The reproductive biology of *Cryptomys hottentotus pretoriae* (Rodentia: Bathyergidae).

by

Lindie Janse van Rensburg

Supervisor : Prof. N.C. Bennett
Co-supervisors: Dr. M. van der Merwe
Dr. A.S. Schoeman

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"I have the strength to face all conditions by the power that Christ gives me."

- Philippians 4: 13.

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Summary

The subterranean mole-rat, *C. h. pretoriae* occurs on the verdant grasslands of the highveld regions of South Africa. The highveld mole-rat is a co-operatively breeding rodent that exhibits seasonal breeding and a reproductive division of labour. Evidence from reproductive tract morphometrics, ovarian histology and plasma oestrogen and progesterone concentrations strongly support that the highveld mole-rat is a seasonal breeder. Although the birth of the offspring is confined to the months of May through to November, qualitative analysis of ovarian histology revealed that females retained reproductive function during the summer non-breeding period (December – March). Seasonal differences were found in ovarian morphometrics in addition to progesterone and oestrogen concentrations which are associated with enhanced follicular activation in April and May and subsequent conceptions from May through to November during the breeding period.

The non-breeding period coincides with the period of maximal dispersal opportunities in the summer rainfall areas inhabited by the highveld mole-rat. Non-reproductive females exhibited follicular development but failed to ovulate in the confines of the colony, as evidenced by a lack in the production of corpora lutea. The endocrinological data supports the lack of ovulation in these socially suppressed non-reproductive females.

Reproductive tract morphometrics, testicular histology and plasma testosterone concentrations for the males suggests that there is a gradual increase in testicular mass and volume with increasing proximity to the breeding season, but after September, the testicular parameters begin to fall. Seminiferous tubule diameter are significantly greater in reproductive males but there is no obvious change with season. In general, testosterone concentrations are higher in the reproductive males, with the highest titres occurring around July and August. All available evidence supports a continuance of reproductive activity during the non-breeding season. It is speculated that reproductive activation in

the non-reproductive males may facilitate inter-sexual recognition and hence facilitate bond formation for independent reproduction.

Bimonthly sampling of males to investigate sperm motility, revealed no significant difference between the sperm kinematics of reproductive (n = 14) and non-reproductive males (n = 17).

Both the follicular and testicular parameters studied indicate that there are two main periods of reproductive activity, these being May/July and September. It is possible that for an animal with an estimated gestation of two months, that the reproductive potential of producing two litters during the breeding season arise with pups being born at the end of May through to November.

The non-breeding season for the highveld mole-rat coincides with the summer rainfall period on the highveld regions of South Africa. Moist, workable soils facilitate the dispersal of previously non-reproductive animals from their natal colonies and subsequent colony genesis arises from previously suppressed females and males.

Examining the age structure within colonies, it was expected that the founding animals would be the oldest animals within the confines of the colony. The age structure within colonies were examined, where all individuals were assigned to nine relative age classes. The reproductive animals were amongst the oldest as well as the heaviest members of the colony. Males tended to be the heavier in the colony as a whole. There was a positive relationship between body mass and increasing age for all the mole-rats studied.

In addition to age determination, morphometric measurements of skulls were performed. Morphometric analyses showed an absence of sexual dimorphism. Cluster, principal components and discriminant analyses revealed two distinct groupings amongst the nine relative age classes. The results of the morphometric data together with the age determination data exhibited a clear 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 acted as an intermediate age class, that consisted of both reproductive and non-reproductive individuals. The statistical analysis of the morphometric data form different geographic localities indicated no distinct differences between them.

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. Due to their subterranean nature, mole-rats are exposed to little light on an infrequent basis, if ever. Yet these animals exhibit a rhythm of melatonin secretion similar to that of other mammals. However, it is not known whether the melatonin rhythm effectively reflects different daylengths.

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). Blood samples collected in the dark period yielded significantly higher melatonin secretion, compared to blood samples collected in daylight. However, the circadian pattern of melatonin secretion in LD did not differ from the pattern observed in SD. Thus, while a circadian rhythm of melatonin secretion exists in *C .h. pretoriae,* the secretion of melatonin cannot be used as a means of distinguishing between different daylengths. It is postulated that in this subterranean rodent mole-rat, other factors such as seasonal changes in temperature and precipitation patterns may be the ultimate cues that the mole-rats respond to for the timing of reproduction.

Thus, in conclusion I suggest that the highveld mole-rat is a seasonal breeder, with the proposed breeding period lasting from May through to November. Evidence suggest that these animals are capable of producing two litters per breeding season. Increased reproductive activities of the males coincides with that of the females, although the males tend to keep their testes functional out of the breeding period.

It is suggested that the highveld mole-rat cannot effectively utilise the photoperiodic signal and thus may use other seasonal *zeitgebers* such as temperature changes or seasonal rainfall as cues for dispersal and the onset of reproduction.

The small colony size (12 individuals) of this species of *Cryptomys* is probably the result of frequent dispersals. The founding animals of a colony become the reproductive force and it is proposed that these individuals are the oldest or amongst the oldest individuals in a colony. Males tend to be heavier than females, but no sexual dimorphism occurs between the sexes with regard to their skull morphometrics. Definite

age groupings are evident within the colony structure, placing the reproductive animals in the oldest age classes.

Chapter 1

General introduction

Seasonal reproduction

Interactions between organisms and their physical environment as well as with other species result in many species exhibiting a restricted breeding period. Reproduction confined to a specific season, requires an animal to be able to time reproduction to ensure that the young are born at the most favourable time of the year, to promote rapid growth and maximal survival (Ims 1990). Seasonal reproduction involves the association of a series of physiological events from gonadal growth, steroidogenesis, gametogenesis, mating and finally the appearance of the young, all occurring within a particular season of the year (Jameson 1988).

Great advantages accompany seasonal reproduction. For example the musky rat-kangaroos, *Hypsiprymnodon moschatus,* produce their young when the two main food resources in their diet, litter fauna and fruits, are in great abundance (Dennis & Marsh 1997). Furthermore, regression of the reproductive organs during the non-breeding period can also be of benefit to an animal. Gonadal regression allows the animal to utilise energy for other activities, such as food gathering, rather than maintaining reproductive organs when not in use (Woodall & Skinner 1989).

Two main groups of cues are used for the onset of seasonal reproduction, namely internal and environmental cues. Endogenous rhythms constitute the internal cues, while environmental cues include factors such as food availability, humidity, temperature and photoperiod.

Mole-rats may use the onset of rainfall and photoperiod to trigger reproduction (Jarvis 1969; Moolman *et al.* 1998; Pevet *et al.* 1984). Temperature is yet another environmental cue that may be important for the onset of reproduction within the

subterranean mole-rats. Although temperature fluctuations in the burrow systems of mole-rats are muted compared to the seasonal ambient fluctuations, there is still some seasonality to the fluctuations (Bennett *et al.* 1988). The foraging burrows of mole-rats do not exceed depths of 30 cm and fluctuations in surface air temperature, influence soil temperatures (Gates 1962; Jarvis 1969; Bennett *et al.* 1988). Furthermore, Bennett *et al.* (1988) found marked seasonal differences in the mean burrow temperatures of mole-rats occurring in mesic and arid habitats, while in the tropics little seasonality in burrow temperature occurred. The presence or absence of seasonal temperature changes in the burrows may be an important determinant in the onset of reproduction in seasonally reproducing mole-rats (Bennett *et al.* 1988).

Another important indirect environmental cue is rainfall (Jarvis 1969). Recrudescence of digging results from rainfall, the result of such excavation being surface mounds of freshly displaced soil. The mole-rats extend their burrow systems and this increases the opportunities for non-reproductive animals to disperse from their natal colony to form their own colonies (Moolman *et al.* 1998). In addition, rainfall influences vegetation growth, thereby affecting availability of food resources (Dennis & Marsh 1997).

Of all the environmental cues, photoperiod is perhaps the most commonly used synchronising agent determining the breeding period of animals, as day length stays constant from year to year (Karsch *et al.* 1984; Gardiner *et al.* 1999). Since mole-rat species are subterranean most individuals are rarely, if ever, exposed to light. Individuals of the blind mole-rat species (*Spalax ehrenbergi*), for example, only receive light stimulus during winter, when they extend their burrow systems, pushing excavated soil to the surface (Shanas & Terkel 1996). However, the light stimulus in winter would not be enough for these animals if the development of their reproductive system were dependent on it. It is necessary for females to be exposed to light stimulus in summer to determine the onset of the reproductive process, to ensure that these animals would be able to breed during the winter months. Light impulses are received during the summer period, thus emphasising the importance of the mole-rat's reproductive circannual rhythm (Shanas & Terkel 1996; Shanas *et al.* 1995).

Trends in reproduction within the subterranean rodents

In solitary subterranean rodents there is sole occupancy of the burrow system. Plural or multiple occupancy arising only during the mating period or when the mother has offspring (Bennett & Jarvis 1988). The pups remain in the maternal burrow system for a short period that may not exceed 60 days in most cases. After this time, the mother will actively and aggressively expel the pups from the system (Bennett & Jarvis 1988; Bennett *et al.* 1991). The social subterranean rodents differ from the solitary species in that the breeding animals share a burrow throughout the year, the offspring do not disperse but rather share it with the parents throughout the year or extended periods of time. Indeed, they become extended families and show varying degrees of philopatry.

World wide, subterranean rodents are typically highly xenophobic and aggressively defend their natal burrow system. The presence of a single animal in a burrow system means that for breeding to take place, the extremely strong barriers of territoriality and aggression must be broken down. Advertisement of sex, status and the intention to breed must also be conveyed to conspecifics. This is achieved usually by seismic communication, which can take on a variety of forms. The blind mole-rat, *Spalax ehrenbergi*, uses head drumming (Rado *et al.* 1987; Heth *et al.* 1987) whereas incisor tapping is used in the rhizomyid *Tachyoryctes splendens* (Jarvis 1969). Hind foot drumming has been reported in the Geomyidae (the gophers; O.J. Reichman pers. comm.) and in three bathyergids, *Georychus capensis* (Bennett & Jarvis 1988), the Namaqua and Cape dune mole-rat (Bennett *et al.* 1991; Jarvis & Bennett 1991). This long distance communication allows members of the opposite sex to come together to procreate.

In contrast, the social species of subterranean rodents can reproduce when the occasion arises. There is no need for a seismic component to reproduction since the breeding animals occur together in the same burrow system. Courtship in the social bathyergids is less abrupt than that which occurs in the solitary species. Because the animals co-habit the same tunnels, pair bonds are formed by the breeding animals. There is considerable foreplay prior to mounting and mating. In seasonally breeding social species mounting and attempted mating is observed outside of the breeding season and this may strengthen the pair bond (Bennett *et al.* 1999).

Social suppression of reproduction in colonial bathyergids

The African mole-rats are fascinating since they exhibit both a range of social organisation and mechanisms to restrict reproduction to a single breeding female (Bennett *et al.* 1997).

Amongst outbred social mole-rats that inhabit mesic habitats, where the opportunities for dispersal and of becoming reproductive in a new colony are potentially high, one would predict that the staying incentives offered by the breeders to subordinates would be higher than in areas with strong environmental constraints. In these species incest avoidance would be the underlying form of monopolisation of reproduction and physiological suppression would be absent (Bennett *et al.* 1999).

In marked contrast to the mesic species, the naked mole-rat inhabits areas in which the ecological constraints are high and therefore theoretically the reproductive female need offer few, if any staying incentives. Naked mole-rats exhibit the most extreme form of social suppression found in the Bathyergidae, with there being physiological suppression in both sexes (Faulkes *et al.* 1990; 1991). The absence of incest avoidance necessitates a stringent reproductive control in the form of physiological suppression.

Bennett *et al.* (1997) suggested that within the family Bathyergidae, there is a continuum of socially-induced infertility occurring amongst different species inhabiting regions of varying degrees of aridity. Hence, in mesic habitats where opportunities for dispersal and of becoming reproductively active in new colonies are great, there is predominantly an incest avoidance component to social suppression and colony members are obligate outbreeders. In marked contrast, the naked mole-rat is an obligate inbreeder, exhibits physiological suppression in both sexes of non-reproductives and shows dominant control. The Damaraland mole-rat (*Cryptomys damarensis*), an obligate outbreeder, lies between these two extremes in that non-reproductive females have both a behavioural and physiological suppression operating upon them (Bennett *et al.* 1996).

The study animal: *Cryptomys hottentotus pretoriae* (Faulkes 1997)

Within the family Bathyergidae a sociality continuum exists, with species ranging from strictly solitary through to the true eusocial species (Jarvis & Bennett 1991; 1993). The genus *Cryptomys* has a wide geographical distribution throughout Africa and contains social representatives that range from loosely social species, such as *Cryptomys hottentotus hottentotus* to the true eusocial mole-rat, *C. damarensis* (Jarvis & Bennett 1991; De Graaff 1964). According to Jarvis *et al.* (1994) the frequency of rainfall and the degree of aridity in the habitat, plays a role in the social organisation of each mole-rat species. Indeed two very important ecological factors, the amount of precipitation per year and the distribution and abundance of food, are directly linked to the social status of each species (Jarvis *et al.* 1994). Thus, eusocial species, such as *C. damarensis* and *Heterocephalus glaber* occur in very arid habitats. These animals reproduce throughout the entire year, with no seasonal component linked to their reproduction. Solitary species such as *G. capensis* are known to occur in the more mesic regions of the Cape and reproduce seasonally. *Cryptomys hottentotus pretoriae,* the highveld mole-rat, is of special interest in that the individuals lead a colonial lifestyle and yet occur in a habitat with fairly predictable rainfall that should select for a solitary lifestyle (Moolman *et al.* 1998).

The highveld mole-rat is a social, subterranean, rodent mole-rat that occurs in colonies of up to twelve individuals (L. Janse van Rensburg, unpubl. data). The highveld mole-rat is phylogenetically closer to *Cryptomys hottentotus natalensis* than to *C. h. hottentotus* (Faulkes *et al.* 1997). They occur on the verdant highlands of South Africa, characterised by cold dry winters and hot moist summers (South African Weather Bureau).

The highveld mole-rat occur in colonies comprising of one reproductive female and one or two reproductive males that are responsible for the procreation of the new colony members (Moolman *et al.* 1998). Non-reproductive females have functional ovaries, suggesting these animals are only reproductively quiescent and not sterile. The non-reproductive males are not physiologically different from the reproductive males in

that their testes are of similar size and both groups undergo spermatogenesis. Non-reproductive males are behaviourally suppressed preventing them from reproducing with the reproductive female (Bennett *et al.* 1994).

It is still unclear what triggers the onset of reproduction in the highveld mole-rat. As suggested by Moolman *et al.* (1998) it is most likely that the highveld mole-rat uses rainfall as a cue for dispersal, since rainfall is frequent and predictable in these habitats (South African Weather Bureau). Opportunities therefore frequently arise for non-reproductive animals to disperse and form their own colonies. The small colony sizes of the highveld mole-rat tend to suggest that emigration from the colony is a common affair. Photoperiod is a potential *zeitgeber* that may have an important influence on the reproduction of the highveld mole-rat and this cue was also investigated during the course of the study.

Aims of the thesis

Knowledge regarding the reproductive biology of *C. h. pretoriae* is extremely fragmentary. A study on the molecular phylogeny by Faulkes *et al.* (1997) and a study undertaken on the social structure and dominance hierarchy by Moolman *et al.* (1998) provide us with the only information about this sub-species. The primary aim of this thesis is to investigate the reproductive cycle of the highveld mole-rat. The common mole-rat, a close relative of the highveld mole-rat, occurs in the winter rainfall region of the Cape province. It is a seasonal breeder that produces two litters per annum (Spinks *et al.* 1999). The highveld mole-rat in comparison occurs in the summer rainfall regions on the escarpment of South Africa, but to date we do not know if it is a seasonally or aseasonally breeding animal.

In chapter 2 the reproductive activity of both males and females are discussed. Intensive histological procedures were used to establish the reproductive cycle of the highveld mole-rat to elucidate whether it is a seasonal of aseasonal breeder. In addition hormone assays were executed to establish if the data obtained would co-incide with the data found during the histological part of the study. Sperm data for the males were

analysed to determine whether any patterns might occur and whether these patterns concur with the reproductive pattern found in the females. In chapter 3 relative age classes, mass and reproductive status was taken into account to determine if the oldest animals in a colony are the breeders. Dispersal and the establishment of new colonies by these vertebrate alates is believed to involve the older and stronger individuals within the colony. Thus, it is suggested that these animals would be the oldest or amongst the oldest members within the colony. Morphometric data was included to determine if sexual dimorphism is present. In addition the morphometric data was analysed for four separate localities, to determine any differences that might occur between different populations from the four localities. The possible role of photoperiod as a cue for the onset of reproduction was investigated in chapter 4, where mole-rats were exposed to two different light regimes. Due to the subterranean nature of this mole-rat it is interesting to explore the possible affect photoperiod might have on reproduction and whether or not it might be some other environmental factor such as rainfall that might play a more important role in the onset of reproduction.

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Chapter 2

Seasonal breeding in the highveld mole-rat, *Cryptomys hottentotus pretoriae*

ABSTRACT

Cryptomys hottentotus pretoriae is a co-operatively breeding rodent that exhibits seasonal breeding and a reproductive division of labour. Body mass, reproductive tract morphometrics, ovarian histology and plasma oestrogen and progesterone concentrations were studied in 189 females from 49 colonies. Although the birth of the offspring is confined to the months of May through to November, qualitative analysis of ovarian histology revealed that females retained reproductive function during the summer non-breeding period (December – March). Seasonal differences were found in ovarian morphometrics and progesterone and oestrogen concentrations which are associated with enhanced follicular activation in April and May and subsequent conceptions from May through to November during the breeding period.

The continuance of ovarian function during the non-breeding period as evidenced by the production of corpora lutea in reproductive females parallels the situation found in the male members of the colony. Interestingly, the non-breeding period coincides with the period of maximal dispersal opportunities in the summer rainfall areas inhabited by the highveld mole-rat. Non-reproductive females, while exhibiting some follicular development failed to ovulate in the confines of the colony, as evidenced by a lack of corpora lutea. The endocrinological data supports the lack of ovulation in these socially suppressed non-reproductive females.

Body mass, reproductive tract morphometrics, testicular histology and plasma testosterone concentrations were studied in 92 males from 37 colonies. The available evidence suggests that there is a gradual increase in testicular mass and volume with

increasing proximity to the breeding season, but after September, the testicular parameters begin to fall. Seminiferous tubule diameter was significantly greater in reproductive males but there was no obvious change with season. In general, testosterone concentrations are higher in the reproductive males, with the highest titres occurring around July and August. All available evidence supports a continuance of reproductive activity during the non-breeding season. It is speculated that reproductive activation in the non-reproductive males may facilitate inter-sexual recognition and hence facilitate bond formation for independent reproduction.

Bimonthly sampling of males to investigate sperm motility, revealed no significant difference between the sperm kinematics of reproductive (n = 14) and non-reproductive males (n = 17).

The available data suggests that there are two main periods of both follicular and testicular activity, these being June and September. It is possible that for an animal with an estimated gestation of two months, that the reproductive potential of producing two litters during the breeding season arises with pups being born at the end of July through to November.

Evidence from this study suggests that during the non-breeding season, moist workable soils facilitate the dispersal of previously non-reproductive animals from their natal colonies and subsequent colony genesis arises from previously suppressed females and males.

INTRODUCTION

Reproduction is important to the biology of all organisms and is the means by which an individual perpetuates copies of its genes. Considering the fundamental role of reproduction in organismal biology, it is surprising that little research has been afforded to subterranean rodent moles (Bennett *et al.* 2000). Reproduction in subterranean rodents is ecologically constrained by the burrow environment. Indeed, subterranean rodents rarely, if ever, venture onto the surface and therefore many of the common proximate cues normally utilised by seasonal breeders, such as photoperiod are precluded from use.

In the labyrinths of the burrow system, other environmental cues may trigger the onset of breeding such as thermoperiod, changes in soil moisture content or the associated sudden flush of vegetation associated with good precipitation (Bennett *et al.* 1988). Seasonal rainfall, which results in a softening of the soil may facilitate the extension of existing burrows as well as enabling opposite sexed conspecifics to come together for the process of procreation.

Members of the Bathyergidae are unique in that they display a broad spectrum of social organisation ranging from strictly solitary through to eusocial representatives (Jarvis *et al.* 1994; Bennett & Faulkes 2000).

Many subterranean rodents are strictly solitary, highly xenophobic and aggressively defend their natal burrow system from conspecifics (Nevo 1979). In these solitary species, reproduction is usually a brief affair, during which strong barriers to aggression are broken down and mating is thus short-lived (Bennett & Jarvis 1988). In solitary species, plural occupancy of the burrow occurs briefly during the breeding season or when the female has young (Bennett & Jarvis 1988).

Along the gradient of social organisation lies a number of social species of the genus *Cryptomys*. All solitary species of Bathyergidae reproduce seasonally, whereas the majority of social mole-rats exhibit no seasonal component to their reproduction, thus reproducing throughout the year (Bennett *et al.* 1991). However, to date one social species from the winter rainfall region of the western and northern Cape Province has been found to be a seasonal breeder (Spinks *et al.* 1997; 1999). Spinks *et al.* (1997; 1999) have shown that the social common mole-rat, *C. h. hottentotus*, exhibits a marked seasonality to reproduction, rearing young during the southern hemisphere summer (late November through to January).

The highveld mole-rat, *C. h. pretoriae* occurs in the summer rainfall regions of the highveld in South-Africa. The sub-species *C. h. pretoriae* is phylogenetically closely related *to C. h. hottentotus*, the common mole-rat, also a social, seasonal breeder (Faulkes *et al.* 1997). The highveld mole-rat occurs in colonies of similar size as the common mole-rat, 2-12 individuals per colony (Moolman *et al.* 1998; L. Janse van Rensburg, unpubl. data). The warm wet summers and dry cool winters of the highveld show a distinct seasonality. This seasonal component and the close phylogenetic relationship

between the common mole-rat and the highveld mole-rat suggests that this social subterranean rodent may also exhibit the potential for seasonal reproduction.

Moolman *et al.* (1998) found the highveld mole-rat to be a loosely social species with no distinct dominance hierarchy. While colonies exhibit a marked reproductive division of labour, with up to two males and one female being responsible for procreation, no secondary work division of labour is apparent (Moolman *et al.* 1998). This is in contrast to the situation found in the Damaraland mole-rat (*Cryptomys damarensis*) (Bennett & Jarvis 1988). Given that the environment in which the highveld mole-rat occurs, exhibits an annual seasonal component to it in the form of a change in both temperature and precipitation, my *a priori* prediction was that this social subterranean rodent mole should exhibit a seasonality to reproduction both in the production of young and also in the recrudescence and regression of the gonads.

In order to address this question, I adopted an approach of sampling a population of highveld mole-rats in the Gauteng Province of South Africa, on a monthly basis for an entire calendar year. Post-mortem examinations have proven invaluable in determining whether subterranean rodents have a seasonal component to reproduction (see Bennett *et al.* 2000 for review). Basic reproductive parameters, such as duration of pregnancies, physical dimensions of reproductive organs and patterns of follicular development and sperm production have been quantified for the tuco tuco, Ctenomyidae (Malizia & Busch 1991), the pocket gophers, Geomyidae (Miller 1946; Smolen *et al.* 1980), the African mole-rats, Bathyergidae (Jarvis 1969; van der Horst 1972; Bennett & Jarvis 1988) and the mediterranean mole-rats, Spalacinae (Redi *et al.* 1986).

Social subterranean rodents have reproduction monopolised by a single female, with all other females being reproductively quiescent or exhibiting socially-induced infertility (Bennett *et al.* 1993; 1994b; 1996; 1997). The reproductive status of an animal is readily identifiable and consequently an examination of complete colonies allows the determination of the reproductive state of this individual at any point in the year. However, the repression of reproduction in non-reproductive females also provides an opportunity to investigate whether relaxation of suppression occurs at any particular part of the season in these otherwise behaviourally infertile females.

In the social bathyergid mole-rats suppression of reproduction in non-reproductive males does not appear to be physiological in nature. Faulkes *et al.* (1994) found no apparent suppression of sperm production or sperm motility in non-reproductive Damaraland mole-rats. However, they found that suppression of reproductive hormones in *Heterocephalus glaber* might correlate with reduced fertility in the non-reproductive males, because the majority of these males produced fewer, non-motile spermatozoa than the reproductive males. However, Faulkes *et al.* (1994) also found that a number of non-reproductive males were reproductively active and thus potentially fertile. Whenever non-reproductive males were removed from their natal colonies, their body and testes size increased and they produced a greater number of motile spermatozoa. In this study, the presence of sperm production and sperm motility, in both reproductive and non-reproductive males throughout the study period were examined to determine the potential non-reproductive males may possess to successfully reproduce when they disperse from their natal colony. I also aimed to determine if any relationship might occur between increased sperm motility and increased testes volume and mass.

The aims of this chapter were threefold. 1) to determine if the highveld mole-rat is a seasonal breeder, 2) to assess if there is relaxation of reproduction in non-reproductive members of the colony at any point in the year and 3) to determine if seasonality of breeding (if present) can be linked to rainfall.

MATERIALS AND METHODS

Capture

A minimum of three colonies of the highveld mole-rat were caught on a monthly basis using modified Hickman live traps (Hickman 1979). The capture period lasted from January 1998 to April 1999 and a total of 126 males and 260 females from 55 colonies constituted the source of my study. Ninety-six of the animals caught were used for a melatonin study (Chapter 3). The animals were captured on golf courses and in gardens in the environs of Gauteng: Pretoria (25°45'S 28°10'E) (Tygerpoort, Monumentpark golf course, Dienste golf course), Johannesburg (26°12'S 28°05'E) (Johannesburg

Countryclub, Modderfontein golf course, Bryanston golf course, Esselinpark golf course), Krugersdorp (26°06'S 27°46'E) (Palm nursery), Vanderbijlpark (26°42'S 27° 49'E) (Industrial areas and small holdings).

Colonies were caught out in their entirety, a colony being deemed fully trapped out if open sections of the burrow where animals had been trapped were not blocked with soil after 3 days.

Housing

The animals were housed in plastic crates (49.5cm x 28cm). Wood shavings and paper towelling were provided as nesting material. The mole-rats were fed sweet potato, gem squash, carrots and apples on a daily basis. The animals were kept in a climate room at a constant temperature of 25 ± 1°C. The animals were maintained in the laboratory for as short a time as possible. However, to ensure that post-mortem examination was as accurate as possible, functionally complete colonies were maintained together for a minimum of a week after all individuals in a system had been trapped out.

Determination of reproductive status

In the laboratory, the animals were sexed and their reproductive status determined. Prominent axillary teats were one of the characteristics used to identify the reproductive female from non-reproductive females. Many of the females caught, exhibited a perforate vagina, irrespective of whether they were reproductive or non-reproductive. During histological procedures confirmation of the reproductive status of the females was supported by the presence of foetuses and placental scars present on the uterine horns, as well as by the presence of corpora lutea or corpora albicans in the ovary. The reproductive male was discerned based on its physical size and its observed copulation with the reproductive female in the laboratory whenever possible.

Histological procedures

After capture and prior to being killed each individual was weighed using a Sartorius 1213MP scale (max = 3000.0g, Zeiss, Germany). The animals were then deeply anaesthetised with halothane, causing death. Blood samples were obtained by

exsanguination from the heart. The blood was centrifuged at 3000rpm and the plasma fraction obtained was immediately stored at -20°C.

The animals were then dissected in order to remove the reproductive tracts. The material was fixed in Bouins fluid for approximately 16 hours prior to being rinsed and stored in 70% ethanol. Prior to being processed, the fixed gonads were weighed using a Sartorius scale (max = 100g, d = 0.1mg, Zeiss, Germany). Both ovaries of the females and both testes of the males were weighed. For statistical analyses the mean of the gonads for each individual was determined.

