PUP ONTOGENY AND FACTORS INFLUENCING BEHAVIOURAL AND MORPHOLOGICAL VARIATION IN NAKED MOLE-RATS, *HETEROCEPHALUS GLABER* (RODENTIA, BATHYERGIDAE)

by

Mannus Justin O'Riain

Thesis presented for the degree of
Doctor of Philosophy

Zoology Department, Faculty of Science
University of Cape Town

March 1996

DECLARATION

This thesis reports the results of original research I conducted under the auspices of the Zoology Department, University of Cape Town, between 1989 and 1995. All assistance that I received has been fully acknowledged. This work has not been submitted for a degree at any other university.

Signed by candidate

Mannus Justin O'Riain

DEDICATION

I dedicate this thesis to my four parents who have provided me with the love, support and encouragement that have made it all worthwhile...... and to the little pink 'uns whose mysterious ways have enthralled and intrigued me for so many years at the expense of their freedom.

ABSTRACT. O'Riain, M.J. 1996. Pup ontogeny and factors influencing the behavioural and morphological variation in naked mole-rats, *Heterocephalus glaber* (Rodentia, Bathyergidae). PhD thesis, University of Cape Town.

A long term behavioural study was undertaken on laboratory-reared naked mole-rats. The main objective of the study was to provide a detailed qualitative and quantitative description of the behavioural and morphological development of naked mole-rats from birth through to adulthood. This study laid an empirical foundation for the subsequent testing of hypotheses pertaining to within and between colony conflict as well as for a study on the existence of a rare dispersal phenotype within colonies.

The overall pattern of development of the pups is similar to other social and solitary mole-rats, but naked mole-rats exhibit a delayed onset in the maturation of both behavioural and morphological attributes. Development was divided into four continuous periods on the basis of motor, sensory and social capabilities. The incorporation of pups into the social organisation of the colony was characterised by acts of both cooperation and conflict. Thus the parents and older offspring donated faeces, groomed pups, maintained the burrow system and brought food back to the nest, - acts which facilitated pup-survival. However, they also physically bullied the pups by shoving them (pup-pushing) thus ensuring the establishment of a social hierarchy within colonies, in which parents dominate the older siblings, who in turn dominated their younger siblings. A positive effect of offspring philopatry to the parents, was the gradual take-over of the colony work activities by the offspring, which enabled the parents to devote more of their time and energy to reproduction. Despite seldom foraging, the breeding female nevertheless maintained a monopoly on food when the food supply was experimentally limited. Offspring always attempted to feed first before provisioning the communal nest, suggesting that the cooperative act of foraging is subject to the selfish precedent of satiation.

The effects of inter-litter hierarchies were evident in the differential growth patterns of successive litters recruited to breeding pairs. Older siblings attained a greater body mass at a given age and there was an inverse trend between asymptotic body mass and litter order. Similarly, the magnitude of the growth response following the death or removal of the breeding male or older siblings was greater for older litter members. Dramatic divergence in the growth trajectories of litter-mates was associated with a divergence in their respective behavioural repertoires.

The social organisation within mature colonies was characterised by a clear primary division of labour between the breeders and their non-breeding offspring. There was only a weakly defined secondary division of labour amongst the non-breeders with most of the variation being attributed to the performance, or lack thereof, of key sexual and dominance related behaviours. These behaviours were perfromed by the older colony members who were also the most likely heirs to reproductive rights within the colony. There was no evidence for morphological specialisation amongst the non-breeders or the breeding male. In contrast the breeding female exhibited a marked lengthening of the vertebrae associated with the attainment and maintenance of reproductive tenure. Furthermore, only the breeding female had a qualitatively distinct behavioural repertoire, ceasing her participation in work and being the sole individual to bear young and lactate. The most salient component of the division of labour amongst non-breeders was that of task fixation. Mole-rats typically remained faithful to a particular task and location within the burrow system as long as they received positive feedback. This effectively ascribed the correct number of individuals to particular tasks as well as ensuring that individuals maintained flexibility in the nature of the tasks that they performed. This combines the benefits of a specialist with that of a generalist, an important adaptation in an environment which is predictably unpredictable.

Naked mole-rats were observed to aggressively defend their burrow systems from the intrusion of foreign conspecifics. Colony defence was primarily performed by the largest colony members. These individuals were well equipped for defence having large broad incisors and well developed temporal muscles. Smaller colony members that intercepted an intruder typically alerted colony members by emitting a shrill alarm call. Colony member recognition was apparently achieved through recognition of familiar colony odours. Both the nest and toilet area were identified as likely sources of colony odour. It is suggested that a cocktail of the odours of all the individuals in a colony form a unique colony odour label. This label serves as a reliable cue for the recognition of colony and non-colony members from genetically similar neighbouring colonies.

A small percentage of the captive population of mole-rats in this study were not aggressive towards foreign conspecifics but rather attempted to solicit them sexually. All but one of these individuals were male and displayed a strong urge to leave their natal burrow system. These individuals were behaviourally, morphologically and physiologically distinct from their siblings. Laden with large deposits of subcutaneous fat, sexually primed, and fulfilling the criteria for outbreeding I suggest that these individuals represent a rare dispersive morph within otherwise highly inbred colonies of naked-rats.

TABLE OF CONTENTS

SECTION I

SECTION II

ACKNOWLEDGEMENTS

I thank 'all' of my parents and grandparents for providing me with the educational and financial platform to initiate a tertiary education. The FRD is thanked for financial assistance during the course of this study.

Professor Jennifer U.M. Jarvis is thanked for the opportunity to study naked mole-rats and to reap the benefits of her many years of research and wealth of both scientific and animal husbandry knowledge. I am indebted to Jenny for the enthusiasm, guidance and encouragement (and nutrition!) that she provided at all stages of this study.

Rachel Alexander is thanked for her good humoured and professional assistance with the X-raying of the 'noo-noos'. Neville Eden is thanked for his patient assistance with all photography. Thanks to Joseph Booysen and Mary Vosloo who were of great help in the care and maintenance of the study animals. Numerous people helped with the mind numbing process of collating behavioural data. My thanks to Rene Welby-Cooke, Felicity Elmore, Anton Wolfaardt, Karin Pilz, Nicola Stegmann and Schula Croeser. The Animal Unit at WITS University's Phyisology Department is thanked for the cooperation and help in measuring the fat content of naked mole-rats.

Andrew Spinks, Stephen Telford, Casper De Villiers, Nigel Bennett, Jonathen Bloomer, Daniel Polakow, Paul Sherman, Christian Peeters, Anthony Hazel and Sioban Munro are thanked for useful comments on rough drafts of various chapters. Lisa Kruger is thanked for the excellent job she did in proof reading final versions of certain chapters. Gonzalo Aguilar was of invaluable help in facilitating my understanding of certain statistical analyses and models. Thanks also to the 'mole-rat group' for numerous interesting discussions, especially Andrew Spinks who was always prepared to 'chew the theoretical cud' ad-infinitum.

Professor Anne Rasa, while at the University of Pretoria, is thanked for providing me with a baseline of essential 'methodological and theoretical tools' for the study of animal behaviour.

Thanks to my friends and colleagues for keeping my 'left brain' active throughout this study, especially Deon Durholtz who was always there to 'lead me astray' and who provided support, companionship and 'warm' beverages in equally replenishable quantities. Thanks too to Casper De Villiers for his motivation throughout this study.

A very special thanks to Karin Pilz for 'keeping my wheels on' over the latter stages of the write-up. Her patience, care and encouragement were an invaluable source of both stability and emotional upliftment.

GENERAL INTRODUCTION

Historical perspective and current social status

Studies on the naked mole-rat (*Heterocephalus glaber*) have contributed substantially to our understanding of the organisation and functioning of social and cooperatively breeding vertebrate systems (Sherman *et al.* 1991; Jarvis *et al.* 1994; Jennions and McDonald 1994). Furthermore, their inclusion in the 'eusocial invertebrate fold' (Jarvis 1981) has encouraged an emphasis on ecological correlates, in the search for factors favouring the evolution and elaboration of eusociality in animal societies (Alexander *et al.* 1991; Jarvis *et al.* 1994).

Animal societies were traditionally categorised as eusocial if they satisfied the following three criteria (Wilson 1971): an overlap of at least two generations, a reproductive division of labour and the cooperative care of the young produced by the breeders. Prior to Jarvis (1981), who demonstrated conclusively that naked mole-rats met all three of these criteria, this definition effectively served as a semantic barrier between theoretical considerations of social invertebrate and vertebrate societies.

In retrospect, exactly why the naked mole-rat proved to be the exception was not clear, for studies on other social vertebrates, for example the dwarf mongoose, *Helogale parvula* (Rasa 1977; Rood 1978), the wild dog, *Lycaon pictus* (Frame *et al.* 1979) and Florida scrub jays, *Aphelocoma coerulescens* (Woolfenden 1975) had previously satisfied the same criteria stipulated in the definition and yet these species were never referred to as eusocial. Superficially, the large colony size of naked mole-rats, up to 300 individuals (Brett 1991; Braude *pers. comm.*), their construction of a permanent nest and burrow system together with their cooperative digging and provisioning behaviour, may have encouraged comparisons with eusocial invertebrates. These similarities may thus have been influential in giving naked mole-rats a 'eusocial status', despite none of these attributes being stipulated in the original definition.

The constraints of a subterranean habitat and large colony size are nevertheless very important to theories pertaining to the elaboration of social systems, for they influence the selective environment of the society. In this regard comparisons with invertebrates such as termites that live in relatively safe, expandable underground fortresses (Roisin 1994), or ponerine ants that live in groups ranging from a few dozen to a few hundred individuals per colony (Peeters, in press) are more realistic than comparisons with free ranging social vertebrates (e.g. the dwarf mongoose, Rasa 1987) which live in smaller groups without permanent, expandable nests or burrow systems. Comparisons between groups as taxonomically different as vertebrates and invertebrates have the further advantage of elucidating common selective factors without the associated 'phylogenetic noise' of a close common ancestry. It is for these reasons that many of the arguments that I develop in this thesis (especially Chapter 3 on social organisation), focus more attention on comparisons with social invertebrates than with social vertebrates.

Recently, Sherman *et al.* (1995) have attempted to dismantle the semantic barriers that exist between eusocial and cooperatively breeding animal species, suggesting the use of a "eusociality continuum", based on an index of reproductive skew (*sensu* Vehrencamp 1983). Sherman *et al.* (1995) argue for the recognition of the fundamental similarities between all social species, and arrange them according to the degree of variation in individual lifetime reproductive success within a breeding group. Thus wild dogs, naked mole-rats and honey bees are united under a "single theoretical and terminological umbrella", facilitating comparisons between them. In this scenario the semantic dilemma between naked mole-rats and other social vertebrates falls away.

In contrast to Sherman *et al.* (1995), Crespi and Yanega (1995) have argued for a restriction of the term eusocial to those species characterised by individuals that become "irreversibly behaviourally distinct (i.e. castes) at some point prior to reproductive maturity". In contrast, cooperative breeders are referred to as species with alloparental care but without castes. This effectively restricts eusociality to a small subset of the invertebrates which includes certain termites, *Macrotermes* and honey bees, *Apis*, while once again aligning naked mole-rats with other social vertebrates as well as some invertebrates. This approach provides greater resolution thus serves to emphasise the closer parallels that exist between certain of the primitive ant species (*e.g. Ponerini*) and vertebrates both of which are classified as cooperative breeders.

Both studies represent an important contribution to our understanding of the taxonomic correlates of sociality. However, I consider Crespi and Yanega's (1995) approach to be more heuristic, for as they point out, the irreversibility of castes represents an evolutionary threshold: "once an individual enters a caste, natural selection of its behaviour has become circumscribed in its effects to modification within a specific limited range of behaviours". Such specialisation in behaviour evolves only under selective conditions substantially different from those that have produced non-eusocial systems. The elucidation of those conditions that have led to the evolution of castes is thus one of the main thrusts of studies on insect social behaviour. Furthermore, the approach of Crespi and Yanega encourages a closer consideration of the fundamental *differences* and similarities that exist between various social species. In contrast Sherman *et al.'s* (1995) approach emphasises the *similarities* in only one aspect of social life, namely reproductive inequalities.

Scope and objectives of the thesis

Much of our knowledge concerning the naked mole-rat is summarised in the book, *The Biology of the Naked Mole-Rat* (Edited by Sherman, Jarvis and Alexnder 1991). In this comprehensive collection of individual studies, which range from systematics to behaviour, it was apparent that the single largest gap in our understanding of naked mole-rat sociobiology, was that of ontogeny and the division of labour within colonies (Jarvis *et al.* 1991; Lacey and Sherman 1991; Lacey and Sherman, in press). Previous studies on the social organisation of naked mole-rats have focussed almost solely on behavioural and morphological variation amongst adult individuals. This mirrors the historical trend in the vast majority of ethological studies, in which the behavioural patterns of adults are considered to be representative of the species under consideration (*sensu* Bekoff 1972). Consequently, the developmental aspects of such behaviours have largely been disregarded, effectively resulting in a restrictive set of explanatory concepts for the social organisation of a given species. So as to fully understand adult social behaviour we must know something of its ontogeny. Thus the study of development cannot be separated from the study of the functions of the adult animal (Tinbergen 1951).

A study of the behavioural and morphological ontogeny of naked mole-rat pups was thus initiated, to address this imbalance. The study was subsequently extended into a long-term behavioural project (total duration of six years), when it became clear that in order to relate the ontogeny of pups to their adult behaviour data had to be collected over a significant portion of an individual's life. These data permitted the first detailed study of factors influencing the behavioural and morphological variation of adults within mature colonies of naked mole-rats. Furthermore, these data laid the foundation for the formulation and testing of hypotheses pertaining to other organisational aspects within mature colonies. More specifically the dynamics of conflict, xenophobia and recognition cues within and between colonies were investigated. In addition, the discovery of a rare dispersal phenotype within colonies, led to an in-depth study of the possible existence of selfish outbreeders within otherwise highly inbred colonies (Faulkes *et al.* 1990; Reeve *et al.* 1990; Honeycutt *et al.* 1991).

Organisation of thesis

The thesis is logically divided into two sections, each with three chapters. The first section, includes chapters 1 to 3, and focuses on the following aspects of naked mole-rat biology. Chapter 1 provides a qualitative and quantitative description of pup ontogeny and early colony development. Chapter 2 is a detailed comparative study of factors that affect the growth of individuals, within and between litters. Chapter 3, uses information collected in Chapters 1 and 2, in addition to detailed behavioural and morphological data for mature colonies, to describe the formation and maintenance of the social organisation within colonies.

Section two (chapters 4-6), draws largely on the knowledge gained in section one, and sets out to test several hypotheses pertaining to group-living. Chapter 4 is a brief study in which I used competition for limited food resources within colonies, as a bioassay for determining the dynamics of conflict within colonies. In Chapter 5, I explore the behavioural response of colony members to foreign conspecifics, and attempt to elucidate how recognition of colony members is achieved. The final chapter (Chapter 6) tests the hypothesis that outbreeders exist within captive naked mole-rat colonies. Verification of this hypotheses initiated a series of detailed measurements on the morphology, behaviour and physiology of outbreeders.

Although each chapter has been written in a manner that effectively allows it to be read in isolation, I make frequent reference to relevant sections in other chapters. This, and the fact that many chapters build on previous chapters means that the thesis needs to be read in its entirety for a full understanding of the issues covered. My approach has forced some repetition of aspects relating to the general biology of the species, but only where it was deemed necessary to enable the reader to obtain a better understanding of the hypotheses being tested. In addition, the detailed discussions, and the summaries provided at the beginning of each chapter, have largely negated the need for an elaborate concluding synthesis. The latter is thus brief and attempts to draw out the most salient findings of the whole study.

Brief overview of the habitat and phylogeny of naked mole-rats

Naked mole-rats inhabit the arid and semi-arid regions of central East Africa. Here they live in groups ranging in size from just a few individuals (n=3 adults in nascent colonies, Braude, pers. comm.) to ca. 300 individuals (Brett 1991). The soils, in which they live and search for food are typically very hard, imposing extremely high energetic costs (McNab 1966) on survival. Both Jarvis (1979; 1985) and Lovegrove and Wissel (1988) have suggested that these high costs, in addition to the risks associated with foraging for a food source that is widely dispersed and patchy in distribution, have been instrumental in leading to the evolution of group living, in both the naked mole-rats and the other social Bathyergidae. Thus, only those mothers that tolerated the presence of their adult offspring in the natal burrow system, and those adult siblings that tolerated one another, would have survived in this arid and unpredictable environment (Jarvis *et al.* 1994). A necessary consequence of this extended family, is the high relatedness within groups, which is further exacerbated by inbreeding in naked mole-rats.

Recently Bruford and Faulkes (pers. comm.) have provided a comprehensive phylogenetic tree for all the genera of the bathyergid family. It is evident from this tree that within the Bathyeridae there is an apparent continuum from solitary through to social genera. However, an early phyletic divergence of naked mole-rats from other social and solitary Bathyergidae provides support for the independent evolution of sociality in the two social genera as suggested by Jarvis and Bennett (1993). Thus where possible comparisons are made between the social system of naked mole-rats and the other genera of social mole-rats, the cryptomids.

Plate I: **The naked mole-rat.**

Naked mole-rats are small, virtually hairless, poikilothermic rodents of the family Bathyergidae. Being completely subterranean they have greatly reduced eyes and ear pinnae. Their bodies are cylindrical in shape with a loose skin and short limbs. Prominent extrabuccal incisors protrude from the mouth and the lips are large and fleshy. Sensory vibrissae are found over most of the body (including the tail) and prominent whiskers are apparent in the snout region, above the eyes and protruding from each cheek. The outer edges of the hind feet are fringed with stiff hairs.

SECTION I

CHAPTER ONE

PUP ONTOGENY AND EARLY COLONY DEVELOPMENT.

SUMMARY

This study describes and quantifies the behavioural and morphological development of 40 captive-born naked mole-rats, recruited in six litters to two breeding pairs (three litters/pair). Development is divided into four continuous periods on the basis of motor, sensory and social capabilities. The overall pattern of development is similar to other social and solitary bathyergids, but naked mole-rats exhibited a delayed onset in the maturation of both behavioural and morphological attributes. Pup mortality rates were highest during the first week postpartum and were directly attributed to failed nursing attempts. Allocoprophagy served as an important nutritional bridge for pups during weaning from milk to solids. Cooperative and agonistic interactions between parents and their offspring and between siblings varied significantly between litters. The functional significance of pup-related activities (such as pup-pushing) to the colony social structure is discussed. The overall work rate at the colony level increased exponentially with recruitment, whereas the amount of work done by the breeders showed a concomitant decrease. Offspring thus aid in the reproductive success and survival of the colony through their contribution to foraging and burrow maintenance tasks in addition to the care of new born siblings. The frequent performance of both agonistic and cooperative social interactions highlight the dual nature of naked mole-rat societies with the need for cooperation in a harsh ecological habitat contrasting sharply with the selfish needs of the individual.

INTRODUCTION

The importance of the naked mole-rat to understanding the origins of eusociality has stimulated research on factors responsible for the evolution and maintenance of their social organisation (Alexander. *et al.* 1991; Lacey and Sherman 1991; Jarvis *et al.* 1994). Much of this research has targeted behavioural and morphological variation of adults within established colonies with little consideration of the developmental aspects of these attributes

(Jarvis and Sale 1971; Jarvis 1981; Isil 1983; Brett 1986; Jarvis *et al.* 1991; Lacey *et al.* 1991). Since adult morphology is the product of development, the developmental systems that generate different adult forms and behaviour are fundamental to the understanding of social behaviour.

Studies on naked mole-rats that include information on development have largely relied on few qualitative data (Jarvis 1991; Lacey and Sherman 1991). The major limitation to a quantitative approach has been the difficulty of rearing successive, large litters in captive colonies. Here I attempt to address this imbalance in our knowledge concerning naked mole-rat biology through the establishment of colonies from reproductive pairs, in conjunction with minimal interference during the early stages of pup-nursing. Success with this approach enabled me to attempt the first detailed study on the behavioural and morphological ontogeny of naked mole-rats.

The primary aims of this research were to:

1) Determine qualitatively the characteristics of the maturing infant mole-rat's behaviour and morphology, and in particular, to characterise the course of its development.

2) Obtain a quantitative account of the developing mole-rat's behaviour and morphology as a function of age.

3) Record the dynamics of parent-offspring/offspring-offspring interactions in colonies established by philopatry.

Observations on entire litters from birth through to adulthood should emphasise developmental factors that influence the behaviour and body size of adult mole-rats, and hence the social organisation within and amongst litters in long established (> three years) colonies. The latter aspect necessitates a longitudinal behavioural study of the same individuals, an area of research hitherto not covered in naked mole-rat sociobiology (see Chapter 3). Here the results of the initial findings (from colony inception to ca. one year) of this longitudinal study are presented. In addition, the effects of offspring recruitment (as alloparents) on the overall work rate of the reproductive pair, and on the development of the colony as a functional unit, suited to survival in a harsh ecotope, are assessed.

METHODS AND MATERIALS

Three breeding pairs (all multiparous females and their mates) of naked mole-rats were established in separate artificial burrow systems. Each system comprised a three and a half metre network of clear Perspex burrows (45mm wide by 53mm high) linked to a nest, food and toilet chamber. The floors of all chambers were covered with wood shavings and a mixture of woodwool and shredded paper toweling was supplied as nesting material. Mole-rats were fed daily (*ad libitum*) on a variety of fruit and vegetables. Their diet was supplemented with moistened Pronutro^{TM} (a commercially prepared nutritionally balanced grain cereal). Toilet chambers were cleaned daily and partially filled with fresh wood shavings. The systems were located in sound proof rooms with humidity and temperature kept at ca. 50% and 30°C respectively. To facilitate thermoregulation, a lamp with a 40 W bulb was provided as a localised heat source along a section of the burrow system. Laboratory conditions and the husbandry methods used are described in detail by Jarvis (1991).

One hundred days after their introduction to the burrow systems all three breeding pairs had produced a litter. The number of individuals in each litter was noted within 24 hours after birth. Care was taken not to disturb the breeding pair until the pups were weaned, as disturbance (*e.g.* loud noises or handling) typically results in recruitment failure (Jarvis 1991). Prior to weaning (and individual identification) it was not possible to obtain individual behavioural profiles, and thus the early developmental variables are a mean for the whole litter and not the average of the individual totals within the litter. Both qualitative descriptive data and quantitative frequency data were recorded for members of each litter born during the study period. This enabled me to provide a detailed account of the ontogenetic sequence of development in addition to changes in the frequency of performance of specific behaviours during the first three weeks of life. The temporal onset of a specific behaviour was taken to be the first time the behaviour was performed by a member of the litter. Data were collected by recording the behaviour of all pups (sequentially) in each colony, every two minutes (scan sampling, Altmann 1974) for a minimum of one hour per day. In addition, approximately one hour of *ad lib.* sampling, divided into four 15 minute intervals during the course of each day, was recorded per litter. This facilitated the detection of the onset of specific behaviours.

Pups that survived to weaning were sexed, weighed and toe-clipped to enable permanent identification. Data concerning sex ratios and mass at birth were previously recorded by Jarvis (1991) in a study that did not necessitate long term observations. Post-weaning data were collected by marking each mole-rat with permanent ink to facilitate individual identification. A check-list was used to record the behaviour and location of each animal within the burrow system. Behaviours that were recorded were identified from previous observations and are largely consistent with Lacey *et al.*'s (1991) ethogram (see Appendix I). Interactive behaviours, such as toothfencing, were scored twice since they involved two animals performing the same behaviour concurrently. Each observation period lasted one hour during which the activity of each colony member was recorded at two minute intervals. Rare behaviours (*e.g.* mating) were recorded continuously. Observations were made randomly between 08h00 and 22h00 on a daily basis.

To investigate possible developmental trends during the first nine weeks following birth, data were combined to provide weekly averages. Thereafter (the next 21 weeks) data were pooled into monthly averages to assess the affects of recruitment on colony ergonomics. Pup-related activities (*e.g.* pup-pushing, allocoprophagy, pup-carrying) and pup-pup interactions (*e.g.* biting and toothfencing) were grouped from the weaning of one litter to the birth of the next. This ensured that data collected on pup-related behaviours were analysed with respect to a specific age range that was comparable across litters. Mole-rat behaviour was recorded for a total of 336 hours in two of the study colonies (169 hrs and 167 hrs for colony 100 and 7400 respectively).

The physical development (tooth eruption, eye opening and opening of auditory meatus) of the pups was monitored daily, while post-weaning body mass (to the nearest 0.1 gram) was determined approximately weekly.

Data analyses

All behavioural data were converted to the frequency of performance per hour. Interactive behaviours were analysed with respect to the mean number of times a behaviour was either initiated or received by both breeders (sorted by gender) and their offspring (sorted by litter). No gender-specific analyses were performed for offspring since previous studies (Faulkes *et al.* 1991; Jarvis *et al.* 1991; Lacey and Sherman 1991), and preliminary analyses in this study, demonstrated the absence of any sex-biased performance in behaviour. Behaviours that were infrequently performed (*e.g.* carrying and manipulation of nest material) were not included in the analyses.

To investigate possible developmental trends, the total frequency of occurrence of a behaviour was correlated with time using Spearman rank order correlations, where age is represented by a maximum of nine age periods. It should be however, be noted that a behaviour would correlate with age only if the frequency of occurrence of that behaviour consistently increased or decreased in successive age periods (Sokal and Rohlf 1969). This rule largely precluded statistical analysis of many developmental variables (*e.g.* feeding on solids). However, because developmental trends are largely descriptive, few statistical procedures are relevant to their interpretation.

Kruskall Wallis ANOVA's of differences between successive litters were conducted by pooling the data for each age cohort over the last sampling interval in both colonies.

RESULTS

Although all three breeding pairs gave birth to litters within 100 days of their establishment, only two of the colonies reared sufficient pups per litter for a statistical comparison within and between litters. Consequently, while growth and morphological development was monitored for individuals born to the third colony (colony 2000) no data on behavioural development were recorded in this colony. The ontogenetic results are thus from 40 pups recruited in three successive litters in colony 100 (23 pups) and colony 7400 (17 pups).

No effects of either litter order or litter size were apparent for either behavioural or morphological development in both colonies (Table 1: ANOVA $F_{2,36}=0.005$, $P=0.995$, colony 100; $F_{2,36}=0.009$, $P=0.992$, colony 7400). Consequently, developmental profiles were generated using combined data for the two colonies (Litters 1-3, in colonies 100 and 7400).

Early pup development

Naked mole-rat development can be divided into four periods - neonatal, transitional, social and juvenile (modified from Williams and Scott 1954), distinguished on the basis of differences in motor, sensory and social capabilities. Table 1, gives the mean age at which the various behavioural and morphological attributes were apparent for members of the litters born to colonies 100 and 7400.

The neonatal period is from birth to day three when the first overt behaviour patterns, not evident in the first 24 hours after birth, were noted. At birth the pup was a bright pink-red colour and the abdominal viscera were clearly discernible through the translucent skin. Cranial sutures, the fontanel in the skull and much of the skeleton were also visible. True to the adult form, the only hairs on the body were the scattered vibrissae ($\pm$1.2 mm in length). The eyes were covered by a layer of skin and the auditory meatus was closed. The incisors erupted within the first day after birth. The digits were fused but discernable and the claws blunt and covered with a transparent layer of skin. The pups responded to tactile and olfactory stimuli (presence of breeding female) and thus vocalised (cf. juvenile squawk, Pepper *et al.* 1991) when displaced from the nest.

The predominant activities performed by pups during this period were sleeping, sucking and locomoting within the nest, in order to locate the breeding female, maintain contact with littermates and, when there were subsequent litters present, to remain at the top of the nest huddle. When sleeping, the pups twitched spasmodically and displayed rudimentary grooming behaviour (hind-limb scratch). Sucking was less frequent in the first week after birth than in the second week as the breeding female was restless and assumed the nursing position for only short periods of time in the first three days postpartum. In addition, the pups were initially inefficient at both locating the breeding female and subsequently securing a nipple. Naked mole-rat pups are able to crawl from birth, although the gait is uncoordinated. The hindlimbs are laterally splayed and cannot support the body. The ventral surface of a pup is thus in constant contact with the substrate. In most instances the forelimbs provide the means of propulsion while the hindlimbs were held at right angles to

Table 1. The effects of litter order and litter size on the physical and behavioural development of naked mole-rats born to the first three litters of colony 100 and 7400. First observed occurrence of physical changes and selected pup and adult behaviours are presented for each litter.

	Age in days			
Character	Lit 1 (N=4)	Lit 2 (N=13)	Lit 3 (N=5)	$\bar{X}$±S.D.
Colony 100				
Mouth nest material	12	12	14	12.7±1.15
Solid food	13	14	14	13.7±0.57
Leave nest	15	17	13	15.0±2.0
Biting	15	18	15	16.3±1.53
Tooth-fence	18	16	20	18.0±2.0
Allocoprophagy	19	18	20	19.0±1.0
Gnawing	20	19	23	20.7±2.08
Sweeping	22	21	19	20.7±1.53
Carry nest material	25	23	23	23.7±1.15
Carry food	30	30	34	31.3±2.31
Autocoprophagy	33	38	35	35.3±2.52
Weaned	37	38	34	36.3±2.08
Eyes open	50	52	51	51.0±1.0
Cumulative age/lit	307	316	314	
Growth rate @ 78 days	0.19	0.16	0.17	
Colony 7400	(N=5)	(N=4)	(N=8)	
Mouth nest material	13	12	14	13.0±1
Solid food	15	14	13	14.0±1
Leave nest	12	17	13	14.0±2.65
Biting	15	18	15	16.0±1.73
Tooth-fence	18	18	20	18.0±2.0
Allocoprophagy	20	18	20	19.3±1.15
Gnawing	22	19	20	20.3±1.53
Sweeping	22	24	18	21.3±3.05
Carry nest material	25	23	23	23.7±1.15
Carry food	30	31	34	31.7±2.08
Autocoprophagy	33	34	35	34.0±1
Weaned	39	38	34	37.0±2.65
Eyes open	48	52	56	52.0±4.0
Cumulative age/lit	312	318	315	
Growth rate @ 78 days	0.21	0.21	0.19	

the body and appeared to serve as stabilisers to prevent the pup from rolling over onto its back. Pups only crawled in a forward direction. The most characteristic mode of locomotion during this period was climbing, where the pups displayed a strong positive geotactic response, continually striving to attain the highest point within their immediate surrounds. This locomotion was accompanied by pivoting (*sensu* Kaplan & Hyland 1972), where the forelimbs pushed and the posterior part of the body was used as a pivot. Pups were frequently rolled over by the movements of adults and other pups within the nest but were able to right themselves easily, in part because of the heterogeneous nature of the nest substrate. Pups that landed on their backs on a flat surface required great effort to right themselves. This was typically accomplished by a lateral twisting of the spine, with all four limbs paddling the air and a rotation of the pelvic region from side to side.

Social interactions appeared to be essentially tactile and thermal, including nursing interactions with the breeding female and huddling with siblings. Detection of the breeding female was almost certainly by means of olfaction, as naked mole-rat pups are blind and the breeding female did not appear to vocalise when assuming a nursing position (lying on her back in the nest). On day three, the first bouts of pup-pushing by the breeding pair and older siblings were noted. This behaviour occurs when a juvenile or adult animal places the blunt, anterior end of its muzzle against the body of a pup and shoves the pup (Lacey *et al.* 1991). Pup-pushing peaked in week four (Fig. 1D), which corresponded with the peak in locomotory activities (Fig. 1B) as the pups attempted to escape from their aggressors.

The transitional period (days 4 - 13) was characterised by an improved performance of behaviours present since birth, in addition to the emergence of adult sensory and behavioural patterns. The skin became more opaque and paler lighter in colour (pink-brown), although the abdominal viscera were still clearly visible. Pups were slightly darker dorsally than ventrally, as are the adults. The webbed digits became progressively more distinct and the claws became visible as small black points at the end of each toe.

Locomotory abilities improved rapidly and by day 10 the limbs were capable of supporting the body for short periods. The hindlimbs were still not held directly beneath the body and although the carriage was visibly more stable, the pups still tripped regularly. Forward progression was limited due to the continuous pivoting action. Thus pups that were displaced more than 10cm from the nest were incapable of returning by themselves and moved in circles until carried back to the nest by an older mole-rat.

The first behaviours to make their appearance (on day 4) were, grooming, which consisted of rather poorly developed face-washing movements after sucking, and freezing either in response to anogenital grooming of the pup or in response to pup-pushing. Behaviours relating to allocoprophagy were first observed on day seven when the pups had successfully located an adult animal involved in autocoprophagy and, after intense sniffing

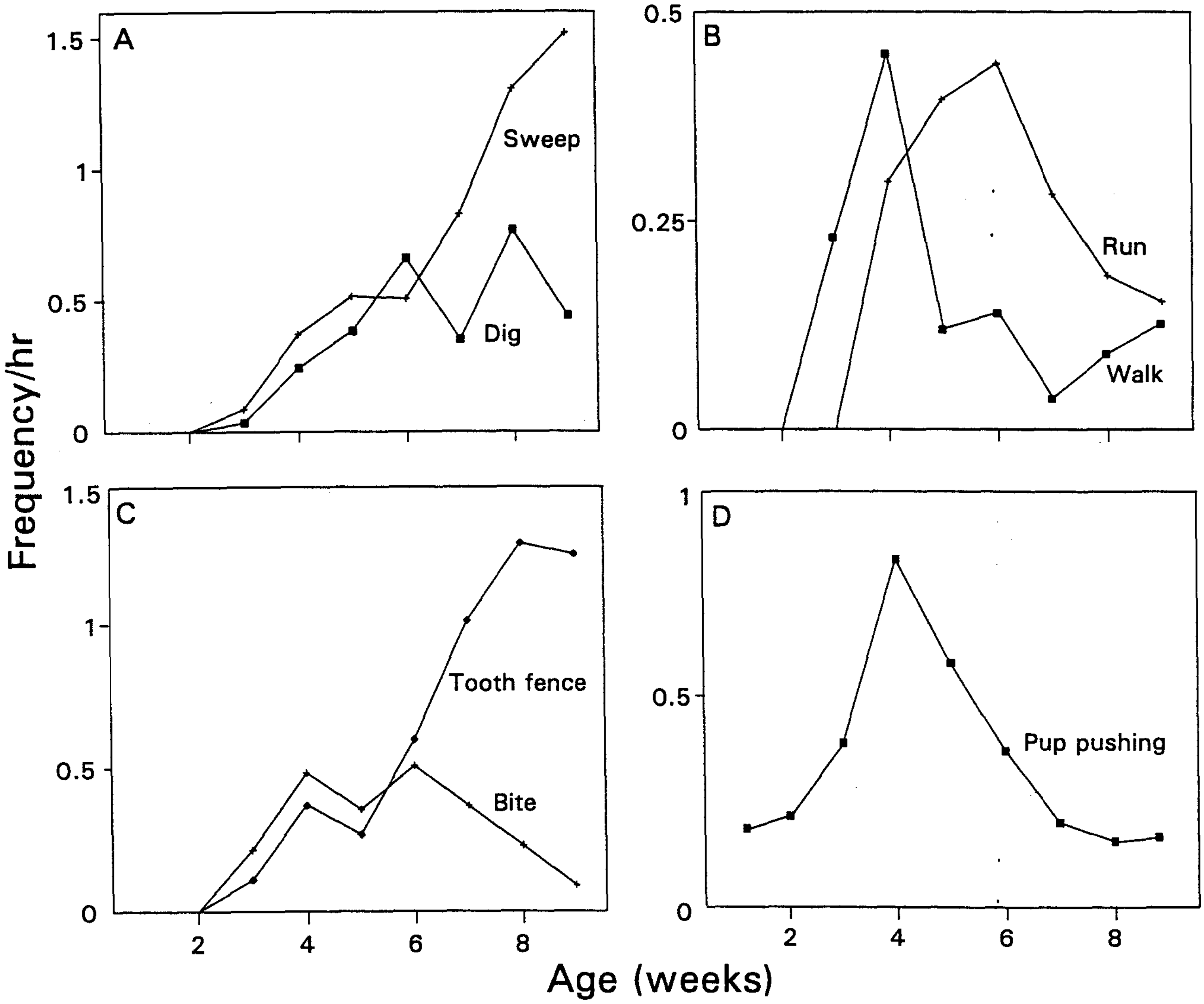


Figure 1. Changes in the average frequency (per hour) of seven key developmental behaviours over the first nine weeks of pup development: (A) work activities - dig and sweep, (B) locomotion outside the nest - walk and run, (C) play - bite and toothfence, (D) adult aggression - pup-pushing.

the pups typically licked the adult's mouth as it chewed the faeces. True allocoprophagy (from the anus) was however, only observed from the third week (Fig. 2). The frequency of performance of this behaviour increased considerably in the fourth week but then decreased thereafter as the pups fed more on solids and with the onset of autocoprophagy. Mouthing of nesting material and food items first occurred on day 12 and both behaviours increased significantly ($r_S=0.88$, $P=0.013$; $r_S=0.962$, $P=0.006$, respectively) during the study period. The onset of these latter behaviours corresponded to the occlusion of the incisors. The eyes remained closed but the auditory meatus appeared to be open. This coincided with the pups first visible reaction (day 14) to external noises, such as the vibrations created by routine cleaning of the burrow systems.

The onset of agonistic and soliciting behaviours heralded the initiation of the social period (days 14 - 26). Biting of siblings and adults (to a lesser extent) was the first agonistic interaction displayed (on day 16), with wrestling and toothfencing being added to the repertoire on day 18 (Fig. 1C). Initially, biting was the predominant play behaviour and appeared to serve as a precursor to other forms of play behaviour. Towards the end of the nine week period however, toothfencing increased dramatically while biting gradually decreased and was rarely observed thereafter (Fig. 1C). Together, play-related behaviours showed a significant increase over the study period ($r_S=0.96$, $P=0.006$, for biting and toothfencing combined). The pups now vocalised (cf. freezing) to appease mole-rats that were pushing them and to solicit faeces during allocoprophagy. The first signs of weaning were noted on day 14 when pups licked and nibbled fresh pieces of food in the nest, in addition to soliciting caecotrophs from older siblings and the reproductives (Fig. 2). Interest in solid food increased as access to suckling decreased. Sucking decreased rapidly from day 21, although the pups were only considered to be fully weaned (no more nursing attempts noted) after day 42. Allocoprophagy would appear to be an important nutritional bridge at the time of weaning, as it increased dramatically between days 21 to 35, peaking at day 28, approximately one week before the frequency of feeding on solids reached a maximum (Fig. 2). The subsequent decrease in the frequency of feeding on solid food resulted from improved handling and ingestion of food items, due to the improved feeding musculature of the pups.

Well-coordinated, rapid locomotion was evident by day 19 (Fig. 1B), and on day 21, the pups were first observed to walk backwards. These developments coincided with the pups' first voluntary departure from the nest. By day 24 the adult style of locomotion appeared to be firmly established. Pups that left the nest typically ran directly to one of the locations in the burrow system that was externally heated by a light source and went to sleep. The frequency of running gradually decreased as the pups began to explore the burrow system further (Fig. 1B) and did not simply shuttle between the nest and the heated area. Other behaviours observed for the first time during this period, included the work behaviours of

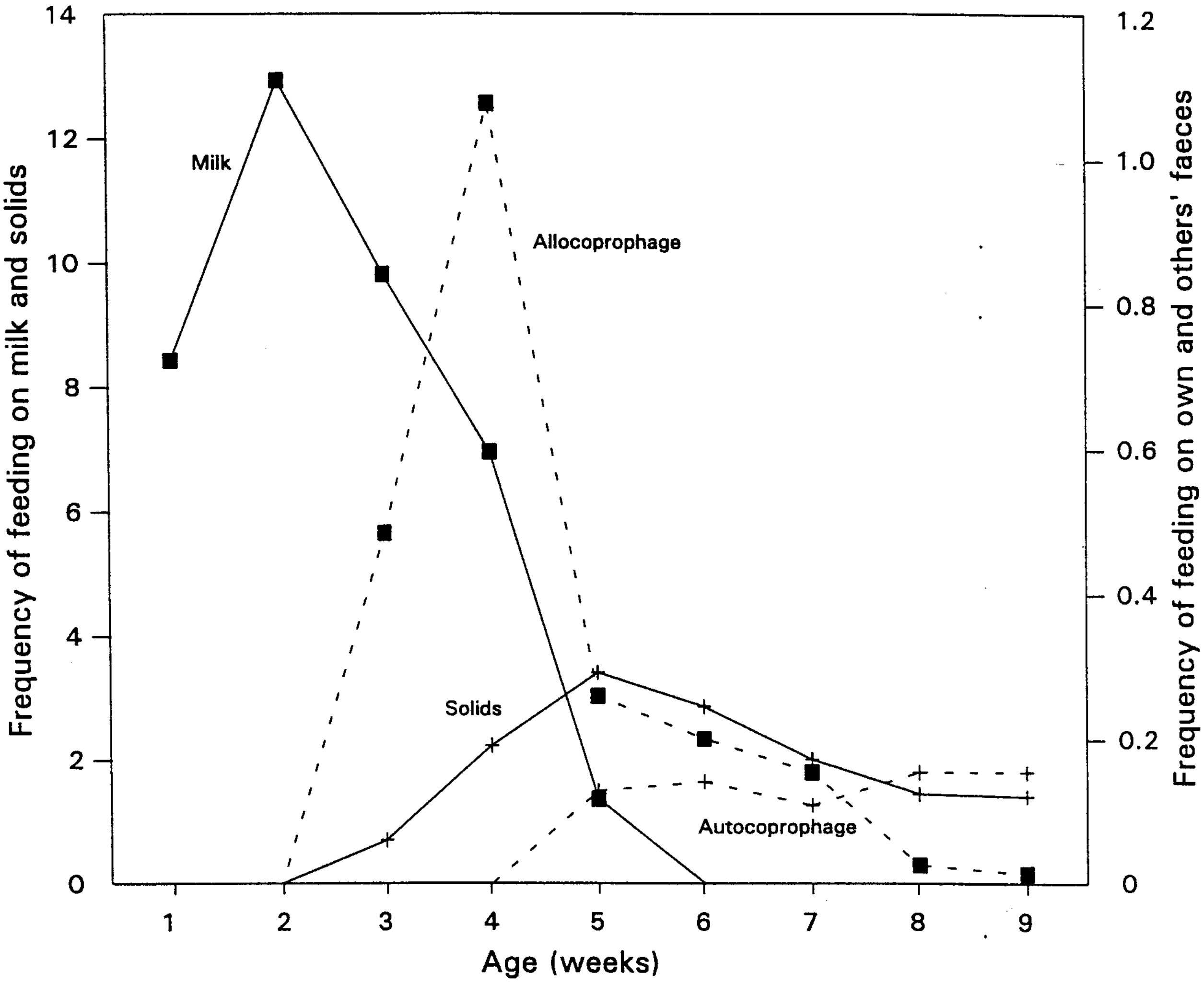


Figure 2. Changes in the average frequency (per hour) of behaviours relating to nutrition (sucking, feeding on solids, allocoprophagy and autocoprophagy) over the first nine weeks of life.

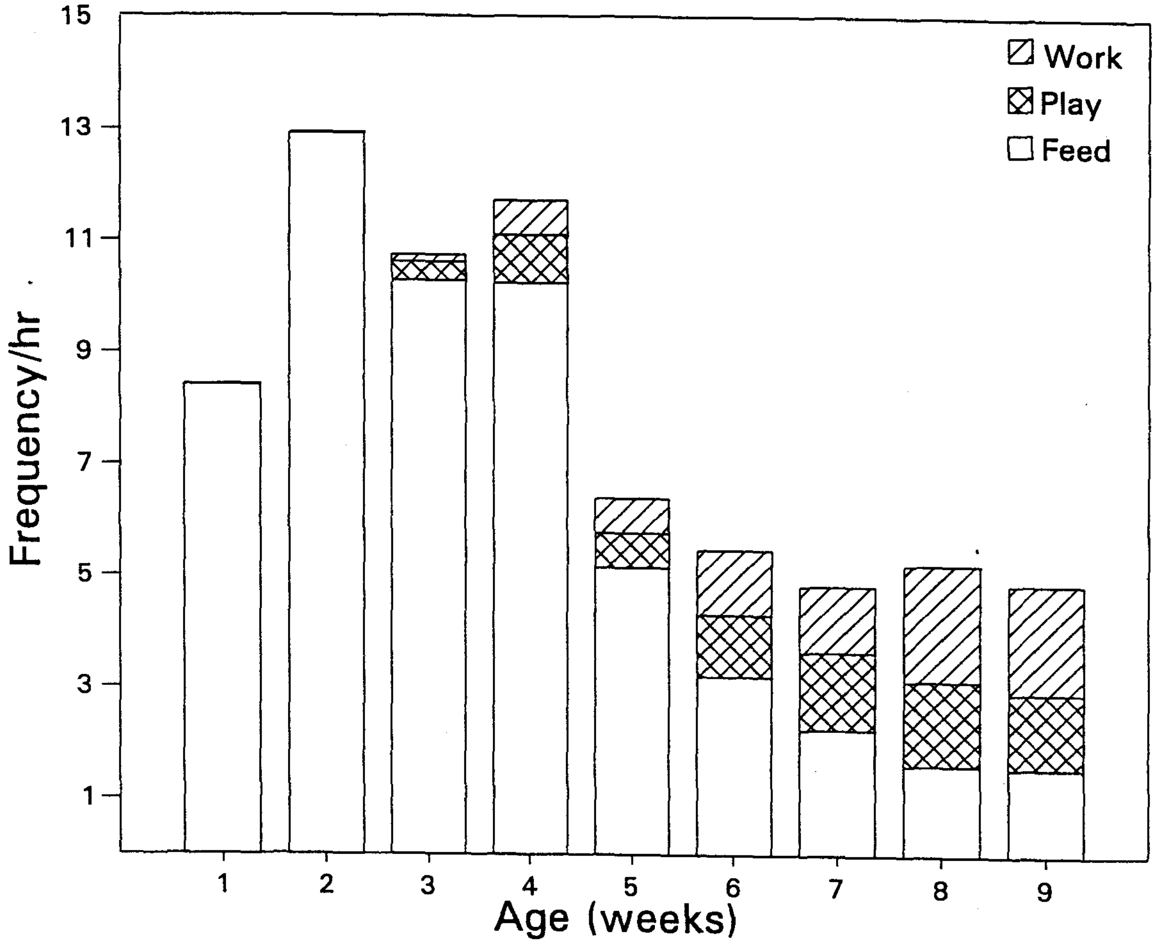


Figure 3. Changes in the relative frequency of the three major behavioural categories, namely, feeding, play and work, over the first weeks postpartum.

digging with the forelimbs (day 17 - claws on the forelimbs more marked than on hind-limbs), the carrying of nest material (day 23) and sweeping (day 21). Both sweeping and digging behaviours (Fig. 1A) showed a significant positive relationship with time (r_S=0.98, P=0.006; r_S=0.91, P=0.014, respectively). Pups were first observed to urinate in the tunnel system on day 20. Prior to this, pup excreta was consumed by adults within the nest. Interestingly, pup excretion appeared to be dependant on grooming of their anogenital region by adults and consequently pups isolated from the colony typically suffer from constipation.

The juvenile period was from day 27 to the birth of the following litter on ca. day 80. During this period the behavioural repertoire became increasingly adult-like and towards the end of this period pup-pushing and allocoprophagy were rarely observed, while the frequency of walking, running and biting all decreased. At the same time work-related behaviours, in particular sweeping, increased steadily (Fig. 1A). The first behaviours to make their appearance were food carrying (day 30) and autocoprophagy on day 33. The latter increased significantly over time (r_S=0.9, P=0.011) and corresponded to a decrease in the frequency of allocoprophagy. The age at which the pups' eyes opened varied within a given litter (ranging from 52-66 days). The mean (±SD) age at which eyes opened across all litters was 60.5±6.99. There was no apparent relationship between the age at which eyes opened and litter order within a colony (Table 1).

A summary of the relative participation in the three major behavioural categories, feeding, play and work is presented in Figure 3. Data on resting was omitted from this graph to facilitate comparisons of the active behavioural repertoire of the pups. Feeding showed a significant decline with age (r_S=-0.9624, P=0.0065), whereas both play and work increased significantly (r_S=0.9, P=0.0109 and r_S=0.929, P=0.0086 respectively). Play and work related behaviours thus dominated at the end of this period, with the pups becoming incorporated into the colony work schedule, prior to the birth of the next litter.

Pup mortality

The survival rate of pups (Table 2) was lowest during the first week postpartum ($\bar{X}$=61.6%±22.5 for colony 100; $\bar{X}$=43.1%±13.2(SD) for colony 7400). While cannibalism of live pups has been observed in other colonies it was not seen to occur in the first three litters in any of the three study colonies. The majority of deaths were due to starvation during the first week as a result of the failure to feed successfully. Dead pups were occasionally cannibalized by the breeders and other colony members. Thereafter pup mortality was rare, with a mean survival rate of 93.3%±11.5 and 95.8%±7.2 for colony 100 and 7400 respectively, and was attributed solely to either gastrointestinal or respiratory infections.

Table 2. The number of pups surviving after one week and one year postpartum. Data are from 6 litters born to 2 colonies (100 & 7400). The numbers in parentheses refer to the percentage survival.

	# born	# survived	
		week 1	1 year
Colony 100			
litter 1	9	5 (55.5)	4 (80)
litter 2	15	13(86.6)	13(100)
litter 3	14	6 (42.8)	6 (100)
X ± SD % survival		61.6±22.5	93.3±11.5
Colony 7400			
litter 1	12	5 (41.7)	5 (100)
litter 2	13	4 (30.7)	4 (100)
litter 3	14	8 (57.0)	7 (87.5)
X ± SD % survival		43.1±13.2	95.8±7.2

Parent/alloparent-pup interactions.

The birth of the first litter was observed for two of the three colonies and provided an opportunity to observe the parental behaviour of the breeders on their own. Previous recordings of birth have always taken place in the presence of alloparents.

The breeding female gave birth (breach and head first) to pups in both the tunnel system and the nest. Apart from consuming the umbilical cord (which is of direct nutritional value), no other direct attention was given to the pups by the breeding female within the first few hours after birth. In contrast, the breeding male was observed to carry pups lying in the tunnels to the nest. Subsequent care of pups was limited to nursing and grooming of the anogenital region by both breeders. This latter behaviour, while apparently preventing constipation in the pups, may also serve to recycle nutrients from the pups back to the reproductives. The breeding female did not seek out the pups in the nest, but typically chose to sleep in contact with the breeding male. Feeding was therefore dependant on the pups' ability to locate the breeding female when she was asleep in the nest. Maternal behaviour was initially limited to a strong predisposition of the female to lie on her back when sleeping, thereby providing access to her nipples.

Both breeding females in this study had 13 nipples (6L:7R). Pups were observed to preferentially suck from the axillary nipples, with 92% of all initial attempts, at the onset of a nursing bout, made on the first three axillary nipples on either side.

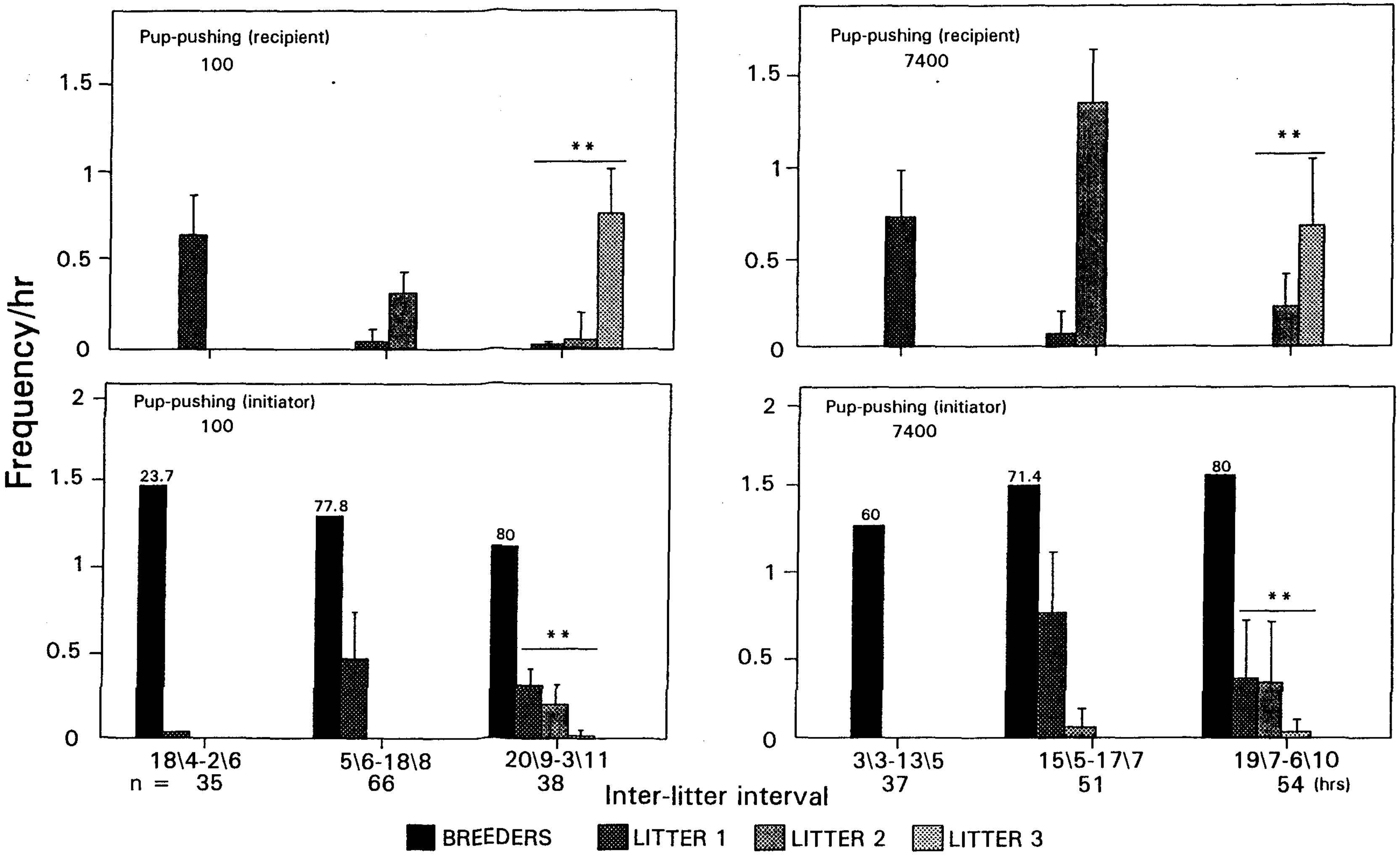


Figure 4. The mean (±SE) frequency per hour of pup-pushing initiation and receiving in both colony 100 and 7400 for the breeders and the first three recruited litters. Data were divided into three successive periods corresponding to the inter-litter birth interval of approximately 80 days between litters 1, 2 and 3. The number above the 'breeders bar' denotes the total contribution by the breeding female, expressed as a percentage of the total for the breeders.

** $P<0.05$

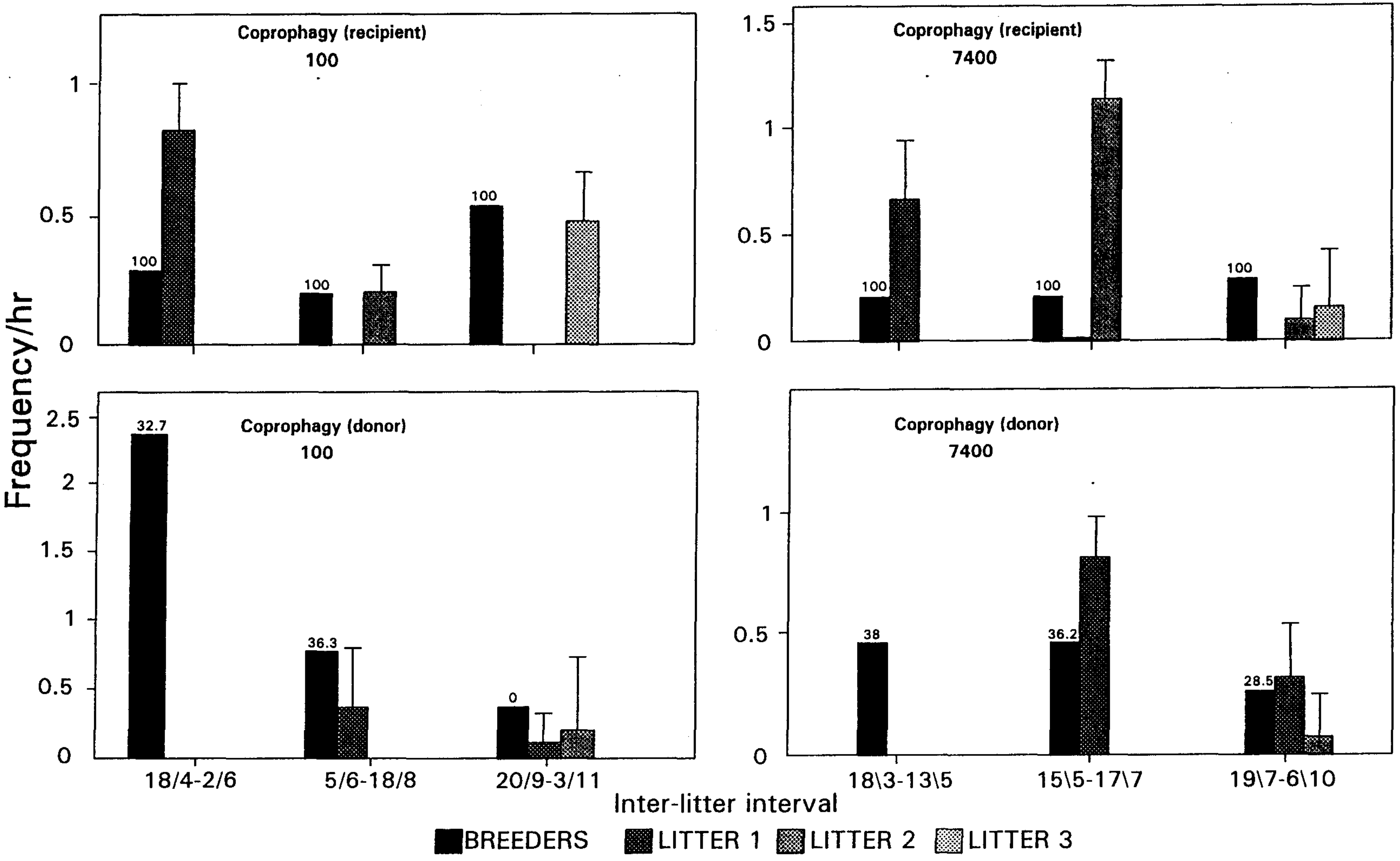


Figure 5. The mean (±SE) frequency per hour of receiving and donating caecotrophs by breeders and members of the respective litters in both colony 100 and 7400. Data were divided into three successive periods corresponding to the inter-litter birth interval of approximately 80 days. The number above the 'breeders bar' denotes the total contribution by the breeding female, expressed as a percentage of the total for the breeders. Sample sizes are the same as for Figure 4.

Inguinal nipples were sucked only when access to the axillary nipples was limited either by other pups or by the posture of the breeding female. Despite obvious competition for axillary nipples, no pup was ever observed to succeed in physically displacing another pup, once it had secured a nipple.

