Chapter 1

General Introduction

The Bathyergidae

The family Bathyergidae (derived from the Greek *bathys* meaning deep and *ergo* to work) comprises subterranean hystricomorph rodents that are endemic to the continent of Africa. The family shows a wide spectrum of social structure ranging from strictly solitary species such as those belonging to the genera *Bathyergus*, *Heliophobius* and *Georychus* through to social and truly social species in the genera *Cryptomys*, *Fukomys* and *Heterocephalus* (Jarvis and Bennett, 1990; 1991).

The bathyergids occupy a range of habitat types from mesic through to arid environments (Jarvis & Bennett, 1990). However, a common underlying prerequisite for their occurrence is the presence of the underground storage organs of geophytes, for which there is a propensity in Africa (Jarvis & Bennett, 1991; Faulkes *et al*., 2004). In mesic regions the soil is workable for much of the year and thus there is essentially no necessity for mole-rats to be social, however, in more arid regions the rainfall is sporadic and unpredictable and in such instances there are few opportunities available to successfully burrow towards the food resources. These brief windows of opportunity require a number of mole-rats to excavate and tunnel to locate and harvest sufficient food resources.

Indeed, sociality in the African mole-rats has been hypothesized to have evolved in response to patterns of rainfall, the subsequent effects on food distribution and size and the consequent costs and risks of foraging (Jarvis *et al*., 1994; Faulkes *et al*., 1997; Bennett & Faulkes, 2000; Burda *et al*., 2000).

The Aridity Food Distribution Hypothesis (AFDH) has been put forward in an attempt to explain how sociality in African mole-rats may have evolved among some mole-rat species but not in others. The premise is that cooperative behaviour arose in some mole-rat species where the energetic costs of burrowing increase as the rainfall pattern becomes more unpredictable and the available food resource more clumped (Jarvis *et al*., 1994). Although Burda *et al.,* (2000) argued against a causal relationship between cooperative foraging for food resources and the evolution of sociality in mole-rats [see Faulkes & Bennett (2007)] and O'Riain & Faulkes, (in press) for further commentary and debate), the AFDH has support from a number of empirical studies including long term field studies and associated molecular studies (Faulkes *et al*., 1997; Jarvis *et al*., 1998; Burland *et al*., 2002, Hess, 2004).

Sociality in mole-rats

The solitary species of southern and eastern African mole-rats (*Georychus* and *Bathyergus*, *Heliophobius*) generally inhabit environments that exhibit a marked seasonality or higher rainfall pattern (Jarvis & Bennett, 1990, 1991; Bennett & Faulkes, 2000; Šumbera *et al.*, 2003a). A number of species of mole-rat that exhibit some degree of social behaviour may also occur in these mesic environments, but also in drier areas (Spinks *et al.*, 1999; Janse van Rensburg *et al*., 2002). The truly social mole-rats occur in semi-arid and arid regions where rainfall is sporadic and unpredictable and hence the times available for successful foraging are restricted and a large workforce is required to sufficiently excavate the tunnel system to locate these patchily distributed food resources.

The species belonging to the genus *Cryptomys* (restricted to southern Africa) are generally composed of small family groups that incorporate up to three successive litters (Bennett, 1989). Colonies are usually around 8-10 animals and are transient in nature, with animals exhibiting regular periods of dispersal from the natal colony Spinks, (1998). The use of microsatellite libraries have revealed that multiple paternity of litters and extra-pair copulations are common in these environments where ecological constraints are not great Bishop *et al*., (2004). In the more highly social mole-rat species *Fukomys damarensis*, colonies have been found to number up to 40 animals but are usually around 12 -14, with reproduction restricted to small cohorts of breeding animals that constitute a single reproductive female and a small group of reproductive males, which may or may not be present in the colony Burland *et al*., (2004).

Burrow structure

Mole-rats are herbivorous and feed mainly on swollen tubers and underground storage organs of geophytes which are encountered and harvested during burrowing activity. Underground storage organs are an exceptional food resource to harvest since they have a long shelf life, they can be obtained throughout the year, are biologically stable and there is little competition from other consumers (Bennett & Faulkes, 2000). Geophytes which are harvested by mole-rats may be either consumed *in situ* or else they are stored in carefully sculptured food chambers which are periodically visited by mole-rats.

A number of studies have examined burrow architecture within various species of mole-rat (Hickman, 1977; Davies & Jarvis, 1986; Spinks *et al*., 2000a; Šumbera *et al*., 2003c; Herbst

& Bennett, 2006). The majority of these studies have concentrated on solitary species in which there is only plural occupancy of the burrow during the breeding season or when the mother has young. In general the burrow systems of solitary species are relatively simple being linear in nature with some sex differences in architecture [see Herbst *et al*., (2004)]. Relatively few studies have examined the burrow structure of social mole-rats, and where this has been done the sample sizes are typically very small. Spinks *et al*., (2000a) examined seven burrow systems of the common mole-rat, *Cryptomys hottentotus hottentotus* at two different trapping localities, whereas Le Comber *et al*., (2002) examined 25 burrow systems from seven species of which three were social (viz. *C. h. hottentotus*, *Fukomys darlingi* and *Heterocephalus glaber*). The burrow architecture of social mole-rats shows a marked complexity with numerous tunnels radiating off from a central nest area [see Spinks *et al*., (2000a)].

There is absolutely no information pertaining to the changes that result in burrow architecture between the rainy and the dry seasons in social mole-rats. The only known study to investigate seasonal changes in burrow architecture is that of Šumbera *et al*., (2003c) who documented significant changes in the burrow systems of the solitary silvery mole-rat, *Heliophobius argenteocinereus*.

