Aspects of the sociality, ecology, reproductive biology and genetic relatedness of colonies of the highveld mole-rat, *Cryptomys hottenetotus pretoriae*.

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genetic variation more explicable in terms of social structure, effective population size and stochastic processes like genetic drift.

Summary

Although relatedness estimates are slightly higher than expected for outbred first-order relatives, it can be stated with reasonable confidence that colonies of the highveld mole-rat consist of first-order relatives (mother-offspring/full siblings). Colonies are composed of highly related individuals, although it is unlikely that they are simply monogamous family groups, it is more likely that colonies are composed of extended family groups.

The higher than expected estimates of relatedness can be explained by low sample size (in terms of the number of individuals, colonies and loci used in the study), limited gene flow or a hypothesised mating system where close relatives breed.

The identification of a reproductive female in colonies by morphological characteristics is reliable in this species, while the identification of the breeding male is unreliable when using morphological characteristics. The breeding male(s) do not appear to be resident in the colonies; when and where mating takes place is unknown at this time.

Offspring of the reproductive female appear, in most cases to be full siblings, although multiple paternity was identified in a number of colonies. Whether multiple paternity is within or between litters is unknown at this time. Individuals which were identified as not being the offspring of the reproductive female generally are thought to be her offspring or full siblings but were mistyped or had a null allele or had genotypes that differed from the expected alleles by a single base pair (evidence of mutation).

Chapter 1

Introduction

Introduction

The endemic African family, the Bathyergidae, comprises five genera of rodent moles exhibiting the widest range of body mass (35 – 1200 g) and social structure (solitary to eusocial) of any of the seven families or sub-families of subterranean rodents. Representatives are found in a diversity of habitats (mesic to arid) and in a wide variety of soils, altitudes and climates. Although they have a ubiquitous distribution throughout much of Africa, little is known of many of the species and particularly those outside of the borders of South Africa (Jarvis & Bennett 1990, Bennett & Faulkes 2000).
The subterranean habitat imposes very similar constraints on all subterranean mammals (Nevo 1979, 1982). Consequently, considerable convergence occurs worldwide in subterranean mammal species with very diverse evolutionary histories. The Bathyergidae show much convergence with other subterranean rodents but also show noteable exceptions, particularly with respect to sociality.

Fossil record

Hystricomorph families appear to have diverged as early as the mid-Cretaceous period before dinosaur extinction (approximately 109 million years ago) (Bennett & Faulkes 2000). Various fossils resembling extant Bathyergidae have been found in fossil beds of the Miocene era (25 mya). Bathyergids appear to have an ancient African origin with the closest outgroup among fossils being the Eocene Ctenodactyloids (Bennett & Faulkes 2000).

Mitochondrial DNA analyses by Honeycutt *et al.* (1987) show wide genetic distances between bathyergids and related taxa that infer that the bathyergids have an ancient origin or a rapid rate of molecular evolution. Three genera of fossils have been found in beds of the early Miocene in East Africa and Namibia (Lavocat 1978). These are the *Bathyergoides, Proheliophobius* and *Paracryptomys* fossil genera.

Taxonomy

The Bathyergidae are a family of entirely subterranean rodents that are endemic to sub-Saharan Africa. They are a monophyletic group, whose closest relatives are the Rock rats (Petromuridae), Cane rats (Thryonomyidae) and Old World Porcupines (Hystricidae). This was shown by parsimony analysis of sequence differences in the mitochondrial 12S rRNA gene (Nedbal *et al.* 1994). The taxonomy of the Bathyergidae and in particular the genus *Cryptomys* is currently under review. There is considerable genetic variability amongst the *Cryptomys* from Zambia and the province of Gauteng in South Africa, in which several yet undescribed species of *Cryptomys* have been found.

The bathyerid family is classed into two sub-families; the Bathyerginae (consisting monotypically of the *Bathyergus* genus) which are separated from the Georychinae (consisting of *Heterocephalus*, *Heliophobius*, *Georychus* and *Cryptomys*) by the presence of *grooved* extrabuccal incisors (De Graaff 1981, Roberts 1951).

Phylogeny within the Bathyergidae

There are currently15 recognised bathyergid species whose phylogeny to one another has been studied in terms of their sequence differences in the mitochondrial 12S rRNA and cytochrome-b genes (Faulkes *et al.* 1997). The *Cryptomys* genus is essentially composed of two groups or sub-clades that are identified on features of the infraorbital foramen and the analysis of the cytochrome-b percentage differences of the mitochondrial DNA (Faulkes *et al.* 1997). One subclade, containing *Cryptomys damarensis* has a thick walled infraorbital foramena (at least 6 species), whereas the other clade containing *Cryptomys hottentotus*, possesses an elliptically shaped and thin walled infraorbital foramena (at least three species).

Sociality

Bathyergids occupy a wide range of habitats (mesic to arid) and member species show a continuum of sociality; ranging from strictly solitary (*Bathyergus suillus, Bathyergus janetta, Georychus capensis* and *Heliophobius argentiocinereus*) through to two eusocial species (*Heterocephalus glaber* and *Cryptomys damarensis*) (Jarvis 1981, Jarvis & Bennett 1993 and Faulkes *et al.* 1997). In general, solitary and weakly social species occupy mesic areas, whereas the two eusocial species occur in arid regions. Most subterranean mammals are solitary and strongly xenophobic towards conspecifics (Nevo 1979).

Sociality in the Bathyergidae is different from that exhibited by other 'social' subterranean rodents in that colony members remain in the social unit until conditions become optimal for dispersal. Colony growth occurs by offspring retention in their natal colony (Jarvis & Bennett 1990). The Cururo, *Spalacopus cyanus* is the only other social subterranean rodent that shows similar features of sociality (Jarvis & Bennett 1990).

The majority of solitary subspecies show a marked seasonal reproduction. The two social species *Cryptomys hottentotus hottentotus* and *Cryptomys hottentotus pretoriae*, which inhabit environments similar to the solitary species, also exhibit marked seasonal reproduction (Spinks 1998, Janse van Rensburg, 2000). In these environments there are marked precipitation and temperature changes and it is believed that these are important to herald reproductive activities.

Cryptomys is by far the most specious genus of the family with all currently recognised species being social. The genus *Cryptomys* is an interesting group that contains a number of species that exhibit a broad spectrum of sociality. *Cryptomys damarensis* is the only species within the genus that has been recognised as being truly eusocial based on data from lifetime reproductive success. It shares this position with the naked mole-rat, *Heterocephalus glaber*. Eusociality has apparently evolved independently in the two genera *Cryptomys* and *Heterocephalus* (Jarvis & Bennett 1993).

A strong correlation exists between aridity/food distribution and the level of sociality exhibited. In general as aridity increases (and food distribution becomes less predictable) the level of sociality increases (Jarvis 1985, Lovegrove & Wissel 1988, Bennett 1988, Lovegrove 1991 and Jarvis *et al.* 1994). As aridity increases, so rainfall decreases. This results in the soil being harder to dig in and for the mole-rats to successfully throw up the characteristic mounds of subsurface excavated soil. Both of these factors make the energetic cost of burrowing greater and the risk of unsuccessful foraging higher (Bennett 1988, Lovegrove 1991 and Jarvis *et al.* 1994). These factors together with a high risk of dispersal (a high chance of predation and a low chance of mating successfully subsequent to dispersal) are thought to select for group living, co-operative foraging and communally caring individuals.

Dispersal risks between habitats are influenced by group size and resource characteristics (Lovegrove 1991). Other factors that are thought to influence dispersal risks are body size, thermoregulation, metabolic rate, soil conditions and caste structure (Lovegrove 1991). A large group size is formed to offset the high foraging and dispersal risks (Lovegrove 1991). Foraging within a habitat also poses certain risks, the most important being the temporal dependence on rainfall (Lovegrove 1991). Dispersal risks in turn affect the preferred mating system (inbreeding or outbreeding). When risks increase, the genetic relatedness amongst colony members is seen to be higher (Lovegrove 1991).

In most co-operatively breeding species, it is proposed that severe ecological constraints imposed on the opportunity to breed underlies the evolution of familial helping and group living (Emlen 1982). There are two situations when offspring will have very low probabilities of successfully reproducing on their own. The first situation arises when ecological requirements are such that suitable breeding locations are restricted and marginal habitats are rare. This results in a shortage of breeding openings (Brown 1974). The second situation arises from inhabiting a changing, unpredictable environment; the erratic changes in carrying capacity creates functional equivalents of breeding openings and closures. Individuals frequently faced with environmentally harsh conditions will

have low chances of reproductive success when the costs of breeding independently are high (Brown 1974). In both cases, the energetic costs of independent breeding may be prohibitive and younger, less experienced individuals delay breeding and postpone dispersal (Brown 1974).

The prolonged bonds that are therefore formed in familial groups cause partitioning within the groups, which favours kin selection (Emlen 1982). Social competition exists in any structured social unit where the frequency of interactions and conflicts are high (Emlen 1982). It is expected that each individual in a group (depending on its social position) would behave in such a way as to maximise its inclusive fitness (Emlen 1982).

Dominance

As with any gregarious species, variations in behaviour will inevitably result in certain individuals appearing stronger than others. This "assertiveness" (Bennett & Faulkes 2000) may be evident in the individual being given priority to resources or high quality resources (usually food). These individuals are thought of as being dominant to other individuals in the group. Dominance in a social group of individuals may be linear (A dominates B, B dominates C, A dominates C) or non-linear (A dominates B, B dominates C, C dominates A).

Dominance may come about in several ways specific to the species behavioural repertoire. For example, in social mole-rats, dominance is thought to be achieved by agonistic (aggressive) interactions – the dominant individual performing the agonistic behaviour whilst the less dominant individual accepting the behaviour of the more dominant individual (ie: not challenging the more dominant individual). An individual's position in the dominance hierarchy of the group is thought to be closely linked to its competitive ability and hence the individual's fitness (Dewsbury 1982).

The social mole-rats, especially *C. h. hottentotus* and *C. h. pretoriae*, show a wide variety of behavioural activities which could be involved in dominance hierarchy formation and

maintenance within their colonies. Behaviours of both an aggressive and submissive nature are exhibited by all individuals in the colony. The eusocial naked mole-rat has been shown to have a distinct dominance hierarchy, with the largest and heaviest individuals possessing the premium rank in the colony (Clarke & Faulkes 1997).

Ecology

All members of the Bathyergidae are herbivorous; feeding on the underground storage organs of geophytic plants (Bennett & Faulkes 2000). African mole-rats rely on these storage organs for all of their nutritional requirements, including hydration requirements, as no free water is consumed. Using geophytic storage organs as a food resource has many advantages. There is little competition in the subterranean niche for these food resources that happen to be available for most of the year (except when the plant is flowering) (Bennett & Faulkes 2000). More importantly, geophytes have a higher concentration of nutrients contained within their storage organs when they are not flowering (Bennett & Faulkes 2000). Although geophytes seem to be relatively unpalatable and even toxic to other mammals, as they contain cardiac glycosides, mole-rats seem to be immune to these factors (Bennett & Faulkes 2000).

It is increasingly apparent that different strategies are employed while feeding. Thus in the Bathyergidae the size of the geophyte determines how it will be handled and harvested (Bennett & Faulkes 2000). Two components are very important in any species feeding strategy (here foraging strategy being excluded); handling time and consumption time. Handling time is especially important in the Bathyergidae as the outer husks of geophytes are usually completely removed prior to consumption. This activity represents an energy loss to the animal as no energy is gained during this process. Only when consumption occurs is energy actually being gained by the individual. It is therefore expected that larger geophytes would be preferred over smaller geophytes as more energy would be gained relative to the cost of dehusking. Larger geophytes have been shown to have a greater amount of total energy content than smaller geophytes (Jarvis *et al.* 1998). Studies on the eusocial Damaraland mole-rat *Cryptomys damarensis* have shown that

handling time decreases as the size of the geophyte increases (Barnett 1991), although in Barnett's (1991) study it was found that small geophytes were consumed relative to the geophytes of a larger mass.

In terms of foraging strategy, there appears to be no definite trend evident regarding patch profitability and choices made by individuals. According to the Marginal Value Theorem (Charnov 1976), high quality patches should be exploited in preference to poorer areas. Studies on the social common mole-rat (*Cryptomys hottentotus hottentotus*) and the eusocial Damaraland mole-rat (*Cryptomys damarensis*) have shown that empty patches are still worked by the mole-rats but are exploited less than patches containing geophytes (Bennett & Faulkes 2000).

Reproduction and Development

In non-gregarious mammals, induced ovulation seems to be the norm (Zarrow & Clarke 1968). Also, many social subterranean rodents such as *Ctenomys talarum* (Weir 1974) are induced ovulators. The method of ovulation used by solitary bathyergids is as yet unknown (Bennett & Faulkes 2000) but these species are expected to be induced ovulators. Social bathyergids are intruiging since within the *Cryptomys* genus both spontaneous and induced ovulation takes place. The common mole-rat (*Cryptomys hottentotus hottentotus*) is an induced ovulator (Spinks *et al.* 1999) whereas the Damaraland mole-rat (*Cryptomys damarensis*) appears to be spontaneous (A.J. Molteno & N.C. Bennett unpubl.).

Hystricomorph rodents appear to have relatively long gestation periods (Weir 1974), this is also evident in the Bathyergidae. Solitary bathyergids have shorter gestation periods (44 - 52 days) when compared to that of social species (56 – 110days) (Bennett 1989, Bennett *et al.* 1994 and Bennett & Aguilar 1995).

Bathyergid pups are nidicolous and altricial (Bennett & Faulkes 2000) and are therefore reminiscent of other subterranean rodents (Bennett *et al.* 1994). Bathyergid pups tend to

develop slowly, similar to that of other rodent species living in a thermally stable (subterranean) environment (Bennett *et al.* 1991).

Postnatal growth rates in bathyergid pups seems to be dependent of the species level of sociality; pups of eusocial species growing the slowest. Bathyergid pup maximum growth rate ranges from 0.207 $gday^{-1}$ (*Heterocephalus glaber*) to 3.340 $gday^{-1}$ (*Bathyergus suillus*) (Brett 1991 and Bennett *et al.* 1991 respectively).

Genetic Relatedness

The two main factors that are thought to influence the genetic structure of mole-rat populations are i) the subterranean niche which the mole-rats have become adapted to and ii) the social structure of individual populations, especially in co-operative breeders (Bennett & Faulkes 2000). These two factors, along with genetic drift and fluctuating population sizes, are stochastic processes associated with the genetic patterns evident in subterranean mammals.

The subterranean niche has a limited capacity for gene flow, especially for those subterranean species that are gregarious in nature. With few dispersal events ever being recorded for bathyergids, it is unlikely that gene flow among populations is prevalent to any large degree. Studies on *Spalax*, a solitary and subterranean myomorph rodent, have shown that limited gene flow can lead to the evolution of several local forms that are morphologically very similar due to convergent evolution and subterranean adaptation (Bennett & Faulkes 2000). It appears that there may be similar forces acting on bathyergids.

The social structure of co-operative breeders can also potentially influence the genetic structure of their populations (Bennett & Faulkes 2000). In bathyergid rodents that have varying degrees of sociality and reproductive skew, one would expect varying patterns of relatedness in the different species.

Although not favoured by several authors (e.g. Sage *et al.* 1986), the 'niche width genetic variation' hypothesis was proposed by Nevo *et al.* (1990) in order to explain genetic patterns in subterranean mammals. This hypothesis, unlike the stochastic processes outlined above, explains the reduced genetic variation in subterranean mammals (when compared to small above-ground mammals) in terms of the subterranean environment that is stable and predictable.

The genetic relatedness of individuals within colonies of social bathyergids has only recently begun to be explored. It is imperative, in order to understand how social systems in bathyergid species evolve and are maintained, that the genetic structure of populations and the genetic relatedness within social groups is understood. With the assistance of genetic markers such as microsatellites as well as relevant software for analysis, parentage (especially paternity) analysis can be routinely undertaken in order to determine genetic relatedness of colony members.

The naked mole-rat (*Heterocephalus glaber*) has been shown to have the highest mean coefficient of inbreeding that has been reported for any natural mammal population (Reeve *et al.* 1990). Other bathyergids have been shown to be obligate outbreeders (for example *Cryptomys damarensis*, Bennett & Faulkes 2000) and a lower mean relatedness is expected, although not yet investigated.

The study species

The highveld mole-rat, *Cryptomys hottentotus pretoriae* is a group living bathyergid. Mean body mass ranges from 88g (females) to 106 g (males) (Jarvis & Bennett 1991). The species inhabits regions with seasonal precipitation patterns (Janse van Rensburg 2000) enabling sufficient burrow excavation during the wet season. The aridity food distribution hypothesis (Jarvis *et al.*1994) predicts that in such an environment, the mole-rats should be solitary or weakly social, only coming together during the breeding season. However, colonies of the highveld mole-rat can exceed 12 animals (G.P. Malherbe and L. van der Walt, pers. obs.).

Knowledge pertaining to the general ecology, reproductive biology and genetic relatedness within colonies is unknown. A study by Faulkes *et al.* (1997) showed that the highveld mole-rat is indeed a sub-species of *Cryptomys hottentotus*. Janse van Rensburg (2000) has demonstrated that the highveld mole-rat is a seasonal breeder that cues its reproduction to seasonal rainfall. Moolman *et al.* (1998) has shown the highveld mole-rat to be social with a non linear ordinal dominance hierarchy.

Aims of the thesis

The primary aim of this thesis was to increase the dearth of knowledge currently known about this social subterranean rodent mole. In chapter 2 I investigated the cardinal dominance hierarchy of the highveld mole-rat. Cardinal dominance determination is the most appropriate measure that can be used on the mole-rat social system. In chapter 3, the handling and consumption time of the geophyte *Ornithogalum secundum* of varying size was investigated. Foraging in terms of size– and density-dependent utilisation and foraging patterns was investigated in light of optimal foraging theory. In chapter 4 the general reproductive biology of the highveld mole-rat was discerned with particular attention to the method of ovulation and growth rates of the offspring. Finally, in chapter 5, the genetic relatedness of colonies of the highveld mole-rat was investigated in order to elucidate the composition of colonies in terms of parentage and relatedness as well as possible mating systems employed in order to be social. Before this could be investigated, it was necessary to determine whether microsatellite primers developed for use on other bathyergid species as well as a universal mammal species could be employed in the study species.

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Chapter 2

Dominance

Abstract

The highveld mole-rat, *Cryptomys hottentotus pretoriae*, is a group-living bathyergid. Species of the Bathyergidae exhibit varying degrees of sociality; from solitary to eusocial species. Bathyergid sociality and dominance has commonly been assessed using ordinal procedures. Using these procedures, the highveld mole-rat is classified as being loosely social. These methods are often subjective and can not deal with ties in rank and other complications. This study uses an objective and more robust cardinal assessment method in order to assign dominance ranks to individuals of specific colonies. It was found that the highveld mole-rat is indeed loosely social with no correlations being observed between individual dominance rank and sex, mass or reproductive status respectively. It is proposed that this method of dominance assessment be used in future dominance related assessments, especially within the Bathyergidae.

Introduction

There have been various attempts in the literature to define dominance relationships amongst individuals as well as many attempts to measure it. The concept of dominance is notoriously inadequately defined (Richards 1974). Attempts to measure dominance include the assessment of priority to incentives, agonistic behaviour, approach/retreat and avoiding behaviour, mating success, display behaviour, grooming behaviour, posture and gait, distance from the alpha male, general spacing and associative attention (physical contact, grooming and play) (Richards 1974). Historically, it has proven difficult to rank individuals reliably on a single measure or to correlate between ranks when using several measures (Richards 1974). For example, Bernstein (1970) failed to establish correlations between behaviours and suggested that social responses were not derived from a "single social mechanism". Contradictions abound in the literature and confused statements are reported where data has been obtained that does not fit traditional theories of social dominance (Richards 1974). Circular arguments are also common where, for example, priority of access to resources leads to dominance but where it is also stated that dominance leads to priority of access to resources (Chance 1956). Lack of correlations may be due to methodological problems or because of other behavioural (including social) or physiological factors (Richards 1974).

The most inclusive and representative definition of dominance relationships amongst individuals has been proposed by Boyd & Silk (1983). These authors suggest that dominance relationships are characterised by three structural properties, namely; stability, transitivity and linearity. Stability in a group of individuals exists as long as individual A always beats individual B in an encounter. Richards (1974) defines group stability as when a group has lived together for a relatively long period of time and no changes are evident in the group composition and that there is no sign of a decline in the health of any individual. Chase (1974) associates increasing group stability with a decreasing frequency and intensity of interactions. It is suggested that this is dependent upon the time that the group has been together. Transitivity exists in a group of individuals when individual A is dominant over individual B and individual B is dominant over individual

C while individual A is dominant over individual C (Richards 1974, Chase 1974). In order for a linear dominance hierarchy to exist, all triads must be transitive. If these three properties are present in a group of animals then unambiguous ordinal dominance ranks can be assigned to each individual in the group and the rank will correspond to the number of individuals in the group that each individual dominates. All three of these properties must be present in a group of individuals in order for ordinal dominance ranks to be assigned. This is unlikely to be the case in mole-rat colonies. Assessing dominance in groups where individuals interact infrequently also proves to be a difficult task (Richards 1974).

The process of assessing dominance relationships involves several steps in order to construct a dominance matrix. These steps include: (i) identifying a behaviour or set of behaviours that are clearly associated with dominance, (ii) establishing a set of criteria to determine the unambiguous identification of winners and losers of dyadic interactions and (iii) collecting observations and assessing the temporal consistency of outcomes (Boyd & Silk 1983). If these steps can be done without deviation then the resulting matrix would allow the individuals in the group to be assigned ordinal dominance ranks (Boyd & Silk 1983). However, it is only very occasionally that these steps are followed without deviation and/or further assumptions. It is sometimes very difficult to determine which behaviour(s) should be used to assess dominance. Hierarchies based on different behaviours do not always agree (Boyd & Silk 1983). Ties in dominance rank are sometimes encountered and further problems are encountered when all individuals in the group do not interact with each other (Boyd & Silk 1983). Dominance relationships between pairs of individuals may be ambiguous such that individual A sometimes dominates individual B but individual B sometimes dominates individual A. If a single pair of individuals in the group show ambiguity in their dominance relationship, individuals cannot be ordered in a linear hierarchy (Boyd & Silk 1983).

Ordinal measures of dominance fail to include the level of ambiguity between pairs of individuals (Boyd & Silk 1983). Using this technique it is also difficult to assess the significance of temporal or contextual fluctuations in the dominance relationship of two

individuals (Boyd & Silk 1983). The use of ordinal measures may distort or obscure the actual relationship between dominance and other variables of interest (Boyd & Silk 1983).

Two types of ranks are evident from the literature. A basic rank is one in which a dominant-subordinate relationship exists between two individuals, not interfered with by the group to which they belong (Richards 1974). The construction of a linear social dominance hierarchy is not possible between dyads tested in this way. A dependant rank is a dominant-subordinate relationship between two individuals that is modified by other individuals or by group dynamics (Richards 1974). As Carpenter (1954) and Hinde (1971) state "dominance relationships have a pervasive effect on group organisation". Thus, for social dominance hierarchies to be used in studies of social behaviour and for social interactions to be predicted accurately, the concept and definition of dominance needs to be adequate (Richards 1974).

Another branch of dominance measures has been debated in the literature and is used in this study. This method of assigning ranks to individuals in a social group is a method that involves using cardinal indices and not ordinal dominance ranks. Unlike the ordinal method, wins, losses as well as ties can be dealt with (Boyd & Silk 1983). This method is based on the method of pairwise comparisons (Boyd & Silk 1983). Cardinal methods express the amount of dominance rather than its order (Boyd & Silk 1983) allowing a measure to be calculated which shows by how much an individual is dominant over another for each behavioural act used. This is possible as the method assigns individuals to a straight line so that the distance between points for each individual represents the amount by which one individual dominates another. The amount that one individual (i) dominates another (j) is defined in terms of the probability that i beats j in any given encounter (P_{ij}). This definition relies on the assumptions that probabilities are constant during observations and that the outcome of an encounter is probabilistically independent of outcomes of previous encounters (Boyd & Silk 1983). Independence means that the probability that i wins any number of consecutive encounters = Pij_n (Boyd & Silk 1983).

The assumptions of the cardinal method of dominance assessment formalize the notion that the outcome of any encounter has a deterministic and random component (Boyd & Silk 1983). This assumes that a deterministic component is stable during the observation period and that random effects on different contests are independent (Boyd & Silk 1983). The assumptions are likely to be violated when dominance relationships are being established or challenged and are likely to be valid when group dynamics are stable (Boyd & Silk 1983).

The use of the cardinal dominance index (CDI) method of assessment does not rely on subjective weightings of different behavioural acts as the method results in an index of dominance and not a rank. This index of dominance can then be ordered, resulting in a dominance rank for each individual in the colony.

The aim of this study was to re-evaluate the dominance hierarchy of the highveld mole-rat using an alternative dominance assessment method to that previously undertaken by Moolman *et al.* (1998) and to determine whether animal mass, sex or reproductive status was correlated to individual dominance position in the colony. It is hypothesised that colonies of the highveld mole-rat would be loosely social and that neither reproductive status, animal mass nor sex would provide reliable correlates to dominance.

Methods and Materials

Behavioural sampling was conducted on each of two colonies (consisting of seven and eight individuals respectively) of the highveld mole-rat. Behavioural sampling for each colony consisted of scan sampling of dyadic interactions for each individual in each colony, each minute. Each colony was observed for a period of 50 hours giving a possible 3000 observations for each individual, although each individual was not always observed to be undergoing a dyadic interaction. The 50 hours of observations for each colony were conducted over a 2 month period between 1999-2000 allowing 30 minutes – 3 hours of behavioural sampling per day (depending on the level of activity in the colony). Animals were only observed when at least half of the individuals were active. Data therefore consisted of counts, for each individual, for each type of interactive behaviour commonly observed.

Ten dyadic interactive behaviours were identified as being common to both colonies and in sufficient frequency to allow individual calculation (each individual had to have at least one interaction per behaviour type). The ten behaviours are listed in Table 1, along with the assumption as to whether they were considered to be dominant or submissive behaviours. An ethogram of the common behaviour types as well as most other behaviours is given in the Appendix.

Table 1: Behaviours used in the analysis of dominance and whether each behaviour was assumed to be dominant or submissive.

Dominant Behaviour	Submissive Behaviour
Clash	Hop
Pass Over	Pass Under
Push	Paw
Tail Bite	Retreat
	Squeak
	Tail Lift

Data Collection

Data was recorded solely by the author by hand on custom made data sheets which made it possible to record data at the rate referred to above. In all cases, individual observations of behaviour were recorded. No winners or losers were identified due to the subjective nature of these decisions. A series of examples follow which illustrate how the behaviour was recorded:

If two individuals clash, then the antagonist and the other individual are recorded with a clash. If the clash continues into the next minute, a further clash is recorded for each animal. This continues until the clashing activity stops.

If an individual lifts its tail while being passed over by another animal, the passing animal is recorded with a pass over while the animal that lifts its tail is recorded with a tail lift. Using ordinal dominance methods, this interaction would have been recorded as a win for the passing animal.

Data was analysed in order to determine whether dominance rank and sex, mass or reproductive status were significantly correlated. Results were compared to that of Moolman *et al.* (1998) whose study was based on a single colony using factor analysis (ordinal dominance methods).

Cardinal Dominance Indices (CDIs) were calculated instead of other ordinal dominance ranks (using factor analysis). The methods for calculating the CDIs were written in SASS by M. van der Linde (Statistics Dept., University of Pretoria). The programming follows the methods laid out by Boyd & Silk (1983).

Using 50 hours of observations for each colony, matrices were constructed for each of the behaviours listed above. The matrices consisted of the total number of observations of behaviour for each individual and its partner as only dyadic interactions were recorded.

For each behaviour and colony a CDI was calculated for each individual (using the constructed matrices and programming by M. van der Linde). An associated probability value was also determined during this procedure. CDI's were recorded when the associated probability values had become constant. In most cases, less than 100 iterations were necessary to achieve constant associated probability. For those behaviours where probability values still varied at 100 iterations, the CDI was taken at the 101 iterations stage.

The CDI's were then ranked for each individual (per colony and behaviour) where the highest index was given the highest rank (one) and considered to be the most dominant individual in the colony, for that behaviour; and the lowest index was given the lowest rank (seven or eight, depending on the number of individuals in the colony) and was considered to be the least dominant individual for that behaviour.

In order to combine behaviours so that a single dominance hierarchy could be constructed, rank frequencies were determined for all behaviours so that the individual

with the highest frequency for each rank was assigned that rank in the colony. Using an ANOVA procedure, it was possible to analyse mass vs rank.

Results

Individuals within each colony were ranked by ordering rank frequencies obtained from the cardinal dominance index for each behaviour. A resulting dominance hierarchy was constructed together with individual mass, sex and reproductive status (Table 2). As individual 6 from colony 2 occupied both position 5 and 6 in the rank frequency analysis, her rank was assigned midway between the two rank values (5.5).

Table 2: Final dominance hierarchies resulting from behaviour matrix and rank frequency analysis using the cardinal dominance index (CDI) method.

COL	RANK	SEX	STATUS*	MASS (g)	COL	RANK	SEX	STATUS*	MASS (g)
1	1	F	NR	111	2	1	M	R	115
1	2	F	NR	81	2	2	M	NR	93
1	3	F	NR	52	2	3	M	NR	109
1	4	F	R	102	2	4	F	R	91
1	5	M	NR	161	2	5.5	F	NR	89
1	6	F	NR	52	2	7	F	NR	85
1	7	M	R	165	2	8	M	NR	82

*: NR = non-reprodcutive individuals, R = reproductive individuals.

The dominance hierarchy was analysed using an ANOVA procure (for each colony) for correlations between individual rank and mass (Figure 1 and 2). No correlation was evident in the linear regression analyses for colony 1 (R^2=0.13, p=0.44), while there was a statistically significant correlation for colony 2 (R^2=0.68, p=0.02).

Visually assessing the dominance matrix, it appears that no relationship exists between individual rank and reproductive status or sex. Although in colony 2 the heaviest male (assumed to be the reproductive male) is the most dominant individual, this does not seem to be the case across the two colonies, since colony 1 does not show that the heaviest male is the most dominant individual in the colony.

It is evident that the individual that was determined to be the reproductive female in each colony exhibits an intermediate rank – not being overtly dominant but not being overtly submissive either. The relative ranks of the two reproductive females in each colony is intermediate; at position 4. Although both reproductive females are occupying this position in the dominance hierarchy, it is probable that the equal relative ranking of these females occurred by chance.