In both colonies the breeding female was the chief pup-pusher in five of the six litters recruited (Fig. 4), a trend which strengthened over time in both colonies. The frequency of pup-pushing between members of different litters varied inversely with age, and was significantly different between litters (H=11.8, P=0.003, colony 100; H=9.49, P=0.009, colony 7400) in both colonies (Fig. 4). Conversely, the recipients of pup-pushing bouts were always the youngest colony members, with the frequency varying significantly (H=16.02, P=0.0003, colony 100; H=9.45, P=0.008, colony 7400) among litters in both colonies (Fig. 4). It was further evident that litter size may influence the average pup-pushing rate. Members of the largest weaned litter (litter 2, colony 100) received the lowest number of pushes per individual, while those from the smallest weaned litter (litter 2, colony 7400) were pushed most frequently.

The apparently altruistic behaviour of donating caecotrophs was performed most often by the breeding male, and in colony 7400, by members of the first litter (Fig. 5). The primary recipients of caecotrophs from these breeders and older siblings were always the youngest colony members. Interestingly, the breeding female was the only individual observed to allocoprophage as an adult (see exception in Chapter 3), soliciting caecotrophs almost exclusively from the breeding male. In mature colonies the breeding female will also solicit caecotrophs from her adult offspring.

Pup-carrying (Fig. 6) was performed primarily by the breeders in both colonies, with the breeding female being initially responsible for most instances of pup-carrying (except in the first few hours postpartum). With subsequent recruitment of offspring, the frequency of pup-carrying by the breeders decreased with a concomitant increase in pup-carrying by the pups' older siblings. In both colonies members of the first litter carried pups more frequently than members of the second litter, although this difference was only significant in colony 7400 (Mann-Whitney U test, U=3.42, P=0.003).

Play behaviours

Play with peers is one of the first activities not directed at the mother, to appear early in life (Fig. 3). The two most commonly performed play-related behaviours were biting (the teeth of one animal close over another animal without causing any physical damage) and tooth-fencing (two mole-rats face one another and either spar with or lock their incisors together). Biting almost always preceded a bout of tooth-fencing, with the latter becoming the dominant behaviour by the end of the juvenile period (Fig. 1C). Figures 6 and 7 demonstrate that these play behaviours varied significantly between litters in

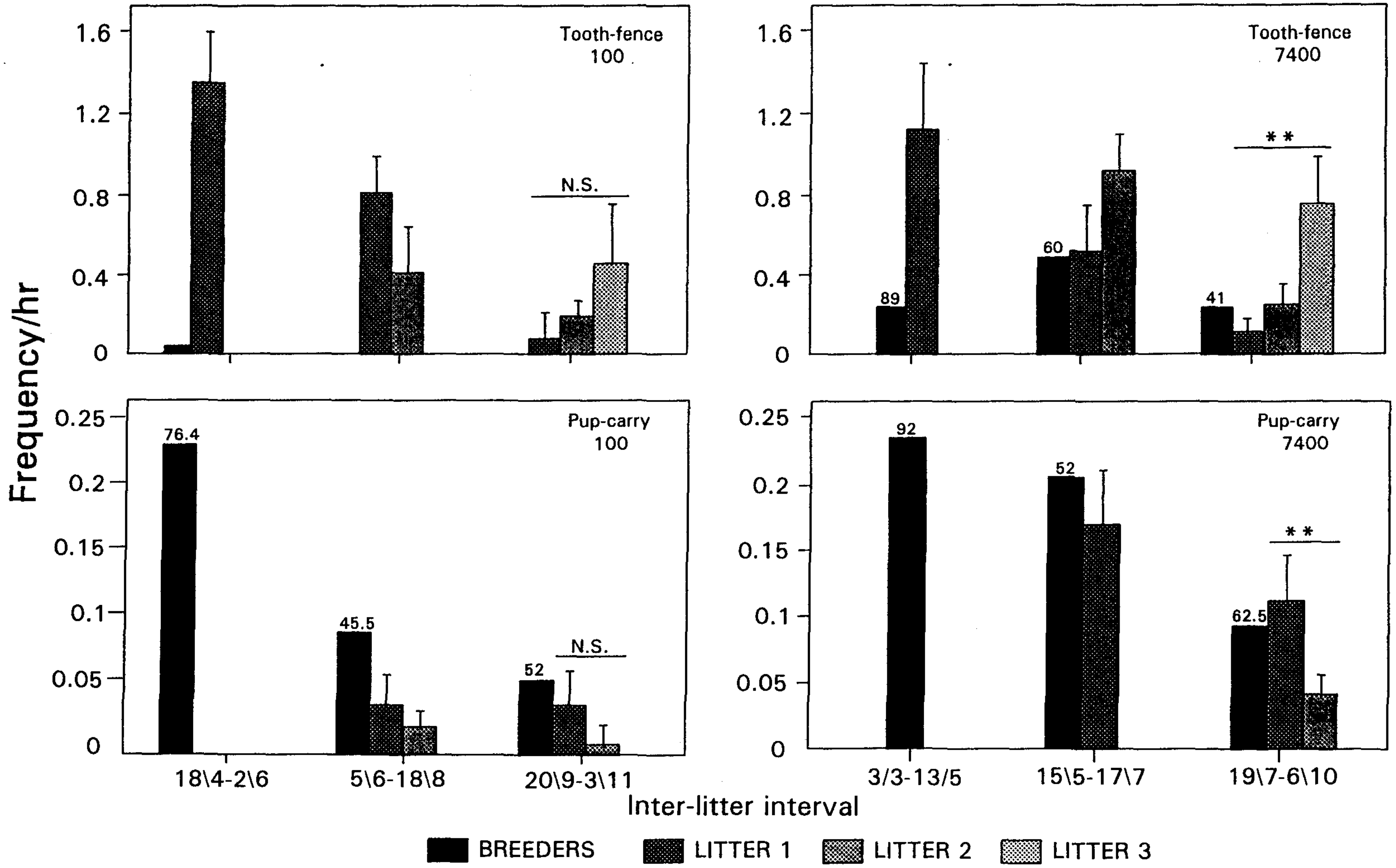


Figure 6. The mean (±SE) frequency per hour of tooth-fencing and pup-carrying by breeders and members of the first three litters recruited to colony 100 and 7400. The number above the 'breeders bar' denotes the total contribution by the breeding female, expressed as a percentage of the total for the breeders. Sample sizes are the same as for Figure 4.

** $P<0.05$

N.S. not significant

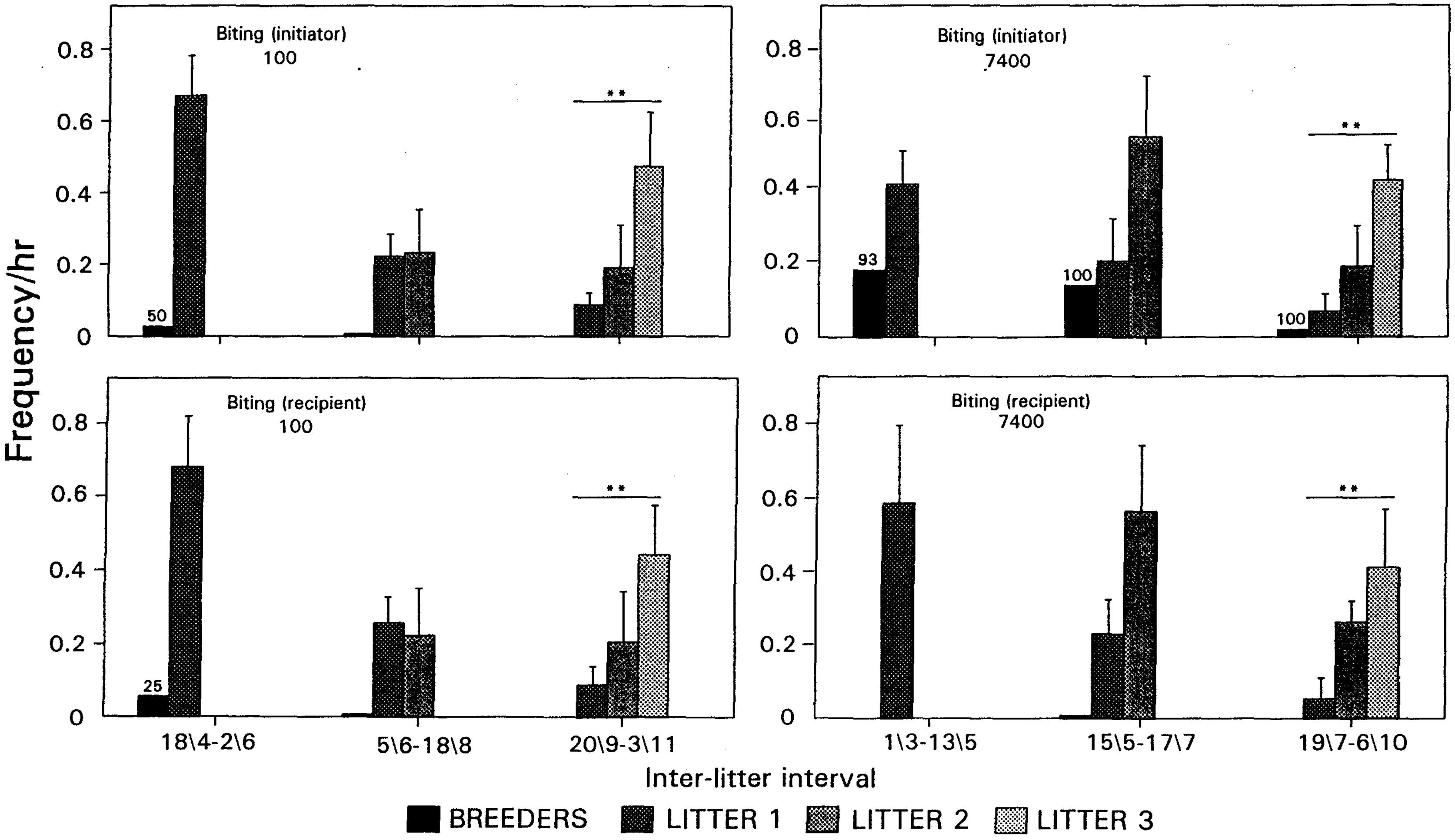


Figure 7. The mean (±SE) frequency per hour of biting and being bitten by breeders and members of the respective litters of colony 100 and 7400. Data were divided into three successive periods corresponding to the inter-litter birth interval of approximately 80 days. The number above the 'breeders bar' denotes the total contribution by the breeding female, expressed as a percentage of the total for the breeders. Sample sizes are the same as for Figure 4.

** $P<0.05$

both colony 100 (H=9.68, P=0.008, (biting); H=11.42, P=0.003, (bitten); H=8.43, P=0.014, (tooth-fencing)) and colony 7400 (H=8.29, P=0.016, biting; H=12.15, P=0.002, (bitten)). The only exception to this was in colony 7400 where the frequency of tooth-fencing did not differ significantly between litters (H=1.479, P=0.48) although a similar trend with the youngest colony members exhibiting the highest frequency of performance was apparent. Participation in both tooth-fencing and biting thus decreases with age (*e.g.* litters 1 & 2 in Figures 6 and 7). It is interesting to note that in colony 7400 the breeding female participated in both tooth-fencing and biting with pups.

While play and social development are undoubtedly linked (Bekoff 1972), it was not possible to establish intra-litter social hierarchies based on the performance of these behaviours. The frequency of 'winning' and 'losing' by pups in dyadic play bouts were approximately equal.

Even though jaw musculature and teeth are well developed in juveniles, tooth-fencing bouts never resulted in physical injury. Towards the end of the juvenile period the pups frequently engaged in prolonged bouts of tooth-fencing, involving up to six individuals. These team fencing bouts typically took place within a blind ending section of the burrow system and were characterised by two groups of three mole-rats, either in tiers or standing abreast, sparring with each other. Although members of each tier behaved as a unit, advancing and retreating together against the other group, allegiances were frequently swopped in the course of these bouts, reinforcing the play motivation of this behaviour. The functional importance of this behaviour is evident by its regular performance by adults during inter-colony fights (Lacey and Sherman 1991; Chapter 5) and in defence against predators (Lacey and Sherman 1991).

The ontogeny of work-related behaviours.

It is clearly evident (Figures 8 and 9) that in the early stages of colony development, the reproductive pair contributed substantially to the primary work-related behaviours associated with foraging and colony maintenance. As pups were recruited however, so the reproductives' combined contribution to work (digging, sweeping and food carrying) exhibited a significant decline in both colonies (r_S=-0.878, P=0.013 for colony 100; r_S=0.69, P=0.037 for colony 7400). In contrast, the work rate of pups within each litter showed a sharp increase with time, before either decreasing or levelling off (*e.g.* litter 1, Fig. 9). Analysis of variance for data in the last sampling interval revealed that only the frequency of food carrying varied significantly between litters in colony 7400 (H=9.72, P=0.009). Results for digging and sweeping were (H=1.87, P=0.39; H=1.65, P=0.44), respectively. In colony 100 however, only the frequency of digging showed a significant difference (H=6.27, P=0.043), while sweeping (H=4.69, P=0.095) and food carrying (H=5.2, P=0.1) showed no significant difference. The overall

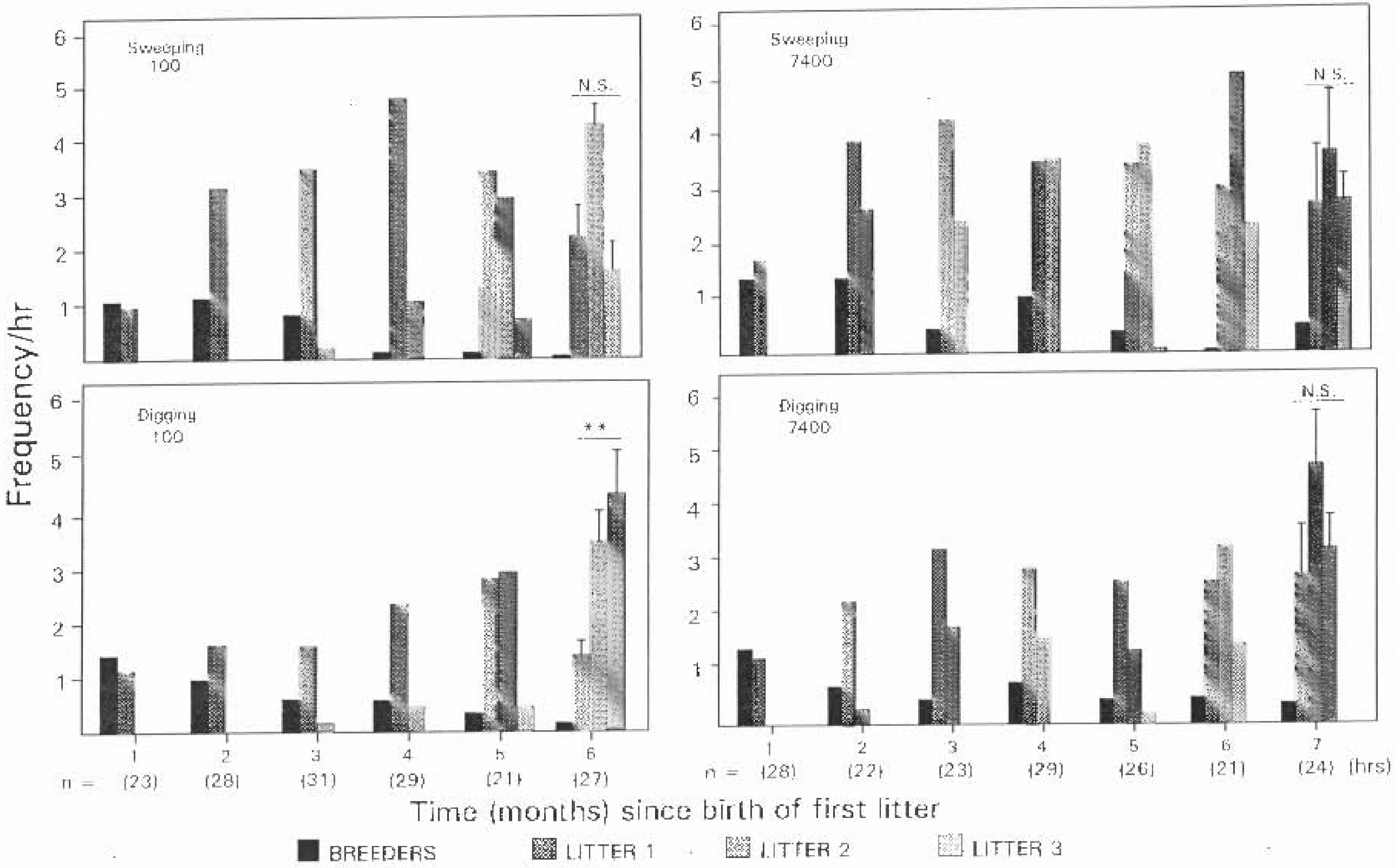


Figure 8. The mean (± SE) frequency per hour of sweeping and digging by the breeders and members of the first three litters recruited to colony 100 and 7400.

** $P < 0.05$

N.S. not significant

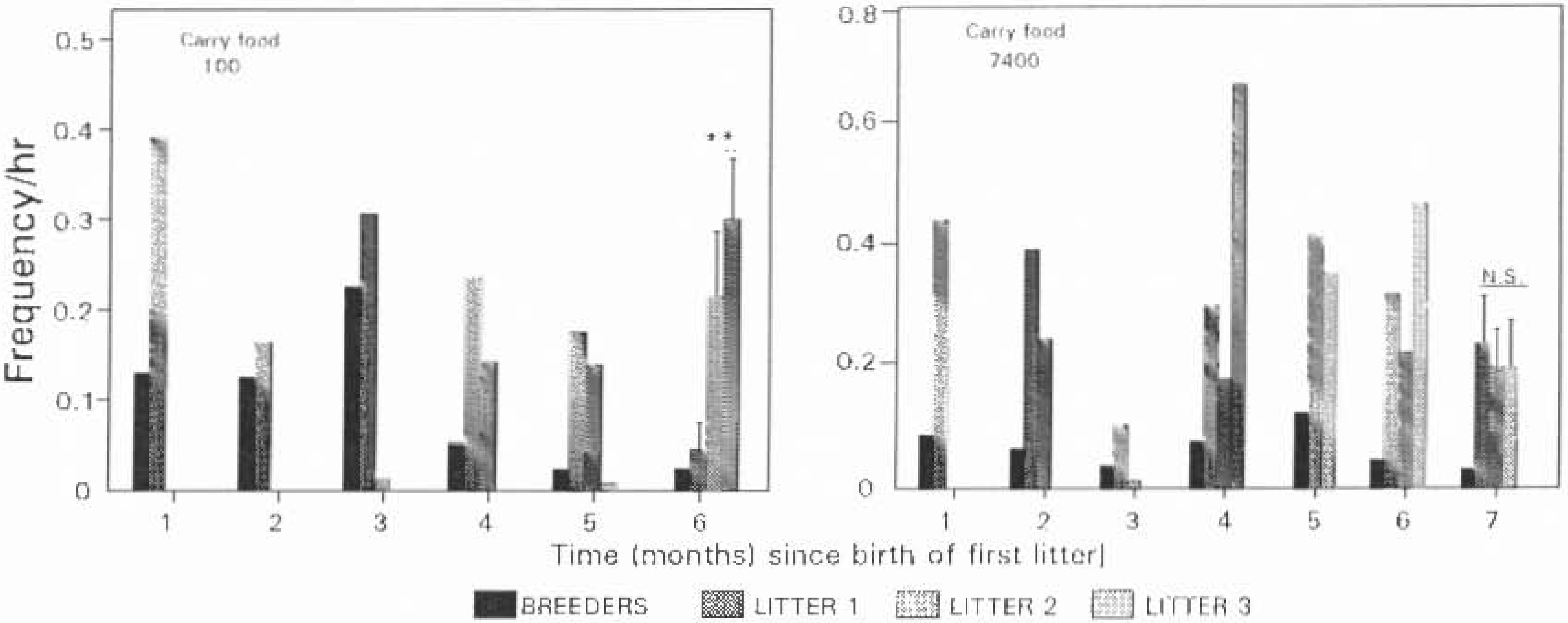


Figure 9. The mean (±SE) frequecy per hour of food carrying by breeders and members of the first three litters recruited to colony 100 and 7400.

Sample sizes are the same as for Figure B.

** $P < 0.05$

N.S. not significant

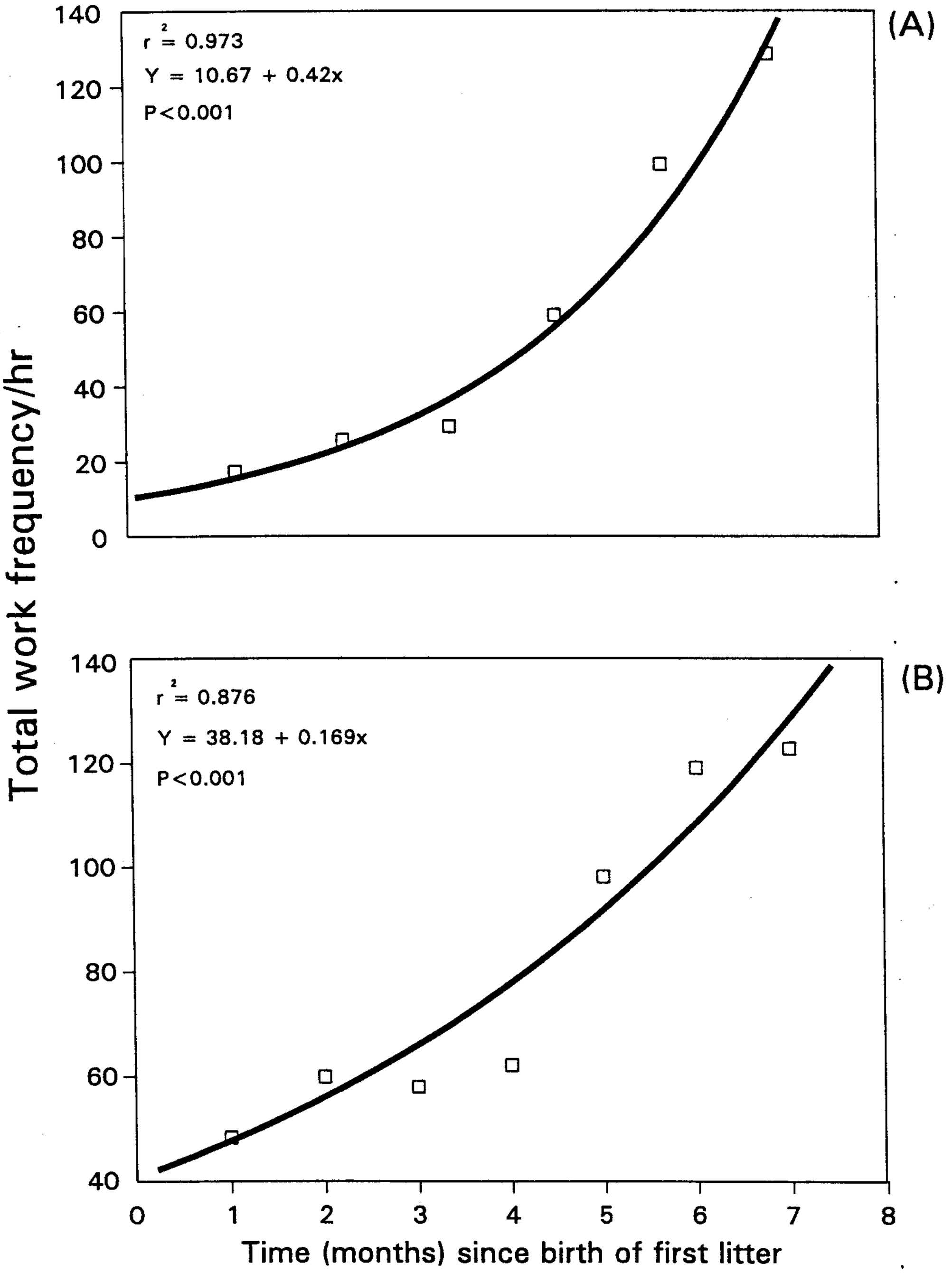


Figure 10. The regression between the frequency of performance of colony maintenance and provisioning behaviours (digging, sweeping, carrying food and nest material) and time in months, for colony 100 and 7400.

frequency per hour in the performance of four work behaviours (digging, sweeping, carrying nest material and food) within the colony showed a significant (exponential) increase with time in both colonies (Fig. 10).

DISCUSSION

The development of naked mole-rat pups follows the general pattern seen in the other social bathyergids (Bennett *et al.* 1991). Naked mole-rats are comparatively altricial at birth however, and consequently differences that are noted can be related to the delayed onset of both behavioural and morphological developmental attributes. It is possible that the production of altricial young enables the breeding female to produce numerically larger litters (up to 27 pups) than other social bathyergids (*e.g. C. damarensis* has a maximum litter size of six).

The survival of altricial young is in turn facilitated by the greater protection and enhanced thermoregulatory properties associated with a communal nest, replete with alloparents. Together, these factors may translate into significantly higher annual recruitment rates for naked mole-rat reproductive females than for those of other social and solitary bathyergids. It is thus possible that natural selection has favoured the production of altricial young to enable breeding females to maximise recruitment in a population characterised by an extreme 'reproductive skew' where the majority of females at any point in time are reproductively quiescent (Jarvis 1981; Faulkes *et al.* 1989).

Case (1978b) states that the greatest energetic expense of the mother occurs after the birth of altricial young, whereas this peak occurs before birth in precocial species. The production of altricial young therefore allows the major portion of the energetic cost to be shared by a second party, usually the father, and/or in the case of cooperative breeders, older siblings.

It is interesting to note that although the Damaraland mole-rat (*C. damarensis*) has a fundamentally similar social system to the naked mole-rat (Jarvis and Bennett 1993), the single breeding female in the colony produces relatively small litters of precocial pups (maximum five pups). This and the higher dispersal rate of colony members in *C. damarensis* colonies, may explain why the average colony size in wild *C. damarensis* (Jarvis and Bennett 1993) is consistently lower than those of wild *H. glaber* (Brett 1991) populations. It further suggests that while the production of altricial young and large litter sizes are not prerequisites for survival in a harsh ecotope, these traits may be indicative of the greater (or different) selective pressures for group living, that naked mole-rat colonies faced in evolving towards sociality.

Case (1978a) suggested that the possession of a secure underground nest may promote the slow postnatal development rates of mammalian young. He also suggested that selective pressures operating on the mother will favour altricial offspring if: 1) the nest site is safer for an embryo to complete development than inside the mother's body, or 2) whenever foraging demands so much skill and experience that the young, no matter how precocial, could not effectively compete with the adults in the population. Both of these suggestions are supported by current knowledge of the naked mole-rat. It is however important to note that these points are satisfied by other social bathyergids, some of which have precocial young and all of which have small litter sizes. Thus it would appear that while these conditions may have facilitated the evolution of altricial young they are not the sole determinants.

One behaviour that was well developed at birth and readily performed by naked mole-rat pups in the early neonatal period, was climbing. Climbing, stimulated by a marked positive geotactic response, ensured that pups remained on top of the pile of huddling mole-rats in the nest. Failure to climb was deleterious to pup survival for two reasons. Firstly, the breeding female typically assumed the nursing position at the top of the pile and thus pups that were there ensured an opportunity to feed. Secondly, the constant jostling of animals in the huddle posed a serious threat of suffocation to pups at the bottom of the pile. Thus, when pups were not either sucking or sleeping they adjusted their position (relative to older colony members) within the nest.

During the early postnatal periods (neonatal and transitional), activities not related to feeding or thermoregulation were maintained at low levels of performance. Major changes became evident in the third week (social period) when pups exhibited a sharp increase in behavioural patterns relating more to social, than to nursing or homeostatic functions. The development of efficient locomotion, and the initiation of the weaning process freed the pups from the necessity of maintaining almost constant contact with their littermates and mother.

Throughout this study there were few observable changes in the topography of any of the behaviours recorded. At the point of onset most behaviours were performed in the adult form, although they were seldom executed as competently as the adults. This finding is not unique to the naked mole-rat (*e.g.* see Bekoff 1977; Meaney and Stewart 1981). There appears to be little evidence that the ability to execute a specific behaviour is modified by early experience. Rather, it is the probability of a specific behaviour being executed within a particular context, that is experientially modified (*sensu* Harlow 1969; Bekoff 1972). That is, the behaviour appears to become more appropriate to the salient stimuli in the environment, and especially to stimuli from other mole-rats. This process of social learning is facilitated by the prolonged infancy of naked mole-rats relative to other bathyergids, which provides increased opportunities to develop the necessary social and communicative skills for integration into a comparatively complex society.

Previous studies on mole-rats have ascribed a specific day delimiting weaning (Bennett and Jarvis 1988; Burda 1989; Bennett *et al.* 1991). For most mammals however, weaning is gradual and involves a progressive reduction in the rate of milk transfer from mother to young, accompanied by an increased intake of solid food by the offspring (Martin 1984). This is true for naked mole-rat pups, although a further dimension is added through the process of allocoprophagy. Allocoprophagy is the intake of faecal matter (caecotrophs) from both the breeders (primarily the breeding male) and older colony members. The peak in allocoprophagy (Fig. 2) prior to the cessation of sucking, or the peak in feeding on solids, suggests that coprophagy represents an important nutritional bridge between milk and vegetable diets. This corresponds to the period when direct parental investment shifts from the breeding female to other colony members (alloparents). The frequency of allocoprophagy decreases with an increase in feeding on solid food and with the pups' ability to practice autocoprophagy; it was seldom observed between adult individuals. In both study colonies the breeding females proved to be the pups' main competition for caecotrophs, especially from the breeding male (Fig. 5).

The effect of this nutritive supplement on the subsequent growth of juveniles is unknown. However, since coprophagy is an essential part of nutrition of some lagomorphs, rodents, and termites (see Lacey *et al.* 1991), it seems likely that naked mole-rats also obtain nourishment by consuming faeces (their own and that of others). Indeed, Mendez (pers. comm.) reported that pups denied access to caecotrophs from alloparents remained small in size. Following the re-introduction of alloparents, the pups frantically begged for faeces and exhibited a concomitant growth surge. In addition, Porter (1957) suggested that juvenile allocoprophagy serves a vital role in facilitating the transfer of the endosymbiotic gut biota, that greatly enhance the ability to digest fibre and cellulose.

One behaviour that appears relatively early in the lives of the pups and remains to be explained from a functional viewpoint, is that of pup-pushing. It is clearly evident (Fig. 4) that the breeders are the principal pup-pushers and that the focus of this behaviour is the youngest litter. Pup-pushing was not associated with any specific activity, or lack thereof. Pups were pushed while sucking, sleeping or locomoting in and out of the nest. Interestingly, this behaviour was occasionally observed to be directed at the anogenital region of the breeding female. Since naked mole-rats are blind, this observation suggests that the cue for pup-pushing is one of odour and that this odour cue is one shared by all pups and the anogenital region of the breeding female.

Initially pups responded to being pushed by attempting to escape from their aggressor. This response gradually changed however, as the social interactions of the pup improved. A more submissive attitude of freezing was initially adopted and, if the shoving continued, a vocalisation, termed the 'juvenile chevron' (Pepper *et al.* 1991) was made. This typically halted the pushing bout and provided the pup with a brief respite and the chance to move away.

There are currently four theories as to the functional significance of pup-pushing. Lacey *et al.* (1991) suggest that it may function to 1) move pups rapidly to the edge of the nest, thereby preventing them from being trampled by other colony members, 2) move pups quickly out of the way of predators, 3) enhance the peristaltic gut action of pups, or 4) help to enforce social dominance among colony members. My observations in this and other studies (Chapters 2 and 3) support only the fourth hypothesis, where newborn pups are physically dominated by all older siblings and their parents. This serves the interests of the older colony members directly. Parents that allow their offspring to remain within the natal territory need to ensure that they maintain the sole rights to reproduction and gain preferential access to limited resources (*i.e.* food, see Chapter 4). The breeding female has been shown to actively suppress reproduction in her offspring through her physical presence and possibly through a variety of agonistic dominance-related interactions (*i.e.* shoving, Faulkes and Abbott 1993). In addition, it has been shown (Chapter 4) that when the colony food supply was experimentally exhausted, the reproductive female gained primary access to any small food items subsequently introduced to the colony. Schieffelin and Sherman (1995) also demonstrated that a feeding hierarchy, based on body mass, exists amongst colony members when competition for food is increased. Furthermore, previous studies (Jarvis 1991; Lacey and Sherman 1991) indicate that the probability of succeeding either of the reproductives is directly related to the age/size of the non-reproductives (see also Chapter 3). Pup-pushing by older siblings may thus serve to establish a dominant/subordinate relationship at a time when the pups are physically incapable of retaliation. This would facilitate the formation of a hierarchy amongst the non-reproductives that dictates future access to both food and possibly reproductive opportunities. In this scenario, litter order would have a profound effect on the relative position of individuals within the colony's social structure.

Although every pup within each litter was pushed, members of the first litter were never pushed by older siblings and they were observed to push members of all subsequent litters. This may explain the previous finding by Jarvis *et al.* (1991) and Chapter 2, that members of the first, and occasionally the second litter, attain the greatest adult size. In addition, further studies on these two colonies (see Chapter 3) revealed that in all cases of either breeder removal or illness, the replacement reproductives were members of the first litter. Litter order may thus be the single most important factor influencing the social structure and reproductive division of labour within recently founded colonies of naked mole-rats.

I found no evidence to suggest that pup-pushing is performed to enhance pup survival, as suggested by the first three hypotheses of Lacey *et al.* (1991). Indeed, pup-pushing has several detrimental effects on pups. The first of these was the physical displacement of pups while sucking, so that nursing bouts were frequently interrupted by non-reproductive 'helpers' and the breeding male. In addition pup-pushing was observed to displace pups, not only from their preferred position on top of the communal huddle, but also from the nest itself. This effectively increased the chance of pups being trampled at the nest entrance or being displaced to the bottom of the pile, away from the preferred nursing position and with increased chances of suffocation. I furthermore, refute the hypothesis, that pup-pushing may serve to move pups quickly out of the way of predators. Not only did it occur frequently in the absence of predators, but it was also never observed when the colony was disturbed by the threat of predation. Older siblings responded to the threat of predators or other disturbances by picking up the pups with their incisors and carrying them, within the tunnels, until the threat passed. I conclude therefore, that pup-pushing is initiated by adult breeders and non-breeders, to behaviourally stress the pups and thus ensure that they remain reproductively inactive and assume a subordinate role within the colony's social hierarchy.

It was evident throughout this study that direct parental involvement in pup-care was minimal in naked mole-rats when compared with other rodent species {*e.g. Rhabdomys pumilio* (Brooks 1982); *Aethomys kaiseri* (Cheeseman 1981); *Lophuromys sikapusi* (Cheeseman 1981); *Rattus norvegicus* (Meaney and Stewart 1981)} or cooperatively breeding vertebrates {*e.g. Helogale parvula* (Rasa 1977; Rood 1978); *Lycaon pictus* (Frame *et al.* 1979); *Marmotus pennsylvannicus* (Armitage 1986). In particular, the maternal behaviour of the breeding female was almost entirely limited to a behavioural predisposition to assuming a nursing posture within the nest when resting.

In addition to nursing, previous studies have included the following behaviours in pup-care: carrying, grooming, nudging, pushing, allocoprophagy (Jarvis 1991; Lacey *et al.* 1991) and sweeping of pups (Lacey *et al.* 1991). The validity of including pup-pushing, sweeping and nudging as pup-care behaviours remains questionable. Pup-pushing does not appear to be in the direct interests of the pup, an implicit assumption in the term 'pup-care'. Pup-sweeping, which involves an older colony member kicking a pup down the length of the tunnel with its hind feet, frequently results in the death of the pup from physical trauma and is thus difficult to reconcile with pup-care. Pup-nudging on the other hand remains to be interpreted from a functional viewpoint and its inclusion as a pup-care behaviour is therefore probably premature. Pup-grooming appears to be an important pup-care activity, by stimulating pups to defecate and urinate, thus preventing constipation. It is possible that this behaviour is also beneficial to the groomer who consumes the waste matter in a manner similar to allocoprophagy. A similar finding was obtained for female rats, who consumed physiologically significant quantities of urine, which contributed to the groomers' overall water balance (Gubernick and Klopfer 1981).

Thus, the only behaviours, other than nursing that are of direct benefit to pups, while of no immediate benefit to the performer are pup-carrying and allocoprophagy. Initially, both breeders are primarily involved in the performance of these behaviours. With subsequent recruitment however, the contribution of the breeding female decreased and, in neither study colony, was she observed to allow allocoprophagy by the pups, while the breeding male did so only infrequently towards the end of the study period. These direct pup-care behaviours thus became the duty of the alloparents.

It was not possible to test the effects of helpers on the survival rate of pups or the overall rate of recruitment to the colony. However, because of the gradual shift in responsibility of pup-care activities from the breeders (excluding suckling) to their offspring, it seems reasonable to predict that recruitment will depend increasingly on those pup-care activities performed by older siblings (*e.g.* allocoprophagy and pup-carrying). Furthermore, the risk of predation to the breeders is greatly reduced, as the high risk tasks of foraging and defence are performed by the helpers. The survival of any unborn young is thus secured. Helpers may therefore contribute significantly to colony growth and survival in nascent colonies, and may also enhance the inclusive fitness of colony members as in the manner of cooperatively breeding birds (Brown 1975; Koenig 1978; Ligon and Ligon 1978; Woolfenden and Fitzpatrick 1978, 1990; Emlen 1982; Mumme and de Queiroz 1985; Brown 1987; Russel and Rowley 1988; Zahavi 1990) and mammals (Rasa 1977, 1989; Moehlman 1979; Malcolm and Marten 1982; Rood 1990; Solomon 1991).

As successive litters were recruited by the breeding pair, so the overall work rate within the colonies showed an exponential increase (Fig. 10), enabling the reproductives to devote their energies almost solely to reproduction and the social manipulation of the work force. A similar trend has been noted for many of the eusocial (Wilson 1971; Holldobler and Wilson 1990) and cooperatively breeding (Robinson 1992; Peeters, in press) insect societies. While recruitment may initially place an extra burden on the colony's available food resources, it may ultimately serve to enhance the foraging efficiency of the colony. Food resources of naked mole-rats are randomly distributed and typically either small and clumped, or large and solitary (Brett 1991). The risk to the colony, in their 'blind' search for food, is thus one of a low probability of encounter (Jarvis 1978; Lovegrove 1991). Once located however, the same characteristics that make food difficult to locate translate into a large stable food reserve (*i.e.* a large patch of small foods or a single large food item). Thus any initial extra nutritional burden is largely offset by the reduced risk of communal foraging (Lovegrove and Wissel 1988; Jarvis *et al.* 1994). There would therefore be strong selective pressure for large initial recruitment to nascent colonies in an attempt to shorten the high risk period associated with a small work force. Indeed, Alexander *et al.* (1991) and Jarvis *et al.* (1994) suggested that it was only those breeders that tolerated their adult offspring in their natal burrow, that would have survived the high risks associated with foraging in an arid environment. This would place heavy selective pressure on colony formation and ultimately favours the evolution of cooperative breeding.

Once groups had formed, selection could then act on the dynamics within the colony, favouring the rapid recruitment of functionally sterile offspring who serve the vital role of helpers in raising siblings and reducing the risks associated with foraging underground in an arid habitat. The combination of aggression (*e.g.* pup-pushing) and cooperation may serve to establish hierarchies within the context of mutually interdependent colony members, thus ameliorating the potential for conflict and competition for limited resources such as reproduction and food.

CHAPTER TWO

THE DYNAMICS OF GROWTH IN NAKED MOLE-RATS - THE EFFECTS OF LITTER ORDER AND CHANGES IN SOCIAL STRUCTURE.

SUMMARY

In this study factors that influence the plasticity in growth and adult body mass in the naked mole-rat are explored. Growth functions (Gompertz transformation), revealed that naked mole-rat pups have the slowest mean maximum growth rate (0.207 $g.day^{-1}$) of all the Bathyergidae. Growth functions varied significantly between litters and there was an inverse trend between asymptotic body mass and litter order. Similarly, the magnitude of the growth response following the death of the breeding male or older siblings was greater for older litter members. There was a significant correlation between body mass and age at all stages of the colony's history although this relationship weakened with time. It is suggested that dominance interactions between old and young mole-rats within the colony are important factors influencing the growth and reproductive potential of all colony members. Changes in body mass were linked to the behaviour of the individual within the colony providing a mechanism for the apparent match between large body size and colony defence and small body size and work frequency. Despite low genetic variation within colonies there is considerable variation in the size of colony members suggesting that plasticity in growth is the product of different ontogenetic histories of colony members that may serve as the basis for variation in size amongst adults in naked mole-rat colonies.

INTRODUCTION

The ability of a single genotype to produce alternative phenotypes under varied environmental conditions is referred to as phenotypic plasticity (Stearns 1989) and has been shown to occur in a variety of different organisms. These include a range of invertebrates: *e.g.* termites (Nijhout 1981) and ants (Keller and Ross 1993) and vertebrates: *e.g.* fish (Dentry and Lindsey 1978; Wainright *et al.* 1991); amphibians (Alford and Harris 1988;

Berven 1990; Pfennig 1990) and mammals (Moore 1965; Weaver and Ingram 1969). Phenotypic plasticity is a character that may evolve in a species as a result of an adaptive, dynamic response to spatial or temporal variation in key environmental factors such as food abundance (West-Eberhard 1969, 1986, 1989; Bernado 1993). It is also an important feature for the evolution of complex insect societies (Wheeler 1986; West-Eberhard 1989; Keller and Ross 1993); where differences in adult size and function (*e.g.* queen/worker) can result from variation in extrinsic factors such as temperature (Plowright and Jay 1977) and/or intrinsic factors such as larval nutrition and dominance interactions within colonies (Wheeler 1991).

Unlike social invertebrates, in which the body form of adult colony members is typically fixed at eclosion (Wilson 1971; Holldobler and Wilson 1990), most vertebrates exhibit some degree of plasticity in body size and shape for much of their adult life (Case 1978).

Variation in body size within mammalian social groups has important effects on the dynamics of interactions within these groups, particularly in the formation of dominance hierarchies. Thus body size typically shows a positive correlation with social rank in, for example primates, such as the gorilla (Schaller 1963) and chimpanzee (Goodall 1975); carnivores such as the dwarf mongoose (Rasa 1977) and lion (Bertram 1978), Gittleman 1985); rodents such as the laboratory rat (Dewsbury 1982) and the naked mole-rat (Schieffelin and Sherman 1995). This has important direct fitness consequences (*e.g.* access to mates).

Factors affecting body size in social organisms are also important to discussions concerning group ergonomics (*sensu* Oster and Wilson 1978). Ultimately, the division of labour within colonies may be viewed as a product of selection for efficiency in the performance of essential tasks.

Here those factors that influence the plasticity in growth and adult body form in captive colonies of naked mole-rats (*Heterocephalus glaber*) are explored. These rodents exhibit a large degree of variation in adult body size and shape (Jarvis *et al.* 1991). This variation culminates in a clear morphological dimorphism between the breeding female and all other colony members, the former exhibiting a marked elongation of vertebrae following the attainment of reproductive status (see Chapter 3).

While previous studies have provided both qualitative and quantitative evidence of variation in growth in naked mole-rats (Jarvis *et al.* 1991; Lacey and Sherman 1991), inter-litter comparisons within colonies have in the past been limited by a paucity of captive colonies with successful recruitment. In addition, few data exist on the growth responses of mole-rats to social perturbations. Here we attempt to quantify the effects of litter order and changes in the social structure on individual growth patterns.

These data represent an important addition to previous studies on factors that affect body mass in this rodent, and the relationship between work and body mass of adult mole-rats

(Jarvis *et al.* 1991; Lacey and Sherman 1991). They also provide a basis for comparison with the other social bathyergids, particularly *Cryptomys damarensis* (Bennett and Jarvis 1988) and *Cryptomys hottentotus hottentotus* (Bennett 1989) in which a similar relationship exists between the amount and type of work performed by colony members and their body mass. Since body size affects both the basal and working metabolic rate, of naked and Damaraland mole-rats (Lovegrove 1990), it is possible that the variation in the size of adults has important consequences for colony ergonomics.

METHODS AND MATERIALS

The same two breeding pairs described in Chapter 1 were used in this study. Initially, they were housed in burrow systems comprised of a geometrically identical three and a half metre network, of clear Perspex burrows (45mm wide by 53mm high), linked to a nest, food and toilet chamber. Following the recruitment of successive litters, the burrow systems, and chambers that connect them, were enlarged to ameliorate the possible effects of limited space on growth variables.

The number of pups in each litter was noted within 24 hrs of birth. Care was taken not to disturb the breeding pair until after pups were weaned, as disturbance (*e.g.* loud noises or handling) often results in the death of the pups (Jarvis 1991). The only exceptions to this procedure were the pups born to the first litter of colony 100. These individuals were weighed from day one through to weaning so as to enable growth comparisons of pups with published data for other species of the bathyergid family (Bennett *et al.* 1991).

Pups surviving to weaning were sexed, weighed and then permanently marked. Data concerning sex ratio and body mass at birth have been recorded in another study (Jarvis 1991). Post-weaning body masses were measured to the nearest 0.1 gram using a digital top-loading balance. Measurements were taken approximately weekly, except during the period immediately after the birth of a litter. After 730 days, the two pairs had raised sufficient pups in successive litters, to enable a statistical analysis of the effects of litter order on growth and adult body mass.

Pup growth comparisons

Large litter sizes and the regular weighing of all pups, enabled an investigation of the growth rates of successive litters in the two focal colonies (100 and 7400) over the first 72-80 days postpartum. This period corresponds to the average inter-litter interval, and was the same interval used by Bennett *et al.* (1991) for other species of Bathyergidae. In addition, we generated growth functions from weaning to day 80 for all the pups born to the first litter of colony 100 (n= five pups) which allowed for statistically valid comparisons of growth functions (*e.g.* the growth constant, $K=day^{-1}$) with other social and solitary bathyergids (see Bennett *et al.* 1991). In addition, the effects of litter size on pup growth (at 72-80 days of age) were investigated using a range of litter sizes (2-20 pups) from five colonies (including the two focal colonies in this study).

Adult growth comparisons.

To facilitate inter-litter comparisons of growth curves sigmoidal growth models were fitted to 14 litters recruited to the two colonies (colony 100, litters 1-6 and colony 7400 litters 1-8). Differences in the number of litters recruited to each colony were a consequence of the death of the breeding male in colony 100, at the end of the first year of the study. Approximately two reproductive cycles were missed by the breeding female before she began mating with one of her offspring.

Growth parameters, including asymptote (A), inflection point (I) and growth constant (K) were also derived for litters 1-6 for colony 100. The absolute ages over which the growth parameters were derived was standardised for each individual (approximately day 72 to day 365). This standardisation was necessary because variation in the absolute age at weighing was found to have a significant effect on the parameters estimated. These growth parameters were used to illustrate characteristics of growth which may be obscured when simpler measures of growth (*e.g.* average growth rates) are considered.

Inter-litter comparisons of body mass at specific ages.

To assess the effects of litter order on body mass, we calculated the mean body mass of the mole-rats in the first eight litters of both colonies at specific ages (n=59 individuals). Three different ages were chosen for all comparisons: 1) six months (days 180-190), 2) one year (days 360-370) and 3) two years (days 730-740). Variation in the age at weighing was a consequence of mole-rats differing in absolute age at any given weekly weighing. More frequent weighing to eliminate this variation would have resulted in excessive disturbance to the colonies. Body mass values were corrected, by dividing body mass by the absolute age, to provide the average gain in mass per day since birth. Data for same-aged individuals were pooled, to provide a mean corrected mass for each litter.

Changes in social structure and growth

Detailed records of colony history enabled us to explore the relationship between individual growth patterns and perturbations to the social structure of both colonies. These perturbations were the result of natural (*i.e.* without specific intervention on my behalf) events and included the death of the breeding male and the death of large non-breeders. By superimposing the occurrence of these events on both the predicted and actual growth trajectories of mole-rats within a colony it was possible to explore the effects of these perturbations on the growth of various litters. It was not possible to explore the effects of the death of the breeding female (colony 7400) on the growth of non breeding colony members. This was because at the time of her death many colony members were still exhibiting an appreciable growth spurt in response to the death of the breeding male. There was thus no baseline data (*i.e.* a long period of 'no change' in the social structure) with which to compare subsequent growth. Such changes have, however, previously been documented by Jarvis *et al.* (1991).

Data analyses

Both empirical and theoretical growth curves are included in this study. Although empirical data may be sufficient to assess the suitability of using growth curves to age individuals in a species, it is necessary to have growth rate (K) values, provided by standardized models, for comparison between species, and comparison between different groups within species (*e.g.* between litters in this study or growth data from other studies and this study).

Growth functions were derived using regression analyses of age and mass employing Gompertz, von Bertalanffy and Logistic transformations, according to Ricklefs (1967) and Zullinger *et al.* (1984). Growth models were fitted using the derivative-free non-linear regression programme (AR) from the BMDP statistical package (Dixon *et al.* 1990), with fixed A values and floating K and I values. BMDP-AR estimates the parameters of non-linear functions, by least squares method, with a pseudo-Gauss-Newton iterative algorithm. The goodness of fit of a particular model to the growth data was measured by three criteria: 1) the smallest deviation between the growth inflection point visually determined from a plot of the data and the point fixed by the model, 2) random distribution of residuals over time (no autocorrelation of residuals), and 3) the smallest residual sum of squares (RSS) for the estimated parameter. Using these criteria the Gompertz model was chosen for all subsequent analyses. A mean maximum growth rate was thus obtained for the respective litters using the Gompertz equation (Ratowsky 1983):

$$W = A/ \text{ x } \exp [-\exp(-K \text{ x } (t-t_i))]$$

where W = predicted weight; A = asymptote; exp = exponential function; K = growth rate constant; t = age and t_i = a parameter indicating inflection time. The growth rate constant K (day-1) was converted to maximum growth rate by multiplying K by $A.e^{-1}$ (estimated mass at the inflection point) to make the growth measures comparable across species and litters.

Inter-litter statistical comparisons were performed using a one-way ANOVA. Statistically significant differences were explored post-hoc, with a Tukey multiple range test. Linear regression analysis was performed to explore the relationship between age and body mass at different stages in the history of the colony.

RESULTS

The birth date, litter size and sex ratio of all pups recruited to the study colonies are provided in Table 1.

Table 1. Details of recruitment to the two focal study colonies (100 and 7400) commencing on February 6, 1989.

Colony	Litter no.	Birth date	No. survived to weaning	
			M	F
7400	1	06-02-89	3	2
	2	26-04-89	2	2
	3	18-07-89	4	4
	4	09-10-89	3	1
	5	28-12-89	2	3
	6	24-03-90	1	4
	7	03-06-90	3	1
	8	28-10-90	2	1
total			20	18
100	1	21-03-89	4	1
	2	04-06-89	10	3
	3	21-08-89	4	2
	4	22-01-90	3	1
	5	09-04-90	1	2
	6	25-06-90	5	1
total			27	10

Pup growth comparisons

The computed growth curves for the naked mole-rat pups from the first litter are presented in Figure 1. The Gompertz model provided an excellent fit to the growth data ($r^2 = 0.9967$, $P<0.00001$). The mean growth rate constant (K) was 0.017 days^{-1}, and the mean maximum rate of growth ($K.A.e^{-1}$) was 0.207g/day.

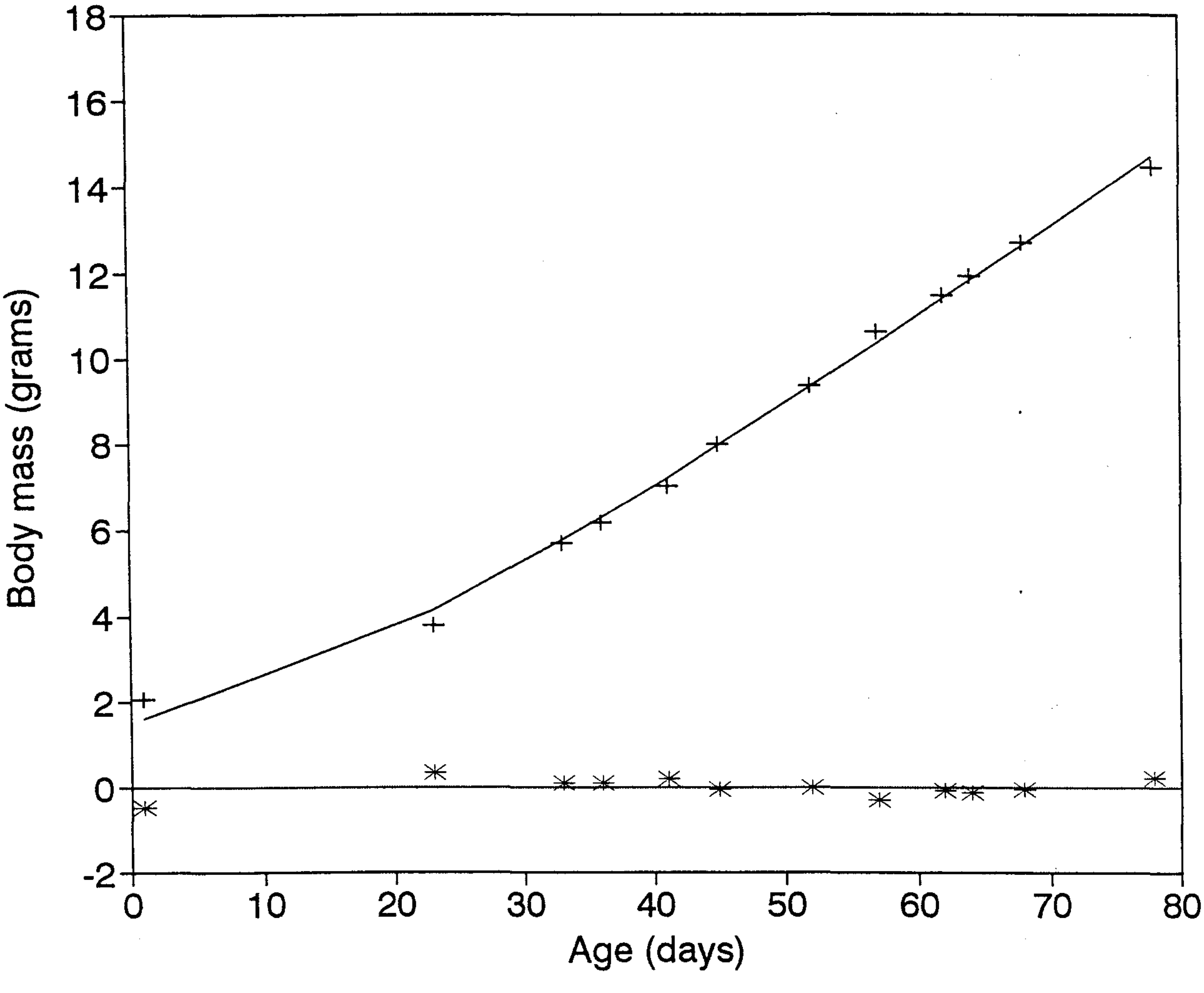


Figure 1. Generated Gompertz plots for postnatal mean body mass against age for five pups in the first litter born to a breeding pair (colony 100). Actual data points are represented by a plus symbol. A plot of the residuals (star symbols) for the 'goodness of fit' (deviations from the zero line) of the Gompertz equation is provided at the bottom of the graph.

Table 2. Inter-litter comparisons of body mass at four specific ages within colonies 100 and 7400.

Colony	Age	F	P	Tukey test (litters)
100	72-80 da.	$F_{5,35}=419.1$	0.2102	N.S.
	6 mo.	$F_{5,34}=419.1$	0.0191	1-6, 1-7
	1 yr	$F_{7,33}=92.94$	<0.000	1-6, 1-7, 1-8 2-6, 2-7, 2-8
	2 yr	$F_{7,31}=63.51$	<0.000	1-5, 1-6, 1-7 1-8, 2-5, 2-6 2-7, 2-8
7400	72-80 da.	$F_{7,28}=4.93$	0.001	1-7
	6 mo.	$F_{7,27}=6.123$	0.0002	1-2, 1-3, 1-5 1-6, 1-7, 1-8
	1 yr	$F_{7,27}=6.384$	0.0002	1-2, 1-5, 1-6 1-7, 1-8
	2 yr	$F_{7,26}=3.94$	0.0047	1-7, 1-8

Comparisons of the mean litter body mass of pups at age 72-80 days (Table 2), revealed that growth was very similar, for all pups in all litters, in colony 100 ($F_{6,35}=419.1$, $P=0.21$). Only litters one and seven of colony 7400 differed significantly ($F_{7,27}=6.12$, $P<0.05$, Tukey test), with the former being significantly heavier.

Linear regression analysis of litter size with the average growth rate (at 72-80 days), for 18 litters in five colonies, revealed a negative non-significant relationship (Corr. coeff= -0.1213, $F_{1,17}=0.253$, $P=0.62079$).

Adult growth comparisons

Growth models

The Gompertz model provided a good fit to the growth data for all litters (r^2 (range) = 0.878-0.997; $P<0.001$ for all regressions). In both colonies, members of the first litter showed the greatest mean maximum growth rate ($K.A.e^{-1}$) with the general trend being a decrease with subsequent litters (Figs 2 and 3). It was further evident that growth over the first 70 days was very similar for all litters (see also above). This suggests that differences in absolute body mass of adult individuals is largely attributable to differences in growth after day 70.

Variation in the parameters of the growth models among litters (colony 100) are illustrated in Figure 4. Both the asymptote and inflection time showed significant differences between litters ($F_{5,34}=4.037$, $P<0.005$; $F_{5,34}=4.76$, $P<0.005$, respectively). Litter 1 and 2 had significantly higher asymptotes and inflection times than litter 7 (Tukey test). In contrast there was no significant difference among litters in the growth rate constant ($F_{5,34}=1.917$, $P>0.05$).

Inter-litter growth comparisons at specific ages.

Litter order has a profound effect on the mean body mass of successive litters at specific ages in both colony 100 and 7400 (Table 2). In both colonies there was a significant difference in the mean body mass between litters at six months, one year and two years. In colony 100, members of the first and second litters had significantly greater mean body masses than mole-rats from the last four litters. In colony 7400, only members of the first litter had a significantly greater mean body mass than subsequent litters. Interestingly, these differences became more marked with time in colony 100 (six months versus two years) whilst in colony 7400 the reverse pattern was evident with all but litter 7 showing some form of 'catch up growth' with litter 1.

Table 3. Regression analyses of age and body mass in colony 100 from six months to four years post-inception.