The material was sequentially dehydrated and embedded in paraffin wax. The ovaries and testes were serially sectioned at 7µm, mounted on glass slides, stained in Ehrlich's haemotoxylin and counter stained in eosin (Drury & Wallington 1967). Using a vernier calliper, the maximum length and width of each testes and ovary was measured. Ovarian volume as well as testicular volume was calculated using the formula for the volume of an ellipsoid, as described by Woodall & Skinner (1989); $V = 4/3\pi ab^2$, where a = ½ maximum length and b = ½ maximum breadth.

Female histology

Each ovary (n = 180) was sectioned in its totality and mounted. Sections were then examined in consecutive order using a light microscope at the following magnifications: x100, x200 and x400. Ovarian follicles were categorised and counted based on follicular development and atretic changes. Bloom & Fawcett (1962) and Bennett *et al.* (1994a) were used as a guide to identify and categorise the various follicular stages. The following follicle counts were made during the study:

1. Primordial follicles (P) were numerous and located at the periphery of the ovary, just interior to the tunic albuginea (Plate 1).
2. The development of primordial follicles into primary follicles (Pr) is characterised by the transition of the flattened or squamous follicular cells into cuboidal cells (Plate 2). The primary follicle is much larger than the primordial follicle and contains an enlarged oocyte surrounded by one or more layers of cuboidal cells.

3. The transition from primary to secondary follicles is marked by the appearance of several irregular spaces in the stratum granulosum, filled with clear liquor folliculi (Plate 3). An increase in the amount of this liquid causes the cell to increase in size and develop into the next follicular stage.
4. The Graafian follicle was defined as having a large continuous fluid filled antrum with the oocyte pressed to one side of the follicle (Plate 4).
5. A ruptured Graafian follicle, named the corpora haemorrhagicum is another distinct feature of a female in a state of ovulation. The ovum loosens itself from the cumulus oophorus and as soon as the follicular wall ruptures the ovum is released (Plate 5).
6. During an organisms lifetime only a fixed amount of ova are discharged during ovulation. The remainder of the follicles degenerate and disappear. This involution of a follicle is termed "atresia" (A) (Plate 6).
7. After the Graafian follicle ruptured it is transformed into a corpus luteum (Cl) (Plate 7). The corpus luteum serves as a source of progesterone during pregnancy.
8. After pregnancy the corpus luteum will regress until it is reduced to a scar, the corpus albicans (Plate 8).

In addition to the follicular count the presence of placental scars and foetuses were recorded, including the presence of very young animals within colonies.

Male histology

Only a few selected sections, from the mid region of the testes were mounted. Sections were then examined using a light microscope at the following magnifications: x100, x200 and x400. Thirty randomly selected, cross-sectioned seminiferous tubules from each male specimen, were chosen and their diameter measured with the aid of an eyepiece micrometer (Vickers instruments).

The following was studied during the histological part of the study using Bloom & Fawcett (1962) as a reference:

1. The seminiferous tubules are lined by the seminiferous epithelium (Se), which consists of two types of cells the supporting cells of Sertoli and spermatogenic cells (Plate 9).
2. The epididymis is an elongated organ attached to the posterior surface of the testis (Plate 10). It is made up of the convoluted proximal part of the excretory duct system.

The results are based on the assumption that any changes in size that might have occurred as a result of fixation were constant across all the samples measured. Thus, all measurements are relative and not absolute (Spinks *et al.* 1997).

Sperm collection

Male highveld mole-rats were put down by halothane inhalation on a bimonthly basis. The testes and epididymis were dissected free from the surrounding connective tissue and fat. The material was placed in a 35mm plastic petri dish filled with preheated Ham's F10 (Sigma Cat No. N6635) culture medium with L-glutamate and supplemented with 1.2 g/l sodium bicarbonate. This medium is used to activate and preserve sperm mobility. By puncturing the vasa deferens the sperm appeared as a thick white fluid and were collected using a pipette. A volume of 10µl of sperm suspension was transferred to an object slide and covered with a cover slip. The slide was placed on a microscope stage pre-heated to 36°C and 5-10 minutes of free-swimming sperm were videotaped using a VHS recorder at 320x magnification.

After the extraction of the sperm, the testes and epididymis were fixed in Bouins fluid for 16 hours, rinsed and stored in 70% ethanol for further histological studies (See male histology, materials and methods).

Tracking sperm movement

Images were recorded at 30 frames per second and played back at 1/10 of the normal speed. Frame by frame analysis was facilitated by coupling the videotape – output to a computer based image analysis system. (Sperm Motility Quantifier, Wirson Scientific and Precision Equipment, Auckland Park, Johannesburg). As the sperm moved across

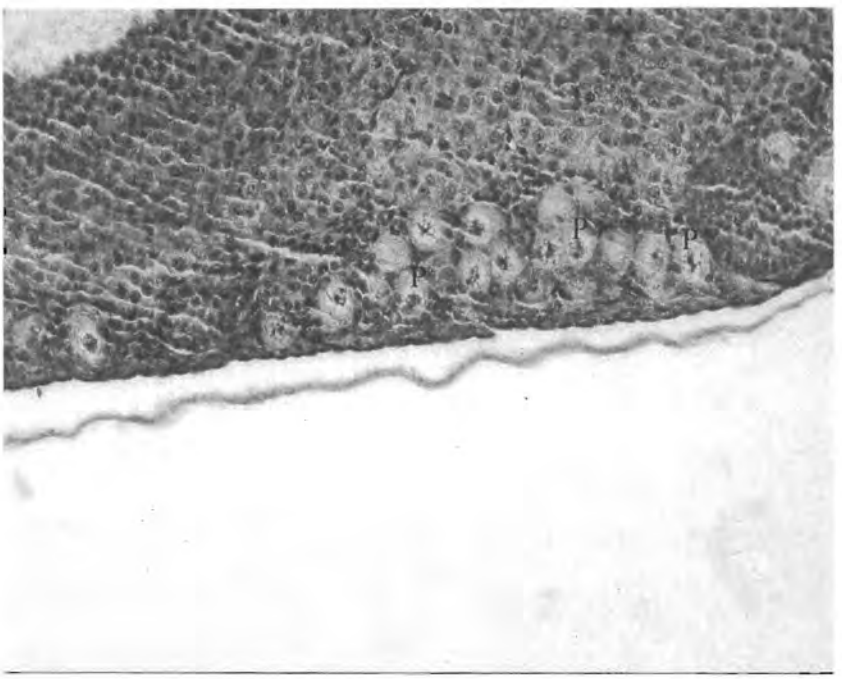

Plate 1. Primordial follicles.

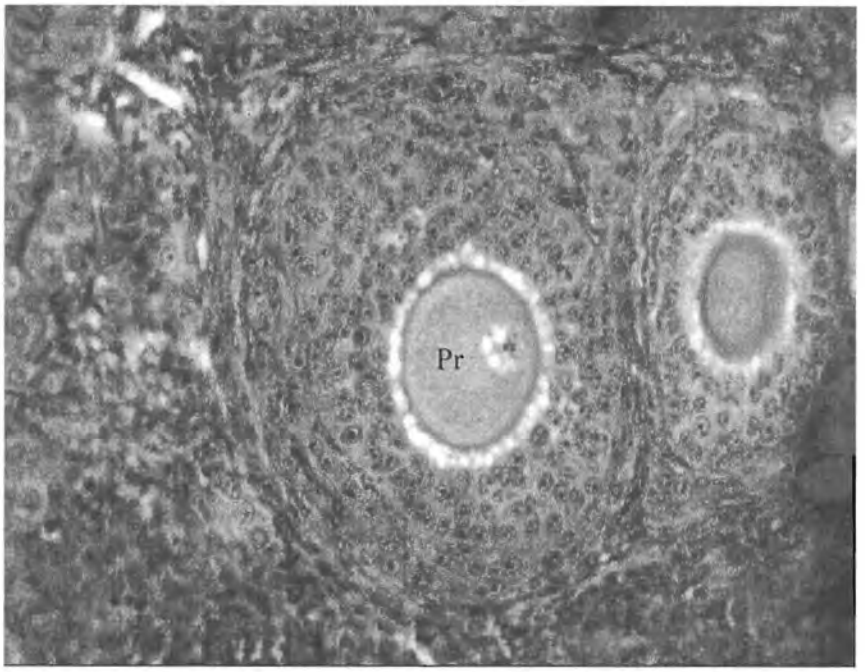

Plate 2. Primary follicle.

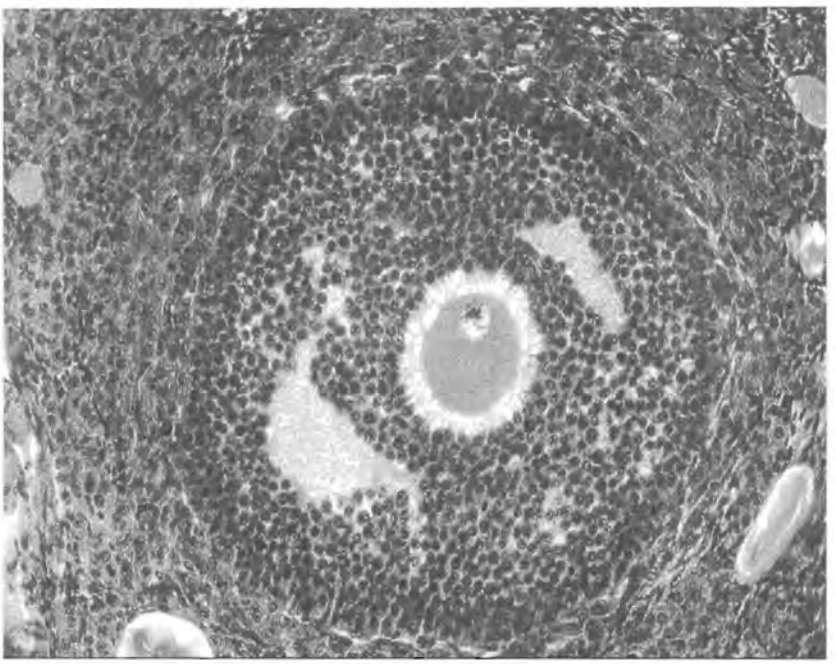

Plate 3. Secondary follicle.

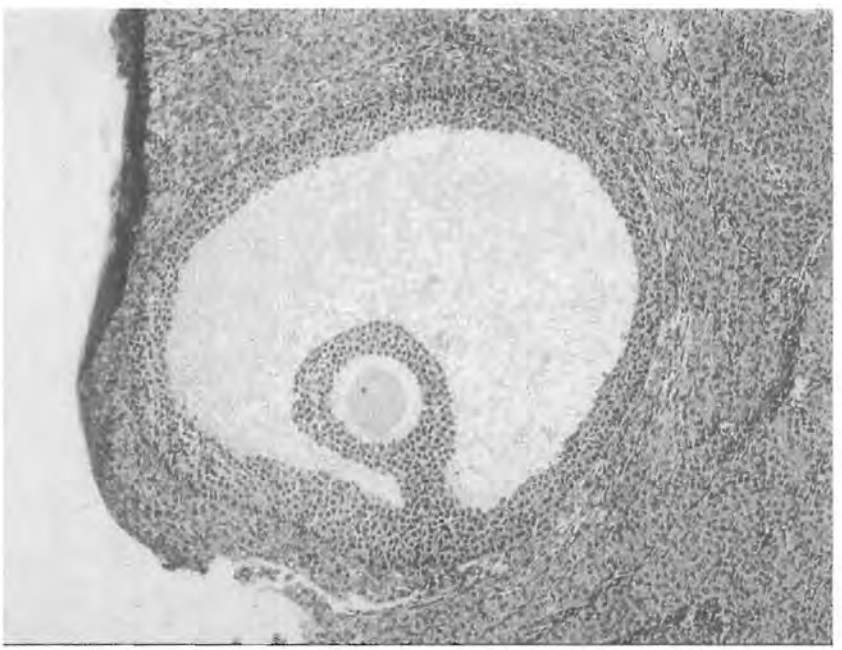

Plate 4. Graafian follicle.

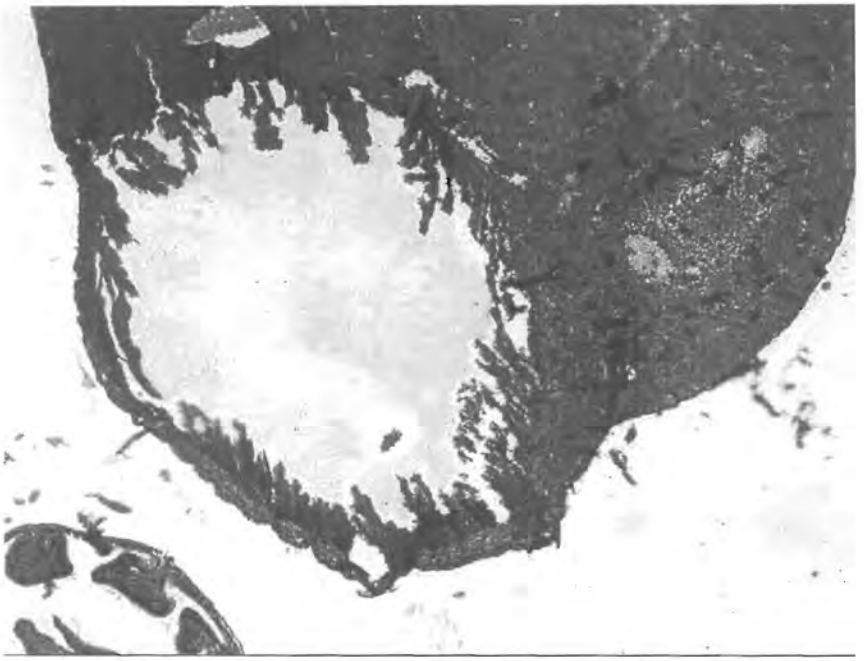

Plate5. Corpus haemorachicum.

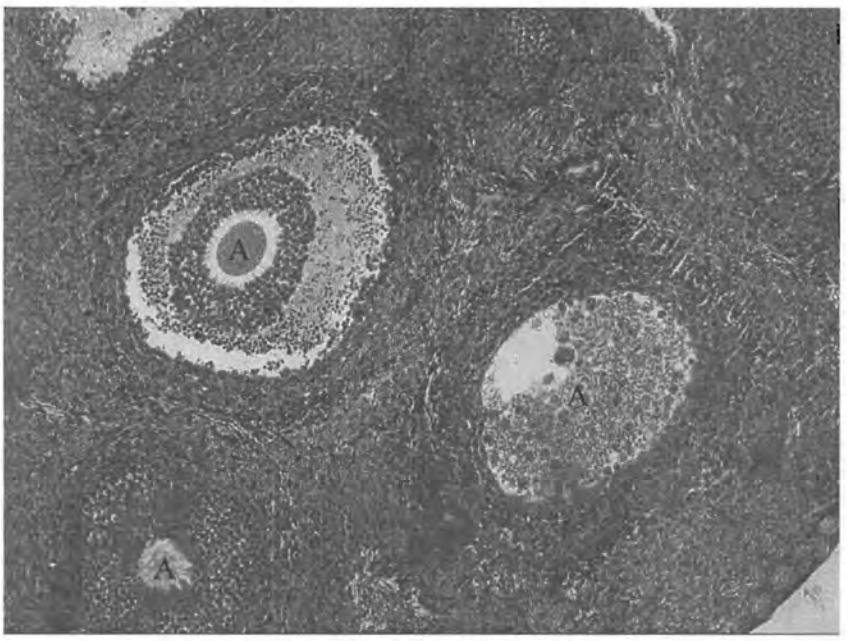

Plate 6. Atretic follicles.

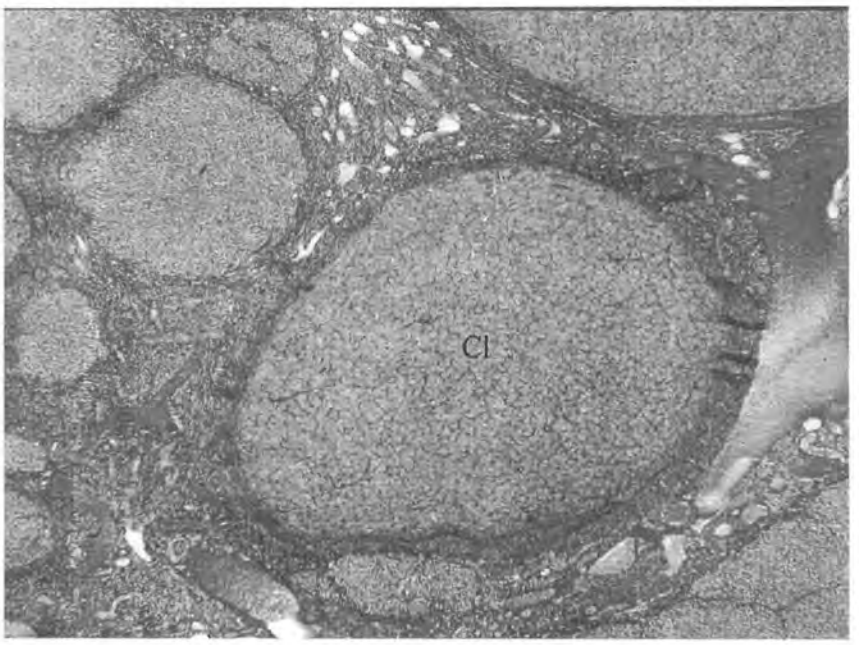

Plate 7. Corpora luteum.

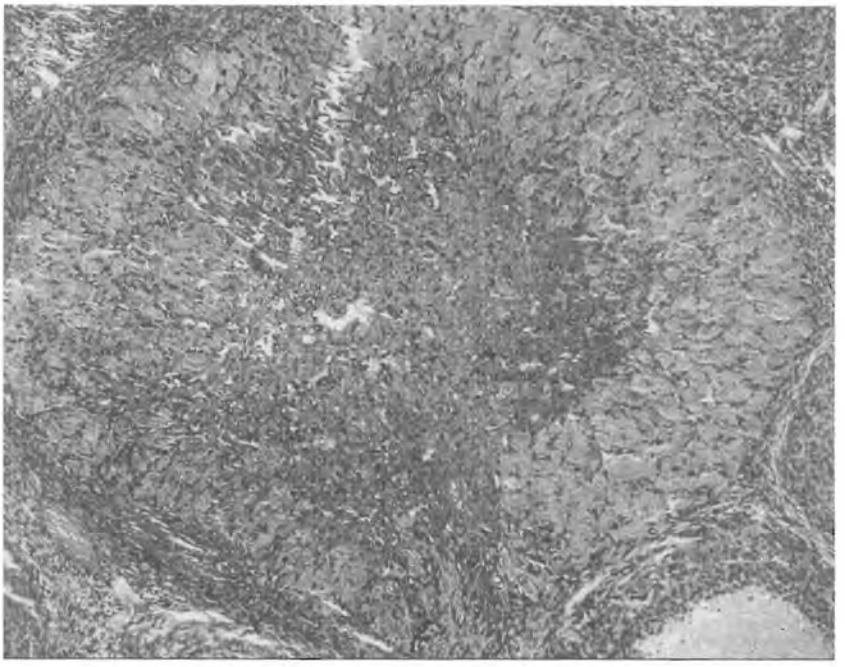

Plate 8. Corpus albicans.

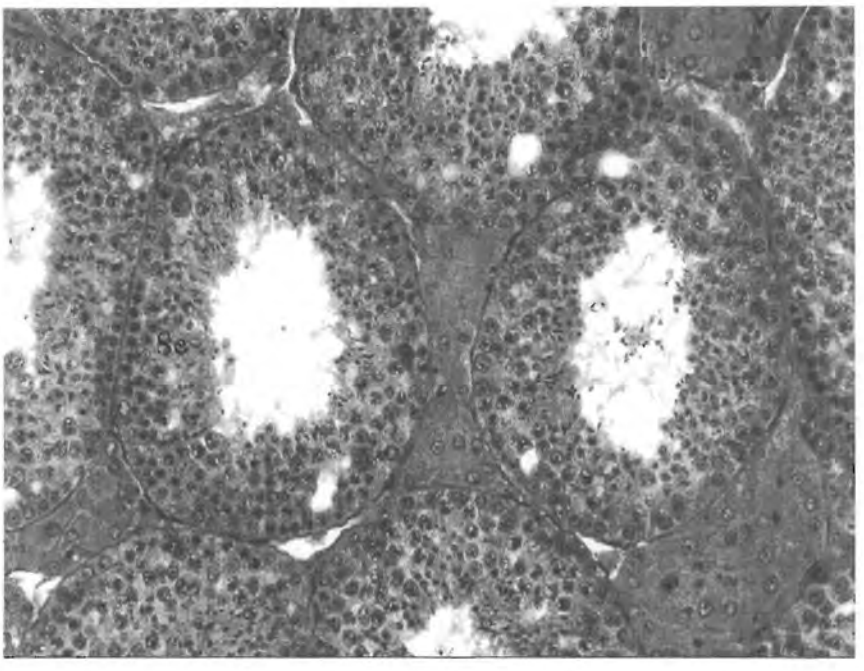

Plate 9. Seminiferous tubules.

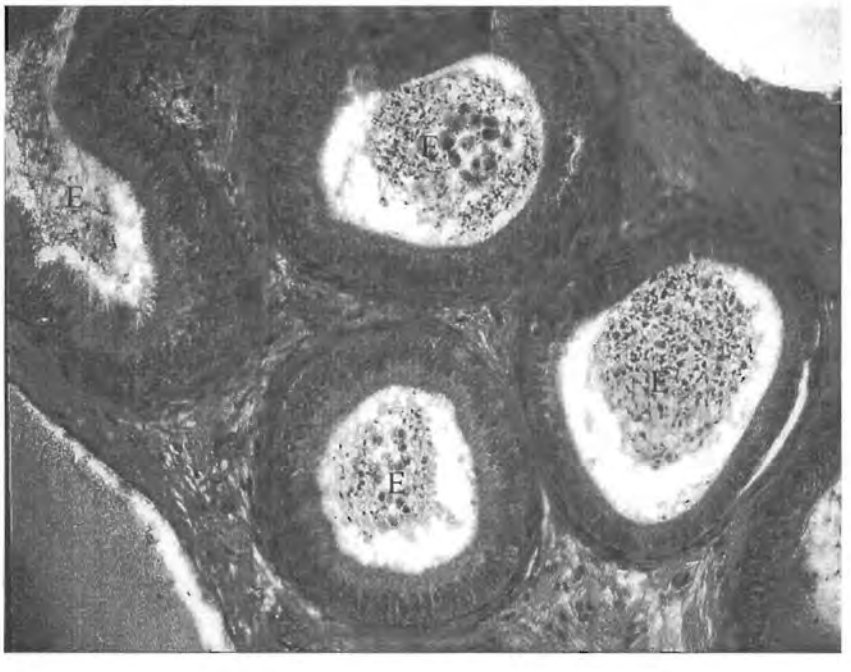

Plate 10. Epididymis

the screen the computer automatically tracked the position for the sperm head/midpiece junction for each frame.

The sperm recordings were captured with a frame skip of zero at an analysis rate of 50Hz: 25 frames were analysed to monitor the sperm trajectory. All tracings complete, the computer automatically scaled the distances between points to microns and calculated all motility parameters (Olds – Clarke 1986).

The following sperm motility parameters as defined by Katz (1991) were measured:

1. Curvilinear velocity (VCL): time-averaged velocity of sperm head along its actual path or curvilinear trajectory.
2. Straight-line velocity (VSL): time averaged velocity of sperm head as projected along the straight line between its first and final detection positions.
3. Average path velocity (VAP): time-averaged velocity of sperm head projected along its spatial average trajectory.
4. Beat cross frequency (BCF): the beat cross frequency of the sperm head.
5. Linearity (LIN): a ratio of projected length of the curvilinear trajectory. (LIN = VSL/VCL).
6. Amplitude of lateral head displacement (ALH): maximum amplitude of lateral distances of the sperm head trajectory about its spatial average path.
7. Wobble (WOB): ratio of VAP to VCL and is an expression of the degree of oscillation of the curvilinear path about its spatial average path (WOB = VAP/VCL).
8. Straightness (STR): ratio of VAP to VSL and is an expression of the straightness of the average path. (STR = VAP/VSL).
9. Dance (DNC): defined by the product of VCL an ALH, and describes sperm motion as the space occupied by the sperm head path during one second. DNC = VCL x ALH.
10. Radian (RAD): the radius of the circle of which the total curvilinear track is an arc. By using the radian, circling sperm can be detected (RAD = (radius/π) x 180^{o}).
11. Curvature (CURV): it reflects the progressiveness of movement (CURV = 1 – (VSLpath/VCLpath).

Progesterone determination

Progesterone was measured in 29 reproductive females (RF) and 154 non-reproductive females (NRF). The plasma progesterone determinations were undertaken on duplicate 100µl samples using the coat-a-count progesterone kit (Diagnostic Products Corporation, USA).

The antiserum is highly specific for all naturally occurring steroids with a cross reactivity of <0.5% except for 20 α dihydroprogesterone and 11-deoxycortisol for which it was 2% and 2.4% respectively. Steroids were not purified and separated by chromatography. The assay is a non-extraction assay.

Validation for progesterone

All the hormone assays were validated for use in the highveld mole-rat as described by Bennett *et al.* (1994b). Plasma progesterone samples from a reproductive female provided a displacement curve parallel to the standard curve when serially double diluted (over the range 1:1 to 1:64). The slope of both curves did not differ significantly (ANCOVA, $F = 2.27$, $p>0.05$; Fig.1), following a log-logit transformation of the data (Chard 1987). The intra assay coefficient of variation for a plasma pool was 7.9% ($n = 10$). The sensitivity of the assay was 0.159nmols/l.

Oestradiol determination

Oestradiol was measured in 23 reproductive (RF) and 145 non-reproductive females (NRF). Oestradiol determinations were performed using a Coat-A-Count Oestradiol kit (Diagnostic Products Corporation, USA). This method requires neither extraction nor chromatography. The antiserum is highly specific for oestradiol, with a very low cross reactivity to any other compounds present in the plasma samples.

Validation for oestradiol

A serial dilution of plasma oestradiol from a reproductive female paralleled the reference preparation, thus the slopes did not differ significantly (ANCOVA, $F = 0.66$, $p>0.05$, Fig. 2), following a log-logit transformation of the data (Chard 1987). The intra assay

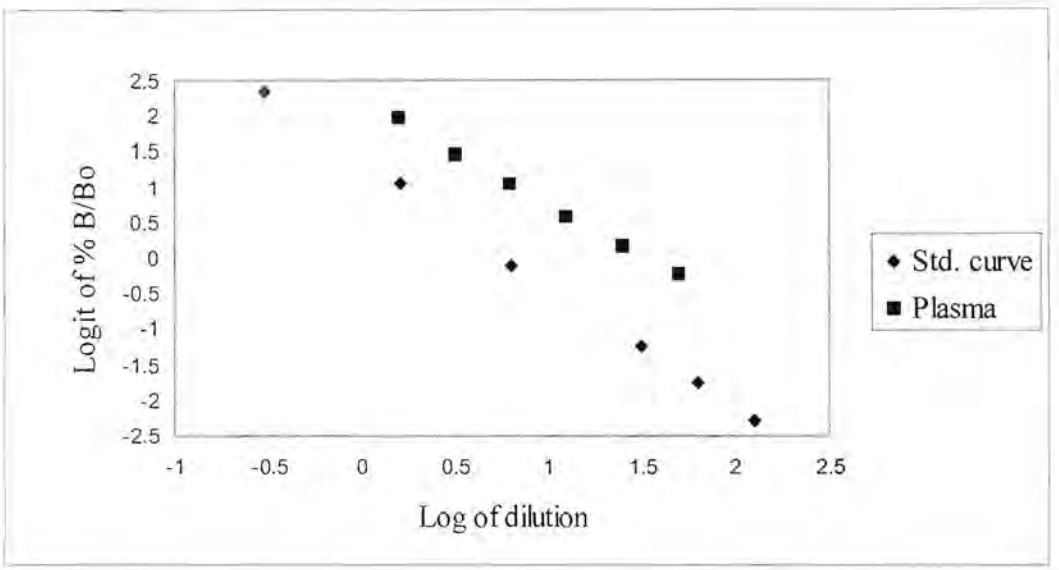

Fig. 1. Reference progesterone preparation (♦) and serial doubling dilution (■) of highveld mole-rat plasma, showing parallelism.