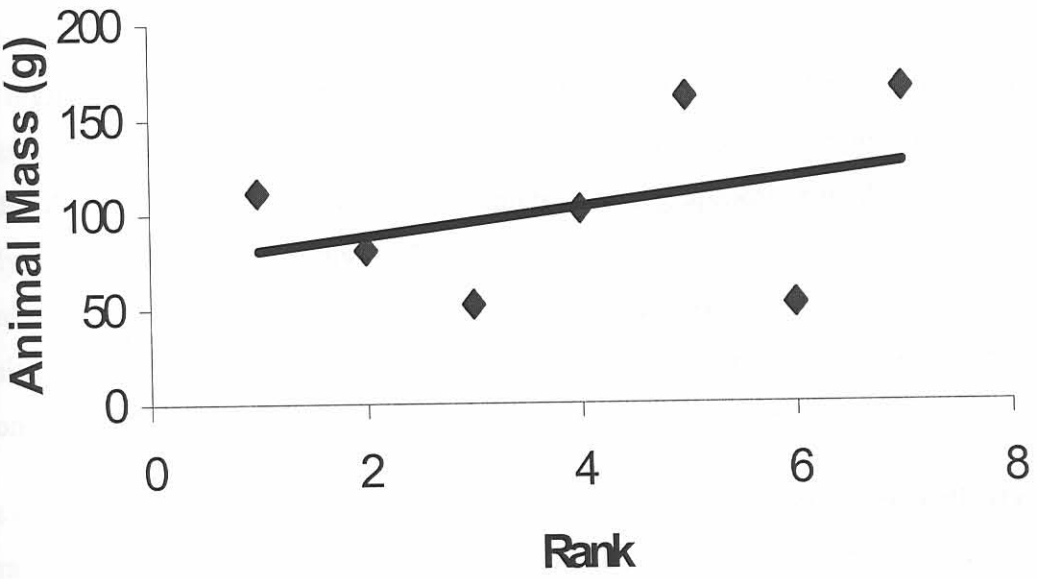

Figure 1: The resulting straight line from a regression analysis of individual rank vs mass (g) for individuals belonging to colony 1. The regression coefficient was calculated to be 0.13 and p=0.44.

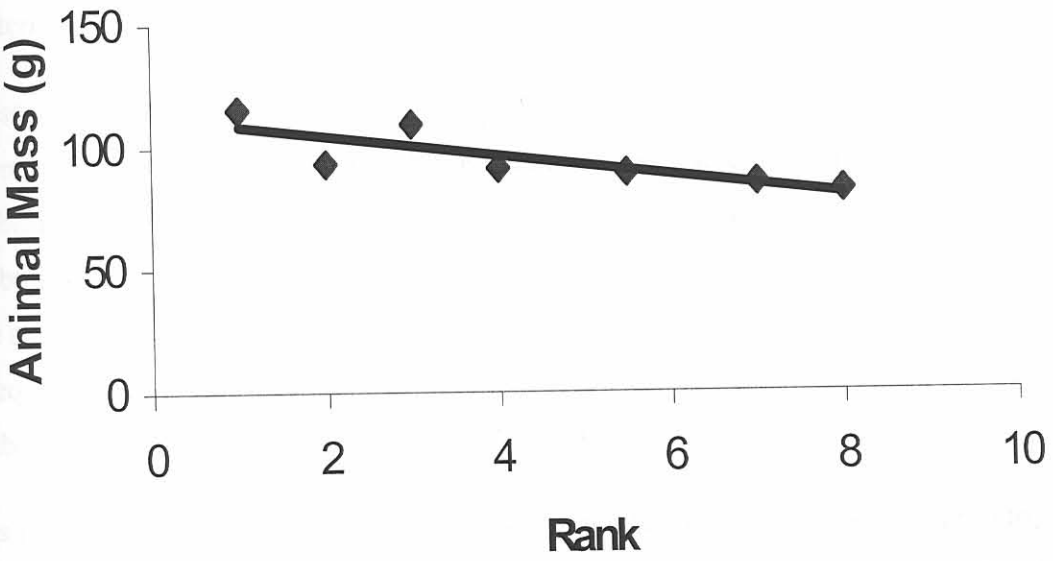

Figure 2: The resulting straight line from a regression analysis of individual rank vs mass (g) for individuals belonging to colony 2 The regression coefficient was calculated to be 0.68 and p=0.02.

Discussion

The highveld mole-rat (*Cryptomys hottentotus pretoriae*) is a social bathyergid that lives in familial groups. Although social, the highveld mole-rat does not appear to have a predictable social colonial dominance hierarchy. From graphical as well as visual analyses, it is evident that individuals do not show any predictable relationship between dominance rank and individual mass, sex or reproductive status. Although there was a significant correlation between mass and rank for colony 2, this was not evident for colony 1 and this result can therefore not be used to reach any broad conclusions.

The dominance hierarchy that results from the Cardinal Dominance Index method of hierarchy determination uses available behavioural data to determine the relative dominance index of each individual in each colony. The behavioural data that was used comprised the most common behaviours in each colony (see Table 1). The assumptions that were made using these behavioural acts were whether the behaviour could be considered to be an act of dominance or an act of submissiveness. These assumptions were thought to be valid as logic (from preliminary observations) played a role in the determination.

A second important assumption made was that an inverse relationship existed between dominance and submissiveness. Hence the most submissive individual (for a particular submissive behaviour) could also be thought of as the least dominant individual (for that submissive behaviour). It was therefore possible to use behaviours that were assumed to be submissive in the study. The individuals were arranged, before the final rank frequency analysis, so that the results were in terms of dominance and not submissiveness.

As Richards (1974) states, it is sometimes difficult to assess which behaviour(s) to use in order to assess dominance in a social group. Using different behaviours ultimately leads to different resulting dominance hierarchies (Boyd & Silk 1983). It was therefore decided, in this study, to incorporate as many behavioural acts (whether assumed to be submissive or dominant) as was possible depending on individual and colony activity.

In the early stages of a study of dominance, it is important to state, categorically, what behaviour is considered to represent a win and which behaviour is considered to be a loss, to an individual (Boyd & Silk 1983). As removing subjectivity was a part of the goal of this study, wins and losses were not identified and individuals were not scored in this way. Individuals were observed on their absolute dyadic interactions and the data was therefore representative of counts of behaviour types between individuals within a colony. Although the assumption of whether a behavioural act is dominant or submissive could be seen to be subjective, it is believed that these assumptions hold - especially after preliminary observations were undertaken.

Boyd and Silk (1983) suggest that three structural properties characterize dominance relationships; stability, transitivity and linearity. Group stability in their view exists as long as individual A always (without exception) beats individual B (Boyd & Silk 1983). However, as wins and losses were not determined in this study (as "beats" is a loaded term – representing something positive to the individual) this definition of group stability was not appropriate. Richards' (1974) definition of group stability is not as subjective, and states that group stability is achieved when the group has been together for a relatively long period of time with no changes evident in group composition or individual's health. The definition of Chase (1974) was also used in this study where increasing group stability was associated with decreasing interaction frequency and intensity. Individuals were only observed after a period of three weeks of being placed in the artificial tunnel system and interactions had become relatively infrequent.

It is likely that mole-rat colonies do not meet the requirements for the ordinal method of dominance assessment since group transitivity is unlikely. For a group to be transitive, with individual A dominating B, and B dominating C – A would have to dominate C (Richards 1974). As this situation is unlikely group linearity is also thought not to exist in colonies of the highveld mole-rat, as linearity only exists where all triads are transitive (Chase 1974). Thus, colonies of *C. h. pretoriae* do not fit the generally accepted models of social dominance hierarchy assessment, except for the fact that they can become stable, and ordinal methods were therefore not used.

The studies carried out on other social bathyergids to date have all used ordinal dominance methods to assess dominance hierarchies (and resulting social structure) of colonies. This study therefore takes a unique standpoint on the social structure of social bathyergids in terms of the above considerations. It also, unfortunately, makes detailed comparisons of results difficult. In what follows, I will discuss other bathyergid species whose social structure has been studied but one must bear in mind that they were carried out using an ordinal ranking technique.

The Bathyergidae exhibit a broad spectrum of sociality (Jarvis & Bennett 1991) – the genus *Cryptomys* is not only the most specious but also exhibits a continuum of sociality from social to a eusocial species (Jarvis & Bennett 1991). Of the cryptomid species that have been studied to date, all are social and include *C. hottentotus hottentotus* (Bennett 1989), *C. darlingi* (Gabathuler *et al.* 1996) and *C. mechowi* (Wallace & Bennett 1998). Most colonies consist of a single reproductive pair that are generally the largest and most dominant colony members (*Cryptomys hottentotus hottentotus;* Bennett 1989, *Cryptomys darlingi*; Bennett *et al.* 1994 and *Cryptomys mechowi*; Wallace & Bennett 1998). In the case of the eusocial Damaraland mole-rat (*Cryptomys damarensis*), it is thought that up to two reproductively active males could inhabit a single colony (Jarvis & Bennett 1993). Colonies of the common mole-rat, *C. hottentotus hottentotus*, exhibit a loose social structure (Rosenthal *et al.* 1992.) and are thought to have frequent dispersal events (Rosenthal *et al.* 1992). Dominance in the Damaraland mole-rat (*C. damarensis*) was shown to be positively correlated to body mass but not related to sex (Gaylard *et al.* 1998).

Colonies of the highveld mole-rat (*C. h. pretoriae*) are thought to consist of a single reproductive pair. This was determined by histological examination (Moolman *et al.* 1998). The species is thought to be transiently social (Moolman *et al.* 1998). Moolman *et al.* (1998) also showed that the highveld mole-rat lacks a distinct dominance hierarchy, colonies are short-lived and colony fragmentation frequently occurs. Similar conclusions can be made from the results of this study. It was found that the highveld mole-rat was loosely social as predictable dominance hierarchies could not be constructed on the basis

of sex, mass or reproductive status. The strongest dominance hierarchies and social structures are found in the eusocial bathyergids; the naked mole-rat (*Heterocephalus glaber*) and the Damaraland mole-rat (*Cryptomys damarensis*). In colonies of these species, strong dominance hierarchies are evident with the reproductive individuals of both sexes appearing to show dominance over the rest of the colony.

The highveld mole-rat appears to be a very loosely social bathyergid, fitting into the cryptomid social continuum at the lower end of the social scale. This is verified by the fact that lone animals as well as pairs of animals are frequently caught following good rains. The aridity food distribution hypothesis (Jarvis *et al.* 1994) predicts that soil aridity (and, covariantly, food distribution) are important factors favouring the evolution of sociality. For most bathyergid species, this theory holds true with solitary species being found in areas with more predictable precipitation and the eusocial species occurring in areas with unpredictable, and low, annual precipitation (Jarvis *et al.* 1994). The highveld mole-rat is interesting as the species is found in a summer rainfall region with relatively high and predictable levels of annual precipitation. This habitat would, according to the hypothesis, select for a more solitary existence since soil hardness (an important factor to foraging and individual dispersal) would not be limiting in a region with sufficient, seasonal rainfall.

It is thus an important finding that the highveld mole-rat is a loosely social bathyergid, also being captured individually as well as in pairs. Pairs and lone animals are usually common in the early part of the year (Janse van Rensburg 2000, G. Malherbe pers. obs.) that coincides with the summer rainfall months. It is perhaps an interesting question; is eusociality the culmination of evolutionary forces or is eusociality an early part of a diverging family's history? In more mesic regions with more predictable and higher precipitation, could a solitary existence not be selected for in place of sociality? In this species it appears as though colonies break up frequently and that being social is not selected for as in eusocial species which occur in less predictable environments.

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Appendix

In the ethogram that follows, a description is given of those behaviours used in the analysis of dominance for this study as well as other behaviours that were observed.

Dominant Behaviours

Pass Over

One animal passes another animal by walking over it from its anterior or posterior region. This behaviour can occur even when both animals are very large; the passing mole-rat seems to force itself over the other animal. The passing mole-rat usually uses all its limbs in the process. The receiver may also lift its tail. The receiving animal may try and inhibit the passing animal by bracing itself with its limbs on the sides of the tunnel or by lifting itself so the tunnel is effectively blocked. The event is only recorded if the passing animal is successful.

Push

One animal pushes another animal by a backwards sweeping action of its hind limbs. The action is similar to that used when the animal is cleaning the burrow or moving food and other debris from one chamber to another. The receiving animal can either be facing the pusher or with its anterior towards the pusher. The receiver occasionally locks itself in position in the burrow system by bracing itself on the sides of the tunnel or simply allows itself to be pushed along the tunnel. The antagonist may also brace itself on the sides of the tunnel while pushing. The action is usually followed by the pusher moving (walking or running) forward again (away from the receiver).

Tail Bite

The antagonist approaches the receiver from the receiver's posterior and bites or nips at the receiver's tail. Occasionally the receiver lifts its tail in response at which time the antagonist either nips or bites at the tail again or does not. Occasionally, the receiver would turn around in the tunnel to face the antagonist. A nip was defined as a short bite at the tail while a bite was defined as an extended hold on the receiver's tail. A tail bite could also be followed by a passing behaviour.

Clash

The antagonist usually comes into contact with another animal's anterior, retreats slightly, and then pushes the other animal with its buccal cavity open so that the extrabuccal incisors are agape. The animals then lock extrabuccal incisors gently while both animals gain and loose ground (move forward or backward from their original positions). The clash usually ends with one of the animals moving away. A clash between two individuals can last for several minutes, by far the longest dyadic interaction amongst colony members. Retreats can occur between clashing bouts.

Submissive Behaviours

Pass Under

One animal passes another animal by walking under the other animal from its anterior or posterior region. This behaviour can occur even when both animals are very large; the passing mole-rat seems to force itself under the other animal. The passing mole-rat usually uses all its limbs in the process. The receiver may also lift its tail. The receiving animal may try and inhibit the passing animal by bracing itself with its limbs on the sides of the tunnel. The receiving animal may also allow easier passage by lifting itself so the tunnel is effectively open. The event is only recorded if the passing animal is successful.

Retreat

An animal that retreats usually does so quickly in response to meeting another individual in the tunnel or chamber. The action is usually associated with a fast backward running motion followed by the animal turning around (if in a tunnel) and then moving forward more slowly than the initial retreat.

Tail Lift

An animal usually lifts its tail from a horizontal to a vertical position in response to an approach to the animal's posterior by another individual. An animal may also lift its tail in response to any of the dominant behaviours listed above or during a pass under event.

Squeak

This type of vocalisation is defined as being a relatively prolonged sound of lower pitch and longer duration than another type of vocalisation (the chirp). The squeak is more common than the chirp. Squeaking can be from any animal and is usually heard in response to another animals action(s). Squeaking can be in response to any of the dominant behaviours listed above or during a pass under event. Squeaking is done by very young individuals (birth to a few days old) if the mother is not present, they are left alone or they are attemptng to attach to a teat.

Hop

This behaviour is characterised by a series of vertical jumping movements by the animal. The animal can be on all fours or can be in a submissive posture on its back with its limbs in the air. The animal can be approaching another or can be the animal being approached. Occasionally, the hopping motion is simultaneously carried out with a series or chirps (which are high pitched and short duration vocalisations).

Paw

An animal paws another at the receiver's posterior region above the tail. Pawing occurs using either or both forelimbs and can last for a relatively long period of time. The receiving animal may respond with a tail lift action. The pawing action may precede a pass over or pass under action.

Description of other behaviours observed.

Sniff

An animal investigates another individual's posterior. The receiving animal usually lifts its tail in response.

Urine Lapping

This behaviour can be done by the urinating animal or the urine of another animal is lapped. Unusually, during this behaviour, the tongue of the individual is visible external to the buccal cavity. This behaviour has been very rarely observed (three times in the entire experimental period).

Copulation

An individual mounts another individual from behind and exhibits pelvic thrusting activity. This behaviour has been observed between reproductive animals (of different sexes) as well as between two males and two females, although rarely. Normal copulation is also rarely witnessed.

Table 3: Biostatistics of the individuals belonging to each of the colonies used in this study.

Colony 1	Sex	Assumed Reproductive Status*	Mass (g)	Colony 2	Sex	Assumed Reproductive Status*	Mass (g)
42S	F	R	108	RP	F	R	87
	F	NR	92		F	NR	87
	F	NR	89		F	NR	85
	F	NR	86		F	NR	83
	F	NR	65		F	NR	74
	M	R	137		M	R	115
	M	NR	150		M	NR	94
					M	NR	52

*R=reproductive, NR=non-reproductive

Chapter 3

Ecology

Abstract

The highveld mole-rat occurs in a region of seasonal precipitation. Burrow extension and active foraging for new food resources (bulbs) can therefore only occur in summer when the soil moisture content is high enough. In this resource limited environment, these subterranean rodents would be expected to be energy maximizers and preferences should be evident relating to bulb size and patch density. It was expected that preferences would be in favour of bulbs that gave the most reward per unit handling time. Feeding trials investigated which bulb sizes were handled most efficiently in terms of animal size and sex. These bulbs were then expected to be preferred during foraging trials which gave animals choices between patch densities, bulb sizes and combinations thereof. Animal sex did not affect handling time while animal mass did. Larger animals spent a greater proportion of time handling bulbs. As bulb size increased from 1-2g to >2g, the proportion of time spent handling bulbs decreased. This can be translated into an increased proportion of time spent actually consuming the bulbs (acquiring energy). Less costs (handling time = no energy gain) mean greater profits (energy acquisition). However, although these bulbs were expected to be preferred during foraging trials, empty patches were generally preferred. Thus, individuals can not be strictly classified as short-term energy maximizers. In the long-term however, the further exploration of empty patches may lead to energy maximization as further resources could be discovered once patches containing bulbs have been found.

Introduction

A large branch of animal behaviour concerns the study of how animals, whether they are herbivores or carnivores, generalists or specialists, forage for their energy and nutrient requirements in the wild and how (if at all) animals optimise their search for food resources in terms of the benefits of feeding. These benefits include energy gain, nutrient gain and, in some cases, water gain and are evaluated relative to the cost of locating the food (energy expenditure, time and predation risk). The process of locating a food resource that will give maximum benefits to the animal at the least expenditure is termed optimal foraging. Optimal Foraging Theory is an important field of behavioural ecology. There are several definitions used by ecologists to describe optimal foraging theory most pertaining to the maximization of the rate of energy gain and energy acquisition and how natural selection shapes foraging behaviour in this way (Barnett 1991, Nevo 1979, Vleck 1981, Kaufman & Collier 1981). Several predictions have been made regarding Optimal Foraging Theory including predictions with regard to optimal diet, optimal foraging space, optimal foraging period and optimal foraging group size (Nevo 1979). As this study deals only with the diet of the animals, the other predictions will be ignored. Optimal Foraging Theory relating to diet predicts that an animal should never specialise on lower-ranked food regardless of abundance while when the food is in higher abundance increased specialisation should be found (Nevo 1979). In other words, when there are fewer resources available, animals should generalise. When food abundance is low, food generalists are favoured (Nevo 1979) as they can make use of a more diverse range of food. Food generalism in subterranean mammals is primarily related to low net energy harvestable (Nevo 1979).

In the subterranean environment the energetic costs of finding food are very high as soil is a dense and cohesive medium (Vleck 1979). Although bathyergids have a lower resting metabolic rate than that of other rodents their digging metabolic rate may be five times higher than their resting metabolic rate (Spinks *et al.* 1999). The energetic cost of burrowing is proportional to the mass of soil removed and therefore to burrow diameter

(Spinks *et al.* 1999). This places an evolutionary limit on animal size. It has also been found that soil moisture content plays a major role in determining when bathyergids extend their burrow system in search of new food resources as they dig with their extra-buccal incisors which, although growing continuously, are worn down faster than their replacement rate (Jarvis & Bennett 1990).

Studies on the bathyergids suggest that resting metabolic rate scales independent of body mass (Lovegrove & Wissel, 1988) that is unlike any other rodent group. Other subterranean rodents tend to forage above ground and it is thought that the metabolic rate of African mole-rats has been selected for in response to a high energy cost and low probability of finding widely distributed food resources by random burrowing (Lovegrove & Painting 1987). Geophytes are the primary food resource of these subterranean rodents. Geophytes typically have a clumped and patchy distribution due to their predominantly asexual mode of reproduction (Barnett 1991). In the subterranean environment foraging is blind and animals are unable to distinguish distance, direction or quality of an unexploited food patch (Barnett 1991 and Jarvis *et al.* 1998).

Because of the high energetic cost of excavation, it would be advantageous to select food of high nutrient quality since this would increase foraging efficiency (Bennett & Jarvis 1995). Geophytes have concentrated nutrients and low fibre (especially after handling which removes the outer husk) and are essential in maintaining a positive water balance (Bennett & Jarvis 1995) in animals that do not drink any free water.

There are several ways in which foraging in the subterranean environment can be optimised. Where food is heterogeneously distributed (patchy), animals optimise foraging by concentrating their search in the most favourable resource patches (in other words, if all else is constant, the animals will concentrate their efforts in the patch with the highest density of resources) (Barnett 1991). Central place foraging assumes that foragers optimise energy delivery to the central place (store) (Barnett 1991). The Orians-Pearson Model of Central Place Foraging has four main assumptions (Hegner 1982). The animal can search simultaneously for prey of many types or energy values, prey is encountered

randomly in a Poisson manner (this means that the expected time to encounter a specific item is independent of length of time foraging in the area), prey handling time is trivial and the animal is attempting to maximise the rate at which energy is delivered to the central place. These assumptions are generally thought to hold for social bathyergids except for the assumption regarding handling time. Determining whether handling times are indeed trivial was investigated in the present study as handling time is thought to be expensive. If a choice for the animal exists, it would be more efficient to pursue a food item that would require less handling time, if handling time is not trivial. Orians-Pearson predict that there would be an increase in load size (and therefore foraging time) for an animal using a patch of a given quality at an increased distance from the central place. This infers that optimal exploitation of a patch is dependent upon the distance between the patch and the central place (Hegner 1982). When the density of food is increased (a patch is encountered), foragers can change their food search from being blind to being directed (Barnett 1991). This is often evident in bathyergids when a sudden branching of the tunnel system is observed. Thus, foraging efficiency can be optimised with food and soil conditions (Barnett 1991). The optimal diet model predicts that selectivity should decrease as search costs increase (Barnett 1991), thus no longer choosing diets that give a maximum energy yield per unit foraging time in these conditions. Animals should choose large bulbs over smaller bulbs as, for equivalent foraging times, larger bulbs are a greater source of energy (Barnett 1991). These last few ways of optimising efficiency are based on an animal's ability to judge a food resource item accurately (Kaufman & Collier 1981).

However, it is unlikely that true optimal foraging is found in nature as evolution results from a series of compromises (Smith & Sweatman 1974). Also, intraspecific variation (Gustafsson 1988) could be high in an environment where a series of decisions regarding the selection of a food item must be made. It must be kept in mind that optimal foraging theory describes typical individuals (Gustafsson 1988). In natural populations, feeding behaviour can differ between animals of different sex and age (Gustafsson 1988) and other factors such as size. There are three main sources of individual variation. Firstly in a patchy environment with different patches, different behaviours could be applicable to

each patch. Secondly, there can be phenotypic differences (including individually acquired skills) that affect the most appropriate behaviour for each individual to use. Finally, if individuals have identical phenotypes, behaviour can depend on the presence of other individuals. Individual differences are probably not mutually exclusive (Gustafsson 1988) especially in a closed, social environment.

In this study, optimal foraging was studied into two ways. Firstly, the response of animals to different sized geophytes (quantified as components of total consumption time) as well as how individual variation (sex and size) played a role in determining the components of total consumption time. It was predicted that larger animals, especially males, would feed on larger bulbs as well as being more efficient at handling the bulbs presented to them. Secondly, patch preference was investigated with patch density and bulb size being varied independently. It was predicted that patches containing the highest density of bulbs would be favoured and that large bulbs would be selected over smaller bulbs. This was investigated in terms of patch visitation as well as excavation distance.

Methods and Materials

Feeding

Three colonies containing a total of 23 individuals of variable body mass were used in the feeding trials. On the day of a feeding trial, animals were housed in separate chambers and deprived of all food for a period of at least six hours. Feeding trials were conducted at approximately the same time of day at which the animals were normally fed. Although it was not possible to standardize motivational states completely (as it was not known when each animal ate last), it was possible, by depriving the animals of food for six hours prior to each study, to state that none of the animals had eaten for at least six hours prior to each study.

A feeding trial consisted of presenting an individual with a naturally occurring geophyte (*Onithogalum secundum*), of a certain size class (mass). Once the individual had accepted the geophyte, two parameters were recorded; handling time (the time taken to handle and/or prepare the geophyte for consumption; excluding mastication) and total consumption time which is the total time taken by an individual to handle and consume the bulb; from the beginning of handling to the end of consumption. Total consumption time = handling time + consumption time, and it was therefore possible to calculate consumption time (the time taken by an individual to eat the bulb beginning at mastication).

Bulbs of three size classes were used, namely; less than 1 g, 1 – 2 g and more than 2 g. Geophyte size classes were determined according to availability. Most of the 23 individuals were subjected to 15 trials; 5 repeats for each size class for statistical integrity. It was not possible to conduct 15 trials for five animals due to their level of stress. The number of repeats for each individual and test were never less than three.

The data was converted to proportions of total consumption time (handling time relative to total consumption time and consumption time relative to total consumption time).

Mean ± SD's (mostly n = 5) were then calculated for each individual and bulb size class. All data analyses were conducted on the proportion of time spent handling the food item relative to total consumption time (ht/tct) as the proportion of time spent consuming the geophyte (ct/tct) would simply be 1 - (ht/tct) and relationships analyzed would all be relative. It is important to note here that all results are thus in a ratio format (thus no units being given).

Handling time was analysed for significant differences between animal mass, animal sex and bulb mass respectively. Before the data could be analysed, an arcsine transformation was carried out. Statistical procedures were carried out by the Statistics Department at the University of Pretoria that consisted of analysis of variance (ANOVA) using a LS MEANS technique. In order to differentiate between results of the ANOVA, a multiple comparison procedure was undertaken. F statistics are only reported for major distinctions while probability values are reported for all tests undertaken.

Foraging

Two functionally complete colonies were used in all foraging trials. The colonies consisted of seven and eight individuals respectively. Foraging trials were conducted in an apparatus specifically designed for the trials; and is illustrated below (Figure I). The apparatus consisted of four foraging trays each comprising 250 cm of available packed soil to burrow. This distance was not limiting. Each tray was filled to capacity with a sandy soil typical of the region. The soil is a red, fine sandy/clay soil. Prior to use, the soil moisture content was standardized to 16% (of wet mass) as soil moisture limits burrow excavation (Jarvis *et al.* 1994).

Each tray was subjected to varied density and size class combination treatments of the geophyte *Ornithogalum secundum* during the experimental procedure. Control runs were undertaken to determine any directional or sensory preference to the four trays; of which no preference was evident (see Figures 7a and 7b). The control procedures were

undertaken for each colony separately and consisted of soil-filled trays but with no geophyte content in any tray.

At the colony's usual feeding time, all individuals of a single colony were placed in the central arena and were left for a few minutes (not longer than 3 minutes) in order to reduce their stress levels. Scan sampling was conducted on the four trays each minute, a single day session lasting 90 minutes. Data gathered consisted of counts of the number of individuals *actively* digging/excavating each of the trays as well as the total distance excavated in each tray after the 90-minute session each day. Each colony and experimental procedure was repeated three times for statistical integrity.

Bulb size classes that were used when varying size class were < 1 g, 1 - 2 g and > 2 g while when varying density were <1 g and >2 g. Similarly to bulb size classes, tray geophyte density was assigned as a function of geophyte size class availability. Tray densities that were used were 2 m^{-1}, 4 m^{-1}, 6 m^{-1} and 0 m^{-1} respectively.

Thus, each colony was subjected to the same series of encounters with the soil arenas varying in bulb density and bulb size class *independently*. Data was analyzed for significant differences between mean (n=3) patch visitation ± SE (count data) using an analysis of variance (ANOVA) technique comparing LS MEANS that were executed by the statistics department of the University of Pretoria. In the same way, data was analyzed for significant differences between mean excavation distances (n=3). This method of comparison employs a multiple comparison technique in order to differentiate between significantly different statistical outcomes of the ANOVA. It is important to note here (foraging section only) that only those statistical outcomes that had $p < 0.025$ were considered to be significant. F statistics are only reported for major distinctions while probability values are reported for all tests undertaken.

Table 1: The following patch density/bulb size class combinations were experimentally investigated.

Experimental Condition	Tray 1 Density (m^{-1})	Tray 2 Density (m^{-1})	Tray 3 Density (m^{-1})	Tray 4 Density (m^{-1})	Bulb Size Class (g)
Control	0	0	0	0	No bulbs used.
Vary Size	2	4	6	0	<1
	2	4	6	0	1-2
	2	4	6	0	>2
Vary Density	2	2	2	0	<1
	4	4	4	0	<1
	6	6	6	0	<1
	2	2	2	0	>2
	4	4	4	0	>2
	6	6	6	0	>2

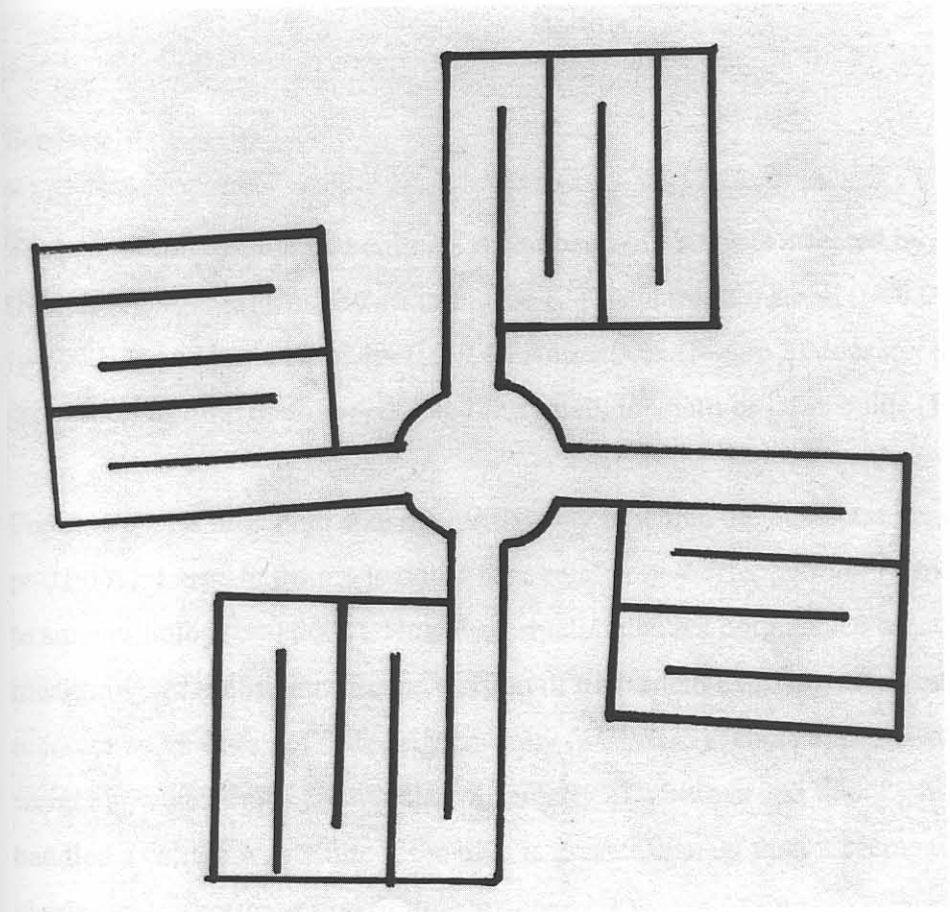

Figure I: Schematic representation of the apparatus used in the foraging trials. Each tray consisted of 250 cm of available burrowing distance. Each tray lead from a central arena into which each entire colony was placed at the beginning of each trial.