Time since inception	Regression equation	r^2	F	P
6 mo.	y= -0.86+0.183	95.23	$F_{1,21}=419.1$	<0.000
1 yr	y= +2.94+0.129	82.29	$F_{1,21}=92.94$	<0.000
2 yr	y= +9.34+0.006	69.40	$F_{1,28}=63.51$	<0.000
3 yr	y= +18.51+0.032	54.26	$F_{1,28}=33.22$	<0.000
4 yr	y= +18.02+0.022	44.81	$F_{1,35}=28.41$	<0.001

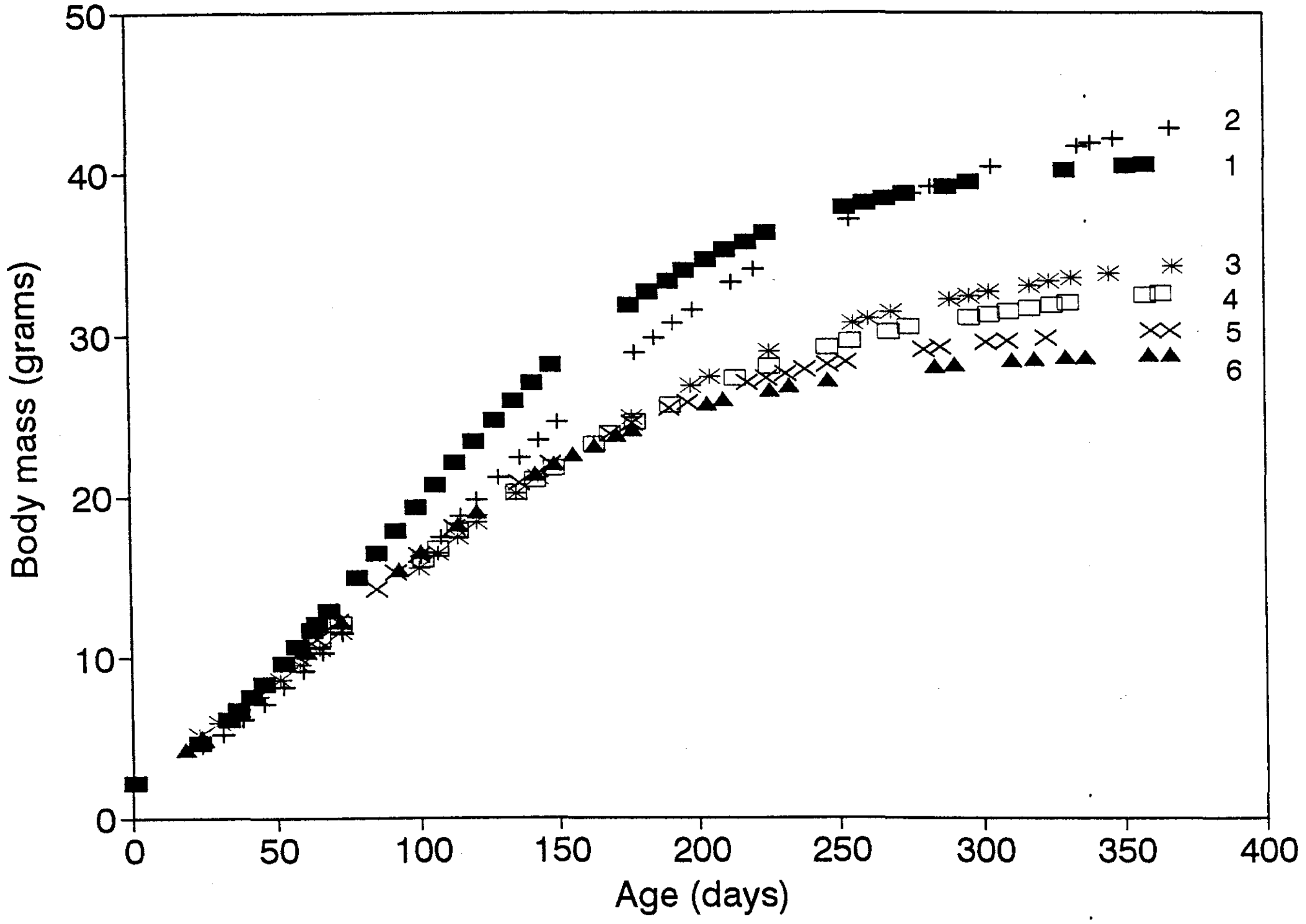


Figure 2. Predicted growth curves (estimated from the Gompertz equation) for litters 1-6 in colony 100. Body mass estimates were derived from actual body masses at ages 72-365 days.

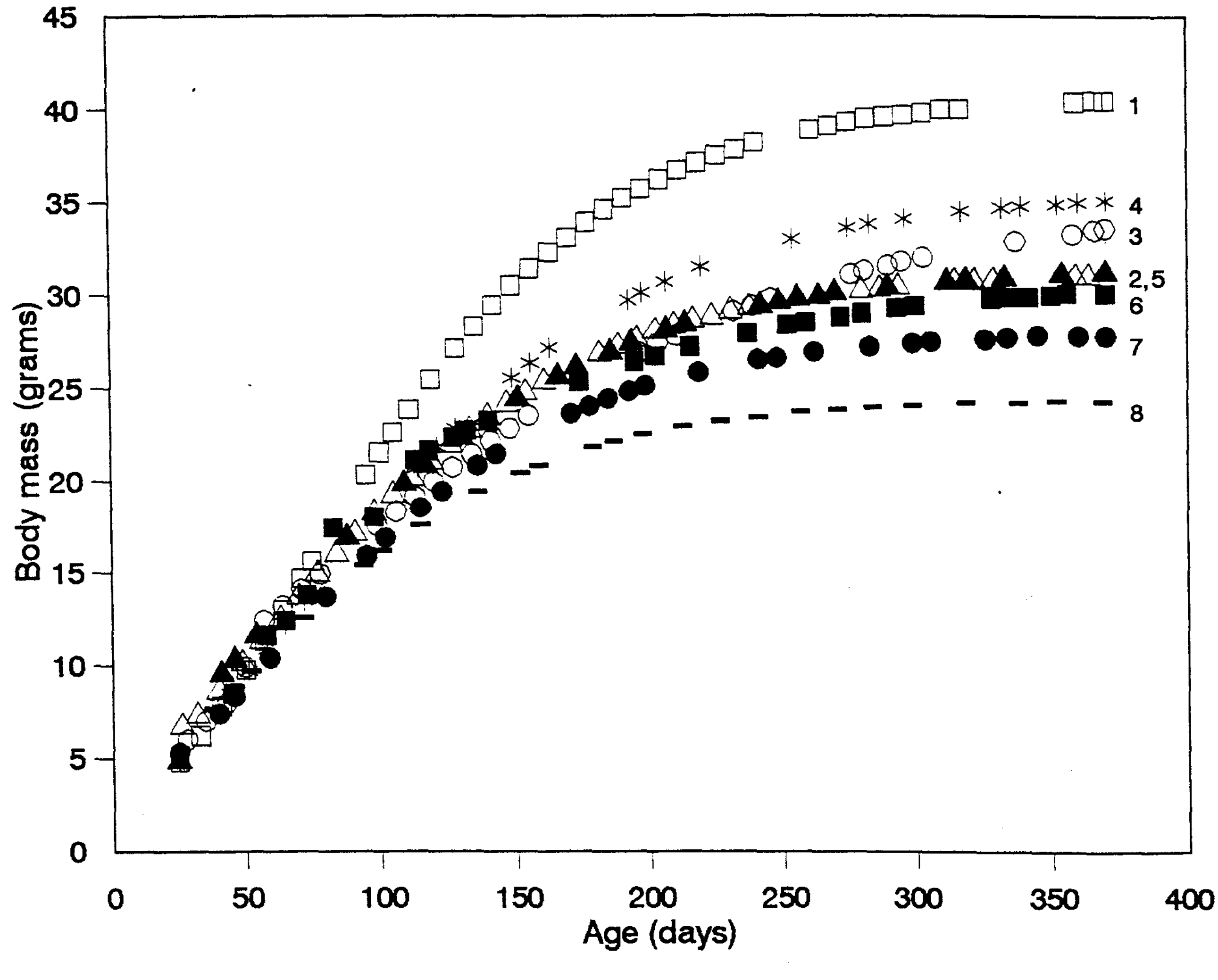


Figure 3. Predicted growth curves (estimated from the Gompertz equation) for litters 1-8 in colony 7400. Body mass estimates were derived from actual body masses at ages 72-365 days.

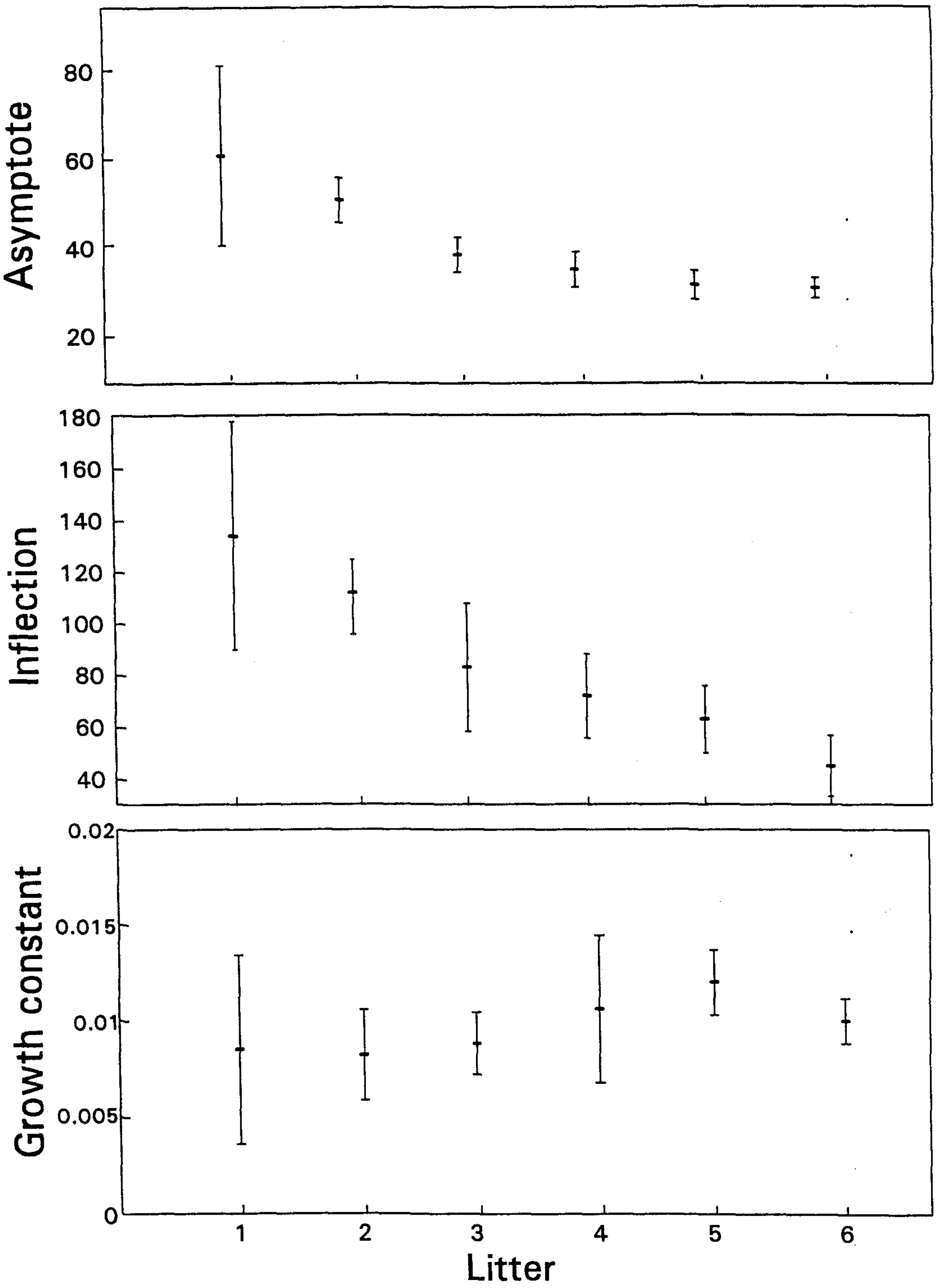


Figure 4. Parameters of Gompertz growth model for litters 1-6 in colony 100: (a) asymptote; (b) inflection time; and (c) growth-rate constant. The central horizontal marker represents the mean and the standard deviation is denoted by the vertical line.

These differences between the respective age cohorts resulted in a positive and significant relationship between age and body mass within both colonies (Table 3). Interestingly, this relationship weakened (r^2=95.23 at 6 months to r^2=44.81 at four years) as the colonies matured suggesting that the reliability of estimating relative ages from the body masses of both captive and wild caught mole-rats may decrease with time since colony inception.

Growth and changes in social structure.

Death of breeding male: Following the death of the breeding male in colony 100, there was a marked change in the growth of individuals from the first litter (Fig. 5). Individual #7, exhibited an enormous growth spurt, whereas individual #9 showed an immediate asymptote of body mass. It was not possible to explore the effects of litter order or individual sex in this example as litter 1 was all male and the only litter to have approached asymptotic body mass at the time of the perturbation. Fortunately, this was possible in colony 7400, where the death of the breeding male occurred at a later stage in the developmental history of the colony. For the purpose of clarity only data for the first, fourth and eighth litters (*i.e.* the oldest, youngest and middle-aged colony members) are provided. Here we see that members of the first and fourth litters exhibited a sharp increase in body mass, deviating markedly from the predicted growth trajectory, following the death of the breeding male (Fig. 6). In contrast, members of the 8th litter exhibited almost no change in their growth trajectories over the same period, with the observed growth tracking the predicted trajectory closely. Overall the magnitude of the growth response varied inversely with litter order, with members of litter 7 being the only other litter not to exhibit a clear growth surge.

Death of older siblings: In colony 7400, the breeding female attacked and killed six of the largest and oldest mole-rats over a period of six months (see Chapter 3 for details of this event). Victims of the breeding female were all members of the first and second litters effectively precluding their inclusion in the results. Data are presented for the two oldest and youngest surviving litters in the colony, excluding the fourth litter because of a small sample size (n=2) at the time of the perturbation.

Almost all colony members exhibited a concomitant increase in body mass (Fig. 7). Once again the magnitude of the response varied inversely with litter order, with individuals in the third and fifth litters showing a large increase in body mass, while members of the seventh and eighth litters showed little response. It is important to note that despite these individuals being appreciably older (ca. two years) than when the first perturbation occurred (death of the breeding male) they were still capable of exhibiting a substantial growth spurt.

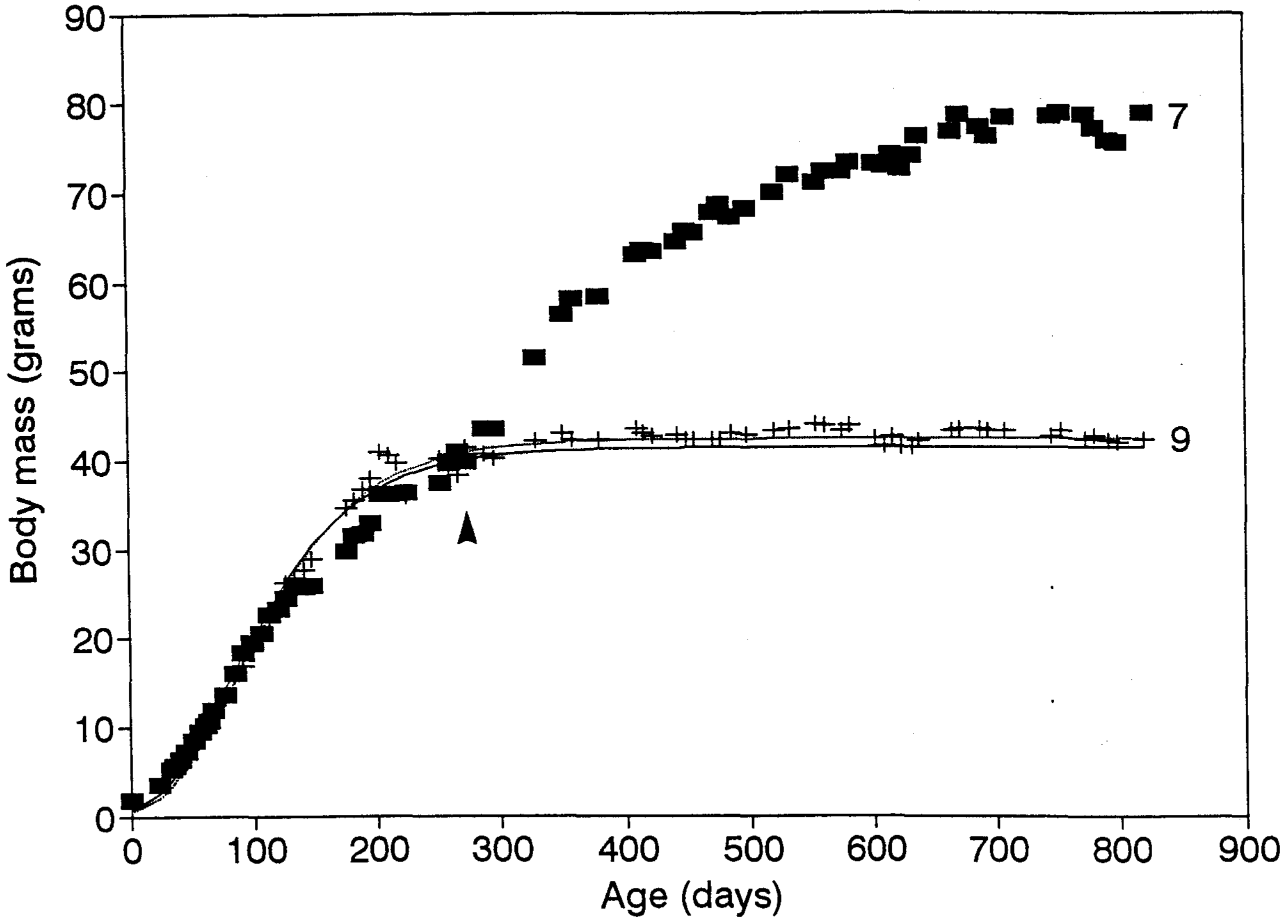


Figure 5. Growth curves for two individuals from the first litter of colony 100, before and after, the death of the breeding male. The arrow depicts the age at which the breeding male died. Symbols reflect the actual growth trajectory of the two individuals while the lines represent the predicted growth trajectory based on the parameters derived from the Gompertz model prior to the death of the breeding male. ■ individual #7, + individual #9. The solid line is the predicted growth trajectory (prior to perturbation) for individual #7; dashed line is predicted trajectory (prior to perturbation) for individual #9.

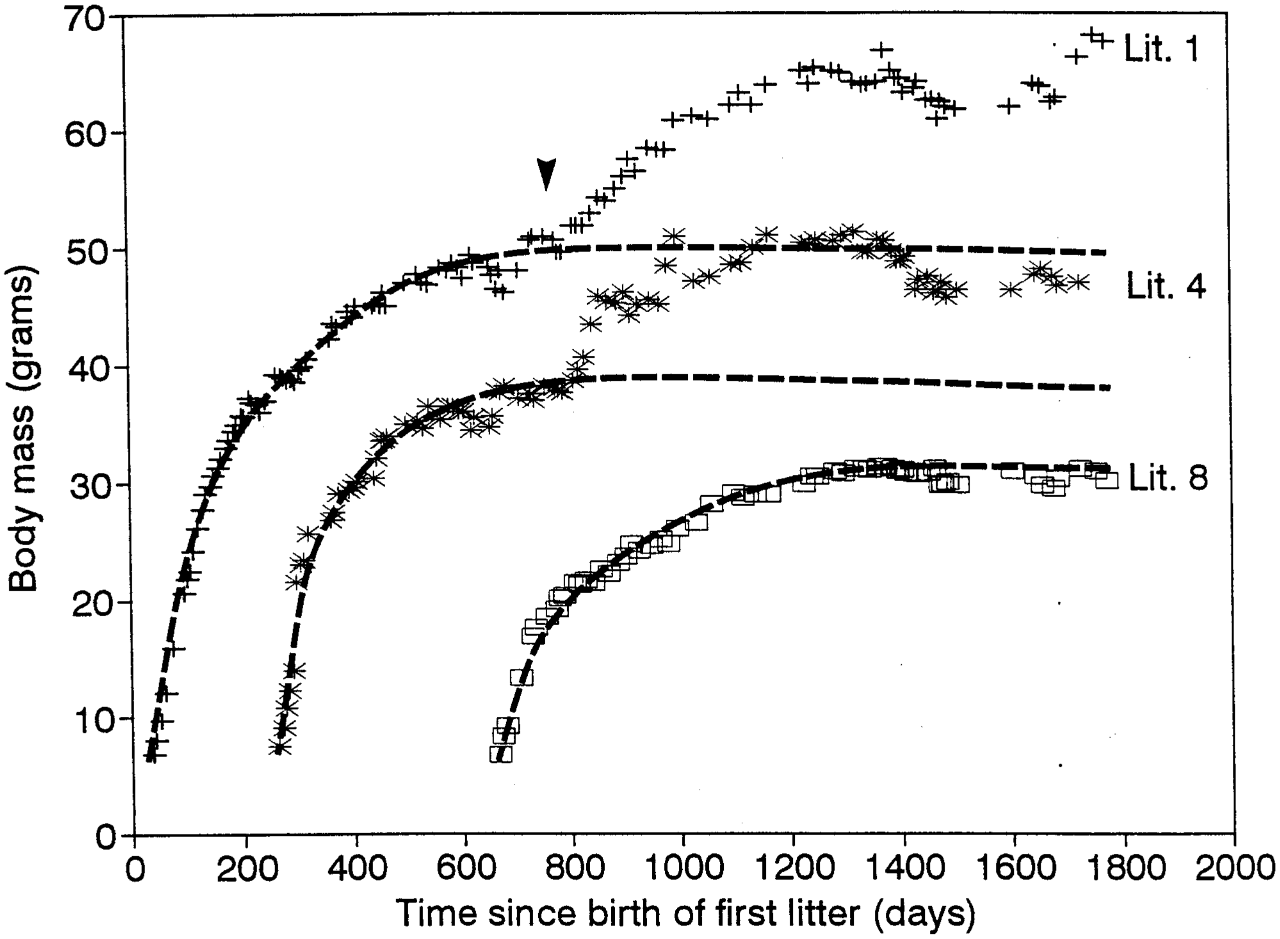


Figure 6. Mean growth curves for individuals in the first (n=5 individuals), fourth (n=4 individuals) and eighth (n=3 individuals) litters of colony 7400, before and after the breeding male died. The arrow depicts when the breeding male died. Symbols reflect the actual growth trajectory of the respective litters while lines represent the predicted growth trajectories based on the parameters derived from the Gompertz model, prior to the death of the breeding male. The x-axis represents the time since the birth of the first litter and not the absolute ages of the respective litter members.

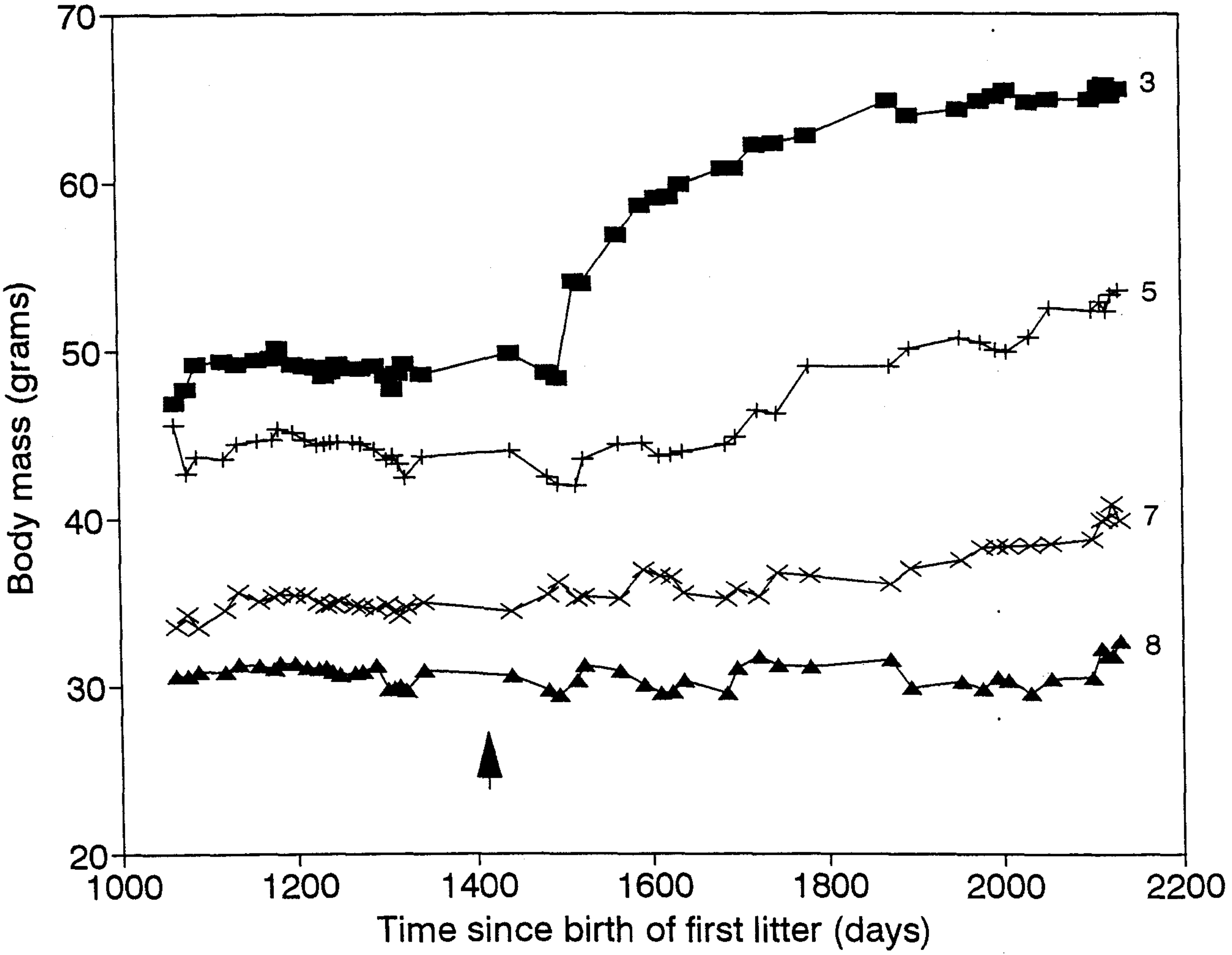


Figure 7. The mean growth trajectories for individuals in the third (n=6 individuals), fifth (n=5 individuals), seventh (n=4 individuals) and eighth (n=3 individuals) litters of colony 7400, following the onset (see arrow) of the death of older siblings (litters 1 and 2). The x-axis represents the time since the birth of the first litter and not the absolute ages of the respective litter members. The numbers on the graph correspond to the litter number.

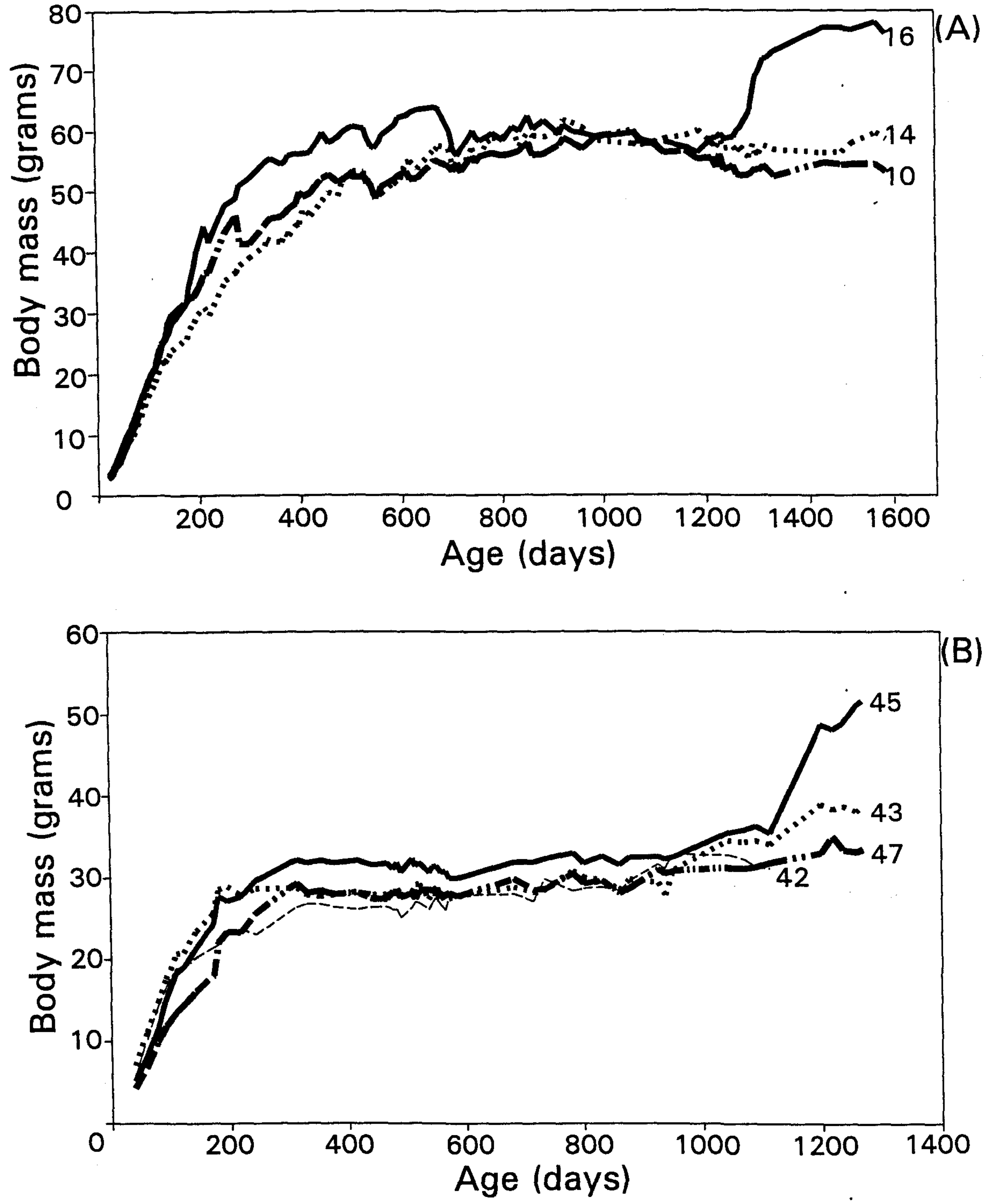


Figure 8. Individual growth trajectories for three individuals in litter 2 (A) and four individuals in litter 7 (B). These litters represent the oldest and youngest litters respectively, within colony 100, which are characterised by both dispersing (individuals #16 and #45) and non-dispersing (individuals #10, 14, 42, 43 and 47) siblings.

Growth spurts of dispersers

The growth trajectories of individuals subsequently identified as dispersers (see Chapter 6) in colony 100, differed from those of their non-dispersing litter-mates (Figs 8A & 8B). All of the six dispersers, identified in Chapter 6, exhibited a noticeable growth spurt at some point in their ontogeny. Thus, for example, individual #16 in the second litter (*i.e.* the oldest disperser, Fig. 8A) and individual #45 in the seventh litter (*i.e.* the youngest disperser Fig. 8B), exhibited a rapid gain in body mass, at some point in their life history.

DISCUSSION

Comparisons of naked mole-rat growth parameters with those of solitary and other social Bathyergidae (Bennett *et al.* 1991) reveal that, during the first 80 days, naked mole-rat pups have the slowest mean maximum growth rate of all the bathyergid species studied to date. Thus despite comparable growth rate constants in three social bathyergids (0.017 days^{-1} for naked mole-rats and K = 0.015 days^{-1} for *C.damarensis* and *C.h.hottentotus*), the mean maximum rate of growth for naked mole-rats (0.207g/day) was notably slower than for either *C.h.hottentotus* (0.461g/day) or *C.damarensis* (0.427g/day) (Bennett *et al.* 1991). Bennett *et al.* (1991) further showed that the mean maximum growth rates of solitary bathyergids were higher than for their social counterparts. Thus naked mole-rats exhibit the slowest mean maximum growth rate (over the first 80 days) of all the Bathyergidae.

The slow growth rate of naked mole-rats is possibly related to their poikilothermic traits and associated low basal metabolic rates (McNab 1966; Withers and Jarvis 1980; Lovegrove 1986; Buffenstein and Yahav 1992). It has been shown that for many mammal species there is a positive relationship between basal metabolic rate and prenatal growth rate (McNab 1980; Nicoll and Thompson 1987).

Considerable variation in growth and asymptotic body mass exists between litters within a colony of naked mole-rats, reared in the laboratory under conditions of *ad lib.* food. Much of this variation may be accounted for by litter order and changes in social structure within colonies. Individuals from the first and sometimes second litter of a nascent colony attain a significantly greater asymptotic body mass than individuals in later litters. Similarly, these older individuals exhibit a greater growth response following the death or removal of the breeding male, or other large siblings.

These findings provide support for the existence of a size/age correlation with social rank, in colonies of naked mole-rats (Schieffelin and Sherman 1995; Chapter 3). The older individuals attain a greater asymptotic body mass at a given age which enables them to out-compete younger/smaller siblings for access to limited resources such as food (Schieffelin and Sherman 1995; Chapter 4) and mating opportunities (Chapter 3).

Perhaps the most interesting aspect of growth within naked mole-rat colonies is that, although individuals may remain relatively constant in size for prolonged periods, they are still capable of exhibiting growth surges and changes in absolute body mass purely in response to changes in social structure within the colony. The dramatic deviations of the actual and the predicted growth trajectories of the siblings in litter 1 of colony 100 (Fig. 5) suggests that these growth changes are closely linked to the behaviour of the individual. Here the divergent morphologies of these two brothers were mirrored by their divergent behavioural repertoires, following the death of the breeding male. Individual #9 became the replacement breeder and exhibited a concomitant asymptote in body mass as predicted by previous findings (see Jarvis *et al.* 1991). In contrast, individual #7 grew extremely fast, became notably robust and was almost solely responsible for all defence-related activities within the colony. Further examples of within-litter divergence of growth trajectories were noted for those litters in which certain individuals became dispersers. In these examples, dispersing individuals exhibited a sudden rapid increase in body mass, that was not correlated with any one particular exogenous cue (*i.e.* a change in the social structure). Furthermore, dispersers in different litters displayed growth surges at different stages in the colony's history, suggesting that no single cue is responsible for triggering the change in body mass. It is possible that this rapid increase in body mass may be attributed to the deposition of fat, as dispersers, while not skeletally more robust from non-dispersers, had significantly higher percentages of body fat than same-aged non-dispersers (see Chapter 6). Such variation in body mass between littermates and the variable growth response of different aged individuals to the same stimuli, suggests caution should be excercised when using body mass as an indicator of age in wild caught colonies.

Growth spurts were further evident for mole-rats in colony 7400 (Fig. 6), where all littermates (males and females) within the first five litters (only litters one and four shown in the Figure) exhibited a marked increase in body mass following the death of the breeding male. In this example, none of the offspring became established as a replacement breeding male, and consequently there was no intra-litter divergence in growth trajectories. Interestingly, individuals in the seventh and eighth litters (only litter eight represented in the Figure) showed no response to the same stimulus suggesting that there is an overriding effect, of older siblings, on their growth.

Variation in the size and shape of measured characters, such as adult body size, is a common feature of most animal species (Travis 1988). Much of this variation is attributable to differences in the state of some environmental variable experienced during sensitive periods of development (West-Eberhard 1989). The most extreme examples of variation in adult body size amongst animals is evidenced by the eusocial invertebrates.

For example, discrete caste polyphenisms exist amongst the sterile helpers (*e.g.* workers and soldiers) of many eusocial hymenopteran species. These differences are determined by nutritional treatments set by the older workers as a function of caste ratios (*e.g.* Wheeler and Nijhout 1983; Wheeler 1986). Although comparable levels of morphological variation have not been reported for other social mammals, the effects on adult body size, of social factors, such as older siblings and litter/group size, have been documented, primarily for small mammals *e.g.* Krebs and Myers (1974); Cameron (1973); Batzli *et al.* (1977); Case (1978a); Koepel and Hoffman (1981); Rickart (1982); Derrickson (1988); Bennett and Navarro, in press). The effects of genetic variation, (Moore *et al.* 1970; Calvert *et al.* 1985) and maternal life history (Aberle and Doolittle 1976; Derrickson 1988) on the body size of adults have also been documented. Given that naked mole-rats show a high degree of inbreeding and that individuals within and between naked mole-rat litters in this study are siblings from the same mother, it is unlikely that growth differences can be attributed to intrinsic/genetic differences. A more plausible explanation is that, similar to those results obtained for most other invertebrate and vertebrate species, these growth differences are attributable to some extrinsic environmental or social variable.

In an important study, Christian (1970) demonstrated that both crowding and a limited food supply influenced growth of siblings. In this study, I attempted to control for the effects of food by ensuring an *ad lib.* supply. However, it is possible that despite attempting to control for the effects of crowding, by extending the absolute size of the burrow systems and nests, there remains a density-dependant effect on the growth of pups in successive litters, based on the philopatric nature of the species.

As mentioned in Chapter 1, observations of inter-litter interactions revealed that older individuals behaviourally dominated younger mole-rats by pup-pushing at an age when the latter were incapable of retaliation. Since the breeders and all older mole-rats engage in the violent shoving of pups and juveniles, it stands to reason that, as the colony grows numerically, so the members of each successive litter will be shoved at a higher frequency. If pup-pushing induces stress in mole-rats, then it is possible that this stress may retard the reproductive and somatic maturation of young mole-rats. While reproductive suppression of offspring through the establishment of clear dominant/subordinate relationships has been suggested (Faulkes *et al.* 1990), similar arguments for suppression of growth in naked mole-rats have not been advanced. The social environment has however, been shown to strongly influence growth and reproduction in other small mammals such as young microtine rodents (Batzli *et al.* 1977). Furthermore, it has been shown that, when released from the effects of reproductive suppression, 'new' breeding female naked mole-rats, exhibit an enormous increase in growth prior to, and after producing their first litter (Jarvis *et al.* 1991, Lacey and Sherman 1991). Older, larger individuals are usually the first (four out of five female takeovers documented by me) colony members to show external signs of reproductive activity (perforate vagina and large nipples), suggesting that they are less suppressed than the younger females in the colony, who frequently show no signs of either sexual activity or growth (O'Riain, unpubl. data). Thus it is possible that mole-rats recruited to large colonies remain small in size as a consequence of the negative effects on growth due to sexual suppression and the failure to undergo puberty. My data indicate that sexual suppression and growth are so interlinked that neither should be considered on its own.

The trends, illustrated in Figures 2 and 3 and Table 2, in which growth of successive litters is suppressed, may be explained by the formation of inter-litter hierarchies. Similar trends between successive age-cohorts have been noted in the cooperatively breeding Damaraland mole-rat (Bennett and Navarro, in press), whose social system is characterised by a linear size-based, dominance hierarchy (Jacobs *et al.* 1991)

Variation in worker size in social insects has been interpreted as a consequence of selection for an improved match between task and size (*e.g.* large, physically robust soldiers versus smaller foragers in *Pheidole dentata*, Oster and Wilson 1978). Ultimately, this results in improved colony efficiency, serving to maximise the inclusive fitness of the individual colony members. Although variation in body size of mole-rats within a colony is continuous (Chapter 3), there is a similar relationship between size and the nature of the tasks performed. Burrow maintenance tasks are inversely correlated with body mass (Jarvis 1981; Faulkes *et al.* 1991; Jarvis *et al.* 1991; Lacey and Sherman 1991), whereas colony defense activities are typically performed by a small subset of the heaviest colony members (Lacey and Sherman 1991; Chapters 3 and 5). My study has shown that it is the members of the first born litter that attain the greatest body mass; they are thus the colony members best suited for the physical task of ousting conspecifics and predators. By contrast, I have shown that the individuals recruited to well established colonies will not grow as large, consequently they will impose lower energetic costs (McNab 1979; Lovegrove 1991) on the colony when performing maintenance tasks. Together, this translates into improved ergonomic efficiency and, similar to arguments for insect societies, improved inclusive fitness of all colony members.

Lovegrove (1991) has argued, that because the daily energy expenditure of a colony will increase with an increase in average body mass of the colony members, it will impose a theoretical upper limit on the number of large mole-rats per colony. It is perhaps for this reason that even once recruitment stops for a prolonged period, colonies of naked mole-rats maintain a scatter of body masses, with many individuals showing an early asymptote in body mass (Chapter 3; Jarvis *et al.* 1991). This ensures that colonies do not become too 'top heavy' and ergonomic efficiency is thus maintained.

That energetic constraints affect body mass is also borne out by field studies in which Jarvis (1979) and Brett (1991) found that mean body mass of wild-caught colony members correlated with food availability in different geographic localities. The mean size of colony members from areas with a low food availability was appreciably smaller (27.9±5.05 g) than that of individuals from regions with a more abundant food supply (31.5±10.9 g).

The above arguments suggest that there are several inter-linked factors affecting body size in naked mole-rat and despite a limited genetic base (Faulkes *et al.* 1990; Reeve *et al.* 1990; Honeycutt *et al.* 1991), a variety of phenotypes are produced with the most appropriate morphology for the prevailing ecological and/or social conditions.

CHAPTER THREE

THE SOCIAL ORGANISATION WITHIN NAKED MOLE-RAT COLONIES.

SUMMARY

In this Chapter factors that influence the social organisation within captive colonies are investigated. It was found that morphology, behaviour and development in naked mole-rats may all exhibit plasticity enabling short term behavioural, as well as long term morphological adjustments to changing environmental and social conditions. The lack of temporal worker castes, roles or morphological specialisation of non-breeders reveals a generalised social system, that is strongly influenced by dominance interactions, initially between breeders and non-breeders, but also among non-breeders. Only the breeding female provides evidence of morphological and behavioural specialisation, with a qualitatively distinct behavioural repertoire and marked lengthening of her body. Both these adaptations are associated with the reproductive role, facilitating the production of large litters, thus maximising the colony's reproductive output. Reproductive inhibition of non-breeders by breeders, is thus the only apparent mechanism of 'role' determination in naked mole-rats, and is maintained through the physical presence of the queen and her regular performance of aggressive behaviours. Importantly, each individual born to a colony is a potential reproductive and thus capable of exhibiting a qualitative change in both morphology and behaviour. Replacements are however, typically from the ranks of the largest, most dominant non-breeders within a colony. Systems in which reproductive control and division of labour of non-breeders is effected through behavioural means (*i.e.* dominance) are capable of rapid responses to events such as the loss of the queen or a colony defender. Amongst the non-breeders, differences between individuals in their threshold levels of behavioural response to specific work and defence stimuli and their relative status in the dominance hierarchy, provide the key elements to the organisation of colony duties. The social organisation in naked mole-rat colonies allows for the immediate response of the colony to temporary modifications of both foraging and defense exigencies, long term modifications following the death of colony members, and irreversible changes in female morphology to enhance the breeding female's fecundity and thus colony

productivity. This flexibility enhances the resilience of the colony, especially to short term environmental fluctuations, in an otherwise predictable environment.

INTRODUCTION

Understanding the mechanisms that integrate the behaviour of individuals into a functional colony is a central issue in the study of social organisms (Robinson 1992). Predictably, most progress in this field has been made in studies of the more diverse social insects. Here the regulation of division of labour has been shown to be influenced by a variety of factors, both intrinsic *e.g.* genes (Frumhoff and Baker 1989; Robinson *et al.* 1989) and hormones (Jaycox *et al.* 1974; Wheeler and Nijhout 1981; Robinson *et al.* 1989) and extrinsic *e.g.* nest architecture (Seeley 1982; Kolmes 1985; Tofts and Franks 1992) and social environment (Davidson 1978; Winston 1982; Winston and Punnett 1982; Bonavita-Cougouran and Morel 1988; Wilson and Holldobler 1988).

Cooperatively breeding and eusocial species, from all taxa, are characterised by a division of labour which falls into two broad categories. A primary division of labour between reproductives and workers and a secondary division of labour among workers (Wilson 1971; Michener 1974; Jennions and MacDonald 1994). The existence of subordinate or sterile workers is the most important aspect of this organisation because a complicated social structure may emerge with even a relatively simple repertory of individual worker behaviour (Oster and Wilson 1978).

Amongst the workers of insect societies, specialisation has been achieved primarily through temporal castes, with physical castes being largely restricted to the ants (Wilson 1971; Hölldobler and Wilson 1990). Both types of labour organisation result in improved efficiency of task performance, because individuals specialise on fewer tasks (Oster and Wilson 1978).

Knowledge of the proximate evolutionary forces that have shaped and maintain the patterns of labour organisation, is well documented within both eusocial and cooperatively breeding invertebrate (*e.g.* Batra 1966; Wilson 1971; Oster and Wilson 1978; Seeley 1982; Peeters and Crewe 1985; Bourke 1988; Lachaud *et al.* 1988; Hölldobler and Wilson 1990; Villet and Crewe 1990; Reeve 1991; Robinson 1992; Peeters in press) and vertebrate societies (*e.g.* Rasa 1977; Rood 1978; Frame *et al.* 1979; MacDonald 1980; Bekoff and Wells 1982; Harrington *et al.* 1983; Brown 1987; Calderone and Page 1988; Agren *et al.* 1989; du Plessis 1990; Emlen 1991; Jennions and MacDonald 1994).

Detailed laboratory studies of the naked mole-rats' social organisation, which arguably represents the pinnacle of mammalian sociality, have also been performed (Jarvis 1981; Isil 1983; Faulkes *et al.* 1991; Jarvis *et al.* 1991; Lacey and Sherman 1991). A distinctive feature of these studies, when compared to studies on other cooperatively breeding vertebrates, is the adoption of terminology originally developed for describing the division of labour within insect societies. Thus, perhaps as a consequence of their 'eusocial status', Jarvis (1981) used both the terms 'caste' and 'age polyethism' to describe non-breeding naked mole-rats with different growth trajectories and work rates. Where caste, as defined by Wilson (1971) is defined as "a particular type, or age group, or both, that performs specialised labor in the colony" (p. 462) and age polyethism as "the regular change in labour roles by colony members as they age" (1971, p.467). While comparisons with social invertebrates is considered heuristic, given the close parallels that exist between their work environments (a permanent expandable nest/burrow system), use of such value-laden terms is potentially misleading given the lack of adequate data concerning the temporal shift of task performance in successive age cohorts, and adequate detail of the dynamics of morphological variation within colonies. A consequence of this is that researchers in this field differed in both their interpretation and application of such terms to the organisation of work activities within naked mole-rat colonies. These differences are described in the book on naked mole-rats (Sherman, Jarvis and Alexander 1991) and may be briefly summarised as follows. Lacey and Sherman (1991) suggest that labour is organised according to the age of the non-breeders, justifying the use of the term age polyethism. Furthermore, the apparent (based on few known-aged individuals) correlation between the age and size of mole-rats led Lacey and Sherman (1991; in press) to modify the term age polyethism, to age/size polyethism when describing the organisation of labour within colonies. However, the findings of Jarvis *et al.* (1991) do not support the use of this term, because considerable variation is apparent in the amount of work performed by individuals of a similar age and size. Furthermore, it has not been shown that mole-rats perform distinct roles (roles being implicit in the definition of age polyethism) within the colony, where 'role' refers to a set of closely linked behavioural acts with a high transition probability (Hõlldobler and Wilson 1990). In the absence of such evidence, use of this term must be considered as premature and thus potentially misleading.

In essence, both studies (Jarvis *et al.* 1991; Lacey and Sherman 1991) suffered from a lack of long term ontogenetic data of same-aged individuals which would enable an adequate evaluation of the applicability of these terms to naked mole-rat societies. Ontogenetic studies are not only essential to a full understanding of adult social behaviour, but also make it possible to compare the ontogenetic sequences within such taxonomically disjunct groups such as naked mole-rats and insects. Furthermore, it is important to consider form and function separately at the level of individual ontogeny, until it can be shown that there are no important differences between the mechanisms guiding the two (Bateson 1977).

The results of a six year study on the social organisation and morphological variation of naked mole-rats are presented here. The successful recruitment of 25 litters to the two focal colonies studied in Chapters 1 and 2 has enabled a critical evaluation of the nature of both the primary and secondary division of labour within colonies. The applicability of value-laden terminology such as age polyethism, physical polyethism and soldiers derived from studies on social invertebrates, is also evaluated.

METHODS AND MATERIALS

Study animals

Individuals from two captive bred colonies (colonies 100 and 7400, see Chapter 1) were used in this study. Both colonies were provided with a nest, toilet chamber, food store and a digging tray. These functionally distinct parts of the burrow system were covered with glass and joined with clear perspex tubes to enable behavioural observations. The size of the burrow system was increased as the colonies grew numerically. To ensure that the work environment remained constant across observation periods the mole-rats were provided with approximately equal quantities of fresh food and toilet/nest material at the same time each day. Behavioural observations were not recorded for at least eight hours after the introduction of these items. Digging behaviours were recorded in all blind-ending sections of the burrow system and these, as well as the digging tray, provided a high density of work sites for the colony. At no stage in either colony's history were the number of mole-rats working at any one time greater than the number of potential work sites. This is an important consideration, for if such opportunities to work were limiting, then the amount of work that one individual could perform might have been limited by the behaviour of other colony members. Such data would then not conform to the assumptions of independence inherent in the statistical analyses used in this study.

Behavioural profiles of colony members

Behavioural data were recorded for all individuals in both colonies for four of the six separate years - 1989, 1992, 1993 and 1994. The first sampling period coincided with the data collected for Chapter 1 and permitted an analysis of the behavioural profiles of mole-rats within newly established colonies. Subsequent sampling, at the end of each calendar year (1992-1994), commenced after the successful recruitment of at least eight litters to each colony. The latter criterion was necessary to allow for a quantitative investigation of the effects of age on the behavioural profile of colony members. Outside of these quantitative sampling periods, both colonies were monitored on a daily basis and all mole-rats were weighed approximately once per week.

For behavioural analyses a minimum of 40 hours of behaviour (maximum of 91 hours) were recorded for each colony in each sampling interval. The total number of hours recorded for colony 7400 was 218 hours and for colony 100, 167.5 hours. Two different sampling methods were used in the study (after Altmann 1974). The principle method was scan (instantaneous) sampling, in which the activity and location of each mole-rat in the colony was monitored every two minutes for a minimum period of one hour per observation session. In this way a 'snapshot' of the activities of the whole colony was obtained in the shortest possible time. *Ad-libitum* sampling was performed to record all occurrences of rare behaviours (*e.g.* mating). Here every behaviour was treated as an event and no information on its duration was recorded. All sampling sessions were performed randomly between 09h00 and 18h00.

To eliminate immediate repeat recordings of an animal, to control for bias towards particular areas or mole-rats, and to ensure that all parts of the burrow system were observed with equal frequency, the pattern of observations was standardised. This was achieved by starting each two minute scan at one end of the burrow system and then following an identical path through all areas of the system to the other side of the burrow. This sampling protocol allowed the establishment of a schema of the activities for each individual, and consequently the reconstruction of the social activity of the colony. Furthermore it enabled the construction of a behavioural repertoire for each individual within the colony. These were then used to establish group subdivision using agglomerative hierarchical cluster analysis. In this manner it was possible to separate workers from reproductives objectively.

A matrix of the total frequency with which each behaviour was performed by each colony member was constructed, and analysed using PRIMER, the multivariate procedure of agglomerative hierarchical cluster analysis (Clarke and Warwick 1995). This method computes similarity coefficients for each pair of mole-rats in the colony. Starting with the original data matrix of frequencies of behaviours, the similarity between any pair of individuals can be defined in an analogous way to that for samples involving comparisons of the ith and lth row (individuals) across all j=1,...,n columns (behaviours). The Bray-Curtis similarity between individuals i and l is:

$$S'_{il} = 100\left\{1 - \frac{\sum_{j=1}^{n} |X_{ij} - X_{lj}|}{\sum_{j=1}^{n} (X_{ij} - X_{lj})}\right\}$$

where S' is the similarity coefficient between the i and lth individuals. The coefficient is an algebraic measure of how close the behavioural profiles are for each individual, averaged over all individuals, and defined such that 100% similarity represents total similarity and 0%, total dissimilarity. Similar methods have been used in numerous studies investigating the social organisation of invertebrates (Gadagkar 1980; Fresneau *et al.* 1982; Corbara *et al.* 1989; Fresneau and Dupuy 1988; Villet 1990; Theraulaz *et al.* 1992) and at least one vertebrate society (Rasa 1977).

The behavioural relationship between all individuals within the colony is represented by a dendrogram, which links individuals in hierarchical groups on the basis of the behavioural similarity of individuals in each cluster. The ethograms (and sizes and ages) of the individuals within each cluster were subsequently examined to see whether groups were performing specific roles and, whether there was any evidence for physical polyethism.

Prior to all analyses the data were root-root transformed to reduce the effect of very high and very low values (frequencies of behaviours) that may otherwise bias the results. An analysis of similarity (ANOSIM, *sensu* Clarke and Warwick 1995) was performed *a priori* on all age cohorts (that consisted of at least three individuals) within each colony. This analysis is designed to enable one to detect whether the behavioural repertoires of same-aged individuals are more similar to one another than to other age-cohorts.

It was not possible to confirm the results obtained for the cluster analysis with, for example, discriminant function analysis. The advantage of the latter technique is that one can ascertain those behavioural variables that account for differences between the respective litters. However, the assumptions inherent in these statistical procedures (*i.e.* balanced samples) were violated by the biological reality of the data (litters/samples are naturally unbalanced).

Temporal division of labour

To test whether the 'behavioural work profiles' of naked mole-rats are age-linked two approaches were followed. Firstly, agglomerative hierarchical cluster analyses were performed on those work behaviours for which the frequency scores were sufficiently large to allow for statistical analyis (digging, sweeping, carrying food and carrying nest material) in both focal colonies. To test for significant differences in the 'work profile' of different age cohorts ANOSIMS were performed. Secondly, changes in the frequency of performance of the four main work behaviours were plotted against time (1989-1994) for four different litters (the two oldest and the two youngest) of colony 7400, to investigate evidence for temporal shifts in the division of labour.

Spatial division of labour

The spatial division of labour within the burrow system was examined by summing the time each individual spent on a particular task at the different work localities within the burrow system. Ten individuals were chosen randomly, from each age cohort (litters 1-10), in colony 100 (1993 sampling interval) and a Chi-squared test performed to determine if the distribution of this pattern differed significantly from random.

Morphological variation and physical polyethism

Body size

Size-frequency distributions of body mass (a measure of size) were determined for colony 100 and two large ($n > 70$ individuals) well established (> 4 years old) colonies. To investigate the effects of both colony age and recruitment on distribution patterns, data were plotted at different stages of each colony's history.

Morphometric study

Two large colonies (Colony 100, $n=42$ animals; Colony 1000, $n=31$ animals) were chosen for this study. Both were functionally complete with a breeding pair and at least eight successive litters. Mole-rats were anaesthetised by Halothane inhalation and then X-rayed (dorsally and ventrally) using a Shimadzu medical X-ray unit. Mammography film was used to provide detailed outlines of both hard and soft body tissues. X-ray dosage was kept to a minimum (40 kilovolts; 25 milliampere) and the entire procedure lasted less than five minutes. No mole-rat suffered any short or long-term effects. A measurement of incisor width, was taken directly from the anaesthetised animals (maximum width in a horizontal plane across upper incisors).

Six skeletal measurements were made from the X-rays (see results; see Appendix II for details). They were chosen on the basis of two criteria. Firstly, that they provided a measure of body size - viz. skull length, skull width and body length. Secondly, that they could be measured accurately and reliably from the X-rays. All measurements were made using digital calipers, accurate to 0.01 mm. Three separate readings were taken for each measurement and averaged for each individual.

Size-frequency distributions of incisor width and lumbar vertebral length were plotted for both colonies and compared with the size-frequency distribution of body mass. To test for the presence of polymorphism (*sensu* Tschinkel 1988) the normal scores for each of these variables were plotted against the actual values. One way ANOVA's were used to ascertain whether differences in the three morphological variables which provided the best estimate of body mass (width across zygomatic arch, width of incisors and length of lumbar vertebrae) were age-linked. Multiple comparisons between all litters were performed using a Tukey test. This test was chosen because it the most robust, with respect to any departures from population normality and homogeneity of variance, of this family of statistical procedures (Zar 1977).

Physical polyethism

To investigate the correlation between the division of labour within colonies and individual size, morphological data (body mass, incisor width, width across the zygomatic arches and vertebrae length) were superimposed on the results of the cluster analysis of the behavioural repertoires of individuals in colony 100 (1994 data). Six clusters were identified at the 70% similarity level, and nine clusters at the 75% similarity level (see dotted lines on Figure 1D). Only clusters containing more than two individuals could be used in the ANOVA. This restricted the first analysis (70% level) to a comparison of four groups, and the second analysis to five groups.

Dominance interactions and reproductive succession

Matrices of dominance relationships, based on agonistic encounters (shoving and pup-pushing) between all mole-rats, both during and after a contest for reproductive dominance (following the death of the breeding female in 1992), were constructed for colony 7400. The probability of linearity within hierarchies was calculated according to the method of Appleby (1983). Only one mole-rat (#15), a chronically thin individual) was not observed to engage in agonistic encounters with other mole-rats. The frequency of performance of the key interactive (shoving and pup-pushing) and sexual (naso-anal grooming) behaviours were plotted for each litter in 1992 during the competition for reproductive rights (*i.e.* in the absence of the original breeders, both of which had died previously). This gave a clear indication of the distinguishing behaviours of the reproductives and non-breeders and also gave insight into differences in the non work-related behavioural repertoires of individuals of different ages.

RESULTS

Behavioural profiles of colony members

Dendrograms constructed for colonies 100 and 7400 over four years (Figures 1A-D & 2A-D), enable a detailed interpretation to be made of the social organisation within the colonies at specific points in time, as well as comparisons across years. It is further possible to follow the behavioural ontogeny of all colony members. Because most individuals did not show an appreciable difference in their behavioural repertoires with time however, an analysis of each colony member was considered to be of little heuristic value. Consequently, only those individuals that exhibited distinct changes in their behaviour over the course of the study period were followed. These individuals were identified as replacement reproductives (R), potential reproductives (P), soldiers (S) and dispersers (D). Although all colony members are in effect potential reproductives, here P refers to individuals that are engaging in sexual behaviours but have not reproduced. To enable comparisons across years these individuals have been identified on the dendrograms by their individual numbers and by a code which identifies them as a member of one of the above groups (see Figure 1 legend for details).

General trends in both colonies

The most salient feature of these dendrograms is the clear separation of breeders (or potential breeders) and non-breeders, with the latter showing a marked tendency to cluster according to their age. Furthermore, older individuals clustered consistently closer to the reproductives, and the pups (when present) clustered at the extreme opposite side of the dendrogram. Results from the analyses of similarity, given in Tables 1 and 2, provide support for these age-related trends - significant differences were often apparent between the behavioural profiles of different aged non-breeders in both colonies during each study interval. Multiple comparisons revealed that most significant differences were between the oldest and the youngest individuals, and not between temporally adjacent age-cohorts, which show considerable overlap in their behavioural repertoires (see Figs 1A-D & 2A-D). Exceptions to these trends were however evident in both colonies at all stages, with individuals from markedly different litters sometimes grouping together. In some cases older individuals clustered with members from younger litters (see member of litter 3, in Fig. 1B), while a few younger individuals were grouped closer to the reproductives than some of their older siblings (see members of litters 10 and 11 in Fig. 1D). By referring to the behavioural catalogue of all litters and the breeders (Table 3 and Figures 6A & B) it can be seen that differences in behavioural profiles are largely attributed to the performance of behaviours related to reproduction and dominance. The breeders and members of the first two litters were the exclusive participants in reproductive anddominance behaviours, while individuals from the youngest litters were characterised by subordinate behaviours. All non-breeding individuals and the breeding male performed all task-related activities. In contrast, the breeding female was never observed to perform certain work-related activities such as digging and sweeping.

Table 1. Summarised results of the analyses of similarity (ANOSIM) in colony 100, in each of the four study periods. Comparison of the behavioural profiles of individuals in different age cohorts was only possible for those litters consisting of at least three animals.

