# Chapter 3

## Age determination of *Cryptomys hottentotus pretoriae* and the relation to reproductive status

## ABSTRACT

Tooth eruption and wear on the molars of 178 females and 96 males were used to assign the animals into nine distinct relative age classes. The reproductive animals were amongst the oldest in addition to being the heaviest members of the colony.

In addition to age determination, morphometric skull measurements were taken for 140 females and 71 males. Morphometric analyses showed an absence of sexual dimorphism. Cluster, principal components and discriminant analyses revealed two distinct groupings amongst the nine relative age classes. A comparison of the morphometric data with that of the age determination data revealed a distinct pattern. The young individuals were assigned to age classes 1 to 4, no reproductive animals were present within this group. The older individuals, including all the reproductive animals, were grouped in age classes 6 to 9. Age class 5 comprised both reproductive and non-reproductive individuals. No distinct morphological differences could be observed between mole-rats collected from four different geographical localities.

## INTRODUCTION

The highveld mole-rat is an African subterranean mole-rat occurring in colonies of up to twelve individuals (L. Janse van Rensburg, unpubl. data). This social, rodent mole-rat exhibits a marked division of labour in which a single reproductive female and

potentially one or two males are responsible for the procreation of new colony members. The remaining non-reproductive males and females are not sterile but are reproductively quiescent. The non-reproductive animals mainly contribute to colony maintenance (Moolman *et al.* 1998). Non-reproductive animals cannot be divided into distinct working groups based on their sex or body mass, unlike *Cryptomys damarensis* (Bennett 1988; Bennett & Jarvis 1988; Bennett 1990). Burrow maintenance behaviours are thus not performed by distinct working groups but by all colony members (Moolman *et al.* 1998).

Colonies of the highveld mole-rat have a non-linear dominance hierarchy, with the reproductive female and one or two reproductive males at the apex of the hierarchy. The reproductive female was ranked the second most dominant animal. Interestingly, the reproductive pair are amongst the heaviest animals in the colony. A similar trend was found in the common mole-rat where the reproductive pair were amongst the heaviest animals in the colony and ranked at the top of the non-linear dominance hierarchy (Bennett 1989). This pattern seems to be common to all species of *Cryptomys* studied to date (Bennett 1988; Jacobs *et al.* 1991; Wallace & Bennett 1998).

Within colonies of the Damaraland mole-rat, *C. damarensis* there is a very distinct division of labour, where colony members can be divided into frequent and infrequent workers (Bennett & Jarvis 1988). In many instances, the frequent workers are significantly smaller when compared to the other members of the colony. The frequent workers may retain their small stature for as long as they occupy this social ranking. Thus, body mass is not a good indicator of age (Bennett *et al.* 1990). Chaplin & White (1969) also concluded from their study on Fallow deer (*Dama dama*) that weight is not suitable for age estimation. A further study on growth and age determination in the hyrax (*Procavia capensis*) by Fairall (1980) concluded that mass is easily influenced by the environment and thus is not a good criterion for age determination. In conclusion we can say that there are too many factors such as food availability and the quality and energetic content that may influence an animals weight (Morris 1972). In the genus *Cryptomys* the position of an animal in the hierarchy as well as the social status can influence the mass of an animal and hence body mass would appear to be a poor and unreliable indicator of age within the genus *Cryptomys* (Bennett 1988; Bennett *et al.* 1990).

Age determination is one of the most difficult parameters to measure (Fairall 1980) and various methods exist to determine age. The traditional method of ageing deer is based on the eruption and wear of the molar teeth (Chaplin & White 1969). Taylor *et al.* (1985) devised a similar method for ageing mole-rats based on the same principal of Chaplin & White (1969) using tooth eruption and wear of molariform teeth.

The mole-rat is equipped with two protruding front incisors on both the lower and upper jaw. The incisors are specifically adapted for digging and biting, whilst the molars which are situated inside the mouth are adapted for grinding and pounding food (Bloom & Fawcett 1962). Both the upper and lower jaw are equipped with two rows of four molar teeth each, one row on each side of the jaw. The incisors are constantly growing due to the extreme wear these teeth undergo during the digging and feeding process. Thus, the number of erupted molars and the amount of wear on these permanent molar teeth are the most reliable indicators of age. However the homology of the molariform teeth in the Bathyergidae is not clear (Roberts 1951; De Graaff 1964), hence the molar teeth are referred to as cheek teeth throughout the chapter. The hard portions of these cheek teeth consist of three different tissues: dentin, enamel and cementum. Throughout a mole-rats lifetime, these tissues are worn down and this wear together with the number of erupted cheek teeth can be used to determine the relative age of a mole-rat.

This chapter describes a method by which the relative ages of animals are determined using the criteria of sequential cheek tooth eruption patterns and the amount of wear upon the cheek teeth in entire colonies of field captured mole-rats, trapped throughout an entire year. It should be stressed here that absolute chronological ages are not reported in this chapter, since no known age wild animals were obtained.

Sexual dimorphism was investigated between males and females and amongst geographical regions using 21 cranial measurements.

The aim of this study was to determine if the oldest animals in the colony are the breeders, by using the cheek tooth eruption and wear patterns to provide relative ages for the animals. My *a priori* prediction being that the reproductive female would be the oldest female, but that because of male biased dispersal, breeding males may possibly not be the oldest but amongst the oldest of the males.

Skull measurements were taken to establish if any sexual dimorphism occurred within the sampled population of the highveld mole-rat and to determine if there might be any distinct groupings of the age classes. In addition the study aimed at investigating morphometric differences between animals sampled from different localities.

## MATERIALS AND METHODS

*Capture and housing*

A monthly collection of *C. h. pretoriae* was undertaken from January 1998 until April 1999. Each month, a minimum of three colonies were captured using modified Hickman (1979) live traps. The animals were kept in climate rooms at a constant temperature of 25 ± 1°C. Each month the animals were put down by halothane inhalation. The animals were weighed, sexed and the reproductive status determined. The reproductive tracts were removed for further histological studies (See chapter 2, Materials and Methods for detailed description on capture and housing).

The heads of the animals were removed just below the foramen magnum, labelled and placed in separate cooking bags. The heads were heated to near boiling in water for approximately two hours, removed from the bags and hand cleaned. The skulls were bleached and left to dry. Care was taken not to boil the heads thus preventing any cranial distortion.

*Age determination*

Age determination was based on the eruption and wear of the cheek teeth. A stereo microscope was used to examine cheek tooth eruption and wear on each skull. Nine skulls were chosen as references for the respective tooth classes to which the remaining skulls would be compared to (see below). Using eruption and tooth wear, nine relative dental age classes were distinguished. Each individual was assigned to a specific age class based on the number of cheek teeth surfaced, amount of wear on each tooth, cusp wear and diameter, the degree to which the dentine was scooped and the presence or absence of grooves in the enamel and the depth of the grooves.

To define the nine relative age classes, the cheek tooth wear and eruption patterns were assessed for the upper right row of each individual's upper jaw, as described by Taylor *et al.* (1985). This method did not prove to be viable in most instances, since a number of skulls had teeth missing from the right upper row, thus the left upper row of teeth were also used to determine wear and eruption. According to Taylor *et al.* (1985) there are no differences in eruption and wear between the upper and lower jaws or between the left and right sides. To exclude any bias, the skulls were aged without prior knowledge of the individual's reproductive status or sex.

The age classes are described as follows:

*Class 1*

Only two cheek teeth are completely erupted with a cavity where the third tooth is about to emerge. The dentine is not scooped and there is little sign of wear on the teeth. Very deep grooves are found on the tooth surface. The cusps are narrow and curved (Plate 1).

*Class 2*

Three completely erupted cheek teeth are present. The first two teeth show little signs of wear, whereas the third tooth shows none. The dentine is not scooped and the diameter of the cusp is narrow. The cusps are rounded and very deep grooves are found on the tooth surface (Plate 2).

*Class 3*

Three erupted cheek teeth are visible, with a very small cavity where the fourth tooth will originate. The dentine of tooth number one is slightly scooped. The cusp of the first tooth is slightly flattened but the cusp diameter of all the cheek teeth is narrow. Deep grooves cover the tooth surfaces (Plate 3).

*Class 4*

Three completely erupted cheek teeth are visible, with the fourth one starting to surface. The dentine of tooth number 1-3 is barely scooped. The cusps of all three teeth are

almost flat (round on the side nearer to the tong and flat on the outside). The cusp diameter is much wider than class 3. Deep grooves are exhibited on tooth surfaces (Plate 4).

*Class 5*

Four completely erupted cheek teeth are observed. The fourth tooth shows no sign of any wear. The dentine of teeth number 1,2 and 3 is slightly scooped. The cusps of teeth 1-3 are almost flat and only a little rounded on the inside of tooth number 3. There is a wide cusp diameter. The grooves are shallow but easily observed (Plate 5).

*Class 6*

Four cheek teeth are visible with tooth number 4 exhibiting little wear. The dentine of tooth number 1 and 2 are slightly more scooped than tooth number 3 and 4. The cusps have a wider diameter than class 5 but are not completely flat. The grooves are shallow but easily observed (Plate 6).

*Class 7*

Four cheek teeth are visible. The dentine of teeth number 1-3 are deeply scooped. Tooth number four exhibits a fair amount of wear but the dentine is not completely scooped. The cusps are flat and have a wider diameter than age class 6. The grooves are barely visible (Plate 7).

*Class 8*

The dentine of all four cheek teeth is deeply scooped. The cusps are completely flat, smooth and well polished. The cusp diameter of the first tooth is very wide. Dentine of the fourth tooth is deeply scooped and grooves on the surface almost completely smooth (Plate 8).

*Class 9*

The four erupted cheek teeth looks completely deformed due to heavy wear. The enamel of teeth number 1-3 is worn with dentine completely exposed. Tooth number 4 is

heavily worn with the dentine deeply scooped. The teeth are reduced in height due to wear (Plate 9).

*Age determination statistical analyses*

One-way analysis of variance (ANOVA) (Zar 1984) was used to determine any significant differences that might occur between males and females with regard to their ages and masses. Where significant differences occurred, a *post hoc* Tukey HSD test (Zar 1984) was performed to determine between which age groups significant differences occur. Mann Whitney-U tests (Zar 1984) were used to determine significant differences between reproductive and non-reproductive males and females respectively. No distinction was made between males and females caught from different localities. The morphometric data from all mole-rat skulls were pooled to investigate if reproductive animals tended to be the oldest individuals in the colony. All statistical tests were performed using Statistica version 5.0™.

*Morphometric analyses*

*Skull measurements*

For this particular part of the study males (M) and females (F) were sampled from four different localities Pretoria (25°45'S 28°10'E) (M = 28; F = 64), Johannesburg (26°12' 28°05'E) (M = 36; F = 62), Vanderbijlpark (26°42'S 27°49'E) (M = 4; F = 8) and the Krugersdorp (26°06'S 27°46'E) (M = 3; F = 6) areas. Enabling us to check for any geographical differences between populations, sampling was done at four separate geographic locations. Twenty-one cranial measurements were recorded (to the nearest 0,05mm) for each skull, using Mitutoyo$^{R}$ digital callipers. All the measurements are illustrated in Plates 10 a - e.

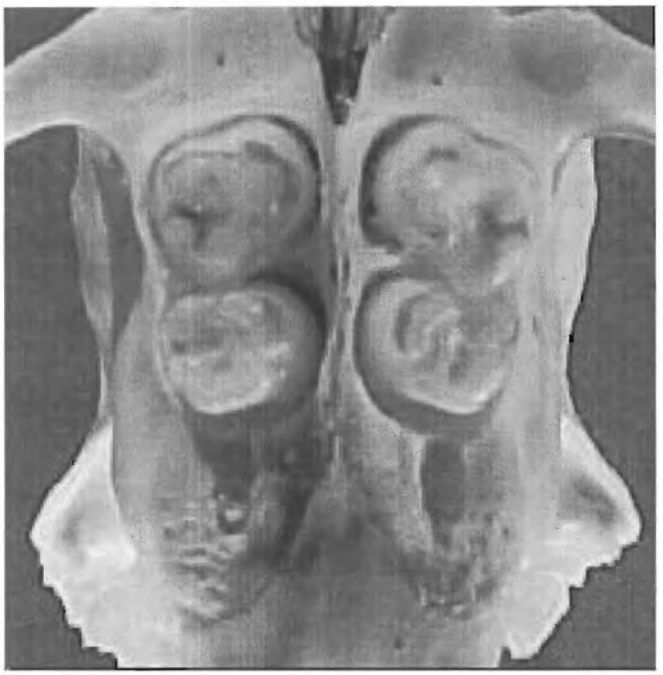

Plate 1. Relative age class 1.

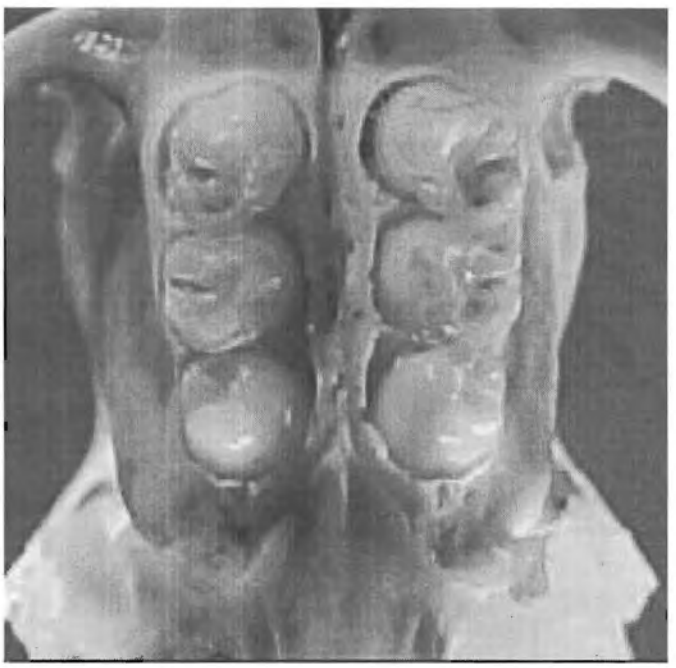

Plate 2. Relative age class 2.

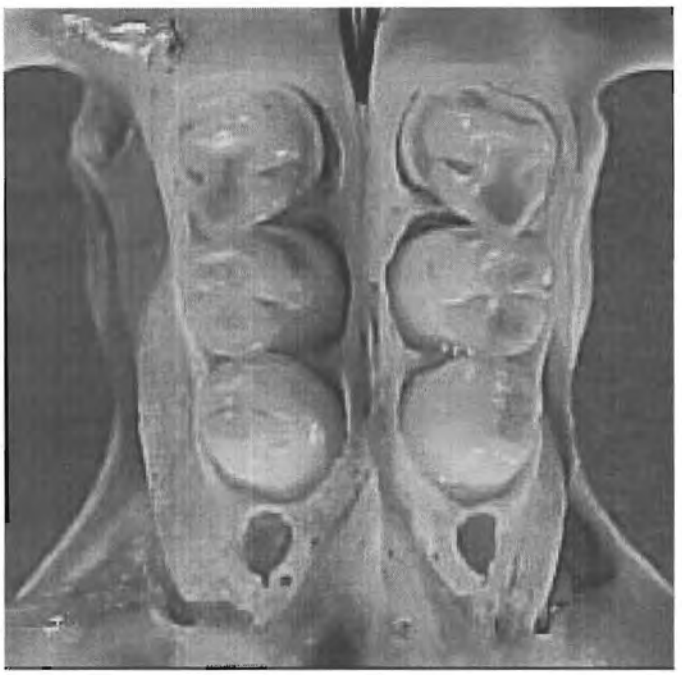

Plate 3. Relative age class 3.

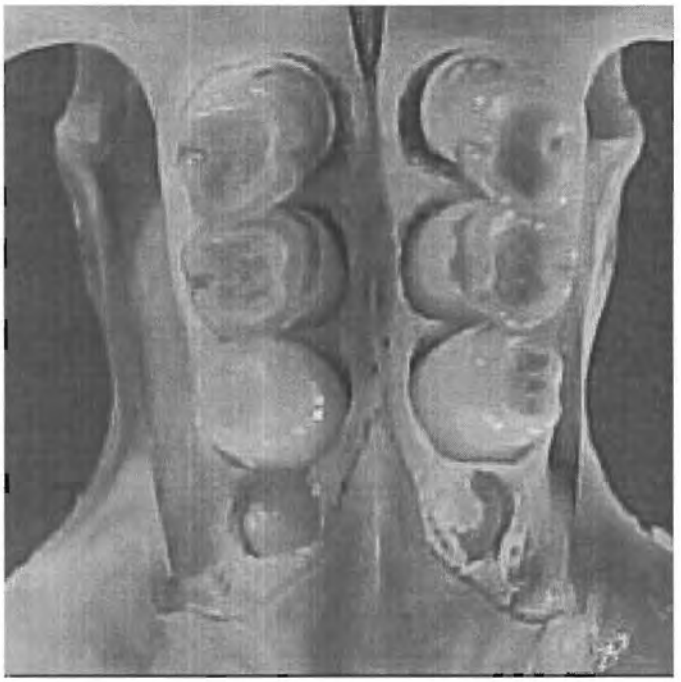

Plate 4. Relative age class 4.

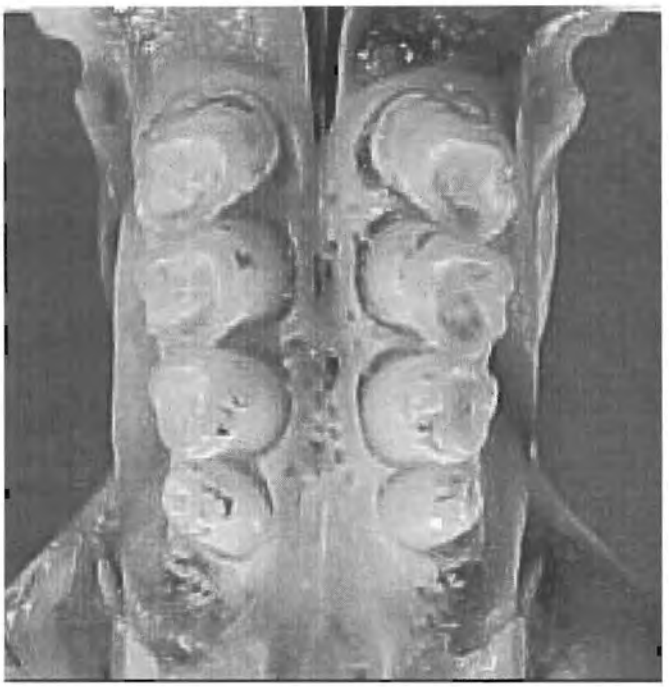

Plate 5. Relative age class 5.

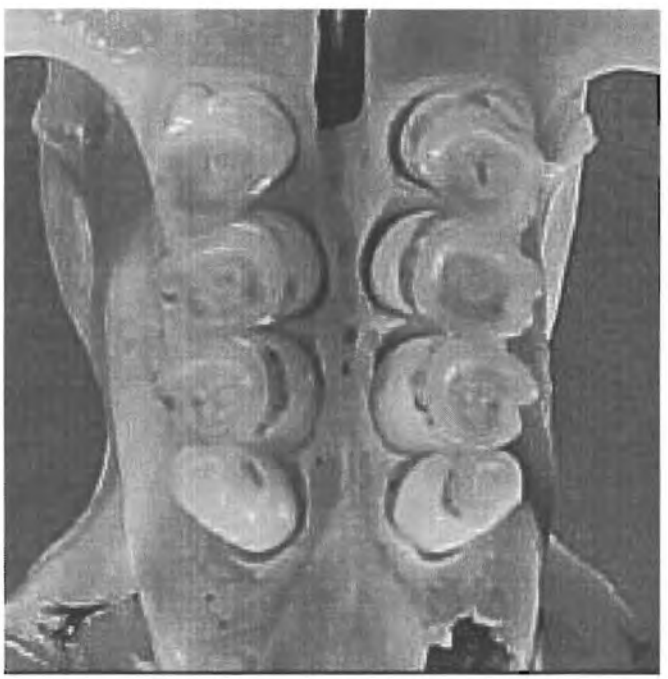

Plate 6. Relative age class 6.