Results

Feeding

The proportion of time that animals spend handling bulbs is affected by animal size ($F=28.29$, $p<0.0001$), as shown in Figure 1. This is true for small ($p=0.0020$), medium ($p=0.0024$) and large bulbs ($p=0.0014$). Animal sex (Figure 2) does not affect the proportion of time that is spent handling small, medium or large bulbs ($F=0.82$, $p=0.37$).

Figure 4 shows how bulb size affects the way in which the bulbs are handled ($F=45.96$, $p<0.0001$). Large bulbs are handled for a relatively shorter period of time than compared to smaller bulbs ($p<0.0001$). However, small bulbs are not handled any differently to medium sized bulbs since the proportion of time spent handling small bulbs compared to medium bulbs does not differ significantly ($p=0.0235$). There appears to be a cut-off weight at which bulbs are handled differently. If a bulb is less than 2g in mass it is handled a certain way while if the bulb is greater than 2g then it seems to be handled for a significantly shorter period of time.

Small animals spend the same proportion of time handling small and medium sized bulbs ($p=0.1233$) (Figure 3 and Figure 6). This is also true for large animals ($p=0.1028$). Small animals spend a greater proportion of time handling small bulbs when compared to large bulbs ($p<0.0001$). This is also true for large animals ($p<0.0014$). Small animals also spend a greater proportion of time handling medium bulbs when compared to large bulbs ($p<0.0001$). This is also true for large animals ($p<0.0001$).

Figure 5 shows how males spend the same proportion of time handling small bulbs as they do medium bulbs ($p=0.0891$). This is also true for females ($p=0.1549$). Males spend a greater proportion of time handling small bulbs when compared to large bulbs ($p<0.0001$). Males also spend a greater proportion of time handling medium bulbs when compared to large bulbs ($p=0.0005$). These trends are similar for females ($p<0.0001$ and $p<0.0001$ respectively).

Absolute values

Overall, small animals handle geophytes for about a third of total consumption time, whereas large animals handle geophytes for slightly longer. Differences in the proportion of time spent handling geophytes is independent of animal sex for both animal size classes.

All bulbs were handled for less than half of total consumption time with the greatest proportion of handling time being used by large animals on small and medium sizes geophytes (0.46 ± 0.10). The smallest proportion of time spent handling geophytes was performed by small animals who fed on large geophytes (0.24 ± 0.09).

Time spent handling geophytes thus varied between 24 % and 46 % of total consumption time. The proportion of handling time was always less than half of total consumption time.

Foraging

In order to test whether individuals had a preference to certain trays, a control experiment was carried out. In these experiments, the four trays were filled with soil only geophytes being absent in all trays. It was important to test the null hypothesis for the control experiment. This hypothesized that there would be no preference to certain trays in terms of visitation (quantified as counts and total distance excavated). If the null hypothesis was rejected, showing that there was a preference to certain trays, this could possibly be explained by odour trails or directional preference when foraging. In this study, there was no evidence that there was any preference to any tray(s) when geophytes were absent from the four trays (Figure 7). Counts varied between 71 and 97 ($p=0.0838$). Individuals excavated a total distance of between 117 and 144 cm ($p=0.2367$).

The trend observed was that no preference existed between the four trays in terms of visitation (count and total excavation distance). This result allowed further,

uncomplicated investigations into possible preferences to trays varying in geopyhte size and patch density independently as well as various combinations of geophyte size and patch density.

When comparing the preferences that individuals showed to patch density, independent to geophyte size, it was evident that there was no statistically significant preference to any of the patches of different geophyte density. This trend was equally evident when using either of the methods of quantification of visitation (count and total excavation distance; see Figure 8 and 9 respectively). Counts varied between 64 and 80 individual visitations that excavated a total distance of between 100 and 128 cm.

When comparing the preferences that individuals showed to geophyte size, independent to patch density, it was evident that there were statistically significant preferences to several of the patches of different geophyte size. This trend was not equally evident when using either of the methods of quantifying visitation (count and total excavation distance).

In terms of count data (Figure 10), it was apparent that individuals preferred empty patches (86.4 ± 5.6) when compared to patches containing medium sized geophytes (58.9 ± 9.7) ($p=0.0089$). Also, individuals apparently preferred empty patches when compared to patches with large sized geophytes (66.4 ± 4.7) ($p=0.0074$). No preference was evident between patches where different sized geophytes were present.

In terms of total excavation distance (Figure 11), it was apparent that individuals preferred empty patches (133.6 ± 8.6) when compared to patches containing medium sized geophytes (87.9 ± 13.3) ($p=0.0048$). Although this trend correlates well to the preference showed by count data, individuals showed no preference to large sized geophytes when compared to empty patches ($p=0.0405$). However, individuals did show a preference to small sized geophytes (123.3 ± 7.5) when compared to medium sized geophytes ($p=0.0211$).

When combining geophyte size together with geophyte patch density it is possible to determine whether certain geophyte size/density patch combinations are preferred over others. In undertaking this analysis, it was evident that very few combinations are preferred over other combinations. A total of four statistically significant preferences were observed. Only one of these combination comparisons was confirmed with both methods of visitation quantification (count and total excavation distance). The other three statistically significant preferences were only evident for either count or total excavation distance data.

When geophyte size and density are combined as a factor for comparison, it is evident that, for large geophytes, empty patches are preferred over patches with a density of 2 geophytes m^{-1} ($p=0.0224$). This is only evident when analysing excavation distance data (Figure 13). Similarly, for large geophytes, empty patches are preferred over patches with a high density of geophytes (6 geophytes m^{-1}) ($p=0.0205$).

When patch densities are 2 geophytes m^{-1}, it is evident that patches with small geophytes are preferred over those with medium geophytes. This observation is confirmed for both methods of visitation quantification (count data; $p=0.0067$ and total excavation distance; $p=0.0046$).

Finally, when preference is compared in low-density patches (2 geophytes m^{-1}) it is evident that patches with small geophytes are preferred over those patches that contain large geophytes ($p=0.0218$). This is only evident for count data (Figure 12).

Visitation quantification correlation

Figure 14 shows an assessment that was made to determine whether any correlation existed between the two methods used to quantify patch visitation, and ultimately, patch preference. The methods that were compared to each other were count data (number of animals in a patch) and the total distance that was excavated in that patch. Strong correlations were found to exist. The linear correlation and power correlation gave

correlation coefficients of 0.758 and 0.805 respectively. The exponential correlation was found to provide the best-fit to the data; giving a correlation coefficient of 0.814.

Although not perfect correlations, either of the correlations could be used to estimate one type of data provided that the other is known. Note that these correlations were estimated on colonies of seven and eight individuals respectively.

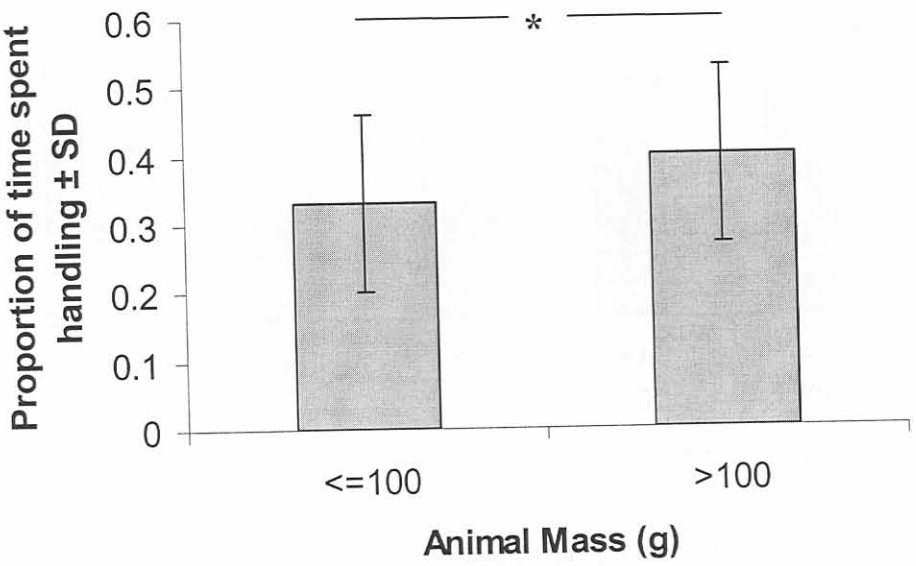

Figure 1: The mean proportion of time (± standard deviation) different sized animals (both sexes) spent handling geophytes. *=significant difference detected.

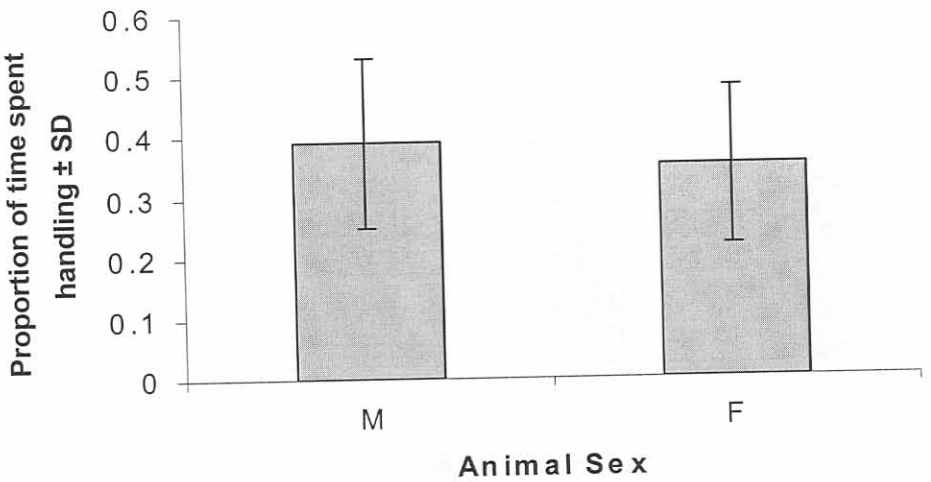

Figure 2: The mean proportion of time (± standard deviation) that animals of different sex (all animal size classes) spent handling geophytes. No significant differences were detected.

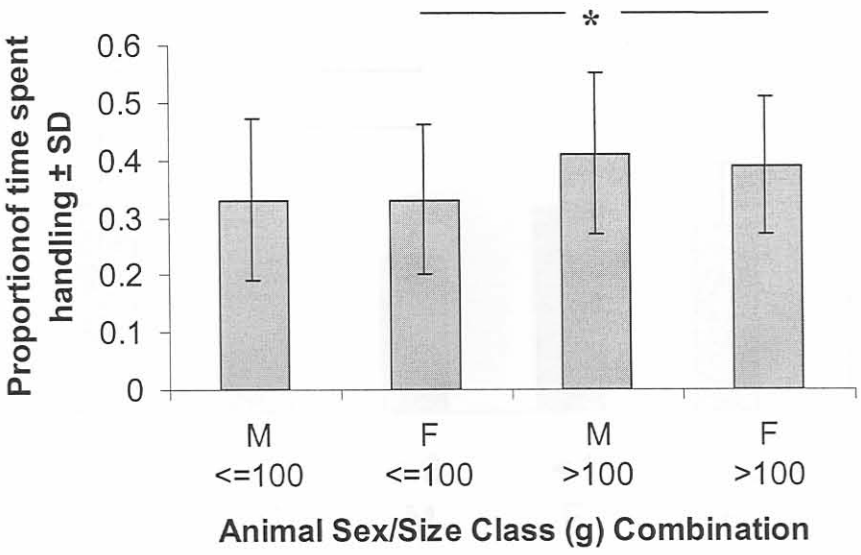

Figure 3: The mean proportion of time (± standard deviation) that different sized animals (of each sex) spent handling geophytes. *=significant differences detected.

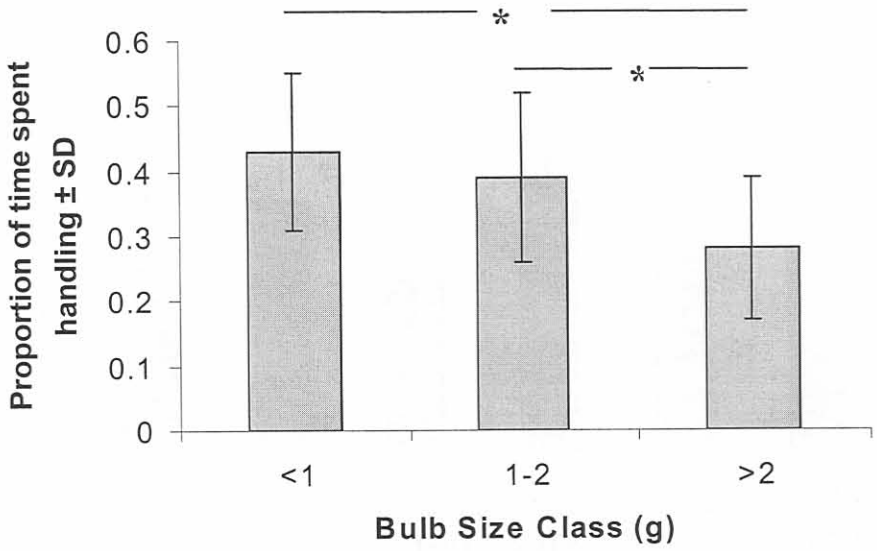

Figure 4: The mean proportion of time (± standard devaition) that was spent (for all animals) on different sized geophytes. *=significant differences detected.

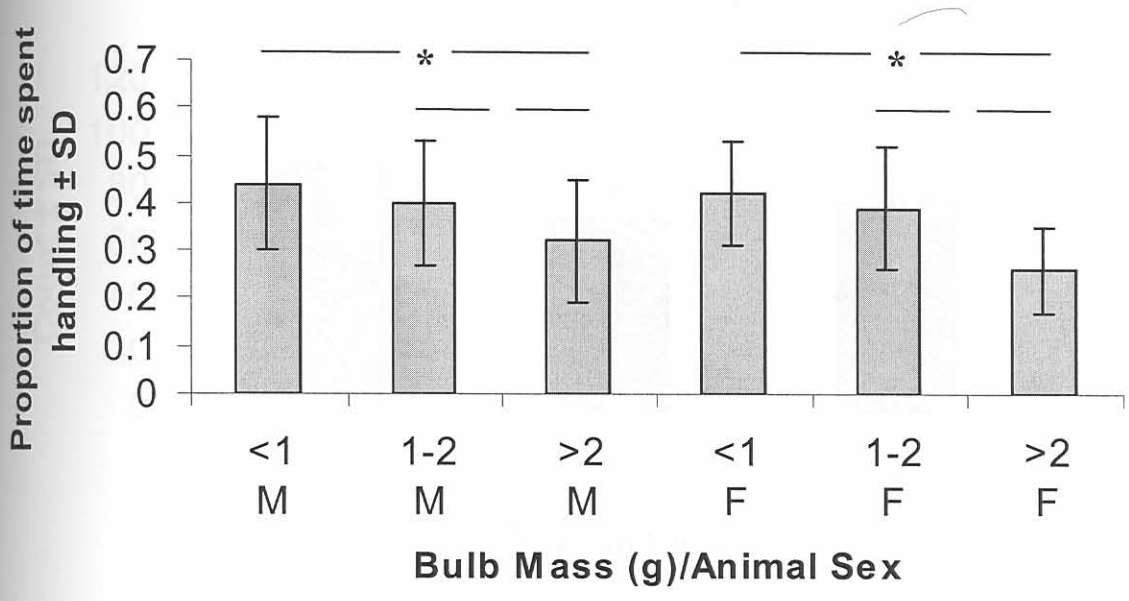

Figure 5: The mean proportion of time that animals of different sex spent handling different sized geophytes. *=significant differences detected.

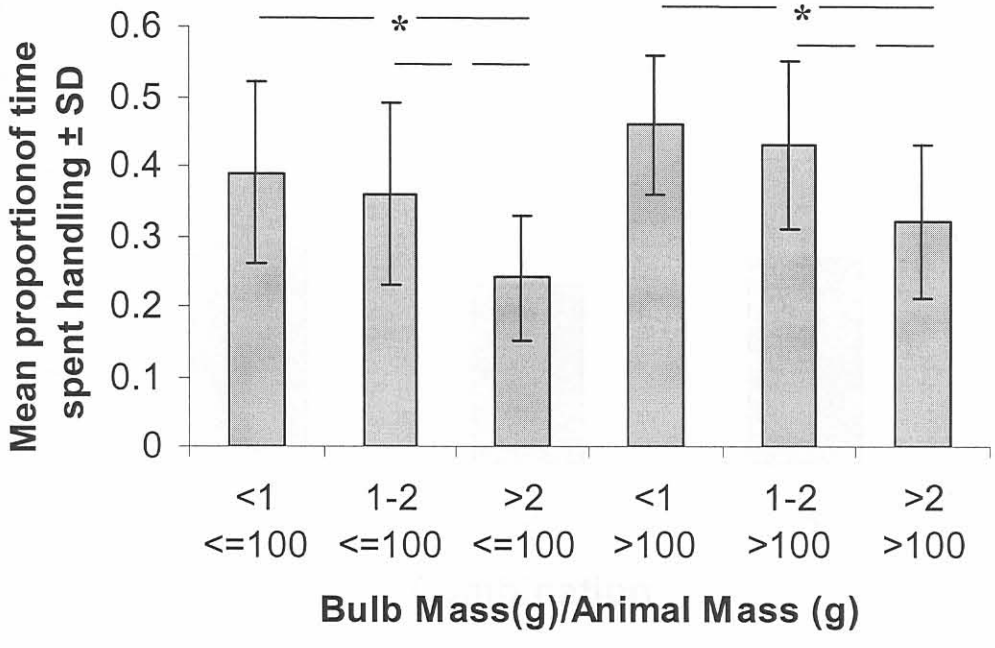

Figure 6: The mean proportion of time (± standard deviation) that different sized animals (of both sexes) spent handling different sized geophytes. *=significant differences detected.

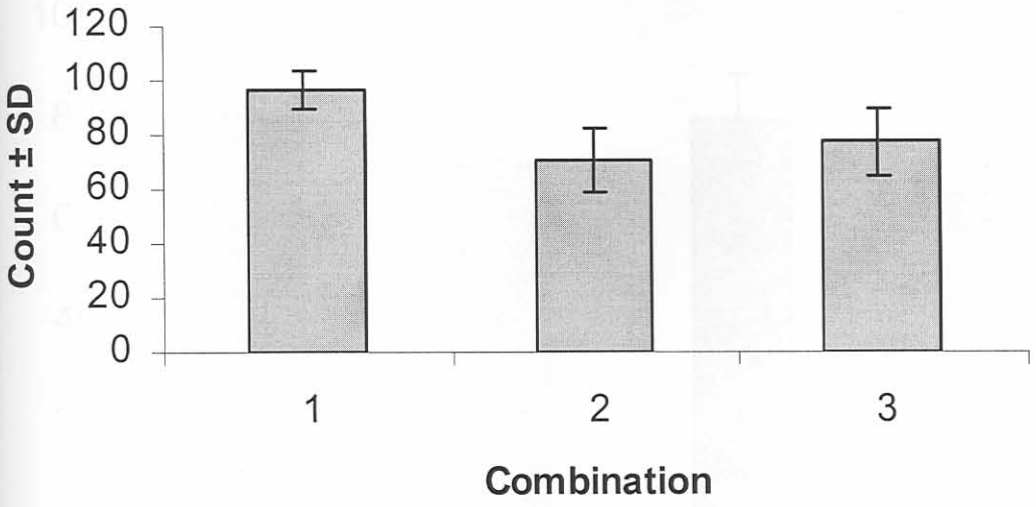

Figure 7a: Mean count (± standard deviation) of individuals observed in each soil filled tray (devoid of any geophytes). No significant differences were detected.

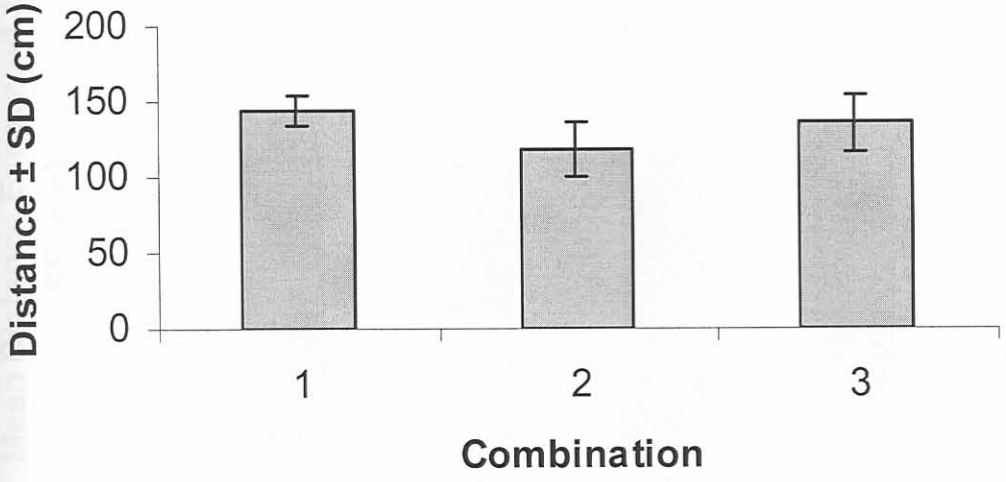

Figure 7b: Mean excavation distance (± standard deviation) dug by animals in each soil filled tray (devoid of any geophytes). No significant differences were detected.

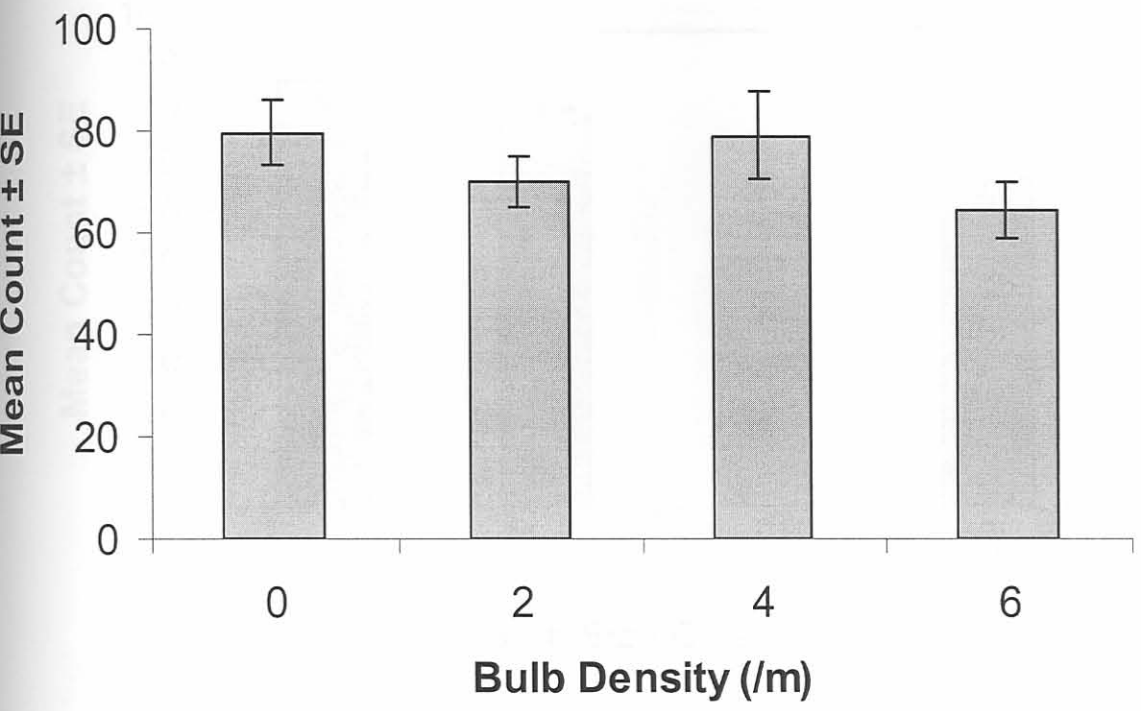

Figure 8: Mean count (± standard error) of animals observed in patches of different geophyte densities (for all bulb sizes). No significant differences were detected.

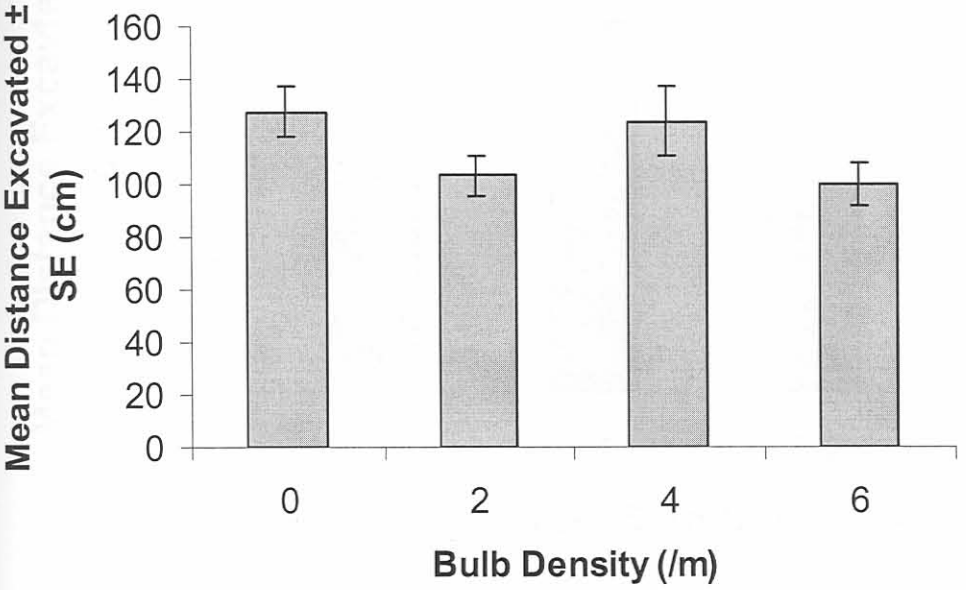

Figure 9: Mean excavation distance (± standard error) dug by individuals in patches of different geophyte densities (for all bulb sizes). No significant differences were detected.

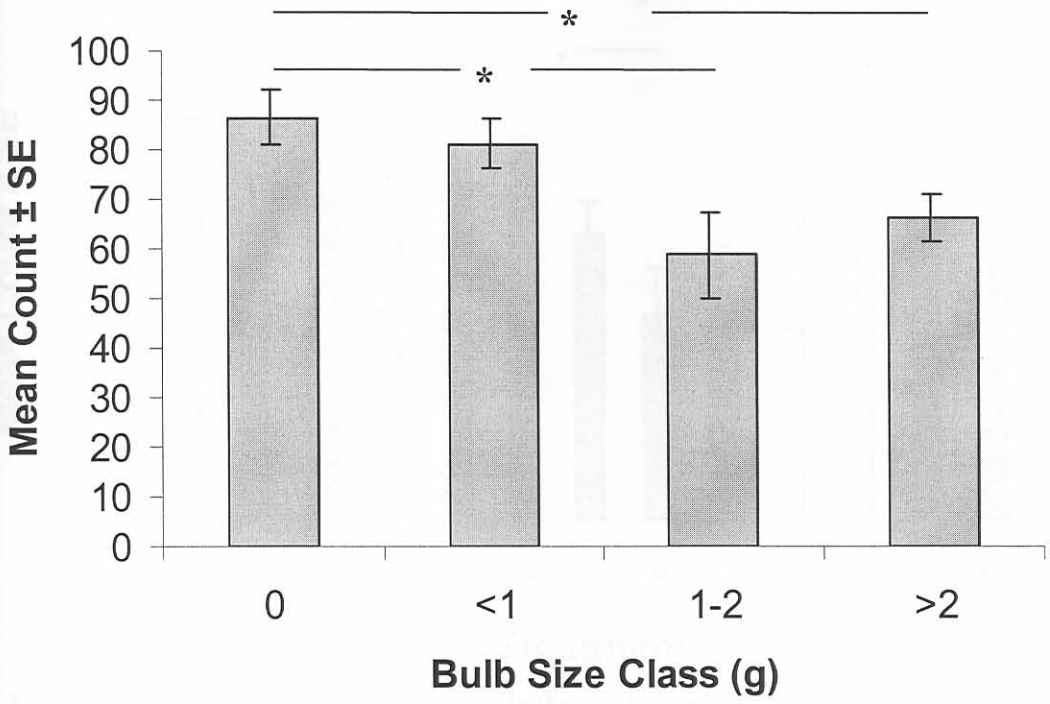

Figure 10: Mean count (± standard error) of animals observed in patches of different geophyte sizes (for all bulb densities). *=significant differences detected.

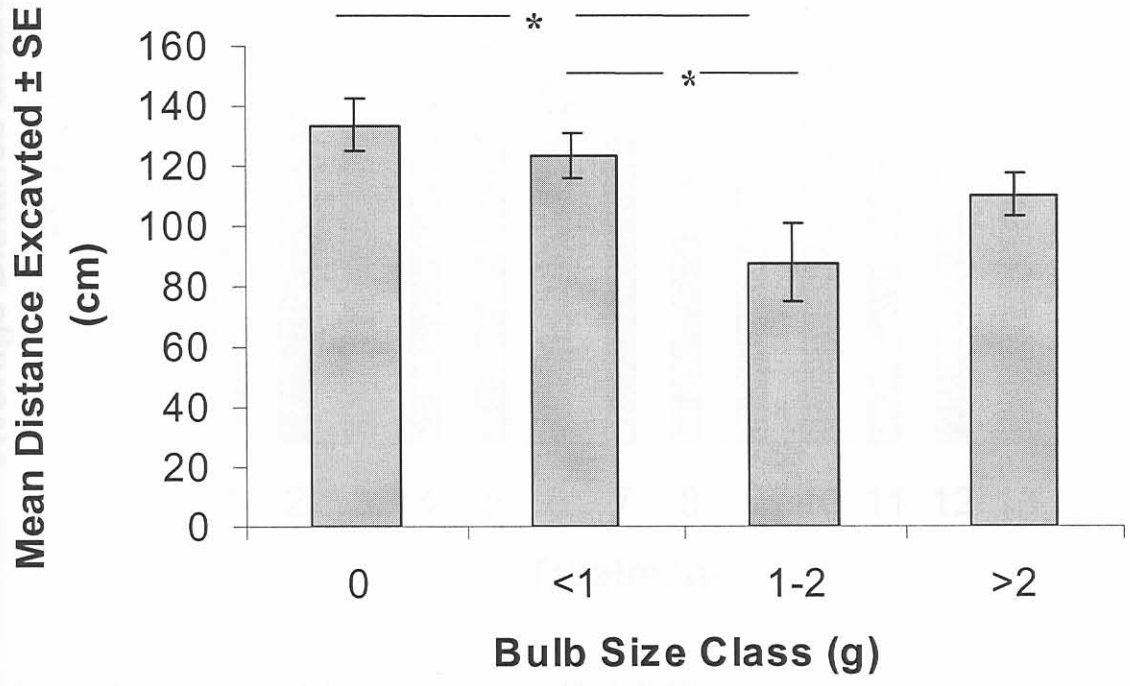

Figure 11: Mean excavation distance (± standard error) dug by individuals in patches of different geophyte sizes (for all bulb densities). *=significant differences detected.