Burrow fractal dimensions

Most studies of burrow architecture have examined metrics ranging from burrow shape, burrow system area, number of segments, linearity, turn angle, number of branches, segment length and branch length, (Romañach & Le Comber, 2004; Romañach *et al.*, 2005; Le

Comber *et al.*, 2006). However, due to the lacking of clarity in variables, recent studies have begun to use fractal dimension to provide a single measure sure of shape that has the advantage of being independent of burrow length Le Comber *et al.*, (2006). Fractal dimension is a formidable choice of measure of burrow shape because it is mainly a measure sure of the extent to which a one dimensional structure fills a plan with low fractal dimension (close to 1.0), describing a burrow that explores relatively little of surrounding area and high fractal dimension (close to 2.0) describing a burrow which explores the surrounding are more thoroughly Le Comber *et al*, 2006. In addition, it is an important tool in burrow system analysis because it further examines the relationship between burrow dimensions and demonstrates that burrows with high fractal dimension are more successful in locating food (Le Comber *et al.* 2002; Le Comber *et al.*, 2006). This detailed study in a single social mole-rat clarifies the assumptions underlying the Aridity Food Distribution Hypothesis (AFDH).

Colony size

In the social mole-rats the size of a colony is dependent upon a number of factors, the size of the territory held by a colony, the degree of risk that the colony members face if attempting to disperse and establish their own colonies, the rainfall pattern and the time the substratum is workable. Colony size also does not remain static but is dynamic, with immigration and emigration occurring under suitable burrowing conditions. Usually following good rainfall, some colony members disperse and colony size is reduced or comprises newly established nascent pairs (Jarvis & Bennett, 1993).

In general the naked mole-rats of eastern Africa set the record with the largest colonies with a mean of around 80 animals from wild captured colonies (Brett, 1986), however, there are rare occasions of when up to three hundred individuals have been captured from a single burrow system, but these are normally in agricultural areas where abundant rootstocks are available Brett, (1986). In the genus *Fukomys*, colony size ranges from small groups of around 5 individuals (*Fukomys darlingi*) through to colonies exceeding 40 individuals (*Fukomys damarensis*), but colonies rarely exceed 20 individuals (Jarvis and Bennett, 1993; Bennett & Faulkes, 2000). In the genus *Cryptomys* colonies rarely exceed 14 animals in size (Bennett, 1989; Janse van Rensburg *et al.*, 2002). Spinks (1998) examined colony size in two populations of the common mole-rat, *Cryptomys hottentotus* that inhabit different regions with contrasting rainfall. Spinks (1998) found that the degree of aridity of the habitat did not affect the mean (5 animals) or range (2-14) of the colony size. The giant mole-rat is interesting in that it occurs in a mesic habitat where rainfall is high and predictable. Consequently it is hypothesized that colonies of these mole-rats should be smaller in size than their arid, but similar in size to their mesic counterparts of southern Africa.

Colony composition

The use of tooth-wear on its own in mammals has been shown to be a poor ageing method (Hall *et al.* 1957; Keiss, 1969; Morris, 1972). However, by studying a single population where diet is fairly uniform the application of tooth-wear on the cusps of the molariform teeth can be used as a relative rather than absolute variable for ageing a population. The time of tooth eruption is less variable and can serve as a valuable marker of relative age. This method can in conjunction with skull morphometrics be used to investigate potential sexual

dimorphism and age in populations of mammals. Indeed, Bennett *et al*., (1990) successfully used this method to investigate sexual dimorphism in two species of social mole-rat, they found that within colonies it was absent in the common mole-rat, *C. h. hottentotus* but distinctly marked in the Damaraland mole-rat, *F. damarensis*.

Colonies of the social mole-rats belonging to the genera *Cryptomys*, *Fukomys* and *Heterocephalus* are generally familiar with some immigrants that have subsequently joined the colony over time. There is usually one, but very rarely two reproductive females that are responsible for the production of young that are recruited to the natal colony (Bennett & Faulkes, 2000). There may be between one to three putative reproductive males, these are usually the founding members of colonies (Bishop *et al*. 2004; Burland *et al*., 2002; Burland *et al*., 2004). A number of successive litters are incorporated into the colony of varying relative age structure, some of which may have opportunities to disperse and establish their own colonies (Bennett *et al*., 1990; Janse van Rensburg *et al*., 2004).

Reproductive strategies in the Bathyergidae

Reproduction in mole-rats is ecologically constrained by the burrow environment (Jarvis & Bennett, 1990). In solitary dwelling mole-rats reproduction is predominantly seasonal. Instead of cueing into photoperiod the mole-rats use changes in temperature, changes in soil moisture content or sudden flushes of vegetation associated with precipitation to herald the onset of reproduction (Bennett, Gutjahr & Faulkes, 2007). Two social species of mole-rat show a marked seasonal component to reproduction. The common mole-rat, *Cryptomys hottentotus hottentotus* and the highveld mole-rat, *Cryptomys hottentotus pretoriae* are the

only two social species of bathyergid studied to date that exhibit a marked seasonality of reproduction (Spinks *et al*., 1997; Janse van Rensburg *et al*., 2002, 2003). These two species inhabit environments which are relatively mesic and have a predictable season of rainfall. In contrast, members of the genera *Fukomys* and *Heterocephalus* do not show a seasonal component to reproduction and produce young throughout the year (Bennett *et al*., 1991, 1994, Bennett & Aguilar, 1995). The majority of these findings have come from laboratory based studies where colonies of mole-rats have been housed under artificial lighting conditions and fed on *ad libitum* diets. To date there is a paucity of information that has arisen from field studies where postmortems have been performed on entire colonies throughout the calendar year (Bennett, Faulkes & Molteno, 2000).

