Reproductive biology of the Cape dune mole-rat, *Bathyergus suillus* (Schreber 1782).

By

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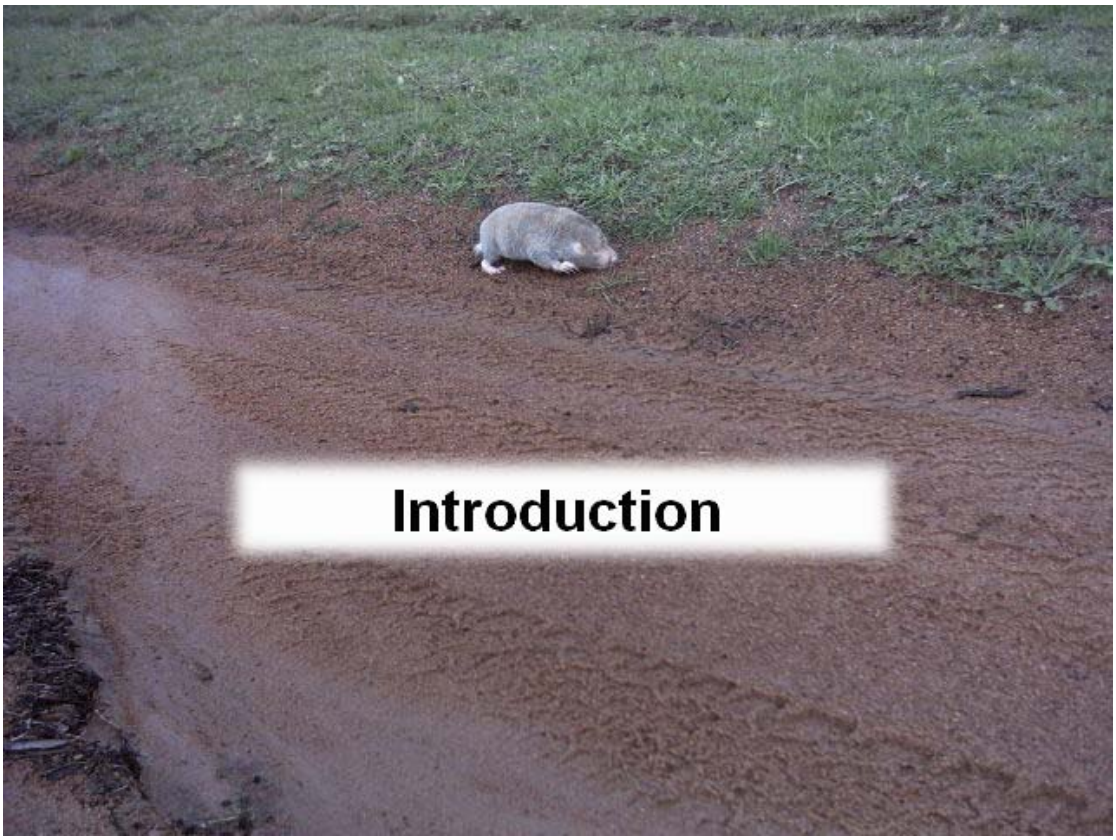
Filippense 4:13 "Ek is tot alles in staat deur Hom wat my krag gee"


Introduction

Seasonal breeding: Seasonal reproduction occurs in many mammalian species and entails the restriction of the reproductive phase to a specific time of year (Karsch et al. 1984). Indeed, in most mammals, reproduction is timed to ensure that birth occurs at a time that maximizes growth and survival of the offspring (Ims 1990; Fitzgerald and McManus 2000).

A number of environmental factors are important for the regulation of seasonal reproduction these include temperature, rainfall but by far the most important cue to above ground mammals is the light dark cycle transition in the morning and the evening (Karsch et al. 1984). These cues allow an animal to anticipate the optimal time for reproduction. Photoperiod is believed to be the most important factor for the regulation of seasonal reproduction (Karsh et al. 1984; Reiter and Follett, 1980). Because of the subterranean nature of mole-rats, individuals are rarely exposed to the light dark cycle. The only time that exposure to light could occur to a limited degree is when the burrow system of the mole-rat is being expanded and excess soil is pushed to the soil surface.

Temperature changes are yet another common regulating factor in seasonal reproduction. Temperature fluctuations are reduced in burrow systems compared to ambient and soil surface temperatures. Marked seasonal differences have been found however in the burrows of mole-rats occurring in both mesic and arid habitats (Bennett et al. 1988) that may contribute to seasonal timing of reproduction. Another important factor in the regulation of seasonal reproduction is rainfall and the associated sudden flush of vegetation (Jarvis 1969). When soil conditions are favourable (i.e. easily workable after rainfall) mole-rats extend their burrow systems

and seek out potential mates or disperse and establish their own colonies. In those species that display xenophobia this may be a very important factor in the regulation of seasonality in reproduction.

The Cape Dune mole-rat, Bathyergus suillus: The focus of this study was to investigate the reproductive biology of the solitary Cape dune mole-rat, *Bathyergus suillus*, occurring in the coastal Western Cape Province of South Africa. The Cape dune mole-rat is a subterranean hystricomorph rodent that belongs to the family Bathyergidae (Greek: *Bathys,* deep and *ergo,* work), and due to the fact that it exhibits a solitary lifestyle individuals of this species are highly xenophobic towards conspecifics. Prior to the onset of reproduction these strong xenophobic barriers need to be broken down. The most common way for these mole-rats to come together for procreation is to signal their sex and intention to mate with neighbouring conspecifics through seismic communication. This form of communication may be achieved through incisor tapping (*Tachyorychetes splendens*; Jarvis 1969), head drumming as seen in the blind mole-rat, *Spalax ehrenbergi* (Rado et al. 1987) and hind foot drumming as employed by other bathyergids such as the Namaqua dune mole-rat, *Bathyergus janetta,* the Cape mole-rat *Georychus capensis* and the Cape dune mole-rat, (Bennett & Jarvis, 1988; Bennett et al. 1991; Jarvis and Bennett 1991). Hindfoot drumming is generally initiated by the male of the species where after the female responds at a different frequency.

An extensive extermination programme was conducted the Cape Town International airport during the period 2003 to 2005 and this provided all data pertaining to this study into the reproductive strategies of the Cape dune mole-rat.

Seasonal reproduction has been reported in a sister taxon of the Cape mole-rat, the Namaqua dune mole-rat, *Bathyergus janetta* (Herbst et al. 2004) and the closely related solitary dwelling Cape mole-rat, *Georychus capensis*; (Bennett and Jarvis 1988a). Many of the social mole-rat species from central Africa are aseasonal breeders (Bennett and Jarvis 1988b; Bennett et al. 1994; Bennett and Aguilar 1995; Jarvis 1981); here photoperiods are more constant throughout the year and temperatures more equable. The social common (Spinks et al. 1997, 1999) and highveld mole-rats (Janse van Rensburg et al. 2002) exhibit seasonal reproduction and interestingly inhabit regions that show a marked seasonality in both rainfall pattern photoperiod and temperature change.

To date there is little information on the breeding biology of the Cape dune mole-rat. A preliminary study by van der Horst (1972) proposed that males may exhibit seasonality to breeding but the results were equivocal. This study sets out to fill this void in our knowledge of bathyergid reproductive biology.

Thesis structure and content: Chapter 1 of the thesis investigates the skull morphology and wear on the molariform teeth of the mole-rats. Age class allocation is based on the eruption and wear of molar teeth as described for the highveld mole-rat by Janse van Rensburg et al. (2004). Sexual dimorphism on the basis of lean body mass is known to occur in the Cape dune mole-rat, but to date this has not been discerned on general skull morphometrics. A total of 22 skull measurements were made to investigate whether sexual dimorphism is indeed present and to what extent sexual dimorphism arises within the different age classes.

Chapter 2 investigates the general reproductive biology of the Cape dune mole-rat that is assessed from post-mortem examination of the morphological,

anatomical and hormonal characters of both sexes of dune mole-rats collected over an entire calendar year. In this chapter data are presented on the general ovarian histology as well as the percentage of females captured pregnant or lactating each month for the entire year. In males, the mean size of the testes and diameter of the seminiferous tubules are discerned. Hormone profiles of testosterone in males and the progesterone/ oestradiol 17β profiles in females were used to complement the histological data.

The general period of breeding was found to be related to the relative frequency and amount of monthly rainfall. The results clearly demonstrate that the Cape dune mole-rat is a seasonal breeder that cues its reproduction into periods of good rainfall when the soils are workable and opposite sexed conspecifics can come together for the process of procreation.

In chapter 3 the concentrations of circulating basal LH were monitored in males and females both in and out of the breeding season. Furthermore the pituitary response to an exogenous overdose of GnRH was investigated to determine if pituitary down regulation of GnRH receptors occurred in the pituitaries of both males and females out of the breeding season.

Chapter 4 involved an anatomical investigation and characterisation of the gonadotrophin releasing hormone (GnRH) neuronal system of both sexes of the Cape dune mole-rat. The production and secretion of LH is controlled by GnRH. Gonadotrophin releasing hormone is produced in the hypothalamus by GnRH releasing cells and then transported via cell fibres to the pituitary where the production and secretion of LH is controlled (Schwanzel-Fakuda and Pfaff 1989).

The GnRH system was characterised by using anti-GnRH antibodies and immunohistochemistry. I investigated the size, distribution and number of these

GnRH perikarya during the reproductively active and quiescent periods of the year. The total number, size and distribution of GnRH releasing cells were determined in both sexes both in and out of the breeding season.

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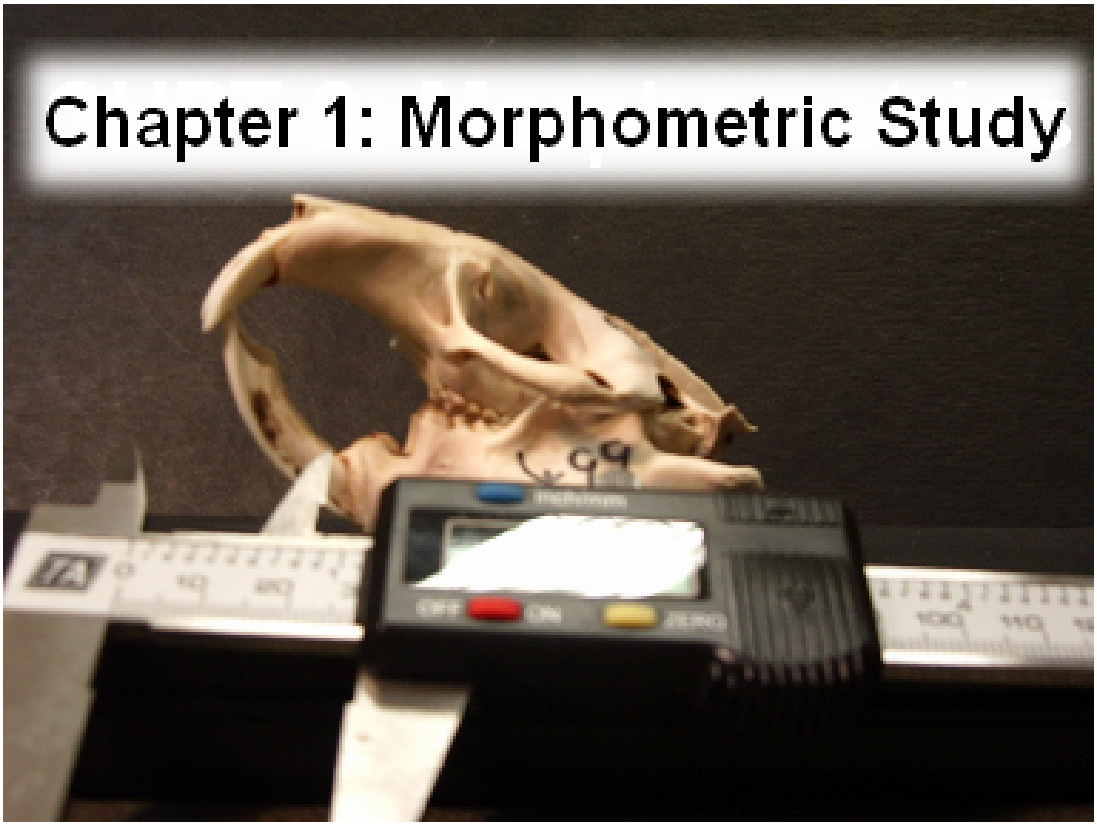

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SEXUAL DIMORPHISM AND AGE VARIATION IN THE CAPE DUNE MOLE-RAT, *BATHYERGUS SUILLUS* (RODENTIA: BATHYERGIDAE) FROM SOUTH AFRICA

The nature and extent of maxillary molar toothrow eruption and wear were used to assign individuals of the solitary Cape dune mole-rat, *Bathyergus suillus* from a population in Cape Town, Western Cape Province, South Africa into 9 relative age classes. Cranial morphometric analysis of this single opportunistic population that emanated from an extermination program at Cape Town International Airport allowed us to investigate the nature and extent of sexual dimorphism and age variation in this little studied species of mole-rat. Both univariate and multivariate analyses showed the separation of age classes 2–3 and 6–9, with age classes 4–5 being intermediate between the two age class groupings, suggesting that individuals of age classes 4 and 5 may be at a point on a visualized hypothetical growth curve where it begins to stabilize. Apart from indicating a progression of age-related increase in size, the results in this study showed the absence of sexual dimorphism in younger individuals of age classes 2–5, and its presence in older individuals of age classes 6 – 9. These results are discussed with reference to reproductive status, and together with an envisaged microsatellite study may assist in providing an insight into our current understanding of social structuring in the little studied solitary Cape dune mole-rat.

Key words: age variation, *Bathyergus suillus*, Cape dune mole-rat, molar morphology, morphometrics, sexual dimorphism

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The Cape dune mole-rat, *Bathyergus suillus* is a solitary, subterranean rodent endemic to coastal Western and Eastern Cape Provinces South Africa (Skinner and Chimimba 2005). To date, however, there have been very few studies on the Cape dune mole-rat, such that a recent extermination program at Cape Town International Airport, Cape Town, South Africa provided an ideal opportunity for a reproductive physiological study of the species. The present study, therefore, forms part of this broader investigation, and attempts to morphometrically assess the nature and extent of sexual dimorphism and age variation in the Cape dune mole-rat.

Previous studies on other African mole-rats based on body mass, external and/or cranial measurements, with reference to age structure and reproductive status found the existence of sexual dimorphism in the social Damaraland mole-rat (*Cryptomys damarensis*), and in the solitary Namaqua dune mole-rat (*Bathyergus janetta*), Davies and Jarvis 1986; Bennett et al. 1990). In contrast, no sexual dimorphism occurs in the social *Cryptomys hottentotus hottentotus* and *C. h. pretoriae*, the social *Heterocephalus glaber* and the solitary Cape mole-rat (*Georychus capensis*), (Hagen 1985; Taylor et al. 1985; Bennett et al. 1990; Janse van Rensburg et al. 2004).

Of fundamental importance in the kind of studies cited above is the difficulty associated with the direct ageing of individuals. While other workers have used body mass to estimate age, it has been found that it may not be suitable for estimating the age of an individual (Bennett 1988; Bennett et al. 1990; Janse van Rensburg et al. 2004). This is because, apart from high degrees of individual variation, body mass and hence external measurements, may potentially be influenced by the availability, quality, and energy content of food, as well as an individual's social rank (Morris

1972; Bennett 1988, 1989; Jacobs et al. 1991; Wallace and Bennett 1998; Janse van Rensburg 2004).

Consequently, various other methods for estimating age have been proposed, and include the degree of molar eruption and wear that are considered to be more reliable estimators of relative age (Janse van Rensburg 2004), and have been applied to a wide range of mammals (Chaplin and White 1969; Gilbert et al. 1970; Dippenaar and Rautenbach 1986; Chimimba and Dippenaar 1994), including mole-rats (Taylor et. al. 1985; Janse van Rensburg et al. 2004). The present study, therefore, uses the degree of molar eruption and wear as indicators of relative age, with reference to cranial morphometric data and sexual dimorphism in the Cape dune mole-rat from South Africa.

Of fundamental importance in the evaluation of sexual dimorphism and age variation, however, relates to how the derived data are analyzed in order to partition these components of non-geographic variation. While these have in the past been assessed using a range of univariate analyses (for a review, see Chimimba and Dippenaar 1994), the partitioning of the percent contribution of the sum of squares (*% SSQ*) of each source of variation to the total *SSQ* is considered to be the most appropriate (Leamy 1983). Nevertheless, there has been concern about the utilization of univariate analyses because of the number of variables that must be significant before concluding on overall significance (Willig et al 1986). Consequently, multivariate analysis of variance (MANOVA – Zar 1996) that utilizes rather than ignores correlations among variables has been recommended as the most appropriate for evaluating overall differences (Willig et al. 1986). The present study, therefore, comparatively uses analysis of variance (ANOVA – Zar 1996), *% SSQ*, and a series of

multivariate analyses to morphometrically evaluate sexual dimorphism and age variation in the Cape dune mole-rat from South Africa.

MATERIALS AND METHODS

Study animals were obtained daily between 2003 and 2005 during an opportunistic mole-rat extermination program at Cape Town International Airport, Cape Town, Western Cape Province, South Africa (33°58'S 18°37'E; 87males, 100 females) using modified vice traps. Animals were captured on a daily basis with the use of modified vice traps. 3mm thick rubber strips were wrapped around both serrated edges of the trap and secured in place with cable ties. While this greatly reduced the efficacy of the traps and necessitated frequent checking it had the desirable effect of limiting damage to the animals' limb upon capture. Traps were checked every 20 minutes and captured animals were immediately removed from their burrow, placed in a 20 litre plastic bucket and euthanased with an overdose of chloroform as an anaesthetic. All sacrificed animals will subsequently be prepared as voucher specimens and will be deposited in the mammal collection of the Transvaal Museum (TM), Pretoria, South Africa.

All procedures conform to the guidelines of the American Society of Mammalogists (ASM; http//mammalogy.org/committees/index.asp; Journal of Mammalogy 79:1416–1431) and the animal ethics committee of the University of Pretoria.

Relative age was estimated by assigning individuals to 9 relative tooth-wear classes using molar eruption and wear on the maxillary toothrow based on the same 9 tooth wear classes (Plate 1) in the highveld mole-rat (*C. h. pretoriae*) defined by Janse

van Rensburg (2000). Unfortunately no individuals of tooth wear class 1 were present so individuals were only assigned to age classes 2 to 9. Individuals were assigned to age classes 2 to 9 based on the following criteria:

Age class 2: Three cheek teeth have completely erupted. Only the first two teeth show any signs of wear whereas there is no sign of tooth wear on the third check tooth (Plate 1 AC 2).

Age class 3: Three cheek teeth have completely erupted and a cavity is present where the fourth tooth is about to surface (Plate 1 AC 3).

Age class 4: Three cheek teeth have completely erupted and the fourth check tooth has started to surface (Plate 1 AC 4).

Age class 5: All four cheek teeth have erupted and there is no sign of wear on tooth number 4 whereas the dentine on the first three cheek teeth is slightly scooped (Plate 1 AC 5).

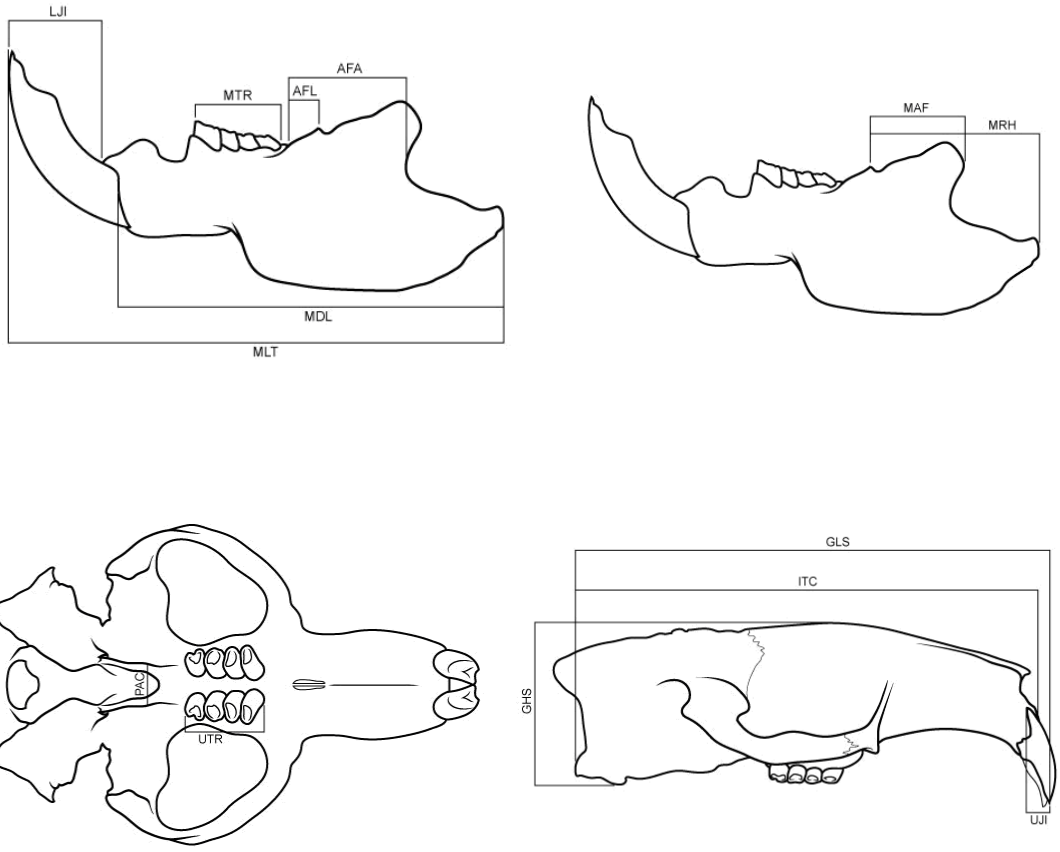
Age class 6: All four cheek teeth have erupted and there are signs of little wear on the fourth cheek tooth (Plate 1 AC 6).

