The biology and population ecology of the Namaqua dune mole-rat, *Bathyergus janetta* from the Northern Cape Province, South Africa

by

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Abstract

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Bathyergus janetta is a solitary subterranean rodent that occurs in the very restricted areas of Namaqualand. This bathyergid is able to survive the arid environment due to the predictable winter rainfall that gives rise to the high abundance and diversity of geophytes in the area. Burrowing dynamics and burrow system configuration was studied by the monitoring of mound production and the excavation of six burrow systems in mole-rats which had been marked by toe-clipping. *Bathyergus janetta* burrows are about 45cm below ground and comprise nesting chambers, food stores, defecation sites and bolt holes. Males have a more linear shaped burrow system than females, probably to increase home ranges in search of a possible mate. Burrow systems undergo consistent excavation and re-excavation of tunnels within the home range and it appears that mole-rats have an optimum burrow length that ranges from 71.2m to 165m with an average home range of $805.86 \pm 375.52 m^2$.

Bathyergus janetta is a seasonal breeder. Females exhibit an elevation in urinary progesterone and oestradiol-17β concentrations and males a rise in testosterone that correlates with seasonal rainfall figures. After the winter rainfall, the soil is soft and moist and easily excavated. *Bathyergus janetta* makes use of this opportunity not only to extend their burrow systems but also to search for possible mates. Plural occupancy by adult mole-rats was observed during the onset of the winter and pups and pregnant females were caught at the end of October and November.

Microsatellites were used in an attempt to determine parentage and elucidate relatedness within a population of *B. janetta*. It was not possible to assign parentage within the scope of the statistical program CERVUS due to the large number of putative parents, although it seems that multiple paternity of litters may occur. Individual *Bathyergus janetta* exhibit low relatedness values suggesting that the population is outbreeding and comprises related and unrelated individuals. A highly significant correlation between the isolation of genetic and geographical distances was found which supports the assumption that the subterranean niche poses a limitation on the dispersal abilities of mole-rats. A comparison between populations of *B. janetta* and *B. suillus* shows a clear distinction between the two species. Surprisingly the two populations of *B. suillus* (although 15-20km apart) show a distinct genetic differentiation and a high genetic diversity within the populations when compared to the *B. janetta* population.

Keywords: Namaqua dune mole-rat, *Bathyergus janetta*, burrowing dynamics, seasonal breeder, microsatellites, relatedness

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"Trust in the Lord with all your heart,
and lean not unto your own understanding.
In all your ways, acknowledge Him,
and He will direct your path."
Proverbs 3:5-6

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Chapter 1 General Introduction

The family Bathyergidae

The African mole-rats, family Bathyergidae are endemic to the sub Saharan African continent (Jarvis & Bennett 1990, 1991) and are widely distributed from the southern tip of Africa to about 10° N of the equator (De Graaff 1981). In contrast to their name, mole-rats are neither moles (insectivores) or rats (murids); in fact they are more closely related to porcupines than any other mammal (Skinner & Smithers 1990). The family name Bathyergidae is derived from the Greek words *bathys* and *ergo* which means deep and work respectively and refers to their subterranean nature. What makes these small mammals so interesting is the fact that the family comprises at least 18 species (Honeycutt, Allard, Edwards & Schlitter 1991) that occur over a wide range of habitats and exhibit a broad spectrum of social structures (Bennett, Faulkes & Jarvis 1999).

Eusociality has interested biologists since Darwin explained how it might have evolved through natural selection in his popular book *The Origin of Species* (Darwin 1859). It has been used to describe the condition found within the social insects (Hymenoptera and Isoptera) where there is (i) a reproductive division of labour, (ii) an overlap of at least two generations, and (iii) cooperative care of the young (Michener 1969; Wilson 1971). Jarvis (1981) described the first eusocial mammal, the naked mole-rat, *Heterocephalus glaber* as eusocial (Jarvis 1981). Since then, laboratory and field studies have shown that two of the five genera in the Bathyergidae show a degree of sociality (Bennett 1989, 1990; Bennett & Jarvis 1988; Jarvis & Bennett 1990, 1993). African mole-rats are a key model to study the evolution of sociality since they exhibit a wide range of social structures that ranges from aggressive solitary animals through to highly social species (naked mole-rats and Damaraland mole-rats, *Cryptomys damarensis*) (Jarvis 1981; Bennett & Jarvis 1988; Jarvis & Bennett 1993).

The earliest descriptions of mole-rats date back to the seventeenth-century (Schreber 1782). Mole-rats are subterranean rodents that live in extensive underground burrow

systems. They are herbivorous and feed on swollen tubers and underground geophytes, which they encounter during burrowing activity (Jarvis & Bennett 1990). They are adapted to their subterranean nature in many ways and have cylindrical shaped bodies with short limbs that facilitate movement in the narrow tunnels of their burrow systems. Except for the naked mole-rat, all other members of the family have a thick pelage with long sensory hairs or vibrissae that stand out from the pelage. The vibrissae are the only hairs still visible on the naked mole-rat (Jarvis & Bennett 1990). Mole-rats have reduced eyes (Cei 1946; Eloff 1958) with very poor eyesight (Oosthuizen 2002). They usually keep their eyes closed and only open them when they are disturbed or alarmed. The visual centres in the brain are reduced (Pilleri 1960) and apparently they cannot form images (Jarvis & Bennett 1990). No ear pinnae are visible but they can hear well and their sensory and olfactory organs are highly developed (De Graaff 1960). Probably the most characteristic feature of mole-rats is their prominent extrabuccal chisel-like incisors with lips that meet behind. The lips separate the incisors from the oral cavity and thus keep soil out of the mouth and digestive tract while the animals are digging. Mole-rats have short tails with stiff fringed hair, which they place on the load of excavated soil, preventing it from falling onto their backs.

The family Bathyergidae comprises five genera, which are grouped into two subfamilies based on their dental characteristics (Roberts 1951; De Graaf 1981). The subfamily Georychinae consists of four genera i.e. *Cryptomys*, *Heterocephalus*, *Heliophobius* and *Georychus*. These mole-rats are small and weigh up to 550g. Their upper incisors are ungrooved and they mainly use their teeth to excavate their burrow systems. There is only one genus and two species in the subfamily Bathyerginae. The Cape dune mole-rat, *Bathyergus suillus* is the largest mole-rat with a body mass of up to 1200g (Jarvis & Bennett 1990). They occur in the sandy areas of the southern and western Cape (Skinner & Smithers 1990). The Namaqua dune mole-rat, *Bathyergus janetta* is slightly smaller (up to 750g) and is more localized in the sandy alluvials of Namaqualand and the southern parts of Namibia (Skinner & Smithers 1990). Both of these species are characterised by their grooved upper incisors and ungrooved lower incisors as well as their strong front claws, which they use for digging their burrow systems.

The evolution of eusociality

Most studies on the behaviour of social animals suggest that eusociality is an evolutionary endpoint that arises from a solitary ancestor (Bennett & Faulkes 2000). Although recent studies have shown that the evolution of eusociality can be bi-directional (social ancestors evolved to solitary animals) (Wcisto & Danforth 1997), the case in the African mole-rats seems to be that sociality in the family has evolved from a solitary ancestor (Jarvis & Bennett 1991). The reasoning behind this includes the finding that most subterranean mammals are solitary (Nevo 1979) and fossorial bathyergids were found to be large (Lavocat 1978). Today all solitary mole-rats are large as well. If this is true, eusociality in the family Bathyergidae may have evolved twice! Firstly, in the naked mole-rat, and of more recent evolutionary origin in the Damaraland mole-rat (Jarvis, O'Riain, Bennett & Sherman 1994). Mole-rats are the ideal model to study the evolution of cooperative care in animals, since there is multiple occurrence of sociality in the family (Bennett & Jarvis 2000). In the naked mole-rat, inbreeding was thought to be the most important factor in the evolution of sociality but after the discovery that all bathyergids seem to outbreed and exhibit interesting inbreeding avoidance mechanisms, research has shifted from relatedness studies to the ecological constraints that drive social evolution.

Several hypotheses have been proposed for the advantage of social behaviour and group living in the Bathyergidae. Burda (1990) proposed that female bathyergids (*C. hottentotus* and *H. glaber*) have relatively long gestation periods, altricial young and slow postnatal growth (all hystricomorph traits) as well as no fat storage for gestation and lactation periods. Therefore sociality is an adaptive trait because reproductive females are totally dependant on colony members for food provisioning. Bennett & Faulkes (2000) argue that low fat storage capacity in females could rather be a response to sociality, rather than the cause. Another hypothesis by Alexander (1991) proposes that group living and sociality evolves as a result of predator avoidance and the subterranean burrow system represents a protective environment for the young. However this does not explain why natal philopatry or overlapping generations should follow (Bennett & Faulkes 2000). There is a definite relationship between sociality and rainfall, as well as rainfall and the distribution of food resources such as geophytes (Bennett & Faulkes 2000). Lovegrove and Wissel proposed a model to explain sociality based on cooperative care reducing the risks of unsuccessful foraging

(Lovegrove & Wissel 1988, Lovegrove 1991). Recently, more support has been given to the food-aridity hypothesis. Social mole-rats occur in more arid environments where rainfall is unpredictable, food resources clumped, are of lower digestibility and more individuals required for successful foraging requirements (Bennett & Jarvis 1995). In contrast, solitary species are found in the more mesic and temperate regions where rainfall is predictable, food resources widely distributed and of a high digestibility (Bennett & Jarvis 1995).

Social organisation and reproduction in the family Bathyergidae

Solitary subterranean rodents are typically xenophobic and very aggressive towards conspecifics (Nevo 1979; Bennett & Jarvis 1988). In order to breed, the strong territoriality and xenophobic nature must be broken down and receptivity, sex and status must be conveyed towards conspecifics. Being underground and solitary, most communication is brought about through long distance seismic transmission. The rhizomyid, *Tachyoryctes splendens* (Jarvis 1969) uses incisor tapping, the blind mole-rat *Spalax ehrenbergi*, uses head drumming (Rado, Levi, Witcher, Intrator, Wallberg & Terkel 1987; Heth, Frankenberg, Raz & Nevo 1987) whereas Geomyidae (gophers) and three bathyergids, *Georychus capensis* (Bennett & Jarvis 1988), Cape dune mole-rat and Namaqua dune mole-rat use hind feet drumming (Bennett & Jarvis 1988; Bennett, Jarvis, Aguilar & McDaid 1991; Jarvis & Bennett 1991).

A solitary subterranean rodent mole is the sole occupant of its burrow system and it is only during the short breeding season when the mother has offspring that multiple occupancy arises (Bennett & Jarvis 1988). Courtship and mating is brief and the pups remain in the natal colony for a short period of up to sixty days (Bennett & Jarvis 1988; Jarvis & Bennett 1990), whereafter the mother aggressively expels them from the system (Bennett & Jarvis 1988; Bennett *et al.* 1991). Social subterranean mole-rats differ from solitary species in that the reproductive animals share a burrow system and can reproduce when the occasion arises. There is considerable courtship and foreplay and the reproductive animals form a pair bond. Their offspring remain in the natal colony for extended periods of time.

Within the social species of mole-rats a continuum of socially induced infertility arises from predominantly behavioural suppression in mesic adapted species to

complete physiological suppression in arid adapted species (Bennett *et al.* 1999). The eusocial naked mole-rat occurs in an arid environment where the risks in dispersing are high. The offspring remain in the natal colony and seldom disperse. Recent evidence from field and laboratory studies suggest that naked mole-rats might outbreed more frequently than was previously expected, however, the success of these founding pairs is not reported (Braude 2000; Ciszek 2000). Inbreeding tends to outweight the few outbreeding attempts and with the loss of one of the reproductive individuals reproductive succession within the colony follows (Clarke & Faulkes 1997). Therefore colonies of naked mole-rats are highly inbred and xenophobic towards conspecifics. Strong physiological and behavioural suppression occurs in both sexes (Faulkes 1990; Faulkes, Trowell, Jarvis & Bennett 1994; Bennett *et al.* 1999).

All species of *Cryptomys* appear to outbreed (Jarvis *et al.* 1994; Burda 1995; Bennett, Faulkes & Molteno 1996; Bennett, Faulkes & Spinks 1997; Faulkes, Bennett, Bruford, O'Brien, Aguilar & Jarvis 1997). Social colonies arise from a reproductive pair and their subsequent offspring. These non-reproductive subordinates benefit from delaying reproduction until conditions are optimal for them to disperse. In mesic areas, where dispersal events are more likely, non-reproductive individuals are only behaviourally restrained from reproduction and the potential reproductive success of the non-breeders is high. In the case of the Damaraland mole-rat that occurs in a more arid environment, dispersal events are less frequent and the reproductive success of non-breeders is lower. To maintain a high reproductive skew in the colony the non-reproductive females are both physiologically and behaviourally suppressed from reproduction, whereas the non-reproductive males are only behaviourally suppressed (Bennett *et al.* 1996; Richard & Bennett 1997).

Solitary mole-rats

Most of the research on mole-rats has concentrated on the mechanisms of evolution of sociality in the Bathyergidae and social organisation within species. There is a paucity of information on the ecology and population biology of solitary species of mole-rats. The Namaqua dune mole-rat occurs in the very restricted area of the Northern Cape Province (Namaqualand and the extreme south of Namibia) (Skinner & Smithers 1990). The ranges of the Cape dune mole-rat (*B. suillus*) and Namaqua dune mole-rat

shows no overlap. Both species are usually found in sand dunes and substrates of sandy alluvials. The Cape dune mole-rat may show some overlap in distribution with the Cape mole-rat (*Georychus capensis*) and all exhibit sympatry with the common mole-rat, *Cryptomys hottentotus*. The solitary mole-rats are strictly seasonal breeders. The Namaqua dune mole-rat is of particular interest in the evolution of sociality since it has evolved the ability to occur in a very arid habitat (sociality in mole-rats is linked to aridity and solitary mole-rats are usually found in the more mesic and temperate regions).

Brief introduction to study area

Namaqualand was the known magisterial district within the old Cape Province and is well known for its magnificent flower displays in spring months. In physiographical and biographical terms it is described as the strongly winter rainfall part of the Succulent Karoo Biome (Rutherford & Westfall 1986; Milton, Yeaton, Dean & Vlok 1997). The area is now defined as the Namaqualand Namib Domain of the Succulant Karoo floristic region (Jürgens 1991). The area is divided into several bioregions (Hilton-Taylor 1996) i.e. the Knersvlakte, Southern Namib desert, Kamiesberg, Richtersveld, Sandveld and Hardeveld. The area is characterised by a very predictable annual rainfall (Hoffman & Cowling 1987) and moderate temperatures throughout the year. This leads to the unique plant ecological features of the area such as: communities dominated by dwarf to low, shallow rooted, short lived and drought sensitive leaf succulent shrubs; and a high diversity and abundance of geophytes (Cowling, Esler & Rundel 1999). The annual rainfall of the area ranges from 50mm in the north west to more than 400mm in the Kamiesberg area, although most of the area receives less than 150mm per year.

The aims of the study

The Namaqua dune mole-rat is a solitary species and occurs only in the very restricted area of Namaqualand, Sandveld, sandy alluvials of the Hardeveld and the southern parts of the Namib desert (M. Herbst, pers. obs.). This mole-rat is an enigma since it is a solitary species and yet can survive in some of the most arid regions of southern Africa. To date there is very little known about the general biology or population ecology of the species. Since the Namaqua dune mole-rat is one of the most endangered species of African mole-rats (listed in the Red data book) further research

is urgently needed to understand its unique life history traits and to assist in decisions made on the conservation of this mole-rat.

In Chapter 2 the biology and population ecology of the Namaqua dune mole-rat is discussed. Intense field studies were conducted to investigate the population structure and density as well as to gather demographic statistics such as body mass measurements and sex ratio. The burrowing dynamics and food preferences were also assessed by the excavation of six burrow systems. In Chapter 3 the reproductive biology was investigated by monitoring reproductive hormones. Urine samples were collected throughout the study period and used in hormonal assays to assess differences in progesterone and oestrogen concentrations in females and testosterone concentrations in males to establish whether there is a distinct breeding season or not. The population genetics are determined in Chapter 4. The use of newly developed microsatellite primers for the Family Bathyergidae were used to look at population dynamics including dispersal events, parentage and the degree of relatedness within the population as well as relatedness between a population of *B. janetta* and two populations of *B. suillus*.

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Chapter 2 The biology and burrowing dynamics of the Namaqua dune mole-rat, *Bathyergus janetta*

Introduction

The family Bathyergidae (African mole-rats) is a well-studied group of subterranean rodents and probably best known for their unique and complex social structures (Jarvis & Bennett 1990, 1991). The family possesses species that ranges from solitary through to eusocial animals (Bennett & Faulkes 2000). The food-aridity hypothesis suggests that sociality in the Bathyergidae has evolved as a result of habitat aridity and the subsequent consequences of food availability and distribution (Bennett & Faulkes 2000; Faulkes, Bennett, Bruford, O'Brien, Aguilar & Jarvis 1997; Jarvis, O'Riain, Bennett & Sherman 1994). Social bathyergids (*Cryptomys*) occur in mesic as well as arid regions where cooperative living reduces the risk of unsuccessful foraging. The solitary mole-rats (*Georychus*, *Bathyergus* and *Heliophobius*) occur in the more mesic and temperate environments where food resources are widely distributed and of high digestibility. The Namaqua dune mole-rat, *Bathyergus janetta* is very unusual since it is a solitary species and yet can survive in some of the most arid regions within South Africa (Skinner & Smithers 1990).

The Namaqua dune mole-rat favours the soft and sandy dunes of the north-west coast of Southern Africa, although some populations do occur in the sandy alluvials more inland between the rocky outcrops of the Kamiesberg. It is also found in the southern parts of Namibia (Skinner & Smithers 1990) (Figure 1). Namaqualand is a mild desert biome characterized by very hot and dry summer months and a cold winter with an average rainfall of about 150mm annually (Cowling, Esler & Rundel 1999). This highly predictable winter rainfall is responsible for the unique flora, which includes a high abundance and diversity of geophytes (Cowling *et al.* 1999; Hoffman & Cowling 1987). Mole-rats are herbivorous and feed mainly on swollen tubers and underground storage organs of geophytes which they encounter during burrowing activity.

Underground geophytes are an ideal food resource, since there is apparently little competition from other foragers; it is available throughout the year, biologically stable and has high concentration of nutrients (Bennett & Faulkes 2000). Many geophytes are unpalatable and toxic for most animals (Watt & Beyer-Brandwijk 1962) or have thick tunics and spinous coverings (Lovegrove & Jarvis 1986), but mole-rats seem to be immune to cardiac glycocides contained in these storage organs and their large incisors are able to penetrate the protective coverings. Mole-rats are adapted to a high fibre, herbivorous diet although there are anecdotal reports of the consumption of invertebrates that are commonly found in burrow systems by the giant mole-rat (Burda & Kawalika 1993; Bennett & Faulkes 2000) but this is the exception rather than the rule.