Patterns of ovulation in the Bathyergidae

Ovulation is essentially achieved through two methods either spontaneously (seasonally or continuously throughout the year) or by induction following mating behaviour and subsequent coitus (Milligan, 1980). The solitary species of mole-rats essentially employ induced ovulation where mechanical stimulation of the vagina and cervix by the penis of the male is essential for ovulation to take place. The solitary male mole-rats are characterized by a penis that has elaborate epidermal spines on the shaft and glans Parag *et al*., (2006). Interestingly, the loosely social mole-rats of the genus *Cryptomys* exhibit induced ovulation (Malherbe *et al*., 2004a; Jackson & Bennett, 2005) and the males possess penile protrusions (rounded, raised structures) rather than spines over the entire surface of the penis Parag *et al*., (2006). In the two eusocial species *Fukomys damarensis* and *Heterocephalus glaber* the penis lacks spines and protrusions altogether and possesses smooth ridges along the length

of the penis. Both of these eusocial species exhibit spontaneous ovulation (Faulkes *et al*., 1990; Snyman *et al*., 2006). As a result of the extreme natal philopatry that is experienced in these social mole-rats it may be of no selective advantage for induced ovulation and as a consequence not necessity for elaborate ornamentation to the penis Parag *et al*., (2006).

The giant mole-rat, Fukomys mechowii

The giant mole-rat which was originally placed in the genus *Georychus*, is one of the largest of the social species and has the lowest chromosome number 2n=40, FN = 80 of all the mole-rats examined to date Macholan *et al*., (1993). It is a large stout mole-rat and is the largest species in the genus *Fukomys*. The pelage is short and dense in nature with a colour that is age dependent being dark slate grey (neonates), through to greyish-brown (weaned young), brown (juveniles and sub-adults) and golden ochre (adult animals). The head is large and may possess a white spot of forehead (except very small spot in some individuals). The giant mole-rat has a wide distribution being endemic to central Africa. The mole-rats are recorded from N. Zambia, S. and E. Zaire, central Angola (and perhaps N. Malawi). The mole-rats are found in Savanna bush-land, cultivated and abandoned fields, gardens, dambos (temporary swamps) and dense *Acacia* woodland. They burrow in a variety of soil types from quite stony to pure sand and clay. The area of distribution is characterized by an annual rainfall of more than 1,100 mm Scharff *et al*., (2001).

The giant mole-rat excavates extensive burrow systems comprising a deep nest (60-160 cm deep) with 3 or 4 entrances, food stores and toilet areas Scharff *et al*., (2001). Giant mole-rats have a low resting metabolic rate 0.6 $\pm$ 0.08 $cm^3O_2g^{-1}h^{-1}$ (96% of expected), low body

temperature 34 ± 0.4°C, and a low thermal conductance 0.09 ± 0.01 $cm^3O^2g^{-1}h^{-1o}C^{-1}$ (Bennett *et al*., 1994).

Food in non-cultivated areas includes grass rhizomes, roots, bulbs and tubers of diverse weeds, shrubs and trees; in cultivated areas; they probably feed on crop plants such as sweet potatoes, cassava and groundnuts Scharff *et al*., (2001]. Giant mole-rats are unusual amongst bathyergids, because they supplement their diet with invertebrate and vertebrate commensals found in their burrows (Burda & Kawalika, 1993; Scharff *et al*., 2001).

The mole-rats occur in colonies ranging from 2- 20 colony members strong. It has been suggested that the colonies can exceed 40 or more individuals (Burda & Kawalika, 1993; Scharff *et al*., 2001). Sex ratio within colonies appears to be in favour of females Scharff *et al*., (2001). The colony comprises a founding reproductive pair and non-reproductive offspring from several litters (Burda & Kawalika, 1993; Wallace & Bennett, 1998; Scharff *et al*., 2001). The reproductive animals are the most dominant, and the non-reproductive males are more dominant than females. The non-reproductive members of the colony cannot be placed into clearly defined work-related groups based on body mass (Wallace & Bennett, 1998). Giant mole-rats are very vocal compared to other species of *Cryptomys* and *Fukomys* (Burda & Kawalika, 1993; Credner *et al*., 1997).

Giant mole-rats appear to breed aseasonally from laboratory and fragmentary field observations, producing up to three litters per annum (Burda & Kawalika, 1993; Bennett & Aguilar, 1995; Scharff *et al*., 1999). The gestation period is long 112 (89-118) days. Mean

litter size is around 2-3 (range 1-4). At birth, young weigh around 19.6 (12.6-27.7) g. First solid foods eaten ca. Day 14 but the pups are weaned ca Day 90. Inter-sibling sparring begins at Day 10 (Bennett & Aguilar, 1995; Scharff *et al*., 1999).

Apart from humans, no predators are known which have specialized on giant mole-rats. Ectoparasites have not been found on animals or in the nests of giant mole-rats. Endoparasties include three species of cestodes (*Inermicapsifer madagascariensis*, *Raillietina* sp.,) and two species of nematodes (*Protospirura muricola*, *Cappilaria* sp.). Endoparasitic load (34%, n = 35) is relatively low compared to most other rodents (Scharff *et al*., 1997; Scharff *et al*., 2001).

Plate 1.1 The giant mole-rat *Fukomys mechowii*

Aims of study

The giant mole-rat (*Fukomys mechowii*) is one of the little studied species of bathyergid in sub-Saharan Africa, with earlier research focusing on the general biology and some laboratory studies on the sociality and reproduction based on small sample sizes with observations from few colonies. Many of the documented reports on colony composition of the giant mole-rat are based on hear say from local hunters.

