Aspects of memory in the Damaraland mole-rat, *Cryptomys damarensis*: spatial learning and kin recognition

by

Marna S. Costanzo

Supervisor: Professor N.C. Bennett

Submitted in partial fulfillment of the requirements for the degree of Master of Science (Zoology)

in the

Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

November 2005

Abstract

Supervisor:
Professor N.C. Bennett, Department of Zoology and Entomology,University of Pretoria, Pretoria, 0002, South Africa

African mole-rats (Bathyergidae) exhibit a wide range of social structures ranging from solitary to eusocial. This allows for studies looking at links between sociality and measurable characteristics such as spatial learning and kin-recognition. Furthermore, the existence of species with differing level of sociality allows for comparison between the highly social species and the solitary species.

The existence of differences in spatial learning ability and memory between the sexes has long been debated. Eusocial Damaraland mole-rats (*Cryptomys damarensis*) and solitary Cape mole-rats (*Georychus capensis*) were tested to see if there were sex or species differences in the ability to locate food in an artificial maze task with the express purpose of investigating spatial learning and memory. Measurements of the time taken to complete the task, the distance travelled, wrong turns taken, and the average velocity at which animals travelled were used to compare performance between animals. Both sexes in each of the species exhibited learning and a decay in memory over time. The Damaraland mole-rat exhibited superior learning and memory retention when compared to the Cape mole-rat. Male Cape mole-rats had superior learning and longer term memory retention when compared to females of the same species. There was no significant difference in learning curves between male and female Damaraland mole-rats, but this species did exhibit a tendency for females to show better medium term memory retention while males performed better on long term memory trials. Species differences are likely to be linked to social organization and possibly the resultant burrow-structure in the natural environment, while sex-differences may be due to differing life histories.

Kin-recognition is important in maintaining the social structure and hierarchy in the eusocial species of African mole-rat, *Cryptomys damarensis*. Opposite sex sibling pairs from reproductively quiescent colonies were tested to see if exposure to colony urine odour would reinforce recognition of opposite sex siblings and the concomitant incest avoidance. Control sibling pairs from the same colonies were exposed to water. Mating, social, and non-social behaviours were measured in all sibling pairs. In addition, urinary levels of cortisol, progesterone and testosterone were measured to examine the interaction between four factors: colony olfactory cues (urinary odour), hormone levels, mating behaviour and relatedness. Exposure to urinary odour reinforced recognition and was correlated to a decrease in mating behaviour. Hormonal assays suggest that female hormone levels are modified based on exposure to colony urine odour, while males are not affected. Olfactory cues such as colony urinary odour is linked to the alteration and correlation of hormone levels and mating behaviour.

The Damaraland mole-rat and other species in the family Bathyergidae provide a useful system for investigating aspects of learning and memory, as well as the potential correlation between cognitive processes and sociality.

Keywords: *Cryptomys damarensis*, *Georychus capensis*, sociality, spatial learning, spatial memory, sex differences, species differences, sibling recognition, urinary odour, mating behaviour, hormone levels

Acknowledgements

I would like to thank my supervisor, Professor Nigel Bennett, for his support and guidance and also for allowing me the freedom to explore my areas of interest, even when they did not completely align with his. I thoroughly enjoyed working with you, and am grateful that I was advised by someone whose personal worth extends well beyond his considerable scientific contributions.

I would like to thank my husband, Cory, for his love and support, for countless pages of proofreading, and for many chopped sweet potatoes. Your confidence in my abilities is what inspires me and the encouragement you provide continues to sustain me.

I would like to thank my family for their help and interest in my work. Thank you to my dad for building apparatus and for trapping mole-rats. Thank you to my mom for all her help, especially for all the assistance with administrative tasks. Thank you to my brother for his interest and amusing insights.

Thank you to Dr. Mike van der Linde for the SAS programming used in the statistical portion of this work and also for his forbearance with my project. Thank you also to Sollie Millard for assistance with the statistical methods used.

Thank you to the Plant Pathology department for allowing me the use of their equipment.

Thank you to Professor Nigel Bennett, the NSF, and the University of Pretoria for the funding of my studies.

Thank you to Mrs. Babsie Potgieter for her help in the lab. Thank you to Marietjie Oosthuizen and also to Nigel's other students for their insights and for taking care of my mole-rats periodically.

"Die Here is my sterkte
en my beskermer,
op Hom het ek vertrou.
Hy het my gehelp.
Daarom is ek bly
en wil ek Hom loof…"
Psalm 28:7

Glossary

TTENTRY – Time taken to enter the maze from the start chamber.

TTGENTRY – Time taken to reach the goal chamber.

TTFOOD – Time taken until the first time that food was handled with the mouth.

WTURNS – The number of wrong turns the animal took before reaching and handling the food.

DISTANCE – The distance travelled before handling the food

VELOCITY – The average velocity with which the animal travelled during the trials as determined by DISTANCE / TTFOOD

MTIME – A mean was then taken of all the time variables (TTENTRY, TTGENTRY, and TTFOOD)

WTURNS (G), DISTANCE (G), VELOCITY (G), WTURNS (T), DISTANCE (T), VELOCITY (T) –

In the trials performed using the Cape mole-rat, the variables WTURNS, DISTANCE and VELOCITY were determined twice to reflect each of these measures as observed when the animal first reached the goal (G) and when the animals first handled the food. A mean between these two observations was then determined to give WTURNS, DISTANCE and VELOCITY for the Cape mole-rat.

OSS – Opposite Sex Sibling. Paired opposite sex siblings from the same colony that were either both experimental animals or both control animals.

SSS – Same Sex Sibling. Siblings of the same sex from the same natal colony where one is part of an experimental pair and the other is part of a control pair.

EM – Experimental Male(s). A male mole-rat subjected to experimental conditions (exposed to colony urine).

CM – Control Male(s). A male mole-rat subjected to control conditions (exposed to water).

EF – Experimental Female(s). A female mole-rat subjected to experimental conditions (exposed to colony urine).

CM – Control Female(s). A female mole-rat subjected to control conditions (exposed to water).

MB – Mating Behaviour. Specified behaviour shown to be associated specifically with mating.

SB – Social Behaviour — Behaviour involving interaction with another animal and which is thought to have a social function.

OB – Non-Social Behaviour. — Other behaviour falling outside the categories of mating or social behaviour.

Abbreviations in Tables

CZG – Refers to the name assigned to group of data in SAS.

Dm, Df, Gm, Gf – Refers to Damaraland males, Damaraland females, Georychus males, and Georychus females respectively.

Tables

The data represented in tables throughout the thesis correspond to the following parameters. Where more than one number is combined it refers to the average of all the parameters listed (e.g. D111213 is the average of D11, D12, and D13).

	Damaraland Females		Damaraland Males		Georychus Females		Georychus Males
F1	Name	**M1**	Name	**D1**	Name	**E1**	Name
F2	Sex	**M2**	Sex	**D2**	Sex	**E2**	Sex
F3	Date	**M3**	Date	**D3**	Date	**E3**	Date
F4	Day	**M4**	Day	**D4**	Day	**E4**	Day
F5	Trial	**M5**	Trial	**D5**	Trial	**E5**	Trial
F6	Time	**M6**	Time	**D6**	Time	**E6**	Time
F7	Species	**M7**	Species	**D7**	Species	**E7**	Species
F8	W-norm	**M8**	W-norm	**D8**	W-norm	**E8**	W-norm
F9	W-test	**M9**	W-test	**D9**	W-test	**E9**	W-test
F10	Tttentry	**M10**	Tttentry	**D10**		**E10**	
F11	Ttgentry	**M11**	Ttgentry	**D11**	Tttentry	**E11**	Tttentry
F12	Ttfood	**M12**	Ttfood	**D12**	Ttgentry	**E12**	Ttgentry
F13	D-test	**M13**	D-test	**D13**	Ttfood	**E13**	Ttfood
F14	W-turns	**M14**	W-turns	**D14**	D-test	**E14**	D-test
F15	Dist	**M15**	Dist	**D15**	W-turns	**E15**	W-turns
F16	Velocity	**M16**	Velocity	**D16**	W-turns(G)	**E16**	W-turns(G)
				D17	W-turns(T)	**E17**	W-turns(T)
				D18	Dist(G)	**E18**	Dist(G)
				D19	Dist(T)	**F19**	Dist(T)
				D20	Velocity	**F20**	Velocity

1 Chapter One – Introduction

The influence of an animal's environment on all aspects of its life history is, in evolutionary terms, hugely influential. The environment to which the animal must adapt and respond is perceived through various sensory mechanisms. In mammals, these include sight, sound, smell and touch. Variations in behaviour may result as a consequence of these sensory perceptions. Animal memory (and its influence on ecological and reproductively significant behaviours) represents an important aspect of an animal's interaction with its environment, whether its influence is ecological or social. Memory may be further examined to identify any adaptive value gained from the possession and retention of sensory information. This study deals with aspects of memory in *Cryptomys damarensis*, the Damaraland mole-rat, as the African mole-rat, adapted to a subterranean existence and exhibiting a range of social constructs, provides a valuable system for the study of features of animal memory.

1.1 Animal cognition and behaviour

Animal cognition may be broadly defined as including: "…perception, learning, memory, and decision making, in short all ways in which animals take in information about the world through the senses, process, retain and decide to act on it" (Shettleworth 2001). Some debate exists on how much of animal behaviour is due to active and intelligent processing of information and how much is due to relatively simple processes. Behavioural ecologists have established hypothetical rules of thumb that explain behaviour such as optimal foraging, while proponents of animal cognition might assume that the animal is actually equipped to continually monitor its energy gain through some neural mechanism (Dyer 1994). As with many debates, the possibility exists that some types of behaviour may be governed by simple mechanisms while others rely on specialized neural adaptations, supporting both theories under different circumstances. In addition, more advanced examples of cognition are likely to have evolved from the adaptation of one or more simple processes, making the wisest route that of evaluating animal behaviour on a case by case basis.

While rules of thumb are often behaviour-specific, cognitive interpretations have been generalized into a few proposed dogmas. One of the most influential

systems, the computational-representational model, characterises animal cognition as possessing three stages, namely encoding, computation and representation (Gallistel 1990). Encoding is the perception of information through sensory input, which is translated into electrical signals in an animal's nervous system. Computation is analogous to the transmission of these signals along modifiable neural pathways, which result in learning. Representation corresponds to the resulting permanent configuration of these pathways, i.e. memory (Real 1994). An individual animal's perception and knowledge of its environment, both ecologically and socially, can be expected to forcefully impact its behaviour, thus allowing animal cognition and behaviour to be fruitfully combined in research.

Cognitive abilities in animals are likely to have evolved due to species-specific selection pressures. A common problem in the analysis of animal cognition is that the performance of a given animal in a specific test is a result of both the animal's innate abilities and of the particular situation with which the animal is presented (Kamil 1994). In order to productively research aspects of a particular behaviour, it is important to take into account the animal's ecology and natural history. Leslie Real (1994) states that: "…the only successful way to think about behaviour is to unite its internal mechanistic foundations with its external effects in specific ecological settings." An individual animal's behaviour in aspects such as mating, predation, and dispersion, immediately impacts the population in which it occurs, and, therefore, the study of individual behaviour may lead to greater understanding of higher levels of ecological organisation (Real 1994). Before attempting to investigate cognition and behaviour, an understanding of both the species and the mechanism of the behaviour should be attained.

1.2 African mole-rats: an introduction to the Bathyergidae

The bathyergids are a family of subterranean rodents containing 18 species with five genera and occurring across Africa in a wide spectrum of habitats (Bennett & Faulkes 2000). There are two genera of social species, namely *Heterocephalus* and *Cryptomys,* and three genera of solitary mole-rats, *Heliophobius, Bathyergus,* and *Georychus* (Bennett *et al.* 1999). Species of *Bathyergus, Georychus*, and *Cryptomys* occur in South Africa with habitats ranging from the mesic regions of the Western Cape to the arid Kalahari Desert. The concomitant range of sociality observed in the

bathyergid mole-rats, from solitary to eusocial, have caused the bathyergids to be the subject of many studies on ecology, social structure, and reproduction.

All African mole-rats show specific adaptation to subterranean life and the burrow systems in which they live, including a streamlined body shape and the existence of continually growing extra-buccal incisors used in digging, specifically in the subfamily Georychinae (Jarvis & Bennett 1991). A typical mole-rat burrow system contains a chamber for sleeping, food storage and a toilet chamber. Teeth (Georychinae) or forefeet (Bathyerginae) are used for digging tunnels while foraging for the underground storage organs of geophytes that constitute the diet and only source of hydration for the African mole-rat. Sensory adaptations include a degenerate visual system, which is compensated for by the presence of sensory hairs (vibrissae) used for perceiving tactile cues (present in all genera except *Georychus*) and the use of olfactory and auditory cues (Jarvis & Bennett 1991, Bennett & Faulkes 2000).

The variety of social constructs within the Bathyergidae also constitutes an environmental adaptation and has been linked to the ecological niches occupied by the various species in the "food-aridity" hypothesis. This hypothesis has been developed in order to explain the correlation between social group size and the aridity of the habitat (Faulkes *et al.* 1997, Bennett *et al.* 1999, Bennett & Faulkes 2000). The "food-aridity" hypothesis is based on three premises: (i) geophytes are larger in size but more sparsely distributed in more arid areas; (ii) mole-rats forage for these geophytes "blindly" so that the chance of finding food increases with the number of individuals foraging in arid areas; (iii) the energetic costs of burrowing is higher when the soil is compact and dry, and mole-rats limit their burrowing to times when the soil can be easily excavated after rainfall has occurred (Bennett *et al.* 1999, Jarvis & Bennett 1991, Bennett & Faulkes 2000). Lovegrove and Wissel (1988) have suggested that, in order to increase the chance of finding randomly distributed food resources it would be advantageous for the mole-rats to increase the number of individuals in a colony while decreasing the body size of individuals. A greater number of foragers makes it more likely to find rare and randomly distributed food sources, but a small body size will keep the energy requirements for individuals low, so that the energy demand in the colony as a whole will remain comparative to that of a single large animal (Bennett & Faulkes 2000).

Another theory for the explanation of eusociality in mole-rats postulates that a monogamous mating system and high levels of genetic relatedness among colony members has shaped social evolution (Burda *et al*. 2000). Monogamy and a tendency toward monogyny may very well play an appreciable role in maintaining colony integrity as successive litters of offspring remain full siblings and this increases return on inclusive fitness. Dependence on other animals to provide food during pregnancy and lactation, as well as a slow growth rate in offspring, is further suggested as causes rather than consequences of eusociality (Burda *et al.* 2000).

In addition to ecological aspects of mole-rat biology, reproductive skew and breeding systems represent another intriguing avenue for research. The two eusocial species of African mole-rat, the naked mole-rat and the Damaraland mole-rat, have been exceptionally well-studied in terms of reproductive division of labour. The naked mole-rat, *Heterocephalus glaber*, first popularised sociality studies on mole-rats when it was described as exhibiting a severe form of mammal sociality that has been equated to eusociality in invertebrates such as honeybees and termites (Jarvis 1981). Subsequently, work done on the Damaraland mole-rat, *Cryptomys damarensis*, has shed light on the various fascinating aspects of social organisation and reproduction in this species.

The social (e.g. *Cryptomys darlingi*) and eusocial species (*Heterocephalus glaber* and *Cryptomys damarensis*) of African mole-rats exhibit a high level of reproductive skew (Cooney & Bennett 2000, Clarke *et al.* 2001, Greeff & Bennett 2000, Jarvis 1981). Two models have been proposed to explain how reproductive skew is maintained, namely the dominant control model and the self-restraint model. The dominant control model involves reproductive suppression imposed by a dominant animal through mechanisms such as aggression, blocking of ovulation by pheromone cues, active interference with copulation and even infanticide (Clarke *et al.* 2001). This is the model that has been proposed to explain reproductive skew in the naked mole-rat, where inbreeding is also known to be common (Faulkes *et al.* 1997, Jarvis *et al*. 1994). The self-restraint model does not involve aggression toward subordinate animals in order to maintain reproductive skew, but rather relies on incest avoidance for breeding to be delayed until a non-related mate and other resources become available (Bennett *et al.* 1996, Clarke *et al.* 2001). The self-restraint model seems to be more appropriate to describe the system that is operational in *C.*

damarensis (Clarke *et al.* 2001) and in *C. darlingi* (Greeff & Bennett 2000) where incest avoidance has been observed and outbreeding is obligate.

1.3 The Damaraland mole-rat and the importance of spatial memory

1.3.1 Ecology

The sub-family Georychinae consists of the genera *Georychus* and *Heliophobius,* which are solitary, and *Cryptomys* and *Heterocephalus,* which contain social species (Bennett & Faulkes 2000). The main study animal in these experiments was *Cryptomys damarensis*, the eusocial Damaraland mole-rat, with the solitary Cape mole-rat, *Georychus capensis*, used as a comparative species in the spatial learning and memory experiments. The eusocial Damaraland mole-rat is found in arid areas of south-western Africa and lives in colonies containing from two to upwards of 40 animals, with a mean colony size of eleven.

The Damaraland mole-rat appears to exhibit sexual dimorphism in that the mean body mass of males across colonies (147.5g±38.8g) compared to that of females (116.5g±22.2g) is significantly higher (Bennett & Faulkes 2000). Division of labour occurs by size and sex, with smaller mole-rats engaging more in maintenance work, such as digging and carrying food, than larger mole-rats. Female mole-rats are also more often engaged in foraging and maintenance with males more often engaging in burrow defence (Bennett 1990). There is a linear dominance hierarchy headed by the breeding male. The breeding female is second in dominance and for the rest of the colony males are dominant over females and dominance is also related to body mass (Jarvis & Bennett 1991).

The Damaraland mole-rat lives in complex burrow systems, usually in sandy soil, with a mean depth below the surface of 40cm and a mean diameter of 6.5cm. The fact that these tunnel systems are found relatively deep underground attests to the extremes of ambient temperatures, ranging from -6 to 44°C. During this same period, temperatures within the tunnel system ranged from 19.6-29.3 °C and mean gas values of 20.4% O_2 and 0.4% CO_2 were measured (Roper *et al.* 2001; N.C. Bennett unpubl.). The arid habitat and sparse availability of geophytes provides support for the food-aridity hypothesis as an explanation for the evolution of eusociality in *C. damarensis*.

A colony of 16 Damaraland mole-rats (combined biomass of 2.2kg) were estimated to have excavated 2.6 tonnes of soil in less than two months according to Bennett and Faulkes (2000), which may be converted to around of 2km of new tunnels. Social species of mole-rats have much larger burrow systems than solitary species, a phenomenon that also seems to be related to food availability and large colonies of *C. damarensis* may have home ranges of up to 13,000 m^2 .

Mole-rats seem to forage blindly for geophytes (Jarvis *et al.* 1998) which results in large, sprawling burrow systems being dug as the animals search for food. Foraging animals exhibit different behaviours based on the size of an encountered geophyte (bulb, corm or tuber). Several studies have suggested that small geophytes are consumed preferentially during foraging because of the projected cost involved in storing a small geophyte, while larger geophytes may either be stored or "farmed". "Farmed" geophytes are usually very large tubers or bulbs that are eaten from by the mole-rats and then packed with soil to allow the tuber to continue growing (Bennett & Faulkes 2000).

1.3.2 Mechanisms of spatial memory

Pearce (1997) defines the study of animal memory as being concerned with how information acquired at a particular time is able to influence future behaviour. In particular, questions arise pertaining to the type of information that is acquired and how long it can be retained. Animals may be expected to gain from remembering three aspects of an event: *what* the event was, *where* it took place, and *when* it was experienced (Flaherty 1985). Remembering *where* an event took place falls into the category of spatial memory. If one follows the predictions of the computational-representational model discussed above, a spatial representation implies that there is an ability to measure and manipulate geometric relations among locations in the environment (Gallistel 1990).

It is clear that animal learning and memory, as well as the mechanisms associated with acquiring and implementing this memorised information, is very important in studying animal behaviour. In particular, animals have been demonstrated to have a large capacity for memory associated with food (Pearce 1997). A prime example is Clark's nutcracker, which has been demonstrated to be able to identify close to 3000 previously visited cache locations. In terms of the robustness of animal memory, rats

have been demonstrated to retain conditioned responses for 60 days, which is a considerable length of time taking into account an expected lifespan of three years.

Memory may be divided into long and short-term memory, or as it has been more recently classified, inactive and active memory (Flaherty 1985, Pearce 1997). The rehearsal or consolidation theory is concerned with the mechanism of information storage in long-term memory retention and stresses that rehearsal of new information increases the chance of it being recalled correctly. A second theory, known as retrieval theory, posits that information is immediately and correctly stored and that forgetfulness is due to a failure to retrieve a particular memory at the required time. This theory also states that cues present while the information is required will later be able to stimulate the recalling of the stored information (Pearce 1997).

Short-term, or active memory is a type of information retention that subjects are only able to utilize for a restricted period (Pearce 1997) and is more easily accessible and malleable than inactive memory (Flaherty 1985). Active memory is the type of memory that is being accessed and used during learning (Flaherty 1985). An example of this can be seen in rats' responses to an eight arm radial maze where animals removed halfway through the task of recovering food pellets, were only able to remember which pellets had been collected for up to four hours after the first half of the task had been performed (Pearce 1997). After this time, a rat's recollection of which parts of the maze had been previously visited declines dramatically.

As with memory, the ability to navigate is invaluable for many animals, particularly as related to finding food and returning home. There are a variety of cues and sources of directional information available to animals, and it is likely that many animals make use of the redundancy of information (Pearce 1997). Methods of navigation for short distance travel include the following: pheromone trails, dead reckoning or path integration, piloting with a single landmark, piloting with multiple landmarks, use of geometric relations, and cognitive maps. For long-range navigation and migration, animals use methods such as magnetic fields, air pressure, polarized and ultraviolet light, and landmarks (Pearce 1997, Dyer 1994). True navigation usually refers to animals travelling large distances, while short distance travel makes use of orientation and homing (Able 1980). For the purpose of this study short range methods are most important (excluding those involving landmarks due to the fact that the mole-rats used in this study have reduced visual acuity).

Pheromone trails have been described in ants and silkworms, and mammals such as the loris urinate on their hands and feet in order to create a trail as they move through branches in the forest (Pearce 1997). Dead reckoning is a way of navigating without the use of landmarks; it involves keeping track of one's position in respect of some point of reference other than a landmark. This type of navigation has been identified in ants, bees, and gerbils (Pearce 1997, Dyer 1994). A cognitive map is a mental representation of a space that an animal possesses and, as such, is most often tested for by seeing if an animal can take a detour to reach a familiar goal (Pearce 1997). Other non-visual methods of orientation include the use of the Earth's magnetic field, wind direction, acoustic and odour cues. In terms of odour cues, studies have suggested that even in animals as adept at homing as pigeons, olfactory cues play a significant role. The experimental severing of the olfactory nerves rendered homing pigeons disorientated, pointing to the use of olfactory cues along with other navigational indicators (Able 1980).