Year	Litter	F-test	P	Multiple comparison tests
1989	1 2 3	$F_{3,19}=0.841$	0.000	1 vs 2 1 vs 3 2 vs 3
1992	1 2 4 6 7 8	$F_{6,32}=0.482$	0.000	1 vs 2 1 vs 4 1 vs 6 1 vs 7 1 vs 8 2 vs 6 2 vs 7 2 vs 8 4 vs 7 4 vs 8 6 vs 7 6 vs 8 7 vs 8
1993	1 2 4 6 7 8	$F_{6,31}=0.186$	0.013	1 vs 6 1 vs 7 1 vs 8 2 vs 6 2 vs 8 4 vs 7 4 vs 8 6 vs 8
1994	2 6 7 8 11 12 13	$F_{7,34}=0.287$	0.01	2 vs 8 2 vs 11 2 vs 13 6 vs 8 6 vs 11 6 vs 13 7 vs 13 8 vs 11 8 vs 13

Table 2. Summarised results of the analyses of similarity (ANOSIM) in colony 7400, in each of the four study periods. Comparison of the behavioural profiles of individuals in different age cohorts was only possible for those litters where n≥3.

Year	Litter	F-test	P	Multiple comparison tests
1989	1 2	$F_{2,9}=0.025$	0.31	n.a.
1992	1 2 3 4 5 6 7 8	$F_{8,28}=0.416$	0.000	1 vs 5 1 vs 6 1 vs 7 2 vs 5 2 vs 6 2 vs 7 2 vs 8 3 vs 5 3 vs 6 3 vs 7 3 vs 8
1993	1 2 3 4 5 6 7 8	$F_{6,29}=0.425$	0.000	1 vs 3 1 vs 5 1 vs 6 1 vs 7 2 vs 4 2 vs 5 2 vs 6 2 vs 7 2 vs 8 3 vs 5 3 vs 6 3 vs 7 3 vs 8 5 vs 8
1994	3 5 6 7 9 11	$F_{6,24}=0.167$	0.025	3 vs 9 5 vs 9

Table 3. A presence/absence list of individual work, dominant, subordinate, reproductive and maternal behaviours performed by members of each successive age-cohort (litter 1 = oldest) and the breeders of colony 7400 (n=37) in 1993. Asterisks (*) indicate that a particular behaviour was performed (present) by members of the respective litter or breeder. Individual behaviours are combined in the respective categories to provide an overview of the repertoire of each age-cohort and the breeders. The letter at the front of each behaviour corresponding to one of the categories below. In addition, the total number of unique behaviours (never observed being performed by any other individual) is provided at the bottom of the table. *1 denotes the age cohort or breeder that clearly performs a given behaviour at the highest frequency (>10% more frequently than any other).

Behavioural code	Non-breeders (litter order)								Breeders	
	1	2	3	4	5	6	7	8	BF	BM
W-Digging	*	*	*	*1	*	*	*	*		*
W-Sweeping	*	*	*	*1	*	*	*	*		*
W-Carry nest material	*	*	*	*	*	*	*	*1		*
W-Carry food	*	*	*	*	*	*	*	*1	*	*
W-Carry pups	*	*	*	*	*	*	*	*		*
W-Groom pups	*	*	*	*	*	*	*	*		*
S-Shoved	*	*1	*	*	*	*	*	*		*
S-Pup-pushed							*	*1		
S-Bitten		*	*	*	*	*	*	*1		
D-Shove	*	*	*	*	*	*	*	*	*	*
D-Pup-push	*	*							*1	*
R-Allogroom genitals	*	*							*1	*
R-Genitals groomed	*	*							*1	*
R-Autogroom genitals	*								*	*1
R-Mutual genit. groom	*	*							*1	*
M-Nurse									*	
Work	6	6	6	6	6	6	6	6	1	6
Subordinate	1	2	2	2	2	2	3	3	0	1
Dominant	2	2	1	1	1	1	1	1	2	2
Reproductive	4	3	0	0	0	0	0	0	4	4
Maternal	0	0	0	0	0	0	0	0	1	0
Unique behaviours	0	0	0	0	0	0	0	0	2	0

She was also the only adult to bear young, lactate and solicit caecotrophs from other adults. The only exception to this latter privilege, was a chronically thin individual (#15, colony 7400), which was observed to successfully allocoprophage from siblings. This individual was the only colony member not observed to autocoprophage, suggesting that it was incapable of doing so. It is further apparent, from Table 3, that the most frequent workers were never the oldest individuals, thus members of litters four (digging and sweeping behaviours) and eight (transporting food and nest material) performed work-related activities with the highest frequency.

The breeding female was the principal participant in most reproductive activities, while the breeding male's behavioural repertoire was effectively the same (excluding copulation) as members of the first litter.

Ontogenetic shifts

There was considerable variation in cluster compositions with time. Two individuals that were behaviourally the most similar in one year were seldom the closest in other years (barring the reproductives). Furthermore, individuals identified as either soldiers or dispersers in the later study periods were seldom grouped together in earlier years. It was, however, apparent that both replacement reproductives and principal colony defenders came from the ranks of the first three litters recruited by the original breeding pair.

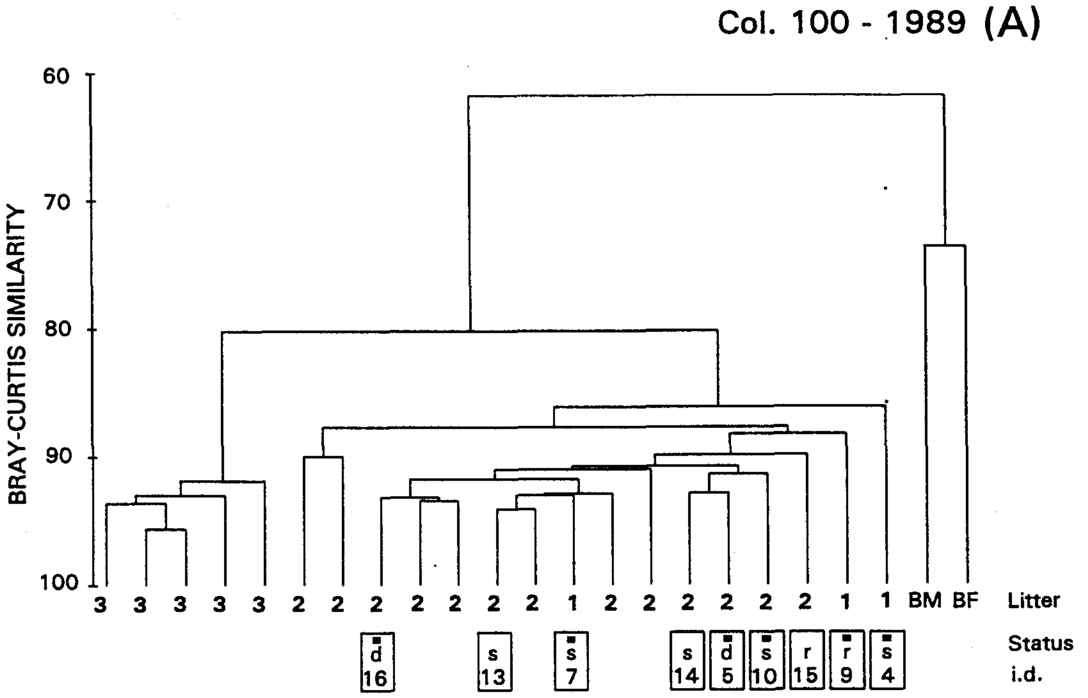
Given the independent histories of the two colonies and the influence of specific events on the ontogeny of their members, the results for each colony will be presented separately.

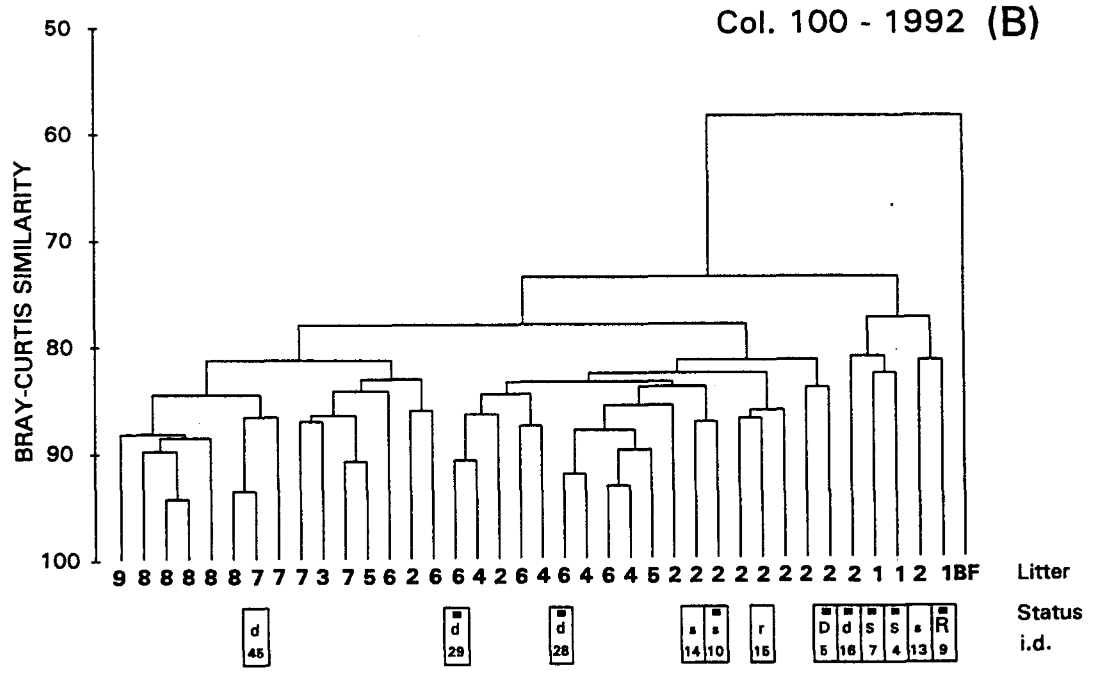
Colony 100

Two events that influenced the ontogeny of non-breeders within the colony were the death of the original breeding male, and the temporary loss in physical vigour of the breeding female (in 1989). Both events coincided with an infection that caused the death of the breeding male and four pups and produced severe swelling of the breeding female's feet that persisted for about 70 days. This left her greatly weakened and unable to either patrol the burrow system or physically dominate (by shoving) her offspring.

The replacement male (#9 from litter 1) was one of three oldest non-breeding males in the colony. There were no clearly discernable behavioural (Fig. 1A) or size (see Chapter 2) differences between these three males at the time of the breeding male's death, although individual #7 appeared to be behaviourally less similar than the others to the reproductives (Fig. 1A). Following the death of the breeding male there was a rapid divergence in both the behaviour and body size (see Chapter 2) of these male siblings with #4 and #7 specialising in colony defence activities (the latter

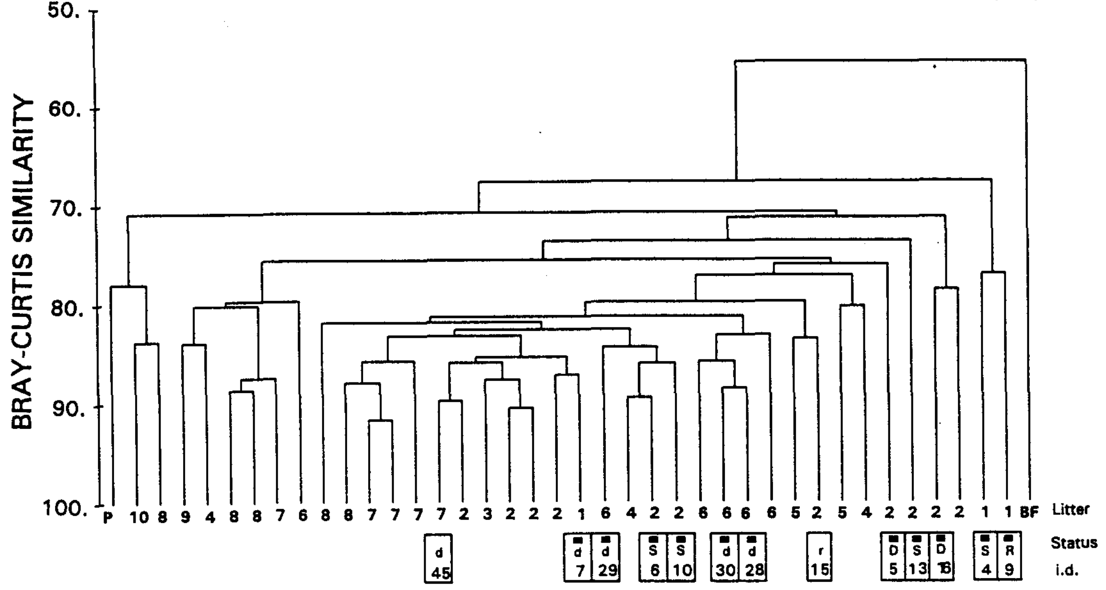
Figures 1A-1D. The results of the hierarchical cluster analyses for the behavioural repertoires (Appendix I) of all colony members in colony 100 in 1989, 1992, 1993 and 1994, respectively, are presented in dendrograms (Clarke and Warwick 1995) The x-axis represents the full set of individuals within the colony, and the y-axis defines the similarity level at which any two individuals are considered to be the same (see branches parallel to the x-axis). Numbers along the x-axis of each dendrogram represent a single individual, the value of which indicates the litter to which that individual belongs, BF denotes the original breeding female and BM the original breeding male. Length of the vertical lines of the dendrogram represents the distance between groups. Data enclosed within boxes at the bottom of each graph provide information on the status, sex and individual identity number of individuals that at some stage in the study exhibited a qualitative change in their behavioural repertoires. These changes are coded and include the following descriptive categories: R replacement reproductive, P potential reproductive, S soldier and D disperser. Letters with a horizontal line above are males. Lower case letters (r,p,s and d) indicate that the individual is not currently characterised by these behaviours but that they manifested themselves later in their ontogeny. This enabled an interpretation of possible behavioural antecedents leading to these changes. The dashed lines in Fig. 1D denote the 70 and 75% levels of similarity chosen for the statistical evaluation of size polyethism. The numbers denote those groups, intersected by the dashed line (where N≥3 individuals), which were compared statistically (see Tables 5 and 6).



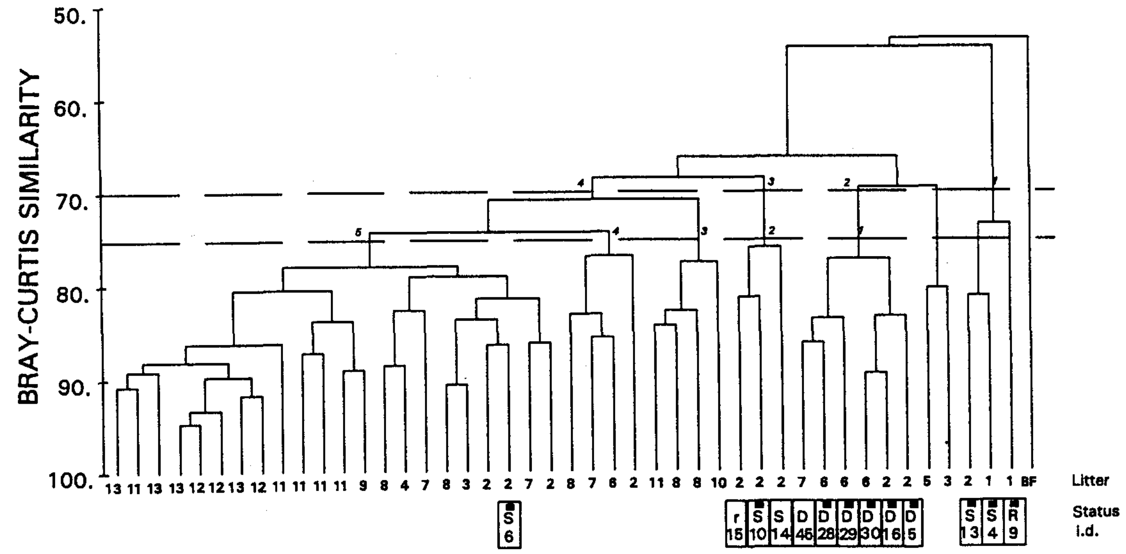


Figures 1A-1D. The results of the hierarchical cluster analyses for the behavioural repertoires (Appendix I) of all colony members in colony 100 in 1989, 1992, 1993 and 1994, respectively, are presented in dendrograms (Clarke and Warwick 1995) The x-axis represents the full set of individuals within the colony, and the y-axis defines the similarity level at which any two individuals are considered to be the same (see branches parallel to the x-axis). Numbers along the x-axis of each dendrogram represent a single individual, the value of which indicates the litter to which that individual belongs, BF denotes the original breeding female and BM the original breeding male. Length of the vertical lines of the dendrogram represents the distance between groups. Data enclosed within boxes at the bottom of each graph provide information on the status, sex and individual identity number of individuals that at some stage in the study exhibited a qualitative change in their behavioural repertoires. These changes are coded and include the following descriptive categories: R replacement reproductive, P potential reproductive, S soldier and D disperser. Letters with a horizontal line above are males. Lower case letters (r,p,s and d) indicate that the individual is not currently characterised by these behaviours but that they manifested themselves later in their ontogeny. This enabled an interpretation of possible behavioural antecedents leading to these changes. The dashed lines in Fig. 1D denote the 70 and 75% levels of similarity chosen for the statistical evaluation of size polyethism. The numbers denote those groups, intersected by the dashed line (where N$\geq$3 individuals), which were compared statistically (see Tables 5 and 6).

Col.100 - 1993 (C)



Col. 100 - 1994 (D)



exhibiting a marked growth spurt), while #9 reproduced with his mother, never engaged in colony defence and ceased growing.

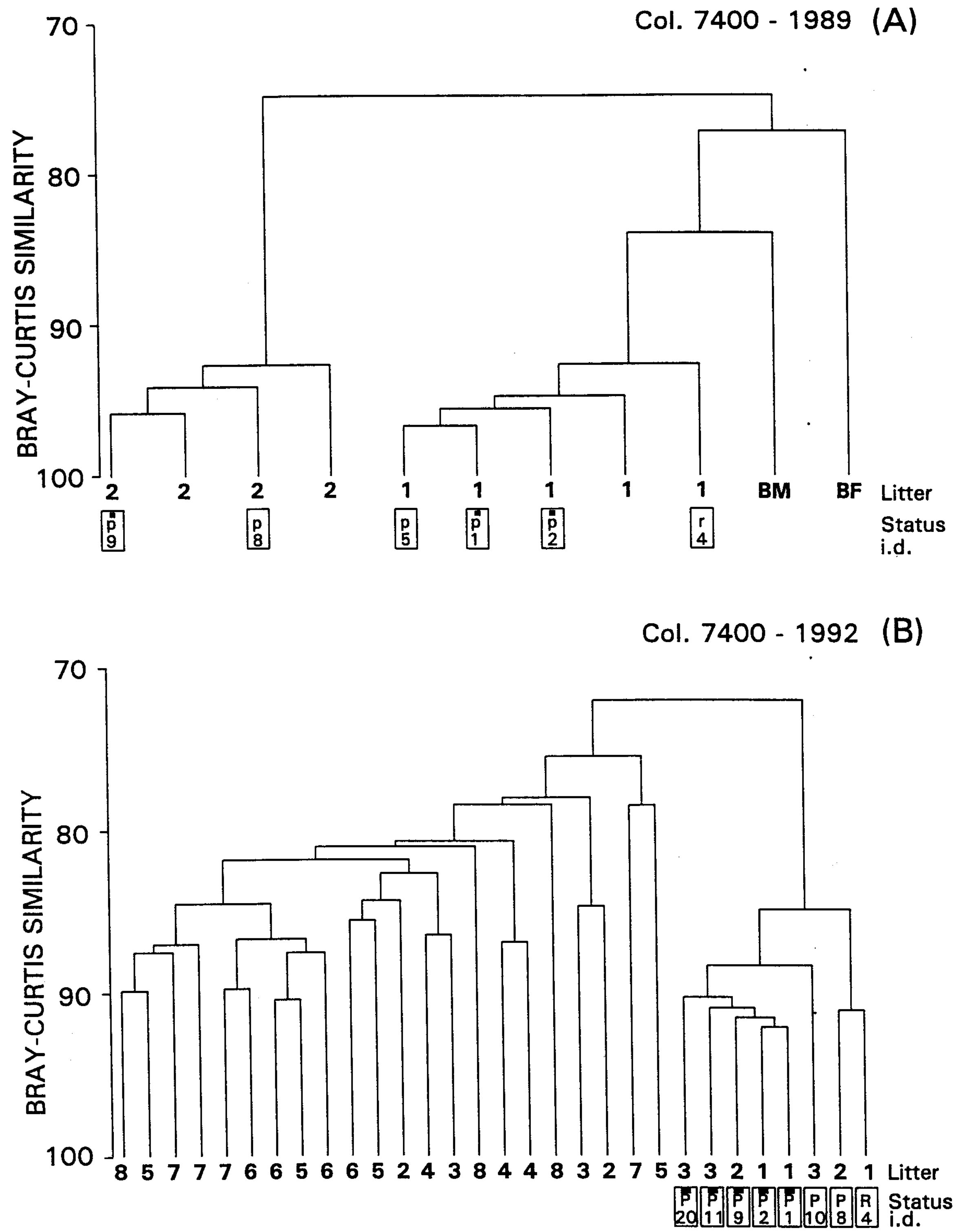
Following the physical incapacitation of the breeding female, individual #15, one of the four oldest non-breeding females, became sexually active and produced a single litter of four pups (none of which survived). However, following the return to health of the breeding female, #15 did not breed again. Interestingly, #15 was behaviourally more similar (although only marginally so) to the reproductives than any other female in the colony prior to this event (Fig. 1A).

During the 1994 study period, six dispersers (shown in Figure 1D) were identified in the colony (see Chapter 6). These individuals are all clustered together in the dendrogram, although they came from three different litters (2, 6 & 7). Examination of dendrograms from earlier years reveal that these individuals were all dissimilar to one another and, barring the clustering of #28 and #30 in 1993 (Fig. 1C), never clustered closely together. Note that although individuals #7 and #29 are adjacent to one another on this dendrogram, they are not clustered together.

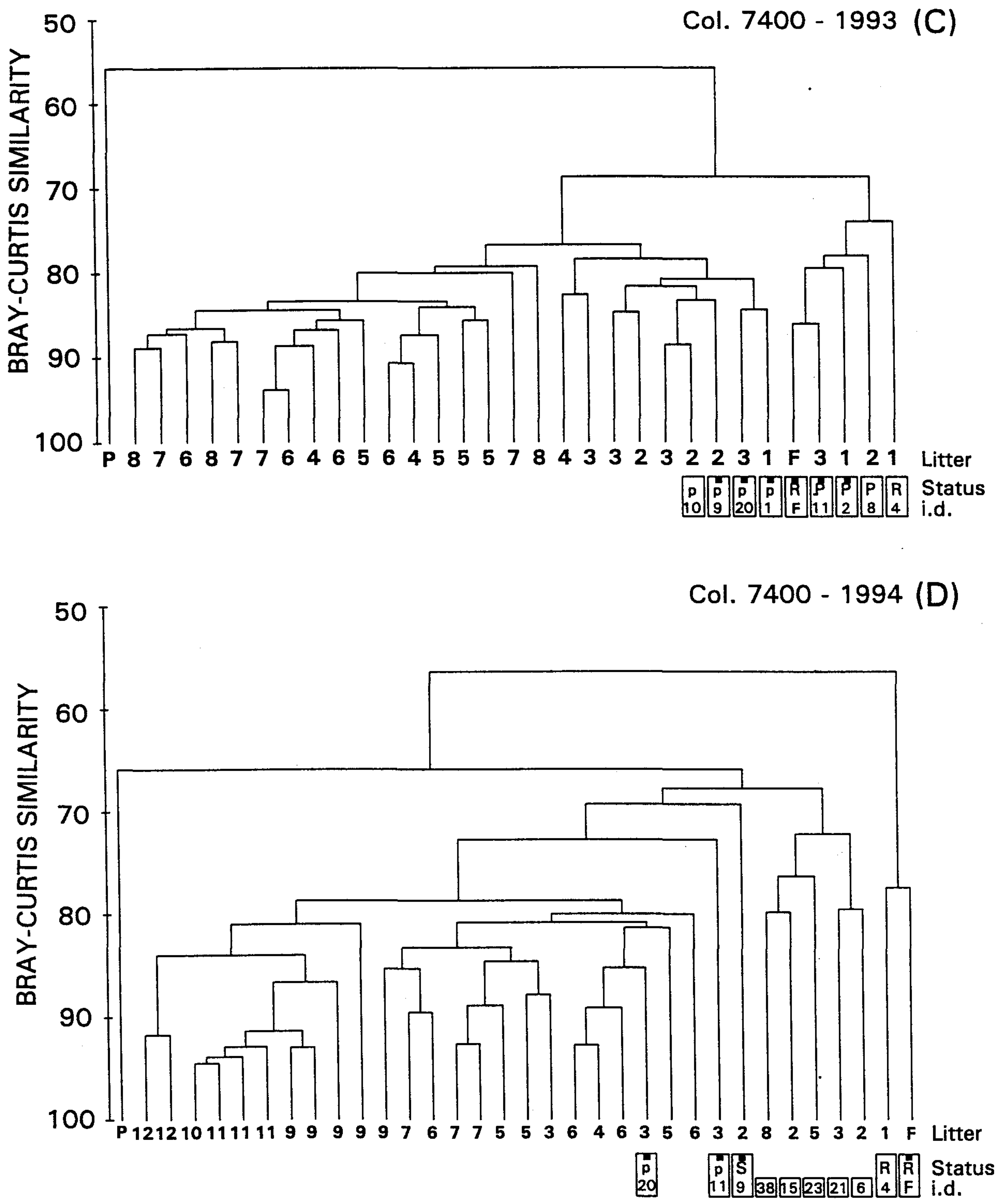
Principal colony defenders were all from the first two litters, but were never grouped together in the dendrograms. This suggests that, unlike reproductives and dispersers, colony defenders may have markedly different behavioural profiles, and hence colony defence does not constitute a distinct behavioural role.

Colony 7400

Both the breeding male and female from this colony died, allowing male and female non-breeders to compete for reproductive status. The breeding female bred only once following the death of the breeding male (January 1992) and prior to her own death (August 1992). Unfortunately, copulation was never observed between her and any of her sons so it was not possible to determine the identity of the temporary breeding male. Following her death, the position of breeding female was viciously contested by the two oldest non-breeding females within the colony (#4 and #5, litter 1). Fighting between these two continued for five months until #4 eventually killed #5. Once again, prior to the death of the breeding female, the replacement breeder (#4) was behaviourally more similar to the reproductives than #5 (Fig. 2A). Having achieved reproductive maturity, #4 refused to copulate with her brothers despite their repeated attempts and her otherwise frequent participation in other pre-copulatory behaviours (*e.g.* naso-anal grooming). This behavioural pattern persisted for over eight months and explains the large group of individuals within the 'reproductive cluster' in 1992 (Fig. 2B). These individuals comprised #4 and her two next oldest sisters, #8 and #10. Individuals #1, 2, 9, 11 and 20 were all males consorting in varying degrees with these three females with #4 and #8 receiving the greatest amount of sexual attention.



Figures 2A-2D. Dendrograms (group-average linking) from Bray-Curtis similarities of the behavioural repertoires of all colony members in colony 7400 in 1989, 1992, 1993 and 1994, respectively. See Fig. 1 legend for details. Individuals #15 and #38 were chronically ill during the whole study while individuals #23, #21 and #6 were all killed by the replacement breeding female during the study. These individuals cluster close to the reproductives on the basis of a limited participation in all work behaviours.



Figures 2A-2D. Dendrograms (group-average linking) from Bray-Curtis similarities of the behavioural repertoires of all colony members in colony 7400 in 1989, 1992, 1993 and 1994, respectively. See Fig. 1 legend for details. Individuals #15 and #38 were chronically ill during the whole study while individuals #23, #21 and #6 were all killed by the replacement breeding female during the study. These individuals cluster close to the reproductives on the basis of a limited participation in all work behaviours.

Following the introduction of a foreign male (F) in 1993 (Fig. 2C), reproduction ensued almost immediately, with #4 giving birth within 100 days (gestation is approximately 80 days). Only males #2 and #11 and female #8 remained in the reproductive group following this onset of reproduction. By 1994 (Fig. 2D), the typical pattern of clear separation of the reproductive pair and the non-breeders, had been re-established. This was achieved by the breeding female (#4), killing all of these individuals except for #11. These killings all occurred during 1994, and individuals #6, #21 and #23 had all been killed by the end of the study. Importantly, these latter individuals all clustered together on the dendrogram (Fig. 2D), and were behaviourally more similar to the reproductives than to all the other adult colony members.

Temporal shifts in work behaviours

All non-breeders from colony 7400, engaged in all work activities (Fig. 3, Table 3). This suggests that only a limited amount of the variation responsible for the pattern of clustering in the dendrograms (Figs 1 & 2) can be explained by differences in work activities of different aged individuals. To test this suggestion hierarchical cluster analyses was conducted on all work behaviours performed by members of the respective age-cohorts (Fig. 4). If task performance was dependant on the absolute age of individuals then one would predict that individuals from different litters would cluster together. Furthermore, if colony members were performing a distinct subset of the work behaviours (*i.e.* a specific role) irrespective of their age, then one would predict that distinct groups, with a low percentage similarity to other groups, would emerge in the dendrograms.

In both colonies it was clear that while the breeding female was different from the rest of the colony, there was very little variation amongst the non-breeders; most individuals were grouped at the 80% level, and with no consistent clustering of same-aged individuals (Fig. 4A, colony 7400 and Fig. 4B, colony 100). One way ANOSIM confirmed this result, with no significant differences evident between individuals from different age cohorts ($F_{7,34}$=-0.037, P=0.645; $F_{6,24}$=0.029, P=0.15, for colony 100 and 7400, respectively). Interestingly, the breeding male in both colonies was grouped with the non-breeders, emphasising the relatively greater divergence of the breeding female with respect to reproductive role and associated lack of participation in work-related activities.

To investigate evidence of temporal changes in the performance of work activities (*i.e.* temporal polyethism) of non-breeders the frequency of performance of the four main work behaviours was plotted over different years (Figs 5A-D) for members of different age-cohorts.

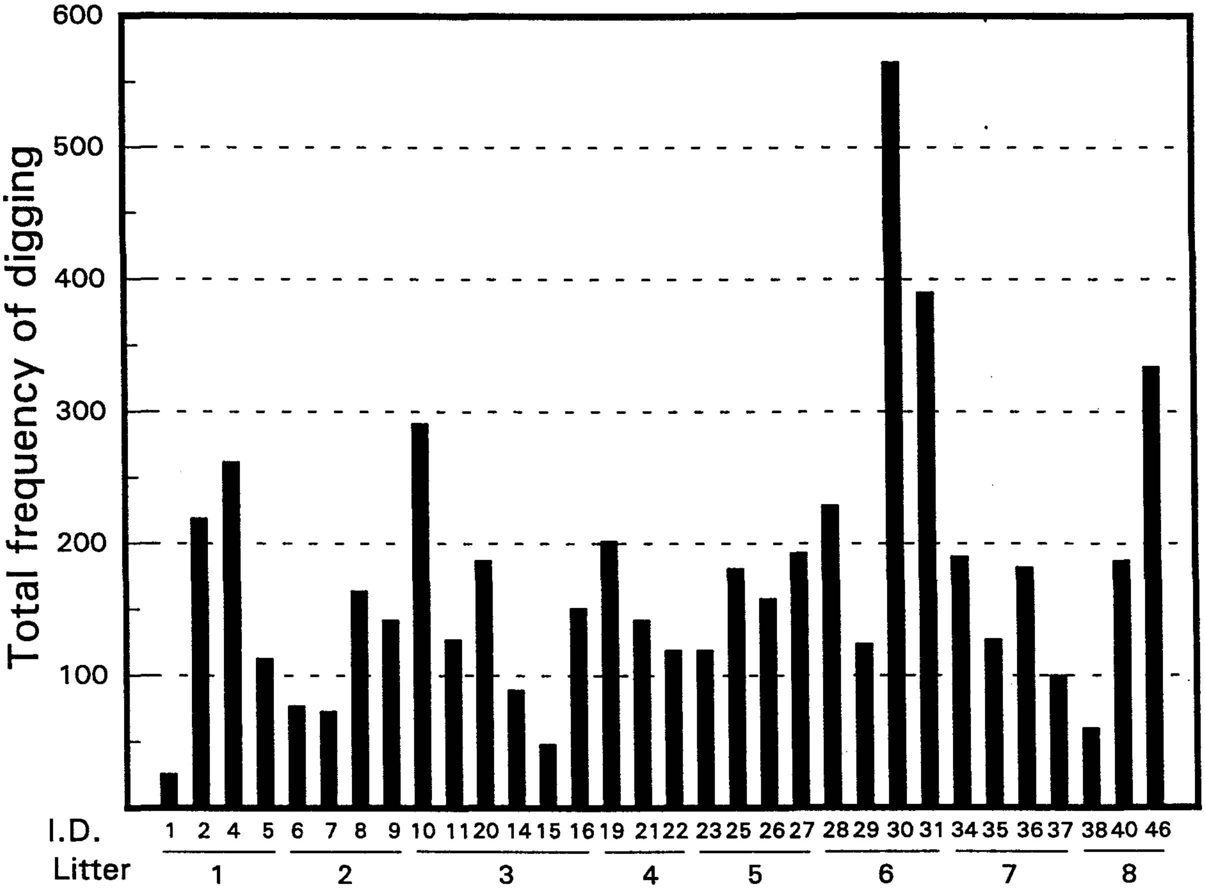
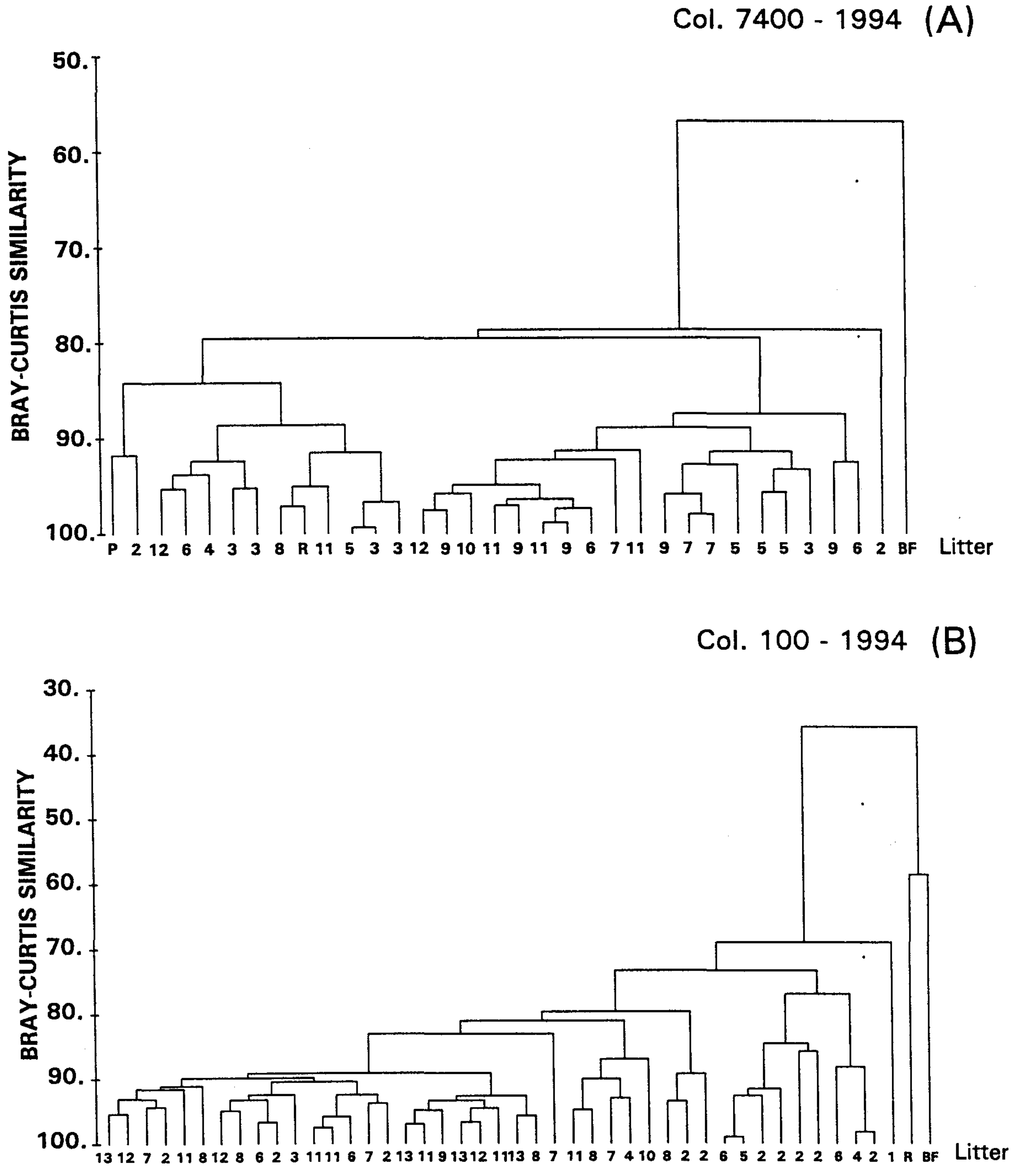
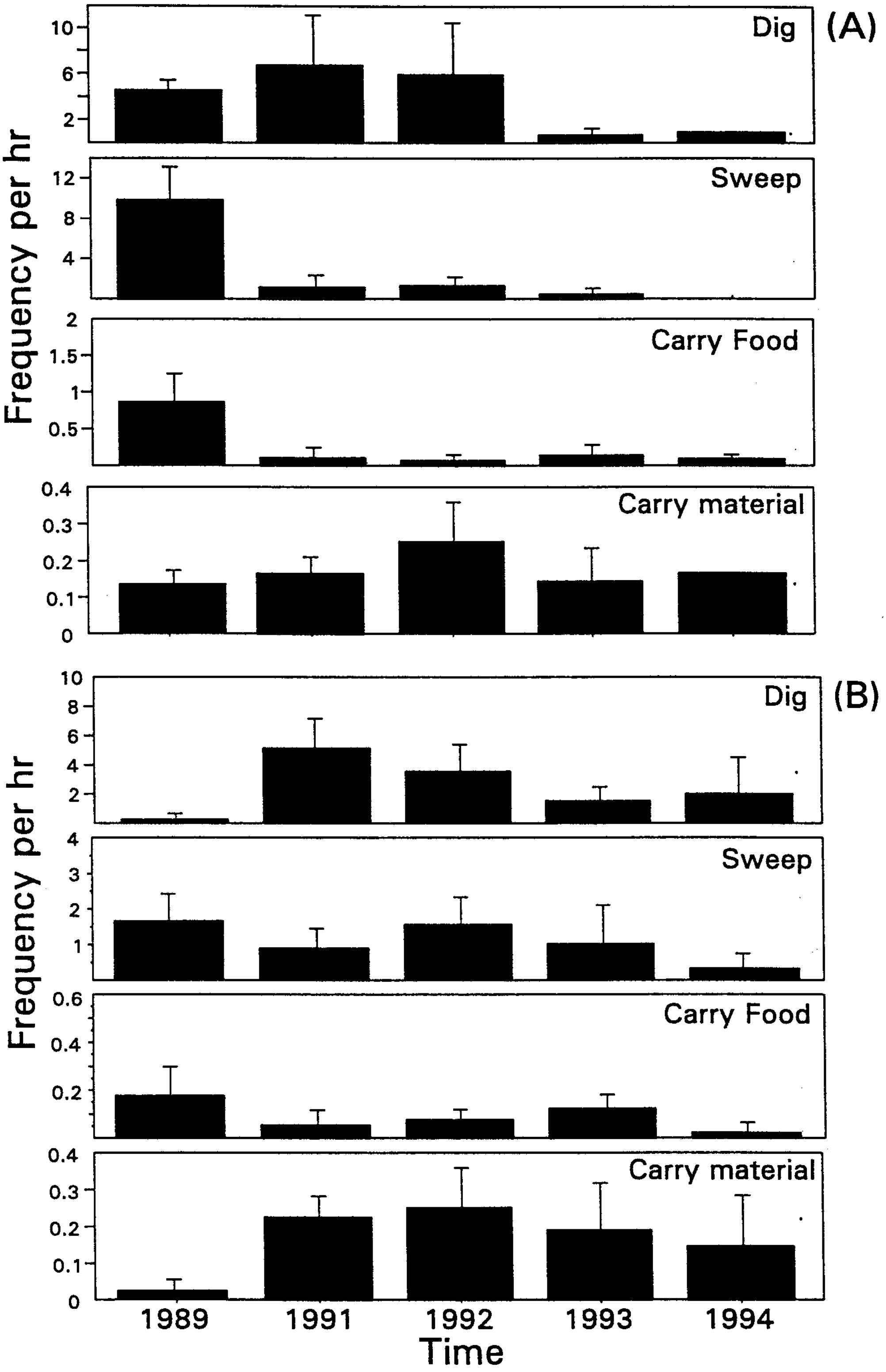


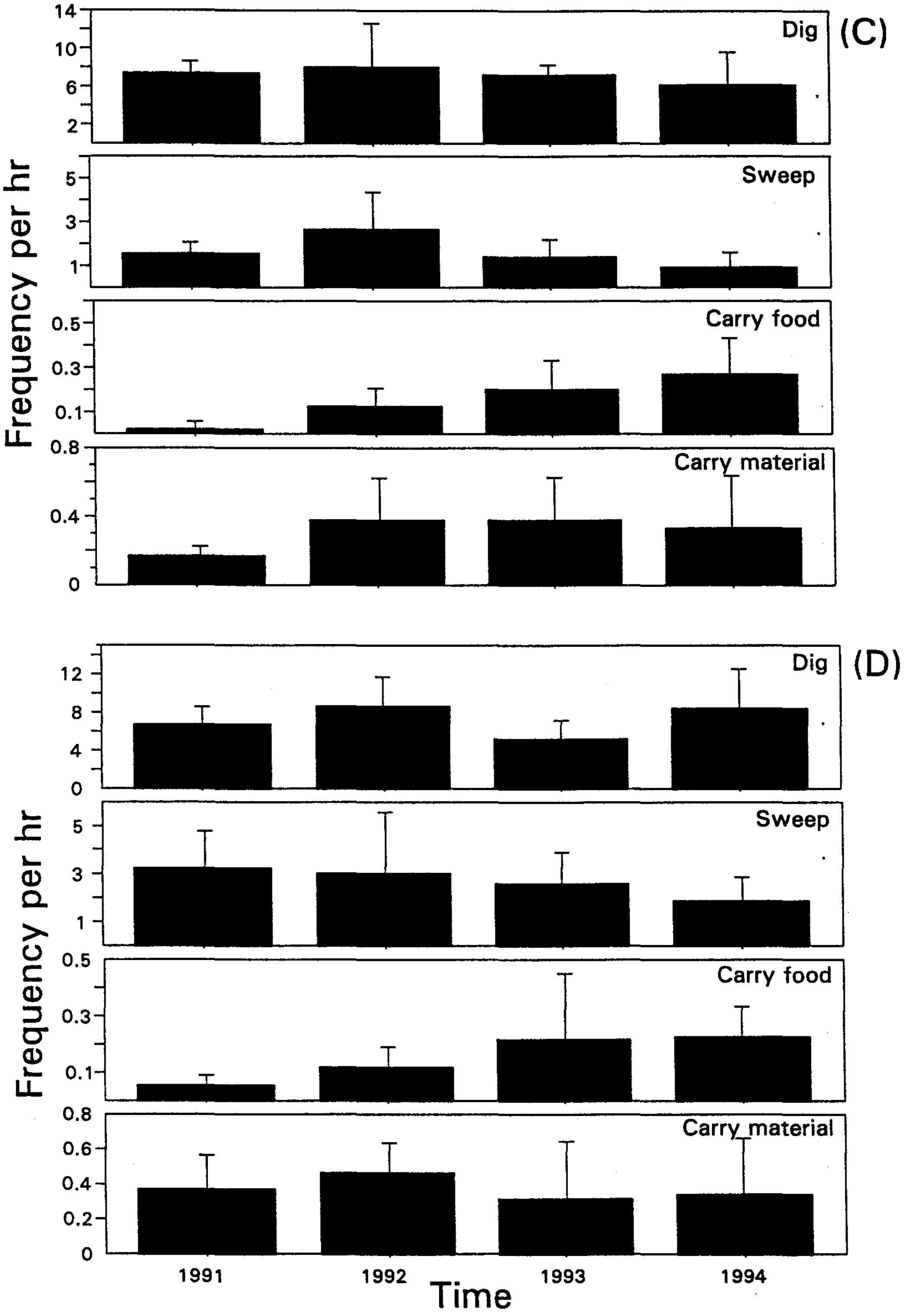
Figure 3. The total frequency of digging behaviour for all the nonbreeding mole-rats within colony 7400 in 1993. Individual identity numbers, and their corresponding litter i.d., are plotted on the x-axis.



Figures 4A & 4B. Dendrograms of the work repertoires only (digging, sweeping, carrying food and carrying nest material), of all the individuals in colony 7400 and 100, respectively. Data were from the last sampling period (1994) for each colony. Numbers on the bottom of each graph represent a single individual, the value of which indicates the litter to which that individual belongs. BF denotes the original breeding female and R the replacement breeding male.



Figures 5A & 5B. The frequency of performance of each of the four main work behaviours, by members of the first two litters recruited to colony 7400, at five different stages in the colonies history (1989-1994, excluding 1990). Similar results were obtained for all other litters, but in the interests of brevity and clarity only the oldest (Figs. 5A & 5B) and youngest litters (Figs. 5C & 5D) are presented.



Figures 5C & 5D. The frequency of performance of each of the four main work behaviours, by members of the last two litters recruited to colony 7400, at five different stages in the colonies history (1991-1994). See also Fig. 5A & 5B legend.

For the sake of clarity only data for the two oldest (Figs 5A & 5B) and the two youngest litters are presented (Figs 5C & 5D). Overall there was no evidence to suggest that members of different age-cohorts perform particular work behaviours, or that they showed predictable changes in the frequency with which they perform each work behaviour with time. Two exceptions to this generalisation were however apparent.

Members of the first litter showed a clear decrease in the performance of work activities with age (excluding carrying nest material). This occurred however, because the only individual from this litter that survived to 1994 was #4, the replacement breeding female. The only other trend amongst the respective litters was the consistent increase with age in the frequency of food carrying by members of the 5th and 7th litters. A similar pattern was not apparent for members of the 2nd litter.

Spatial division of labour

Observations of animals actively digging showed that within a single working bout (uninterrupted period of work) individuals focussed their attention at one (83.6% of observations, n=780 bouts), or occasionally two work sites (17.2% of observations, n=780 bouts) as long as the site remained unchallenged by other workers. Furthermore, the spatial division of labour across all sampling periods was clearly non-random with a highly significant difference (Chi-squared test, $P<0.00001$) between observed and expected frequencies of digging at given sites within the burrow system for all individuals tested (Table 4). Most individuals (48%) concentrated their efforts in the food chamber (A), and the junction leading to the food chamber (B: 24.3%), although six of the ten sites, where mole-rats were observed digging at least once, were the primary focus of digging behaviour for at least one individual. It was further evident that certain individuals limited their digging activity almost entirely to one or two areas (*e.g.* individual #6, spent 95.89% and individual #100, 88.62% of their time actively digging at A). Other individuals were more generalised in the overall spatial distribution of their digging activities (*e.g.* individual #20, >5% at six different sites). However, within any one given work bout these individuals focussed their activity on a maximum of only three sites.

Dominance and sexual interactions

The initiators and recipients of dominant aggressive behaviours (*i.e.* shoving) amongst non-breeders were the oldest individuals (Fig. 6A). In contrast, the primary recipients of the foremost subordinate behaviour (pup-pushing) were the youngest colony members (Fig. 6A). However, the relatively high proportion of circular (*i.e.* A>B>C>A) as opposed to linear (A>B>C, and A>C) dominance

Table 4. The percentage time that each individual, within colony 7400, engaged in digging behaviour at a particular site within the burrow system. The total number of times each site was the preferred working site for all mole-rats within the colony is provided in the bottom row of the table. Chi-squared statistics, and the associated probabilities, are provided for ten different-aged individuals within the colony. This statistical test was performed to test whether the spatial distribution of an individuals' digging activities, deviated significantly from a purely random distribution.

	Work site											
Individual	A	B	C	D	E	G	N	0	Q	T	Chi-squ.	P-value
4	5.88	35.29	0.00	0.00	0.00	17.65	0.00	0.00	0.00	41.18	228.3	P<0.0001
7	64.29	17.86	0.00	1.79	1.79	14.29	0.00	0.00	0.00	0.00		
9	0.00	33.33	0.00	0.00	0.00	33.33	0.00	0.00	0.00	33.33		
3	9.26	0.00	0.00	0.00	53.70	29.63	1.85	0.00	5.56	0.00	366.2	P<0.0001
5	56.07	25.71	0.00	0.00	7.86	0.00	0.00	0.00	0.36	10.00		
6	95.89	4.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
10	17.99	55.40	0.72	1.44	0.72	23.02	0.00	0.00	0.00	0.72		
12	78.88	13.36	0.00	0.00	2.59	1.72	0.00	0.00	3.02	0.43	541.9	P<0.0001
13	43.75	9.38	3.13	0.00	3.13	28.13	0.00	0.00	6.25	6.25		
14	12.09	32.97	0.00	8.79	32.97	8.79	0.00	1.10	0.00	3.30		
15	29.59	21.89	0.00	1.18	34.91	0.59	0.00	0.59	2.96	8.28		
16	36.00	16.00	0.00	0.00	4.00	28.00	0.00	0.00	4.00	12.00		
17	27.12	22.03	1.69	9.60	0.00	20.34	0.00	1.13	1.13	16.95		
18	86.67	0.61	0.00	0.00	0.61	10.30	0.00	0.00	0.61	1.21	662.1	P<0.0001
23	79.26	17.78	0.74	0.00	1.48	0.00	0.00	0.00	0.00	0.74		
100	88.62	10.57	0.00	0.00	0.00	0.81	0.00	0.00	0.00	0.00		
21	60.26	11.86	0.00	0.32	8.97	4.49	0.00	0.00	13.46	0.64	305.4	P<0.0001
20	17.53	11.51	0.00	1.92	5.21	36.44	2.19	0.00	18.36	6.85		
22	53.04	20.87	0.87	0.00	20.87	0.00	0.00	0.00	0.00	4.35		
2	35.42	17.36	0.69	0.69	1.39	0.00	0.00	0.00	0.00	44.44		
25	40.23	21.84	1.15	0.00	0.00	8.05	0.00	0.00	10.34	18.39		
26	17.20	13.98	2.15	1.08	16.13	4.30	0.00	0.00	36.56	8.60	118.6	P<0.0001
27	62.82	23.72	0.00	2.56	1.92	7.05	0.00	0.00	0.00	1.92		
28	69.76	3.46	0.18	0.36	7.10	1.82	0.00	0.00	15.30	2.00		
29	45.56	44.79	0.39	0.39	1.93	0.77	0.39	0.00	0.00	5.79		
30	9.02	72.18	0.00	2.26	10.53	2.26	0.00	0.00	0.75	3.01	442.2	P<0.0001
31	51.28	23.08	2.56	2.56	0.00	7.69	0.00	0.00	2.56	10.26		
42	26.59	38.15	0.00	4.05	5.78	1.73	0.00	0.00	3.47	20.23		
43	51.98	33.05	0.00	1.41	9.60	0.28	0.00	0.00	2.54	1.13	289.6	P<0.0001
44	16.67	21.49	0.00	6.14	50.88	1.32	0.88	0.00	2.19	0.44		
45	2.54	30.08	0.42	15.68	2.12	5.51	0.00	0.00	0.42	43.22		
47	77.19	8.77	0.00	0.00	4.39	4.39	0.00	0.00	1.75	3.51		
48	21.59	22.73	1.14	14.77	7.95	0.00	0.00	0.00	12.50	19.32		
49	14.02	40.19	0.93	5.61	23.36	0.93	0.00	0.00	6.54	8.41	150.6	P<0.0001
50	18.72	17.45	0.00	6.81	7.23	0.00	0.00	0.00	35.74	14.04		
51	34.11	40.31	0.00	0.78	2.33	4.65	2.33	0.00	11.63	3.88		
52	0.00	42.50	0.00	0.00	25.00	7.50	0.00	0.00	15.00	10.00		
53	17.28	33.95	0.00	3.70	17.28	1.23	0.00	0.00	17.28	9.26	114.9	P<0.0001
Most popular	18	9	0	0	4	1	0	0	2	3		

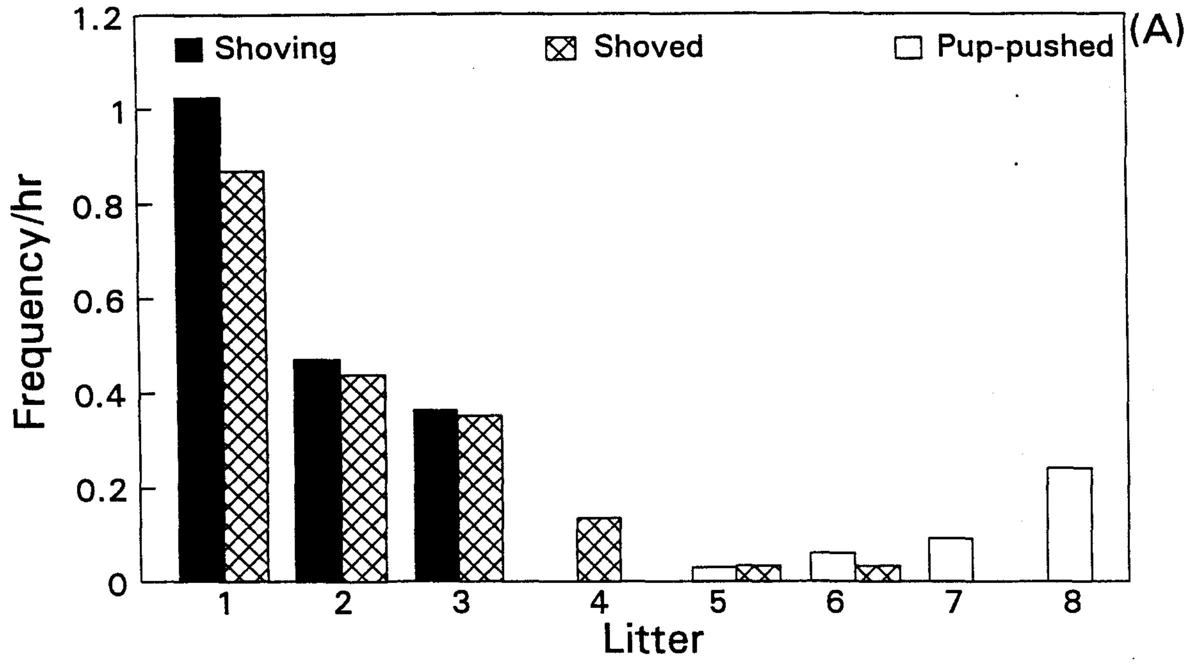


Figure 6A. The total frequency of performance of the key dominant (shoving) and subordinate (shoved and pup-pushed) behaviours, averaged for members of each of the eight litters recruited to colony 7400 by 1992. Both the original breeding male and female had died, prior to this point in time.

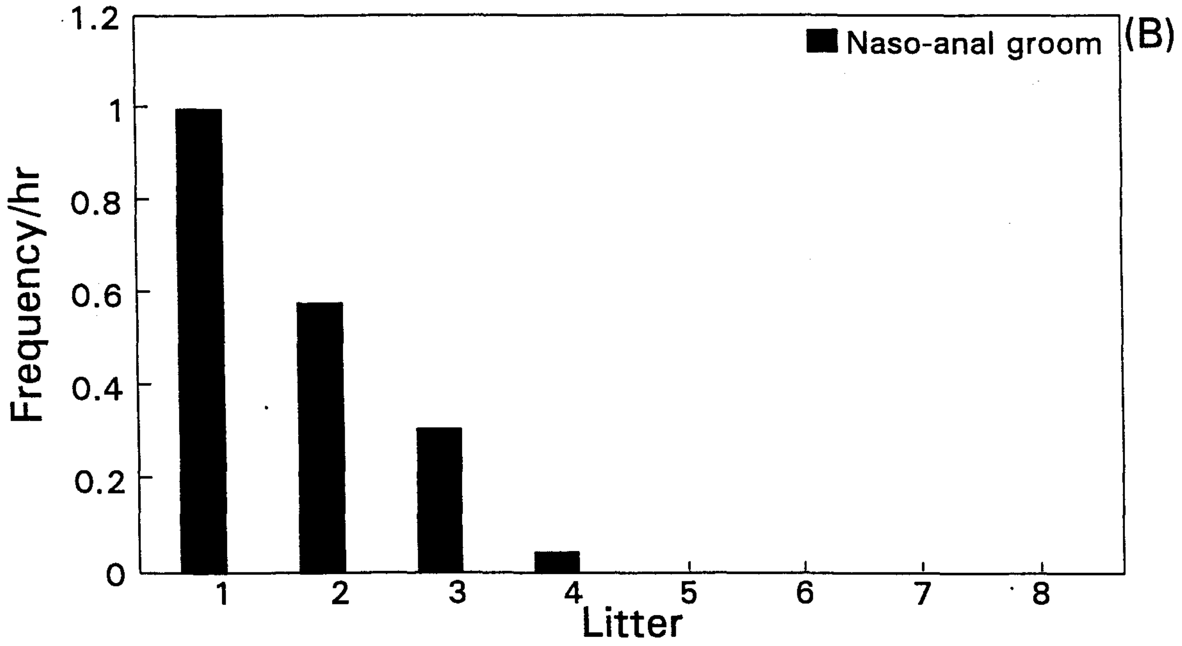


Figure 6B. The total frequency of performance of the key sexual behaviour (naso-anal interactions), not directly related to reproduction. Data were averaged for members of each of the eight litters recruited to colony 7400 by 1992. Both the original breeding male and female had died, prior to this point in time.

triads effectively obfuscates the empirical evaluation of a dominance hierarchy in naked mole-rat colonies. Thus, within colony 7400 it was not possible to objectively rank the non-breeders, due to the high number (more than half of the non-breeders) of tied rank scores. This was confirmed using Appleby's (1983) method for determining the transitive dominance amongst group members. Here the linearity indices were low and non-significant, both in the absence of, and following the emergence of, a breeding female (Linearity index K=0.126, $X^2=49.2$, $P>0.05$ in 1992; K=0.361, $X^2=159.88$, $P>0.05$, in 1995, respectively). Importantly, the highest ranked individual in both cases was the eventual breeding female (#4), whereas the lowest ranked individuals were always the youngest colony members.

Table 4. The percentage time that each individual, within colony 7400, engaged in digging behaviour at a particular site within the burrow system. The total number of times each site was the preferred working site for all mole-rats within the colony is provided in the bottom row of the table. Chi-squared statistics, and the associated probabilities, are provided for ten different-aged individuals within the colony. This statistical test was performed to test whether the spatial distribution of an individuals' digging activities, deviated significantly from a purely random distribution.

The patterns associated with dominance and age were mirrored for the key sexual activities performed by non-breeding colony members (litters 1-4, Fig. 6B). Hence, naso-anal grooming, the most frequently performed sexual activity, varied inversely with age, and members of the last three litters were never observed engaging in either this or any other sexual acts.

Morphological variation and physical polyethism

Body size frequency distributions

It is evident from Figure 7, that although incipient colonies (6 months) have the smallest modal body mass (18.6g), the modal mass of colony members increases rapidly and at one year the mode was already 41g. The size-frequency distribution became more positively skewed with time, due to the growth of individuals that were clearly larger than the modal class. The proportion of these larger individuals remained constant from two years and the majority remained in the 30-50g body mass size classes. The negative skew at one year can be attributed to the presence of two newly recruited litters, that were clearly smaller than the mode.