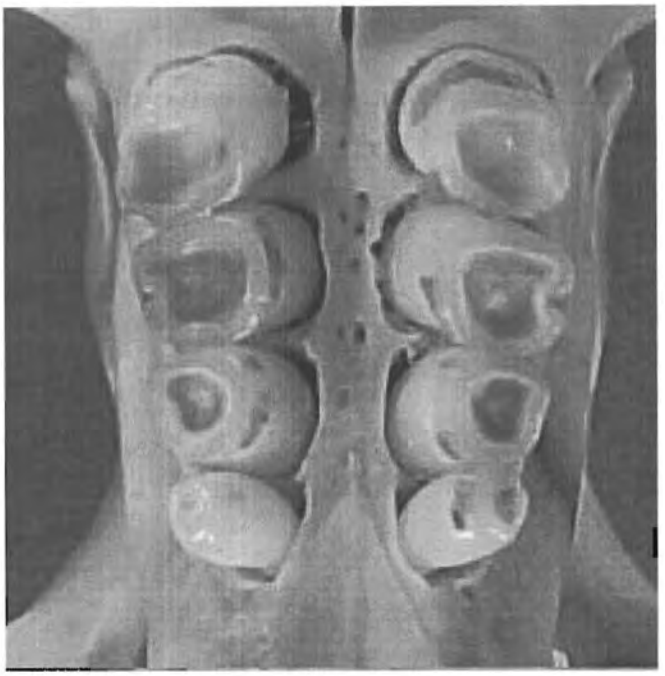

Plate 7. Relative age class 7.

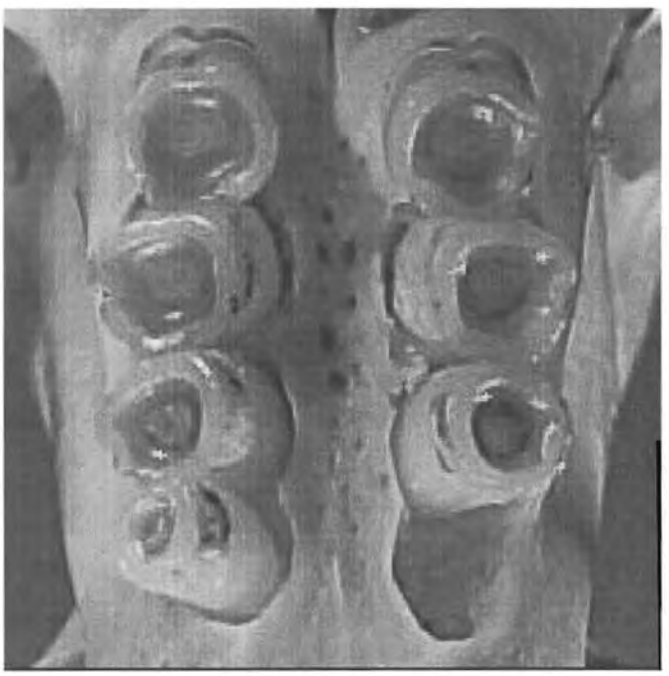

Plate 8. Relative age class 8.

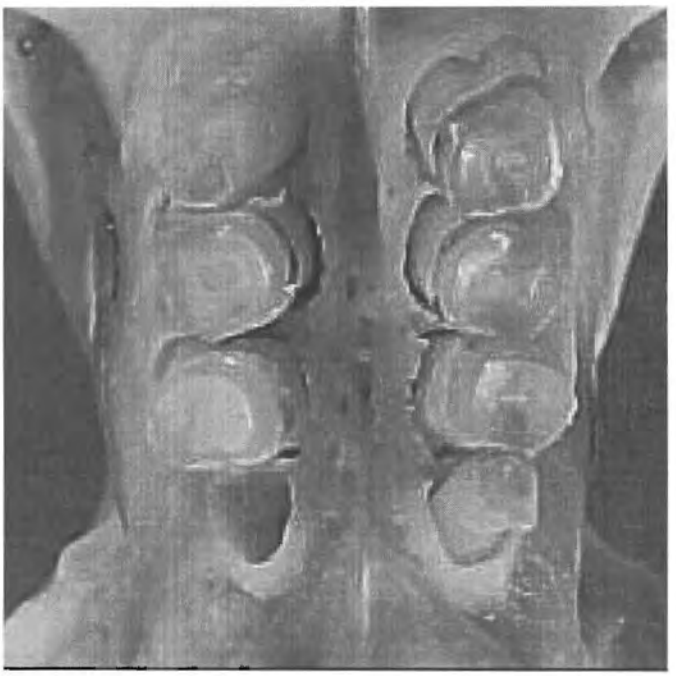

Plate 9. Relative age class 9.

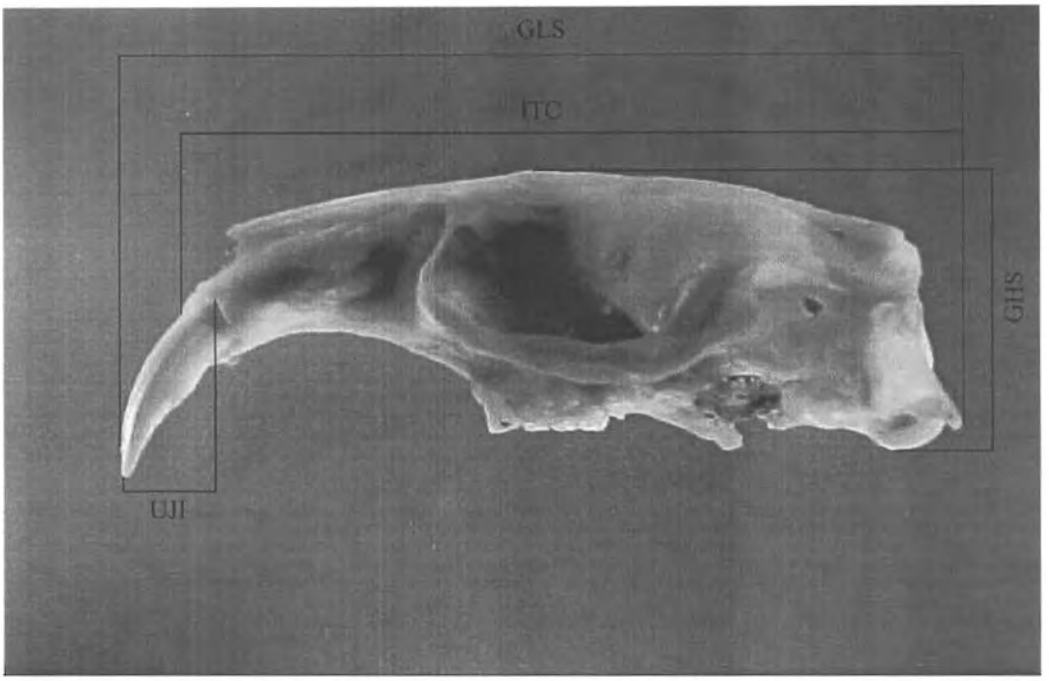


Plate 10 a.

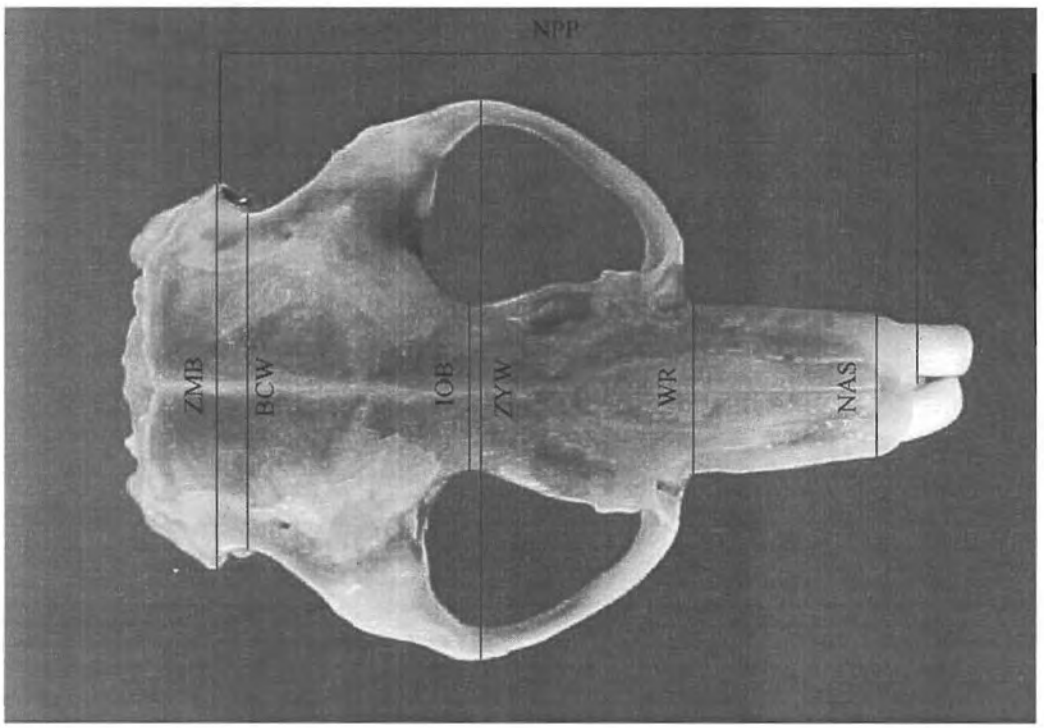


Plate 10 b.

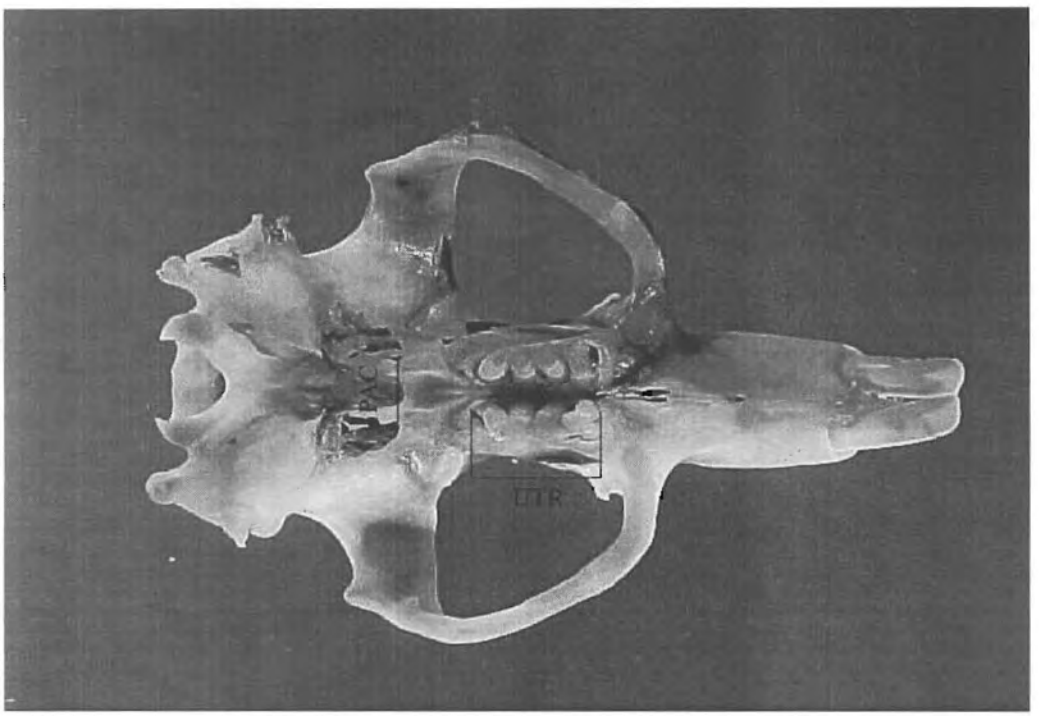


Plate 10 c.

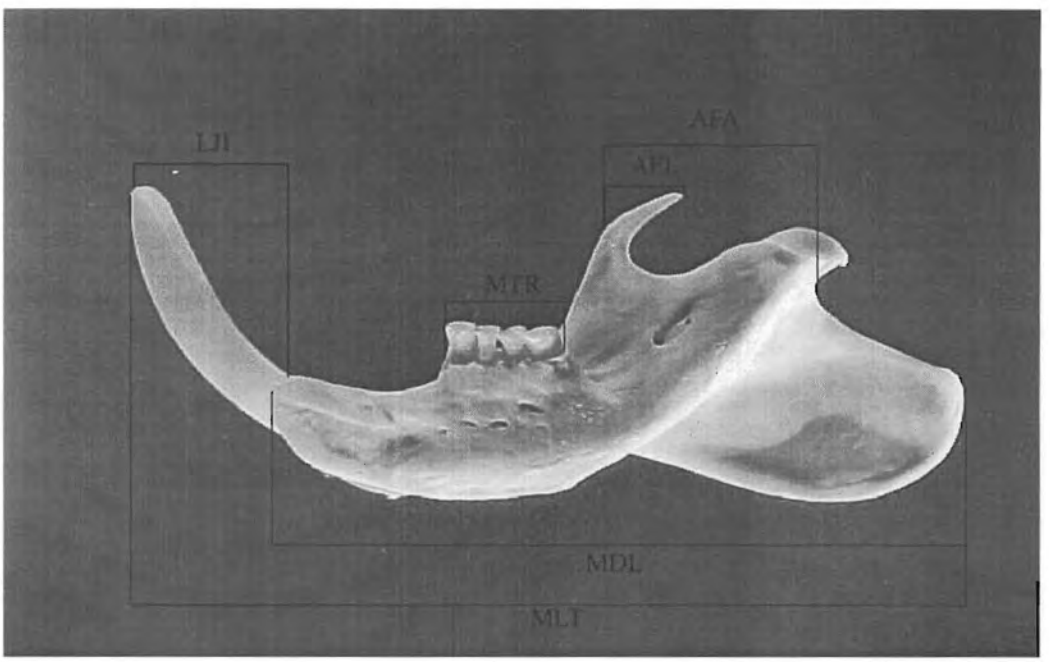


Plate 10 d.

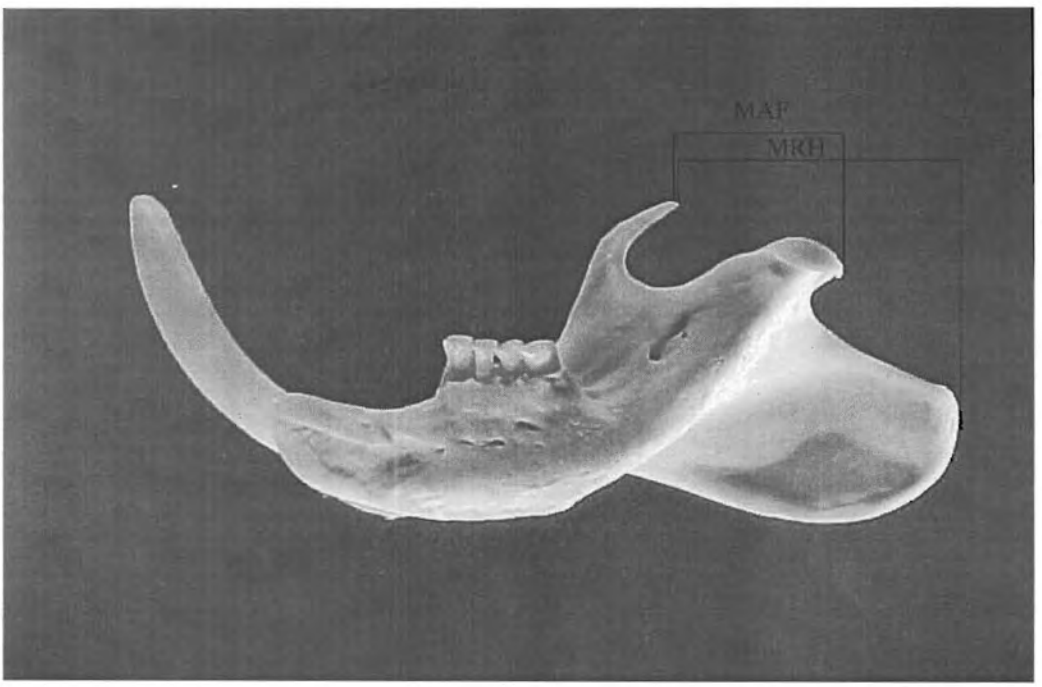


Plate 10 e.

Description of measurement taken (Plates 10 a – e):

1. GLS: Greatest length of skull, from the tip of the front incisors to the posterior part of the skull.
2. ITC: Incisor to condyle length, from anterior surface of incisor at alveolus to most posterior projection of the occipital condyle.
3. BCW: Brain case breadth, the widest measurement of the brain case taken dorsally.
4. ZMB: Zygomatic breadth, greatest width of skull, taken between zygomatic processes of squamosals, in dorsal view.
5. ZYW: Greatest zygomatic width, between outer margins of zygomatic arches, perpendicular to longitudinal axis of skull.
6. IOB: Least breadth of interorbital constriction, least distance dorsally between orbits.
7. WR: Width of the rostrum.
8. NAS: Nasal width, at anterior most point where nasals join premaxillae.
9. UTR: Crown length of maxillary tooth row, from the anterior edge of first molar to the posterior edge of the last molar.
10. PAC: Hard palate width at point of constriction immediately posterior to the last molar.
11. NPP: Distance from anterior edge of nasals to anterior edge of posterior part of zygomatic arch.
12. GHS: Greatest height of skull, perpendicular to horizontal plane through bullae.
13. MLT: Greatest length of mandible, including the teeth, from posterior surface of condylar process to the tip of the incisor.
14. MDL: Greatest length of mandible (excluding teeth), from posterior surface of condylar process to anteroventral edge of incisor alveolus.
15. MTR: Mandibular tooth row length, from anterior edge of the first molar alveolus to posterior edge of the last molar alveolus.
16. AFL: Articular facet length to posterior edge of molar number four.
17. MAF: Mandibular foramen-articular facet length, from the ventral edge of mandibular foramen to midposterodorsal edge of articulating facet.

18. AFA: Articular facet to the middle of the angular process.
19. MRH: Mandible-ramus height, from dorsal edge of coronoid process to ventral edge of angular process.
20. UJI: Upper jaw incisor length, measured from the tip of the incisors to the base, where the teeth connect to the skull.
21. LJI: Lower jaw incisor length, measured from the tip of the incisor to the base, where the teeth connect to the skull.

After data screening it was decided that only age class 6, 8 and 9 from Pretoria and age class 6 from Johannesburg could be used to determine sexual dimorphism. Since only these specific age classes had the required number of males and females to prove the analyses viable. This selection was subjected to one way analyses of variance (ANOVA) (Zar 1984). Ideally, we would have preferred to include age class 8 and 9 from Johannesburg to the analyses, but insufficient males hampered the analyses.