It is difficult to quantify the significance of olfaction in navigation and orientation, not because it plays an immaterial role, but rather because animals often deploy all their senses in order to accomplish the task of navigating. A salmon's ability to locate its home stream during migrations has been linked to olfactory cues and olfactory homing has been identified in frogs and newts. Rodents are known to possess acute olfactory senses and may therefore also use odour as a cue in navigation. Olfaction has also been identified as playing a dominant role in foraging among nocturnal, crepuscular blind and burrowing species, as well as cave-dwelling species with good vision (Stoddart 1980).

1.3.3 Aims of this study

Based on the previously discussed ecology of the Damaraland mole-rat it is obvious that navigation in the burrow system must play an integral role in the mole-rats finding previously located food resources, as well as locating the dedicated sleep and toilet chambers. Judging by the complexity of these burrow structures such a navigational system must be well-developed and one could suggest that the animals will also be able to rapidly learn new spatial configurations, as the burrow system may change very suddenly after a period of rainfall. The need to navigate an extensive burrow system without visual cues poses a unique challenge to the mole-rat.

This study aims to first explore the species' general learning curve and memory retention and then attempt to identify proximate mechanisms for orientation in the Damaraland mole-rat. Due to sexual dimorphism, as well as division of labour, one may expect to find different levels of spatial ability among male and female animals. The spatial ability of the eusocial Damaraland mole-rat is compared to that of the solitary Cape mole-rat in order to establish possible correlations with social structure and ecology.

This research is important in terms of tying animal spatial cognition and memory to the natural life history and ecology of a specific animal, thus providing grounds for proposing possible evolutionary adaptations in the expression of a specific level of spatial consciousness.

1.4 Reproductive skew in the Damaraland mole-rat and the importance of kin-recognition

1.4.1 Eusociality in the Bathyergidae

Heterocephalus glaber:

Perhaps the most fascinating reproductive system found in the African mole-rat is that of *Heterocephalus glaber*, the naked mole-rat. These animals exhibit a degree of sociality that conforms to the classic definition used to describe invertebrate eusociality (Michener 1969, Wilson 1971), where there is an overlap of generations, reproductive division of labour, and cooperative care of young (Jarvis 1981). Colonies consist of a breeding pair and their offspring, and colonies exhibit a high degree of relatedness: the mean colony relatedness being R=0.81±0.1 (Reeve *et al.* 1990). Naked mole-rats have furthermore been found to inbreed in the absence of unrelated animals and the succession to reproductive status most often happens from within the colony (Reeve *et al.* 1990, Bennett *et al.* 1999), which in turn explains the high level of relatedness among wild colonies.

Non-breeding female naked mole-rats are reproductively suppressed, which is a state that is maintained by both behavioural and physiological means (Faulkes *et al.* 1991). Non-reproductive females have underdeveloped ovaries (Faulkes *et al.* 1990, Faulkes & Abbott 1993) and show low levels of LH concentrations, as well as a lack of sensitivity to GnRH challenges (Faulkes *et al.* 1990). The reproductive suppression is, however, reversible, with females becoming reproductively active

within eight days after the death of the queen or removal from their natal colony (Bennett *et al.* 1999).

Male naked mole-rats also show reproductive suppression in non-breeders, with non-reproductive males showing lower levels of spermatogenesis in addition to non-motile sperm (Faulkes *et al.* 1994). Non-reproductive males have also been found to exhibit significantly lower levels of urinary testosterone and circulating LH and a decreased sensitivity to GnRH challenges in comparison to breeding males (Faulkes & Abbott 1993, Faulkes *et al.* 1990).

Cryptomys damarensis:

The Damaraland mole-rat is the other species of African mole-rat that exhibits sociality to the extent that it has been described as eusocial. Phylogenetic studies have shown that eusociality in *Cryptomys* evolved separately from eusociality in *Heterocephalus* (Allard & Honeycutt 1992). Wild colonies of Damaraland mole-rats consist of an unrelated breeding pair (Faulkes *et al*. 1997, Burland *et al*. 2002, Bennett & Faulkes 2000), the offspring of the breeding female and possibly a few unrelated males with an average colony size of between eleven and sixteen animals (Bennett *et al.* 1999, Faulkes *et al.* 1997, Jarvis & Bennett 1993, Bennett & Navarro 1997, Bennett & Faulkes 2000). The mean relatedness for breeding pairs is R=0.02±0.04 and the mean relatedness among colony members is R=0.46±0.01 (Burland *et al.* 2002). Offspring of the Damaraland mole-rat are philopatric and colonies display a very high reproductive skew with only 8% of individuals ever achieving direct reproductive success (Jarvis *et al.* 1994). However, in stark contrast to the naked mole-rat where inbreeding has been observed, the Damaraland mole-rat is an obligate outbreeder (Bennett *et al.* 1996, Cooney & Bennett 2000).

Female non-breeders are reproductively suppressed and this state seems to arise from social pressures (Bennett *et al.* 1999), as well as physiological suppression in the presence of the breeding female (Bennett *et al.* 1993, Bennett *et al.* 1999, Molteno & Bennett 2000). Non-reproductive female Damaraland mole-rats exhibit more follicular development in their ovaries than do non-reproductive naked mole-rats (Bennett *et al.* 1994). Like naked mole-rats, non-reproductive Damaraland mole-rat females also show reduced levels of LH concentrations and a decreased sensitivity to GnRH challenges when measured against reproductive females (Bennett *et al.* 1993), although both of these effects are lessened with their removal from their natal

colony (Bennett *et al.* 1996, Molteno & Bennett 2000). Thus, while reproductive suppression is reversible in the Damaraland mole-rat, this does not occur within the natal colony as is possible with naked mole-rats. Queenless colonies of the Damaraland mole-rat remain quiescent after the death of the queen, barring a dispersal event or the introduction of an unrelated animal, and show severe incest avoidance and a high resistance to inbreeding (Bennett *et al.* 1996, Jarvis & Bennett 1993, Bennett *et al.* 1999, Cooney & Bennett 2000).

In non-breeding male Damaraland mole-rats sperm production and motility does not seem to be repressed (Faulkes *et al.* 1994). Furthermore, circulating levels of testosterone and LH, as well as the responses to GnRH challenges, do not differ significantly between breeding and non-breeding males (Bennett *et al.* 1994, Bennett *et al.* 1993). It thus seems that males are socially suppressed and are behaviourally barred from mating due to incest avoidance (Bennett *et al.* 1994, Bennett *et al.* 1993, Jacobs *et al.* 1998).

1.4.2 Mechanisms of kin-recognition

E.O. Wilson defines kin recognition as "the recognition of and discrimination toward various categories of kin" (Wilson 1987). According to this definition the phenomenon of kin recognition can be divided into two parts, namely (i) the "recognition" of kin, involving the mechanisms and cues used to evaluate relatedness and (ii) the "discrimination" toward kin, related to the value and function of being able to recognize kin. This follows the general trend in animal behaviour which often revolves around identifying both the immediate mechanism as well as the evolutionary function associated with the particular behaviour being studied (Fletcher & Michener 1987). Animal recognition may further be divided into three levels of recognition: (i) discrimination between one's own group and a foreign group, such as species discrimination or discrimination between colony members and non-colony members in mole-rats, (ii) discrimination among different classes in a group, such as between siblings and cousins or between different castes in a social system, and (iii) discrimination of individuals, such as mate recognition and parent-offspring recognition.

In order for kin-recognition to exist, the animal must be able to classify other individuals as kin or non-kin by ways of a neural mechanism, which, in turn, leads to

discriminatory behaviour towards different classes of conspecifics (Barnard *et al.* 1991). There are four proposed mechanisms of kin recognition, namely spatial location, familiarity, phenotype matching and recognition alleles (Tang-Martinez 2001, Fletcher & Michener 1987). Spatial memory is probabilistic depending on site specific cues and is useful if an animal is likely to only encounter close relatives at a particular site. Parent birds may use spatial memory by identifying the chicks in its nest as being offspring. Familiarity is also probabilistic and is based on social interactions that are unequivocally associated with relatedness. In this manner interactions between parents and offspring or between siblings in a nest or burrow may lead to familiarity.

Phenotype matching can be used when phenotype correlates strongly with genotype and does not require any previous interaction with the other animal. This requires the comparison of the individual being evaluated to a learned template formed either from the self or a familiar relative. Lastly, recognition alleles encode both the phenotypic cues as well as the ability to recognize them in the absence of any learned template (Fletcher & Michener 1987, Tang-Martinez 2001). It has been argued that familiarity is the only real mechanism of kin-recognition, with differences only appearing in the cues that are used to gain familiarity (Tang-Martinez 2001). The goal of this study is, however, not to prove that specialized pathways for kin-recognition exist but rather to look at the role that urinary odours play as a possible method of kin-discrimination, regardless of whether it is learned or genetic.

There are different classes of cues available in order to facilitate kin-recognition. Self-generated cues, being mainly olfactory or auditory cues, may be used as grounds of comparison in order to distinguish between kin and non-kin (Heth *et al.* 1998, Todrank *et al.* 1998, Tang-Martinez 2001). In the presence of such cues, animals would not need a long term memory of their own phenotype as they would always be able to compare "self" to "other", but animals using direct familiarisation will exhibit "forgetting" after a period of separation (Mino & Tang-Martinez 1999). Another possibility is the use of group-specific cues gained by close association with conspecifics, in which case the animal compares an individual to a known group template (Tang-Martinez 2001). Lastly, there is the possibility of individual recognition, where the animal becomes habituated to a certain individual and consequently classifies that phenotype as known and familiar (Tang-Martinez 2001, Bekoff 1981).

Kin recognition is often defined as arising due to two ultimate causes, namely kin selection and mating preference. Kin selection theory predicts that an animal will be inclined to improve its inclusive fitness and that this will influence its behaviour toward kin and non-kin. Mating preference is associated with inbreeding avoidance in order to avoid the accumulation of deleterious effects associated with incest, although optimal inbreeding or optimal outbreeding may perhaps present the most beneficial genetic results (Fletcher & Michener 1987). Kin-recognition has been described in several species of mammal. Previous studies on Hamadryas baboons have indicated that males are able to assess the relationship between another male and female (Bachmann & Kummer 1980, Kamil 1994). Cheney and Seyfarth (1980) also found that vervet monkeys seem to be cognisant of the relationship between a female and her offspring.

Social signals may be detected by visual, acoustic, tactile and olfactory channels and may be used to distinguish the identity of individuals, families and populations within a species (Stoddart 1980). Odours, including urinary odours, may thus play a role in recognition in some species. For example, in guinea pigs (*Cavia porcellus*), a hystricomorph rodent, urine has been found to play an important role in terms of social odour, specifically during courtship where both males and females are observed to spray the opposite sex with urine (Brown & Macdonald 1985). Rats have been found to be able to discriminate sex based on urinary odours as well as being able to discriminate between two samples from different individuals (Stoddart 1980). Female laboratory mice have been shown to prefer the scent of urine and faeces of a non-related male from the same strain over those of a brother or male from a different strain. This preference may reflect the higher genetic risks of inbreeding (with a brother) or uncontrolled outbreeding (with a different strain) (Gilder & Slater 1978, Stoddart 1980, Todrank *et al.* 1998). It has been demonstrated that that male guinea pigs are able to discriminate the sex of conspecific urine samples as well as differentiate between species, based on the time spent sniffing various samples (Brown & Macdonald 1985). Furthermore, these males showed the ability to discriminate self from other, even though no conclusive evidence was found in terms of being able to identify individuals. This differentiation between different urine samples may be affected by diet and differences in metabolism between the sexes. It is consequently clear that in many rodents the recognition of an individual and

characteristics of that individual may be achieved by odour cues, including urinary odours.

Some effort has been made to identify characteristics of urine that could be related to recognition. Non-volatile compounds (400g/mol or greater) in guinea pigs' urine were found to be important and animals performed poorly when urine was placed out of tactile reach (Brown & Macdonald 1985). It was also found that the early olfactory environment was important in olfactory imprinting which later affects mate-choice in guinea pigs (Beauchamp & Wellington 1981, Brown & MacDonald 1985). Other species of hystricomorph rodents, such as the agoutis (*Dasyprocta punctata*), also urinate in a social context specifically occupying significance between courting animals and mothers and offspring (Kleiman 1974, Rozhnov & Rozhnov 2003).

1.4.3 Aims of study

Social knowledge among group living organisms would be expected to be of the utmost importance in order to establish dominance hierarchies or evaluate the availability of a potential mate. Memory of such social information would be very important as it negates the need to redefine social relationships repeatedly every time another individual is encountered. But, as with all other resources, there may be a predicted equilibrium where the length of retention of social knowledge would be weighed against the likelihood of needing to access that knowledge again. In this manner one would predict that memory resources will be wasted if the particulars of a relationship with an animal that had died is remembered. The length of social memory may thus be a function of how often it is reinforced by encounters with the other individual. In the case of these experiments on the Damaraland mole-rat, the social memory is that of recognising a sibling, while the reinforcement is either colony urinary odour or physical contact with the familiar animal.

Damaraland mole-rat colonies are known to remain quiescent after the death or removal of the queen. As animals exhibit incest avoidance, even when the individual and the colony as a whole lose reproductive fitness, one would expect a sound system of recognition to be in place. Incest avoidance can be used as a measure of recognition, as animals will refrain from engaging in mating behaviour with siblings as long as they are recognised as siblings. Urine has been identified as an important cue in mating and recognition in other rodents. Urinary odour is a candidate for a

mechanism of recognition in mole-rats (O'Riain & Jarvis 1997), although it is very possible that more than one mechanism may exist.

These experiments constitute a preliminary probe into the possibility of urinary odours acting as a proximate mechanism of discrimination between kin and non-kin, and as such functioning as a parameter in the ultimate maintenance of incest avoidance and the maintenance of colony integrity.

1.5 Bibliography—Chapter One

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2 Chapter Two – Spatial Learning and Memory

2.1 Introduction

The need for an efficient method of spatial orientation and memory has been comprehensively discussed in the blind mole-rat, *Spalax ehrenbergi* (Kimchi & Terkel 2001, 2004) and other underground rodents, such as the tuco-tuco, *Ctenomys talarum* (Scleich & Antinuchi 2004). It is posited that due to the increased cost of locomotion during excavation, in contrast to locomotion above ground, subterranean rodents would minimise energy expenditure by choosing the most direct and energy-efficient pathway around an obstacle. Previous studies on the blind mole-rat, *Spalax ehrenbergi*, have indeed found that these subterranean rodents exhibit a well-developed mechanism of spatial orientation and memory. As the blind mole-rat does not have access to external visual landmark cues, path integration has been proposed as a possible mechanism for spatial orientation (Kimchi *et al*. 2004).

Golden hamsters (*Micocrecetus auratus*) have been found to rely on path integration in the absence of other cues, while the presence of landmark cues override internal (idiothetic) signals (Etienne *et al.* 2004). Path integration, also called "dead-reckoning", or the utilisation of the earth's magnetic field are both highly likely mechanisms of orientation used by the Bathyergidae. Magnetic orientation has in fact been demonstrated to exist at both the behavioural and neuronal level in *Cryptomys anselli,* the Zambian mole-rat (Nemec *et al.* 2001).

Kimchi and Terkel (2001) conducted maze learning and memory experiments on three species of rodents that utilise subterranean tunnel systems, namely the laboratory rat (*Rattus norwegicus*), the Levant vole (*Microtus guentheri*), and the blind mole-rat (*Spalax ehrenbergi*), and found that the blind mole-rat exhibited superior spatial memory skills when compared to the other two species. It was concluded that the blind mole-rat requires a better sense of spatial orientation and memory as it inhabited more complex tunnel systems than the other species and spends its entire life underground in the absence of any landmark cues. Subterranean rodents also seem to make use of tactile cues in orienting themselves in mazes, while above-ground species do not seem to be able to use tactile cues as efficiently (Kimchi & Terkel 2004).

In the current study a comparative approach was taken in examining two closely related species with different social systems and differing levels of complexity in their tunnel systems. The Cape mole-rat, *Georychus capensis*, is a solitary species of African mole-rat and has far simpler tunnel geometry than many of the social species (Bennett 1988) (Fig 2.1a). The Damaraland mole-rat, *Cryptomys damarensis*, on the other hand, is a eusocial species with a more complex burrow system, similar to the design found in other species of social *Cryptomys* (Bennett 1988) (Fig 2.1b). Group living marmosets have also been found to possess long-term spatial memory (Menzel & Juno 1985, Real 1994), setting a precedent for investigating the relationship between sociality and spatial memory.

Notwithstanding the discrepancy in both social and burrow structure, both species feed on underground tubers and bulbs and forage in similar ways, thus creating a system that is appropriate for close comparison.

Figure 2.1a Schematic drawing of a *Georychus capensis* burrow system (used with permission from Bennett 1988). Scale in meters.

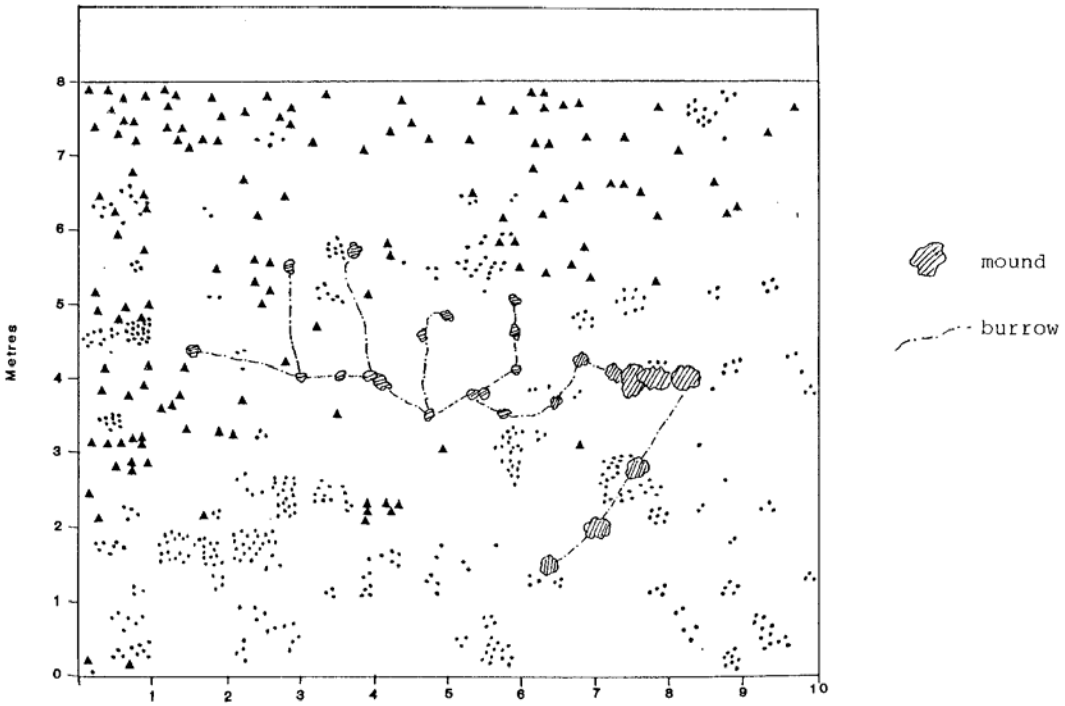
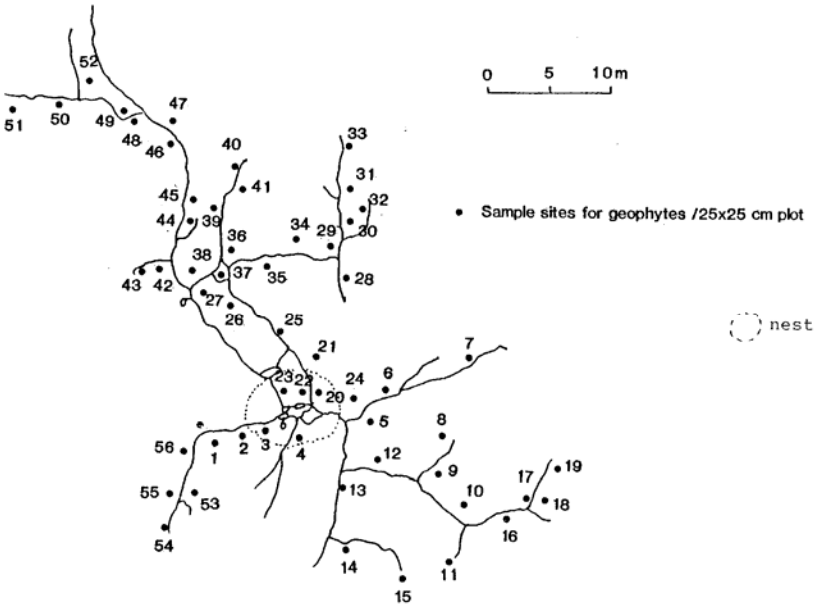


Figure 2.1b Schematic drawing of a *Cryptomys hottentotus* burrow system with similar architecture to *Cryptomys damarensis* burrow systems (used with permission from Bennett 1988).



In yet another species of African mole-rat, the naked mole-rat, odour trails have been implicated in providing pathways to food sources for other colony members, and individual animals were found to recruit colony members to food sources (Judd & Sherman 1996). The concept of odour trails may contribute significantly to the location and re-location of food sources, and as such this was taken into account during the planning of these experiments.

According to the representational-computational model of animal cognition, spatial representation implies the ability to measure and manipulate relationships among landmarks or other cues (Gallistel 1990, Real 1994). This means that care should be taken to match a test with the natural history and ecology of an animal in order to represent a problem that the animal is likely to encounter under natural circumstances. In these trials the maze is an appropriate setting for spatial learning and memory trials precisely because mole-rat burrow systems closely correspond to a maze.

Mole-rats are unable to form a visual representation with the structures needed for image formation. Vision is degenerated by almost 90%, but mole-rats do possess circadian rhythms similar to those of above-ground rodents (Cooper *et al.* 1993, Bronchti *et al*. 1989). Both the naked mole-rat and the Zambian mole-rat have been shown to possess structures beyond those needed for the maintenance of circadian rhythms, but these structures remain much less developed than in more sighted mammals, suggesting that vision is not very important to these fossorial rodents

(Mills & Catania 2004, Nemec *et al.* 2004). This implies that orientation is likely to occur using non-visual cues.

During the design of these experiments circadian rhythms were taken into account to ensure that experiments were conducted during the most active times for each of the species. The solitary Cape mole-rat, *Georychus capensis*, exhibits nocturnal activity patterns (Lovegrove & Papenfus 1995) whereas *Cryptomys damarensis* exhibits diurnal locomotory and feeding behaviour (Lovegrove *et al*. 1993).