Frequency distributions of body mass for individuals from large colonies were normally distributed (Fig. 8). In both colonies these distributions were strongly positively skewed. In colony 300 the recruitment of 50 individuals between 1991 and 1995 ensured that the smallest mass class remained 15-20g, while the largest increased from 60-65g to 65-70g, with a clear increase in the number of individuals

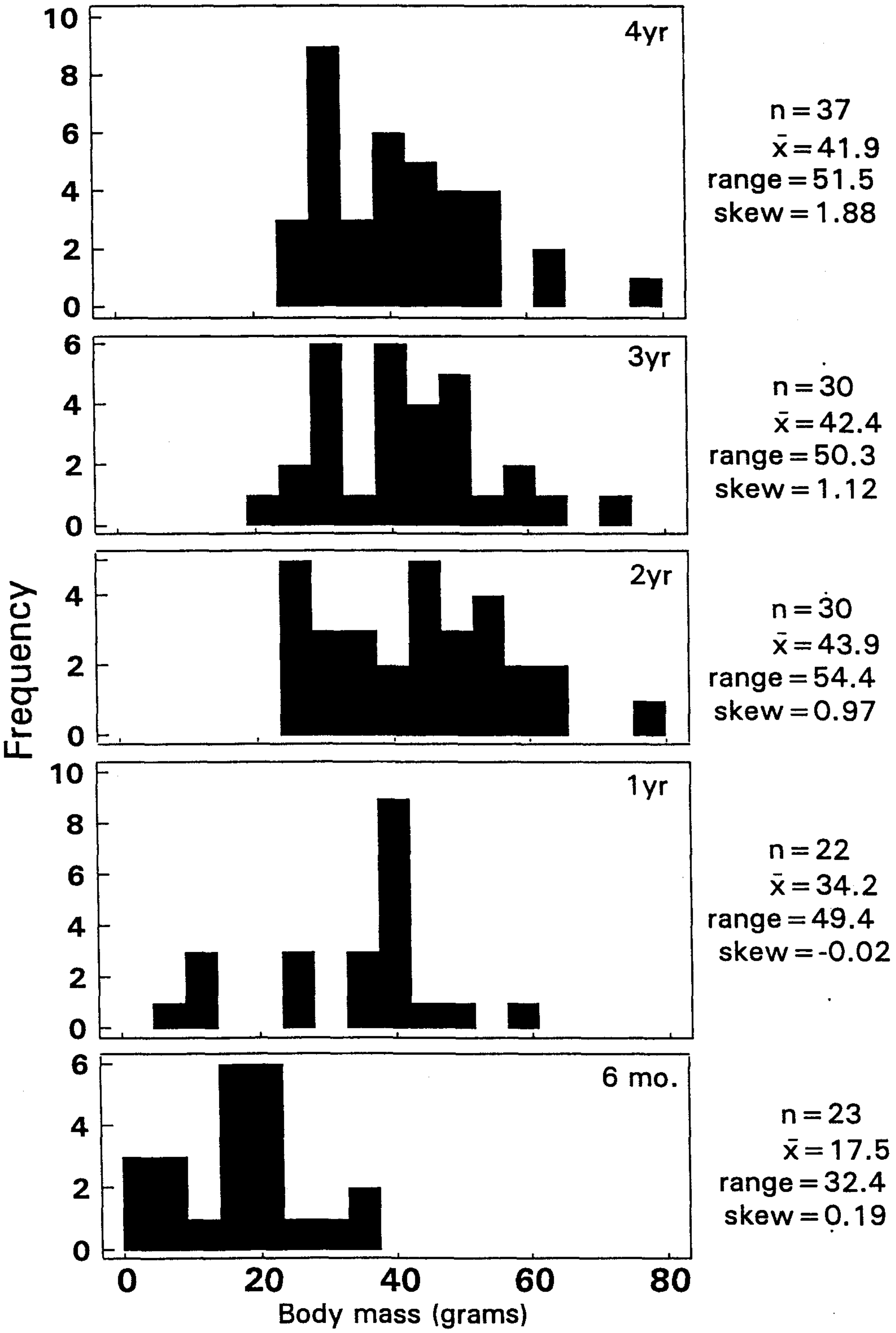


Figure 7. Size-frequency distributions of body mass, for all colony members in colony 100, over the first four years following colony inception. Most of the changes took place within the first year, thereafter the distribution was normal but consistently skewed towards the largest individuals.

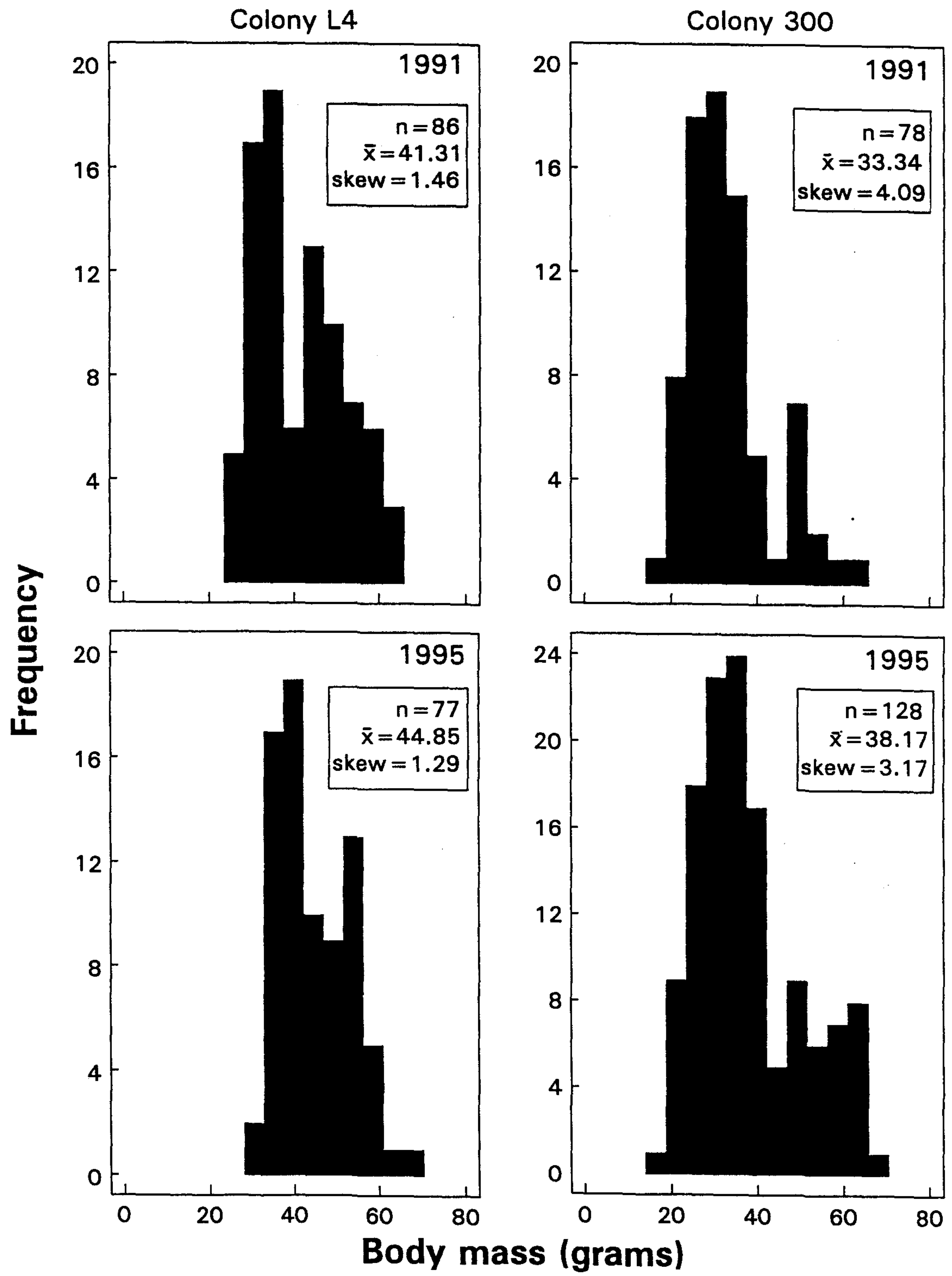


Figure 8. Body mass size-frequency distributions for two large colonies over time. Colony 300 increased numerically from 78 individuals in 1991 to 128 individuals in 1995. The mean±SE increased from 33.34±1.08 (range 18.6-62.2) to 38.17±1.09 (range 18-68). Both distributions were positively skewed. Colony L4 decreased in colony size from 86 to 77 individuals, as a result of fighting and subsequent deaths amongst the large colony members, and no recruitment to the colony. The mean±SE body mass increased from 41.31±1.14 (range 24.1-63.7) to 44.85±0.96 (range 31.6-44.85).

ranging in size from 40-60g. In contrast, the number of individuals in colony L4, decreased between the two sampling periods, following the death of nine large-sized individuals. Despite there being no recruitment over the four year period, the mass frequency range of 40 grams in 1991 (25g-65g) was maintained and the lower and upper limits increased by only 5g (30-70g).

Morphometrics

Allometric regressions of skeletal measurements against body mass (Table 5) show that relative growth of these parameters are related by monophasic allometry (*i.e.* nonisometric), with the allometric constant a̲ consistently less than unity (*sensu* Holldobler and Wilson 1990).

Size-frequency distributions of body mass and incisor width were strongly positively skewed (Fig. 9A & 9B). Visual inspection suggests the existence of two overlapping modal groups. Objective analysis using normal score plots refuted this however, with a single 'straight' line providing evidence for the existence of only a single normal population (however, see below). Non-breeding colony members are thus uniform in morphology, and there was nothing to indicate the presence of morphologically distinct subgroups (*i.e.* soldiers) within the colony. It is however important to note that the size-frequency distribution for incisor width provides evidence of a discontinuity, with a few individuals having notably broader incisors (Plate 1a). Intriguingly these individuals all exhibited heightened participation in colony defence activities. There was strong evidence for the existence of dimorphism between the breeding female and all other colony members, as noted in the size-frequency distribution of vertebral length (Fig. 9C; Plate 1b). The normal score plot suggested that

Table 5. Allometric constants relating body mass to the seven morphological variables measured from the x-rays of all individuals (n=29), except the breeding female, of colony 1000. The allometric equation is of the form $y=a+bx$.

Variable	a	b	r	$F_{1,27}$	P<
Zygomatic arch	12.99	0.131	0.945	229.3	0.000
Incisor width	1.66	0.039	0.920	149.1	0.000
Vertebrae length	2.17	0.040	0.935	188.6	0.000
Pelvis length	10.47	0.095	0.903	119.3	0.000
Body length	7.36	0.102	0.850	70.4	0.000
Skull length	19.74	0.166	0.900	115.9	0.000
Skull width	9.43	0.023	0.651	19.8	0.000

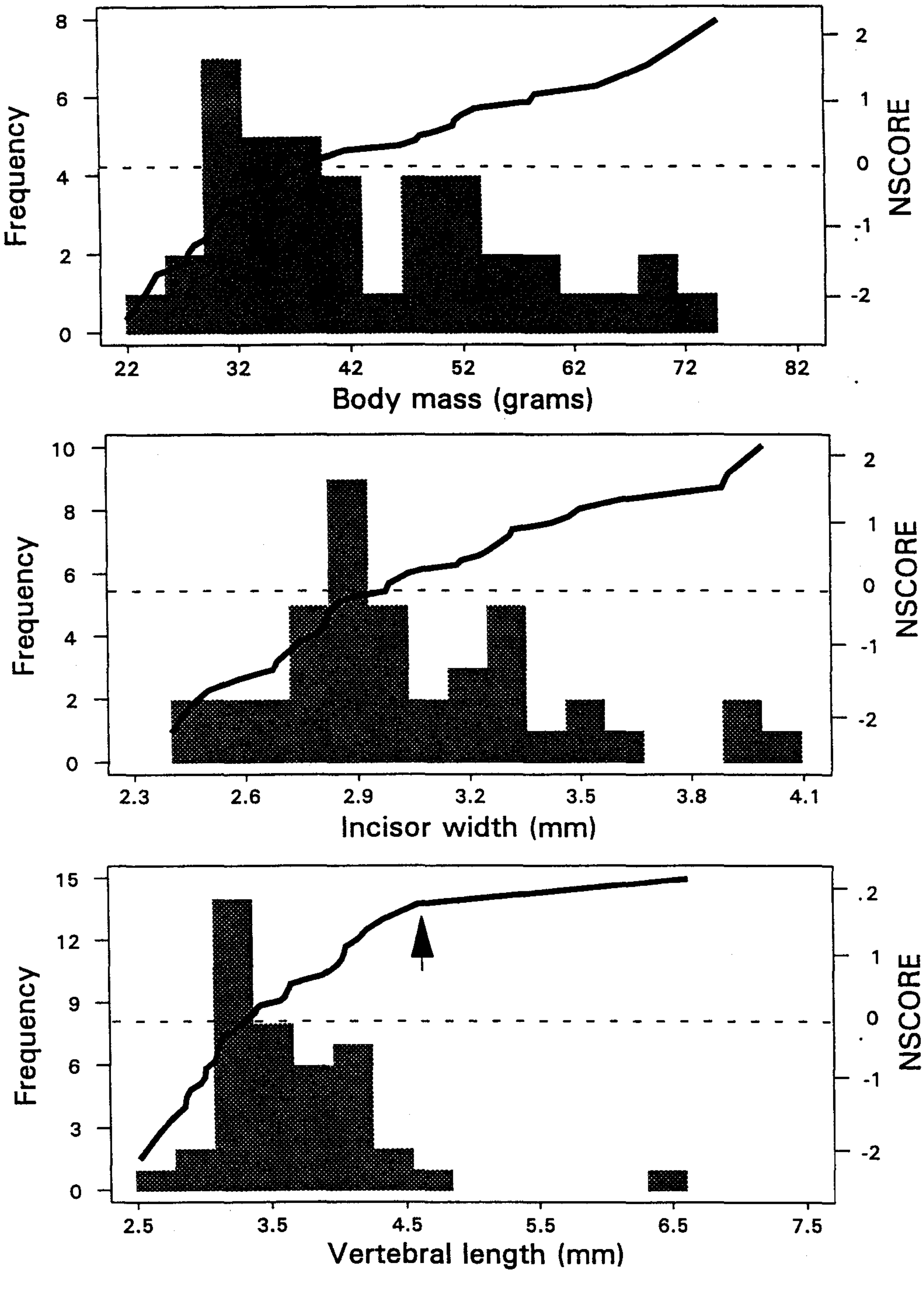


Figure 9. Histograms showing the size-frequency distributions of three morphological measurements, body mass, incisor width and vertebral length, for all the individuals in colony 100. Each variable is positively skewed towards a few large individuals. The normal scores for each distribution are also plotted for each variable (see line running through each of the histograms). Only vertebral length shows evidence of a kink (see arrow) in the normal score plot, indicating that the underlying distribution is the sum of two normal curves (*sensu* Tschinkel 1988). The smaller individuals are all non-breeders and the breeding male, while the largest individual is the breeding female. The normal score plots for body mass and incisor width, suggest a single normal population, despite the strong positive skewing of the distribution.

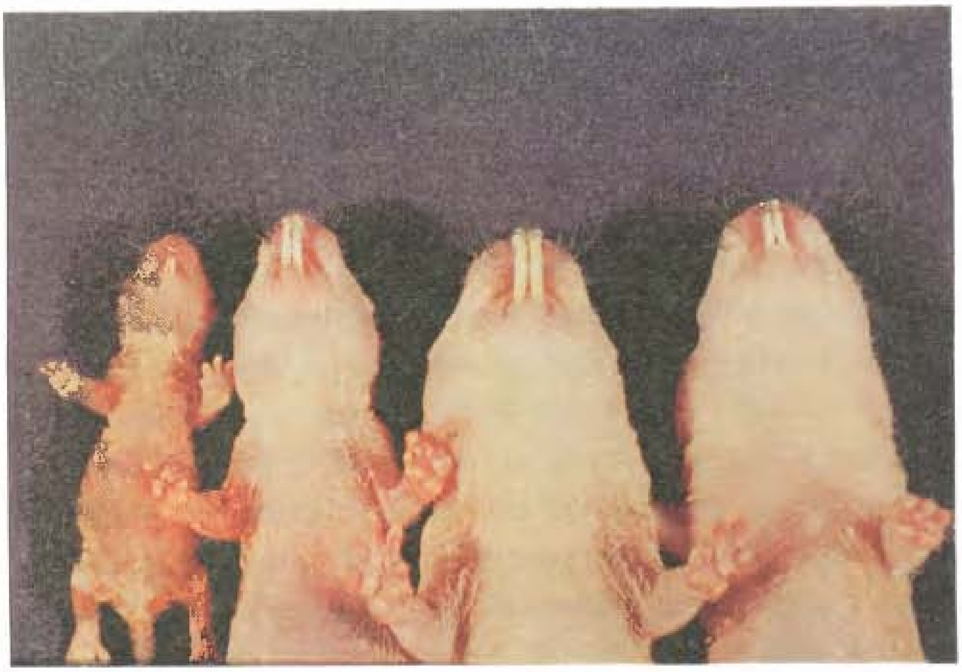


Plate 1a. A ventral view of the head and neck region of a (from left to right) pup, small non-breeding adult, large non-breeding adult and the breeding female from a single colony (#1000) of naked mole-rats. Note the robust incisors of the large non-breeder (3rd from left). This individual was the principal colony defender against foreign conspecifics.

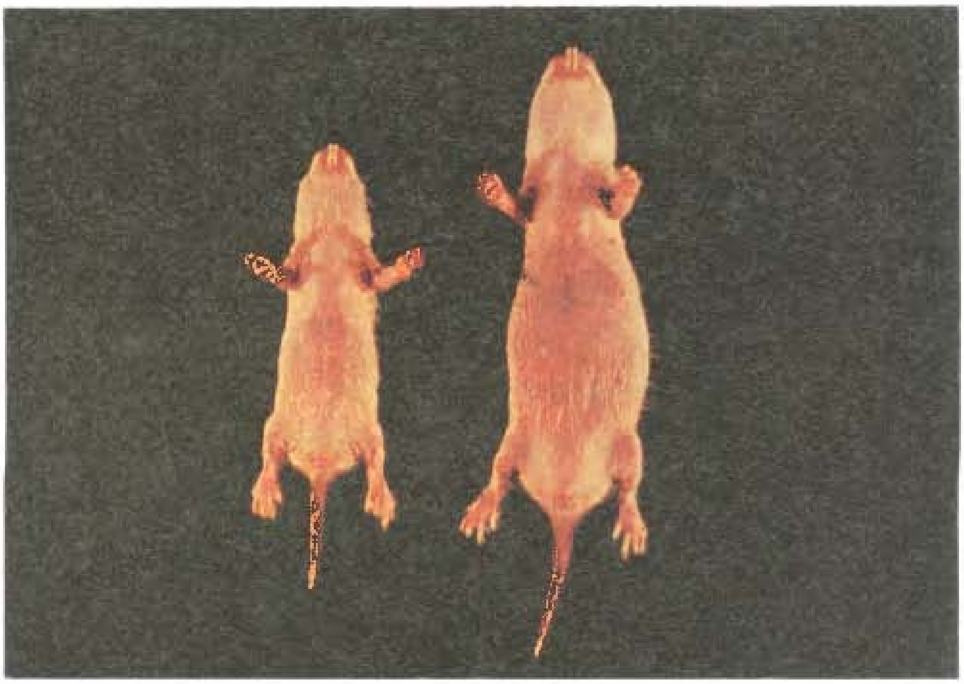


Plate 1b. A ventral view of a non-breeding (left) and breeding female (littermates) showing the tremendous elongation of the body associated with the reproductive role.

the skewed distribution was actually the sum of two separate distributions, resulting in the pronounced 'kink' (see arrow, Fig. 9C) in the normal score curve. These differences are a result of the elongation of the breeding female's vertebrae, posterior to the cervical vertebrae.

Allometric regression of vertebral length (a reliable indicator of body length) against zygomatic arch width (a reliable indicator of head width) (Fig. 10A) showed that these measurements were significantly correlated in both focal colonies ($r^2=0.849$; $F_{1,38}=214.1$, $p<0.0001$, for colony 100; $r^2=0.899$; $F_{1,29}=259.4$, $p<0.0001$, for colony 7400). By superimposing the data for the breeding females it is evident that they both form distinct outliers and therefore that the allometric expression for these variables is not applicable to them. By superimposing litter numbers on the data points for members of colony 100 (Fig. 10B), it can be seen that while there is a clear trend for the oldest and youngest colony members to group at opposite ends of the regression, there is considerable overlap in the data from litters lying between them. One way ANOVA's on the morphological differences between individuals in different litters (where $n\geq3$ individuals) revealed significant differences ($P<0.05$) for all variables measured (Table 6). Multiple comparison tests (Tukey test) revealed that most significant differences were between members of litter 2 and all other litters. This emphasises the morphological overlap between successive age-cohorts. It is important to note that the non-breeding female in colony 100 (#15) that bred once, (see above), was the only individual to fall outside the 95% confidence limits of the regression line (see boxed #2, Fig. 10B).

Physical polyethism

Comparisons of the morphological characters of individuals from colony 100 that were grouped behaviourally at the 70% and 75% similarity levels (Fig. 1D) are presented in Tables 7 and 8, respectively. There were six distinct groups at the 70% similarity level. Only four of these were sufficiently large ($n\geq3$ individuals) to allow for statistical comparisons however. All morphological measurements (except zygomatic arch width) considered showed significant differences between the clusters. Tukey tests revealed that these differences were attributed to the grouping of reproductives and soldiers (groups 1 and 3), dispersers (group 2) and all other mole-rats (group 4). The dispersers (group 2) had large subcutaneous fat deposits (see Chapter 6), while group 4 consisted primarily of younger colony members, with little or no subcutaneous fat. Significant differences in incisor width were apparent between groups 3 and 4. Two members of group 3 were colony defenders (#10 and #14), while the third individual (#15) was a large sized sibling.

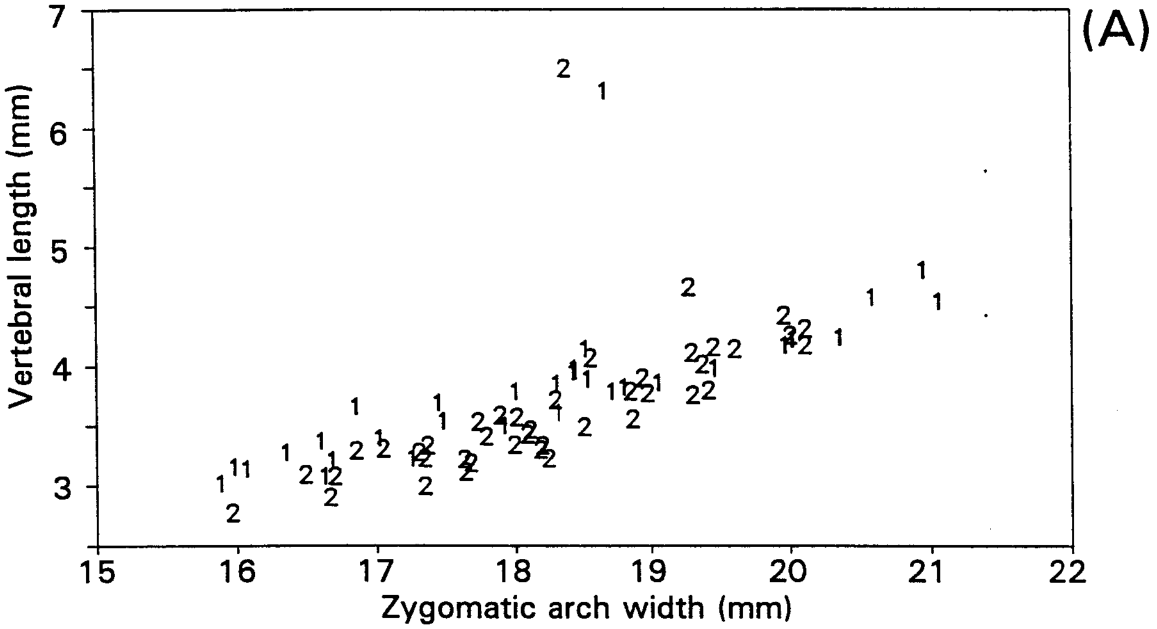


Figure 10A. Plot of two skeletal measurements (width across the zygomatic arch and vertebral length) for all the individuals within two colonies of naked mole-rats. Data points denoted with a #1 are individuals from colony 1000, while those denoted with a #2, are from colony 100. The two outliers (one from each colony) are the breeding females, and were excluded from all analyses. The allometric equation for colony 1000, excluding the breeding female, is y=-1.535+0.365x ($F_{1,29}$=259.39, P<0.0001, r^2=0.899). The equation for colony 100 is y=-3.101+0.365x ($F_{1,38}$=214.07, P<0.0001, r^2=0.849).

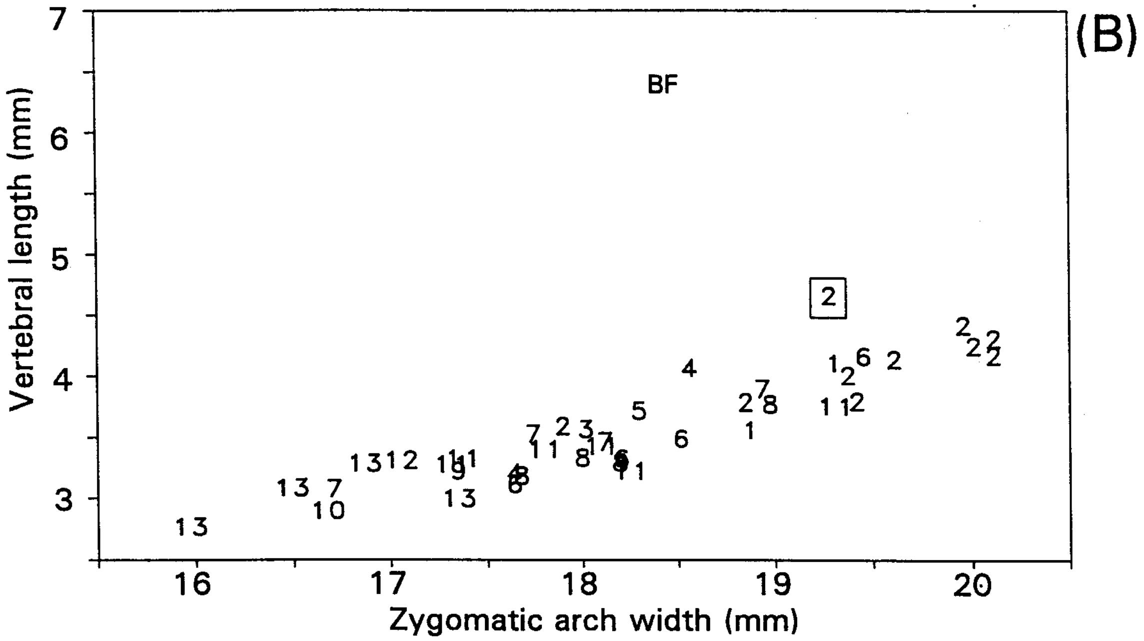


Figure 10B. Plot of zygomatic arch and vertebral length for the individuals of colony 100. Data points are represented by numbers denoting the respective litters of each individual within the colony. BF is the breeding female, and the data point enclosed within a square is individual #15, the only female non-breeder to have reproduced.

Table 6. Statistical comparisons of morphometric variables between age cohorts (where n≥3 individuals) within colony 100. Data are from x-rays taken for all colony members in 1995.

Variable	Litter	Mean±SE	$F_{5,26}$	P	Tukey test
	2	19.47±0.24			
	6	18.47±0.38			2 vs 7
Zygomatic arch (mm)	7	17.89±0.46	9.99	0.000	2 vs 11
	8	18.23±0.28			2 vs 13
	11	18.03±0.29			6 vs 13
	13	16.68±0.29			
	2	4.12±0.10			
	6	3.53±0.23			2 vs 6
Vertebrae length (mm)	7	3.51±0.17	9.55	0.000	2 vs 7
	8	3.41±0.13			2 vs 8
	11	3.42±0.08			2 vs 11
	13	3.05±0.11			2 vs 13
	2	3.55±0.10			2 vs 6
	6	3.12±0.07			2 vs 7
Incisor width (mm)	7	3.05±0.09	17.06	0.000	2 vs 8
	8	2.90±0.04			2 vs 11
	11	2.78±0.02			2 vs 13
	13	2.57±0.09			6 vs 13
					7 vs 13
	2	56.9±3.20			
	6	50.0±5.92			2 vs 8
	7	48.3±8.67	7.50	0.002	2 vs 11
Body mass (g)	8	38.2±2.32			2 vs 13
	11	33.7±1.47			
	13	29.8±1.48			

Although this cluster did not include all the principal colony defenders it does emphasise the result obtained for the size-frequency distribution, namely that defenders appear to have larger incisors than colony non-defenders. There were five groups with n≥3 individuals at the 75% similarity level. One-way ANOVA revealed similar trends to those obtained at the 70% similarity level. Dispersers (group 2) were significantly heavier than non-dispersing members of groups 3 and 5, while the three individuals of group 3 (two soldiers and a ex-replacement breeder, #15) were clearly more robust than non-breeders in groups 4 and 5, despite the presence of a single soldier, #6, in group 5.

Table 7. Comparisons of morphological variables for individuals in the four groups ($n \geq 3$ individuals) identified at the 70% similarity level of Figure 1D.

Variable	Groups	Mean±SE	$F_{3,35}$	P	Tukey test
Body mass (g)	1 2 3 4	53.97±3.29 56.86±6.03 51.60±3.61 39.31±2.30	4.55	0.0086	2 vs 4
Zygomatic arch (mm)	1 2 3 4	19.09±0.48 18.78±0.45 19.45±0.09 17.95±0.19	3.57	0.0234	n.s.
Vertebrae length(mm)	1 2 3 4	4.78±0.89 3.72±0.23 4.21±0.25 3.45±0.07	6.913	0.0009	1 vs 2 1 vs 4
Incisor width (mm)	1 2 3 4	3.37±0.33 3.24±0.11 3.57±0.17 2.92±0.06	5.167	0.0046	3 vs 4

Group 3 also had significantly longer vertebrae than members of groups 3, 4 and 5. This can be attributed to the correlation between vertebrae length and body mass (Table 5), with the three individuals of group 3 being large, older individuals. The low sample size of the breeders (n=2) precluded their incorporation in this statistical analysis. It is apparent however, that the only differences between non-breeding individuals in the different behavioural groups occur between dispersers and soldiers, and all other non-breeding individuals.

Table 8. Comparison of morphological variables for individuals in the five groups ($n \geq 3$ individuals) identified at the 75% similarity level of Figure 1D.

Variable	Groups	Mean±SE	$F_{4,31}$	P	Tukey test
Body mass (g)	1	56.40±4.95	3.67	0.0147	1 vs 3
	2	51.60±3.61			1 vs 5
	3	30.23±2.58			
	4	37.78±2.52			
	5	40.24±2.99			
Zygomatic arch (mm)	1	18.79±0.36	2.77	0.045	n.s.
	2	19.45±0.09			
	3	17.25±0.29			
	4	17.76±0.36			
	5	18.04±0.25			
Vertebrae length (mm)	1	3.73±0.18	3.58	0.016	2 vs 3
	2	4.21±0.25			2 vs 4
	3	3.15±0.13			2 vs 5
	4	3.34±0.10			
	5	3.49±0.09			
Incisor width (mm)	1	3.19±0.09	3.57	0.017	2 vs 3
	2	3.57±0.17			2 vs 5
	3	2.77±0.05			
	4	3.01±0.11			
	5	2.93±0.08			

DISCUSSION

The results of this study provide strong evidence in support of a primary division of labour between the reproductive and the non-reproductive members of naked mole-rat colonies. The reproductives formed a distinct subgroup in all multivariate procedures. In contrast, there was little evidence of a clear secondary division of labour. Neither age nor size showed a consistent relationship with helper activities. At all stages of colony development there were, however, significant differences in the behavioural repertoires of colony members of different ages in both study colonies (Tables 1 & 2). These differences were attributed to both sexual and dominance-related activities, associated with the primary division of labour, rather than to work. This is consistent with findings for cooperatively breeding social invertebrate species in which dominance interactions dictate the overall division of labour - the winners specialise in reproduction and the losers in defence and work. Thus, in the queenless ants (*Diacamma* sp.) and the cooperatively breeding wasp, *Polistes dominulus*, an individual's social rank determines its behavioral profile and is an important organisational mechanism within the colony (Theraulaz *et al.* 1992; Peeters and Tsuji 1993; Heinze *et al.* 1994).

It is further evident that all work behaviours are performed by all non-breeding individuals within the colony and, apart from a tendency for certain younger mole-rats to perform heightened levels of work in well established colonies (Fig. 3, Jarvis 1981; Jarvis *et al.* 1991; Faulkes *et al.* 1991; Lacey and Sherman 1991), there are no consistent age-related trends. If different age-cohorts within the colony have identical work repertoires, then division of labour cannot be explained in terms of age-castes (*sensu* Seeley 1982; Kolmes 1985; Hölldobler and Wilson 1990; Robinson 1992; Pratt *et al.* 1994). Furthermore, there was little evidence in favour of a temporal shift in the frequency of performance of tasks and no changes in the type of tasks performed by members of different age-cohorts were evident (Fig. 5).

The important distinction between naked mole-rat colonies and most insect societies is thus that the mole-rats are not performing different roles, only different amounts of the same tasks. The task repertoires of all non-breeding naked mole-rats are thus the same and differences observed are quantitative, in contrast to the qualitative differences between temporal castes in both eusocial (Oster and Wilson 1978) and cooperatively breeding (Lachaud *et al.* 1988; Pratt *et al.* 1994) insects. It is possible that these differences reflect the simple and generalised work repertoire of the naked mole-rat, which is geared primarily towards foraging, as compared to that of insect societies with a large proportion of highly specialised brood care activities, in addition to foraging.

The Task environment

A fundamental difference between insect societies and naked mole-rat colonies is the degree of complexity of the 'task environment'. Both eusocial insects (Wille and Michener 1973; Oster and Wilson 1978; Seeley 1982) and naked mole-rats (Jarvis *et al.* 1985, Brett 1991) inhabit microhabitats characterised by a spatial separation of functionally different areas. Indeed, as mentioned in the introduction to the thesis, it is this very aspect of their biology that make comparisons with social insects more insightful than those with other social vertebrate species (*e.g.* dwarf mongooses). Such comparisons have obvious limitations however, which have still to be considered. The major limitation is the fact that naked mole-rats construct very simple nests, contiguous with a physically uniform network of interconnecting burrows. There are no functionally different areas within the nest itself, and resting as well as sexual activities, feeding and nursing, all occur anywhere within the confines of the nest.

This contrasts with even the most primitive ant species in which there are always functionally discrete areas within the nest for the care of eggs, larvae and pupae (Holldobler and Wilson 1990). Furthermore, in these and other eusocial insect species, the broods are physically separated according to their relative developmental state.

Apart from a very generalised work environment, the task repertoire of naked mole-rats is small (six behaviours, Lacey *et al.* 1991, or five behaviours, see discussion in Chapter 1) and is primarily involved with extending (foraging) and maintaining the burrow system. Helping behaviours involving the care of pups in the nest area are performed infrequently and there are often long periods in both field (Brett 1991) and laboratory colonies (Sherman *et al.* 1991) when there are no pups to tend. In contrast, insect societies have larger (ca.15-38, Wilson 1976) and more elaborate task repertoires, with an emphasis on specialised brood care activities. Because there are typically many eggs, larvae and cocoons to be cared for at any one time, these activities are the main focus of the colony's total activity budget (Robinson 1992).

These differences have important consequences for comparisons between social invertebrates and vertebrates. Seeley (1982) suggests that the spatial layout of social insect nests set the stage for the evolution of the age polyethism schedule. He argues that age polyethism within honeybee colonies reflects a compromise between selection for efficiency in performing tasks and selection for efficiency in locating tasks, a suggestion supported by Hölldobler and Wilson (1990) for ants.

Task specialisation by workers in a structured nest environment leads to a more efficient performance of essential tasks by colony members and minimises the mean distance travelled between functionally related tasks (Wilson 1976). Naked mole-rats colonies do not have the same degree of predictability in their task environment and hence task specialisation would lead to worker redundancy. The emphasis in their colonies is thus for flexibility, hence the lack of specific roles and/or age-castes.

A typical pattern for an age-based division of labour such as in ants, bees and wasps is for young workers to be initially occupied with tasks such as brood and queen care within the safe confines of the nest (Franks 1987). As they age and approach physiological senescence, they first change to a stand-by role in defence and then switch to foraging and finally territorial patrolling (West-Eberhard 1981; Wilson 1985). This is construed as adaptive since it postpones the most hazardous labour, such as foraging and defense, to late in a worker's life when it is more expendable (Oster and Wilson 1978).

More recently, Tofts and Franks (1992) have developed a 'foraging for work' algorithm, providing further support for Seeley's (1982) conclusion that among social insects, the age polyethism schedule is functionally related to nest architecture so as to minimise travel between tasks, and maximise efficiency of task location. This paper was also of interest because it was the first attempt to make direct comparisons with ants, bees and naked mole-rats, suggesting that common selective factors may have influenced their respective labour patterns. By taking a closer look at the algorithm proposed by Tofts and Franks (1992) we can critically assess whether Seeley's hypothesis can be applied to naked mole-rat colonies.

Tofts and Franks (1992) propose that species with a wide array of brood care and housekeeping tasks, can be expected to have more temporal castes than those with relatively simple repertoires. Furthermore, the more dispersed the tasks for which temporal castes are specialised, the more likely is the labour to be discretised. They also assume that individuals in insect colonies are born into the first task located on the brood pile and that they only change tasks once the next age cohort has emerged and passively displaced them. The role of the cohort therefore changes passively with time *i.e.* temporal polyethism. How does this compare with naked mole-rats ?

Tasks in a naked mole-rat colony are not ordered. A juvenile mole-rat is gradually assimilated into the task force as it develops the requisite motor and sensory capabilities (see Chapter 1). Because of its small size, the 'new recruit' is initially ineffective, and although it responds to the full range of adult work contingencies, is incapable of adequately fulfilling the requirements of a given task within the colonies work schedule. With increasing age, task performance efficiency of juveniles improves and, while there is no qualitative change in their behavioural repertoire, marked quantitative changes are observed (see Chapter 1). Interestingly, juveniles often solicit digging or gnawing sites that are frequently 'worked' by experienced adults. Unlike ants (McDonald and Topoff 1985) however, this process is not passive. A juvenile solicits the work site by physically encroaching on the work space of an adult while simultaneously vocalising (loud chirp associated with competition for a resource in adults). This behaviour has important consequences for colony ergonomics, as the new work-force recruits then focus their energies on tasks prioritised by more experienced workers. It also reveals a learned component of the task schedule, a feature not apparent for most insects and one which effectively precludes a correlation between age and the spatial division of labour in naked mole-rats. These findings contrast markedly with those for the social hymenopterans (*e.g.* ants and bees) in which the young worker emerges as a fully functional adult capable of performing specific tasks as efficiently as older colony members.

Oster and Wilson (1978) predicted that in the absence of evolutionary constraints a social species should have as many castes, or roles, as there are tasks. The lack of roles amongst non-breeding naked mole-rats, which live in colonies of a size comparable to many social insect species, suggests that there may be insufficient different tasks to make such specialisation energetically feasible. Thus it may be more economical to be less efficient at a wider variety of tasks than to specialise on improving the performance of one or two. It is also important because of the length of time that would be required to replace specialised non-breeders should they die, which would effectively leave the colony with critical gaps in its' work force.

An important consideration with regard to these arguments is that behavioural distinctions in captive naked mole-rats may be 'blurred' because of the lack of adequate work stimuli in the captive environment. This problem, however, is currently insurmountable because of the subterranean habits of free-living mole-rats.

It is evident that there is a low degree of variability in the behavioural profiles of individual naked mole-rats. Besides very young animals, these differences are not closely associated with either the age (Table 3, Fig. 4) or size (Tables 7 & 8) of non-breeders, and this points to a lack of either physical or temporal subcastes amongst the non-breeders. It is also clear from the preceding discussion that workers do not change tasks in a predictable fashion and that division of labour within naked mole-rat colonies is not functionally related to nest/burrow architecture. In the absence of any distinct age-related trends or inter-individual variation in the behavioural repertoire, use of the term age or age/size polyethism (*sensu* Lacey and Sherman 1991) must be regarded as inappropriate and potentially misleading. This is not to say that there is absolutely no effect of age on the overall division of labour; but where there is considerable variation in individual ontogeny, with a high degree of behavioural idiosyncrasies among individuals (Fig. 3) of a given age-group, age can only at most be loosely correlated with work in naked mole-rat colonies.

An alternative explanation must therefore be invoked to explain how the performance of tasks within a naked mole-rat colony is organised. While the absence of distinct roles in naked mole-rat colonies allows for greater flexibility in worker response, it raises the problem of organisational efficiency, and in particular of assigning the correct number of workers to essential tasks. However, if one looks closely at the way in which tasks are performed in captive colonies, it is possible to detect what appears to be clear organisational principles that may enhance the efficiency of work performed within the colony, despite a lack of distinct roles amongst workers.

Task fixation and the spatial division of labour

The most salient component of the work schedule of naked mole-rats is task fixation, where an individual typically performs a given task at a particular position in the burrow system, as long as it receives positive feedback *i.e.* the task remains unfinished. This may help to assign the appropriate number of workers to a task and ensure that workers do not randomly change tasks. Brett (1991) provides circumstantial field evidence in support of task fixation. Using telemetry he found that specific individuals consistently worked in a single locality of the burrow system, both during and in subsequent work bouts.

Task switching, in captive individuals occurred within a single functional type (*e.g.* digging and sweeping), rather than between types. Possible advantages of this include minimising travelling and waiting time between tasks, as has been shown for *A. mellifera* (Seeley 1982). This effectively reduces the costs associated with locomotion between tasks and also the time taken to find other tasks.

A further characteristic of the organisation of labour within naked mole-rat colonies is that at any given moment only a small fraction of individuals are actively working ca. 17.9% (5.93±2.3 individuals out of 33 non-breeders, n=40 random samples over 2 months in colony 7400, 1992). This apparent laziness is not necessarily a selfish strategy on behalf of the lazy individuals but may serve to minimise the total energy expenditure of the colony while ensuring the presence of a large reserve force. Brett (1991) also found that in wild colonies of naked mole-rats certain individuals spend at least 80% of their daily time budget, inactive within the nest.

The importance of a large reserve force of inactive individuals is evidenced when a rare stimulus such as a blocked burrow or damp easily worked soil is met with a rapid increase in the colony work rate. Naked mole-rat colonies, like some insect societies, may therefore alter their behaviour in response to different circumstances by changing the behaviour of individual workers. These changes include modification of activity levels, re-allocation of effort, or some combination of both.

Inactivity may thus be construed as adaptive. Resting workers serve as a reservoir for the foraging population, and thereby impart a flexibility to the labour schedules that allow the colony to adapt quickly to capricious changes in their environment.

The presence of certain individuals (*e.g.* #30, Fig. 3) that performed heightened work levels may reflect the existence of temporally stable individual idiosyncrasies, or elitism (*sensu* Oster and Wilson 1978) which may further enhance the complexity and efficiency of labour within the colony. Otto (1958), working on the ant *Formica polyctena*, noted that certain individuals work far more than others, or focus considerably more of their energies on certain tasks than others do, for much of their adult life. These hard working individuals are not specialists (*sensu* Tofts and Franks 1992) as they do not restrict their work to a single task, but rather task fixation is an integral component of all work performed. This combines the benefits of a specialist worker, who tends to remain at one task and therefore minimises time spent between tasks, with that of a generalist, who is capable of performing all tasks - thus ensuring a flexible colony response to changes in the environment. It is possible that workers that specialise on specific tasks, or on the locality of that task within the burrow system, improve their efficiency (*sensu* Seeley 1982; Jeanne 1986) although this has yet to be tested in naked mole-rats. Workers are thus not only able to abandon their current work and switch to new tasks when the needs of the colony vary, but they may also alter the amount of time that they allocate to a particular task.

Morphological differences within colonies

Size variation of workers within eusocial ant colonies has been shown to increase with colony size (Wilson 1971; Tschinkel 1988; Hölldobler and Wilson 1990). In addition, size-frequency distributions of body mass become progressively more skewed with time from colony inception, resulting from distinct polymorphisms amongst the workers of the colony (Hölldobler and Wilson 1990). Although similar trends were apparent for changes in body mass with time in naked mole-rats, the normal score plots of mass and incisor width (Fig. 9) for these individuals suggest that these skewed distributions were all part of a single normally distributed population, pointing to a lack of polymorphism amongst non-breeders. These patterns were further apparent in the size-frequency distributions of body mass in the two large, well established colonies.

It is important to note that in these colonies the size variation did not increase with time despite the differences in recruitment to the two colonies. This suggests the existence of an upper limit to body size of non-breeders, that is achieved relatively early in the colony's history (ca. 2 years, Fig. 7). The size-frequency distributions of colony L4, provide support for the findings presented in Chapter 2, namely, that even in the absence of recruitment over prolonged periods, a wide range in body size is maintained. These findings afford strong evidence against Lacey and Sherman's (1991) suggestion that *all* individuals increase in size with age, forming the basis of an age/size based polyethism. Both recruiting and non-recruiting colonies maintain an appreciable size range, largely through the suppression of growth of younger colony members (see Chapter 2). This is construed as adaptive for, in the absence of such a process, colonies would become 'top heavy', imposing energetic constraints on the colony as a whole; the global energetic cost of digging is lower for small sized fossorial individuals than for large (McNab 1979).

Morphological divergence between the breeding female and all other colony members.

In contrast to the findings for body mass and incisor width, the size-frequency distribution of vertebral length provided evidence for strong dimorphism in naked mole-rats. Two morphologically distinct groups were apparent, with no intermediates between them. This was confirmed by the distinct 'kink' in the normal score curve (*sensu* Tschinkel 1988). As far as can be ascertained, this represents the first evidence for the existence of a morphologically specialised reproductive phenotype within social vertebrates.

The regression plot of width across the zygomatic arch and vertebral length, highlights the morphological divergence of the breeding females from all other colony members in both colony 100 and 1000. Importantly, the only other individual that falls outside of the 95% confidence limits for this curve is #15 of colony 100, which as previously mentioned, is the only female to have temporarily escaped reproductive suppression and reproduced. That this individual is still morphologically more similar to the non-breeders than either of the breeding females suggests that continued reproduction is necessary to fully develop an elongated body.

The breeding female and non-breeders in naked mole-rats, differ morphologically, largely as a result of morphological adaptations for efficient reproduction. Thus a greatly elongated body allows the breeding female to produce large litters (up to 27 pups) and still move through the narrow confines of the burrow system. This represents an intriguing parallel with the termite queen, who prior to the onset of reproduction is morphologically indistinguishable from unmated alates. It is only through the process of reproduction itself that the characteristically enlarged abdomen of the queen develops.

However, it is important to point out that unlike other eusocial insects (e.g. honeybees), dimorphic adult females are not produced by divergent developmental pathways prior to adulthood. Rather, reproductive differentiation in naked mole-rats occurs in the adult stage. This is more similar to the cooperatively breeding wasps, bees and ponerine ants, than to eusocial species. It is thus preferable not to refer to either breeding females or workers as members of distinct castes in naked mole-rats, for as Peeters and Crozier (1988) and Peeters and O'Riain (in prep) point out, it is more useful to restrict the use of the term 'caste', to morphological divergence following pre-adult differentiation.

What are the limits to morphological specialisation amongst the non-breeders?

The potential for all non-breeding naked mole-rats to assume a reproductive role (following the death of a breeder), may offset any advantages (increased inclusive fitness) associated with extensive or irreversible morphological specialisation for improved efficiency within the colony, as noted for certain hymenopteran insect societies. A similar argument has been proposed by Bourke (1988) who suggested that there may be a conflict in the worker caste of cooperatively breeding invertebrates, between further morphological specialisation and sterility on the one hand, and the retention of ovaries, and thus the ability of individuals to produce at least some offspring themselves, on the other hand. Furthermore, although morphologically specialised non-breeders, such as defenders in naked mole-rat colonies, would be highly efficient in their particular duties, they would be poorly adapted for other tasks. By comparison, as discussed earlier, mole-rats showing behavioural specialisation can still retain a great deal of flexibility and can switch tasks should the need arise. Thus morphologically unspecialised mole-rats can meet environmental exigencies through behavioural flexibility. Similar arguments have been advanced for eusocial hymenopteran species characterised by a monomorphic work force. Here the system of gyne determination may limit or constrain the evolutionary possibilities of further subdivision of females into new morphological castes (Wheeler 1988). Thus no eusocial bees or wasps have evolved morphologically complex worker castes (Wilson 1971).

Colony defense - a special case

When the function of specific invertebrate colony members is that of partly or wholly fighting on behalf of the colony, they are often referred to as soldiers (Hölldobler and Wilson 1990). According to these criteria and to the evidence presented in both Chapter 5 and by Lacey and Sherman (1991), that the ousting of intruders was performed primarily by the largest and oldest non-breeders, it seems appropriate to use the term soldier in naked mole-rat colonies.

The subterranean niche and narrow confines of the tunnels that naked mole-rats inhabit provide the opportunity for individual non-breeders to perform heroic acts when either predators or foreign conspecifics enter their burrows. Alexander *et al.* (1991) suggested that such acts may select for specialised worker-soldier phenotypes. Intriguingly, there is slight evidence for polymorphism among non-breeders for incisor width (Fig. 9), the weapons of defence. Size (body mass) variation between large and small individuals is however continuous, suggesting that heroic acts do not select for marked phenotypic specialisation.

An additional morphological feature that appeared to correlate with colony defence was that of jaw musculature. Primary colony defenders were characterised by hypertrophied temporal musculature, resulting in a more square-shaped head, readily discernable to the trained eye. Unfortunately, it was not possible to quantify these differences and they were not manifest in any of the skeletal variables investigated.

Suggestions of subtle morphological specialisations for defence must however be viewed with caution because there were other large individuals that showed an equal propensity to engage in defence-related activities, that were not grouped together by their incisor width. Furthermore, in the absence of large-sized individuals within the colony smaller colony members will attack a foreign conspecific (O'Riain unpubl. data).

If defence is largely a behavioural specialisation, the performance frequency for which simply correlates positively with body size, then 'defenders' can also perform other tasks when the need arises. In these circumstances the physical features that make them efficient defenders may translate into making them exceptionally powerful diggers during brief periods of optimal digging opportunities, such as after prolonged rains (Jarvis *et al.* 1994). See Chapter 5 for further arguments on the close relationship between defence-related activities and reproductive potential of non-breeders.

Dominance interactions and reproductive succession

In contrast to the linear dominance pattern observed for some other social bathyergid species (Jacobs *et al.* 1990; Bennett and Jarvis 1991; Rosenthal *et al.* 1991) the dominance structure among naked mole-rat colony members typically consists of a single dominant individual (the breeding female) and several subordinate individuals, with a high degree of circular triads resulting in a low linearity coefficient. A similar pattern is found in the cooperatively breeding wasp *Ropalidia marginata* (Chandrashekara and Gadagkar 1991).

In a wide variety of social animals, dominance orders are established through agonistic encounters between group members and hence usually reflect the competitive abilities of individuals, with an individual's fitness being closely linked to its rank within the group (Dewsbury 1982). In the eusocial insects, particularly those with strongly differentiated queen and worker castes, the queen usually controls reproduction by producing inhibiting pheromones rather than by overt aggression with other colony members (Fletcher and Ross 1985; Wheeler 1986; Hôlldobler and Wilson 1990; Heinze *et al.* 1994). Furthermore, eusocial systems are typified by a dichotomy between workers or queens, precluding the former from mating.

In contrast, within simple insect social systems which lack morphological queens/worker castes and where all females are potential queens (Wheeler 1986), the struggle for control of the reproductive physiology of nest mates is resolved entirely during the adult stage by direct aggressive behaviour towards other colony members (West-Eberhard 1969; Ito 1993; Peeters and Tsuji 1993; Peeters *et al.* 1993; Heinze *et al.* 1994). Support for this mechanism is evident in the positive correlation between dominant status and ovarian development/reproductive success in both cooperatively breeding social insects (Pardi 1948; Gadagkar 1980; Peeters in prep) and mammals. The latter includes examples where dominant individuals maintain their reproductive rights over subordinate colony members through direct physical interference. For example, in captive wolves the alpha female interrupts or prevents subordinate females from mating (Zimen 1971), while in the dwarf mongoose, the male frequently interrupts attempts by subordinates to copulate (Rood 1990). The dominant females in captive marmosets suppresses the oestrus cycle of subordinates so that they fail to ovulate (Abbot and Hearn 1978). It has been suggested that naked mole-rats are similar to marmosets since breeding females are able to interfere with and suppress reproduction in both female and male colony members through aggressive behaviour which has an inhibitory effect on the endocrine system (Faulkes *et al.* 1991).

An important question therefore, is what determines whether or not an individual becomes a breeder? When attempting to provide an answer to this question it is important to consider those factors that prevent every female from becoming a breeder on the one hand, and also those that promote the chances of any one individual within a group becoming the replacement breeder. The former category includes inhibition by the reigning breeding female through signaled-dominance inhibition (*i.e.* shoving), while the latter includes factors that promote the chances of an individual becoming a breeder, such as its relative age and size.

Although the frequency of aggressive interactions between naked mole-rat colony members was not sufficient to determine the social rank of all individuals in the colony, it was clearly evident that, similar to the ponerine ant *P. sublaevis* (Ito and Higashi 1991) the oldest colony members were the primary recipients and instigators of overt dominance interactions (*i.e.* shoving). In contrast, although the younger members were never shoved they were the main subjects of pup-pushing by older members (Fig. 6A, see also Chapter 1). While both these behaviours may serve the ultimate function of establishing dominance within the colony, it is important to note that the breeding female is the primary initiator of shoving and rarely engages in pup-pushing. Furthermore, mole-rats that are frequently pup-pushed (*i.e.* of a low social rank) but never shoved, typically fail to show any signs of sexual activity following the death or removal of the breeders, whereas those individuals that are most frequently shoved are usually the first to show external signs of reproductive activity (*e.g.* a perforate vagina in females).

These results suggest the existence of dual functionality with respect to aggressive/dominance interactions within naked mole-rat colonies. On the one hand shoving behaviour may serve to advertise the breeding female's reproductive vigour and effectively suppresses the reproduction of close competitors, while pup-pushing may serve to establish a dominance hierarchy amongst non-breeding individuals (see Chapter 1). In the case of the most recent born litters, this has the additional effect of suppressing both their growth (see Chapter 2) and reproduction. Experiments in which the breeding females died or were removed from the colony, clearly demonstrated that breeding female dominance inhibited reproduction in those individuals that were the primary recipients of agonistic encounters (*i.e.* top-ranking non-breeders) (Faulkes *et al.* 1991; Jarvis 1991; this study).

These findings are similar to those for the social wasps of the genus *Polistes*, in which dominance is positively correlated with age (Pardi 1948; Dew and Michener 1981; Strassman and Meyer 1983). When reproductive naked mole-rats were removed from the colony the oldest non-breeders present took over.

Dominance may be correlated with age in small colonies of social insects and mammals because it is intrinsic to a system lacking castes, where individuals never completely lose the ability or chance to become a replacement reproductive. Such systems where 'reproductive control' is focussed in the adult stage have the important advantage of flexibility in the face of disaster to the colony (Jeanne 1972). Breeding females that die can be quickly replaced from a pool of emergent adults. Thus, to preserve maximum responsiveness, queen/breeding female control must occur as late in the development (adult versus egg/foetus) as possible.

SECTION II

COMPETITION FOR LIMITED FOOD RESOURCES REVEALS DYNAMICS OF CONFLICT IN COLONIES OF THE NAKED MOLE-RAT.

SUMMARY

In this study the response of individuals, within 13 captive colonies of naked mole-rats, to conditions of limited food availability, is described and quantified. The results highlight the dual nature of interactions within colonies with the apparently altruistic behaviour of food-provisioning being subject to the selfish precedent of satiation. Benefits associated with cooperative foraging accrued disproportionately to breeding females who, despite seldom finding food, invariably fed from a limited food supply. It is suggested that while opportunities for selfish foraging by subordinate non-breeders exist in the wild, breeding females successfully parasitise the foraging efforts of their offspring.

INTRODUCTION

Group living may arise whenever the fitness benefits that accrue to a group of individuals, performing cooperative behaviours such as foraging and defence, are greater than for solitary individuals (Alexander 1974). Although cooperation underlies much of the success of these groups, competition and conflict are usually also present. Indeed, the benefits associated with group living typically accrue disproportionately to a few dominant individuals within the group at the expense of others (Vehrencamp 1983). The degree of bias in benefits that dominant animals can impose on subordinates is in turn linked to ecological constraints on the opportunities, to disperse and breed independently, of the subordinates (Emlen 1991).

In groups that form as a result of familial philopatry, parents are invariably dominant over their offspring (Maynard Smith 1978). This is true for a variety of social mammalian species *e.g.* dwarf mongoose, *Helogale parvula* (Rasa 1977); wild dog, *Lycaon pictus* (Malcolm and Marten 1982); wolf *Lupis canis* (Zimen 1971) and bird species *e.g.* juncos, *Junco hyemalisin* (Fretwell 1969); acorn woodpeckers, *Melanerpes formicivorus* (Stanback 1994), in which the benefits to the parents include primary access to food and a monopoly on reproduction. Conflict situations that arise through competition for limited resources (*e.g.* food) may thus be resolved through existing social hierarchies in which the parents, who are at the apex of the hierarchy, dominate their subordinate offspring. Parents may thus benefit considerably from their ability to 'parasitise' the foraging efforts of their adult offspring and channel this nutritional leverage into improved reproduction. Selection thus acts simultaneously on the dominant individuals, to secure more resources for themselves at the expense of the subordinates, and on the subordinates to leave the group, when excessively manipulated, if they can do better elsewhere (Vehrencamp 1983). Individuals that remain at home can maximise their inclusive fitness by 'altruistically' helping to raise close relatives (Hamilton 1964) and/or by selfishly competing with group members for limited resources that are important for future direct fitness benefits (*e.g.* fat stores for dispersers, see Chapter 6, and large body size amongst competitors, see Chapters 2 & 3).

The naked mole-rat, which lives in numerically large colonies (ca. 70 animals) typically comprised of parents, their offspring and siblings, provides an excellent model to test this hypothesis. Reproduction within these groups is monopolised by an omnipotent subset (typically the parents), the female of which is principally involved with the physical bullying and harrassment of other colony members. These actions would appear to actively suppress subordinate individuals through behaviourally mediated stress (Faulkes *et al.* 1990; Faulkes and Abbott 1993). Death of the breeding female typically results in the subordinates aggressively competing for the reproductive vacancy.

In this study an attempt is made to establish whether this observed conflict in reproductive interests extends to conflict over access to food resources. This is an important question as it serves to establish whether the non-reproductive offspring, which are the primary foragers (Jarvis *et al.* 1991; Lacey and Sherman 1991), behave selfishly or altruistically, especially under conditions of limited food availability. In addition, it provides an empirical test of whether the observed skew in reproduction (Jarvis 1981; Jarvis 1991) is in fact mirrored by a skew in access to food, in favour of the behaviourally dominant parents.