Age class 7: All four cheek teeth have erupted and there are signs of a fair amount of wear on the fourth cheek tooth. The dentine on the first three teeth is deeply scooped (Plate 1 AC 7).

Age class 8: All four cheek teeth have erupted and the dentine on all four teeth is deeply scooped (Plate 1 AC 8).

Age class 9: All four cheek teeth have erupted and the dentine is deeply scooped. All four cheek teeth are deformed and are reduced in height due to heavy wear (Plate 1 AC 9).

Similarly, twenty-one linear cranial measurements defined by Janse van Rensburg (2000) as well as WI (measured as the greatest width of the incisor where the incisor meets the premaxillae) were recorded by 1 observer (LH) to the nearest 0.05 mm using a pair of Mitutoyo^{R} digital calipers (Mitutoyo American Corporation, Aurora, Illinois) (Figure 1).



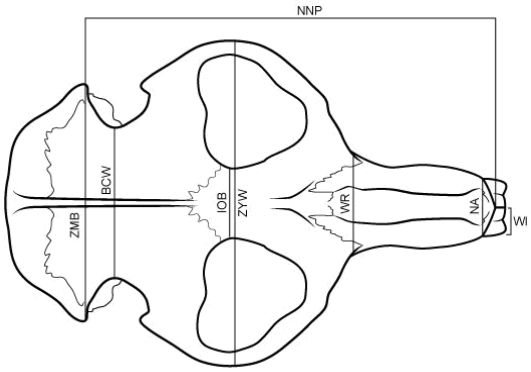


Figure 1. Abbreviations and reference points of skull measurements as defined by Janse van Rensburg 2000: GLS: Greatest length of skull, from the tip of the front incisors to the posterior part of the skull; ITC: Incisor to condyle length, from the anterior surface of the incisor at alveolus to most posterior projection of the occipital condyle; BCW: Widest measurement of brain case breadth; ZMB: Zygomatic breadth, greatest width of skull, taken between zygomatic processes of sqaumosals, in dorsal view; ZYW: Greatest zygomatic width, between outer margins of zygomatic arches, perpendicular to longitudinal axis of skull; IOB: Least breadth of the interorbital constriction; WR: Width of the rostrum; NA: Anterior width of nasal where it joins up with the premaxillae; UTR: Crown length of maxillary tooth row, from the anterior edge of first molar to the posterior edge of the last molar; PAC: Hard palate width at point of constriction immediately posterior to the last molar; NNP: Distance from anterior edge of nasals to anterior edge of posterior part of zygomatic arch; GHS: Greatest height of skull, perpendicular to horizontal plane through bullae; MLT: Greatest length of mandible, including teeth, from posterior surface of condylar process to the tip of the incisor; MDL: Greatest length of mandible (Excluding teeth), from posterior surface of condylar process to anteroventral edge of incisor alveolus;

MTR: Mandibular tooth row length, from anterior edge of the first molar alveolus to posterior edge of the last molar alveolus; AFL: Articular facet length to posterior edge of molar number four; MAF: Mandibular foramen-articular facet length, from ventral edge of mandibular foramen to midposterodorsal edge of articulating facet; AFA: Articular facet to the middle of the angular process; MRH: Mandible-ramus height, from dorsal edge of coronoid process to ventral edge of angular process; UJI: Upper jaw incisor length, measured from the tip of the incisors to the base, where the teeth connect to the skull; LJI: Lower jaw incisor length, measured from the tip of the incisor to the base, where the teeth connect to the skull; WI: Width of the incisor where the incisor meets the premaxillae.

Sexual dimorphism and age variation were first univariately assessed simultaneously using Model I 2-way analysis of variance (ANOVA–Zar 1996) of samples of tooth wear classes 2–9 (there were no individuals of tooth wear class 1), and were undertaken after tests for normality and homogeneity of variances showed that the data satisfied the assumptions of ANOVA (Zar 1996). Where statistically significant age differences were detected, non-significant subsets ($P > 0.05$) were identified by the Student-Newman-Keuls (SNK – Gabriel and Sokal 1969; Sokal and Rohlf 1981) test of ranked means. Estimates of *% SSQ* of four sources of variation (sex, age, sex-age interaction and error (= residual)) were computed directly from the derived ANOVA tables by dividing the *SSQ* associated with each source of variation by the total *SSQ*.

Sexual dimorphism and age variation were also multivariately assessed using the unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis and principal components analysis (PCA) of standardized variables (Sneath

and Sokal 1973). While the former analysis was based on Euclidean distances and correlation coefficients among groups, the latter was based on correlation coefficients among variables (Sneath and Sokal 1973). Additional multivariate analyses included canonical variates analysis (CVA – Sneath and Sokal 1973) of the sexes and/or age classes, followed by MANOVA to test for statistically significant differences between group centroids. All morphometric analyses were based on the 22 cranial measurements, and were undertaken using algorithms in STATISTICA version 5.0 (StatSoft, Inc. 2002).

RESULTS

Univariate analyses: Since individuals of age class 1 were not available for examination in this study, all univariate as well as multivariate results are based on individuals of age classes 2–9. *F*-values from a Model I two-way ANOVA of the sample show that almost all measurements have statistically significant *F*-values for age, sex, and the interaction between the two components of non-geographic variation (Table 1). However, despite 16 out of 22 measurements showing significant sexual dimorphism at either $P < 0.01$ or $P < 0.001$, the largest *F*-values are generally associated with the age component rather than sex and the interaction between age and sex (Table 1), and all except two measurements (BCW and IOB) are highly statistically significant at $P < 0.001$.

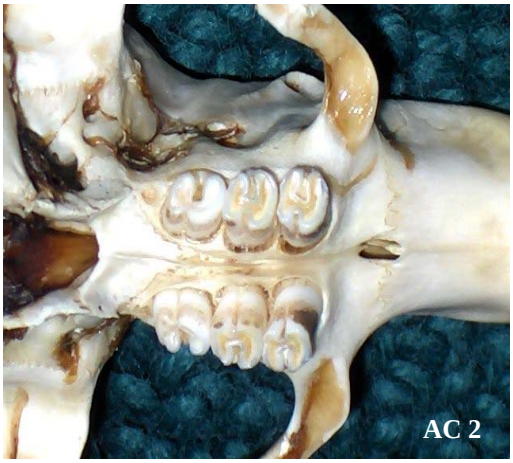
The importance of age variation is also shown by the generally higher *% SSQ* for age (mean *% SSQ* = 43.16%; range = 6.30–63.12%) than for sex (3.05%; 0.27–7.16%) and the interaction (5.45%; 1.06–8.08%) between the two components of variation (Table 1). Although 14 out of 22 measurements show significant interaction

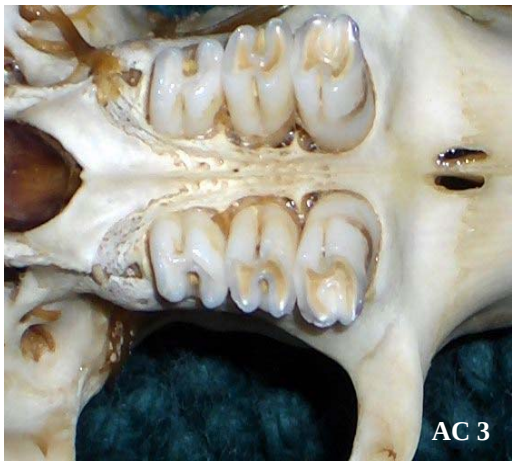
between age and sex dimorphism at either $P < 0.01$ or $P < 0.001$, the mean *% SSQ* associated with the error (= residual) component (45.99%; range = 6.56–91.05) are slightly higher than those for age (43.16%; 6.30–63.12%) (Table 1).

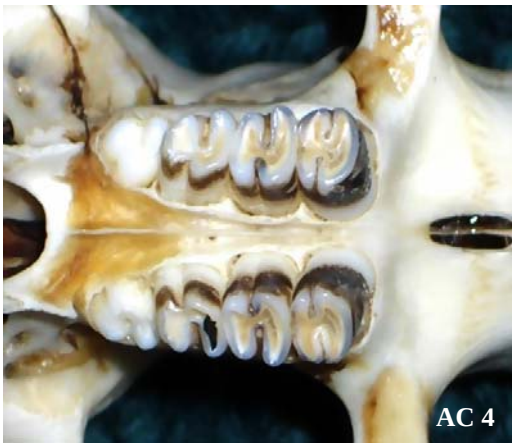
The SNK tests undertaken to identify non-significant subsets where statistically significant age differences were detected revealed three contrasting trends (Appendix I). The first and major pattern in the order of ranked means involved 12 out of 22 measurements analyzed that show an orderly increase in size with increasing age from age classes 2–9. The second pattern involved 8 measurements consistently placed individuals of the younger age classes 2 and 3, and those between the older age classes 4–9 in the same non-significant subsets. The third pattern involving 2 measurements only showed no statistically significant differences between all 8 age classes analyzed. In 12 cases where there was an orderly increase in size with age, individuals of age class 4 were grouped with individuals of the younger age class 3 in 4 measurements. The overall pattern in univariate analyses is also evident in standard descriptive statistics of the sample analyzed (not illustrated but obtainable from Leanne Hart on request) that show a direct relationship between measurement magnitude and age.

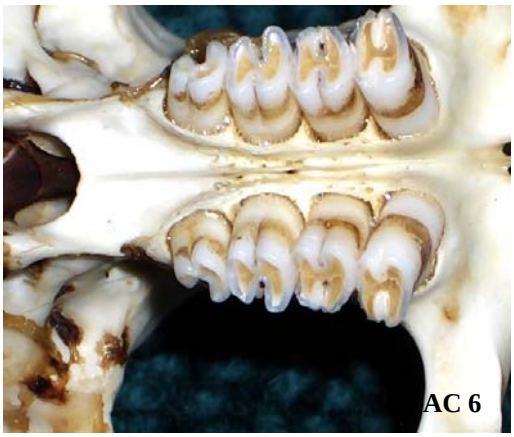
Multivariate analyses: The first two principal components from the analysis of the sample are given in figure 2. Because of the large number of individuals involved in the analysis, the PCA scattergram was first examined with reference to age. Tooth wear class 2 and 3 largely separate from those of tooth wear classes 4–9 on the first size-related rather than the shape-related principal component axes with extensive overlaps between the latter age classes. The scattergram also shows a tendency for individuals of age classes 4 and 5 to plot intermediate between those of age classes 2–3 and those of age classes 6–9 on the first PCA axis.

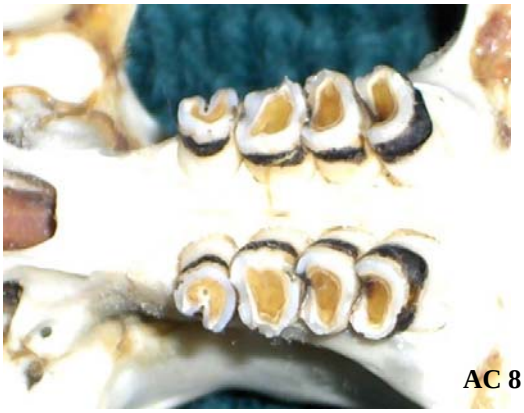
Plate 1.

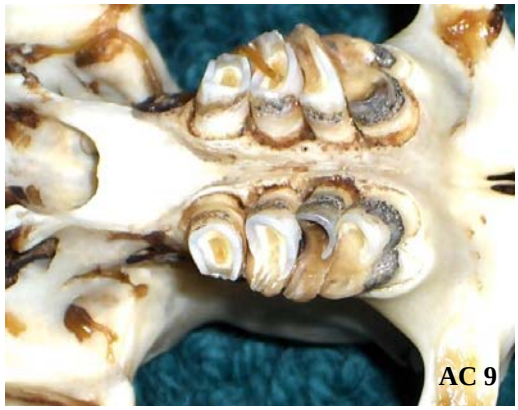












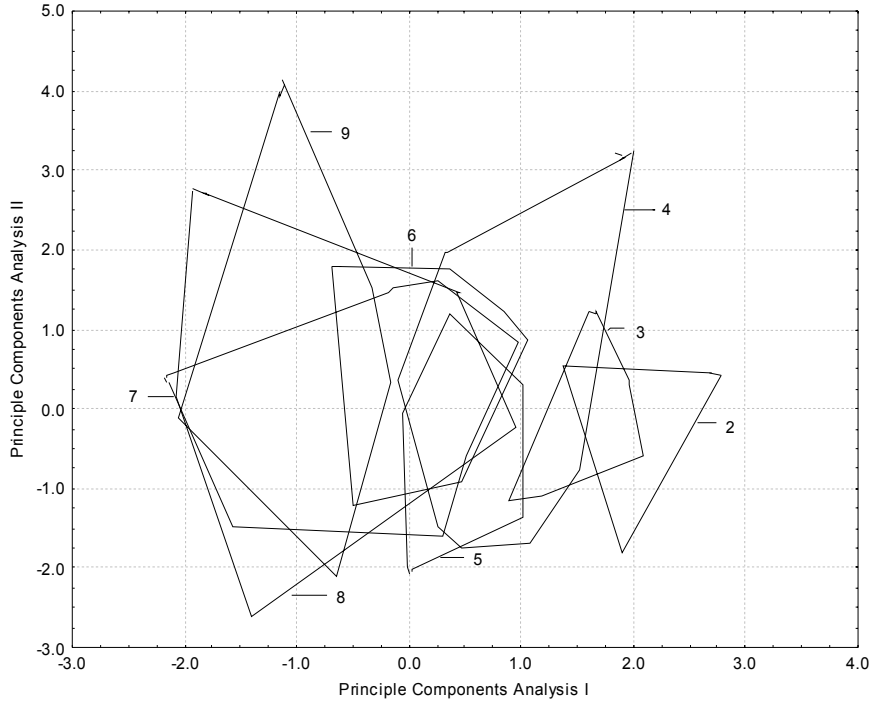


Figure 2. The first two axes from a principal components analysis of males and females of eight tooth wear classes (2–9) of the Cape dune mole-rat, *Bathyergus suillus* from Cape Town International Airport, Cape Town, South Africa. Minimum convex polygons enclose individuals of each tooth wear class. For illustrative clarity, the scatter of individuals in multivariate space and their associated sexes have been omitted.

An examination of the remaining derived PCA axes (3–22) did not reveal any further separation of age classes as well as sexual dimorphism. The first principal component that explains 67.2% of the total variance generally had high and negative loadings on most measurements (Table 2) that also featured either as statistically significantly different in the ANOVA or contributed highly towards the total % SSQ.

Only one measurement (IOB) had a relatively high loading on the second principal component axis that only explains 5.5% of the total variance (Table 2).

Because of the cluttered nature of the PCA scattergram which made the assessment of sexual dimorphism difficult as well as the relatively low level of variation explained by the first two principal components axes (72.7%), the sample was also examined by UPGMA cluster analysis. A distance phenogram (Appendix II) showed three relatively discrete clusters. The first cluster showed a combination of largely male and female individuals from age classes 2–3, with some individuals of age classes 4–5. The second cluster largely consisted of individuals of age classes 6–9, with some individuals of age classes 4–5, and with some minor subclusters within this cluster comprising individuals of the same sex, while the third cluster only included males of age classes 7–9, which are larger than females.

Collation of the results of ANOVA, SNK tests, PCA and the cluster analysis strongly suggest the separation of age classes 2–3 and 6–9, with those of age classes 4–5 being intermediate between the two age class groupings, suggesting a progression of age-related increase in size on the first PCA axis. More importantly, the cluster analysis showed the absence of sexual dimorphism in younger individuals of age classes 2–5 (not illustrated by obtainable from LH on request), but indications of sexual dimorphism in older individuals of age classes 6 – 9, and is best illustrated by a distance phenogram derived from a UPGMA cluster analysis of these age classes only (Figure 3).

Table1. *F*-values and percent sum of squares (*% SSQ*) of each source of variation from a Model I two-way analysis of variance (ANOVA) of eight tooth wear classes (2–9) of male and female Cape dune mole-rats, *Bathyergus suillus* from Cape Town International Airport, Cape Town, South Africa. Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ (For description of measurements see figure 1).

Measurement	*F*-value			*% SSQ*			
	Age (A)	Sex (S)	A x S	Age (A)	Sex (S)	A x S	Error
NNP	58.87***	24.64***	6.65***	62.98	3.76	7.12	26.14
ZMB	34.67***	15.78***	3.85***	53.18	3.46	5.90	37.47
BCW	1.69	2.06	0.42	6.30	1.09	1.55	91.05
IOB	2.14*	3.10	1.74	7.45	1.54	6.05	84.97
ZYB	50.87***	10.89**	5.50***	63.12	1.93	6.83	30.31
WR	43.34***	9.59**	2.80**	60.24	1.90	3.90	33.96
NA	20.95***	7.55**	3.25**	42.14	2.17	6.54	49.14
WI	33.92***	6.85**	3.83***	58.82	1.55	6.06	38.68
PAC	4.49***	2.86	1.63	14.50	1.20	5.28	78.90
UTR	22.14***	9.02**	4.10***	42.61	2.48	7.89	47.02
LJI	23.64***	28.43***	4.58***	41.68	7.16	8.08	43.08
MLT	58.59***	25.38***	6.87***	62.65	3.88	7.35	26.12
MDL	53.97***	22.90***	4.63***	62.54	3.79	5.37	28.31
MTR	9.31***	0.64	0.36	27.22	0.27	1.06	71.46
AFI	6.09***	1.43	1.23	19.07	0.64	3.85	76.45
AFA	32.03***	12.05***	3.87***	51.62	2.78	6.25	39.37
MAP	25.88***	22.35***	2.59*	46.14	5.69	4.62	43.55
MRH	33.71***	14.65***	3.67**	52.75	3.28	5.75	38.22
GLS	44.37***	28.07***	3.57**	58.10	5.25	4.67	31.98
ITC	53.76***	35.56***	4.21***	61.46	5.81	4.81	27.93
UJI	9.87***	3.57	2.44*	26.50	1.37	6.54	6.56
GHS	11.42***	16.88***	1.74	28.54	6.03	4.36	61.07
Mean				43.16	3.05	5.45	45.99

Table 2. Loadings of measurements on the first two components from a principal components analysis of the Cape dune mole-rat, *Bathyergus suillus* from Cape Town International Airport, Cape Town, South Africa. (Measurements defined in figure 1.)

Measurement	Principal Components axes	
	I	II
NPP	-0.97	0.045
ZMB	-0.932	-0.025
BCW	-0.389	-0.451
IOB	-0.182	-0.810
ZYB	-0.965	0.080
WR	-0.915	0.010
NA	-0.876	-0.016
WI	-0.893	0.096
PAC	-0.430	-0.475
UTR	-0.728	-0.120
LJI	-0.878	0.014
MLT	-0.985	0.073
MDL	-0.971	0.065
MTR	-0.542	-0.069
AFI	-0.572	0.164
AFA	-0.927	0.113
MAP	-0.898	-0.008
MRH	-0.915	0.095
GLS	-0.934	0.078
ITC	-0.943	0.046
UJI	-0.730	0.067
GHS	-0.798	-0.146
% trace	Axis I = 67.20%	Axis II = 5.50%

The absence and the potential presence of sexual dimorphism in younger and older individuals, respectively was further examined by independent ANOVA and *% SSQ* of individuals of age classes 2–5 and age classes 6–9. *F*-values from a Model I two-way ANOVA of individuals of age classes 2–5 show 19 of the 22 measurements to have statistically significant *F*-values for age, but none for sex, with only 7 being significant in the interaction between the two components (Table 3). The importance of age variation rather than sexual dimorphism in the younger age classes 2–5 is also shown by the generally higher *% SSQ* for age (mean *% SSQ* = 35.93%; range = 2.94–

58.46%) than for sex (0.68%; 0.00–3.13%) and the interaction (5.37%; 1.07–12.79%) between the two components of variation (Table 3). Similar to the *% SSQ* analysis of age classes 2–9, the analysis of age classes 2–5 echoes the higher mean percent contribution of the error component (58.02) (Table 3). *F*-values from a Model I two-way ANOVA of individuals of age classes 6–9, however, showed 17 of the 22 measurements to have statistically significant *F*-values for age, 19 to be sexually dimorphic with males being larger, and 7 to be significant in the interaction between the two components (Table 4). Unlike the earlier ANOVAs, this analysis also showed that sex had significantly higher *F*-values for sex than for age (Table 4).

The importance of sexual dimorphism rather than age variation in the older age classes 6–9 is also shown by the generally higher *% SSQ* for sex (mean *% SSQ* = 20.24%; range = 0.35–37.85%) than for age (9.47%; 0.68–18.13%) and the interaction (3.23%; 0.38–7.74%) between the two components of variation (Table 3).