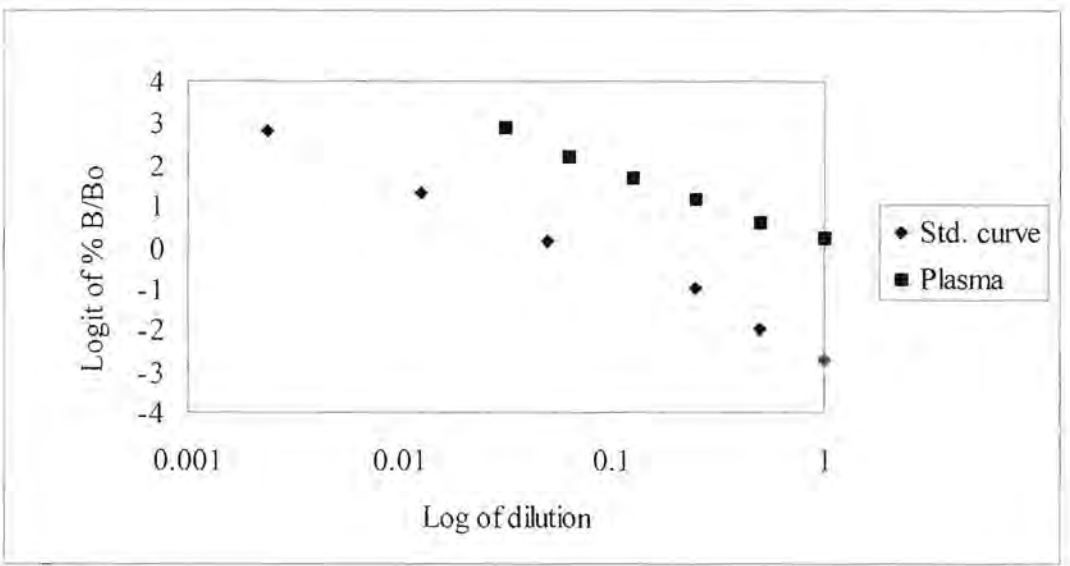

Fig. 2. Reference oestradiol preparation (♦) and serial doubling dilution (■) of highveld mole-rat plasma, showing parallelism.

coefficient of variation had a value of 17.38 (n = 6). The sensitivity of the assay was 0.002nmols/l.

Testosterone determination

Testosterone was determined for 43 reproductive males (RM) and 55 non-reproductive males (NRM). Total testosterone concentrations were measured using a Coat-A-Count total testosterone kit (Diagnostic Products Corporation, USA). Due to the simplicity of this method, neither extraction nor chromatography is required. The antiserum is highly specific for testosterone, with very low cross reactivity to other compounds. Crossreactivity with dihydrotestosterone is less than 5%.

Validation for testosterone

A serial double dilution of reproductive male plasma testosterone paralleled with the standard curve, thus validating the assay (ANCOVA, $F = 0.18$, $p>0.05$, Fig. 3). A log-logit transformation of the data was followed (Chard 1987). The intra assay coefficient of variation was equal to 4.9 (n = 6). The sensitivity of the assay was 0.011nmols/l.

Statistical analyses

Statistical analyses were performed using Statistica version 5.0™ and Prism, GraphPad Software™. The non-parametric, Mann Whitney U-test (Zar 1984), was used for comparisons between males and females with regard to their reproductive status.

After testing for normality within the hormone data sets, Kruskal Wallis and Dunn's multiple range tests (Zar 1984) were used to determine significant differences between reproductive and non-reproductive animals within months. Comparative testing between reproductive and non-reproductive male sperm was undertaken using a one way analysis of variance (ANOVA), after testing for homoscedacity. The sperm motility characters for reproductive and non-reproductive males were analysed using a Generalised Linear Model (GLM) (Zar 1984). 95% degree of confidence applies to all statistical tests.

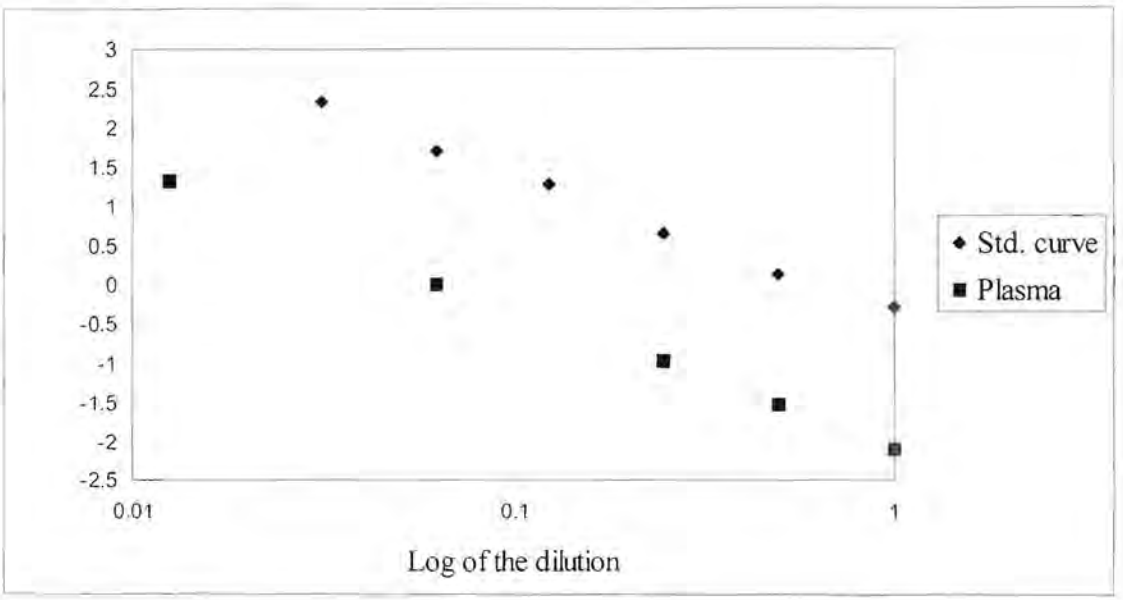

Fig. 3. Reference testosterone preparation (♦) and serial doubling dilution (■) of higveld mole-rat plasma, showing parallelism.

RESULTS

The ovarian histology

All follicle counts, measurements and hormone concentrations are expressed as mean ± S.E. (standard error) throughout for all animals studied. No S.E. bars are indicated on graphs, where sample size (n) = 1.

The number of primordial follicles was significantly higher for the non-reproductive females than for the reproductive females (NRF = 206.21 ± 9.02; RF = 115.29 ± 21.41) (Mann Whitney U-test, U = 961.000 $p<0.001$, n(NRF) = 150, n(RF) = 29), throughout the entire sample period (Fig. 4). There was no significant difference in the number of primary follicles between reproductive and non-reproductive females throughout the sampling period (NRF = 20.82 ± 1.26; RF = 25.47 ± 4.07) (Mann Whitney U-test, U = 2023.00, $p>0.05$, n(NRF) = 151, n(RF) = 29) (Fig. 5). No pattern was observed in the distribution of secondary follicles between reproductive and non-reproductive females throughout the year (NRF = 0.54 ± 0.01; RF = 0.59 ± 0.19) (Fig. 6).

The number of Graafian follicles were not significantly higher in the reproductive females when compared with non-reproductive females (NRF = 0.14 ± 0.03; RF = 0.10 ± 0.05) (Mann Whitney U-test, U = 2164.50, $p>0.05$, n(NRF) = 151, n(RF) = 29) (Fig. 7).

The reproductive females possessed Graafian follicles from February through to May with a peak observed during August (0.30 ± 0.35, n = 2). No Graafian follicles were found during June and July or for September through to January. In the non-reproductive females, Graafian follicles were present for nine months of the year, excluding the months of May, July and September (Fig. 7).

Reproductive females have a significantly higher mean number of atretic follicles than the non-reproductive females (NRF = 12.92 ± 0.62; RF = 19.53 ± 1.88) (Mann Whitney U-test, U = 14459.00 $p<0.001$, n(NRF) = 151, n(RF) = 29). Higher follicular activity in the reproductive females is evident from the statistical analyses ($p<0.05$) across the months of June to December (Fig. 8).

Corpora lutea were not present in any of the non-reproductive females sampled. However, reproductive females examined had corpora lutea present in their ovaries

throughout the sampling period, excluding the month of January (RF = 1.220 ± 0.21) (Fig. 9). Corpora albicans were only observed in August and October in the reproductive females (Fig. 10). During the study two reproductive females gave birth to three young each, in captivity within the months of May and July. The presence of very young individuals with masses not exceeding 40g (age class 1), were sampled from May through to December, excluding June, October and November.

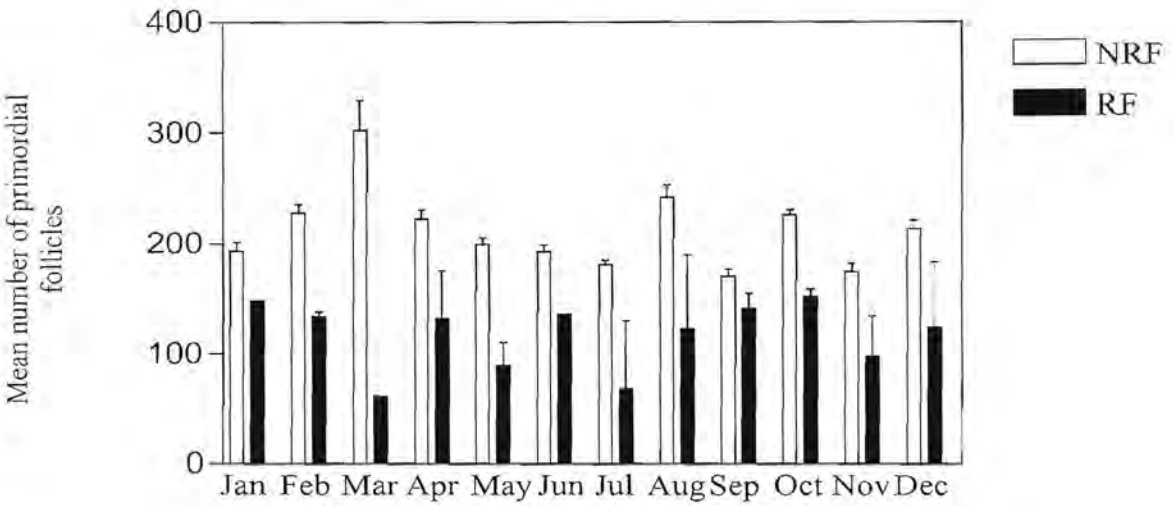

Fig. 4. The mean ± S.E. of primordial follicles in reproductive (RF) and non-reproductive female (NRF) ovaries.

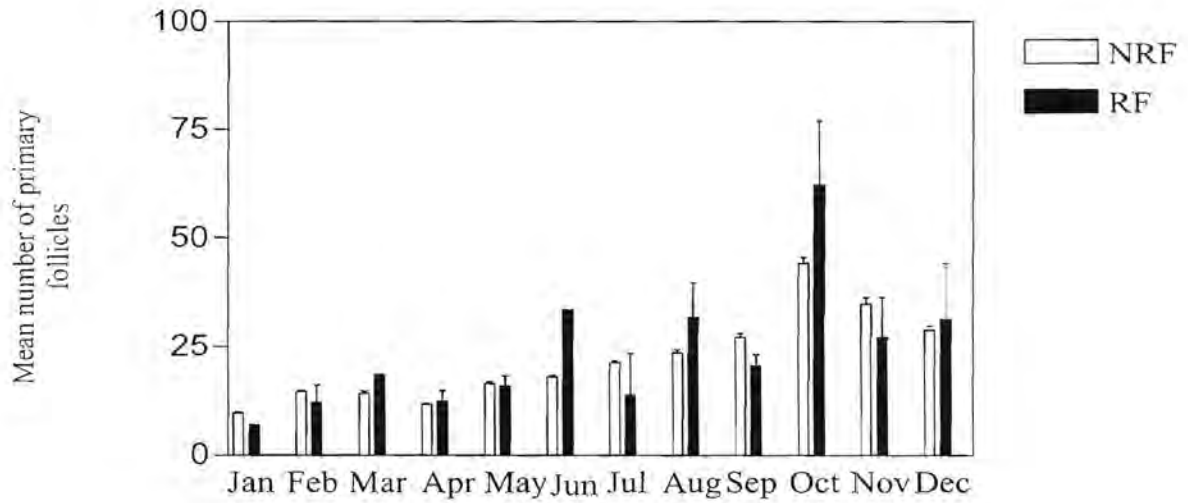

Fig. 5. The mean ± S.E of primary follicles in the reproductive (RF) and non-reproductive female (NRF) ovaries.

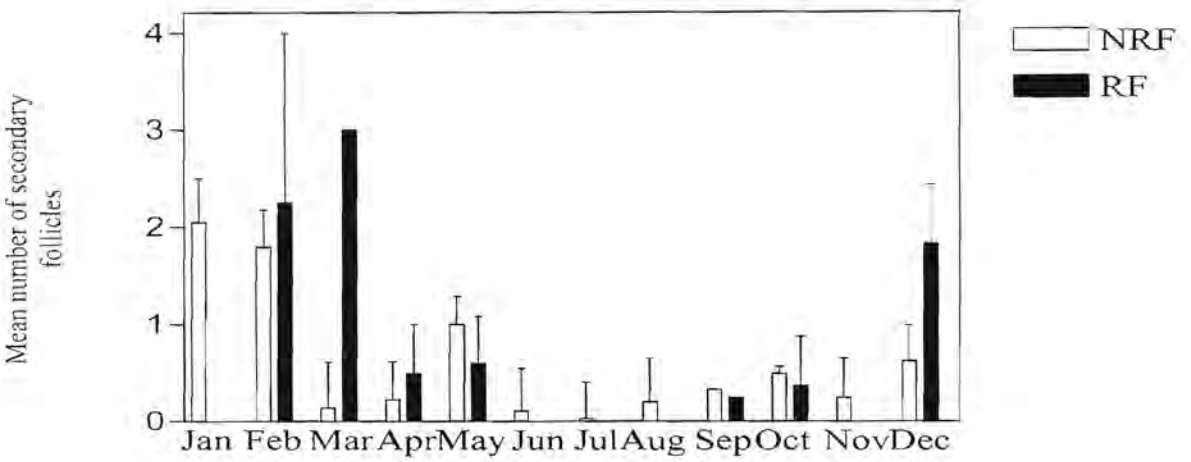

Fig. 6. The mean ± S.E. of secondary follicles in reproductive (RF) and non-reproductive female (NRF) ovaries.

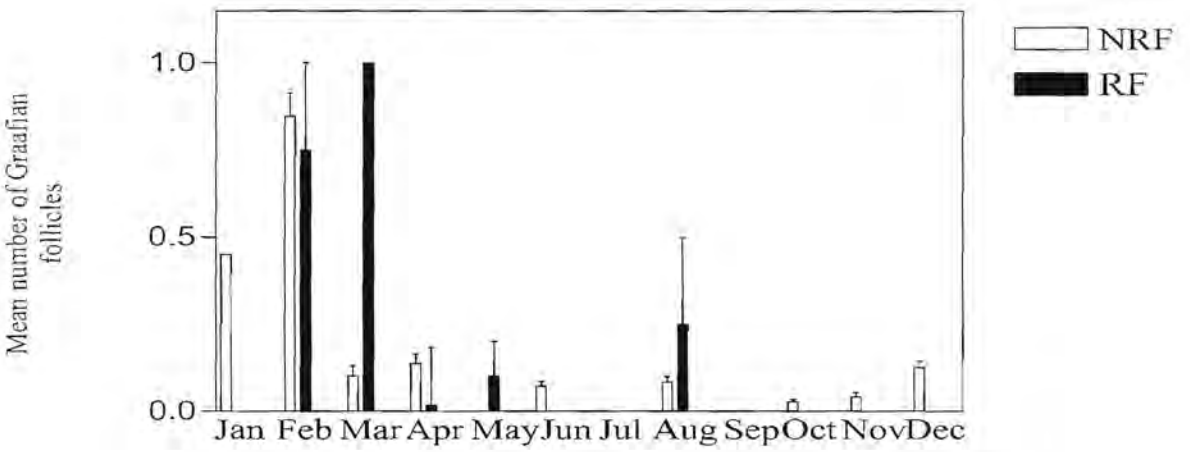

Fig. 7. The mean ± S.E. of Graafian follicles in the reproductive (RF) and non-reproductive female (NRF) ovaries.

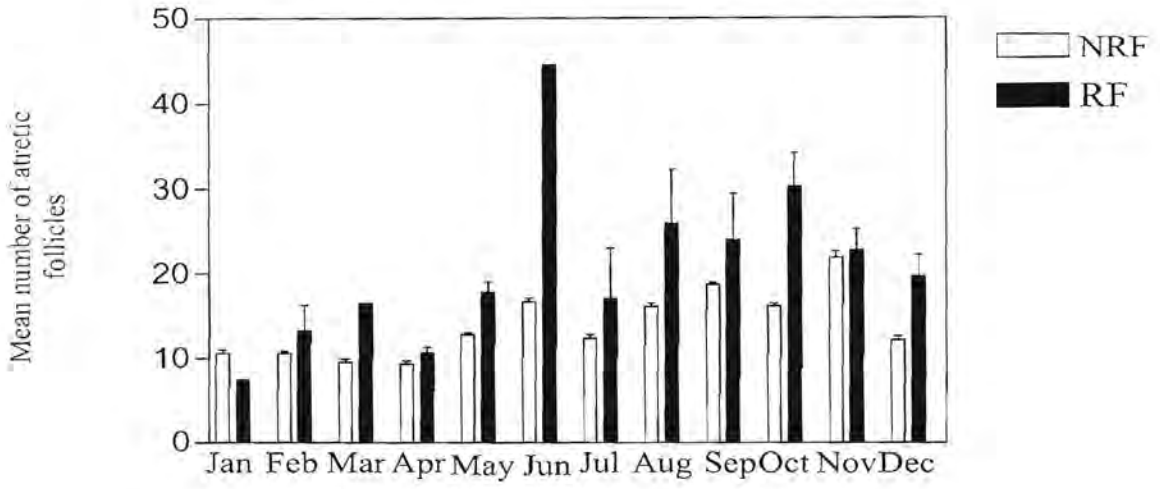

Fig. 8. The mean ± S.E. of atretic follicles in reproductive (RF) and non-rerproductive female (NRF) ovaries.

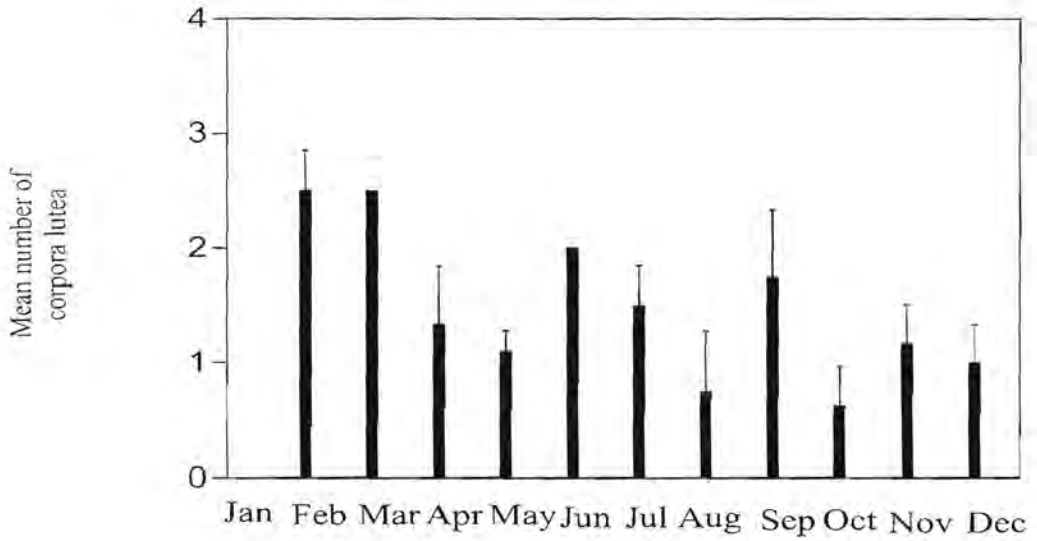

Fig. 9. The mean ± S.E. of corpora lutea counted in reproductive female (RF) ovaries.

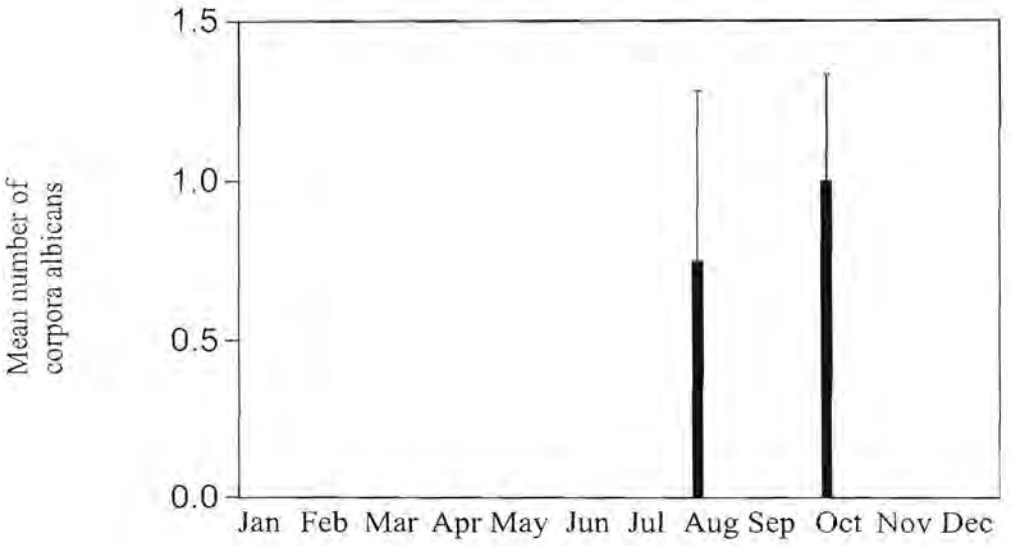

Fig. 10. The mean ± S.E. of corpora albicans in reproductive female ovaries.

Ovarian mass and volume

Mean ovarian mass in reproductive females exhibited a steady increase from April (0.06g ± 0.02, n = 3) to a peak value in July (0.14g ± 0.08, n = 2). A second, very subtle peak was observed in September (0.08g ± 0.01, n = 3) (Fig. 11). No ovarian mass and volume data were available for any females during February or any reproductive females during March (due to technical problems). The mean ovarian mass of non-reproductive females showed little fluctuation throughout the year. Reproductive females had a greater mean ovarian mass when compared to non-reproductive females (RF = 0.08g ± 0.01; NRF = 0.03g ± 0.00). (Mann Whitney U-test, U = 14.50, $p<0.05$, n(NRF) = 135, n(RF) = 26). The mean ovarian volume exhibited a trajectory of development similar to ovarian mass (NRF = 5.33mm³ ± 0.30, n = 154; RF = 18.61mm³ ± 3.38, n = 24) and reproductive females peaked in June (46.32mm³, n = 1) and July (44.73mm³ ± 28.85, n = 2) with the month of September (14.45mm³ ± 2.67, n = 3) showing a slightly greater volume than the month of August (13.95mm³ ± 8.97, n = 2) (Fig. 12).

Testicular histology

The mean seminiferous tubule diameter for reproductive males was significantly higher than that for the non-reproductive males (NRM = 149.28µm ± 4.71; RM = 183.32µm ± 4.02) (Mann Whitney U-test, U = 501.00, $p<0.01$, n(NRM) = 63, n(RM) = 39) (Fig. 13). Although highly significant differences occurred between reproductive and non-reproductive males, neither of the two male groups exhibited appreciable differences, between months within each of the groups (Fig. 13).

Testicular mass and volume

The mean testicular mass of the reproductive males increased albeit steadily from January (0.09g ± 0.05, n = 3) and reached a peak mass in July (0.34g ± 0.04, n = 2) (Fig. 14). A second peak in reproductive male testicular mass occurred in September (0.29g ± 0.02, n = 6), but then decreased towards December (0.16g ± 0.04 n = 4) (Fig. 14). The mean testicular mass of the reproductive males (0.21g ± 0.01) was significantly higher than the testicular mass of the non-reproductive males (0.10g ± 0.01) (Mann Whitney U-test, U= 10.00, $p<0.05$, n(NRM) = 51, n(RM) = 39).

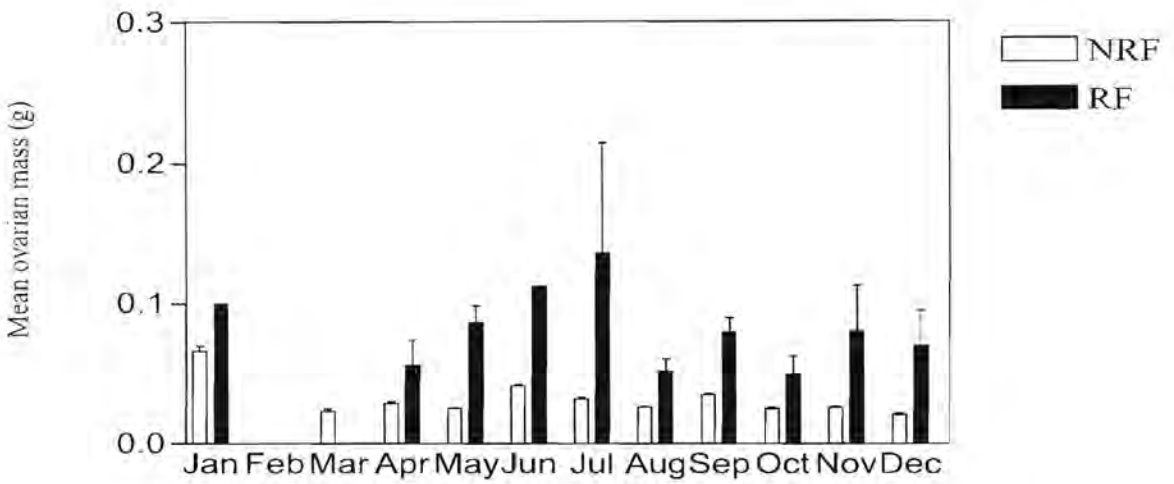

Fig. 11. The mean ± S.E ovarian mass for reproductive (RF) and non-reproductive females (NRF).