METHODS AND MATERIALS

Manipulations involved 13 laboratory-reared colonies, each comprising a single breeding female, 1-3 breeding males and non-breeders of both sexes. Colonies were housed in artificial burrow systems constructed from perspex. While burrow system layout and length varied between colonies, each colony had only a single food and toilet chamber and members of each colony always used a single nest despite typically having a choice of two nesting chambers. Prior to experimental trials colonies were deprived of food for 36 hours. This interval was sufficiently long to ensure that no food was present within the burrow system for at least 12 hours prior to the introduction of a food item, while short enough not to adversely affect the health of any colony members. At the end of this period a single piece of sweet potato ($1cm^3$) was introduced into the food chamber and the behavioural responses of colony members recorded. Trials were terminated when the food item had been totally consumed.

A total of 31 trials were performed on these 13 colonies from 15/11/91 to 14/12/92 with a minimum of 60 days between successive trials for any one colony. Both within and between colony variance in response to food deprivation, was tested in four colonies of similar size (29-38 individuals/colony) in 6, 6, 5 and 5 trials, respectively. Based on the responses of these colonies a series of one-off trials were subsequently performed on a further nine colonies to further examine variation in response between colonies. During the period between trials, colonies were habituated to an *ad libitum* food supply. This long time interval between food trials was imposed to minimise the effects of habituation to food deprivation, and as an attempt to make any within-colony replicates appear as 'stochastic events'. Prior to data analyses colony members were assigned to one of three readily identifiable functional groups (non-breeders, breeding female and breeding male(s)) within each colony. Results were analysed using a Chi-squared goodness of fit test, in which the expected frequencies were calculated according to the probability of members of each functional group either encountering, feeding first, or feeding at all, from an introduced food item. The expected frequency of encounter was a product of the number of trials and the relative proportion of individuals within a functional group (*i.e.* in a colony of 31 individuals, 29 were non-breeders, one was a breeding female and one a breeding male, thus the probability of one of the non-breeding animals finding food was 29/31 or 93.5%, which translates into an expected probability of 5.61 in 6 trials). Assuming a first-come-first-serve basis, the expected probability of feeding was a direct function of having found the food first.

RESULTS

It was clearly evident that the numerically superior non-breeders were primarily responsible for the discovery of a food item (Fig. 1). Indeed, the observed frequency of success in finding food did not differ from the expected probability within all 4 colonies (Table 1, Chi-square tests, $P>0.05$ for all colonies). In contrast breeding females, despite seldom finding a food item, obtained a disproportionate share of a limited food supply (Table 1, Chi-square tests, $P<0.05$ for all colonies). There was no difference between the

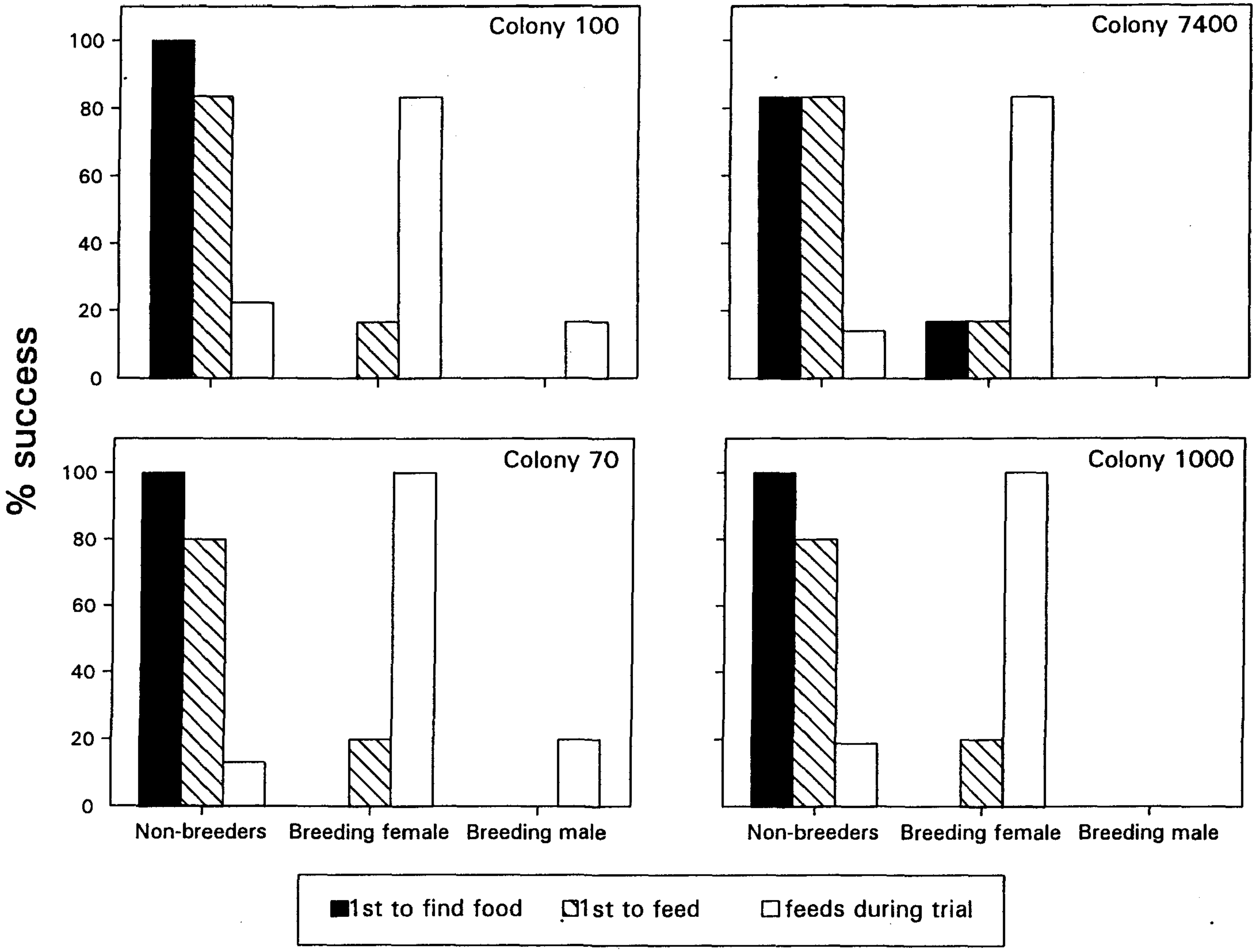


Figure 1. The percentage success that members of the respective functional groups (non-breeders, breeding female and breeding male(s)) achieved in three food related activities, following food deprivation.

Table 1. Data are presented as the total number of times a member of the respective functional groups within the four colonies were successful in three different aspects of feeding following food deprivation. Chi-squared statistics and the resultant probability (p) are presented for the expected versus the observed data for each functional group within each feeding related category. For all colonies there was a single breeding male and female, except colony 70 which had 3 breeding males.

Colony	Feeding variable	NB	BF	BM	Chi-squared	p
100 (n=31)	1st to find	6(100%)	0(0%)	0(0%)	0.084	0.959
	1st to feed	5(83.3%)	1(16.7%)	0(0%)	1.14	0.565
	Feeds	7(24.1%)	5(83.3%)	1(16.6%)	26.14	<0.0001
7400 (n=36)	1st to find	5(83.3%)	1(16.7%)	0(0%)	0.681	0.711
	1st to feed	5(83.3%)	1(16.7%)	0(0%)	0	1
	Feeds	6(17.6%)	5(83.3%)	0(0%)	8.17	<0.02
70 (n=38)	1st to find	5(100%)	0(0%)	0(0%)	0.176	0.916
	1st to feed	4(80%)	1(20%)	0(0%)	1.17	0.558
	Feeds	5(13.1%)	5(100%)	1(33.3%)	26	<0.0001
1000 (n=32)	1st to find	5(100%)	0(0%)	0(0%)	0.061	0.969
	1st to feed	4(80%)	1(20%)	0(0%)	1.17	0.558
	Feed	6(20%)	5(100%)	0(0%)	25.17	<0.0001

NB = Non-breeders; BF = Breeding female; BM = Breeding male

* data do not sum to 100% as it was possible for more than one individual to feed on a given food item in each trial

expected and the observed frequencies for feeding first, that is, individuals that found a food item typically were the first individuals to feed from it.

Despite the fact that non-breeders were more numerous and were the first individuals to encounter the food item in most trials, they often enjoyed an only brief opportunity to feed. In contrast breeding females, who were seldom first to encounter the food item in any trials, still succeeded in feeding from the food item in most trials. Breeding males only enjoyed a small success rate in feeding and were never the first individuals to encounter the food item. A similar pattern emerged for the nine one-off trials with non-breeders finding the food item in 100% of all trials and only feeding in 75% of these trials. Again breeding females, despite never finding the food first, successfully fed from the single food item in all trials. Breeding males never found the food first and only enjoyed a feeding success of 11.1% for the nine trials. Importantly, breeding females achieved this level of success despite being lighter in body mass and markedly less physically robust than many of the non-breeders. In 12 of the 13 colonies the breeding female was lighter than at least two of the non-breeders suggesting that their competitive superiority is not a function of physical size or robustness.

Upon encountering food, foraging non-breeders tended to carry it to the nest (the preferred site of food consumption) and attempt to feed (63% of all trials). Other colony members rapidly became aware of the presence of the food item resulting in frequent tugs-of-war (*sensu* Schieffelin and Sherman 1994) between non-breeders. Here at least two individuals (maximum observed was four) bite into the food item and attempt to wrest it from one another by pulling in opposite directions. These contests were always accompanied by vocalisations (*i.e.* loud chirps, *sensu* Pepper et al. 1991). If an individual eventually won a tug-of-war it would immediately attempt to eat. In situations in which the breeding female intervened, she was never heard to vocalise, invariably won the contest (see Fig. 1) and was seldom challenged (3% of all trials) while feeding. Interestingly, those foraging non-breeders that managed to consume part of the food item, tended not to return to the nest (32% of all trials) but rather selected a dead-end section of the burrow system, into which they would face while feeding. This had the two-fold effect of restricting access to other non-breeders and delaying detection by the breeding female; thus affording them the opportunity to feed.

DISCUSSION

It was clearly evident that, under simulated conditions of limited food availability, conflict over access to food arises. While non-reproductive naked mole-rats frequently provision the nest with food when fed *ad libitum* (Jarvis 1969; Lacey and Sherman 1991), it was apparent that every mole-rat that encountered a food item when hungry, attempted to eat first. The apparently cooperative behaviour of provisioning thus appears to be subject to the selfish precedent of being satiated. This finding suggests that this ecological source of selection can trigger selfish behaviour by non-breeding offspring. The breeding female, despite seldom foraging for food, nevertheless maintains primary access to a limited food resource, reinforcing the notion (as outlined in the introduction) that the breeding female benefits from the establishment of a social hierarchy, in which her superior status dictates both reproductive and feeding dominance.

Overt aggression in the form of biting or shoving was not observed in any of the trials despite their frequent performance by the breeding female during routine patrols of the burrow system, under conditions associated with an *ad libitum* food supply. Here selection seems to have favoured the reduction of within-colony aggression despite the obvious competition for food. Reduced aggression is also noted during competition over access to digging sites (Brett 1991; Jarvis *et al.* 1991; Pepper *et al.* 1991).

In a recent fairly similar study, Schieffelin and Sherman (1995) reported a feeding preference in favour of larger individuals, usually the reproductives. Interestingly, Schieffelin and Sherman (1995) did not find that breeding females dominated breeding males in tugging contests, despite their overall behavioural dominance of the breeding males (Jarvis 1981; Faulkes 1991; Lacey and Sherman 1991; Reeve and Sherman 1991; Reeve 1992). This may be a consequence of differing methodologies and the different colony histories of the colonies studied in the respective laboratories. In particular, the breeding males in Scheiffelin and Sherman's colonies had fairly recently attained breeding status whereas in the colonies used in this study, all the breeding males were well established. Both Jarvis *et al.* (1991) and Lacey and Sherman (in press) state that whereas replacement breeding males are often initially amongst the largest non-breeders, they show a progressive decrease in body size with continued reproductive tenure. Thus the breeding males in this study were never the largest colony members. Given that dominance in tugging contests over food was shown to be correlated with body mass by Schieffelin and Sherman, it perhaps not surprising that in their colonies the breeding males were so successful. This serves to highlight the importance of colony history and social structure in predicting the outcome of social interactions within group living organisms.

Scieffelin and Sherman (1995) also suggested that small non-breeders would be unsuccessful as selfish foragers. Although the results presented here suggest that non-breeders do in fact succeed as selfish foragers, it is important to note that in both this study and that of Schieffelin and Sherman (1995) small, readily portable, cubes of sweet potato were used as a food source, whereas in the wild a large proportion of the mole-rats' food supply constitutes single, large (up to 50kg) 'immovable' tubers (Brett 1991). Foraging mole-rats that encountered large food items in captivity always fed *in situ* and were never observed to remove pieces for transport to the nest. Hungry foraging mole-rats that encounter large tubers in the wild would thus have ample opportunity to feed on the food item *in situ* because this would typically occur fairly far from the nest. Importantly, this would often be in the absence of the breeding female or other larger, competitively superior colony members.

Two further scenarios may possibly influence both selfish and manipulative behaviour by the non-breeders and the breeding female, respectively, in the wild. Firstly, small foragers may benefit inadvertently from the small diameter of primary foraging burrows (Brett 1991) that would physically restrict access by the breeding female when she is in a gravid state. Secondly, the reliance of all mole-rats on the communal nest for efficient thermoregulation (Withers and Jarvis 1980) ensures that all colony members are brought into close physical and olfactory contact. The monitoring of all colony members, and their subsequent manipulation by the breeding female, may be achieved simply by monopolising nest entrances. Both Lacey and Sherman (1991) and Jarvis (1991) have shown that this behaviour, termed 'facing out', was typically performed by breeders and the largest non-breeders wihin a colony. It is possible that this behaviour serves both to facilitate the rapid departure of large colony defenders from the nest to intercept intruders (*i.e.* nest-guarding, *sensu* Lacey and Sherman 1991) and to enable the breeders (who are seldom involved with colony defence (see Chapter 5) to monitor the recent activities (*i.e.* foraging success) of returning subordinate non-breeders. Indeed, when non-reproductive females physically contested a reproductive opening in their colony, most of the aggressive interactions were initiated at the nest entrance (O'Riain, unpubl. data), and contestants vigorously competed to monopolise this site.

In summary therefore, juvenile naked mole-rats may benefit from philopatry by avoiding the high costs associated with dispersal (Lovegrove 1986; Brett 1991; Sherman *et al.* 1992) but pay by assuming a subordinate role within the colony. The breeding female is clearly the most dominant individual within the colony and monopolises both the right to reproduce and access to limited food resources. The fact that the breeding female constantly enforces her dominance over her potentially selfish offspring suggests that altruism in naked mole-rats is greatly overshadowed by their meaner Malthusian nature.

COLONY MEMBER RECOGNITION AND XENOPHOBIA.

SUMMARY

The ability of naked mole-rats to discriminate between familiar and unfamiliar conspecifics and their response to intruders was investigated. Odour cues used by mole-rats in recognition contexts were identified using a three-way choice apparatus and 'decision rules' for accepting or rejecting conspecifics were explored in a series of odour manipulation experiments. Results indicated that naked mole-rats are highly xenophobic, even to closely related foreign conspecifics, and that a division of labour amongst the non-breeders exists in colony defence. The principal mechanism of recognition appears to be distinct colony odour labels that are contributed by each colony member and are distributed among, and learned by, all colony members. Differences in the mixture of these odours provide, even genetically similar colonies, with a unique odour label. These odours persist despite controlling for exogenous cues. Fitness consequences of these phenomena are interpreted with respect to the need for closely related neighbouring colonies to maintain autonomy and the importance of excluding foreign competitors from within colony rivalry for reproductive succession.

INTRODUCTION

Kin bias is a feature of social organisation in many species (Gadagkar 1985; Sherman and Holmes 1985; Fletcher and Michener 1986; Blaustein and Porter 1990; Hepper 1991; Pfennig and Sherman 1995) and can occur for a number of reasons, some involving kin discrimination, others reflecting incidental consequences of discrimination at other levels (Barnard 1991). In social organisms bias towards close kin in the form of colony mates is particularly important where it serves to facilitate cooperation amongst colony members and decrease the chances of social parasitism, theft of brood and food stores or killing of the colony (Michener 1974; Crosland 1990; Fishwild and Gamboa 1992). Social insects typically have well developed nestmate recognition abilities by which they admit nestmates, and exclude non-nestmates from their colony (Buckle and Greenberg 1981; Gamboa *et al.*

1986; Fletcher and Michener 1987; Venkataraman *et al.* 1988). In these instances discrimination may be effected at the level of group member recognition rather than the degree of kinship (Barnard 1991). If groups tend to be comprised of relatives (*e.g.* parent-offspring associations) such discrimination will necessarily be correlated with genetic similarity. According to Grafen (1990) kin discrimination at this level does not represent kin recognition (see however, Blaustein *et al.* 1991; Byers and Bekoff 1991; Stuart 1991).

Evolutionary explanations for kin bias are often based on the assumption that the animals discriminate among conspecifics according to their genetic relatedness (Hepper 1991). It is argued that it is only by responding differentially on the basis of genetic similarity that individuals can obtain the fitness benefits as espoused by Hamilton's kinship theory (Hamilton 1964a; 1964b). Experimental tests of this assumption and the mechanisms that enable such genetic discrimination are thus essential to theories of kin-selection.

Colonies of the naked mole-rat, a cooperatively breeding subterranean rodent (Jarvis 1981), have been shown to exhibit a marked xenophobic response to foreign conspecifics in the laboratory (Lacey and Sherman 1991). Intriguingly, neighbouring colonies, the most likely source of foreign conspecifics within naturally occurring populations, are also close genetic relatives (Faulkes *et al.* 1990; Reeve *et al.* 1990). Such high inter-colony relatedness is due in part to the high viscosity (low vagility) of naked mole-rat populations, with limited dispersal and the apparent formation of new colonies by fissioning (Brett 1991). Their close physical proximity makes them the most likely competitors in any interactions while their extremely high relatedness would suggest a limited potential for the use of genetic cues in inter-colony recognition. This raises interesting questions as to the mechanisms used by naked mole-rats to successfully recognise colony members and reject genetically similar (*i.e.* siblings), but foreign conspecifics.

The aims of this study were to investigate (1) the ability of the naked mole-rat to discriminate between foreign conspecifics and resident members of a colony in a homogeneous environment, (2) how kinship and familiarity affect recognition ability, and (3) the possible sources of cues used in discrimination and the maintenance of colony integrity.

METHODS AND MATERIALS

All animals used in these experiments were captive born and all experiments were performed in the laboratory. Except where noted, housing and maintenance procedures are the same as those given in Chapter 1.

Transfer experiments

The behavioural responses of resident mole-rats to a foreign mole-rat were investigated by transferring individuals from one experimental colony to another. The transferred mole-rat is refered to as the foreigner and the colony into which it is transferred as the resident colony. Foreigners were chosen at random (amongst non-breeders) from their colony and then introduced into the toilet chamber of the resident colony within one minute of their removal. The toilet chamber was chosen as the site of introduction as it was the least frequented location within the resident burrow system thus enabling the introduction of the foreigner with minimal disturbance to resident colony members. In addition, the latency time for foreigners leaving the toilet chamber was much less than for foreigners leaving either food chambers or digging arenas. This effectively decreased the time from introduction to contact between foreigner and resident mole-rats, minimising the duration of each trial.

Two 'relatedness classes' of foreigners were used in all experiments to explore the effects of familiarity versus relatedness. (1) Foreign kin, which were mole-rats that had been removed from their natal colony (the resident colony) at least six months prior to the onset of these experiments and housed together in small groups of same sexed individuals. (2) Foreign non-kin, which were mole-rats that were born and reared in a completely separate colony to resident mole-rats. While the exact degree of relatedness between foreign non-kin and resident mole-rats was not measured (*i.e.* by DNA fingerprinting), previous studies (Faulkes *et al.* 1990; Reeve *et al.* 1991) have shown that intra-colony relatedness is consistently higher than inter-colony relatedness.

A total of 80 trials were performed on four resident colonies (Fig. 2). These included 16 foreign kin trials, (4 foreign kin to each of 4 resident colonies), 44 foreign non-kin trials (11 foreign non-kin to each of 4 resident colonies) and, 20 control trials (5 resident mole-rats to their respective resident colonies). The latter experiment was designed to control for the experimental effects of handling and transferring mole-rats. The overall experimental design ensured that all resident colonies were exposed to a similar cross-section of foreigners, to minimise the possible effects of variation in inter-colony recognition/aggression. In addition, the random selection of individuals within source colonies ensured variation in the body mass of foreigners thus allowing an analysis of the effects of body size on the acceptance or rejection levels.

Diet and nest-bedding were the same in all colonies, minimising the possible influence of exogenous odour sources and thus allowing for the assessment of recognition responses in a relatively homogeneous odour environment.

The behaviour of the transferred individual, as well as its interactions with the colony residents it encountered, was recorded for a maximum of 10 minutes. Only 3 of the 60 trials involving foreigners lasted longer than 30 secs, and in all of these trials the foreign mole-rat was accepted. Acceptance or rejection of the transferred animal in each trial was noted on the basis of either aggressive (*i.e.* biting and toothfencing, *sensu* Lacey and Sherman 1991) or non-aggressive interactions (*i.e.* sniffing and ignoring) by resident colony members. No animals sustained any injuries during these trials as it was possible to intervene at all stages of the encounters. Each introduced mole-rat was usually tested against several members of the resident colony because more than one animal was usually active in the burrow system at the time of introduction. Control experiments were performed by removing an individual (< 1 minute) and then re-introducing it to its own colony.

Although it was not possible to control for behavioural idiosyncrasies of the introduced mole-rats, their behaviour was not observed to vary much. Foreign mole-rats typically advanced cautiously along the section of burrow that leads directly from the toilet chamber and it was here that all aggressive interactions between them and resident colony members were observed to take place. This section of burrow was designed to allow the experimenter immediate access to the animals, to ensure that resident mole-rats did not physically harm the introduced mole-rat.

Odour choice experiments

Nestmate recognition cues were investigated in a three-way choice test employing a T-maze (Fig. 1). All the non-breeders from two colonies (colonies 100 & 7400, n=45 and n=37, respectively) were used in this study. Each trial involved removing an individual from its colony and after a five minute adjustment period, introducing the mole-rat into the T-maze. Each mole-rat was presented with the choice of an odour source from its own colony, a foreign colony and a blank (a chamber containing nesting or toilet material that had not previously been in contact with any mole-rats). Two colony odour sources were used separately: the soiled litter of the communal toilet and the soiled bedding material of the nest. Each test lasted 10 minutes during which preference was inferred from the frequency and duration of attempts to gain access (by gnawing at the perforated partition) to an odour source. Each animal was tested once only, to minimise the effects of familiarity and learning on the results. The position of the different odour sources relative to one another was randomized to control for any directional bias amongst members of the two colonies. The entire apparatus was cleaned, wiped down with 70% alcohol and then rinsed with water between each trial to remove residual odours from previous animals.

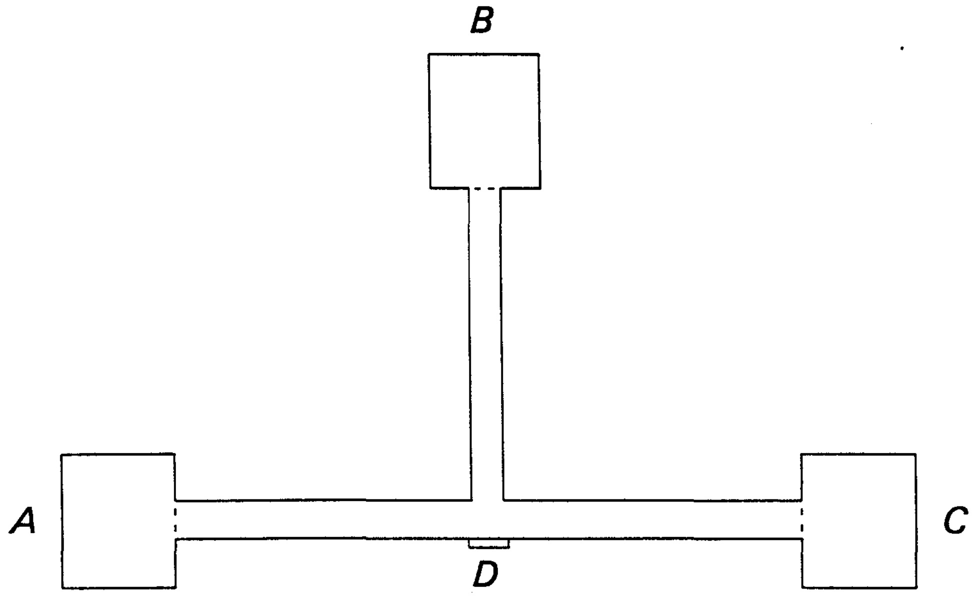


Figure 1. Odour choice apparatus. Individual mole-rats were introduced into the apparatus at D. A,B and C represent chambers into which the different odour sources were introduced. Perforations at the end of each arm enabled olfactory detection of th odour sources in each chamber. The arms of the apparatus were all interchangeable.

Odour manipulation experiments

To investigate the relative importance of an individual's odour versus the colony odour in nestmate recognition experiments we manipulated the odour of individual non-breeding mole-rats. The odours of individuals were altered by exposure to different odour sources. Forty-five trials were conducted (15/colony) using 15 individuals from each of three colonies. Individuals from each colony were subjected to three different odour environments, two experimental and one control. Experimental odours involved exposing individuals (5 individuals per odour) to either a foreign colony odour source or a blank colony odour source for a period of 12 hours and then re-introducing them into their natal burrow system. Exposure to a foreign colony's odour source was accomplished by introducing the mole-rat to a section of the burrow system of a foreign colony that was temporarily sealed-off from its inhabitants (to prevent physical contact). A mixture of soiled material from both the nest and toilet of the foreign colony were introduced to this sealed section to ensure that the animal was exposed to a diverse compliment of foreign colony odour cues. Blank colony odour sources were obtained by placing the mole-rat into a section of previously unused burrow provided with clean toilet (woodshavings) and nesting material (wood-wool). Control experiments were performed by confining an animal to a section of its own burrow system and thus exposing it to its own colony odour sources. When these animals, who had been exposed to these three different odour environments in separate trials were returned to their colony, the behavioural response of the resident mole-rats to these individuals was recorded. The aggression rate (and acceptance/rejection) was determined as the number of aggressive acts (*i.e.* shoving) per manipulated individual per trial.

Data Analyses

Acceptance or rejection of foreigners in transfer experiments was recorded as a categorical variable with two states, accept or reject. Here a single attack on the foreigner was sufficient for a trial to be categorised as reject. It was not possible to accurately record the aggression rate due to ethical considerations requiring that no physical harm be experienced by any of the interactants. This necessitated that the observer intervene whenever the potential for serious injury existed, effectively preventing an assessment of the aggression rate. Differences in the proportion of rejected versus accepted foreign kin and foreign non-kin, and males and females, were collated for all colonies and tested using the Fisher exact test for comparing proportions (Zar 1984). Differences in the body mass of accepted versus rejected mole-rats were explored using the Mann-Whitney U test for unpaired samples.

The reduced severity of aggressive encounters between interactants in odour manipulation trials enabled an assessment of the rate of aggression. These latter data were thus analysed using a multifactoral ANOVA with aggression rate as the dependant variable, odour treatment, colony and sex as factors and the manipulated individuals body mass as a co-variate. To determine the effects of an intruder on the general activity levels within the colony we compared the levels of activity of all non-breeders within the colony before the introduction and immediately after the removal of a foreign individual using a paired t-test. The relationship between the behaviour of resident mole-rats and their body mass were analysed using Spearman's rank correlation tests. All odour trials were analysed using the Friedman's analysis of variance model for related samples.

Ethical considerations

Preliminary experiments were performed to determine the most effective and yet least stressful means of testing the proposed hypotheses. Given the potentially harmful design of the transfer experiments it is important to point out firstly, that alternatives were considered before proceeding with the final protocol, and once adopted, precautions were taken to prevent the physical injury of animals in all trials.

I first attempted placing foreign individuals in separate containers that could be placed in olfactory but not physical contact with resident colonies. However, the results from these trials showed that there was no measurable response to either controls or foreigners. Resident mole-rats gnawed at the perforated dividing wall for both controls and foreign conspecifics. There was no evidence of threats (*i.e.* open mouthed gaping), and obviously no chance of mole-rats performing any other aggressive interactions in their natural repertoire. Furthermore, while mole-rats chose their own colony odours over foreign odours in the odour choice trials they never displayed any agonistic response to the foreign odour. Given these limitations it was decided to adopt a more direct method of measuring inter-colony aggression, however not without ensuring that no individuals were subjected to physical harm.

Of paramount importance was our ability to terminate any given experiment at the point when the foreigner and the residents had initiated the ritualised act associated with conspecific aggression but prior to the occurrence of any potentially injurious aggression. The former took the form of shoving, open mouthed gapes, incisor fencing, toothlocking and biting, typically followed by a rapid retreat by the foreign individual. Here biting as defined by Lacey *et al.* (1991:237) "the jaws of one animal close over the body of another animal" - does not imply that the skin is broken, indeed breeding females regularly bite subordinate colony members, most of which are her offspring. By ensuring that interactants could be immediately accessed at all stages during the experiment (by removing the roof of the burrow) I was able to intervene prior to the escalation of ritualised aggression. When the roof of the burrow is removed all mole-rats freeze, enabling the researcher to immediately terminate any interactions. I intervened in every experiment in which I witnessed an escalation in the ritualised form of aggression (*i.e.* all rejections). In this way no individual in any of the experiments sustained any physical injuries (*i.e.* the skin was never broken).

The apparently excessive duration of transfer trials (maximum of 10 minutes) was a function of the fact that foreign mole-rats, in preliminary trials, occasionally remained undetected in the resident colony for periods up to seven minutes. The decision to make the total time 10 minutes, was thus an attempt to cater for these instances. Once olfactory/physical contact had been made between a foreigner and a resident then the trial was invariably terminated within 30 seconds.

Transferred individuals in odour manipulation trials were typically shoved following their return to their natal colony. Other behaviours included biting (see above) and toothfencing. No physical injuries were sustained by any individuals. Transferred individuals were always monitored as this was an integral part of the experiment. Observations were terminated once colony activity and the behaviour of the transferee had returned to normal (usually within 10 minutes of re-introduction). A further point was that mole-rats exposed to foreign odours were not observed to show any overt signs of distress and all their exhibited behaviours were part of their normal behavioural repertoire when in their natal burrow system.

RESULTS

Transfer experiments

Naked mole-rats were highly xenophobic to foreign conspecifics. Resident mole-rats aggressively rejected foreign mole-rats (non-kin) in 93.2% of all trials (Fig. 2). There was no significant effect of sex ($Z=0.514$, $P>0.05$, Fisher exact test) on acceptance or rejection. Similarly there was no significant difference in the body mass of rejected versus accepted mole-rats (Mann-Whitney test, $U=0.102$, $P=0.978$).

Resident mole-rats showed similar levels of rejection ($Z=0.916$, $P>0.2$, Fisher exact test) of both foreign non-kin (93.2%) and foreign kin (100%) (Fig. 2). These results suggest that acceptance or rejection is dependent on recent association and familiarity of odours and not genetic relatedness.

Given that naked mole-rats live in the dark, have poor visual acuity and that introduced animals were always sniffed following physical contact, it seems likely that discrimination was achieved through olfaction. In all colonies discrimination and aggressive rejection of foreigners was positively correlated with the body mass of the aggressors (Spearman rank correlation test, $P<0.05$, for data presented in Figs 3 A-D); most foreigners being rejected by a small group of highly aggressive, physically robust individuals.

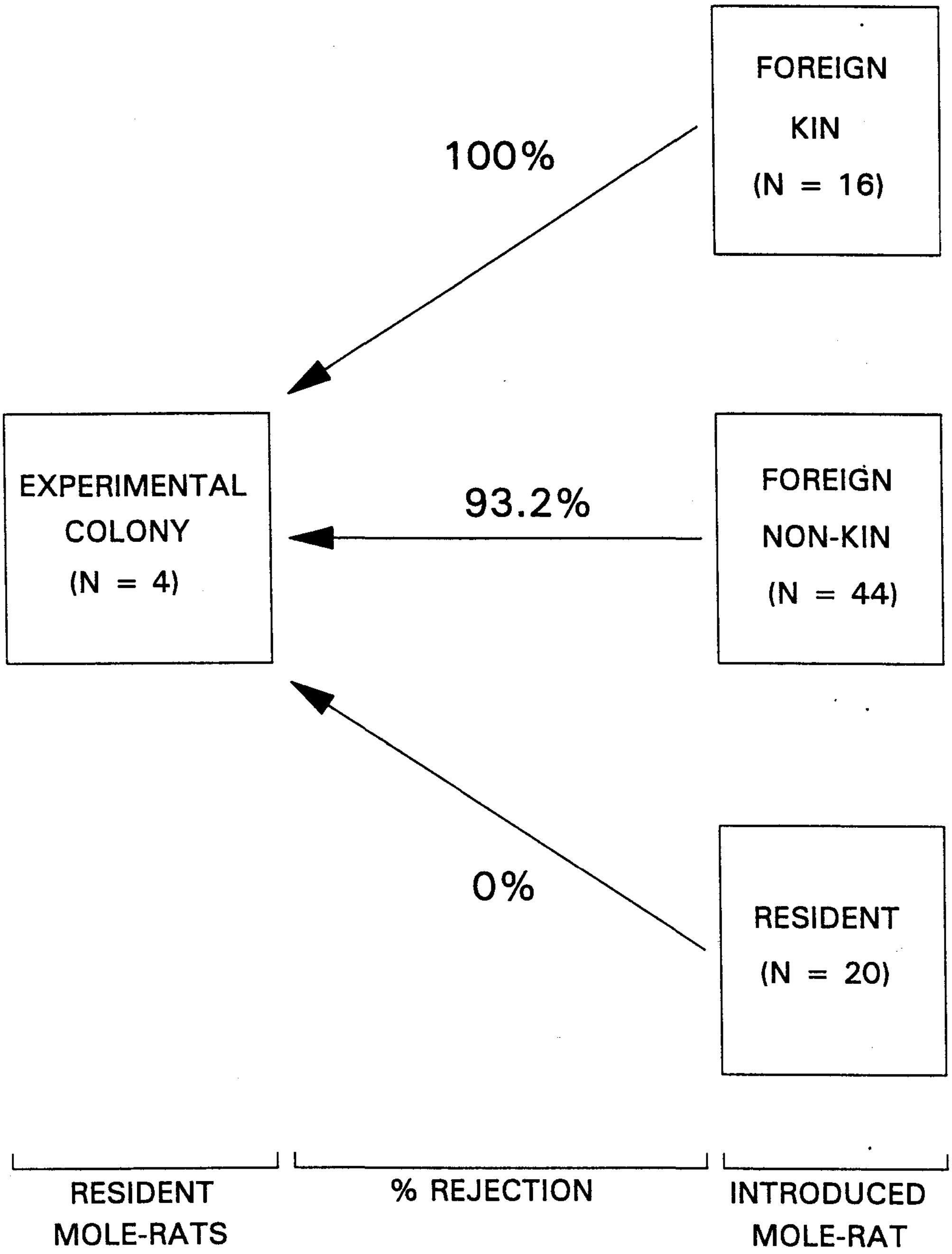


Figure 2. Types of introductions performed in transfer experiments and their subsequent percentage rejection by resident colonies. The number of foreign and control mole-rats for each 'relatedness/familiarity' class is provided in parentheses.

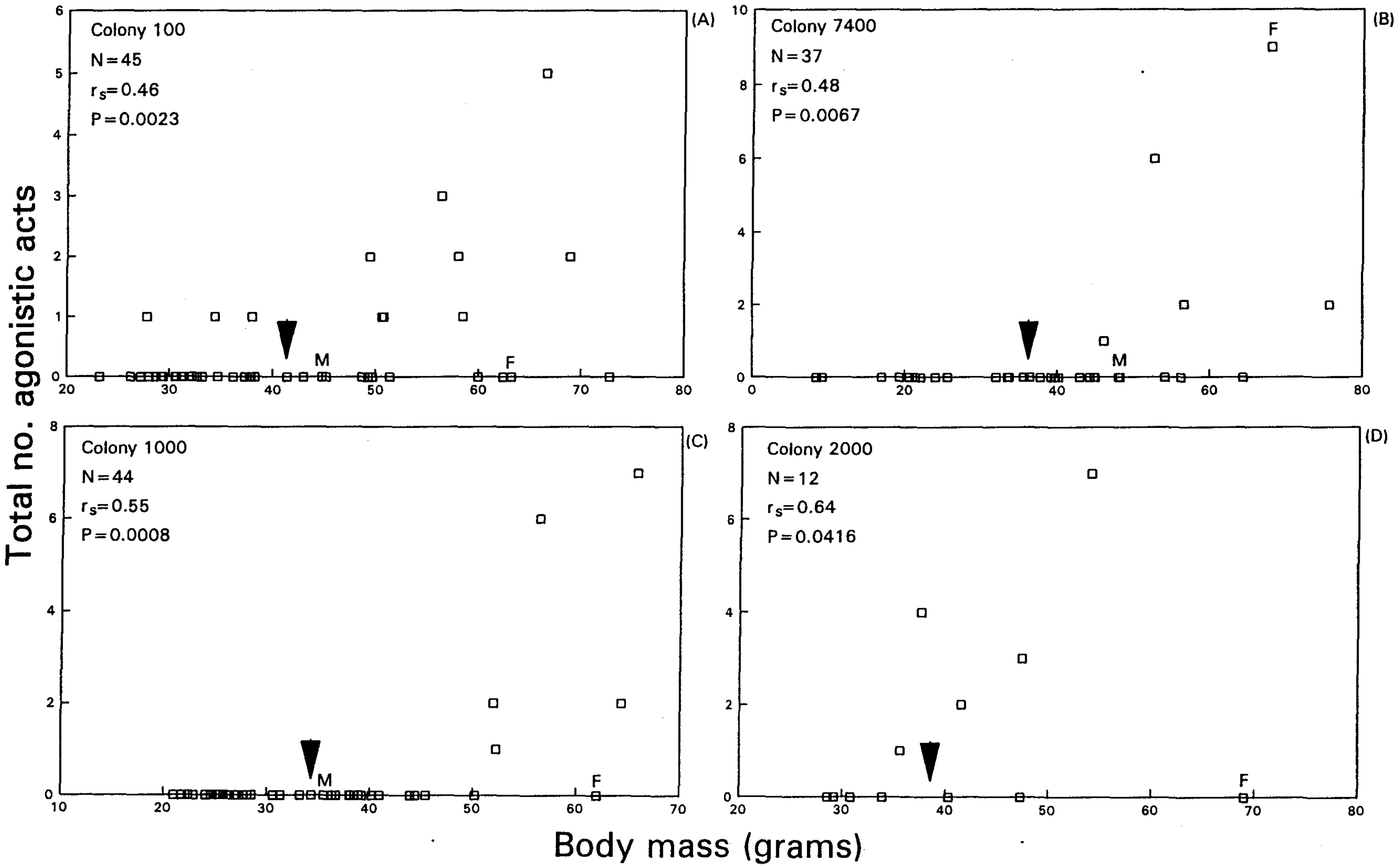


Figure 3. The relationship between aggression towards foreigners and the body mass of resident mole-rats. The mean body mass for each colony is indicated by the arrow. F = breeding female and M = breeding male.

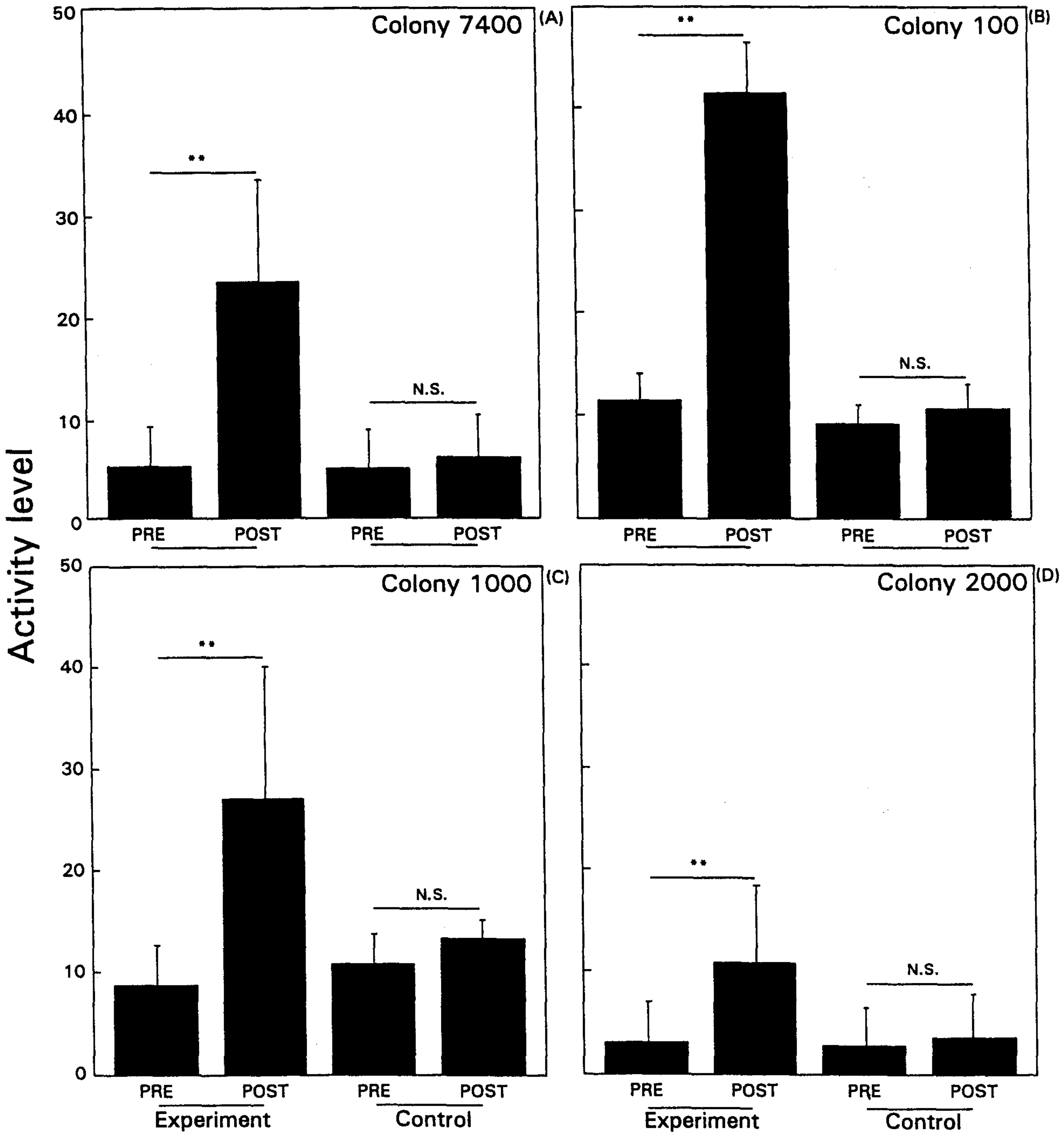


Figure 4A-D. The mean number (±SD) of mole-rats active within the burrow system before and after the introduction of a foreign (experimental) and resident (control) mole-rat. Experimental data: colony 7400, t=-5.17 $P<0.001$; colony 100, t=-14.68, $P<0.001$; colony 1000, t=-6.69, $P<0.001$; colony 2000, t=-13.56, $P<0.001$. Control data: $P>0.05$ for all colonies.

** $P<0.001$

N.S. not significant

Interestingly, despite being the principal colony defenders, these larger individuals were seldom the first to encounter introduced animals (see also Lacey and Sherman 1991). Smaller more active colony members were responsible for the majority of first encounters, a probabilistic outcome of their heightened activity in the burrow (Jarvis *et al.* 1991). Upon encountering a foreigner these individuals typically (92% of trials) retreated and emitted an alarm call (described by Pepper *et al.* 1991). Alarm callers invariably returned directly to the communal nest to alert colony members to the presence of an intruder. This behaviour resulted in a significant increase (paired t-test, $P<0.05$ for all colonies) in the number of mole-rats actively patrolling the burrow system (Figs 4 A-D), in addition to mobilising the colony defenders.

Reproductive animals, despite being amongst the largest individuals in the colony were, not observed to engage in colony defence against foreigners in three of the four study colonies (Figs 3 A, C and D). In contrast, the reproductive female in colony 7400 (Fig. 3B) was responsible for the majority of aggressive acts associated with the ousting of intruders. Controls for all transfer experiments indicate that introduction procedures did not significantly influence the behaviour of the resident colony members (paired t-test, $P>0.05$ for all colonies).

Odour choice experiments

The odour choice tests showed that mole-rats spent significantly more time (Friedman ANOVA, $P<0.001$ for all trials) attempting to access their own colony odours than foreign colony odours. Results were consistent for both colonies and for both experimental odour sources (Figs 5 A-D). Mole-rats in both colonies spent more time on average attempting to access the odour source from the nest than from the toilet. Close olfactory contact with soiled toilet material appeared to cause mild irritation of the mole-rats nasal region with many individuals grooming their nose between efforts to access the odour source. This behaviour reduced the total time that mole-rats spent attempting to access this odour source, as reflected in Figures 5C and 5D.

Odour manipulation experiments

There were no significant interaction effects between odour treatment, colony or sex, and consequently the main effects were calculated without them. Furthermore, body mass did not co-vary significantly ($F_{1,44}=0.005$, $P=0.947$) and was thus excluded from the analysis. There was a significant difference in the aggression rate between odour treatments ($F_{2,44}=20.46$, $P<0.0001$) with mole-rats subjected to foreign and blank odour environments being shoved significantly more frequently than mole-rats exposed to their own colony odour (Figs 6 A, B, C; Tukey multiple range test). There was no significant difference in the aggression rate of individuals

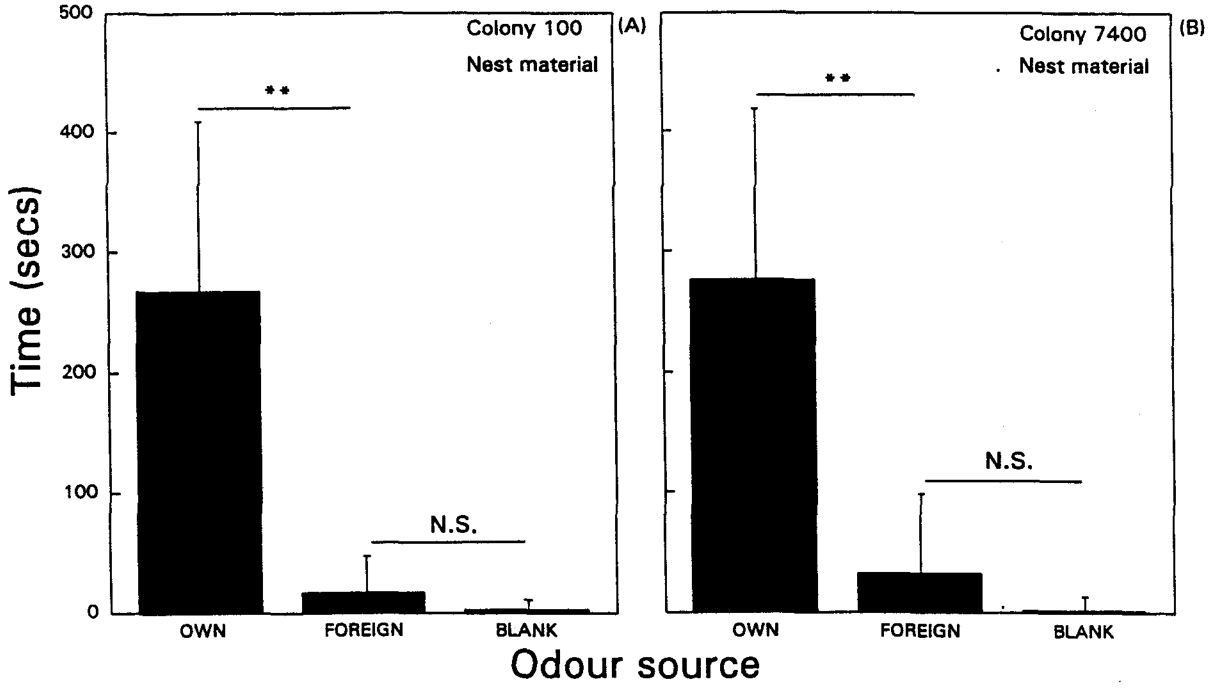


Figure 5A & 5B. The mean amount of time (±SD) mole-rats spent actively attempting to access the respective odour sources. Friedman ANOVA, $Xr^2=65.89$, $P<0.001$ for colony 100; Friedman ANOVA $Xr^2=50$, $P<0.001$. ** $P<0.001$, N.S. not significant. Odour source = soiled nest material.

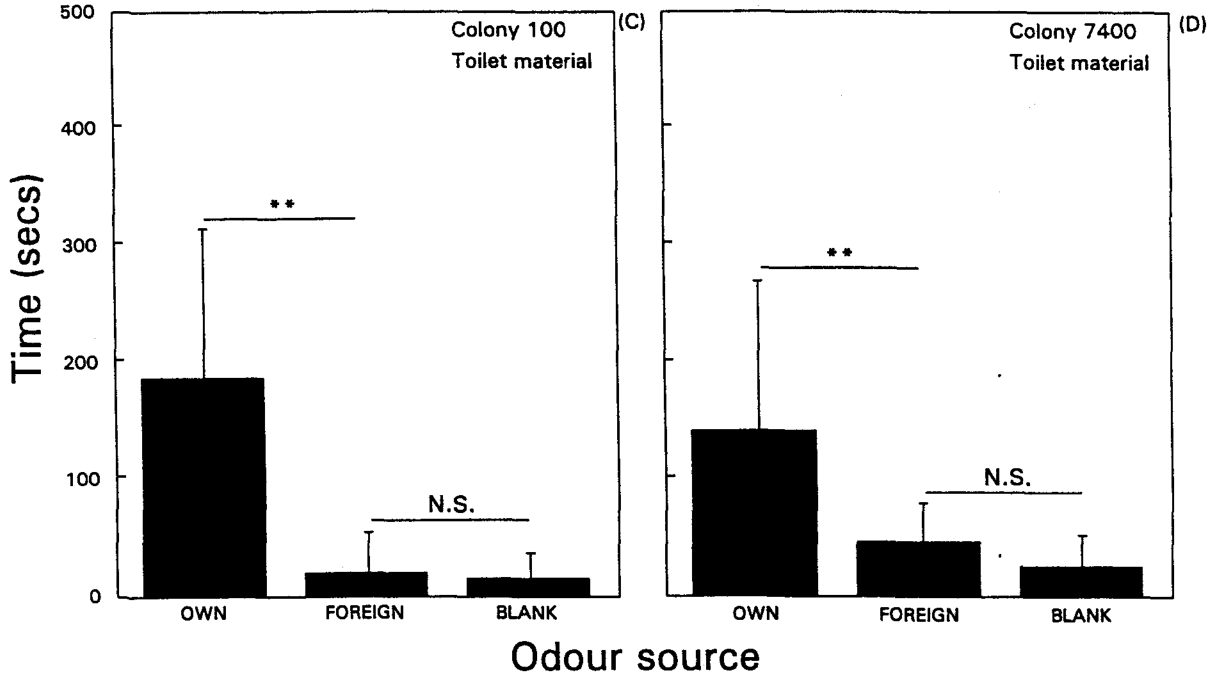


Figure 5C & 5D. The mean amount of time (±SD) mole-rats spent actively attempting to access the respective odour sources. Friedman ANOVA, $Xr^2=41.05$, $P<0.001$ for colony 100; Friedman ANOVA $Xr^2=32.76$, $P<0.001$. ** $P<0.001$, N.S. not significant. Odour source = soiled toilet material.

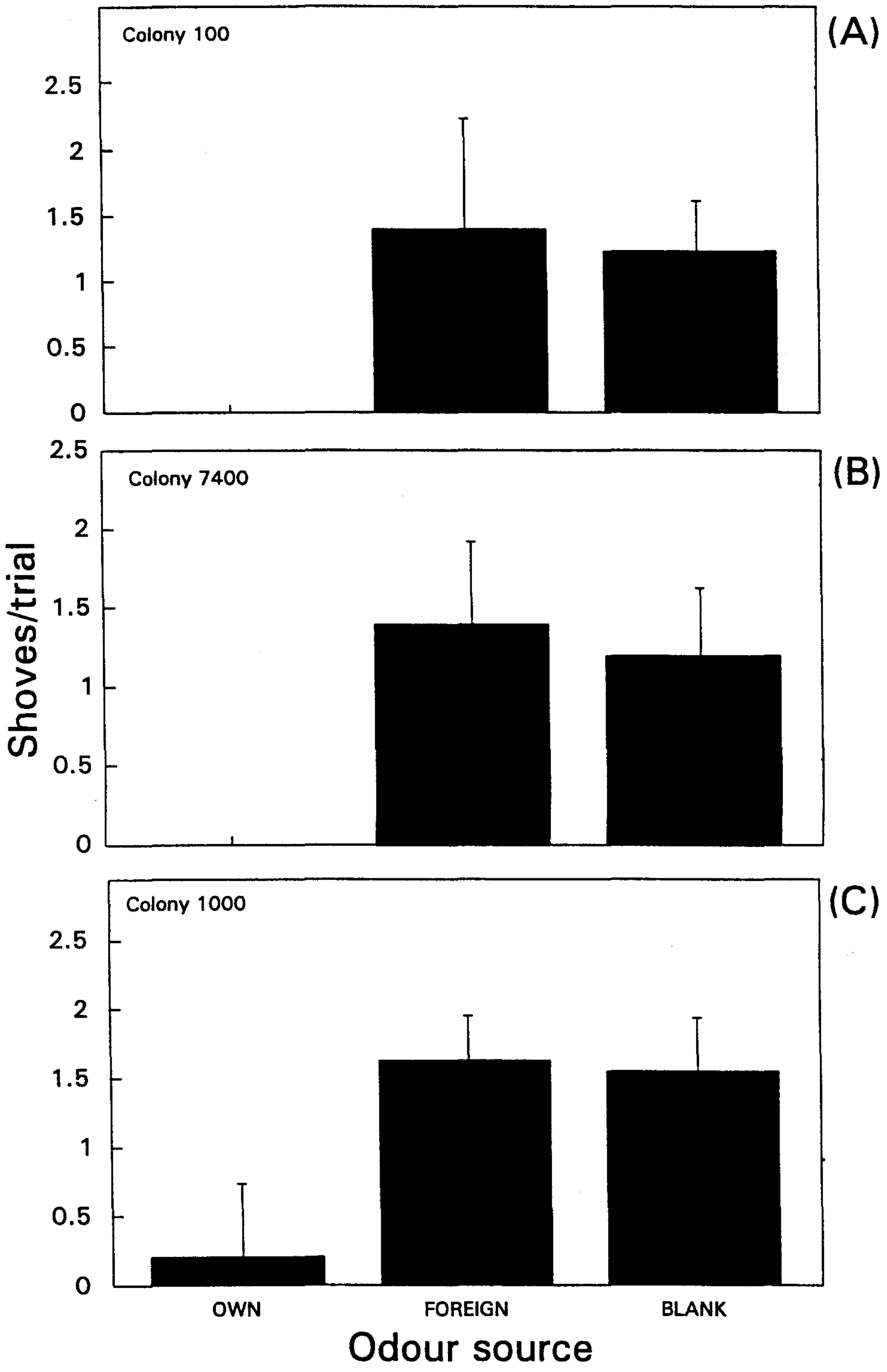


Figure 6. The mean number (±SD) of agonistic acts (shoves) performed by resident mole-rats on individuals that were returned to their colonies after separate exposure to three different odour sources.

exposed to either foreign or blank odour sources (Figs 6 A, B, C; Tukey test). There was a significant difference in the rate of aggression ($F_{2,44}=4.308$, $P=0.02$) between colonies 100 and 1000 (Tukey test), with the latter exhibiting the highest levels of aggression. The sex of manipulated individuals did not have a significant effect on aggression rates ($F_{1,44}=2.97$, $P=0.09$). All experimental animals were accepted back into their resident colony within 10 minutes (*i.e.* no more shoving or tugging at the skin).

DISCUSSION

These results demonstrate that naked mole-rats recognise colony members and aggressively discriminate against foreign conspecifics. This ability persisted despite controlling for exogenous cues in all colonies. Naked mole-rats however, fail to discriminate between foreign kin (siblings no longer resident in the colony) and foreign non-kin, and reject both. This indicates that genetic similarity *per se* is not the criterion used for discrimination. It is possible that because inter-colony relatedness in populations of naked mole-rats is high, foreign colony recognition systems based on a direct assessment of relatedness would lead to ambiguity. Furthermore, because naked mole-rats are predictably more likely to associate spatially and/or temporally with kin, and/or are more likely to be unfamiliar with non-kin, colony recognition mechanisms need not involve an ability to recognise genetic relatives (Sherman 1980; Davis 1982; Barnard 1991). The finding that colony members interact aggressively even with kin that have been removed from their natal burrow system for a short period (12 hours), suggests that odour familiarity in naked mole-rats needs to be continually reinforced if it is to serve as an effective mechanism of colony member recognition. This may be mediated by some of the communal behaviours of naked mole-rats. Thus all colony members are dependant on the communal nest for efficient thermoregulation and routinely use the communal toilet area. In both these places they may contribute to and acquire distinctive colony odours.

These results suggest therefore, that colony member recognition in the naked mole-rat is achieved through the mixing of individual odours to form a unique and dynamic recognition cue between colonies of closely related individuals. Thus, physically separated groups of siblings will contribute to and acquire a unique colony odour or 'badge' (*sensu* Linsenmair 1987). Similarly, newly acquired foreign odours are capable of inducing aggression in otherwise non-aggressive colony members. Similar findings have been reported for the spiny mouse *Acomys cahirinus* (Porter and Wyrick 1979) and house mouse *Mus musculus* (Hurst *et al.* 1993). In contrast, in the absence of familiarity, both Richardson's ground squirrels *Spermophilus richardsonii* (Davis 1982) and the white-footed mouse *Peromyscus leucopus* (Grau 1982) can still recognise their siblings.

Odour choice experiments suggest that both the nest and toilet areas are distinctively marked with colony odours. When individuals are given a choice between their own and a foreign colony odour, they are able to distinguish between them and preferentially choose their own colony odours. Because these odour sources are derived from all members of the colony, it is likely that colony odours themselves are a combination of individual odour cues. This 'cocktail' of odours is shared among all the members of a colony but would appear to be different from that of other, even closely related, colonies (such as those formed by siblings). The colony-specific group labels are thus reliable cues for the recognition of familiar colony members but convey no direct information on genetic relatedness.

The most likely recognition mechanism in naked mole-rats would thus appear to be through the sharing of familiar odours that are spread through close physical association. Holmes and Sherman (1982) state that sometimes animals treat as kin, those conspecifics with whom they have a close association during their life. Naked mole-rats thus exhibit kin bias, because under natural conditions there is normally a reliable correlation between genetic relatedness and the spatial/temporal component of association. This provides support for Barnard's (1991) theoretical prediction that local populations that tend to be viscous, with little immigration and dispersal, may use familiarity with individuals as a guide to relatedness and thus to decisions about behavioural bias.

This recognition mechanism in naked mole-rats closely parallels nestmate recognition cues in some social insects. Various authors have suggested that nestmate recognition in many social insects may have both genetic and environmental components (Wilson 1971; Jutsum *et al.* 1979; Carlin and Holldobler 1983; Page and Breed 1987; Gastreich *et al.* 1991; Holldobler and Wilson 1991; Venkataraman *et al.* 1992), and that colony odours, derived from the mixing of individual odours or a common environmental odour, may have evolved as the simplest and most effective means of facilitating nestmate recognition (Holldobler and Michener 1980).