Similar to the *% SSQ* analysis of age classes 2–9, and of age classes 2–5, the analysis of age classes 6–9 also showed higher mean percent contribution by the error component (67.05) (Table 3). The results obtained from these univariate as well earlier multivariate analyses of tooth wear classes 2–9 are all broadly similar to those obtained from combined CVA and MANOVA of males, females and tooth wear classes, and independent PCAs of age classes 2–5 and age classes 6–9 (not illustrated but obtainable from LH on request).

The latter series of analyses, whose scattergrams were less cluttered than those of age classes 2–9 also showed indications of the lack of sexual dimorphism in the younger tooth wear classes 2–5 and its presence in the older tooth wear classes 6–9.

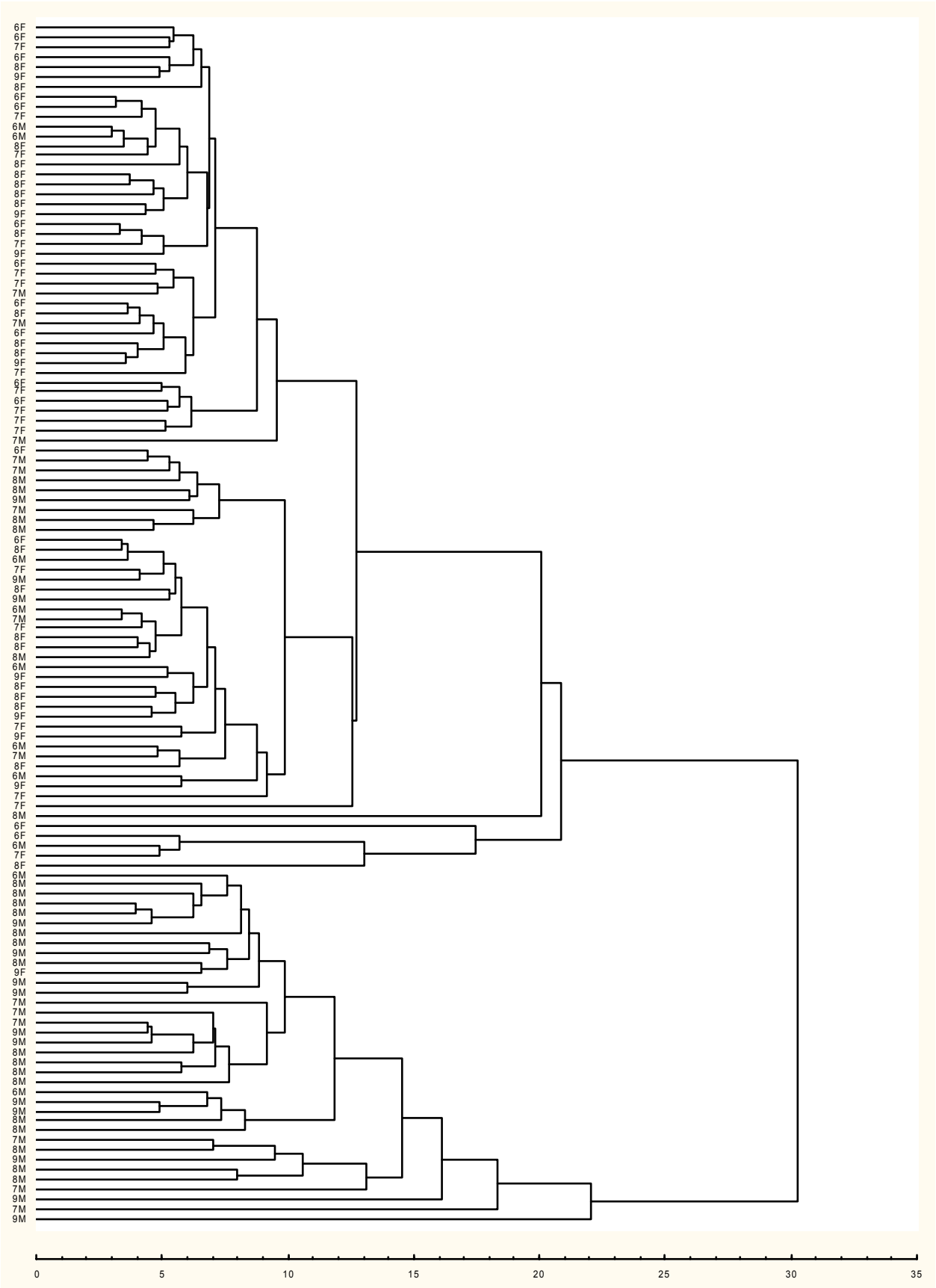


Figure 3. A distance phenogram from an unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis of male and female of four older tooth

wear classes (6–9) of the Cape dune mole-rat, *Bathyergus suillus* from Cape Town International Airport, Cape Town, South Africa. The sexes (M = males; F = females) and tooth wear classes (6–9) of all individuals analysed are indicated.

Table 3. *F*-values and percent sum of squares (*% SSQ*) of each source of variation from a Model I two-way analysis of variance (ANOVA) of four younger tooth wear classes (2–5) of male and female Cape dune mole-rats, *Bathyergus suillus* from Cape Town International Airport, Cape Town, South Africa. Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. (Measurements are defined in figure 1.)

Measurement	*F*-value			*% SSQ*			
	Age (A)	Sex (S)	A x S	Age (A)	Sex (S)	A x S	Error
NNP	34.11***	0.04	5.38**	58.31	0.02	9.19	32.48
ZMB	29.38***	0.18	1.82	58.46	0.12	3.62	37.80
BCW	1.00	0.33	0.33	4.90	0.54	1.63	92.93
IOB	0.67	0.98	2.93*	2.94	1.42	12.79	82.85
ZYB	20.71***	0.68	1.27	50.24	0.56	3.09	46.10
WR	17.13***	0.12	1.02	46.06	0.11	2.75	51.08
NA	8.61***	0.02	0.21	30.95	0.02	0.75	68.29
WI	11.90***	0.60	1.94	35.77	0.59	5.83	57.81
PAC	43.41***	0.12	1.44	17.49	0.16	5.79	76.55
UTR	32.23***	0.33	5.23**	56.98	0.19	9.24	33.59
LJI	11.84***	3.21	22.25	34.65	3.13	6.59	55.63
MLT	32.46***	0.05	1.48	61.31	0.00	2.79	35.89
MDL	30.97***	0.17	0.40	7.87	0.27	1.90	89.96
MTR	29.65***	0.14	1.56	59.00	0.09	3.10	37.81
AFI	2.13	0.01	0.23	9.98	0.01	1.07	88.93
AFA	18.67***	1.07	1.23	47.55	0.91	3.14	48.40
MAP	19.27***	1.29	3.67*	45.47	1.02	8.67	44.84
MRH	33.50***	0.17	2.17	13.71	0.22	8.82	77.25
GLS	22.69***	2.00	3.66*	49.31	1.45	7.96	41.29
ITC	20.85***	2.13	3.12*	47.61	1.62	7.39	43.38
UJI	10.60***	0.33	3.04*	32.38	0.34	9.27	58.01
GHS	4.90**	1.61	0.70	19.51	2.13	2.80	75.56
Mean				35.93	0.68	5.37	58.02

Table 4. *F*-values and percent sum of squares (*% SSQ*) of each source of variation from a Model I two-way analysis of variance (ANOVA) of four older tooth wear classes (6–9) of male and female Cape dune mole-rats, *Bathyergus suillus* from Cape Town International Airport, Cape Town, South Africa. Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. (Measurements are defined in figure 1.)

Measurement	*F*-value			*% SSQ*			
	Age (A)	Sex (S)	A x S	Age (A)	Sex (S)	A x S	Error
NNP	9.14***	91.66***	3.03*	11.33	37.85	3.75	47.07
ZMB	4.67**	60.58***	1.08	7.30	31.58	1.68	59.44
BCW	0.27	3.37	0.62	0.68	2.81	1.54	94.97
IOB	4.09**	3.12	0.37	9.43	2.40	0.86	87.32
ZYB	9.34***	54.10***	3.99**	18.13	0.35	7.74	73.79
WR	9.42***	42.59***	1.52	14.98	22.59	1.98	60.45
NA	5.10**	29.99***	3.6	9.00	17.63	6.36	67.01
WI	5.76**	35.28***	2.41	9.94	20.30	4.17	65.59
PAC	2.18	6.79*	2.22	4.89	5.06	4.97	85.08
UTR	1.49	42.14***	1.95	2.70	25.31	3.52	68.47
LJI	8.30***	53.82***	4.03**	12.15	26.28	5.91	55.66
MLT	11.35***	86.75***	3.61*	13.86	35.32	4.41	46.41
MDL	9.94***	69.41***	2.80*	13.45	31.32	3.79	51.44
MTR	1.80	2.61	0.15	4.40	2.13	0.38	93.09
AFI	1.30	6.78*	1.46	3.01	5.26	3.40	88.32
AFA	6.72***	53.25***	0.36	10.69	28.25	0.57	60.49
MAP	7.34***	49.25***	0.77	11.74	26.26	1.23	60.77
MRH	5.68**	48.30***	1.27	9.30	26.37	2.08	62.25
GLS	7.28***	63.87***	1.50	10.70	31.27	2.20	55.82
ITC	11.27***	93.57***	2.69*	13.56	37.51	3.23	45.70
UJI	3.25*	13.77***	1.84	6.81	9.63	3.85	79.71
GHS	5.96***	34.09***	2.02	10.39	19.82	3.52	66.27
Mean				9.47	20.24	3.23	67.05

DISCUSSION

Both univariate (ANOVA; *% SSQ*) and multivariate (UPGMA cluster analysis; PCA; CVA) analyses in this study clearly showed statistically significant age variation, but no statistically significant sexual dimorphism among individuals of age classes 2 – 5. In these age classes sex only contributed a total of 0.66% to the total

variance observed, whereas the contribution of age towards the total variance amounted to nearly 36%. The analysis of individuals of age classes 6 – 9, however, showed statistically significant differences in both age classes and the sexes. In this grouping age contributed only 9.47% of the total variance whereas sex contributed a total of 20.24%. These results strongly suggest the presence of sexual dimorphism in adult individuals rather than in juvenile and sub-adult individuals. Our results are similar to those of a study by Hon–Tsen Yu and Yao–Sung Lin (1999) who found body mass to be sexually dimorphic in older rather than juvenile and sub-adult individuals of the spiny rat (*Niviventer coxingi*). The yellow-pine chipmunk, *Tamias amoenus*, also displays sexual dimorphism, where the females of this species are significantly larger than the males (Schulte- Hostedde and Millar 2000). A more recent study by Schulte- Hostedde et al. (2001) investigated the presence of sexual dimorphism with respect to body size and composition in deer mice, *Peromyscus maniculatus* Wagner, bushy-tailed wood rats, *Neotoma cinerea* Ord, and red-backed voles, *Clethrionomys gapperi* Vigors. For all these species it was found that males had more lean dry mass relative to body size when compared to the females.

The first principle components axis and the distance phenogram suggest that the presence of sexual dimorphism and age differences observed, with the males being larger than the females, are largely due to overall size rather than shape. Since male–male interactions are expected to increase during the reproductively active phases of the life cycle, when Cape dune males compete for reproductive opportunities (Bennett and Faulkes, 2000), it may be advantageous for a male to invest more resources into its growth and the maintenance of its body condition. An increase in body morphometrics, as seen for males of age classes 6 to 9 when compared to other individuals may thus be advantageous with respect to mating opportunities. Dissection

of male mole-rats showed extensive fatty tissue deposits around the neck region (Leanne Hart pers. obs). It is possible that the fatty tissue deposits may cushion damage from the extra-buccal teeth in male–male aggression during the reproductive phase of the male's life cycle.

Statistical analyses (univariate and multivariate) suggests that there is an increase in size from tooth wear class 2 to 4 and multivariate analyses suggest that this increase may even include tooth wear class 5. Individuals of tooth wear classes 4 and 5 may thus be at the stabilizing point of a hypothetical growth curve.

The use of tooth eruption and wear to age animals have been shown to be unreliable indicators of age in some mammals such as bats, the elk and the white–tailed deer (Hall et al. 1957; Keiss 1969; Morris 1972; Gilbert and Stolt 1970). However, the assessment of single populations as well as relative rather than absolute ages such as in the present investigation may be appropriate. Similarly, body size has also been shown to be a poor indicator of age in other mammals (Cameron-Smith 1965; Chaplin and White 1969).

Even though analyses showed that there are indeed age differences between all 8 age classes and sexual dimorphism, albeit only from age class 6 to 9 large error components due to the contribution of age and sex was shown by the *% SSQ* analyses this fact suggests that there may be other more important mediating factors leading to the observed non-geographical variation displayed by the Cape dune mole-rat. We propose a further investigation to partition these potentially influential population factors.

While the results found in this study based on a single population may be valid, there is a need to assess additional populations before generalizations can be made for the Cape dune mole-rat. Once these generalizations are concluded, these

together with future molecular studies (e.g. using microsatellites) may be instrumental in providing additional tools to improve our current understanding of social structuring within colonies of the Cape dune mole-rat.

ACKNOWLEDGEMENTS

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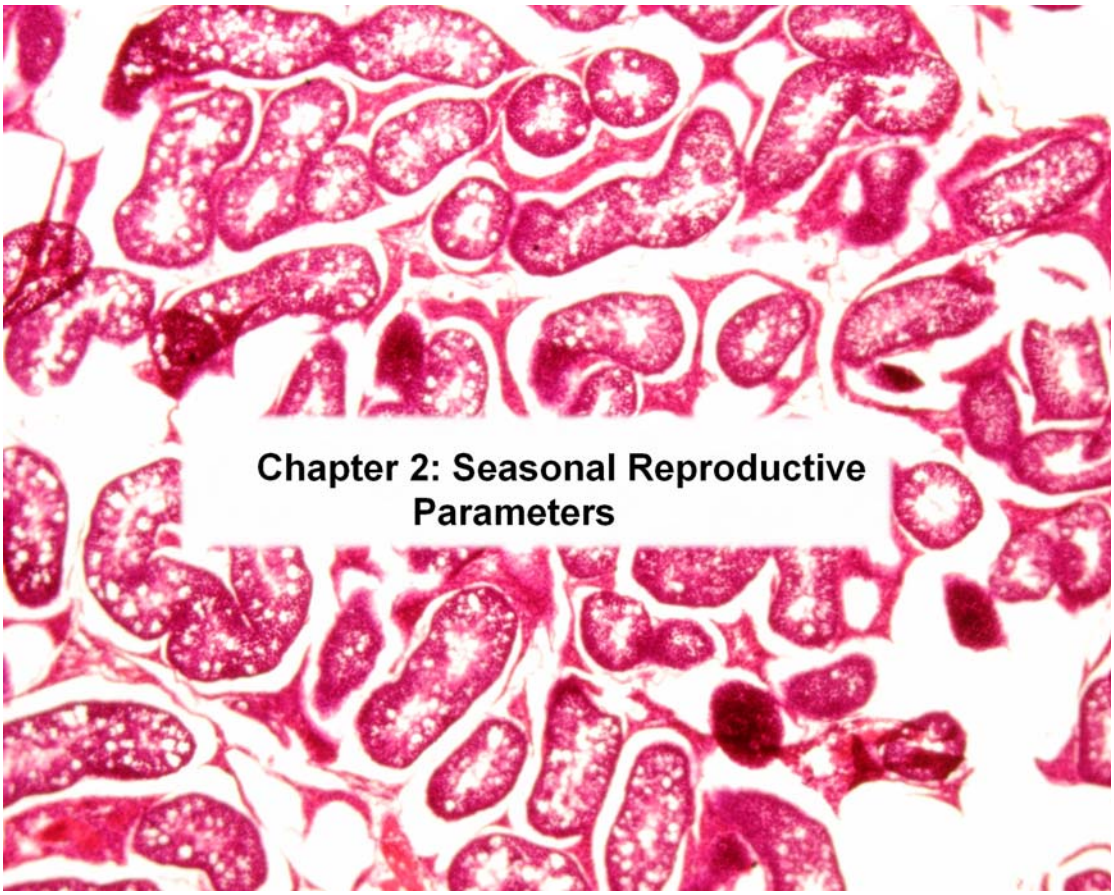

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IS THE CAPE DUNE MOLE-RAT *BATHYERGUS SUILLUS* (RODENTIA: BATHYERGIDAE) A SEASONAL OR ASEASONAL BREEDER?

The Cape dune mole-rat, *Bathyergus suillus* is a solitary subterranean rodent. To date no study has succeeded in obtaining an adequate sample size to fully document seasonal changes in the reproductive biology of this species. In this study we present data on 1315 free-living animals that were sampled at different times of the year within one study area. We captured 581 males and recorded their body mass in addition to performing morphometrics on their gonads. A subset of 253 males was used to examine testicular histology and plasma testosterone concentrations following the onset of the breeding season (April to May). We noted a gradual increase in testicular mass in addition to an increase in the diameter of the seminiferous tubules in males as the breeding season approached (April to May), but thereafter (October) these testicular parameters diminished in size. Mean testosterone concentration exhibited a concomitant increase in May, peaking between June and August.

Body mass and gonadal morphometrics were obtained for 734 females. A subset of 250 females were used to study ovarian histology, plasma oestrogen and progesterone concentrations. Pregnant females were only captured during the period June to February with the highest percentage of pregnancies (65%) recorded in September. Qualitative analyses of ovarian histology revealed that females retain the potential for ovulation and subsequent production of corpora lutea during early winter and into spring (June to October). Seasonal differences were found in ovarian morphometrics and hormone concentrations that are associated with enhanced follicular development. The percentage of lactating females was highest around October. In contrast, mean progesterone concentrations of females were elevated

throughout the year showing three distinct peaks in March, June and September, which would encompass many of the periods of pregnancy. The current data support a strong seasonality in the reproduction of the Cape dune mole-rat with a reproductive peak that is linked to the period of maximal rainfall within the distributional range of this species.

Key words: Bathyergidae, Cape dune mole-rat, Hormone concentration, mole-rat, Seasonal reproduction.

INTRODUCTION

Seasonality of reproduction is not limited to either solitary or social species and may be variable within families (Busch et al. 2000; Hansen 1960; Vaughan 1962; Verts and Carerway 1991; Rado et al. 1992). The onset of reproduction in seasonally breeding rodents has long been attributed to changes in photoperiod, however, for mammals spending the majority of their life underground other environmental cues such as thermoperiod, changes in soil moisture content or sudden flushes of vegetation associated with good precipitation may be far more important in triggering reproduction.

The majority of subterranean rodents are strictly solitary and xenophobic, aggressively defending their burrow system from foreigners (Nevo 1979). Courtship and mating is usually a brief affair that together with nursing (by the female) necessitates a temporary reprieve from the marked xenophobia towards conspecifics (Bennett and Jarvis 1988a; Herbst et al. 2004).

The Bathyergidae is one of a few families of rodents that have both solitary and social representatives (Jarvis and Bennett 1990; Bennett and Faulkes 2000). Indeed, African mole-rats are unusual in that they display a broad spectrum of social organization ranging from strictly solitary through to eusocial representatives (Jarvis et al. 1994; Bennett et al. 1999).

Studies on the reproductive life history patterns of the social mole-rats have dominated the last decade of research on the Bathyergidae (Bennett et al. 1996, 1997, 1999; Janse van Rensburg et al. 2002, 2003; Spinks et al. 1997, 1999). Most social African mole-rat species exhibit a pattern of aseasonal reproduction (Bennett and Jarvis 1988a; Bennett et al. 1994; Bennett and Aguilar 1995; Jarvis 1981). To date, only two exceptions are apparent, viz. *Cryptomys hottentotus hottentotus* and *Cryptomys hottentotus pretoriae* (Spinks et al. 1997, 1999; Janse van Rensburg et al. 2002). By contrast our knowledge of the reproductive biology of solitary species is most comprehensive for only two species, viz. the Cape mole-rat, *Georychus capensis* (Bennett and Jarvis 1988b) and the Namaqua dune mole-rat, *Bathyergus janetta* (Herbst et al. 2004). That for the Cape Dune mole-rat lacks information on hormonal profiles and ovarian histology. There is some evidence that the male Cape dune mole-rat may be seasonal breeders but the evidence is equivocal (Jarvis 1969, van der Horst 1972). All attempts to breed these strictly solitary Cape Dune mole-rats in the laboratory have been unsuccessful. Consequently there is a great paucity of information on a number of parameters of their reproductive biology (Bennett and Jarvis 1988a; Bennett and Faulkes 2000; Bennett et al. 2000; Sumbera et al. 2003).

In this paper parameters such as the duration and frequency of pregnancy, physical dimensions of reproductive organs, and patterns of follicular development

have been augmented with endocrine profiles to provide insights into circannual reproductive events.

These data have been obtained from a large sample of wild captured male and female *B. suillus* and are used to examine: (1) whether the Cape dune mole-rat is indeed a seasonal breeder, (2) what the duration of the breeding period is, and (3) possible cues for the onset of reproduction.