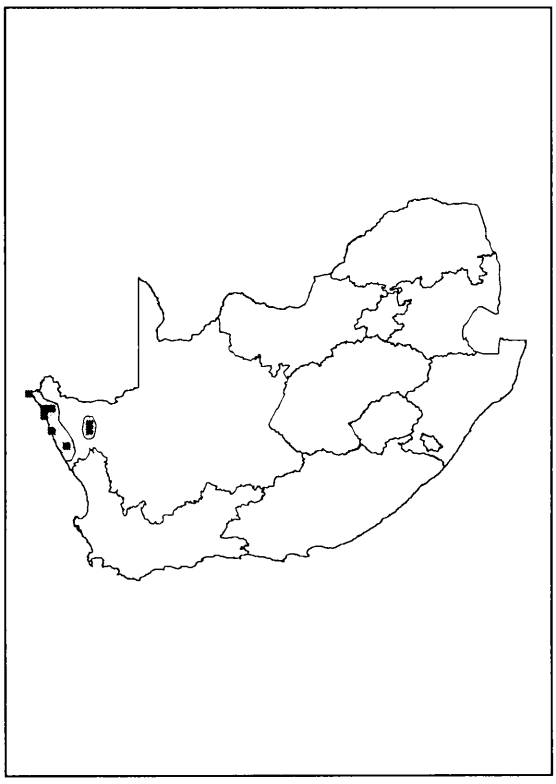

Figure 1 Distribution map of *Bathyergus janetta*

Geophytes are stored in food chambers (De Graaff 1964, 1981; Genelly 1965; Du Toit, Jarvis & Louw 1985; Davies & Jarvis 1986; Lovegrove & Jarvis 1986; Bennett 1988). Mole-rats tend to prune the buds and sprouting shoots from corms and tubers (Bennett &

Faulkes 2000), whereas the Mediterranean blind mole-rat, *Spalax,* does the same with *Oxalis* bulbs but however eats the shoots (Galil 1967). It has also been observed that mole-rats "farm" geophytes (Jarvis & Sale 1971; Bennett 1988; Spinks 1998; Bennett & Faulkes 2000) by partially consuming the geophytes and then replugging the consumed section with soil so that it will regenerate.

Mole-rats drink no free water but take their water in through their food via the metabolic pathways. Geophytes have a high water content of 77-80% by weight (Bennett & Jarvis 1995) and the humidity in their burrow systems are high (>95%) therefore water loss is low (Buffenstein & Yahav 1991). The size of the geophytes is also important and indeed in arid areas where there is a decrease in geophyte density there tends to be an increase in the average size of food items (Bennett 1988; Spinks 1998).

The dune mole-rats (*Bathyergus*) excavate their burrow systems in the soft sand dunes of the Cape Province. These mole-rats are characterized by a large mean body size (*B. suillus* up to 1800g and *B. janetta* 750g) and strong front claws used for digging the burrow systems (Bennett & Faulkes 2000). The burrow system represents a tremendous energetic investment (the cost of excavating in soil is 360-3400 times as great as moving the same distance across the surface (Vleck 1979)). Mole-rats spend their entire lives in the confines of their burrow system. The burrows serve as protective residences and the tunnels as foraging bases (Jarvis & Bennett 1990). A mole-rat burrow system comprises deep tunnels, which includes the nest, food stores and defecation sites, as well as shallower foraging tunnels (Bennett & Faulkes 2000). Habitat type is reflected in burrow architecture and it appears that large social groups of mole-rats explore the surrounding area much more efficiently than solitary species (Le Comber, Spinks, Bennett, Jarvis & Faulkes 2002).

The objective of this study was to determine the demographic statistics of a population, including the mean body mass of each sex and variation in body mass. The sex ratio of the population, density and home ranges were also assessed. Burrowing dynamics were determined by the excavation of six *B. janetta* burrow systems. This enabled us to

determine foraging strategies used by the mole-rats for food collection. The calculation of soil turnover enables the determination of the amount of excavation that is possible during optimum burrowing conditions. The burrow system, depths, positions and contents of nests, defecation sites and food stores were also recorded.

Materials and Methods

Capture and data collection of *B. janetta*

Mole-rat burrow systems were located by the detection of discrete rows and groups of mounds on the surface. The Namaqua dune mole-rat was found only in soft sandy arenosols surrounded by rocky outcrops. The mole-rats were caught with modified snaptraps anchored to a stake in the ground or by using modified Hickman live traps (Hickman 1979). The traps were placed inside an open section of the burrow system, baited with sweet potato and checked every 30 minutes.

The following data was collected from the 71 mole-rats captured at the study site: sex, body mass (g), body length from nose to tip of tail (mm), reproductive condition and any distinguishing features. Captured animals were anaesthetized with Halothane stabilized with thymol 0.01% m/m, toe-clipped and released to their burrow systems. A urine sample was collected for hormone concentration analysis (Chapter 3) and the small section of toe placed in DMSO and stored at -20°C for later DNA analysis (Chapter 4).

Study area

The study was undertaken on the farm Kardou (30°9'S 17°56'E) just outside the town of Kamieskroon. The mole-rats *Bathyergus janetta* and *Cryptomys hottentotus hottentotus* were detected by the presence of rows and groups of mounds on the surface. *Bathyergus janetta* was found mostly in the sandy valley and sand dunes where the soil is softer and easier to dig in. *Cryptomys hottentotus hottentotus*, a social species of mole-rat was found in the more consolidated soils. It has been found that where the soil is intermediate the mole-rats occur sympatrically. The population of *B. janetta* was studied by the mark recapture method during August 2000 to September 2001. The vegetation on the farm is typically Lowland Succulent Karoo, comprising mainly of dwarf succulent and species-rich scrubland dominated by leaf succulent members of the Mesembryanthemaceae and Crassulaceae (Cowling *et al.* 1999). The area was characterized by soft sandy soils dominated by grasses and *Euphorbia* scrubs.

Mapping of study site

The approximate area covered by mole-rats was determined from the positions of mole-rat mounds and confirmed by the capturing of the uniquely marked mole-rat at each burrow system. Individual burrow systems could easily be detected by rows of mounds and the residency of the mole-rat confirmed by capture. All positional data were recorded on graph paper by using a tape measure, lengths of string or by pacing.

Molehills

Nine mole-rat burrow systems (five male and four female) were selected and weekly the positions of all newly formed mounds produced were recorded over the twelve-month period. Individual mole-rat mounds were measured by gathering up soil in a bucket and subsequently weighing it (g). In total 27 molehills were weighed.

Excavations of mole-rat burrow systems

In April 2001 six mole-rats (three male and three female) were removed from their burrow systems to prevent any further changes being made to the burrow system. The burrow systems were then excavated and the position, dimensions (distance from floor to top of burrow) and depth (distance from surface to floor of burrow) were noted. The dimensions, position, depth and contents of the nesting areas, food stores and defecation sites were also recorded. Home ranges were determined by calculating the area enclosing each burrow system and its associated mounds. The perimeter of each home range was defined as approximately 2m beyond the burrows and mounds (Davies & Jarvis 1986). The excavated burrow systems were chosen at random and were not adjacent to each other.

Results

Bathyergus janetta (Plate 1) was the dominant mole-rat species in the area and *C. hottentotus* occurred only in the more consolidated soil near rocky outcrops. In total 71 mole-rats comprising 28 males, 26 females and 17 juveniles were caught in the study site of 1557 km^2. Average adult mass of males was 439.36 ± 191.84g while that of females was 330.38 ± 58.86g. Sexual dimorphism is present with males larger than females. All recorded data is summarized in Table 1. All positions of captured animals were recorded on graph paper, coordinates were determined and plotted onto a graph (Figure 2).

Table 1 Average body measurements of all adult *B. janetta* individuals caught in Kamieskroon

	Body mass (g) ± SD	Length (mm) ± SD	Total
Male	439.36 ± 191.84	283.57 ± 39.24	28
Female	330.38 ± 58.86	273.23 ± 32.02	26
Average	384.87 ± 125.35	278.23 ± 35.63	54

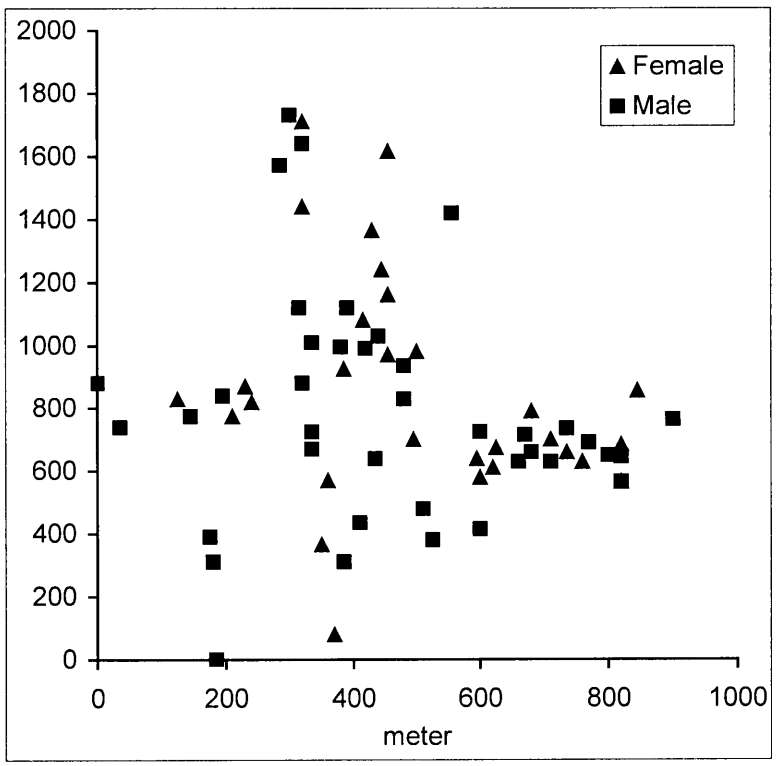

Figure 2 Map of all recorded mole-rats caught in study site

Mound production and activity

Mound building activity increased after good showers of rain. Figure 3 indicates the total amount of recorded freshly produced mounds of 9 burrow systems and the annual rainfall figures recorded for Kamieskroon (rainfall data with permission of SA Weather Bureau). It is evident that mound production activity increases when the soil is moist. Indeed one day after good rainfall, several new mole-rat mounds were observed in the study site. The correlation between mound production and monthly rainfall was significant ($r = 0.85$, $p < 0.05$).

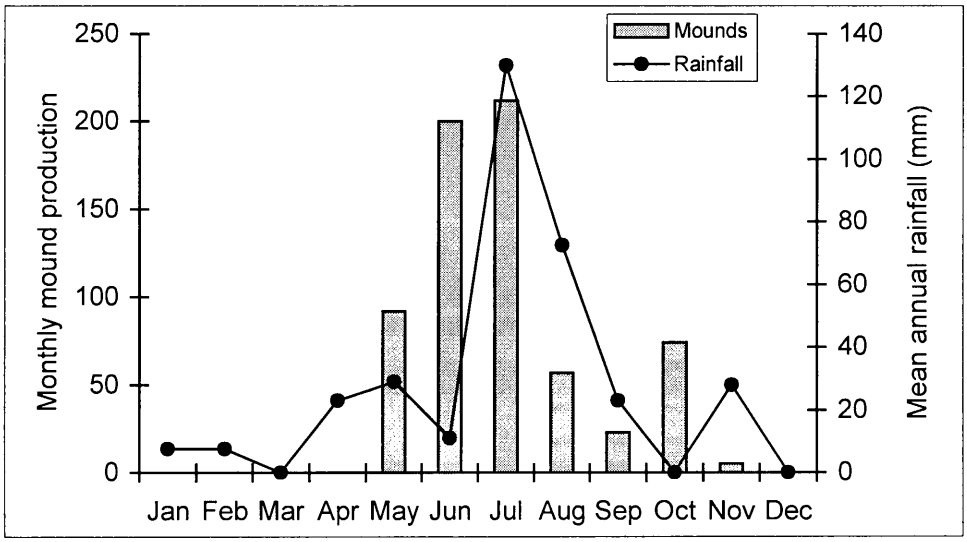

Figure 3 Total amount of molehills extruded during the year 2000/2001 with the corresponding rainfall figures (SA Weather Bureau).

In total, 27 molehills produced by *B. janetta* were weighted from 9 burrow systems. The mean averaged weight (15.52 ± 6.49kg) of mole-rat mounds produced by the nine mole-rats is summarized in Table 2. Mean tunnel diameter at the base of the molehills was 12cm. During the study period a total of 663 molehills were produced, equivalent to about 419.06kg of moist soil. New mounds were produced in the same positions as previous molehills that were several months old, suggesting that this may have resulted from the re-excavation of parts of the burrow system. The majority of fresh mole-rat mounds were produced during the night or early in the morning.

Table 2 Mole-rat mound production and tunnel measurements

Burrow system	Occupant		Total number of new mounds 2000/2001	Average mass of single mound (kg)	Average diameter of tunnel system (cm)	Total amount of soil excavated (kg)
	Sex	Mass				
1	M	463g	117	13.67	14.17	1599.39
2	M	708g	76	11.48	15.83	872.48
3	M	395g	57	17.16	13.5	978.12
4	M	200g	44	18.37	9.17	808.28
5	M	344g	49	16.01	11.83	784.49
6	F	307g	103	10.42	9.83	1073.26
7	F	315g	83	20.91	13.33	1735.53
8	F	380g	65	19.07	9	1239.55
9	F	351g	69	12.51	9.33	863.19
Average			73.7	15.52 ± 6.49	11.78	1106.03 ± 350.09

The burrow system

Mole-rat burrow systems comprised a single main tunnel with shorter and shallower side branches. Nests and food chambers consisted of small hollow rooms excavated directly out of the main tunnel or slightly deeper than main tunnel. Main burrow tunnels were at an average depth of 45cm whereas side tunnels were shallower and abruptly ended in dead ends. Figure 4 and 5 are a schematic representation of the six excavated burrow systems (Plate 2.1).

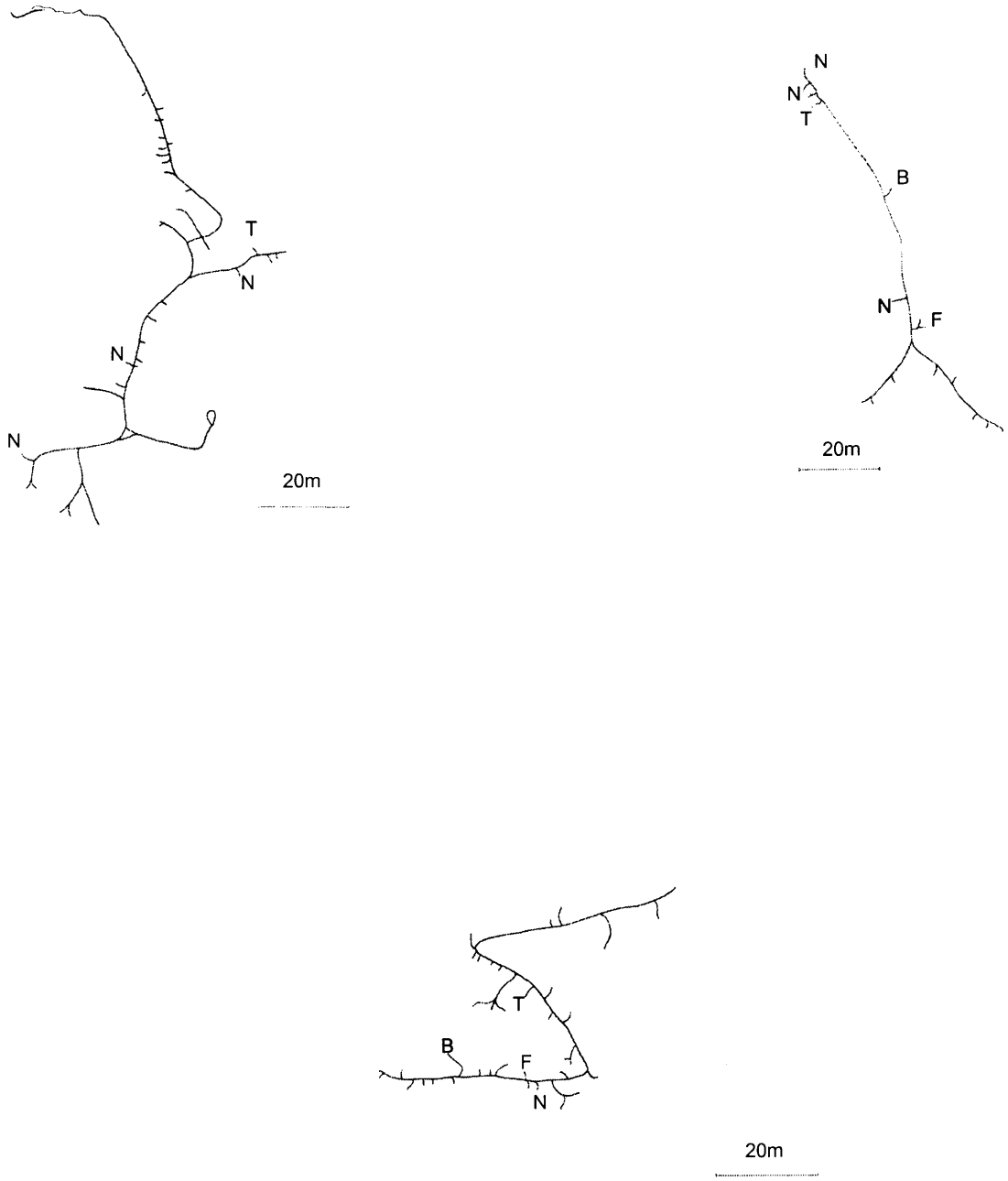

Figure 4 Schematic representation of the excavated burrow systems of *B. janetta* males indicating (N) nesting areas, (T) defecation sites, (F) foodstores and (B) bolt holes

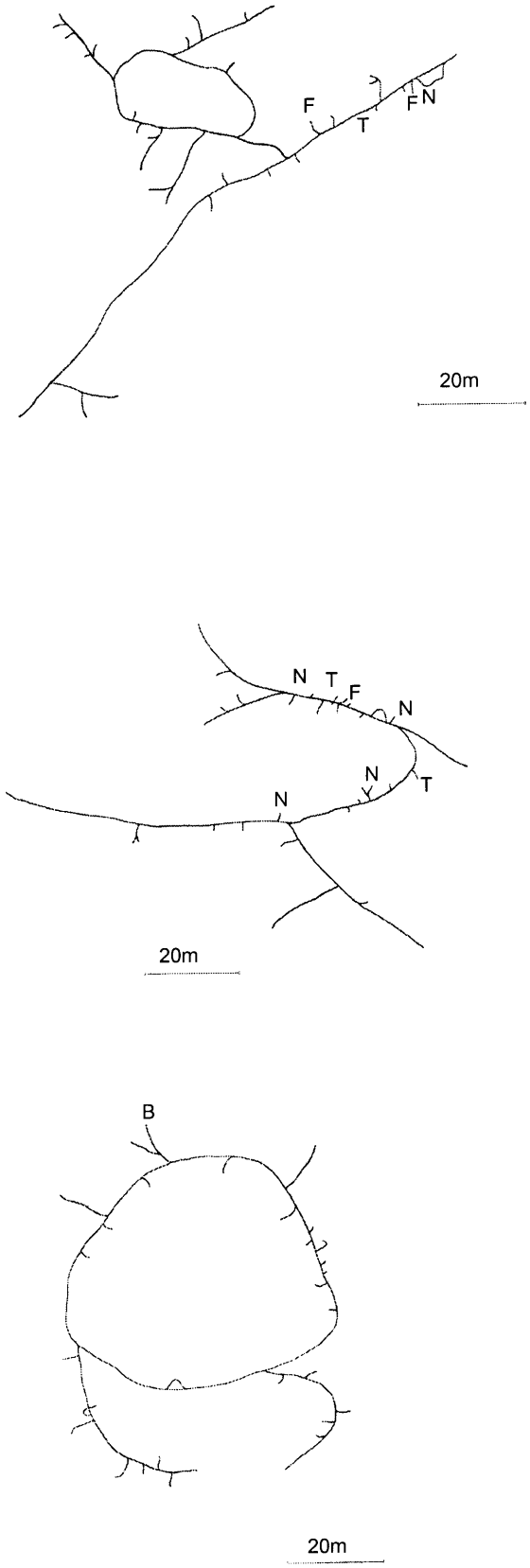

Figure 5 Schematic representation of the excavated burrow systems of *B. janetta* females indicating (N) nesting areas, (T) defecation sites, (F) foodstores and (B) bolt holes

Table 3 Burrow system statistics

System	Sex	Mass (g)	Total length (m)	Nests	Food stores	Defecation sites	Bolt hole depth (cm)
1	M	297	160.92	5	1	1	-
2	M	451	+71.2	3	1	1	145
3	M	261	79.6	1	1	1	150
4	F	371	165	1	2	1	-
5	F	499	141.6	4	1	1	160
6	F	396	149	-	-	-	165
Average			127.89	2.8	1.2	1	155

Nests

A total of 15 nests were found during the excavations of six burrow systems. The nests tended to be near the center of the burrow system. *Bathyergus janetta* nests were in hollowed out chamber with a single entrance in the walls of the main tunnel, or a short blind ending side branch. Nests were mostly situated in the deeper tunnels. The deepest nest of *B. janetta* was at 58cm while the shallowest was at 40cm.