The evolution of group living in naked mole-rats through natal philopatry, itself a consequence of severe ecological constraints which restrict the opportunities to disperse and found new colonies (Lovegrove and Wissel 1988; Jarvis *et al.* 1994), ensures that individuals in a given colony were more closely related to one another than to mole-rats in neighbouring colonies. Rejection of closely related foreign conspecifics may thus serve to ensure the maintenance of maximal levels of relatedness within colonies and thus increase the inclusive fitness benefits that accrue when cooperating with relatives. If the penalties for failing to exclude foreign conspecifics were trivial, colony-specific recognition and colony defence would have a limited selective advantage. However, both the existence of a highly efficient defence system in naked mole-rat colonies and the existence of a reliable colony member recognition mechanism, suggest that protection of the burrow system and its resources from potential usurpers is of paramount importance to their continued survival. Indeed the existence of a defence-based division of labour, with small individuals recruiting large colony defenders with alarm calls, suggests an evolutionary history of defence against conspecifics. Interestingly, foreigners never gave alarm calls while in the resident burrow system or following physical contact with a resident individual. These foreigners did however (94 % of all trials) give alarm calls following their return to their natal burrow system, suggesting that the alarm call of the naked mole-rat is context specific, serving to alert only colony members to the presence of foreign conspecifics.

A further important selective advantage of xenophobia in naked mole-rats may be that of the selfish 'desire' to reproduce. Despite reproduction being monopolised by a minority of dominant animals in the colony, every mole-rat born to that colony has the potential to be a reproductive (Jarvis *et al.* 1991) and colony members are therefore principal competitors in fulfilling this role. The acceptance of a foreign conspecific would only serve to diminish an individual's chances of attaining reproductive status and gaining the associated direct fitness benefits. Dispersal to avoid this competition is a costly alternative for naked mole-rats and one which appears to have been selected against, judging from its lack of occurrence (Brett 1991) and the fact that inbreeding is common both in the field (Faulkes *et al.* 1990; Reeve *et al.* 1990) and the laboratory (Jarvis 1991).

This suggested reproductive threat is supported by the finding that colony defence against foreign conspecifics is predominantly performed by the largest/oldest members of the colony (Figs 2 A-D). These individuals are the most likely reproductive replacements in the event of the death of either of the colony's reproductives (see Chapter 3). In another series of experiments designed to investigate reproductive succession in naked mole-rats, the new reproductives, both male (n=4) and female (n=4) were consistently amongst the largest (upper 5%) individuals in their colony (O'Riain, in prep.). Their large size and experience makes them worthy contestants for what is often a bloody battle to the death for reproductive rights within the colony (Jarvis 1991). It is these individuals, therefore, that stand to lose the most, in potential direct fitness terms, by accepting a foreign conspecific into their social hierarchy (tantamount to giving away free tickets in a small lottery). Furthermore, while colony defence is positively correlated with body mass in naked mole-rat colonies, the breeding female, typically one of the largest animals in the colony, rarely participates in colony defence (n=3 this study). The exception to this observation was in colony 7400 (Fig. 4B) in which the reproductive female had only recently acquired her sexual status through intense physical competition with colony members. This breeding female was consistently the most aggressive defender of the colony against foreigners, emphasising the close link between colony defence and reproductive opportunity.

The ability to maintain colony integrity, in the midst of closely related neighbouring colonies, is obviously important and reflects an evolutionary history of repeated contacts between colonies. I suggest that the use of a recognition system in which the individual odours are mixed to form a unique colony odour may have evolved to function primarily as a mechanism to ensure the acceptance of colony members and the rejection of non-colony members, and as a mechanism to permit the segregation of genetically similar kin in different neighbouring colonies. This is essential for the efficient functioning of individual colonies as separate entities (Stuart 1992). Naked mole-rat colonies are essentially independent functional units with a characteristic composition of a reproductive pair, their nonreproductive offspring and other close kin. Their efficient functioning may be dependant on this composition and their ability to maintain colony autonomy, in the midst of closely related neighbouring colonies, may be essential for survival.

CHAPTER SIX

FAT, PROMISCUOUS DISPERSERS IN NAKED MOLE-RAT COLONIES - OUTBREEDING IN AN INBRED SPECIES ?

SUMMARY

Behavioural, morphological and physiological evidence is presented for the existence of a dispersal phenotype within captive colonies of naked mole-rats. Potential dispersers were identified by providing all mole-rats within each colony with repeated opportunities to escape from their natal burrow system and interact with foreign conspecifics. Contrary to the xenophobic response noted for non-escapees, frequent escapees showed a significant preference for foreigners in both triplet associations and mate choice (using a T-maze) experiments. Furthermore, when presented with the choice of foreign breeding versus non-breeding females, both escapees and sexually active males showed similar responses, namely a significant preference for the reproductive female. Thus a strong urge to escape from the natal burrow and a significant preference for foreign conspecifics provides strong evidence for the existence of dispersers within naked mole-rat colonies. Hierarchical cluster analysis showed that these dispersers were behaviourally more similar to one another, within the colony context, than to any other colony members.

Whilst dispersers were significantly heavier than a random sample of same aged non-dispersers, there was no significant difference in the skeletal robustness (excluding incisor width, which was greater for non-escapees) between the two. This mass difference appeared to be a consequence of a significantly higher percentage body fat of dispersers as determined from EM-SCAN analyses. Dispersers had significantly higher levels of circulating luteinising hormone than non-dispersers and showed a significant increase in LH levels following the injection of 1.0 μl of GnRH. This supports behavioural observations that dispersers are sexually primed before attempting to disperse from their natal colony. Together, these findings support recent field evidence for the occasional formation of nascent colonies from pairs and small groups containing unrelated (by common parentage) individuals and suggest that, although rare, dispersal and outbreeding may occur within naked mole-rat populations.

INTRODUCTION

Localised populations of naked mole-rats exhibit the highest known coefficient of inbreeding (F=0.45) yet recorded among free-living mammals (Reeve *et al.* 1990). While it is possible that this value reflects historical genetic bottlenecks, or strong selection for survival in a specialised niche (Nevo 1979), close inbreeding (parent-offspring and sib-sib matings) has frequently been observed in captive colonies. Such inbreeding has largely been attributed to the prohibitive costs associated with dispersal and outbreeding and the limited survival probabilities of small nascent colonies (Jarvis 1985; Lovegrove and Wissel 1988; Brett 1991; Jarvis *et al.* 1994).

Close inbreeding is known for a variety of mammalian species *(e.g. Marmota flaviventris*, Schwartz and Armitage 1980, Armitage 1986; *Microtus pennsylvannicus*, Selander 1970; *Ochotona princeps*, Smith and Ivins 1987; *Procavia johnstoni*, Hoeck 1982), in which the high probability of mortality during dispersal makes helping and delayed maturation a relatively secure fitness option. Inbreeding is usually associated with lowered fitness such as inbreeding depression and susceptibility to pathogens (Ralls *et al.* 1986) and it is generally suggested that inbreeding behaviour is strongly selected against in most species (Dobzhansky 1970; Bengtsson 1978). Indeed, in all of the above mentioned examples of inbreeding, dispersal and inbreeding avoidance mechanisms do exist to promote some degree of outbreeding (Smith 1993). Similarly, in certain eusocial insects, cycles of close inbreeding are typically punctuated with a dispersal phase and obligate outbreeding (Bartz 1979) although the functional significance of this remains controversial (see Myles and Nutting 1978).

In contrast, genetic evidence suggests that naked mole-rats are obligate inbreeders (Reeve *et al.* 1990), and Brett's field data (1986;1991) indicate that new colonies form through fission from the parent colony. While occasional inbreeding is especially deleterious in normally outbred species, due to the exposure of deleterious homozygotes to selection; constant ecological pressure selecting for inbreeding results in these deleterious alleles being 'weeded out' of the genome and inbreeding becomes progressively less costly. Thus naked mole-rats routinely inbreed with no apparent negative consequences.

The apparent lack of a disperser phenotype in naked mole-rat colonies was discussed by Dawkins (1989) who noted that the most puzzling feature of naked mole-rats is that, although they are like social insects in so many ways, they seem to have no equivalent caste to the winged reproductives of ants and termites. This was so surprising to him that he speculated that a dispersal phase would indeed be discovered. He proposed (rather tongue in cheek) that this 'caste' of dispersing individuals might take the form of warm blooded (naked mole-rats show poikilothermic traits) hairy individuals, whose emergence would be triggered by particular environmental conditions that have not been realised this century - hence dispersers have remained undiscovered.

The apparent lack of incest avoidance in naked mole-rats, the absence of any obvious dispersal phase in captive colonies, the high inbreeding coefficients of wild populations, and circumstantial field evidence, have all suggested that colonies usually formed through fission. However, recent discoveries in our laboratories of behavioural and morphological idiosyncrasies amongst captive bred individuals, in addition to Braude's (*pers. comm.*) finding that in Northern Kenya a few nascent colonies contained unrelated animals, suggest that outbreeding may sometimes occur.

MATERIALS AND METHODS

Study animals

All the mole-rats used in this investigation were housed under the same laboratory conditions (see Jarvis 1991 for details). Mole-rats were fed daily on a diet of mixed fruit and vegetables (*ad-libitum*), supplemented with a commercially prepared, nutritionally balanced, grain cereal (Pronutro^{TM}). Individuals from 16 colonies were used in this study. Colony size varied from 2 to 91 individuals. Potential dispersers were identified from three of the larger colonies (see below), whilst the remaining 13 colonies provided source animals for choice experiments.

Dispersal and outbreeding opportunities.

Three large colonies (Col. 100, n=45; Col. L4, n=86 and Col. L2, n=41 individuals) with a history of persistent escapees were studied. All three colonies were functionally complete (*i.e.* breeders and non-breeders present) and reproductively active, although only colony 100 had successfully raised offspring in the six months prior to this study.

The mole-rats in these colonies were provided with multiple opportunities to escape through a single small opening in the burrow system. As a general behavioural rule naked mole-rats avoid open spaces and refrain from leaving the 'safe' confines of the burrow system. Mole-rats that exited their burrow system were caught in pit-fall traps (depth 10cm, diameter 45cm) partially filled with wood shavings. Traps were checked approximately every hour, over 12 hour periods from 09h00 to 17h00 and the sex, body mass and identity of all mole-rats found in the traps were recorded before they were returned to the burrow system. At the end of each 12 hour period the opening in the burrow system was closed.

Individuals that repeatedly escaped (*i.e.* $\geq$3 escapes) over three consecutive days, were used in a series of triplet associations to assess whether they exhibited xenophobic (*sensu*

Lacey and Sherman 1991; Chapter 5) or affiliative behaviour when presented with a foreign conspecific or, as a control, an animal from their own colony. Triplet associations were thus comprised of two colony members, the frequent escapee (hereafter refered to as the escapee) and a non-escapee and a mole-rat from another colony.

The experimental procedure was as follows: frequent escapees were removed from their colony and placed alone in a circular test arena (35cm diameter) for 10 minutes. A mole-rat from the escapees' own colony and a foreign mole-rat, both of the same sex and similar in size, were then introduced simultaneously to the arena. The behavioural interactions of all individuals were recorded on a checklist over a five minute period. Interactions were classified as aggressive (alarm call, hissing, shoving, open mouthed gape) or affiliative/sexually solicitous (sniff without aggression, mating call, anogenital and body nuzzling, *sensu* Lacey *et al.* (1991)). Care was taken to ensure that no individuals were harmed during any of the experiments.

Frequent escapees that exhibited a preference for foreign conspecifics over familiar colony mates were then subjected to the following manipulations in an effort to quantify their response to foreign conspecifics (versus the response of non-escapees) and to characterise their phenotype.

Mate choice experiments

A series of three-way choice experiments (*sensu* Chapter 5) were used to quantify the finding that frequent escapees showed a preference for foreign, unrelated conspecifics versus familiar, related individuals. In addition, the response of frequent escapees was compared to that of sexually primed males. Sexually primed males were males that had been removed from their natal colonies at least 6 months prior to these experiments. Faulkes *et al.* (1990) have shown that males housed alone are reproductively active (having similar LH profiles to breeding males within colonies), and show sexually solicitous behaviour toward foreign conspecifics.

Escapees and control non-escapees were presented with a choice of a foreign male and a familiar female (sibling) to test the effects of familiarity on choice. Preliminary choice experiments had revealed that both foreign non-breeding males and females were equally attractive to escapees ($F_{2,10}=3.02$, $P>0.05$). However, Westlin *et al.* (1994) have clearly demonstrated that there is considerable variation in non-breeding female sexual activity/receptivity. Thus to eliminate this potential source of error, only foreign males were used to assess the effects of familiarity on choice. Secondly, escapees and singly housed reproductive males were presented with the choice of a foreign breeding female and foreign non-breeding female. Foreign animals, excluding breeding females, were chosen randomly from 13 possible source colonies.

Each test lasted 10 minutes during which preference was deduced from the frequency and duration of attempts to gain access to either of the source mole-rats (*sensu* odour choice experiments in Chapter 5). Test subjects were then allowed access to the individual of their choice to enable an interpretation of the motivational context of preference. Each animal was tested once in both experiments, so familiarity and learning effects did not influence the results. The entire apparatus was washed with biocide^{TM} and then wiped down with 70% alcohol, between each trial, to remove residual odours from previous animals. Control experiments were performed by providing animals with a choice between two similar animals (*e.g.* escapees were provided with the choice of two same-sexed foreign individuals). Variation in response of escapees and non-escapees was analysed using a one-way analysis of variance model (ANOVA). Comparisons of individual means were made *post-hoc* using Tukey's multiple range test.

Behaviour and morphology of escapees versus non-escapees within the colony context.

Behaviour: Behavioural data were recorded for all members of colony #100 (n=45 individuals) which contained six frequent escapees (identified in the above experiment, see Fig. 1). All mole-rats were distinctively marked with permanent ink. The behaviour (see Appendix I) and location of each animal within the burrow system was recorded at two minute intervals (Scan sampling, Altmann 1974), for minimum periods of one hour. These periods were distributed randomly between 09h00 and 19h00 hours. In addition, rare behaviours (*e.g.* mating) were recorded continuously until completed. Behaviours were recorded for 40 hours over a period of one month. This included parturition and thus incorporated important behaviours relating to pup-care and postpartum oestrus and mating.

A minimum of 1200 acts were recorded per colony member (54 000 acts for the whole colony). From these data, individual behavioural profiles were compiled and sub-groups of individuals within the colony were identified using hierarchical cluster analysis (with group average linking), on the basis of percentage similarities between individual behavioural profiles. Prior to all analyses, the data were root-root transformed to avoid biasing the results in favour of those behaviours performed at either very high or low frequencies. Analyses were performed using PRIMER (Clarke and Warwick 1994), a statistical software package designed for multivariate analysis of community data. No behaviours relating to either triplet associations or escape frequencies were included in these analyses as they would obviously bias the results. Behaviours included were thus all 'normal colony behaviours'.

Morphology: All colony members were weighed weekly and X-rayed at the end of the study. Prior to X-raying, the mole-rats were anaesthetised by Halothane inhalation and then placed prostrate, ventral surface down, on the X-ray plate. All radiography was done with a Shimadzu medical X-ray unit. Mammography film was used to provide detailed outlines of both hard and soft body tissues. The lowest possible dosage was used for all X-rays (40 Kilovolts; 25 milliampere). The entire procedure lasted about five minutes per mole-rat, and no mole-rat suffered any short or long term debilitating effects. Body length (tip of nose to base of tail) and body girth (immediately posterior to the forelimbs) were measured from the anaesthetised animals using a piece of calibrated (in mm) inflexible nylon.

Seven skeletal variables were measured from the X-ray of each individual (see Appendix II): width of upper incisors, distance between zygomatic arches, length of skull, length of lumbar vertebra (number five counting from the posterior), length of pelvis, width of skull (parietals) and length of body (excluding tail). These variables were chosen according to two different criteria, (1) accuracy of measurement, and (2) functional significance (*e.g.* robustness of skull, in particular those bones relating to digging). Measurements for escapees and non-escapees of a similar mean body mass were then compared in a series of paired t-tests.

Fat store analysis

Frequent escapees differed in appearance from non-escapees in having what appeared to be large folds of subcutaneous fat, primarily in the neck and thoracic region. These differences were also evident in soft tissue outlines on X-rays (see Plate 1). To test whether escapees do in fact have significantly greater percentage body fat than non-escapees the following method was used.

The percentage body fat of a random sample (drawn from a hat) of escapee (n=7) and a random sample of similarly sized (n=10), similarly aged non-escapee (from the three colonies used in the above experiment) was measured with an EM-SCAN Model SA-1. This non-invasive technique for the determination of lean body mass and fat stores has been used successfully for birds and mammals in a size range of 40-600g (Walsburg 1987). All the mole-rats used were within this size range (Range = 44-72g). Care was taken to ensure that all study animals were maintained under similar environmental conditions (temperature and humidity) immediately prior to all measurements (*sensu* Walsburg 1987).

Each mole-rat was anaesthetised by Halothane inhalation. Body shape was estimated by measuring body length (nose to tail base) and girth (body circumference immediately posterior to the forelimbs) prior to placing the animal on the sled of the instrument. All EM-SCAN measurements were made with each animal placed on its dorsum with spine straight and aligned along the long axis of the chamber. Five one second measurements were made over a one minute period and an average EM SCAN reading obtained. Rectal temperatures were monitored on all anaesthetised animals to ensure that body temperatures did not vary by more than $1^{O}C$ between samples. Differences in percentage fat were analysed using an unpaired t-test. The distribution of body fat was also examined through dissection of a single deceased escapee.

Blood sampling

To assess possible differences in reproductive activity, levels of circulating luteinising hormone (LH) were measured in a random sample of escapees (n=6 males), similarly sized non-escapees (n=10 males) and breeding males (n=2). The small sample size for breeding males was a consequence of the difficulty of obtaining unequivocal evidence on breeding status for males (*i.e.* observations of mating). It was thus not possible to include breeding males in statistical analyses but hormonal data were included to enable qualitative comparisons with the other two groups.

In addition, LH responses to administration of exogenous gonadotrophin releasing hormone (GnRH) were determined in an attempt to compare the degree of reproductive suppression in the three groups. In an attempt to standardise sampling between colonies the challenges were given at approximately the same stage in the reproductive cycle of the breeding females within each colony (Westlin *et al.* 1994). All blood samples were taken between 14h00 and 16h00. Individuals were hand held and blood was obtained from veins on the dorsal surface of the foot (with a sterile needle). Approximately 300-400 microlitres of whole blood were collected using heparinized micro-haematic tubes before, then 20 minutes after, subcutaneous administration of 1.0μg GnRH in 200μl saline (*sensu* Faulkes *et al.* 1991). LH was measured using a mouse leydig cell bioassay (Van Damme *et al.* 1974), as previously described and validated for naked mole-rats by Faulkes *et al.* (1990).

RESULTS

Escaping and outbreeding opportunities.

Only a few (13.3% col. 100; 4.6% col L4; 9.7% col L2) mole-rats within all three study colonies exhibited a marked escape response from their natal colonies (Fig. 1). Thirteen of the of 14 frequent escapees were males. No frequent escapees responded aggressively to foreigners in triplet associations, indeed the majority of them displayed sexually solicitous behaviour towards foreigners despite often being the same sex as the foreigner. In contrast, control non-escapees always behaved aggressively towards foreign conspecifics. There was no sexually solicitous behaviour between the individuals from the same colony (escapees and non-escapee controls).

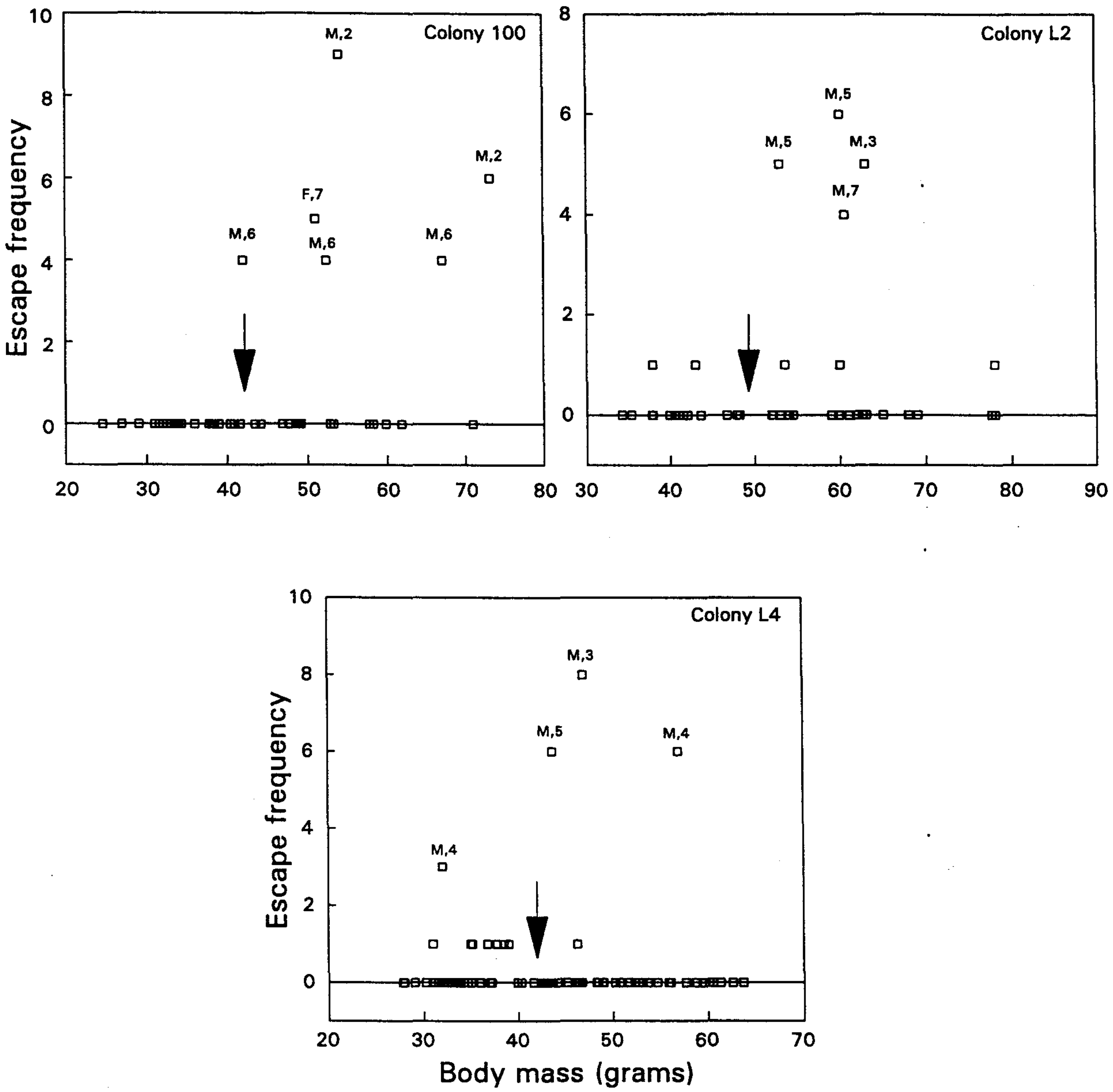


Figure 1. The frequency with which individuals from three different colonies (N>40 animals/colony) escaped from their burrow system over a period of three consecutive days. Individuals that left their burrows were caught in pitfall traps positioned about 30cm from the point of escape. M=non-breeding males and F=non-breeding females. The number adjacent to these letters corresponds to the respective litter of each frequent escapee (≥3 escapes). The arrow denotes the average body mass for each colony.

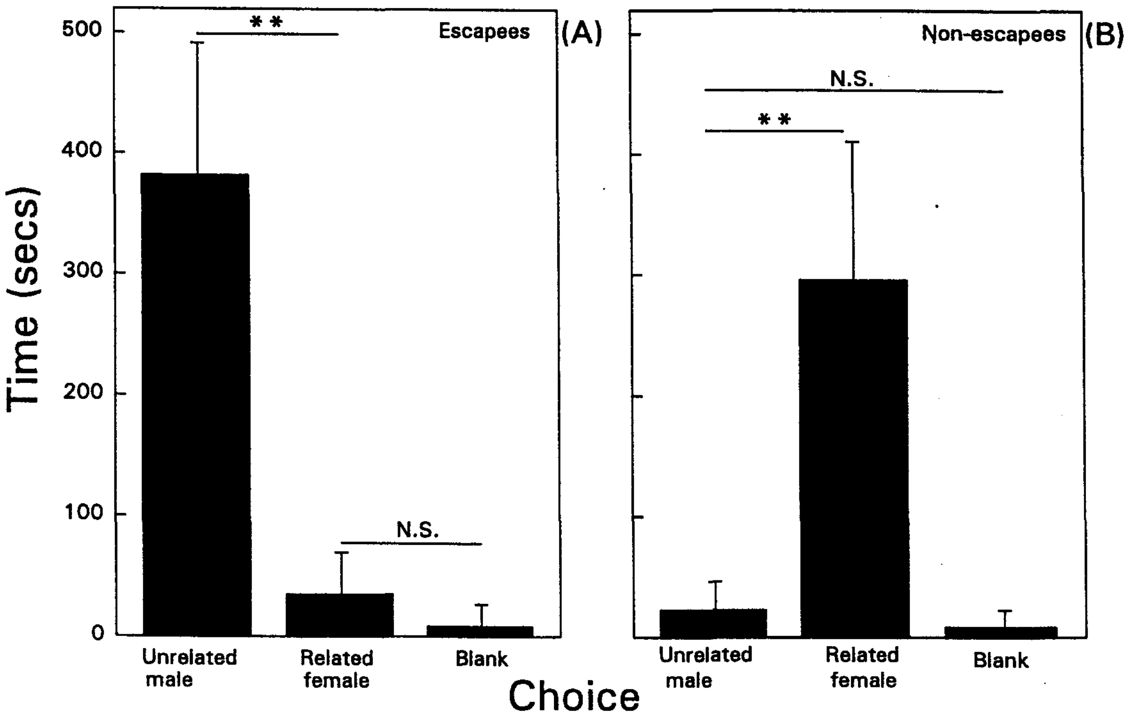


Figure 2A & 2B. Results from a three-way choice experiment in which the mean amount of time (±SD) escapees (n=19) and non-escapees (n=16) from the same colony spent attempting to access chambers containing either unrelated or related individuals or an empty chamber. ** P<0.05, N.S. not significant.

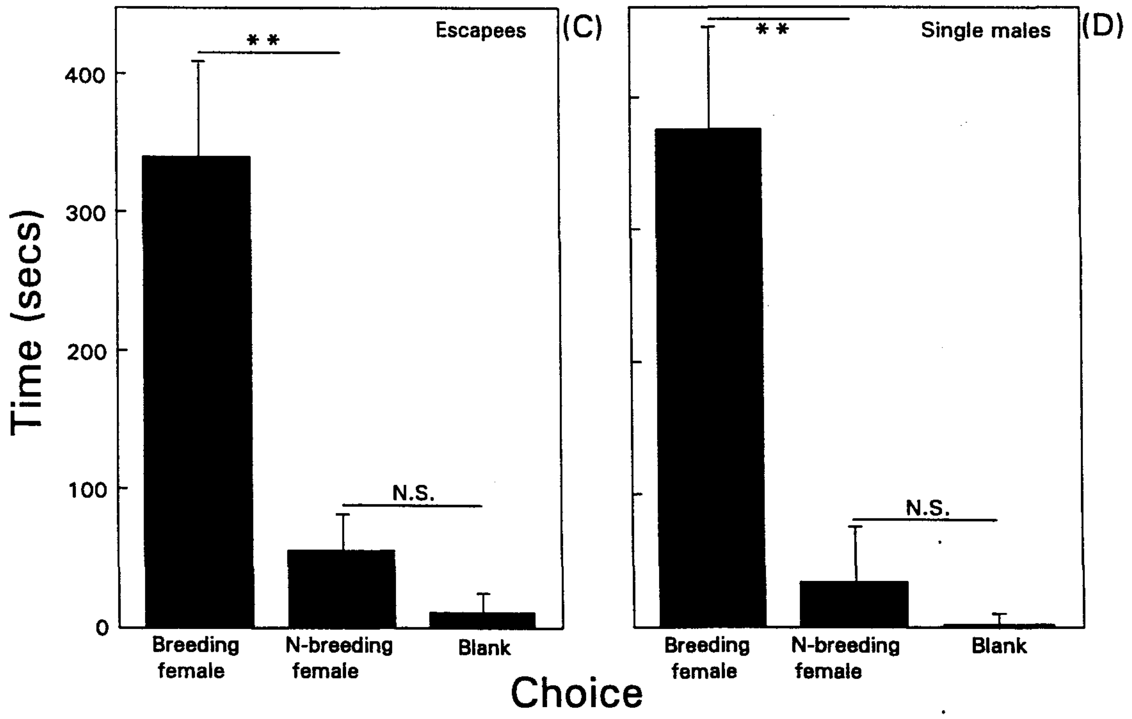


Figure 2C & 2D. The mean amount of time (±SD) escapees (n=16) and singly-housed males (n=19) spent actively attempting to access chambers containing a breeding female, a non-breeding female from the same colony or an empty chamber (blank).
** P<0.05, N.S. not significant.

Mate choice experiments

Frequent escapees spent significantly more time attempting to access the chamber of a foreign mole-rat than that of a familiar colony member or a blank chamber ($F_{2,18}=64.73$, $P<0.0001$, Fig. 2A). Non-escapees in contrast spent significantly more time attempting to access familiar colony members than either a foreign mole-rat or the blank chamber ($F_{2,15}=30.09$, $P<0.0001$, Fig. 2B). All frequent escapees showed solicitous behaviour, and attempted to copulate with foreign mole-rats, despite the fact that foreigners were all male, and typically behaved aggressively toward them.

The response of singly housed males and frequent escapee males to a choice of breeding versus non-breeding females was similar (two-way ANOVA $F_1=0.01$, $P=0.9225$). Both groups showed a significant preference for foreign breeding females over foreign non-breeding females ($F_{2,15}=89.141$, $P<0.0001$, single males; $F_{2,18}=107.92$, $P<0.0001$, frequent escapee; Figs 2C & 2D). There was no significant difference between the amount of time spent attempting to access non-breeders and blanks (empty chambers) for both groups.

Behaviour and morphology of escapees within the colony context.

Behaviour: A litter of about 12 pups was born during this study. Although no pups survived longer than one week postpartum, all colony members were in contact with the pups, enabling an assessment of their participation in pup-care (*e.g.* pup-carrying). Mating was observed nine days postpartum, and the behaviour of the breeding female and all those who consorted with her was noted over approximately two and a half hours of *ad-lib.* sampling. Only male #9 was observed to successfully copulate with the breeding female. This individual was the son of the breeding female and is thus an example of close inbreeding within a naked mole-rat colony. Only one other male (#17, her son) attempted to mate the breeding female, he repeatedly mounted her but did not achieve intromission. None of the escapees attempted to mate her, despite passing over her whilst she was both unattended and in full lordosis (n=8 observations).

Results from the hierarchical cluster analysis indicate that the behavioural profiles of escapees are different from those of similarly aged and/or similarly sized non-escapees. Individuals 5,16,30,29,28 and 45 who were all classified as frequent escapees in the dispersal trials, all clustered within the same sub-group on the dendrogram (see Fig. 3). This suggests that the behavioural profiles of these individuals are more similar to each other than to any other colony member. This was confirmed by performing an analysis of similarity (*sensu* Clarke and Warwick 1995, see Chapter 3 for details), with escapees having significantly different behavioural profiles to all other colony members ($F_{2,44}=0.45$, $P=0.001$).

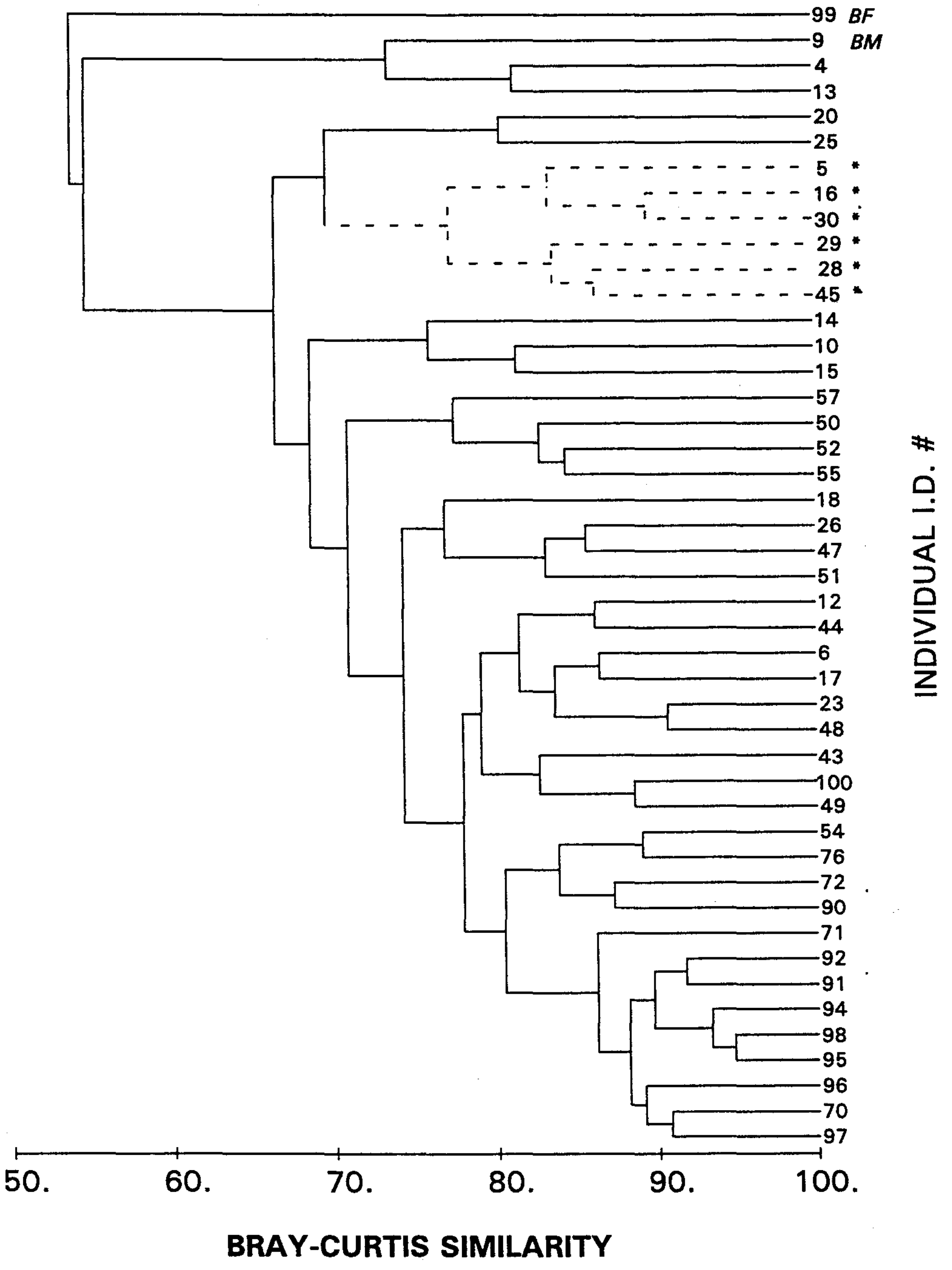


Figure 3. Dendrogram derived from an agglomerative hierarchical cluster analysis of the behavioural profiles of 45 individuals within colony 100, including the breeding pair. Each number represents an individual. Numbers denoted with a * that are joined together with a dashed line are those individuals identified in escape trials as frequent escapees. BF=breeding female; BM=breeding male.

Subsequent comparisons revealed that these six individuals exhibited significantly higher mean frequencies (X=0.72 acts/hr, S.D.=±0.58) of locomotion (walking and running) within the burrow system when compared to a random sample of same aged non-escaping individuals (X=0.21 acts/hr, S.D.=±0.065) (two tailed t-test, t=2.95; P=0.014). They also spent significantly more time eating (two tailed t-test, t=3.125; P=0.01) than similar sized non-escapees. In contrast cooperative behaviours (*e.g.* sweeping and the transport of nest material) were performed at a lower frequency, although this difference was not significant (X=0.037±0.02 acts/hr for frequent escapees; X=0.233±0.226 for non-escapees; two tailed t-test, t=-2.13, P=0.058). There was no significant difference (two tailed t-test, t=-0.15, P=0.88) in the number of dominant aggressive encounters with the breeding female (*i.e.* she did not shove them more) experienced by escapees versus non-escapees.

Morphology: Escapees in colony 100 (n=6) were significantly heavier than a random sample of same sexed and same aged non-escapees (X±S.D.=56.57±11.45g for escapees; X±S.D.=40.67±5.57g for non-escapees; t=2.889, P=0.016). Controlling for this effect of body mass (t-test for mean masses of escapees versus non-escapees: t=0.22, P=0.823), there was a trend for non-escapees to exhibit greater robustness in all skeletal variables measured. However, only one skeletal variable, incisor width, differed significantly between non-escapees and escapees (X±S.D.=3.63±0.342mm for non-escapees; X±S.D.=3.2±0.236mm for escapees; t=1.27, P=0.028). Interestingly, all escapees showed evidence of a sharp increase in body mass (see Chapter 2) at some point prior to this study. It is possible that this rapid increase in mass is associated with the deposition of fat stores, as suggested by the findings below. There were insufficient escapees/colony to test this relationship in either colony L4 or L2.

Fat store analysis

Escapees had a significantly higher percentage body fat than similar sized non-escapees (t-test, t=2.58, P=0.02, Fig. 4A). Furthermore, escapees exhibited a significantly greater girth/body length ratio (t-test, t=6.04, P=0.00005, Fig. 4B), resulting in a qualitatively different body shape (short and stout, see Plate 1). Dissection of a deceased escapee revealed large deposits of subcutaneous fat in the neck region, immediately lateral (dorsal surface) to the lumbar vertebrae and between the base of the tail and the upper part of the hindlimbs (dorsal surface).

Plasma LH levels

Escapees had significantly higher levels of circulating plasma LH (pre-GnRH challenge) than non-escapees (X=8.31±6.57 and X=2.203±2.09 mi.μ./ml respectively; Mann-Whitney U-test, U=-1.97, P=0.049, see Fig. 5). Both escapees and non-escapees

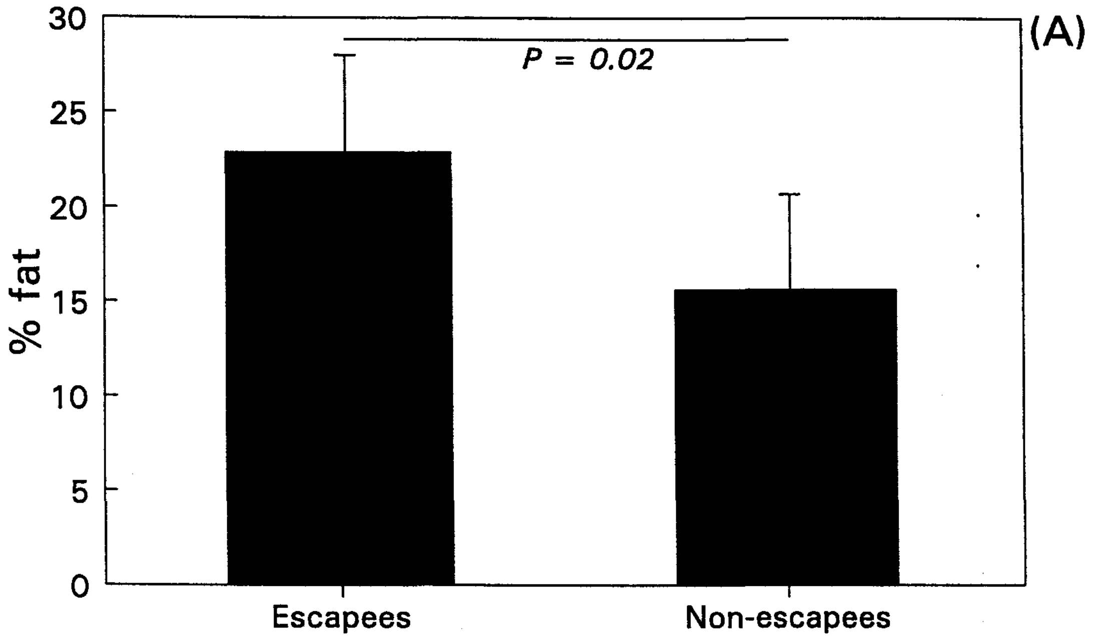


Figure 4A. The mean percentage body fat (±SD) of a random sample of escapees compared with a random sample of similar-sized (body mass) non-escapees.

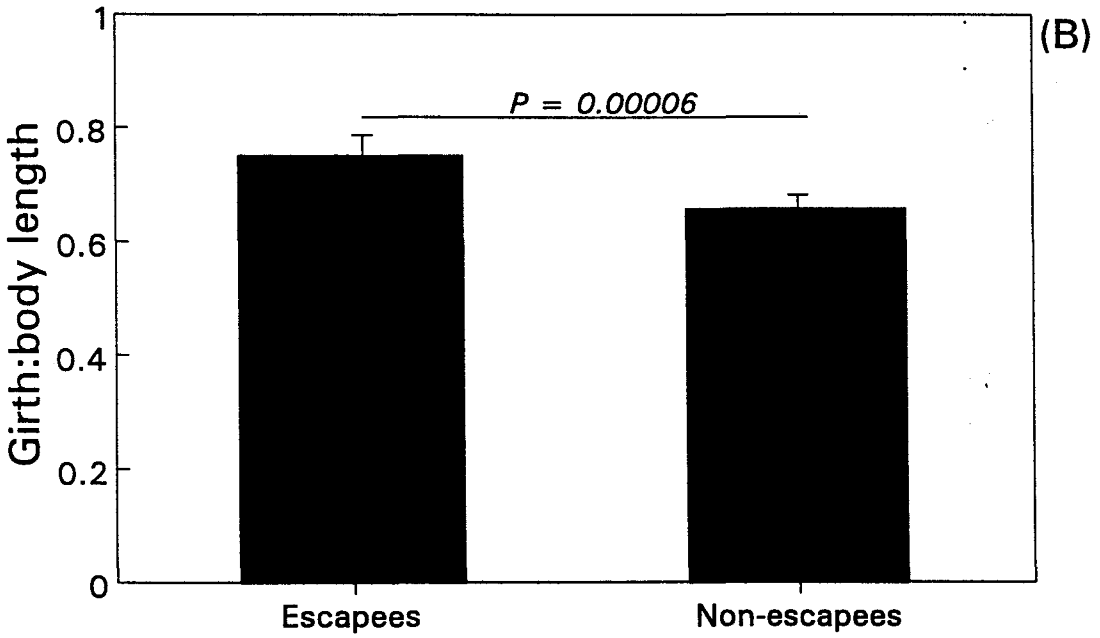


Figure 4B. The mean girth to body length ratio (±SD) of a random sample escapees compared with a random sample of similar sized nonn-escapees.

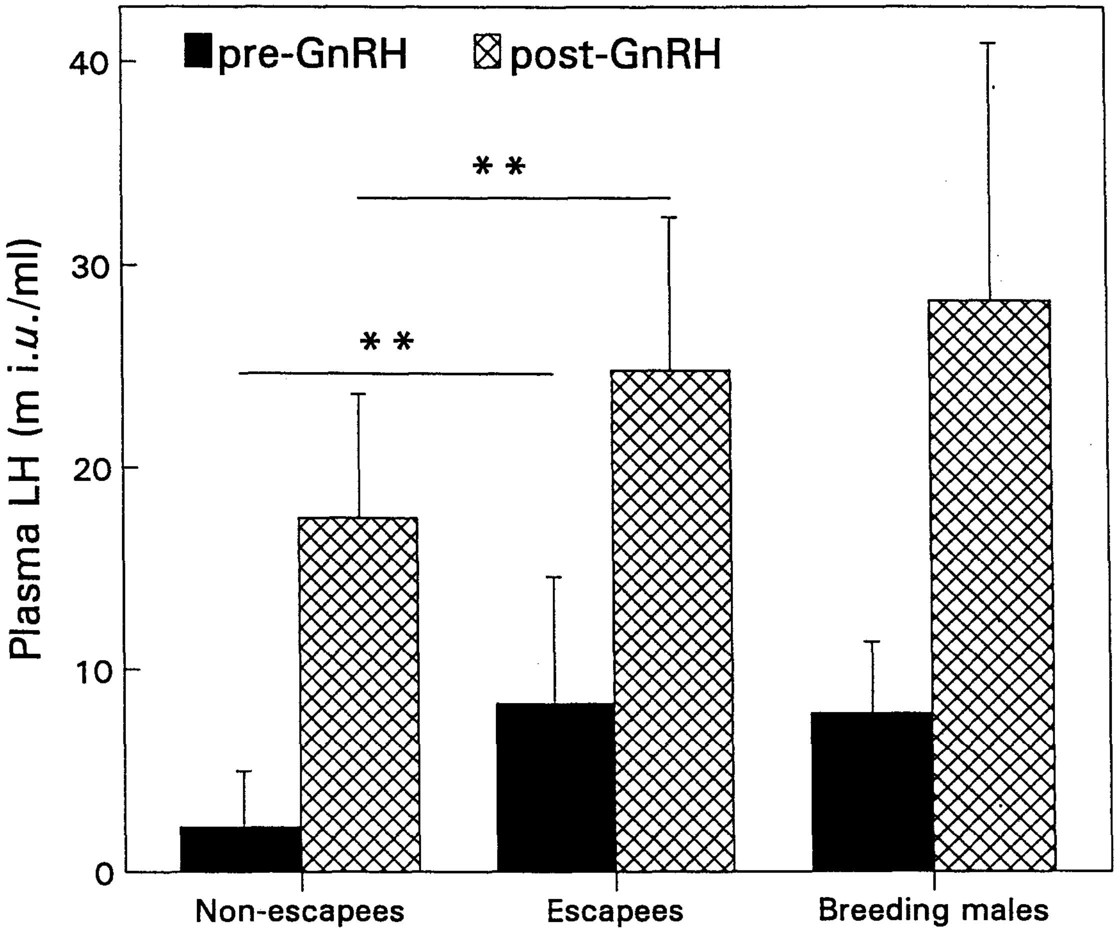


Figure 5. Mean concentrations (±SD) of plasma luteinising hormone (LH) in non-escapees (n = 10), escapees (n = 7) and breeding males (n = 2), before and after a single injection of 1μg GnRH. Statistical tests did not include the breeding males.

** P<0.05

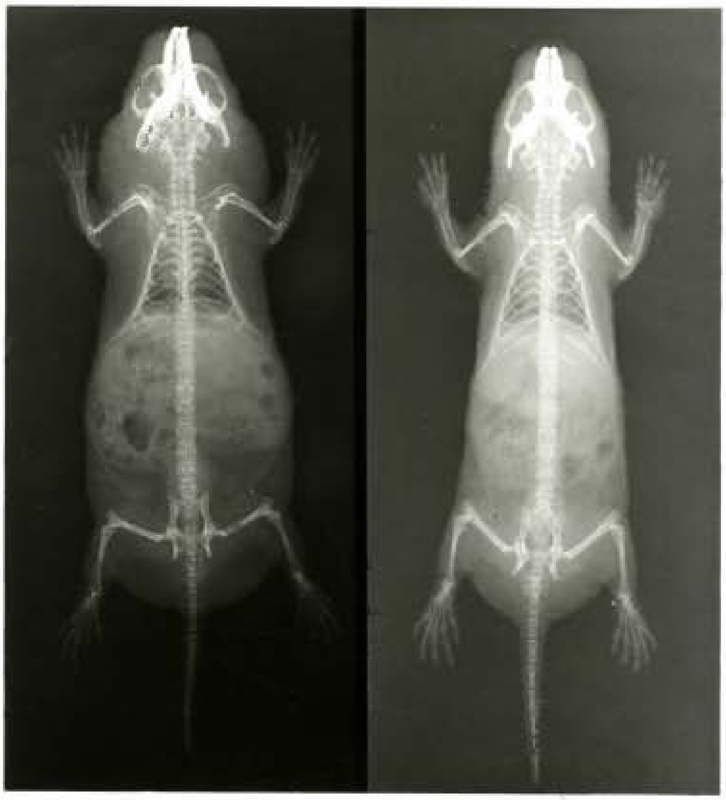


Plate 1. X-ray images of a frequent escapee (left) and a non-escapee littermate provide evidence for a distinct dispersive morph. Note the thick neck region and overall rotund appearance of the escapee. This is largely a consequence of subcutaneous fat deposits.

showed a significant increase in plasma LH following the administration of exogenous GnRH (Wilcoxan signs test, T=2.04, P=0.041 for escapees; Wilcoxan signs test, T=2.846, P=0.0043 for non-escapees). Post-GnRH levels of circulating LH were highest for breeding males. Escapees had higher mean post-GnRH levels of circulating LH than non-escapees ($\bar{X}$=24.84±8.89 and $\bar{X}$=17.52±6.6 mi.μ./ml respectively; Mann-Whitney U test *U*=-1.82, P=0.103).

DISCUSSION

This study provides the first empirical evidence for the existence of a possible dispersal phenotype within captive colonies of the naked mole-rat. Previously, the apparent lack of incest avoidance (Jarvis 1991; Jarvis *et al.* 1994), high inbreeding coefficients (Faulkes *et al.* 1990; Reeve *et al.* 1990), the marked xenophobic response of colony members to foreign conspecifics (Lacey and Sherman 1991; Chapter 5) and the paucity of small nascent colonies in the wild (Brett 1991), indicated that naked mole-rats were obligate inbreeders and that new colonies were only formed by colony fissioning (Brett 1991). However, the inbreeding avoidance mechanisms (dispersal and discrimination against kin) displayed by individuals in this study, and also recent field studies (Braude, pers. comm.) in which nascent colonies were found to contain at least some individuals from more than one colony, suggest that outbreeding, although rare, occurs in naked mole-rats.

Frequency of occurrence of dispersers

Only six of the 48 captive colonies at the University of Cape Town contained individuals with strong dispersal tendencies. Interestingly, all of these colonies were large (≥40 individuals) and well established (> four years since inception, with a minimum of 10 successfully recruited litters). While there were also large colonies without obvious dispersers, there were no small colonies with dispersers suggesting a lower limit to colony size for the emergence of dispersers. Furthermore, in the three colonies selected for this study only a few individuals attempted to disperse.

Dispersal ultimately enables individuals to reproduce and exploit new areas of the habitat. However, such movement may expose them to an increased probability of mortality (Bekoff 1977; Swingland 1984). Thus, whilst natural selection within animal populations is usually against dispersal (because of the increased chances of mortality), successful dispersers may accrue sufficient reproductive benefits to ensure that selection for dispersers within the population is maintained (Stenseth 1984). The apparent rarity of dispersers within both captive (this study) and wild (Brett 1991; Braude pers. comm.) naked mole-rat populations, may well reflect these opposing selective pressures. The fossorial habit and high foraging risks of naked mole-rats (Jarvis 1985; Lovegrove and Wissel 1988, Lovegrove 1991; Jarvis *et al.* 1994) bias selection strongly in favour of philopatry and inbreeding.

Sex bias in dispersal tendencies

Dispersal in the three study colonies was strongly biased in favour of males (92.8%). This finding conforms to data for other small mammals (Gaines and McClenaghan 1980; Greenwood 1980) and is frequently interpreted as an inbreeding avoidance mechanism (Dobson 1982; Pusey and Packer 1987; Ralls *et al.* 1986).

Greenwood and Harvey (1982) suggested that a male bias in dispersal is usually associated with a mate defence mating system, and a female bias with a resource defence mating system. Furthermore, Greenwood (1984) suggested that in mammals, high maternal investment has predisposed females to a greater incidence of cooperative behaviours that may in turn facilitate philopatry. An exception to this general mammalian pattern is the wild dog, *Lycaon pictus* in which females are the main dispersers (Frame *et al.* 1979).

In most polygynous and promiscuous mammals, juvenile males are the predominant dispersers, while in most monogamous species, juveniles of both sexes disperse (Dobson 1982). None of these theories adequately explain the male-biased dispersal in captive naked mole-rats, which are not strictly characterised by either a resource or mate defence mating system, and who form essentially monogamous (or polyandrous) bonds (Jarvis 1991). Furthermore, non-breeding males and females participate equally in the cooperative behaviours (Lacey and Sherman 1991; Jarvis *et al.* 1991; Faulkes *et al.* 1991) that may predispose a particular sex to philopatry or dispersal. Whilst it is difficult to explain the occurrence of male-biased dispersal in naked mole-rats along traditional lines of reasoning, it is important to note that females vigorously contest breeding vacancies in their natal colony, whereas males show little or no competition for the same opportunity (Jarvis 1991). This suggests that natal breeding rights and philopatry are primarily female biased, effectively selecting against female dispersal.

Behavioural and morphological profile of dispersers

I was fortunate in having both developmental and detailed behavioural profiles for all the individuals within one of the study colonies used in these experiments (colony 100). This allowed for a preliminary investigation into possible behavioural and morphological differences that may exist between dispersing and non-dispersing individuals within the colony context. It was clearly evident from the hierarchical cluster analysis that the six most frequent dispersers within this colony formed a behaviourally distinct group that participated little in cooperative maintenance tasks and displayed a heightened frequency of both locomotory and feeding activities.

Together these results suggest that behavioural antecedents may exist, that could be used to identify potential dispersers. Although data were insufficient to enable a thorough analysis, there was no evidence to suggest that litter order or litter sex ratio were important variables to consider. Dispersers were from the 2nd, 6th and 7th litters which had both male (2nd and 6th) and female (7th) biases.

It has been suggested (Harrison 1979) that the most likely maintenance mechanism for intraspecific differences in movement is that of genetic variation in the threshold response of individuals to environmental factors. Thus, when particular environmental factors become either favourable (*e.g.* more food) or unfavourable (*e.g.* crowding), they act as stimuli to influence ontogeny and cause some individuals to develop into migratory forms (Kennedy 1975). While attempts were made to control for the effects of food, by ensuring an *ad-libitum* supply to all colonies, it is possible that colony size is an important variable to consider in future studies. The probability of any one individual inheriting the breeding rights within the colony has to decrease as a function of increasing colony size. This may promote the emergence of dispersers in large colonies.

Dispersing individuals were not subjected to heightened parental or sibling aggression despite their selfish disposition, suggesting that dispersers are not forced to leave their natal colony (*sensu* Christian 1970; Moore and Ali 1984; Wasser 1985), but do so of their own volition.

This result contrasts with the model developed by Commins, Hamilton and May (1980) which suggest that a conflict of interest occurs between parents and offspring and that it is in the parents best interest to disperse some of their offspring, despite heavy mortality during dispersal. It is possible that in naked mole-rats, strong selection pressure favouring the parental retention of offspring as helpers outweighs selection for parents forcing a subset of their offspring to disperse. Following the death of the parents, siblings typically exhibit heightened aggression that may lead to group fission (see Lacey and Sherman, in press). In this scenario competitively superior individuals, typically the oldest and largest colony members, may serve their interests better by staying and contesting the breeding vacancy, whereas younger siblings may benefit from accepting the costs of migration in return for a habitat in which there is less competition. Juveniles may be prevented from dispersing due to their small size and lack of energy reserves in the form of subcutaneous fat. This may explain why dispersers in all three colonies studied, were never the oldest or the youngest colony members.

Previously, it has been suggested that the breeding female and her few male consorts maintain a monopoly on reproduction by actively suppressing their offspring through various dominance interactions (Jarvis 1991; Lacey and Sherman 1991; Reeve and

Sherman 1991; Faulkes *et al.* 1991). It was thus surprising that dispersers, despite having higher basal levels of plasma LH than non-dispersers, were not the focus of behaviourally mediated acts of suppression within their natal colonies. It is possible that levels of LH do not provide the cue for the initiation of aggressive encounters between the breeding female and subordinate male mole-rats, allowing certain individuals to escape suppression. Alternatively, dispersers may simply be better able to avoid aggressive encounters with the breeding female. Thus heightened levels of locomotory activities, in particular running, may be an attempt to avoid contact with the breeding female and so escape suppression.

Importantly, dispersers within colony 100, despite physiological and behavioural evidence of reproductive activity, ignored the breeding female when she was in oestrus. Thus, inbreeding avoidance mechanisms in dispersers may limit the breeding opportunities of these individuals while at home, negating the need to actively suppress them. Obviously, higher basal levels of circulating plasma LH are important to dispersers who benefit from being reproductively primed before encountering foreign, potential mates.

Although few studies have directly measured the relative proportion of fat in dispersing versus non-dispersing vertebrates, it is frequently assumed that the significantly heavier body masses of dispersers reflects the amount of energy stored as fat (*e.g.* Holekamp 1984). The finding that dispersers were generally less robust skeletally than a sample of similar sized (body mass) non-dispersers, together with significantly higher percentages of body fat, suggests that heavier body mass may be indicative of fat stores.

Initially, it was thought that the insulative subcutaneous fat of dispersing mole-rats may offset thermoregulatory problems (in these poikilothermic animals) during dispersal. However, preliminary comparisons (O'Riain, unpubl.) between the Ta/Tb regression slopes for dispersers versus non-dispersers over a range of low ambient temperatures (20-25^{o}C) revealed no significant differences. The high percentage fat of dispersers may serve as a nutritional safeguard against starvation during dispersal and colony establishment, in a manner similar to the fatty reserves typical of the winged alates of termites.

Outbreeding and mate choice in dispersers

Naked mole-rats are known to be highly xenophobic (Lacey and Sherman 1991; Chapter 5); discrimination against foreign individuals and recognition of colony members appears to be achieved through the presence or absence of familiar colony odours (see Chapter 5). However, when given a choice, dispersers chose to associate with foreigners significantly more frequently than colony members. Moreover, they sexually solicited the foreign animal, despite the foreigner being of the same sex and aggressive. In addition, dispersers showed a consistent preference for foreign breeding females over foreign non-breeding females, whilst in colony 100, none of the 6 dispersers showed any sexual interest in the resident breeding female, even when she was in oestrus. Interestingly, foreign breeding females showed no aggression to dispersers (or to foreign reproductively active males) when paired after the choice experiment. Indeed, they readily consorted with these males, engaging in mutual naso-anal nuzzling, although no copulation was observed. This was in contrast to non-breeding females, who responded aggressively and attempted to flee their advances.

A problem that inevitably faces all dispersers is acquiring a mate or being accepted by a foreign colony and integrating with another social hierarchy. We (O'Riain and Jarvis, unpubl. data; Chapter 3) have observed two instances in the laboratory where foreign males were not only accepted by a colony, but became the reproductive males. Furthermore, Braude (pers. comm.) has found a marked naked mole-rat moving between different colonies and several instances of nascent colonies in the wild, containing unrelated individuals.

These findings, together with the non-aggressive behaviours displayed by breeding females in choice experiment pairings, suggest that occasionally males may indeed achieve reproductive success by successfully dispersing from their natal burrow system into a foreign system.

It is my suggestion that, despite the existence of dispersers within the population, the enormous costs and risks associated with dispersal may effectively preclude successful establishment of new colonies from pairs of individuals. Of the six nascent colonies found by Braude in N. Kenya, none survived for more than a year. Outbreeding opportunities may thus be limited to the transfer of individuals (predominantly males) between colonies. More favourable environmental conditions associated with increased rainfall may temporarily improve the success of dispersers and the establishment of small nascent colonies. However, for the most part, the harsh ecological conditions are likely to favour the establishment of new colonies through budding (Jarvis *et al.* 1994); thus effectively restricting mate choice to those within each colony. In effect, all matings between members of neighbouring colonies would therefore represent close inbreeding. Ultimately, continuous inbreeding increases the genetic homogeneity of a local deme (Shields 1982), which may explain the high inbreeding coefficients recorded for naked mole-rats within specific geographic locales.