The study first focused on providing a description of the general burrow architecture of the giant mole-rat from 32 burrow systems excavated in their entirety and the colony composition of the colonies captured during excavation. On average 3 burrows were excavated monthly for a period of one year. Fractal analysis was employed on the mapped burrow systems to answer three general questions relating specifically to the assumptions of the AFDH: (1) Is burrow fractal dimension higher in the rainy season, as might be predicted if either or both the energetic costs of digging, or differences in patterns of food distribution, vary between seasons? (2) Do larger colonies have burrows with higher fractal dimensions? This might be the case if, as the AFDH suggests, foraging is more efficient in larger, cooperatively foraging colonies. (3) Is higher fractal dimension associated with a greater mass of food in the burrow? (Le Comber *et al.,* 2006) showed, using computer simulations, that burrows with high fractal dimension located more food; here, we test whether this is reflected in larger food stores in a natural situation.

Postmortem examination of entire colonies collected during the project were used to investigate the mean colony size, relative age structure based on tooth wear and eruption

patterns of entire colonies over a period of one year. Furthermore, cranial morphometric analyses were performed to ascertain whether (i) the species is sexually dimorphic and (ii) if sexual dimorphism is evident does it arise early in the life history or following maturation and the attainment of adulthood. Using the animals collected from the study we further investigated whether the giant mole-rat is an aseasonal or seasonal breeder. The final component of the project investigated whether ovulation in non-reproductive females removed from the confines of the colony results from spontaneous or induced ovulation.

Chapter 2

Materials and methods

Study site

Locations

The study was undertaken over a period of 10 months from September 2005 to June 2006, on two adjacent farms, Kakalo and Mushishima, located 25km south of Chingola in the Copper Belt Province of Zambia ($10^{o}40$'S and $20^{o}85$'E). A total of 32 discrete burrow systems, out of c. 80, were excavated from areas of disturbed natural vegetation, that is, natural vegetation that is disturbed intermittently by agricultural practices. The mean (±SD) annual rainfall at Chingola is 1029.9 ± 233.8mm/year^{-1}, with 6.05 ± 0.94 months/year^{-1} with more than 25mm of rain [the amount required to soften the soil at the depth of foraging tunnels (Jarvis *et al*., 1994)].

Study animals

Burrow architecture

Mole-rat burrow systems were located by the presence of discrete rows and groups of molehills on the surface. The distance between individually excavated colonies was deliberately maintained at 1000m to ensure that they represented separate burrow systems. The burrow systems were excavated in agricultural fields, grassland and dambos (shallow wetlands) within a variety of soil types ranging from clay to sandy loams. On average, 3 burrow systems were excavated every month using the hoe method Jarvis, (1991b) and the burrow was traced for its entire length. Capturing of mole-rats continued until the entire burrow system was excavated. The burrows were measured using a tape measure and string and plotted on graph paper relative to the magnetic north. The dimensions, depth and contents of the nesting areas, food stores and toilet chambers were also recorded.

Burrow excavations were assigned to either the rainy season or the dry season, according to the observed onset/end of the annual rains. The transition between seasons is abrupt and unambiguous and in some cases burrows that were excavated in the same month (November 2005; April 2006) were assigned to different seasons. The animals were captured by manually digging out the burrows leading to the centrally positioned nest, food stores and latrines. In most cases, the mole-rats retreated into the bolt holes (blind ending tunnels) from where they were captured; as the animals emerged, the tunnel was blocked and mole rats were captured by hand. Burrow systems took between 3-6 days to be excavated in their entirety. The positions of all tunnels and chambers were surveyed and mapped for each burrow.

Burrow fractal dimensions

To quantify burrow shape in a way that was independent of size, we used fractal dimension, which offers a useful measure of the extent to which a burrow explores the surrounding area, especially in fossorial mammals in which the burrow is used for foraging (Le Comber *et al*., 2002, 2006; Šumbera *et al*., 2003a; Romañach & Le Comber, 2004). Burrow fractal dimension was estimated by calculating the box-counting or capacity dimension, DB (Block *et al.*, 1990), using methods adapted from Le Comber *et al*., (2002) and Romañach & Le Comber (2004). Burrow maps were photocopied to a standard size, h (256mm along the longest axis), and redrawn to ensure constant line thickness. Taking h=256mm, grids of h/20, h/21, h/22, h/23 . . . h/27 (the practical limit of resolution) were superimposed on the burrow maps, and the number of grid squares covering the burrow was counted for each grid size in turn. Thus, the grid size was successively halved, from a maximum of 256mm to a minimum of 2 mm. For a straight line, as the grid size is halved, the number of grid squares

required to cover the burrow increases by a factor of 21; that is, it doubles. For a plane, the number of grid squares increases by a factor of 22=4. This can be represented by regressing log N(e) against-log (1/e), where e is the length of the grid square and N(e) is the number of squares of length e required to cover the image, with the fitted line constrained to pass through the origin. For a perfectly linear structure, the slope of this line equals 1; for a perfectly planar structure, the slope equals 2. Thus, the slope of the fitted line corresponds to the line's fractal dimension. Variation in the depth of different parts of the burrows was ignored; this tends to be slight in comparison with burrow length (Bennett & Faulkes, 2000).

Aseasonality

Climatology, animal composition and classification

Zambia has 3 major seasons: (i) a cold dry season from mid-May to July; (ii) a hot dry season from August to October; and (iii) a wet season stretching from mid-November through April, sometimes extending to the second week of May. From the first to second week of November, just before rains commence, a minor dry/cool season occurs. Our sampling period thus covered all three seasons.