Further statistical analyses included *a posteriori* Student Newman-Keuls (SNK) tests (Zar 1984) for maximally non-significant subsets ($p<0.05$, SNK). The analyses were done separately for each location. Due to the absence of sexual dimorphism (See results) males and females were pooled together for the SNK analyses. This univariate analyses proved to be inconclusive and as a result of variation due to sex and age, the data was examined using principal component analyses (PCA) and unweighted pair-group arithmetic average cluster analyses (UPGMA) (Zar 1984).

Unlike univariate analyses, the multi-range tests, UPGMA and PCA enabled us to analyse all of the age classes including the poorly represented classes in all four of the localities. Both statistical tests were performed to visualise the data in such a way that the broader pattern would be exhibited. The UPGMA cluster analyses proved to be helpful in determining the groupings of age groups and the sexes. Once established that no definite groupings of males and females existed, discriminant analyses were performed to determine the groupings of the relative age classes. Canonical variates analyses (CVA) of the age classes were done for each separate locality. All the analyses were based on the 21 measurements taken for each individual. Standard statistics tables

for all 21 measurements were drawn for each locality separately. All statistical analyses were undertaken using Statistica version 5.0™.

## RESULTS

*Age determination*

All age determination and morphometric data are expressed as mean ± S.E. (standard error). All mass and age data collected for both reproductive (n = 69) and non-reproductive (n = 266) animals from four localities (as mentioned in Materials & Methods, Morphometric analyses) were pooled. A separate mean value for reproductive and non-reproductive animals was calculated for each of the monthly samples. The values were plotted (Fig. 1), to determine any relationship that might exist between the mass of an animal, the age class it belongs to and its reproductive status. The non-reproductive animals and the reproductive animals grouped separately, into two defined clusters. The entire group of reproductive animals exhibited an increased mass and were all allocated to age classes 5 to 9 (Fig. 1).

During sampling, the sex ratio was always in favour of females. Separating the male and female mass and age data (Fig. 2), revealed that the mean mass of the males (99.78 ± 33.51, n = 96) was significantly higher than the mean mass of the females (90.95 ± 25.62, n = 184) (MANOVA, $F = 46.53$; $p<0.001$), although the sample size for the males (n = 96) was much smaller, than that of the females (n = 184).

The total number of males (n(M) = 96) and females (n(F) = 184) sampled, were grouped together with regard to their masses and relative ages (Fig. 3). The graph shows an increase in mass synonymous with an increase in age. Statistical analyses indicate that there is a significant difference with regard to mass between the nine age classes (Mann Whitney-U test, $U = 7316.50$, $p<0.001$). A *post hoc* Tukey HSD test was performed to determine between which age groups a significant difference occurred (Table 1.1). Results of the statistical test revealed no significant differences between age class 1-3, but highly significant differences occurred between the latter and the remainder of the age

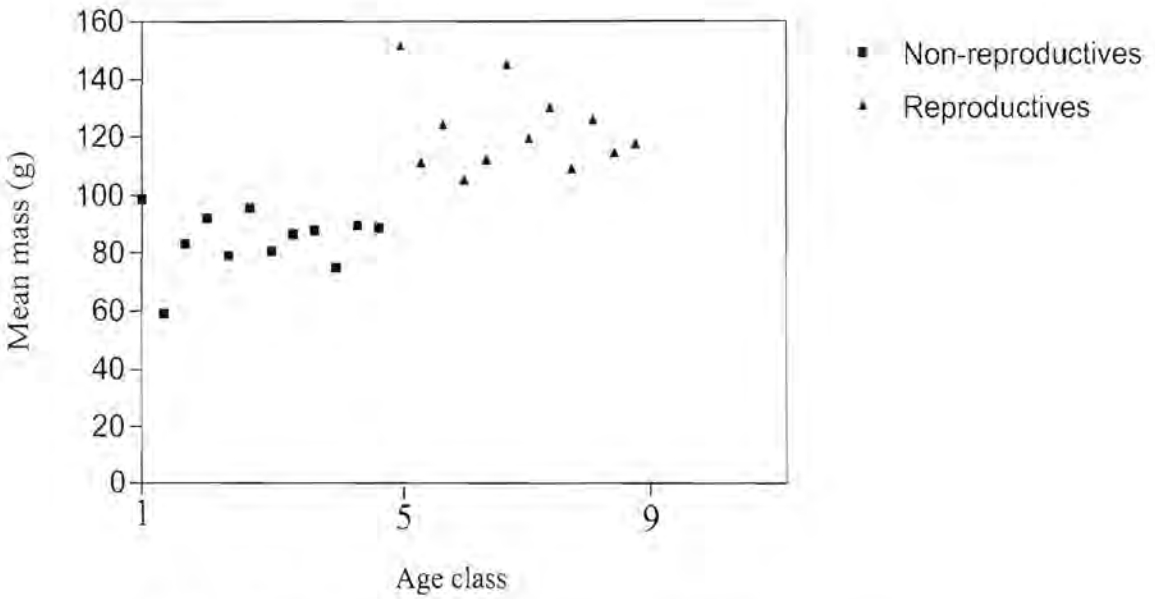


Fig. 1. The difference in mass for both non-reproductive and reproductive animals form different age classes.

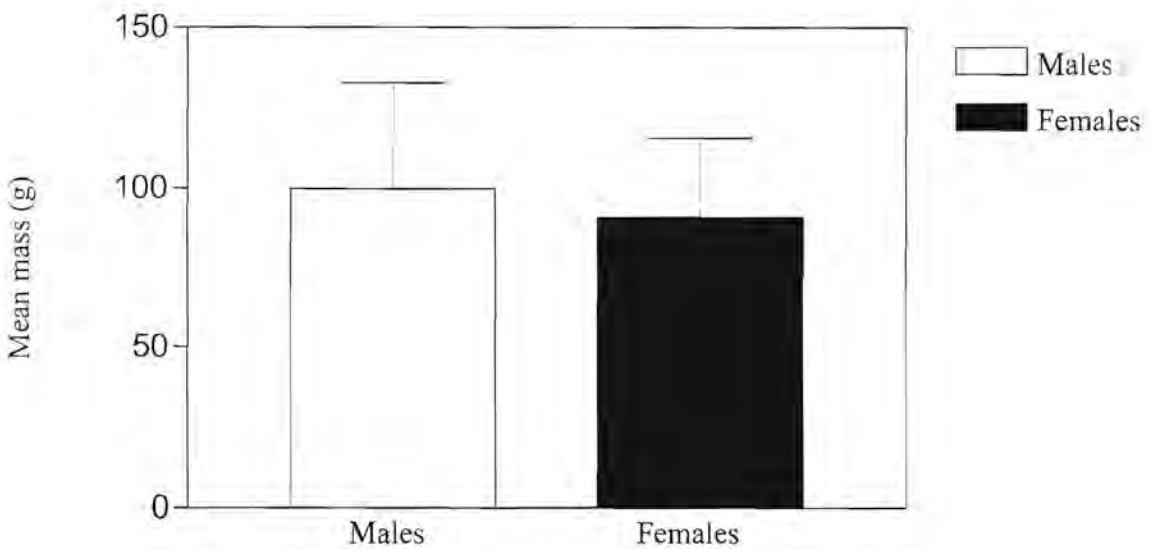


Fig. 2. The mean ± S.E. body mass of all males and females.

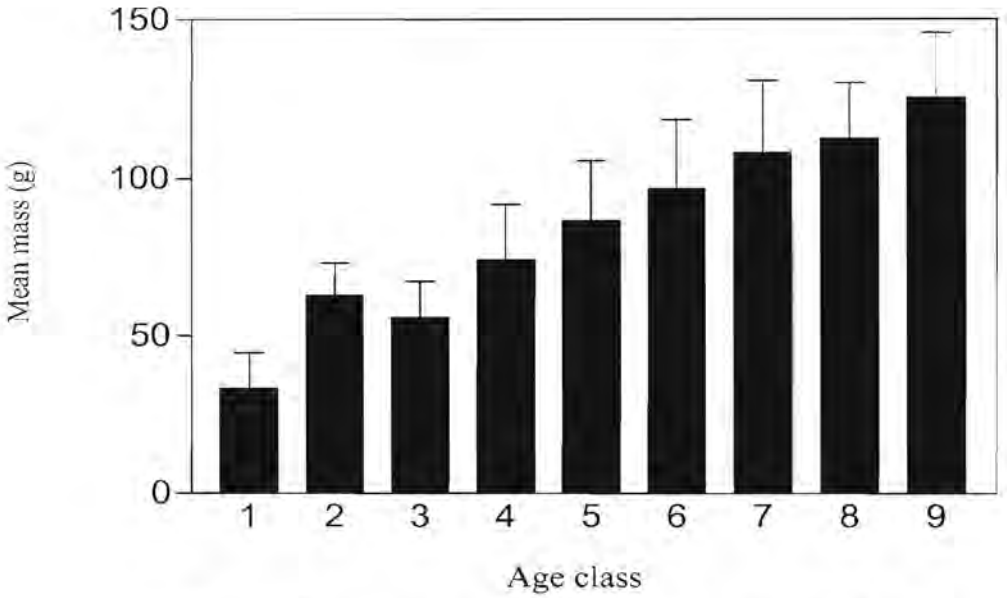


Fig. 3. The mean mass ± S.E. in both males and females from different age classes sampled during 1998/1999.

**Table 1.1.** The mean ± S.E. for both sexes of *C. h. pretoriae*. (Tukey HSD test, no letters in common denote significant differences).

| Age class | n | Mean ± S.E. | |
|---|---|---|---|
| 1 | 10 | 33.61 ± 3.52 | a |
| 2 | 5 | 62.96 ± 4.74 | ad |
| 3 | 16 | 55.93 ± 2.90 | ac |
| 4 | 29 | 74.38 ± 3.26 | dce |
| 5 | 49 | 86.71 ± 2.70 | ef |
| 6 | 66 | 96.92 ± 2.66 | f |
| 7 | 32 | 108.12 ± 4.00 | fg |
| 8 | 47 | 112.65 ± 2.55 | gh |
| 9 | 23 | 125.61 ± 4.21 | h |

classes. No significant differences occurred between age class 4 and 5, 6 and 7, 7 and 8 or 8 and 9) (Tukey HSD test, $p<0.05$).

Separating the data for males and females, it was found that the females had a continuous increase in mass with an increase in age (Fig. 4). Highly significant differences occurred between reproductive ($n = 29$) and non-reproductive females ($n = 151$) with regard to mass (Mann Whitney-U test, $U = 741.50$, $p<0.001$) and age (Mann Whitney-U test, $U = 653.50$, $p<0.001$). Significant differences that occurred between age classes are presented in Table 1.2 (Tukey HSD test, $p<0.05$).

In Fig. 5. reproductive males ($n = 40$) have a significantly higher mean mass (Mann Whitney-U test, $U = 142.50$, $p<0.001$) than non-reproductive males ($n = 56$). Highly significant differences occurred between reproductive and non-reproductive males with regard to age (Mann Whitney-U test, $U = 420.50$, $p<0.001$). The heaviest males were all grouped in age class 8, unlike Fig. 4 where the heaviest females were all grouped in age class 9. Age class 2 for males showed an increased mass, higher than the mass increase of age class 3, however this difference was not statistically significant (Fig. 5). A similar trend was observed in females and again this difference proved not to be statistically significant (Fig. 4). The presence of statistically significant differences between age classes are presented in Table 1.3 (Tukey HSD test, $p<0.05$).

*Morphometric analyses*

*Univariate analyses*

Skulls ranging from age classes 1 to 9 were available for this part of the study, however, because of damage to several skulls only a select few could be used for morphometric measurements. Samples collected from Johannesburg included age classes 2-9, from Pretoria age classes 3-9, from Vanderbijlpark age classes 5-9 and from Krugersdorp age classes 5-9.

The results of the one-way ANOVA on the skull measurements of age class 6, 8 and 9 of mole-rats sampled in Pretoria and age class 6 of mole-rats sampled in Johannesburg, revealed no apparent sexual dimorphism in the highveld mole-rat colonies (Table 2).

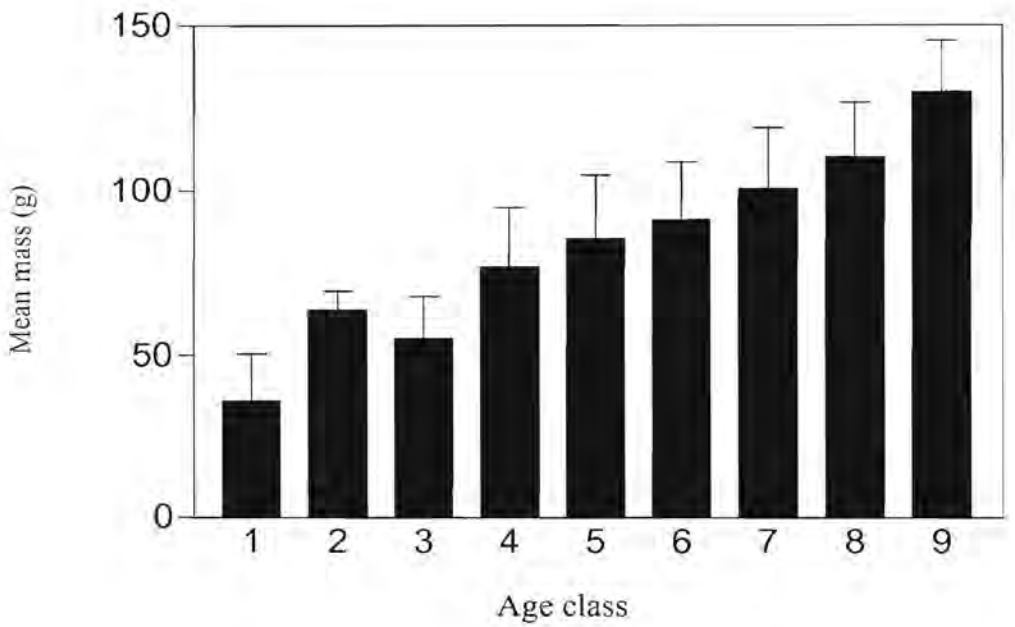


Fig. 4. The mean ± S.E. mass of females from nine relative age classes.

**Table 1.2.** The mean ± S.E. for all females sampled (Tukey HSD test, no letters in common denote significant differences).

| Age class | n | Mean ± S.E. | |
|---|---|---|---|
| 1 | 5 | 35.92 ± 6.48 | a |
| 2 | 2 | 64.05 ± 4.05 | abc |
| 3 | 10 | 55.22 ± 4.10 | a |
| 4 | 19 | 76.65 ± 4.20 | b |
| 5 | 36 | 85.33 ± 3.26 | b |
| 6 | 45 | 91.03 ± 2.63 | bc |
| 7 | 21 | 100.61 ± 4.03 | cd |
| 8 | 31 | 110.34 ± 2.92 | de |
| 9 | 10 | 129.70 ± 5.02 | e |

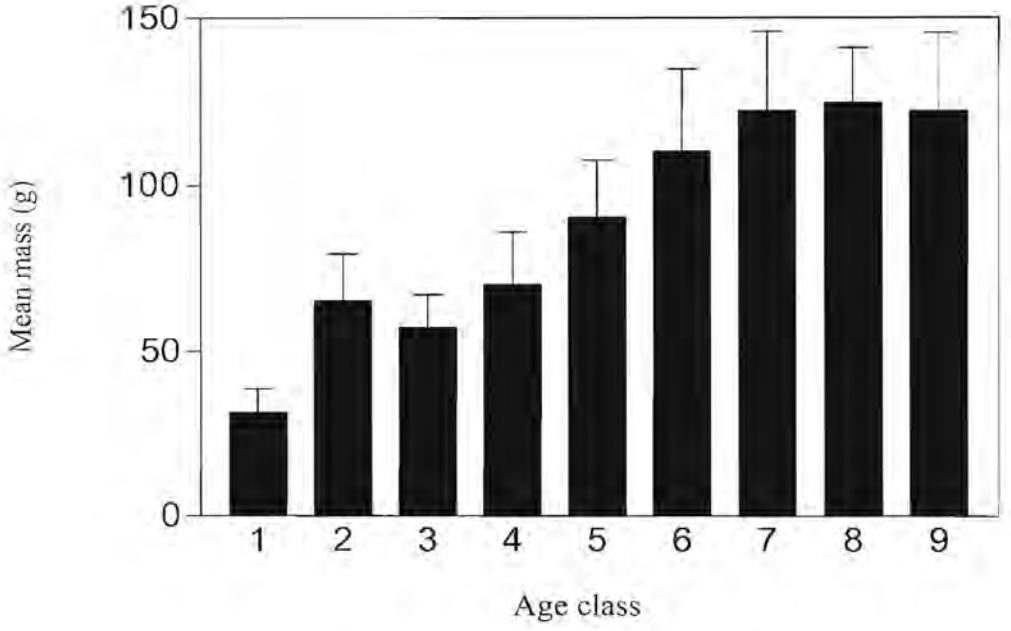


Fig. 5. The mean ± S.E. mass of males from nine relative age classes.

**Table 1.3**. The mean ± S.E. for all males sampled. (Tukey HSD test, no letters in common denote significant differences).

| Age class | n | Mean ± S.E. | |
|---|---|---|---|
| 1 | 5 | 31.30 ± 3.34 | a |
| 2 | 3 | 65.23 ± 8.28 | abc |
| 3 | 6 | 57.12 ± 4.04 | abc |
| 4 | 10 | 70.06 ± 5.07 | bc |
| 5 | 13 | 90.53 ± 4.73 | cd |
| 6 | 20 | 110.14 ± 5.52 | de |
| 7 | 11 | 122.45 ± 7.12 | e |
| 8 | 15 | 124.94 ± 4.17 | e |
| 9 | 13 | 122.47 ± 6.41 | e |

**Table 2** Results of one-way analyses of variance (ANOVA), to indicate significant differences (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$) between all measurements within age classes 6,8 and 9 from Pretoria (PTA) and age class 6 from Johannesburg (JHB). Measurements are defined in Plates 10a -e.

| | Class 6 PTA | Class 8 PTA | Class 9 PTA | Class 6 JHB |
|---|---|---|---|---|
| Measurements | F-values | F-values | F-values | F-values |
| GLS | 3.38 | 3.10 | 0.00 | 5.42 * |
| ITC | 3.28 | 5.61 * | 0.28 | 2.78 |
| BCW | 2.51 | 1.53 | 1.00 | 0.19 |
| ZMB | 0.53 | 0.18 | 0.22 | 0.04 |
| ZYW | 2.84 | 12.03 ** | 0.01 | 4.42 * |
| IOB | 0.69 | 0.87 | 0.01 | 0.59 |
| WR | 5.67 * | 11.34 ** | 1.99 | 6.74 * |
| NAS | 5.14 * | 15.54 *** | 3.45 | 7.87 ** |
| UTR | 0.86 | 0.32 | 0.32 | 1.53 |
| PAC | 0.78 | 0.18 | 1.55 | 0.82 |
| NPP | 0.11 | 5.84 * | 0.00 | 5.80 * |
| GHS | 5.12 * | 8.21 ** | 0.06 | 2.40 |
| MLT | 2.02 | 12.17 ** | 1.64 | 3.56 |
| MDL | 1.88 | 12.64 ** | 0.92 | 2.80 |
| MTR | 0.08 | 0.40 | 0.02 | 0.02 |
| AFL | 0.07 | 0.67 | 2.36 | 5.16 * |
| MAF | 2.71 | 8.86 ** | 1.45 | 0.09 |
| AFA | 0.46 | 15.72 *** | 0.01 | 4.61 * |
| MRH | 4.10 | 1.64 | 1.16 | 10.47 ** |
| UJI | 1.88 | 0.51 | 3.73 | 1.72 |
| LJI | 3.28 | 0.46 | 4.17 | 6.94 * |

The absence of sexual dimorphism amongst the sexes allowed the pooling of data for further analyses. The SNK test showed that to a large extent age classes 1 to 5 grouped together, while age classes 6 – 9 grouped together. In a few instances age class 5 grouped with age classes 6 – 9, whereas age group 6 sometimes grouped with age classes 1 – 5 (Table 3: Johannesburg). No animals from age class 1 were caught in Johannesburg. A similar trend was found in the mole-rats sampled from Pretoria (Table 3: Pretoria). Measurement IOB, PAC and GHS show no significant differences between any of the age classes in Pretoria (Table 3: Pretoria). No animals from age class 1 or 2 were sampled during fieldwork in the Pretoria area.