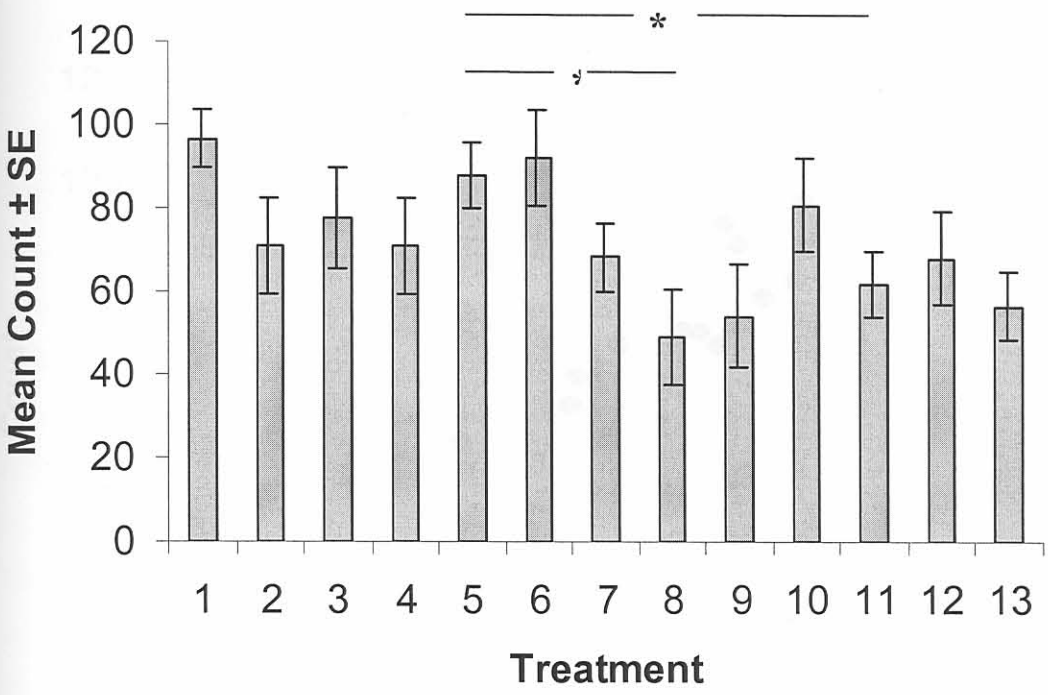

Figure 12: Comparison of the number of individuals observed (count ± standard error) in patches with different geophyte size/density combinations. *=significant differences detected.

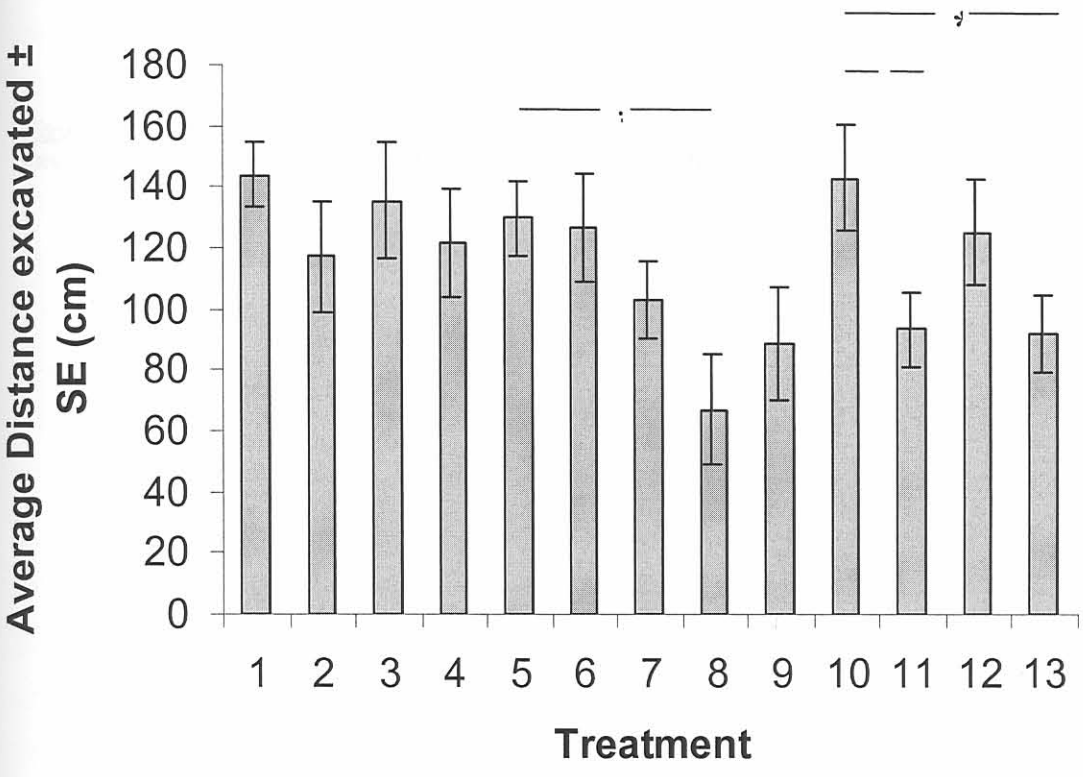

Figure 13: Comparison of the distance excavated by individuals (excavation distance ± standard error) in patches with different geophyte size/density combinations. *=significant differences detected.

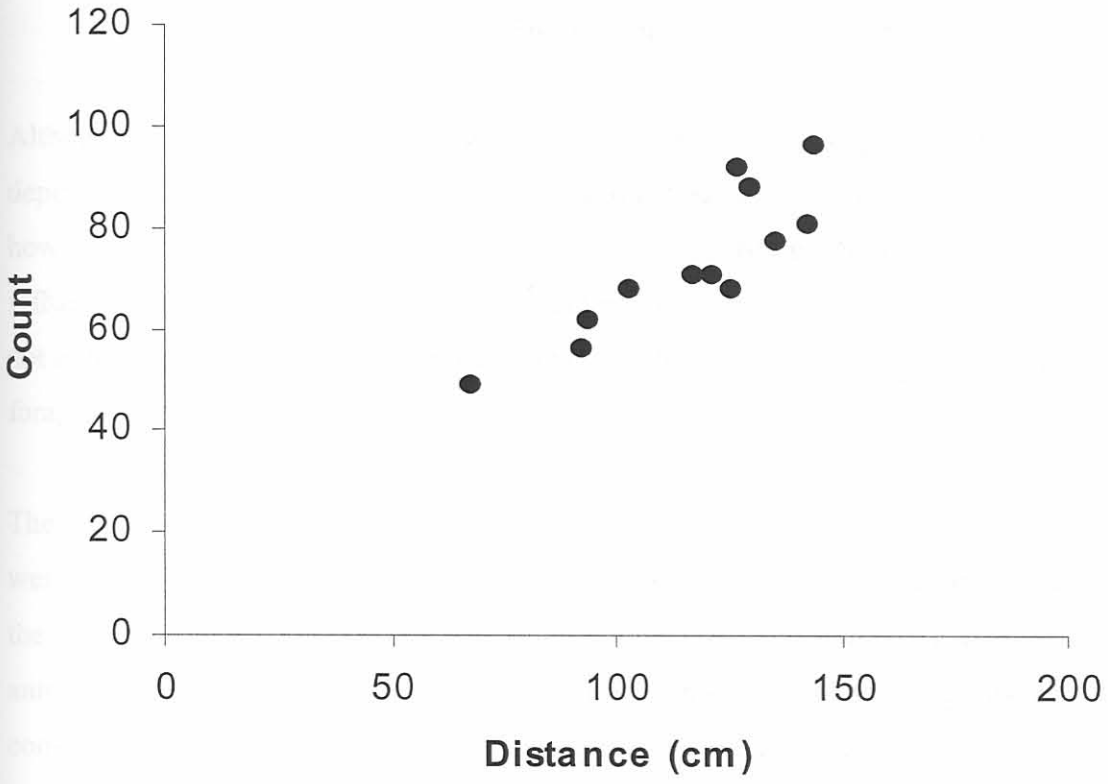

Figure 14: A comparison of the quality of data used in this study in order to determine whether any correlation exists between the two types of patch visitation quantification.

Discussion

Although it was not possible to study optimal foraging *per se*, as optimal foraging theory depends on the individual's resulting fitness and natural selection (Sih 1982), it was however possible to estimate feeding efficiency and identify possible factors which influence feeding behaviour. Using these factors, it was possible to estimate whether or not individuals within colonies of the highveld mole-rat could be classified as optimal foragers.

The responses that animals of different size and sex showed to geophytes of varying size were quantified in terms of the time which animals spent handling geophytes relative to the total consumption time. Total consumption time was measured as the time that an animal took to deal with a geophyte in its entirety. This included handling time as well as consumption time. Handling time is an energetically expensive activity as time is taken (and energy used) in order to make the geophyte suitable for consumption. Energy is only acquired once the geophyte has been consumed and for this reason handling time was assumed to be more important than consumption time as the energy cost of consumption time is minimal compared to the amount of energy that is acquired – while no energy is acquired during handling. Barnett (1991) showed in the eusocial *Cryptomys damarensis*, that handling time was expensive and important in determining resource selection.

Feeding

In natural populations, feeding behaviour can vary intra-specifically (Gustafsson 1988). In birds, an individual's sex (eg Selander 1966 cited in Gustafsson 1988), age (e.g.Partridge & Green 1985 cited in Gustafsson 1988) as well as other individual traits (e.g. Grant *et al.* 1976 cited in Gustafsson 1988), such as an individual's position in a social hierarchy and its mass, can influence feeding behaviour. Since bathyergid age determination is difficult to accomplish non-invasively, individual sex and mass were only used to investigate differences in feeding behaviour.

It was evident that there was no relationship between animal sex and the proportion of time spent handling geophytes. It was expected that males, being the more aggressive of the sexes, would handle geophytes more efficiently. Females apparently spend the same proportion of time handling geophytes of different sizes as males do.

Animal size appears to play a role in determining the proportion of time an individual spends handling a particular sized geophyte. It was predicted that larger animals, because of their assumed older age, would handle geophytes more efficiently as experience is assumed to increase with age. It was further postulated that this would be evident from a difference in handling times where larger animals would handle geophytes for a relatively shorter period of time than younger animals. There was a significant difference in the proportion of time that larger and smaller animals spent handling geophytes of different sizes but was not as expected. It was evident that larger animals spend a greater proportion of their total consumption time handling the geophytes than smaller animals do. However, this can be explained by rather looking at this trend in terms of consumption time. A larger animal would consume a bulb of a certain size faster than a smaller animal simply due to the volume of the oral cavity. Therefore, if a large animal spends a smaller proportion of its time consuming than a smaller animal, the opposite trend will be evident when comparing handling times and a larger proportion of time will be seen being invested in handling when compared to smaller animals.

It was apparent that geophytes of varying size were handled differently. Small geophytes were handled for a longer time relative to total consumption time when compared to large ones. In both males and females, the trend observed was that as geophyte size increased, so the proportion of time spent handling the geophyte decreased. Animal size had no effect on this trend, since both small and large animals showed the same pattern. This trend was expected as a larger bulb has more tissue for consumption compared to the smaller bulb. Thus, it would be expected that a greater proportion of time (and energy) would be spent on handling smaller bulbs than larger ones. This is an interesting finding since this trend would favour optimal foraging when an abundance of geophytes of varying sizes is available. Individuals should favour larger bulbs, since less time (and

energy) would be spent on preparing the geophyte for consumption and energy acquisition. Also, once the geophyte has been handled and made suitable for consumption, more energy would be acquired than if a smaller bulb had been handled. This observation leads to the formation of an energy maximizing hypothesis which could result in the animals being termed optimal foragers. In an abundant resource (geophyte) patch, individuals should preferentially select larger geophytes over smaller ones. This hypothesis is tested in the next section (foraging).

It is interesting that there appears to be a cut-off mass at which size classes of bulbs are handled differently. Bulbs of less than 2g (small and medium bulbs) showed no significant difference in the proportion of time that animals spent handling them, while geophytes of greater than 2g were handled for shorter periods of time. Also, the proportion of time spent handling geophytes was always less than the amount of time spent consuming the geophyte – evidence that the consumption of the geophyte is more important in terms of energy acquisition as the greatest amount of time should be spent on acquiring energy.

It is therefore concluded that the proportion of time that an individual spends handling a geophyte is a function of bulb and animal size. Animal sex played no role in determining the way in which geophytes are handled. Larger bulbs are handled for a relatively shorter period of time than smaller bulbs and larger animals handle bulbs for a relatively longer proportion of time than smaller animals. It is hypothesized that, given an abundant patch of resources (geophytes) varying in size, individuals should preferentially select larger geophytes over smaller ones as the handling time of a larger bulb is less and energy acquired from a larger bulb is greater than that obtained from a smaller geophyte. It is therefore predicted that individuals would be energy maximizers in a resource abundant patch.

Foraging

A foraging animal is assumed to exhibit two major patterns of behaviour; travelling between patches and feeding within patches (Arditi & Dacorogna 1998). If patches have not yet been found, subterranean consumers should forage blindly (randomly) until prey patches are encountered (Glasser 1984). Once prey patches are encountered, foraging should become directed as the probability of finding food increases (Barnett 1991). Consumers are faced with choices when foraging in particular patches (Hirakawa 1997). These choices determine which prey item to consume, which patch to visit, how long to stay in a particular patch and the best route to take in order to get to the patch (Hirakawa 1997). The first two of these choices are important in this study, while the length of time in a patch as well as the route taken to the patch are not investigated in this study.

Two questions have been identified concerning how prey patches are used by foragers: (1) do foragers concentrate their efforts in more profitable patches? and, (2) do the decisions that foragers make follow rules predicted by optimal foraging theory ? (Sih 1982). This study attempts to answer these questions. It is important to note, again, that these questions do not test optimality of patch use but the efficiency of patch use by foragers (Sih 1982). Studies concerning optimality need to take fecundity, growth rate and survivorship (fitness) into account (Sih 1982).

Patch preference was not evident when all four experimental trays were filled only with soil (no geophytes). Hence, no directional or sensory preference played a role in preferences that were evident with different geophyte size/patch density combinations. Results can therefore be explored in a meaningful way – the preferences evident only being attributable to geophyte size, density or a combination of the two.

It is apparent that patch density, when analysed independent of geophyte size, plays no role in determining geophyte patch preference while geophyte size, when analysed independent of patch density does play some part in determining patch preference.

It was shown that individuals generally prefer empty patches when compared to either patches containing medium or large geophytes. This was evident from an analysis of visitation (count) data and excavation distance data. It was also evident from excavation distance data that there was a preference for small geophytes when compared to large geophytes.

When comparing preferences of geophyte size/patch density combinations, it was evident that very few preferences existed. The only trend that can be discussed is that, generally, individuals tend to prefer empty patches when compared to patches with geophytes size/density combinations present (particularly for large geophytes at densities of 2 m^{-1} and 6 m^{-1}). Also, at low patch densities (2 geophytes m^{-1}), small geophytes are preferred over medium and large geophytes.

Although it has been found that foragers generally follow the qualitative predictions of optimal foraging theory, foragers also appear to fall somewhat short of being energy maximizers (Sih 1982). This is thought to be due to other foraging or fitness considerations being taken into account (Sih 1982). For example, studies on rats have shown that they show a preference for food items with the lowest handling cost but this was not an absolute preference (Kaufman & Collier 1981). It has also been shown that differences in the increase of handling time between small and large prey items might alter profitability and reward rate of different size classes during feeding (Barnett 1991).

Bathyergids have lower resting metabolic rates than other rodents; resting metabolic rate being independent of body mass (Lovegrove & Painting 1987). Lower resting metabolic rates are thought to have been selected for in response to high energy costs of burrowing and the low probability of finding widely dispersed geophytes by random burrowing (Lovegrove & Painting 1987). From the results presented from this study it appears as though individuals of the highveld mole-rat are not maximizing their immediate energy consumption. If individuals were foraging in order to maximize their immediate net energy intake it would be expected that they would show preferences to patches with large bulbs, a high density of bulbs, or a combination of high density/large geophyte

patches. This appears not to be the situation since patches with no geophytes occurring within them appeared to be favoured.

As the definition of optimal foraging takes into account that individuals would attempt to maximize their net energy intake (independent of fitness requirements mentioned above), the highveld mole-rat cannot be classed as an optimal forager. Rather, it appears, individuals prefer patches with the lowest density of geophytes when other patches that contain geophytes have been investigated. These trends were evident using both methods of preference quantification; count data as well as excavation distance data.

In an environment with a patchy food distribution, a forager may sample a number of patches prior to more intensive exploitation (Hodapp & Frey 1982). This appears to be just what is happening with the highveld mole-rat. It appears as though all of the patches in the study were explored and then choices were made that caused more intense exploration of patches where no geophytes had yet been located. This foraging strategy may be allowing individuals in the colonies to "hedge their bets". In other words, once patches have been located which contain geophytes (irrespective of the size or density of the geophytes) other patches are explored which have not yet given evidence of geophytic presence. These patches are then explored further in order to determine whether a new source of geophytes can be found not far away from the other patches. Patches that contain geophytes will be used as a resource while exploring the other (empty), less profitable patches.

It has also been shown that in dominance-related social contexts and when constraints of limited feeding time exist, the problem of choosing the optimum balance between exploring food arenas and exploitation of food arenas is intensified because of competition (Hodapp & Frey 1982). In other studies it has also been shown that an increase in activity was found in patches of lower food quality and quantity (Hodapp & Frey 1982).

Although optimal foraging theory can predict and explain food choice and food patch choice by animals, for an animal that does not forage widely and whose food selection abilities may be limited, regulation of ingestive and digestive processes may be an especially important component of a strategy to optimize feeding energetics (Barnett 1991). This could be the case for the highveld mole-rat where very low resting metabolic rates and restricted food selection abilities select for the foraging strategy that has been observed in this study.

Correlation of quantification methodologies

It is interesting to observe that a relatively strong correlation exists between the two types of methods of preference quantification used, namely; individual count data and excavation distance data. It appears that either of these methods can be used to estimate patch preference with reasonable accuracy. This correlation also shows that excavation distance is positively correlated to the number of animals found to be in a patch at any particular time.

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Chapter 4

Ovulation and Postnatal Growth

Abstract

In those eutherian species which need either the physical presence of a male (induced ovulators) or his pheromones (spontaneous ovulators) to ovulate, female activation of oestrous is the result of hypothalamic-pituitary stimulation of oestrogen production. Spontaneous ovulation could be an advantage in harsh environments (particularly with low mating success/skewed sex ratios) but is an energy expensive activity. Induced ovulation could also be advantagous in harsh environments (particularly if seasonal) where resources (food) are low and could be an energy saving mechanism. Bathyergids and other hystricomorphs studied to date appear to be spontaneous ovulators with a suppression of reproduction at high densities being enforced by a breeding female or male (either behaviourally, chemically or both). This study investigated whether the female highveld mole-rat is an induced or spontaneous ovulator (when suppression is removed); both quantitatively (progesterone profiles) and qualitatively (histologically). The female highveld mole-rat appears to be an induced ovulator shown by increased urinary progesterone as well as the presence of corpora lutea, while in the physical presence of a novel male. Copulatory behaviour and postnatal growth and development were also investigated. The highveld mole-rat has similar copulatory behaviour to other loosely social bathyergids and pups have a slow mean maximum growth rate which fits the species position in the bathyergid sociality continuum.

Introduction

In some mammals, male pheromonal cues activate oestrous in females (Fadem 1989) and female pheromones may play a role in the suppression of reproduction in social groups. In others, the physical presence of males may activate oestrous and ovulation (Fadem 1985). Furthermore, male presence as well as male-related pheromones can stimulate oestrous and ovulation in females (Hinds *et al.* 1992). It has been hypothesized that the pheromonal activation of oestrous in eutherian mammals is the result of hypothalamic-pituitary stimulation (a neuroendocrine response) of ovarian oestrogen production (Fadem 1989, Flint *et al.* 1997). The mechanism by which the male pheromones cause ovulation in the female is by alteration of gonadotrophin secretion and subsequent oestradiol release in the female (Fadem 1985). It has been shown that both spontaneous and induced ovulators are responsive to the oestrous-activating properties of male pheromone(s) (Fadem 1985).

Ovulation in eutherian species can be either spontaneous or induced; there are advantages and disadvantages to each method of ovulation. Spontaneous ovulators show a continuous cycling of reproductive hormones and subsequent ovulation with and without the physical and/or chemical cue(s) of a male, whereas induced ovulators only show the cycling of reproductive hormones and ultimately ovulation in the physical presence of males. This physical presence usually involves coitus. Induced ovulators fail to undergo hormonal cyclicity in the absence of a male as a result of the lack of coitus.

In eutherian species that show major fluctuations in population density, the stimulation of oestrous by males is a characteristic (Fadem 1985). Whitten (1966) and Milligan (1980) state that induced ovulation is relatively common among eutherian species. Conaway (1971) found that physiological responsiveness to social cues is closely related to the ecology of a species. In harsh environments where males are difficult to locate and/or the chance of successful mating is low, spontaneous ovulation would be a great advantage.

Spontaneous ovulation can also have significant advantages where mates are aggressive towards each other or, for other reasons, do not stay in contact for extended periods, or where sex ratios are skewed in favour of females and where males have to move on to other females in order to ensure reproductive success. By ovulating continuously (except while gravid) females can ensure reproductive success with mating occurring quickly as priming of female reproductive hormones by a male is unnecessary. This would obviously be an advantage in aseasonal breeders. However, this places continuous stress on the female that, in a resource limited and/or seasonal breeding niche, can not be afforded. Whitten and Bronson (1970), as well as Stoddart (1980), concluded that for solitary species where males and females are separated physically, activation of oestrous by close contact with males would not be advantageous. This is in contrast to the theory outlined above for spontaneous ovulators.

The highveld mole-rat (*Cryptomys hottentotus pretoriae*) occurs in a fossorial niche that is limited in terms of food availability and the opportunity to forage. Foraging can only occur when the soil is soft enough to permit burrowing and hence the obtaining of food reserves (typically geophytes). Food reserves are unevenly distributed and if the soil is not workable may not even be found (burrowing is achieved using extra-buccal incisors which are themselves restricted by their growth rate – the incisors being eroded much faster when soil is less workable). A female in the colony that continuously ovulates would be using energetically demanding resources that have been acquired. In this seasonally breeding mole-rat (Janse van Rensburg 2000), spontaneous ovulation would be impractical and place an unnecessary demand on stored energy reserves.

Induced ovulators require priming by the physical contact of a male prior to ovulation taking place. It would therefore be expected that a successful mating would take relatively longer to achieve. It is evident that males of induced ovulating species are also paired with the female for an extended period.

The naked mole-rat (*Heterocephalus glaber*; Bathyergidae) (Faulkes *et al.* 1990) as well as many other hystricomorph rodents are spontaneous ovulators (e.g. the guinea pig and

chinchilla) (Weir and Rowlands 1974). Indeed, most hystricomorph rodents studied to date have been shown to ovulate spontaneously (Weir 1974). However, the naked mole-rat is an aseasonal breeder that produces several litters per year. Because of increasing population densities in regions with limiting constraints, there is often a suppression of reproduction in many rodent species, which may be the resultant effect of urinary semio-chemicals or primer pheromones. Social interactions affect reproductive function in a wide range of species (Flint *et al.* 1997). Within group living species such as *C. h. pretoriae* an optimal group size should exist, since, if too many animals were to reproduce it could negatively affect survival, especially in a fossorial environment where food resources are limiting. A colony is founded by an unrelated pair of mole-rats that procreate, all other colony members being the offspring of the reproductive pair. Thus, suppression of reproduction in these colonies is important to ensure heterozygozity since close relatives should not produce offspring due to the possible negative deleterious effects of inbreeding depression. A dominance hierarchy is thought to exist in eusocial colonies where dominant animals are the breeding animals. Thus, ovulation in non-breeding animals is suppressed by the dominant, breeding female. A suppression of female reproduction can be manifested in several ways; through puberty delay, suppression of oestrous, suppression of ovulation or a block to embryo implantation (Faulkes *et al.* 1990).

Subterranean rodents are usually solitary in nature and thus xenophobic towards conspecifics. These rodents typically communicate via seismic signalling initially in order to announce their presence and intention (Bennett & Faulkes 2000). It is in this way that two solitary individuals could come together for breeding purposes without fatal mutual aggression.

Seismic communication has been reported in the Geomyidae as well as in five species of the Bathyergidae (Bennett & Jarvis 1988a, b, Jarvis & Bennett 1990, 1991). These species appear to use their hind limbs in order to drum on the substrate. In other species such as *Tachyoryctes splendens* (Rhizomyidae) and *Spalax ehrenbergi* (Spalacidae), the

incisors or head are used to tap on the burrow system in order to communicate (Jarvis 1969a; Heth *et al.* 1987; Rado *et al.* 1987 cited in Bennett & Faulkes 2000).

After the two individuals have accepted each other, copulation may take place. It is evident that solitary subterranean rodent species exhibit very similar copulatory behaviour that includes thrusting during copulation, multiple intromission and multiple ejaculation (Bennett & Faulkes 2000). Copulation is usually followed by grooming behaviour.

Limited data suggests that subterranean rodents have great variability in terms of their gestation period. Gestation period can range from as little as 18 days in the pocket gopher (*Thomomys talpoides*) to 120 days in the tuco-tuco (*Ctenomys talarum*) (Schramm 1961 and Weir 1974 respectively). Social bathyergids as well as ctenomyid species tend to have longer gestation periods than solitary bathyergids, geomyids or spalacids (Bennett & Faulkes 2000).

To date, studies of bathyergid gestation periods reveal that solitary species can have gestation lengths ranging from 44 days (Cape mole-rat) to 52 days (Cape dune mole-rat). Whereas in the social species such as the Mashona mole-rat and common mole-rat, gestation period can fall between 56 to 66 days (Bennett 1989 and Bennett *et al.* 1994a). Interestingly, the social giant Zambian mole-rat has a very long gestation period of 111 days (Bennett & Aguilar 1995 and Burda 1989).

Litter sizes of subterranean rodents are generally very small (Bennett *et al.* 1991 and Malizia & Busch 1991). In geomyids, litter size can range from 1- 13 (Hanson 1960, Miller 1946 and Howard & Childs 1959). In ctenomyids litters can range from 1-5 (Weir 1974) while in spalacids 1-5 (Shanas *et al.* 1995 and Gazit *et al.* 1996). In the bathyergids the solitary species may have larger litters (1-10) (Bennett & Faulkes 2000) compared to social species (1-5) (Bennett & Faulkes 2000).

There is limited data available documenting the postnatal development of bathyergids. (Bennett & Jarvis 1988a, b, Bennett 1989, Burda 1989). Jarvis (1978) showed that the eusocial mole-rat, *Heterocephalus glaber*, had a very long period of postnatal development. Thereafter, a further two articles were published on various species of bathyergids; both solitary and social species (Bennett *et al.* 1991 and Bennett & Navarro 1997). These studies generally found that the social bathyergids have a much slower rate of postnatal growth than solitary species (Bennett *et al.* 1991). Members of the genus *Cryptomys* have extremely low mean maximum growth rates compared to solitary and non-bathyergid solitary subterranean and fossorial rodents (Bennett *et al.* 1991). The long postnatal development of social bathyergids appears to be characteristic of the Bathyergidae. It has been hypothesized that, because the pups of social species are more altricial when born than those of solitary species, and due to the fact that these pups are incorporated into the colony (do not disperse as quickly as pups of solitary species), pups of social species tend to grow slower than pups of solitary species (Bennett *et al.* 1991).

This study was undertaken in order to elucidate aspects of the reproductive biology and development of the highveld mole-rat; concentrating on ovulation method and postnatal development (growth). It was hypothesized that non-reproductive females, in the absence of other colony females, would be induced to ovulate and that postnatal development would fall within ranges expected for social bathyergids.

Methods and Materials

Ovulation

Experimental design

In order to determine whether females were induced or spontaneous ovulators, nine females were used in this study. The study was carried out from the middle of May 2000 (which is within the breeding season, Janse van Rensburg 2000). Nine non-reproductive females were placed in isolation from possible chemical and behavioural stimulation of males for a period of five weeks. This time period was chosen for all experiments, since bathyergid oestrous cycles are thought to be around one month in duration (see Faulkes *et al.* 1990). The time period for a complete oestrous cycle in *Heterocephalus glaber* is around 34 days (Faulkes *et al.* 1990). Six large males (three of which had been vasectomized) were also placed in isolation. After the five-week isolation period, the nine females were left in isolation for a further five weeks and urine samples were collected from them every second day. These samples constituted the control samples. After this five-week period, three of the females ('alone-separated' females coded AS) were placed in chemical contact with large, unvasectomized males and urine was collected every second day as before. Also, three females ('alone-contact' females coded AC) were placed in chemical and behavioural contact with vasectomized males; urine being collected every second day. The final three females were left in isolation as control animals (`alone' females coded A). Data thus consisted of five weeks of control data for six females (control period) and 10 weeks of control data for two females (control period and experimental period). One female died during the study, thus limiting the control data during the experimental period to two females. The amount by which data would be limited by the death of this female was thought to be insignificant.

Sample collection

Urine samples were collected throughout the sampling day (09h00 – 14h00 every second calendar day) whenever urine was observed to be present in the collecting dish of the experimental chambers. The chambers had a smooth mesh floor to allow urine to fall through to the collecting dish whilst not allowing faecal contamination. Urine in the collecting dish was removed and frozen at –40 °C. All urine collected for a single female was combined for the sampling day. Each female was fed the same amount of food at the same time each day (11h00). Urine volume that was collected during each sampling day was variable (due to variable indirect fluid intake) but usually in excess of that required for analysis (240 μl).

Creatinine determination

All urine samples were analysed for progesterone concentration. These concentrations had to be corrected, since the urine concentration was variable due to varied fluid intake. The correction was undertaken by analysing each urine sample for creatinine concentration. Creatinine is used to correct for urine concentration as it is a breakdown product of tissue proteins and is excreted at a relatively constant rate. Final results are therefore not expressed as ng progesterone/ml urine but rather as ng progesterone/mg creatinine. This method therefore gives an index by which progesterone concentration can be measured; standardising all progesterone concentrations to that of 1 mg creatinine.

The creatinine concentration of each sample was determined using a modified Jaffe reaction (Folin 1914). Creatinine standards were made up at concentrations of 0, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 mg creatinine/ml distilled-deionized water (DDIW) from a stock solution of 3 mg creatinine/ml DDIW. Ten microlitres of standard or sample was added to the wells of a microtitre plate (in duplicate) leaving two wells empty as a duplicate blank. To each well (including the blanks) 300µl of picrate reagent was added. Picrate reagent was made up fresh and consisted of saturated picric acid solution, alkaline triton and DDIW (1:1:10). The alkaline triton was, in turn, made up of 4.2 ml triton X-

100, 12.5 ml 1N NaOH and 66.0 ml DDIW. Alkaline triton was only used when homogenous by continuous stirring. The microplate was then left in the dark, at room temperature, to allow colour development for a period of 1.5 hours. The absorbance of the standards, controls, blanks and samples were measured at an optical density of 492 nm using a microplate reader.