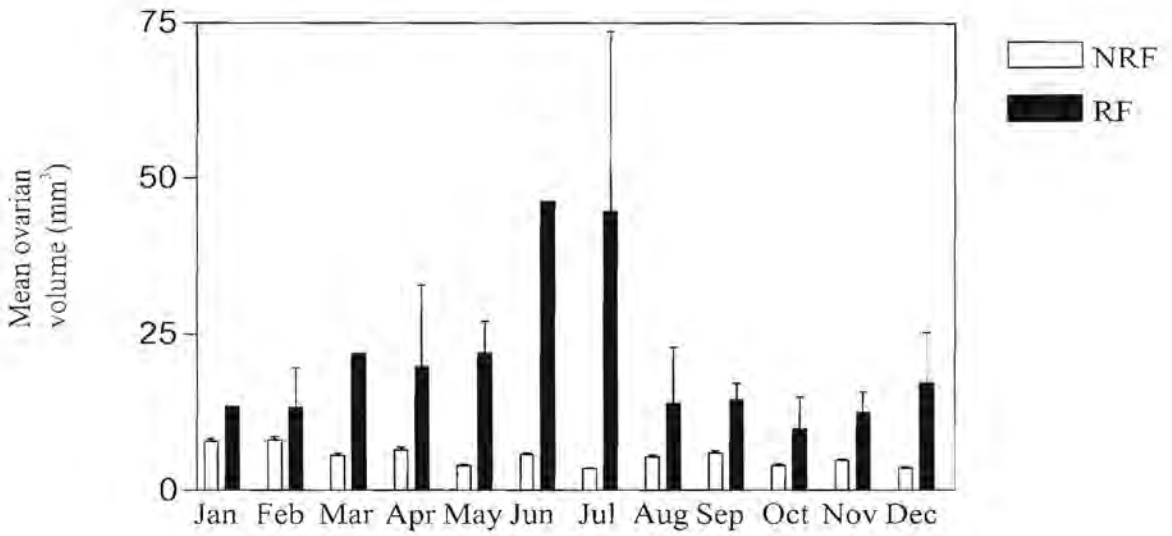

Fig. 12. The mean ± S.E ovarian volume for reproductive (RF) and non-reproductive females (NRF).

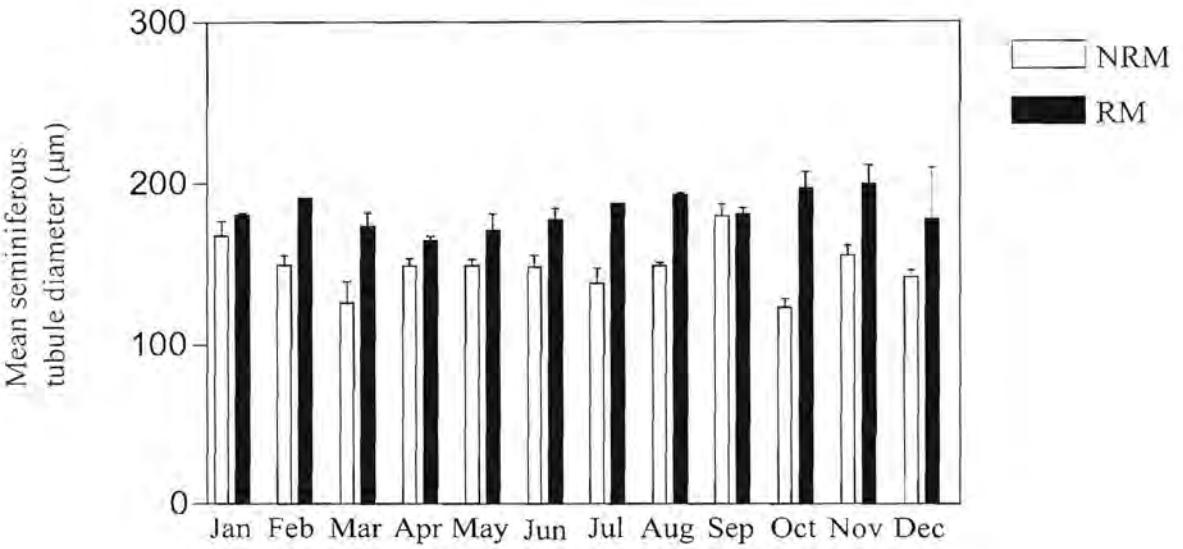

Fig. 13. The mean ± S.E. seminiferous tubule dimater for reproductive (RM) and non-reproductive males (NRM).

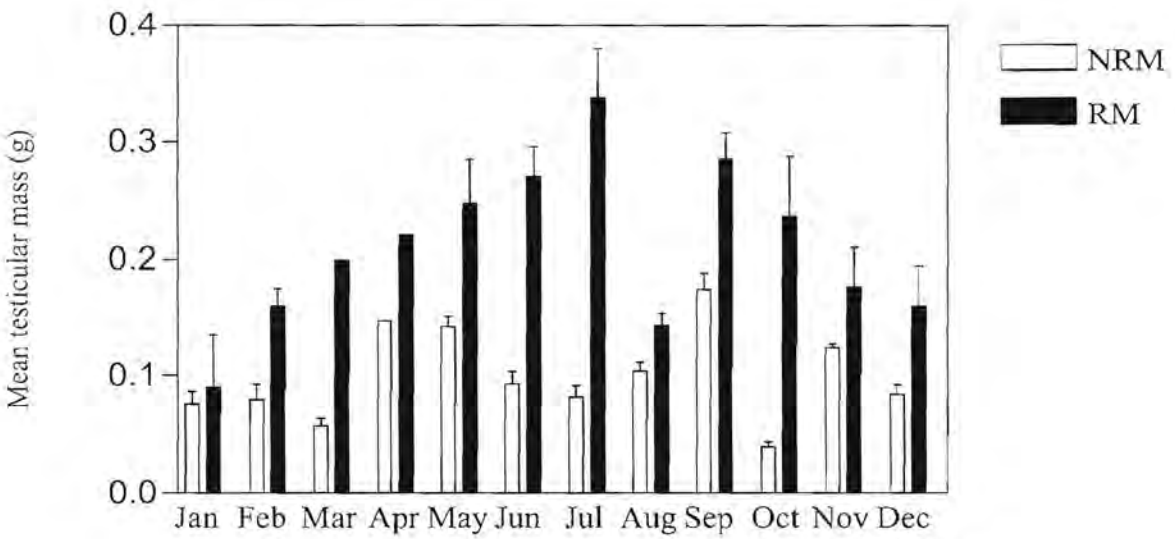

Fig. 14. The mean ± S.E. testicular mass for reproductive (RM) and non-reproductive males (NRM).

The testicular volume showed the same trend (Fig. 15). The first peak in testicular volume of the reproductive males (101.40mm^3 ± 16.17, n = 2) coincided with the first peak in ovarian mass for reproductive females in July (Fig. 11). In contrast to the testicular mass, the second peak in testicular volume occurred in October (99.48mm^3 ± 35.35, n =3) and not in September (Fig. 15).

Sperm motility

In table 1 the various sperm motility parameters were recorded for both reproductive and non-reproductive males. Without exception all the parameters showed no significant difference between reproductive and non-reproductive males. In addition, comparing the mass and age of the animals, as well as the number of sperm observed for both reproductive and non-reproductive males as co-variates in a Generalised linear model (GLM) only a significant difference could be observed for the beat cross frequency (BCF) variable between the masses of reproductive and non-reproductive males (GLM, $F = 4.65$, $p<0.05$) (Table 2). The remaining variables revealed no statistical significant differences. No significant differences between the kinematic parameters between reproductive and non-reproductive males were observed for either age or the number of sperm observed.

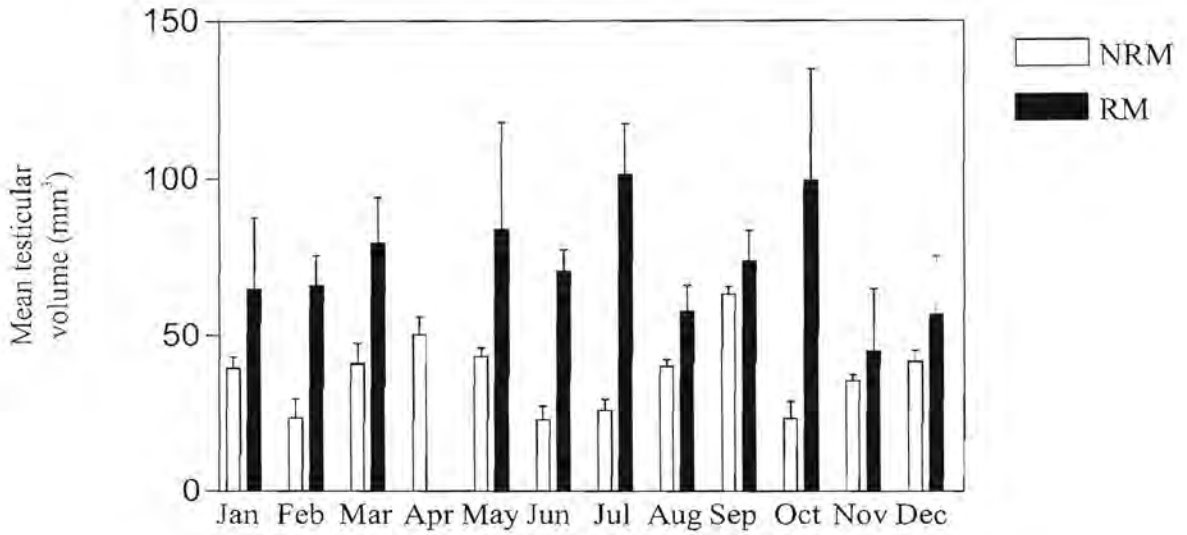

Fig. 15. The mean ± S.E. testicular volume for reproductive (RM) and non-reproductive males (NRM).

Table 1 Comparative sperm motility characteristics for highveld mole-rats of differing reproductive status. RM = Reproductive males (n=14), NRM = Non-reproductive males (n=17). The mean and standard error are indicated.

Variable	RM	NRM	F value	*p*
VCL	108.50 ± 5.05	107.76 ± 5.40	0.29	0.60
VSL	81.21 ± 3.91	82.19 ± 3.66	0.55	0.46
LIN	73.72 ± 2.17	75.72 ± 2.76	0.16	0.70
Mean ALH	1.44 ± 0.14	1.36 ± 0.21	4.22	7.16
Maximum ALH	3.92 ± 0.28	3.86 ± 0.45	1.01	0.32
BCF	27.32 ± 1.65	23.94 ± 1.94	1.43	0.24
DNC	368.08 ± 56.83	415.87 ± 142.72	4.22	4.91
DNC mean	11.96 ± 2.01	10.37 ± 2.52	0.32	0.58
VAP	91.68 ± 3.90	91.55 ± 3.55	0.96	0.34
WOB	0.85 ± 0.01	0.86 ± 0.02	0.38	0.54
STR	0.86 ± 0.02	0.87 ± 0.01	0.00	0.96
RAD	1.54 ± 0.09	1.50 ± 0.09	1.24	0.28
CURV	0.39 ± 0.02	0.34 ± 0.03	3.50	0.07

VCL = curvilinear velocity; VSL = straight line velocity; LIN = percentage linearity; ALH = amplitude of lateral head displacement; BCF = beat cross frequency; DNC = dance; VAP = average path velocity; WOB = Wobble; STR = straightness; RAD = Radian; CURV = curvature.

Table 2 Comparing reproductive (n=14) and non-reproductive males (n=17) with regard to mass, age and number of sperm observed (Count), to determine statistical differences. Generalised Linear Model ($p<0.05$).

	Mass		**Age**		**Count**	
Variable	F value	*p*	F value	*p*	F value	*p*
VCL	0.49	0.49	0.08	0.77	1.06	0.31
VSL	1.83	0.19	0.42	0.52	0.11	0.75
LIN	0.75	0.39	1.78	0.19	1.15	0.29
Mean ALH	0.07	0.79	0.05	0.82	0.08	0.79
Maximum ALH	0.16	0.69	0.04	0.84	0.20	0.66
BCF	4.65	0.04	1.63	0.21	1.48	0.23
DNC	0.10	0.75	0.40	0.53	0.28	0.60
DNC mean	0.02	0.88	0.18	0.68	0.45	0.51
VAP	2.65	0.12	0.18	0.68	0.46	0.50
WOB	1.42	0.24	1.52	0.23	0.82	0.37
STR	0.00	0.96	1.62	0.21	1.02	0.32
RAD	0.59	0.45	0.11	0.75	0.17	0.68
CURV	0.58	0.45	0.02	0.90	0.91	0.35

(Abbreviations - See table 1)

Hormones

The mean circulating progesterone concentrations measured in the reproductive females were significantly higher than in the non-reproductive females (NRF = 2.17nmol.l^{-1} ± 0.30; RF = 60.78 nmol.l^{-1} ± 16.30) (Mann Whitney U-test, U = 391.00, $p<0.01$, n(NRF) = 154, n(RF) = 29). Within the first three months of 1998 (January to March) the progesterone concentrations of the reproductive females were very low (5.51 nmol.l^{-1} ± 1.83, n = 4). These levels, however, increased and were much higher from April through to December (69.62nmol.l^{-1} ± 18.35, n = 25) (Fig. 16). Two peaks can be observed: the first peak in April (55.50nmol.l^{-1} ± 51.03, n = 3) and a second peak in September (163.41nmol.l^{-1} ± 39.03, n = 3). Within the months of May through to November a significant difference was found between reproductive females and non-reproductive females within months (Kruskal Wallis test, H = 78.58, $p<0.001$; Dunn's multiple range test, $p<0.05$).

Oestradiol is a steroid hormone, secreted principally by the ovarian follicles and also by the corpora lutea, placenta and adrenals in the female. No clear trend was observed for the oestradiol concentrations (NRF = 0.33nmol.l^{-1} ± 0.08, n = 140; RF = 23.52nmol.l^{-1} ± 11.13, n = 28) (Fig. 17). The oestradiol concentrations for January through to March were very low for both the reproductive and non-reproductive females (NRF = 0.32nmol.l^{-1} ± 0.02, n = 23; RF = 1.06nmol.l^{-1} ± 0.63, n = 4). In April (64.76 nmol.l^{-1} ± 64.74, n = 3) and May (75.13nmol.l^{-1} ± 45.29, n = 5) very high oestradiol concentrations were observed for the reproductive females. Reproductive females obtained in the remaining months (June to December) had oestradiol concentrations which never exceeded 25 nmol.l^{-1} (NRF = 0.36nmol.l^{-1} ± 0.11, n = 93; RF = 5.28 nmol.l^{-1} ± 2.70, n = 16). In May, August, September and November significant differences were found between the reproductive and non-reproductive females (Kruskal Wallis test, H = 66.29, $p<0.001$; Dunn's multiple range test, $p<0.05$).

The testosterone concentrations of the reproductive males always exceeded that of the non-reproductive males in each respective month (NRM = 1.21nmol.l^{-1} ± 1.46; RM = 15.27 nmol.l^{-1} ± 1.87) (Mann-Whitney U-test, U = 635.00, $p<0.001$, NRM = 55 RM = 42). In both the reproductive and non-reproductive males the highest testosterone concentrations were recorded in June (NRM = 16.10nmol.l^{-1} ± 8.09, n = 4; RM = 28.90

nmol.l^{-1} ± 9.49, n = 5) and August (NRM = 18.18nmol.l^{-1} ± 1.86, n = 3; RM = 24.10nmol.l^{-1} ± 10.92, n = 7) (Fig. 18). Analysis of the all months sampled, revealed a highly significant difference between reproductive males and non-reproductive males in October (NRM = 1.49nmol.l^{-1} ± 1.23, n = 3; RM = 12.77nmol.l^{-1} ± 1.73, n = 3) (Kruskal Wallis test, H = 44.11, $p<0.01$; Dunn's multiple range test, $p<0.05$, NRM = 3, RM = 3). The testosterone concentrations for both reproductive and non-reproductive males show two distinct peaks, the first in June and a second in August. These peaks coincide with the progesterone peaks of the females (Fig. 16).

Rainfall

Rainfall data for the various regions in which trapping was undertaken were obtained from the South African Weather Bureau (Fig. 19). Single animals were captured only during months in which rainfall occurred (Fig. 20).

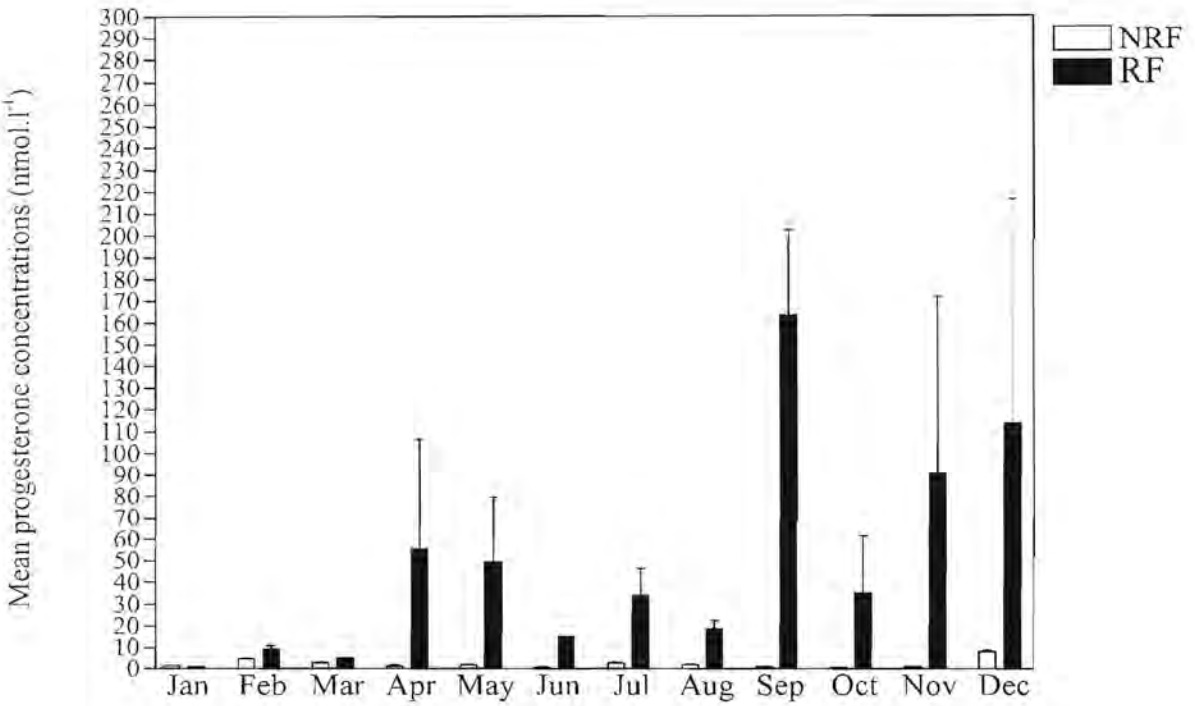

Fig. 16. The mean ± S.E. progesterone concentrations for reproductive (RF) and non-reproductive females (NRF).

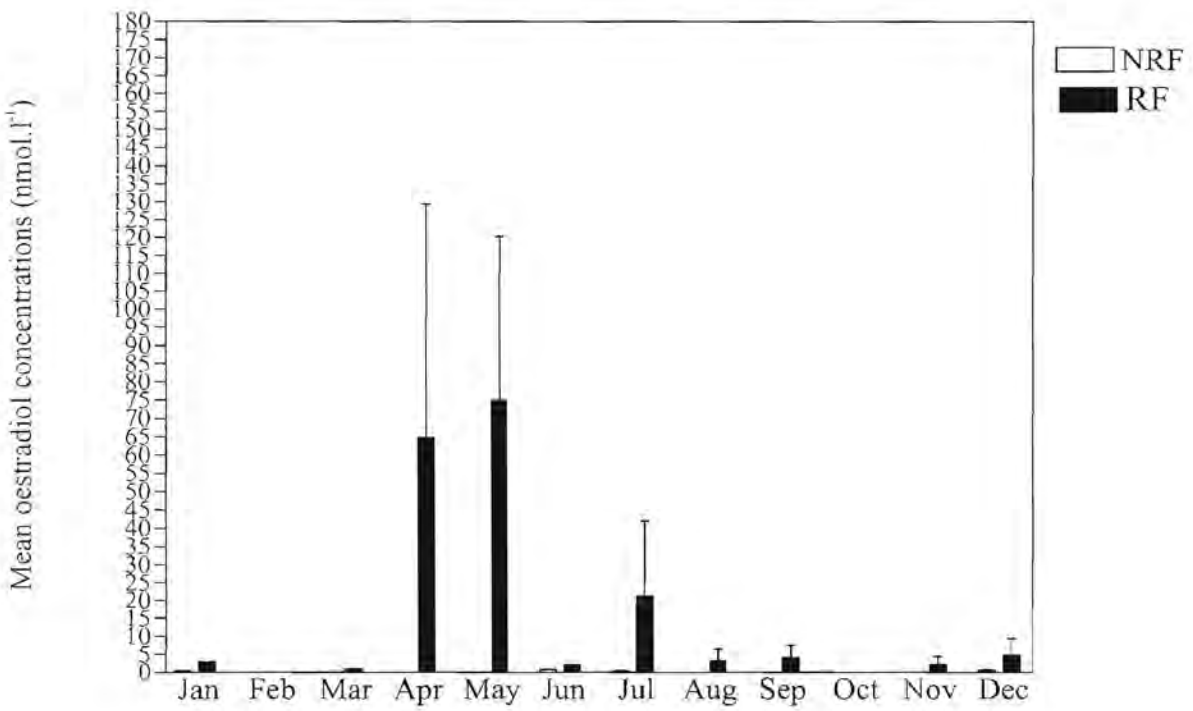

Fig. 17. The mean ± S.E. oestradiol concentrations for reproductive (RF) and non-reproductive females (NRF).

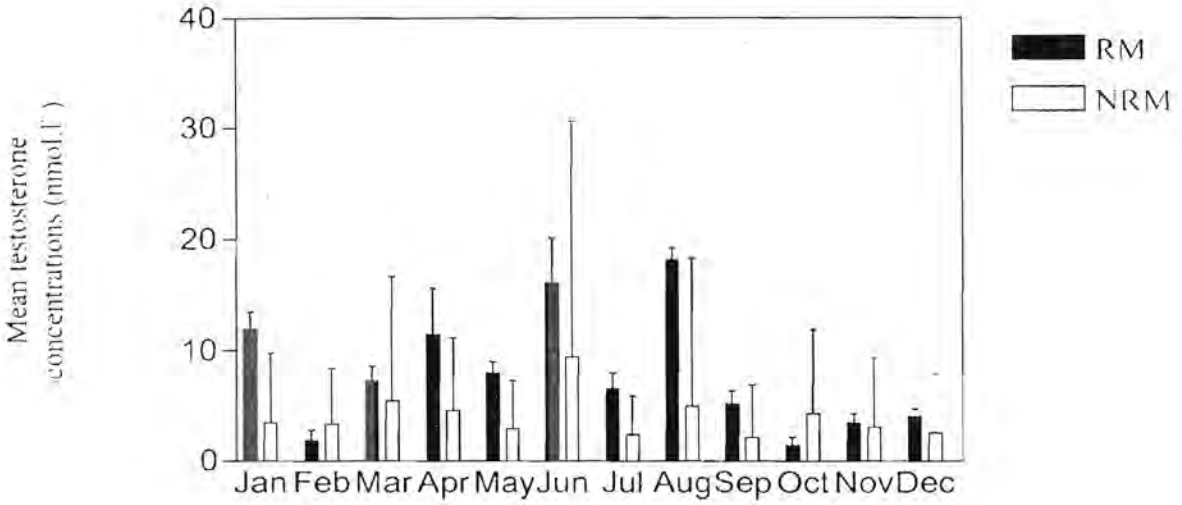

Fig. 18. The mean ± S.E testosterone concentrations for reproductive (RF) and non-reproductive males (NRM).

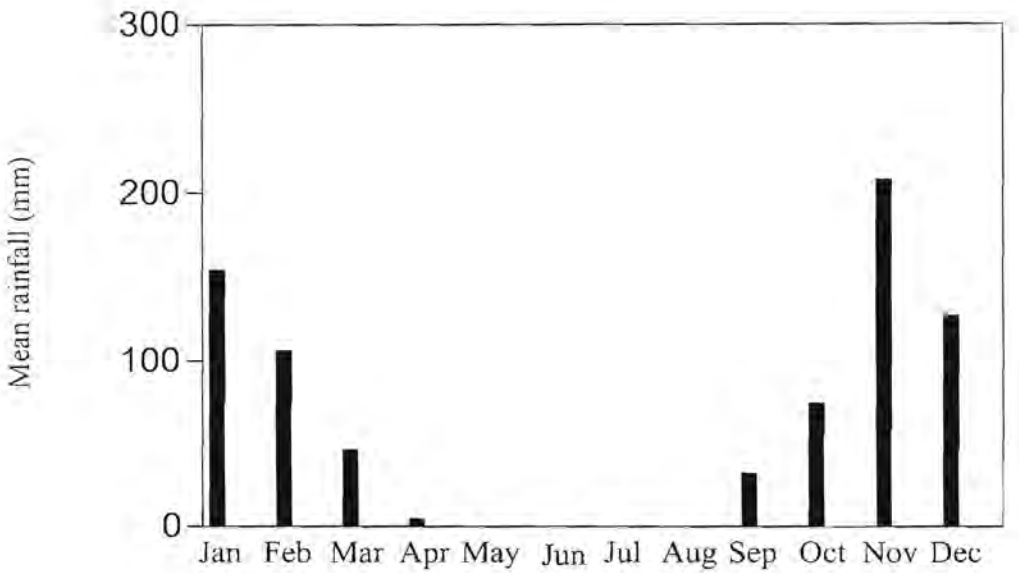

Fig. 19. The mean rainfall (mm) for each month during 1998 (South African Weather Bureau).

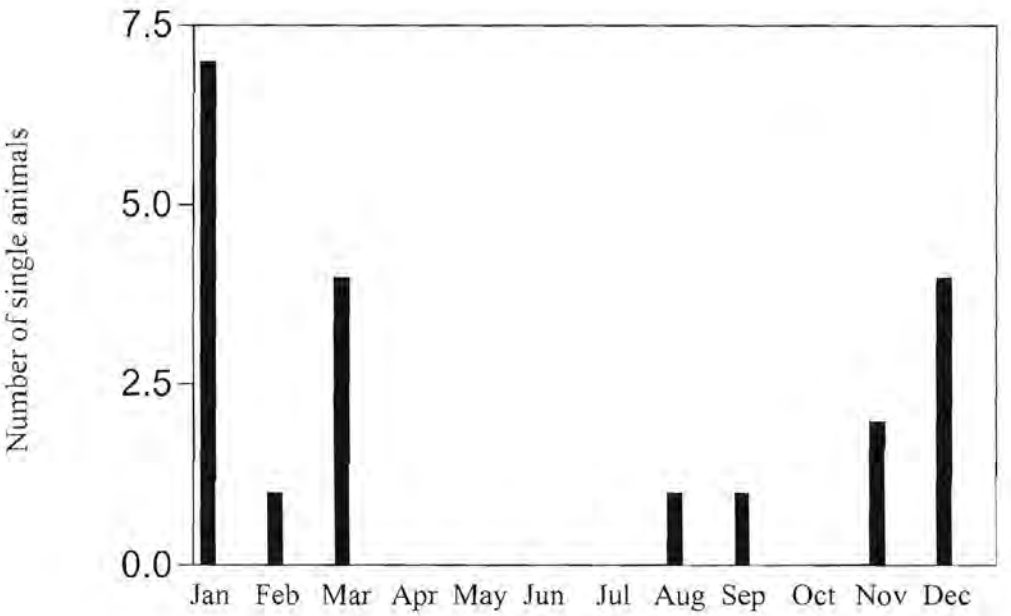

Fig. 20. The number of single animals sampled during 1998.

DISCUSSION

It is very likely that reproduction has been subjected to more evolutionary pressures than any other physiological system (Weir 1974). As a consequence a wide range of reproductive strategies are displayed in the animal kingdom. The family Bathyergidae is one such diverse group that incorporates solitary species reproducing seasonally to co-operatively breeding individuals that predominantly reproduce aseasonally.

Seasonal breeding represents an adaptation of animals to ensure maximum survival of their young (Louw 1993). Offspring are born at a time when environmental conditions are most favourable for growth and survival (Hickman *et al.* 1993).