No animals from age classes 1, 2, 3 and 4 were collected in Vanderbijlpark (Table 3: Vanderbijlpark). Due to the small sample sizes (n = 1) of age class 5 and 9 no mean or standard error could be calculated for these particular age classes. The following measurements: ZMB, UTR, PAC, NPP, MTR, MAF and LJI, showed no significant differences between the various age classes.

In the fourth location, Krugersdorp, no animals from age class 1,2,3, and 4 were captured (Table 3: Krugersdorp). Only one animal was sampled for age class 5 and 7 respectively and only one male and one female sampled for age class 6. No significant differences were present in any of the measurements.

The SNK values for Vanderbijlpark as well as Krugersdorp supports the trend found in Johannesburg and Pretoria. Although only values for age class 6, 7 and 8 for Vanderbijlpark and values for age class 6, 8 and 9 are available for Krugersdorp, these groups almost always showed no significant differences between them with regard to the various measurements (Table 3: Vanderbijlpark and Krugersdorp).

The standard statistics for 21 measurements per locality for each age class are presented in Appendix 1 (Johannesburg), Appendix 2 (Pretoria), Appendix 3 (Vanderbijlpark), Appendix 4 (Krugersdorp).

*Multivariate analyses*

The relatively large sample size from Johannesburg and Pretoria were the focus for the multivariate assessment, since these samples included almost all of the age groups and both sexes were represented sufficiently.

**Table 3.** Multiple range SNK tests of age classes in *C. h. pretoriae* from a) Johannesburg, b) Pretoria, c) Vanderbijlpark and d) Krugersdorp. The sample size (n), mean ± S.E. (standard error) for each each measurement are indicated. NS = no significant difference between the age classes. No letters in common denote significant differences at $p<0.05$. Measurements defined in Plates 10a-e.

**a) Johannesburg**

| Measurement | Age class (n) | Mean ± S.E. | | Measurement | Age class (n) | Mean ± S.E. | |
|---|---|---|---|---|---|---|---|
| GLS | 2 (2) | 32.30 ± 1.07 | a | GHS | (2) | 12.74 ± 0.58 | NS |
| | 3 (3) | 33.90 ± 1.31 | ac | | 3 (3) | 13.53 ± 0.23 | |
| | 4 (11) | 35.74 ± 0.78 | bc | | 4 (11) | 13.83 ± 0.18 | |
| | 5 (30) | 36.57 ± 0.34 | bc | | 5 (30) | 13.93 ± 0.21 | |
| | 6 (30) | 38.08 ± 0.37 | bd | | 8 (8) | 14.80 ± 0.25 | |
| | 8 (8) | 39.69 ± 0.69 | f | | 7 (8) | 14.90 ± 0.25 | |
| | 7 (8) | 40.09 ± 0.70 | f | | 6 (30) | 15.15 ± 0.87 | |
| | 9 (6) | 40.48 ± 0.52 | f | | 9 (6) | 15.29 ± 0.38 | |
| ITC | 2 (2) | 28.75 ± 1.06 | a | MLT | 2 (2) | 29.13 ± 1.72 | a |
| | 3 (3) | 30.37 ± 0.74 | ac | | 3 (3) | 30.73 ± 0.73 | ab |
| | 5 (30) | 32.44 ± 0.41 | bc | | 4 (11) | 32.54 ± 0.95 | ace |
| | 4 (11) | 32.65 ± 0.89 | bc | | 5 (30) | 33.71 ± 0.39 | be |
| | 6 (30) | 33.91 ± 0.36 | b | | 6 (30) | 35.32 ± 0.48 | cef |
| | 8 (8) | 35.24 ± 0.55 | b | | 8 (8) | 37.79 ± 0.95 | f |
| | 9 (6) | 35.69 ± 0.68 | b | | 7 (8) | 38.21 ± 0.75 | f |
| | 7 (8) | 35.95 ± 0.59 | b | | 9 (6) | 38.98 ± 0.89 | f |
| BCW | 2 (2) | 15.68 ± 0.47 | a | MDL | 2 (2) | 22.47 ± 2.51 | a |
| | 4 (11) | 17.09 ± 0.40 | bc | | 3 (3) | 23.44 ± 0.26 | ab |
| | 3 (3) | 17.52 ± 0.81 | bd | | 4 (11) | 24.97 ± 0.64 | ace |
| | 5 (30) | 17.90 ± 0.18 | bcde | | 5 (30) | 25.90 ± 0.32 | be |
| | 6 (30) | 18.05 ± 0.18 | bcde | | 6 (30) | 26.97 ± 0.34 | cef |
| | 8 (8) | 18.44 ± 0.15 | bcde | | 8 (8) | 29.12 ± 0.88 | f |
| | 7 (8) | 19.10 ± 0.29 | de | | 7 (8) | 29.47 ± 0.84 | f |
| | 9 (6) | 19.55 ± 0.42 | e | | 9 (6) | 29.62 ± 0.87 | f |
| ZMB | 2 (2) | 14.58 ± 0.12 | a | MTR | 2 (2) | 5.15 ± 0.41 | a |
| | 3 (3) | 15.05 ± 0.27 | ab | | 3 (3) | 5.52 ± 0.35 | a |
| | 4 (11) | 15.50 ± 0.27 | ab | | 4 (11) | 6.36 ± 0.22 | b |
| | 5 (30) | 15.96 ± 0.32 | ab | | 7 (8) | 6.41 ± 0.15 | b |
| | 8 (8) | 16.05 ± 0.40 | ab | | 9 (6) | 6.44 ± 0.15 | b |
| | 6 (30) | 16.16 ± 0.15 | ab | | 8 (8) | 6.55 ± 0.14 | b |
| | 7 (8) | 16.30 ± 0.16 | ab | | 6 (30) | 6.64 ± 0.04 | b |
| | 9 (6) | 17.06 ± 0.68 | b | | 5 (30) | 6.67 ± 0.05 | b |
| ZYW | 2 (2) | 19.98 ± 0.98 | a | AFL | 2 (2) | 0.38 ± 0.27 | a |
| | 3 (3) | 21.60 ± 0.50 | ab | | 3 (3) | 0.32 ± 0.18 | b |

| | | | |
|---|---|---|---|
| | 4 (11) | 22.24 ± 0.54 | ab |
| | 5 (30) | 23.38 ± 0.31 | bc |
| | 6 (30) | 24.84 ± 0.31 | cd |
| | 8 (8) | 26.12 ± 0.68 | de |
| | 7 (8) | 26.48 ± 0.48 | de |
| | 9 (6) | 27.55 ± 0.72 | e |
| IOB | 5 (30) | 7.73 ± 0.08 | NS |
| | 4 (11) | 7.78 ± 0.09 | |
| | 6 (30) | 7.78 ± 0.06 | |
| | 7 (8) | 7.98 ± 0.19 | |
| | 8 (8) | 7.82 ± 0.23 | |
| | 9 (6) | 8.05 ± 0.09 | |
| | 3 (3) | 8.14 ± 0.24 | |
| | 2 (2) | 8.25 ± 0.95 | |
| WR | 2 (2) | 5.52 ± 0.39 | a |
| | 3 (3) | 6.30 ± 0.25 | b |
| | 4 (11) | 6.31 ± 0.15 | ab |
| | 5 (30) | 6.80 ± 0.09 | bc |
| | 6 (30) | 7.04 ± 0.11 | bc |
| | 9 (6) | 7.43 ± 0.31 | c |
| | 8 (8) | 7.47 ± 0.29 | c |
| | 7 (8) | 7.60 ± 0.21 | c |
| NAS | 2 (2) | 4.29 ± 0.23 | ac |
| | 3 (3) | 4.86 ± 0.37 | abc |
| | 4 (11) | 4 97 ± 0.12 | abc |
| | 5 (30) | 5.50 ± 0.08 | bde |
| | 6 (30) | 5.81 ± 0.11 | df |
| | 7 (8) | 6.29 ± 0.20 | df |
| | 9 (6) | 6.38 ± 0.31 | df |
| | 8 (8) | 6.59 ± 0.38 | fe |
| UTR | 3 (3) | 5.35 ± 0.13 | a |
| | 2 (2) | 5.54 ± 0.08 | a |
| | 4 (11) | 6.65 ± 0.15 | b |
| | 7 (8) | 6.76 ± 0.14 | b |
| | 5 (30) | 6.77 ± 0.06 | b |
| | 8 (8) | 6.78 ± 0.15 | b |
| | 6 (30) | 6.87 ± 0.07 | b |
| | 9 (6) | 7.04 ± 0.15 | b |
| PAC | 2 (2) | 3.36 ± 0.47 | NS |
| | 4 (11) | 3.43 ± 0.13 | |
| | 7 (8) | 3.49 ± 0.23 | |
| | 6 (30) | 3.71 ± 0.11 | |
| | 5 (30) | 3.77 ± 0.08 | |
| | 9 (6) | 3.84 ± 0.30 | |
| | 3 (3) | 3.91 ± 0.26 | |
| | 8 (8) | 4.23 ± 0.42 | |

| | | | |
|---|---|---|---|
| | 4 (11) | 6.91 ± 0.31 | b |
| | 5 (30) | 7.39 ± 0.13 | b |
| | 6 (30) | 7.68 ± 0.15 | b |
| | 7 (8) | 8.00 ± 0.27 | b |
| | 9 (6) | 8.12 ± 0.50 | b |
| | 8 (8) | 8.22 ± 0.36 | b |
| MAF | 2 (2) | 6.85 ± 0.17 | a |
| | 3 (3) | 7.21 ± 0.42 | ab |
| | 5 (30) | 7.77 ± 0.12 | adf |
| | 4 (11) | 7.85 ± 0.20 | ace |
| | 6 (30) | 8.26 ± 0.17 | bcd |
| | 8 (8) | 8.40 ± 0.17 | bcf |
| | 7 (8) | 8.43 ± 0.32 | bce |
| | 9 (6) | 9.39 ± 0.32 | df |
| AFA | 2 (2) | 8.24 ± 0.21 | a |
| | 3 (3) | 9.12 ± 0.41 | ab |
| | 5 (30) | 9.72 ± 0.13 | be |
| | 4 (11) | 9.89 ± 0.19 | bde |
| | 6 (30) | 10.36 ± 0.16 | def |
| | 8 (8) | 10.51 ± 0.27 | def |
| | 7 (8) | 10.92 ± 0.38 | def |
| | 9 (6) | 11.38 ± 0.34 | f |
| MRH | 2 (2) | 12.69 ± 0.56 | a |
| | 3 (3) | 13.46 ± 0.38 | ab |
| | 4 (11) | 14.12 ± 0.46 | ac |
| | 5 (30) | 14.91 ± 0.24 | bc |
| | 6 (30) | 15.89 ± 0.28 | cd |
| | 8 (8) | 16.73 ± 0.58 | d |
| | 7 (8) | 17.25 ± 0.39 | d |
| | 9 (6) | 17.61 ± 0.66 | d |
| UJI | 3 (3) | 7.37 ± 0.32 | ac |
| | 2 (2) | 7.46 ± 0.27 | a |
| | 5 (30) | 8.87 ± 0.16 | bc |
| | 4 (11) | 9.18 ± 0.30 | b |
| | 8 (8) | 9.22 ± 0.52 | b |
| | 6 (30) | 9.33 ± 0.24 | b |
| | 7 (8) | 9.67 ± 0.29 | b |
| | 9 (6) | 10.13 ± 0.41 | b |
| LJI | 2 (2) | 11.77 ± 0.08 | a |
| | 3 (3) | 12.45 ± 0.38 | ab |
| | 4 (11) | 13.65 ± 0.70 | acd |
| | 5 (30) | 13.88 ± 0.18 | ade |
| | 6 (30) | 14.53 ± 0.27 | bdf |
| | 8 (8) | 15.74 ± 0.69 | cef |
| | 9 (6) | 15.75 ± 0.76 | cef |
| | 7 (8) | 15.83 ± 0.59 | cef |

| | | | | | |
|---|---|---|---|---|---|
| NPP | 2 (2) | 20.66 ± 1.25 | a | | |
| | 3 (3) | 22.49 ± 0.69 | ab | | |
| | 4 (11) | 23.11± 0.62 | bc | | |
| | 5 (30) | 24.12± 0.29 | bd | | |
| | 6 (30) | 25.08 ± 0.29 | cd | | |
| | 8 (8) | 25.86 ± 0.51 | ed | | |
| | 7 (8) | 27.50 ± 0.66 | e | | |
| | 9 (6) | 27.72 ± 0.70 | e | | |

b) **Pretoria**

| | | | | | | |
|---|---|---|---|---|---|---|
| GLS | 3 (5) | 31.43 ± 5.69 | a | GHS | 3 (5) | 2.33 ± 0.38 a |
| | 4 (8) | 33.95 ± 0.50 | b | | 5 (5) | 13.10 ± 0.25 ad |
| | 5 (5) | 34.60 ± 0.49 | bc | | 4 (8) | 13.22 ± 0.27 ab |
| | 6 (24) | 36.03 ± 0.32 | c | | 6 (24) | 13.96 ± 0.17 bd |
| | 7 (14) | 37.99 ± 0.39 | d | | 7 (14) | 14.44 ± 0.20 de |
| | 8 (26) | 38.94 ± 0.38 | de | | 8 (26) | 14.82 ± 0.16 df |
| | 9 (10) | 40.06 ± 0.64 | e | | 9 (10) | 15.30 ± 0.30 ef |
| ITC | 3 (5) | 27.93 ± 4.92 | a | MLT | 3 (5) | 28.07 ± 2.97 a |
| | 4 (8) | 30.26 ± 0.44 | b | | 4 (8) | 30.33 ± 0.65 a |
| | 5 (5) | 31.33 ± 0.73 | b | | 5 (5) | 31.07 ± 0.71 a |
| | 6 (24) | 32.37 ± 0.43 | b | | 6 (24) | 32.05 ± 1.10 a |
| | 7 (14) | 34.28 ± 0.41 | ce | | 7 (14) | 35.93 ± 0.54 b |
| | 8 (26) | 35.38 ± 0.41 | cd | | 8 (26) | 36.82 ± 0.42 b |
| | 9 (10) | 36.46 ± 0.59 | de | | 9 (10) | 38.75 ± 0.95 b |
| BCW | 3 (5) | 15.78 ± 2.39 | a | MDL | 3 (5) | 21.35 ± 1.62 a |
| | 4 (8) | 16.73 ± 0.34 | ab | | 4 (8) | 23.32 ± 0.53 b |
| | 5 (5) | 16.82 ± 0.32 | ab | | 5 (5) | 23.76 ± 0.35 bc |
| | 6 (24) | 17.75 ± 0.22 | bc | | 6 (24) | 25.61 ± 0.51 cdf |
| | 7 (14) | 18.52 ± 0.26 | c | | 7 (14) | 27.37 ± 0.44 def |
| | 8 (26) | 18.67 ± 0.28 | c | | 8 (26) | 27.98 ± 0.36 ef |
| | 9 (10) | 19.08 ± 0.37 | c | | 9 (10) | 30.22 ± 0.72 g |
| ZMB | 3 (5) | 14.01 ± 1.88 | a | MTR | 3 (5) | 5.01 ± 1.86 a |
| | 4 (8) | 14.88 ± 0.17 | b | | 4 (8) | 5.57 ± 0.22 b |
| | 5 (5) | 15.03 ± 0.14 | b | | 7 (14) | 6.12 ± 0.08 c |
| | 6 (24) | 15.38 ± 0.14 | bc | | 6 (24) | 6.20 ± 0.06 c |
| | 7 (14) | 15.72 ± 0.18 | bd | | 8 (26) | 6.29 ± 0.08 c |
| | 8 (26) | 15.80 ± 0.18 | be | | 5 (5) | 6.31 ± 0.12 c |
| | 9 (10) | 16.23 ± 0.14 | cde | | 9 (10) | 6.39 ± 0.11 c |
| ZYW | 3 (5) | 20.18 ± 2.86 | a | AFL | 3 (5) | 5.64 ± 5.00 a |
| | 4 (8) | 21.27 ± 0.42 | ab | | 4 (8) | 6.22 ± 0.18 ab |
| | 5 (5) | 22.11 ± 0.49 | bc | | 5 (5) | 6.39 ± 0.28 a |
| | 6 (24) | 23.21 ± 0.25 | c | | 6 (24) | 7.02 ± 0.11 bce |
| | 7 (14) | 25.06 ± 0.39 | c | | 7 (14) | 7.30 ± 0.22 cd |
| | 8 (26) | 25.77 ± 0.36 | c | | 8 (26) | 7.80 ± 0.18 de |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 9 (10) | 27.19 ± 0.50 | c | | 9 (10) | 8.30 ± 0.37 | e |
| IOB | 5 (5) | 7.42 ± 0.14 | ac | MAF | 4 (8) | 7.16 ± 0.12 | a |
| | 3 (5) | 7.43 ± 0.29 | a | | 3 (5) | 7.18 ± 1.84 | a |
| | 4 (8) | 7.63 ± 0.08 | ac | | 5 (5) | 7.54 ± 0.25 | a |
| | 6 (24) | 7.64 ± 0.08 | ac | | 6 (24) | 7.77 ± 0.13 | a |
| | 7 (14) | 7.85 ± 0.09 | ac | | 7 (14) | 8.54 ± 0.16 | b |
| | 8 (26) | 7.90 ± 0.07 | bc | | 8 (26) | 8.60 ± 0.13 | b |
| | 9 (10) | 7.92 ± 0.11 | bc | | 9 (10) | 8.66 ± 0.25 | b |
| WR | 3 (5) | 5.43 ± 0.44 | a | AFA | 4 (8) | 8.84 ± 0.21 | a |
| | 4 (8) | 5.87 ± 0.13 | ab | | 3 (5) | 8.84 ± 1.69 | a |
| | 5 (5) | 6.14 ± 0.20 | b | | 5 (5) | 9.05 ± 0.26 | a |
| | 6 (24) | 6.42 ± 0.08 | b | | 6 (24) | 9.69 ± 0.11 | a |
| | 7 (14) | 6.95 ± 0.01 | c | | 7 (14) | 10.44 ± 0.20 | b |
| | 8 (26) | 7.31 ± 0.12 | c | | 8 (26) | 10.56 ± 0.13 | b |
| | 9 (10) | 7.39 ± 0.21 | c | | 9 (10) | 11.17 ± 0.45 | b |
| NAS | 3 (5) | 4.13 ± 0.82 | a | MRH | 3 (5) | 12.29 ± 1.36 | a |
| | 4 (8) | 4.65 ± 0.14 | ab | | 4 (8) | 12.89 ± 0.34 | a |
| | 5 (5) | 5.00 ± 0.22 | bc | | 5 (5) | 13.75 ± 0.42 | ab |
| | 6 (24) | 5.24 ± 0.07 | cd | | 6 (24) | 14.73 ± 0.20 | bc |
| | 7 (14) | 5.18 ± 0.11 | e | | 7 (14) | 16.11 ± 0.28 | de |
| | 8 (26) | 6.04 ± 0.12 | e | | 8 (26) | 16.20 ± 0.38 | cd |
| | 9 (10) | 6.36 ± 0.23 | e | | 9 (10) | 17.73 ± 0.58 | e |
| UTR | 3 (5) | 5.43 ± 0.69 | a | UJI | 3 (5) | 6.95 ± 2.41 | a |
| | 4 (8) | 6.05 ± 0.19 | b | | 5 (5) | 7.53 ± 0.45 | ac |
| | 6 (24) | 6.32 ± 0.07 | bd | | 4 (8) | 7.81 ± 0.45 | ab |
| | 5 (5) | 6.42 ± 0.12 | bc | | 6 (24) | 7.91 ± 0.12 | ace |
| | 7 (14) | 6.48 ± 0.10 | be | | 7 (14) | 8.63 ± 0.17 | bc |
| | 8 (26) | 6.56 ± 0.07 | cde | | 9 (10) | 8.66 ± 0.45 | bc |
| | 9 (10) | 6.85 ± 0.10 | cde | | 8 (26) | 9.00 ± 0.19 | be |
| PAC | 3 (5) | 3.17 ± 1.26 | a | LJI | 3 (5) | 10.45 ± 1.99 | a |
| | 4 (8) | 3.53 ± 0.13 | ab | | 4 (8) | 12.33 ± 0.63 | b |
| | 5 (5) | 3.71 ± 0.14 | b | | 5 (5) | 12.48 ± 0.32 | b |
| | 7 (14) | 3.79 ± 0.10 | b | | 6 (24) | 13.09 ± 0.25 | b |
| | 6 (24) | 3.79 ± 0.10 | b | | 7 (14) | 14.71 ± 0.44 | c |
| | 8 (26) | 3.92 ± 0.09 | b | | 9 (10) | 14.87 ± 0.67 | c |
| | 9 (10) | 4.09 ± 0.14 | b | | 8 (26) | 14.99 ± 0.24 | c |
| NPP | 3 (5) | 19.72 ± 1.88 | a | | | | |
| | 4 (8) | 21.74 ± 0.48 | b | | | | |
| | 5 (5) | 22.25 ± 0.53 | bc | | | | |
| | 6 (24) | 23.49 ± 0.28 | c | | | | |
| | 7 (14) | 25.34 ± 0.37 | d | | | | |
| | 8 (26) | 26.15 ± 0.33 | de | | | | |
| | 9 (10) | 27.21 ± 0.45 | e | | | | |