A straight line was plotted (absorbance vs creatinine concentration) for the means of the standards. Using simple linear regression, the equation of the straight line was determined. Rearranging the equation for the standard curve and substituting absorbance values for y-values, independent values were interpolated (creatinine concentration) for each sample. Descriptive statistics (mean ± SD) were calculated.

Progesterone determination

Using a plasma-based solid phase ^{125}I RIA (radioimmunoassay) technique designed for the direct, quantitative measurement of progesterone in serum or plasma, (Diagnostic Products Corporation 1998), progesterone concentration in each urine sample was determined. The validity of using the test kit for the detection of progesterone in urine samples was determined by Molteno (1999) who found serial dilutions of urine and plasma to parallel the standard curve

^{125}I-labelled progesterone competes for a fixed time (3 hour incubation period at room temperature; Basic Procedure) with progesterone in the sample for antibody sites. The antibody is immobilized to the supplied polypropylene tubes (1 per sample) and therefore, by decanting the supernatant, competition can be terminated thus isolating the antibody-bound fraction of the radiolabelled progesterone of the tubes. Placing the polypropylene tubes in a gamma counter and counting for a period of 60 s results in a number (counts/min) which by means of a calibration curve could be converted to progesterone concentration (uncorrected for creatinine).

An advantage of this progesterone kit is that decanting the supernatant from the polypropylene tubes can be done vigorously without the loss of antibody-bound material. This results in negligible non-specific binding (NSB) (Coast-a-Count Progesterone Manual, Diagnostic Products, California). During in-house testing of the Basic Procedure (500 tubes) it was found that coefficients of variation (CVs) were low and uniform, the assay being able to detect progesterone concentrations as low as 0.02 ng/ml. The antiserum (rabbit) has a high specificity for progesterone as well as very low cross-reactivity to other naturally occurring steroids present in the samples. Other in-house tests conclude that the assay is accurate over a broad range of progesterone values (evident in extensive spiking recovery and linearity under dilution experiments). Due to the high specificity of the tracer, total counts approximate 70 000 cpm (counts/min) at iodination; maximum binding is approximately 50% (35 000 cpm) for the Basic Procedure.

Four uncoated tubes were labelled in duplicate for NSB and Total Count determination. Fourteen coated tubes were used for seven duplicated standards used to construct the standard curve from which results were interpolated. Standard solutions consisted of 0, 0.1, 0.5, 2.0, 10.0, 20.0 and 40.0 ng/ml. A volume of 100 µl of each standard solution was pipetted into each of the respective tubes. A further 100 µl of 0 ng/ml standard was pipetted into each of the duplicate NSB tubes. A volume of 100 µl of sample was pipetted into each labelled sample tube. A volume of 1.0 ml of ^{125}I Progesterone was added to every tube. The tubes were incubated at room temperature for a period of 3 hours. Following decantation of the tubes, the tubes were placed in a gamma counter for a period of 60 s.

Results of NSB and Total Counts were compared to that given in the kit manual. Using the following calculations, the binding of each pair of tubes could be determined as a percent of maximum binding:

Net count = average count (cpm) – average NSB (cpm)

Percent bound = (net count/net maximum binding count) * 100

Taking the NSB-corrected counts for the 0 ng/ml standard as having a 100% binding, the percent bound of each of the other standards could be determined.

By plotting log (concentration of standard) against the percentage bound, a standard curve was fitted to all the standard concentrations except for the 0 ng/ml as log(0) = undefined. As three assays were undertaken, three standard curves were fitted to each set of points; yielding three standard curves which were in turn used in interpolation for respective samples. An equation was determined for the straight line fitted using simple linear regression with correlation coefficients exceeding 94%.

Corrected progesterone determination

Using the results of the progesterone and creatinine determinations it is possible to correct the progesterone concentrations for the dilution of the sample. Progesterone concentrations (ng/ml) were divided by creatinine concentrations (mg/ml) for each sample. This results in a final, corrected progesterone concentration expressed as ng progesterone/mg creatinine.

Data analysis

Descriptive statistics that were employed in data reduction were the mean ± SD. These statistics were calculated without the outlier data values (<3 % of data eliminated). Means ± SD were calculated for all animals separately for each stage of the experiment therefore giving mean ± SD for eight animals both before (control) and after (experiment) experimental manipulation. Means of each female that were in chemical contact with males and those in contact with males were then compared to the data of females being entirely separated from males; using a series of 1-sample t-tests.

Histology

Qualitative observations were made of the ovaries of the females used in the study. One sample could not be sectioned as preservation had been unsuccessful. Ovaries were removed from the females directly after the last urine sample had been taken. The ovaries were placed in Bouins fixative for preservation. Standard histological techniques were employed to dehydrate, section, stain and mount the ovarian samples (Drury and Wallington 1967). Ovaries were stored in Bouins solution for 2 months prior to being transferred into 70 % ethanol before sequential dehydration in increasing ethanol concentrations. Entire ovaries were then embedded in paraffin wax. Sections of 7 μm thickness were then mounted on slides and stained with Ehrlich's haemotoxylin. Counter-staining was then achieved using eosin. The qualitative analysis was primarily concerned with the absence or presence of follicular development within the ovaries and the presence (or absence) of corpora lutea. Of the sections that were successfully prepared, the clearest of sections were observed, thus the nature of the qualitative approach used in this study.

The process of successful ovulation involves the development and maturation of follicles that produce and support the oocyte prior to an egg (mature oocyte) being released for possible fertilisation. If the egg is fertilised, the corpus luteum will develop further to support pregnancy. If ovulation is unsuccessful, various stages of follicular development are still evident in the ovary but the corpus luteum will degenerate. The presence of primordial follicles indicates early follicular development, these structures being interior to the tunic albuginea in the Highveld mole-rat ovary (Janse van Rensburg 2000). Primordial follicles then proceed through various stages of development. These stages include the development of primordial follicles into primary, secondary and Graafian follicles. The primary follicle is differentiated from primordial follicles by an increase in size as well as a cellular shape change (squamous cells become cuboidal) (Janse van Rensburg 2000). An oocyte will usually be evident within the structure of a primary follicle. Secondary follicles result from further development of primary follicles. The

secondary follicles, although even larger than the primary follicles, undergo a morphological change that makes them clearly identifiable. Areas filled with liquor folliculi appear in the stratum granulosum of the secondary follicle (Janse van Rensburg 2000). Once this fluid-filled space increases in size, the oocyte becomes forced to one side of the follicle. It is at this stage that the follicle can be recognised as being Graafian (Janse van Rensburg 2000). If the follicle, with its oocyte matures further, ovulation will occur - the mature oocyte being released for possible fertilization. A corpus luteum is formed from the mature follicle following successful ovulation. If successful ovulation does not occur, the Graafian follicle, secondary follicle or primary follicle will degenerate – evident by the presence of atretic follicles (Janse van Rensburg 2000).

Gestation period and development

Gestation period was estimated from two pairs of individuals and was considered to be from the time of observed coitus of each of the pairs to birth (days). Two animals were paired on the 12th October, the male separated on the 15th October. A further two animals were paired on the 17th October.

Postnatal Growth

Three pups (two males and a female) were used in this study. Pups were weighed daily prior to feeding (between 11h00 ad 13h00) until individuals reached a relatively constant mass. This stage was evident when the curve of mass vs age reached a plateau. Attempts were made to weigh pups from day of birth (day 1) but this usually resulted in a decline of pup health and an increase in mother-pup disturbance. The weighing of pups could therefore only be started when pups were not constantly with the mother. Pups were always kept with the same animals in the holding chamber as at birth. Mass was rounded to the nearest gram as daily variation in weight due to feeding time was evident.

Several different growth models have been employed to analyse the growth data of bathyergid species. These techniques include the use of the Gompertz, Logistic and von

Bertalanffy mathematical models. To date, the most successful model has been the Gompertz equation due to the least residual sum of squares for bathyergid species comparison (Bennett *et al.* 1991, Bennett & Navarro 1997). From the Gompertz equation, it is possible to estimate maximum growth rate, age at maximum growth rate, mass at maximum growth rate and maximum mass. Maximum growth rate allows various taxa to be compared to one another (Bennett *et al.* 1991). Zullinger *et al.* (1984) found the Gompertz model to be the best compromise; since there is minimal difference in the models except at early and late growth periods where the Gompertz model is superior. In one study (Zullinger *et al.* 1984) the von Bertallanfy model was shown to be superior, intraspecifically, while the Gompertz model was shown to be the superior model when comparing species. As it was a goal of the study to compare the growth rate of the study species with other bathyergids, the Gompertz model was chosen.

Due to the difficulty of breeding subterranean, fossorial rodents successfully in the laboratory very few complete sets of individual growth data could be obtained. Although in the time allocated for the study there were numerous births in the laboratory, a large proportion of the pups did not survive. This study concentrates on the postnatal development (growth) of the loosely social mole-rat *Cryptomys hottentotus pretoriae* (Moolman *et al.* 1998). It was predicted that, due to the social status of the species, pups would show slow postnatal development evident in a slow maximum growth rate.

As two of the three pups were only first weighed when they were three days old, the mass at birth could not be accurately calculated. Another important advantage of using the Gompertz function is the reliability when the y-intercept values (day 0=day of birth) is unknown (Zullinger *et al.* 1984).

Parameters that could be estimated from the Gompertz function were: maximum mass (A), the maximum potential for growth (B) and immediate growth rate (R). The Gompertz function, for this study, is represented as:

$$Y(T) = A * e^{-B * R^{T}}$$

Other parameters which could be estimated from the data, using the Gompertz function, were: maximum growth rate (GR), age at maximum growth rate (TM) and mass at maximum growth rate (YTM). All of these parameters that were estimated could be statistically analysed for significant sexual dimorphisms, even though there were only three growth curves. These statistical analyses were undertaken by means of bootstrapping.

Bootstrapping

The technique of bootstrapping is considered to be a useful, valid and an important tool in statistical analyses of data sets that fall short in terms of sample size for other statistical procedures to be employed. In the case of growth data, bootstrapping uses each of the original data sets in order to allow descriptive statistics to be estimated for that particular data set. For each of the three data sets, 400 points were chosen at random around the area of the curve that showed the highest growth rate. Each of these points was subjected to an analysis that estimated the rest of the curve based on that point. This resulted in 400 'curves' being estimated from 400 randomly chosen points of the original curve. Descriptive statistics could then be calculated based on these 400 curves therefore allowing comparisons between pups, and sexes, to be made. Thus, for each of the three original growth curves, parameters were estimated from 400 curves obtained from bootstrapping of the original curve.

Data Analysis

Two of the three curves had data for a period of longer than one year. These two curves were therefore constructed and analysed for data of a 365 day period. Anomalous variation in one of these curves after 365 days prohibited analysis of data beyond the 365 day period. All three curves had data for a period of 318 days. Therefore, a second,

separate analysis was undertaken which included all three data sets. The results of these two separate analyses were then compared to each other in order to determine whether the 365 day data set could be eliminated so that all results could be based on the 318 day data sets.

Comparisons were also made pertaining to the six variable parameters listed above. Comparisons were made between all three sets of data as well as between sexes.

Bathyergid species comparisons were made on mean maximum growth rates only. Where mean maximum growth rates were not reported, calculations were made to estimate this parameter. Providing that the maximum mass (A in g) and the growth rate constant (K in $days^{-1}$) were reported, the mean maximum growth rate (MGR in $gday^{-1}$) could be calculated as follows:

$$\mathbf{MGR = K * A * e^{-1}}$$

In order to use mean maximum growth rate as an interspecies comparative tool, the assumption is made that this rate of growth is constant over the animal's entire growth period until it has reached its adult mass (Bennett *et al.* 1991).

Results

Ovulation

Control

The control data (before experimental manipulation) revealed that non-reproductive females had a basal corrected progesterone concentration below 32 ng progesterone/mg creatinine. Mean basal concentrations ranged from 13.5 to 31.1 ng progesterone/mg creatinine.

Experimental manipulation

Females that were only in chemical (pheromonal) contact with novel males showed no visually significant increase in corrected progesterone concentration compared to basal concentration (that when alone). Each mean for the female placed in chemical contact was statistically tested against the mean basal concentration. No significant differences were evident. See Table 2 for a summary of the statistical outputs.

Females that were in physical contact with novel males all showed a highly significant result when comparing mean basal concentration (that where alone) Mean progesterone values measured for these females ranged between 47.5 ng progesterone/mg creatinine to 57.5 ng progesterone/mg creatinine. However, these values are means over the entire experimental period for each of the females. These values provide no indication of how the progesterone varies and the data could be seen as being a straight line along these mean values. With the use of descriptive statistics, standard deviations from the mean were calculated for each of the females placed in physical contact with a male. From these standard deviations a range for progesterone concentration were obtained showing that progesterone concentration is indeed highly variable and the variance may be an order of magnitude within a single individual during a relatively short period of time. The standard deviations associated with each of the means are reported in Table 1.

Table 1: Descriptive statistics calculated for each of the females that were placed in physical contact with novel males.

Individual	Highest Concentration	Lowest Concentration	Mean ± SD	2*SD = Range
AC1	82.31	26.29	54.30 ± 28.01	56.02
AC2	80.79	34.29	57.54 ± 23.25	46.50
AC3	70.70	24.35	47.53 ± 23.17	46.35

Table 2: Results of 1-sample t-tests concerning corrected progesterone concentration. Mean corrected basal concentration (n=10) of separately housed non-breeding females compared to experimental data for each experimentally manipulating non-breeding female. The arithmetic mean of the basal concentration was 19.30 ng progesterone/mg creatinine. The critical t-statistic for each test was 2.26. *=significant rejection of Ho.

Statistical Test	Test Statistic |Tcalc|	Result (Reject/ Do not reject Ho)	Associated probability
AS1 EXPT VS MEAN BASAL CONC	0.32	Do not reject	0.753
AS2 EXPT VS MEAN BASAL CONC	1.01	Do not reject	0.324
AS3 EXPT VS MEAN BASAL CONC	1.05	Do not reject	0.307
AC1 EXPT VS MEAN BASAL CONC	18.19	Reject	< 0.001*
AC2 EXPT VS MEAN BASAL CONC	19.87	Reject	< 0.001*
AC3 EXPT VS MEAN BASAL CONC	14.67	Reject	< 0.001*

From individually constructed progesterone profiles (Figures 1 – 3), it is evident that changes in corrected progesterone concentration only occurred in those females who were placed in physical contact with vasectomized novel males. The changes in corrected progesterone concentration were also only evident after experimental manipulation. This gives further credibility to the results.

If females were spontaneous ovulators, it would be apparent that they would be cycling to some continuous, regular and predictable degree in the corrected progesterone concentrations while the females were alone. From the data that were obtained there appears to be little, if any, cycling pattern evident that would be conclusive to mapping an oestrous cycle. This appears to be the case in individuals that were housed alone and those in chemical contact with males. Females that were in physical contact with males

exhibit a cycle. However, it was not possible, from this data, to map an oestrous cycle accurately.

Table 3: Results of the qualitative histological analysis showing the presence or absence of various stages of follicular development and whether ovulation has occurred (shown by the presence of corpora lutea).

Female ID	Primordial Follicles	Primary Follicles	Seondary Follicles	Graafian Follicles	Lutenized Unruptured Follicles	Atretic Follicles	Corpus Lutea
A2	Present	Present	Absent	Absent	Present	Present	**Absent**
A3	Present	Present	Present	Absent	Present	Present	**Absent**
AS2	Present	Present	Absent	Present	Present	Present	**Absent**
AS3	Present	Present	Absent	Present	Absent	Present	**Absent**
AC1	Present	Absent	Absent	Absent	Present	Present	**Present**
AC2	Present	Present	Absent	Present	Absent	Present	**Present**
AC3	Present	Present	Absent	Absent	Present	Present	**Present**

All individuals (except AC1) had primordial as well as primary follicles present. Secondary follicles were generally absent. Graafian follicles were only found in AS females as well as a single AC female. They were not present in A animals. Luteinized unruptured follicles were present in most samples, except AS3 and AC2. All females showed evidence of follicular regression as there were atretic follicles present in all samples. Most importantly, corpora lutea were only found in AC females.

Courtship and copulation

Precopulatory behaviour is initiated by a head-to-head confrontation between the two reproductive animals. The male then sniffs and nuzzles the female around her flanks and the ano-genital area and whilst doing so he emits a chip chip chip call. The female raises her tail and emits a series of high pitched squeals resembling iz-iz-iz, kicks her hind feet backwards into the face of the male. The male grooms the rump of the female by gently nibbling her fur with his incisors. Courtship can last between 60 and 180 seconds. Following the foreplay and if the female is receptive, the male slides over the back of the female and intromission proceeds. During a successful intromission, the female remains

stationary, ceases vocalising, lifts and thrusts her posterior into the face of the male and adopts a lordodis-like posture with her tail upturned against her back. The female braces herself against the burrow using her forepaws. The male grips her lumbar region with his forepaws and thrusts at a rate of between 3-4 thrusts per second. Thrusting becomes slower, but deeper towards the end of copulation that terminates with the female emitting a high pitched prolonged squeal. At the end of copulation the male and female separate and groom their genitalia with their forefeet and mouth. Mating almost always takes place in the burrow.

Gestation and Litter Size

Few copulation events were observed in the laboratory. Two animals were paired on the 12th October and the male separated from the female on the 15th October 2000. A pup was born on the 18th December, 2000 providing a gestation period of between 63 and 66 days. A further two animals were paired on the 17th October, 2000 producing a litter of two pups on the 16th December 2000, providing a maximum gestation period of 64 days.

Litters born in the laboratory ranged from a single pup to three pups; usually two pups were born to a female (1.6 ± 0.8, n=6).

Postnatal Development

New born pups are 2-2.5cm long, hairless and pink, except for purple pigmentation around the cheeks. The eyes and auditory meatus are closed, the digits of the feet are well formed and clawed and the incisors have erupted. If placed on their backs, the pups can right themselves with difficulty. The pups produce high frequency cries and mewing sounds when suckling.

By day 8 the pups have a slatey grey fur that is darker around the saddle and head region. Solid foods are consumed on day 14. Sibling-sibling sparring occurs around day 40. By

day 14 the pelage is shaggy, slatey grey to charcoal in colour and has a whorled appearance. The pups were fully weaned around day 32.

The bootstrapping technique allowed descriptive statistics to be calculated for the parameters of each growth data set. Two of these parameters (growth potential; B and immediate growth; R) were not analysed statistically as they revealed little information. Descriptive statistics for these parameters were however calculated and are presented in Table 4. Data collected for the 365 day period are not used further in this study.

Table 4: Mean ± standard deviation from bootstrapping analyses of parameters from the Gompertz equation not shown in Figures 5 - 6; B=growth potential and R=immediate growth.

Data Set	Sex	Data up to (days)	N	B ± SD (ratio)	R ± SD (ratio)
1*	Female	318	295	2.12 ± 0.07	0.996 ± 0
2**	Male	318	295	2.38 ± 0.04	0.996 ± 0
3	Male	318	299	2.83 ± 0.08	0.996 ± 0
4*	Female	365	341	2.13 ± 0.03	0.996 ± 0
5**	Male	365	341	2.30 ± 0.02	0.995 ± 0

*Data set 1 and 4 are from the same individual.

**Data set 2 and 5 are from the same individual.

Table 5: Results of statistical comparisons of relevant data sets. Values indicated are probabilities. The null hypothesis was rejected at $p<0.05$.

Data Sets Compared	**MGR**	TM	YTM
1* and 2**	0.000***	0.747	0.001***
1 and 3	0.000***	0.021***	0.000***
2 and 3	0.013***	0.016***	0.092
4* and 5**	0.000***	0.229	0.000***

*Data set 1 and 4 are from the same individual.

**Data set 2 and 5 are from the same individual.

***The null hypothesis is rejected.

Maximum growth rates ranged from 0.23 gday-1 to 0.39 $gday^{-1}$. Males had a maximum growth rate of 0.36 ± 0.00 $gday^{-1}$ and 0.39 ± 0.01 $gday^{-1}$ (Figure 6). The maximum

growth rate of the female was lower (0.23 ± 0.00 $gday^{-1}$). These results were based upon between 295 and 299 data points that were calculated for each curve using bootstrapping techniques. Maximum growth rates calculated using 341 data points do not significantly differ from using the slightly shorter data sets. The maximum growth rates from males and females using 341 data points are 0.23 ± 0.00 $gday^{-1}$and 0.35 ± 0.00 $gday^{-1}$ for the female and male respectively (Figure 6).

It is evident that there are significant differences in maximum growth rates for all relationships investigated. The maximum growth rates of males appear to be higher than that of females. However, when comparing data sets 2 and 3 (both males) it would be expected that the null hypothesis would be accepted as the maximum growth rates for the males are very similar. When statistically comparing these means and their standard deviations, it is evident that there is a significant difference between the data sets of the males in terms of maximum growth rate. This result should be treated with caution due to the very low standard errors inherent in using 400 randomly chosen data points for bootstrapping analyses.

Males and females reached their maximum growth rates at different masses. For males, maximum growth rate was achieved at a mass of 88.7 g (318 day data) and 107.8 g (365 day data). These results were not statistically different from one another. When the mass at maximum growth rate for the female is compared to that of the males (for 318 and 365 day data respectively) it is evident that the mass at which a female attains her maximum growth rate (61.6 g or 62.1 g) is statistically different from that of males.

The age at which males and females reach their maximum growth rates does not appear to be strictly attributed to sex. Males reached their maximum growth rate at ages of 211.1 ± 15.5 days old and 287.9 ± 27.98 days old (for 318 days of data) and 184.0 ± 7.0 days old (for 365 days of data). This gives a range of ages where males can reach their maximum growth rate of between 184.0 ± 70 days old and 287.9 ± 27.9 days old. The female reached her maximum growth rate at 201.6 ± 25.11 days old (318 days old) and 204.2 ± 15.3 days old (for 365 days of data). Although there was a significant difference

between the age at which males and females reached their maximum growth rates (for the 318 day data), there was also a significant difference between the age at which males reached their maximum growth rate (for 318 days of data). Also, there was no significant difference between age of maximum growth rate when comparing males and female data for 365 days.

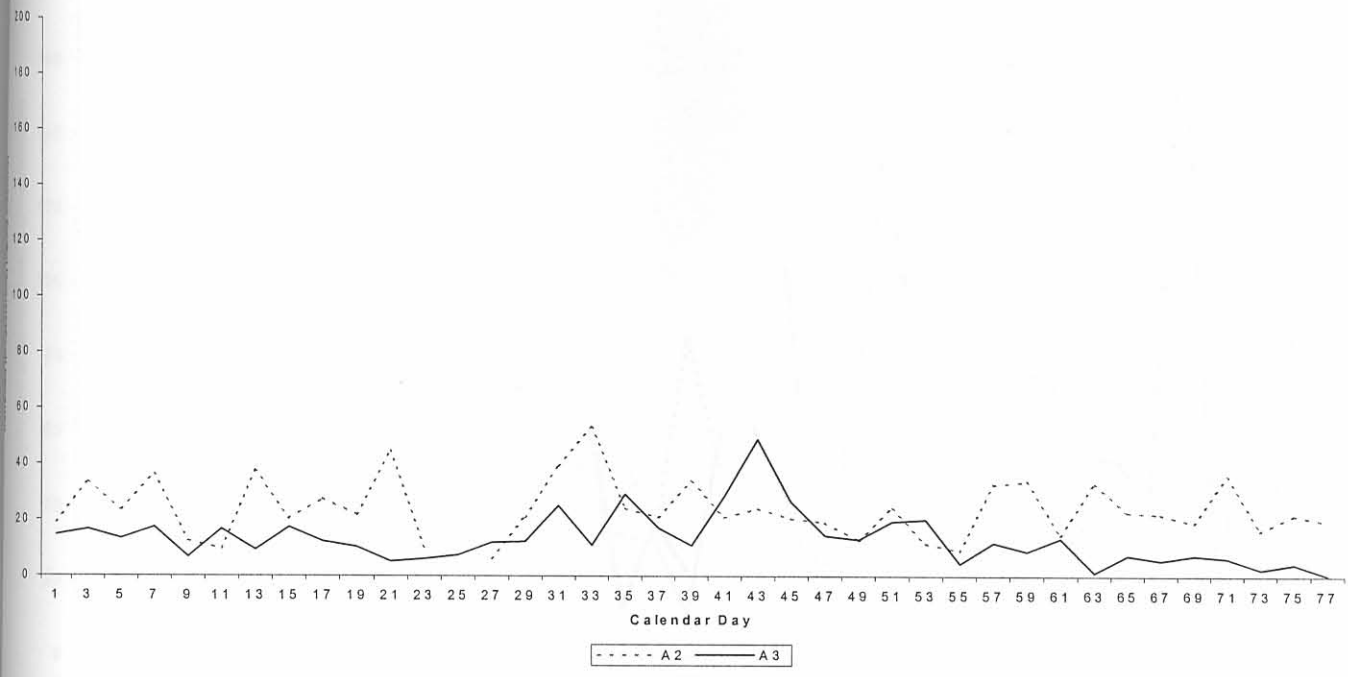

Figure 1: Progesterone profiles for non-reproductive females (n=2) while housed completely on their own for the entire duration of the study.

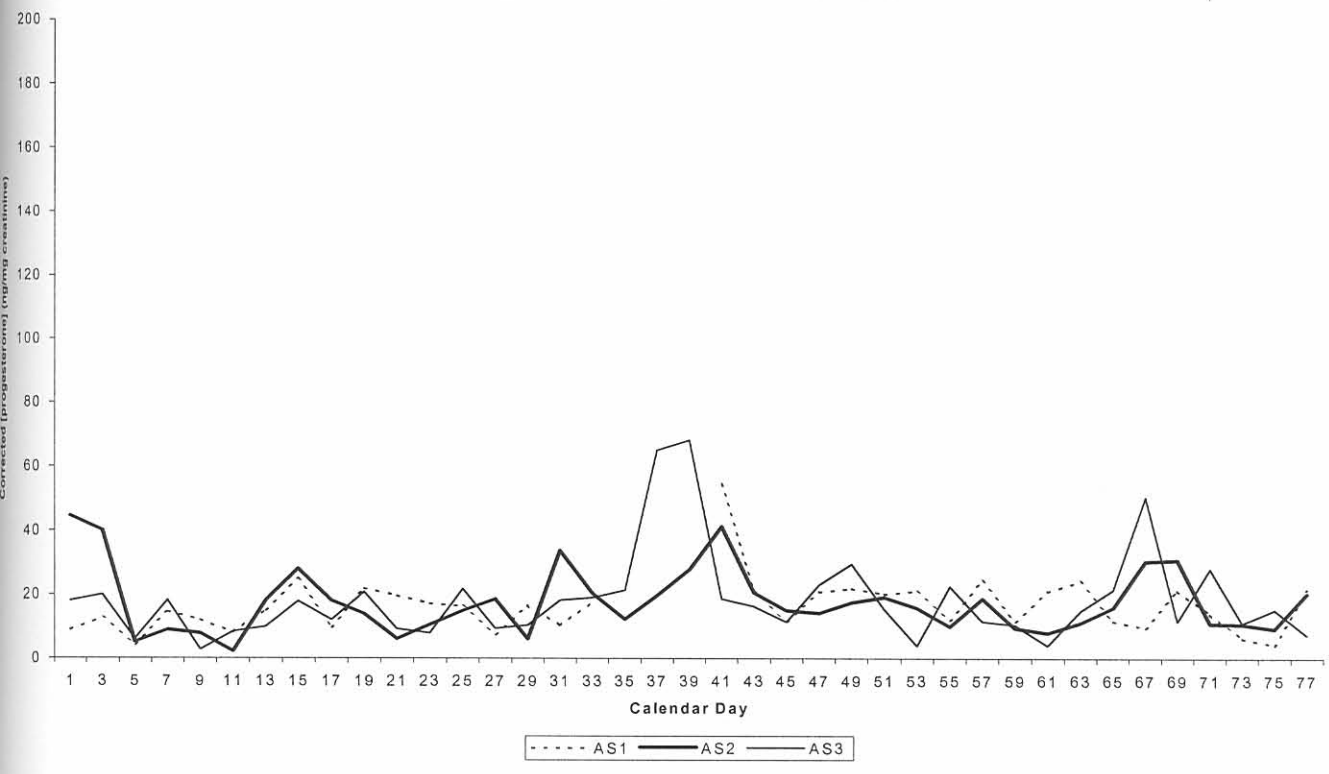

Figure 2: Progesterone profiles for non-reproductive females (n=3) while housed completely on their own (day 1 – 40) and then physically separated from a novel male (day 41 – 77).

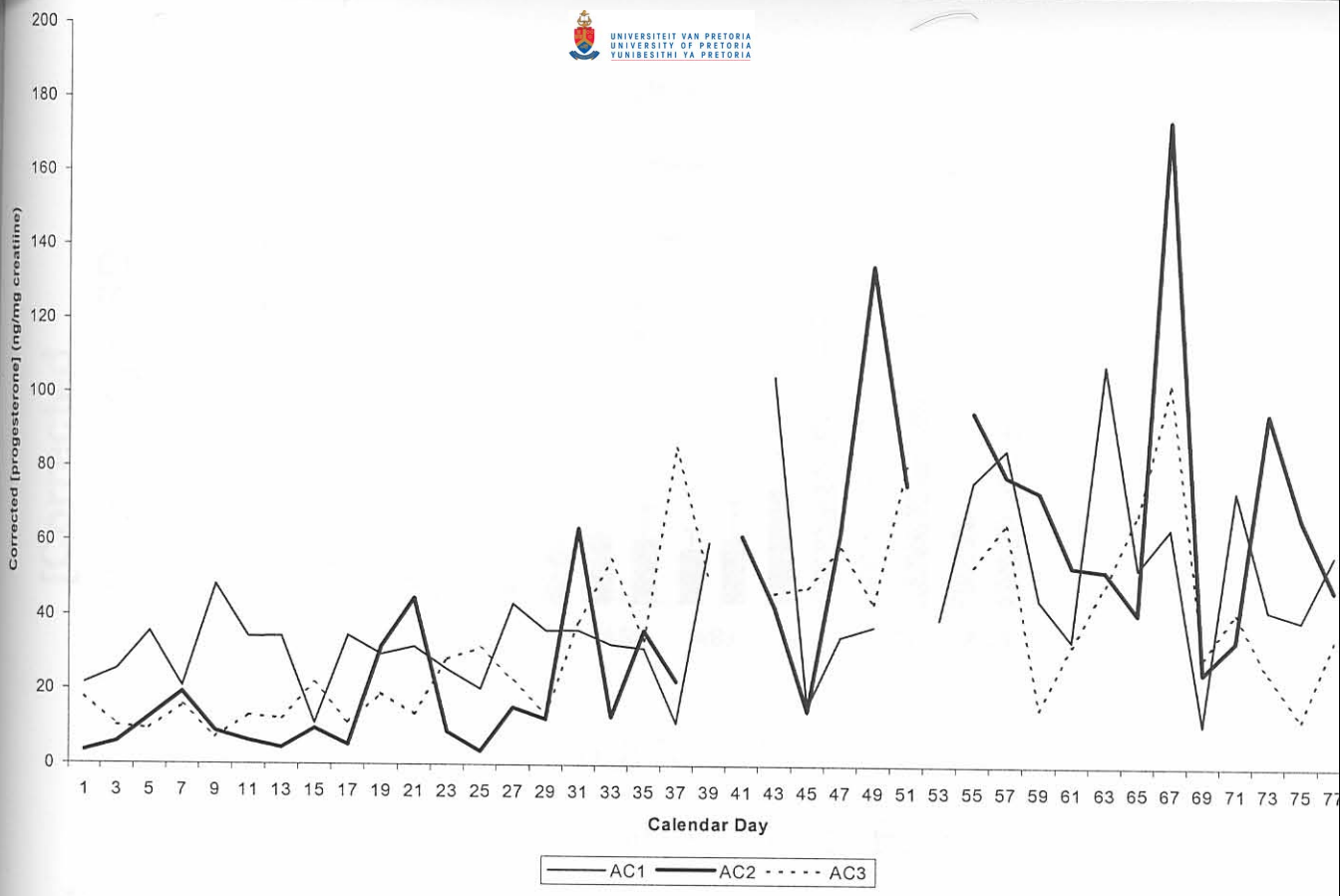

Figure 3: Progesterone profiles for non-reproductive females (n=3) while housed completely on their own (day 1 – 40) and then placed in physical contact with a novel, vasectomised male (day 41 – 77).