Within the bathyergids, seasonal reproduction is usually confined to solitary species, such as *Georychus capensis* and *Bathyergus suillus* occurring in the mesic habitats of the Cape (Taylor *et al.* 1985; Bennett *et al.* 1991). The social species *C. damarensis* and *H. glaber*, exhibit a reproductive division of labour as well as distinct working groups within the confines of the colony. These mole-rats inhabit the arid regions of eastern and southern Africa (Faulkes *et al.* 1991; Jacobs *et al.* 1991) and thus, living in social groups enable these mole-rats to withstand very harsh environmental conditions and still maintain a high rate of reproductive success (Lovegrove 1988).

Within the hierarchical groups of co-operatively breeding mammals, reproductive suppression is a common phenomenon. Dominant individuals inhibit sexual activity in non-reproductive animals through behavioural or physiological suppression (Abbott 1987; Abbott *et al.* 1988). Such reproductive inhibition occurs in the naked mole-rats, *H. glaber* (Faulkes *et al.* 1990; 1991; Abbott 1984), wild dogs, *Lycaon pictus* (Malcolm & Marten 1982; Creel *et al.* 1997) and the dwarf mongooses, *Helogale parvula* (Rood 1980; Creel *et al.* 1992).

Within a mole-rat colony only one female and usually one or potentially two males are responsible for the procreation of new colony members (Bennett & Jarvis 1988; Faulkes *et al.* 1990; 1991; Jarvis & Bennett 1990; 1991; Bennett *et al.* 1997). Reproductive animals suppress the non-reproductive animals from reproducing (Faulkes *et al.* 1990; 1991; 1994; Bennett *et al.* 1993; 1994b; 1997; Spinks *et al.* 1997). Bennett *et al.* (1997) suggest that there is variation in the mechanisms of reproductive modulation

which can be correlated with environmental factors. The arid species (e.g. *H. glaber*) enforces physiological suppression, exhibiting extreme socially induced infertility where both non-reproductive males and females are physiologically suppressed from reproducing (Faulkes *et al.* 1990; 1991). Similarly *C. damarensis* exhibits physiological suppression as well as behavioural inhibition in the non-reproductive females with behavioural inhibition present in non-reproductive males (Bennett *et al.* 1994a; Bennett *et al.* 1994b; 1996). The mesic species (e.g. *Cryptomys darlingi*) practices behavioural suppression, in which incest avoidance is solely responsible for the maintenance of reproductive skew.

Mesic environments have frequent and predictable periods of rainfall and thus cater for frequent dispersal opportunities. Colonies that occur in these areas are very small, for example the Mashona mole-rat with a colony size of up to nine individuals (Bennett *et al.* 1997) and the common mole-rat colonies ranging from two to fourteen individuals (Spinks *et al.* 1997). Within these colonies it is of the utmost importance that the method used for reproductive suppression be quickly discarded when the opportunity for dispersal arises and the animals have the opportunity to procreate.

Until recently only the common mole-rat, *C. h. hottentotus* had been identified as being a social mole-rat, displaying seasonal reproduction (Bennett *et al.* 1991; Jarvis & Bennett 1991; Spinks *et al.* 1997). Spinks *et al.* (1997) suggested that *C. h. hottentotus* displays a cyclical reproduction because of invading a seasonal habitat. The common mole-rat occurs in the winter rainfall regions of the western and northern Cape Province. Long-term mark and recapture studies, revealed that the common mole-rat occurs in colonies of up to fourteen individuals and produces a maximum of two litters per annum (late November through to January) (Jarvis & Bennett 1991; Spinks 1998). This pattern of reproductive periodicity is typical of mammals, both surface dwelling and subterranean, that occur in seasonal habitats (Page *et al.* 1994; Mills *et al.* 1992; Kaplan & Mead 1994).

The invasion of *C. h hottentotus* into a seasonal, mesic habitat, which is predominantly inhabited by solitary mole-rats may be explained as a survival strategy that decreases or eliminates competition with other social mole-rat species and therefore increases its own survival and reproductive success.

The highveld mole-rat is phylogenetically closely related to the common mole-rat (Faulkes *et al.* 1997) and likewise, inhabits a seasonal habitat. According to Bennett *et al.* (1999) and Jarvis & Bennett (1991) the change in temperature and rainfall due to seasonality are important determinants of seasonal breeding in the solitary bathyergids. It would seem that these same environmental cues are responsible for seasonal reproduction in the common mole-rat that produce young during the southern hemisphere summer when the soil is workable and the food resource is readily harvested. I suggest that the highveld mole-rat uses the onset of the first rains during spring as a cue for dispersal and mating and then produce young during the southern hemisphere winter months (May to July).

Ovarian histology

The presence of primordial, primary and secondary follicles serve as an indicator of follicular development. According to Bloom & Fawcett (1962) primordial follicles are more abundant within the ovaries of non-reproductive females. This trend was consistent throughout the sampling period. The presence of primary and secondary follicles in both reproductive and non-reproductive females suggest that the ovaries undergo normal follicular development, supporting the assumption that non-reproductive females are not sterile but only reproductively quiescent in that ovulation does not occur. According to Weir (1974) and Bloom & Fawcett (1962) most follicles in both reproductive and non-reproductive ovaries do not survive maturation nor ovulation and degenerate to become atretic follicles and is a feature common to all mammalian ovaries (Mossman & Duke 1973). My study on the highveld mole-rat revealed that reproductive females possessed a higher follicular development, which leads to increased numbers of atretic follicles present in the ovaries. Similarly, non-reproductive females displayed high numbers of atretic follicles, which again supports the assumption made that the non-reproductive females have functional and active ovaries.

According to Clarke (1981) and Gorman & Stone (1990), seasonally breeding animals exhibit a regression of follicular development during the non-breeding time, thus no secondary or Graafian follicles are present in the ovaries. However, Spinks (1998) found that *C. h. hottentotus* shows follicular development during the non-breeding period

and thus suggested that reproductive function does not regress. The same trend is encountered in the highveld mole-rat. The predictability of the rainfall is an important determinant of reproduction, consequently a constant state of reproductive readiness would be advantageous, since the first rains might be advanced or delayed relative to the norm.

The high number of Graafian follicles in the ovaries of non-reproductive females out of breeding season may indicate a readiness in reproductive physiology for the anticipation of dispersal during the summer, with the onset of the first rains. Only non-reproductive females possessed Graafian follicles during June, October, November and December (breeding season). Most non-reproductive females were perforate, which would suggest that the non-reproductive females are not sterile but only inhibited from reproducing. Shanas *et al.* (1997) found a similar trend in the blind mole-rat, *Spalax ehrenbergi*. Although a seasonal breeder, the ovaries of the blind mole-rat females kept in a laboratory were found to be active out of the breeding season.

The reproductive females of the highveld mole-rat had a larger number of Graafian follicles at the beginning of the breeding season. Using the presence of corpora lutea as and indication of ovulation and pregnancy, the breeding season can be delineated as occurring from April until December. If one assumes that the gestation period is comparable to the sister taxon *C. h. hottentotus* of approximately 60 days, then it is feasible for the highveld mole-rat to produce two litters per annum.

Ovarian mass and volume

According to Weir & Rowlands (1974) the most striking feature of hystricomorph ovaries is the development of large amounts of luteal tissues and the presence of large corpora lutea. Weir & Rowlands (1974) suggested that the luteal tissues are developed to extend the gestation period or as in the chinchilla, it may serve as an extra source of progesterone.

Data obtained on the highveld mole-rat females show that the corpora lutea fill the ovary of a reproductive female during pregnancy. This gives the reproductive female ovary a granular-like surface appearance, unlike the smooth surface of the non-reproductive female ovaries. Besides the granular surface appearance of the ovaries of

reproductive females, they also exhibit very thick uteri as well as placental scars or foetuses. In contrast, the non-reproductive female ovaries were small and smooth. The uteri of the non-reproductive females are very thin and have a flaccid appearance.

In seasonally breeding mammals, temporal changes in the ovarian and uterine dimensions occur. An increase in ovarian size, mass and volume of reproductive females during the breeding season has been found in the European rabbits, *Oryctolagus cuniculus*, corn mice, *Calomys musculinus* and the red giant flying squirrels, *Petaurista petaurista* (Boyd & Myhill 1987; Mills *et al.* 1992; Lee *et al.* 1993). Spinks (1998) found a similar trend in the common mole-rat, *C. h. hottentotus*, where reproductive females showed an increase in ovarian mass and volume during the breeding season. The higher level of follicular development in the reproductive females correlated to a higher ovarian mass.

The present study shows that at the peak of the breeding season in the highveld mole-rat a large number of foetuses are present and the ovaries attain the greatest mass, due to the presence of large corpora lutea filling the stroma. Closer examination of the data reveals two subtle peaks in the mean number of corpora lutea present, occurring during June and September, which suggests that the highveld mole-rat may have the potential to produce two litters within a breeding season. The ovarian volume verifies the results of the ovarian mass, showing a higher ovarian volume in June, however, a second peak cannot be readily discerned.

Female hormones

Peaks in plasma oestradiol 17β concentrations occurred in April and May, indicative of enhanced follicular development at the beginning of the breeding season. Oestradiol concentrations for the reproductive females drastically decreased for the remainder of the breeding months. Whereas, progesterone concentrations, showed an increase within these months indicating ovulation or pregnancy. During pregnancy a lack of follicular development was observed in the highveld mole-rat, unlike that observed in the Cape porcupine (*Hystrix africaeaustralis*), where the number of large follicles increased with an extension of the gestation period (Van Aarde & Skinner 1986) and in the plains viscacha (*Lagostomus maximus*), where follicles develop throughout pregnancy to

ovulatory size (Weir 1971). Within the highveld mole-rat progesterone reached its highest concentrations in the breeding season (April to December), whereas very low concentrations were recorded during the non-breeding period (January to March). Two peaks are present in the progesterone data for reproductive females, the first in April and the second in September. The progesterone profiles in conjunction with the ovarian histological data suggest the highveld mole-rat being a seasonal breeder.

Testicular histology

Assuming the breeding season occurs from the beginning of April and ends in December and that the non-breeding season lasting for approximately 3 months (January – March), it would be advantageous to keep the testes functional and the seminiferous tubule diameter at a constant size. This would facilitate an increase in sperm production to enable the males to be reproductively functional for the breeding season (Spinks 1998). The present study reveals that although the seminiferous tubule diameter of the reproductive males are significantly larger than that of the non-reproductive males, they both maintained the seminiferous tubule diameter at constant levels throughout the year. During the breeding season the reproductive males exhibit two very subtle peaks in seminiferous tubule diameter which coincides with the months of the two litters of the reproductive females (June and September). Spinks (1998) found a similar trend in the common mole-rat males. There was no regression in spermatogenesis and a lack of any seasonal periodicity in male reproduction. The maintenance of reproductive activity is uncommon amongst seasonally breeding animals. However, male mole-rats need a ready supply of sperm throughout the year to seize any opportunity for reproduction that might arise during its lifetime.

Testicular mass and volume

Reproductive males show a gradual increase in testicular mass and testicular volume towards and during the breeding season, which reaches a peak within the month of July and also coincides with the peak reproductive month for the females. A second peak in testicular mass was observed in September, again this coincides with the second peak found in the progesterone concentrations for the reproductive females as well as the

number of corpora lutea. In turn, the testicular volume shows a very subtle peak during the month of October.

Sperm motility

Sperm motility is essential for the process of normal fertilisation (Katz *et al.* 1989). This demands the successful migration of the sperm to the ovum and then penetrating the cumulus oophorus and zona pellucida of the egg (Green 1988; Katz *et al.* 1989).

During the study no statistically significant differences were found between the sperm motility parameters of reproductive (n = 14) and non-reproductive males (n = 17). Neither mass, age nor the number of sperm had an influence on the sperm motility of reproductive and non-reproductive males. A similar trend was observed in the common mole-rat and the Damaraland mole-rat, where both reproductive and non-reproductive males exhibited no significant differences in their sperm motility (Spinks 1998; Faulkes *et al.* 1994). Thus, no apparent suppression of sperm motility exists in the non-reproductive males (Faulkes *et al.* 1994).

Due to the loosely social structure of the colonies, it may be possible for males to be in constant competition for the rights to breed with the reproductive female. Therefore by keeping the testes functional and sperm motile, males have the possibility of attaining reproductive rights. The frequent dispersal of the non-reproductive males would also favour males with highly motile sperm, ensuring the procreation of the species.

Male hormones

Reproductive males being the most dominant in the colony (Moolman *et al.* 1998) exhibited the highest testosterone concentrations throughout the entire sampling period of the study. However, no significant differences were found between the testosterone concentrations of reproductive and non-reproductive males, with the exception of October. In contrast to the male naked mole-rat, *H. glaber*, there are no physiological differences nor any distinct hormonal differences (Bennett *et al.* 1994b), since all males undergo spermatogenesis and exhibit similar testosterone levels. On closer examination, both reproductive and non-reproductive highveld mole-rat males displayed higher

testosterone concentrations a month prior to each of the two prominent reproductive months (July and September).

The approaching breeding season may result in increased aggression (due to high testosterone concentrations and reproductive competition) between males as they compete for the exclusive right to breed with the reproductive female or newly acquired females. The increase in testosterone occurs twice, just before each of the reproductive peaks found in the reproductive females. Presuming higher aggression between all the sexually mature males in a colony, with an increase in testosterone approaching the breeding season, I suggest that competition occurs twice between males for the right to be the breeding male. Further, I suggest that this might be because of no distinct dominance hierarchy found within the highveld mole-rat colonies (Moolman *et al.* 1998), which might lead to continuous competition for being the breeding male.

The importance of rainfall

The highveld mole-rat occurs in the summer rainfall regions in the highveld of South Africa. The rainfall data obtained from the South African Weather Bureau indicates that the months during which single animals were caught coincides with the months in which rainfall occurred. Thus, it is possible that these animals disperse towards the last months of the breeding season (September/October), right through to the beginning of the next breeding season (April), using rainfall as a cue for dispersal. Dispersing during the wet period, when the soil is workable, will optimise the distance over which these animals will be able to dig and minimise the energetic costs associated with burrowing (Jarvis & Bennett 1991). Food is readily available during the wet period and is not seen as a limiting factor.

Thus, in conclusion I suggest that the highveld mole-rat is a seasonal breeder, with a proposed breeding period lasting from April up to the end of December, with no reproductive activity during January, February or March. In addition a proposal is made that two litters are born during the breeding season in May/July and September.

Dispersal occurs with the onset of the first rains in September/October and continues up to the beginning of the breeding season in April/May promoting the establishment of new colonies.

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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.

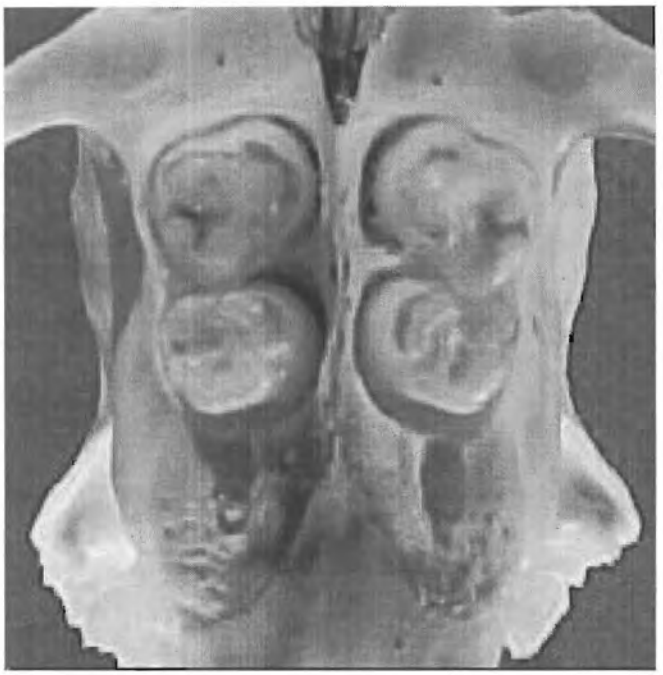

Plate 1. Relative age class 1.

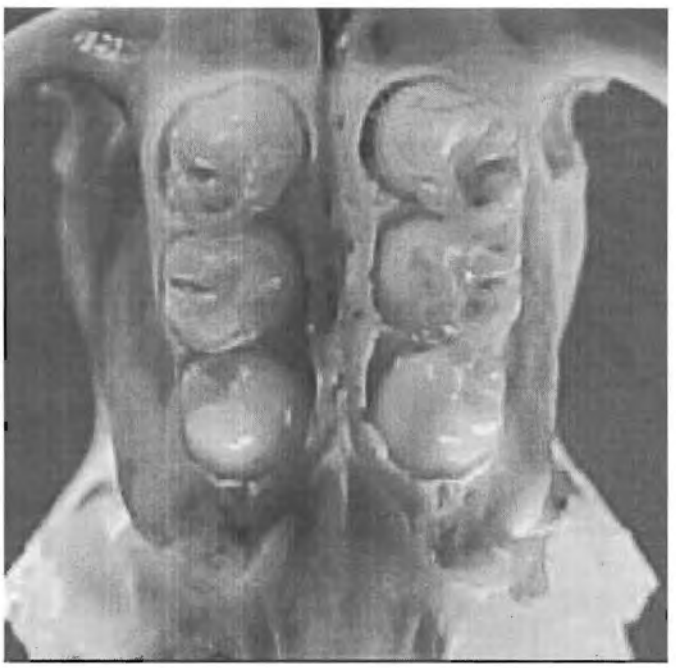

Plate 2. Relative age class 2.

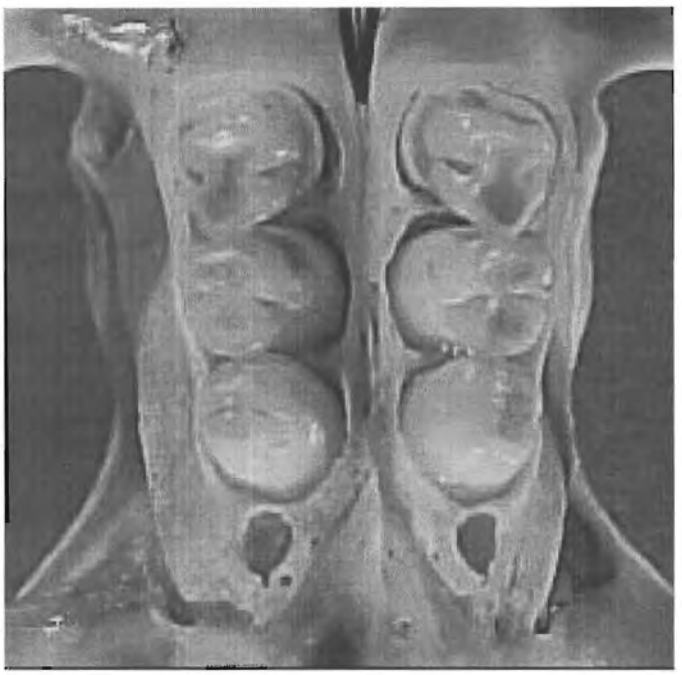

Plate 3. Relative age class 3.

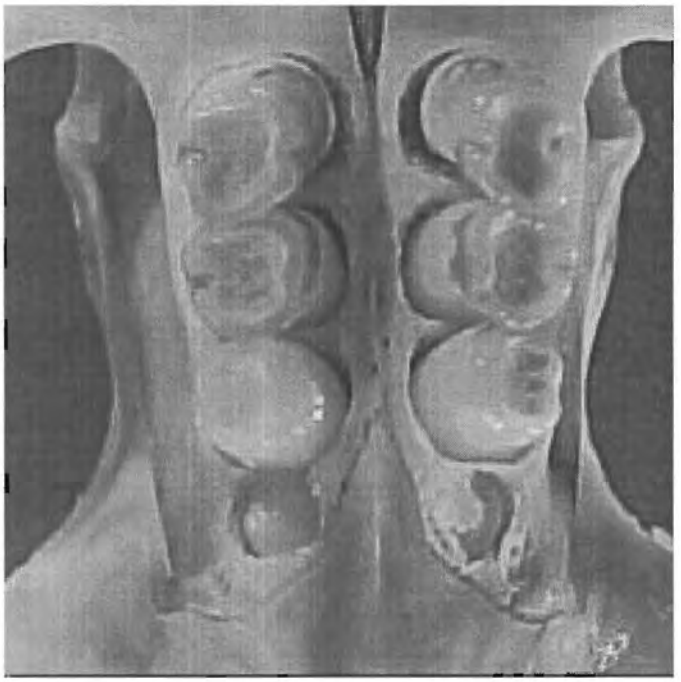

Plate 4. Relative age class 4.

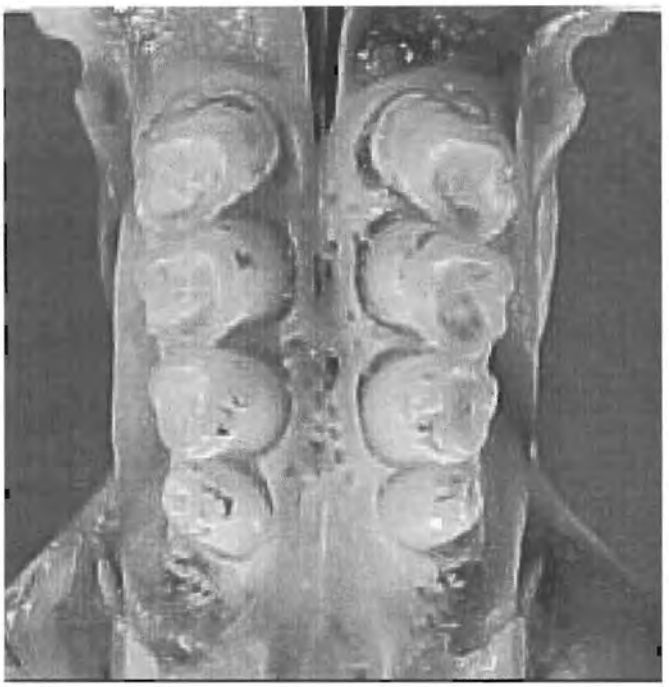

Plate 5. Relative age class 5.

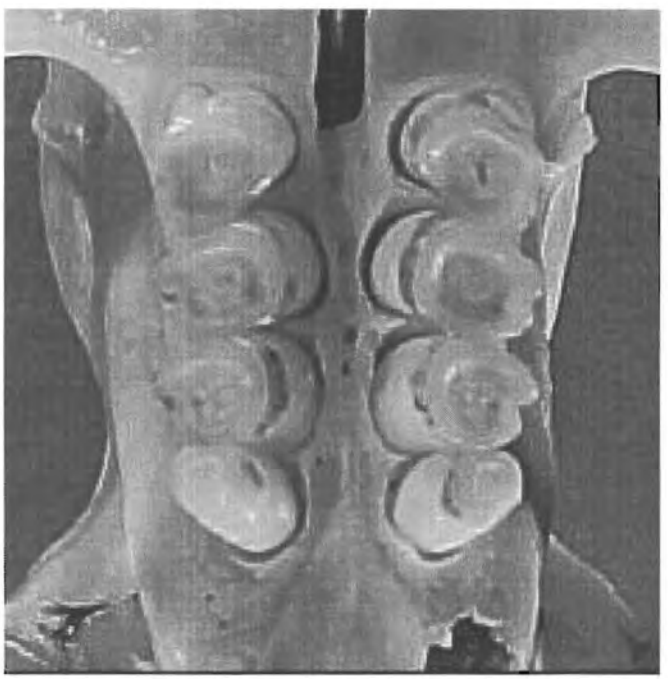

Plate 6. Relative age class 6.

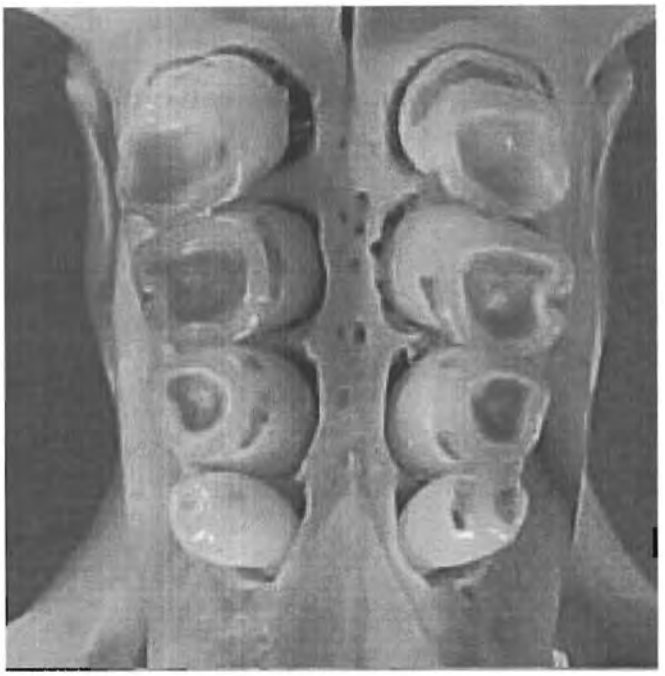

Plate 7. Relative age class 7.

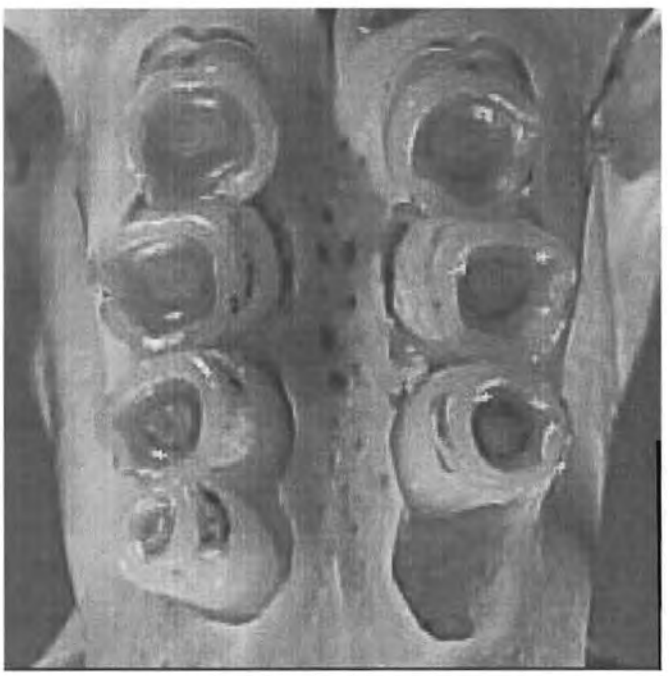

Plate 8. Relative age class 8.

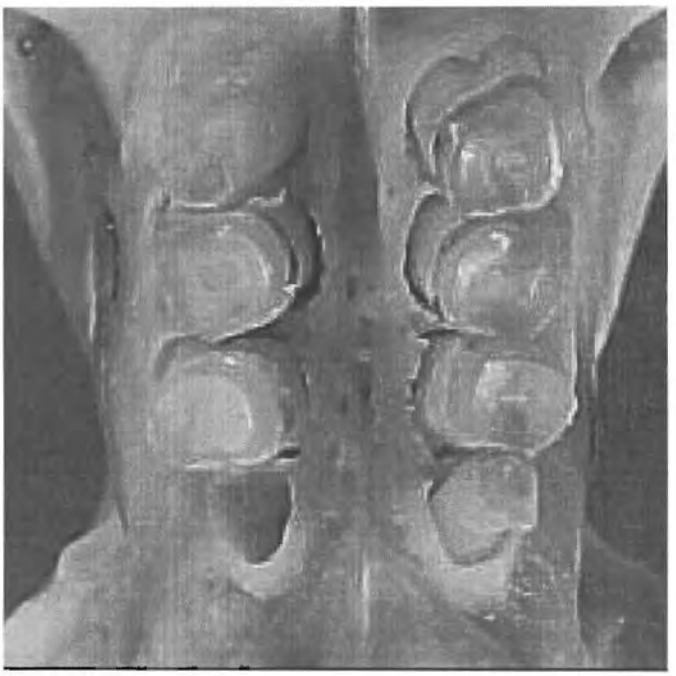

Plate 9. Relative age class 9.