Nests were used mainly as sleeping areas and only one nest contained two small bulbs. The rest of the nest contents comprised pieces of grass and the outer leaves of *Oxalis* bulbs (Plate 2.2). There was very little variation in the nest contents of the six excavated burrows systems. Details of nests and content are describe in Table 4.

Table 4 Summary of the nest contents of 15 *B. janetta* nests

Burrow system	Number nests	Average depth (cm)	Average mass contents (g)	Description
1	5	48.6	71.24	Blind ending
2	1	51	94.8	side branch in
3	1	58	9.68	main tunnel
4	5	49.4	81.41	
5	3	47	70.87	

Food stores

Food stores were located in close proximity of the nesting chambers in a blind ending side branch. Food was tightly packed into the end of the tunnel. A few of the bulbs were mixed with sand and one food store was blocked off. Table 5 summarizes the description of food stores. The food stores of the six burrow systems totaled a mass of 590.3g (average 98.38g per burrow system) and comprised mainly *Oxalis* bulbs. Other minor food items included *Lachenalia* and *Homeria* bulbs. Grass may constitute a large part of the mole-rats diet and it has been observed that grass roots are a favoured food item as well as the tubers of *Grielum bimaculatum*. Mole-rats cut up grass into approximately 2-5cm long pieces and then start eating the grass from top until it is finished. There were no signs of disbudding of growing shoots and all tubers were dormant. Some of the *Oxalis* bulbs were very small being less than 2cm in diameter (Plate 3.1).

Home ranges

The average home range of *B. janetta* was 805.86 ± 375.52m^2 with a minimum size of 397.66m^2 and a maximum size of 1438.1m^2. In Kamieskroon, *Bathyergus janetta* has a patchy distribution since mole-rats only occur in the softer and sandy soils. The study site (Plate 3.2) was a huge sandy alluvial valley occurring between the rocky outcrops of the Kamiesberg and in total 71 mole-rats were caught. The burrow systems of the males were almost always linear whereas those of the females had more circular home ranges. It is very difficult to determine the density of mole-rats in

the area since mole-rats occur as near as 5m from each other whereas others may be more than 200m away.

Table 5 Summary of the contents of the food stores found in *B. janetta* burrow systems.

Burrow	Number stores	Depth (cm)	Mass contents (g)	Contents	Description
1	1	46	55.7	37 *Oxalis* bulbs 4 roots	Side branch filled with food
2	1	55	158.4	186 *Oxalis* bulbs 2 roots 6 *Lachenalia* bulbs 1 *Homeria* bulb	Side branch filled with food and blocked off with sand
3	1	40	197	107 *Oxalis* bulbs 2 *Lachenalia* bulbs 1 *Homeria* bulb	Side branch filled with food
4	2	51 50	6.1 62.8	8 *Oxalis* bulbs 1 *Babiana* sp. 132 *Oxalis* bulbs 1 root 4 *Homeria* bulbs	Side branch filled with food Blind ending tunnel
5	1	52	110.3	41 *Oxalis* bulbs 1 root 19 *Lachenalia* bulbs 2 *Homeria* bulbs 2 unknown tubers	Side branch filled with food

Burrow permanence and extensions

Overall positions of burrow systems remain more or less fixed although on a small scale there were a lot of changes. During some months no evidence of changes or extensions occurred especially during the dry summer months. Extensions made in both directions and evidence of new mounds between old mounds suggested a general cleaning of the burrow system. Average extensions to the burrow system varied between 1-3.5m per day but these were exceptions and long periods occurred without any activity. Highest burrowing activity occurred towards end of rainy season when the soil is soft and moist.

Discussion

There is surprisingly little information on the biology of the Namaqua dune mole-rat. This solitary subterranean rodent occurs in the semi desert area of Namaqualand where it is endemic to the region (Skinner & Smithers 1990). The dune mole-rats (*Bathyergus*) are the largest bathyergids. Solitary subterranean mammals are typically xenophobic and highly aggressive. Generally body mass in the family Bathyergidae decreases with an increase in sociality (Jarvis & Bennett 1990) accompanied by an increase in habitat aridity. The Namaqua dune mole-rat is an exception to this rule and due to the predictability of winter rainfall in the Namaqualand that leads to the high abundance and diversity of geophytes, this solitary mole-rat can survive in the dry area of Namaqualand.

The solitary mole-rats (*Bathyergus, Heliophobius* and *Georychus*) are generalist feeders with a more catholic diet, who feed on grasses, geophytes and aerial vegetation which is pulled into their burrow systems. On the other hand, the social mole-rats (*Cryptomys hottentotus* and *C. damarensis*) are specialist feeders consuming geophytes which they tend to store in large food caches.

The burrow systems of the dune mole-rat appear to be very large and deep. A comparison between five bathyergids and rhizomyids reveal that the larger mounds of the dune mole-rat can be misleading (Table 6). Clearly the two smallest mole-rats, *C. hottentotus* and *Heterocephalus glaber* have the smallest biomass per metre of burrow system and thus the longest burrow of any fossorial rodent (Davies and Jarvis 1986). In contrast, the solitary mole-rats have relatively shorter burrow systems. Therefore it can be seen that there is a correlation between burrow length, diet and food availability. *Bathyergus suillus* and *Georychus capensis* are generalist feeders and occur in the higher rainfall areas of the South West Cape compared to *B. janetta*, which occurs in a very arid area but with a high abundance of bulbs and geophytes and therefore also relatively short burrow systems. *Tachyoryctes splendens*, a generalist feeder in the moist areas of East Africa also tends to have a shorter burrow system compared to *H. glaber* and *Heliophobius argenteocinereus* from the arid regions. The giant mole-rat *Tachyoryctes macrocephalus* and *T. splendens* from Ethiopia collect food from the surface and have considerably

shorter burrow systems than either *B. suillus* or *B. janetta* from South Africa. It has also been shown that the pocket gopher, *Thomomys bottae,* in areas with a high productivity generally has shorter burrow systems.

Mole-rat activity appears to be highest after rainfall when the soil is soft and easy to burrow in. Indeed when comparing monthly mound production in *B. janetta* with the average rainfall figures for the area there is a positive correlation. This has also been observed in other bathyergids (De Graaff 1964; Du Preez 1984). However these studies do not consider plugging activity below ground. Davies & Jarvis (1986) suggested that *B. suillus* extend their burrow systems throughout the year and that moist soil does not lead to an increase in activity to extent burrow systems and food collection. In all other bathyergids it may be advantageous to extend burrow systems whilst the soil is moist and to store excess food for times of the year when the cost of burrowing would be high. It is evident from this study that there is a consistent change in the burrow system on a micro scale and that plugging and blocking of old tunnels occurs throughout the year. It may be that burrow extensions and changes are consistent throughout the year and that the increase in mole-rat mounds during the wet season is simply because the same amount of energy is needed to push up wet soil and plugging when the soil is soft. I believe that *B. janetta* does use the advantage of softer soil to extend the burrow systems in the wet winter seasons. This burrowing frenzy in the winter months may be to extend their burrow systems in optimal digging conditions to search for food or conversely it might be undertaken in order to facilitate the search of a potential mate. *Bathyergus janetta* is a seasonal breeder that initiates breeding in the winter months. The softer soil is beneficial in the search for a new mate but also presents ideal conditions for the pups when they start dispersing in early spring to establish their own burrow systems following parental eviction.

A very large burrow system is not always advantageous. The six excavated burrow systems ranged in length from 71.2m to 165m. The average length of 127.9m is well within the range for that measured in *B. suillus* burrow systems (107 - 420m) (Davies & Jarvis 1986) and it is suggested that when comparing burrow length of subterranean

mammals the optimum working length and cost of maintenance of long burrow systems needs also to be considered. The little variation in burrow length in this study would tend to suggest that *B. janetta* has an optimum burrow length.

Evidence from the excavation of burrow systems of *B. suillus* show that the burrow length is not linked to the sex of the occupant. Although the average length of male burrow systems was less than females, burrow system 2 was not fully excavated and the sample size was too small to show any statistical correlation. In solitary mole-rats, sexual dimorphism does occur and burrow diameter may be linked to sex. In *B. janetta* males are larger than females and thus need more space to move through their tunnel systems. The average burrow diameter for males was 13cm whereas that of females was 10cm and did not differ significantly, although the samples size was very small. Schultz (1978) found no correlation between sex and burrow diameter in *B. suillus* but in view of sexual dimorphism Davies & Jarvis (1986) do not agree.

The subterranean niche of the mole-rats is relatively stable with very little temperature flux inside the burrow system (Bennett & Jarvis 1988). These burrow systems are a very expensive investment. Vleck (1979) showed it to be 340-3600 times energetically more expensive to burrow than to move the same distance above ground. This might explain why mole-rats remain in a very well defined area. However by no means is the burrow system fixed and foraging burrows are constantly shifting. Shultz (1978) found no correlation between the extent of a burrow system and the total amount of mounds produced. By keeping an optimum burrow length *B. janetta* would always be able to respond to breaks in the system, blocking it up before any predators can enter. It has also been found in this study as well as by Davies and Jarvis (1986) for *B. suillus,* that these mole-rats remain in an area, working and reworking the system by continuously digging new foraging burrows and blocking old ones. These observations are substantiated by the frequent appearance of new mounds next to older mounds and therefore suggests the mole-rats may maintain a balance between vegetation eaten and the rate of regeneration (Davies & Jarvis 1986).

The shape and form of burrow systems appears to conform with the general findings of several other studies on subterranean rodents in that the male seems to have a more linear shaped burrow system in relation to females (Figure 4 and 5). Davies & Jarvis (1986) found that the burrow system of a male *B. suillus* that was surrounded by the burrow systems of two females was shorter and distinctively more linear. In *Thomomys bottae* male burrow systems were found to be significantly longer and more linear than other individuals (Reichman, Whitham & Ruffner 1982). A more linear burrow system in male mole-rats may increase the potential of the animal finding a mate. Solitary bathyergids are aggressive and highly xenophobic towards conspecifics, it is only during the short breeding season and when the mother has young that plural occupancy arises. Aggressive behaviour in solitary mole-rats often leads to intense fighting with resultant death (Bennett & Faulkes 2000).

The depth of the tunnel system depends upon the soil characteristics as well as the size of the animal. It is suggested that the depth of tunnels correlate with the depth of the geophytes (De Graaff 1964). This seems to be true for most bathyergids (Lovegrove & Jarvis 1986). Smaller bathyergids also occur in areas with more consolidated soil and shallow tunnels are less likely to collapse. The dune mole-rats however, are found at depths far deeper than their food resources. *Bathyergus janetta* has a main tunnel at an average depth of 45cm and shallower foraging tunnels. The softer sand dunes and larger body size forces the dune mole-rats to dig deeper burrow systems to prevent them from collapsing (Davies & Jarvis 1986; M. Herbst pers. obs.).

Nests

The underground burrow system of mole-rats is a stable and protective environment against both temperature extremes and predators. Previous reports suggested that the evidence of old nesting material in mole-rat mounds (Skaife 1963) could indicate the close proximity of nests. However, in this study it was not found to be the case. During excavations of burrows, old nests were found to be sealed up with sand. The ejection of nesting material onto the surface would involve unnecessary energy expenditure (Davies & Jarvis 1986). Nests were found to have a single entrance, be almost circular and of a

diameter of approximately 25cm at a depth of 50cm. Each nest was filled with predominantly pieces of grass and *Oxalis* leaves. The nest chamber of *B. janetta* is primarily a sleeping area. Very few small bulbs were found in the nesting chambers and no faeces or waste material was found. A total of 15 nests were found in the six excavated burrow systems (average of 2.2 nests per burrow). None of the nests were found underneath vegetation, suggesting that above ground vegetation provides little shelter or protection at this depth since environmental conditions are stable. Davies & Jarvis (1986) suggested that the use of more than one nest simultaneously may reduce the distance an animal has to travel to rest or sleep. However, the use of multiple nests may also reduce infestation of parasites. Evidence suggests that areas prone to flooding have nests, which tend to be located on higher ground (Roberts 1951; Lovegrove & Jarvis 1986; Nevo 1961), although nests are also found at deeper levels than tunnels (De Graaf 1962; Hickman 1979; Davies & Jarvis 1986). It is suggested that the location of nests is dependent on the water drainage properties of the area (Jarvis & Sale 1971). In this study, no flooding of *B. janetta* burrow systems occurred, suggesting that burrows are located in areas with good drainage, although rainfall for this area is considerably lower than that for *B. suillus* and it may just be that rainfall is not high enough to cause flooding.

Food stores

The excavation of six food stores in this study is of particular interest. Food storage in *B. suillus* has been found to be rare and only occurs under adverse conditions. It has been suggested that if an animal is a generalist feeder it does not need to maintain food stores (Davies & Jarvis 1986). The Namaqua dune mole-rat occurs in an arid area and this may be one of the reasons why they keep food stores. All other bathyergids do keep larders and it seems that the geophytes in the food store normally exhibits some species selectivity (Bennett & Faulkes 2000). Mole-rats have been shown to exhibit a preference for particular geophyte species (Lovegrove & Jarvis 1986; Bennett & Faulkes 2000). Food stores represent small energy refuges, which can be used during times of food scarcity or they may be used by the breeding female when she has young. However, food stores have been found in both male and female burrow systems. Despite the fact that

Bathyergus janetta is a generalist feeder, which exists mainly on grass and geophytes, *Oxalis* bulbs were by far the most dominant geophytes and food resource in these caches.

Defecation sites

During the excavation of the six burrow systems five defecation sites were found. There is very little information on defecation sites in bathyergids (Genelly 1965; Jarvis & Sale 1971; Hickman 1979; Davies & Jarvis 1986). The Namaqua dune mole-rat produces relatively large defecation sites filled with faeces and sand. This organic waste must make a small contribution to the nutrient pool in this region, such as the case in the *B. suillus* mole-rat in the Fynbos region (Davies & Jarvis 1986). Hickman (1979) suggested that toilet areas might serve to confuse predators, but this is highly unlikely.

Bolt holes

Bolt holes are not uncommon in the burrow systems of subterranean rodents. Bolt holes are tunnels suddenly extending deep down and serve as a place of retreat when the animals are alarmed or threatened. In *Tachyoryctes* the nest is used for multiple functions and the bolt hole is always found in close proximity of the nest, whereas *Heliophobius* only sleeps in the nest and the bolt hole is located elsewhere (Jarvis & Sale 1971). Bolt holes have also been recorded for *Geomys* (Scheffer 1940) and *Talpa* (Godfrey & Crowcroft 1960). In *B. suillus* bolt holes exceed two metres where depth made further excavation impossible (Davies & Jarvis 1986). Indeed in the six burrow systems excavated, four bolt holes have been found and exceed 155cm but were not followed to the end due to difficulty in digging in soft sand.

Summary

There are many similarities to the methods of burrowing and the pattern of burrow systems of mole-rats from the family Bathyergidae. Dissimilarities can be explained due to differences in habitat type, food resources and social structure. *Bathyergus janetta* is a solitary rodent that is able to survive in the arid regions of the Northern Cape Province of South Africa. Predictable rainfall gives rise to a high abundance and diversity of geophytes. The burrow system of *B. janetta* has an optimum length, which is smaller than

B. suillus, but undergoes consistent excavation and re-excavation of tunnels in its home range. The Namaqua dune mole-rat makes use of optimum burrowing conditions to extend the tunnel system and search for potential mates. This is substantiated by the large amount of new mounds produce during the rainfall season.

Burrow systems may contain several nesting chambers, food stores and defecation sites. It is proposed that the organic matter and contents of burrow systems may contribute to the nutrients in the soil. Food stores are uncommon for *B. suillus* but not for *B. janetta*. Both species of mole-rats are generalist feeders and utilize mainly the underground storage organs of geophytes and above ground vegetation that they pull into their burrow systems. Food storage in *B. janetta* is important, especially during the hot and dry summer months. Bolt holes are common in many subterranean rodents and in *B. janetta* exceed more that 1.5m in depth and serve as places of refuge when alarmed or threatened.

Table 6 A comparison of the burrowing dynamics in Bathyergidae and Rhizomyidae subterranean rodents (Modified from Davies & Jarvis 1986)

	Average adult mass (g)		Number in burrow system	Average mass per burrow system	Average burrow length	Biomass	SD	Number systems in sample	Homerange
	Male	Female							
Bathyergidae									
Bathyergus suillus	933	635	1	877	256	3.8	1.84	7	1390-3496
B. janetta	439	330	1	379	128	3.3	1.16	6	398-1438
Georychus capensis	181		1	169	48	4.5	1.88	14	272
Heliophobius argenteocinereus	160		1	100	47	2.1	0.34	4	172
Cryptomys hottentotus	83	58	2-14	132-810	464	0.8	0.22	4	3922
Heterocephalus glaber	21		60	1250	595	2.1	-	1	5401
Rhizomyidae									
Tachyoryctes splendens	250	218	1	231	36	7.7	3.17	6	100
T. s. ruandae	240	212	1	226	20	11.3	-	2-50	36
T. macrocephalus	300-1000		1	300-1000	34	-	-	1	-

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Plate 1 Namaqua dune mole-rat, *Bathyergus janetta*

Plate 2.1 Excavated burrow system

Plate 2.2 Contents of *B. janetta* nest

Plate 3.1 Contents of *B. janetta* food store

Plate 3.2 Study site on farm Kardou just outside town of Kamieskroon

Chapter 3 The reproductive biology of the Namaqua dune mole-rat, *Bathyergus janetta*

Introduction

To reproduce is the ultimate objective of all life processes. All living organisms are capable of giving rise to new generations similar to themselves. Reproduction is the most important activity to ensure the continued existence of a species. Considering the subterranean nature of African mole-rats (Bathyergidae) and the ecological constraints imposed by their burrow environment, it is not surprising that there is so little information on the reproductive biology of solitary mole-rats (Bennett & Faulkes 2000; Bennett, Faulkes & Molteno 2000).