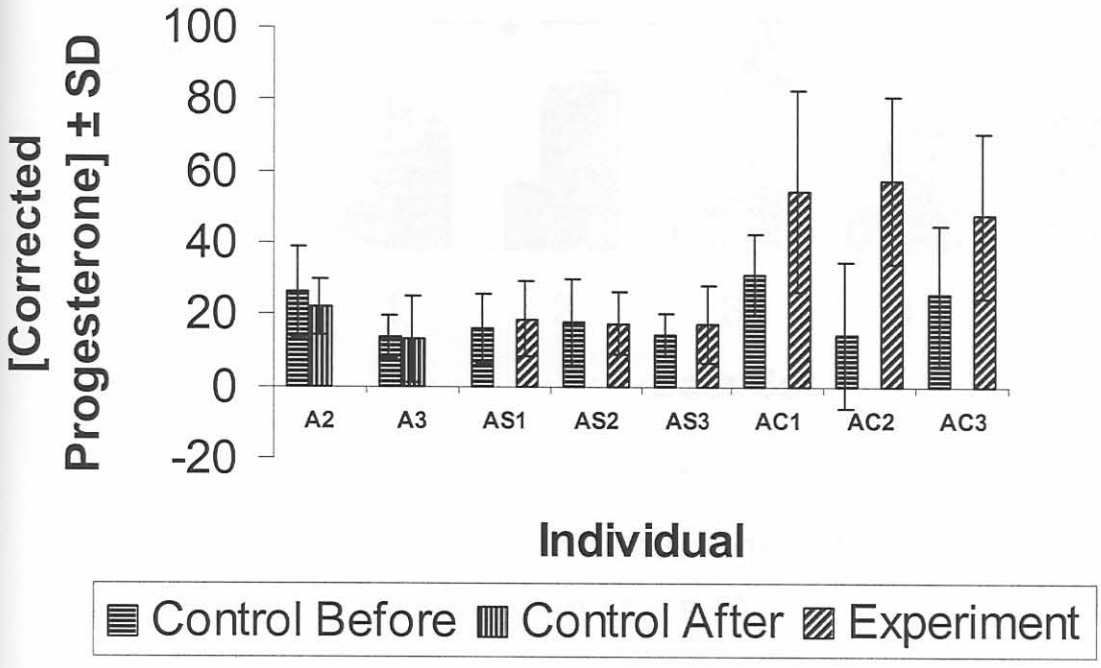

Figure 4: Corrected progesterone concentrations for each non-reproductive female for each part of the study; while totally alone (control before and control after) and while physically separated or in contact with novel males (experiment).

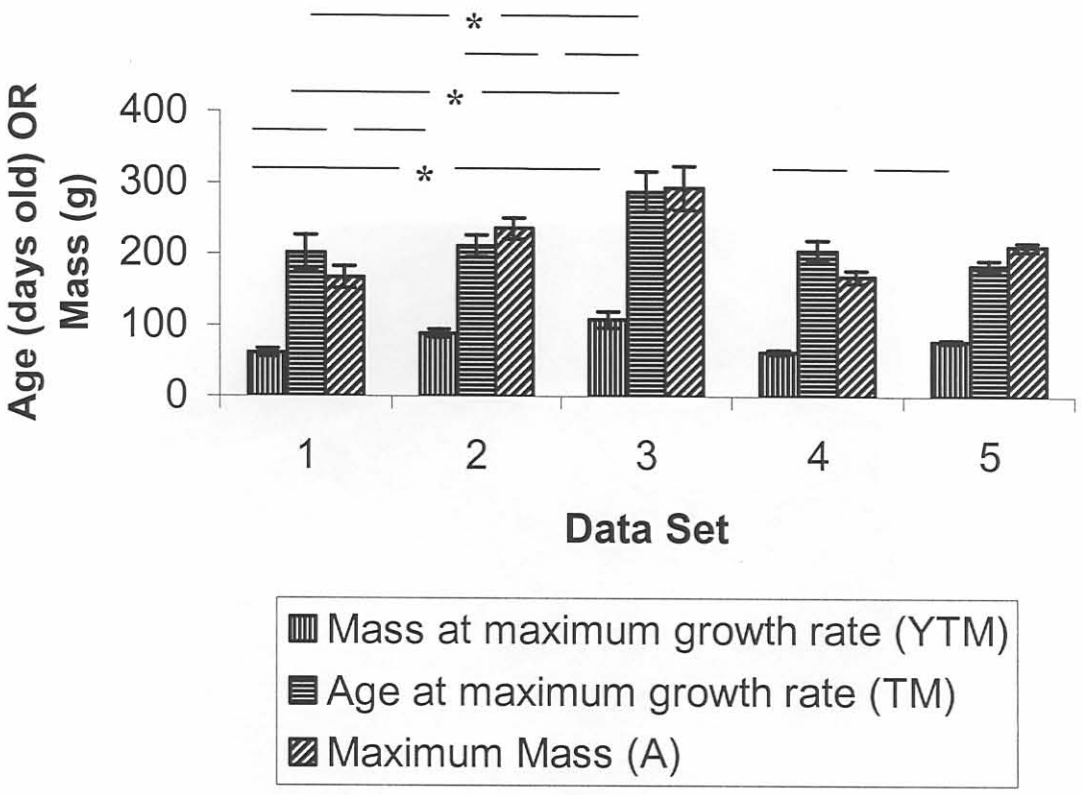

Figure 5: A comparison of results obtained from the different data sets. Data sets varied in terms of the amount of data used in the analyses. Parameters that are compared are mass at maximum growth rate (YTM), age at maximum growth rate (TM) as well as maximum mass (A).

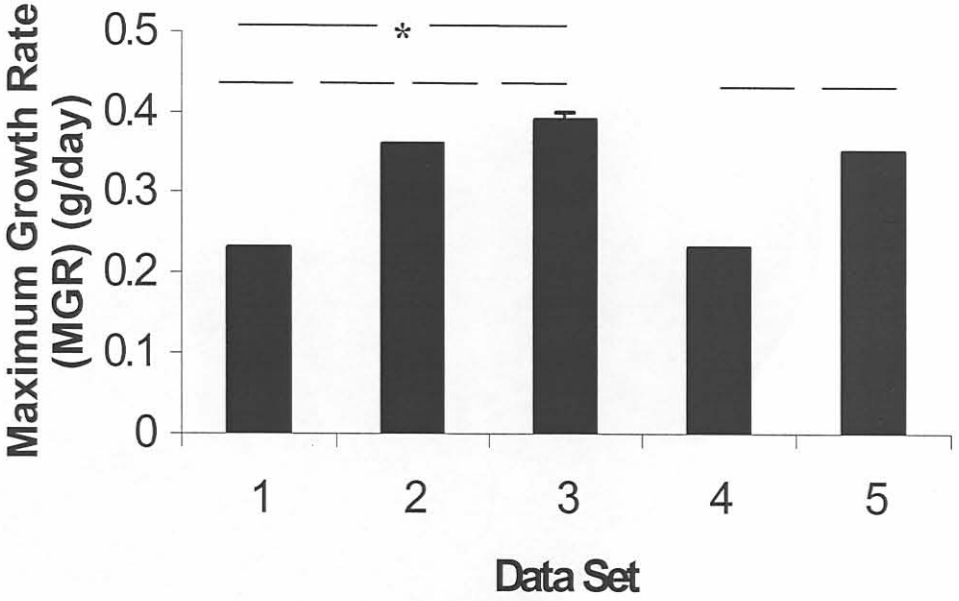

Figure 6: A comparison of results obtained from the different data sets. Data sets varied in terms of the amount of data used in the analyses. The parameter that was compared is maximum growth rate (MGR).

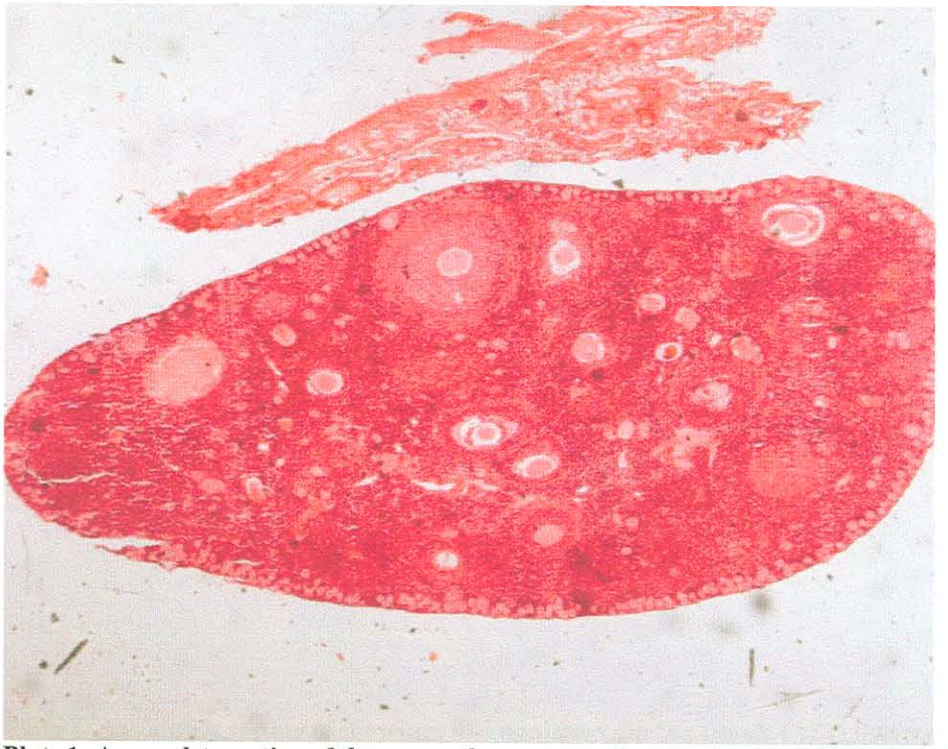

Plate 1: A complete section of the ovary of a non-reproductrive female (A1) that was housed alone for the entire duration of the study. Note the absence of a corpus luteum. Magnification = 550X.

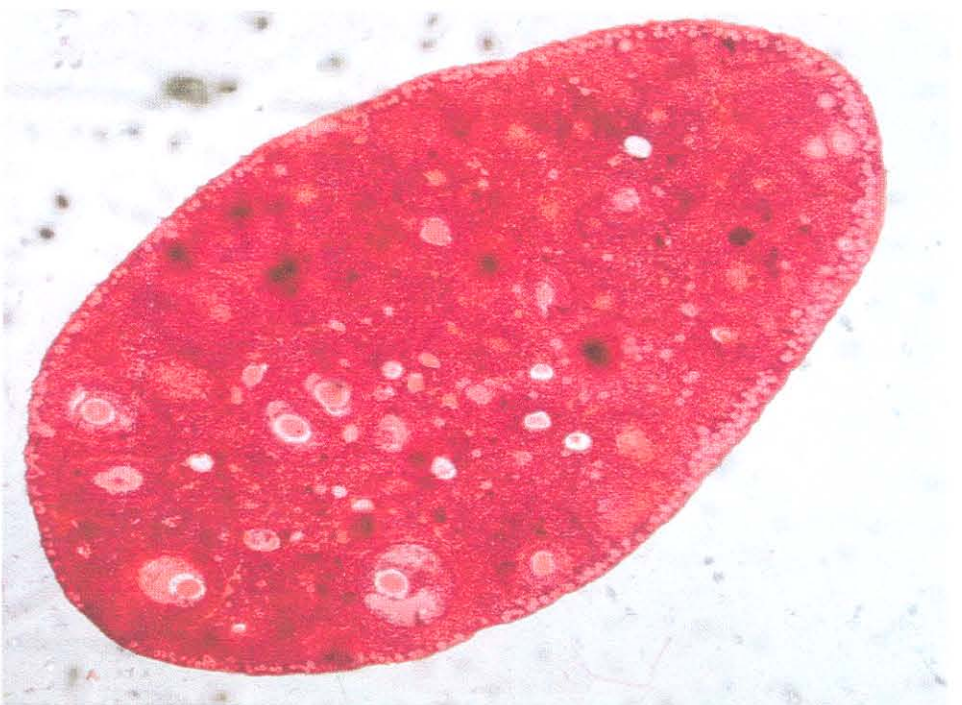

Plate 2: A complete section of the ovary of a non-reproductive female (A2) that was housed alone for the entire duration of the study. Note the absence of corpus luteum. Magnification = 550X.

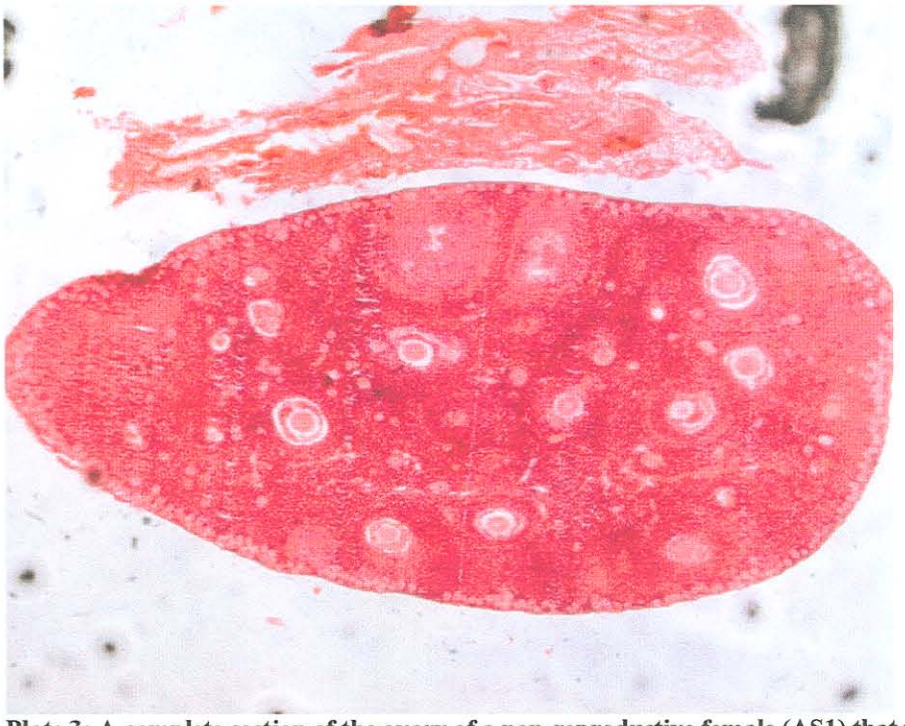

Plate 3: A complete section of the ovary of a non-reproductive female (AS1) that was physically separated from a novel male for the latter half of the study. Note the absence of corpus luteum. Magnification = 550X.

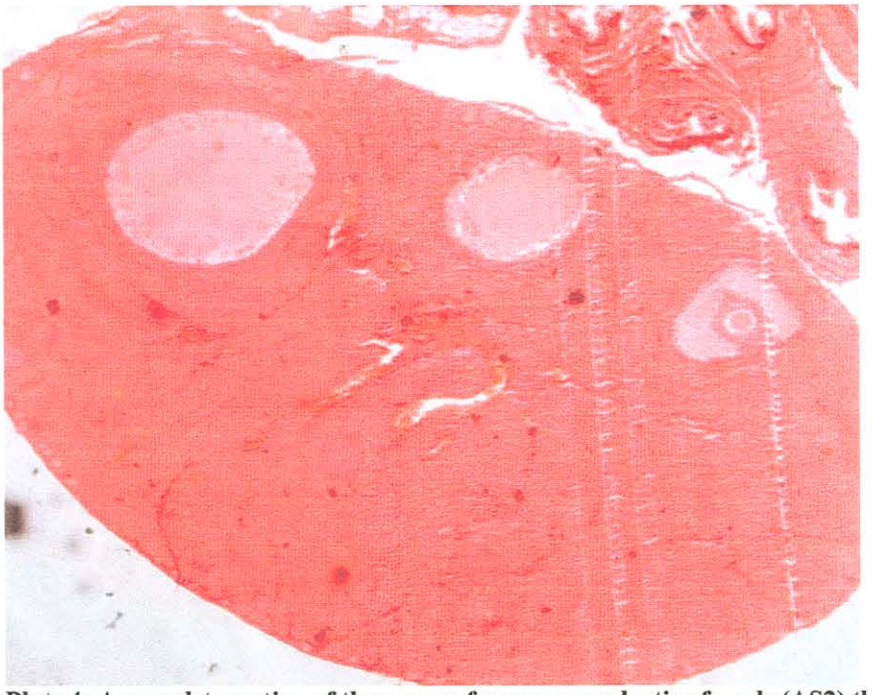

Plate 4: A complete section of the ovary of a non-reproductive female (AS2) that was physically separated from a novel male for the latter half of the study. Note the absence of corpus luteum. Magnification = 550X.

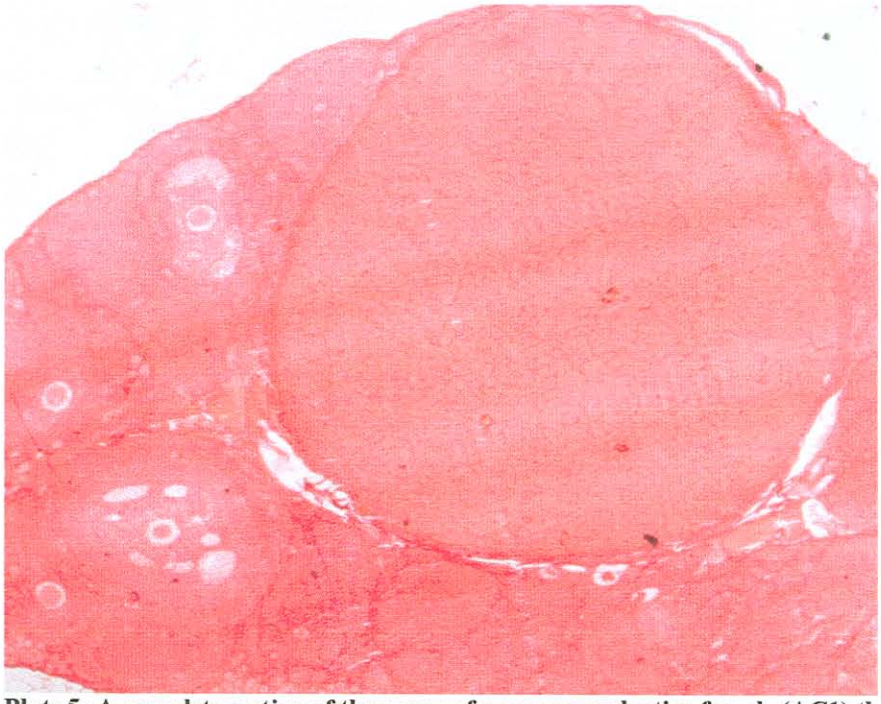

Plate 5: A complete section of the ovary of a non-reproductive female (AC1) that was placed in physical contact with a novel, vasectomised male for the latter half of the study. Note the presence of corpus luteum. Magnification = 550X.

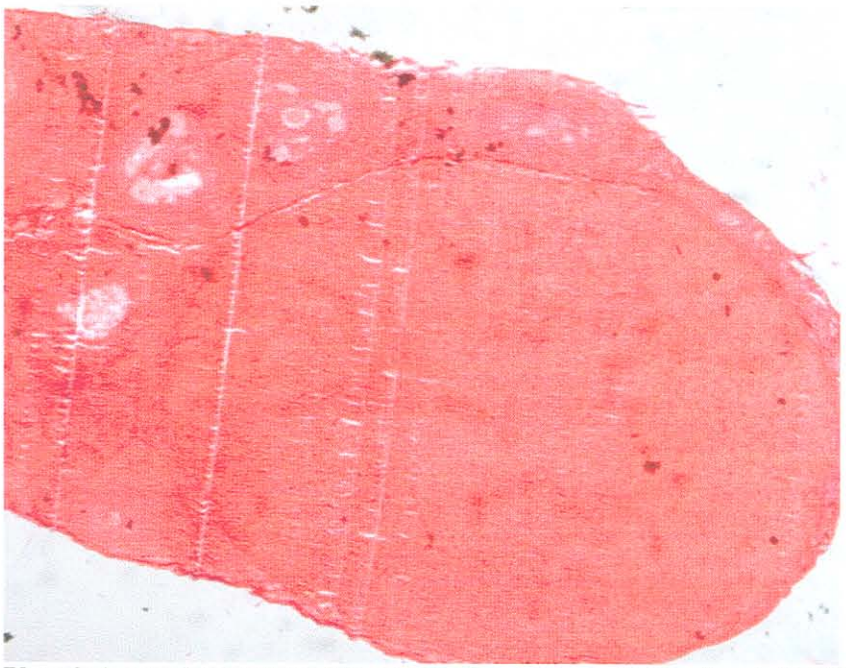

Plate 6: A complete section of the ovary of a non-reproductive female (AC2) that was placed in physical contact with a novel, vasectomised male for the latter half of the study. Note the presence of corpus luteum. Magnification = 550X.

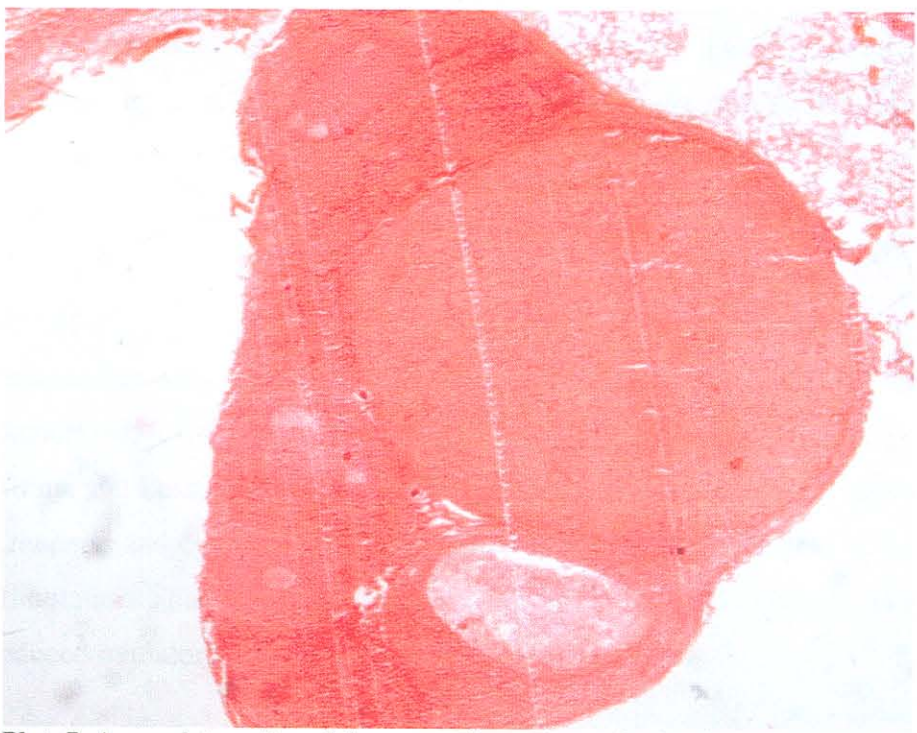

Plate 7: A complete section of the ovary of a non-reproductive female (AC3) that was placed in physical contact with a novel, vasectomised male for the latter half of the study. Note the presence of corpus luteum. Magnification = 550X.

Discussion

The family Bathyergidae comprises a range of species that exhibit a broad spectrum of social systems. Indeed, they are one of the few truly subterranean mammals that exhibit species that are social (Jarvis & Bennett 1990). Similarly, they exhibit a wide range of reproductive patterns that span the realms of strict incest avoidance as is the case in the Mashona mole-rat through to complete physiological suppression as is evidenced in the eusocial naked mole-rat (Bennett & Faulkes 2000).

Many solitary subterranean rodents appear to be induced ovulators. It would seem that induced ovulation is the rule in solitary mammals (Zarrow & Clark, 1968). Among the Ctenomyidae, *Ctenomys talarum* is known to be an induced ovulator (Weir, 1974). Altuna and Lessa (1985) studied the penile morphology of a Uruguayan species of *Ctenomys* and concluded that the spines on the glans provided cervico-vaginal stimulation. Shanas *et al.* (1995) has suggested that *Spalax ehrenbergi* may also be an induced ovulator.

In the female brush-tailed bettong (*Bettongia penicillata*) ovulation is induced by the male. This is evident from the absence of corpora lutea in the ovaries of separated females (Hinds & Smith 1992). The male is thus important as a stimulus for follicular growth and subsequent ovulation (Hinds & Smith 1992).

McClintock (1983), working on rats, reported that the number of intromissions could be correlated with successful pregnancy. Copulation acts on the neuroendocrine reflex in the female with a resultant surge in prolactin and increased progesterone levels required to maintain pregnancy (McClintock 1983).

To date, in social bathyergids, there are few data pertaining to the method of ovulation. Faulkes, Abbott & Jarvis (1990) and Faulkes *et al.* (1990) found the naked mole-rat to exhibit spontaneous ovulation, since if a non-breeding female was removed from a

colony and housed singly, she underwent a normal oestrous cycle. It appears from this study that the seasonally breeding social highveld mole-rat is an induced ovulator. This method of ovulation probably has evolved as a consequence of living in an environment with a marked seasonality to it.

The progesterone data revealed that, unlike many hystricomorph rodents outside of the bathyergid family (such as the guinea pig and chinchilla) and the naked mole-rat (*Heterocephalus glaber*), the highveld mole-rat shows elevated progesterone concentrations (relative to creatinine) only when females are in direct, physical contact with males.

Basal progesterone concentrations were generally low in non-reproductive females housed alone. The females that remained alone during the experimental manipulation as well as those that were in chemical contact with males revealed little evidence of any change in progesterone concentration.

Histological examinations revealed that successful ovulation had occurred in the females that had been placed in direct, physical contact with males. This was evident by the presence of a single, large corpus luteum in each ovary examined. In females where no corpora lutea were observed, there was evidence of follicular development. Follicular development was characterized by the presence of primordial and primary follicles. Graafian follicles were observed in females that were placed in chemical contact with males (AS females). Atretic follicles resulting from incomplete follicular development were evident in all females. Hence follicular development occurred in all females removed from the colony, yet ovulation was blocked by the lack of coitus.

It is interesting that females placed in chemical contact with males showed increased follicular development when compared to females housed alone. All of these females (AS females) had an ovarian histology that was more developed than that of females housed alone. This was evident from the presence of Graafian follicles. It appears as though an olfactory stimulation of the female by the male is enough to stimulate the female to

produce further stages of follicular development but the stimulation is not sufficient to cause successful ovulation, since corpora lutea were absent in the ovaries of these females.

The naked mole-rat and Damaraland mole-rat are the only bathyergids currently found to be spontaneous ovulators. (Faulkes *et a.l.* 1990 and Molteno 1999). Spontaneous ovulation is expected in an aseasonal breeder since females must be fertile all year round. In a seasonal breeder such as the highveld mole-rat, induced ovulation is the predicted method of ovulation. The Bathyergidae is an interesting taxon as member species appear to exhibit different methods of ovulation.

The restriction of reproduction to a single female in a colony is a feature common to all social bathyergids (Bennett & Faulkes 2000). Courtship and pre-copulatory behaviours at the onset of the breeding season shows a trend within the Bathyergidae. In the solitary *G. capensis,* precopulatory behaviours at the onset of the breeding season may possibly be directed at breaking down the strong aggression normally exhibited towards conspecifics. Courtship is initiated by the male signalling to the female through the soil substratum separating their burrows by drumming with the hindfeet. (Bennett & Jarvis 1988a). In the common mole-rat, *C. h. hottentotus*, foreplay is again initiated by the male, however, since the animals occupy the same burrow system for the whole year there is low intensity interaction by both animals. These interactions include vocalizations and the smelling of the genitalia prior to copulation (Bennett 1989). In the Damaraland mole-rat, *Cryptomys damarensis*, the situation is reversed, and the female initiates courtship (Bennett & Jarvis 1988b). While in the naked mole-rat once again it is the female who is the solicitor (Jarvis 1981). When copulation was observed during this study, it was evident that courtship is initiated by the male highveld mole-rat consisting of sniffing, nuzzling, vocalization as well as precopulatory grooming. This display of behaviour by the highveld mole-rat fits the trend of males of loosely social and solitary bathyergid species initiating courtship, while in the more social species, courtship is initiated by the reproductive female.

The relatively long gestation period of the highveld mole-rat of approximately 66 days is comparable to that of the phylogenetically closely related common mole-rat (Bennett 1989). The gestation period of *G. capensis* is relatively short 45-50 days (Bennett & Jarvis 1988a), when compared to that of the naked mole-rat 66-74 days (Jarvis 1991); Damaraland mole-rat 78-92 days (Bennett & Jarvis 1988b) or Giant mole-rat 111 days (Bennett & Aguilar, 1995). It is unusual for small mammals to have such long gestation periods and supports the close taxonomic affinity that the bathyergids have with the hystricomorphs (Bennett & Jarvis 1988a).

The litter size of the highveld mole-rat is within that expected for a social bathyergid (and subterranean rodent) with litter size being approximately 2 pups. Litter sizes of subterranean rodents are generally very small (Bennett *et al.* 1991, Malizia & Busch 1991); ranging from 1 –13. Solitary bathyergid species may have larger litters (1-10) (Bennett & Faulkes 2000) compared to social species (1-5) (Bennett & Faulkes 2000).

There appears to be no overall pattern in the development of pups of the bathyergids. The young are all relatively altricial. They move out of the nest within a few days of birth and begin to supplement their food intake with solids from day 14 (Bennett *et al.* 1991). Although pups of eusocial bathyergids seem to eat solids at a relatively early age (when compared to social and solitary bathyergids), there is no clear trend evident. Pups of the highveld mole-rat begin consuming solids at approximately day 14; which is similar to that of the eusocial *H. glaber* (day 14), the social *C. darlingi* (day 14) and the solitary *B. janetta* (day 13) (Bennett & Faulkes 2000). Weaning in the highveld mole-rat occurs at about day 32, which is similar to *C. darlingi* and *G. capensis* (solitary).