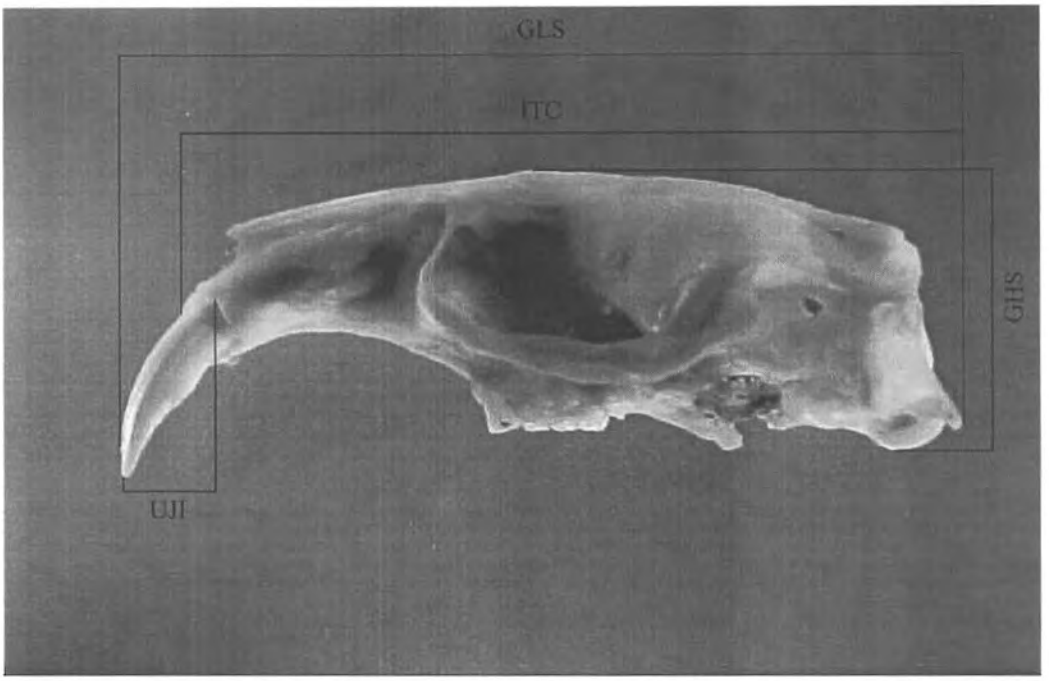

Plate 10 a.

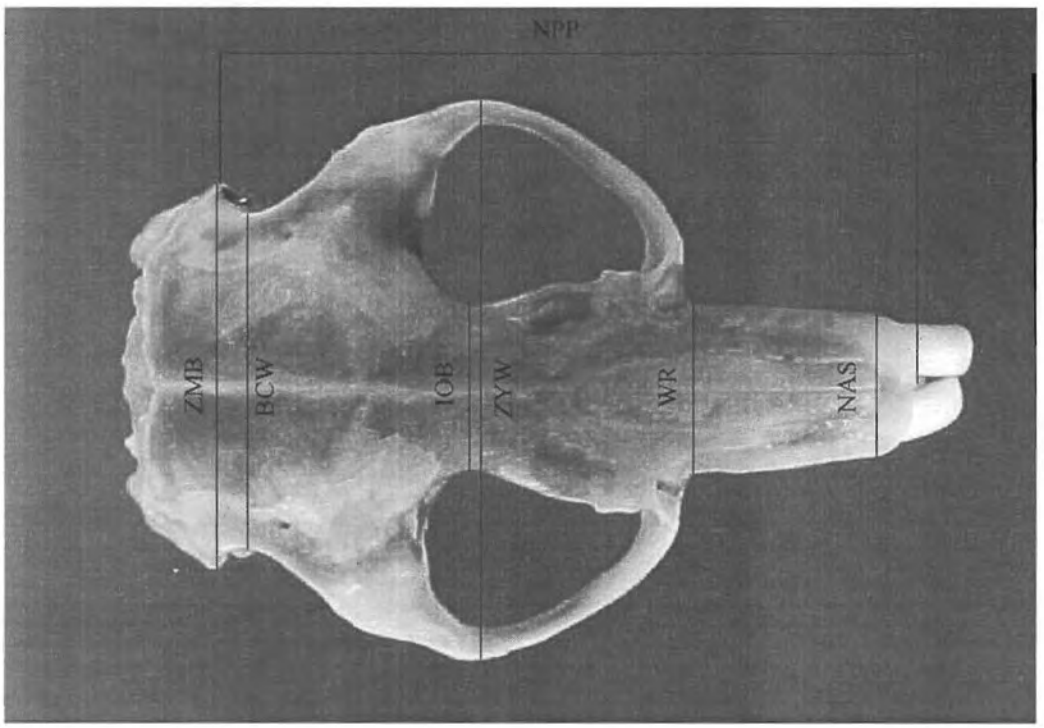

Plate 10 b.

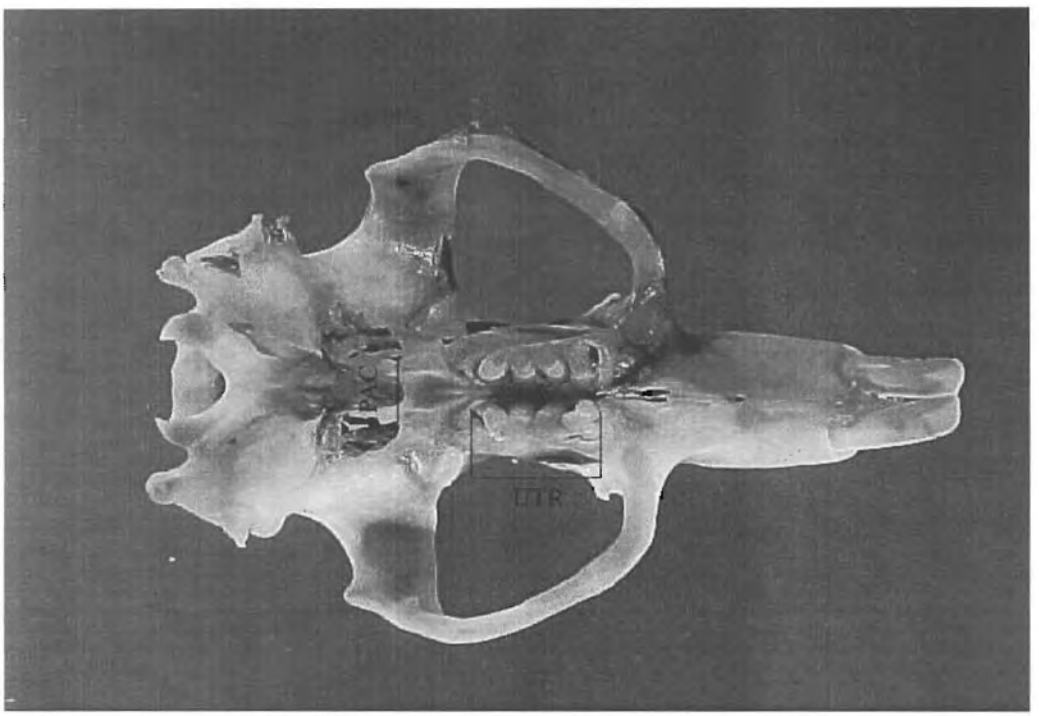

Plate 10 c.

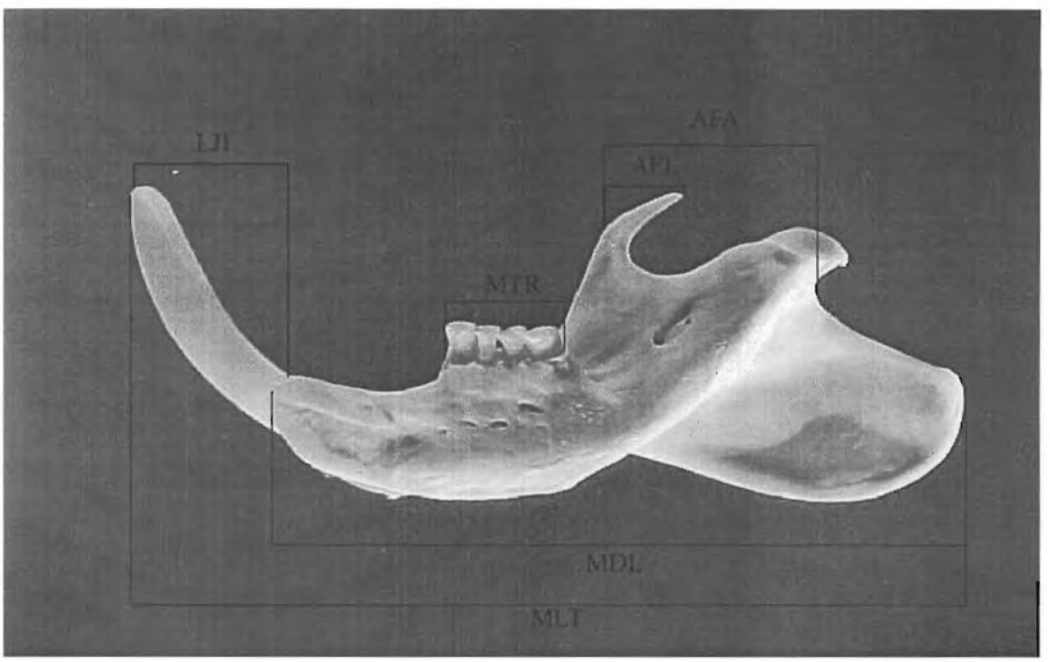

Plate 10 d.

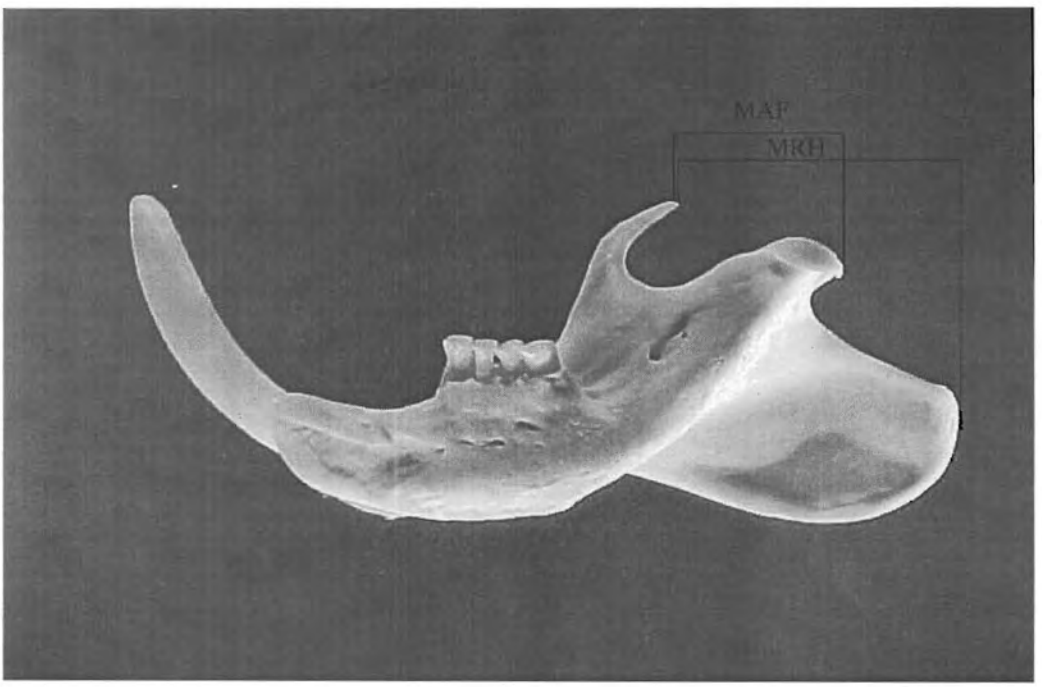

Plate 10 e.

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

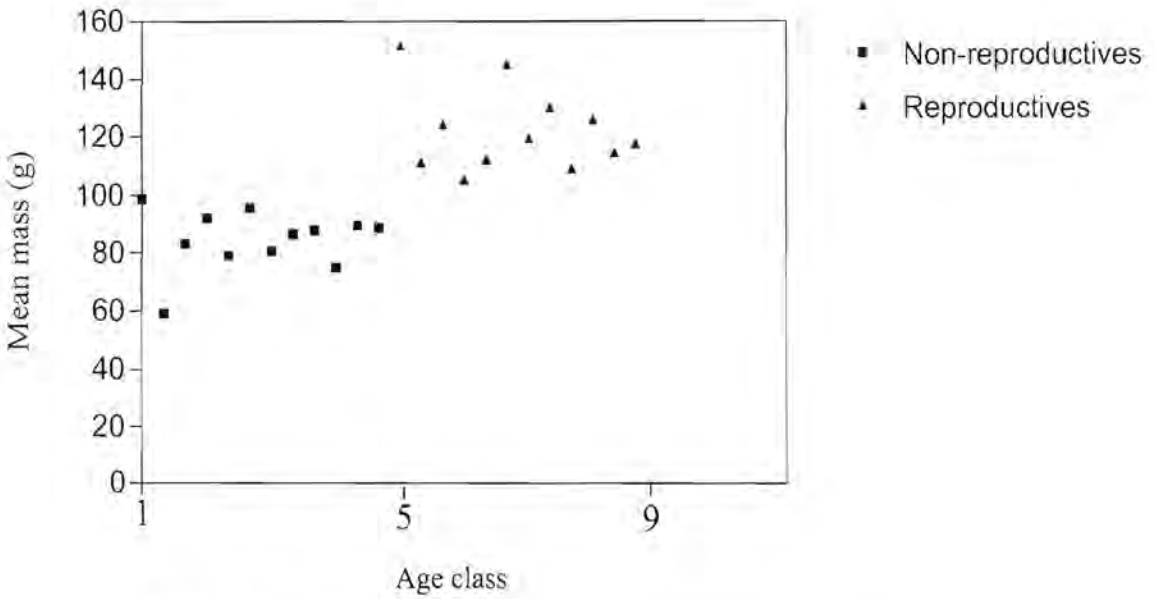

Fig. 1. The difference in mass for both non-reproductive and reproductive animals form different age classes.

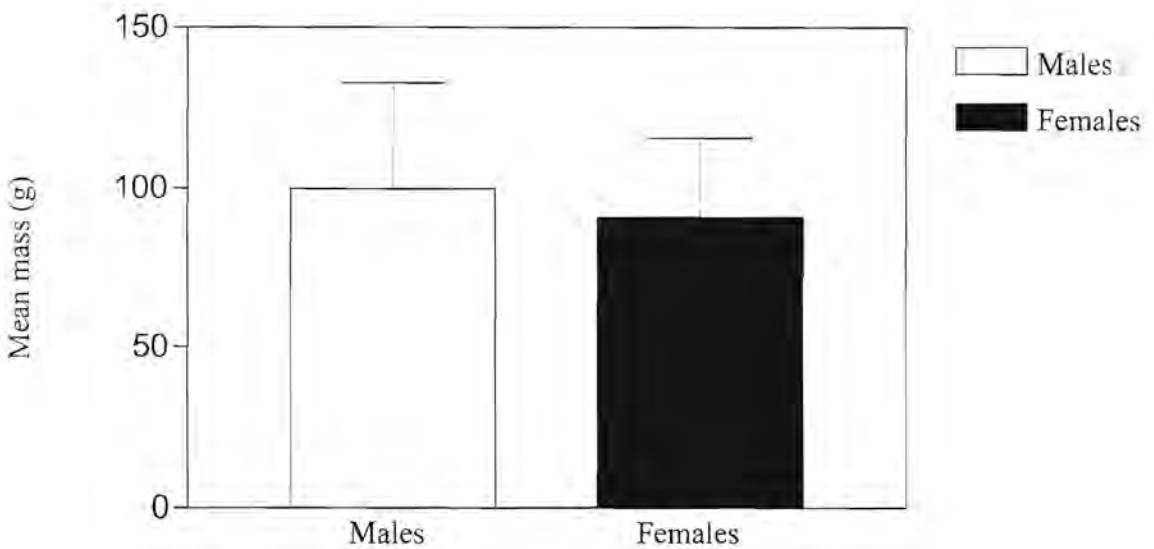

Fig. 2. The mean ± S.E. body mass of all males and females.

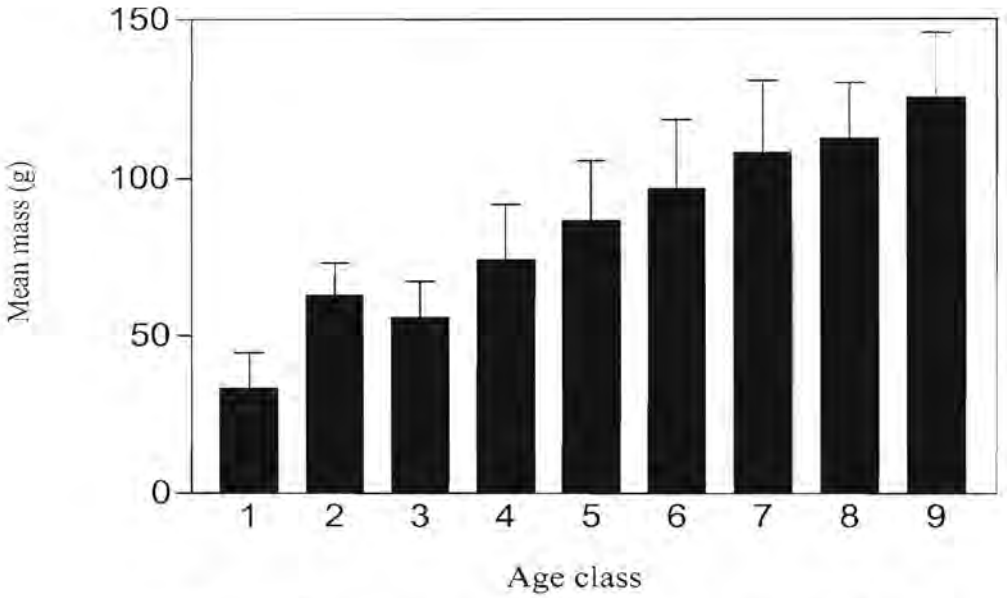

Fig. 3. The mean mass ± S.E. in both males and females from different age classes sampled during 1998/1999.

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).

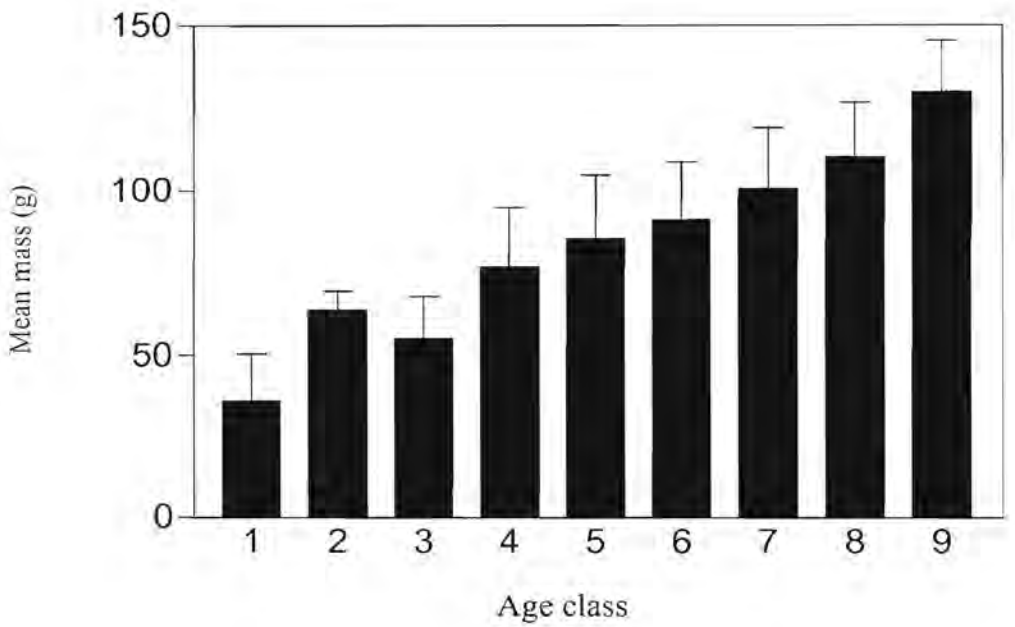

Fig. 4. The mean ± S.E. mass of females from nine relative age classes.

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

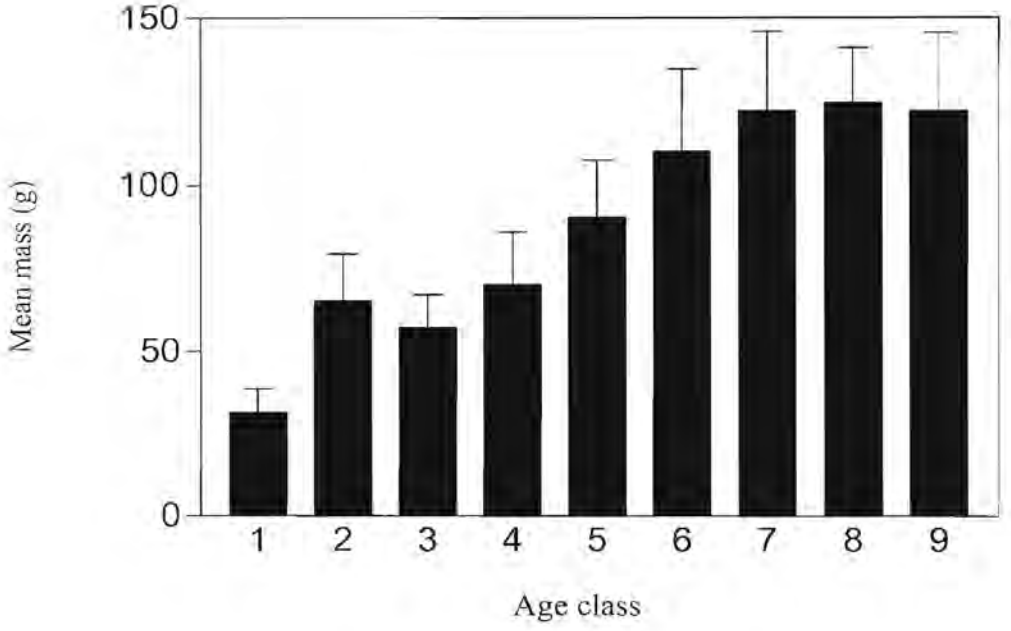

Fig. 5. The mean ± S.E. mass of males from nine relative age classes.

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.

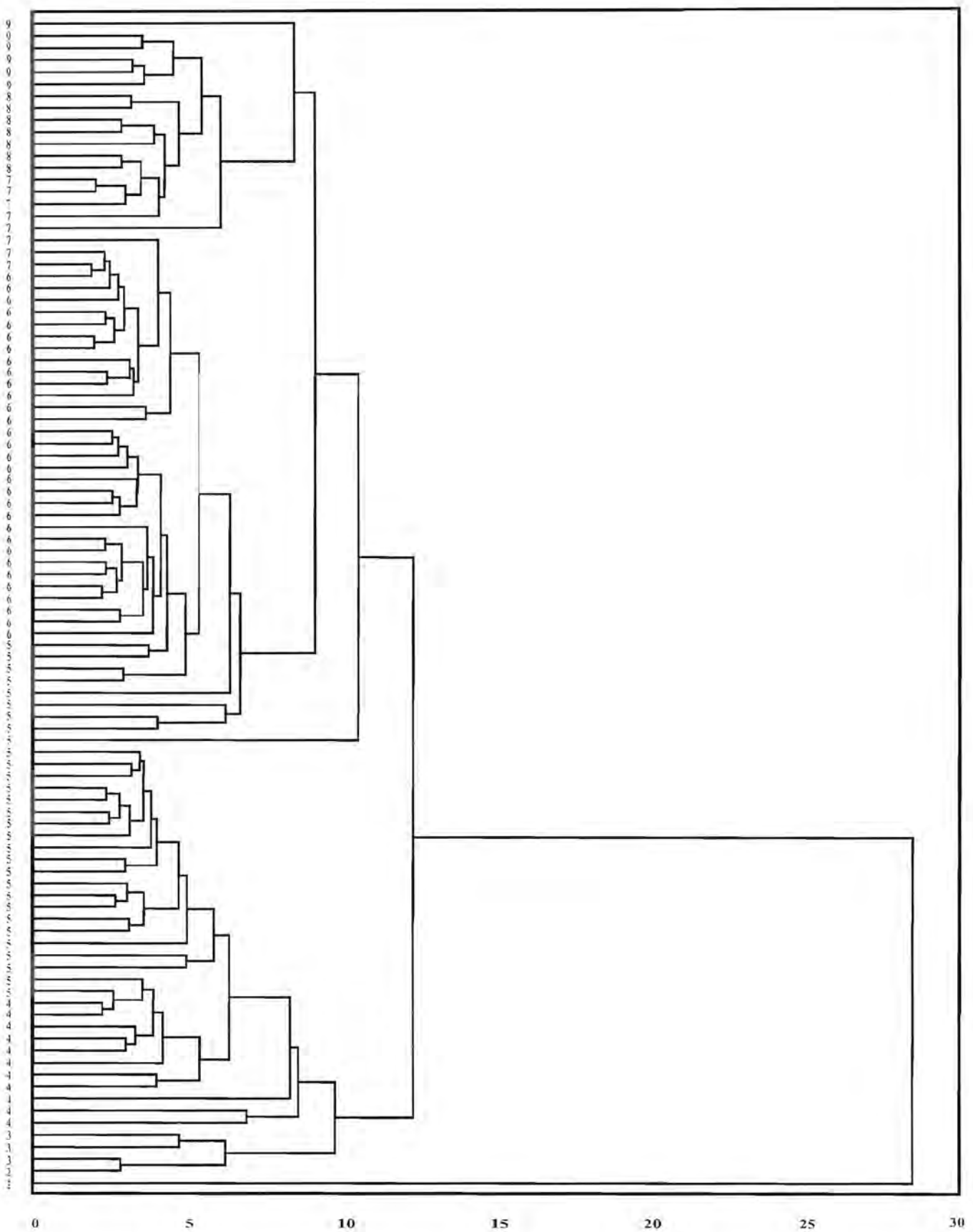

Fig. 6. Euclidean distance phenogram from a UPGMA cluster analysis of *C. h. pretoriae* from Johannesburg. Relative age classes 2 - 9 are indicated for 98 individuals.