Since all animals used in these experiments were subjected to the same conditions and the geometry of the maze was kept consistent, the results obtained from the two species may be used for comparisons of spatial learning and memory both between the sexes within a species and between eusocial and solitary species.

In the Bathyergidae the different species not only exhibit different levels of sociality, but they also exhibit different levels of complexity in the geometry of their tunnel systems. The question therefore arises as to whether social species with more intricate burrow systems constructed by many individuals would exhibit a superior level of spatial learning and memory when compared to solitary species that possessed a simpler burrow system. To explore this possibility, learning curves and memory retention were investigated under similar conditions in both the Cape and Damaraland mole-rats and were compared to determine if a significant difference existed between both the species and sexes.

2.2 Materials & Methods

2.2.1 Study Animals

Cape mole-rats (*Georychus capensis*) were captured at Darling in the western Cape Province, South Africa and Damaraland mole-rats (*Cryptomys damarensis*) at Hotazel in the northern Cape Province, South Africa, and had been resident in the laboratory for a period of at least a month prior to the experiment. Individual Damaraland mole-rats and Cape mole-rats were housed in plastic containers containing wood shavings and fed a diet of sweet potato, carrot, gem squash and apple *ad libitum* (Bennett 1988). The animals were kept in a temperature-controlled room (25°C±1°C) and maintained on a 12L:12D light cycle.

For the Damaraland mole-rat both learning (acquisition) and memory (retention) trials were conducted during the light component of the 12L:12D light

cycle, as this is the time when the Damaraland mole-rat is known to be active (Lovegrove *et al*. 1993; Oosthuizen et al. 2003). Five mole-rats were tested at every period of memory retention. The Cape mole-rat was conditioned and tested during the dark cycle, since they are nocturnal (Lovegrove & Papenfus 1995). A single red light was used to facilitate observation by the experimenter in the dark. Animals appeared to be undisturbed by the presence of this weak light source. Three animals of each sex were used for every period of retention. This smaller sample size was due to the limited availability of study animals.

2.2.2 Apparatus

A clear Perspex maze (Figure 2.2) comprising interlocking sections (approximately 20cm in length with a diameter of 8cm) was used. A similar Perspex apparatus was used to conduct behavioural experiments on Damaraland mole-rat colonies (Cooney & Bennett 2000) and is an appropriate approximation of natural tunnel systems. The maze was cleaned using 30% ethanol prior to commencing each of the animal's two days of habituation and before the memory trial. The maze was not cleaned between trials on the same day. This was done to allow the animal to become habituated to the maze using natural mechanisms of learning, including olfaction. The sections of the maze could be interchanged between animals and experiments so as to avoid any bias that could possibly arise by using the same sections of tunnel in the same position repeatedly.

2.2.3 Procedure

Control females had an average weight of 90.8g (stdev=11.7g) and experimental females had a mean weight of 89.6g (stdev=14.9). For males the mean weight was 108.4g (stdev=23.9) in the control group and 113.8g (stdev=29.7). All animals were paired in order to minimize weight discrepancies.

Animals were deprived of food 24 hours prior to habituation and memory experiments (Kimchi & Terkel 2001). In between the two days of habituation animals were fed enough to keep their body mass stable. The body masses of the animals recovered fully after the experiments. Food deprivation was necessary in order to motivate the animals to explore. Mole-rats used in preliminary trials that had not been deprived of food tended to respond poorly to exploratory behaviour and rather engaged in behaviours such as digging or sleeping showing only a passing interest in

the food chamber. Terkel and Kimchi (2001) found a similar pattern of behavioural response in voles, laboratory rats, and *Spalax ehrenbergi*, and they also used food deprivation to attain a motivational state conducive to maze trials. Food deprivation was also necessary in order to create the conditions for a consistent trend to explore; animals could be expected to be highly motivated to find a particular chamber in the maze, thus minimizing random exploration of the maze.

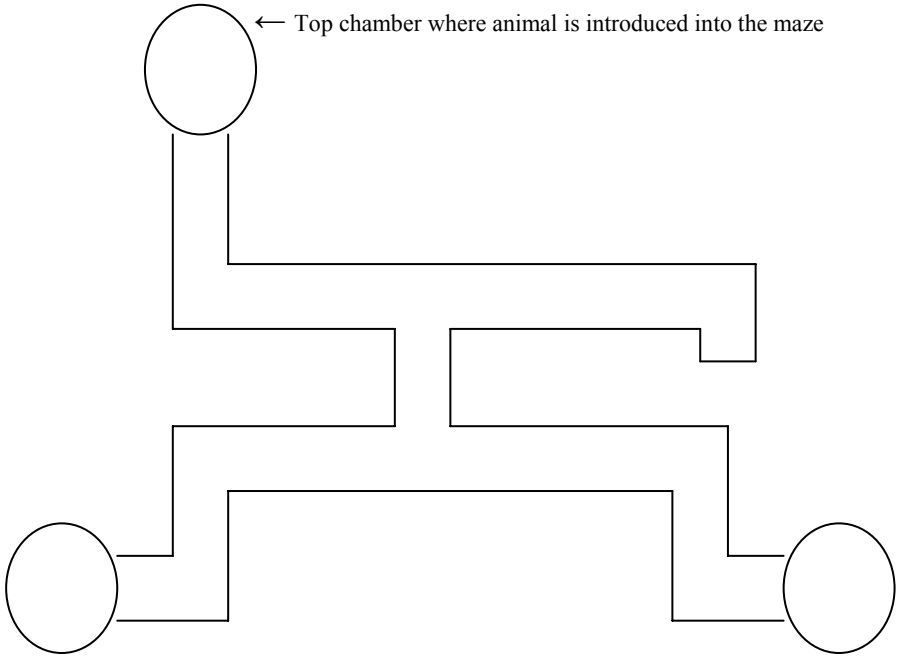
Animals were introduced into the top chamber by means of a small transfer jar or by the use of handling tweezers, while food (a piece of apple) was placed in the bottom left chamber. Care was taken not to cause undue stress in the transfer of the animal and most of the mole-rats seemed undisturbed by the transfer. Animals were timed using a Timex stopwatch and the data were hand recorded on a custom-made data sheet. Once an animal completed the maze, the food chamber was separated from the rest of the maze using a metal trapdoor that was manually inserted in the tunnel leading to the food chamber.

The animal was allowed to eat for 15 seconds, thus ensuring that it was indeed aware of the presence of the food and that hunger was a motivating factor. After 15 seconds the trial was terminated, and the animal was removed and returned to its home cage while the maze was reset. This took approximately 2 minutes, after which the animal was placed in the top chamber again. Conditioning trials were repeated eight times a day for two consecutive days, after which the animals were left undisturbed in their home cages for their respective test periods. Conditioned animals were then subjected to a single trial in order to ascertain memory retention after a period of one, seven, or fifteen days respectively

The following parameters were measured during the trials: Time taken to enter the maze from the start chamber (TTENTRY), time taken to reach the goal chamber (TTGENTRY), time taken until the first time that food was handled with the mouth (TTFOOD), the number of wrong turns the animal took before reaching and handling the food (WTURNS), the distance travelled before handling the food (DISTANCE) and the average velocity with which the animal travelled during the trials (VELOCITY), as determined by DISTANCE / TTFOOD. A mean was then taken of all the time variables (TTENTRY, TTGENTRY, and TTFOOD) to produce MTIME. In the trials performed using the Cape mole-rat, the variables WTURNS, DISTANCE and VELOCITY were determined twice to reflect each of these measures as observed when the animal first reached the goal (WTURNS (G), DISTANCE (G), VELOCITY

(G)) and when the animals first handled the food (WTURNS (T), DISTANCE (T), VELOCITY (T)). A mean between these two observations was then determined to give WTURNS, DISTANCE and VELOCITY for the Cape mole-rat. This was done due to the careful nature of these mole-rats who, unlike the Damaraland mole-rat, very rarely consumed the food upon first discovery but tended to assess the maze further and then return to the food.

Figure 2.2—Maze



2.2.4 Statistical Analysis

All statistical tests were performed using SAS 8.0 (SAS Institute Inc. 1999) and referencing some of the methods that had been used by Kimchi and Terkel (2001) to describe learning and memory in the blind mole-rat. In order to describe the learning curve for each species and gender, regression analysis was run on all the measured parameters against the combined variable Day-Trial (for example, Day-Trial 15 is Trial 5 on Day 1 and Day-Trial 21 is Trial 1 on Day 2) and the inverse of Day-Trial. For all measurements comparisons were only made within a species or within a sex.

Learning experiments

In order to compare the performance in the learning trails between the two species and different sexes, the coefficients obtained from the non-linear regressions

were tested for equality using the following equation to obtain an F-value by performing the Chow test (Davidson *et al.* 1993):

$$F_{k,\, N+M-2k} = \frac{(ESS_R - ESS_{UR})/k}{ESS_{UR}/(N + M - 2k)}$$

where ESS_R is the restricted error sum of squares, ESS_{UR} is the unrestricted sum of squares (which is the sum of the error sums of squares of the individual equations), N + M - 2k represents the combined degrees of freedom for both equations, and k equals the number of restrictions in the model.

Memory experiments

Medium-term memory was determined by finding the difference between performance on the first trial of the second day (trial 9) and the last trial of the first day (trial 8). A large positive value with a significant p-value indicates poor medium-term memory as the animal's performance in trial 9 was significantly worse than in trial 8. A significant negative value would indicate improvement in performance over the gap between trials, with an animal's performance in trial 9 being better than its performance in trial 8. The closer the difference is to 0, the smaller the change in performance was. This is true for all variables except velocity (a higher velocity value equals a faster performance) where a large positive value indicates improvement and a negative value indicates a poor medium-term memory.

Long-term memory was determined by comparing the learning rate as (a-c)/a where a = performance in the first learning trial and c = performance in the last (16^{th}) learning trial to the rate of memory retention (a-b)/a where a = performance in the first learning trial and b = performance in the single memory trial (on Day 4, 9, or 17). A large positive value in the learning rate would indicate that there was a large learning effect. Similarly, a large positive number in the memory retention rate would indicate that a large learning effect could still be observed at the time of memory testing. If the learning rate is significantly higher than the rate of memory retention, it can be concluded that the effect of learning had decreased significantly over the time between the last learning trial and the single memory trial.

To determine whether there were differences between the two species and differences in performance by the different sexes, a general linear model (GLM) was used to determine the effect of the species and sex (SPGN), memory type (MTYPE-learning or memory recall) and the day of the memory trial (MEMDAY). The Type III

results were used, and the Bonferroni correction was used in the reading of all single-point values within GLM results. The Bonferroni correction can be used when several dependent statistical tests are being performed simultaneously (as in the GLM procedure) in order to adjust the alpha value (α=0.05) to reflect the number of comparisons being made (Weisstein 1999). This correction calls for each comparison to be evaluated at a level of α/n, where n is equal to the number of comparisons made.

2.3 Results

2.3.1 Foraging behaviours

A variety of behaviours were observed during the maze trials, including digging (gnawing with teeth against tunnel walls), sleeping, urinating and pumping. All of these behaviours are normally observed in colonies and have been described in the Damaraland mole-rat (Bennett 1988).

The Cape mole-rat was observed more often to exhibit alarm behaviours such as pumping. These animals were also more prone to start digging with their teeth while in the experimental tunnel system, and several slept for long periods of time. In contrast, the most common behaviour observed in the Damaraland mole-rat was urination.

2.3.2 Learning: Time

Time (in seconds) was measured at several stages during the maze experiments, namely Time to Tunnel Entry, Time to Goal Entry and Time to Food. Further measures were Number of Wrong Turns and Distance Travelled (in centimetres). Distance Travelled was divided by Time to Goal in order to determine Velocity (centimetres per second).

In both the Damaraland and Cape mole-rats linear equations for the separate time measurements were fitted to the mean values obtained for each class of animal (Fig.2.3a, 2.3b, 2.3c, 2.3d). The lines fitted to the separate data points provided values of p smaller than 0.05 (see Table 3.1), while the means of the data points generally did not generate significant p-values (data not shown). In addition, linear equations failed to describe the data series adequately, with R^2-values of less than 0.30.

Table 2.1 Mean Time against Day-Trial

Graph Name	Data represented	Intercept (c)	Slope (m)	p-value	R^2
CZG67	Dm M101112	21.35758	156.79858	<.0001	0.3851
	Df F101112	22.01494	169.77973	<.0001	0.3582
	Gm E111213	73.55447	209.64488	<.0001	0.2385
	Gf D111213	42.63565	331.65930	<.0001	0.3887
CZG68	Dm MM10	1.44323	16.59609	<.0001	0.7959
	Df MF10	1.24831	11.59475	<.0001	0.8083
	Gm ME11	-0.55059	38.67806	<.0001	0.8316
	Gf MD11	-11.40119	135.29514	<.0001	0.8958
CZG69	Dm MM11	16.29190	191.67319	<.0001	0.8274
	Df MF11	18.14830	213.02560	<.0001	0.8201
	Gm ME12	109.77347	211.74672	<.0001	0.7221
	Gf MD12	89.34201	369.05216	<.0001	0.7047
CZG70	Dm MM12	47.75081	259.78907	<.0001	0.7622
	Df MF12	53.75885	276.00222	<.0001	0.7026
	Gm ME13	136.02179	358.96338	<.0001	0.7255
	Gf MD13	97.53366	451.30776	<.0001	0.7194

* Please see glossary for explanation of data represented in tables

Time measurements plotted against the inverse of Day-Trial were better fits to the data and generated significant p-values ($p<0.001$) in all cases, and R^2-values above 0.7 for the means of the time variables. It was decided to use the mean values for a specific species and gender, as the study did not attempt to identify individual differences in performance, but rather differences along species and gender lines. The following graphs reflect the equations fitted to the mean data for each species and gender.

Figure 2.3a illustrates that the Cape mole-rat generally takes longer to complete the task than the Damaraland mole-rat. Males in general tend to be faster than females within a particular species.

Figure 2.3a: CZG67
Y: Mean Time (Tunnel Entry, Goal, & Food) (s) against X: 1/ Day-Trial

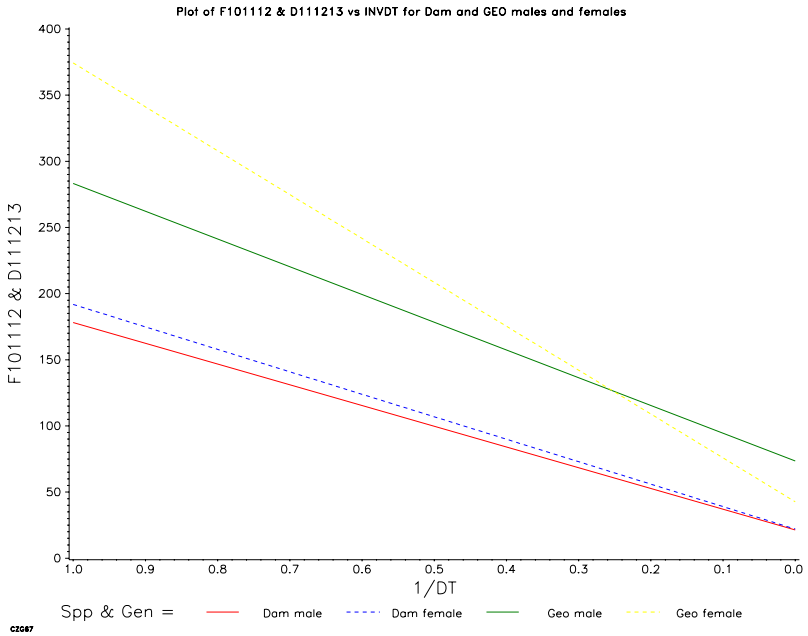


Figure 2.3b shows the mean time to tunnel entry with the Damaraland mole-rat still performing more quickly than the Cape mole-rat, although there does not seem to be a clearly significant pattern. Female Cape mole-rats are an outlier, being much slower to enter the tunnel than any of the other animals.

Figure 2.3b: CZG68
Y: Mean Time to Tunnel Entry (s) against X: 1/ Day-Trial

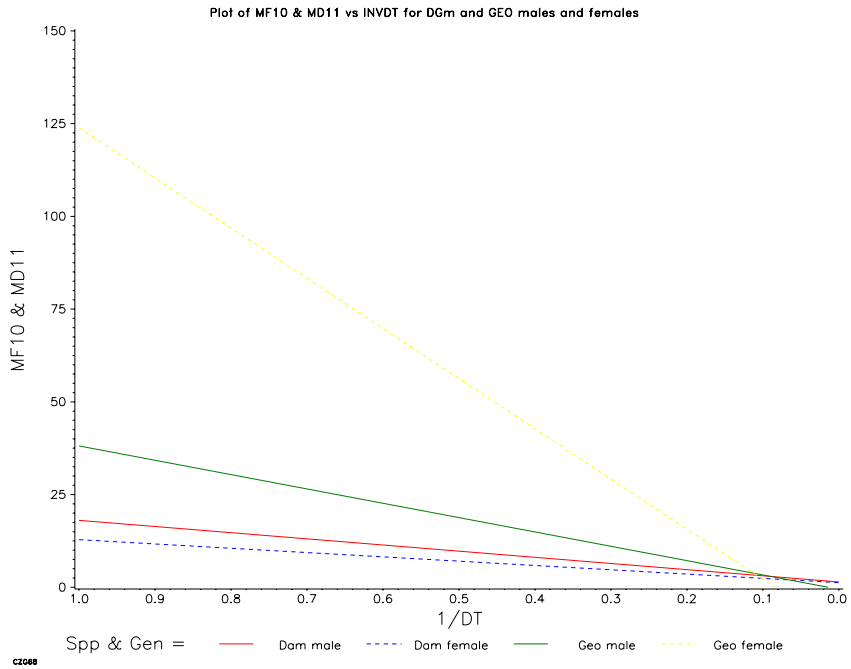
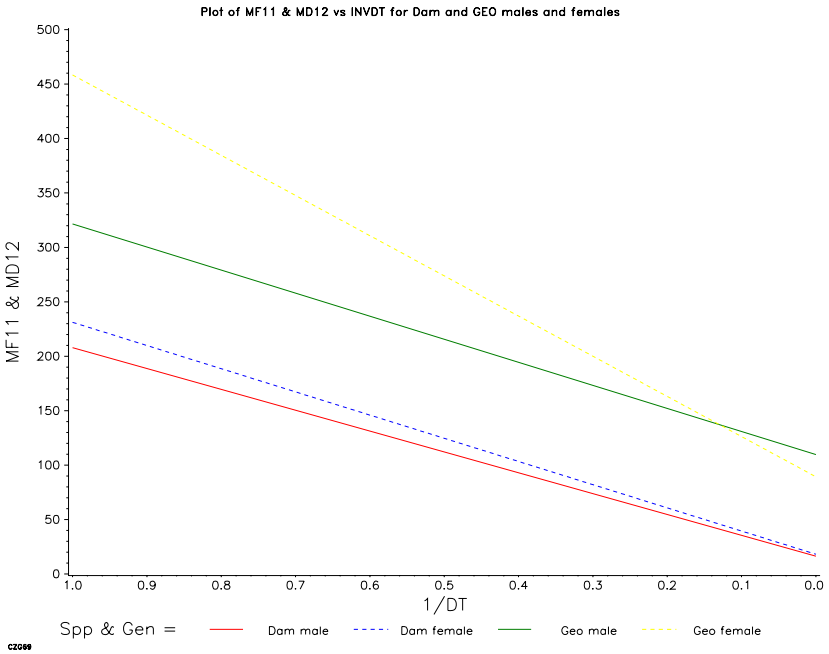


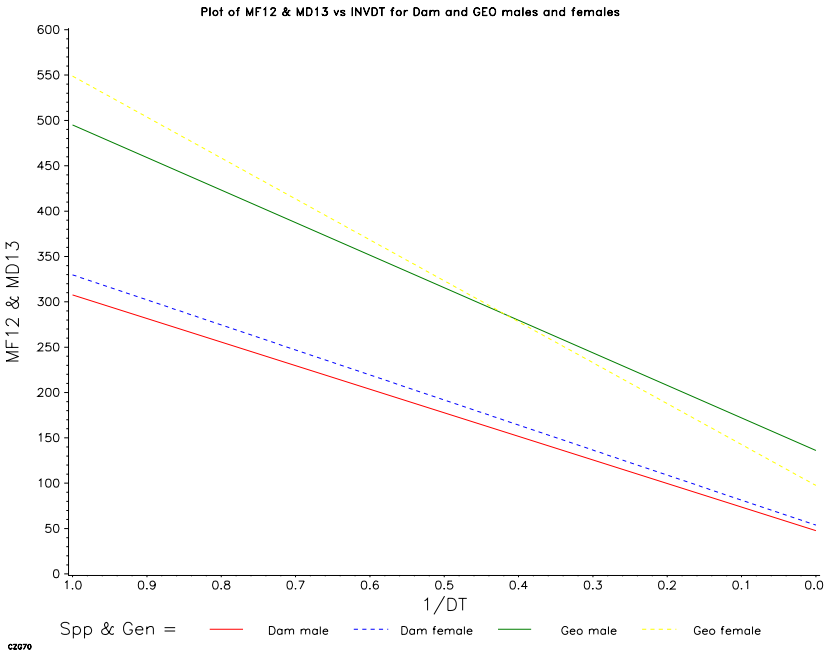
Figure 2.3c illustrates that the Damaraland mole-rat generally reached the goal more quickly than the Cape mole-rat, and once again males were slightly faster than the females of the same species.

Figure 2.3c: CZG69
Y: Mean Time to Goal (s) against X:1/ Day-Trial



Damaraland mole-rats were quicker to start eating the food than the Cape mole-rat on completion of the task, with no clear trend between the sexes (Figure 2.3d).

Figure 2.3d: CZG70
Y: Mean Time to Food (s) against X: 1/ Day-Trial



2.3.3 Learning: Distance travelled

The distance travelled was measured in centimetres and describes the entire path travelled by a mole-rat, including retracing its steps. Linear equations were fitted

to the distance measurements for all groups. Graphs obtained from fitting lines to points plotted for distance versus day-trial did not fit particularly well. The best R^2-values were around 0.6, and most values fell under 0.3 (Table 2.2a). Graphs where distance was plotted against the inverse of day-trial also did not fit particularly well (Table 2.2b) implying that the relationship may be more accurately described by a non-linear equation.