A total of 32 colonies comprising 317 animals were collected following complete excavation of the burrow systems. On capture, mole-rats were euthanased using chloroform (see 'Ethical Note'), weighed, toe-clipped for use in separate genetic studies and sexed. Their likely breeding status was determined, with breeding females identified by elongated teats and a perforate vagina and breeding males characterized by a stained mouth area, prominent, bulging abdominal testes and large body size (Bennett & Aguilar, 1995). Animals were categorised into three age classes based on laboratory studies of growth

(Bennett & Aguilar, 1995) as follows: (i) juveniles (weighing from 1-100g, aged approximately 100-150 days); (ii) sub-adults (101-200g, aged approximately 1 year); or (iii) adults (201g and above, aged approximately 1 year and above). Body masses of adults were compared statistically using a *t* test. After dissection, the genetic materials of kidney, liver and heart were sampled and stored in 70% Ethanol. The number of foetuses per pregnant female and juveniles per every colony were recorded throughout the capturing period.

Sexual dimorphism and age variation

Tooth-wear age classes and cranial measurements

Out of a total of three hundred and seventeen (317) animals captured, two hundred and sixty five (265) of which 151 were females and 114 males had undamaged skulls, on which the tooth-wear and eruption and cranial measurements were based. Molar teeth for animals were thoroughly cleaned and micrographs made for the assessment of age variation (Fig. 2.1). Twenty two (22) cranial measurements were also taken for the examination of sexual dimorphism of the animals (Fig. 5.2).

The estimation of relative age was adopted and modified from Janse van Rensburg *et al.* (2004) and Hart *et al.* (2007) as follows: *Tooth-wear class 1* – only two cheek teeth fully erupted; an emerging third cheek tooth still in a cavity; little sign of tooth-wear on teeth; *Tooth-wear class 2* – three cheek teeth fully erupted; only the first two cheek teeth showing signs of tooth-wear; *Tooth-wear class 3* – three cheek teeth fully erupted; an emerging fourth cheek tooth still in a cavity; *Tooth-wear class 4* – three cheek teeth fully erupted, with the fourth cheek tooth starting to surface; *Tooth-wear class 5* – all four cheek teeth erupted

with no sign of tooth-wear on the fourth cheek tooth; *Tooth-wear class 6* – all four cheek teeth erupted; fourth cheek tooth showing little signs of wear; *Tooth-wear class 7* – all four cheek teeth erupted; fourth cheek tooth showing a fair amount of wear; *Tooth-wear class 8* – all four cheek teeth erupted, with deeply scooped dentine on all four cheek teeth; and *Tooth-wear class 9* – all four cheek teeth erupted with deeply scooped dentine on all four cheek teeth that are also deformed and reduced in height due to heavy tooth-wear (Fig. 2.1).

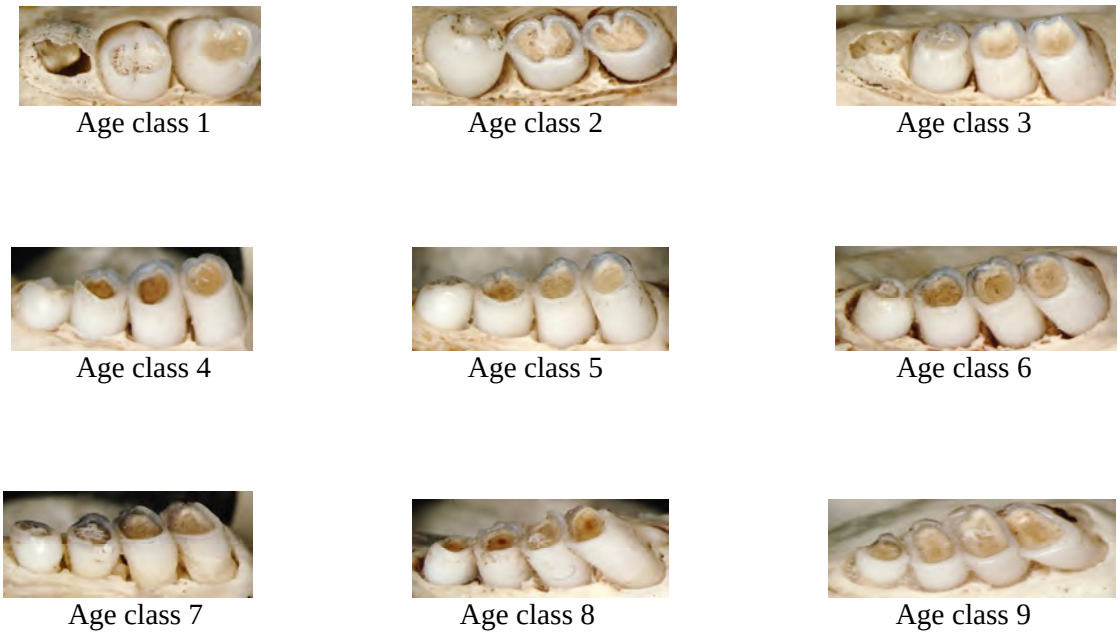

Figure 2.1. Right maxillary molar tooth row of the giant mole-rat *Fukomys mechowii* illustrating nine tooth-wear classes as adopted and modified from Janse van Rensburg *et al*., (2004) and Hart *et al.*, (2007) and described in the text.

The assessment of craniometric sexual dimorphism and variation in relative age within the giant mole-rat was based on 22 linear cranial measurements adopted and modified from Janse van Renseburg *et al.*, (2004) and Hart *et al.*, (2007) (Fig. 2.2). All cranial

measurements were recorded by a single observer (AMS) to the nearest 0.05mm using a pair of Mitutoyo digital calipers (Mitutoyo American Corporation, Aurora, Illinois, USA.).