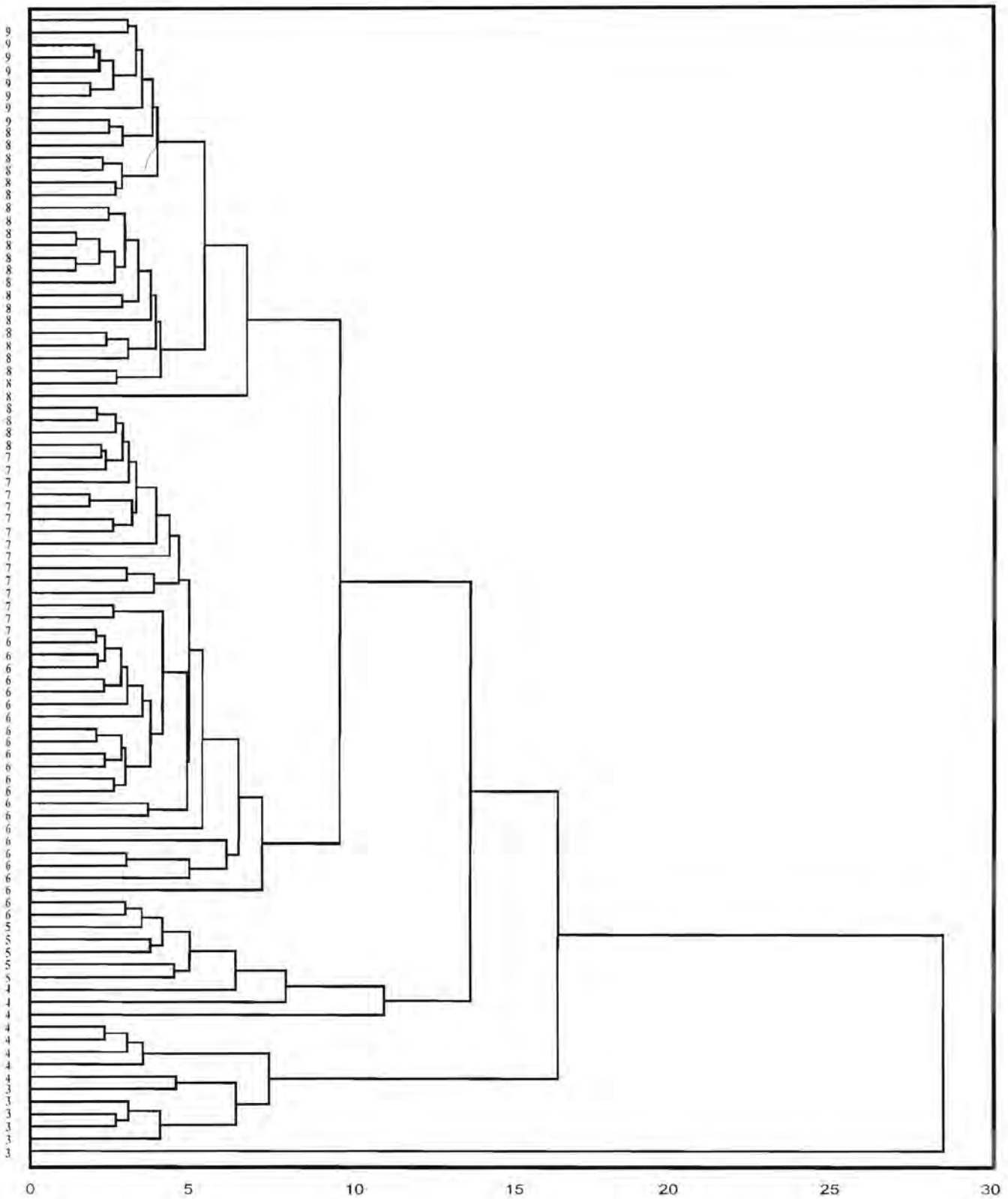

Fig. 7. Euclidean distance phenogram from a UPGMA cluster analysis of *C. h. pretoriae* from Pretoria. Relative age classes (3 - 9) for 92 individuals are indicated.

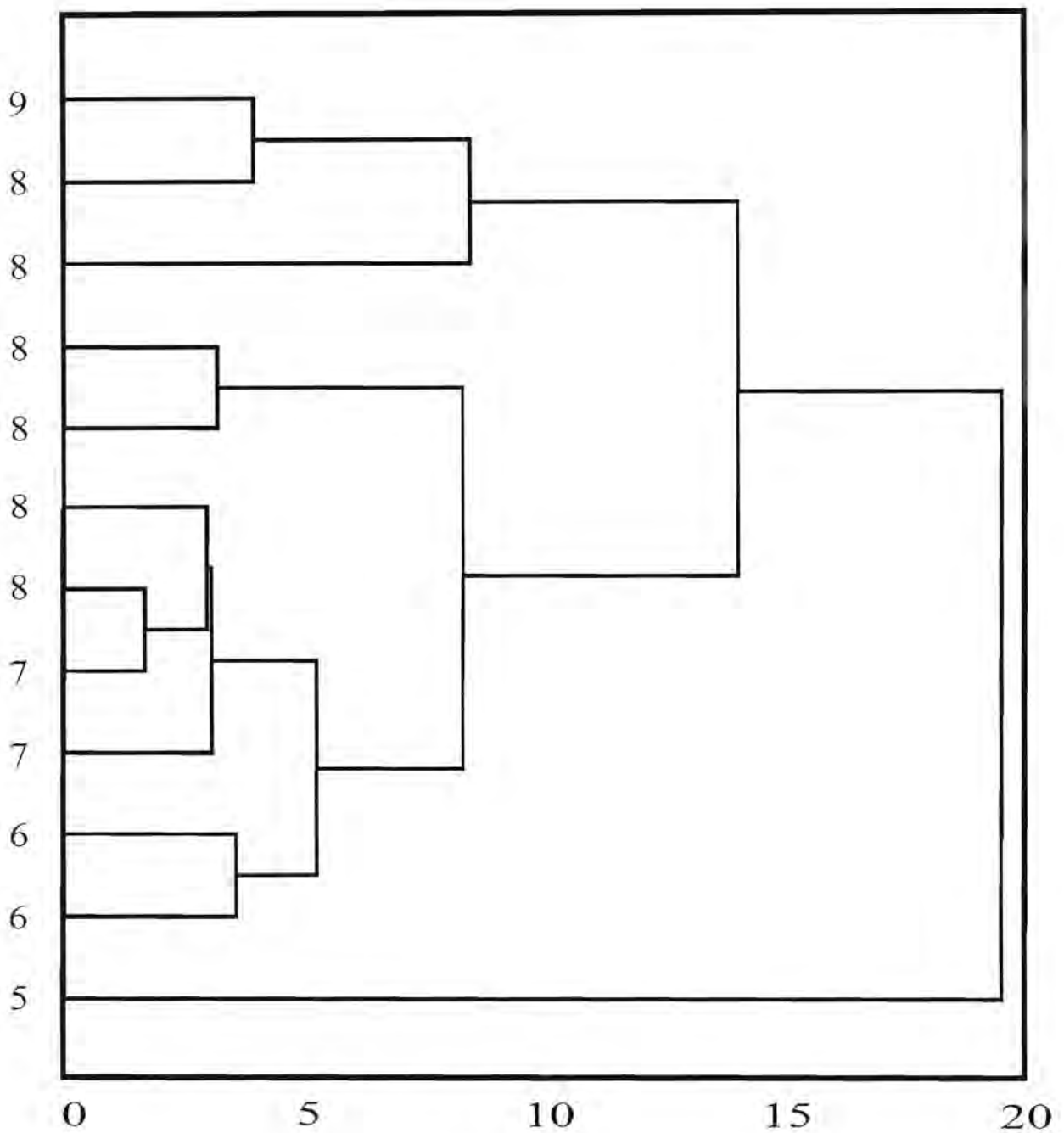

Fig. 8. Euclidean distance phenogram from a UPGMA cluster analysis of *C. h. pretoriae* from Vanderbijlpark. Relative age classes(5 - 9) for 12 individuals are indicated.

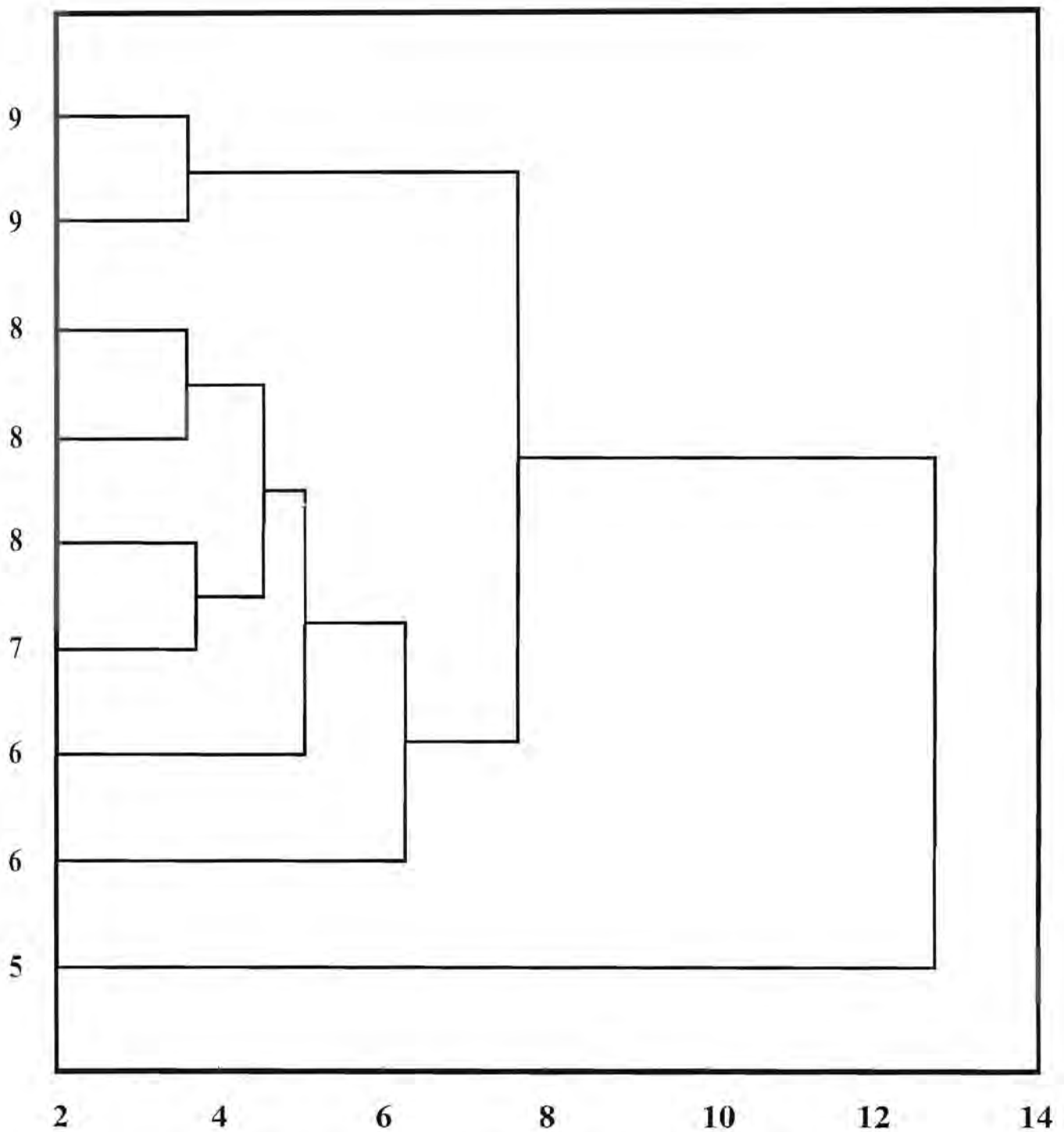

Fig. 9. Euclidean distance phenogram from a UPGMA cluster analysis of *C. h. pretoriae* from Krugersdorp. Relative age classes (5 - 9) for 9 individuals are indicated.

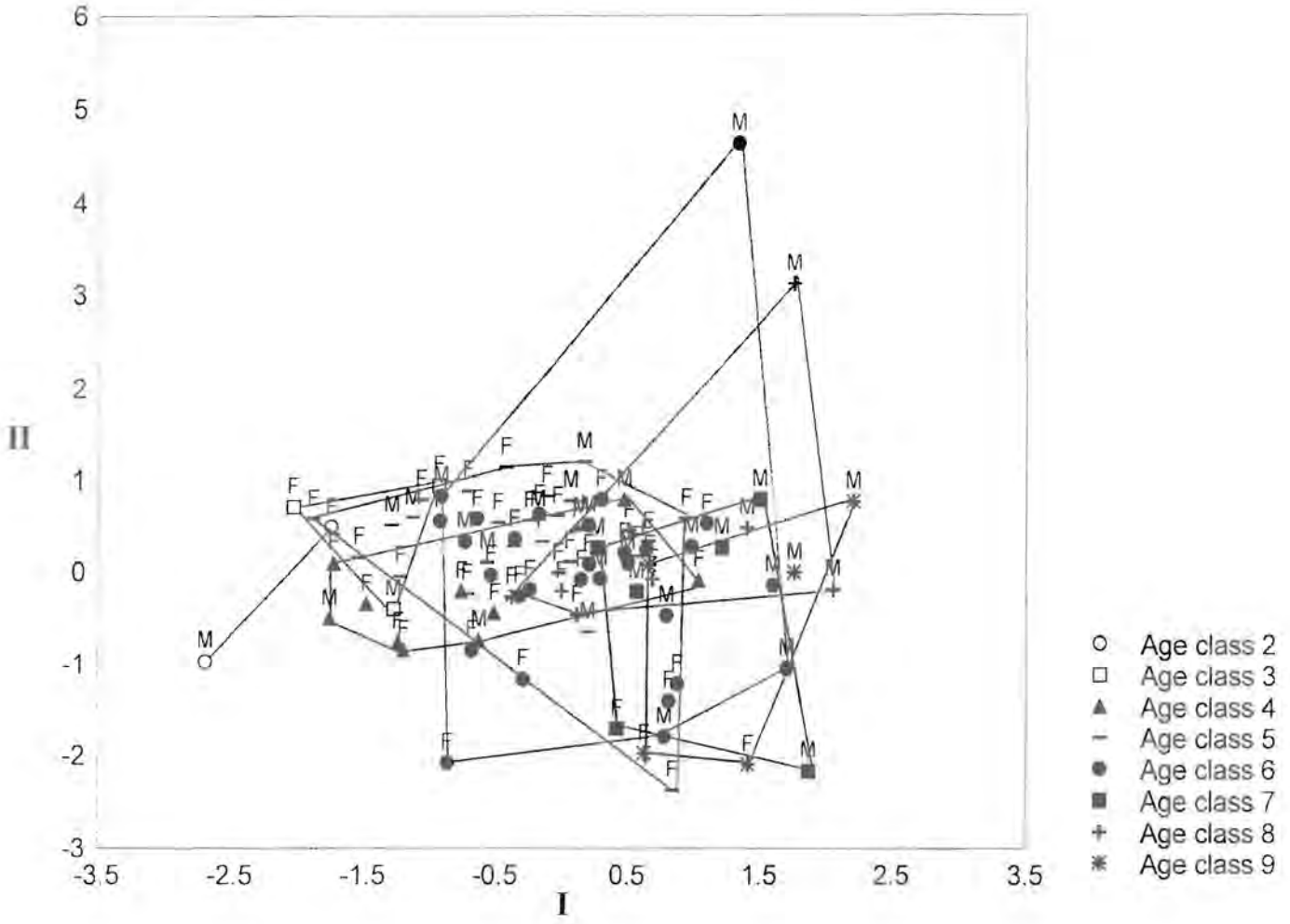

Fig. 10. The first two axes from a principal component analysis of *C. h. pretoriae* from Johannesburg. The sex (M = males; F = females) and age classes of 98 individuals are indicated.

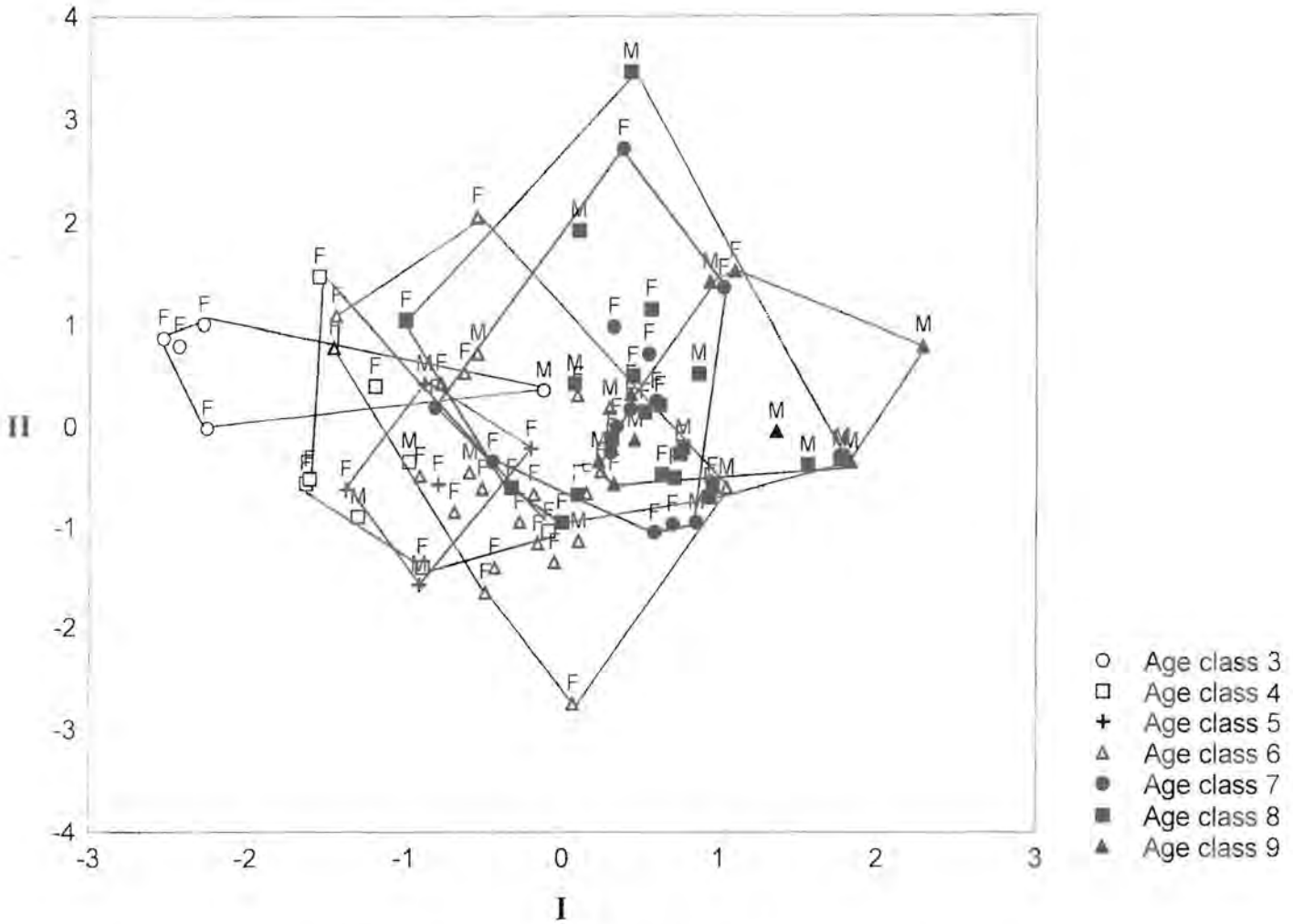

Fig. 11. The first two axes from a principal component analysis of *C. h. pretoriae* from Pretoria. The sex (M = males; F = females) and age classes of 92 individuals are indicated.

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%

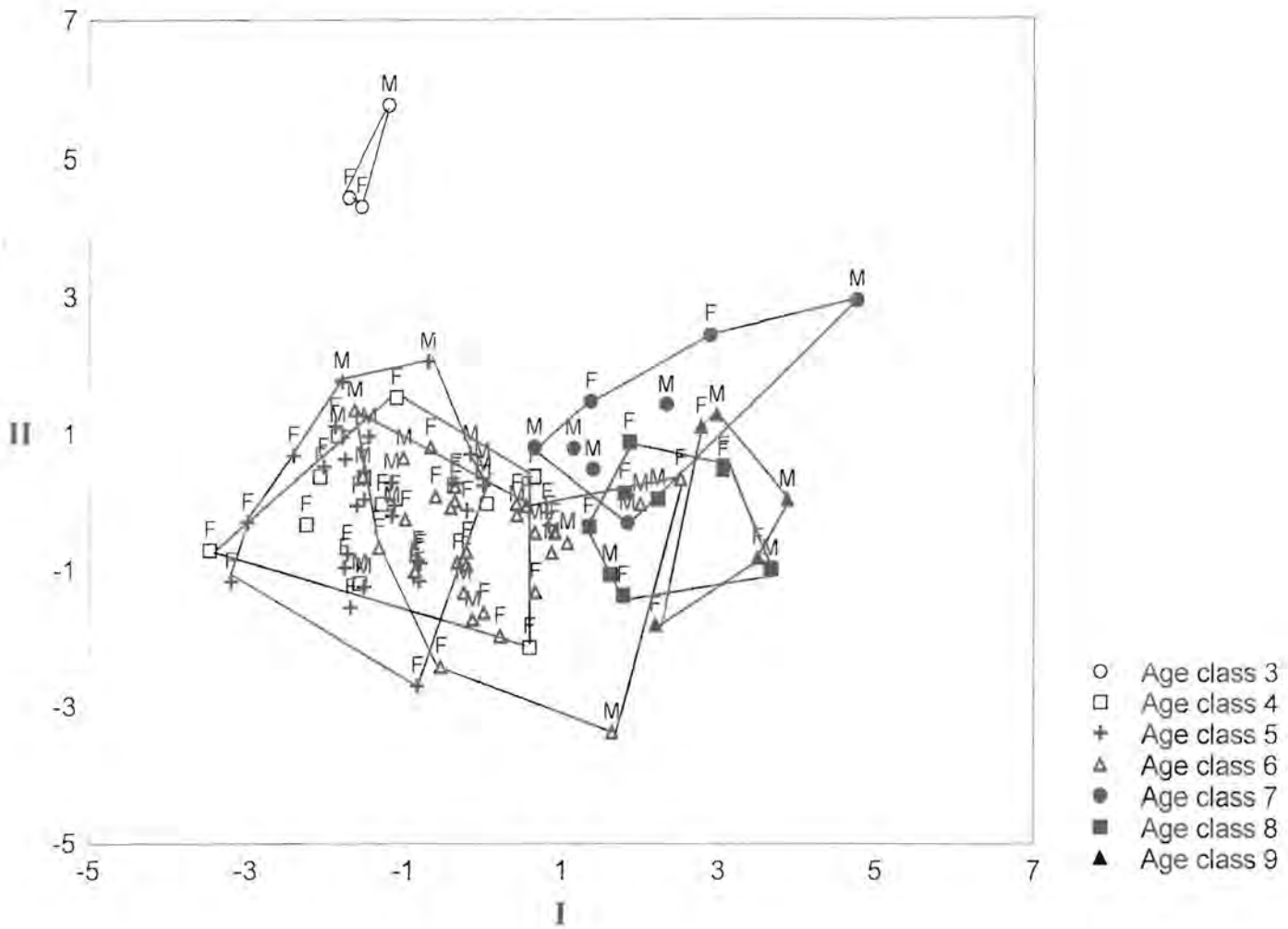

Fig. 12. The first two axes from a discriminant analysis of age class 3 - 9 in *C. h. pretoriae* from Johannesburg. The sex (M = males; F = females) and age classes of 98 individuals are indicated.

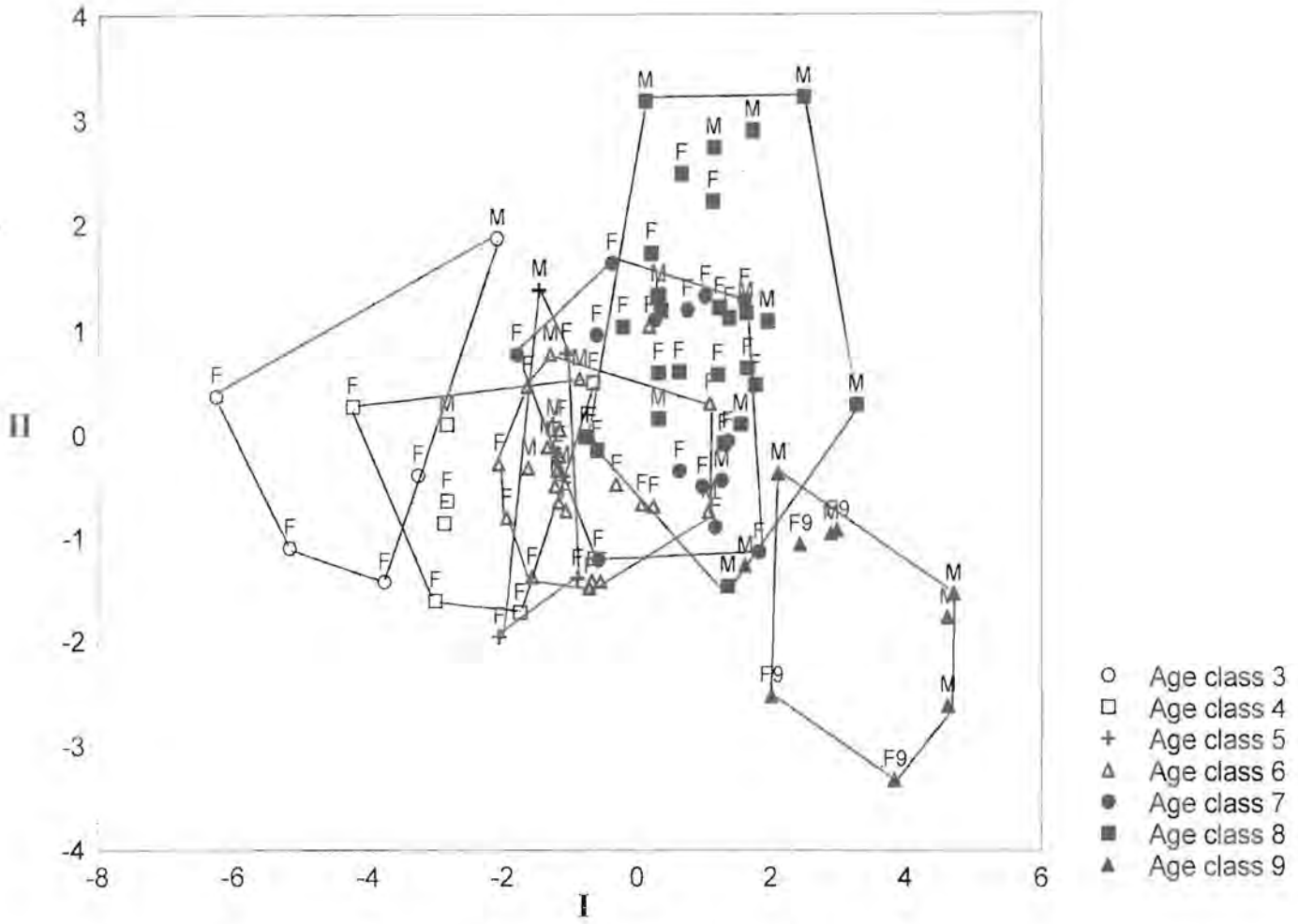

Fig. 13. The first two axes from a discriminant analysis of age class 3 - 9 in *C. h. pretoriae* from Pretoria. The sex (M = males; F = females) and age classes of 92 individuals are indicated.

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

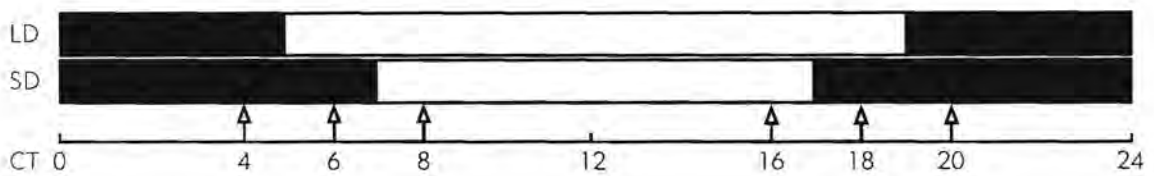

Fig. 1. Diagrammatic summary of the experimental design. LD = long days. SD = short days. CT = circadian time. Solid bar represents subjective night (darkness). Open bar represents subjective day (light). Blood samples were collected via cardiac puncture from eight animals per *zeitgeber* time, indicated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

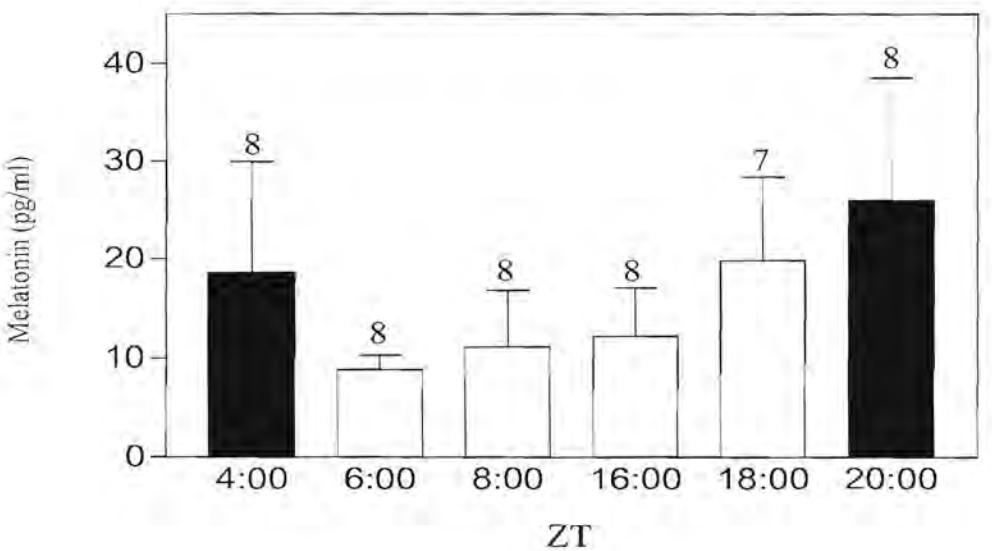

Fig. 2a. Melatonin secretion in *C. h. pretoriae* during long day (n = 47). Open bars denote subjective day (light) and solid bars represent subjective night (dark). *Zeitgeber* times (ZT) are indicated.