The interactions between species and their environmental conditions can result in restricted breeding seasons. The post mortem examination of many species collected throughout the year indicates that many subterranean rodents exhibit a seasonal breeding pattern: e.g. Ctenomyidae (Reig, Busch, Ortelis & Contreras 1990), Geomyidae (Smolen, Genoways & Baker 1980), Rhizomyidae (Flynn 1990), Bathyergidae (Bennett & Jarvis 1988a) and Spalacidae (Simson, Lavie & Nevo 1993). Seasonal breeding allows animals to time their reproduction such that young are produced only when environmental conditions are favourable and thus maximise the offspring survival (Ims 1990). The Musky rat-kangaroo (*Hypsiprymnedon moschatus*) produce their young when the main diet (litter fauna and fruit) is in great abundance (Dennis & Marsh 1997). Regression of reproductive organs during non-breeding periods allows animals to utilise more energy for activities such as food gathering (Woodall & Skinner 1989).

The onset of reproduction and the subsequent breeding season depends on two main cues. Internal cues such as endogenous clocks and external cues such as: photoperiod, temperature, food availability and humidity. In subterranean rodents that reproduce seasonally there is limited information on which environmental factors control reproduction. Photoperiod is probably the most important and commonly used agent to

synchronise breeding patterns in animals (Karsch, Bittman, Foster, Legon & Robinson 1984; Gardiner, Boyd, Follett, Racey & Reijnders 1999). In subterranean mole-rats with small reduced eyes, photoperiod may not play a role. Although, the blind mole-rat, *Spalax ehrenbergi* can still entrain its daily activity pattern to a circadian light-dark cycle even with vestigial eyes that are totally subcutaneous (Cooper, Herbin & Nevo 1993). Another important environmental cue may be temperature. Burrow temperatures within mole-rat burrow systems may be less variable than ambient above ground temperatures (Gates 1962; Bennett & Faulkes 2000), but the shallow foraging tunnels are exposed to diurnal and seasonal fluctuations in temperature. Solitary high latitude subterranean rodents do show marked breeding patterns and this may be due to seasonal changes in temperature of their burrow systems. Rainfall is another environmental cue that leads to increased vegetational growth and food availability (Dennis & Marsh 1997). The moistening of soil promotes burrowing activity and dispersal in solitary African mole-rats (Bennett & Faulkes 2000). Indeed, in all three Cape mole-rats (*Georychus capensis, Bathyergus suillus* and *B. janetta*) offspring are born in early spring when the soil is still moist, food is widely distributed and the soil is optimal for the establishment of independent burrow systems. Tropical mole-rats inhabiting lower latitudes lack distinct breeding seasons. This may be due to the absence of environmental cues and the stable underground niche of their burrow systems (Bennett & Faulkes 2000).

Most solitary subterranean rodents are aggressive and highly xenophobic towards conspecifics. To breed, this aggression has to be broken down. Solitary animals communicate to conspecifics in order to convey information such as sex, status and the intention to breed. Most underground communication is of a seismic nature (Bennett & Faulkes 2000). Vibrations are transmitted through the soil at levels an order of magnitude greater than auditory signals (Narins, Reichman, Jarvis & Lewis 1992). The mole-rats of the family Bathyergidae use hind foot drumming to communicate (Bennett & Jarvis 1988a,b; Jarvis & Bennett 1990,1991). In captive colonies of Cape mole-rats hind foot drumming is initiated by males with vibrations of a frequency of about 0.035s per pulse. Females respond by producing slightly slower vibrations of a frequency of 0.05s per pulse. In the field drumming can be heard 10m away from the source (Bennett & Jarvis

1988a). The Rhizomyid, *Tachyoryctes splendens* uses incisor tapping and the blind mole-rat *Spalax ehrenbergi* uses head drumming to convey messages underground (Jarvis 1969a; Heth, Frankenberg, Raz & Nevo 1987; Rado, Levi, Hauser, Witcher, Alder, Intrator, Wollberg & Terkel 1987). Social bathyergids communicate within the confines of their burrow system. Seismic signals are less important whereas tactile, vocal and olfactory cues are more prominent and are used in courtship and mating behaviour. Seismic communication may be important during dispersal. Once solitary mole-rats come together then tactile, olfactory and vocal communication may become more important.

Since solitary mole-rats are so aggressive, it is often difficult to observe mating and courtship behaviour, even under laboratory conditions. Most solitary subterranean rodents exhibit very similar courtship and mating behaviours. Courtship in *S. ehrenbergi* (Nevo 1961), *Tachyoryctes splendens* (Jarvis 1969b), *G. capensis* (Bennett & Jarvis 1988a), *Ctenomys pearsoni* (Altuna, Francescoli & Izquierdo 1991), *Thomomys bottae* (Andersen 1978) and *Geomys* (Schramm 1961) is usually initiated by the male. Seasonality can be monitored through urinary hormone concentrations. In *G. capensis,* urine samples collected from captive males and females throughout the year revealed an increase in oestrogen and testosterone concentrations during May to September. This correlated to the same time period that courtship, copulation and subsequent pregnancies were observed in the field (Bennett & Jarvis 1988b). In social bathyergids courtship behaviour is a prolonged activity and mating can be observed over consecutive days (Bennett 1989; Bennett & Jarvis 1988b; Bennett & Faulkes 2000). In the common mole-rat, males initiate courtship (Bennett 1989) and in the Damaraland and naked mole-rats courtship are initiated by the females (Bennett & Jarvis 1988b; Bennett 1990; Jarvis 1991).

Solitary subterranean rodents appear to be induced ovulators (Bennett *et al*. 2000). This translates to ovulation being triggered by the act of mating itself, as seen in the Ctenomyid, *Ctenomys talarum* (Weir 1974). Induced ovulation appears to be the general rule in non-gregarious mammals (Zarrow & Clarke 1968) and an adaptive trait in solitary species relying on brief, chance encounters for mating. In the family Bathyergidae it is still unknown if the solitary species are induced or spontaneous ovulators. The social

common mole-rat *Cryptomys hottentotus* is an induced ovulator (Spinks, Bennett & Jarvis 1999), whereas the two eusocial mole-rats, the naked mole-rat and Damaraland mole-rat are spontaneous ovulators (Faulkes, Abbott & Jarvis 1990; Molteno & Bennett 2000).

Gestation time in bathyergids is long and provides support for their close hystricomorph affinities (Bennett & Faulkes 2000). Litter size is generally small and characteristic of most subterranean rodents (except the naked mole-rat) (Bennett, Jarvis, Aguilar & McDaid 1991; Malizia & Busch 1991). All seasonally breeding, solitary mole-rats produce up to two litters annually (Jarvis 1969a,b; De Graaff 1981; Bennett & Jarvis 1988a; Bennett 1989; Bennett *et al.* 1991; Malizia & Busch 1991). Since solitary mole-rats can reproduce twice a year, it would appear as if recruitment of young per female might exceed that of social mole-rats. Females will always try to maximise their genetic contribution to future generations and therefore should produce as many young as possible (Bennett & Faulkes 2000).

Since there is so little information on the reproductive biology of solitary mole-rats and it is so difficult to examine their reproductive behaviour, the aim of this study was to amass information on the reproductive biology of the Namaqua dune mole-rat, *Bathyergus janetta.* Intense field observations and the collection of urine throughout the year was conducted and the subsequent measurement of urinary hormone concentrations undertaken to determine if the mole-rat had an established breeding season. The capture of mole-rats throughout a period of twelve months enabled the detection of times of plural occupancy of burrows as well as the reproductive status of females and the times that pups were caught. This Chapter describes the reproductive behaviour, breeding season and dispersal of pups and adults in their natural environment.

Materials and Methods

Namaqua dune mole-rats were caught in Kamieskroon (30°9'S, 17°56'E), Northern Cape Province during July 2000 through to September 2001. The mole-rats were caught with modified snaptraps, Hickman live-traps (Hickman 1979) or by cutting of their retreat with a hoe when they came to block an open section of their tunnel systems (Jarvis & Sale 1971). Standard body measurements and body mass were taken.

To investigate urinary hormone profiles (testosterone, progesterone and oestradiol-17β) urine samples were collected from every mole-rat caught during the time of the study. Urine was used in hormone analysis since it could be obtained via a non-invasive procedure. Animals were placed in plastic containers with a pre-cleaned floor until they had urinated, after which they were returned to their burrow systems. Namaqua dune mole-rats void between 2 and 4 ml of urine. The urine was collected using a Pasteur pipette and subsequently stored at –20°C until required for radioimmunoassays.

Field observations

The reproductive status of animals was noted by placing an animal in a category of either adult or juvenile. Juveniles were recognised as animals weighing less than 200g and adults more than 200g. Teat size was determined by arbitrary observations and classified as small or large in size. Times of plural occupancy of burrow systems were also noted as well as times that pups were present in burrow systems.

Chemical analysis

Creatinine analysis

Urinary hormone concentrations may vary due to differences in fluid intake. To standardise all urinary hormone concentrations, creatinine concentrations in the urine were determined. Creatinine is a breakdown product of tissue proteins and is excreted at relatively constant rates. All urinary hormones are expressed as hormone concentration in terms of millimoles of creatinine excreted. Concentrations of creatinine in each sample were determined using the Jaffe reaction (Folin 1914). The creatinine concentration in the urine was determined from 5µl of thawed urine by the use of a microplate reader at a

wavelength of 492 nm as described by Clark (1999) and Malherbe (2001) (see Appendix 1). The values of all standards were plotted on a graph for absorbance against creatinine concentrations (Figure 1). By using a simple linear regression, the equation of a straight line was determined and the corrected creatinine concentrations for each sample were determined.

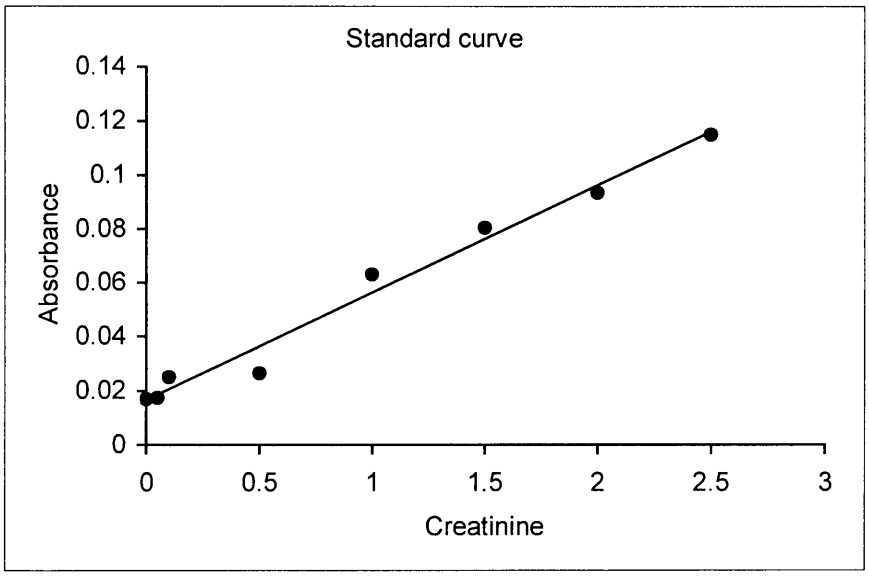


Figure 1 Standard curve for correct creatinine concentrations

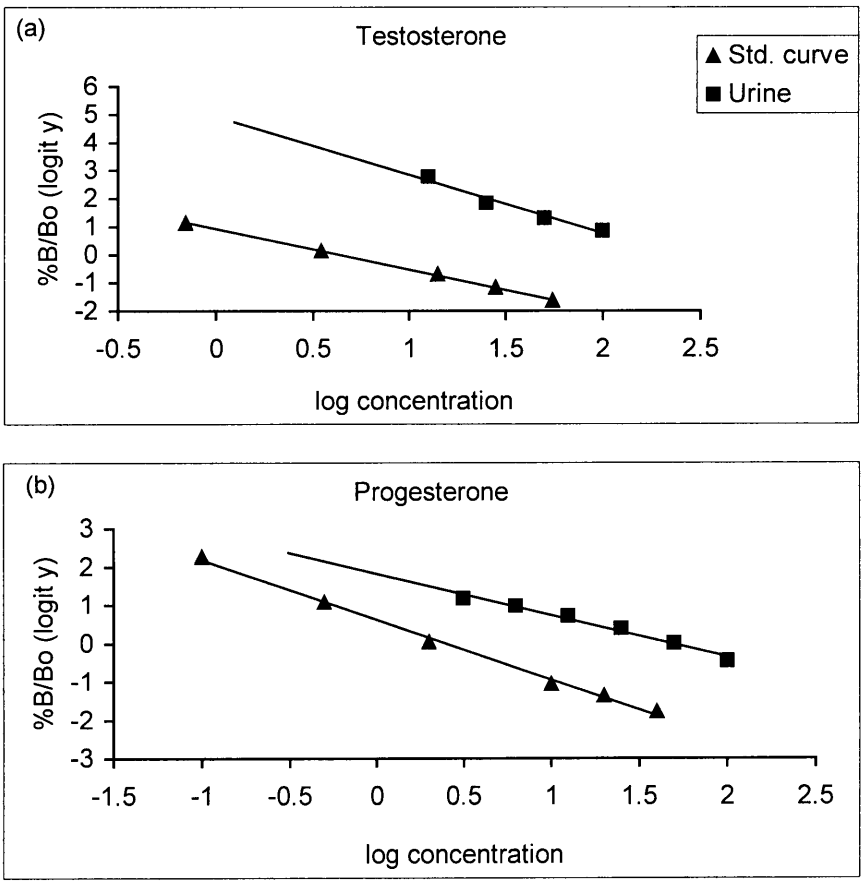
Hormone assays

Testosterone, progesterone and oestradiol-17β bioassays were performed using a *Coat-A-Count Kit* (Diagnostic Corporation, USA) as per manufacturers instructions. The procedure is a solid phase radioimmunoassay based on a hormone specific antibody immobilised to the walls of polypropylene tubes. For the testosterone immunoassay 50µl of urine was used. The ^{125}I-labeled testosterone competes for a fixed time of 3 hours at 37°C with the testosterone in the sample for antibody sites. For the progesterone and oestrogen immunoassay 100µl of urine was used and incubated for 3 hours at room temperature (25°C). The tubes are then decanted thoroughly and counted on a Cobra II

gamma counter (Packard Instrument Company, Meriden, USA). The amount of hormone present in each sample was determined from a calibration curve.

Validating the hormone immunoassays

Each radioimmunoassay was validated for use in the Namaqua dune mole-rat by serially diluting the urine over a range of 1:1 to 1:32 with a matrix similar to that used to prepare the ligand. This produced a curve with a slope that could be compared to a standard curve. For all radioimmunoassays the sample double dilutions paralleled the standard curve (Figure 2). By following log-logit transformation of data (Chard 1987) the slopes of the lines were compared using STATISTICA computer package (Statsoft, Tulsa, USA) and found not to differ significantly (ANCOVA Testosterone: $F = 0.68$, $P > 0.5$; Progesterone: $F = 1.57$, $P > 0.25$ and Oestradiol-17β: $F = 0.3$, $P > 0.62$).



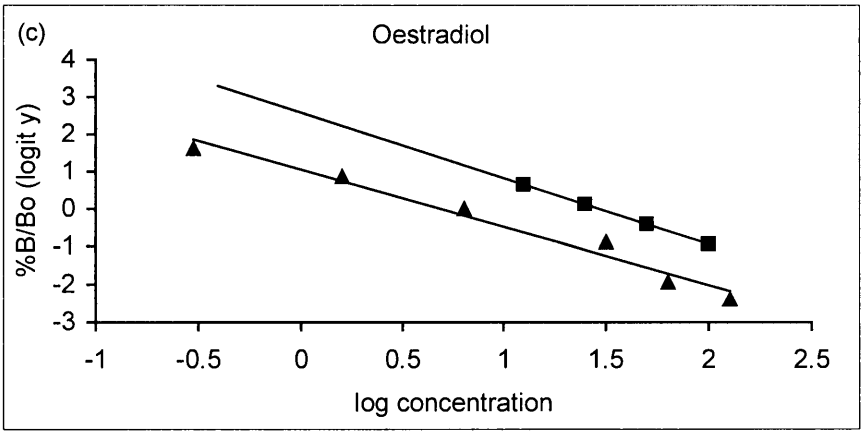


Figure 2 Serial double dilutions of unextracted *B. janetta* urine and standard curve showing parallelism for (a) testosterone; (b) progesterone; (c) oestradiol-17β concentrations.

Results

Observational data

The total numbers of animals caught during July 2000 – September 2001 are summarised in Table 1 with the reproductive status of individuals during each month. A total of nine pups were caught during the spring months after the winter rainfall of Namaqualand. Female teat size tends to increase from August indicating that females are pregnant, lactating or have young in the burrow system. Plural occupancy may result from either the presence of pups in the burrow system or the observation that two adult animals were in the same burrow system.

Table 1 Reproductive data of animals caught during July 2000 – September 2001

Month	Number adults	Number juveniles	Pregnant	Teat size Small/large	Plural occupancy
January	18	-	-	S	No
February	12	1	-	S	No
March	16	1	-	S	No
April	10	-	-	S	No
May	20	-	-	S	Yes
June	15	-	-	S	Yes
July	24	-	-	S	Yes
August	22	-	4	L	Yes
September	18	-	3	L	Yes with pups
October	12	2	1	L	Yes with pups
November	23	6	2	L	No
December	16	1	-	L	No
Total	206	11	10		

Hormone profiles

Testosterone

Testosterone concentrations in males showed an increase during July and August as well as in October and November months (Figure 3). This is the time of the year when Namaqualand receives winter rainfall and the soil is still soft and moist. An increase in the number of mole-rat mounds was also observed (Chapter 2) and it is suggested that digging conditions were favorable for mole-rats to extend their burrow systems and search for potential mates to breed with. Seasonal breeding has been described in solitary mole-rats and an elevation in urinary hormone concentrations in *B. janetta* males occurs during the season with high rainfall

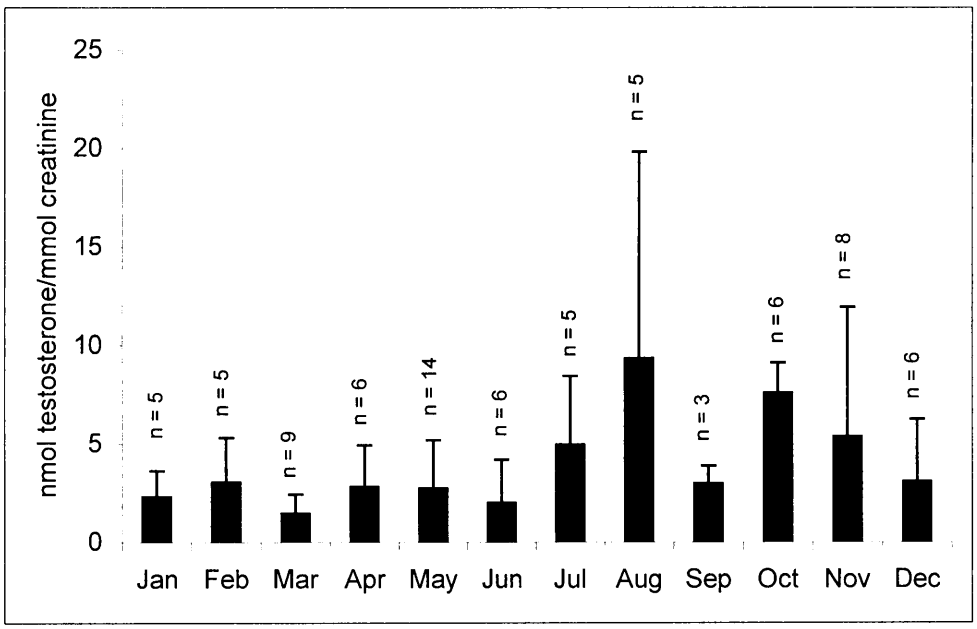


Figure 3 Urinary testosterone concentrations (nmol/mmol creatinine) collected from males during the study.