c) **Vanderbijlpark**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| GLS | 6 (2) | 34.90 ± 0.06 | b | GHS | (2) | 13.54 ± 0.02 | NS |
| | 7 (2) | 37.08 ± 0.37 | ab | | 7 (2) | 14.30 ± 0.23 | |
| | 8 (6) | 38.87 ± 0.49 | a | | 8 (6) | 14.49 ± 0.17 | |
| ITC | 6 (2) | 31.63 ± 0.20 | NS | MLT | 6 (2) | 31.46 ± 0.62 | NS |
| | 7 (2) | 32.10 ± 0.33 | | | 7 (2) | 34.41 ± 0.53 | |
| | 8 (6) | 34.43 ± 0.44 | | | 8 (6) | 36.57 ± 0.86 | |
| BCW | 7 (2) | 18.52 ± 0.02 | NS | MDL | 6 (2) | 23.92 ± 0.12 | b |
| | 6 (2) | 18.57 ± 0.51 | | | 7 (2) | 27.01 ± 0.97 | a |
| | 8 (6) | 19.19 ± 0.13 | | | 8 (6) | 27.84 ± 0.36 | a |
| ZMB | 6 (2) | 15.41 ± 0.42 | NS | MTR | 6 (2) | 5.46 ± 0.15 | NS |
| | 7 (2) | 15.86 ± 0.39 | | | 7 (2) | 5.74 ± 0.05 | |
| | 8 (6) | 16.04 ± 0.04 | | | 8 (6) | 5.91 ± 0.13 | |
| ZYW | 6 (2) | 23.05 ± 0.53 | b | AFL | 6 (2) | 7.05 ± 0.60 | b |
| | 7 (2) | 25.10 ± 0.02 | ab | | 7 (2) | 7.98 ± 0.27 | ab |
| | 8 (6) | 25.93 ± 0.32 | ac | | 8 (6) | 8.74 ± 0.33 | ab |
| IOB | 6 (2) | 7.09 ± 0.04 | b | MAF | 6 (2) | 6.99 ± 0.35 | NS |
| | 8 (6) | 7.56 ± 0.09 | ab | | 8 (6) | 7.94 ± 0.23 | |
| | 7 (2) | 7.75 ± 0.07 | a | | 7 (2) | 8.03 ± 0.29 | |
| WR | 6 (2) | 5.80 ± 0.13 | b | AFA | 6 (2) | 9.41 ± 0.29 | bc |
| | 7 (2) | 6.60 ± 0.19 | a | | 7 (2) | 10.11 ± 0.23 | abc |
| | 8 (6) | 6.75 ± 0.10 | a | | 8 (6) | 10.14 ± 0.14 | bc |
| NAS | 6 (2) | 4.85 ± 0.04 | NS | MRH | 6 (2) | 14.27 ± 0.21 | NS |
| | 7 (2) | 5.51 ± 0.01 | | | 7 (2) | 15.78 ± 0.70 | |
| | 8 (6) | 5.86 ± 0.17 | | | 8 (6) | 16.37 ± 0.32 | |
| UTR | 6 (2) | 5.77 ± 0.30 | NS | UJI | 6 (2) | 7.96 ± 0.38 | NS |
| | 7 (2) | 6.26 ± 0.17 | | | 7 (2) | 8.83 ± 0.17 | |
| | 8 (6) | 6.40 ± 0.16 | | | 8 (6) | 9.20 ± 0.27 | |
| PAC | 7 (2) | 3.80 ± 0.39 | NS | LJI | 6 (2) | 11.81 ± 0.55 | NS |
| | 6 (2) | 3.99 ± 0.31 | | | 7 (2) | 12.66 ± 0.07 | |
| | 8 (6) | 4.27 ± 0.09 | | | 8 (6) | 15.21 ± 1.28 | |
| NPP | 6 (2) | 22.83 ± 0.29 | NS | | | | |
| | 7 (2) | 24.45 ± 0.17 | | | | | |
| | 8 (6) | 29.35 ± 2.27 | | | | | |

d) **Krugersdorp**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| GLS | 6 (2) | 38.49 ± 1.55 | NS | GHS | 6 (2) | 14.12 ± 0.17 | NS |
| | 8 (3) | 39.18 ± 0.57 | | | 8 (3) | 14.46 ± 0.36 | |
| | 9 (2) | 42.08 ± 1.47 | | | 9 (2) | 15.31 ± 0.32 | |
| ITC | 6 (2) | 34.41 ± 1.09 | NS | MLT | 6 (2) | 34.34 ± 2.33 | NS |
| | 8 (3) | 35.78 ± 0.93 | | | 8 (3) | 36.68 ± 0.42 | |
| | 9 (2) | 37.63 ± 1.74 | | | 9 (2) | 39.30 ± 1.57 | |
| BCW | 6 (2) | 18.08 ± 0.46 | NS | MDL | 6 (2) | 25.74 ± 2.20 | NS |
| | 8 (3) | 18.23 ± 0.20 | | | 8 (3) | 27.96 ± 0.63 | |
| | 9 (2) | 18.95 ± 0.43 | | | 9 (2) | 30.08 ± 0.61 | |
| ZMB | 6 (2) | 15.02 ± 0.35 | NS | MTR | 6 (2) | 6.36 ± 0.01 | NS |
| | 8 (3) | 15.16 ± 0.08 | | | 8 (3) | 6.38 ± 0.21 | |
| | 9 (2) | 15.88 ± 0.10 | | | 9 (2) | 6.66 ± 0.03 | |
| ZYW | 6 (2) | 23.35 ± 0.85 | NS | AFL | 6 (2) | 6.82 ± 0.63 | NS |
| | 8 (3) | 24.97 ± 0.30 | | | 8 (3) | 7.13 ± 0.04 | |
| | 9 (2) | 26.61 ± 1.18 | | | 9 (2) | 8.38 ± 0.59 | |
| IOB | 6 (2) | 7.48 ± 0.42 | NS | MAF | 6 (2) | 7.94 ± 0.22 | NS |
| | 8 (3) | 7.66 ± 0.23 | | | 9 (2) | 8.64 ± 0.11 | |
| | 9 (2) | 7.89 ± 0.17 | | | 8 (3) | 8.86 ± 0.56 | |
| WR | 6 (2) | 6.65 ± 0.21 | NS | AFA | 6 (2) | 9.72 ± 0.12 | NS |
| | 8 (3) | 7.10 ± 0.33 | | | 9 (2) | 11.14 ± 0.20 | |
| | 9 (2) | 7.84 ± 0.21 | | | 8 (3) | 11.38 ± 0.54 | |
| NAS | 6 (2) | 5.43 ± 0.30 | NS | MRH | 6 (2) | 14.85 ± 1.42 | NS |
| | 8 (3) | 5.86 ± 0.24 | | | 8 (3) | 15.98 ± 0.41 | |
| | 9 (2) | 6.65 ± 0.43 | | | 9 (2) | 17.45 ± 1.01 | |
| UTR | 6 (2) | 6.96 ± 0.01 | NS | UJI | 6 (2) | 8.67 ± 0.06 | NS |
| | 9 (2) | 7.37 ± 0.26 | | | 8 (3) | 9.32 ± 0.63 | |
| | 8 (3) | 7.62 ± 0.35 | | | 9 (2) | 10.11± 0.42 | |
| PAC | 6 (2) | 4.04 ± 0.45 | NS | LJI | 6 (2) | 14.74 ± 1.56 | NS |
| | 8 (3) | 4.40 ± 0.29 | | | 9 (2) | 16.55 ± 1.54 | |
| | 9 (2) | 4.41 ± 0.13 | | | 8 (3) | 18.55 ± 1.03 | |
| NPP | 6 (2) | 25.35 ± 1.54 | NS | | | | |
| | 8 (3) | 26.05 ± 0.69 | | | | | |
| | 9 (2) | 27.11 ± 1.40 | | | | | |

Firstly, the data were subjected to cluster analyses and principal components analyses. These analyses were used to identify age groupings within a geographical area and to determine if there might be a difference between males and females.

Samples were examined using cluster analyses to determine any age class groupings or the grouping of males and females. The cluster analysis for Johannesburg (Fig. 6), Pretoria (Fig. 7), Vanderbijlpark (Fig. 8) and Krugersdorp (Fig. 9), exhibited exactly the same trend, in which no sexual dimorphism occurred between males and females. Secondly, two distinct age class clusters could be observed for all of the above mentioned localities. The first cluster combines all the young individuals from age class 1 to 4, while the second cluster, groups the older individuals together from age class 6 to 9. Age class 5 can be regarded as an intermediate age class, which tends to fall in both of the clusters.

The principal component analyses undertaken for both Johannesburg (Fig. 10) and Pretoria (Fig. 11) exhibits a large overlap with respect to males and females. No discrete groupings of the sexes could be observed, but definite groupings of age classes were present. Thus in all the PCA graphs there was no indication of sexual dimorphism. The principal components analysis on the samples collected from Johannesburg show that principal component I (60.7% of variance) has high positive loadings on all measurements (Table 4), suggesting that size may be important. Principal component II contributes to 7.3% of the variance, with high negative loadings on more than half of the measurements.

A similar trend is followed by the samples collected in Pretoria, principal component I (66.8% of variance) exhibit high positive loadings on all the measurements (Table 5), again indicating size may be of importance. Principal component II contributes to 6.3% of the variance with negative loadings on nearly half of all the measurements.

These results, together with the univariate results led us to pool the sexes for discriminant analyses of the age classes.

The mole-rats from Vanderbijlpark and Krugersdorp were excluded from the discriminant analyses due to the small sample sizes. There is a distinct grouping pattern of all the age classes resulting from the canonical variates of the age classes in the

highveld mole-rats sampled in Johannesburg (Fig. 12). A similar trend is followed by the discriminant analyses for the highveld mole-rats collected in Pretoria (Fig. 13). The canonical scores for both canonical variates number one and two for Johannesburg and Pretoria are shown in Table 6 and 7 respectively.

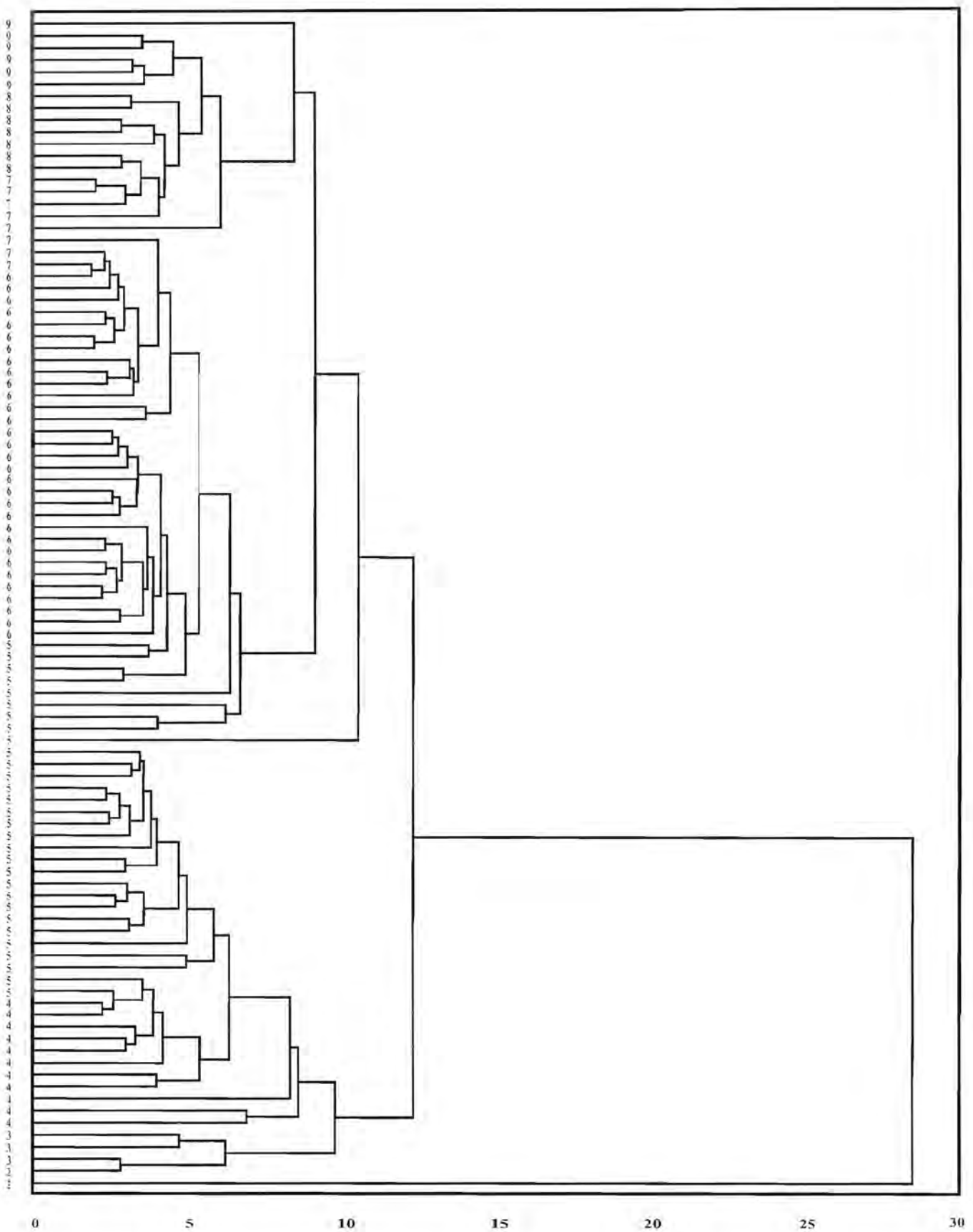


Fig. 6. Euclidean distance phenogram from a UPGMA cluster analysis of *C. h. pretoriae* from Johannesburg. Relative age classes 2 - 9 are indicated for 98 individuals.

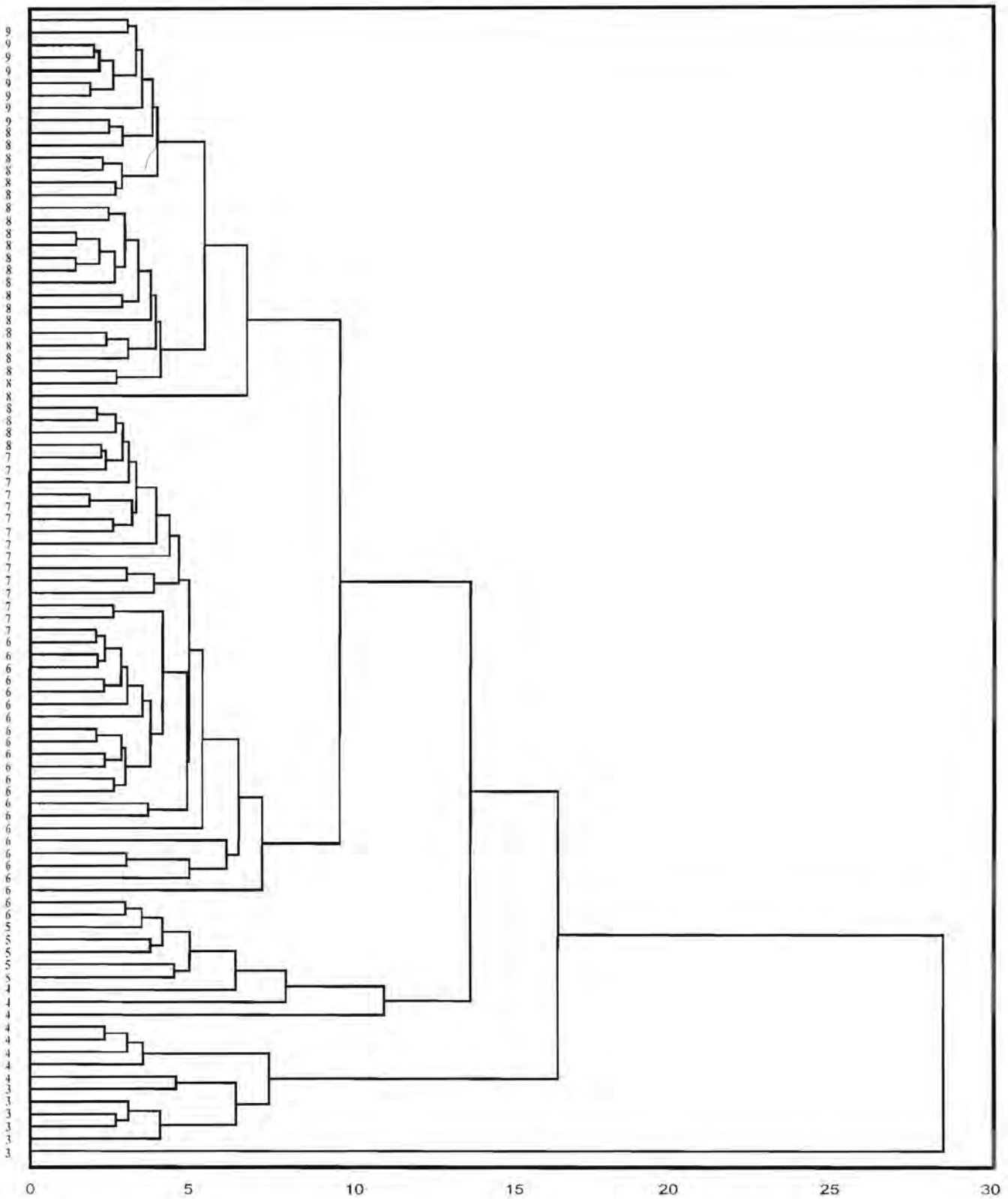


Fig. 7. Euclidean distance phenogram from a UPGMA cluster analysis of *C. h. pretoriae* from Pretoria. Relative age classes (3 - 9) for 92 individuals are indicated.