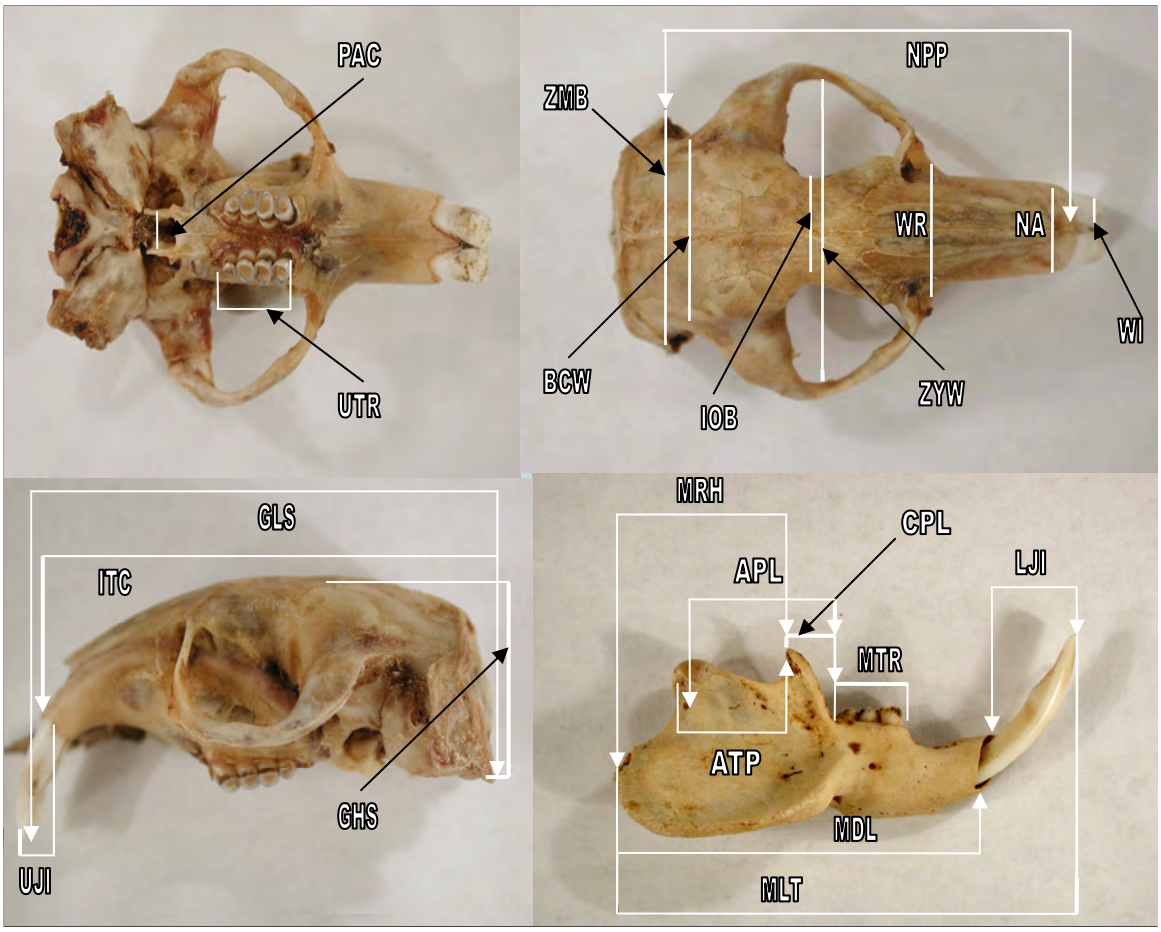

Figure 2.2. Abbreviations and reference points of 22 cranial measurements used in the present study as adopted and modified from those defined and illustrated by Janse van Rensburg *et al*., (2004) and Hart *et al.*, **(**2007) : (1) GLS – greatest length of skull, from the tip of the front of the incisors to the posterior part of the skull; (2) ITC – incisor to condyle length, from the anterior surface of the incisor at alveolus to the most posterior projection of the occipital condyle; (3) BCW – widest measurement of braincase breadth; (4) ZMB – zygomatic breadth - parietal width, (5) ZYW – greatest zygomatic width, between outer margins of the zygomatic arches, perpendicular to longitudinal axis of the skull; (6) IOB –

least breadth of the interorbital constriction; (7) WR – width of the rostrum ; (8) NA – anterior width of nasal where it joins with the premaxillae; (9) UTR – crown length of maxillary tooth row, from the anterior edge of the first molar to the posterior edge of the last molar; (10) PAC – hard plate width at point of constriction immediately posterior to the last molar; (11) NPP – the distance from anterior edge of nasals to the anterior edge of posterior part of the zygomatic arch; (12) GHS – greatest height of skull, perpendicular to horizontal plane through bullae; (13) MLT – greatest length of mandible, including teeth, from the posterior surface of condylar process to the tip of incisor; (14) MDL – greatest length of mandible (excluding teeth), from the posterior surface of condylar process to anteroventral edge of the incisor alveolus; (15) MTR – mandibular toothrow length, from the anterior edge of the first molar alveolus to the posterior edge of the last molar aveolus; (16) CPL-coronoid process length to posterior edge of fourth molar; (17) ATP – articular process length, from the ventral edge of mandibular foramen to mid-posterodosal edge of the coronoid process; (18) APL – angular process length of the middle mandible; (19) MRH – mandibular-ramus height, from the dorsal edge of the coronoid process to the ventral edge of angular process; (20) UJI – upper jaw incisor length, measured from the tip of the incisors to the base, where the teeth connect to the skull; (21) LJI – lower jaw incisor length, measured from the tip of the incisor to the base, where the teeth connect to the skull; and (22) WI – width of the incisor where the incisor meets the premaxilae.