MATERIALS AND METHODS

Acquisition of animals: All gonadal tissues and plasma used in this study were obtained from mole-rats captured at the Cape Town International airport (33°58'S 18°37'E) as part of an eradication program conducted in conjunction with the University of Cape Town. A total of 581 males and 734 females were captured during the period of January 2003 to January 2004. Animals were captured on a daily basis with the use of modified vice traps. 3mm thick rubber strips were wrapped around both serrated edges of the trap and secured in place with cable ties. While this greatly reduced the efficacy of the traps and necessitated frequent checking it had the desirable effect of limiting damage to the animals' limb upon capture. Traps were checked every 20 minutes and captured animals were immediately removed from their burrow, placed in a 20 litre plastic bucket and euthanased with an overdose of chloroform as an anaesthetic. The animals were weighed and sexed, following which the abdominal cavity was opened along the ventral midline. Blood was taken directly from the ventricles of the heart using heparinized syringes. The blood was immediately centrifuged and the plasma fraction initially frozen at -20 °C and stored at -70 °C until radioimmunoassay for the respective steroid hormones. The gonads

were removed and preserved in 10% buffered formalin. All females were checked for pregnancy (presence of uterine embryos) and lactation (prominent nipples surrounded by a small circle of matted fur).

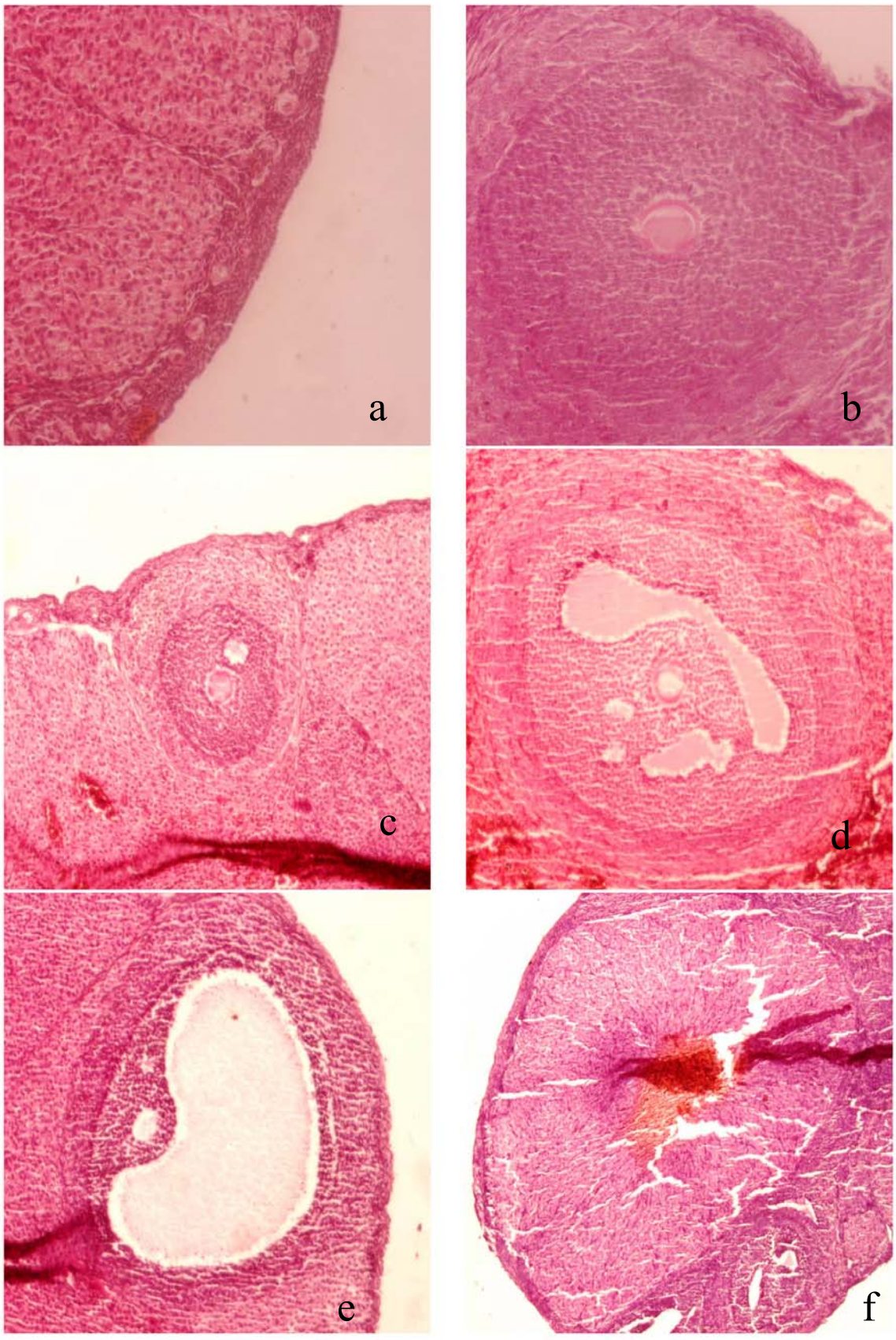
Gonads: The gonads from 471 males and 623 females were collected. Excess fatty tissue was removed from the gonads after which the maximum length and width were recorded using a Vernier calliper (Mitutoyo American Corporation, Aurora, Illinois). Gonad mass was weighed to the nearest 0.1g on a Sartorius 1213MP (Zeiss, Germany) scale. The measurements obtained from the paired gonads were averaged. From these data it was possible to determine the average gonadal volume for each individual using the equation for the volume of an ellipsoid: $V = 4/3\ (\pi ab)^2$ where a = ½ max length and b = ½ max width (Woodall and Skinner 1989).

A sub-sample of 253 males and 250 females was used for the endocrine and histological study. The testes, ovaries and corresponding plasma samples for each individual were used. Standard histological procedures were applied to all gonads. Briefly, gonads were first dehydrated through a series of alcohol baths and then imbedded in a paraffin wax cube. The gonads were sectioned on a microtome at a thickness of 7µm and sections mounted on microscope slides. Sections were not mounted serially and sub-samples for each individual were taken. For males sections were only taken from the middle part of each testis. A minimum of 60 sections per testis per male were examined. For females a total of 120 sections where examined for each ovary. Sections were stained with haematoxylin and counter stained with eosin. For a detailed description of this staining process see Drury and Wallington (1967). After staining, the sections were examined under a light microscope at magnifications of x40 and x80. In males the diameters of the seminiferous tubules were determined for 66 tubules from each testis with the use of an eyepiece micrometer.

Ovarian sections were examined for the presence and number of different follicles and corpora lutea. Follicle and corpora lutea counts were averaged for both ovaries from the same individual to give us the relative number of follicles per section of ovarian tissue. The condition of the ovarian tissue after the staining process was not always optimal and because some of the sections were damaged the identification of certain structures proved to be difficult. For this reason we excluded the Corpora heamorachicum and Corpora albicans follicles from the analyses. The identification and classification of follicles were done according to that ascribed by Bloom and Fawcett (1962) and Bennett et al. (1994) (Plate 1):

- Primordial follicles: These follicles were numerous and found at the perimeter of each section (Plate 1a).
- Primary follicles: These follicles were significantly larger than the primordial follicles and characterised by the presence of a large nucleus (Plate 1b).
- Secondary follicles: Secondary follicles resembled primary follicles with the only difference being the presence of a small fluid filled antrum (Plate 1c).
- Tertiary follicles: The tertiary follicles had a much larger fluid filled antrum than the secondary follicles (Plate 1d).
- Graafian follicles: In these follicles the fluid filled antrum has increased throughout the whole section of the follicle with the nucleus anchored to the one side of the follicle (Plate 1e).
- Corpora lutea: After the release of the egg cell the fluid filled antrum is rapidly filled with cells and forms a corpora luteum (Plate 1f).
- Atretic: These follicles can occur during any stage of the development of a follicle and are characterised by the presence of degraded cells, which had ceased to develop into the next follicle stage also diagnostic is the presence of the crumpled zona pellucida

Plate 1.



Hormonal overview: Plasma from 253 males and 250 females was used to measure circulating concentrations of testosterone and progesterone respectively. A total of 225 of the 250 plasma samples obtained from the females were also used to determine the oestradiol 17β concentrations. Hormonal profiles for each of the sex steroids were constructed on a monthly basis for the samples collected over the calendar year.

Progesterone radioimmunoassay: Progesterone assays were performed as described by Bennett et al. (1994) using a coat-a-count Progesterone kit (Diagnostic Products Corporation, Los Angeles, USA). The antiserum is highly specific for progesterone with cross-reactivity to all naturally occurring steroids <0.5%, with the exception of 17α hydroxyprogesterone (3.4%), 11-deoxycorticosterone (2.4%), 5β-pregnan-3, 20 –dione (3.2%) and 5α-pregnan-3, 20-dione (9%). Standard curve concentrations used to perform the progesterone radio immunoassay ranged from 0.3 to 127.2 nmols/l. The assay was validated for *B. suillus* plasma by testing the slope of the curve produced using serial dilutions of un-extracted mole-rat plasma obtained from a pregnant female (over the range 1:1 – 1:32) against the standard curve. Following logit-log transformation of the data (Chard, 1987) the slopes of the lines were compared using the Statistica package (STATSOFT 2002) and found not to differ significantly from the reference preparation (ANCOVA, $F_{1,7} = 0.005$, $p > 0.05$). The minimum detection limit of the assay was 0.36nmols/l. Intra- and inter assay coefficients of variation for repeated determinations of a quality control were 4.4 % and 7% respectively.

***Oestradiol-17β determination*:** Oestradiol 17β assays were performed as described by Herbst et al. (2004) using a coat-a-count Oestradiol 17β kit (Diagnostic Products Corporation, Los Angeles, USA). Cross-reactivity of the antibody to all naturally occurring steroids was 10% with oestrone, <5% with oestriol, oestrone-β-D-glucoronide, oestrone-3-sulphate, d-equilenin, 17β-oestradiol-3-monosulphate, testosterone and androsterone. The assay was validated for *B. suillus* plasma by testing the slope of the curve produced using serial dilutions of un-extracted mole-rat plasma obtained from a female with high oestradiol concentrations (over the range 1:1 – 1:32) against the standard curve. Following logit-log transformation of the data (Chard 1987) the slopes of the lines were compared using the Statistica package (STATSOFT 2002) and found not to differ significantly from the reference preparation (ANCOVA, $F_{1,6} = 0.087$, $p > 0.05$). The sensitivity of the assay was 10pg/ml. Intra- and inter assay coefficients of variation for repeated determinations of a quality control were 5 and 11% respectively.

***Testosterone determination*:** Testosterone samples were assayed using Coat-a-Count Testosterone kits (Diagnostic Products Corporation, Los Angeles, USA). Cross reactivity of the antibody was 16% with 1-ketotestosterone, <5% with dihydrotestosterone and 19-hydroxyandrostendione and 1% with aldosterone, androstendione, cortisol, corticosterone, oestrone, methyltestosterone and progesterone. The assay was validated for *B. suillus* plasma by testing the slope of the curve produced using serial dilutions of un-extracted mole-rat plasma obtained from a male with high testosterone concentrations (over the range 1:1 – 1:32) against the standard curve. Following logit-log transformation of the data (Chard 1987) the slopes of the lines were compared using the Statistica package (STATSOFT 2002)

and found not to differ significantly from the reference preparation (ANCOVA, $F_{1,5}$ = 2.96, $p > 0.05$). The sensitivity of the assay was 20ng/l. Intra- and inter assay coefficients of variation for repeated determinations of a quality control were 8 and 12% respectively.

The research was cleared by the Animal Ethics Committee, University of Pretoria (AUCC 040702-015). All procedures followed American Society of Mammalogists guidelines as set out in the Journal of Mammalogy http://www.mammalogy.org/committees/index.asp; *Journal of Mammalogy* 79:1416-1431).

Statistical analysis*:* An ANCOVA was used to determine the degree of parallelism between regression lines when the radioimmunoassays were validated. Preliminary analysis of the data showed that there was a clearly defined reproductive period during the year (from June up to and including October). The reproductive parameters focussed on in this study were thus grouped into 'breeding season' and 'out of breeding season' groups and an unrelated t-test or Mann Whitney U-test was performed (depending on whether the data were normally distributed or not). All analyses were performed on the Statistica Ver 6.0 computer program.

RESULTS

Pregnancy and lactation*:* A general trend was observed in the percentage of females captured that were pregnant and/or lactating (Figure 1). No pregnant or lactating females were captured from March through to May. From June through to

September there was a steep increase in the percentage of pregnant females captured with more than 60% of the females captured in September being pregnant.

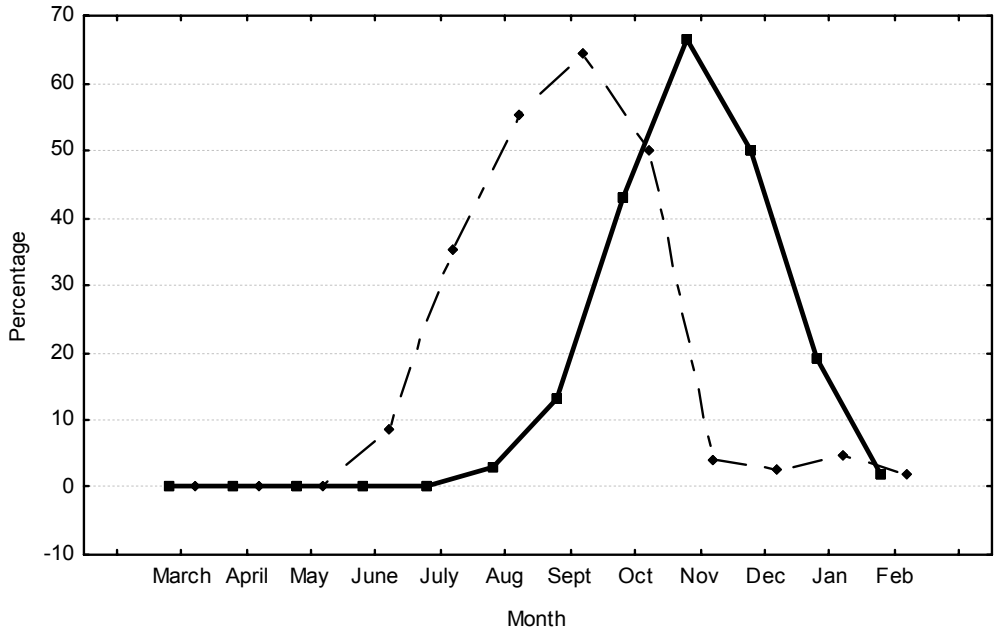


Figure 1. The percentage of Cape dune mole-rat females captured that were pregnant and/or lactating from January 2003 to January 2004. Dashed lines denote pregnant females and solid lines lactating females.

In October approximately 50% of all females sampled were pregnant but this declined sharply during the months of November through to January. Lactating females were first captured during August and lactation was evident through to February. The percentage of lactating females captured increased steadily from August to a peak in November where about 65% of all females sampled were lactating. The percentage of females lactating first decreased in December (when only 50% were lactating) and dropped markedly in January and February.

***Ovarian histology*:** A distinct succession was evident in the proliferation from one follicular stage to its next form (Table 1). The number of primordial follicles per section increased from January through to March (19.60 ± 6.10). This decreased to 3.16 ± 0.99 in December. Primary follicles, although occurring in much lower numbers than the primordial follicles, showed much the same trend except for another sharp increase in the numbers during July.

Table 1. Succession of follicles from primordial to Graafian for Cape dune mole-rat females captured during January 2003 and January 2004. All numbers are given as mean number of follicles ± standard error per section of ovarian tissue

	n	Primordial	Primary	Secondary	Tertiary	Graafian
January	9	3.90 ± 1.35	0.55 ± 0.19	0.06 ± 0.03	0.09 ± 0.07	0.18 ± 0.07
February	26	15.07 ± 2.90	0.97 ± 0.14	0.02 ± 0.01	0.10 ± 0.02	0.17 ± 0.04
March	22	19.60 ± 6.10	1.22 ± 0.13	0.06 ± 0.02	0.22 ± 0.05	0.23 ± 0.04
April	24	17.26 ± 3.17	1.16 ± 0.16	0.09 ± 0.03	0.15 ± 0.03	0.28 ± 0.06
May	37	15.53 ± 1.92	0.91 ± 0.10	0.10 ± 0.02	0.18 ± 0.04	0.36 ± 0.05
June	43	9.65 ± 1.20	0.76 ± 0.07	0.12 ± 0.02	0.16 ± 0.02	0.27 ± 0.04
July	5	10.27 ± 2.29	1.14 ± 0.23	0.19 ± 0.07	0.19 ± 0.08	0.39 ± 0.14
August	17	5.92 ± 0.66	0.83 ± 0.12	0.13 ± 0.03	0.22 ± 0.05	0.28 ± 0.05
September	16	2.63 ± 0.35	0.44 ± 0.06	0.05 ± 0.01	0.08 ± 0.02	0.23 ± 0.05
October	7	3.03 ± 0.64	0.49 ± 0.10	0.06 ± 0.03	0.07 ± 0.03	0.21 ± 0.05
November	16	3.44 ± 0.89	0.44 ± 0.06	0.06 ± 0.02	0.11 ± 0.03	0.26 ± 0.06
December	16	3.16 ± 0.99	0.32 ± 0.08	0.03 ± 0.01	0.04 ± 0.03	0.07 ± 0.02

Secondary follicles increased in number from January (0.06 ± 0.03) to a peak in July (0.19 ± 0.07) and then again gradually decreased to a mean of 0.03 ± 0.01 in December. The mean number of tertiary follicles doubled from February to March

and then maintained a stable value through to August from where it then again decreased to 0.22 ± 0.05 follicles per section in September.

Although the trend in the numbers of Graafian follicles was not as robust as that previously seen for some of the aforementioned follicles there was a doubling in the number of Graafian follicles from January (0.18 ± 0.07) to May (0.36 ± 0.05). This was subsequently followed by a decrease in June and then another increase in July. There was no significant difference (Mann-Whitney *U* test, $U = 6092$; $p > 0.05$) in the number of Graafian follicles produced per section between the breeding (0.27 ± 0.03; $n = 88$) and non-breeding (=0.24 ± 0.02; $n = 150$) periods. The slight reduction in number in July may be due to ovulation and the subsequent development into a corpora lutea of pregnancy.

Corpora lutea were present throughout most of the year with three distinct peaks observed during the months of April, July and October (Figure 2). There was a significant increase (Mann-Whitney *U* test, $U = 5580.50$; $p < 0.01$) in the number of corpora lutea in the breeding season (0.04 ± 0.01; $n = 88$) compared to out of the breeding season (0.02 ± 0.01; $n = 150$). Finally the number of Atretic follicles increased dramatically from January to March, this was then followed by a decrease from April to December (Figure 3).

Ovarian Mass and Volume: The corrected mass of the ovaries of the Cape dune mole-rat increased from January (0.40 ± 0.06mg/g) to a peak value in May (0.59 ± 0.1mg/g) (Figure 4). A gradual decrease then followed with the lowest value obtained in October (0.25 ± 0.07mg/g). From October to December the mass of the ovaries again increased to 0.37 ± 0.05mg/g body mass. Ovarian volume was fairly stable throughout the year ranging from 198.8 ± 19.6mm^3 to 250.8 ± 17.4mm^3, except

for the months of April to June where the volume of the ovaries was greatly reduced (range 141.1 ± 16.00mm^3 to 154.2 ± 13.20mm^3) (Figure 5).

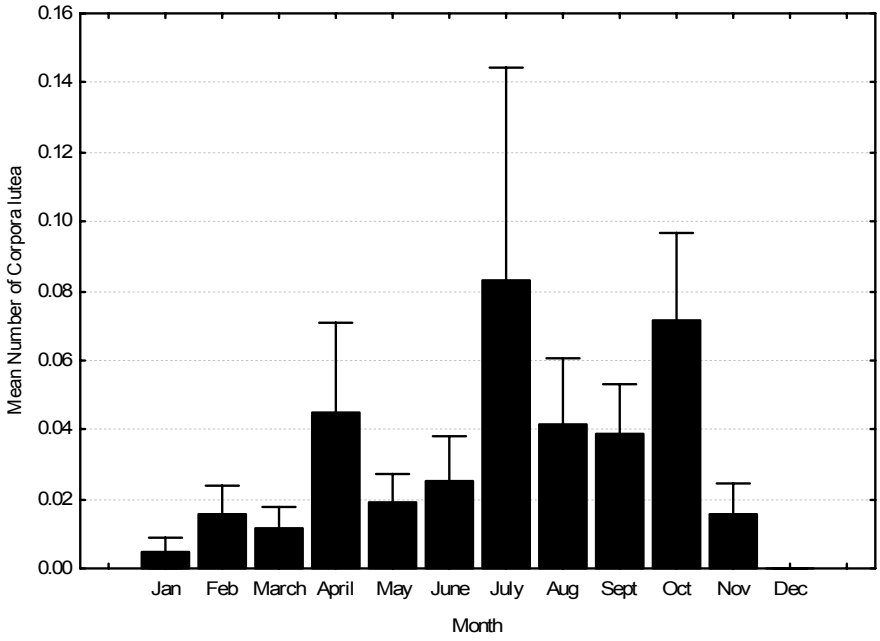


Figure 2. Number of corpora lutea (Mean ± SE) per section of ovarian tissue for Cape dune mole-rat individuals captured during January 2003 and January 2004.