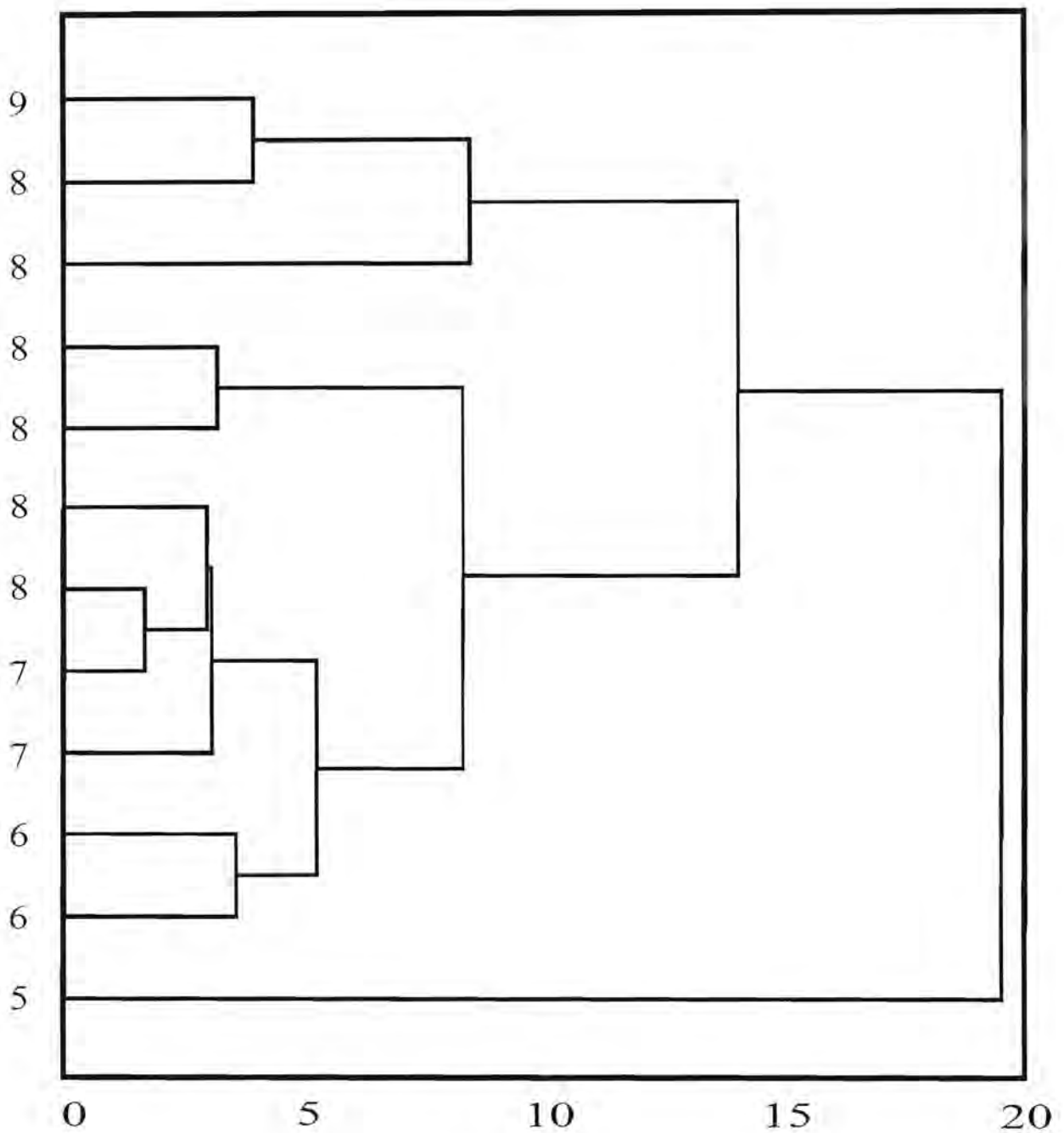


Fig. 8. Euclidean distance phenogram from a UPGMA cluster analysis of *C. h. pretoriae* from Vanderbijlpark. Relative age classes(5 - 9) for 12 individuals are indicated.

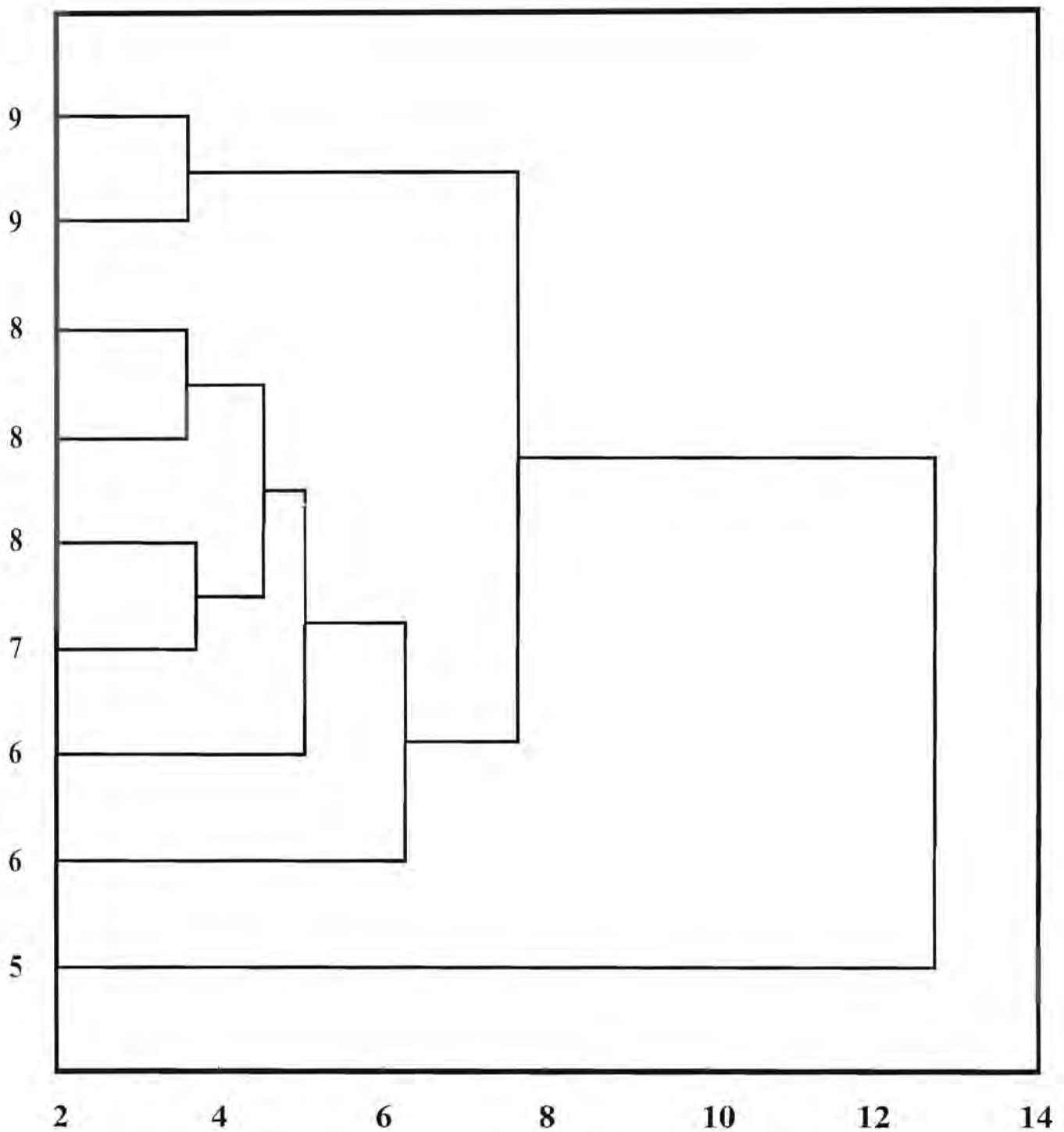


Fig. 9. Euclidean distance phenogram from a UPGMA cluster analysis of *C. h. pretoriae* from Krugersdorp. Relative age classes (5 - 9) for 9 individuals are indicated.

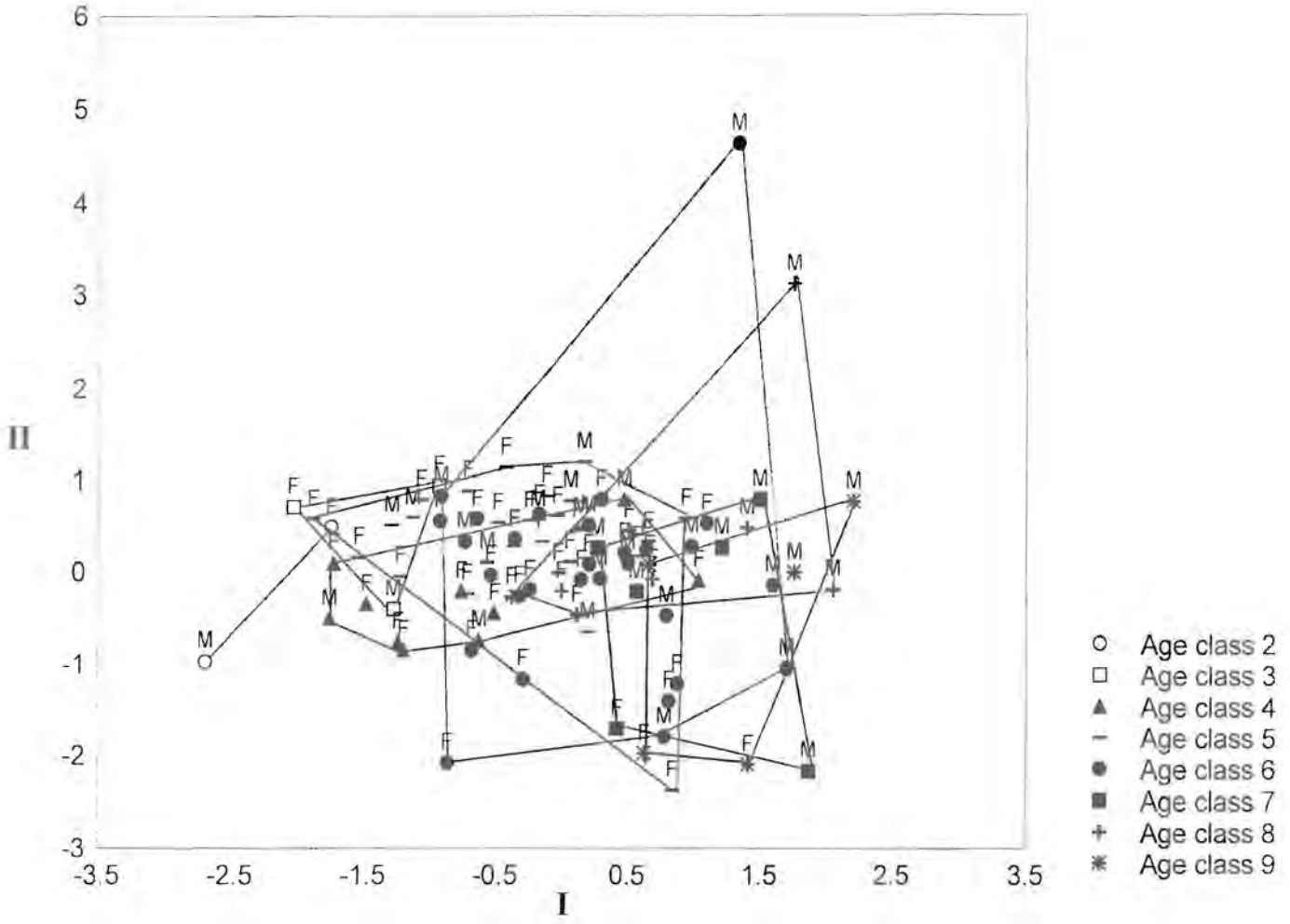


Fig. 10. The first two axes from a principal component analysis of *C. h. pretoriae* from Johannesburg. The sex (M = males; F = females) and age classes of 98 individuals are indicated.

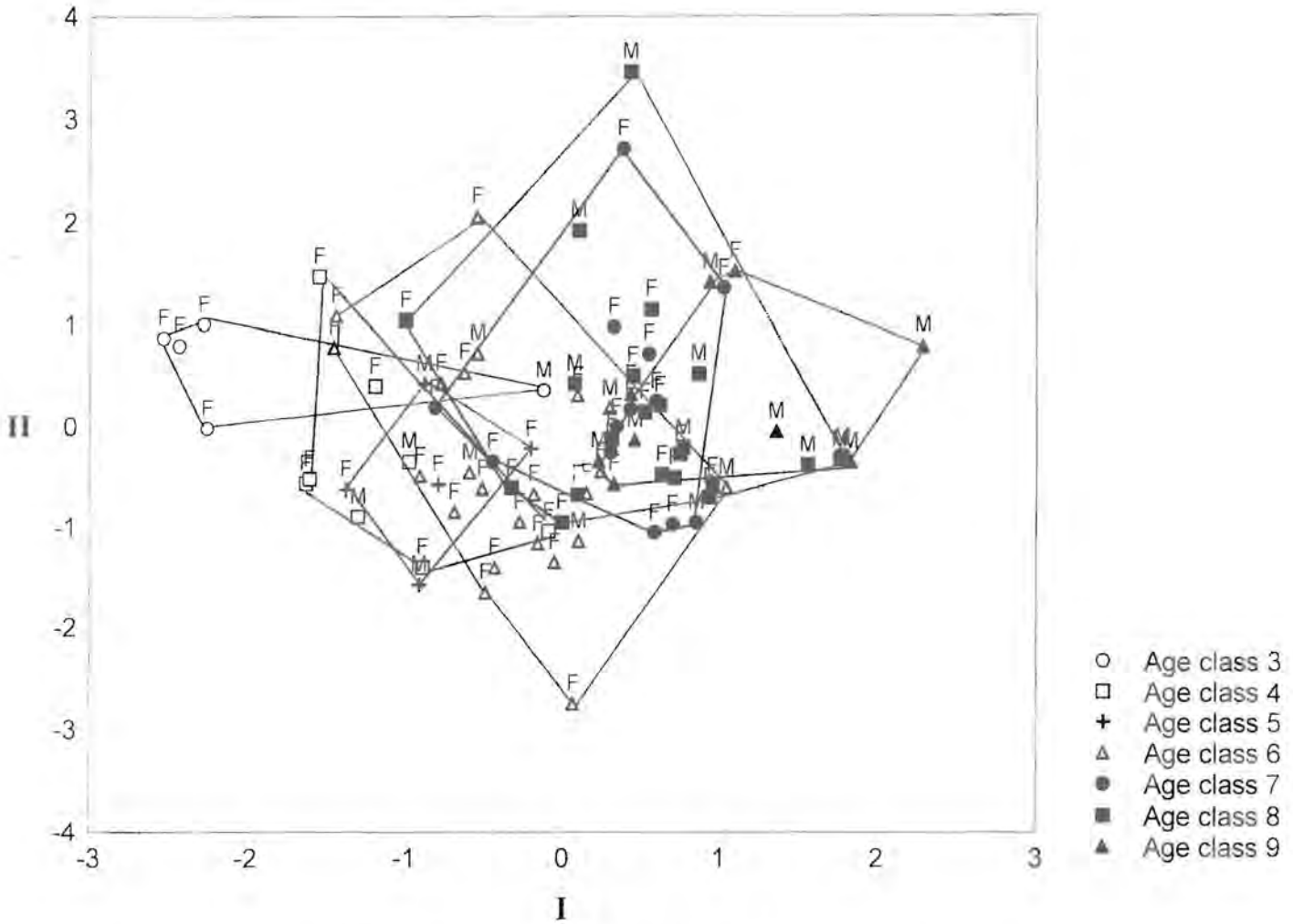


Fig. 11. The first two axes from a principal component analysis of *C. h. pretoriae* from Pretoria. The sex (M = males; F = females) and age classes of 92 individuals are indicated.