Table 2.2a Distance Travelled against Day-Trial

Graph Name	Data represented	Intercept (c)	Slope (m)	p-value	R^2
CZG179	ME17	628.80151	15.78660	0.0610	0.2286
	MD17	432.65179	40.98617	0.0245	0.3120
CZG180	ME18	1262.67651	-0.39932	0.9732	0.0001
	MD18	935.92262	23.96402	0.2559	0.0911
CZG184	ME1718	945.73901	7.69364	0.3625	0.0595
	MD1718	684.28720	32.47510	0.0901	0.1914
CZG188	MM15	1049.06075	-22.87912	0.2014	0.1138
	ME18	1262.67651	-0.39932	0.9732	0.0001
CZG189	MM15	1049.06075	-22.87912	0.2014	0.1138
	ME1718	945.73901	7.69364	0.3625	0.0595
CZG195	MF15	1034.78765	-10.25143	0.6530	0.0148
	MD1718	684.28720	32.47510	0.0901	0.1914

Table 2.2b Distance Travelled against Inverse Day-Trial

Graph Name	Data represented	Intercept (c)	Slope (m)	p-value	R^2
CZG204	ME17	785.82157	-108.06631	0.5400	0.0274
	MD17	832.56310	-243.87105	0.5337	0.0283
CZG205	ME18	1223.36193	170.00047	0.4642	0.0389
	MD18	1128.10509	54.48164	0.8985	0.0012
CZG209	ME1718	1004.59175	30.96708	0.8550	0.0025
	MD1718	980.33409	-94.69471	0.8123	0.0042
CZG213	MM15	642.72645	1002.67976	0.0009	0.5591
	ME18	1223.36193	170.00047	0.4642	0.0389
CZG214	MM15	642.72645	1002.67976	0.0009	0.5591
	ME1718	1004.59175	30.96708	0.8550	0.0025
CZG220	MF15	785.15489	769.04414	0.0713	0.2138
	MD1718	980.33409	-94.69471	0.8123	0.0042

2.3.4 Learning: Wrong turns

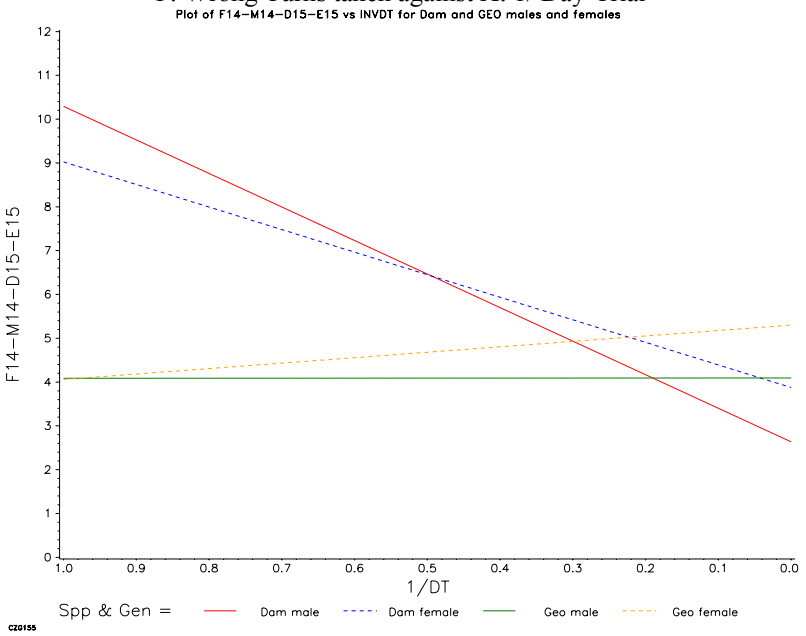
Neither the lines fitted to wrong turns versus day-trial nor those fitted against the inverse of day-trial gave good fits, yielding several p-values larger than 0.05 and no R^2-values better than 0.2 (see Table 2.3). It appears as if the Damaraland mole-rat starts with more wrong turns which decrease over Day-Trial, while the Cape mole-rat starts with fewer wrong turns initially with the number of wrong turns increasing slightly over Day-Trial. However, linear graphs did not adequately represent the data points on which they are based (see R^2 in Table 2.3), and were thus not useful in describing trends.

Table 2.3 Wrong Turns against Day-Trial

Graph Name	Data represented	Intercept (c)	Slope (m)	p-value	R^2
CZG104	Dm M14	5.79537	-0.18090	0.0077	0.0267
	Df F14	5.57758	-0.06760	0.4323	0.0024
	Gm E15	3.54258	0.06586	0.2192	0.0070
	Gm D15	2.57186	0.30069	0.0005	0.0578
CZG107	Dm M14	5.79537	-0.18090	0.0077	0.0267
	Df F14	5.57758	-0.06760	0.4323	0.0024
	Gm E16	6.28685	0.03605	0.6458	0.0010
	Gf D16	5.06974	0.22883	0.0447	0.0198
CZG155	Dm M14	2.63481	7.65583	<.0001	0.1252
	Df F14	3.87612	5.14871	0.0016	0.0384
	Gm E15	4.09444	-0.00856	0.9935	0.0000
	Gm D15	5.30041	-1.24038	0.4579	0.0027

It is clear on the inverse graph (Figure 2.4) that the Damaraland mole-rat appears to reduce the number of wrong turns taken as time progresses, while male and female Cape mole-rats exhibit similar numbers of wrong turns initially, but female Cape mole-rats increase the frequency of wrong turns over time.

Figure 2.4: CZG155
Y: Wrong Turns taken against X: 1/ Day-Trial



2.3.5 Learning: Velocity

Velocity was measured as centimetres travelled per second. Linear equations for velocity plotted against day-trial did not provide good fits, although the fit improved when the averages for all animals were used as opposed to individual data points for each animal (Table 2.4a). When velocity was plotted against the inverse of day-trial, fit improved in most instances to a p-value of less than 0.05 and a R^2-value of larger than 0.6 (Table 2.4b).

Table 2.4a Velocity against Day-Trial

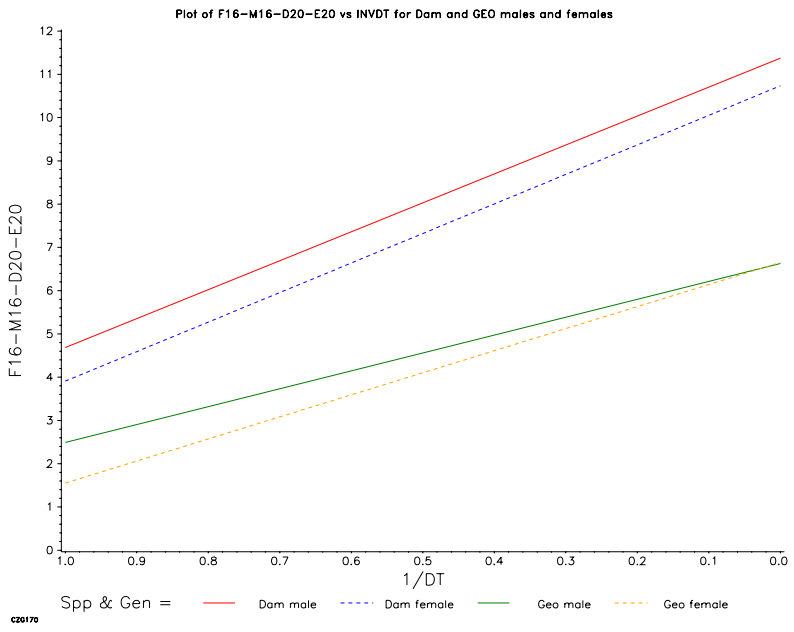
Graph Name	Data represented	Intercept (c)	Slope (m)	p-value	R^2
CZG152	Df F16	10.73408	-6.82601	<.0001	0.1675
	Gf D19	7.08700	-5.26090	<.0001	0.0846
CZG153	Df F16	10.73408	-6.82601	<.0001	0.1675
	Gf D20	6.65107	-5.09832	<.0001	0.0938
CZG154	Df F16	10.73408	-6.82601	<.0001	0.1675
	Gf D1920	6.83372	-5.09311	<.0001	0.0889
CZG167	Dm M16	11.37405	-6.68805	<.0001	0.1491
	Df F16	10.73408	-6.82601	<.0001	0.1675
	Gm E19	6.98774	-4.91520	<.0001	0.1689
	Gf D19	7.08700	-5.26090	<.0001	0.0846
CZG170	Dm M16	11.37405	-6.68805	<.0001	0.1491
	Df F16	10.73408	-6.82601	<.0001	0.1675
	Gm E20	6.62649	-4.13442	<.0001	0.1581
	Gf D20	6.65107	-5.09832	<.0001	0.0938
CZG190	MM16	7.48045	0.29398	0.0033	0.4710
	ME19	3.89302	0.24483	0.0003	0.6227
CZG191	MM16	7.48045	0.29398	0.0033	0.4710
	ME20	4.10504	0.19765	0.0011	0.5422
CZG192	MM16	7.48045	0.29398	0.0033	0.4710
	ME1920	3.93513	0.22063	0.0003	0.6205
CZG196	MF16	6.05643	0.38406	<.0001	0.7851
	MD20	2.80369	0.33994	0.0001	0.6560
CZG197	MF16	6.05643	0.38406	<.0001	0.7851
	MD1920	3.00215	0.33922	0.0001	0.6644

Table 2.4b Velocity against Inverse Day-Trial

Graph Name	Data represented	Intercept (c)	Slope (m)	p-value	R^2
CZG210	ME1920	6.77186	-4.54976	<.0001	0.6751
	MD1920	7.03999	-5.46361	0.0050	0.4411
CZG206	ME19	7.02760	-4.98591	0.0001	0.6608
	MD19	7.28706	-5.62219	0.0061	0.4268
CZG207	ME20	6.67932	-4.23220	0.0002	0.6361
	MD20	6.85234	-5.48605	0.0053	0.4372
CZG215	MM16	11.40167	-6.73189	0.0002	0.6320
	ME19	7.02760	-4.98591	0.0001	0.6608
CZG216	MM16	11.40167	-6.73189	0.0002	0.6320
	ME20	6.67932	-4.23220	0.0002	0.6361
CZG217	MM16	11.40167	-6.73189	0.0002	0.6320
	ME1920	6.77186	-4.54976	<.0001	0.6751
CZG221	MF16	10.78819	-6.94417	0.0001	0.6568
	MD20	6.85234	-5.48605	0.0053	0.4372
CZG222	MF16	10.78819	-6.94417	0.0001	0.6568
	MD1920	7.03999	-5.46361	0.0050	0.4411

It appears that both male and female Damaraland mole-rats travel at a higher velocity than the Cape mole-rats (Figure 2.5). Furthermore, males travel at a slightly higher velocity than females. There also appears to be a larger discrepancy between male and female Damaraland mole-rats when compared to Cape mole-rats of the same sex, though not significantly so. Overall, the velocity of all animals increases as the number of learning trials increase.

Figure 2.5 Y: Velocity in cm/s against X: inverse Day-Trial



2.3.6 Medium-term memory

Table 2.5

VARIABLES	MODEL	1&2	1&3	2&4	3&4
TTENTRY	0.0843	0.6042	0.6450	**0.0154**	0.1234
TTGENTRY	0.4189	0.1052	0.4125	0.2673	0.7048
TTFOOD	0.1820	0.0533	0.8836	0.3515	0.4029
MTIME	0.7008	0.4472	0.6836	0.2673	0.4339
WTURNS	**0.0027**	0.4377	0.2114	**0.0007**	0.0649
DISTANCE	**0.0108**	**0.0456**	0.6701	**0.0010**	0.3930
VELOCITY	0.3223	0.1770	0.3373	0.9852	0.3151

Within the model (Table 2.5), SPGN had the largest influence on WTURNS and DISTANCE (for definitions of the variables discussed here, please refer to Materials and Methods). There is a significant difference between female Damaraland and female Cape mole-rats for TTENTRY, with female Cape mole-rats exhibiting a significantly higher mean time than female Damaraland mole-rats. There is a tendency for female Damaraland mole-rats to perform better than male Damaraland mole-rats in terms of TTFOOD, with female Damaraland mole-rats having improved their performance from trial 8 to trial 9. Female Cape mole-rats perform significantly better than female Damaraland mole-rats in terms of WTURNS, improving their performance between trial 8 and trial 9. The female Damaraland mole-rats exhibit a significantly higher mean DISTANCE on trial 9 than conspecific males. Female Damaraland mole-rats also exhibit a significantly greater mean DISTANCE on trial 9 than on trial 8 as compared to female Cape mole-rats, with these females performing better on trial 9 than they did on trial 8 and thus appearing to improve their memory overnight.

2.3.7 Long-term memory

Table 2.6

VARIABLES	**MODEL**	**MTYPE**	**MEMDAY**	**MTYPE *MEMDAY**	**SPGN**	**MTYPE *SPGN**	**MEMDAY *SPGN**	**MTYPE *MEMDAY *SPGN**
TTENTRY	0.5837	0.1320	0.4383	0.4991	0.3633	0.7607	0.7683	0.4213
TTGENTRY	0.9073	0.7615	0.2065	0.9271	0.5319	0.8623	0.8221	0.8888
TTFOOD	0.5712	*0.0836*	0.2278	0.9886	0.2459	0.8404	0.8818	0.9421
MTIME	0.1366	**0.0186**	*0.0549*	0.8685	0.1620	0.4399	0.6585	0.7669
WTURNS	**0.0122**	0.2427	0.4622	0.5682	0.3741	0.3448	*0.0624*	0.3699
DISTANCE	**0.0029**	0.2939	0.4269	0.4540	0.1301	0.1508	*0.0611*	0.2794
VELOCITY	**<.0001**	**<.0001**	**0.0207**	0.7343	**<.0001**	0.1313	**0.0106**	0.2936

Table 2.6 contains a summary of the p-values obtained by GLM analysis in SAS with significant values in **bold** and values approaching significance in *italics*. The table can be read as a grid and provides each dependent variable on the left with a

p-value, which indicates the significance of the relationship to the various independent variables. From top to bottom the dependent variables are: Time to tunnel entry (TTENTRY), Time to goal entry (TTGENTRY), Time to food (TTFOOD), Mean time (MTIME), Wrong turns taken (WTURNS), Distance travelled (DISTANCE), and Velocity (VELOCITY). From left to right the independent variables are Memory type (MTYPE - long or medium-term), Memory day (MEMDAY - number of days since last learning trial), Memory type and memory day (MTYPE*MEMDAY), Species-sex (SPGN - a combined variable with four possible values that indicates an animal's species and sex), Memory type and Species-sex (MTYPE*SPGN), Memory day and Species-sex (MEMDAY*SPGN), and Memory type as well as Memory day and Species-sex (MTYPE*MEMDAY*SPGN).

2.3.8 MTIME: Long-term memory recall as measured by time

In table 2.6 the only significant difference for MTIME at the model level was for MTYPE, where the value of MTYPE significantly influenced the value obtained for MTIME across all values of MEMDAY and SPGN. The influence of MEMDAY-values on MTIME approached significance.

In order to be able to compare MTYPE 3 (memory recall) between genders, it has to be determined that there is no significant difference in the MTYPE 2 (percentage learned by 16th learning trial) of groups being compared. As can be seen in Table 2.7a, it was found that animals showed no difference in the percentage learning attained during the learning trials. This allows for the further comparison of MTYPE 3 memory between both species and sexes.

Table 2.7a

MTYPE 2 (p-values for GLM comparison)	*C. damarensis* males	*G. capensis* females
C. damarensis females	0.6892	0.8092
G. capensis males	0.6635	0.3412

Three significant p-values were obtained at the level of single comparisons when MTYPE and MEMDAY were kept constant with SPGN being the only variable parameter. In comparing Cape mole-rat males and females for MTYPE 2 on MEMDAY 17 (11&12) it was found that the females tested on day 17 had performed significantly better on learning than the males tested on day 17. Comparing MTYPE 3 and MEMDAY 9 between Damaraland mole-rat males and Cape mole-rat males (17&19) showed that Damaraland mole-rat males performed significantly better than

male Cape mole-rats in terms of memory recall on day 9. Males of both the Damaraland mole-rat and Cape mole-rat also showed a significant difference for MEMTYPE 3 and MEMDAY 17 (21&23) with males of the Damaraland mole-rat performing significantly better than male Cape mole-rats on memory recall on day 17 (see Table 2.7b).

Table 2.7b

MTYPE=, MEMDAY=	Dam males & Dam females	Dam males & Geo males	Dam females & Geo females	Geo males & Geo females
2,4	1&2*	1&3	2&4	3&4
	0.7907	0.7626	0.7905	0.8029
2,9	5&6	5&7	6&8	7&8
	0.6735	0.4233	0.9245	0.8143
2,17	9&10	9&11	10&12	11&12
	0.1460	0.6598	0.7912	**0.0386**
3,4	13&14	13&15	14&16	15&16
	0.9983	0.8482	0.7058	0.8570
3,9	17&18	17&19	18&20	19&20
	0.6175	**0.0221**	0.9079	0.1458
3,17	21&22	21&23	22&24	23&24
	0.3467	**0.0248**	0.8163	0.2505

*The numbers above the p-value refer to the cell in the GLM table where the value was found.

Table 2.7c compares learning and memory performance (MTYPE 2 and MTYPE 3) between the three MEMDAY-values (4, 9 and 17) for each value of SPGN. For each MTYPE separately there are no differences between different MEMDAY-values for a specific SPGN, although two points are approaching significance. Male Cape mole-rats exhibit a tendency to perform faster on MEMDAY 4 than on either MEMDAY 9 or MEMDAY 17 (see Table 2.7c).

Table 2.7c

MTYPE, MEMDAY	*C. damarensis* males	*C. damarensis* females	*G. capensis* males	*G. capensis* females
2,4&9	1&5	2&6	3&7	4&8
	0.7410	0.6574	0.2486	0.4859
2,4&17	1&9	2&10	3&11	4&12
	0.1888	0.7234	0.1051	0.7991
2,9&17	5&9	6&10	7&11	8&12
	0.3234	0.3859	0.5300	0.3023
3,4&9	13&17	14&18	15&19	16&20
	0.8612	0.5223	*0.0510*	0.7823
3,4&17	13&21	14&22	15&23	16&24
	0.8676	0.2950	*0.0567*	0.4684
3,9&17	17&21	18&22	19&23	20&24
	0.9935	0.6817	0.9562	0.6767

The only significant difference between MTYPE for the same SPGN and the same day is for female Cape mole-rats, where the animals tested on MEMDAY 17 performed significantly worse during the memory trial than during the last learning trial (Table 2.7d).

Table 2.7d

MTYPE, MEMDAY	*C. damarensis* males	*C. damarensis* females	*G. capensis* males	*G. capensis* females
2&3,4	1&13	2&14	3&15	4&16
	0.6838	0.9120	0.4769	0.4861
2&3,9	5&17	6&18	7&19	8&20
	0.8014	0.7397	*0.0786*	0.7825
2&3,17	9&21	10&22	11&23	12&24
	0.4558	*0.0908*	0.2766	**0.0446**

2.3.9 WTURNS: Long-term memory recall as measured by wrong turns

Table 2.8a

MTYPE *SPGN	3*DAM M	3*GEO M	3*GEO F	2*DAM F	2*GEO M
3*DAM F	0.7995		0.1219	0.6606	
3*GEO M	0.8738				
3*GEO F		**0.0409**			
2*DAM M	0.8898	0.7499		0.9597	0.6119
2*GEO M		0.8243			
2*GEO F			**0.0291**	0.7841	0.7204

Long-term memory in male and female Cape mole-rats differed significantly when measured by WTURNS —females made significantly more wrong turns than males. Female Cape mole-rats also performed significantly worse on memory recall (MTYPE 3) as measured by WTURNS than they did on the last learning trial (MTYPE 2) (Table 2.8a).

Table 2.8b

MTYPE=, MEMDAY≠	*C. damarensis* males	*C. damarensis* females	*G. capensis* males	*G. capensis* females
2,4&9	1&5	2&6	3&7	4&8
	0.9978	0.3900	0.6075	0.6498
2,4&17	1&9	2&10	3&11	4&12
	0.8292	0.9490	0.7803	**0.0002**
2,9&17	5&9	6&10	7&11	8&12
	0.8271	0.3077	0.7560	**0.0002**
3,4&9	13&17	14&18	15&19	16&20
	0.9609	0.8341	0.5995	0.8853
3,4&17	13&21	14&22	15&23	16&24
	0.9576	0.9855	0.9947	0.7033
3,9&17	17&21	18&22	19&23	20&24
	0.9967	0.8316	0.5494	0.5878

There is a significant difference between MEMDAY 17 and the other days (MEMDAY 4 and MEMDAY 9) for the amount learned by female Cape mole-rats as

measured by WTURNS. The amount learned (MTYPE 2) for animals later tested on MEMDAY 17 is significantly less than the amount learned for the animals later tested on the other days. It is thus not possible to compare MTYPE 3 between these days, as the starting points differ significantly (Table 2.8b).

2.3.10 DIST: Long-term memory recall as measured by distance

Table 2.9a

MTYPE=, MEMDAY=	Dam males & Dam females	Dam males & Geo males	Dam females & Geo females	Geo males & Geo females
2,4	1&2	1&3	2&4	3&4
	0.7487	0.8050	0.9193	0.8812
2,9	5&6	5&7	6&8	7&8
	0.2373	0.6618	0.3119	0.3940
2,17	9&10	9&11	10&12	11&12
	0.9266	0.6458	**<.0001**	**<.0001**
3,4	13&14	13&15	14&16	15&16
	0.7694	0.8161	0.5844	0.9959
3,9	17&18	17&19	18&20	19&20
	0.8558	0.3434	0.4682	0.2620
3,17	21&22	21&23	22&24	23&24
	0.8689	0.7982	0.4891	0.7793

Female Cape mole-rats tested on MEMDAY 17 differed significantly from both female Damaraland mole-rats and male Cape mole-rats in terms of performance on the last learning trial (MTYPE 2) as measured by DISTANCE. Female Cape mole-rats are thus not further compared for performance and memory of animals tested on MEMDAY 17, as these animals did not perform sufficiently in the learning trial and the trial is likely to contain biased data (Table 2.9a).

Table 2.9b

MTYPE=, MEMDAY≠	*C. damarensis* males	*C. damarensis* females	*G. capensis* males	*G. capensis* females
2,4&9	1&5	2&6	3&7	4&8
	0.8042	0.2299	0.6508	0.1645
2,4&17	1&9	2&10	3&11	4&12
	0.7395	0.9367	0.7148	**<.0001**
2,9&17	5&9	6&10	7&11	8&12
	0.9325	0.1576	0.9082	**0.0020**
3,4&9	13&17	14&18	15&19	16&20
	0.9086	0.7217	0.4957	0.6889
3,4&17	13&21	14&22	15&23	16&24
	0.9203	0.9587	0.8850	0.9264
3,9&17	17&21	18&22	19&23	20&24
	0.9882	0.7343	0.3406	0.5893

Table 2.9b confirms that the learning exhibited by the female Cape mole-rats eventually tested on MEMDAY 17 is abnormal, as it differed significantly from the learning of other female Cape mole-rats that were eventually tested on MEMDAY 4

and MEMDAY 9. Learning can be expected to not vary largely within a SPGN-group, as is confirmed by the high p-values observed for all other groups.