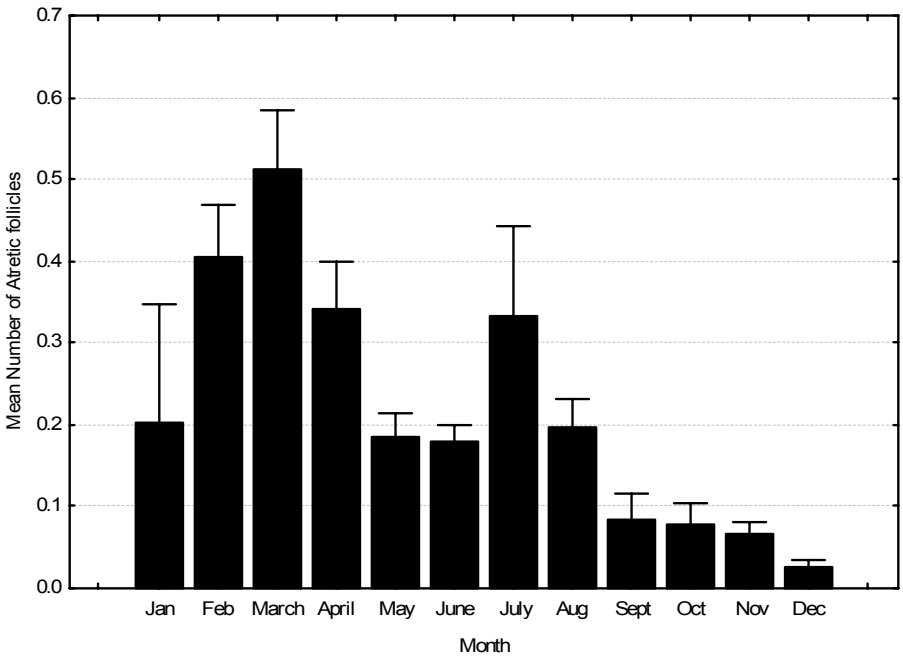


Figure 3. Number of atretic follicles (Mean ± SE) per section of ovarian tissue for Cape dune mole-rat individuals captured during January 2003 and January 2004.

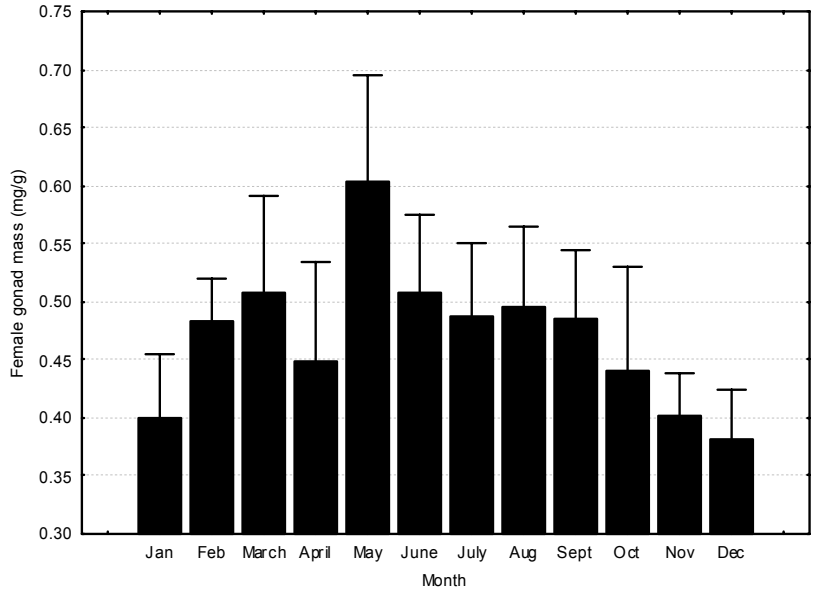


Figure 4. Corrected mass of the ovaries of the Cape dune mole-rat during the period of January 2003 to January 2004. Corrected mass is given in mg/g body mass

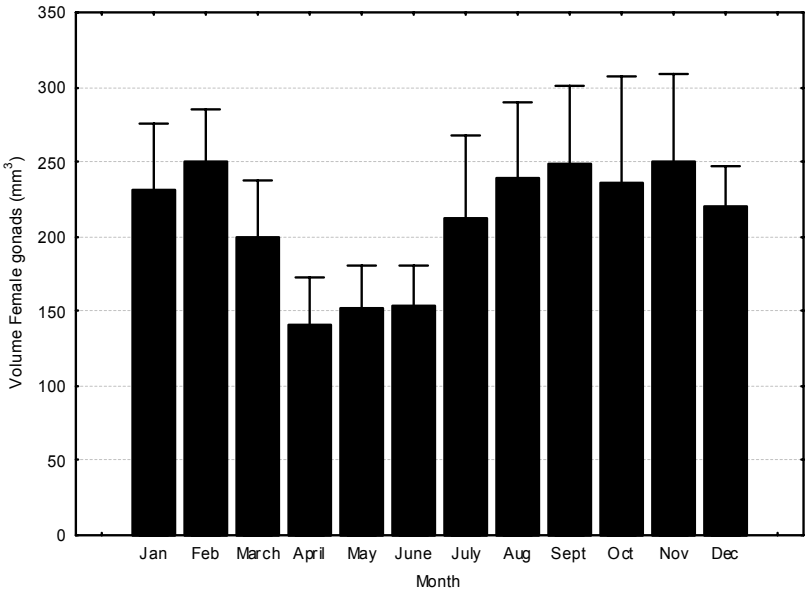


Figure 5. Fluctuation in the volume (mm^3) of the ovaries of the Cape dune mole-rat captured during the period of January 2003 to January 2004.

Testicular Mass and Volume: The testicular mass of male Cape dune mole-rats was stable from January to April ranging from 0.42 ± 0.13mg/g to 0.57 ± 0.31mg/g body mass (Figure 6). This was followed by a sharp increase to 1.22 ± 0.94mg/g body mass in July. Testicular mass then finally decreased to a value of 0.58 ± 0.27mg/g body mass in December. The volume of the testes showed a dramatic increase from April (277.5 ± 48.9mm^3) to May (546.0 ± 64.2mm^3) (Figure 7). The testicular volume also changed throughout the year in a pattern closely mirroring the testes mass changes. Volume of the testes was fairly constant from January to April. A peak value in the volume of the testes was reached in July (758.4 ± 75.1mm^3). This was followed by a decrease in the volume to 509.7 ± 75.4mm^3 in September. In October there was another increase in the volume (645.7 ± 89.1mm^3) followed by a gradual decrease during the months of November and December (371.7 ± 89.4mm^3).

Seminiferous tubule diameter: The seminiferous tubule diameters of the Cape dune mole-rat were fairly constant during the months of January through to April (Figure 8). In May there was a sudden increase in the diameter of the tubules. This increase remained relatively stable until July where after tubule diameter decreased. A sudden increase occurred again during October where the highest value for seminiferous tubule diameter was measured. The months of November and December showed seminiferous tubule diameters that were comparable to that of January through to April. A significant difference was evident between the reproductively active (19.3 ± 0.5µm; n = 69) and reproductively quiescent (16.3 ± 0.5µm; n = 136) periods of the year ($t = 4.12$; $df = 203$; $p < 0.001$).

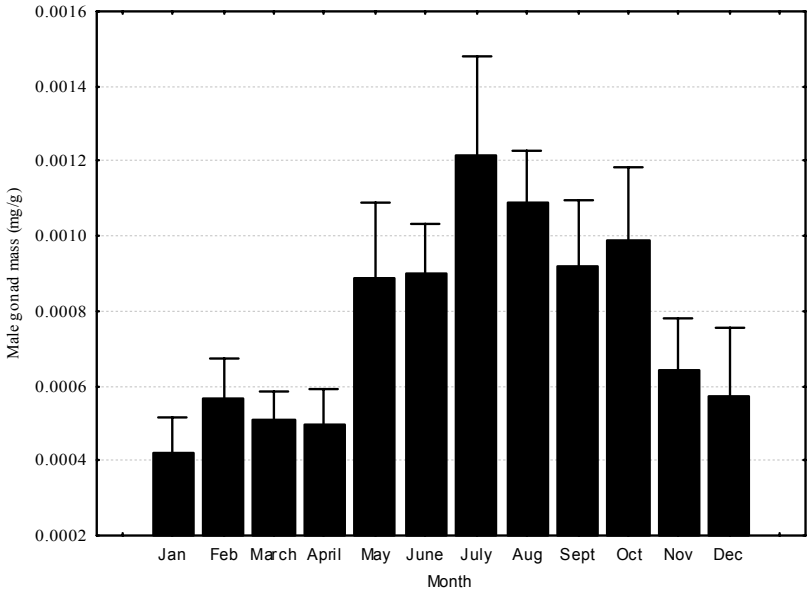


Figure 6. Corrected mass (mg/g) of the testes of the Cape dune mole-rat during the period of January 2003 to January 2004 (Mean ± SE). Corrected mass is given in mg/g body mass.

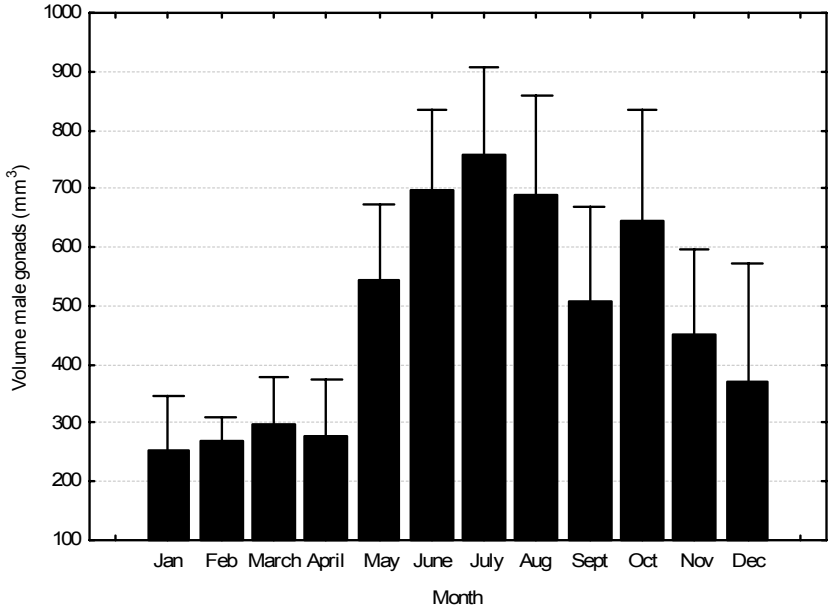


Figure 7. Fluctuations in the volume (mm^3) of the testes of the Cape dune mole-rat captured during January 2003 to January 2004.

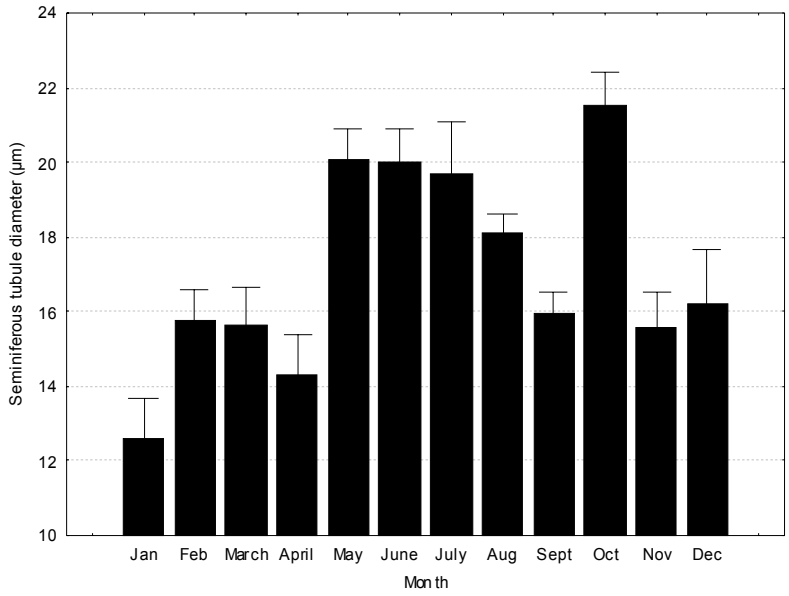


Figure 8. Fluctuations in the seminiferous tubule diameter (µm) of the Cape dune mole-rat, *Bathyergus suillus*, captured during the period of January 2003 and January 2004 (Mean ± SE).

Hormones: Testosterone hormonal levels in males showed two distinct peaks, the first in June (422.0 ± 122.2 nmols/l) and then August (371.9 ± 124.00 nmols/l) (Figure 9). During the rest of the year the basal testosterone levels were relatively low. Testosterone levels were significantly (Mann-Whitney U test, U = 5478. 00; $p < 0.001$) higher in the breeding period (243.6 ± 41.8 nmols/l; n = 99) compared to the non-breeding (94.0 ± 14.2 nmols/l; n = 154) period of the year.

Progesterone concentrations were elevated throughout the year but increased dramatically from February (Figure 10). Three clear peaks of progesterone concentration occurred in March (71.0 ± 8.9 nmols/l), June (78.6 ± 8.2 nmols/l) and September (76.0 ± 9.7 nmols/l).

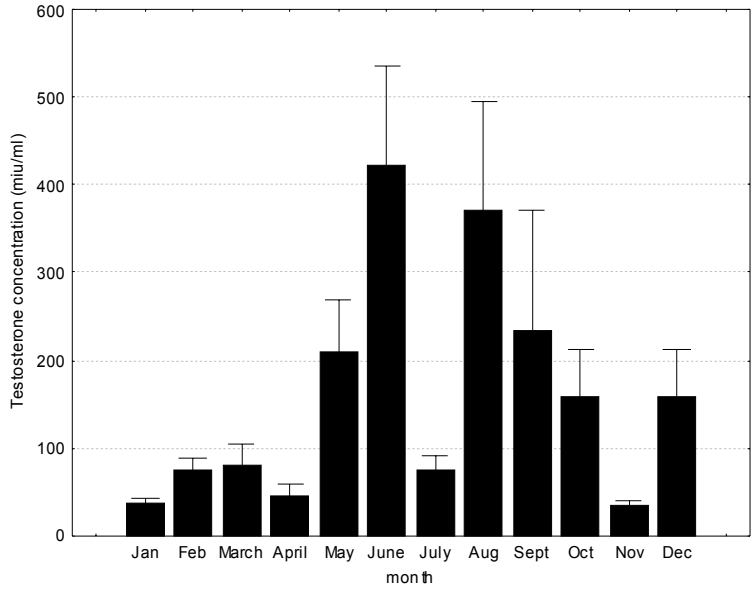


Figure 9. Fluctuation in the testosterone concentration (miu/ml) in male Cape dune mole-rats captured at the Cape Town International Airport during the period of January 2003 to January 2004 (Mean ± SE).

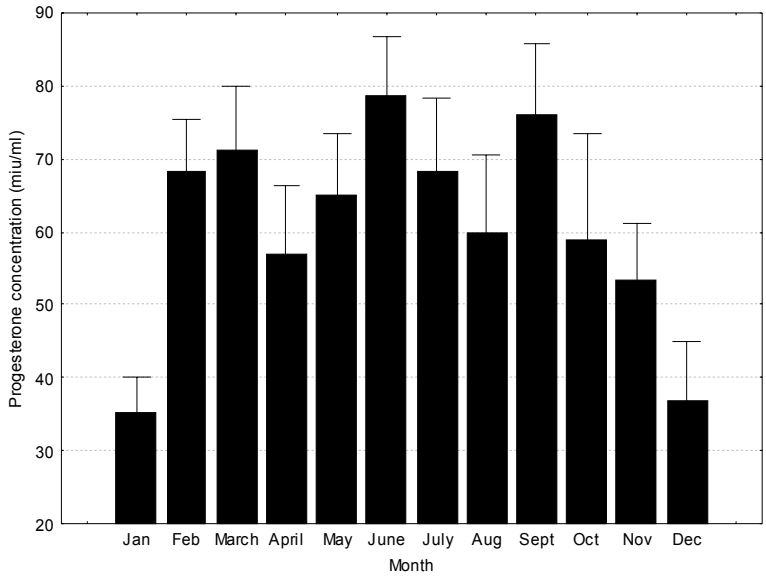


Figure 10. Fluctuation in the progesterone concentration (miu/ml) in female Cape dune mole-rats captured at the Cape Town International Airport during the period of January 2003 to January 2004 (Mean ± SE).

A slight decrease occurred from October (58.8 ± 14.6 nmols/l) to December (37.0 ± 8.2 nmols/l). Progesterone concentrations were significantly higher during breeding (70.7 ± 4.5 nmols/l; n = 96) when compared to the non-breeding season (56.5 ± 3.2 nmols/l; n = 154) (Mann-Whitney U test, U = 6068.00; $p < 0.05$).

Oestradiol 17β concentrations showed a slight increase during the months of January (27.75 ± 8.03 pg/l) to July (262.7 ± 80.8 pg/l) (Figure 11). In August these values increased markedly to a value of 608.6 ± 214.5 pg/l and these elevated levels were maintained through to November (832.0 ± 239.4 pg/l).

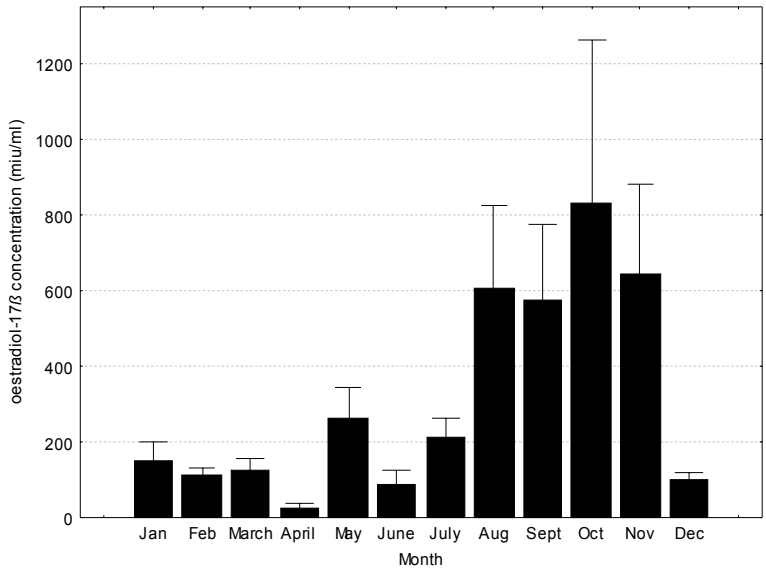


Figure 11. Fluctuation in the oestradiol-17ß concentration (miu/ml) in female Cape dune mole-rats captured at the Cape Town International Airport during the period of January 2003 to January 2004 (Mean ± SE).

After November oestradiol levels again decreased to a value corresponding to that of the mean values between January and July (98.0 ± 23.4 miu/ml). No significant difference (Mann-Whitney *U* test, $U = 5695.00$; $p > 0.05$) was found between oestradiol concentrations during (379.0 ± 76.5 pg/l; $n = 90$) and outside of the breeding season (215.8 ± 42.5 pg/l; $n = 135$).

Rainfall data: Mean monthly rainfall for the two-year duration of the study period is provided in Figure 12. In both years the rainfall peak was in August with most rainfall measured between March and October. The driest months in both years were November through to February.

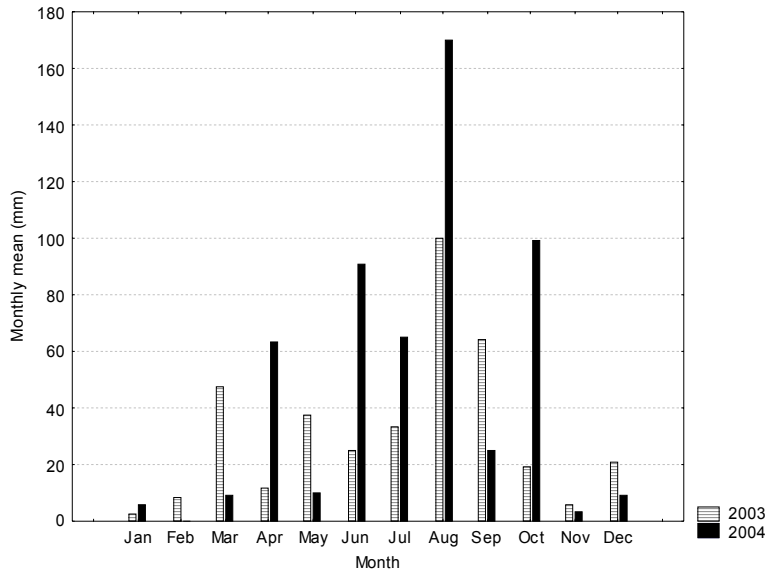


Figure 12. Mean monthly rainfall (mm) for the period January 2003 – December 2004 measured at the Cape Town International Airport. Solid bars denote rainfall measured during 2003 and while stripped bars denote rainfall data measured during 2004.

DISCUSSION

The Cape dune mole-rat is clearly a seasonal breeder whose reproduction is closely tied to the winter rainfall period of the Western Cape. Male Cape mole-rats signal to females at the onset of the breeding season with hind foot drumming (Bennett and Jarvis 1988a), a behaviour that may well communicate sexual readiness and which may act as a proximate cue for the onset of reproduction in the female. Testicular recrudescence occurs in May with an increase in the absolute size of the testes and an associated increase in the diameter of the seminiferous tubules. The titres of testosterone similarly rise in May and are maintained until October. The Cape mole-rat, *Georychus capensis* occurs sympatrically with the Cape dune mole-rat and male *G. capensis* show a similar pattern of gonadal and hormonal recrudescence associated with the onset of hind foot drumming (Bennett and Jarvis 1988a). Testicular regression and reduced testosterone secretion at the end of October is accompanied by a reduction in testes mass and seminiferous tubule diameter. The seasonal pattern of testosterone and testicular recrudescence/regression is closely mirrored by trends noted in *G. capensis* (Bennett and Jarvis 1988a).