**Table 4** Loadings of variables on components I and II from a principal components analysis of *C. h. pretoriae* caught in Johannesburg.

| Measurements | Principal components | |
|---|---|---|
| | I | II |
| GLS | 0.97 | 0.00 |
| ITC | 0.86 | -0.11 |
| BCW | 0.78 | -0.01 |
| ZMB | 0.62 | -0.31 |
| ZYW | 0.96 | -0.03 |
| IOB | 0.41 | -0.01 |
| WR | 0.94 | 0.09 |
| NAS | 0.89 | 0.16 |
| UTR | 0.64 | 0.02 |
| PAC | 0.39 | 0.74 |
| NPP | 0.92 | -0.08 |
| GHS | 0.41 | 0.44 |
| MLT | 0.93 | -0.11 |
| MDL | 0.92 | 0.01 |
| MTR | 0.57 | 0.29 |
| AFL | 0.81 | 0.38 |
| MAF | 0.73 | -0.54 |
| AFA | 0.84 | -0.30 |
| MRH | 0.95 | 0.01 |
| UJI | 0.65 | -0.16 |
| LJI | 0.70 | 0.09 |
| % Trace | 60.7% | 7.3% |

**Table 5** Loadings of variables on components I and II from a principal components analysis of *C. h. pretoriae* sampled in Pretoria.

| Measurements | Principal components | |
|---|---|---|
| | I | II |
| GLS | 0.96 | 0.04 |
| ITC | 0.96 | -0.03 |
| BCW | 0.88 | -0.23 |
| ZMB | 0.80 | -0.35 |
| ZYW | 0.96 | 0.15 |
| IOB | 0.69 | -0.30 |
| WR | 0.95 | 0.05 |
| NAS | 0.93 | 0.11 |
| UTR | 0.69 | -0.31 |
| PAC | 0.56 | -0.30 |
| NPP | 0.96 | 0.06 |
| GHS | 0.93 | -0.13 |
| MLT | 0.80 | 0.04 |
| MDL | 0.90 | 0.18 |
| MTR | 0.67 | -0.37 |
| AFL | 0.82 | -0.20 |
| MAF | 0.74 | 0.50 |
| AFA | 0.81 | 0.45 |
| MRH | 0.83 | 0.24 |
| UJI | 0.06 | -0.28 |
| LJI | 0.76 | 0.18 |
| % Trace | 66.8% | 6.3% |

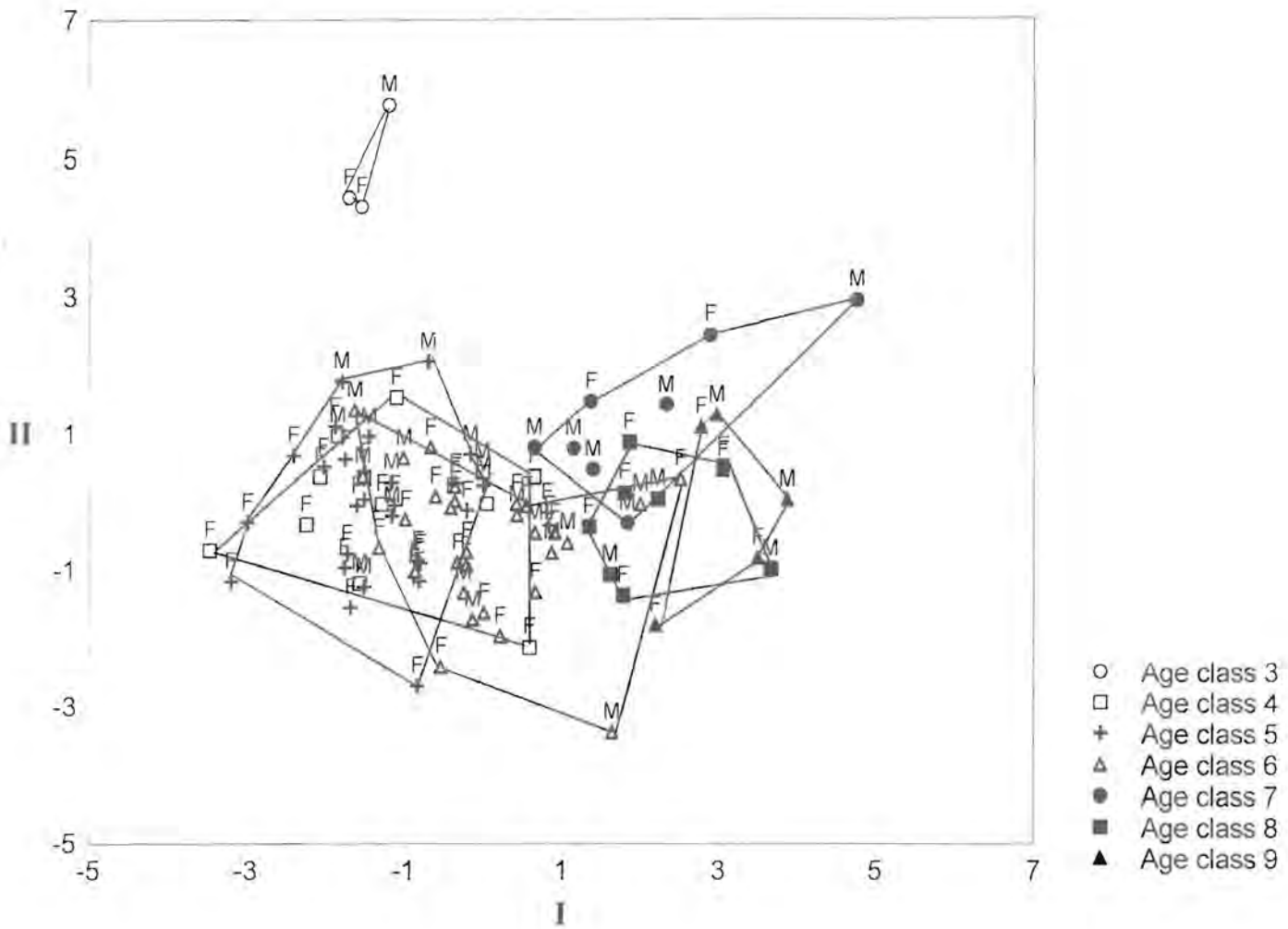


Fig. 12. The first two axes from a discriminant analysis of age class 3 - 9 in *C. h. pretoriae* from Johannesburg. The sex (M = males; F = females) and age classes of 98 individuals are indicated.

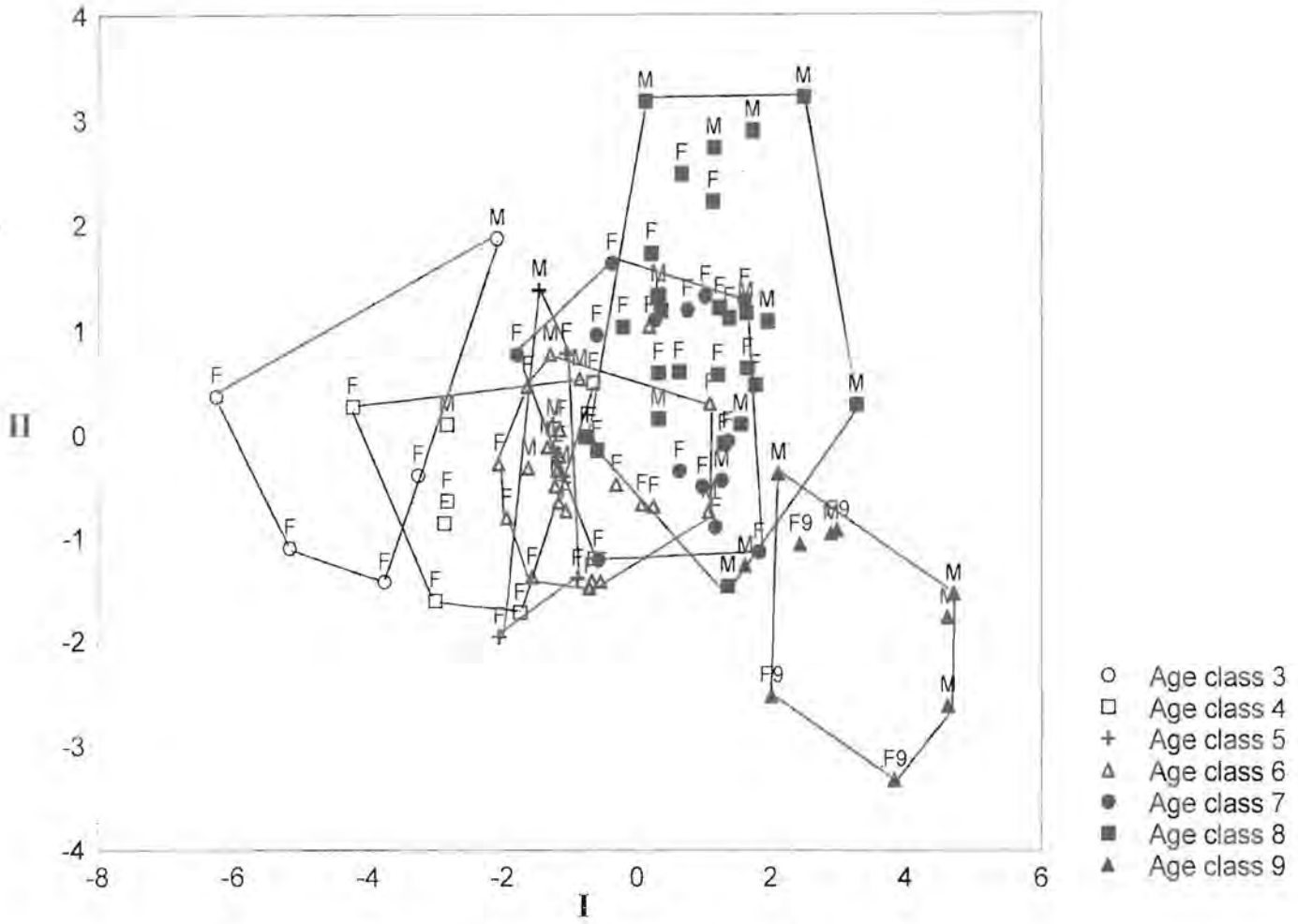


Fig. 13. The first two axes from a discriminant analysis of age class 3 - 9 in *C. h. pretoriae* from Pretoria. The sex (M = males; F = females) and age classes of 92 individuals are indicated.

**Table 6** Loadings of variables on all the canonical variates from a canonical variates (discriminant) analysis on 21 skull measurements taken for *C. h. pretoriae* sampled in Johannesburg.

| Variable | Canonical variate I | Canonical variate II |
|---|---|---|
| GLS | 0.53 | -0.19 |
| ITC | 0.43 | -0.13 |
| BCW | 0.37 | 0.04 |
| ZMB | 0.15 | -0.11 |
| ZYW | 0.61 | -0.16 |
| IOB | 0.11 | 0.19 |
| WR | 0.40 | -0.08 |
| NAS | 0.51 | -0.16 |
| UTR | 0.17 | -0.62 |
| PAC | 0.07 | -0.01 |
| NPP | 0.54 | -0.06 |
| GHS | 0.11 | -0.07 |
| MLT | 0.58 | -0.15 |
| MDL | 0.55 | -0.14 |
| MTR | -0.02 | -0.50 |
| AFL | 0.29 | -0.12 |
| MAF | 0.36 | -0.15 |
| AFA | 0.42 | -0.13 |
| MRH | 0.50 | -0.13 |
| UJI | 0.22 | -0.22 |
| LJI | 0.37 | -0.11 |

**Table 7** Loadings of variables on all the canonical variates from a canonical variates (discriminant) analysis on 21 skull measurements taken for *C. h. pretoriae* sampled in Pretoria.

| Variable | Canonical variate I | Canonical variate II |
|---|---|---|
| GLS | 0.71 | 0.32 |
| ITC | 0.63 | 0.29 |
| BCW | 0.41 | 0.18 |
| ZMB | 0.38 | 0.05 |
| ZYW | 0.73 | 0.26 |
| IOB | 0.23 | 0.18 |
| WR | 0.60 | 0.42 |
| NAS | 0.63 | 0.28 |
| UTR | 0.42 | 0.01 |
| PAC | 0.24 | 0.02 |
| NPP | 0.72 | 0.32 |
| GHS | 0.52 | 0.18 |
| MLT | 0.50 | 0.23 |
| MDL | 0.63 | 0.10 |
| MTR | 0.36 | 0.08 |
| AFL | 0.49 | 0.14 |
| MAF | 0.43 | 0.35 |
| AFA | 0.52 | 0.17 |
| MRH | 0.57 | 0.08 |
| UJI | 0.27 | 0.32 |
| LJI | 0.46 | 0.38 |

# DISCUSSION

*Age determination*

According to Gilbert & Stolt (1970) the use of tooth wear on its own has been shown to be an unreliable ageing method. Taylor *et al.* (1985) mentions that due to variable individual teeth characteristics, the use of tooth eruption and particularly of tooth wear entail some degree of error. Variability in tooth characteristics can result from genetic differences, soil hardness or nutritional differences (Morris 1972; Taylor *et al.* 1985). In this study, tooth eruption and wear was used as a method of ageing according to Taylor *et al.* (1985), however the data obtained was only used as a relative measure of age.

Body mass, in contrast, has been shown to be a poor indicator of age in small mammals (Chaplin & White 1969) and specifically within the genus *Cryptomys* (Bennett 1988; Bennett *et al.* 1990). Body mass is readily influenced by health and diet and therefore not a reliable method of ageing (Morris 1972; Chaplin & White 1969). Age was not determined using body mass in this study, but rather, I aimed to determine if there might be any relationship between an animal's relative age, its mass and its reproductive status.

A study by Bennett & Jarvis (1988) on the division of labour within *C. damarensis* colonies, showed that frequent workers were lighter in body mass than infrequent workers. They found a change in the social role of some workers as soon as new pups were recruited to the working force of the colony. These mole-rats started to show an increase in mass, while the frequent workers did not. They suggest that body mass appears to be independent of age and is rather linked to the social status of the individual in a colony. However, body mass seems to be dependent on age in the reproductive animals within a colony (Bennett & Jarvis 1988). Within *C. damarensis* colonies the reproductive pair are the heaviest and oldest animals in a colony, with the breeding male being the heaviest and most dominant animal in the colony as a whole (Bennett & Jarvis 1988). Clarke & Faulkes (1997) found that the reproductive *Heterocephalus glaber* females were the oldest and heaviest within their colonies. Whereas a study by Bennett (1989) showed the reproductive males in *C. h. hottentotus*

colonies to be heavier than all the other animals in a colony including the reproductive females. According to Moolman *et al.* (1998), the reproductive female is either the largest or amongst the largest of the females, whereas the reproductive male is usually the largest animal in the colony.

In the highveld mole-rat colonies, the reproductive males as well as the reproductive females are either the heaviest and oldest or amongst the heaviest and oldest animals in a colony. Moolman *et al.* (1998) suggests that within the genus *Cryptomys,* size may be an important determinant of dominance position within a colony. Moolman *et al.* (1998) found no distinct dominance hierarchy within highveld mole-rat colonies, which may explain why in my study I found no significant differences between the ages of the reproductive males and reproductive females. However, a significant difference in mass was evident between the two reproductive sexes. A higher mean mass was exhibited by the reproductive males, thus reproductive males tend to be the heaviest in the highveld mole-rat colonies. Similarly it was shown in *C. h. hottentotus*, that the reproductive pair did not clearly stand out as the older mole-rats within the colony (Bennett 1989).

*Morphometric analyses*

Males tend to be heavier than females, both univariate and multivariate results however, showed a lack of sexual dimorphism within the cranial measurements of the highveld mole-rat. A similar trend is shown by *C. h. hottentotus,* that exhibits an overall lack of sexual dimorphism, even though the breeding males tend to be heavier than the breeding females and are the heaviest animals within a colony (Bennett *et al.* 1990). I suggest that the reason for highveld mole-rat males weighing more than females, may be as a result of males possessing a higher ratio of muscle tissue than fatty tissue, which result in them weighing more than the females (Eckert *et al.* 1996).

With regard to age variation, the univariate SNK tests provided ample information to which relative age classes group together, being age class 1 - 4/5 and age class 5/6 - 9. Similar results with regard to age class groupings were found for all the study areas. Thus, it can be claimed with certainty that no differences in cranial measurements between the sexes occur between the four different populations.

In addition, the multivariate analyses conclusively showed two distinct age groupings within the array of relative age classes. The first group consists of all the young non-reproductive individuals in the relative age classes 1 – 4. The second clearly defined group is individuals belonging to age group 6 – 9. This group consists of both reproductive and non-reproductive individuals. Age class 5 is an intermediate age class, with only a few reproductives occurring within this class. If the relative age classes are visualised as sections of unknown lengths on a hypothetical growth curve, age class 5 can be visualised as the section on the turning point of the graph, just as the curve begins to stabilise (Chimimba & Dippenaar 1994). Thus individuals belonging to age class 5 are in the process of becoming reproductively mature.

In conclusion, using both the wear on the cusps of the molariform teeth and the eruption of particular teeth, nine sequential developmental age classes in the highveld mole-rat can be discerned. The reproductive animals were found to be amongst the oldest individuals of the colony. This is the first study where the application of age can with certainty be applied to the allocation of relative age to reproductive members of the genus *Cryptomys*.

Twenty-one cranial measurements were performed on the skulls of 71 males and 140 females. It was found that sexual dimorphism is absent in the highveld mole-rat both within and amongst populations from particular geographical boundaries.

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# Chapter 4

## Can the highveld mole-rat (*Cryptomys hottentotus pretoriae*), regulate the rhythm of melatonin secretion to measure changes in daylength?

## ABSTRACT

Melatonin secretion in mammals has a circadian rhythm, the period of which is dependent on the daylength. Circannual changes in the period of the melatonin rhythm can be used as a neurochemical index of season in order to time reproduction. Subterranean mammals are exposed to light infrequently, if ever, yet their circadian rhythm of melatonin secretion is similar to that of other mammals. However, it is not known whether the melatonin rhythm effectively reflects different daylengths. I hypothesize that the circadian pattern of melatonin secretion in the highveld mole-rat cannot be regulated to reflect different photoperiods. The highveld mole-rat was used to compare the pattern of melatonin secretion in two different photoperiodic regimes, namely long days (LD, 14L:10D) and short days (SD, 10L:14D). Melatonin secretion was significantly higher in blood samples collected in the dark, compared to those collected in daylight. However, the circadian pattern of melatonin secretion in LD did not differ from the pattern observed in SD. Thus, although a circadian rhythm of melatonin secretion exists in the highveld mole-rat, melatonin secretion cannot be used as a means to distinguish between different daylengths. It is postulated that in this subterranean rodent mole, seasonal changes in temperature and precipitation patterns may be the ultimate cues that the mole-rats respond to for the timing of reproduction.

# INTRODUCTION

Changes in photoperiod are constant from year to year and thus, can be claimed to be a major environmental *zeitgeber* governing seasonal activity such as reproduction in animals (Legan & Karsch 1983), which is primarily linked to the cyclical secretion of melatonin.

A wide variety of organisms exhibit circadian rhythms of activity and hormone secretion, regulated by internal clocks that are entrained primarily by the alternating cycle of light and darkness. Secretion of the hormone melatonin is far greater at night, when animals are in the dark, than during the day, when animals are exposed to light (Reiter 1991). Thus, melatonin secretion has a circadian rhythm, the period of which is determined by the length of day. As circannual changes in daylength are predictable, the period of the melatonin rhythm can be used as a neurochemical index of season (or *zeitgeber*).

For the melatonin rhythm to be an effective *zeitgeber*, the period of the rhythm needs to be differentially regulated at a resolution that is equal to, or greater than, the average seasonal change in daylength. While some species can detect changes in photoperiod as small as 40 minutes (Hau *et al.* 1998), other species fail to respond even to large changes (Lewy & Newsome 1983; McConnell 1987). The ability to use melatonin secretion as a *zeitgeber* allows animals to time processes such as reproduction to occur at the most appropriate time (Brainard *et al.* 1982), which is advantageous in seasonally variable environments. The majority of animals live in environments that are subjected to seasonal changes in important variables such as climate (Pevet *et al.* 1984). On the other hand, species that do not appear to respond to photoperiod are usually adapted to constant or unpredictable habitats (e.g., Heideman & Bronson 1993) in which restricting reproduction to a particular time is not an advantage. However, there are environments, such as subterranean habitats, where regulating reproduction may be advantageous, but where the photoperiodic signal is inappropriate or deficient (Heideman & Bronson 1994). For example in the subterranean, blind mole-rat (*Spalax ehrenbergi*), which is a summer breeder and only exposed to light during the winter months when excavated soil are pushed to the surface during burrow extensions (Shanas *et al.* 1997).

Harsh environments are a characteristic of many habitats occupied by small mammal species in southern Africa. These environments are predominantly characterised by irregular and unpredictable rainfall, with food and water availability coinciding with the rainfall patterns. Due to these changing environments many of the small mammals tend to be seasonal breeders, optimising their survival with regard to the available resources during the breeding season (White & Bernard 1996). Factors such as protein or water intake may directly influence the timing of reproduction in these animals (White & Bernard 1996). These factors often interact with, or override photoperiodic information (Nelson *et al.* 1997). According to Bronson (1989), photoperiod is an important cue for the onset of reproduction in animals occurring in the northern hemisphere. However, the unpredictability of rainfall reduces the efficiency with which photoperiod can be used as a cue for reproduction (White & Bernard 1996). Rainfall has an extreme effect on the vegetation of semi-arid and arid regions within southern Africa (Neal 1984; Happold & Happold 1992). Thus, rainfall may be playing a more important role than photoperiod in the reproductive cycles of South African mammals.