Progesterone

Concentrations of urinary progesterone in females were very low throughout much of the year (excluding July to September). From July through to September there was a very

large increase in progesterone concentrations in females (Figure 4). This is correlated with female teat size increase and plural occupancy of adult animals in burrow systems (Table 1). Due to very low progesterone concentrations during summer months it is suggested that that *B. janetta* mole-rats have a very strict breeding season ranging from July to the end of September.

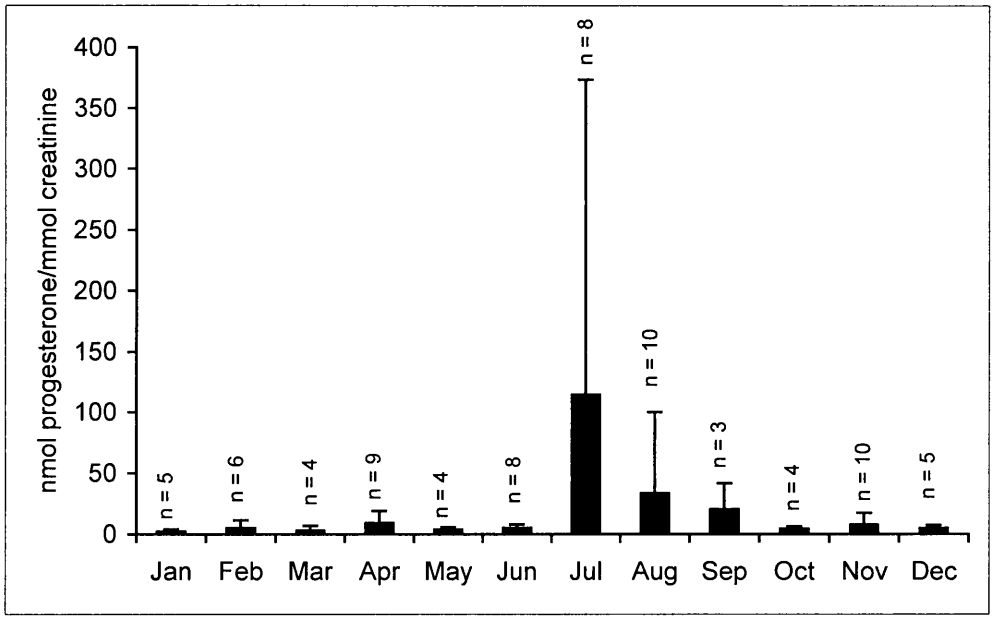


Figure 4 Urinary progesterone concentrations (nmol/mmol creatinine) collected from females during the study.

Oestradiol-17β

Oestradiol-17β concentrations in *B. janetta* females were very high during the breeding season and it was necessary to dilute the samples 1:16 times. This suggests that the particular individuals were ovulating. Elevated urinary oestradiol-17β concentrations in females were observed during July and August and correspond to the patterns observed for female progesterone and male testosterone concentrations (Figure 5). However the slight elevation in oestradiol-17β during March to May is an anomaly.

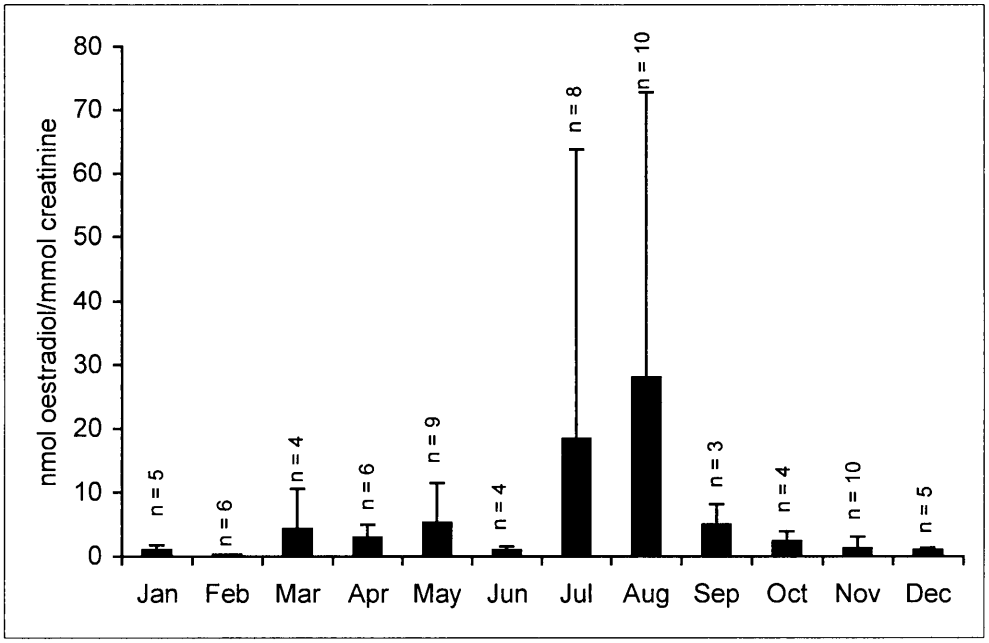


Figure 5 Urinary oestradiol-17β concentrations (nmol/mmol creatinine) collected from females during the study.

Correlation with rainfall

Urinary hormone concentrations correlate with seasonal rainfall figures (testosterone: $r = 0.43$, $p < 0.05$; progesterone: $r = 0.94$, $p < 0.05$ and oestradiol-17β: $r = 0.8$, $p < 0.05$). Thus it appears as if rainfall might be an important environmental cue for the onset of reproduction in *B. janetta* (Figures 6 & 7).

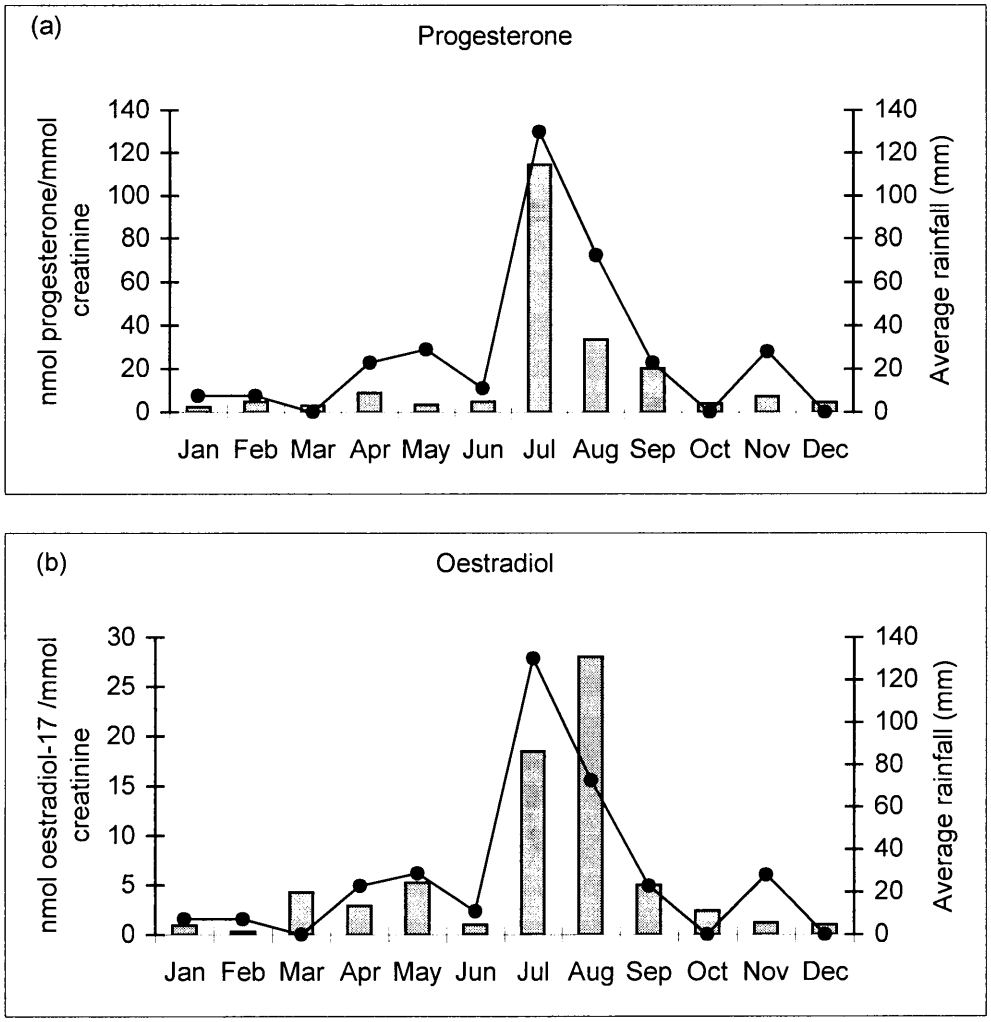


Figure 6 Correlation of average monthly rainfall (mm) with (a) urinary progesterone concentrations (nmol progesterone/mmol creatinine) and (b) urinary oestradiol-17β concentrations (nmol oestradiol-17β/mmol creatinine) in *B. janetta* females.

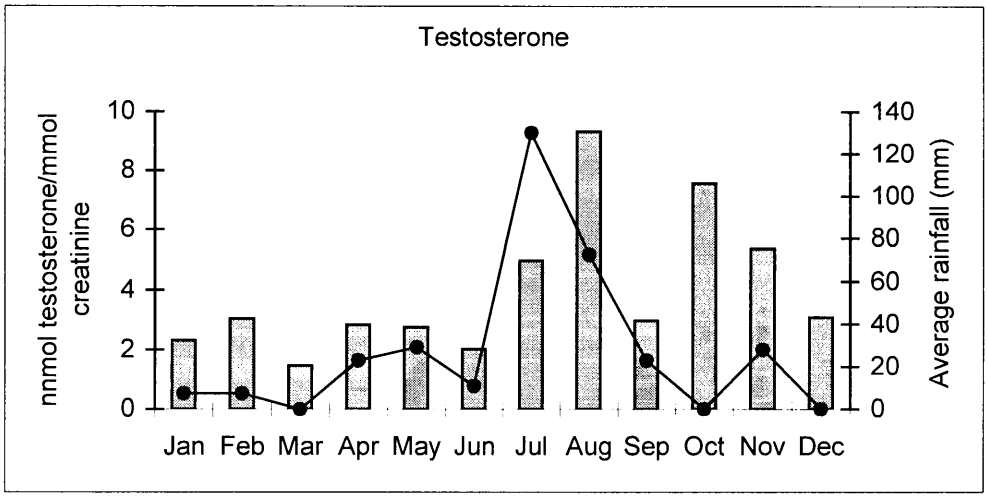


Figure 7 Correlation of monthly average rainfall (mm) with urinary testosterone (nmol testosterone/mmol creatinine) concentrations in *B. janetta* males.

Behavioural observations

Attempts to establish reproductive pairs for observations of reproductive behaviours such as courtship, mating, gestation times and development of pups were not successful since mole-rats were very aggressive towards conspecifics even during the breeding season. It is suggested that drumming of the hind feet are important pre-courtship behaviour and convey messages such as reproductive status of the animal.

Discussion

Bathyergus janetta is a solitary subterranean rodent that is for most of the year the sole occupant of a burrow system. The burrow systems of solitary mole-rats can be in very close proximity, and less than two metres away from each other, but the systems are not interlinked (Bennett & Jarvis 1988a). Solitary mole-rats are highly aggressive towards each other and when two individuals are placed together (of either sex) it results in fighting that can eventually lead to the death of an animal (Bennett & Faulkes 2000). When two *B. janetta* mole-rats were put together, both animals initiated drumming with their hind legs and after a few seconds lunged forward producing high pitched sounds and attacking one other with their extrabuccal incisors.

Field observations have shown an increase in the amount of drumming during July to November and on two occasions, drumming could be heard in the field (M. Herbst pers. obs.). Drumming in mole-rats is a method of seismic communication. Being solitary subterranean animals, mole-rats have to convey messages, such as reproductive status and territoriality to conspecifics. Drumming has also been observed in other families of subterranean mammals such as the Geomyidae (Bennett & Jarvis 1988a) and other bathyergids, *B. suillus, G. capensis, C. hottentotus* and *C. damarensis* (Bennett & Jarvis 1988a,b; Jarvis & Bennett 1990,1991). Drumming is achieved by simultaneously striking both hind feet on the burrow floor at a very fast tempo, while supporting the body with the front feet (Bennett & Faulkes 2000). In *G. capensis,* males initiate drumming and the females respond by drumming with a slightly slower tempo (Bennett & Jarvis 1988a). Other means of communication by subterranean rodents include incisor tapping (the Rhizomyd, *Tachyoryctes splendens*) (Jarvis 1969a) and head drumming (the blind mole-rat, *Spalax ehrenbergi*) (Heth *et al.* 1987; Rado *et al.* 1987). Bennett & Jarvis (1988a) proposed that drumming signals produced by fossorial mammals might not only be used in precopulatory signalling, but also when the animals are alarmed or as a means to defend their territory. It is suggested that drumming in *B. janetta* plays an important role in breaking down any hostile behaviour before mating. On several occasions two mole-rats of different sex were put together during the breeding season but it always resulted in fighting.

Copulatory behaviour in all solitary subterranean rodents is very similar. Courtship is initiated by the male and involves an initial period of long distance communication before animals meet *e.g. Spalax ehrenbergi* (Nevo 1979), *Thomomys* (Anderson 1978), *Geomys* (Schramm 1961), *Tachyoryctes splendens* (Jarvis 1969a) and *Georychus capensis* (Bennett & Jarvis 1988a). Copulation in *B. janetta* has not been observed, but in each case where two adults animals were observed in one burrow system on checking the following day, only one animal was caught with no signs of a second animal coming to block the open section of the burrow system. Thus it seems as if copulation may be very brief. Mating in subterranean rodents involves repeated mounting sequences with short periods of grooming, particularly around the genitalia. Extended periods of courtship and copulation in *S. ehrenbergi* and pocket gophers (*Thomomys talpoides* and *T. bottae*) may reflect the relative safety that a subterranean burrow system provides (Andersen 1978). Interestingly male mole-rats were caught in female burrow systems as well as females in male burrow systems. It is not known in African mole-rats how they disperse in search for a possible mate but it seems as if burrow systems are indeed interlinked during the short period of mating.

Sexual dimorphism in *Bathyergus* is extremely marked (Taylor, Jarvis & Crowe 1985; Jarvis & Bennett 1991). It may be possible that only large and aggressive males mate and that matings are polygynous (mating with several females), such as the case in the geomyid *T. bottae* (Reichman, Whitham & Ruffner 1982). Male Cape dune mole-rats are characterized by a thick pad of skin on the ventral surface of the neck (Davies & Jarvis 1986) and badly injured animals have been caught in the field during breeding seasons (Jarvis & Bennett 1991). Indeed Bennett & Faulkes (2000) have found two interlocking skulls of male Namaqua dune mole-rats in the field. Both animals dying in mortal combat been locked together!

The presence of pups in the burrow systems was observed at the end of the rainy season towards the early summer months (October and November). On one occasion a mother with four of her pups was caught in the same burrow system. The mother was lactating

and the pups still drinking from her. The pups averaged a mass of 61g and were already able to block up open sections in the burrow system. When given slices of sweet potato to eat the pups did try to break small pieces off, but were not able to succeed. A long gestation time is a characteristic of hystricomorph rodents (Weir 1974). Gestation time in bathyergids ranges from 44 days in *G. capensis* (Bennett & Jarvis 1988a) to 111 days in *Cryptomys mechowi* (Jarvis 1991) (Table 2).

In solitary bathyergids the pups are forced to leave the burrow system and establish their own territory at an early age. In *G. capensis* pups disperse by extending the maternal burrow system or to move short distances above ground (Jarvis & Bennett 1991) and it seems that dispersal in the dune mole-rats (*Bathyergus*) are very similar (Bennett & Faulkes 2000). The mothers of solitary bathyergids become very intolerant towards juveniles and the pups start dispersing at approximately 60 days of age (Bennett pers. comm.).

Seasonal breeding in *B. janetta* is shown in urinary hormone profiles collected throughout the year from a wild population. Urinary testosterone concentrations in males were relatively high throughout the year but show a heightened increase starting from May through to November (Figure 3). All hormone concentrations in females were very low. Both progesterone and oestrogen show a sharp increase in July and August (Figures 4 & 5). Oestrogen concentrations in a few females were very high during July and August and had to be diluted 1:16 times, suggesting that the females were ovulating. Female teat size starts to increase in August with pregnant and lactating females being caught during August to November. All field observation data and urinary hormone concentrations support a very strict breeding season, which correlates with the rainfall pattern of the area (Figure 6 & 7). The breeding season in *B. janetta* is restricted to a very short period just after the first winter rainfall. Seasonal breeding allows animals to time the reproduction of young when environmental conditions are favourable and thus maximise the offspring survival (Ims 1990). Solitary bathyergids (*Georychus* and *Bathyergus*) adult females may have the opportunity to breed twice a year and thus have a higher recruitment of pups in

comparison to social species of mole-rats (Table 2). Females should produce as many young as possible therefore maximising their genetic contribution to future generations.

Summary

Reproduction in a wild population of *B. janetta* is described as a result of catching and re-catching marked animals throughout the year. *Bathyergus janetta* is a solitary mole-rat that is highly xenophobic towards conspecifics. This aggression needs to be broken down in order to breed. Mole-rats convey messages such as territory and reproductive status by low frequency seismic drumming. Recrudescence of breeding occurs in July when plural occupancy of adult animals was observed on several occasions in the same burrow systems. Mating appears to be brief whereafter the male leaves the female to rear the pups on her own. Urinary hormone concentrations of testosterone in males and progesterone and oestrogen in females showed a marked elevation in the months of July to September, which correlates to the rainfall pattern of the area. After the winter rainfall, the soil is soft and moist and easily excavated. *Bathyergus janetta* makes use of this opportunity not only to extend their burrow systems but also to search for possible mates. Pups were observed from September - October when the soil is moist and excavation of their own burrow systems is possible on expulsion from the maternal burrow system by the mother.

Table 2 A comparison of factors associated with reproduction and parturition for the family Bathyergidae (Adapted from Bennett & Faulkes 2000)

Species	No. in burrow	Breeding season	Gestation (days)	Max. no. of litters per year	Mean litter size	Birth mass (g)	Mean annual recruitment
Bathyergus suillus	1	Jul – Oct	52	2	2.4 (1-4)	34	5
Bathyergus janetta	1	Aug – Dec	-	2	3.5 (1-7)	15.4	7
Georychus capensis	1	Aug – Dec	44 – 48	2	6 (4-10)	5-12	12
Heliophobius argenteocinereus	1	Apr – Jun	87	-	- (2-4)	7	-
Cryptomys h. hottentotus	<14	Oct – Jan	59 – 66	2	3 (1-6)	8-9	6
Cryptomys damarensis	<41	All year	78 – 92	4	3 (1-5)	8-10	12
Cryptomys amatus	<25	All year	100	3	2 (1-2)	7.8-8.1	6
Cryptomys darlingi	<11	All year	56 – 61	4	1.7 (1-3)	6.9-8.2	8
Cryptomys machowi	<8	All year	97 – 111	3	2 (1-3)	15-21	6
Heterocephalus glaber	<295	All year	66 - 74	4	13 (1-27)	1.8	52

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Chapter 4 Population genetics and relatedness in the Namaqua dune mole-rat, *Bathyergus janetta*

Introduction

An understanding of animal behaviour and the causes of social organisation require very intensive and long-term field observations. The subterranean lifestyle of mole-rats makes it difficult to obtain data and knowledge of the mating system, patterns of dispersal, gene flow and the relatedness among individuals of the same population. The use of genetic techniques has revolutionised population biology in so many new and exciting ways. It is now possible to determine genetic variation in populations, parentage and relatedness (Bourke, Green & Bruford 1997; Amos, Schlotterer & Tautz 1993; Girman, Mills, Greffen & Wayne 1997; Burland 1998).