Trends can be seen in the growth rates of pups within the Bathyergidae (as well as between bathyergids and other subterranean mammals and hystricomorph rodents). The pups of solitary species channel energy into rapid growth. They disperse at an early age and for this it is advantageous to be large and strong so as to be able to dig effectively and defend their own burrow. The pups of social species do not have such selection

pressures operating upon them, instead the pups must fit into the colony hierarchy and eventually join the colony work force.

Subterranean mammals adaptively converge on a variety of traits (Nevo 1979). However, contrasting lifestyles caused by different constraints lead to disparate patterns of development of young and associated growth rates (Bennett *et al.* 1991). Most subterranean mammals (including hystricomorphs) have long breeding cycles (Burda 1989); bathyergids having long periods of postnatal development (Bennett & Faulkes, 2000). Young of mammalian species can be altricial (under developed at birth) or precocial (fully developed at birth other than size) and nidifugous (feed own once weaned) or nidicolous (parentally fed). One or more of these traits can accurately describe the way that a pup is likely to behave. Solitary species usually have precocial and nidifugous young, while social species tend to have altricial young. Altricial species nest almost exclusively below ground, in burrows, caves or tree holes (Bennett *et al.* 1991).

Pups of solitary bathyergid species are strong, large and ready to disperse and establish themselves within several months of birth, whereas pups of social species are usually altricial and remain in their natal colony indefinitely (Bennett *et al.* 1991). Pups of social species thus rely more on their close relatives for foraging and care of themselves. These factors have implications on growth (Bennett *et al.* 1991; Bennett & Navarro, 1997).

Mammalian species have two main mechanisms that cause a variation in growth rates; a physiological mechanism (which can be limiting to growth or allowing an increase in growth) as well as the mechanism of the selective values of differential growth rates (Case 1978).

There are two ways that growth rates can be adjusted metabolically, namely through rates of energy assimilation and the efficiency at which assimilated nutrients are converted into living tissue (Case 1978). It has been hypothesized that the slowest growth rates should be found in precocial, nidifugous young as a greater proportion of their energy

requirements are invested in foraging and temperature regulation (Case 1978). This is because they usually have larger birth weights and thus more tissue to support metabolically.

Body size and growth rates have been found to be highly heritable traits; thought to be a function of the potency of growth hormone from the anterior pituitary. Species specific growth rates are genetically determined and are adjusted adaptively in order to meet the forces of the environment (Case 1978). Selection pressures acting on the mother usually favour altricial offspring particularly when the nest is a safer environment for a pup to complete development in, than when compared to the womb (Case 1978). Another case where altricial young would be favoured occurs when foraging requires so much skill and experience that no matter how precocial the pup is, the pup could not effectively compete with adults (Case 1978).

There are several factors which could lead to the selection of a, usually, faster growth rate. For example, faster growth rates could be selected for in order to reduce the risk of infants to predation (Williams 1966 and Lack 1968).

All *Cryptomys* species are social and have a slower mean growth rate than their solitary bathyergid counterparts (Bennett *et al.* 1991). Social bathyergids have a lower resting metabolic rate than solitary species (Bennett *et al.* 1991) and this may result in a lower postnatal growth rate. Several socio-biological factors are thought to act as forces on the selection of a slower postnatal growth rate in social bathyergids. Young are not forced to take full responsibility for their own well-being from birth as pups are incorporated into a hierarchy which cooperate in meeting the foraging needs of the colony (Bennett *et al.* 1991).

Table 6: Summary of maximum growth rates for several bathyergid species.

Species	Social Status	Mean Maximum Growth Rate ($gday^{-1}$)	Reference
Bathyergus suillus	Solitary	3.3	Bennett *et al.* 1991
Bathyergus janetta	Solitary	1.68	Bennett *et al.* 1991
Georychus capensis	Solitary	1.22	Bennett *et al.* 1991
Cryptomys sp. (anselli)	Social	0.08 – 0.63*	Burda 1989
Cryptomys hottentotus pretoriae	Social	0.23 – 0.39**	This study
Cryptomys hottentotus hottentotus	Social	0.229	Bennett *et al.* 1991
Cryptomys damarensis	Eusocial	0.233	Bennett *et al.* 1991

*dependent on sex, colony environment (whether with or without parents) and stage of development (of which three stages were identified).

**dependent on sex.

Table 7: Summary of maximum growth rate for other subterranean mammals.

Species	Mean Maximum Growth Rate ($gday^{-1}$)	Reference
Tachyoryctes ruandae	1.35	Zullinger *et al.* 1984
Cannomys badius	2.01	Zullinger *et al.* 1984
Thomomys talpoides	2.21	Zullinger *et al.* 1984

Table 8: Summary of growth rates of other hystricomorph rodents (cited in Case 1978).

Species	Growth Rate ($gday^{-1}$)	Reference
Cavia sp.	3.2 – 4.5*	Brody 1945, Rood 1972
Proechimys semispinosus	2.1 – 2.5**	Tesh 1970
Hoplomys gymnurus	2.4	Tesh 1970, Weir 1974
Microcavia australis	1.9	Rood 1970, 1972
Erethizon dorsatum	21.2	Walker 1964, Weir 1974
Galea musteloides	2.4	Rood 1972

*dependant on species.

**dependant on sex.

It is clear that social bathyergids have a much slower maximum growth rate compared to solitary bathyergids, hystricomorph rodents and subterranean rodents (non-hystricomorphs). The study species, *C. h. pretoriae*, exhibits the trend found for social bathyergid species in which an increase in sociality is met with a decrease in maximum growth rate. With a maximum growth rate of 0.23 – 0.39 $gday^{-1}$, it is apparent that the altricial pups of this species have a very low maximum growth rate like other social bathyergids; which is much lower than that estimated for solitary bathyergids, other hystricomorph rodents as well as other subterranean rodents (non-hystricomorphs).

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Chapter 5

Relatedness

Abstract

Animal societies can be composed of related and/or unrelated individuals. Field observations are difficult especially in subterranean species. Molecular techniques (genetic markers) are proving indispensable in cases where individuals are difficult to observe, mark of follow in the wild and where intensive fieldwork would have been required to achieve the same result. Social bathyergid groups are interesting in that there is usually a single reproductive pair responsible for procreation in each colony. The reproductive pair is assumed to be unrelated individuals in order to ensure genetic heterozygozity. Other colony memebers are thought to be the offspring of the original reproductive pair. The genetic composition of assumed breeders and other colony members is investigated in this study. The project was initially undertaken in order to test microsatellite primers developed for other bathyergid species on the study species, *Cryptomys hottentotus pretoriae*. Single phalange samples were used to determine inter- and intracolonial relatedness and to identify parental individuals. Of nine microsatellite primers tested, six gave positive and scorable autoradiographs. These primers had been develop for use in related species. A universal mammal primer was also used successfully. Conditions for the successful PCR for each primer are reported. It was found that most individuals in each colony were related on a first order level (R=0.5), although estimates of relatedness were exaggerated (low sample size). Breeding males identified morphologically were found not to be the paternal contributor of offspring alleles in any of the colonies. The paternal contributor of alleles in offspring was not identified in any of the colonies and it is therfore assumed that he is not present. Morphological idenitification of breeding females proved to be reliable as those females were identified genetically as being the maternal contributor to offspring alleles. Thus, colony individuals appear to be the offspring of the breeding female and are full-siblings. Evidence of mulitple paternity was found in three of the five colonies. Studies are needed to further elucidate aspects of bathyergid social organization.

Introduction

History

The arrangement of a social system in animals is intriguing in terms of the way that the social system is formed, how it functions as a cohesive unit as well as which individuals comprise the social unit. The latter aspect of social organisation is interesting in that social units can be made up of related and/or unrelated individuals. Historically, members of a social unit were identified by observing and recording matings and births in the social unit (which neonates belong to which female/male pair and the fate of those neonates). Over several generations, one could reliably predict which individuals would make up a social unit for a particular species and which would be excluded.

However, the use of these procedures requires intensive fieldwork providing that the species is easy to observe in the field and that specific individuals can be identified in each group. The identification of individuals requires individual-specific recognition signatures (marks, scratches, and notches) or the use of a marking procedure. It is also important to add that the fieldwork has to be intensive as if an animal went missing from a group then, without intensive field observations, it would not be clear if the individual left the group willingly, under duress or whether the animal died.

The use of genetic markers has revolutionised biology in numerous ways. In the case of animal social systems, the use of genetic markers has been very useful in identifying specific individuals as well as determining the relatedness of individuals within specific social units (eg Amos *et al.* 1993, Morin *et al.* 1994, Altmann *et al.* 1996, Girman *et al.* 1997). The use of genetic techniques is being increasingly shown to be advantageous in situations where multiple matings are a part of the species reproductive behaviour. While using genetic marker techniques, it has been found that, in baboons, multiple matings in females are far more frequent than ever predicted (Altmann *et al.* 1996).

Incompatability of bathyergid social research and field observations

Bathyergid social systems range from being asocial (solitary species) to eusocial (highly organised, group living species) (Jarvis 1981, Bennett *et al.* 1988, Jarvis & Bennett 1993, Faulkes *et al.* 1997). The social system of bathyergids is, however, a continuum (Lovegrove & Wissel 1988, Lovegrove 1991) and there are species that can be classified as being loosely social (occasionally group living but not highly organised). The Highveld mole-rat is a loosely social bathyergid (Moolman *et al.* 1998, this sudy).

Bathyergids occupy a fossorial, subterranean niche (Bennett *et al.* 1988, Faulkes *et al.* 1997) and it is thought that individuals very seldom (if at all) go above ground. It is therefore obvious that intense field observations would prove impossible. The only method that has been adopted to attempt to follow the changing structure of a group of mole-rats in the field is a long-term study (Bennett & Jarvis, pers. comm. 2000) with much effort and resources being employed on an annual basis. This method is carried out by live-trapping all individuals in known colonies and toe-clipping each individual. Annual comparisons are then made with regard to which individuals are new members of each colony as well as which individuals are still present in each colony. However, it is not known, unless a marked individual is captured elsewhere, whether missing individuals have dispersed or have died. Identifying the breeding female is relatively simple but it is assumed that the largest, heaviest male is the breeding male (Bennett pers. comm. 2000). In several studies on other mammalian species, the breeding male identified by morphology has been excluded as a parent through genetic marker techniques (Morin *et al.* 1994).

Advantages of genetic marker techniques

Employing techniques that involve the use of genetic markers would therefore be of considerable advantage to determining the composition of social units of the social (and eusocial) bathyergid species. The innovative approach of using microsatellite DNA as a genetic marker is of utmost importance to bathyergid social research if anything is to be

discovered regarding the composition of social units of social bathyergids. As well as allowing estimates of relatedness to be calculated within colonies, the microsatellite approach also allows the determination of parentage by exclusion analysis; especially the determination of paternity if maternity is already known (Bruford & Wayne 1993, Amos *et al.* 1993, Morin *et al.* 1994, Altmann *et al.* 1996 and Girman *et al.* 1997)

Characteristics of Microsatellites

Microsatellites are tandem repeat units that are widely dispersed throughout eukaryotic genomes (Queller *et al.* 1993). They are also referred to as variable number tandem repeats (VNTRs) and, as this name suggests, consist of tandemly repeated base pairs (Hamada *et al.* 1982, Tautz & Rentz 1984 and Tautz *et al.* 1986). The length of the repeat can vary from 1 base pair to 10 base pairs, but are generally less than 5 base pairs long (Bruford & Wayne 1993). Microsatellites are often highly polymorphic (Queller *et al.* 1993) due to the variation in the number of repeat units (Bruford & Wayne 1993). They are co-dominant and are inherited in a Mendelian fashion (Jarre 1996). They are considered to be neutral (Jarre & Agoda 1996). The use of microsatellites yields a high information content of genetic data such as sexual selection, mating systems and social organisation (Goodnight & Queller 1999). Microsatellites are important due to their abundance and ubiquitous distribution in animal genomes.

The mode of evolution of microsatellites is highly contentious. One theory is that microsatellites evolve via a stepwise mutation model, which assumes that mutation either increases or decreases the allele size by one repeat unit (Machugh *et al.* 1994 and Estoup *et al.* 1995a) or due to intra-allelic polymerase slippage during replication (Goldstein *et al.* 1995 and Tautz 1989). This model predicts that allelic length can arise from homology (alleles being identical-by-descent) or size homoplasy (convergence, parallelism or reversion) (Estoup *et al.* 1995b). However, the infinite allele model has also been presented to explain the process of the evolution of microsatellites (Shriver *et al.* 1993 and Di Rienzo *et al.* 1994). This model suggests that any allele arising by mutation is different from those previously present in the species (Machugh *et al.* 1994 and Estoup *et*

al. 1995a). There is, as yet, little consensus regarding which model is more appropriate to explain the evolution of microsatellites and neither model takes into account the possibility that mutations may not be asymmetrical (Jarre 1996). However, Forbes (1995) suggests that allele frequency distributions fit the stepwise mutation model more than the infinite allele model.

There are a number of drawbacks in using microsatellites. In particular, the quality of information that can be obtained using microsatellites depends upon the consistency and objectivity of gel-scoring, how accurate observed variation is and how representative the results are of the entire population (Queller *et al.* 1993). Another problem is the occurrence of null alleles. Null alleles are non-amplifying alleles (Paetkau & Strobeck 1995) and are recognised by apparent non-inheritance of parental alleles in known parent-offspring pairs (Pemberton *et al.* 1995). The presence of null alleles cause heterozygotes to be scored as homozygotes. This would result in heterozygote deficiencies relative to Hardy-Weinberg expectations (Brookfield 1996 and Pemberton *et al.* 1995). The expected frequency of null alleles being encountered are low in some species (Brookfield 1996). In other taxa, the ocurrence of null alleles is relatively high (Pemberton *et al.* 1995 and Callen *et al.* 1993). Using microsatellites from related taxa, although cost effective, requires caution as the use of degenerative primers may increase the probability of encountering null alleles. Varying the conditions of the Polymerase Chain Reaction as well as the concentration of template DNA mitigates this (Malherbe pers. obs. 2000).

The level of genetic relatedness amongst pairs of individuals (R) is defined as the expected proportion of alleles that are identical-by-descent that are shared amongst those individuals (Bourke 1997). Two individuals that are related as parent-offspring will share at least one allele per locus (PalsbØll 1999 and Goodnight & Queller 1999). But this could happen by chance in unrelated individuals and is even more likely for siblings (PalsbØll 1999). To minimise such random matches, it is important to use a sufficient number of loci in the study (PalsbØll 1999). The calculation of the estimate of R for each relationship is carried out by using excellent software available on the internet (KINSHIP and RELATEDNESS, Queller & Goodnight 1989). Previously, estimates of R were made

using correlation and regression coefficients (Pamilio & Crozier 1982 and Crozier *et al.* 1984). These methods, however, only allow the estimation of R across groups and are affected by small sample size.

Characteristics of social bathyergids

The highveld mole-rat (*Cryptomys hottentotus pretoriae*) lives in a region of seasonal precipitation (summer rainfall region). Rainfall is thought to be sufficient to allow extensive burrowing to take place when the soil is at its most workable. The species is also a seasonal breeder (Janse van Rensburg 2000) with two breeding peaks in a year; May/July and September (Janse van Rensburg 2000). Between one and three pups are born to a single female in each colony (Malherbe pers. obs. 1999, 2000).

As it is thought that in most social bathyergid systems there is seldom more than a single breeding female present in each colony (Bennett et al., 1999; Faulkes *et al.* 1997; Lovegrove 1991), the breeding female can be identified with a high level of accuracy (using morphological characteristics, in particular the presence of teats). This means that through exclusion analysis paternal alleles can be identified and it can be inferred as to whether there is more than one father in the colony as well as which individuals they are. This is extremely powerful in bathyergid social research as the information can be used to challenge the theories of the social evolution. Other information relating to the causes and consequences of social evolution which are obtainable from parentage and relatedness information includes mating systems, dispersal patterns and gene flow among groups (Burland 1998).

The effect of a small population size (Paetkau & Strobeck 1994) is affected by non-random mating and where there are significant differences in the survival of young (Spencer *et al.* 1995). Non-random mating could play a role in social bathyergid populations.

Aims of the study

As Girman *et al.* (1997) suggest, one could use molecular genetic data to test hypotheses and predictions that follow from observations of social structure. Microsatellite genotype data will be used in order to identify pedigree and other relationships within colonies of the highveld mole-rat, *Cryptomys hottentotus pretoriae*. The identification of relationships within these colonies will also allow an investigation into possible alternative mating systems and social organisation that may become apparent during the analysis.

This component of the project will serve to complement other research undertaken concerning *Cryptomys hottentotus pretoriae*, specifically answering questions regarding social organisation and the mating system that has evolved in this social bathyergid.

Also, it is hoped that results from this study will allow a deeper understanding of how social units are formed in a loosely social bathyergid and how this species actually fits into the continuum of Cryptomid (and bathyergid) sociality.

It was hypothesised that colonies of the highveld mole-rat consist of a single breeding pair (one breeding female and one breeding male) and that all other colony members would turn out to be the offspring of that reproductive pair.

Methods and Materials

General

A total of five colonies were used in this study and named uniquely; L03, L01, GRP, GAS, G42S. These colonies consisted of 13, 10,10,10 and 6 individuals respectively. Of these 49 individuals, one sample was not properly preserved (from L03) and two samples gave impure genomic DNA when analysed for genomic DNA concentration with a spectrophotometer. All other samples (the remaining 46) were properly preserved and contained pure genomic DNA. For further details concerning sample individuals (sex, colony status and genotype) see the Appendix.

Breeding females were identified morphologically due to their size and the presence of conspicuous teats. This method of identification was thought to be accurate as in other bathyergid species. Breeding males were also identified morphologically, purely by size. In other bathyergid species, this method of identification has been shown to be generally accurate (eg *Cryptomys damarensis*, Bennett & Jarvis 1988) although being atypical of eusocial societies (Jarvis *et al.* 1994).

Genomic DNA sampling

Genomic DNA was obtained from single phalange samples or entire toes samples which were obtained from toe clipping techniques as animals needed to be permanently marked in this way for another study. Phalange samples were preserved at –40 °C in a solution of dimethylsulfoxide (DMSO) saturated with sodium chloride (NaCl). Due to NaCl saturation, the samples did not freeze at this temperature. The samples were couriered to the University of London and stored at –20 °C.

Genomic DNA extraction

The samples were subjected to whole genomic DNA extraction techniques. Each sample was cut in a sterile environment so that approximately a single phalange or an entire toe was available for use. Extraction was undertaken by a commonly performed extraction technique, outlined in the Appendix.

DNA concentration determination

The concentration of genomic DNA was determined accurately on randomly selected samples using a spectrophotometer. Of the samples, only two (individuals 1308 and 1333) showed impure extracts but were used in further analysis so as to compare the results of impure extractions with those of pure extractions as well as to see what results an impure extraction gave. However, the results of the scoring of these samples were not used in any analytical procedures.

DNA amplification

The success of extraction was determined by a 1% agarose gel checking procedure (see Appendix for recipe and method).

Once it was clear that there was sufficient genomic DNA in the sample, the DNA was amplified using the Polymerase Chain Reaction (PCR). Each sample was subjected to separate PCR's for each primer that was tested a total of nine primers being tested. The primers that were tested consisted of three developed for *Cryptomys hottentotus hottentotus* (VB11, DD4 and EF12; Burland *et al.* in press.), five developed for *Cryptomys damarensis* (DMR2, DMR3, DMR4, DMR5, and DMR7; Burland *et al.* in press.) and one universal mammal primer (NCAM; Moore *et al.* 1998). The DMR primers were made using an enriched technique while the EF12 primer was made using a non-enriched technique (pers. comm. Burland 2001). Unfortunately, due to time constraints it was impossible to develop primers specific to the study species. Also, it did

not seem necessary to develop primers specific to the study species as there were a number of primers already available for other closely related species.

Several primers produced excellent results without any modification of original DNA whereas other primers needed PCR conditions and/or DNA dilution(s) to be altered for positive results. Three primers (DMR2, VB11 and DD4) were excluded from further analysis as, after several attempts, their use proved to be unsuccessful.

Final PCR conditions and template DNA concentrations that resulted in positive, scorable banding patterns for each locus are presented in Table 2 (Results section).

Microsatellite allele size determination

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

Polyacrylamide gels were vacuum dried, placed on autoradiograph film and left for varying time periods depending on the level of radioactivity and the type of film used. The film was processed using an automated process and scored. Size markers were used to determine the absolute sizes of alleles for each locus. For DMR3 a size standard could not be run and the sizes of the alleles were named arbitrarily.

Scoring of gels

The banding of each individual for each locus was compared to its relevant size standard (except for DMR3). For individuals where banding was unclear, the PCR products were run on a separate gel again and usually resulted in the individual being successfully scored. Unclear banding could be due to several reasons (Weber 1990). Strands can migrate at different mobilities or the Taq polymerase enzyme (*Thermophilus aquaticus*)

could add a single non-complementary base to the 3' end of a fraction of the newly replicated models resulting in bands differing by a single base pair. A third reason could be due to Taq polymerase skipping or occasionally adding repeats during elongation. Another situation where scoring could become difficult arises when heterozygotes differ by one base pair and when these individuals are compared to homozygotes with skipping as described above.

Calibration of R estimates

KINPOP software estimates the number of loci necessary to provide consistent and robust estimates of relatedness (Altmann *et al.* 1996, Girman *et al.* 1997). The software accomplishes this by a rarefaction analysis that is carried out in the following way (Girman *et al.* 1997). Relatedness is calculated from the microsatellite genotype data from a single locus selected at random. A second locus is selected at random and, without replacement of the first locus already used, relatedness is re-calculated. This relatedness value is thus based on the microsatellte genotype data of both loci. This process of addition of loci without replacement is continued until all loci have been added. The difference between successive relatedness values is expressed as a function of the total number of loci used. This process is repeated 100 or 1000 times and the mean difference values of relatedness are calculated for each locus addition. A curve can then be constructed of mean difference (dependant axis) versus number of loci (independent axis). If it is observed that the curve asymptotes towards the independent axis, it is evident that even if several more loci are used the estimate of relatedness would not improve significantly for there to be sufficient reason to use more loci. This allows cost and time saving.

In the rarefaction analysis in this study, genotype data was used form 46 individuals from five colonies of the highveld mole-rat. Both 100 and 1000 simulations were carried out in order to determine whether the number of simulations used altered the outcome of the

analysis. The mean difference in relatedness (± SD) between n and n+1 loci was expressed as a function of n+1 loci for 100 and 1000 repetitions respectively.

Identification of mother- and father-offspring pairs

The KINSHIP 1.3 software package assigns parentage and other hypothesised pedigree relationships to individuals using microsatellite genotype data via exclusions (Goodnight & Queller 1999). Single locus markers (microsatellites) can be consistently scored across gels, and are used for unbiased estimates (for example; estimates of relatedness) and likelihood methods can be used to identify parentage and other relationships (Goodnight & Queller 1999). KINSHIP 1.3 codes each type of pedigree relationship in terms of the probability that the individuals in question share an allele identical-by-descent from the maternal or paternal line (Goodnight & Queller 1999).

It was thus possible to identify mother-offspring as well as father-offspring pairs. The KINSHIP software package uses microsatellite genotypes to calculate the likelihood that a dyad is related by a specified pedigree (Goodnight & Queller 1999). KINSHIP also calculates a likelihood ratio for any pair of hypothesised pedigrees (Goodnight & Queller 1999). The program attaches statistical significance to the results by executing a given number of simulations. Type II error rates (false negatives) are also calculated (Goodnight & Queller 1999). This refers to the rate of dyads which are of the defined pedigree relationship are not identified as such by KINSHIP 1.3 (Goodnight & Queller 1999).

KINSHIP allows one to set hypothesised r_m and r_p values for each hypothesis. The r_m value refers to the probability that half of the focus individual's alleles are identical by descent to the putative mother, while the r_p value refers to the probability that half of the focus individual's alleles are identical by descent to the putative father. In order to identify mother-offspring pairs, the null hypothesis was set at $r_m = 0$, $r_p = 0$ while the primary hypothesis was set at $r_m = 1.0$, $r_p = 0$. This analysis made it possible to determine the number of paternal alleles within a colony (among the mother's offspring which were

identified from the maternal analysis above) that in turn gave evidence in determining whether multiple paternity had occurred.

Once the maternal contributor of alleles had been identified, an extra column was added to the original input file to give identified offspring a label referring to the identity of their mother. This input file was used in the subsequent likelihood analysis that would identify father-offspring pairs. For this analysis, the null hypothesis was not changed from the maternal analysis but the primary hypothesis became; $r_m = 0$, $r_p = 1.0$.

Calculating Pairwise Relatedness

The RELATEDNESS 5.0.7 software package weights each individual allele by its frequency in the population (Girman *et al.* 1997) using microsatellite genotype data. This means that rare alleles receive a relatively higher weight than more common alleles. Output values from RELATEDNESS 5.0.7 can range from +1 to –1. A relatedness output value of 0.5 is expected for outbred first order relationships such as mother-offspring and full sibling dyads (Table 1). The output of RELATEDNESS is based on the following expression (Girman *et al.* 1997).

$$R_{dyad} = \Sigma\,\Sigma\,(Py - P^*)/\,\Sigma\,\Sigma\,(Px - P^*)$$

Where: Px and Py = the frequency of each allele in individual x and y respectively.
P* = the population frequency of each allele excluding the compared individuals.

Using the RELATEDNESS 5.0.7 software package it was possible to calculate pairwise relatedness estimates between each individual (dyad) in each colony.

Table 1: Expected relatedness estimates for outbred predigree and other relationships at Hardy-Weinburg equilibrium (after Rasmuson 1993).

Relationship	Relatedness Estimate
Mother-offspring	0.5
Father-offspring	0.5
Full siblings	0.5
Half siblings (maternal allele shared)	0.25
Half siblings (paternal allele shared)	0.25

Calculating Mean Relatedness

Mean relatedness was calculated within colonies using the RELATEDNESS 5.0.7 software package. Mean estimates of relatedness were calculated for the following categories of colony members: amongst males, amongst females, amongst offspring, amongst all colony members, amongst mother-offspring pairs, males vs females and mother vs not-offspring,. These individuals were identified from the KINSHIP analyses. Not-offspring refers to those individuals in the colony that were not identified as offspring of the breeding female using KINSHIP analysis.

Results

Polymerase Chain Reaction

Table 2: Results of Polymerase Chain Reaction condition manipulation. The conditions outlined below resulted in successful amplification of microsatellite genomic DNA for further analysis.

Locus Tested	Target Taxon (Degree of Sociality)	Dilution of original* DNA	PCR Conditions	Number of alleles identified
EF12	*Cryptomys hottentotus hottentotus* (social)	1 in 10	Initial denaturation @ 94 °C (3 min) 5 cycles of: Denaturation @ 94 °C (45 s) Annealing @ 57 °C (1 min) Extension @ 72 °C (1 min) Then 25 cycles of: Denaturation @ 94 °C (45 s) Annealing @ 55 °C (1 min) Extension @ 72 °C (1 min) Final extension @ 72 °C (10 min)	6
DMR3	*Cryptomys damarensis* (eusocial)	Original	Initial denaturation @ 94 °C (3 min) 30 cycles of: Denaturation @ 94 °C (45 s) Annealing @ 55 °C (1 min) Extension @ 72 °C (1 min) Final extension @ 72 °C (10 min)	16
DMR4	*Cryptomys damarensis* (eusocial)	1 in 5 **or** 1 in 10**	As DMR3	11
DMR5	*Cryptomys damarensis* (eusocial)	Original	As DMR3	8
DMR7	*Cryptomys damarensis* (eusocial)	Original **or** 1 in 10**	As DMR3	8
NCAM	Mammalia	Original	As DMR3	4

*where original refers to the concentration of DNA after extraction and resuspension in tissue quality water. No further dilution was made.

** sample dependant.

Parentage Analysis

It was assumed that there was a single reproductive female in each colony. This was verified by morphological characteristics, with the reproductive female having enlarged teats as well as, occasionally, penetrated vaginas. Reproductive females were therefore identified morphologically. Using the reproductive females as a known factor, it was possible to determine whether the reproductive female was the maternal parent of any of the other colony members. In all of the five colonies, it was apparent that most individuals in each colony were offspring of the reproductive female that was identified. Across all five colonies, there were only seven exceptions to this. These were individuals 1284, 1285 (from colony L01); 1316 (from colony GRP); 1326, 1328 (from colony GAS) and 1332, 1334 (from colony G42S) (see Appendix). All mother-offspring relationships that were identified, in each colony, were significant at $p < 0.001$. Of the seven individuals that did not appear to be offspring of the reproductive female, six individuals shared alleles with the reproductive female at all but one locus. The seventh exception, individual 1326, only shared maternal alleles at three of the six loci.

The paternal parent was also morphologically identified. This identification was then compared to paternal analyses using the maternal alleles already identified. In all cases, the male that was identified morphologically turned out not to be the breeding male (see Relatedness section). This was evident by the null hypothesis not being rejected in all cases where the putative father was compared with offspring that were identified during the maternal analysis. All of the males morphologically identified as the breeder in the colony were also identified by KINSHIP as being offspring of the breeding female in that colony.

Paternal allele analysis

The paternal allele analysis showed evidence of multiple paternity for all colonies except L01 and GAS. Multiple paternity was identified using manual exclusion analysis, basing the analysis on the identified maternal alleles. If more than two alleles (excluding those

of the mother) were present in a colony, for a particular locus, it was assumed that the extra alleles came from a second father (Table 3).

Table 3: Loci identified, for each colony, in which there were more than two paternal alleles (evidence of multiple paternity).

Colony	Locus giving evidence of multiple paternity	Numeber of alleles identified not of breeding female (possible paternal alleles) for each locus giving evidence of multiple paternity
LIDIA01	None	-
LIDIA03	DMR3	3
GLENRP	DMR4, NCAM	4, 3
GLENAS	None	-
GLEN42S	DMR3	4

Rarefaction Analysis

It was shown by rarefaction analysis (Figure 1) that as the number of loci used to calculate estimates of relatedness increases, so the mean difference in the estimate of relatedness decreases. Note that the actual difference between successive additions of loci also decreases. Using five loci to calculate an estimate of relatedness results in the difference in the mean estimate of relatedness being around 0.08 while when using six loci, the difference in the mean estimate of relatedness is around 0.06. The standard deviations related to each estimate of the difference in mean relatedness also decrease as the number of loci being used increase. In this analysis, the estimates were based on whether 100 or 1000 repetitions were used in order to calculate the mean ± SD. Whether 100 or 1000 repetitions were invoked, there was negligible differences between the two curves (Figure 1).

Relatedness

Figure 2 shows that mean relatedness across colonies is very high for most relationships investigated. All relationships investigated had mean relatedness estimates of around 0.6, except for the mother-not offspring relationship. Not-offspring refers to those individuals in the colony that were not identified as offspring of the breeding female using KINSHIP analysis.