Exploratory analyses of the derived craniometric measurements revealed the data to be normally distributed. The nature and extent of sexual dimorphism and age variation were first simultaneously univariately assessed by a two-way (ANOVA Zar, 1996) of samples of age classes 1–9 after establishing that tests for normality and homogeneity of variances satisfied the assumptions of ANOVA tests Zar, (1996). Where statistically significant age differences were detected by the ANOVA, non-significant subsets ($P > 0.05$) were identified by the *post hoc* Student-Newman-Keuls test (SNK; Gabriel & Sokal, 1969; Sokal & Rohlf, 1981) of ranked means. The derived two-way ANOVA table was in turn used to estimate the

%*SSQ* of the four potential sources of variation in the data, namely, sex, age interaction, and error (= residual) by dividing the *SSQ* associated with each source of variation by the total SSQ.

The nature and extent of sexual dimorphism and age variation within *F. machowii* was also multivariately assessed by an unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis and principal component analysis (PCA) of standardized variables (Sneath & Sokal, 1973). UPGMA cluster analysis was based on Euclidean distances and correlation coefficients among groups, while the PCA was based on correlation coefficients among variables (Sneath & Sokal, 1973). Additional analyses included the computation of standard descriptive statistics. Since exploratory analyses showed that data on body mass were not normally distributed, body mass data within sexes and age classes were evaluated using the non-parametric Mann-Whitney *U* test Zar, (1996). All statistical analyses in the present study were based on all the 22 cranial measurements recorded, and were undertaken using the statistical software programme STATISTICA, version 8.0 (STatTSoft Inc. 2008).

Ovulation methods

Experimental design, Creatinine and Progesterone determination

The mole-rats used in the study were separately trapped from two farms, Kakalo and Mushishima, located 25 km south of Chingola in the Copper Belt province of Zambia (10^{o} 40'S and 20^{o} 85'E). Animals were trapped by the hoe method (Bennett & Faulkes, 2000). All females used in the study had a mass exceeding 250g and males had a mass exceeding

350g to assure sexual maturity. Six non- reproductive females were collected from 6 different colonies, twelve adult males were also collected from 6 different colonies 1000m apart. Non-reproductive females were discerned by their non-perforate vagina and used for the experiment. Animals were then shipped to Pretoria and housed in a temperature-controlled room maintained at between 26 and 28^{o}C with a relative humidity of between 50 and 60% and a lighting regime of 12L: 12D. Wood shavings were used as nesting material. Mole-rats were fed sweet potatoes, chopped gem squash and apple daily; no free water was provided since they obtain all of their water requirements from the food resource to maintain a positive water balance.

Experimental manipulations and collection of urine took place from October 2006 until August 2007. In captivity, 6 non-reproductive females removed from their natal colonies were housed individually in plastic containers 1x0.5x0.5m, without a male for 18 weeks from 4th October, 2006 to 7th February, 2007. This same period included the first 6 weeks of acclimatization period from 4th October to 15th November, 2006 and experiment 1 (control), from 16th November, 2006 to 7th February, 2007. Subsequently, experiment 2 (chemical) started from 8th February through to 31st March, 2007 and was subdivided with the first 3.6 weeks (control) with females on their own and 3.7 weeks (chemical) with females being allowed non-physical contact with males but only separated by a mesh wire. The non-reproductive females were given a period of isolation for two and half months prior to commencement of the 12 weeks experiment 3 from 3rd June to 25th August 2007 and which inclusively covered the initial 6 weeks of control period with females alone and the subsequent 6 weeks when females were physically paired with vasectomised males. Urine

samples were collected every after one day for all experimental periods and urine profiles generated. This study was mainly aimed at elucidating the ovulation methods of the giant mole-rat.

Experimental design

Experiment 1. Six non-reproductive females of the giant mole-rat were identified from six separate colonies in Kakalo and Mushishima Farm blocks. Similarly twelve (12) matured males with dark colourations around the mouth were trapped from 6 other colonies located 1000m distant from where the females were collected. Females were constantly kept individually until they were shipped to Pretoria where they were placed individually in plastic containers (1 x 0.5 x 0.5m) and subsequently subjected to experimental manipulation. Urine samples were collected every second day for period of 83 days as described below. The samples collected for this period served to detect if the non-reproductive females were producing cyclical progesterone profiles.

Experiment 2. The same six females were then transferred to plastic containers sized (0.6 x 0.3 x 0.35m) and provided with a wire mesh (3mm grid) separation for preventing any physical contact but allowing chemical communication. A gonadally complete male was placed in the second compartment of the holding cage for the following 26 days. Urine samples were collected throughout this experiment every second day as described below and served to detect if the physical presence of a male stimulated spontaneous ovulation.

Experiment 3. After two and half months of isolation, the same six non-reproductive females were housed individually in plastic containers each with a vasectomised male and

allowed to physical contact and behavioural interactions. Urine samples were taken throughout this experiment every second day as described below.

Surgery

Six males were vasectomized three months prior to being placed in physical contact with one of the six females. All vasectomies were completed three months prior to pairing up to ensure clearance of sperm from the vas deferens as well as a full recovery after surgery. Males were anaesthetized using iso-fluorane gas induction and maintenance with a mask. The vas deferens and epididymis were removed from each of the testis. Vasectomy was only performed to ensure that physical contact alone and not hormonal changes resulting from fertilization were recorded.

Sample collection

The non-reproductive females were placed in urine collection chambers every second day between 08h00 and 14h00 during which urine samples were collected. The chambers were cylindrical and had a smooth mesh floor to allow urine to fall through to a collecting dish. This set up prevented faecal contamination of the urine. The mole-rats were checked hourly throughout the day and urine samples were collected by sterile pipette being stored in plastic epindorph tubes with the animal number and date. The plastic tubes were stored at -35^{o}C. On completion of collection the mole-rat was returned to the holding cage. The volume of urine varied between 500µl and 2500µl. The required amount for progesterone and subsequent creatinine determination was 240µl. In the event of no urine being voided by a mole-rat, it was treated as a missing point this happened very infrequently and had no effect on the overall progesterone profile.