The only other significant values obtained for measurements of DISTANCE as a quantifying dimension for memory retention is that of female Cape mole-rats who also differed significantly from all other groups for MTYPE 2 for the animals tested on MEMDAY 17. Other groups did not differ within or between groups for any values of MTYPE or MEMDAY.

2.3.11 VELOCITY: Long-term memory recall as measured by velocity

Table 2.10a

MTYPE *MEMDAY	1: 3*4	2: 3*9	3: 3*17	4: 2*4	5: 2*9
2: 3*9	0.4440				
3: 3*17	0.1033	0.3035			
4: 2*4	*0.0577*	0.1745	0.7191		
5: 2*9		**0.0004**		**0.0392**	
6: 2*17			**0.0044**	**0.0165**	0.7341

In general, animals tested on MEMDAY 9 did significantly better during the last learning trial (MTYPE 2) than during their memory trial (MTYPE 3) (Table 2.10a). The same was true for animals tested on MEMDAY 17, but for animals tested on MEMDAY 4 this observation only approached significance. Animals tested on MEMDAY 4 moved significantly faster than those tested on either MEMDAY 9 or MEMDAY 17.

Table 2.10b

MTYPE=, MEMDAY=	Dam males & Dam females	Dam males & Geo males	Dam females & Geo females	Geo males & Geo females
2,4	1&2	1&3	2&4	3&4
	0.8519	0.1182	**0.0002**	**0.0279**
2,9	5&6	5&7	6&8	7&8
	0.7909	0.3363	**0.0010**	**0.0042**
2,17	9&10	9&11	10&12	11&12
	0.1411	0.2348	0.4643	0.1373
3,4	13&14	13&15	14&16	15&16
	0.8084	0.0941	**<.0001**	**0.0014**
3,9	17&18	17&19	18&20	19&20
	0.9742	0.6997	0.7183	0.3190
3,17	21&22	21&23	22&24	23&24
	0.4875	0.9043	0.1061	0.2159

Females of both the Damaraland and Cape mole-rat differed significantly in terms of VELOCITY exhibited for MTYPE 2, with female Damaraland mole-rats moving significantly faster than female Cape mole-rats for the animals tested at MEMDAY 4 and MEMDAY 9 (Table 2.10b). There is no significant difference for MEMDAY 17, but the female Cape mole-rats tested on MEMDAY 17 were found to

display deviant behaviour as measured by WTURNS and DISTANCE, and this result is thus hard to quantify. Female Damaraland mole-rats also performed significantly better than female Cape mole-rats on the memory trial (MTYPE 2) conducted on MEMDAY 4.

Male Cape mole-rats move significantly faster than females as measured by the average VELOCITY obtained for MTYPE 2 for animals tested at MEMDAY 4 and MEMDAY 9. Furthermore, Cape mole-rats also exhibit a sex-based disparity with faster males and slower females for performance on MEMDAY 4.

Table 2.10c

MTYPE=, MEMDAY≠	*C. damarensis* males	*C. damarensis* females	*G. capensis* males	*G. capensis* females
2,4&9	1&5	2&6	3&7	4&8
	0.7126	0.9361	0.4499	0.1828
2,4&17	1&9	2&10	3&11	4&12
	0.7692	0.4376	0.1704	0.0074
2,9&17	5&9	6&10	7&11	8&12
	0.4451	0.3493	0.3433	0.0955
3,4&9	13&17	14&18	15&19	16&20
	0.5751	0.7836	0.2996	**<.0001**
3,4&17	13&21	14&22	15&23	16&24
	0.6554	0.6426	0.0750	**<.0001**
3,9&17	17&21	18&22	19&23	20&24
	0.9035	0.4337	0.3630	0.1982

Female Cape mole-rats were the only group to exhibit significant differences in memory recall between MEMDAY 4 and 9, as well as between MEMDAY 4 and 17 (Table 2.10c). These females did significantly better on the memory trials on MEMDAY 9 and MEMDAY 17 (with the previously mentioned caveat regarding MEMDAY 17) than they did on MEMDAY 4.

The female Cape mole-rats in general performed significantly worse than all other groups. Male Cape mole-rats generally completed the experimental task significantly slower than their Damaraland mole-rat counterparts.

Table 2.10d

MTYPE *SPGN	3*DAM M	3*DAM F	3*GEO M	3*GEO F	2*DAM M	2*DAM F	2*GEO M
3*DAM F	0.4609						
3*GEO M	**0.0303**						
3*GEO F		**<.0001**	**0.0003**				
2*DAM M	0.3856						
2*DAM F		*0.0654*			0.8003		
2*GEO M			**0.0098**		0.2232		
2*GEO F				**<.0001**		**0.0017**	**0.0282**

Within-sex comparisons between both males and females of the two species revealed that Damaraland mole-rats perform significantly better than Cape mole-rats

for MTYPE 3 (Table 2.10d). Within-species the Cape mole-rat shows a significantly better performance for males over females for MTYPE 3, while Damaraland mole-rats shows no such trend. For MTYPE 2, female Damaraland mole-rats perform significantly better than female Cape mole-rats, but this trend did not extend to the males. The female Cape mole-rats also performed significantly worse than male conspecifics on their last learning trial. Male and female Cape mole-rats performed significantly worse for MTYPE 3 than for MTYPE 2, with female Damaraland mole-rats approaching a significant decrease in velocity between MTYPE 3 and MTYPE 2.

2.4 Discussion

2.4.1 Species differences

General behaviour during the experimentation clearly differed between the species. Cape mole-rats were noticeably more cautious and stressed when entering a novel system than Damaraland mole-rats and this was expressed by the increased frequency of pumping sequences and the enhanced likelihood of retracing their steps at any given point during the experimentation. Cape mole-rats were also observed to take a longer time to enter the maze and explored the maze more thoroughly prior to eating even after having located the food source, once again indicating a higher degree of vigilance. Increased digging behaviour may also reflect an attempt to find an alternate route to the food source (Kimchi & Terkel 2003). Urination during foraging occurred in the Damaraland mole-rat and may be a way of scent marking food for both individual and colony retrieval, as has been noted in naked mole-rats (Judd & Sherman 1996). Of particular interest in the current study is the regular occurrence of pumping, which has been classified as an alarm or threat behaviour in both the Damaraland and Cape mole-rats. Pumping usually occurs when an animal is approaching a perceived source of alarm or threat and is preceded by sniffing of the air. The animal then stops with its hind and forelimbs splayed and its head and tail stretched out. The animal then forcibly pumps its hind region up and down. After a few strokes the animal may pause and sniff before pumping again, or may suddenly retreat backwards along the tunnel (Bennett 1988).

These differences in behaviour are also reflected in the quantitative results obtained from the measurements taken during the experiments. There is a general difference in performance between the Cape mole-rat and Damaraland mole-rat in

both learning and memory, with the Damaraland mole-rat generally learning faster and remembering more accurately. As the burrow geometry of the social Damaraland mole-rat is more complex than that of the Cape mole-rat (Bennett 1988), this finding is consistent with results obtained previously indicating that blind mole-rats have superior spatial memory when compared to rodents inhabiting less complex tunnel systems (Kimchi & Terkel 2001). The difference in burrow geometry may be due to the fact that burrow structures in Damaraland colonies are excavated by several different colony members at different times, reflecting the work of many individuals, while the Cape mole-rat lives in a burrow constructed by itself. Another factor may be the differing energetic costs of burrowing and foraging experienced by Damaraland and Cape mole-rats (see Du Toit, Jarvis & Louw 1985, Jarvis, Bennett & Spinks 1998, White 2005). The Damaraland mole-rat clearly exhibited a much faster rate of learning than the Cape mole-rat. This finding may be due to superior spatial skills exhibited by the Damaraland mole-rats, or could potentially be an artefact of the more cautious exploration style of the Cape mole-rat.

While there was no significant difference between the males of the two species for medium term memory retention, female Cape mole-rats showed overall better medium memory retention than female Damaraland mole-rats. The female Damaraland mole-rats nevertheless still moved more quickly than female Cape mole-rats, but did so less efficiently, making a greater number of wrong turns and travelling longer distances. These factors indicate that the Cape mole-rats were achieving good times based on their memory of the layout of the maze, and not because of any behavioural or speed effects.

The overall performances in long-term memory trials were poorer than the performances on the last learning trial for both species. This illustrates that performance, as measured by time, is indeed based on memory, since memory recall improves throughout learning trials but fades over time if it is not reinforced. This general trend is also expected if the type of memory being accessed has changed from short term, active memory into long-term memory (Pearce 1997). Overall, the Damaraland mole-rat exhibits superior long-term memory retention to that of the Cape mole-rat. This is not surprising when one takes into account the complexity of the geometry of the burrow systems that are inhabited by Damaraland mole-rats, where the geometry is constantly in a state of dynamic flux and where several animals are responsible for modifications to the tunnel system (Bennett & Faulkes 2000).

The measurement of wrong turns used as a method of quantifying learning and memory did not appear to be particularly useful in between-species comparisons. There was also no difference within species in terms of the distance travelled to complete the experimental task. There were, however, obvious differences in the velocities obtained by animals of different species but of the same sex, and the time and velocity measured during these trials were most useful for comparison. Female Damaraland mole-rats appeared to be better than female Cape mole-rats at learning the path through the experimental maze, since they travelled faster and were less hesitant about the direction they were taking. This difference in velocity may also be due to female Damaraland mole-rats being less cautious during their exploration of the maze, possibly explained by the fact that the eusocial colony structure equates to there being other mole-rats patrolling and guarding the tunnel system (Bennett & Faulkes 2000), making the burrow system of the Damaraland mole-rat generally safer than that of the solitary Cape mole-rat.

2.4.2 Sex differences

Previous studies have indicated that there are no observable sex differences for spatial ability in the solitary blind mole-rat, *Spalax ehrenbergi*, when confronted with a maze learning trial (Kimchi & Terkel 2001). Other studies did, however, indicate that the blind mole-rat may exhibit some sex-dependent differences in burrow-structure (Marhold *et al.* 2000). The existence of certain sex-dependent differences pertaining to spatial organization and the difficulty of conclusively quantifying such differences through experimentation is reflected in the results obtained with the maze trial conducted on the Damaraland and Cape mole-rats. There were no conclusive differences in the degree of learning between the sexes in either species, although males appeared to perform slightly more efficiently than females in most of the measurements used. This disparity in learning may be linked to the general larger size of males, making them less cautious than females or it could reflect a more cognitive base due to differing spatial abilities. The possibility that female hormonal-levels may play a role also exists, and a connection between hormones and spatial ability had previously been demonstrated in humans (Chabanne, Peruch & Thinus-Blanc 2004).

The observation that female Cape mole-rats are extremely cautious is supported by their performance in learning and shorter and medium term memory

trials as measured by velocity. Female Cape mole-rats move significantly slower than male Cape mole-rats. This effect diminishes as the time between the last learning trial and the memory trial increases but does not necessarily indicate that these females are now moving faster, but rather that male Cape mole-rats become more cautious if they have not visited a certain part of the burrow system for a period of time. The effect could, conversely, be due to a better memory retention on the part of the female Cape mole-rats, which is manifested in a smaller decrease in the general velocity with which they travel. Male Damaraland mole-rats appeared to have significantly better medium-term memory retention than their female counterparts as measured by distance travelled although females tended to move faster to get to the food resource, making it difficult to determine whether one sex truly out performed the other in terms of medium-term memory.

Further comparison between the sexes within a single species gave equivocal results. No absolute difference was found between sexes in either the Damaraland mole-rat or the Cape mole-rat, although certain trends were seen that may reveal more striking differences when further explored. Measurements of wrong turns taken did not fit any simple curve, and were thus not useful in this analysis, while distance travelled did not reveal any disparity between the sexes. Measurements of velocity revealed a superior performance by female Damaraland mole-rats when compared to males of the same species on MEMDAY 4 memory trials. In contrast, male Cape mole-rats completed the maze faster on MEMDAY 4 memory trials than Cape mole-rat females. These findings should be further explored by looking at possible differences between normal behaviour within the sexes, which may require different levels of medium term memory. In the Damaraland mole-rat males are on average larger than females, and it has been observed that larger animals are more frequently involved in colony-defence (Bennett 1990). This ties in to the hypothesis that shorter-term memory is more important for remembering food sources as the location of new tuber sites are likely to change frequently, making longer-term memory investment unnecessary. Conversely, long-term memory is more important in terms of defending the burrow system and remembering where previously threatening situations occurred has long-term benefits.

A recent review of the literature on navigational sex-differences in rats and mice found that, while male rats seem to have a clear advantage over females in most spatial tasks, the existence of sex-differences in mice is much less obvious (Jonasson

2005). Some of these sex-differences appeared to be linked to the specific protocol used, while others were more robust. Measurements from the current experiment generally indicate that there is a significant difference in the performance of the two sexes for the Cape mole-rat but not for Damaraland mole-rat—female Cape mole-rats perform significantly worse than males, while male and female Damaraland mole-rats learn and retain at comparable levels with slight variation in terms of long and medium term memory. As this is the first experiment of this kind comparing Damaraland and Cape mole-rats, the influence that experimental protocols may have on the results is unclear. It is, however likely, given the attention paid to producing a system comparable to the natural environment of these mole-rats, that the sex-difference in the Cape mole-rat is not merely an experimental artefact.

2.5 Conclusion

Among the two species of mole-rat, spatial memory and navigation appears to be correlated with the level of burrow-complexity (Kimchi & Terkel 2001, current study) and sociality (Menzel & Juno 1985, Real 1994). It should be considered that the within species sex-differences noted for the Cape mole-rat may also be influenced by the social system in this species. Further experimentation involving species of intermediate sociality, as well as mazes of differing complexity, may shed light on whether or not sex-differences in spatial ability are present in cooperative mole-rat species and what the specific effect of burrow-complexity is in relation to sociality.

The experiments conducted here focused on identifying whether or not spatial learning and memory differs between species and between the sexes within a species. It is not immediately clear what the cues are that Cape and Damaraland mole-rats use to facilitate learning and memory of spatial information, and whether or not those cues differ between the species and sexes. The representational-computational model of spatial representation (Gallistel 1990, Real 1994) adequately describes the results obtained here, showing that learning and memory is fundamental to the navigational skills of these two species of mole-rats. Further experiments may focus on examining the use of specific cues, such as magnetic orientation (Nemec *et al.* 2001), path integration (Kimchi *et al*. 2004, Etienne *et al.* 2004), and odour cues (Judd & Sherman 1996) in order to facilitate spatial learning and memory.

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3 Chapter Three – Kin-recognition in *Cryptomys damarensis*

3.1 Introduction

Kin-recognition in the social Bathyergidae has previously been explored in both the naked mole-rat, *Heterocephalus glaber* (O'Riain & Jarvis 1997) and the Damaraland mole-rat *Cryptomys damarensis* (Jacobs *et al*. 1998, Jacobs & Kuiper 2000), with emphasis primarily on the well-documented xenophobia exhibited in these species. Jacobs *et al.* (1998) noted that in the Damaraland mole-rat, foreign animals introduced into a colony were readily identified and were either attacked or solicited for mating, depending on the sex of the animals involved. In all cases foreign animals were treated differentially from animals natal to the colony.

Many behavioural studies, including the present one, use the occurrence of kin-discrimination (acting differentially toward kin and non-kin) to provide evidence for the existence of kin-recognition. It is important to bear in mind that, although kin-discrimination is logically always dependent on some method of kin-recognition, kin-recognition only leads to kin-discrimination where the benefits outweigh the costs (Waldman 1988). Thus, while the presence of kin-discrimination demonstrates the existence of kin-recognition, the absence of kin-discrimination does not disprove the existence of kin-recognition.

A number of studies carried out on different families of mole-rats have shown that recognition and discrimination do appear in both the social and solitary species of these subterranean rodents. The subterranean nature of mole-rats, coupled with their reduced visual system, has resulted in olfactory and tactile cues gaining importance in interaction with other animals, including as methods of recognition. Studies conducted on the blind mole-rat, *Spalax ehrenbergi*, indicate that these solitary animals are able to distinguish sex and seasonal breeding cues based on urinary odours of other blind mole-rats (Heth *et al.* 1996). In the social and eusocial species of the Bathyergidae, kin-recognition is predicted to play an even more important role. The naked mole-rat, is able to recognise and distinguish between its own colony odour and both blank and foreign colony odours as represented by nesting and toilet material (O'Riain & Jarvis 1997). Furthermore, the high degree of xenophobia exhibited by both the naked mole-rat (O'Riain & Jarvis 1997) and Damaraland mole-

rat (Bennett & Faulkes 2000, Jacobs *et al*. 1998) when confronted with foreign animals lends support for the existence of some form of kin-recognition.

Previous studies on the Bathyergidae have found evidence for both kin-recognition in the naked mole-rat (O'Riain & Jarvis 1997) and individual recognition in the Damaraland mole-rat (Jacobs & Kuiper 2000). Male Damaraland mole-rats that were separated from each other, but not from the rest of the colony, directed more aggressive behaviour towards each other than towards any other colony member. This study concluded that recognition is more likely to be based on familiarity and learned cues than on recognition alleles or phenotype self-referencing.

As an obligate outbreeder, the Damaraland mole-rat is dependent on a mechanism such as kin-recognition to facilitate incest avoidance. Maximising inclusive fitness (indirect fitness gained through the fitness of close relatives) is important to non-breeding mole-rats, due to their philopatric nature and the limited breeding opportunity available to most individuals in a colony. In fact, Damaraland mole-rats demonstrate kin-recognition based on more than simple spatial location, either exhibiting aggression toward foreign animals (Jacobs *et al*. 1998) or initiating mating behaviour with a foreign animal of the opposite sex (Jarvis & Bennett 1993, Rickard & Bennett 1997) even when the foreign animal is encountered within the confines of the natal colony and tunnel system. In the Damaraland mole-rat, the benefits gained from avoiding inbreeding depression (the accumulation of deleterious genes) have to be weighed against the cost of maintaining familiarity with siblings. There is no utility in remembering a mole-rat that has not been encountered for several weeks, as the other animal is likely to have dispersed or died, and any resources directed towards maintaining a memory that has not been recently reinforced are likely wasted.

While it is clear from the abovementioned publications that the Damaraland mole-rat exhibits some level of kin-recognition, specifically as observed in terms of incest avoidance and xenophobia, the cues used to determine kinship are as yet unclear. There are two possible types of cues that may be used for kin-recognition, namely indirect or probabilistic (based on properties of the circumstance rather than characteristics of the individual itself) or direct (based on perceived traits known to be expressed by kin) (Waldman 1988, Tang-Martinez 2001). The closed burrow system of mole-rats favours the use of a simple mechanism such as spatially-based recognition and shared colony cues due to predictable circumstances such as huddling

in the nest. Yet, the addition of eusociality, a dominance hierarchy, and obligate outbreeding complicate the circumstances under which recognition is likely to take place. In Damaraland mole-rats it is likely that the mole-rats could express direct recognition of kin based on the likelihood that non-kin will sometimes be encountered within the natal burrow system (Bennett & Faulkes 2000), and laboratory studies have demonstrated discrimination directed at unfamiliar animals within a captive colony in a laboratory setting (Jacobs & Kuiper 2000, Jacobs *et al*. 1998). Direct recognition may be further divided between mechanisms relying on the genetic basis of traits (perceived genetic similarity to another animal through self-referent phenotype matching or recognition alleles) and mechanisms relying on habituation (familiarity based on previous association with kin) (Waldman 1988). Kin-recognition based on familiarity requires a process of learning and a mechanism of memory, while kin-recognition based on relatedness may employ, but does not require, mechanisms of learning or memory.

3.1.1 Recognition by descent: genetics, relatedness and phenotype matching

Phenotype matching is based on a learned template (either from self or parents) (Lacy & Sherman 1983, Tang-Martinez 2001). Phenotype matching can be used both for individual recognition (where individuals are recognized for their unique traits) and class recognition (recognition of classes of animals such as close kin or non-kin). Crozier (1987) notes that it seems nearly impossible to definitively determine whether self-referent phenotype matching or recognition alleles are being used when recognition is based on genetic relatedness. It is, however, possible to either support or dispute the existence of a direct genetically-based mechanism for recognition without identifying the exact functioning of that mechanism.

In the Damaraland mole-rat, the life-history of the animal makes the evolution of more than one kind of proximate mechanism of recognition possible. Intra-colony relatedness is higher than inter-colony relatedness (Burland *et al.* 2002), providing a possible basis for genetically-based recognition in situations where burrow systems could overlap. However, colonies with two to three breeding males have been described in the Damaraland mole-rat, hence forming colonies where non-breeders consist of both full and half siblings (Jarvis & Bennett 1993, Burland *et al*. 2004). Recent findings show that conditions exist in the wild where non-breeding females do

come into contact with unrelated males. This is due to immigration into the colony and there have been several observations of multiple paternity (Faulkes *et al.* 2004). Systems in which multiple paternity is observed may lend themselves to the evolution of phenotype matching in order to direct discriminatory behaviour towards siblings or half-siblings. Thus, while colonies where kin-discrimination is based on degree of genetic relatedness have not been described in the Damaraland mole-rat, the conditions that may lead to the evolution of such discrimination do exist. It should be noted, however, that accurate phenotype matching requires the assessment of a large number of genetically-defined traits and may not be selected for in situations where differential association with close kin may be expected (Lacy & Sherman 1983). It is clear that the determination of relatedness among mole-rats used in kin-discrimination studies may be useful in gaining further insight into the mechanisms involved in kin-recognition.

In the case of a colony with a single breeding pair, individual genetic recognition would be difficult, as all animals in a colony (with the exception of the unrelated breeding pair) will be related by an average of approximately 0.5 (reflective of sibling-sibling relatedness or parent-offspring relatedness). This situation will, however, allow class recognition of animals that are closely related (siblings or parents for non-breeders and offspring for breeders) and animals that are not closely related (potential breeding opportunities or breeding threats for non-breeders and breeders alike), which may consequently be used in colony recognition. Because of the system of social cooperation and the possible ambiguity of the class of a closely related animal encountered in the burrow system (sibling, parent or offspring; fitness opportunity or fitness threat), genetically-based individual recognition is likely to be supported by some level of familiarity. Due to spatial overlap in the closed burrow system, close kin predictably huddle together and use the same toilet chamber. This provides a basis for familiarity-based recognition. It may then be predicted that genetically-based individual recognition is likely to exist in conjunction with recognition based on familiarity, but colony recognition, on the other hand, could exist independently from any familiarity.

3.1.2 Recognition by association: familiarity and habituation

Although phenotype matching is possible within the context of the ecology of *Cryptomys damarensis*, colony recognition in Damaraland mole-rat colonies is

apparently based on colony odour and familiarity and not on genetic relatedness (Bennett & Faulkes 2000, Jacobs & Kuiper 2000).