The first incidence of pregnancy in the Cape dune mole-rat was recorded in June, but the month characterised by the most pregnant females was September. The number of pregnant females decreases thereafter with only a few pregnancies recorded in late January and February. Interestingly, the first sign of lactating females was in August and the lactation peak was in November. Given that the gestation period of *B. suillus* is estimated to be 50-60 days (Bennett and Faulkes 2000), this result corresponds closely with the prior peak in pregnancy in September. In *G. capensis* embryos were recorded in females from August through to December (Bennett and Jarvis 1988a). Given that this species has a gestation period of 40-50

days this would suggest that similar to *B. suillus*, courtship and copulation take place in late June. Thus the similar patterns of reproduction in these sympatric but phylogenetically distinct species points to a common ecological cue i.e. rainfall that triggers the onset of reproduction.

The sister taxon of *B. suillus*, is the allopatric Namaqua dune mole-rat, *B. janetta* which inhabits the winter rainfall region of Namaqualand (Skinner and Smithers 1990). Seasonal breeding is clearly demonstrated in this species with urinary testosterone concentrations in males increasing from May through to November. In females, urinary progesterone concentrations were low except for a sharp increase in July and August. Female teat size started to increase from August with pregnant and lactating females being caught in August to November (Herbst et al. 2004).

Together our data support the hypothesis that *B. suillus* is a seasonal breeder and that the onset of reproduction correlates with the winter rainfall period. Rainfall raises the soil moisture content and triggers the growth of below ground plant structures; both of which may provide the mole-rats with reliable external cues for recrudescence (Dennis and Marsh 1997).

Most solitary species of the bathyergid family would thus appear to be seasonal breeders whereas their social counterparts reproduce aseasonally. This might reflect broad differences in the distribution of solitary and social bathyergids and the degree to which environmental conditions vary seasonally. The Western Cape experiences a very dry and hot summer with little to no rainfall for four months of the year. Such conditions are not conducive to digging new tunnels necessary for locating mates and for the young to disperse and may explain the restriction of breeding to the wet winter months. It is possible that the relatively short gestation period of both solitary species reflects selection for the production of two litters within the limited

breeding period. This may explain the clear second peak of many of the reproductive parameters (e.g., number of female corpora lutea and male gonad volume) in October. Females should produce as many young as possible to maximise their reproductive fitness. The solitary species *B. suillus* and *G. capensis* have relatively short gestation periods 50-60 and 40-50 days respectively (Bennett and Jarvis 1988a; Bennett and Faulkes 2000) when compared to their social counterparts that may vary from 78 days in the Damaraland mole-rat, *Cryptomys damarensis* through to 110 days in the giant mole-rat, *Cryptomys mechowi* (Bennett and Jarvis 1988b; Bennett and Aguilar 1995). The relatively short gestation periods in solitary species enables them to have two litters during the breeding season and so increase the yearly number of pups produced.

All previously studied solitary and social mole-rats that occupy seasonal environments have been shown to be induced ovulators and the males of the various species possess epidermal spines or ornamentation on their penises. This intricate morphology may be important in stimulating the onset of ovulation in the female Cape dune mole-rat (Parag et al. in press). It is thus plausible that female *B. suillus* are induced ovulators, which would facilitate rapid onset of reproduction and so enable multiple mating opportunities and potentially multiple litters to be born during the breeding season.

In males there appears to be two distinct phases of maximal testosterone production and secretion; these being in June and August. The increase in testosterone may well be one of the mediating factors leading to the onset of hind foot drumming and subsequent mating. Males of the Namaqua dune mole-rat similarly displayed two distinct peaks in testosterone production in August and October that coincided with hind foot drumming in the field (Herbst et al. 2004).

Female hormonal profiles were not as clear-cut as those found in males, but progesterone concentrations were notably higher from May to October with a second rise in February and March. Corpora lutea were present in a few females outside of the breeding season although no pregnancies were recorded within this period. This might possibly indicate that *B. suillus* are induced ovulators and that these corpora lutea are the result from males occasionally mating with females outside of the breeding period. Oestradiol 17β concentrations were generally higher over the period May to November after which the mean concentrations declined sharply. It has been speculated that the Cape dune mole-rat, although displaying a seasonal component in reproduction, may under favourable environmental conditions exhibit an opportunistic breeding pattern by extending the duration of the breeding season (Hart et al. submitted).

Most hormonal and morphological reproductive parameters explored in this study provide strong evidence for a seasonal reproductive pattern with the potential for females to produce two litters per annum under optimal environmental conditions. Rainfall and the associated increase in primary productivity would appear to be a key ecological variable determining the timing and frequency of reproduction in *B. suillus*.

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THE PITUITARY POTENTIAL FOR OPPORTUNISTIC BREEDING IN THE CAPE DUNE MOLE-RAT, *BATHYERGUS SUILLUS*.

In this paper the effect of sex and season on baseline and post-GnRH challenge luteinising hormone (LH) levels in a solitary, seasonally breeding mole-rat were investigated. Circulating basal concentrations of luteinising hormone (LH) were found to differ significantly with season in both sexes. However, no significant difference was found in circulating basal LH concentration between the sexes either within or out of the breeding season.

The magnitude of the LH response to an exogenous pharmacological overdose of GnRH both in and out of the breeding season in males and females respectively was not significant. This finding suggests that there is no down regulation of GnRH receptors on the pituitary during the non-breeding season. Cape dune mole-rats thus have the potential for opportunistic breeding outside of the typical breeding period. We argue that this represents an adaptation to limited and brief opportunities for mating in this xenophobic and aggressive species.

Key words: luteinising hormone, pituitary, mole-rat, *Bathyergus*, gonadotrophin-releasing hormone

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INTRODUCTION

The Cape dune mole-rat *Bathyergus suillus* (Schreber 1782) is a solitary dwelling rodent that lives in the mesic regions of the Western Cape Province of South

Africa (Bennett et al. 1991). This region is characterised by predictable, high winter rainfall and abundant evenly distributed food resources (viz. the roots and aerial portions of grasses and geophytes) (Bennett and Jarvis 1995; Bennett and Faulkes 2000). Solitary subterranean rodents are typically xenophobic and aggressive towards conspecifics and *B. suillus* is no exception (Bennett and Jarvis 1988; Jarvis and Bennett 1990). As a consequence the pairing of individuals under laboratory conditions has proved difficult and little is known of their reproductive biology. Trapping of wild animals has revealed that they typically produce offspring during the spring months. This has been supported by Van der Horst (1973) who suggested a seasonal breeding pattern based on testicular parameters of male Cape dune mole-rats.

Reproductive biology has been studied in a number of social bathyergids with the trend being that reproduction is restricted to a single breeding female and a number of male consorts (Bennett and Jarvis 1988; Spinks et al. 1999). Non-reproductive members of the colony may be physiologically, behaviourally or environmentally inhibited from reproducing. The reproductive animals within the colony generally reproduce throughout the year (Bennett and Jarvis 1988; Bennett et al. 1994; Jarvis and Bennett 1993; Bennett and Aguilar 1995) with the exception of the social common and highveld mole-rats that breed seasonally (Spinks et al. 1999; Janse van Rensburg et al. 2002).

Despite strong xenophobic behaviour towards conspecifics, Cape dune mole-rats readily signal to one another through the soil by drumming with their hind feet. Drumming and its role in reproduction, has previously been reported in the solitary Cape mole-rat, *Georychus capensis* (Bennett and Jarvis 1988). This seismic communication in *B. suillus* is considered not only important in territoriality but also in triggering the onset of reproductive behaviour and subsequent pairing.

In this paper we investigate evidence for seasonal activation of the hypothalamo-pituitary axis in *B. suillus* through the administration of an exogenous GnRH challenge to individuals of both sexes at two different times of the year. Times (based on data from long-term capture records) were chosen to coincide with the middle of the non-breeding and breeding seasons respectively.

We ask a simple but fundamental question, is the pituitary of the Cape dune mole-rat modulated by the season of the year or is it active throughout the year? We investigated this by challenging the pituitary of freshly caught male and female Cape dune mole-rats with a pharmacological overdose of exogenously administered GnRH and by measuring the response of the pituitary to this challenge. Classical seasonally breeding mammals, which move from the breeding into the non-breeding season typically exhibit a reduced GnRH production and secretion, as well as an increased sensitivity of the hypothalamic region to negative feedback control by gonadal hormones (Lincoln and Short 1980). We postulated that there should be a reduction in LH concentration or even a shut down of LH production out of the breeding season. In addition we predicted that an inhibited or reduced pituitary responsiveness to an exogenous GnRH challenge should occur in both sexes of Cape dune mole-rat.

MATERIALS AND METHODS

A total of 9 males and 13 females were captured in July 2004 and 8 males and 10 females in January 2005 at the Cape Town International Airport (33°58'S 18°37'E) as part of a long-term pest control programme. The study site, although situated in the Fynbos Biome was characterised by alien grass species. Body mass

during the breeding season ranged from 298g to 835g with a mean of 681g ± 181g for females (n = 13) and 390g to 1920g with a mean of 1002g ± 513g for males (n = 9). Out of the breeding season body mass ranged from 620g to 1020g with a mean of 840g ± 140g for females (n = 10) and 840g to 1400g with a mean of 1107g ± 249g for males (n = 8). The animals were captured using vice traps that were modified to catch the animal on the fore or hind limb. Traps were checked every 20 minutes and captured animals were placed in individual 20*l* buckets with fresh grass for food. The animals were then taken through to the University of Cape Town. Blood samples were obtained by puncturing the brachial vein of the foot using a hypodermic needle and drawing a small amount of blood ca. 300-400µl into heparinized micro-hematocrit tubes by capillary action. Blood samples were subsequently centrifuged and the plasma stored at –40^{o}C until determination of LH concentration. The mole-rats were then euthanased with an overdose of Halothane anaesthetic (Zeneca, Johannesburg, South Africa) after the experiment.

***GnRH Administration*:** Exogenous GnRH produced in the laboratory of R.P. Millar was used for the hormone challenges. The hormone was synthesized using solid phase methodology and had a purity >98% homogeneity (Millar et al. 1989). Ampoules of 2µg GnRH in 200µl sterile physiological saline were stored at –70^{o}C until required. To determine the effect of GnRH on the pituitary during and out of the breeding season we administered an exogenous dose of GnRH by subcutaneous injection. In all experiments 2µg exogenous GnRH was administered sub-cutaneously on the dorsal surface of the mole-rat as a bolus 200µl injection and the test subject was left for 20 minutes before the second blood sample was obtained. All

experimental procedures were cleared with the ethics committee of the University of Pretoria (AUCC 040702-015).

Luteinising hormone Bioassay: Basal and challenged plasma LH concentrations were determined using an *in vitro* bioassay based on the production of testosterone by dispersed mouse Leydig cells (Van Damme et al. 1974). The incubation medium comprises 12ml Eagle's basal medium (Highveld Biological, Johannesburg, South Africa), 2.1ml 7.5% sodium hydrogen carbonate (Highveld Biological, Johannesburg, South Africa) 2ml foetal calf serum (Highveld Biological, Johannesburg, South Africa) and 100ml distilled water and is prepared by placing it on ice and gassing it slowly with Carbogen 5 (95% O_2 and 5% CO_2 Afrox, Germiston, RSA). A mouse (NMRI strain, South African Vaccine Producers, Edenvale), ca. 6 weeks old, was killed by cervical dislocation, its testes removed, decapsulated and minced in 5ml incubation medium. After stirring the cell suspension for 5 minutes on a magnetic stirrer, the suspension was filtered through a fine nylon mesh and incubated for 1 h. in a shaking water bath at a temperature of 34^{o}C under a stream of Carbogen 5 gas.

Following incubation the cell suspension was centrifuged at 2500 rev/min for 5 minutes at 4^{o}C. The supernatant was decanted and the cells re-suspended in incubation medium. The process was repeated, after which the cell suspension was slowly mixed on a magnetic stirrer for 5 min.

The number of Leydig cells was counted using a haemocytometer (WSI, depth 0.1mm, 1/400mm^2). Incubation medium was added until the number of cells counted corresponds to the final cell suspension volume (in ml.)

The LH buffer, containing 2.9g disodium hydrogen orthophosphate dodecahydrate, 0.29g sodium dihydrogen orthophosphate dihydrate and 4.38g of sodium chloride (AnalR, BDH Ltd, Poole, U.K.) is made up to 1 litre in deionised dH_20, with 0.1% BSA (Merck, Darmstadt, Germany).

The samples of mole-rat plasma were prepared at a dilution of 1: 20 in LH buffer. The mammalian LH standard (2^{nd} International standard 1988, Code 80/552, N.I.B.S.C. Hertfordshire, U.K.) (Bennett et al. 1993) in LH buffer was serially double-diluted to produce a standard curve over the range 400-3.625 µIU ml/100µl. A volume of 100µl of sample, standard and control or LH buffer (to obtain an estimate of total binding) was added to the bioassay tubes. Standards and total binding were assayed in triplicate.

A volume of 200µl of diluted cell suspension was added to each assay tube, the tubes were incubated in a shaking water bath (34^oC) for 3 h. under Carbogen 5 gas. Placing the tubes in a water bath at 100^oC for 15 min. terminates testosterone production. A volume of 150 µl of phosphate buffered saline was added to each tube. Testosterone production was measured by radioimmunoassay as described by Bennett et al. (1994).

A test for parallelism was used to validate the use of the assay in the Cape dune mole-rat. A volume of 50µl of a known plasma pool high on LH was double-diluted over the range of the standard curve. Serial doubling dilutions of mole-rat plasma collected after GnRH administration were compared to the LH standard curve. These dilutions were parallel to, and not significantly different from the reference preparation (ANCOVA, $F_{1\ 5}$ 2.96, $p > 0.05$). The sensitivity of the assay (determined at 95% binding) was 12.7 µIU/tube or 2.5mIU/ml. Intra-and inter assay coefficients of

variation for repeated determinations of a quality control were 8% and 13% respectively.

***Statistical analysis*:** All data were analysed using a multivariate analysis of variance (MANOVA). Basal LH levels were analysed both between and within the sexes for the two periods. Pre and post administration analyses were investigated using the repeated measures MANOVA. We also analysed the difference in LH levels after the administration of GnRH both within and between the two sexes. All analyses were performed using Statistica version 5.0 computer package.

RESULTS

***Basal LH levels*:** The basal concentrations of circulating LH levels were investigated both within and between the two sexes during the breeding season (July, 2004) and out of the breeding season (January, 2005). Basal LH levels between the males and females for January ($F = 0.50$, $P = 0.51$) and July ($F = 1.94$, $P = 0.20$) were not significantly different. However a significant difference in basal LH concentration was found for males, 3.34miu/ml ± 1.21 (Jan) vs. 14.03miu/ml ± 0.748 (Jul) ($F = 88.01$, $P < 0.001$) and female's 2.3miu/ml ± 1.09 (Jan) vs. 12.39miu/ml ± 0.52 (Jul) ($F = 43.02$, $P < 0.001$) with breeding season. Basal LH concentrations during the breeding season were considerably higher in males (14.03miu/ml ± 0.748) and females (12.39miu/ml ± 0.52) than for the respective sexes out of the season (3.34miu/ml ± 1.21) males and (2.3miu/ml ± 1.09) for females (Figure 1 and 2).

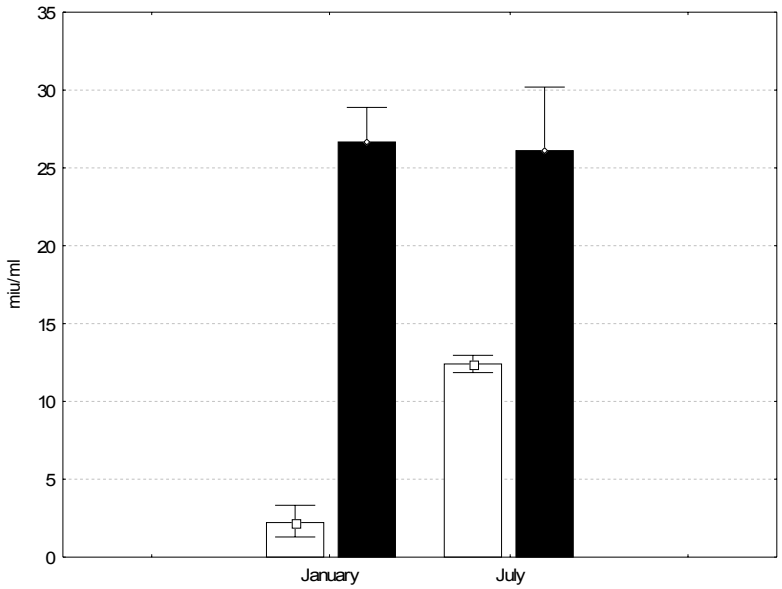

Figure 1. Mean (miu/ml) ± SD of basal concentrations of LH hormone and the response to a GnRH challenge in female *Bathyergus suillus* during January and July. Basal LH levels are denoted by the clear blocks, while post administration LH concentrations are denoted by the filled in blocks. Significant differences were observed in basal levels between January and February. Pre and post administration LH concentrations also differed significantly from one another.

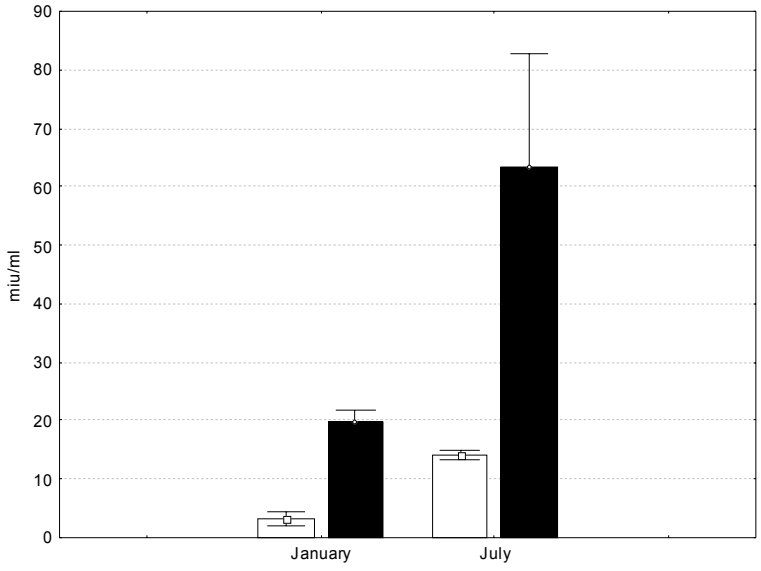

Figure 2. Mean (miu/ml) ± SD of basal concentrations of LH hormone and the response to a GnRH challenge in male *Bathyergus suillus* during January and July. Basal LH levels are denoted by the clear blocks, while post administration LH concentrations are denoted by the filled in blocks. Significant differences were observed in basal levels between January and February. Pre and post administration LH concentrations also differed significantly from one another.

***After the administration of GnRH*:** There was a significant response in both males (3.34miu/ml ± 1.21 (Pre) vs. 19.69miu/ml ± 2.05 (Post)) (F = 138.40, P < 0.001) and females (2.3miu/ml ± 1.09 (Pre) vs. 26.68miu/ml ± 2.17 (Post)) (F = 137.32, P < 0.001) during January to a single challenge of 2µg exogenous GnRH (Figure 3.). Likewise in July both males (14.03miu/ml ± 0.748 (Pre) vs. 63.36miu/ml ± 19.22 (Post)) (F = 6.37, P = 0.04) and females (12.39miu/ml ± 0.52 (Pre) vs. 26.08miu/ml ± 4.17 (Post)) (F = 9.25, P = 0.01) exhibited a significant increase in basal LH levels following administration of exogenous GnRH (Figure 4.)

No significant differences were found in the plasma LH levels following GnRH administration for both periods sampled in either males (F = 2.57, P = 0.16) or females (F = 0.00, P = 0.95).

No significant differences were found between the responses of males vs. females during either the non-breeding (F = 2.90, P = 0.15) or breeding season (F = 3.34, P = 0.11).

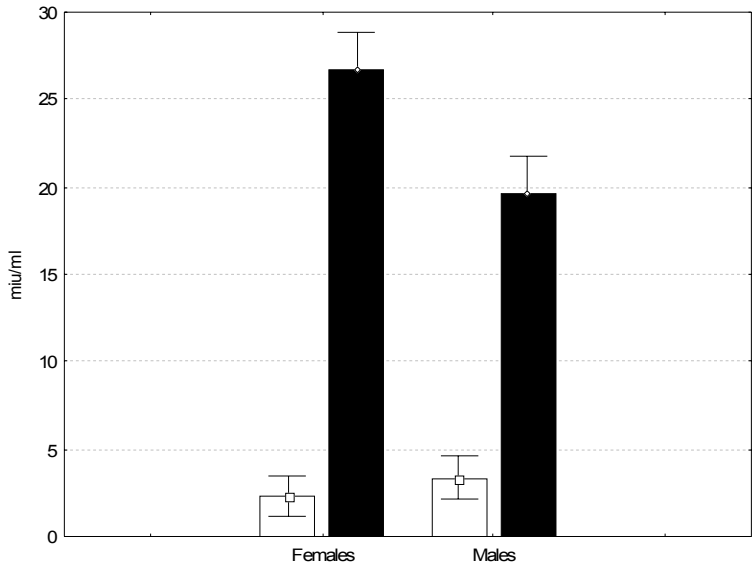

Figure 3. Mean (miu/ml) ± SD of basal concentrations of LH hormone and the response to a GnRH challenge in male and female *Bathyergus suillus* during January. Open blocks denote LH concentrations (basal) before a GnRH challenge has been performed, while the filled in blocks denote post challenge LH concentrations. Although significant differences were found within the sexes with regards to pre and post challenge concentrations, no significant differences were found when relating basal values and response values between the two sexes.