The relatedness values estimated are nearer that expected for first-order relatives such as full sibling relationships or mother-offspring relationships (see Table 1). These estimates (except mother-not offspring) are all very close to the value estimated for the mother-offspring relationship (0.66 in this study) that is a first-order relationship. However, pairwise estimates of relatedness ranged from –0.1 to 0.8; the majority of comparisons however occurred in the 0.5 – 0.6 range (62 %). This is what is expected for mother-offspring relationships (first-order relatives).

Similarly, when the relatedness estimates of offspring are compared to each other (Figure 2) it is evident that relatedness is estimated at 0.65. Using pairwise relatedness estimates, a range of relatedness values are obtained from 0.1 to 0.9. However, the majority of pairwise estimates (64 %) lie between 0.5 and 0.7.

When comparing putative mothers to offspring that were identified through KINSHIP not to be offspring of the putative mother (n=7), most relatedness estimates occur between 0.4 and 0.6 (67 %) when analysing pairwise relatedness estimates. Mean estimates of relatedness are for mother-not offspring relationships. Most estimates therefore fall in a range typical to that expected for first-order relatives.

All of the estimates of relatedness of male individuals lie between 0.5 and 0.7, with the majority of estimates around the 0.5 relatedness category (Figure 2). The range in relatedness estimates (majority) for females was the same as that for males, although not all estimates were within this range. Several estimates occurred between 0.8 – 0.9 and 0.3

– 0.4 (Figure 2). The majority of estimates therefore were within the range expected for first-order relationships. Comparing males to females (or vice versa) it is evident that a similar trend exists although the range of the majority of estimates of relatedness appears to expand to 0.4 to 0.7 (Figure 2).

Possible incidence of mutation and null alleles

Due to the fact that six of the seven individuals excluded as offspring of the reproductive female shared her alleles at most loci (and returned high relatedness estimates when compared to her), it is possible that these individuals were actually her offspring, but were excluded as a result of a mutation event or a null allele.

Table 4: Possible explanation for individuals not showing relatedness to the breeding female.

Colony	Individual	Possible reason for disparity (other than being unrelated)	Locus	Alleles involved
LIDIA01	1284 1285	Null allele Null allele	EF12 EF12	E E
GLENRP	1316	Mutation	DMR4	H mutating to J
GLENAS	1326 1328	None Mutation	 DMR4	 K mutating to L
GLEN42S	1332 1334	Mutation Mutation	DMR3 EF12	C mutating to D OR D mutating to E C mutating to E

It is possible that the genotypes of individuals 1316, 1328, 1332 and 1334 at loci DMR4, DMR4, DMR3 and EF12 respectively are consistent with expectations of allele mutation events. In these cases, the allele that has been scored is only a single base pair different from putative offspring (of the breeding female) genotypes. Individuals 1284 and 1285 had genotypes that appear to have arisen from a null allele belonging to the breeding female whose genotype (at locus EF12) is 'EE'. Individual 1326 appeared to be very

different from the breeding female's genotype; mutation or the presence of a null allele are therefore unlikely. This individual's presence in the colony is interesting.

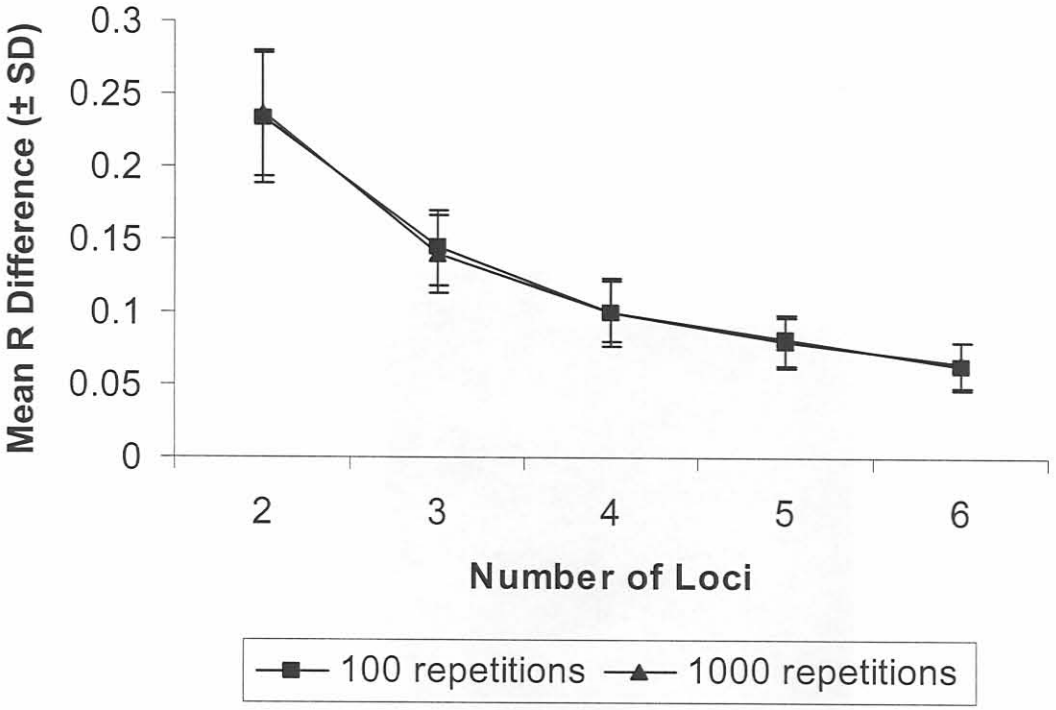

Figure 1: Results of the rarefaction analysis carried out on the estimates of relatedness in order to establish whether six loci were sufficient to carry out further analysis.

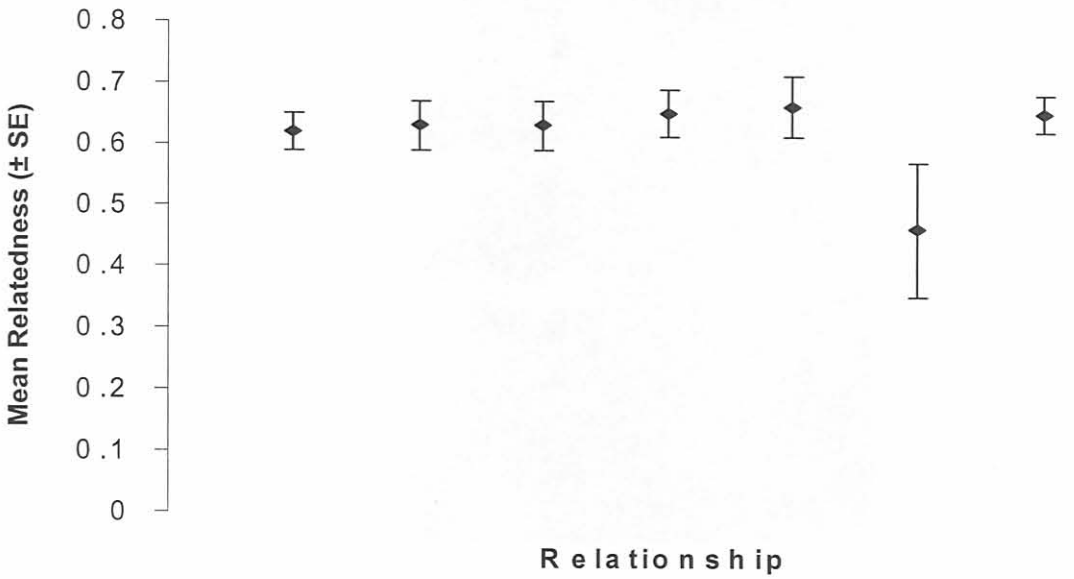

Figure 2: Mean relatedness estimates for each type of relationship investigated in this study. All relationships are near that hypothesized for first-order relatives except mother-not offspring relationships.

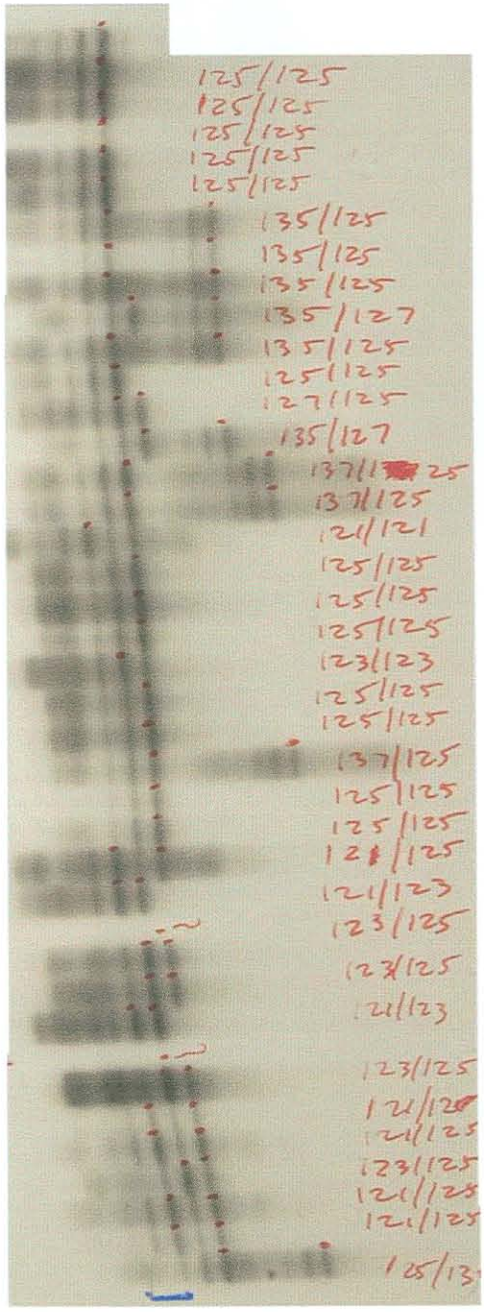

Plate 1: The resulting autoradiograph for the EF12 primer (*C. h. hottentotus*) which was successfully tested at the PCR conditions outlined in the results section. Most bands (which represent individuals) are clearly scorable resulting in homozygoous or heterozygous allele sizes being identified. Size standards were run on separate gels.

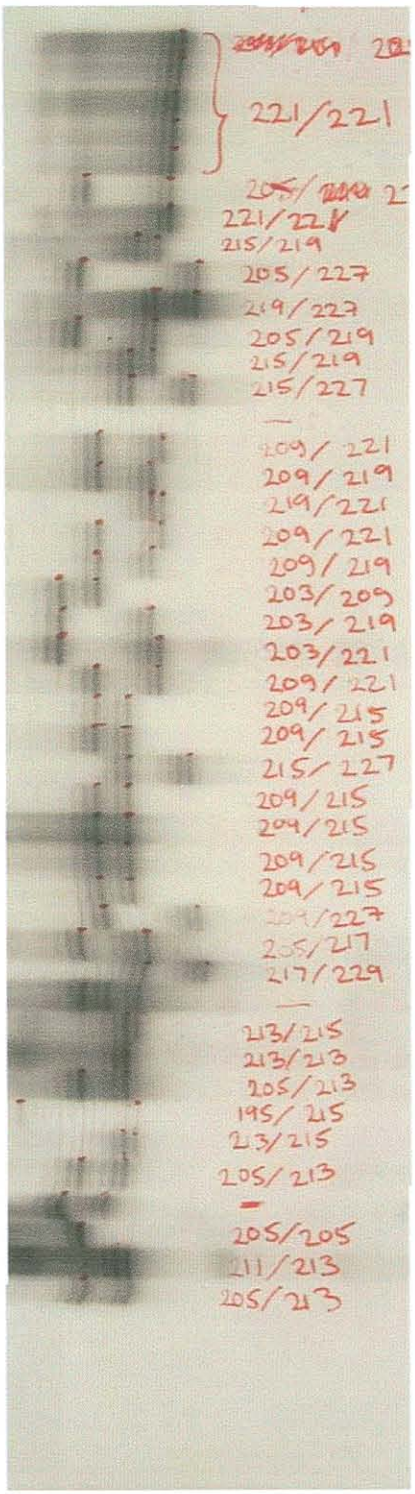

Plate 2: The resulting autoradiograph for the DMR4 primer (*C. damarensis*) which was successfully tested at the PCR conditions outlined in the results section. Most bands (which represent individuals) are clearly scorable resulting in homozygoous or heterozygous allele sizes being identified. Size standards were run on separate gels.

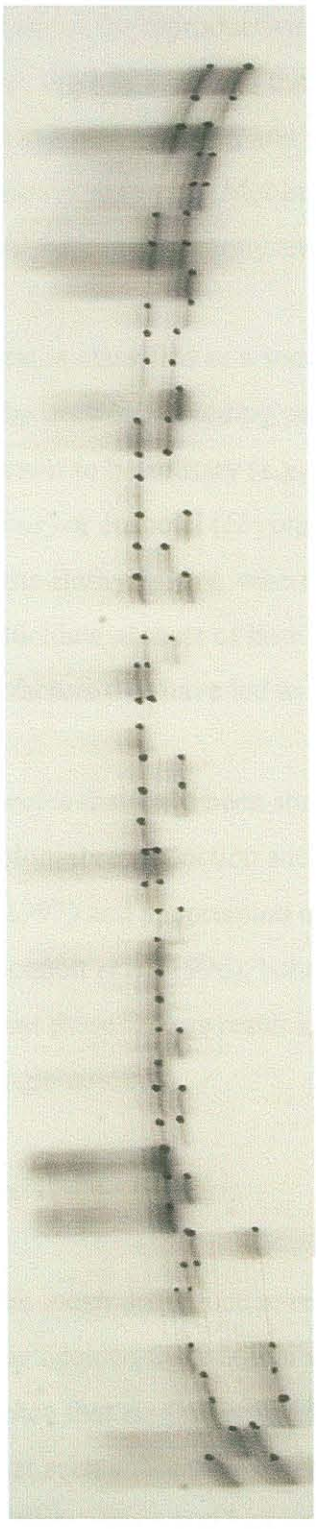

Plate 3: The resulting autoradiograph for the DMR5 primer (*C. damarensis*) which was successfully tested at the PCR conditions outlined in the results section. Most bands (which represent individuals) are clearly scorable resulting in homozygoous or heterozygous allele sizes being identified. Size standards were run on separate gels.

Discussion

Very little is known about the reproductive biology of the highveld mole-rat, *Cryptomys hottentotus pretoriae*. What is known is that the species is a seasonal breeder (Janse van Rensburg 2000) and induced ovulator, and that colonies are made up of a very loose social system/dominance hierarchy (Moolman *et al.* 1998) with no significant correlation in terms of individual rank in the colony and sex or mass.

The highveld mole-rat is classified as a social bathyergid species as it is group living and there is assumed to be only one breeding pair in the colony. Other members of the Bathyergidae are known to be solitary (e.g. *Georychus capensis*), social (e.g. *Cryptomys hottentotus hottentotus*) or eusocial (*Cryptomys damarensis* and *Heterocephalus glaber*). In a family such as the Bathyergidae, where a continuum of sociality exists, it would be very rewarding to elucidate aspects of how social units are comprised and formed in order to understand factors that have led to the evolution of sociality and eusociality.

Other bathyergid species that have been studied to date show that there can be several important cues to colonial reproduction such as incest avoidance (*Cryptomys damarensis*; Rickard & Bennett 1997) and suppression of reproduction (*Cryptomys damarensis*; Bennett 1994 and Bennett *et al.* 1996); behaviourally, chemically or a combination of the two. It is assumed that these factors result in a single breeding pair being responsible for reproduction in a single colony.

Number of loci used

As the estimate of the mean difference in relatedness estimates started to asymptote at six loci (Figure 1), it was decided that six loci would be sufficient for the purposes of this study. Further evidence that six loci would be sufficient for the study was that the standard deviations at each successive locus addition were becoming negligible and that the number of repetitions above 100 had no effect on the estimates (Figure 1). It was concluded that six loci would provide robust estimates of relatedness.

Calibration of relatedness estimates

Mean pairwise relatedness estimates suggest that individuals within colonies are related in a way that approximates first-order relatives (parent-offspring or full siblings). Known first order relationships (mother-offspring pairs identified using KINSHIP) were calculated, using RELATEDNESS, to be 0.66. All other relationships investigated (except mother-not offspring relationships) resulted in relatedness estimates very close to this value. Knowing that our data suggests that first-order relationships approximate 0.6 in our samples, it can be assumed that other first-order relationships might also approximate this value.

It can be concluded that most relationships investigated approximate the estimate for relatedness of a known first-order relationship (0.5; Rasmuson 1993, Bourke 1997). The low relatedness estimate for mother-not offspring comparisons may be due to the inclusion of individual 1326 that appears to be very different, in terms of its genotype, to any other colony member.

Overestimation of relatedness estimates

The overestimation of relatedness estimates can be explained in terms of sample size and assumptions regarding mating system and gene flow.

Although microsatellite primers developed for use on a specific species are considered to be effective in amplifying genomic DNA in other related taxa (Bruford 1999, Schlotterer *et al.* 1991 and Moore *et al.* 1998), three of the nine primers tested resulted in unsuccessful amplification of genomic DNA from the highveld mole-rat. The nine primers were developed from the eusocial *C. damarensis* and the social *C. h. hottentotus*. Thus, only six microsatellite primers were used in this study. Although from rarefaction analyses it appears clear that six loci were sufficient for reliable estimates of relatedness, it also appears that six would be the minimum number of loci acceptable for reliable

estimates of relatedness (Figure 1), as although the mean difference in relatedness estimates is low at six loci (0.06), the curve is only beginning to asymptote. The highveld mole-rat is colonial (Moolman *et al.* 1998 and Malherbe pers. obs. 1999) and it was only possible to investigate five colonies (consisting of 49 individuals). A low number of loci, a low number of individuals and colonies contribute to a relatively low sample size. Furthermore, colonies were caught from geographically separate areas, up to 30 km apart. As above-ground dispersal has seldom been recorded for any social bathyergid (pers. comm. Bennett), it is unlikely that gene flow between areas would be likely. A combination of the low sample size and geographically dispersed sampling may mean that insufficient background data on allele frequencies may be available for these analyses.

It is not likely that these factors affected the results of the KINSHIP analyses, as all mother-offspring relationships were significant at $p < 0.001$. However, it is possible that unrepresentative allele frequencies may have affected the relatedness estimates. This is because there is certainty (from KINSHIP analyses) surrounding the mother-offspring relationships being correct and the estimate of relatedness, which is 0.66, should, as a first-order relationship, be estimated as 0.5. The slightly elevated estimate of relatedness for first-order relatives may also be due to the effects of inbreeding (Shields 1983 and Shields 1993). It is apparent that there is evidence of much homozygosity amongst individuals which may be attributed to a mating system where close relatives breed.

Other relationships investigated using the RELATEDNESS analyses included (offspring, males, females, males vs females) all suggest that individuals within colonies are first-order relatives. Although the estimates of relatedness, for the above relationships, are higher than expected (0.5), the estimates approximate that of mother-offspring pairs whose elevated estimate were explained.

The relationship between mothers and individuals not identified as their respective offspring, although also falling in the 0.4 – 0.6 range of estimates, appears to be lower than the expected estimate for first order relatives. Given that the relatedness of first-

order relatives are overestimated in this study, one can assume that this estimate of relatedness is also overestimated. There appears to be individuals within the colonies to which the respective mother is not related (n=7). However, reaching this conclusion can be misleading as if one analyses the manual exclusion analysis performed on each individual and locus, it is apparent that the cause of these individuals not being related to the breeding female may be due to the occurrence of null alleles at certain loci or due to point mutation during the polymerase chain reaction. Thus, these individuals may be the offspring of the breeding female but due to errors in the PCR process are evident as unrelated individuals. These individuals may also be the full-siblings of the breeding female.

Null alleles and mutation

Most individuals were identified as being offspring of the reproductive female that was identified morphologically (using results from KINSHIP analyses). The majority of exceptions to this (six of seven individuals) were related to the breeding female, and are either the breeding female's offspring or her full-siblings (using estimates calculated by RELATEDNESS). These exceptions can be explained by the presence of a null allele (individuals 1284 and 1285) or by mutation by a single repeat unit (individuals 1316, 1328, 1332 and 1334). Individual 1326 had a very different genotype to any other individual in the colony and its presence in the colony is interesting. This individual may be an immigrant of the colony; possible a male (although the sex of the individual is unknown) and may be the type of individual that is responsible for multiple paternity in colonies.

Origin of paternal alleles

It appears that the paternal alleles are obtained from males external to the colony, as putative fathers morphologically identified were not responsible for the alleles in

offspring of the reproductive female. As no putative fathers were identified in any of the colonies sampled, the identification of the breeding male by morphological characteristics is not reliable in this species. It would be of great value to investigate this aspect of the species reproduction more intensely in order to understand how future fathers come into contact with the breeding female of each colony, as well as how long they remain in colonies. Multiple paternity may also be occurring within colonies (evident by >2 paternal alleles being identified) although pairwise relatedness estimates suggest that the majority of offspring of the reproductive female are full siblings.

Social organisation of colonies of the highveld mole-rat

Colonies of the highveld mole-rat appear to be made up of first-order relatives, specifically mother-offspring relationships. No paternal contributor to offspring allele combination was identified genetically in any of the colonies that were studied. It appears likely that males may migrate into already existing colonies in order to reproduce. However, analagous to this is the fact that known offspring of the reproductive female appear generally to be full siblings of each other – suggesting that they had been fathered by a single male. Studies dwelling specifically on this aspect of the social organisation of the highveld mole-rat would do much to elucidate intricacies of social mole-rat reproductive biology.

The highveld mole-rat's position amongst subterranean rodents

The highveld mole-rat is expected to resemble other subterranean rodents in that the truly subterranean niche typically has a limited capacity for gene flow among populations (Bennett & Faulkes 2000). Population genetics studies of *Spalax*, a blind, solitary myomorph rodent mole of the Middle East, have revealed that limited gene flow has led to the evolution of numerous local forms (Bennett & Faulkes 2000 and Nevo *et al.* 1995). Along with a co-operative breeding strategy, the influence on genetic components and relationships within populations of the highveld mole-rat is thought to be significant in

terms of the effects these factors have on the genetic relatedness of individuals of the species.

There appear to be two main hypotheses regarding genetic patterns evident in subterranean mammals. The first hypothesis concerns stochastic processes that arise from limited gene flow, fluctuating population sizes and genetic drift (e.g, Sage *et al.* 1986). The second hypothesis (Nevo 1979) concerns niche-width-variation. It is thought that the reduced genetic variation seen in subterranean mammals (when compared with small mammal species living above ground, Nevo 1990) results from the narrow subterranean niche which is stable and predictable.

Knowledge of the genetic structure of populations and within groups is crucial if we are to understand the factors involved in the evolution and maintenance of social systems (Bennett & Faulkes 2000), as the social structure of co-operative breeders can potentially influence the genetic structure of mole-rat populations.

In bathyergid rodents that have varying degrees of sociality and reproductive skew, one would expect varying patterns of relatedness in the different species (Bennett & Faulkes 2000). Three bathyergid species have been studied genetically but only the eusocial naked mole-rat *Heterocephalus glaber* (Reeve *et al.* 1990, Faulkes *et al.* 1990 and 1997) has been studied in terms of its genetics relatedness.

The naked mole-rat (*Heterocephalus glaber*) was shown to have a mean intra-colony relatedness of 0.81. The highest mean coefficient of inbreeding that has been reported for a natural mammal population was found for the naked mole-rat (Reeve *et al.* 1990). Other bathyergids to date have been shown to be obligate outbreeders and a lower mean relatedness is expected.

Studies on both the Damaraland mole-rat and the common mole-rat (*Cryptomys hottentotus hottentotus*) give evidence that does not support the niche-width-genetic-variation hypothesis. The three bathyergid species (including *H. glaber*) show patterns of

genetic variation more explicable in terms of social structure, effective population size and stochastic processes like genetic drift.

Summary

Although relatedness estimates are slightly higher than expected for outbred first-order relatives, it can be stated with reasonable confidence that colonies of the highveld mole-rat consist of first-order relatives (mother-offspring/full siblings). Colonies are composed of highly related individuals, although it is unlikely that they are simply monogamous family groups, it is more likely that colonies are composed of extended family groups.

The higher than expected estimates of relatedness can be explained by low sample size (in terms of the number of individuals, colonies and loci used in the study), limited gene flow or a hypothesised mating system where close relatives breed.

The identification of a reproductive female in colonies by morphological characteristics is reliable in this species, while the identification of the breeding male is unreliable when using morphological characteristics. The breeding male(s) do not appear to be resident in the colonies; when and where mating takes place is unknown at this time.

Offspring of the reproductive female appear, in most cases to be full siblings, although multiple paternity was identified in a number of colonies. Whether multiple paternity is within or between litters is unknown at this time. Individuals which were identified as not being the offspring of the reproductive female generally are thought to be her offspring or full siblings but were mistyped or had a null allele or had genotypes that differed from the expected alleles by a single base pair (evidence of mutation).

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Appendix

Genomic DNA extraction

Label sterile tubes; one tube for each sample.

Add 380 µl extraction buffer to each sterile tube.

Extraction buffer consisted of the following reagents: 30 ml 10X TNE, 30 ml 1M Tris pH8, 270 ml dH2O, 20 ml 10% SDS (OR 8 ml 25% SDS). Refrigeration not necessary.

Add a sample to each tube.

Add 20 µl Protinase K to each tube.

Vortex each tube.

Place tubes in a waterbath at 55 °C for 3 hours – overnight.

Vortex occasioanlly during this period.

Picofuge each sample.

Transfer the liquid to labelled sterile tubes.

Add 200 µl 5M NaCl to each tube.

Vortex each tube.

Shake vigorously for approximately 20 minutes.

Add 600 µl IAC (Isoamyl alcohol:Chloroform = 1:24) in a fume cupboard to each tube.

Vortex each tube.

Shake for a further 10 minutes.

Vortex each tube.

Centrifuge at 4000 rpm for 10 minutes.

Transfer the supernatant to labelled sterile tubes (a volume of approximately 400 µl) in a fume cupboard.

Add 650 µl Isopropanol (100 % propan-2-ol) to each tube.

Invert slowly twice, faster 3 times and again slowly twice. DNA may be visible at this stage.

Centrifuge at 12000 rpm for 10 minutes.

Pour off supernatant from each tube.

Add 1 ml cold 70% ethanol to each tube.

Flick each tube to dislodge the DNA pellet.

Invert each tube.

Centrifuge at 12000 rpm for a further 5 minutes.

Pour off the ethanol.

Picofuge each tube.

Remove the remaining ethanol with a micopipette.

Dry each tube (lids off) on a hot plate at 55 °C for 10 – 15 minutes or as soon as dry.

Add 150 µl dH2O to each tube.

Flick each tube to resuspend the extracted DNA.

Picofuge each tube.

Freeze each tube.

1% Agarose Gel

0.8 g Agarose I

80 ml 0.5X TBE

Once the gel had been poured and had set, the DNA was heated at 55 °C for 3 minutes. Five microlitres of 1X dye were added to each well of a microtitre plate lid, along with 2 µl of sample DNA. The gel was run at 125 V for approximately 30 minutes. Once rinsed in water, the gel was placed in ethidium bromide staining medium and quietly shaken for 30 minutes. After another rinse, the gel was placed above UV light and a photograph was taken of the florescent part of the gel.

Table 5: Information related to the samples that were used in the present study.

Colony	Ind ID	Sex	Status*	EF12	DMR3	DMR4	DMR5	DMR7	NCAM
L03	1	F	O	CC	HQ	JJ	BF	BC	AA
	2	F	O	CC	HK	JJ	BF	BC	AB
	3	M	O	AC	HH	JJ	BE	BB	AA
	4	F	R	CC	HQ	CJ	EF	BB	AB
	5	F	O	CC	ND	JJ	EF	BC	AB
	6	F	O	CC	HQ	CJ	BE	BC	AA
	7	F	O	AC	HH	CJ	BE	BC	AA
	8	F	O	AC	HH	JJ	FF	BB	AA
	9	F	O	CC	HH	JJ	BF	BC	AB
	10	M	O	CC	GQ	JJ	BE	BC	AB
	11	F	O	CC	GQ	CJ	BE	BC	AB
	12	M	O	CC	HH	JJ	FF	BB	AA
L01	1	F	O	CE	CC	FH	BE	EE	CD
	2	M	O	CE	CC	CK	BE	BE	DD
	3	F	R	EE	CC	HK	BB	BE	DD
	4	F	O	DE	CC	FK	BB	EE	DD
	5	F	O	CE	CC	CK	BG	BE	DD
	6	F	ND	CC	CC	CH	BG	BE	DD
	7	M	ND	CD	CC	FH	BE	EE	DD
	8	M	O	DE	CC	FK	BG	EE	DD
GRP	1	F	O	BC	ND	DJ	BE	DF	DD
	2	F	R	BC	ND	DH	AB	DF	DD
	3	F	O	BB	HM	HJ	AB	BD	AD
	4	M	O	BC	BH	DJ	AA	DF	AD
	5	M	O	BC	BH	DH	AE	BD	DD
	6	M	O	BC	BH	BD	AE	BF	CD
	7	M	O	BB	HH	BH	AA	BD	CD
	8	ND	ND	BB	BM	BJ	AB	BD	DD
	9	ND	O	BC	ND	DJ	AB	BD	DD
	10	M	O	BD	JP	DF	BD	GG	AC
GAS	1	M	O	BB	JJ	DF	BB	AH	AC
	2	M	O	BD	JP	FK	BB	CG	AC
	3	F	O	BD	FP	DF	BB	AG	AC
	4	F	O	BB	JJ	DF	BD	CG	AC
	5	F	O	BB	FJ	DF	BB	CG	AC
	6	F	O	BB	JJ	DF	BD	AG	AC
	7	F	R	BD	FJ	DK	BD	AG	AA
	8	ND	ND	EF	JL	CG	BB	BC	CC
	9	ND	ND	BD	JJ	GL	BD	AG	AC
	10	ND	O	BB	FP	ND	BB	AC	AC
G42S	1	M	O	CD	EN	EF	DH	BC	CC
	2	M	O	CE	AE	EE	CD	BC	AC
	3	F	ND	CE	AD	CE	BC	BB	AA
	4	F	ND	DE	AE	EF	BH	BB	AC
	5	F	R	CC	CE	CE	CH	BC	AC
	6	ND	O	CC	CO	CE	CH	BB	AC

* O = offspring of reproductive female, R = reproductive individual, ND = not determined.

Table 6: Key (given by locus) to the codes used for allele sizes in the preceeding text.

EF12		DMR3		DMR4		DMR5		DMR7		NCAM	
Code	Allele Size (bp)	Code	Allele Size (bp)	Code	Allele Size (bp)	Code	Allele Size (bp)	Code	Allele Size (bp)	Code	Allele Size (bp)
A	121	A	902	A	195	A	248	A	132	A	238
B	123	B	912	B	203	B	250	B	134	B	244
C	125	C	914	C	205	C	252	C	136	C	248
D	127	D	916	D	209	D	254	D	138	D	250
E	135	E	918	E	213	E	256	E	140	E	
F	139	F	920	F	215	F	258	F	142		
		G	922	G	217	G	260	G	148		
		H	924	H	219	H	268	H	152		
		J	926	J	221						
		K	928	K	227						
		L	930	L	229						
		M	934								
		N	936								
		O	938								
		P	946								
		Q	950								