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

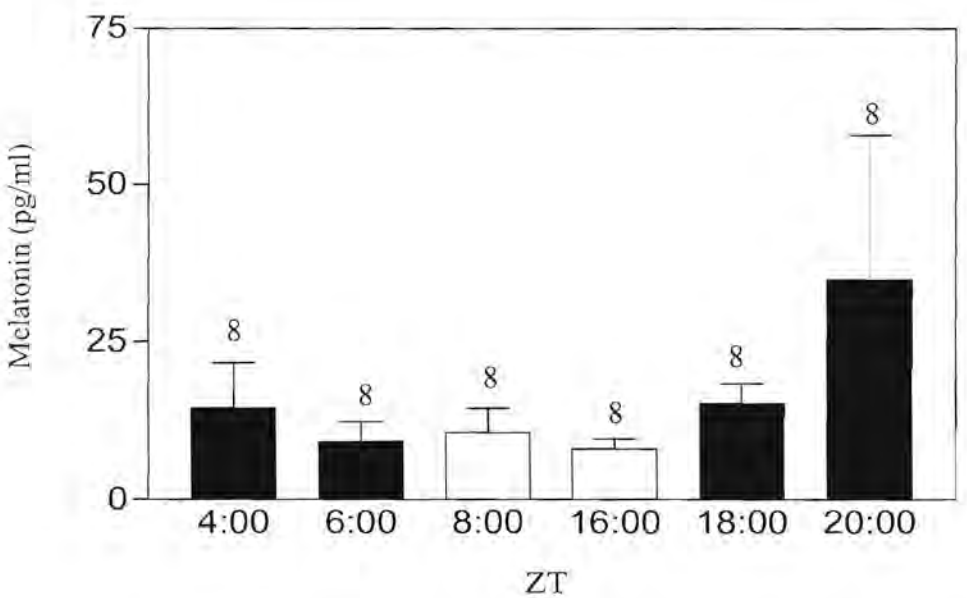

Fig. 2b. Melatonin secretion in *C. h. pretoriae* during short day (n = 48). Open bars denote subjective day (light) and solid bars represent subjective night (dark). *Zeitgeber* times (ZT) are indicated.

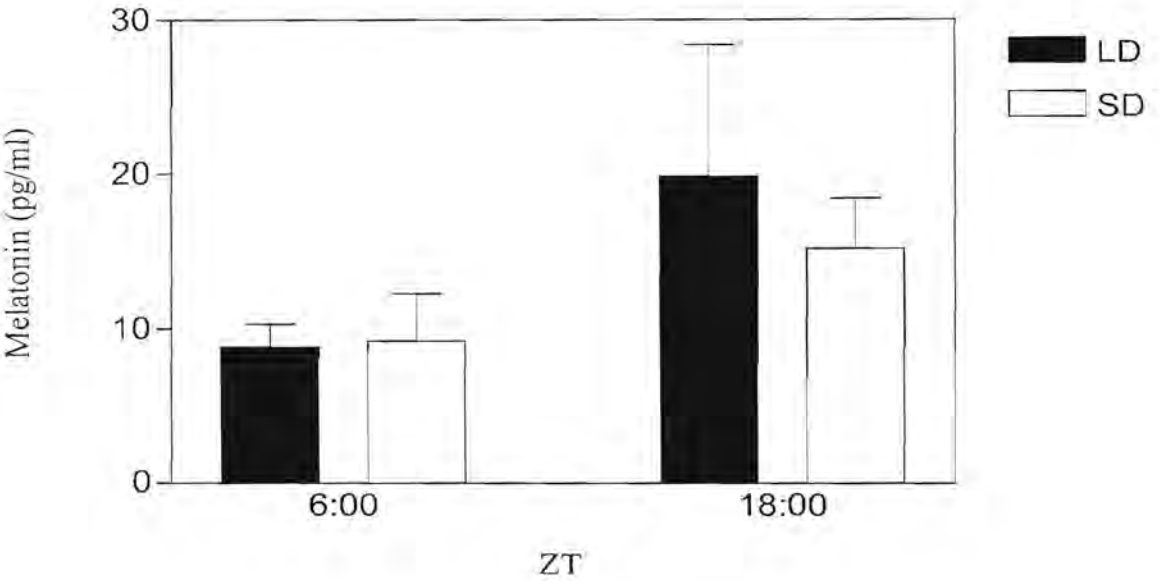

Fig. 3. A comparison of melatonin levels at ZT 6:00 and ZT 18:00 during short days (SD) in the subjective day (open bars) and long days (LD) during the subjective night (solid bars). *Zeitgeber* times (ZT) are indicated.

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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Appendix 1

Standard statistics of 21 measurements of *C. h. pretoriae* males and females sampled in Johannesburg. The sample size (n), mean, standard errors (S.E.) and coefficient of variation (CV) are indicated. Measurements are defined in Plates 10a-e.

			Measurements										
Sex	Age class (n)		GLS	ITC	BCW	ZMB	ZYW	IOB	WR	NAS	UTR	PAC	NPP
Males	4 (3)	Mean	36.12	31.68	16.89	15.81	22.46	7.94	6.35	4.98	6.64	3.50	23.53
		S.E.	1.99	1.42	0.73	0.67	1.28	0.09	0.26	0.26	0.21	0.34	1.10
		CV	9.52	7.76	7.50	7.39	9.89	1.92	7.09	9.02	5.47	16.69	8.11
	5 (9)	Mean	36.81	32.48	17.94	15.91	23.35	7.94	6.87	5.52	6.65	3.81	23.88
		S.E.	0.53	0.61	0.27	0.24	0.44	0.18	0.16	0.13	0.11	0.17	0.48
		CV	4.29	5.60	4.55	4.46	5.63	6.72	6.80	7.32	4.93	13.27	6.09
	6 (11)	Mean	39.15	34.68	18.16	16.19	25.65	7.84	7.39	6.16	6.99	3.84	25.95
		S.E.	0.77	0.77	0.27	0.30	0.59	0.11	0.18	0.17	0.14	0.20	0.51
		CV	6.52	7.38	5.01	6.10	7.66	4.65	7.87	9.36	6.83	17.61	6.50
	7 (6)	Mean	40.92	36.51	19.27	16.23	26.84	8.09	7.83	6.49	6.93	3.76	27.49
		S.E.	0.55	0.62	0.27	0.21	0.56	0.24	0.18	0.19	0.11	0.18	0.67
		CV	3.29	4.16	3.47	3.10	5.07	7.31	5.78	7.30	3.86	11.49	5.96
	8 (3)	Mean	41.75	36.71	18.58	17.22	28.03	8.41	8.39	7.75	7.11	5.00	26.68
		S.E.	0.28	0.47	0.28	0.49	0.83	0.17	0.04	0.40	0.08	1.07	1.33

		CV	1.16	2.21	2.62	4.88	5.12	3.52	0.89	9.04	1.83	36.96	8.65
	9 (2)	Mean	41.74	36.88	20.37	16.80	29.46	8.17	8.25	7.27	7.35	4.17	29.62
		S.E.	1.04	0.69	0.69	0.28	0.38	0.02	0.28	0.28	0.06	0.61	0.26
		CV	3.51	2.65	4.76	2.32	1.80	0.43	4.72	5.55	1.06	20.54	1.22
Females													
	3 (2)	Mean	33.39	30.14	17.20	14.81	21.75	8.11	6.13	4.54	5.47	4.09	22.32
		S.E.	2.09	1.22	1.30	0.20	0.84	0.41	0.32	0.34	0.08	0.33	1.16
		CV	8.85	5.70	10.69	1.91	5.43	7.15	7.27	10.59	2.07	11.41	7.35
	4 (8)	Mean	35.60	33.01	17.16	15.38	22.15	7.73	6.30	4.96	6.66	3.41	22.95
		S.E.	0.87	1.12	0.50	0.29	0.63	0.12	0.19	0.14	0.20	0.15	0.79
		CV	6.93	9.61	8.21	5.35	8.05	4.49	8.38	8.26	8.35	12.15	9.70
	5 (21)	Mean	36.47	32.42	17.88	15.98	23.39	7.65	6.77	5.48	6.82	3.75	24.23
		S.E.	0.44	0.54	0.24	0.45	0.41	0.08	0.11	0.10	0.07	0.09	0.36
		CV	5.57	7.64	6.05	12.87	7.98	4.68	7.48	8.46	4.64	10.51	6.76
	6 (20)	Mean	37.50	33.57	18.01	16.09	24.40	7.74	6.84	5.64	6.80	3.63	24.61
		S.E.	0.32	0.33	0.23	0.17	0.30	0.07	0.12	0.11	0.07	0.12	0.30
		CV	3.76	4.35	5.83	4.84	5.41	3.77	7.79	8.70	4.87	14.40	5.46
	7 (2)	Mean	37.61	34.27	18.60	16.52	25.39	7.65	6.90	5.71	6.24	2.68	27.52
		S.E.	1.06	0.26	0.94	0.22	0.36	0.03	0.22	0.24	0.00	0.36	2.33
		CV	3.99	1.05	7.15	1.88	1.98	0.55	4.41	5.83	0.00	18.77	11.97
	8 (4)	Mean	38.51	34.09	18.31	15.39	24.98	7.39	6.91	5.80	6.55	3.86	25.43
		S.E.	0.68	0.55	0.23	0.28	0.58	0.30	0.19	0.23	0.24	0.13	0.24
		CV	3.55	3.20	2.51	3.70	4.61	8.04	5.55	8.00	7.20	6.81	1.86
	9 (4)	Mean	39.86	35.09	19.15	17.19	26.60	8.00	7.02	5.93	6.88	3.68	26.78
		S.E.	0.32	0.85	0.44	1.07	0.61	0.13	0.23	0.19	0.18	0.38	0.56

		CV	1.62	4.87	4.63	12.43	4.60	3.31	6.64	6.37	5.27	20.71	4.20

			GHS	MLT	MDL	MTR	AFL	MAF	AFA	MRH	UJI	LJI
Males	4 (3)	Mean	14.22	33.10	25.55	6.39	7.14	7.96	9.94	14.48	9.19	13.73
		S.E.	0.38	2.37	1.93	0.48	1.00	0.35	0.50	1.40	0.65	0.59
		CV	4.63	12.43	13.06	13.06	24.17	7.53	8.72	16.80	12.29	7.43
	5 (9)	Mean	14.41	34.01	25.75	6.45	7.34	7.67	9.59	15.08	8.81	14.22
		S.E.	0.59	0.50	0.55	0.10	0.14	0.20	0.21	0.33	0.25	0.26
		CV	12.35	4.41	6.36	4.61	5.69	7.71	6.45	6.56	8.65	5.54
	6 (11)	Mean	16.88	36.45	27.69	6.65	8.10	8.33	10.77	16.91	9.74	15.37
		S.E.	2.33	1.03	0.61	0.08	0.22	0.30	0.25	0.48	0.37	0.45
		CV	45.78	9.34	7.30	3.87	8.96	12.05	7.69	9.45	12.72	9.76
	7 (6)	Mean	15.09	38.96	30.02	6.58	8.26	8.38	10.97	17.64	9.66	16.36
		S.E.	0.26	0.69	1.01	0.15	0.26	0.43	0.52	0.41	0.20	0.44
		CV	4.30	4.34	8.24	5.44	7.81	12.61	11.57	5.69	5.04	6.53
	8 (3)	Mean	15.53	40.58	31.55	6.94	9.33	8.81	11.14	18.34	10.16	16.44
		S.E.	0.21	1.18	0.60	0.21	0.29	0.27	0.35	0.72	0.65	1.22
		CV	2.39	5.05	3.31	5.26	5.38	5.40	5.50	6.78	11.07	12.81
	9 (2)	Mean	16.12	41.64	32.23	6.67	9.65	8.70	11.34	19.60	9.49	16.85
		S.E.	0.02	0.50	0.57	0.01	0.37	0.23	0.72	0.52	0.96	1.46
		CV	0.13	1.70	2.50	0.11	5.42	3.82	8.98	3.72	14.24	12.22

Females

3 (2)	Mean	13.49	30.77	23.29	5.79	6.90	6.94	9.07	13.28	7.14	12.83
	S.E.	0.40	1.26	0.37	0.41	0.16	0.55	0.70	0.58	0.38	0.01
	CV	4.19	5.77	2.25	9.90	3.28	11.11	10.91	6.18	7.53	0.11
4 (8)	Mean	13.69	32.33	24.75	6.35	6.82	7.80	9.87	13.99	9.18	13.62
	S.E.	0.20	1.07	0.62	0.26	0.29	0.26	0.22	0.44	0.37	0.97
	CV	4.10	9.38	7.09	11.68	12.02	9.29	6.17	8.94	11.27	20.05
5 (21)	Mean	13.73	33.58	25.96	6.76	7.42	7.81	9.78	14.84	8.89	13.74
	S.E.	0.15	0.51	0.40	0.05	0.18	0.15	0.16	0.32	0.20	0.23
	CV	4.88	7.00	7.06	3.51	11.05	9.07	7.71	9.76	10.31	7.83
6 (20)	Mean	14.18	34.71	26.62	6.61	7.45	8.22	10.17	15.30	8.97	13.94
	S.E.	0.16	0.40	0.36	0.06	0.18	0.20	0.18	0.24	0.31	0.29
	CV	5.00	5.09	6.05	3.93	10.53	11.10	7.93	7.15	15.70	9.21
7 (2)	Mean	14.33	35.99	27.82	5.89	7.21	8.57	10.80	16.08	9.70	14.24
	S.E.	0.46	1.38	0.85	0.03	0.33	0.05	0.16	0.10	1.30	1.91
	CV	4.49	5.42	4.30	0.84	6.38	0.74	2.03	0.88	18.95	18.97
8 (4)	Mean	14.26	36.26	27.64	6.37	7.50	8.10	9.88	15.89	9.18	16.15
	S.E.	0.21	0.61	1.04	0.10	0.23	0.17	0.13	0.50	0.47	0.38
	CV	2.96	3.38	7.56	3.18	6.18	4.18	2.68	6.24	10.16	4.65
9 (4)	Mean	14.87	37.64	28.31	6.33	7.36	9.73	11.40	16.62	10.45	15.20
	S.E.	0.44	0.42	0.37	0.20	0.13	0.35	0.46	0.25	0.40	0.89
	CV	5.85	2.22	2.63	6.35	3.66	7.21	7.99	3.02	7.62	11.73

Appendix 2

Standard statistics of 21 measurements of *C. h. pretoriae* males and females sampled in Pretoria. The sample size (n), mean, standard errors (S.E.) and coefficient of variation (CV) are indicated. Measurements are defined in Plates 10a-e.

			Measurements										
Sex	Age class (n)		GLS	ITC	BCW	ZMB	ZYW	IOB	WR	NAS	UTR	PAC	NPP
Males	3 (2)	Mean	4.57	30.57	16.88	14.93	21.04	7.35	6.06	4.89	5.98	3.70	22.37
		S.E.	0.52	0.49	0.21	0.32	0.46	0.05	0.00	0.14	0.24	0.05	0.66
		CV	2.15	2.24	1.80	2.98	3.09	0.96	0.12	4.05	5.68	1.91	4.17
	5 (2)	Mean	34.57	30.89	16.22	14.78	22.32	7.61	6.14	4.81	6.40	3.63	21.95
		S.E.	0.00	0.21	0.28	0.21	0.78	0.13	0.03	0.09	0.25	0.38	0.21
		CV	0.00	0.96	2.44	1.96	4.94	2.32	0.58	2.80	5.52	15.02	1.39
	6 (5)	Mean	37.11	33.83	18.40	15.58	23.99	7.77	6.77	5.52	6.46	3.97	23.68
		S.E.	0.68	0.88	0.47	0.38	0.81	0.17	0.19	0.21	0.18	0.23	0.72
		CV	4.08	5.81	5.70	5.48	7.55	4.82	6.26	8.33	6.28	13.23	6.81
	8 (11)	Mean	39.69	36.42	19.08	15.89	26.98	7.97	7.71	6.46	6.51	3.97	27.01
		S.E.	0.62	0.66	0.36	0.36	0.51	0.14	0.21	0.17	0.13	0.16	0.55
		CV	5.14	5.98	10.00	7.49	6.22	5.61	8.94	8.92	6.66	13.02	6.75
	9 (6)	Mean	40.08	36.19	18.78	16.18	27.24	7.92	7.62	6.67	6.81	4.23	27.21
		S.E.	1.05	0.92	0.47	0.16	0.71	0.14	0.30	0.30	0.11	0.20	0.59

		CV	6.41	6.21	6.08	2.44	6.41	4.33	9.75	11.06	3.75	11.68	5.32
Females													
	3 (4)	Mean	30.13	26.47	15.18	13.53	19.43	7.35	5.07	3.69	5.48	2.95	18.61
		S.E.	0.59	0.36	0.05	0.09	0.30	0.13	0.09	0.19	0.34	0.11	0.29
		CV	3.87	2.73	0.67	1.35	3.02	3.39	3.46	9.93	12.18	7.58	3.08
	4 (6)	Mean	33.74	30.16	16.68	14.86	21.35	7.72	5.81	4.57	6.07	3.47	21.53
		S.E.	0.64	0.59	0.45	0.22	0.55	0.06	0.17	0.18	0.26	0..17	0.61
		CV	4.65	4.76	6.67	3.54	6.32	1.99	7.07	9.52	10.37	11.79	6.92
	5 (3)	Mean	34.62	31.62	17.23	15.20	21.96	7.30	6.14	5.13	6.43	3.77	22.46
		S.E.	0.90	1.29	0.33	0.14	0.75	0.21	0.36	0.37	0.16	0.09	0.92
		CV	4.50	7.04	3.39	1.57	5.90	4.95	10.15	12.50	4.37	3.94	7.13
	6 (19)	Mean	35.74	31.99	17.58	15.32	23.01	7.60	6.33	5.17	6.28	3.74	23.45
		S.E.	0.34	0.47	0.23	0.15	0.22	0.09	0.08	0.06	0.08	0.11	0.31
		CV	4.11	6.36	5.79	4.38	4.16	5.26	5.55	5.09	5.72	13.20	5.80
	7 (13)	Mean	37.93	34.13	18.47	15.69	24.96	7.81	6.91	5.79	6.45	3.75	25.27
		S.E.	0.41	0.41	0.28	0.19	0.41	0.08	0.11	0.11	0.11	0.09	0.40
		CV	3.91	4.35	5.41	4.38	5.92	3.83	5.53	7.04	5.82	9.06	5.64
	8 (15)	Mean	38.40	34.62	18.38	15.73	24.89	7.84	7.01	5.73	6.60	3.89	25.52
		S.E.	0.43	0.44	0.24	0.17	0.36	0.08	0.09	0.10	0.09	0.11	0.35
		CV	4.38	4.94	5.13	4.22	5.57	3.80	5.16	6.44	5.06	11.30	5.24
	9 (4)	Mean	40.02	36.86	19.53	16.32	27.12	7.90	7.05	5.90	6.92	3.88	27.21
		S.E.	0.53	0.66	0.60	0.30	0.79	0.21	0.18	0.23	0.20	0.16	0.79
		CV	2.63	3.56	6.15	3.64	5.81	5.26	4.96	7.71	5.79	7.89	5.78

	GHS	MLT	MDL	MTR	AFL	MAF	AFA	MRH	UJI	LJI

Males	3 (2)	Mean	13.45	30.19	23.16	5.59	6.33	6.93	8.25	12.73	8.93	12.41
		S.E.	0.10	1.39	1.06	0.19	0.64	0.24	0.08	1.03	0.35	0.13
		CV	1.05	6.49	6.47	4.81	14.30	4.90	1.46	11.39	5.63	1.48
	5 (2)	Mean	12.75	30.96	23.59	6.25	6.97	7.25	9.14	13.60	8.04	12.82
		S.E.	0.42	0.93	0.11	0.32	0.17	0.42	0.69	0.62	0.37	0.25
		CV	4.66	4.25	0.69	7.24	3.45	8.30	10.68	6.45	6.42	2.81
	6 (5)	Mean	14.65	35.02	26.95	6.23	7.08	8.18	9.84	15.45	8.63	13.93
		S.E.	0.47	0.89	0.84	0.15	0.28	0.43	0.26	0.51	0.47	0.22
		CV	7.18	5.64	6.93	5.33	8.86	11.75	5.99	7.35	12.12	3.55
	8 (11)	Mean	15.30	38.23	29.23	6.23	7.97	8.99	11.06	16.76	9.17	15.18
		S.E.	0.28	0.56	0.45	0.09	0.36	0.18	0.19	0.82	0.36	0.36
		CV	6.15	4.86	5.13	4.96	14.76	6.81	5.83	16.25	13.07	7.84
	9 (6)	Mean	15.36	39.71	30.78	6.41	8.73	8.42	11.14	18.24	9.28	15.83
		S.E.	0.32	1.33	1.08	0.16	0.55	0.30	0.70	0.80	0.44	0.51
		CV	5.08	8.23	8.60	6.16	15.38	8.64	15.45	10.71	11.60	7.92
Females												
	3 (4)	Mean	11.92	26.83	20.39	4.62	5.46	6.72	8.57	11.62	6.67	9.88
		S.E.	0.08	0.22	0.44	0.08	0.25	0.11	0.12	0.08	0.39	0.16
		CV	1.32	1.62	4.25	3.46	9.14	3.10	2.75	1.28	11.56	3.18
	4 (6)	Mean	13.14	30.37	23.37	5.56	6.18	7.24	9.04	12.95	7.43	12.30
		S.E.	0.37	0.81	0.67	0.29	0.18	0.13	0.22	0.38	0.51	0.86
		CV	6.86	6.55	7.01	12.95	7.30	4.49	5.96	7.22	16.89	17.11
	5 (3)	Mean	13.34	31.14	23.88	6.36	6.00	7.73	8.99	13.85	7.19	12.26
		S.E.	0.29	1.17	0.62	0.10	0.25	0.32	0.25	0.67	0.70	0.51
		CV	3.71	6.53	4.54	2.82	7.11	7.21	4.89	8.46	16.95	7.17
	6 (19)	Mean	13.78	31.27	25.26	6.19	7.00	7.66	9.65	14.53	7.96	12.87
		S.E.	0.16	1.31	0.59	0.07	0.13	0.12	0.13	0.19	0.22	0.29
		CV	4.95	18.34	10.17	5.00	7.87	6.86	5.73	5.81	11.98	9.86

7 (13)	Mean	14.36	35.86	27.22	6.11	7.22	8.57	10.46	16.02	8.71	14.83
	S.E.	0.20	0.58	0.45	0.09	0.22	0.17	0.21	0.29	0.17	0.46
	CV	5.04	5.82	6.00	5.13	10.89	6.98	7.25	6.49	6.88	11.21
8 (15)	Mean	14.47	35.78	27.07	6.33	7.67	8.31	10.20	15.79	8.88	14.85
	S.E.	0.13	0.44	0.40	0.13	0.17	0.14	0.19	0.26	0.21	0.34
	CV	3.61	4.74	5.73	7.73	8.66	6.50	4.52	6.40	9.34	8.77
9 (4)	Mean	15.20	37.32	29.37	6.37	7.65	9.02	11.22	16.97	7.72	13.44
	S.E.	0.66	1.06	0.72	0.16	0.18	0.43	0.53	0.81	0.76	1.24
	CV	8.59	5.65	4.92	4.94	4.67	9.45	9.45	9.50	19.51	18.40

Appendix 3

Standard statistics of 21 measurements of *C. h. pretoriae* males and females sampled in Vanderbijlpark. The sample size (n), mean, standard error (S.E.) and coefficient of variation (CV) are indicated.
Measurements are defined in Plates 10a-e.

			Measurements										
Sex	Age class (n)		GLS	ITC	BCW	ZMB	ZYW	IOB	WR	NAS	UTR	PAC	NPP
Females	8 (5)	Mean	39.13	34.55	19.20	16.03	26.13	7.56	6.76	5.95	6.47	4.30	30.27
		S.E.	0.50	0.51	0.15	0.04	0.31	0.11	0.12	0.18	0.17	0.10	2.54
		CV	2.90	3.33	1.78	0.61	2.67	3.34	3.88	6.67	5.68	5.19	18.78
			GHS	MLT	MDL	MTR	AFL	MAF	AFA	MRH	UJI	LJI	
		Mean	14.59	37.01	28.13	5.95	8.73	8.07	10.22	16.57	9.29	15.56	
		S.E.	0.16	0.90	0.25	0.15	0.40	0.24	0.13	0.30	0.31	1.50	
		CV	2.49	5.47	2.01	5.61	10.30	6.52	2.96	4.05	7.59	21.60	

Appendix 4

Standard statistics of 21 measurements of *C. h. pretoriae* males and females sampled in Krugersdorp. The sample size (n), mean, standard errors (S.E.) and coefficient of variation (CV) are indicated. Measurements are defined in Plates 10a-e.

			Measurements										
Sex	Age class (n)		GLS	ITC	BCW	ZMB	ZYW	IOB	WR	NAS	UTR	PAC	NPP
Males	9 (2)	Mean	42.08	37.63	18.95	5.88	26.61	7.89	7.84	0.65	7.37	4.41	27.11
		S.E.	1.48	1.74	0.42	0.09	1.18	0.16	0.21	0.42	0.26	0.13	1.39
		CV	4.96	6.54	3.17	0.89	6.27	2.96	3.70	9.05	5.09	4.17	7.28
Females	8 (3)	Mean	38.20	34.17	17.89	15.02	24.44	7.25	6.53	5.43	7.01	3.89	24.86
		S.E.	0.57	0.93	0.20	0.08	0.31	0.24	0.33	0.24	0.35	0.29	1.69
		CV	2.56	4.71	1.90	0.91	2.16	5.60	8.66	7.80	8.62	13.01	4.78
			GHS	MLT	MDL	MTR	AFL	MAF	AFA	MRH	UJI	LJI	
Males	9 (2)	Mean	15.31	39.30	30.08	6.66	8.38	8.64	11.14	17.45	10.11	16.55	
		S.E.	0.32	1.58	0.61	0.04	0.59	0.11	0.20	1.02	0.42	1.53	
		CV	2.96	5.67	2.87	0.74	9.96	1.88	2.54	8.23	5.95	13.12	
Females	8 (3)	Mean	13.83	35.95	26.87	6.01	7.05	7.90	10.45	15.27	8.24	16.77	
		S.E.	0.36	0.42	0.64	0.21	0.05	0.56	0.57	0.41	0.62	1.03	

CV	4.49	2.01	4.08	6.11	1.10	12.25	8.95	4.64	13.17	10.60