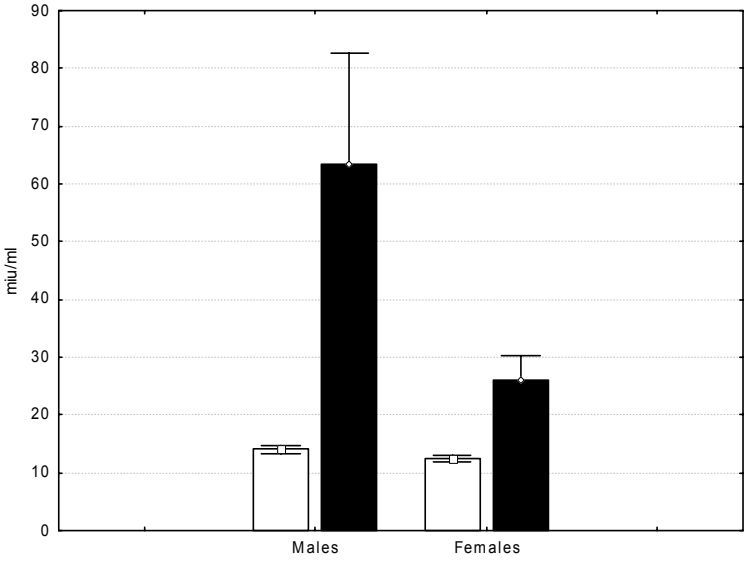

Figure 4. Mean (miu/ml) ± SD of basal concentrations of LH hormone and the response to a GnRH challenge in male and female *Bathyergus suillus* during July. Open blocks denote LH concentrations (basal) before a GnRH challenge has been performed, while the filled in blocks denote post challenge LH concentrations. Although significant differences were found within the sexes with regards to pre and post challenge concentrations, no significant differences were found when relating basal values and response values between the two sexes.

DISCUSSION

Despite strong evidence for being seasonal breeders we did not find a reduction in LH concentration in either males or females out of the breeding season. In addition pituitary responsiveness to an exogenous GnRH challenge was not inhibited or reduced for either sex in the non-breeding period. To date much attention has been drawn towards the basal LH concentrations and the subsequent response to an exogenous GnRH challenge in the reproductive and non-reproductive colony members of social African mole-rats (Bennett et al. 1993; Bennett et al. 1996; Bennett et al. 1997; Bennett et al. 1999; Bennett et al. 2000; Spinks et al. 1999; Spinks et al. 2000; Van der Walt et al. 2001). In particular this has been used to investigate the potential social-suppression at the level of the pituitary in non-reproductive socially occurring mole-rats. Interestingly, no research has been afforded to the solitary species that inhabit the more mesic coastal regions of the northern and western Cape Provinces of South Africa.

The mole-rats of both sexes exhibited comparable basal concentrations both within and outside of the breeding period. However, the circulating basal LH

concentrations for each sex were slightly higher during the breeding, compared to the non-breeding period. Moreover, the mole-rats of both sex exhibited a similar release of stored LH from the pituitary as a result of an overdose of GnRH both in and out of the breeding season.

All of the solitary dwelling mole-rats of southern African breed seasonally, while most of the social species of bathyergids reproduce throughout the year (Bennett et al. 1991). Two exceptions to the latter trend are, the common mole-rat (*Cryptomys hottentotus hottentotus),* which inhabits a winter rainfall region and rears young during the southern hemisphere summer (late November through to January) (Spinks et al. 1997; Spinks et al. 1999); and the highveld mole-rat (*Cryptomys hottentotus pretoriae*), which occurs in a summer rainfall zone and rears young in the winter (early June to August) (Janse van Rensburg et al. 2002; Janse van Rensburg et al. 2003)

In both these seasonally breeding social mole-rats, it was found that males failed to exhibit a regression in spermatogenesis and no periodicity in male reproduction was evident (Spinks et al. 1997; Janse van Rensburg et al. 2002; Janse van Rensburg et al. 2003). The maintenance of reproductive activity is uncommon amongst seasonally breeding animals. Janse van Rensburg et al. (2002) postulated that male mole-rats may need a ready supply of sperm throughout the year to enable them to seize any opportunity for reproduction that might arise.

We propose that a similar scenario may operate in the Cape dune mole-rat. It is possible that unpredictable rainfall patterns due to the El Nino southern oscillations, may have selected against a strictly seasonal reproductive physiology in *B. suillus.* Aseasonal rainfall might allow for reproductive opportunities outside of the normal

breeding season. This would favour a state of constant physiological readiness in both males and females to maximise fitness.

It has been suggested that social mole-rat species engage in the costly retention of reproductive function out of the breeding season to maintain stable pair bonds and to ensure that individuals are sexually primed in the event of stochastic dispersal opportunities. Although stable pair bonds are not a feature of the Cape dune mole-rat's reproductive biology dispersal opportunities are likely to be equally stochastic. Dispersal greatly increases the probability of encountering opposite sexed conspecifics and hence mating opportunities. The observation in this study that the pituitary retains its function throughout the year may represent an adaptation to maximise reproduction in an opportunistic breeding system.

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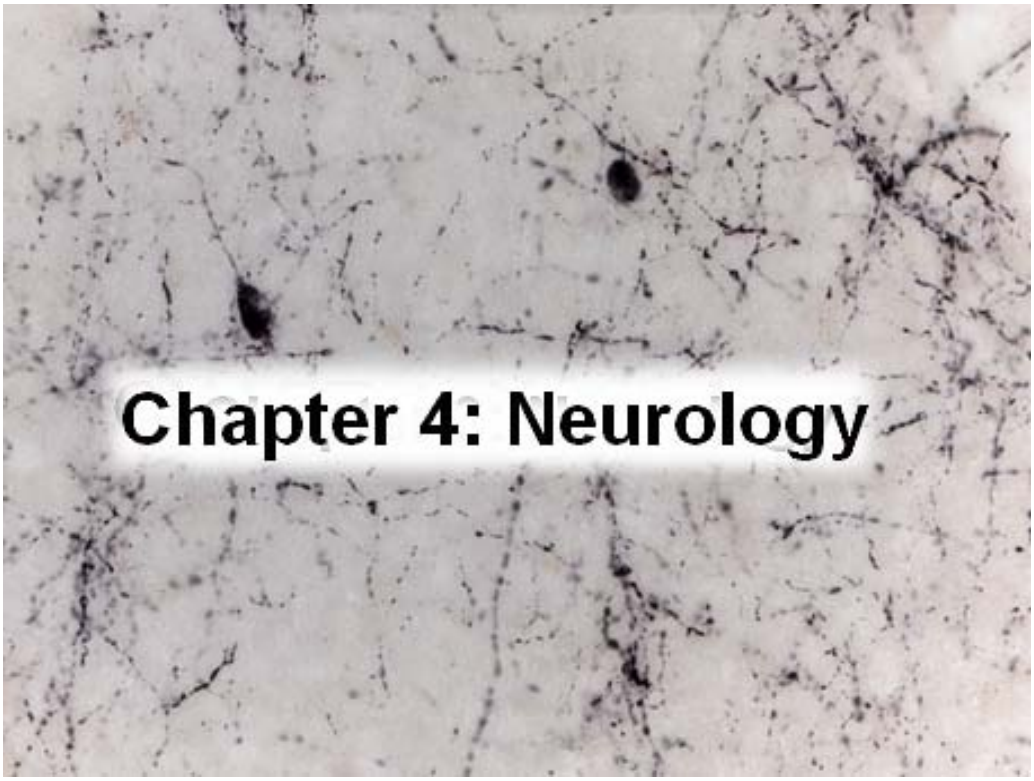

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A NEUROANATOMICAL INVESTIGATION INTO THE GnRH SYSTEM OF THE SEASONALLY BREEDING CAPE DUNE MOLE-RAT, *BATHYERGUS SUILLUS.*

The gonadotrophin-releasing hormone (GnRH) system of the Cape dune mole-rat, a solitary subterranean rodent, was visualised immunocytochemically. The GnRH neurones and fibres were loosely distributed along the septo-preoptico-infundibular pathway. Dense aggregations of GnRH fibres were present in the regions of the organum vasculosum of the lamina terminalis and the median eminence. No neurons were present in the subfornical organ. Total numbers of GnRH-immunoreactive cell bodies in male and female mole-rats did not differ significantly when compared between the breeding- and non-breeding seasons. Similarly there was no difference in the rostro-caudal distribution of the GnRH cell bodies in male and female mole-rats in the breeding season, whereas females out of the breeding season had fewer GnRH neurons in the medial preoptic area compared to the mediobasal hypothalamus.

The average size of GnRH soma did not differ significantly between seasons in male mole-rats. However, in female mole-rats the average GnRH soma was significantly larger in the breeding season than out of the breeding season.

Key words: GnRH, *Bathyergus suillus*, Cape dune mole-rat, immunocytochemistry, neuroanatomical

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INTRODUCTION

The Cape dune mole-rat, *Bathyergus suillus,* is a solitary seasonally breeding rodent mole that displays severe xenophobia towards conspecifics (Jarvis and Bennett 1990). During the breeding season males use seismic communication in the form of hind foot drumming to advertise their intention to mate. The female responds to the male by drumming at a different frequency. The male proceeds to burrow towards the female, brief copulation ensues and the male returns to his own burrow system (Bennett and Jarvis 1988; Bennett & Faulkes, 2000). The breeding period appears to be restricted from May to September (Hart et al. submitted.), a period that coincides with the winter rainfall season of the Cape Province in South Africa.

Mean circulating levels of luteinizing hormone (LH) display significant seasonal differences within each sex, the concentration being higher during the breeding season. Nevertheless, the magnitude of the LH response to exogenous GnRH does not vary seasonally in either male or female Cape dune mole-rats (Hart et al. submitted); this suggests that there is no down regulation of pituitary GnRH receptors during the non-breeding season.

The GnRH neuronal system has been visualised in a number of social mole-rat species such as the Damaraland mole-rat, *Cryptomys damarensis* (Molteno et al. 2004), the common mole-rat, *Cryptomys hottentotus hottentotus* and the highveld mole-rat, *Cryptomys hottentotus pretoriae* (Du Toit et al. 2005). To date there has been no characterisation of the GnRH system for any of the three solitary African mole-rat genera.

In mammals GnRH cells are scattered throughout the forebrain ranging from the olfactory bulbs to the hypothalamus (Schwanzel-Fukuda and Pfaff 1989). As the distribution of GnRH neurons depend on the migration of these neurones from the

olfactory placode (Schwanzel-Fakuda and Pfaff 1989) differences in the distribution is evident between species. Eighty percent of the GnRH cell bodies reach the mediobasal hypothalamus in the mink (Ntoumi et al.1994), whereas only a few GnRH cells do so in the opossum, *Monodelphis* domestica (Schwanzel-Fakuda et al. 1988) The GnRH system is highly plastic, thus, differences in morphological parameters such as soma size and cell number have been found to vary post-mating in the female musk shrew, *Suncus murinus* (Dellovade et al.1995), between the sexes in the springbok (Robinson et al. 1997) and between territorial and non-territorial male cichlid fish, *Haplochromis burtoni* (Francis et al. 1993). In the seasonally breeding Syrian hamster, *Mesocricetus auratus*, the size of GnRH neurones increases during the non-breeding period but their total number remains stable throughout the year (Urbanski et al. 1991). In marked contrast, the white-footed mouse, *Peromyscus leucopus*, displays clear differences in the number of GnRH neurons between the reproductive and non-reproductive seasons (Glass 1986).

The aim of the present study was to characterize the GnRH system of the Cape dune mole-rat by investigating the distribution, number and size of the GnRH perikarya in the forebrain. Our *a priori* prediction was that male and female Cape dune mole-rats do not exhibit a significant difference in the total number of GnRH neurons between breeding and non-breeding seasons. However, we hypothesised that the size of the biosynthetic region of the cell may vary with season.

MATERIALS AND METHODS

As part of an eradication program at the Cape Town International Airport (33°58'S 18°37'E) a total of 6 males and 8 females were captured during the breeding period (July 2004) and 6 male and 6 female out of the breeding period (January 2005).

Body mass during the breeding season ranged from 520g to 835g with a mean of 723g ± 52g for females (n = 8) and 390g to 1920g with a mean of 953g ± 261g for males (n = 6). Out of the breeding season body mass ranged from 680g to 980g with a mean of 821g ± 44g for females (n = 6) and 840g to 1400g with a mean of 1090g ± 101g for males (n = 6). All experimental procedures were cleared with the ethics committee of the University of Pretoria (AUCC 040702-015).

Shortly after capture, individuals were euthanased with an overdose of Halothane anaesthetic (Zeneca, Johannesburg, South Africa). Animals were perfused through the right ventricle of the heart using 200ml saline at 37^{o}C, followed by approximately 200ml of chilled, buffered 4% PFA (paraformaldehyde) solution. Animals were decapitated and the brains removed from the skulls. Brains were stored in a 2% PFA solution at 4^{0}C until processing. Prior to sectioning the brains were placed in a 30% sucrose solution for 48h to 96h. The brains were mounted on dissecting blocks using methylcellulose, snap-frozen with dry ice and cut on a cryostat. The tissue was cut in the coronal plane at 25µm. Every 6th section was sampled. Free-floating sections were placed in anti-freeze solution and stored at –20^{o}C until immunocytochemical staining.

Nissl stain of sections: One set of sections was Nissl-stained as a guide to the general brain anatomy of the Cape dune mole-rat. Free-floating sections were mounted on poly-L-lysine coated slides and left to air dry for approximately 48h. The slides were placed in a toluene blue solution for approx 15 seconds, taken through 70%, 90% and 100% alcohol baths and a xylene wash (3min each), and cover slipped using DPX mount. Sections were viewed under a light microscope and the images were captured digitally.

GnRH immunoreactive staining: One set of sections for each animal was treated immunocytohemically to visualise the distribution and anatomy of the GnRH neurones in the brain. The sections were washed in PBS for 45min, followed by Triton X-100 (0.25ml in 50ml PBS for 30 mins) (v/v; BDH Chemical Company, UK), and 45min in PBS. The sections were placed in a 0.1% H_2O_2 solution (30 min) followed by a 30 min PBS wash. After a 30 min pre-treatment in 2% Normal Donkey serum the sections were placed in a primary anti-rabbit antibody (1:30 000) for 72h at 4^{o}C (Incstar Corporation, USA).

Following this incubation period sections were washed for 2h in PBS. The secondary antibody (biotinilated donkey anti rabbit at 1:750) (Jackson Immunoresearch Laboratories Inc., USA) was then allowed to react with the sections for 2hrs at room temperature, followed by a PBS wash. Thereafter a combination of the primary and secondary antibody (1:1000 in both instances) was allowed to react with the sections for 90 min. This was followed by a 30 min TRIS buffer rinse. Nickel-diaminobenzidine (0.05%) (Trizma 7.6, Sigma Chemical Company, USA) was allowed to react with the sections for 15 minutes, followed by a 2 min Tris rinse. The sections were mounted on non-coated glass slides using an Elvanol solution, air-dried for approximately 48h, cover slipped using a DPX mounting fluid and inspected under a light microscope.

Number and distribution of GnRH Cell bodies: Each section from the rostral preoptic area to the posterior hypothalamus was carefully examined under a light microscope. Digital images were used, in combination with the Nissl stain images, to determine the distribution of the GnRH neuronal system of the Cape dune mole rat.

The total number of GnRH cell bodies was counted for every section and multiplied by six to establish the total number for each animal.

Determination of GnRH cell body size: An image analysis program (ImageJ version 1.30, National Institutes of Health, USA) was used to analyse digital images of single GnRH neurons. This allowed for the determination of the total area occupied by the neuron irrespective of the anatomy and number of fibre projections. Where fibre projections were present the boundary of the cell body was designated as described by Robinson et al. (1997).

Statistical analysis*:* Statistica Ver 6 was used in all statistical analyses (Statsoft, USA). Non-parametric data were analysed by Kruskal Wallis Analyses of Variance (ANOVA), while parametric data were analysed by two-way ANOVAs. Results are presented as the mean and SE The significance level was assumed at $p<0.05$.

RESULTS

***GnRH cell body morphology*:** GnRH-immunoreactive cell bodies were generally spindle shaped and in some cases the nuclei of cells were clearly visible (Figure 1a). Most cells had processes with long intricate associations with the processes of other cell bodies (Figure 1b), while other cells displayed no visible processes (Figure 1c). GnRH-immunoreactive processes had a beadlike appearance and formed intricate associations in some areas of the brain (Figure 1d).

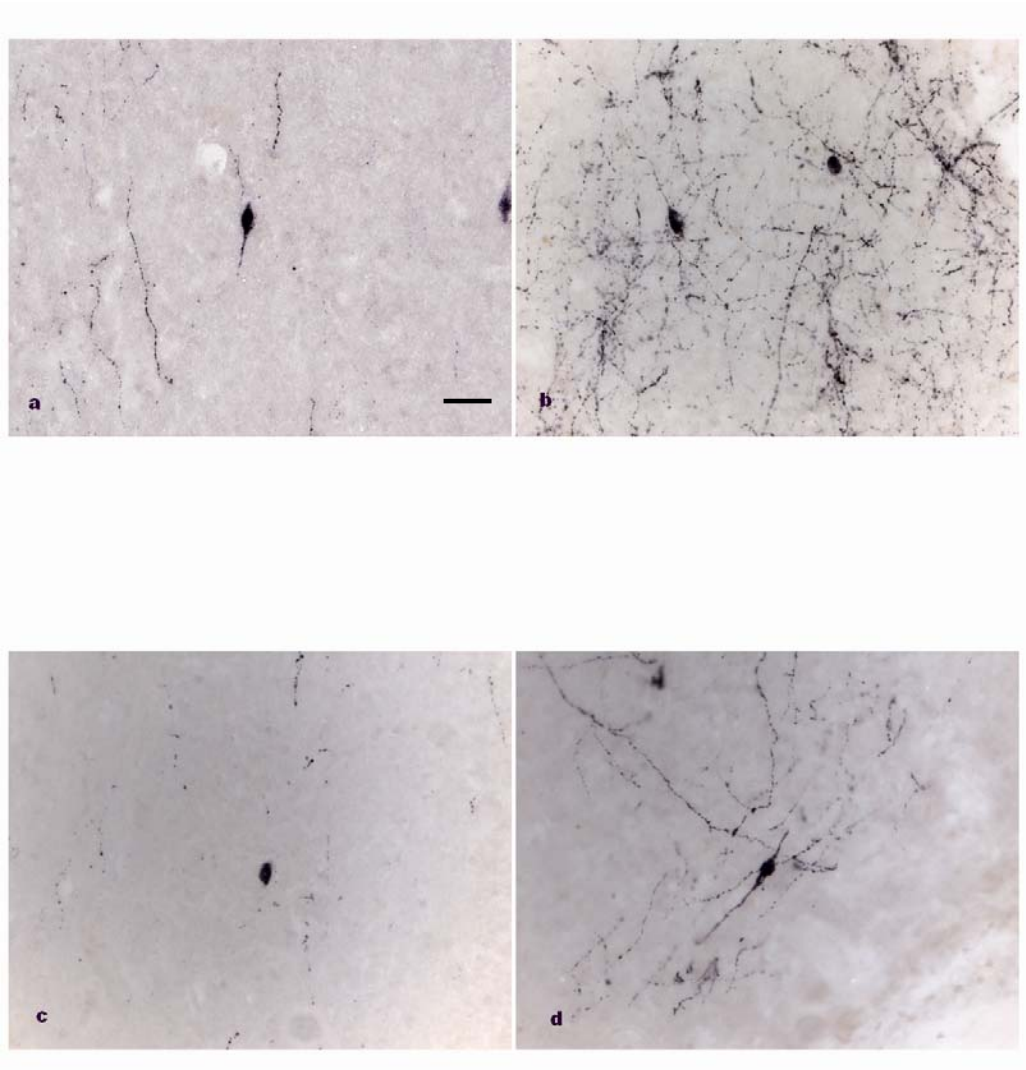

Figure1. Morphology of GnRH neurones in the brain of the Cape dune mole-rat, *Bathyergus suillus*, showing (a) a spindle shaped cell body, (b) projections of fibres from cell bodies and associations with other cells, (c) a cell body without any projections and (d) the beaded appearance of the GnRH fibres. Scale bar = 20µm.

Number and distribution of GnRH cell bodies and processes: GnRH cell bodies were generally distributed in a loose band along the septo-infundibular pathway stretching from the rostral preoptic area (Figure 2 a-d) to the mediobasal hypothalamic area (Figure 2 e-m). They were not observed in the subfornical organ,

olfactory bulb or hippocampus. Marked aggregations of GnRH processes were found at two sites: the region of the organum vasculosum of the lamina terminalis (OVLT; Figure 2c) and the median eminence (ME; Figure 2g-m).

The relative ratio of distribution of GnRH cell bodies between the preoptic area and mediobasal hypothalamus did not differ significantly between females during (0.44 ± 0.05; n = 8) and out (0.51 ± 0.06; n = 6) of the breeding season ($F_{1;\ 12} = 0.737$; p = 0.407). Similarly, males did not display any significant difference with regards to the distribution of cell bodies between the preoptic area and mediobasal hypothalamus areas during (0.477 ± 0.045; n = 6) and out (0.48 ± 0.47; n = 6) of the breeding season ($F_{1;\ 10} = 0.022$; p = 0.88).