Associative recognition in animals may be simplified by dividing it into two levels that are not mutually exclusive, namely kin recognition (recognising a class of individuals, including whether an animal is kin or non-kin) and individual recognition (recognising one particular individual among other, similar specimens). While kin recognition evolves with the ultimate goals of incest avoidance and kin-selection, individual recognition evolves in association with dominance hierarchies or social altruism (for review, see Crozier 1987, Tang-Martinez 2001, Waldman 1988).

The eusocial structure and life-history of the Damaraland mole-rat favours the existence of both individual and kin-recognition. Damaraland mole-rats exhibit a dominance hierarchy (Bennett & Faulkes 2000) and monogamy with examples of monogyny (Jarvis & Bennett 1993, Burland *et al*. 2002, 2004), making the ability to recognise individuals desirable. Individual recognition is, however, not essential—it is possible for an animal to judge dominance based on perceived characteristics such as size. Breeders may also be recognised by the manifestation of differential physical and chemical factors. Social compensation, which demands individual recognition, has not been specifically noted, with little evidence of phenomena such as reciprocal grooming, although allogrooming does take place (Bennett 1988).

In a study by Jacobs and Kuiper (2000), agonistic interactions between males were used as a measure of recognition, and it was found that the Damaraland mole-rat appears to use individually distinct cues rather than cues based on genetic relatedness. Although care was taken to assure that any aggression was due to unfamiliarity, rather than other factors such as dominance hierarchies, the findings of the experiment left room for further evaluation of the type of proximate mechanism of kin-recognition in Damaraland mole-rats. Furthermore, although one may expect any two non-breeding animals within an intact colony to be siblings, genetic data to confirm this supposition was not available. The same study indicated that it is likely that recognition is based on individual-specific cues rather than recognition based on colony-specific cues (as was found in the naked mole-rat), and the design of the present study aims to further explore that finding. In addition, the effect of stress and hormonal fluctuations was beyond the scope of this previous study. The current study aims to build on the existing evidence pointing towards kin-recognition in the Damaraland mole-rat based

on learned spatial or temporal cues rather than on a direct estimation of genetic relatedness.

3.1.3 Smelly relatives: the role of chemical perception in recognition

Every species of vertebrate has a sense aimed at chemical perception and in the Bathyergidae this replaces the secondarily lost capacity to perceive light (Stoddart 1980). In colonies of the naked mole-rat unfamiliar animals are probably detected by olfactory cues due to the lack of visual acuity in mole-rats and the fact that an unfamiliar animal is always sniffed when first encountered in the burrow system (O'Riain & Jarvis 1997).

The olfactory system in vertebrates is relatively simple and is briefly described here (for further detail see Stoddart 1980). An animal is only required to be close enough to the source of odour for a few scented molecules to enter its nose where sensory cells are covered by a thin layer of mucus. The scented molecule is then perceived by the sensory cell, which is linked to the olfactory area of the brain. In mammals the olfactory epithelium consists of a basal membrane supporting receptor cells, ciliated support cells, basal cells, and the mucus-secreting Bowman's glands. An odorant triggers the adsorption site on the cilia and travels towards the knob from where it continues along the neural pathway to the brain. Olfactory bulbs lying beneath the cerebral hemispheres function to process odour signals before they reach the brain proper. In rats it has been found that the pre-optic area (consisting of the hypothalamus, prepiriform complex and amygdaloid nucleus) responds more strongly to urine odours, while the olfactory bulbs respond more strongly to non-urine odours. Sniffing in rats has been found to contribute to the guiding of air that contains odorous molecules towards the olfactory organ by an active sniff cycle (Stoddart 1980).

Self-produced odours in vertebrates are usually aimed at conspecifics and are most commonly derived from elimination procedures such as urination and defecation (Stoddart 1980). In rodents, active marking behaviour includes anal drag, urination on the ground, salivation and scratching of lateral glands. In social mole-rats it has been suggested that behaviour in the toilet chamber may play a role in maintaining familiarity with other colony members (Bennett & Faulkes 2000). Urinary odours have been previously identified as a method of marking in naked mole-rats, and these

mole-rats have been shown to discriminate between urinary odours arising from their own colony and those of different colonies (O'Riain & Jarvis 1997). However, Faulkes *et al.* (1990) found that urinary primer hormones are unlikely to play a role in reproductive suppression, as daily contact with toilet and nest material from the parental colony did not inhibit or delay the onset of ovulation in naked mole-rats. In the blind mole-rat (*Spalax ehrenbergi*), urine has been shown to contain both species- and sex-specific chemosensory cues that vary according to seasonal changes and elicit varying responses from male and female mole-rats (Heth & Todrank 1995). Female blind mole-rats are also able to determine male breeding-status by apparently detecting testosterone levels in urine and blood samples (Gottreich *et al*. 2000) and evidence has been found that urinary odours may be used in phenotype matching in *Spalax ehrenbergi* (Heth & Todrank 2000). It is clear from the abovementioned results that rodents show differential levels of odour discrimination.

Urinary odour cues may be used as a mechanism for both direct recognition (direct evaluation of a urinary odour) and indirect recognition (familiarity with an individual or class urine odour). Studies on rodents such as golden hamsters, *Mesocricetus auratus* (Todrank *et al*. 1998), ground squirrels, *Spermophilus beldingi* (Holmes 1984) and blind mole-rats, *Spalax ehrenbergi* (Heth & Todrank 1996) have shown that several species are capable of recognising their own siblings' odours but fail to distinguish the odour of two closely related but unfamiliar animals. Individual identification within a colony is important in terms of maintaining dominance hierarchies and identifying breeding partners or offspring. Outside a colony or family group, however, identifying an animal as being of the same species, but unfamiliar, or of a different species, is sufficient in order to identify a mating opportunity or threat. Allocating memory resources to a finer discrimination between unfamiliar animals is thus unnecessary. Broad discrimination could be based on one of two proximate mechanisms: kin recognition by direct familiarity (i.e. remembering an individual odour) or kin recognition by phenotype matching (i.e. comparing characteristics of a new odour to a known odour) (Todrank *et al.* 1998).

3.1.4 Ethology: Kin-recognition and mating behaviour in *Cryptomys damarensis*

Although ethology and animal cognition were previously classified as the domains of biologists and psychologists respectively (Shettleworth 2001), much can

be gained from combining aspects of both these approaches to phenomena such as kin-recognition. It is impossible to ascertain the level of awareness that animals experience through the tests conducted in most experiments, but one can deduce the probability that some type of learning has taken place earlier in an individual's experience. In this manner, interactive behaviour in a social species, such as *Cryptomys damarensis*, may reflect earlier social learning.

Differential behaviour toward different classes of animals has been used successfully to identify kin-discrimination and therefore kin-recognition. Two easily observed types of behaviour on which to base the existence of kin-discrimination are agonistic behaviours between conspecifics and mating behaviour between members of the opposite sex. Mating behaviour, easily identifiable and predictably related to a certain class of animals in the Damaraland mole-rat, is preferable due to the ethical considerations of animals sustaining injury during agonistic interactions. The Damaraland mole-rat lends itself to the use of mating behaviour as a method of identifying kin-recognition and kin-discrimination due to the strong expression of incest avoidance.

Colonies of Damaraland mole-rat have been found to remain reproductively quiescent after the death or experimental removal of the breeding female until the introduction of a foreign male or female has resulted in the recurrence of breeding (Jarvis & Bennett 1993, Rickard & Bennett 1997). Jacobs *et al.* (1998) also found that two opposite-sex siblings isolated from the natal colony did not engage in mating behaviour during a span of 36 hours. Furthermore, Bennett *et al.* (1996) found that opposite-sex siblings housed together for up to eight weeks did not engage in pre-copulatory behaviour and that all sexual behaviour was absent. The results of these studies showed that animals will remain incest avoidant even in the absence of other colony members and that incest avoidance is probably due to an innate aversion to inbreeding rather than social pressures. This phenomenon is in severe contrast to the pairing of two unrelated animals of the opposite sex where mating behaviour is observed soon after the animals are introduced to the same cage (Bennett *et al.* 1996, M. Costanzo pers. obs.).

3.1.5 Endocrinology: Hormones as a measure of reproductive activity in *Cryptomys damarensis*

It has been well-documented in the naked mole-rat that urinary progesterone levels reflect circulating levels of plasma progesterone as well as breeding status. Faulkes *et al.* (1991) described progesterone levels in reproductively active females as cycling between undetectable levels of <0.5 ng per mg to 97.8 ng per mg creatinine up to 148 ng per mg creatinine. In non-reproductives they found urinary progesterone to be consistently undetectable. Previous studies on the Damaraland mole-rat have found a significant difference in the levels of progesterone and oestrogen in reproductive and non-reproductive females. The levels of circulating cortisol (both sexes), progesterone (females) and testosterone (males) were determined by urine sampling. Non-reproductive females that have been removed from their natal colony showed increased levels of circulating progesterone (Molteno & Bennett 2000). As physical and emotional stress may reduce fertility and reproduction (Johnson *et al.* 1992), cortisol levels should be measured to examine as a factor influencing the occurrence of mating behaviour. Behavioural stress (Johnson *et al.* 1992) and pheromones secreted by other animals (e.g. Lepri 1986) have been shown to influence reproductive suppression.

3.2 Materials and Methods

The five colonies used in this experiment were queenless colonies that had been in the laboratory for a period of at least one month and had remained reproductively quiescent during this time. These experimental colonies had at least two male and two female mole-rats in order to facilitate paired controls from within the same colony (Table 3.1). Animals were selected to minimise variation in body mass between pairs from the same colony where possible. The colonies were housed in large plastic containers,kept in a temperature controlled room and fed a diet of sweet potato, carrot, gem squash, and apple *ad libitum* (Bennett 1990). After removal from the colony, experimental animals were kept in smaller containers for the duration of the experiment, while the rest of the colony remained intact.

Table 3.1: Designated symbols for experimental and control animals arranged by natal colony

	Experimental Animals		Control Animals	
Colony	**Male**	**Female**	**Male**	**Female**
Gholf	C	A	D	B
Lughawe	I	F	H	E
Drank	L	M	J	K
Colony 5	O	Q	N	P
Colony A	T	X	S	U

Animals were observed for the occurrence of mating behaviour for at least a week prior to the commencement of experiments, and only in the "Gholf" colony was any attempt to mate observed. In that case a male attempted to mount a female and another male but was repulsed by the other mole-rats. The particular male was excluded from the pairs used in this study. Furthermore, the females from the "Gholf" colony remained imperforate, and the female did not become pregnant, suggesting that no actual penetration had taken place.

3.2.1 Experimental protocol

Unless otherwise noted, all experimental animals were housed singly in plastic containers containing wood shavings and maintained as described by Bennett (1990). Recognition experiments conducted on naked mole-rats showed that they are able to discriminate ("recognise") individuals belonging to their own colony and that this seems to be based mostly on colony odour. As animals that had been removed from their colony for a period of time were also subject to aggressive interactions from their colony mates, it was concluded that the colony odour familiarity had to be continually reinforced (O'Riain & Jarvis 1997). The two most probable sources of colony odour were identified as nesting or toilet material. For the purpose of this study, urinary odour was identified as a possible contributor to colony odour, as the urine of mole-rats has a characteristically strong and lingering smell.

In naked mole-rats it has been found that the odour used to recognise colony members is probably a mixture of the odour of all the individual animals in the colony (O'Riain & Jarvis 1997). Because of this finding, urine was collected prior to the start of experimentation and combined into an equal mixture of the urine of all the colony members, including the test animal. While these equal proportions may not give an exact estimation of the composition of colony urinary odour, equal

proportions were chosen as it is nearly impossible to establish the exact percentage that each animal contributed to colony urinary odour.

The mixture of equal parts of all the colony members' urine was prepared and frozen at -20°C to avoid the deterioration of any hormones or other compounds present in the urine. Two to three drops of urine mixture were applied daily onto the noses of the Urine-group (experimental group) as well as 0.5ml urine on a paper towel in the cage. The Water-group (control group) received the same amount of regular tap water both on their noses and on a paper towel to control for any stress response to any disturbance caused by experimental procedure. Tap water was used instead of distilled water as tap water is likely to contain non-specific minerals and chemicals as opposed to the colony specific compounds present in urine. Any differences in results between experimental and control animals would thus not merely reflect a reaction to the presence of compounds in general, but rather suggest a specific influence of the particular compounds present in the urine samples. The experimental group consisted of experimental males (EM) and experimental females (EF) while the control group may be further classified as control males (CM) and control females (CF). The addition of tap water did not control for any irritation caused to the animals due to the smell associated to the urine, but both Water- and Urine-group animals responded by wiping their noses with their front paws immediately after a drop of liquid was delivered, and then resumed their normal behaviour. In this study, the animals receiving the urine did not seem to be any more disturbed than those receiving water, but it has been suggested that the strong odour associated with mole-rat urine may have an irritant effect (O'Riain & Jarvis 1997). The urine and water samples were applied to both the noses of the animals and a small square of paper towel in order to address the possibility of differential responses to touching and smelling odorous stimuli (Heth & Todrank 1995).

3.2.1.1 Ethogram for *Cryptomys damarensis*

Mating Behaviour

Mating behaviour includes all behaviours associated directly with reproductive interaction with the opposite sex in the Damaraland mole-rat. Mating behaviour in the Damaraland mole-rat has been described in detail by Bennett and Faulkes (2000) and is highly ritualised. In the Damaraland mole-rat, the female solicits the male by a

characteristic head-to-head stance, where the animals are standing with their snouts touching. This behaviour may be very brief, if observed at all. Both animals, but most often the female, emit high-pitched squeaky vocalisations. The female then mounts the head of the male (a behaviour described as head-mounting hereafter) and raps her hind feet on the burrow floor. Genital nuzzling may also occur with one or both animals laying on their sides and sometimes accompanied by vocalisations and biting. The male and female then engage in a head-to-tail chase (circle chasing) with the female's tail raised (tail-raising). The male seizes the female by her tail, using his incisors, and mounts the female, holding her around the shoulders with forelimbs and biting her neck. The female responds by the raising of the head, flattening of the body, and copulation then ensues.

Social Behaviour

The behaviours mentioned below may be described as social when another animal is directly involved. Social behaviours in the naked-mole rat makes up about 74% of all behavioural data points observed (Lacey *et al*. 1991) and are similarly common in the Damaraland mole-rat.

Grooming

Grooming is a collective term used to describe the following types of behaviours: cleaning the forefeet or hind feet with incisors, wiping the face and muzzle with both forefeet, scratching regions of the body with the hind foot, grooming the tail with teeth or forefeet, grooming the genitals with the tongue or incisors. In the naked mole-rat, allogrooming (grooming a conspecific) is not observed between adults and autogrooming (grooming self) is observed at a low but consistent frequency of about 1.3% ±0.1% of behavioural data points observed (Lacey *et al.* 1991).

In the Damaraland mole-rat, both autogrooming and allogrooming are observed at regular but thus far unquantified levels. In the Damaraland mole-rat, allogrooming usually consists of nibbling the other animal around the head, neck, shoulders, flank and the forelimbs. The animal being groomed usually responds by moving the area being groomed towards the groomer, sometimes even raising a forelimb so that the groomer can reach the fur around the limbs. The animal being groomed may also close its eyes or emit squeaking noises. Allogrooming is regularly observed in adult

the Damaraland mole-rat and is believed to serve to remove ectoparasites from one another along with constituting a significant social behaviour. Damaraland mole-rat females were found to autogroom more than males and most autogrooming appears to occur after a period of rest (Bennett 1988).

Face biting occurs when one animal gently bites another animal on the head, near the facial region. This behaviour is not agonistic, and is often accompanied by other social behaviour or by mating behaviour.

Resting

Resting is a group of behaviours characterised by lack of motor activity, and specifically includes the presence of yawning, dozing and reclining. Dozing is described as when an animal is literally asleep on its feet, with its head drooping and not being engaged in any other definable activity. Reclining (sometimes described as huddling) is when an animal is lying down on its stomach, back or side with its eyes closed (Lacey *et al.* 1991). In naked mole-rats, reclining equals 56% to 61% of daily activity in non-breeding animals and breeding males, while breeding females reclined less, at 34% to 46%.

In captive colonies of the Damaraland mole-rat, sleeping may occupy up to 80% or 90% of daily activity. The reproductive male spends around 85% to 86% of the time resting in the nest, with the reproductive female spending only 36% to 37% of her time resting. Non-reproductive workers spent between 60% and 80% of their time resting in the nesting area, and no significant difference in sleeping patterns was found between reproductive or worker mole-rats. The most common resting position in the Damaraland mole-rat is lying flat on the stomach, although lying on the back or side is common in social resting (Bennett 1988). Bennett (1988) also found that the reproductive pair in a colony preferentially rested together, an observation, which implies that resting may play a social role. The occurrence of reclining in behavioural trials may thus correspond to an animal's breeding status and, as thus, may be used to support data gained from the observation of mating behaviour. For the purpose of this study, reclining or resting behaviour is generally described as "sleep", and may be a social or non-social behaviour.

Other Behaviour (Non-Social)

Eating is a non-social behaviour. A feeding mole-rat squats on its hind legs and holds the piece of food with its front feet. When consuming a piece of sweet potato the animal will remove the skin with its incisors and then remove small pieces of sweet potato, which it then eats. Sporadically the animal will shake the food with its forefeet and change its grip to start eating a different portion of the piece of food (Bennett 1988).

The act of carrying a variety of materials in the mouth, including food and nesting materials, has been classified as a transportation behaviour in naked mole-rats. Digging, in naked mole-rats, can be divided into three separate behaviours: gnawing (incisors are used to loosen dirt), backshoveling (legs are used to propel dirt behind animal) and foreleg digging (foreleg is scraped over surface to remove small pieces of dirt, etc.) (Lacey *et al.* 1991).

In Damaraland mole-rats a number of colony maintenance behaviours have been noted: digging and gnawing (including gnawing the sides of the cage in captivity), sweeping and transport of soil (or sawdust in captivity), nest-building and the carrying of nesting materials, and food carrying. In this study the aforementioned behaviour is simply classified as working, a non-social behaviour. In the Damaraland mole-rat the reproductive male has been noted to do very little work (0.91% to 1.50% of total work done), while the reproductive female did more work (5.4% to 7.74%), especially spending time with nest building (Bennett 1988).

The most common agonistic behaviour found in the Damaraland mole-rat is sparring, which occurs most regularly between pups or between adults and juveniles. A correlation has also been found between the frequency of sparring and allogrooming, suggesting that both behaviours may be involved in determining a juvenile's rank in the hierarchy (Bennett 1988).

3.2.1.2 Experimental Observation of Behaviour

Animals were allowed physical contact with their paired sibling (water with water, urine with urine) of the opposite sex (opposite sex sibling or OSS) for approximately an hour at a time with increasing increments of time in between. The period between the physical contact experiments was increased from one day to three, six, nine, and finally 12 days for all experimental pairs. With some pairs a final gap

of 18 days of separation in between physical contact was included. The physical contact experiment took place in a neutral container, similar to the ones where the experimental animals were housed, containing sawdust and four pieces of sweet potato. The female was introduced first and immediately afterwards the male was placed in the container. Behaviour was recorded by continuous observation. Behaviours were noted on a specially designed sheet and were classified as mating, social, or non-social behaviours (Bennett 1988, Lacey *et al.* 1991, Bennett & Faulkes 2000) and were measured as behaviour exhibited per minute observed (behaviour/min).

The presence of kin-recognition was measured by observing mating behaviour and changes in sex and stress hormone levels respectively. The mechanism at work is examined by investigating how relatedness, or habituation to an olfactory cue, influences the expression of kin-recognition. Even in the presence of phenotype matching it may be overridden by familiarity cues under certain circumstances (Waldman 1988). However, in the absence of familiarity cues (such as with the control group) genetic evaluation of relatedness would be expected to surface if it does exist.

Several different behaviours were observed during the physical contact trials, of which 19 were noted often enough to be recorded and classified. These behaviours were classified using previously compiled ethograms for the two eusocial species, *Heterocephalus glaber* and *Cryptomys damarensis* (Bennett 1988, Lacey *et al.* 1991, Bennett & Faulkes 2000). The behaviours were roughly classified as ritualized mating behaviour (Bennett & Faulkes 2000), social behaviour or non-social behaviour (Bennett 1988, Lacey *et al.* 1991). These categories of behaviour, as used in this study, are expounded in the following table and in the following sections describing the behaviours specifically.

Table 3.2 Behavioural categories

Mating Behaviour (MB)	head-to-head, vocalisation, tail raising, genital smelling, head mounting, circle chasing, mounting, mating
Social Behaviour (SB)	face biting, grooming (other), sniffing, sleeping (next to), sleeping (on top)
Non-social Behaviour (OB)	eating, grooming (self), sleeping alone, work (dig/move), run/climb out, chase away (agonistic)

3.2.1.3 Classification of Behaviour

For the purpose of this study vocalizations were only classified as mating behaviour if accompanied by one or more of the other behaviours associated with mating. This was done due to the high frequency of vocalizations emitted by the Damaraland mole-rat during normal interaction with conspecifics. Furthermore, mating was never specifically noted and was classified together with mounting. This was done as mounting usually occurred repeatedly and it was difficult to ascertain during which instances copulation actually took place. Other behaviours observed during mating were mounting by the female, similar to that exhibited by the male prior to copulation, genital nuzzling by both animals, and biting.

The social behaviours noted in this study comprised face biting, grooming the other animal, sniffing at another animal and sleeping together (next to or on top of each other). Each of these behaviours is described below:

Face biting is observed in the Damaraland mole-rat and seems to be a social behaviour as opposed to an agonistic behaviour, as it is often accompanied by other social behaviours such as sniffing and may even lead to mating behaviour. Face biting is often accompanied by vocalisations and could possibly be a grooming behaviour.

Animals were often observed sniffing each other's heads or bodies, apart from the previously described genital sniffing. Animals also engaged in prolonged sniffing of the other animal's nose where urine had been applied.

In the current study only dozing and reclining were counted as resting behaviour and were recorded as "sleeping" when an animal was immobile with its eyes closed and appeared to be asleep. Sleeping was then categorized into social sleeping (reclining or dozing in physical contact with another animal by lying next to or on top of each other) and non-social sleeping (engaging in resting behaviour alone). Sleeping alone or autogrooming (as described above) were classified as non-social behaviours.

Feeding behaviours are classified in the current study as any behaviour where food is consumed and thus excludes the moving of food through the use of the incisors or feet. Animals that were eating were not engaged in any other behaviour.

Although paired animals would sometimes be eating at the same time, there was rarely any physical contact with the other animal. Most instances of agonistic behaviour were observed when an eating animal was disturbed.

The moving of food without consumption is classified as a working behaviour for the purpose of this study, as mole-rats commonly create designated food stores in their nests.

There were two locomotor behaviours noted in the current study, namely running (forwards and backwards) and climbing out. Climbing out was when an animal would stand up against the side of the container on its hind legs with its front legs bracing it against the side of the container. This would usually be accompanied by sniffing the air and other seemingly exploratory movements. Walking and standing were not noted specifically as they occurred almost continuously.