There are different genetic techniques that can be used (Box 1) (Queller, Strassmann & Hughes 1993). In this study I used microsatellite loci that have been developed specifically for the family Bathyergidae (Burland, Bishop, O'Ryan & Faulkes 2001). Microsatellite loci are short tandem repeat sequences of DNA that are widely distributed throughout the eukaryotic genome (Bruford & Wayne 1993). They were detected over 20 years ago but regarded as sequences of no particular interest (Tautz & Renz 1984). Since the advent of the Polymerase Chain Reaction (PCR) it was realized that microsatellites might be the most important Mendelian markers ever found (Jarne & Lagoda 1996).

Box 1

1. Allozymes are protein markers that are electrophoresed through a gel. An enzyme specific reaction highlights one locus whose alleles might have to migrate a different distance due to charge differences.
2. Restriction fragment length polymorphisms (RFLPs). DNA is cut with a restriction enzyme and then run on an agarose gel, blotted to membranes and then probed with cloned radio labelled DNA that binds to a single locus. Alleles that differ in the presence or absence of nearby restriction sites will produce different fragment sizes.

3.	Multilocus minisatellites or DNA fingerprints. The method is similar as above (2) but the probe hybridises to a minisatellite sequence. Complex band patterns result from many loci with alleles that vary in the number of repeats.
4.	Single-locus minisatellites or variable number of tandem repeats (VNTRs). The probes that are used are clones that identify a single minisatellite locus at sufficiently high stringency. The clones are obtained by screening a library with the standard minisatellite probe.
5.	Randomly amplified polymorphic DNA (RAPDs) are arbitrary oligonucleotides of about 10 bases used in a PCR reaction and will usually anneal well enough to serve both forward and reversed primer at 3-10 sites. The products are electrophoresed through agarose gel and stained. Bands present in one individual may not be present in another for a variety of reasons, chiefly variation in the primer annealing sites.

Box 1 Segregating genetic markers (Modified from Queller *et al.* 1993)

Characteristics of microsatellites

Microsatellites are co-dominant and inherited in a Mendelian fashion. They are considered as neutral (Jarna & Lagoda 1996). Microsatellites generally comprise of one to five base pairs, that are highly polymorphic in natural populations and they give a high level of genetic information (Goodnight & Queller 1999; Queller *et al.* 1993).

The development of microsatellite loci

These numerous, highly variable, short sequences of DNA became the choice for high-density genome mapping (Hearne, Ghosh & Todd 1992). The development of microsatellite loci involves the cloning of small segments of host DNA, detection of microsatellite loci and the subsequent sequencing in order to find flanking sequences that can be used as locus specific PCR primers (Queller *et al.* 1993). Since the use of microsatellites is fairly new, one of the difficulties in the use of them has been the

necessity to develop markers for the species of interest. In this study, microsatellite loci for the family Bathyergidae have been developed by Burland *et al.* (2001).

To amplify microsatellite loci from any individual the use of the Polymerase Chain Reaction (PCR) is made (Saiki, Gelfand, Stroffel, Higuchi, Horn, Mullis & Erlich 1988). The information received can then be viewed as bands on a polyacrylamide gel. The quality of the information depends on (i) how objectively the products are scored, (ii) how accurately they reflect the underlying genetic variation, and (iii) how representative they are (Queller *et al.* 1993).

One of the drawbacks in the use of microsatellite loci is the occurrence of null-alleles. Null-alleles are non-amplifying alleles (Paetkau & Ströback 1995). They are recognised by apparent non-inheritance of parental alleles in known parent-offspring pairs (Pemberton, Slate, Bancroft & Barrett 1995). In some species the occurrence of null-alleles can be very low (Brookfield 1996) but it can also be relatively high (Pemberton *et al.* 1995). Null-alleles result in a Hardy-Weinberg disequilibrium because heterozygotes are read as homozygotes (Brookfield 1996; Pemberton *et al.* 1995).

The advantages in the use of microsatellites

The use of PCR to amplify the DNA samples is a tremendous advantage over other genetic techniques. Only a small sample of DNA is needed (Queller *et al.* 1993). Microsatellites have been used in several human population genetic studies (Estoup, Garnery, Soluignac & Cornuet 1995; Edwards, Hammond, Jin, Caskey & Chakraborty 1992) as well as the construction of human linkage maps (Litt & Luty 1989; Weissenbach, Gyapay, Dib, Vignal, Morisette, Millasseau, Vaysseix & Lathrop 1992). Microsatellites are also extremely useful in studies on conservation of endangered species, especially in looking at events of inbreeding, social structures, gene flow and dispersal events (Moxon & Wills 1999; Bruford & Wayne 1993; Amos *et al.* 1993; Girman *et al.* 1997). Another advantage of microsatellites and the use of PCR for amplification is the ability to use old and degraded DNA (Roewer, Rieß & Prokop 1991; Ellegren 1991) especially in forensics and criminal cases (Budowle, Giusti, Waye, Baechtel, Fourney, Adams, Presley, Deadman & Monson 1991).

Parentage

The identification of parentage relies on the process of exclusion analysis. This process involves the comparison of candidate parent genotypes against the offspring genotype. Parents are then excluded if they do not share the same alleles with the offspring at each locus. The adults that remain are hence regarded as putative parents. Putative parents may not be the true biological parent (Marshall, Slate, Kruuk & Pemberton 1998). During collection of samples in the field it is not always possible to be 100% sure that all individuals have been sampled. In such cases the confidence of parental identification depends on statistical inferences (Chakraborty, Meagher & Smouse 1988). Where behavioural data are available it can be used to permit further exclusions of possible parents (Girman *et al*. 1997).

Relatedness

The level of relatedness (R) among individuals is defined as the expected proportion of alleles identical by descent that are shared among individuals (Bourke *et al.* 1997). The expected values for R of known relationships in diploid organisms are calculated by the assumption that half of the genetic material of an offspring is inherited from the father and half from the mother. The R value of a true parent to offspring relationship is thus 0.5. Expected R values for known relationships are given in Table 1. Estimated R values using genetic data were determined by the method developed by Queller and Goodnight (1989), which calculates the relatedness within single groups, and between pairs of individuals or dyads.

Relatedness will increase when closely related individuals breed with each other. This is termed inbreeding. Thus inbreeding can be described as the mating between individuals that share a greater common ancestor than if they had been drawn at random from a large population (Chesser & Ryman 1986; Shields 1993). Inbreeding can be beneficial in maintaining kin-selection within a social group (Hamilton 1964). However, inbreeding can also result in a decrease in fitness in individuals through inbreeding depression (Keller, Arcese, Smith, Hochachka & Stearns 1994). Therefore a variety of inbreeding avoidance mechanisms have evolved in animal species (Pusey & Wolf 1996).

Table 1 Expected values of levels of Relatedness (R) for diploid organisms, after Rasmuson (1993) and Bourke (1997).

Relationship	Expected levels of (R)
Parent – Offspring	0.5
Full Siblings	0.5
Half Siblings	0.25
Grandparent – Grandchild	0.25
3rd Descendant	0.125
4th Descendant	0.063
Unrelated	0

Gene flow results in a decrease in relatedness levels. By introducing new genes, not identical by decent into a group, relatedness values will decrease. Outbreeding can be achieved through dispersal events. In most mammalian species it is the males that disperse (Greenwood 1980). In the family Bathyergidae it has been shown that the most dominant males will disperse in an attempt to outbreed (Bennett & Faulkes 2000).

It is known that *B. janetta* is a solitary species that occurs in fragmented populations in Namaqualand through to the southern part of Namibia (Skinner & Smithers 1990). It has also been observed (M. Herbst pers. obs.) that *B. janetta* will very seldom appear on the surface, unlike the Cape dune mole-rat, *B. suillus* that has been reported to disperse above ground (Jarvis & Bennett 1990). The objective of this study was therefore to determine, using genetic techniques, the parentage of young born into the *B. janetta* population and the genetic relatedness of the population. The correlation between genetic and geographical distance will also be assessed to determine the distance that mole-rats can disperse. Tests for genetic differentiation between the *B. janetta* population and two populations of the congeneric species, *B. suillus* will also be conducted.

Materials and Methods

DNA extraction

DNA was extracted from small sections of toes taken from the mole-rats during the field study. The toes were kept in a solution of DMSO at -4°C until further analysis. The toe tissue was sliced with a scalpel blade and digested overnight at 55°C with 20 µl proteinase K in 360 µl extraction buffer. After digestion, 200 µl of 5M NaCl (sodium chloride) was added, and the solution gently mixed for 20 minutes. Following this, 500 µl IAC (chloroform/isoamyl alcohol) was added and shaken again for 10 minutes. The organic and aqueous phase were separated by centrifuging at 4000 rpm for 10 minutes. The aqueous phase (supernatant) was transferred to a new labelled eppendorf. The DNA was precipitated with 600 µl propan-2-ol and pelleted at 12 000 rpm for 10 minutes. After pouring away the supernatant, the pellet was washed with 500 µl of 70% ethanol (EtOH) and then pelleted again by centrifugation at 12 000 rpm for 5 minutes. All remaining supernatant was removed and the DNA dried for 1 hour at 37°C and then re-suspended in 100 µl of sterile and DNA-free 10mM Tris.

DNA amplification

The DNA was amplified using the Polymerase Chain Reaction (PCR). Each sample was subjected to separate PCRs for each primer that was tested. All available primers were tested but only those that gave scoreable results were used. The following primers were used: two developed for *Cryptomys hottentotus* (EF12 and CH3), four developed for *Cryptomys damarensis* (DMR1, DMR3, DMR5 and DMR7) and one universal mammal primer NCAM (Burland *et al.* 2001 & Moore, Hale & Byrne 1998). The primer DMR7 is, however, not the same as the locus DMR7 mentioned in Burland *et al*. 2001. Instead, a microsatellite locus adjacent (within 20 base-pairs) to the one described by Burland *et al*. (2001) was amplified using the primers DMR7 F1 (5' CCTGGGAGAGTCTTTGTTTATACC 3') and DMR7 R2 (5' ACGTGAAGCTAAAGTGCTATG 3') (T. M. Burland, unpublished data). Not all results proved to be useful in the analyses and therefore only the primers with the most reliable results were used i.e. NCAM, DMR5, DMR7B, CH3 and EF12.

Hot microsatellite PCRs Reagent Template

	X 20	X 30	X 40	X 50	X 60
LABEL REACTION					
dH_2O	1.5	2.25	3	3.75	4.5
F PRIMER	1.5	2.25	3	3.75	4.5
PNK B_o (5x)	1	1.5	2	2.5	3
γ 32P	0.5	0.75	1	1.25	1.5
PNK	0.5	0.75	1	1.25	1.5
COLD COCKTAIL (detailing various Mg concentrations)					
dH_2O	124	186	248	310	372
dNTPs	22	33	44	55	66
NH_4 Bo (10x)	20	30	40	50	60
DMSO	20	30	40	50	60
$MgCl_2$ 1.5mM	6	9	12	15	18
R PRIMER	1.2	1.8	2.4	3	3.6
TAQ	2	3	4	5	6

The labelling cocktail was made in a screw cap tube and incubated at 37°C for 1-2 hours. The cold cocktail was made up and Taq added in the end. One microlitre of DNA was aliquoted into a micro titre plate. The labelling reaction was stopped by heating the tube to > 90°C for 3 minutes, then spinning down and kept on ice. The labelling reaction was then added to the cold cocktail and mixed well. Nine microlitres of cocktail was aliquoted to each sample and a small amount of mineral oil added to prevent evaporation. The micro titre plate was finally placed into a PCR machine and the program used as follows:

PCR conditions

94°C for 3 minutes

30 cycles of:

94°C for 30 seconds

Annealing temperature for 45 seconds

72°C for 45 seconds

72°C for 10 minutes

Screening of microsatellite loci

The PCR products were run out on a 6% polyacrylamide gel in order to determine the allele presence and size of each microsatellite loci. Polyacrylamide gels are used instead of agarose gels for size determination since agarose gel has a poor resolution of small fragments (Weber 1990).

After the gel had set, the PCR products were run in a 1200ml 1x TBE buffer solution (120ml 10x TBE and 1080ml H_2O). The polyacrylamide gels were vacuum dried and placed in a cassette with an auto-radiographed film and left for a period depending on the level of radioactivity of the gel. The film was developed using an automated process and scored accordingly.

Scoring of gels

The bands on the gel were scored and given arbitrary sizes. Samples of *B. janetta* and *B. suillus* were run on the same gels so that comparisons could be made. In cases where the bands were unclear and scoring not possible, the PCR products were run on a separate gel that resulted in successful scoring. Alternatively, the PCR was repeated.

Analysis of Hardy-Weinberg and Linkage disequilibrium

Hardy-Weinberg and linkage disequilibrium were tested using the statistical program GENEPOP (Raymond & Rousset 1995). Estimation of the exact probability was carried out using the Markov chain method with the following parameters: dememorisation 1000, batches 100, iterations per batch 1000. All deviations from equilibrium were considered significant at $p<0.05$ after correction for multiple tests (Rice 1989).

Identification of parents

Parentage was only determined in the *B. janetta* population, as this was the only population where parents and offspring were caught and identified. A total of 68 adults and 12 offspring were analysed. Offspring were identified as those mole-rats that weighed less than 200g. The young included in the analysis comprised only those pups caught in the same burrow as the mother and they were confirmed as mother-offspring pairs by direct observation. Parentage was determined by the use of the statistical package CERVUS (Marshall *et al.* 1998). This program is designed for large scale parentage analysis using co-dominant loci. The analysis is broken down into three sequential stages. By using the genotype data the program analysed the allele frequencies, ran appropriate simulations and likelihood-based parentage analysis while testing the confidence of each parentage.

Estimation of relatedness (R)

Relatedness was calculated using the program RELATEDNESS 5.0 (Queller & Goodnight 1989). This program is designed to calculate genetic relatedness among and between demographically defined groups of individuals. The program uses a regression measure of relatedness and is calculated by the equation:

$$R = (P_y - P^*)/(P_x - P^*)$$

P_x is the frequency of the allele in the current individual and for any diploid organism the value will be either 0.5 or 1.0. P_y is the frequency of the allele within the individual(s) being compared to the current individual, whereas P* is the frequency of the allele within the population, excluding all putative relatives of the sub-population to which the individuals compared belong. Alleles are thus weighted accordingly to their frequency, with rare alleles being given more weight.

For mean estimates of relatedness, the numerator and denominator were summed across alleles, loci and individuals and for pairwise estimates the numerator and denominator were summed over the alleles and loci only. The standard error was estimated by jack-knifing over loci (Queller & Goodnight 1989). The relatedness value obtained varies from –1 to 1. Negative values may result when either of the individuals possesses a rare allele (de Ruiter & Greffen 1998) and also when

individuals share fewer genes than the basal level of sharing within the population (McDonald & Potts 1994). Relatedness values were estimated between females, males and mother-offspring pairs in the *B. janetta* population.

Isolation by distance

The statistical program GENEPOP was used to analyse the correlation between genetic distances and geographical distances. The package allows an analysis of isolation by distance between pairs of individuals as describe by Rousset (2000). It computes a semi matrix of F_{ST} or $F_{ST}/(1 - F_{ST})$ values and the positions of individuals are specified as coordinates standing for their names. Each individual is treated as a separate population. This is done using the Mantel test (Mantel 1967) and the principle of the test is to permute lines or columns of the semi matrix. Mantel considers a particular statistic *Z* and approximations of its distribution. This program, however, uses a rank correlation and no approximation. It is expected that the occurrence of isolation by distance will generate a positive correlation between geographical distance and estimates of F_{ST}.

Genetic distances

Genetic distances between *B. janetta* and *B. suillus* were calculated with the statistical program GENETIX. GENETIX is a permutation-based statistical interference procedure and represents an alternative to bootstrapping and jack-knifing. Bootstrapping and jack-knifing provide an estimate of the confidence interval around the observed value, whereas the permutation and exact tests estimate the probability value of departure from the null hypothesis.

Results

DNA extractions and microsatellite loci screening

All extracted DNA was run on an agarose gel and screened under a UV light to confirm that all samples contained suitable DNA for further analyses. A total of 68 *B. janetta* and 69 *B. suillus* samples (Table 2) were screened using 6 polymorphic loci. In total, 822 single-locus genotypes were screened (Raw data of all genotypes in Appendix 2).

Table 2 Summary of the three populations used in this study

Population	Male	Female	Unknown	Total
B. janetta - Kamieskroon	35	33	-	68
B. suillus - Belhar	8	13	4	25
B. suillus - Cruiser Park	13	29	2	44

All microsatellites designed for African mole-rats (Burland *et al.* 2001) were screened and only those with scoreable results were used in this study. (Plate 4) The locus DMR7B was used in place of DMR7, as it was found to be the most polymorphic locus in the two species of *Bathyergus*. Linkage disequilibrium tests revealed that DMR7 and DMR7B are linked and therefore cannot be used as two independent loci (Fishers' method, p = highly significant). The characterization of the 5 microsatellite loci and PCR results are summarized in Table 3 and 4.

Table 3 The optimum PCR conditions and characterization of 5 microsatellite loci used to screen *B. janetta* individuals with observed heterozygosity (H)

Locus	Annealing temp °C	Mg^{2+} [ ] mM	Observed alleles	Observed H
NCAM	57-55	1.5	3	0.51
DMR5	55	1.5	6	0.75
DMR7B	57-55	1.5	6	0.78
CH3	57-55	1.5	3	0.66
EF12	57-55	1.5	2	0.50

* Annealing temperature of 57-55 indicate 5 cycles at 57°C followed by 25 cycles at 55°C

Table 4 The optimum PCR conditions and characterization of 5 microsatellite loci used to screen *B. suillus* individuals with observed heterozygosity (H)

Locus	Annealing temp °C	Mg^{2+} [ ] Mm	Belhar		Cruiser Park	
			Observed alleles	Observed H	Observed alleles	Observed H
NCAM	57-55	1.5	6	17	5	0.33
DMR5	55	1.5	8	21	9	0.41
DMR7B	57-55	1.5	3	7	2	0.25
CH3	57-55	1.5	4	14	4	0.35
EF12	57-55	1.5	8	20	5	0.49

* Annealing temperature of 57-55 indicate 5 cycles at 57°C followed by 25 cycles at 55°C

Hardy-Weinberg equilibrium

Hardy-Weinberg equilibrium values were estimated for each population-locus combination as well as across loci for each population and combined across populations for each loci (Table 5). Deviations from the Hardy-Weinberg equilibrium can result because mating is non-random, the population is genetically subdivided and in addition

the locus may be under selection or the locus carries null-alleles. In this study the populations were genetically subdivided, but it has been accounted for by assigning them to different populations and deviations from the Hardy-Weinberg equilibrium is probably because of the occurrence of null-alleles or natural selection.