The majority of social mole-rats exhibit aseasonal reproduction with the exception of the common mole-rat, which occurs in the winter rainfall regions of the Cape (Spinks *et al.* 1997). It is tempting to suggest that such aseasonal reproduction is due to an inability to interpret season as there is no appropriate photoperiodic signal below ground. Indeed, a common feature of many subterranean animals is the absence of an ocular system, presumably as light penetrates the subterranean environment very infrequently (Cooper *et al.* 1993). However, ocular regression does not mean that circadian rhythms of melatonin secretion are absent (Pevet *et al.* 1984; Jagota *et al.* 1999). In the only other study of melatonin secretion in a fossorial mammal, Reiter *et al.* (1994) found a photoperiodic entrained circadian rhythm of melatonin secretion in the valley pocket gopher (*Thomomys bottae*), but did not determine whether this rhythm could be differentially regulated to reflect different daylengths, i.e. whether the circadian melatonin rhythm could be a *zeitgeber*. The present study addresses this question. Specifically, I hypothesised that the pattern of melatonin secretion in strictly subterranean mammals cannot be regulated to allow a distinction between different photoperiods. The main aim of this study was to assess whether there is a change in the pattern of the

melatonin rhythm of the pineal in the highveld mole-rat in response to a change in daylength.

## MATERIALS & METHODS

*Study animal*

I used the strictly subterranean highveld mole-rat as the study animal. This mole-rat is distributed in the Gauteng Province of South Africa and breeds seasonally (Chapter 2). With the aid of modified Hickman live traps (Hickman 1979) the mole-rats were captured in and around the suburbs of Pretoria (See Chapter 1 for details) and transferred to artificial holding facilities in light- (*c*.200 $\mu W/cm^2$ in daytime, <0.01 $\mu W/cm^2$ at night) and temperature-controlled (25 ± 1°C) rooms. A cohort of 48 animals were exposed to a long-day (LD) photoperiod (14L:10D) for 2 months prior to the first series of blood sampling (see below). A separate cohort of 48 animals were transferred to a short-day (SD) photoperiod (10L:14D) and allowed to habituate for 2 months before completing the experiment.

Attention was paid to several factors in an attempt to maximise the opportunity for detecting differences in the pattern of melatonin secretion between LD and SD. First, as it has been shown that greater magnitudes of change in photoperiod are more likely to elicit photoperiodic responses, the magnitude of change in photoperiod used in this study was relatively large (4 h) and greater than that in the field (2.5hrs). Secondly, as no evidence of *C. h. pretoriae* activity above ground has been found in more than ± 800 hours of field observation (L. Janse van Rensburg & N.C. Bennett, unpubl. data) and burrows are located 20cm below the ground surface, a depth at which no light is detectable (N.C. Bennett unpubl. data) we were confident that the amount of light delivered to the animals during the subjective day in the laboratory far exceeded anything they might encounter in their natural milieu. Thirdly, the animals were given sufficient time (approximately 2 months) to adjust from the LD regime they encountered in natural conditions to the SD photoperiod in the laboratory and the other way around.

*Experimental design.*

The experiment was designed to compare melatonin secretion in mole-rats exposed to LD and SD at the same times of day. Midnight was designated time zero (ZT0). The day was from ZT5 to ZT19 in LD and from ZT7 to ZT17 in SD. Six time points were selected at which blood samples were taken and assayed for melatonin (Fig. 1). These time points were located at ZT4, 6, 8, 16, 18 and 20. Two of these points (ZT4 & ZT20) were in the night (dark) in both LD and SD, while another two points (ZT8 & ZT16) were in the daylight (light) in both LD and SD. However, whether there was light at points ZT6 and ZT18 depended on the prevailing photoperiod. Thus, in LD samples at both ZT6 and ZT18 were taken during the daylight, while both were in the dark in SD. The rationale was that if melatonin secretion is sufficiently different between LD and SD to accurately reflect the difference in the photoperiod, levels of melatonin should be significantly different in samples taken at the time points that change from being in the dark in SD to being in the light in LD (i.e. at ZT6 and ZT18).

*Blood sampling*

All experimental procedures were carried out under the guidelines of the Ethics Committee of the University of Pretoria (Permit #960426-006). Blood samples were collected from the heart by cardiac puncture.

Although the suprachiasmatic nucleus of the highveld mole-rat is relatively insensitive to light (Negroni 1998), precautions were taken while collecting samples in the dark to ensure minimal exposure of the animals to light. Individuals were captured with the aid of a weak (<0.05 $\mu W/cm^2$) red-filtered headlamp. Once captured, an individual was immediately anaesthetised in the dark and once expired, an aluminium foil cap was placed over the head to prevent light acting through the eyes on the pineal. Blood was obtained from the heart within two minutes of the death of the animal.

*Melatonin assay*

Melatonin was assayed in duplicate 100 µl aliquots of plasma, using a previously described, double antibody radioimmunoassay technique (Frazer *et al.* 1983) with an antibody first raised by Tillet and co-workers. (1986) (Heideman *et al.* 1998). Parallelism

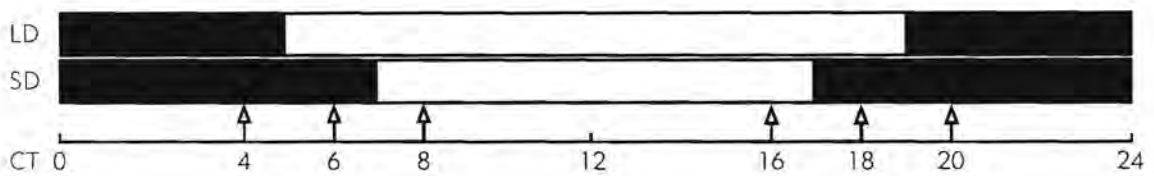


Fig. 1. Diagrammatic summary of the experimental design. LD = long days. SD = short days. CT = circadian time. Solid bar represents subjective night (darkness). Open bar represents subjective day (light). Blood samples were collected via cardiac puncture from eight animals per *zeitgeber* time, indicated by the arrows.

was demonstrated between a standard curve in ovine plasma and a pool of mole-rat plasma with 0, 16, 32, 64 and 128 pg/ml of melatonin added (slope = 0.99, least squares $r^2 = 0.99$, $p<0.05$). The intra- and inter-assay coefficient of variation for the combined assays was 7.7 and 10% respectively. Sensitivity of the assay was 4 pg/ml.

*Statistical analyses*

A Student *t*-test (Zar 1984) was used to determine significant differences between light and dark within LD and SD respectively. The Student *t*-test was also used to determine if there might be any significant differences between the pooled data for LD and SD. To compare melatonin concentrations amongst *zeitgeber* time groups, an ANOVA with a Tukey's HSD post-test was performed (Zar 1984). All statistical tests were performed using the statistical program, Statistica version 5.0™.

## RESULTS

A normal, if weak, circadian rhythm of melatonin secretion in *C. h. pretoriae* was detected. Melatonin levels were generally significantly higher at night (Dark = 19.77 ± 2.07pg/ml (n = 48) *vs* Light = 11.64 ± 0.86pg/ml (n = 47) (Student *t*-test, $p<0.001$). The difference between night- and daytime values was slightly less in SD (9.11, $p<0.05$) than in LD (9.57, $p<0.01$) (Table 1). Melatonin secretion did not increase significantly 2 hours after the onset of night (Figure 2a), with a value of 19.86 ± 3.22pg/ml ($n = 7$) at T18 and a value of 26.13 ± 4.40pg/ml ($n = 8$) at T20 in LD and from 8.13 ± 0.58pg/ml ($n = 8$) at T16 to 15.25 ± 1.13pg/ml ($n = 8$) at T18 in SD (Fig. 2b). In addition the decrease in melatonin secretion after the onset of night was not significant in either of the photoperiods (Table 2).

The levels of melatonin at the time points for which differences might be expected, namely ZT6 (8.88 ± 0.52pg/ml in LD (n = 8) *vs* 9.25 ± 1.08pg/ml in SD (n = 8)) and ZT18 (19.86 ± 3.22pg/ml in LD (n = 8) *vs* 15.25 ± 1.13pg/ml in SD (n = 8)), were not significantly different (Fig. 3).

**Table 1** Melatonin (pg/ml) secretion (mean±S.E.) in *C.h. pretoriae* during the subjective day (light) and night (dark). Blood samples were collected from 48 individuals held in long days (LD, 14L:10D) and 48 individuals held in short days (SD, 10L:14D). *p<0.05, **p<0.01, ***p<0.001 (Students *t*-test). [§]Samples from both long days and short days.

| | Light | **Dark** | **Difference** |
|---|---|---|---|
| **LD** | 12.81 ± 1.12 (*n* =31) | 22.38 ± 3.02 (*n* =16) | 9.57 ** |
| **SD** | 9.38 ± 0.78 (*n* =16) | 18.47 ± 2.71 (*n* =32) | 9.11 * |
| **Pooled**[§] | 11.64 ± 0.86 (*n* = 47) | 19.77 ± 2.07 (*n* = 48) | 8.13*** |

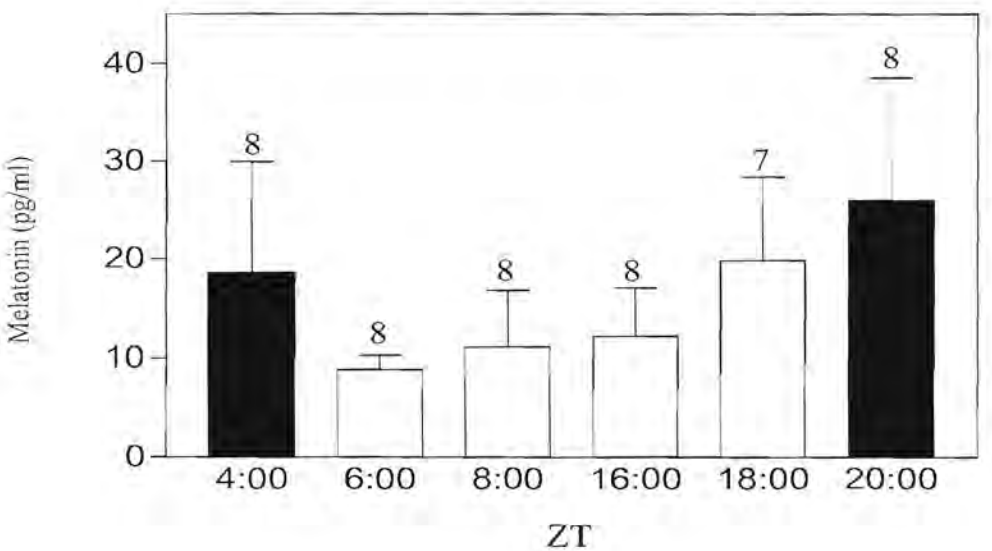


Fig. 2a. Melatonin secretion in *C. h. pretoriae* during long day (n = 47). Open bars denote subjective day (light) and solid bars represent subjective night (dark). *Zeitgeber* times (ZT) are indicated.

**Table 2** The melatonin concentrations (pg/ml) (mean±S.E.) observed for each *zeitgeber* time measured in *C. h pretoriae*. LD, long days (14L:10D) and SD, short days (10L:14D). Different letters designate significant differences between *zeitgeber* times for LD and SD respectively (Tukey HSD test, $p < 0.05$).

| Photoperiod | ***Zeitgeber* times** | | | | | |
|---|---|---|---|---|---|---|
| | T4 | T6 | T8 | T16 | T18 | T20 |
| LD | 18.63±11.33 ac | 8.88±1.46 a | 11.13±5.74 ae | 12.25±4.83 ae | 19.86±8.51 ad | 26.13±12.44 bcde |
| SD | 14.50± 7.21 ae | 9.25±3.06 a | 10.63±3.85 ae | 8.13±1.64 a | 15.25±3.20 ae | 34.88±23.09 bd |

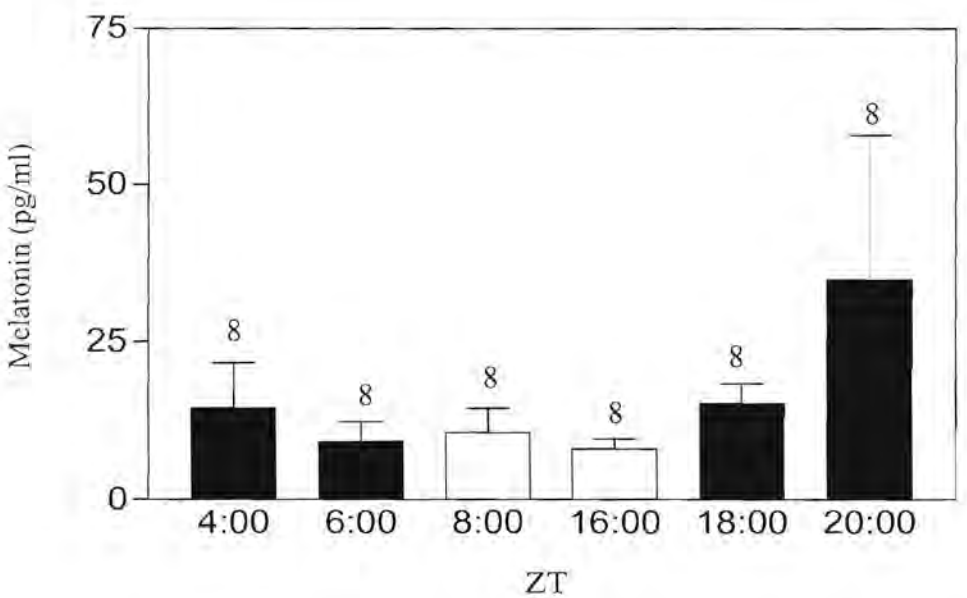


Fig. 2b. Melatonin secretion in *C. h. pretoriae* during short day (n = 48). Open bars denote subjective day (light) and solid bars represent subjective night (dark). *Zeitgeber* times (ZT) are indicated.

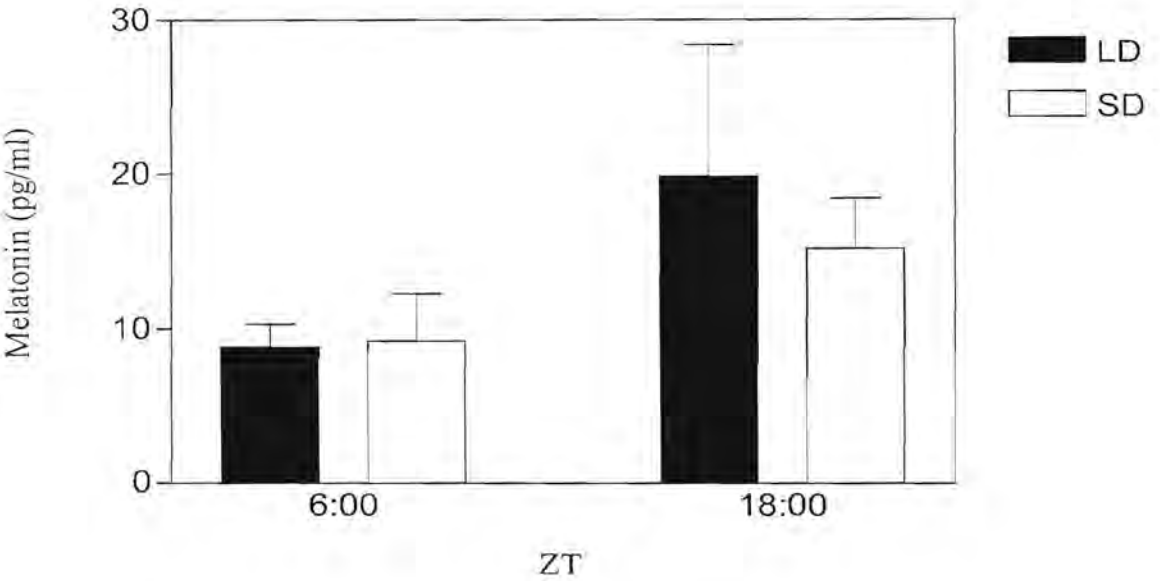


Fig. 3. A comparison of melatonin levels at ZT 6:00 and ZT 18:00 during short days (SD) in the subjective day (open bars) and long days (LD) during the subjective night (solid bars). *Zeitgeber* times (ZT) are indicated.

# DISCUSSION

Melatonin secretion in the strictly subterranean highveld mole-rat, has a normal circadian rhythm with levels of melatonin being higher at night than during the day. This has been reported in troglodytes (Green & Romero 1997) and confirms Reiter *et al.*'s (1994) observations in another species of fossorial mammal (*Thomomys bottae*). While the ocular system of fossorial and subterranean mammals is often reduced (Cooper *et al.* 1993) or absent (Jagota *et al.* 1999) ocular regression is often accompanied by progression in non-visual photic structures that sub-serve photoperiodic functions (Cooper *et al.* 1993). Thus, regression of the ocular system does not mean that the pineal-melatonin system is unresponsive to photoperiod, indeed, photoperiodic responses have been found in blind animals (Pevet *et al.* 1984; Green & Romero 1997). Clearly, the absence of a functional visual system does not preclude photoperiodic responses via non-ocular systems (Lovegrove & Papenfus 1995; Jagota *et al.* 1999).

Despite the presence of a circadian rhythm in melatonin secretion in the highveld mole-rat, only a very small difference between the secretion patterns observed in LD and SD was observed. (9.57 vs 9.11) This supports our hypothesis that the pattern of melatonin secretion does not change sufficiently to allow a distinction between different photoperiods. While these results are based on laboratory-housed animals, the magnitude of change in the two photoperiods used in this study (4 h) is greater than that normally experienced in the field. This suggests that it is unlikely that this species can effectively distinguish different photoperiods in its natural milieu on the basis of melatonin secretion.

The absence of a functional circannual index of daylength has important implications for long-lived animals. If daylength cannot be measured, there is no way to cue reproduction in advance of a changing photoperiod. Thus, presumably the seasonally reproducing highveld mole-rat must cue into other factors such as seasonally changing burrow temperatures (Bennett *et al.* 1988) or increased precipitation and hence changing edaphic factors of the substratum for burrowing. Nelson *et al.* (1997) also mentions that the variability in the yearly onset and offset of the breeding season in the field suggests

that other factors in addition to photoperiod are responsible for the reproductive cycle of mammals.

One of the features of the data collected during the study, is relatively high inter-individual variability. This has been reported for melatonin rhythms in other species (Goldman *et al.* 1997) and is normally taken as evidence of weak photic entrainment of the circadian clock (Tobler *et al.* 1998). As selection reduces variability (Price 1995), the high variability observed here is indirect evidence of a lack of selective pressure for the maintenance of a functional pineal-melatonin system. This would suggest that photoperiodism is not adaptive in a subterranean environment. The fact that the neural system that supports photoperiodism is intact suggests that there is no selective pressure against photoperiodism, but rather that the pineal-melatonin system may be a neutral, relict trait, as has been suggested for some species of cave fish (Green & Romero 1997).

Finally, it is important to note that the level of melatonin secreted at night in the highveld mole-rat is less than two-fold greater than in daylight. This is similar to the pocket gopher (*T. bottae*), a subterranean mammal (Reiter *et al.* 1994), but small compared to that of other rodents (e.g., Brainard *et al.* 1982). However, whether this is of any relevance to the ability of individuals to interpret photoperiod is debatable, as it is the length, rather than the amplitude, of melatonin secretion that seems to be important (Arendt 1995). Differences in the melatonin secretion induced by changes in the length of the night are interpreted by the animal to regulate seasonal changes in physiological processes (Wehr 1997).

In summary, the results of the study showed that there is a normal but relatively weak circadian rhythm of melatonin secretion in the highveld mole-rat. The pineal-melatonin system is clearly intact, but the pattern of melatonin secretion in LD and SD did not change sufficiently to reflect the change in daylength. Thus, we suggest that this subterranean rodent may not rely on a changing photoperiod for its seasonality of reproduction, but rather, it is more likely that seasonal changes in temperature or precipitation may be crucial for the activation of reproduction.

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