The total number of GnRH cell bodies varied between 732 and 2778 for females and 864 and 2064 for males. There was no significant difference between the numbers of cells for females during (1061.28 ± 191.66; n = 8) and out (1558.02 ± 221.31; n = 6) of the breeding season ($F_{1,\ 12} = 2.88$; p = 0.12). Similarly, the total number of cell bodies for males during the breeding season (1557 ± 129.64; n = 6) and out of (1615.98 ± 129.64; n = 6) the breeding season were not significantly different ($F_{1,\ 10} = 0.10$; p = 0.75).

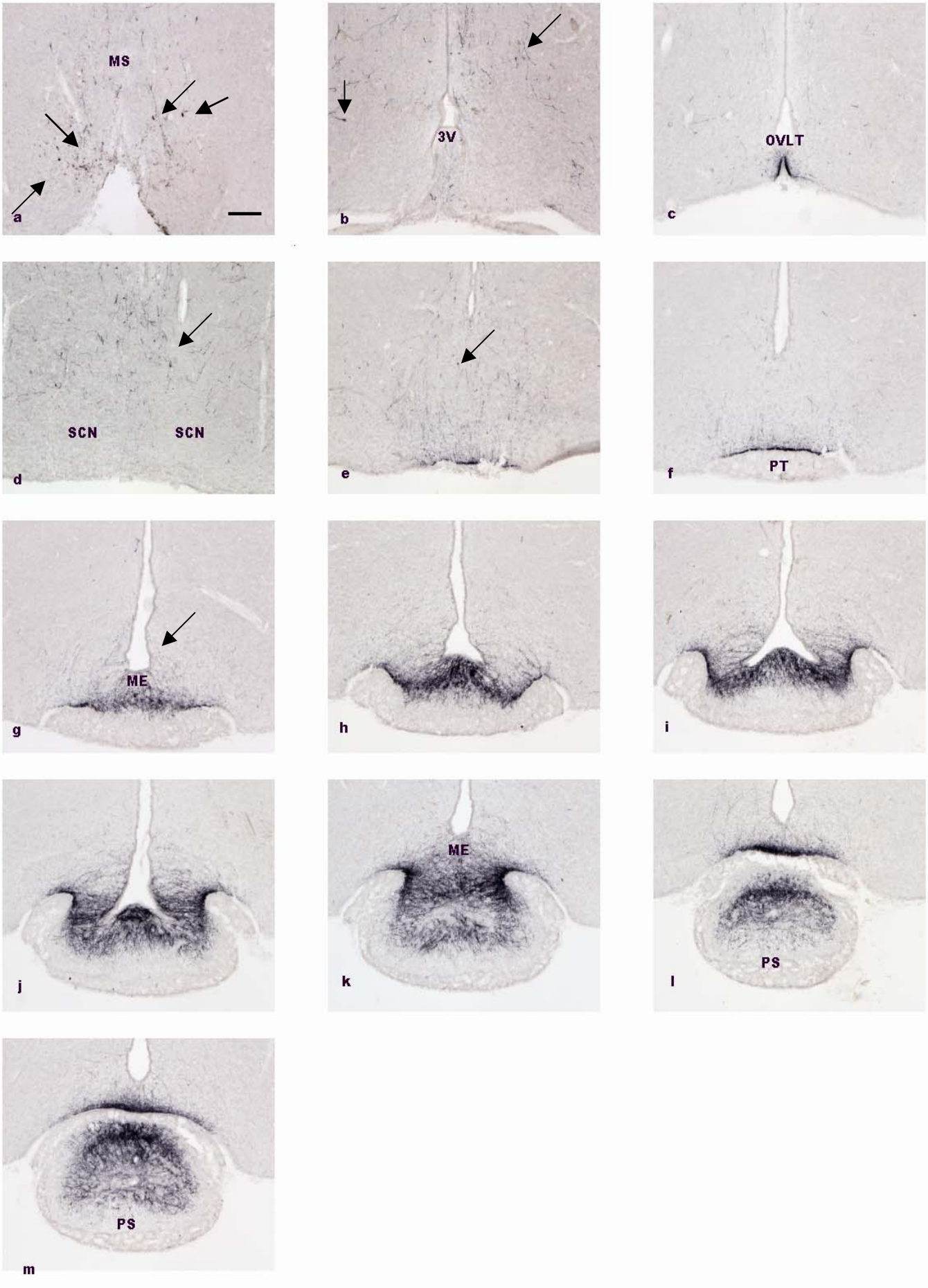
MS
a
3V
b
OVLT
c
SCN
SCN
d
e
PT
f
ME
g
h
i
j
ME
k
PS
l
PS
m

Figure 2. Coronal sections of the forebrain of a Cape dune mole-rat from rostral to caudal (a-m). The preoptic area is seen from slide a-d and the mediobasal hypothalamus from slide e-m. Arrows indicate individual GnRH neurons. GnRH cells and fibres are orientated dorso-ventrally in the medial septum (a) and surrounding the third ventricle (b). Strong interactions between GnRH fibres are seen in the region of the organum vasculosum of the lamina terminalis (OVLT) (c) and the median eminence (ME) (g-m). From the ME GnRH is secreted into the pituitary stalk (PS) (l-m) from where it regulates the production and secretion of luteinizing hormone. Scale bar = 100µm

GnRH cell body size: The average size of GnRH cell bodies varied between 63.30µm^2 and 103.04µm^2. There was no significant difference between males during (89.38µm^2 ± 4.39; n = 6) and out (84.05µm^2 ± 4.74; n = 6) of the breeding season ($F_{1, 11}$ = 0.68; p = 0.43). However, a highly significant difference was found between females during (89.15µm^2 ± 1.93; n = 8) and out (74.07µm^2 ± 2.23; n = 6) of the breeding season ($F_{1,12}$ = 26.13; p < 0.001).

DISCUSSION

The characteristic spindle shape of the GnRH cell bodies seen in the Cape Dune mole-rat is similar to that observed in other species (Robinson et al. 1997; Molteno et al. 2004 Du Toit et al. in press). Processes extending from the cell bodies were found to have a beaded appearance. Most GnRH cell bodies displayed processes however, the number of projections visible in a particular section depended on the orientation of the neurones and the plane of sectioning.

The general distribution of GnRH neurones in the Cape dune mole-rat closely resembles the distribution seen in other mole-rat species (Du Toit et al. in press, Molteno et al. 2004). GnRH immunoreactive neurones were distributed in a band along the septo-infundibular pathway (Figure 2). GnRH neurones were found in both the preoptic area and mediobasal hypothalamus. The relative number of GnRH cells in the preoptic area and mediobasal hypothalamus was similar in males and females irrespective of breeding season. In the Damaraland mole-rat and the highveld mole-rat, however, the percentage of cells in the mediobasal hypothalamus (15% and 13.5%, respectively) was lower than that found in the preoptic area (Molteno et al. 2004; Du Toit et al. in press). In contrast, in the common mole-rat, 56% of the GnRH cell bodies are found in the mediobasal hypothalamus (Du Toit et al. in press). The distribution of these neurones in the brain depends on their migration from the olfactory placode (Schwanzel-Fukuda and Pfaff 1989). In the opossum, *Monodelphis domestica*, very few GnRH cell bodies reach the preoptic area (Schwanzel-Fukuda et al. 1988), while in the mink approximately 80% of reach the mediobasal hypothalamus (Ntoumi et al. 1994).

In the Cape dune mole-rat, the two major aggregations of GnRH-immunoreactive processes were the OVLT and the ME. Radioimmunoassay (Wheaton et al. 1975) and immunocytochemistry (Baker et al. 1975) have shown that the greatest amount of GnRH is stored in the ME prior to release into the hypophyseal portal blood system.

The total number of GnRH neurons in both male and female Cape dune mole-rats did not differ between the breeding and non-breeding seasons. Similarly, studies on the Syrian hamster (*Mesocricetus auratus*) and the Djungarian hamster (*Phodopus sungorus sungorus*) have failed to show a difference in the number of GnRH neurones

between those seasons (Urbanski et al. 1991; Yellon 1989). Nevertheless, in the white footed mouse, *Peromyscus leucopus*, photoperiod-induced gonadal regression is associated with an increase in the GnRH content of the hypothalamus and an increase in the total number of GnRH cell bodies (Glass 1986; Petterborg 1981).

The total number of GnRH cell bodies calculated for the Cape dune mole-rat closely resembles that previously determined for other species such as rats (Silverman et al. 1994), primates (Silverman et al. 1982) and the springbok, *Antidorcas marsupialis* (Robinson et al. 1997). In contrast, in the Damaraland mole-rat the total number of GnRH cell bodies was smaller, approximately 650. Du Toit et al. (in press) found a similar number of cell bodies in the common mole-rat (640 cells), but the related highveld mole-rat displayed 1650 GnRH cells. As in the case of the highveld mole-rat the Cape dune mole-rat also has in excess of a 1000 GnRH cell bodies in the forebrain.

Male Cape dune mole-rats did not display significant differences in the size of GnRH neurons when compared between breeding and non-breeding individuals. Breeding female mole-rats, however, had significantly larger neurones than individuals out of the breeding season.

A recent study performed by Hart et al. (submitted) on the Cape dune mole-rat has shown that there is a significant difference in the basal levels of LH in males and females when comparing individuals in and out of the breeding season. Since LH levels are regulated by GnRH, differences in either the number or size of GnRH neurones could be expected. Nevertheless, no evidence of seasonal differences in the number of GnRH cell bodies was found in this species. In female Cape dune mole-rats seasonal difference with regards to the cell size most probably mediate seasonal differences in LH levels (Hart et al. submitted). Such speculation does not apply to the

males. It is possible that LH in males may be controlled by the selective release of GnRH from storage sites as opposed to a reduction or an increase in the production of GnRH from GnRH neurons.

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Conclusion

CONCLUSION

A study investigating the tooth wear and eruption patterns as well as the associated morphometric measurements of 22 skull parameters on 87 male and 100 female *B. suillus* revealed that sexual dimorphism was apparent in adult Cape dune mole-rat but that it only becomes pronounced with an increase in relative age and hence maturity of the mole-rat. One of the contributing factors to this finding may be related to the fact that males have to compete with rival males for reproductive opportunities. An increase in body size would thus be advantageous to a male, as it would increase the probability of out-competing a rival male in order to secure an opportunity for reproduction. This finding of increased sexual dimorphism with the age of the Cape dune mole-rats is the first study to highlight this differentiation in skull morphology.

The Cape dune mole-rat exhibits a marked seasonal reproduction that appears to be confined to months of good precipitation. This in essence makes sense, since it is only during these periods that the soil has optimal properties for burrowing and hence pairing up of these mole-rats for procreation. The post-mortem examination of 581 male and 734 female Cape dune mole-rats collected over an entire calendar year revealed from anatomical, histological and endocrinological data that the breeding season falls within the months of June to October. Macro anatomical measurements of the gonads showed that the gonads exhibited cyclical changes. In males, both the volume and the mass of the testes increased during the months of May to October. In females the ovarian volume increased from July to November. In contrast, the mass of the ovaries of females in general was reduced during this period. Since the testes of

males are predominantly composed of seminiferous tubules, an increase in the volume of these structures would thus necessitate an increase in the overall volume and mass of the testes. The ovaries of females in contrast are composed of follicles of varying stages and subsequent luteinization. Follicles are important in the production and subsequent release of the ova prior to fertilization and directly thereafter are composed of large fluid filled spaces. Since this fluid is believed to have a lower density than that of the ovarian tissue it is conceivable that an increase in the volume of the ovaries would thus lead to a subsequent decrease in the mass. A histological examination of the gonads showed that the diameter of the seminiferous tubule in males increased during the months of May to October. A clear trend in the proliferation of one follicular stage to another was also evident. Interestingly no significant difference was found in the number of Graafian follicles when comparing individuals during and out of the breeding season. Significantly more corpora lutea were present in the ovaries during the breeding season as opposed to out of the breeding season supporting the notion that the Cape dune mole rat is a seasonal breeder. Hormonal profiles for the period of a year for testosterone in males, and progesterone and oestradiol 17β for females revealed that although there are distinct fluctuations in the basal levels of these hormones, males display a more clearly defined profile compared to females. In hind sight this observation is logical since it is the males that are responsible for the onset of reproductive activity as they are the first of the two sexes to begin the process of hind foot drumming.

The reproductive physiology of the Cape dune mole-rat was further investigated by measuring the differences in the basal luteinizing hormone (LH) concentrations during the breeding period (August) and out of the breeding period (January) with a view to investigating potential opportunistic reproductive events in

this solitary mole-rat. The basal circulating concentrations of LH in both sexes were significantly elevated during the month of August compared to January, providing further evidence of a distinct breeding season in the Cape dune mole-rat. Moreover on challenging the pituitaries of both sexes both in and out of the breeding season with an exogenous gonadotrophin releasing hormone (GnRH) challenge the magnitudes of response of LH were found to be very similar during both the breeding and non-breeding period. We propose that in the Cape dune mole-rat it is possible that unpredictable rainfall patterns due to the El Nino southern oscillations, may have selected for plasticity of the hypothalamo-pituitary axis in this strictly seasonal mole-rat.

Finally a neuroanatomical study of the GnRH neuronal system revealed that there were no significant differences in the distribution of GnRH cell bodies between the medial preoptic area and the mediobasal areas for both sexes during and out of the breeding season. Similarly no significant differences were found in the total number of GnRH neurons between the different seasons for both sexes. Males did not show any difference in the cell body size between different reproductive periods of the year. Females in contrast showed a highly significant difference in cell body-size with those cell bodies during the breeding season being significantly larger than the cell bodies out of the breeding season.

In sum, it would appear that the Cape dune mole-rat from the Western Cape Province of South Africa is a seasonally breeding rodent mole that exhibits the pituitary potential to mate and produce offspring outside of the breeding season if favourable environmental conditions prevail. The differences in LH concentration in and out of the breeding season are not related to either disparate numbers of neurons or types of GnRH neurons.

It is quite possible that this species exhibits a marked polyandry in which one male may service several females and a female in turn may mate with numerous males. A population study currently underway investigating the gene flow in the population and paternity of specific litters of pups may further enlighten us into the fascinating reproductive system operational in the solitary subterranean rodent mole.

Appendices

APPENDIX I

SNK test performed on 8 age classes of *Bathyergus suillus*. Vertical lines denote non-significant differences between age classes. Groups with NS denote those groups where all age classes differed significantly from one another.

	AC(#)	Mean	SD	
NPP	II(5)	37.59	6.76	
	III(15)	42.13	3.43	
	IV(22)	47.28	3.29	|
	V(23)	49.72	3.10	|
	VI(25)	52.62	4.05	|
	VII(31)	55.15	5.65	|
	VIII(43)	57.23	5.49	|
	IX(23)	58.65	4.84	|
ZMB	II(5)	25.54	1.68	|
	III(15)	26.54	1.36	|
	IV(22)	28.90	1.60	|
	V(23)	30.25	1.20	|
	VI(25)	30.90	1.81	|
	VII(31)	31.63	2.69	|
	VIII(43)	32.58	2.04	|
	IX(23)	32.88	2.21	|
BCW	II(5)	19.95	0.75	NS
	III(15)	20.34	0.67	
	IV(22)	20.10	4.09	
	V(23)	21.40	0.70	
	VI(25)	21.39	0.91	
	VII(31)	21.59	1.42	
	VIII(43)	21.97	1.23	
	IX(23)	21.64	4.43	
IOB	II(5)	10.47	0.76	NS
	III(15)	10.17	0.40	
	IV(22)	10.23	0.48	
	V(23)	10.15	0.45	
	VI(25)	10.02	0.43	
	VII(31)	10.18	0.43	
	VIII(43)	10.18	0.42	
	IX(23)	10.48	0.41	

	AC(#)	Mean	SD	
MLT	II(5)	45.58	3.10	|
	III(15)	48.85	2.63	|
	IV(22)	55.73	4.06	|
	V(23)	58.30	3.34	|
	VI(25)	62.51	5.34	|
	VII(31)	64.52	6.38	|
	VIII(43)	68.29	7.03	|
	IX(23)	70.54	6.22	|
MDL	II(5)	32.97	2.11	
	III(15)	36.61	1.72	
	IV(22)	41.29	2.71	|
	V(23)	43.22	2.93	|
	VI(25)	45.50	3.47	|
	VII(31)	46.70	4.13	|
	VIII(43)	49.14	4.32	|
	IX(23)	50.14	3.45	|
MTR	II(5)	10.06	0.71	
	III(15)	10.88	0.80	
	IV(22)	11.98	0.47	|
	V(23)	12.15	0.49	|
	VI(25)	12.64	0.57	|
	VII(31)	12.27	2.28	|
	VIII(43)	12.69	0.63	|
	IX(23)	13.12	0.59	|
AFI	II(5)	6.12	0.52	|
	III(15)	6.06	0.53	|
	IV(22)	6.66	0.77	|
	V(23)	6.81	1.10	|
	VI(25)	7.06	0.83	|
	VII(31)	7.11	0.64	|
	VIII(43)	7.30	0.84	|
	IX(23)	7.51	0.91	|

ZYB	II(5)	30.85	3.15
	III(15)	32.16	1.48
	IV(22)	36.40	3.02
	V(23)	38.11	2.57
	VI(25)	41.03	2.92
	VII(31)	42.17	3.93
	VIII(43)	44.51	4.26
	IX(23)	45.42	3.57

AFA	II(5)	13.46	1.16
	III(15)	14.56	0.92
	IV(22)	15.95	1.21
	V(23)	16.62	0.97
	VI(25)	17.56	1.79
	VII(31)	18.02	2.09
	VIII(43)	19.21	1.94
	IX(23)	19.51	1.59

WR	II(5)	10.78	0.42
	III(15)	11.35	0.54
	IV(22)	12.15	0.60
	V(23)	12.50	0.67
	VI(25)	13.13	0.64
	VII(31)	13.24	0.90
	VIII(43)	13.64	0.76
	IX(23)	14.13	0.61

MAP	II(5)	9.81	0.65
	III(15)	10.98	0.77
	IV(22)	12.14	0.92
	V(23)	12.13	0.98
	VI(25)	12.88	1.33
	VII(31)	13.56	1.72
	VIII(43)	14.39	1.57
	IX(23)	14.54	1.42

NA	II(5)	9.05	0.99
	III(15)	9.20	0.75
	IV(22)	9.88	0.89
	V(23)	10.45	0.61
	VI(25)	10.82	0.91
	VII(31)	11.01	0.92
	VIII(43)	11.45	1.02
	IX(23)	11.76	1.01

MRH	II(5)	18.91	1.58
	III(15)	21.28	1.45
	IV(22)	23.58	1.60
	V(23)	25.14	1.58
	VI(25)	26.28	3.65
	VII(31)	27.83	3.24
	VIII(43)	29.21	3.16
	IX(23)	29.30	2.62

WI	II(5)	2.89	0.36
	III(15)	3.03	0.29
	IV(22)	3.32	0.25
	V(23)	3.51	0.31
	VI(25)	3.77	0.31
	VII(31)	3.95	0.51
	VIII(43)	4.10	0.44
	IX(23)	4.18	0.24

GLS	II(5)	46.44	3.49
	III(15)	48.28	9.72
	IV(22)	56.31	3.98
	V(23)	59.45	3.73
	VI(25)	62.80	5.07
	VII(31)	65.16	6.86
	VIII(43)	68.27	7.10
	IX(23)	70.02	6.07

PAC	II(5)	5.57	0.37
	III(15)	5.74	0.29
	IV(22)	5.94	0.49
	V(23)	6.21	0.53
	VI(25)	6.25	0.73
	VII(31)	6.39	0.59
	VIII(43)	6.23	0.58
	IX(23)	6.59	0.63

ITC	II(5)	46.23	3.26
	III(15)	47.34	9.37
	IV(22)	55.43	3.76
	V(23)	57.64	3.79
	VI(25)	61.28	4.50
	VII(31)	63.36	6.31
	VIII(43)	66.48	5.76
	IX(23)	68.28	5.37

UTR	II(5)	9.21	0.87
	III(15)	10.14	0.70
	IV(22)	10.93	0.48
	V(23)	11.16	0.47
	VI(25)	11.24	0.44
	VII(31)	11.39	0.84
	VIII(43)	11.43	0.69
	IX(23)	11.70	0.68

UJI	II(5)	7.73	0.98
	III(15)	9.46	1.31
	IV(22)	10.89	1.75
	V(23)	11.15	1.34
	VI(25)	12.79	1.89
	VII(31)	12.59	2.37
	VIII(43)	12.80	3.26
	IX(23)	14.93	3.47

LJI	II(5)	15.89	2.74
	III(15)	15.51	2.54
	IV(22)	19.32	2.58
	V(23)	19.49	1.53
	VI(25)	20.93	2.93
	VII(31)	22.39	3.13
	VIII(43)	23.88	4.02
	IX(23)	25.60	4.27

GHS	II(5)	16.63	0.64
	III(15)	17.22	1.56
	IV(22)	17.61	1.06
	V(23)	18.12	0.89
	VI(25)	18.11	1.33
	VII(31)	18.86	1.52
	VIII(43)	19.45	1.39
	IX(23)	19.17	1.23

APPENDIX II