Table 5 Measurements of Hardy-Weinberg equilibrium for each population-locus combination and overall for each population and each locus. All p-values were adjusted for multiple tests using Bonferroni tests (Rice 1989)

Loci	*B. janetta* Kamieskroon	*B. suillus* Belhar	*B. suillus* Cruiser Park	Across loci
NCAM	NS	NS	*	NS
DMR5	NS	NS	**	**
DMR7B	NS	NS	NS	*
CH3	NS	NS	NS	NS
EF12	NS	NS	NS	NS
Across pop	*	NS	**	
NS p> 0.05	* p<0.05	** p<0.01		

Parentage analysis

Parentage could only be assigned to the population of *Bathyergus janetta*, since this was the only population where data on potential mother and offspring pairs was available. In this study there were five known mother and offspring combinations (mother and pups being captured in the same burrow system) as summarized in Table 6.

Table 6 Known mother-offspring pairs of *B. janetta*

Mother	Pup	Weight of pup (g)
16	44	58
16	65	102
51	52	56
51	53	65
51	54	63

In the parentage analyses for candidate mothers, all offspring were tested against a list of 29 putative mothers. For those young where neither parent was known between three and 11 potential candidate mothers could be identified. If it is assumed that pups do not disperse very far away from the mother when they are still small, then all potential mothers more than 300m away could be excluded.

There were no known father-offspring pairs. The parentage analysis of putative fathers was calculated using a list of all offspring and their assigned putative mothers against a list of all potential fathers. This enables an estimation of the most likely father for each of the possible mother-offspring pairs. Table 7 shows all likely combinations of maternal and paternal candidates within 300m of the pups capture site. Some caution should be taken, however, since we do not know how far mole-rats can actually disperse. The LOD score is the product of the likelihood ratios at each locus and is calculated for each candidate parent based on the genotypes of the candidate parent, the offspring and other candidate parent. A positive LOD score implies that the candidate parent is more likely to be the true parent than an arbitrary randomly-chosen individual. A negative LOD score implies that the candidate parent is less likely to be the true parent than any arbitrary randomly-chosen individual or the candidate parent and the offspring share very common alleles at every locus. The most likely candidate parent will have the highest LOD score.

Table 7 Possible combinations of mother and fathers in close proximity of offspring.

Pup	Possible mothers	Possible fathers
44	Known 16	8, 18, 34, 35
65	Known 16	18, 34, 35
49	7*	22*, 14
	38	22
50	38	14
	7*	14*, 22, 15
52	Known 51	4, 22, 15
53	Known 51	4, 22
54	Known 51	15, 14
56	37	-
	12	18, 34
58	60	33
66	48	4, 22
	51*	4*, 22

* indicate combinations of putative mothers and fathers with a positive LOD score

When looking at known mother-offspring pairs, pups 44 and 65 have the same possible fathers, males 18, 34 and 35. However pups 52, 53 and 54 with known mother 16 do not give the same indication and it seems as if multiple paternity may be operational. It was, however, not possible to assign paternities within 95% confidence levels, probably because there were too many putative parents and not enough loci.

Relatedness

Relatedness between known pairs was calculated using the program RELATEDNESS 5.0 (Queller & Goodnight 1989). The average relatedness values for females and males in the population were very low (-0.03 ± 0.018 and 0.03 ± 0.019 respectively) suggesting that

the population is outbred. For known mother-offspring pairs the relatedness values were between 0.27 and 0.46 (average 0.37 ± 0.09) that is slightly below that expected for first order relatives. (Figure 1)

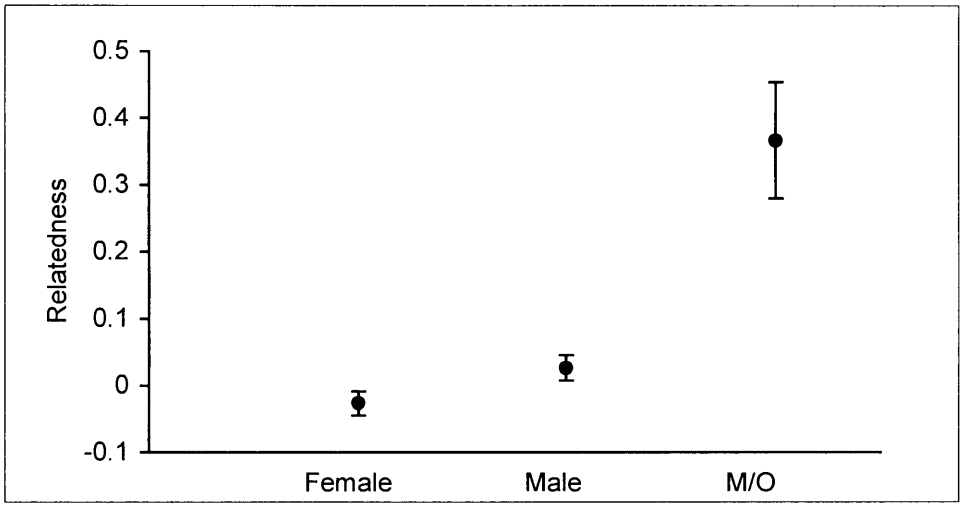


Figure 1 Average relatedness values for females, males and known mother and offspring pairs (M/O) in *B. janetta*.

Genetic and geographical distances

The correlation between genetic distance and geographical coordination was calculated using the statistical program GENEPOP 3.3 (Rousset 2000). The test of isolation by distance was highly significant p = 0.0003 (Figure 2) in *B. janetta*.

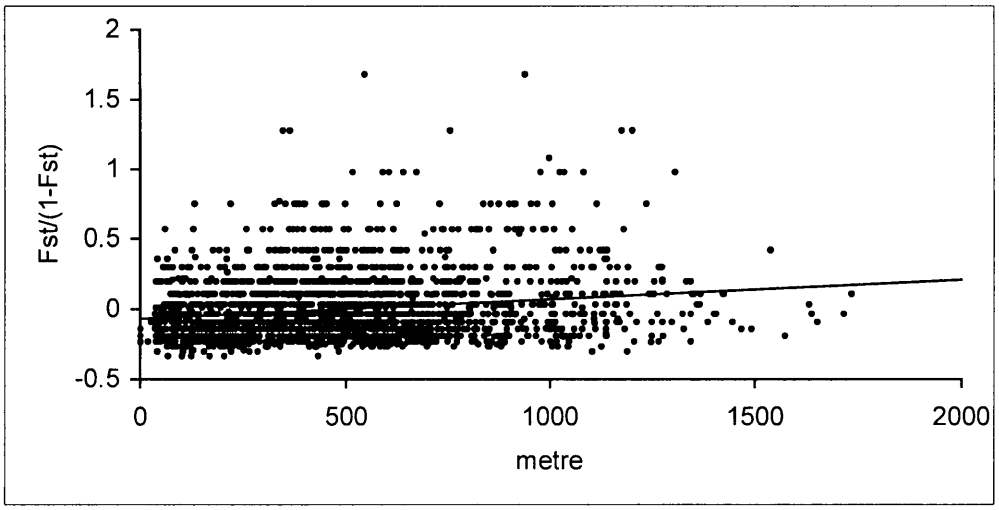


Figure 2 Isolation by genetic and geographic distances

Comparison between the two species

Genetic distances between *B. janetta* and *B. suillus* were calculated with the statistical program GENETIX which shows clearly the differences in the two species, as well as the differences between the two *B. suillus* populations from Belhar and Cruiser Park, which is separated by some 15-20km (J. Jarvis pers. comm.) (Figure 3). There is also a higher genetic diversity within the two *B. suillus* populations compared to that of the *B. janetta* population.

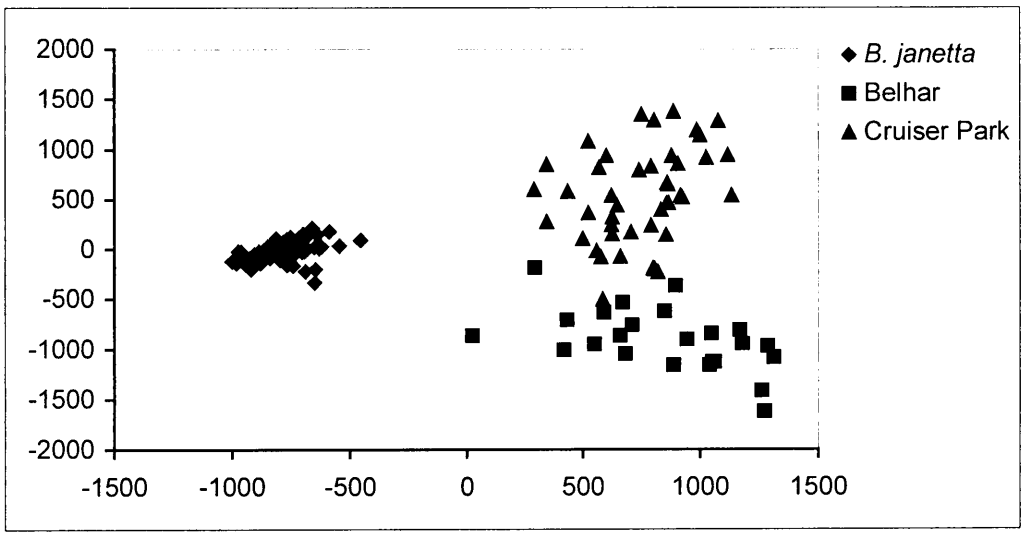


Figure 3 Genetic distances between *B. janetta* and the two *B. suillus* populations

Discussion

Very little is known about the genetic structure or dispersal events that occur in solitary mole-rats. The Namaqua dune mole-rat is a solitary, seasonal breeder that occurs in the winter rainfall region of the semi-desert area of Namaqualand. After rainfall the soil is moist and soft and there is an increase in above ground vegetation. This provides an opportunity for solitary mole-rats to extend their burrow systems (Bennett & Faulkes 2000). It is suggested that mole-rats disperse above ground as evidenced by the large number of open holes occurring in extruded mounds (M. Herbst pers. obs.; Jarvis & Bennett 1990). Although, it is possible that mole-rats dig tunnels towards one another during the breeding season since neighboring burrow systems can be as close as one metre from one another (Chapter 2). Increased vegetation may protect mole-rats from predators such as eagles and falcons whilst they are dispersing above ground, however, no mole-rats were ever observed above ground during daylight hours (M. Herbst pers. obs.).

Identification of parents

Mothers

In this study two mothers could be assigned as true mothers, since mother and young were caught within same the burrow system and the young were still drinking from her. The other potential mothers were assigned maternal status using the statistical program CERVUS, with the assumption that 95% of the mole-rats in the population were caught (Marshall *et al.* 1998). To narrow down the number of putative mothers, geographical distance was included and all mothers greater than 300m away were excluded. This should, however, be read with caution since the assumption that young mole-rats have low dispersal ability is made. However, the identification of isolation by distance within the population strongly supports these assumptions.

Putative fathers

The identification of putative fathers does not provide any significant or convincing results. The lower proportion of young allocated to a father and the high number of putative fathers may result from the fact that they can only be considered if they form a

compatible pair with a putative mother. Assigning fathers on geographical distance also makes the assumption that fathers do not disperse far from mothers and it is not known how far males disperse to mate with females. Table 8 narrows down the number of putative fathers. Although no mother-father-offspring pair can be identified it is very interesting that multiple paternity may occur.

False exclusions of parents

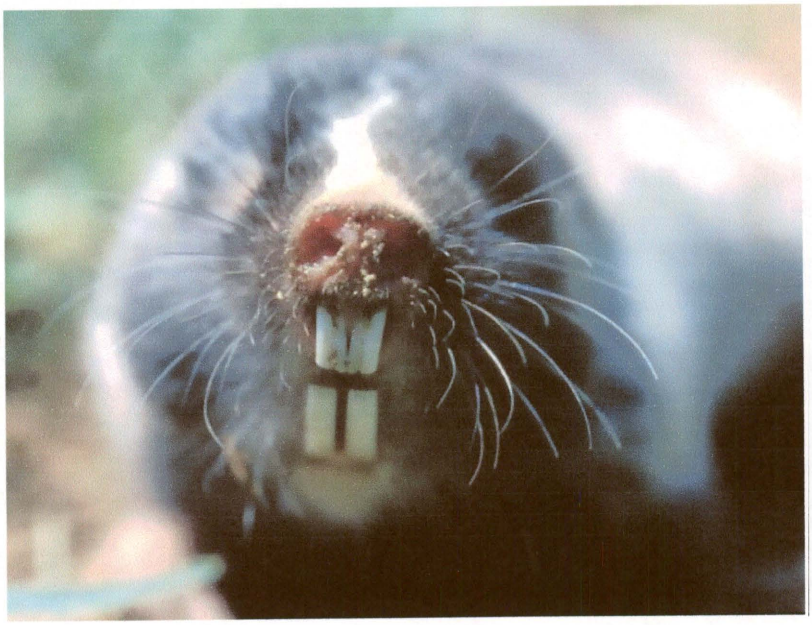
Parents may have been falsely excluded if a mutation in allele size occurred between parent and offspring. However the highest mutation rate recorded for a microsatellite locus was 10^{-3} per gamete per generation (Weber & Wong 1993) and since there were so many potential mothers and fathers per young in this study, it is highly unlikely that parents were falsely excluded.

Calibration of R estimates

There were only two mother-offspring pairs with R values of 0.27 and 0.46 (averaged 0.37 ± 0.09), which is close to first order relatives of 0.5. R values may not be accurate, since only five of the nine microsatellite loci yielded scoreable results. It is apparent that there is high heterozygozity amongst individuals, which may be attributed to a mating system of outbreeding. Hardy-Weinberg estimates revealed no significant differences from equilibrium and provide support of a random breeding population. Relationships that were used in this study were mother-offspring, average females and average males.

Highly significant results were obtained by the isolation of distance geographical and genetic distances. This suggests that mole-rats do not disperse very far and remain in the same area. The subterranean niche and the difficulties in burrowing great distances may hinder mole-rats and prevent them dispersing very far. Indeed mole-rat burrow systems are confined to specific areas, although within the locality there is great deal of activity (Davies & Jarvis 1988; M. Herbst pers. obs.).

To my parents for all their love and support



The Namaqua dune mole-rat

The Namaqua dune mole-rat is predicted to resemble other subterranean rodents in that the truly subterranean niche has a limited capacity for gene flow in populations (Bennett & Faulkes 2000). The distribution and habitat of *B. janetta* also has a limitation on dispersal events and gene flow between populations, especially in the Kamiesberg area where *B. janetta* only occur in patches of soft soil between rocky outcrops. It is already evident that *B. janetta* populations closer to the coast have a lower average body mass and a lighter coat than mole-rats in the Kamiesberg area (Skinner & Smithers 1990).

Most studies on population genetics in subterranean mammals have focused on the blind mole-rat *Spalax* in the Middle East. Limited gene flow between populations has lead to the evolution of numerous local forms. Interestingly there is a correlation between the different chromosomal forms with aridity and it seems that some forms are adapted to environmental stress (Bennett & Faulkes 2000; Nevo, Filippucci, Redi, Simson, Heth & Beiles 1995).

In the African mole-rats (Bathyergidae) the different social structures possessed by the species influence the genetic structure in mole-rat populations. In social species (*Cryptomys* and *Heterocephalus*) the genetic structure within the group is a major factor in determining the kind of behaviour and reproductive strategies expressed by an individual.

Observed genetic patterns in subterranean mammals can be explained by stochastic processes such as limited gene flow, fluctuating population sizes and genetic drift (Bennett & Faulkes 2000). Nevo (1979) has proposed the "niche width variation hypothesis" which explains the reduced genetic variation in subterranean mammals compared to small above ground mammals (Nevo, Filippucci & Beiles 1990) as the result of the subterranean niche which is stable and predictable. However stochastic events are recognized as the predominant factor to explain genetic patterns observed in subterranean mammals (Sage, Contreras, Roig & Patton 1986).

In the Bathyergidae knowledge of the genetic structure is important to our understanding of the evolution and maintenance of social structures. The variation in the degree of sociality coupled with the level of reproductive skew in the Bathyergidae should result in changing patterns of relatedness amongst the different species.

Molecular techniques provide vast amounts of information about social and reproductive biology of a population without the need to directly observe animals for long periods of time. In cases such as subterranean mole-rats or ocean dwelling pilot whales (Amos *et al.* 1993) observation is extremely difficult and molecular techniques have provided a means to study these animals. Molecular techniques, include sequencing analysis can allow the construction of phylogenetic relationships (Allard & Honeycutt 1992; Faulkes, Bennett, Bruford, O'Brien, Aguilar & Jarvis 1997a) or a multilocus approach such as this study in which the use of microsatellites can tell us something about the degree of relatedness (Burland 1998; Malherbe 2001).

Genetic studies at the intra specific level have been carried out on three mole-rat species the Damaraland mole-rat (*C. damarensis*), the common mole-rat (*C. hottentotus*) and the naked mole-rat (*H. glaber*). However the highly inbred naked mole-rat has received by far the most attention (Reeve, Westneat, Noon, Sherman & Aquadro 1990; Faulkes, Abbott & Mellor 1990; Faulkes, Abbott, O'Brien, Lau, Roy, Wayne & Bruford 1997b). Geographical barriers such as rivers and mountains can effectively split neighboring populations. *Bathyergus janetta* populations are effectively split by the Orange River and the Kamiesberg Mountains hence it would be very interesting to compare these different populations that are geographically isolated.

In this study it is clear that the subterranean niche poses a limitation on dispersal opportunities. In the population of *B. janetta* from Kamieskroon the individuals found closer together were naturally more related than those at a further distance. A very interesting result came from the isolation of distance analyses between the two *B. suillus* populations. Although these two populations are separated by 15-20km, (J. Jarvis pers. comm.) there is a clear genetic diversity between the two populations. Solitary mole-rats

outbreed and genetic patterns reveal that dispersal abilities are limited by the subterranean habitat and thus have a limitation on the gene flow between populations.

Summary

Microsatellite markers were used to determine parentage and genetic relatedness in a population of *B. janetta*. It was not possible to assign parentage to offspring born into the population due to the large number of potential parents. A known mother and her offspring (3 pups) were sampled and it appeared that multiple paternity could possibly be in operation, although this was a single case and more research is required to substantiate this statement. *Bathyergus janetta* individuals show low relatedness values suggesting that the population is outbred and comprises of related and unrelated individuals. When looking at geographical distances and genetic distances between individuals a highly significant correlation confirmed that the subterranean niche poses difficulties for gene flow and *B. janetta* do not disperse great distances. More evidence for the limitation of the subterranean habitat on dispersal can be seen when two *B. suillus* populations are compared. Interestingly, there is a higher genetic diversity within the two populations of *B. suillus* than when compared to the *B. janetta* population.