Creatinine determination

Prior to hormone assay all urine samples were analysed for creatinine measurement Booney *et al.,* (1982). Expressing urine hormone concentrations per mmol creatinine takes into account that the concentration of urine may differ depending on the food intake of an animal. Creatinine concentration was determined by using a modified version of the Jaffe Reaction Folin, (1914). The process involves adding 10µl of standard or sample to the well of a microplate, in duplicate, and leaving two wells empty as duplicate control blank. A further 300µl of picrate reagent is added to the wells, including the blanks.

Fresh alkaline picrate was mixed and comprised a saturated picric acid solution, alkaline titron and deionised water (1:1:10). The alkaline triton is composed of 4.2ml triton x-100, 12.5 ml 1N NaOH and 66.0ml distilled deionised water. The microplate is then placed in the dark for a period of 1.5h, at room temperature to allow colour development to occur. A standard curve (R^2 >0.99) was used to determine all sample values.

Progesterone determination.

The hormone progesterone is an important indicator hormone because it rises with increasing follicular development and subsequent production of the corpora lutea of ovulation Espey & Lipner, (1994). The cyclical pattern of progesterone secretion in spontaneous ovulators provides the rationale behind the use of progesterone concentrations for detecting ovulation Bauman, (1981).

The concentration of progesterone in the urine samples was measured using the technique of Bennett *et al.,* (1994). The progesterone concentrations were measured using a coat-a-count kit (Diagnostic Products Corporation, Los Angeles, USA). The antiserum is highly specific for progesterone with a cross reactivity to all naturally occurring steroids <0.5%, with the

exception of 17α hydroprogesterone (3.4%), 11-eoxycorticosterone (2.4%), 5β-pregnan-3, 20-dione (3.2%) and 5α-pregnan-3, 20 dione (9.0%). The concentrations of the standards ranged from 0.3 to 127.2 nmol/l.

The assay has been validated for use in the mole-rat by testing the slope of the curve produced using serial dilutions of un-extracted mole-rat urine obtained from a pregnant female (over the range 1:1 to 1:64) against that of the standard curve. Following logit-log transformation of the data the slopes of the curve were compared using Statistica. The curves were parallel to and did not differ significantly from one another. The minimum detection limit of the assay was 0.36 nmol/l and intra- and inter-assay coefficient of variation was 5.1% (n=6) and 9.3% (n=6) respectively.

Electrone micrograph

Penises were dissected out from frozen material of adult males, thawed and placed in 10% formalin on thawing. This fixing procedure was followed by a series of treatments with a 0.075M phosphate buffer, whereas post-fixation was achieved using a treatment of 1% osmium tetraoxide. Specimens were subjected to a series of dehydration steps using ethanol of increasing concentration (30-100%). Critical point drying (CPD) was reached (CPD from liquid CO_2) and the material was further dehydrated using the BIORAD 3000 critical point dryer (Watford, UK). At this point, the material is effectively coated with dehydrated cells that carry heavy metals (osmium and phosphate fixative) to which minute particles of gold can adhere. A Polaron E5200C (Watford, UK) sputter coater was used to sputter a few nanometers of gold, coating the fixed dehydrated material, which was mounted on a carbon

tape on a lead stage. The gold plated material was then viewed with a scanning electron microscope – J SM-840 (JEOL, Tokyo, Japan) and subsequent images were produced.

Statistical analysis

Wilcoxon signed rank tests, Bonferroni correction and Statistica version 0.8

Progesterone values were averaged for each individual female for the isolation periods and those with chemical contact, seismic communications or physical contact, respectively. For the first experiment, data were divided into two parts to obtain comparative data for both isolation and the contact phase of experiment 2 and 3. The cut-off point was chosen after 30 days corresponding to the length of the latter two experiments. Since data did not satisfy the assumptions for parametric tests, data for the two parts of each experiment where compared employing Wilcoxon signed rank tests. Likewise pairwise comparisons were made between the first part of the different experiments and the second parts. Bonferroni correction was applied to correct for sequential tests and all statistical tests were carried out with SPSS 15.0. However, the Statistica version 8.0 analysis was used in the pairwise comparison of all the 3 experiments for control and experimental.

Ethical note

In this part of the Copper Belt, these animals are treated as pests (Bennett & Faulkes, 2000) and are also used by the local population as an important human food source, comprising a major source of protein. In this study, the animals were collected at the request of local farmers who would otherwise have eradicated them by other methods; after the study, the

remaining colonies from the area (including those in the grassland and dambos) were caught for food by local farmers.

Animals were euthanased with an overdose of chloroform on the evening of the day of capture by experienced workers, and stored in the interim in covered plastic containers with sand, nest material and food; sexing, weighing and tissue collection were carried out post mortem. Tissue from these animals was collected for a variety of other projects, including population genetics, craniometrics and studies of reproductive biology. Carcasses were eaten by trappers and farmers. The project was reviewed and passed by the Ethics Panel of the University of Pretoria (ref. AUCC06509/011). Capture of mole rats was authorized by the Department of Veterinary and Nature Conservation in the Copper Belt Province of Zambia, and all the necessary permits were obtained in Zambia.

All procedures followed the guidelines of the American Society of Mammalogists (ASM; Animal Care and Use Committee 1998; http://www.mammalogy.org/committees/index.asp) and the animal ethics committee of the University of Pretoria, Pretoria, South Africa. Standard data recorded from collected samples included sex and body mass (g) and all specimens were prepared as voucher specimens and will be deposited in the Natural History Museum, Lusaka, Zambia.