Adults very rarely spar, and in this study the only agonistic behaviour noted was that of chasing. Chasing mostly occurred when an animal was eating and was disturbed by another animal. The eating animal would then rush after the other animal. In response to this the other animal would retreat quickly.

3.2.2 Hormonal Assays

In order to establish basal hormone levels, urine samples were taken from all colony members at least three times before the experimental animals were removed from the colony. The samples were collected using urine traps consisting of a cage with a meshed wire bottom and a catching tray (Bennett *et al*. 1994). After collection the urine samples were kept at -20°C until analysis.

Further urine samples were collected from all experimental animals 24 hours prior to physical contact, as well as collecting the first sample given after the termination of physical contact. This varied by animal, but was always within 12 hours after physical contact. Samples were frozen and later analysed using commercial Coat-a-Count hormone assay kits for which parallelism had previously been established (Bennett *et a*l. 1994, Clarke *et al*. 2001).

Creatinine concentrations were determined using a plate reader to measure the absorbance of the urine samples as described in Molteno (1999) in order to standardize the urine concentration. Even though bathyergids do not drink free water, but rather acquire all necessary quantities from their food supply (Bennett & Faulkes 2000), their urine concentration still varies with fluid intake. This in turn influences

the concentration of the hormonal excretion detected in the urine. Creatinine is a breakdown product from tissue proteins, usually formed by the muscles in mammals (Schmidt-Nielsen 1997), and is excreted at a relatively constant rate. All hormone concentrations are thus expressed as hormone/mg creatinine.

A modified Jaffe reaction (Folin 1914) was used to calculate the creatinine concentration for each of the urine samples. The process involves adding ten microlitres of standard or sample to the wells of a microplate, in duplicate, and leaving two wells empty as a duplicate control blank. A further 200µl of picrate reagent was added to all the wells, including the blanks.

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

Cortisol assay

Urinary cortisol concentrations were determined using a commercial radioimmunoassay kit (Coat-a-Count, Diagnostic Products, USA) as described in Molteno (1999). The assay was able to determine cortisol concentrations of between 1.26 nmol/L and 1380 nmol/L. Specificity of the antiserum was assessed by the supplier and cross-reactivity was less than 1% with all naturally occurring steroids, with the exception of 11-deoxycortisol (11.4%), Prednisolone (76%) and Prednisone (2.3%). The assay was validated for urine by testing for parallelism using serial doubling dilutions of mole-rat urine obtained from an individual with high cortisol levels (range 1:1 to 1:64). The slope of the lines did not differ significantly (ANCOVA $F_{1\text{-}6}$ = 4.7 and P<0.05). The sensitivity of the assay (90% binding) was 6.1ng ml^{-1}. The inter and intra-assay coefficients of variation were 2.8% and 2.1% respectively (Clarke *et a*l. 2001).

Progesterone assay validation

Urinary progesterone concentrations were determined using a Coat-a-Count radioimmunoassay kit (Diagnostic Products, USA) as described in Bennett *et al.*

(1996). The progesterone assay was sensitive to progesterone concentrations of between 0.15 ng/ml and 127.2 ng/ml. Pooled urine samples (one pool with expected low concentration from a male and one with expected high concentration from a pregnant queen) were double diluted from 1:1 to 1:64 and assayed. The low pool contained no progesterone (below detection limit), and the high pool contained 15.9 ± 0.54 nmols/mmol creatinine.

In the assay were also included 6 samples at a dilution 1:64 from the pool with expected low concentration of progesterone. To these samples, 100µl of progesterone, in increasing concentrations (0.3, 1.60, 6.40, 31.8, 63.6 and 120.20 nmols/l) was added in duplicate. The curve was perfectly parallel to the standard curve. The assay was validated for the test species by comparing the slope of the curve produced using serial doubling solutions of un-extracted mole-rat urine (over the range 1:1 to 1:64) against that of the standard curve (ANCOVA, $F_{1\text{-}6} = 4.9$, $P>0.05$, $R^2 = 0.98$). The slopes of the lines were compared using the *Statistica* software package (Statsoft 2002) subsequent to logit-log transformation of the data (Chard 1987).

Extracted urine samples from the 2 pools were assayed and compared with non-extracted samples from the pools. There were no significant differences ($P<0.05$) in progesterone concentration between the extracted and non-extracted samples. The intra-assay and inter-assay coefficient of variation for the assay were 7.2 and 11.8% (n=4 assays). The sensitivity of the assay as defined as two standard deviations of the buffered bland was 0.4nmol L^{-1}. The radio-immunoassay has been previously validated for use in the naked mole-rat by Westlin, Bennett & Jarvis (1994).

Testosterone assay

Testosterone assays were carried out using a Coat-a-Count testosterone kit (Diagnostic Products Corporation, USA). The assay was able to determine urinary testosterone levels of between 1.0773 nmol/L and 1600 nmol/L. The assay was validated by testing for parallelism using serial doubling dilutions of unextracted mole-rat urine obtained from an individual exhibiting high testosterone levels over the dilution range (1:1 to 1:64). The slopes of the lines were compared and found not to differ significantly (ANCOVA $F_{1\text{-}6} = 4.3$ and $P<0.05$). The sensitivity of the assay (90% binding) was 0.1 ng ml^{-1}. The intra-assay coefficient of variation was 2.6% (n = 6) (Clarke *et al*. 2001).

3.2.3 Statistical Analysis

All statistical tests were performed using SAS (SAS Institute Inc. 1999). To examine whether there were intra-group hormonal differences between the experimental and control groups, a general linear model (GLM) was used to determine the effects of individual variation within animals, variation over days, and between samples taken before and after experimentation. The Type III results were used, and the models were adjusted by the stepwise removal of levels of comparisons until a significant effect could be identified. A separate GLM was used to determine if there were significant differences in the basal hormone levels determined before the start of experimentation. Paired t-tests were used to compare hormone levels and behaviour between paired siblings from the same colony. Behavioural variation within the control and experimental groups was also determined by GLM for mating, social, and non-social behaviours. Correlations were determined between the levels of the different hormones and different classes of behaviour.

3.3 Results

3.3.1 Behaviour

Individual variation by animal was found in the randomly assigned experimental and control groups for all behaviours (Table 3.3).

Table 3.3 Group comparisons

Group	MB: Mating Behaviour	SB: Social Behaviour	OB: Non-Social Behaviour
EF: Experimental Females	$p < 0.0001$	$p = 0.0059$	$p < 0.0001$
CF: Control Females	$p < 0.0001$	$p = 0.0025$	$p < 0.0001$
EM: Experimental Males	$p < 0.0001$	$p = 0.1120$	$p < 0.0001$
CM: Control Males	$p = 0.0004$	$p = 0.0108$	$p < 0.0001$

Tested at *10 % and **5% confidence levels per group of 10 comparisons. Individual points within the model is measured at * Bonferroni $\alpha < 0.01$, and ** Bonferroni $\alpha < 0.005$. NS indicates a non-significant value.

In the EF-group (experimental females), one out of the five females (female A) exhibited significantly higher levels of MB (mating behaviour) than the other females. In the SB (social behaviour) category, two of the ten pair-wise comparisons showed a significant deviation from the other females in the group (females Q and X). Two females (Q and X) deviated significantly from the EF-group OB-levels (non-social behaviour), but not from each other. In the CF-group (control females), two of the

five females (B and E) differed significantly from the group, but not from each other in levels of MB. In terms of SB, three of the ten pair-wise comparisons differed significantly from the others, and this difference was attributed to female P. Two females (P and U) differed significantly from the group in OB-levels (non-social behaviour), but did not differ significantly from each other.

The EM-group (experimental males) contained one male (C) that showed significantly higher MB-levels than the rest of the animals. SB-levels did not show any animals that deviated significantly from the rest of the group. One male (O) did exhibit significantly different levels of OB in comparison to the other males in the group. Among males in the CM-group (control males), two males (J and S) differed significantly from the other animals, but not from each other. In terms of SB, two of the ten pair-wise comparisons showed a significant difference for male N. Levels of OB indicated that two males (N and S) differed significantly from the group.

Behavioural data analyses further focused on comparisons between SSS (same sex siblings).

Table 3.4 Paired comparisons between SSS

Comparison (Females)	Animals	Sig	Mean	Comparison (Males)	Animals	Sig	Mean
Mating Behaviour	A & B	**	B < A	Mating Behaviour	C & D	**	D < C
	F & E	NS	E > F		I & H	**	H > I
	M & K	**	K > M		L & J	**	J > L
	Q & P	**	P > Q		O &N	*	N > O
	X & U	**	U > X		T & S	**	S > T
Social Behaviour	A & B	**	B > A	Social Behaviour	C & D	NS	D > C
	F & E	*	E < F		I & H	NS	H > I
	M & K	NS	K < M		L & J	**	J < L
	Q & P	NS	P > Q		O &N	NS	N > O
	X & U	NS	U < X		T & S	NS	S < T
Non-Social Behaviour	A & B	**	B > A	Non-Social Behaviour	C & D	NS	D > C
	F & E	**	E > F		I & H	*	H > I
	M & K	NS	K > M		L & J	NS	J > L
	Q & P	NS	P < Q		O &N	NS	N < O
	X & U	**	U > X		T & S	NS	S > T

NS Not significant, * p<0.1, ** p<0.05

Experimental animals were matched with their same-sex control sibling from the same colony and were compared using a paired t-test to determine whether significant differences exist (Table 3. 4). In four of the five colonies SSS males showed significant differences in MB, while the fifth colony showed a tendency toward significance ($p<0.1$). Male SSS from four of the colonies had a CM with higher levels of MB than the EM, with three of these differing significantly ($p<0.05$). In four of the five colonies, females showed significant differences in MB between

SSS. In three of these colonies the CF showed higher levels of MB than the EF, and in one colony (Gholf) the EF showed higher levels of MB than the CF.

Four of the five colonies showed no significant difference in SB between the EM and CM, with only the Drank colony EM having higher levels of SB than the CM. Four of the five colonies showed no significant difference between female SSS, with the CF from Gholf showing more SB than her EF counterpart.

In terms of female OB-levels, three of the five colonies had CF with significantly higher levels of OB than the EF from the same colony. The paired t-tests of male SSS resulted in no significant differences in levels of OB.

Correlations between the occurrences of different types of behaviour are summarized in table 3.5.

Table 3. 5 Correlation between behaviours

Animal	Ctrl/Exp	M/F	Colony	Correlated Behaviours	Correlation	p-value
A	Exp	F	Gholf	Mating and Non-Social	-0.84376	0.0347
F	Exp	F	Lughawe	Mating and Social	0.91922	0.0272
J	Ctrl	M	Drank	Mating and Social	-0.92544	0.0081
K	Ctrl	F	Drank	Social and Non-Social	-0.81653	0.0474
L	Exp	M	Drank	Mating and Social	-0.93206	0.0210
M	Exp	F	Drank	Mating and Social	-0.88119	0.0483

Of the six animals that showed correlations between different types of behaviour, four showed a correlation between MB and SB. Three of these animals were from the Drank colony and showed a strong negative correlation between MB and SB. Of the six animals showing behavioural correlations four were experimental and two were control animals, while four were female and two were male.

3.3.2 Hormones

Basal-level cortisol levels between SSS were expected to be similar, because all experimental animals were non-breeding individuals living in the same colony at the time that these measurements were taken. Any variation that emerges during experimentation would thus be due to experimental conditions rather than pre-existing individual variation in cortisol levels. Females K and M exhibited a significant difference in cortisol levels ($p=0.05$, paired t-test) and were thus excluded from further analyses as individual variation in cortisol levels would obscure any signal due to experimental effects. These females' cortisol levels did, however, follow similar patterns. The only male SSS that showed a significant difference was T (EF) and S

(CF) where T had significantly lower levels of cortisol than S. Subsequent variation in cortisol levels for these two animals was thus not examined due to the existence of significant individual variation.

The p-values obtained through GLM modelling are summarised in table 3.6.

Table 3.6

		Cortisol		Progesterone		Testosterone	
		Basal	Experimental	Basal	Experimental	Basal	Experimental
Experimental Females (A,F,M,Q,X)	Model p-value	**0.0342**	**0.0150**	0.2480	**0.0161**	-	-
	Animal Name (H1)	**0.0342**	**0.0078**	0.2480	**0.0020**	-	-
	Before/After (H3)	-	0.9613	-	0.8622	-	-
	Day (H6)	-	0.1566	-	0.5199	-	-
Control Females (B,E,K,P,U)	Model p-value	**0.0218**	**0.0348**	0.1823	0.1186	-	-
	Animal Name (H1)	**0.0218**	**0.0086**	0.1823	**0.0277**	-	-
	Before/After (H3)	-	0.1525	-	0.2685	-	-
	Day (H6)	-	0.3400	-	0.4956	-	-
Experimental Males (C,I,L,O,T)	Model p-value	**0.0191**	0.0584	-	-	0.6491	0.7446
	Animal Name (H1)	**0.0191**	0.1186	-	-	0.6491	0.5101
	Before/After (H3)	-	0.7319	-	-	-	0.4821
	Day (H6)	-	**0.0459**	-	-	-	0.6186
Control Males (D,H,J,N,S)	Model p-value	**0.0128**	0.1614	-	-	**0.0048**	**0.0274**
	Animal Name (H1)	**0.0128**	0.0933	-	-	**0.0048**	**0.0037**
	Before/After (H3)	-	0.3209	-	-	-	0.8337
	Day (H6)	-	0.4633	-	-	-	0.2476

There was a significant amount of individual variation in the basal cortisol levels of all groups. Although the complete model showed significant variation in the EF-group basal cortisol levels, this was not observed at the level of single comparisons at a Bonferroni significance level of $p<0.005$ ($p<0.05$ for 10 individual comparisons). The CF-group also did not show any significant variation in basal cortisol levels at the level of individual comparisons. The model p-value was thus ignored for the two female groups, as there were no significant differences at any individual point of comparison within the model. However, during experimentation there was significant variation in both the EF-group cortisol levels and the CF-group cortisol levels both at the model level and at the individual points of comparison. In the male groups the EM and CM had one and two out of ten points respectively that showed significant variation from the rest of the group, and the model p-value for the male groups are thus accepted. Neither of the male groups showed variation in cortisol levels during experimentation.

There were no significant within-group differences in basal levels of progesterone in either the EF or CF group, but during experimentation levels of progesterone in both groups of females exhibited significant variations. EM showed no variation in basal testosterone levels or during experimentation. One of the control males (J) exhibited a significant variation in basal levels of testosterone when compared to the other animals in the CM-group, a trend, which continued through experimentation.

During experimentation females from the Lughawe colony (E and F) showed some significant variations when compared to the other females in their respective groups, but not when compared to each other.

A day effect was only observed in experimental males, with mean cortisol levels on day 3 differing significantly from days 1, 6, 12, and 18.

It was found that there was no significant difference in hormone levels in samples taken before and samples taken after physical contact between paired opposite sex siblings. Table 3.7 describes the correlation between hormonal levels.

Table 3.7 Correlation between hormone levels

Animal	Ctrl/Exp	M/F	Colony	Correlated Hormones	Correlation	p-value
A	Exp	F	Gholf	Cortisol and Progesterone	0.84201	0.0355
F	Exp	F	Lughawe	Cortisol and Progesterone	0.97066	0.0060
O	Exp	M	Colony 5	Cortisol and Testosterone	0.89695	0.0154
T	Exp	M	Colony A	Cortisol and Testosterone	0.82134	0.0450

Of the four animals that showed a correlation between hormone levels all four were experimental animals. Two EFs and two EMs showed strong positive correlations between cortisol and progesterone and cortisol and testosterone respectively. No specific colony was strongly represented.

Paired t-tests were also conducted to determine whether significant hormonal differences existed between paired same-sex siblings (Table 3.8 a and b).

Table 3.8a Basal levels

Comparison	Animals	p-value	Sig	Description
Cortisol (F)	A & B	0.0987	NS	B < A
Cortisol (F)	F & E	0.1864	NS	E > F
Cortisol (F)	M & K	0.0520	NS	K > M
Cortisol (F)	Q & P	0.7907	NS	P > Q
Cortisol (F)	X & U	0.3726	NS	U < X
Cortisol (M)	C & D	0.1108	NS	D > C
Cortisol (M)	I & H	0.4838	NS	I > H
Cortisol (M)	L & J	0.2626	NS	L > J
Cortisol (M)	O &N	0.3003	NS	O > N
Cortisol (M)	T & S	0.0466	**	T< S
Progesterone	A & B	0.2028	NS	B < A
Progesterone	F & E	0.0470	**	E > F
Progesterone	M & K	0.1252	NS	K > M
Progesterone	Q & P	0.0319	**	P > Q
Progesterone	X & U	0.1344	NS	U > X
Testosterone	C & D	0.5618	NS	D < C
Testosterone	I & H	0.3218	NS	I > H
Testosterone	L & J	0.6053	NS	L > J
Testosterone	O &N	0.1961	NS	O > N
Testosterone	T & S	0.4828	NS	T < S

There were no significant differences in basal cortisol levels between paired siblings, except for males T and S from Colony A. The control male S exhibited significantly higher levels of cortisol than did T. In the Lughawe colony (females E and F) as well as in Colony 5 (females P and Q), the control females (E and P) had significantly higher progesterone levels than did their experimental counterparts. There were no significant differences in basal testosterone levels.

Table 3.8b Experimental Hormone Levels

Comparison	Animals	p-value	Sig	Description
Cortisol (F)	A & B	0.0759	NS	B > A
Cortisol (F)	F & E	0.3028	NS	E > F
Cortisol (F)	M & K	0.0264	**	K > M
Cortisol (F)	Q & P	0.0172	**	P > Q
Cortisol (F)	X & U	0.7395	NS	U < X
Cortisol (M)	C & D	0.3420	NS	D > C
Cortisol (M)	I & H	0.2722	NS	H > I
Cortisol (M)	L & J	0.2758	NS	J > L
Cortisol (M)	O &N	0.6582	NS	N > O
Cortisol (M)	T & S	0.3011	NS	S > T
Progesterone	A & B	0.0820	NS	B > A
Progesterone	F & E	0.2550	NS	E > F
Progesterone	M & K	0.0194	**	K < M
Progesterone	Q & P	0.0222	**	P > Q
Progesterone	X & U	0.0562	NS	U > X
Testosterone	C & D	0.2064	NS	D < C
Testosterone	I & H	0.2428	NS	H > I
Testosterone	L & J	0.0533	NS	J > L
Testosterone	O &N	0.3780	NS	N < O
Testosterone	T & S	0.2810	NS	S < T

During experimentation K and P, control females in Drank and Colony 5

respectively, exhibited higher levels of cortisol than their experimental counterparts. These same animals also exhibited significantly different levels of progesterone than the experimental counterparts, introducing the question of whether levels of these two hormones are linked. Once again, there was no significant difference in testosterone levels in any of the paired animals.

3.3.3 Hormonal and Behavioural Correlations

Table 3.9 summarises the correlations found between hormonal levels and the exhibited behaviours.

Table 3.9

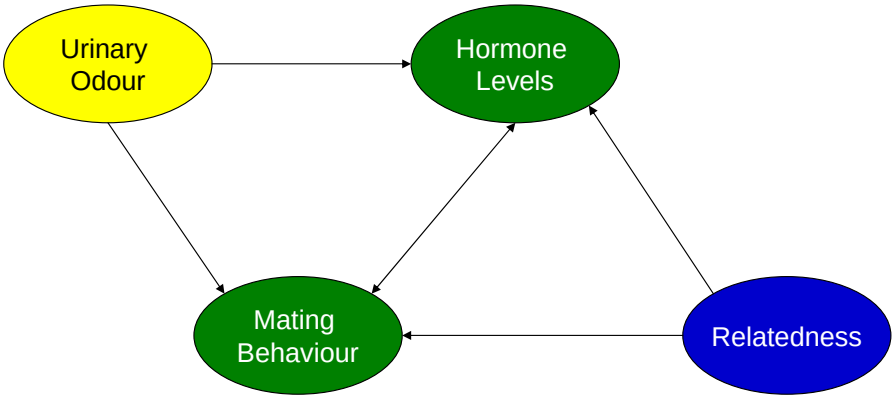
Animal	Ctrl/Exp	M/F	Colony	Correlated Hormone and Behaviour	Correlation	p-value
E	Ctrl	F	Lughawe	Mean Cortisol & Non-social	-0.91994	0.0269
E	Ctrl	F	Lughawe	Mean Progesterone & Social	-0.91880	0.0274
I	Exp	M	Lughawe	Mean Cortisol & Mating	-0.98283	0.0027
U	Ctrl	F	Colony A	Mean Progesterone & Mating	0.90933	0.0120
U	Ctrl	F	Colony A	Mean Cortisol & Non-Social	0.87351	0.0230

Only two colonies, Lughawe and Colony A, exhibited significant correlations between hormone levels and behaviour. Of the five instances where correlation was exhibited between hormones and behaviour, four of the animals were female. There were no significant hormonal-behavioural correlations involving testosterone, which was not surprising since testosterone levels did not seem to be influenced by any experimental manipulations. Control female E showed negative correlation between levels of social behaviour and both progesterone and cortisol levels. Experimental male I revealed a negative correlation between mating behaviour and cortisol levels. Control female U had positive correlations between mating and progesterone levels as well as between cortisol levels and non-social behaviour. Of the five cases where correlation was found, two were progesterone and three were cortisol-related behavioural correlations. All categories of behaviour were represented with two cases of MB, two of SB, and one of OB-related correlation.

3.4 Discussion

As can be seen in Figure 3.1 below, there were four primary variables and their associated relationships explored in this thesis. The following discussion summarises the findings that emerged from exploring those relationships.

Figure 3.1: Interactions between variables that influence mating behaviour



3.4.1 The effect of urinary odour on behaviour

Behavioural experiments were conducted around two null hypotheses: (1) the amount of individual variation between SSS should be the same as within treatment groups, and (2) that there will be no difference in levels of behaviour between experimental animals (exposed to urinary odours) and control animals (exposed to water).

The first null hypothesis was rejected for MB for males and females, as well as OB for females. The hypothesis was accepted for SB in males and females, as well as for OB in males. Significant differences in MB levels of the experimental and control pairs occurred between two to three times more often than behavioural differences within a treatment group, indicating that individual variation in behaviour was not similar between SSS and within treatment groups. This disparity shows that the treatment effect is a larger effect than the effect attributable to the colony of origin. This finding directly correlates to previous research indicating that familiarity prevents incest (Bennett *et al.* 1996) and supports the expectation that exposure to urine reinforces familiarity in the current experiments.

