levels of acceptance for age determination purposes. Similar problems have been encountered in other species, for example, bats (Philips, Steinberg & Kunz 1982), while Hall-Martin (1976), Gasaway, Harkness & Rausch (1978) and Leader-Williams (1979) all encountered problems in the interpretation of the layer structure of cementum of ungulates. Difficulties experienced in defining cementum lines accurately in porcupines, however, are not surprising since Klevezal' & Kleinenberg (1969) indicated that annual layers are not formed in teeth with permanent longitudinal growth.

Earle & Kramm's (1980) account of the use of this technique as a reliable indicator of real age in Canadian porcupines is thus surprising. Reliability in their study was based on the differences between the number of cementum lines in the various teeth within the tooth row without accounting for the variability owing to ramification and resorption. The shortcomings of such an approach are apparent and have been reviewed in detail by Dapson (1980). Well-defined lines were present in the periosteal zone of the humeruses of all purcupines examined but resorption and ramification introduced variability beyond the level of acceptance for age determination purposes.

Combined eye lens weight increased with an increase in age but did not follow the pattern considered characteristic for mammals in general (Morris 1972); this discrepancy being ascribed to the dental age classes, based on eruption patterns, not representing chronological ages. The lines describing the relationship between lens weight and age nevertheless indicate an initial period of rapid growth from birth to 24 months of age, followed by a period of relatively slow growth (Figure 2). The considerable overlap in lens weight within the subadult (II – IV) and adult (VI – IX) age classes is ascribed to the actual ages presented by each age class being continuous rather than discrete. Inequality in the ages represented by each class (see Table 1) contributed to the observed heteroscadasticity (see Dapson 1980) which rendered eye lens weight unsuitable as a criterion for age determination.

Tooth measurements could not be used for age determination owing to the shape of the teeth and their continual longitudinal growth. The sequence of tooth eruption and replacement, however, provided a ready means to determine the ages of porcupines less than 30 months of age. Chronological ages could be assigned to six of the nine dental age classes on the basis of age at eruption of the molars and replacements of the premolars as recorded in captive animals. The sequence of tooth eruption and replacement was also used by Corbet & Jones (1965) and Maguire (1976) to group porcupine skulls into relative age classes. These studies, however, were directed at taxonomic aspects with no reference being made to the real ages represented by each age class. Thus it is clear that without the availability of known-age material representing most of the life-span of porcupines (20 years; Kingdon 1974), precise age determination of adult animals would not be feasible using established techniques.

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References

COLLETT, S.F. 1981. Population characteristics of *Agouti paca* (Rodentia) in Colombia. Publication of the Museum, Michigan State University, Biological Series. 5: 489 – 601.

CORBET, G.B. & JONES, L.A. 1965. The specific characters of the crested porcupines, subgenus *Hystrix*. *Proc. zool. Soc. Lond.* 144: 285 – 300.

DAPSON, R.W. 1980. Guidelines for statistical usage in age-estimation techniques. *J. Wildl. Mgmt* 44: 541 – 548.

EARLE, R.D. & KRAMM, K.R. 1980. Techniques for age determination in the Canadian porcupine. *J. Wildl. Mgmt* 44: 413 – 419.

GASAWAY, W.C., HARKNESS, D.B. & RAUSCH, R.A. 1978. Accuracy of moose age determination from incisor cementum layers. *J. Wildl. Mgmt* 42: 558 – 563.

HALL-MARTIN, A.J. 1976. Dentition and age determination of the giraffe *Giraffa camelopardalis*. *J. Zool., Lond.* 180: 263 – 289.

KINGDON, J. 1974. East African mammals: An Atlas of Evolution in Africa. Vol. 2, Part B. Academic Press, London.

KLEVEZAL', GALINA, A. & KLEINENBERG, S.E. 1969. Age determination of mammals from annual layers in teeth and bones. IPST Press, Jerusalem.

LEADER-WILLIAMS, N. 1979. Age determination of reindeer introduced into South Georgia. *J. Zool., Lond.* 158: 501 – 515.

MAGUIRE, JUDITH, M. 1976. A taxonomic and ecological study of the living and fossil Hystricidae with particular reference to Southern Africa. Ph.D. thesis, University of the Witwatersrand, Johannesburg.

MORRIS, P. 1972. A review of mammalian age determination methods. *Mammal Rev.* 2: 69 – 104.

PHILIPS, C.J., STEINBERG, B. & KUNZ, T.H. 1982. Dentin, cementum, and age determination in bats: a critical evaluation. *J. Mammal.* 63: 197 – 207.

VAN AARDE, R.J. 1984. Reproduction in the porcupine *Hystrix africaeaustralis* Peters. D.Sc. thesis, University of Pretoria, Pretoria.

WILLNER, GALE R., DIXON, K.R., CHAPMAN, J.A. & STAUFFER, J.R. 1980. A model for predicting age-specific body weights of nutria without age determination. *J. appl. Ecol.* 7: 343 – 347.