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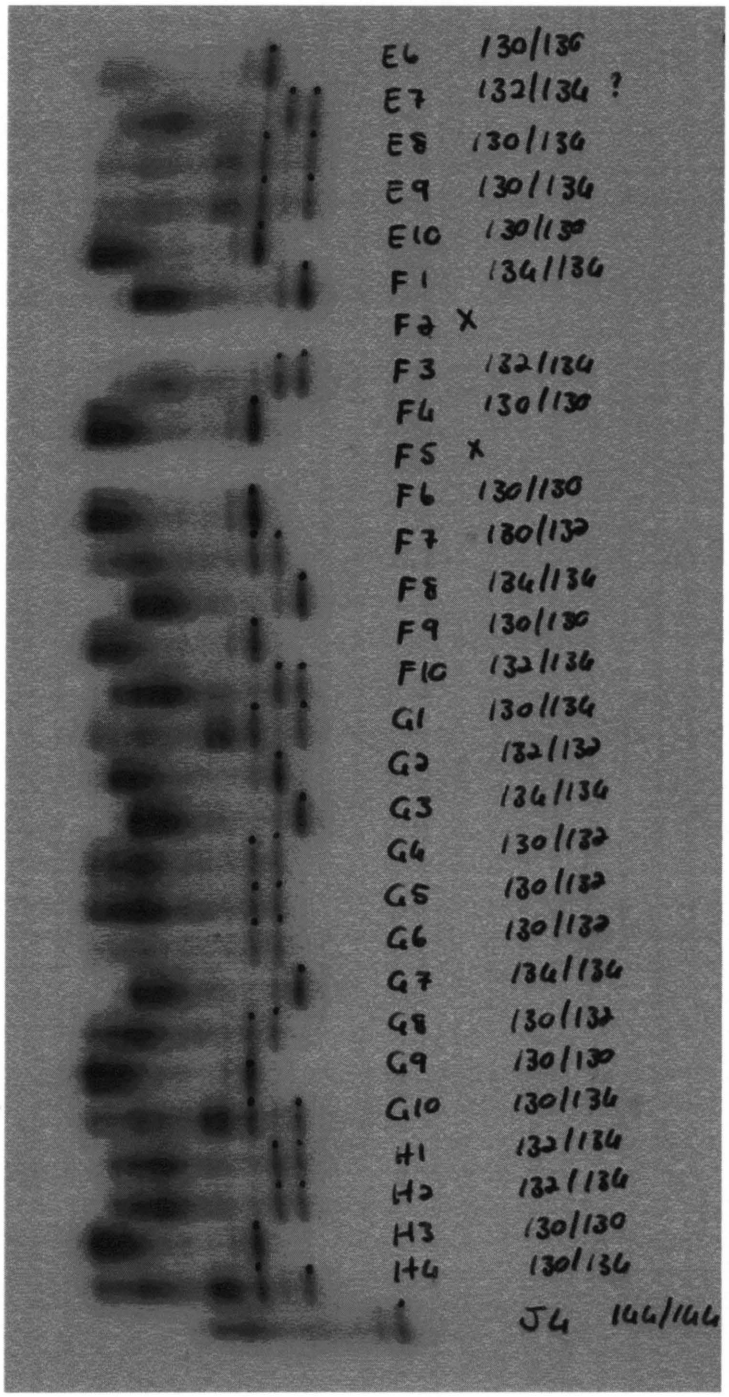

Plate 4 An example of an auto-radiographed film, clearly showing the PCR products

Chapter 5 Synthesis

The Namaqua dune mole-rat, *Bathyergus janetta* (Thomas & Schwann 1904)

The Namaqua dune mole-rat is the second largest completely subterranean mammal in Africa (the largest being *B. suillus* from the Western Cape). *Bathyergus janetta* occurs in sandy soils and differs from *B. suillus* in body size, the distinct colour of the pelage and its geographical distribution. It is an amazing subterranean miner that can push up an estimated 1735kg of soil in a year! Mole-rats in the genus *Bathyergus* use their strong, heavily clawed front feet to burrow and push the excess soil onto the surface with their powerful hind legs. They are continuously busy excavating and re-excavating their burrow systems. *Bathyergus janetta* is the sole occupant of a burrow system (which may be up to 160m in length), comprising a deep main tunnel with nesting areas, food stores and defecation sites and shallower foraging tunnels as well as a bolt hole or escape tunnel exceeding 1.55m in depth. The Namaqua dune mole-rat makes use of optimal burrowing conditions, increasing mound production after rainfall when the soil is moist. The burrow system is in a constant dynamic flux changing continuously and despite the fact that mound production decreases in the dry summer months, re-excavation and filling of old tunnels were observed. This suggests that the mole-rat keeps an optimum burrow length throughout the year. Dune mole-rats are aggressive and highly xenophobic towards conspecifics. They communicate via seismic transmission using hind foot drumming to convey messages concerning their sex, territory and reproductive status.

Distribution and status

The Namaqua dune mole-rat is endemic to the Northern Cape Province, Namaqualand and the extreme south of Namibia (Skinner & Smithers 1990). It favours soft sand dunes and sandy alluvials. It is classified of least concern by the IUCN Red Data book for endangered species, although *B. janetta* is probably the most endangered of all mole-rats due to the fragmented populations and habitat destruction.

Description

Length averages 278 ± 35.63mm and the mean male body mass 439.36 ± 191.84g, whereas females have a mean body mass of 330.38 ± 58.86g. They have a thick coat with long sensory hairs protruding above the coat. The ridge of the coat is black as is much of the head but the flanks of the body are silvery to grey. A small white stripe just above the nose is distinctly visible. They have large front claws with five digits and grooved upper incisors (characteristic of all species in the genus) and lips that close behind. Limbs and tail are short with fringed hairs bordering the tail and edge of hind feet. The eyes are small, ear pinnae absent and the nose is large and flat. The sense of smell, touch and orientation underground is good. They make grunting sounds when threatened, drumming with the hind feet. If provoked they attack by jumping foreword, mouth agape and uttering a high pitch sound.

Burrowing dynamics

The subterranean niche of the mole-rats is relatively stable with very little temperature flux inside the burrow system (Bennett & Jarvis 1988). These burrow systems are an extremely expensive investment (Vleck 1979). *Bathyergus janetta* has a main tunnel at an average depth of 45cm with shallower foraging tunnels. The softer sands of the dunes and larger body size forces the dune mole-rats to excavate deeper tunnel systems to prevent them from collapsing. Burrow systems may contain several nesting chambers, food stores and defecation sites. Food stores are uncommon for *B. suillus* but are standard features of the burrow system of *B. janetta*. Bolt holes are common in many subterranean rodents and in *B. janetta* they can exceed more that 1.5m in depth and serve as places of refuge when alarmed or threatened. The shape and form of burrow system appears to conform with the general findings of several other studies on subterranean rodents in that the male seems to have a more linear shaped burrow system in relation to females (Davies & Jarvis 1986; Reichman, Whitham & Ruffner 1982).

Feeding biology

The Namaqua dune mole-rat is herbivorous and feeds mainly on underground tubers and geophytes as well as above ground vegetation such as Namaqua daisies (*Dimorphoteca*

sinuata), which they pull into their burrow systems. In this study *Oxalis* sp. bulbs were the most favoured food item as was grass roots and *Grielum bimaculatum* tubers (M. Herbst pers. obs). When feeding, *B. janetta* rests on the hind feet, holding the food with its front feet, dis-budding the food prior to consumption (Plate 5). Outer leaves and smaller pieces of grass are used as nesting material. Before eating *B. janetta* will keep the food between the teeth making brushing movements with the front feet. All moisture is obtained from the food alone and mole-rats do not drink water.

Breeding biology

It is a seasonal breeder, mating in winter months (July to August). Urinary hormone concentrations of testosterone in males and progesterone and oestrogen in females showed a marked elevation in the months of July to September, which correlates to the rainfall pattern of the area. Plural occupancy of burrows by adult mole-rats was observed only during July and August. Pups are born in September to October and remain in the maternal burrow system until November to December. Whereafter, they start dispersing by extending the maternal burrow or move short distances above ground. Litters average 2-4 pups and in this study a mother and 4 pups were caught. In this seasonal breeder up to two litters may be produced per season (Bennett & Faulkes 2000).

Genetic analysis

In this study microsatellite loci (Burland, Bishop, O'Ryan & Faulkes 2001) were used in an attempt to try to determine parentage and relatedness within a population of *B. janetta*. It was not possible to assign a single parent based on statistical programs due to the large number of potential parents. Although it seems probable that multiple paternity of litters may occur. Hardy-Weinberg estimates revealed no significant differences from equilibrium and support the idea of a random breeding population. The average relatedness values for females and males in the population were very low (-0.03 ± 0.018 and 0.03 ± 0.019 respectively) suggesting that the population is outbreeding. For known mother-offspring pairs relatedness values were 0.27 and 0.46 (average 0.37 ± 0.09) which is slightly below that expected for first order relatives. A correlation between genetic and geographical distances confirmed that the subterranean niche limits gene flow as well as

dispersal abilities. It would be interesting to compare different *B. janetta* populations separated by extreme physical and geographical barriers such as the Kamiesberge and Orange River that prevent gene flow. It is very evident that *B. janetta* coat colour and body size varies regionally (Skinner & Smithers 1990). The isolation of distance between two populations of *B. suillus* showed a marked genetic differentiation between the two populataions as well as a high genetic diversity within two *B. suillus* populations when compared to the *B. janetta* population.

Conservation status

The Namaqua dune mole-rat can reach high population densities in areas where food resources are unlimited. Therefore, farmers see them as pests due to damage to plants and their burrowing activities in farming lands. The subterranean niche poses a limit on dispersal abilities and fragmentation of populations as well as habitat destruction by mining companies may be the greatest risk to survival of this secretive Namaqualand resident.

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Plate 5 *Bathyergus janetta* eating an *Oxalis* bulb

Appendix 1

Microcreatinine analysis

***Reagents*:**

Alkaline triton X-100 (freshly prepared):

Triton X-100 4.2 ml

NaOH 1N 12.5 ml

DIDW 66 ml

Saturated picric acid

30g picric acid slurry directly into a 1 litre beaker and almost filled with DIDW

Heat to a bout 80°C to dissolve with occasional stirring

Pour whilst hot into a stoppered bottle

Allow to cool and crystallize before use

Standards

Make 0.1N HCl. Weigh out 300mg creatinine out in small glas vail. Dissolve in a few ml of 0.1N HCl and transfer with Pasteur pipette to a 100ml volumetric flask. Make up to 100ml with 0.1N HCl (3.0 mg/ml creatinine stock). Make dilutions for standards as follows

Creatinine (mg/ml)	3 mg/ml stock (ml)	0.1N HCl (ml)
0.0	0	30
0.5	5	25
1.0	10	20
1.5	15	15
2.0	20	10
2.5	25	5
3.0	30	0

Picric reagent

1 volume alkaline triton solution

1 volume saturated picric acid

10 volumes DIDW

Method:

1. Thaw samples and standards and vortex
2. Prepare alkaline triton solution
3. Add 5µl of standards and samples and quality controls in duplicates. Leaving two wells empty for blank.
4. Add 300µl of working picric reagent to each well including blanks. Leave in dark room at room temperature for 1.5-4 hours and read at 492nm on micro-plate reader. (Colour develops progressively over 1.5h and is fairly constant, then declines slowly after 4h.)

	1	2	3	4	5	6
Row A	Blank	Blank	Control	Control	S8	S8
Row B	Std 0.0	Std 0.0	S1	S1	S9	S9
Row C	Std 0.5	Std 0.5	S2	S2	S10	S10
Row D	Std 1.0	Std 1.0	S3	S3	S11	S11
Row E	Std 1.5	Std 1.5	S4	S4	S12	S12
Row F	Std 2.0	Std 2.0	S5	S5	S13	S13
Row G	Std 2.5	Std 2.5	S6	S6	S14	S14
Row H	Std 3.0	Std 3.0	S7	S6	S15	S15

Appendix 2

Genotypes

Individual	Microsatellite primers				
	NCAM	DMR5	CH3	EF12	DMR7
Bathyergus janetta					
1	256/256	252/262	130/132	120/142	128/130
2	248/248	252/264	130/138	120/142	0/0
3	248/256	252/252	130/138	120/142	128/130
4	250/250	252/264	130/132	120/120	128/132
5	248/250	252/264	130/130	120/142	130/132
6	256/256	254/264	130/138	120/120	132/144
7	248/256	252/262	132/138	120/142	130/144
8	248/248	260/262	132/138	120/142	132/144
9	250/250	252/252	130/132	120/142	130/132
10	248/256	252/264	132/138	120/142	130/132
11	248/256	252/262	132/132	120/142	132/132
12	248/256	260/262	132/132	142/142	130/132
13	248/250	264/264	130/130	142/142	128/132
14	256/256	252/252	130/130	120/142	144/144
15	256/256	252/262	130/132	120/142	132/144
16	248/256	252/262	132/138	120/142	132/132
17	248/256	252/262	132/132	120/142	132/144
18	248/248	252/262	130/132	120/120	144/144
19	248/250	252/264	130/138	120/142	132/132
20	248/248	252/262	132/138	120/142	132/144
21	256/256	252/262	130/138	120/142	130/132
22	250/256	252/252	130/130	120/142	132/144
23	248/256	252/264	138/138	120/142	130/132
24	256/256	252/264	130/138	120/120	132/132

25	248/256	252/254	130/130	120/120	130/144
26	248/248	250/262	130/130	120/142	132/144
27	248/248	252/262	130/138	120/142	128/132
28	248/248	252/252	130/130	120/120	130/136
29	248/256	252/252	130/130	120/120	128/132
30	248/248	262/262	130/132	120/142	128/130
31	250/256	252/252	130/132	120/120	128/130
32	248/248	252/262	130/132	120/120	128/130
33	248/256	262/264	132/138	120/142	132/144
34	248/248	252/262	130/132	120/120	144/144
35	248/248	252/262	130/132	120/120	144/144
36	248/256	254/264	132/138	120/142	0/0
37	248/248	262/262	132/132	120/120	130/132
38	256/256	252/262	130/132	120/120	132/144
39	248/256	252/262	132/138	120/142	132/144
40	248/250	252/262	130/138	142/142	128/144
41	248/248	262/262	130/132	120/142	128/144
42	248/256	262/264	132/132	120/120	130/140
43	256/256	252/262	130/138	120/120	128/130
44	248/248	260/262	132/132	120/120	132/144
45	248/256	252/264	138/138	120/142	130/132
46	248/256	252/252	130/130	120/120	128/132
47	248/256	252/264	130/130	120/142	128/132
48	250/256	264/264	132/132	120/120	128/130
49	250/256	252/262	130/138	120/142	144/144
50	256/256	252/252	130/138	120/120	144/144
51	248/250	252/262	0/0	120/120	128/132
52	250/256	252/262	130/132	120/142	128/132
53	250/250	252/262	130/132	120/120	128/144
54	248/256	252/262	130/132	120/120	132/144
56	248/248	252/262	130/132	120/142	130/144

57	256/256	254/262	132/138	120/142	130/130
58	248/256	252/264	132/138	120/120	128/132
59	248/248	252/264	130/132	142/142	128/132
60	250/256	252/252	138/138	120/120	128/132
61	250/250	252/252	132/132	120/142	130/132
63	248/250	262/262	130/130	120/142	128/132
65	248/248	252/262	130/138	120/120	132/144
66	250/250	252/264	130/132	120/120	128/144
67	256/256	260/262	132/138	120/120	128/130
68	248/256	262/264	132/138	120/120	128/130
69	248/250	252/264	132/138	120/142	128/130
70	248/256	252/262	130/132	120/120	0/0
71	248/256	252/262	130/132	142/142	0/0
B. suillus					
Belhar 1	0/0	0/0	130/136	0/0	130/132
Belhar 2	238/238	248/262	136/136	132/132	130/132
Belhar 3	238/238	248/262	136/136	132/132	130/132
Belhar 4	238/238	248/262	136/136	132/142	130/130
Belhar 5	238/244	250/252	130/136	134/142	130/132
Belhar 6	0/0	248/260	0/0	134/142	0/0
Belhar 7	238/239	250/262	134/136	132/138	130/130
Belhar 8	238/238	244/262	136/136	134/142	130/130
Belhar 9	238/244	250/262	136/136	134/138	130/130
Belhar 10	238/238	244/248	130/136	132/142	130/132
Belhar 11	238/244	248/262	130/136	132/138	130/130
Belhar 12	238/239	248/262	134/136	138/138	130/132
Belhar 13	236/244	0/0	130/136	132/134	130/130
Belhar 14	238/240	248/262	130/136	132/134	130/130
Belhar 15	239/244	250/262	136/136	134/138	130/130
Belhar 16	238/240	248/262	130/136	138/138	128/130
Belhar 17	238/240	248/262	130/138	138/140	128/128

Belhar 18	238/240	248/260	136/136	132/148	130/130
Belhar 19	240/244	248/250	136/136	134/138	0/0
Belhar 20	0/0	248/248	134/136	134/138	0/0
Belhar 21	242/244	250/258	136/138	140/144	130/130
Belhar 22	238/242	250/250	136/136	132/140	130/130
Belhar 23	236/244	250/260	136/136	132/140	130/130
Belhar 24	238/240	248/262	134/136	132/134	130/130
Belhar 25	240/244	254/250	130/136	134/146	130/130
Cruiser 1	240/240	250/264	136/136	130/136	130/130
Cruiser 2	238/242	254/254	134/136	132/136	130/132
Cruiser 3	238/238	256/256	134/138	136/138	130/132
Cruiser 4	240/240	258/260	136/136	134/136	130/130
Cruiser 5	240/240	254/258	134/136	134/136	130/130
Cruiser 6	238/239	252/260	136/136	134/136	130/132
Cruiser 7	240/242	256/260	136/144	130/136	130/130
Cruiser 8	238/242	260/260	136/138	134/136	130/130
Cruiser 9	240/240	256/264	136/144	130/136	130/130
Cruiser 10	238/240	252/254	136/136	134/136	130/132
Cruiser 11	238/240	254/264	136/136	132/136	130/132
Cruiser 12	238/238	256/264	136/136	134/138	130/132
Cruiser 13	239/240	252/252	136/136	134/134	130/132
Cruiser 14	240/242	250/258	136/136	134/136	130/132
Cruiser 15	240/240	256/264	136/136	136/136	130/130
Cruiser 16	240/242	252/264	136/138	130/136	130/132
Cruiser 17	238/242	260/260	136/136	134/136	132/132
Cruiser 18	240/240	252/252	136/144	130/136	130/130
Cruiser 19	238/238	252/260	136/136	132/138	130/132
Cruiser 20	240/240	0/0	136/144	130/136	130/130
Cruiser 21	240/242	258/258	136/144	130/136	130/130
Cruiser 22	240/240	252/258	134/136	134/136	130/130
Cruiser 23	240/242	254/256	136/144	136/136	130/130

Cruiser 24	240/240	258/260	136/138	134/136	130/130
Cruiser 25	240/240	256/264	134/138	134/136	130/130
Cruiser 26	238/242	254/260	136/136	0/0	132/132
Cruiser 27	240/242	260/264	134/134	136/136	130/130
Cruiser 28	238/239	256/264	136/136	134/138	130/132
Cruiser 29	239/240	252/252	136/136	134/134	130/130
Cruiser 30	238/242	254/264	136/136	134/136	130/132
Cruiser 31	240/242	264/264	136/138	134/136	132/132
Cruiser 32	238/238	254/260	136/136	134/134	132/132
Cruiser 33	240/240	260/260	138/144	134/136	130/130
Cruiser 34	240/240	262/262	134/144	134/136	130/130
Cruiser 35	240/242	254/264	134/136	136/138	130/130
Cruiser 36	240/242	256/258	134/136	136/136	130/132
Cruiser 37	238/240	254/258	136/136	134/136	130/130
Cruiser 38	240/242	260/266	134/136	136/136	130/130
Cruiser 39	232/240	262/262	134/136	134/136	132/132
Cruiser 40	240/240	262/262	134/138	134/136	130/130
Cruiser 41	0/0	256/256	134/144	136/136	130/132
Cruiser 42	0/0	254/254	134/134	136/138	130/132
Cruiser 43	0/0	260/264	136/138	136/138	130/132
Cruiser 44	0/0	258/266	136/136	134/136	130/132