Exposure to urine (familiarity) exerts a stronger influence on recognition than genetic kinship (relatedness). If urine and urinary odours facilitate recognition in the Damaraland mole-rat, it would be expected that control animals should be more likely to exhibit mating behaviour than their experimental counterparts. In fact, the largest amount of significant SSS differences occurred in levels of MB, with 80% of male and female SSS showing significant differences, implying that the application of urine most strongly influences mating behaviour. Jacobs *et al.* (1998) have shown that animals refrain from mating with siblings, even when both animals had been isolated from the main colony. A later study concluded that colony odour alone is not

sufficient for recognition, and that recognition exists on an individual level that requires periodic reinforcement (Jacobs & Kuiper 2000). In the light of the present experiments it seems that, although individual recognition may be necessary, recognition is also likely to be odour-based. It may thus be concluded that the exhibition of mating behaviour by the control animals, which had only been exposed to water, indicates a breakdown of incest avoidance due to a lack of kin-recognition. This would cause animals to treat the opposite sex sibling (OSS) as an unfamiliar opposite sex conspecific.

The use of urine has been identified as the major source of chemical cues in mice (*Mus musculus domesticus*) and has also been identified as an important olfactory cue in golden hamsters (*Mesocricetus auratus*). Urinary odours have also been demonstrated to induce endocrine and behavioural responses in mice (Johnston 2003). Based on the current experiment, it now appears that the Damaraland mole-rat also utilises olfactory cues in order to identify siblings. Further experiments investigating the compounds found in mole-rat urine would be invaluable in determining the specific role that urine plays in recognition.

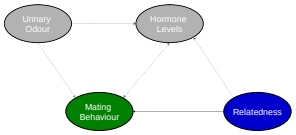
Recognition may play a larger role in work and non-social behaviour in females than in males, as there was no significant difference in levels of non-social behaviour in male SSS, but three female pairs of SSS exhibited a significant difference. This finding is congruent with previous research showing that while non-breeding females are reproductively suppressed, this is not the case for males (Bennett *et al.* 1999). The higher variation in OB between SSS than within treatment groups may be explained by the fact that the CFs are no longer reproductively suppressed and are able to become breeding animals. Bennett (1988) showed that breeding females engage in nest maintenance more often than do either breeding males or non-breeding animals. The higher occurrence of OB in CFs may thus be due to an increase in nesting behaviours due to anticipated breeding and the possible establishment of a colony. Alternatively, the higher occurrence of OB may also be due to the sensory separation from the colony, causing an increase in activities necessary for survival that would previously have been performed by all colony members. This explanation is, however, less likely, as there is no corresponding differentiation in male levels of OB.

But why does exposure to urinary odour exert a stronger effect on MB than on other types of behaviour? As the incentives for colony recognition are intimately tied into both the breeding system of the Damaraland mole-rat and incest avoidance, it is

not surprising that the familiarity effect of urine most strongly influences the occurrence of mating behaviour. In *Spalax ehrenbergi*, mole-rats were able to distinguish between urine collected from animals in breeding season and urine collected out of breeding season (Heth *et al.* 1996). In the current experiments exposure to urinary odour had only a marginal effect on social behaviour of both males and females, possibly because social behaviour is exhibited both between breeding pairs and non-breeding siblings. Social behaviour will thus be exhibited in all situations where the other animal is accepted (either as a breeding partner or a sibling) and not attacked. This is confirmed by the observation that only one pair of female SSS and one pair of male SSS from different colonies exhibited a significant difference in the level of social behaviour, and there were no discernible patterns in levels of social behaviour.

It is clear that the urine treatment influences mating behaviour significantly for both males and females, and it is proposed that the regular exposure of animals to a mixture of their colony's urine affects their levels of mating behaviour. Therefore urinary odour is a likely candidate for facilitating sibling-recognition in *Cryptomys damarensis*.

3.4.2 The effect of relatedness on behaviour

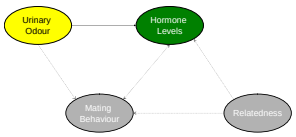


The null hypothesis was that if animals in a colony were all related by a mean of 0.5 and recognition was based on a mechanism measuring relatedness, then levels of behaviour between siblings within a colony should not vary significantly, regardless of treatment group. If, however, unrelated animals were present, or recognition was driven by familiarity rather than relatedness, variations in behaviour will be observed.

As mentioned in the previous section, there were behavioural differences observed between SSS from the same colony, where the experimental pair is assumed to possess the same level of relatedness as the control pair. These variations were more severe than those between members of the same treatment group for MB in both sexes and for female OB. If relatedness between colony members is constant, these experiments show that recognition is not based purely on relatedness and that recognition is strongly influenced by familiarity. This is congruent with previous findings indicating that familiarity plays a significant role in recognition in the Damaraland mole-rat (Jacobs *et al.* 1998). It must be noted that definitive relatedness

data would further flesh out this question and allow for the examination of any correlation between relatedness and recognition, particularly as the presence of unrelated non-breeding animals within wild colonies of Damaraland mole-rat has been demonstrated (Burland *et al*. 2004). Variations in relatedness would then offer another possible explanation for variation in behavioural levels.

3.4.3 The effect of urinary odours on hormone levels



The two null hypotheses for all tests of hormone levels is that (1) the amount of individual variation between SSS should be the same as within treatment groups, and that (2) there will be no difference in levels of behaviour between experimental animals (exposed to urinary odours) and control animals (exposed to water).

Cortisol

According to the null hypothesis, one would expect there to be no difference in levels of stress (as measured by cortisol levels) between SSS. Results from male SSS supported the null hypothesis, and exposure to urine did not seem to have any effect on levels of stress in males. However, in the females two SSS pairs (K & M and P & Q) had a CF with significantly higher cortisol levels than the EF, causing the null hypothesis to be rejected for females. Female SSS K and M did not differ significantly in their basal levels, and a graph of cortisol levels shows that the variation in these two animals shows similar trends. However, females P (CF) and Q (EF) show very different patterns of cortisol levels from day 6 onwards. These results suggest that further experiments may find that females that were exposed to urine from their native colony may experience less stress than females who did not receive familiar olfactory cues.

Due to the cooperative nature of the Damaraland mole-rat social system, an animal that has been isolated from the native colony can expect to have a much lower chance of survival and thus be subject to higher levels of stress. Colonial living is speculated to be highly beneficial, and dispersal very risky. This can be seen by looking at the low percentage (8%) of animals that disperse (Jarvis *et al.* 1994). It is interesting to consider that chemosensory exposure to colony odour (in the form of urine) may be sufficient to reduce stress and indicates the importance of chemosensory cues in Damaraland mole-rat females, but apparently this does not

apply to males. Although this finding is merely preliminary, further studies may show that males do not undergo as strong a stress response as females do when removed from their familiar chemosensory environment. This may perhaps be linked to a stronger disposition toward dispersal for males (Hazell *et al.* 2000).

The variation in cortisol levels was only observed in one pair, although it was a very strong effect (paired t-test for basal cortisol levels p=0.79 and for experimental cortisol levels p=0.02). A recent study looked at cortisol levels in female Damaraland mole-rats after interaction with unfamiliar same-sex conspecifics and found no difference in cortisol levels before and after exposure to unfamiliar conspecifics. This study was conducted with animals still housed within their native colony (Ganem & Bennett 2004).

The aforementioned study differs from the present study, however, in that the current study measured cortisol over a period of up to 18 days while the previous study measured cortisol levels directly after the encounter with a conspecific. It is therefore still conceivable that there may be differing levels of stress between EF and CF that could be detected with further studies. The combined result of the Ganem and Bennett (2004) study with the current experiment suggests that the observed rise in cortisol levels could be linked to the removal from the natal colony. This finding supports the observation that females are more aggressive toward unfamiliar animals than males (Jacobs *et al.* 1998) and may point to more sensitive chemosensory perception or to stronger philopatric behaviour in females, although a previous study on another species of *Cryptomys* found no sex bias in philopatric behaviour (Spinks *et al*. 2000).

Progesterone

Experiments were conducted around the null hypothesis that exposure to either urine or water would not alter females' progesterone levels. If there are significant differences in the levels of progesterone between SSS, then higher average levels of progesterone in control animals would indicate that exposure to colony urinary odours represses the levels of reproductive hormones in female mole-rats. In terms of progesterone, the individual nature of each female's hormonal cycle probably precludes the use of progesterone levels as a measurement over such a short period of time.

There was more individual variation in basal levels of progesterone than had been exhibited in female cortisol levels, an observation that is possibly due to the cycling effect of progesterone levels. The fact that variation within both the CF and EF-groups became significant during experimentation implies that the experimental protocol itself, as well as separation from the colony with or without exposure to urine, impacts circulating progesterone. Findings by Molteno (1999) showed that animals that have been out of the presence of the breeding female for a significant amount of time show an increase in the urinary progesterone levels and subsequent spontaneous ovulation, and these findings are supported by the present results which indicate that other changes in social environment influence progesterone levels. This result is to be expected given the strong impact that the mole-rats' social structure has on reproductive activity. SSS comparison did not reveal any definitive pattern of change in progesterone levels, but the very fact that two of the five SSS did show significant differences during experimentation again implies that progesterone levels do not remain unaffected by environmental manipulations. Future experiments should attempt to determine a female's progesterone cycle so that the cycle before and during experimentation may be compared.

Testosterone

Experiments were conducted around a null hypothesis, which expected the exposure to urinary odours to have no effect on testosterone levels between SSS. A significantly higher level of testosterone for either the CM or EM in any SSS pair would be seen as an indication of reproductive activity, which could then be confirmed by behavioural observations.

Within experimental and control groups only one male varied significantly from the other males in the control group in both basal and experimental testosterone levels, indicating that this variation was pre-existing and not brought on by experimentation. The lack of significant difference in basal or experimental testosterone levels between male SSS further indicates that the null hypothesis is supported. It may therefore be concluded that exposure to urine does not influence testosterone levels in males, as none of the SSS exhibited any differences in testosterone levels.

The results from all the hormonal assays strongly suggest that, while hormone levels in female Damaraland mole-rats are influenced by both removal from the

colony and exposure to colony urine odour, male hormone levels do not seem as susceptible to changes that are linked to the social environment as female hormone levels are. This finding correlates with previous work, which showed that inbreeding avoidance and reproductive inactivity is maintained by the female mole-rats (Bennett *et al*. 1996).

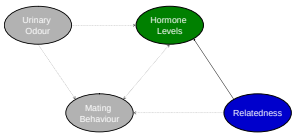
If recognition is in fact on an individual basis, then the animals receiving water and those receiving urine should show similar patterns of mating behaviour. This would be due to the fact that exposure to colony odour (such as urine) does not reinforce the mechanism of individual recognition, but physical contact with a specific individual does. One would then expect to see a strong trend of diminishing recognition as the number of days in between physical contact is lengthened. There was, however, no such trend observed. If recognition was based on self-referent phenotype matching with a purely genetic basis and no environmental influences, no mating behaviour should ever be observed between siblings. This was also not the case, and nearly all control animals did engage in mating behaviour. It is also possible that the mechanism of kin-discrimination changes based on circumstances. For example, while in the presence of the colony, a mole-rat may use colony-specific cues to identify kin or non-kin intruders in the tunnel system. Once removed from the natal colony, the animal may now rely on self-referencing in order to find a mate dissimilar to itself. Conversely, individual recognition may be used in the natal colony, but colony-specific cues or self-referencing may be used during dispersal events.

Exposure to colony urinary odour had a greater overall effect on female hormone levels, and very little effect on male hormone levels. This substantiates previous findings that indicated that, while there are hormonal differences between breeding and non-breeding females, there is no difference between breeding and non-breeding males (Bennett *et al.* 1993).

This observation is congruent with previous findings that indicate that both female progesterone level and behaviour play a significant role in maintaining incest avoidance (Bennett *et al*. 1996, Jacobs *et al.* 1998, Bennett & Faulkes 2000). Furthermore, the effect of urine odour as a mechanism of recognition should not be discounted, as there were observable changes in both behaviour and hormone levels in SSS exposed to water versus those exposed to urine. It has previously been hypothesized that discrete sources of chemical signals contain distinct information,

which may be detected by hamsters and other rodents, as well as containing overlapping information (Johnston 2003). It has also been suggested that the smearing of fluid on exiting the toilet chamber in the Damaraland mole-rat may have significance in familiarising the colony members with one another (Bennett 1988). Further work should focus on more closely identifying the compounds in urine, which may be utilised in facilitating recognition, as well as teasing apart the role of genetic relatedness in recognition.

3.4.4 The effect of relatedness on hormone levels

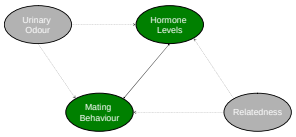


No relatedness data was available to confirm that all animals from a single colony were, in fact, siblings and related by an average of 0.5. Observations were thus purely on whether or not familiarity appeared to influence recognition separately from the question of whether or not genetic mechanisms of recognition are present. If hormone levels are not significantly influenced by the proximity of natal colony members, then one would expect to see intra-colony as well as inter-colony differences in hormone levels, regardless of the fact that both experimental and control animals have the same amount of physical contact with siblings. If relatedness does influence hormone levels and unrelated animals are present in some colonies, but not others, then one would expect to observe significant intra-colony variation in some colonies but not in others. While there was some variation within colonies, this variation was not consistent, and the role of familiarity versus relatedness was not fully tested in these experiments. Since the pairs in these studies were removed from the colonies at the same time, they retained familiarity with one another, and the resultant incest avoidance does not reflect a direct estimate of relatedness by the animals involved. Non-breeding animals from the same colony are likely to be siblings, but this is not always the case.

The results indicate that 20% of SSS do exhibit a significant variation from the hormone levels shown by their paired sibling, with the variation occurring only in progesterone and cortisol levels. This statistic does not allow for any definitive conclusions, but does suggest that the relationship between physical contact with relatives and hormone levels is more complicated than a direct correlation would indicate. While it is well-known that the presence of the breeding female has an impact on hormone levels in non-breeding females (Bennett *et al.* 1996, 1999,

Burland *et al.* 2004), it has also been suggested that environmental factors such as opportunity for dispersal and habitat saturation may impact trends toward sociality (Perrin & Lehman 2001, Le Galliard *et al.* 2005).

3.4.5 The interaction between hormones and behaviour



The null hypothesis was that there would be no sign of any interaction between hormone levels and the behaviour exhibited by animals, regardless of the kind of grouping used to examine the animals. If a correlation was found between the hormones and behaviour within a colony, it could be concluded that hormone and behaviour levels are simultaneously influenced by relatedness or familiarity. If a correlation between hormones and behaviour was found within the experimental group or within the control group, but not within colonies, then it may be concluded that urinary odour simultaneously influences hormones and behaviour.

Correlations were seen both within treatment groups and within colonies, implicating both relatedness and familiarity as factors influencing hormonal and behavioural correlation. Both of the females exhibiting correlations were CF, with female E exhibiting strong negative correlations between progesterone and cortisol levels and levels of social behaviour. This implies that as the occurrence of stress and reproduction goes up, the occurrence of social behaviour may be expected to go down. While there is no clear pattern in the correlation between hormones and behaviour, it is clear that there is some sort of effect being observed, and that further experimentation may find specific correlations between levels of stress (as measured by cortisol) and reproductive activity (specifically as measured by progesterone for females).

3.5 Conclusion

This investigation showed that mating behaviour in the Damaraland mole-rat can be used as a more humane alternative to investigating recognition than aggressive behaviour. It was also found that familiarity does play a role in the maintenance of the social system of the Damaraland mole-rat, as suggested by Jacobs and Kuiper (2000), and that the behaviour exhibited by the mole-rats is not explicable by inherent genetic mechanisms of recognition alone. If recognition were based purely on a genetic mechanism, then habituating animals to an odour should not disrupt recognition - the recognition mechanism should still be intact based on phenotype

matching or recognition alleles. The fact that exposure to urinary odour allows the mole-rats to apparently recognize each other for a longer time period shows that familiarity does play a role. While it does not prove that genetic mechanisms do not also exist, it does imply that familiarity is playing some role in recognition. It is possible that familiarity with the urine odour is merely confusing the inherent genetic mechanisms of recognition, but it still shows that familiarity can influence recognition.

While genetic mechanisms of recognition may be present, this study found that sibling recognition is influenced by familiarity with colony urine odour. Furthermore, the variation in progesterone and cortisol levels between control and experimental animals indicates that these hormones have some form of interaction with the environment. The correlation found between behaviour and hormone levels illustrates the interdependence of these two variables, and suggests that more specific research looking at the interplay will provide fruitful insights.

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4 Chapter Four – Aspects of memory in *Cryptomys damarensis*

It has been proposed that the evolution of cognition should be studied alongside how it functions, as well as studying what the behavioural implications are in applicable ecological situations (Real 1993). The work presented in the current thesis focussed on determining whether or not there is a cognitive mechanism operational in the behaviours associated with reproduction and foraging. The results from the experiments confirm the important role that animal cognition and memory plays in understanding animal behaviour. While these results do not answer questions pertaining to the neural mechanisms involved in learning and memory in mole-rats, it is clear that memory is involved in both social recognition and orientation. It would be difficult to find an alternative explanation for the gradual difference in behaviour exhibited over a span of time. Evaluating the apparent decline in performance in kin-recognition and spatial orientation under the same conditions, with time as the primary variable, supports a mechanism that involves memory and recall.

In the experiments involving sibling-recognition, it appears that memory of siblings is created and maintained on the basis of familiarity. The observation that memory is involved in directing acts of kin-discrimination does not imply that there is a higher-level cognitive function involved in deciding how to act towards another animal. Rather it would appear from the results of this research, as well as previous findings (Jacobs & Kuiper 2000), that familiarity is maintained by constant reinforcement. The current results demonstrate that memory is probably involved, since the behaviour (incest avoidance) does not change immediately following removal of the reinforcement agent (urine odour). The persistence of incest avoidance after the removal of the cue for familiarity suggests either the use of memory or an innate mechanism such as phenotype matching or recognition alleles, which do not require learning (Tang-Martinez 2001). The probable existence of memory has demonstrated, but does not prove, that familiarity is exclusively involved. Further experiments focussed on demonstrating that learning is involved in the development of kin-discrimination, and results from investigations aimed at searching for a genetic basis of recognition are needed to form more definitive hypotheses.

In the spatial experiments learning was clearly present in both the Damaraland mole-rat and the Cape mole-rat as indicated by the slope of the learning curves constructed from the obtained data. Mole-rats were allowed to use external "landmark" cues that in all probability are chemosensory (Judd & Sherman 1996) and tactile in nature (Kimchi & Terkel 2004) given the lack of visual acuity exhibited by these fossorial rodents (Nemec, Burda & Peich 2004). The chemosensory cues were eliminated by washing the maze and removing any traces of semiochemicals, thus forcing the mole-rats to rely on either path integration, magnetic or tactile cues. The role of the earth's magnetic field in orientation may be examined by manipulating the orientation of the maze, or by experimentally altering the magnetic field surrounding the maze. Furthermore, the role of tactile cues could be further investigated by either increasing the diameter of the tunnel system, which makes contact with the walls more difficult or by trimming the vibrissae or sensory hairs used in tactile orientation.

Experiments aimed at examining spatial ability in the Damaraland and Cape mole-rat revealed that species' spatial ability differ in correlation to the intricacy of the architecture of the burrow system. Performance in the maze trials over a period of time is definitively linked to learning and memory. None of the animals exhibited any predictable pattern of exploration, but all improved their orientation performance with experience in the maze system. Longer periods of time between exposure to the maze and tests also correlated with a decrease in performance, as would be predicted when an older memory has to be accessed.

Furthermore it is interesting to note that there may be significant differences in spatial memory within a species, as observed in the Cape mole-rat. Female Cape mole-rats exhibited better longer term memory than males, despite exhibiting slower learning curves. If sociality is a predictor of enhanced spatial memory then it is clear that male and female Damaraland mole-rats inhabit similar social environments, whereas female Cape mole-rats have more social environments than the exclusively solitary males due to the plural occupancy of burrows with young offspring during the breeding season.

Hormones may also be implicated more strongly if their effects on learning, memory, and the neurological basis for these behaviours are more closely examined. It is also likely that hormonal fluctuations are more liable to influence female learning and memory, as females experience greater fluctuations during their reproductive cycle. Progesterone and cortisol have been shown to oscillate with changes in mating

behaviour in the recognition experiments conducted here. Furthermore, in humans the female hormonal cycle influences the ability to navigate. More specifically, when levels of oestrogen and progesterone are low during the menstrual phase the performance of human males and females with respect to spatial tasks are indistinguishable. However, during the post-ovulatory phase, female spatial orientation in the absence of landmarks is impaired (Chabanne, Peruch & Thinus-Blanc 2004). Insulin is another hormone that has been implicated in the modulation of cognitive functions and insulin receptors have been identified in the hippocampus (Park 2001).

It is also intriguing to consider that ecological and life-history conditions may correlate to behaviours such as recognition and burrow orientation. Certainly it is most plausible that these factors influence what could be considered adaptive or selectively advantageous for mole-rats living in mesic or arid environments as single animals or members of social colonies. Although there is, as yet, no conclusive evidence indicating that sociality in itself is linked to improved navigation ability, it is exciting to consider that corticotropin-releasing factor (CRF) has been described as binding differentially in social and solitary vole species in certain parts of the brain, including the hippocampus (Lim, Nair & Young 2005). The hippocampus has been found to be an important structure in the brain for spatial orientation and the learning of complex routes in the environment. Indeed, London taxi drivers have on average much larger hippocampal volumes than the general population (Maguire *et al.* 2000), apparently due to the need to memorise the many routes they drive.

The involvement of the hippocampus in spatial orientation in mammals should result in increased hippocampal volumes in species where spatial learning is more important (Shettleworth 2001). Evidence for this has been found by Sherry *et al.* (1992) who found enlargement of the hippocampus in both birds (see also Reboreda *et al.* 1996) and mammals in response to increased demand for spatial cognition and memory, and predicted that this trait may be selected for under certain circumstances, such as with food-hoarding species and polygynous moles that remember the location of females in the area.

If this prediction holds, both the Damaraland mole-rat and the Cape mole-rat would have circumstances favouring the enlargement of the hippocampus. For Damaraland mole-rats memory of a complex spatial environment would favour increased use of the hippocampus, while the need to remember the location of

potential mates could similarly influence hippocampal size in Cape mole-rats. Both species may, however, use cues that would perhaps limit the necessity for an exceptionally developed hippocampus. The Damaraland mole-rat could use odour cues to locate food sources (Judd & Sherman 1996), although it is unlikely that their chemical communication is varied enough to indicate differentially trials to food, toilet and nesting chambers. Cape mole-rats are known to use seismic signalling to locate other animals (Narins *et al.* 1992), and this may predicate the need to remember the location of other mole-rats in the area.

Clearly the function of memory needs to be further studied in the Bathyergidae. The existence of behavioural and ecological variation implies that there are circumstances under which memory is likely to play a role in modulating animal behaviour. Further consideration should be given to the evolutionary history of the mechanisms involved in memory, as well as investigating the intriguing role that hormones could play in further understanding how memory relates to social systems.

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