Social considerations of inbreeding and outbreeding

Philopatry, leading to inbreeding, may be adaptive due to higher average survivorship of juveniles (through access to parental resources), accessibility to potential mates (siblings and other close relatives) and the maintenance of locally adapted gene complexes (Shields 1987). However, while there is no evidence to suggest that naked mole-rats suffer from the potentially deleterious effects of inbreeding, close inbreeding cannot continue indefinitely (Chesser and Ryman 1986). Although in the medium term (*e.g.* after 50 generations), chronic local inbreeding may be advantageous in diploid animals (because of reduced genetic load), it is likely to become detrimental for a variety of reasons. These include an accumulation of genes with mild deleterious effects (Werren 1993) and the loss of the ability to adapt to changing environments (Maynard Smith 1978). Indeed, Tyson (1984) defined the parameter D_n which is the genetic bias for desertion, or antisocial behaviour, in the n^{th} generation of inbreeders. After this point is reached there would be selection against altruistic alleles favouring cooperation within genetically closed groups and selection for selfish dispersers.

Thus, while both field and laboratory evidence suggest that inbreeding is common in naked mole-rats, the above theoretical predictions support the findings that inbred colonies do sometimes harbour selfish dispersers which avoid close inbreeding. Furthermore, although naked mole-rats are highly inbred, there is little evidence to suggest that this has resulted in greater altruistic tendencies (*e.g.* pup care, see Chapter 1) or more reduced selfish behaviours than their outbred relatives (*e.g.* *C.damarensis*, Jarvis and Bennett 1993) or other mammalian cooperative breeders (*e.g.* dwarf mongoose, *Helogale parvula*, Rasa 1977; wild dog, *Lycaon pictus*, Malcolm and Marten 1982).

The paucity of field evidence for both dispersal and outbreeding events in naked mole-rats may therefore be largely a product of the high somatic fitness costs associated with dispersal for a poikilothermic, subterranean mammal. Inbreeding remains a necessary trade-off between these costs and the limited mating opportunities available at the natal site. More favourable environmental conditions (*e.g.* an increase in rainfall) may serve to promote both the frequency and survival of those dispersers that either invade established colonies or form nascent colonies with a few foreign individuals. Thus, different combinations of morphological, physiological and behavioural characters, which at a proximate level result in certain individuals showing dispersal tendencies, may serve ultimately to ensure optimal levels of inbreeding within localised populations.

SYNTHESIS

This study attempts to improve our understanding of how naked mole-rat colonies form, function and maintain autonomy as cooperatively breeding units. The results obtained have been interpreted within the theoretical framework of the evolution and elaboration of group living within animal societies.

It has previously been suggested that the evolutionary inception of group living in mole-rats was the retention of young within the natal burrow system (Alexander *et al.* 1991; Jarvis *et al.* 1994). Given the importance of this hypothesis to arguments pertaining to the evolution of sociality in vertebrates I have focussed considerable attention on the formation of colonies from breeding pairs. This provides much of the focus for this thesis, *viz.* the formation of colonies from breeding pairs. Behavioural observations on newly formed colonies showed that aggressive interactions occur between parents and their offspring but, contrary to solitary bathyergid species (Jarvis and Bennett 1991), this aggression does not lead to eviction and dispersal from the natal burrow system. Rather, these interactions appear to establish a social hierarchy between the parents and their offspring in which the parents dominate and maintain a monopoly on reproduction and, in the case of the breeding female, control access to limited food resources. Young born to the breeding pair also interact aggressively with one another but, in the presence of their parents, this aggression never leads to a fatality. Intra-litter interactions are characterised by a high frequency of play-related behaviours, whereas inter-litter interactions are typically more aggressive in nature with the older individuals dominating their younger siblings.

These dominant/subordinate interactions form the basis of most of the behavioural and morphological variation evident amongst adult colony members. For example, individuals of the first-born litters grow significantly faster and attain significantly greater body masses at a specific age than those of last-born litters. The death of either of the parents and/or older siblings results in a rapid increase in the body masses of the surviving colony members. The magnitude of these growth surges varies positively with the absolute age of the colony members. Furthermore, any new breeding opportunities are taken by the oldest colony members and, in the absence of the parents, competition between offspring often leads to fatalities.

From the parents' perspective, the retention of progeny appears to be advantageous for a number of reasons. On pair formation, the male and female perform all of the essential duties necessary to maintain the burrow system and obtain food. Following recruitment however, both breeders slow down their work rates, with the breeding female eventually ceasing to participate in most work activities. This effectively allows the parents to devote almost all their energy to reproduction and, particularly in the case of the breeding female, manipulation of offspring to ensure a monopoly on reproduction and priority of access to food. A further nutritional advantage to the breeding female is access to caecotrophs produced by her offspring. This food source is particularly important to her in the latter stages of pregnancy when, due to her greatly enlarged abdomen, she is physically incapable of eating her own caecotrophs. Given the high fibre and cellulose content of the mole-rat's diet, this nutritive aid has important consequences not only for the breeding female's survival during late pregnancy, but also for the number of foetuses she can support. Lastly, the performance of foraging and defence activities by non-breeders reduces the breeders exposure to danger, which further enhances their potential lifetime reproductive output.

Despite the obvious bias in the accruement of benefits to the breeders, it would appear that natural selection has favoured natal philopatry by the offspring. This may be a consequence of the enormous costs and risks associated with dispersal and consequently the minimal chances of independent reproduction. This, together with the fact that naked mole-rats readily inbreed, may predispose offspring to remain at home, and accept the unfavourable bias in direct fitness benefits in the 'hope' of ultimately replacing their parents and inheriting the natal breeding site. Competition amongst the non-breeders for natal reproductive vacancies would thus be expected to be fierce, given the enormous fitness benefits associated with reproductive status. This is readily borne out by observations of bloody battles within colonies between the most likely female reproductive replacements. Interestingly, male-male conflict was never observed, with only a few instances of females attacking males. Indeed, males showed no aggression towards one another even when the breeding female was sexually receptive and, on one occasion, three males were observed taking turns in mounting the breeding female. It is possible that, because the attainment of male reproductive status is largely a result of female choice, male-male competition for reproduction is unnecessary.

Naked mole-rat colonies are essentially discrete units whose success and efficiency is largely dependent on their functioning as a single reproductive unit. The importance of maintaining autonomy is witnessed in the elaborate and efficient recognition and subsequent rejection mechanisms displayed by mole-rats upon encountering foreign conspecifics. The ousting of foreign conspecifics may be attributed in part to natal philopatry and the associated within-colony rivalry for reproductive vacancies. Accepting a foreigner would, in itself, reduce each individual's chances of becoming a reproductive. It could also be argued that the acceptance of foreigners would lower the average relatedness within a colony, thus effectively reducing the inclusive fitness benefits of the non-breeders. Irrespective of the relative importance of these possible proximate selective pressures, the existence of a well organised defence system against foreign conspecifics, replete with alarm calls, bears testimony to an evolutionary history of repeated contacts between neighbouring colonies. Before such a defence system can be effective, there needs to be a reliable means of discriminating colony from non-colony members. This appears to be achieved through the detection of the presence or absence of distinct colony odour labels. These labels are most likely a combination of the individual odours of each colony member and are distributed among, and learned by the entire colony. A recognition mechanism that relies on familiarity with the group's current odour composition (a cocktail of individual odours) serves as a simple yet effective means of discriminating between genetically similar (typical of neighbouring wild colonies) colony and non-colony members.

Prior to this study, colony formation in naked mole-rats was thought to occur solely through colony fissioning, a theory supported by both circumstantial field (Brett 1991) and laboratory (Lacey and Sherman, in press) evidence. Furthermore, xenophobia and routine inbreeding shown by laboratory colonies seemed to suggest that foreign conspecifics were quite simply extraneous for the purposes of reproduction. My finding that a small percentage of our captive population contradict these suggestions is important, for it suggests that selection for selfish dispersers exists in naked mole-rat populations. Field evidence in support of these findings is encouraging and serves to enforce the conviction that although rare, outbreeding, the rule for all the other social bathyergids, does occur in naked mole-rats. It is clear that these outbreeders are morphologically distinct to their closely related siblings, but exactly what cues trigger this divergence remains an intriguing unanswered question.

Dispersers are the only morphologically distinct group of non-breeding colony members. Although individuals involved in colony defence within colonies are typically the largest colony members and possess visibly enlarged temporal musculature, there is a size continuum between them and non-defenders. The lack of other morphologically specialised non-breeders or of temporal castes reveals a generalised social organisation in naked mole-rats, which differs substantially from those of most eusocial insect species (*e.g.* honey bees and higher termites). This suggests that use of 'invertebrate terminology' such as castes and age polyethism should not be used when describing naked mole-rat social organisation.

The only other naked mole-rat within the colony which exhibits morphological and behavioural specialisation is the breeding female, with her unique suite of attributes associated with reproduction. I have suggested that because all adult non-breeders have the potential to reproduce, selection has not favoured the evolution of extensive or irreversible morphological specialisations for improved efficiency in behaviours which do not directly enhance reproduction. Rather, efficiency in the performance of foraging and maintenance tasks is achieved through short-term behavioural specialisation. The advantage of this is that individuals, and consequently whole colonies, retain a great deal of flexibility and can meet capricious changes in environmental and social exigencies very rapidly. The control of non-breeder reproduction by the breeding female is thus the single most important mechanism influencing the behaviour and morphology of colony members. Systems in which reproductive control and the division of labour amongst non-breeders is effected through behavioural means (*i.e.* dominance) are capable of rapid responses to events such as the loss of a breeder or large defenders. Furthermore, the relative positions of the non-breeding individuals within the colony's social hierarchy, and the differences in their threshold responses to various work and defence stimuli, may provide the key elements to the social organisation of naked mole-rat colonies.

Although naked mole-rats appear to exhibit numerous cooperative behaviours that may be construed as altruistic, it is in fact more realistic to think of each colony member as exploiting the colony for food, shelter and the prospect of mating and producing offspring. Acts of altruism are rare within colonies and both cooperative defence and foraging acts are couched within the framework of selfishness. It would appear that individuals remain at home to elevate their personal survival and reproductive rates above those that would accrue from a solitary lifestyle. However, without a sufficient percentage of subordinate individuals to do the work, a colony of large, lazy individuals would soon collapse. Therefore the spread of selfish genes is ultimately limited by the adverse effects on the efficiency of a colony and the fitness of all its members.

The discovery of intriguing similarities between the social organisation of naked mole-rats and certain cooperatively breeding invertebrate species, serve to stress the importance of similar selective pressures acting on organisms as taxonomically diverse as insects and mammals. A more complete understanding of these similarities, and also features which set them apart from the eusocial insects, may prove invaluable in elucidating those factors which initially promoted group living and those which subsequently led to the evolution of morphologically specialised helper castes.

REFERENCES

Abbott, D.H. and Hearn, J.P. 1978. Physical, hormonal and behavioral aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. *J. Reprod. Fertil.*, 53:155-166.

Aberle, E.D. and Doolittle, D.P. 1976. Skeletal muscle cellularity in mice selected for large body size and in controls. *Growth*, 40:133-145.

Agren, G., Zhou, Q. and Zhong, W. 1989. Ecology and social behavior of Mongolian gerbils, *Meriones unguiculatus*, at Zilinhot, inner Mongolia, China. *Anim. Behav.*, 37:11-27.

Alexander, R.D. 1974. The evolution of social behavior. *Annu. Rev. Ecol. Syst.*, 5:325-383.

Alexander, R.D., Noonan, K.M., and Crespi, B.J. 1991. The evolution of eusociality. In: *The Biology of the Naked Mole-Rat.* (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 3-44. Princeton University Press, Princeton, New Jersey.

Altmann, J. 1974. Observational study of behaviour: sampling methods. *Behaviour,* 49:227-267.

Appleby, M.C. 1983. The probability of linearity in hierarchies. *Anim. Behav.*, 31:600-608.

Armitage, K.B. 1986. Individual differences in the behavior of juvenile jellow-bellied marmots. *Behav. Ecol. Sociobiol.*, 18:419-424.

Barnard, C. 1991. Kinship and Social Behaviour: The Trouble with Relatives. *Trends Ecol. Evol.*, 6:310-312.

Barnes, R.H., Fiala, G. and Kwong, E. 1963. Decreased growth rate resulting from prevention of coprophagy. *Nutr. Rev.*, 20:125-128.

Bartz, S.H. 1979. Evolution of eusociality in termites. *Proc. Natl. Acad. Sci. U.S.A.*, 76:5764-5768.

Bateson, P. 1981. Discontinuities in development and changes in the organisation of play in cats. In: *Behavioural development. The Bielefeld Interdisciplinary Project.* (Ed. by K. Immelmann, G.W. Barlow, L. Pednovich and M. Main), pp. 296-337. Cambridge University Press, Cambridge.

Batra, S.W.T. 1966. Nests and social behavior of halictine bees of India. *Indian J. Entomol.*, 28:375-393.

Batzli, G.O., Getz, L.L. and Hurley, S.S. 1977. Suppression of growth and reproduction of microtine rodents by social factors. *Journal of Mammalogy*, 58:583-591.

Bekoff, M. 1972. The development of social interaction, play, and metacommunication in mammals: An ethological perspective. *Quart. Rev. Biol.*, 47:412-434.

Bekoff, M. 1977. Mammalian dispersal and the ontogeny of individual behavioural phenotypes. *Am. Nat.*, 111:715-732.

Bekoff, M. 1987. Group living, natal philopatry, and Lindstrom's lottery: it's all in the family. *Trends Ecol. Evol.*, 2:115-116.

Bekoff, M. and Wells, M.C. 1982. Behavioral ecology of coyotes: Social organization, rearing patterns, space use, and resource defense. *Z. Tierpsychol.*, 60:281-305.

Bengtsson, B.O. 1978. Avoiding inbreeding: at what cost? *J. Theor. Biol.*, 73:439-444.

Bennett, N.C. 1989. The social struture and reproductive biology of the common mole-rat, *Cryptomys h. hottentotus*, and remarks on the trends in reproduction and sociality in the family Bathyergidae. *J. Zool. Lond.*, 219:45-59.

Bennett, N.C. and Jarvis, J.U.M. 1988. The reproductive biology of the Cape mole-rats, *Georychus capensis* (Rodentia, Bathyergidae). *J. Zool. Lond.*, 214:95-106.

Bennett, N.C., Jarvis, J.U.M. and Aguilar, G. 1991. Growth and development in six species of African mole-rats (Rodentia: Bathyergidae). *J. Zool. Lond.*, 225:13-26.

Bennett, N.C. and Navarro, R. (in press). Differential growth patterns between successive litters of the eusocial Damaraland mole-rat, *Cryptomys damarensis*. J. Zool. Lond..

Bernado, J. 1993. Determinants of Maturation in Animals. *Trends Ecol. Evol.*, 8:166-173.

Bertram, B.C.R. 1978. *Pride of Lions*. Scribners, New York.

Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology, 71:1599-1608.

Blaustein, A.R. 1983. Kin recognition mechanisms: phenotypic matching or recognition alleles? *Am. Nat.*, 121:749-754.

Blaustein, A.R. and Porter, R.H. 1990. The ubiquotous concept of recognition with special refernce to kin. In: *Methods, Inference, Interpretation, and Explanation in the study of behaviour*. (Ed. by M. Bekoff and D. Jamieson), pp. 123-148. Westview Press, Boulder.

Blaustein, A.R., Bekoff, M., Byers, J.A. and Daniels, T.J. 1991. Kin recognition in vertebrates: what do we really know about adaptive value? *Anim. Behav.*, 6:1079-1083.

Blouin, S.F. and Blouin, M. 1988. Inbreeding Avoidance Behaviours. *Trends Ecol. Evol.*, 3:230-233.

Bonavita-Cougourdan, A. and Morel, L. 1988. Interindividual variability and idiosyncrasy in social behaviours in the ant *Camponotus vagus* Scop. *Ethology*, 77: 58-66.

Bourke, A.F.G. 1988. Worker reproduction in the higher eusocial Hymenoptera. *Q. Rev. Biol.*, 63: 291-311.

Brett, R.A. 1986. The ecology and behaviour of the Naked mole-rat (*Heterocephalus glaber* Ruppell) (Rodentia: Bathyergidae). Unpubl. Phd thesis, University of London.

Brett, R.A. 1991. The Ecology of Naked Mole-Rat Colonies: Burrowing, Food and Limiting factors. In: *The Biology of the Naked Mole-Rat*. (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 137-184. Princeton University Press, Princeton, New Jersey.

Brett, R.A. 1991. The Population Structure of Naked Mole-Rat Colonies. In: *The Biology of the Naked Mole-Rat*. (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 97-136. Princeton University Press, Princeton, New Jersey.

Brooks, P.M. 1982. Aspects of the reproduction, growth and development of the four-striped field mouse, *Rhabdomys pumilio*. *Mammalia*, 46:53-63.

Brown, J.L. 1975. *The evolution of behaviour*. New York: W.W. Norton.

Brown, J.B. 1987. *Helping and Communal Breeding in Birds*. Princeton University Press, Princeton, New Jersey.

Buffenstein, R. and Yahav, S. 1991. Is the naked mole-rat *Heterocephalus glaber* an endothermic yet poikilothermic mammal? *J. Thermal Biol.*, 16:227-232.

Burda, H. 1989. Reproductive biology (behaviour, breeding and postnatal development) in subterannean mole-rats, *Cryptomys hottentotus* (Bathyergidae). *Z. Saugetierkunde*, 54:360-376.

Byers, J.A. and Bekoff, M. 1991. Development, the conveniently forgotten variable in 'true kin recognition'. *Anim. Behav.*, 41:1088-1090.

Calderone, N.W. and Page, R.E. 1988. Genotypic variability in age polyethism and task specialisation in the honey bee, *Apis mellifera* (Hymenoptera: Apidae). *Behav. Ecol. Sociobiol.*, 22:17-25.

Calvert, C.C., Famula, T.R., Bernier, J.F. and Bradford, G.E. 1985. Serial composition during growth in mice with a major gene for rapid postweaning growth. *Growth*, 49:246-257.

Cameron, G.N. 1973. Effect of litter size on postnatal growth and survival in the desert woodrat. *J. Mamm.*, 54:489-493.

Carlin, N.F. and Holldobler, B. 1993. Nestmate and Kin Recognition in Interspecific Mixed Colonies of Ants. *Science*, 222:1027-1029.

Case, T.J. 1978a. On the evolution and adaptive significance of postnatal growth rates in the terrestrial vertebrates. *Q. Rev. Biol.*, 53:243-282.

Case, T.J. 1978b. Endothermy and parental care in the terrestrial vertebrates. *Am. Nat.*, 112:861-874.

Chandrashekara, K. and Gadagkar, R. 1991. Behavioural Castes, Dominance and Division of labour in a Primitively Eusocial Wasp. *Ethology*, 87:269-283.

Cheeseman, C.L. 1981. Observations on the reproductive biology and early postnatal development of two species of African rodents. *Mammalia*, 45:483-491.

Chesser, R.K. and Ryman, N. 1986. Inbreeding as a strategy in subdivided populations. *Evolution*, 40:616-624.

Christian, J.J. 1970. Social subordination, population density and mammalian evolution. *Science*, 168:84-90.

Clarke, K.R. and Warwick, R.M. 1994. *User Guide to Primer v3.1.* Plymouth Marine Laboratory.

Clarke, K.R. and Warwick, R.M. 1995. *Change In Marine Communities: An Approach to Statistical Analysis and Interpretation.* Plymouth Marine Laboratory.

Commins, H.N., Hamilton, W.D. and May, R.M. 1980. Avoiding inbreeding: at what cost? *J. Theor. Biol.*, 73:439-444.

Corbara, B., Lachaud, J.P. and Fresneau, D. 1989. Individual variability, social structure and division of labour in in the ponerine ant *Ectatomma ruidum* Roger (Hymenoptera: Formicidae). *Ethology*, 82:89-100.

Creel, S.R. and Creel, N.M. 1991. Energetics, reproductive suppression and obligate communal breeding in carnivores. *Behav. Ecol. Sociobiol.*, 28:263-270.

Crespi, B.J. and Yanega, D. 1995. The definition of eusociality. *Behav. Ecol.*, 6:109-115.

Davidson, D.D. 1978. Size variability in the worker caste of a social insect (*Veromessor pergandei* Mayr) as a function of the competitive environment. *Am. Nat.*, 112:523-532.

Davis, L.S. 1982. Sibling Recognition in Richardson's Ground Squirrels (*Spermophilus richardsonii*). *Behav. Ecol. Sociobiol.*, 11:65-70.

Dawkins, R. 1989. *The Selfish Gene*. 2nd Ed., Oxford University Press, Oxford.

Dentry, W. and Lindsey, C.C. 1978. Vertebral variation in Zebrafish (*Brachydanio rerio*) related to the prefertilisation temperature history of their parents. *Can. J. Zool.*, 56:280-283.

Derrickson, E.M. 1988. Patterns of postnatal growth in a laboratory colony of *Peromyscus leucopus*. *J. Mamm.*, 69:57-66.

Dew, H.E. and Michener, C.D. 1981. Division of labour amongst workers of *Polistes metricus* (Hymenoptera, Vespidae): Laboratory foraging activities. *Ins. Soc.*, 28:87-101.

Dewsbury, D.A. 1982. Dominance, copulatory behavior, and differential reproduction. *Quart. Rev. Biol.,* 57:135-159.

Dixon, W.J., Brown, M.B. Engelman, L. and Jenrich, R.I. 1990. *BMDP statistical Software Manual*, Berkeley, University of California Press.

Dobson, F.S. 1982. Competition for mates and predominant juvenile male dispersal in mammals. *Anim. Behav.*, 30:1183-1192.

Dobzhansky, T. 1970. *Genetics of the Evolutionary process*. Columbia University Press, New York.

du Plessis, M.A. 1991. The role of helpers in feeding chicks in cooperatively breeding green (red-billed) woodhoepoes. *Behav. Ecol. Sociobiol.*, 34:291-295.

Emlen, S.T. 1982. The evolution of helping II. The role of behavioral conflict. *Am. Nat.*, 119:40-53.

Emlen, S.T. 1991. Evolution of cooperative breeding in birds and mammals. In: *Behavioural ecology: an evolutionary approach.* (Ed. by J.R. Krebs and N.B. Davies). pp. 231-267. 3rd ed. Blackwell, Oxford.

Faulkes, C.G., Abbott, D.H. and Jarvis, J.U.M. 1990. Social suppression of ovarian cyclicity in captive and wild colonies of naked mole-rats, *Heterocephalus glaber. J. Repro. Fertil.*, 88:559-568.

Faulkes, C.G., Abbott, D.H. and Jarvis, J.U.M. 1991. Social suppression of reproduction in male naked mole-rats, *Heterocephalus glaber. J. Repro. Fertil.*, 91:593-604.

Faulkes, C.G., Abbott, D.H. and Mellor, A.L. 1990. Investigation of genetic diversity in wild colonies of naked mole-rats (*Heterocephalus glaber*) by DNA fingerprinting. *J. Zool. Lond.,* 221:87-97.

Faulkes, C.G., Abbott, D.H., Jarvis, J.U.M. and Sherriff, F.E. 1989. Social suppression of reproduction in male naked mole-rats, *Heterocephalus glaber. J. Reprod. Fertil. Abstr. Ser.*, 3:113.

Faulkes, C.G., Abbott, D.H., Liddell, C.E., George, L.M. and Jarvis, J.U.M. 1991. Hormonal and Behavioural Aspects of Reproductive Suppression in Female Naked Mole-rats. In: *The Biology of the Naked Mole-Rat.* (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 426-445. Princeton University Press, Princeton, New Jersey.

Fletcher, D.J.C. and Michener, C.D. 1986. *Kin recognition in animals.* Wiley, New York.

Fletcher, D.J.C. and Ross, K.G. 1985. Regulation of reproduction in eusocial Hymenoptera. *Ann. Rev. Ecol. Syst.*, 30:319-343.

Frame, L.H., Malcolm, J.R., Frame, G.W. and Van Lawick, H. 1979. Social organization of African wild dogs (*Lycaon pictus*) on the Serengeti plains, Tanzania. *Z. Tierpsychol.*, 50:225-249.

Franks, N.R. 1987. The Organisation of Working Teams in Social Insects. *Trends Ecol. Evol.*, 2:72-75.

Franks, N.R. and Norris P.J. 1987. Constraints on the division of labour in ants: D'Arcy Thompson's cartesian transformations applied to worker polymorphism. *Experientia Suppl.*, 54:253-269.

Fresneau, D. and Dupuy, P. 1988. A study of polyethism in a ponerine ant: *Neoponera apicalis* (Hymenoptera: Formicidae). *Anim. Behav.*, 36:1389-1399.

Fresneau, D., Garcia Perez, J. and Jaisson, P. 1982. Evolution of polyethism in ants: observational results and theories. In: *Social Insects in the Tropics*. (Ed. by I.P. Jaisson), pp. 129-155. Universite Paris Nord, Villetaneuse.

Fretwell, S. 1969. Dominance behaviour and winter habitat distribution in Juncos (*Junco hyemalis*). *Bird-banding*, 40:1-25.

Frumhoff, P.C. and Baker, J. 1989. A genetic component to division of labour within honey bee colonies. *Nature*, 333:358-361.

Gadagkar, R. 1980. Dominance hierachy and division of labour in the social wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae). *Curr. Sci.*, 49:772-775.

Gadagkar, R. 1985. Kin recognition in social insects and other animals: A review of recent findings and a consideration of their relevance for the theory of kin selection. *Proc. Ind. Acad. Sci. (A)*, 94:587-621.

Gaines, M.S. and McClenaghan, L.R. Jr 1980. Dispersal in small mammals. *Anu. Rev. Ecol. Sys.*, 11:163-196.

Gaston, A.J. 1978. Demography of the jungle babbler, *Turdoides straitus*. *J. Anim. Ecol.*, 47:845-870.

Gastreich, K.R., Queller, D.C., Hughes, C.R. and Strassmann, J. 1991. Kin Discrimination in the Tropical Swarm-founding Wasp, *Parachartergus colobopterus*. *Anim. Behav.*, 40:598-601.

Gittleman, J.L. 1985. Function of communal care in mammals. In: *Essays in honour of John Maynard Smith*. (Ed. by P.J. Greenwood, P.H. Harvey and M. Slatkin), pp. 187-205. Cambridge University Press, Cambridge.

Goodall, J. 1975. Chimpanzees of the Gombe National Park: Thirteen years of research. In: *Hominisation und Verhalten* (Ed. by G. Kurth and I. Eibl-Eibesfeldt), pp.74-136. Fischer Verlag, Stuttgart.

Grau, H.G. 1982. Kin recognition in white-footed deemice (*Peromyscus leucopus*). *Anim. Behav.*, 30:497-505.

Greenwood, P.J. 1980. Mating systems, Philopatry and Dispersal in birds and mammals. *Anim. Behav.*, 28:1140-1162.

Greenwood, P.J. 1984. Mating systems and evolutionary consequences of dispersal. In: *The Ecology of Animal Movement* (Ed. by P.J. Greewwood and I.R. Swingland), pp. 116-131. Clarendon Press, Oxford.

Greenwood, P.J. and Harvey, P.H. 1982. The natal and breeding dispersal of birds. *Annu. Rev. Ecol. Syst.*, 13:1-21.

Gubernick, D.J. and Klopfer, P.H. 1981. *Parental care in mammals*. New York Plenum Press.

Halpin, Z.T. 1993. Kin recognition cues of vertebrates. In: *Kin Recognition* (Ed. by P.G. Hepper), pp. 220-258. Cambridge University Press, Cambridge.

Hamilton, W.D. 1964a. The genetical evolution of social behaviour I. *J. Theor. Biol.*, 7:1-16.

Hamilton, W.D. 1964b The genetical evolution of social behaviour II. *J. Theor. Biol.*, 7:17-52.

Hamilton, W.D. 1970. Selfish and spiteful behaviour in an evolutionary model. *Nature, London.*, 228:1218-1220.

Hamilton, W.D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.*, 3:193-232.

Harlow, H.F. 1969. Age-mate or peer affectional systems. In: *Advances in the Study of Behaviour*. (Ed. by D.S. Lehrman, R.A. Hinde and E. Shaw), pp. 333-383. Academic Press, New York.

Harrington, F.D., Mech, L.D. and Fritts, S.H. 1983. Pack size and wolf pack survival: their relationship under varying ecological conditions. *Behav. Ecol. Sociobiol.*, 13:19-26.

Harrison, R.G. 1979. Flight polymorphisms in the field cricket *Gryllus pensylvanicus*. *Oecologia*, 40:125-132.

Heinze, J., Holldobler, B. and Peeters, C. 1994. Conflict and cooperation in ant societies. *Naturwissenschaften*, 81:489-497.

Hepper, P.G. 1991. Recognizing kin: ontogeny and classification. In: *Kin recognition in animals* (Ed. by P.G. Hepper), pp. 259-278. Cambridge University Press, Cambridge.

Herbers, J.M. 1980. On caste ratio in ant colonies: population responses to changing environments. *Evolution*, 34:575-585.

Herbers, J.M. & Cunningham, M. 1983. Social organisation in *Leptothorax longispinosus* Mayr. *Anim. Behav.*, 31:759-771.

Hoeck, H.N. 1982. Population dynamics, dispersal and genetic isolation in two species of hyrax (*Heterohyrax brucei* and *Procavia johnstoni*) on habitat islands in the Serengeti. *Z. Tierpsychol.*, 59:117-210.

Holekamp, K.E. 1984. Natal dispersal in Belding's ground squirrels (*Spermophilus beldingi*). *Behav. Ecol. Sociobiol.*, 16:21-39.

Hölldobler, B. and Michener, C.D. 1980. Mechanisms of identification and discrimination in social Hymenoptera. In: *Evolution of Social Behaviour: Hypotheses and Empirical tests*. (Ed. by H. Markl), pp. 35-58. Verlag Chemie, Weinheim.

Hölldobler, B. and Wilson, E.O. 1990. *The ants*. Cambridge, Massachusetts: Belknap Press of the Harvard University Press.

Hoogland, J.L. 1982. Prairie dogs avoid extreme inbreeding. *Science*, 215:1639-1641.

Honeycutt, R.L., Nelson, K., Schlitter, D.A. and Sherman, P.W. 1991. Genetic variation within and among populations of the naked mole-rat: evidence from nuclear and mitochondrial genomes. In: *The Biology of the Naked Mole-Rat*. (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 195-208. Princeton University Press, Princeton, New Jersey.

Hurst, J.L., Fang, J. and Barnard, C.J. 1993. The role of substrate odours in maintaining social tolerance between male house mice (*Mus musculus domesticus*). *Anim. Behav.*, 45:997-1006.

Isil, S. 1983. A study of social behaviour in laboratory colonies of the Naked mole-rat (*Heterocephalus glaber* Ruppell, Rodentia: Bathyergidae). Unpubl. M.Sc. thesis, University of Michigan.

Ito, F. 1993. Social organization in a primitive ponerine ant: queenless reproduction, dominance hierarchy and functional polygyny in *Amblyopone* sp. (*reclinata* group) (Hymenoptera: Formicidae: Ponerinae). *J. Nat. Hist.*, 27:1315-1324.

Ito, F. and Higashi, S. 1991. A linear dominance hierarchy regulating reproduction and polyethism of the queenless ant *Pachycondyla sublaevis*. *Natuurwissenschaften*, 78:80-82.

Jacobs, D.S., Bennett, N.C., Jarvis, J.U.M. and Crowe, T.M. 1991. The colony structure and dominance hierarchy of the Damaraland mole-rat, *Cryptomys damarensis* (Rodentia: Bathyergidae). J. Zool. Lond., 224:553-576.

Jarvis, J.U.M. 1969. The breeding season and litter size of African mole-rats. *J. Reprod. Fertil.*, 6:237-248.

Jarvis, J.U.M. 1978. Energetics of survival in *Heterocephalus glaber* (Ruppell), the naked mole-rat (Rodentia: Bathyergidae). *Bull Carnegie Mus. Nat. Hist.,* 6:81-87.

Jarvis, J.U.M. 1981. Eusociality in a mammal: Cooperative breeding in naked mole-rat colonies. *Science*, 212:571-573.

Jarvis, J.U.M. 1985. Ecological studies on *Heterocephalus glaber*, the naked mole-rat, in Kenya. *Natl. Geogr. Soc. Res. Rep.*, 20:429-437.

Jarvis, J.U.M. 1991. Methods for Capturing, Transporting, and Maintaining Naked Mole-Rats in Captivity. In: *The Biology of the Naked Mole-Rat*. (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 467-483. Princeton University Press, Princeton, New Jersey.

Jarvis, J.U.M. 1991. Reproduction of Naked Mole-rats. In: *The Biology of the Naked mole-rat*. (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 384-425. Princeton University Press, Princeton, New Jersey.

Jarvis, J.U.M. and Bennett, N.C. 1991. Ecology and Behavior of the Family Bathyergidae. In: *The Biology of the Naked Mole-Rat*. (Ed. by P.W. Sherman, J.U.M. Jarvis & R.D. Alexander), pp. 66-96. Princeton University Press, Princeton, New Jersey.

Jarvis, J.U.M. and Bennett, N.C. 1993. Eusociality has evolved independently in two genera of bathyergid mole-rats - but occurs in no other subterranean mammal. *Behavioral Ecology and Sociobiology*, 33:253-260.

Jarvis, J.U.M. and Sale, J.B. 1971. Burrowing and burrow patterns of East African mole-rats *Tachyoryctes*, *Heliophobius* and *Heterocephalus*. *J. Zool. Lond.*, 163:451-479.

Jarvis J.U.M., O'Riain, M.J. & McDaid, E. 1991. Growth and Factors Affecting Body Size in Naked Mole-rats. In: *The Biology of the Naked Mole-Rat*. (Ed. by P.W. Sherman, J.U.M. Jarvis & R.D. Alexander), pp. 358-383. Princeton University Press, Princeton, New Jersey.

Jarvis, J.U.M., O'Riain M.J., Bennett N.C. and Sherman, P.W. 1994. Eusociality: a family affair. *Trends Ecol. Evol.*, 9:47-51.

Jaycox, E.R., Skowronek, W. and Gwynn, G. 1974. Behavioral changes in a worker honey bee (*Apis mellifera*) induced by injections of a juvenile hormone mimic. *Ann. Entomol. Soc. Am.*, 67:529-534.

Jeanne, R.L. 1972. Social biology of the neotropical wasp *Mischocyttarus drewseni*. *Bull. Mus. Comp. Zool. Harvard Univ.*, 144:63-150.

Jeanne, R.L. 1986. The evolution of the social organisation of work in social insects. *Monitore Zool. Ital.*, 20:119-133.

Jennions, M.D. and Macdonald, D.W. 1994. Cooperative breeding in mammals. *Trends Ecol. Evol.*, 9:89-93.

Jutsum, A.R., Saunders, T.S. and Cherrett, J.M. 1979. Intraspecific aggression in the leaf-cutting ant *Acromyrmex octospinosus*. *Anim. Behav.*, 27:839-844.

Kaplan, H. and Hyland, S.O. 1972. Behavioural development in the mongolian gerbil (*Meriones unguiculatus*). *Anim. Behav.*, 20:147-154.

Keller, L. and Ross, K.G. 1993. Phenotypic plasticity and 'cultural transmission' of alternative social organisations in the fire ant *Solenopsis invicta*. *Behav. Ecol. Sociobiol.*, 33:121-129.

Kennedy, J.S. 1956. Phase transformation in locust biology. *Biol. Res.*, 31:349-370.

Knerer, G. and Atwood, C.E. 1966. Polymorphism in some Nearctic halictine bees. *Science (Wash. D.C.)*, 152:1262-1263.

Koenig, W.D. 1978. Ecological and evolutionary aspects of cooperative breeding in acorn woodpeckers of central coastal california. - Ph.D. dissertation, University of California, Berkeley.

Koeppl, J.W. and Hoffman, R.S. 1981. Comparative postnatal growth of four ground squirrel species. *J. Mamm.*, 62:41-57.

Kolmes, S.A. 1985. A quantitative study of the division of labour among worker honey bees. *Z. Tierpsychol.*, 68:287-302.

Krebs, C.J. and Myers, J.H. 1974. Population cycles in small mammals. *Adv. Ecol. Res.*, 8:267-399.

Kruskal, J.B. and Wish, M. 1978. *Multidimensional scaling*. Sage University Paper series on Quantitative Applications in the Social Sciences, 07-011. Sage Publications, Beverly Hills.

Lacey, E.A. and Sherman, P.W. 1991. Social Organisation of Naked Mole-Rat Colonies: Evidence for divisions of Labor. In: *The Biology of the Naked Mole-Rat.* (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 275-336. Princeton University Press, Princeton, New Jersey.

Lacey, E.A. and Sherman, P.W. (in press). Cooperative breeding in naked mole-rats: Implications for vertebrate and invertebrate sociality. In: Cooperative Breeding in Mammals (Ed. by N. Solomon).

Lacey, E.A., Pepper, J.W., Alexander, R.D., Braude, S.H. and Sherman, P.W. 1991. An Ethogram for the Naked mole-rat. In: *The Biology of the Naked Mole-Rat.* (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander) pp. 209-242. Princeton University Press, Princeton, New Jersey.

Lachaud, J.P., Fresneau, D. and Corbara, B. 1988. Mise en evidence de souscastes comportementales chez *Amblyopone pallipes. Actes Coll. Ins. Soc.*, 4:141-147.

Ligon, J.D. and Ligon, S.H. 1978. Communal breeding in green woodhoopoes as a case for reciprocity. *Nature*, 276:496-489.

Linsenmair, K.E. 1985. Individual and family recognition in subsocial arthropods, in particular the desert arthropod *Hemilepistus reaumuri*. In: Fortschritte der Zoologie, Bd, 31: Experimental Behavioural Ecology. (Ed. by B. Holldobler and M. Lindauer), pp. 411-436. Fischer Verlag, Stuttgart.

Lovegrove, B.G. 1986. The metabolism of social subteranean rodents: adaptations to aridity. *Oecologia*, 69:551-555.

Lovegrove, B.G. 1991. The evolution of eusociality in mole-rats (Bathyergidae) a question of risks, numbers, and costs. *Behav. Ecol. Sociobiol.*, 28:37-45.

Lovegrove, B.G. & Wissel, C. 1988. Sociality in mole-rats: Metabolic scaling and the role of risk sensitivity. *Oecologia (Berl.)*, 74:600-606.

Macdonald, D.W. 1980. Social factors affecting reproduction amongst red foxes. In: *The Red Fox.* (Ed. by E. Zimen), pp. 123-175. The Hague: Junk.

McDonald, P. and Topoff, H. 1985. Polymorphism in army ants related to division of labour and colony cyclic behaviour. *J. Comp. Psychol.*, 1:3-14.

Malcolm, J.R. and Marten, K. 1982. Natural Selection and the Communal Rearing of Pups in African Wild Dogs (*Lycaon pictus*). *Behav. Ecol. Sociobiol.*, 10:1-13.

Martin, P. 1984. The time and energy costs of play behaviour in the cat. *Z. Tierpsychol.*, 64:298-312.

Martin, P. and Bateson, P. 1986. Studying development. In: *Measuring Behaviour an introductory guide*. Cambridge University Press, New York.

Maynard Smith, J. 1974. Recombination and the rate of evolution. *Genetics*, 78:299-304.

Maynard Smith, J. 1978. Optimisation theory in evolution. *Ann. Rev. Ecol. Syst.*, 9:31-56.

McNab, B.K. 1966. The metabolism of fossorial rodents: A study of convergence. *Ecology*, 47:712-733.

McNab, B.K. 1979. The influence of body size on the energetics and distribution of fossorial and burrowing animals. *Ecology*, 60:1010-1021.

McNab, B.K. 1980. On estimating thermal conductance in endotherms. *Physiological Zoology*, 53:145-156.

Meaney, M.J. and Stewart, J. 1981. A descriptive study of social development in the rat (*Rattus norvegicus*). *Anim. Behav.*, 29:34-45.

Michener, C.D. 1974. *The social behaviour of bees*. The Belknap Press of Harvard University, Cambridge, Massachucetts.

Moehlman, P.D. 1979. Jackal helpers and pup survival. *Nature*, 277:382-383.

Moore, J. and Ali, R. 1984. Are dispersers and inbreeding avoidance behaviour related? *Anim. Behav.*, 32:94-112.

Moore, R.W., Eisen, E.J. and Ulberg, L.C. 1970. Prenatal and postnatal maternal influences on growth in mice selected for body weight. *Genetics*, 64:59-68.

Moran, N.A. 1992. The evolutionary maintenance of alternative phenotypes. *Am. Nat.*, 139:971-989.

Mumme, R.L. and de Querioz, A. 1985. Individual contributions to cooperative behaviour in the acorn woodpecker: effects of reproductive status, sex and group size. *Behaviour*, 95:290-313.

Myles, T.G. and Nutting, W.L. 1978. Termite eusocial evolution: a re-examination of Bartz's hypothesis and assumptions. *Q. Rev. Biol.*, 63:1-23.

Nevo, E. 1979. Adaptive convergence and divergence of subterranean mammals. *A. Rev. Ecol. Syst.*, 10:269-308.

Nicoll, M.E. and Thompson, S.D. 1987. Basal metabolic rates and energetics of reproduction in therian mammals: marsupials and placentals compared. *Sym. Zool. Soc. Lond.*, 54:71-88.

Nijhout, H.F. 1981. Physiological control of moulting in insects. *Am. Zool.*, 21:631-640.

O'Riain, M.J. and Jarvis, J.U.M. (in press). Colony member recognition and xenophobia in the naked mole-rat. *Anim. Behav.*

Oster, G.F. and Wilson, E.O. 1978. *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, New Jersey.

Otto, D. 1958. Uber die Arbeitsteilung im Staate von *Formica rufa rufo-pratensis minor Gossw.* und ihre verhaltensphysiologischen Grundlagen: Ein Beitrag zur Biologie der roten Waldameise. *Wiss. Abh. Adtsch. Akad. Landwirtschaftwiss.* (Berl.), 30: 1-169

Packer, C. and Pusey, A.E. 1993. Dispersal, kinship, and Inbreeding in African Lions. In: *The natural history of inbreeding and outbreeding*. (Ed. by N.W. Thronhill), pp. 375-391. University of Chicago Press.

Page, R.E. and Breed, M.D. 1987. Kin Recognition in Social Bees. *Trends Ecol. Evol.*, 2:272-275.

Pamilo, P. 1984. Genetic relatedness and the evolution of insect sociality. *Behav. Ecol. Sociobiol.*, 15:241-248.

Pardi, L. 1948. Dominance order in Polistes wasps. *Physiol. Zool.*, 21:1-13.

Peeters, C. (in press). Morphologically "primitive" ants: comparative review of social characters, and the importance of queen-worker dimorphism. In: *Social Competition and Cooperation in Insects and Arachnids*. (Ed. by J. Choe and B. Crespi), *vol. 2 Evolution of Sociality*. Princeton University Press, Princeton, New Jersey.

Peeters, C. and Crewe, R. 1985. Worker reproduction in the ponerine ant *Ophthalmopone berthoudi* - an alternative form of eusocial organization. *Behav. Ecol. Sociobiol.*, 18:29-37.

Peeters, C. and Crozier, R.H. 1988. Caste and reproduction in ants: not all mated egg-layers are "queens". *Psyche (Camn., Mass.)*, 95:238-288.

Peeters and O'Riain (in prep). Eusociality and Cooperative breeding: reproductive inequalities and social evolution.

Peeters, C. and Tsuji, K. 1993. Reproductive conflict among ant workers in *Diacamma* sp. from Japan: dominance and oviposition in the absence of the gamergate. *Insectes Soc.*, 40:119-136.

Pepper, J.W., Lacey, E.A., Alexander, R.D., Braude, S.D. and Sherman, P.W. 1991. Vocalisations of the Naked mole-rat. In: *The Biology of the Naked Mole-rat*. (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 243-274. Princeton University Press, Princeton, New Jersey.

Pfennig, D. 1990. The adaptive significance of an environmentally cued developmental switch in an anuran tadpole. *Oecologia*, 85:101-107.

Pfennig, D.W. and Sherman, P.W. 1995. Kin Recognition. *Sci. Am.*, 68-73.

Pfennig, D.W., Reeve, H.K. and Shellman, J.S. 1983. Learned component of nestmate discrimination in workers of a social wasp, *Polistes fuscatus* (Hymenoptera: Vespidae). *Anim. Behav.*, 31:412-416.

Plowright, R.C. and Jay, S.C. 1977. On the size determination of bumblebee castes. *Can. J. Zool.*, 55:1133-1138.

Pollock, G.B. 1983. Population viscosity and kin selection. *Am. Nat.*, 122(6):817-829.

Porter, A. 1956. Morphology and affinities of Entozoa and Endophyta of the Naked mole-rat *Heterocephalus glaber. Proc. Zool. Soc. Lond. (B).*, 128:515-527.

Porter, R.H. and Wyrick, M. 1979. Sibling recognition in spiny mice (*Acomys cahirinus*). *Behav. Ecol. Sociobiol.*, 3:61-68.

Pratt, S.C., Carlin, N.F. and Calabi, P. 1994. Division of labor in *Ponera pennsylvannica* (Formicidae: Ponerinae). *Insectes Soc.*, 41:43-61.

Pusey, A.E. and Packer, C. 1987. The evolution of sex-biased dispersal in lions. *Behaviour*, 101:277-310.

Queller, D.C. 1992. Does population viscosity promote Kin Selection? *Trends Ecol. Evol.*, 7:324-344.

Ralls, K., Harvey, P.H. and Lyles, A.M. 1986. Inbreeding in natural populations of birds and mammals. In: *Conservation Biology: The Science of Scarcity and Diversity*. (Ed. by M.E. Soule), pp. 35-56. Sinauer.

Ralston, M. 1988. Derivative non-linear regression. In: *BMDP statstical software manual*. (Ed. by Dixon, W.J., Brown, M.B., Engelman, L., Hill, M.A. and Jenrich, R.I.), pp. 1:389-417. University of California Press, Berkley, L.A..

Rasa, O.A.E. 1977. The Ethology and Sociobiology of the Dwarf Mongoose (*Helogale undulata rufula*). *Z. Tierpsychol.*, 43:337-406.

Rasa, O.A.E. 1989. Helping in Dwarf mongoose societies: An alternative reproductive strategy. In: *The sociobiology of sexual and reproductive strategies*. (Ed. by O.A.E. Rasa, E. Voland and C. Vogel). Croom Helm, Beckenham.

Ratowsky, D.A. 1983. *Nonlinear regression modelling*. New York: Marcel Dekker.

Reeve, H.K. 1991. Polistes. In: *The Social Biology of Wasps.* (Ed. by K.G. Ross and R.W. Matthews), pp. 99-148. Cornell University Press, Ithaca, New York.

Reeve, H.K. 1992. Queen activation of lazy workers in colonies of the eusocial naked mole-rat. *Nature* (*London*), 358:147-149.

Reeve, H.K. and Sherman, P.W. 1991. Intracolonial Aggression and Nepotism by the Breeding Female Naked Mole-Rat. In: *The Biology of the Naked Mole-Rat.* (Ed. by P.W. Sherman, J.U.M. Jarvis and R.D. Alexander), pp. 337-357. Princeton University Press, Princeton, New Jersey.

Reeve, H.K., Westneat, D.F., Noon, W.A., Sherman, P.W. and Aquadro, C.F. 1990. DNA "fingerprinting" reveals high levels of inbreeding in colonies of the eusocial naked mole-rat. *Proc. Natl. Acad. Sci., U.S.A.*, 87:2496-2500.

Rickart, E.A. 1982. Annual cycles of activity and body composition in *Spermophilus lateralis. Can. J. Zool.*, 59:865-871.

Robinson, G.E. 1992. Regulation of division of labor in insect societies. *A. Rev. Entomol.*, 37:637-665.

Robinson, G.E., Page, R.E., Strambi, A. 1989. Hormonal and genetic behavioural control in honey bee colonies. *Science*, 246:109-112.

Rood, J.P. 1978. Dwarf mongoose helpers at the den. *Z. Tierpsychol.*, 48:277-287.

Rood, J.P. 1987. Dispersal and intergroup transfer in the Dwarf mongoose. In: *Mammalian Dispersal Patterns.* (Ed. by B.D. Chepko-Sade and Z.T. Halpin), pp. 85-103. University of Chicago Press, Chicago.

Rood, J.P. 1990. Group size, survival, reproduction and routes to breeding in dwarf mongooses. *Anim. Behav.*, 30:566-572.

Rosenthal, C., Bennett, N.C. and Jarvis, J.U.M. 1991. The changes in the dominance hierarchy over time of a complete field captured colony of *Cryptomys hottentotus hottentotus. J. Zool. Lond.*, 228:205-225.

Russel, E. and Rowley, I. 1988. Helper contributions to reproductive success in the splendid fairy wren (*Malurus splendens*). *Behav. Ecol. Sociobiol.*, 22:131-140.

Schaller, G.B. 1963. *The Mountain Gorilla: Ecology and Behaviour.* University of Chicago Press, Chicago.

Schwartz, O.A. and Armitage, K.B. 1980. Genetic variation in social mammals: The Marmot Model. *Science*, 207:665-667.

Schieffelin, J.S. and Sherman, P.W. 1995. Tugging contests reveal feeding hierarchies in naked mole-rat colonies. *Anim. Behav.*, 49:537-541.

Scott, J.P. 1958. *Aggression.* University of Chicago Press, Chicago.

Seeley, T.D. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav. Ecol. Sociobiol.*, 11:287-293.

Selander, R.K. 1970. Behaviour and genetic variation in natural populations. *Amer. Zool.*, 10:53-66.

Sherman, P.W. 1980. The limits of ground squirrel nepotism. In: *Sociobiology: Beyond Nature/Nurture?* (Ed. by G.W. Barlow and J. Silverberg), pp. 505-544. Westview Press, Boulder, Colorado.

Sherman, P.W. and Holmes, W.G. 1985. Kin recognition: issues and evidence. In: *Experimental behavioural ecology*. (Ed. by B. Holldobler and M. Lindauer), pp.437-460. Fischer verlag, Stuttgart/New York.

Sherman, P.W., Jarvis, J.U.M. and Alexander R.D. 1991. *The Biology of the Naked Mole-Rat.* 1st ed., Princeton University Press, Princeton, New Jersey.

Sherman, P.W., Jarvis, J.U.M. and Braude, S.H. 1992. Naked mole-rats. *Sci. Am.*, 267:72-78.

Sherman, P.W., Lacey, E.A., Reeve, H.K. and Keller, L. 1995. The eusociality continuum. *Behav. Ecol.* 6:102-108.

Shields, W.M. 1982. *Philopatry, Inbreeding and the Evolution of Sex*. State University of New York Press.

Shields, W.M. 1987. Dispersal and mating systems: investigating their causal connections. In: *Mammalian Dispersal Patterns*. (Ed. by B.D. Chepko-Sade and Z.T. Halpin), pp. 3-234. University of Chicago Press, Chicago.

Shields, W.M. 1993. The natural and unnatural history of inbreeding and outbreeding. In: *The natural history of inbreeding and outbreeding*. (Ed. by N.W. Thronhill), pp. 143-169. University of Chicago Press.

Smith, A.T. 1993. The Natural History of Inbreeding and Outbreeding in Small Mammals. In: *The natural history of inbreeding and outbreeding*. (Ed. by N.W. Thronhill), pp. 329-351. University of Chicago Press.

Smith, A.T. and Ivins, B. 1987. Colonisation in a pika population: dispersal vs. philopatry. *Behav. Ecol. Sociobiol.*, 13:37-47.

Smith, P.K., and Connelly, K. 1972. Patterns of play and social interaction in pre-school children. In: *Ethological Studies of child behaviour*. (Ed. by N.G. Blurton Jones), pp. 65-95. Cambridge University Press, Cambridge.

Smith, R.H. 1979. On selection for inbreeding in polygynous animals. *Heredity*, 43:205-211.

Sokal, R.R. and Rohlf, F.J. 1969. *Biometry*. (Ed. by W.H. Freemand and Co.), San Francisco.

Solomon, N.G. 1991. Current indirect fitness benefits associated with philopatry in juvenile voles. *Behav. Ecol. Sociobiol.*, 29:277-282.

Stanback, M.T. 1994. Dominance within broods of the cooperatively breeding acorn woodpecker. *Anim. Behav.*, 47:1121-1126.

Stearns, S.C. 1989. The evolutionary significance of phenotypic plasticity. *Bioscience,* 39:436-445.

Stenseth, N.C. 1984. Causes and consequences of dispersal in small mammals. In: *The Ecology of Animal Movement* (Ed. by P.J. Greenwood and I.R. Swingland), pp. 63-101. Clarendon Press, Oxford.

Strassman, J.E. and Meyer, D.C. 1983. Gerontocracy in the social wasp, *Polistes exclamans. Anim. Behav.*, 31:431-438.

Stuart, R.J. 1991. Kin recognition as a functional concept. *Anim. Behav.*, 41:1093-1094.

Stuart, R.J. 1992. Nestmate recognition and the ontogeny of acceptability in the ant, *Leptothorax curvispinosus. Anim. Behav.*, 30:403-408.

Swingland, I.R. 1984. In: *The Ecology of Animal Movement* (Ed. by P.J. Greewwood and I.R. Swingland). Clarendon Press, Oxford.

Taylor, G.T. 1979. Reinforcement, motivation and intraspecific aggressive behaviour. *Behav. Neur. Biol.*, 27:1-24.

Theraulaz, G., Pratte, M. and Gervet, J. 1990. Behavioural profiles in *Polistes dominulus* (Christ) wasp societies: a quantitative study. *Behaviour*, 113:223-249.

Tinbergen, N. 1951. *The study of instinct.* Oxford University Press, Oxford.

Tofts, C. and Franks, N.R. 1992. Doing the right thing: Ants, honeybees and naked mole-rats. *Trends Ecol. Evol.*, 7:346-349.

Travis, J. 1994. Evaluating the Adaptive Role of Morphological Plasticity. In: *Ecological morphology: integrative organismal biology.* (Ed. by P.C. Wainwright and S.M Reilly), pp. 99-122. University of Chicago Press, Chicago.

Trivers, R.L. 1971. The evolution of reciprocal altruism. *Q. Rev. Biol.*, 46:35-57.

Tschinkel, W.R. 1988. Colony growth and the ontogenty of worker polymorphism in the fire ant, *Solenopsis invicta. Behav. Ecol. Sociobiol.*, 22:103-115.

Tyson, J.J. 1984. Evolution of eusociality in diploid species. *Theor. Pop. Biol.*, 26:283-295.

Van Damme, M.P., Robertson, D.M. and Diczfalsuy, E. 1974. An improved in vitro bioassay method for measuring luteinising hormone (LH) activity using mouse Leydig cell preparations. *Acta. Endorcrinol.*, 77:655-671.

Vehrencamp, S.L. 1983. Optimal Degree of Skew in Cooperative Societies. *Amer. Zool.*, 23:327-335.

Venkataraman, A.B., Swarnalatha, V.B., Nair, P. and Gadagkar, R. 1992. The mechanism of nestmate discrimination in the tropical social wasp *Ropalidia marginata* and its implications for the evolution of sociality. *Anim. Behav.*, 43:95-102.

Villet, M., Crewe, R. and Duncan, F. 1991. Evolutionary trends in the reproductive biology of ponerine ants (*Hymenoptera: Formicidea*). *J. Nat. Hist.*, 25:1603-1610.

Wainright, P.C., Osenberg, C.W. and Mittelbach, G.G. 1991. Trophic polymorphism in the pumpkinseed sunfish (*Lepomis gibbosus*): Environmental effects on ontogeny. *Func. Ecol.*, 5:40-55.

Waldmann, B., Frumhoff, P.C. and Sherman, P.W. 1988. Problems of Kin Recognition. *Trends Ecol. Evol.*, 3:8-13.

Walsburg, G.E. 1987. Evaluation of non-destructive method for determining fat stores in small birds and mammals. *Physiol. Zool.*, 61(2):153-159.

Wasser, P.M. 1985. Does competition drive dispersal? *Ecology*, 66:1170-1175.

Welker, W.I. 1971. Ontogeny of play and exploratory behaviours: A definition of problems and a search for new conceptual solutions. In: *The ontogeny of Vertebrate Behaviour* (Ed. by H. Moltz), pp. 171-228. Academic Press, New York.

Werren, J.H. 1993. The Evolution of Inbreeding Haplodiploid organsisms. In: *The natural history of inbreeding and outbreeding*. (Ed. by N.W. Thronhill), pp. 42-59. University of Chicago Press.

West-Eberhard, M.J. 1969. The social biology of polistine wasps. *Misc. Publ. Mus. Zool. Univ. Mich.*, 140:1-101.

West-Eberhard, M.J. 1981. Intragroup selection and the evolution of insect societies. In: *Natural Selection and Social Behaviour.* (Ed. by R.D. Alexander and D.W. Tinkle), pp. 3-17. Chiron Press, New York.

West-Eberhard, M.J. 1986. Alternative adaptions, speciation, and phylogeny (a review). *Proc. Natl. Acad. Sci. U.S.A.*, 83:1388-1392.

West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Ann. Rev. Ecol. Syst.*, 20:249-278.

Westlin, L., Jarvis, J.U.M. and Bennett, N.C. 1994. Relaxation of reproductive suppression in non-breeding female naked mole-rats, *Heterocephalus glaber. J. Zool. Lond.*, 234:177-188.

Wheeler, D.E. 1986. Development and physiological determinants of caste in social *Hymenoptera*: evolutionary implications. *Am. Nat.*, 128:13-34.

Wheeler, D.E. 1991. The developmental basis of worker caste polymorphism in ants. *Am. Nat.*, 138:1218-1238.

Wheeler, D.E. and Nijhout, H.F. 1981. Soldier determination in ants: new role for juvenile hormone. *Science*, 213:361-363.

Wheeler, D.E. and Nijhout, H.F. 1983. Soldier determination in the ant *Pheidole bicarinata*: Hormonal control of caste and size within castes. *J. Insect Physiol.*, 29:847-854.

Wille, A. and Michener, C.D. 1973. The nest architecture of stingless bees with special reference to those of Costa Rica. *Rev. Biol. Trop.* (San Jose, Costa Rica) (Suppl) 1:1-278.

Williams, E. and Scott, J.P. 1954. The development of social behaviour patterns in the mouse, in relation to natural periods. *Behaviour*, 6:35-64.

Wilson, E.O. 1971. *The Insect Societies.* Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Wilson, E.O. 1975. *Sociobiology.* Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Wilson, E.O. 1976. Behavioural discretisation and the number of castes in an ant species. *Behav. Ecol. Sociobiol.*, 1:141-154.

Wilson, E.O. 1984. The relation between caste ratios and division of labour in the ant genus *Pheidole* (Hymenoptera: Fromicidae). *Behav. Ecol. Sociobiol.*, 16:89-98.

Wilson, E.O. 1985a. The principles of caste evolution. In: *Experimental Behavioural Ecology*. (Ed. by B. Holldobler and C. Lindauer), pp. 307-324. Fischer Verlag, New York.

Wilson, E.O. 1985b. The sociogenesis of insect colonies. *Science, Wash., D.C.*, 228:1489-1495.

Wilson, E.O. and Hölldobler, B. 1988. Dense heterarchies and mass communication as the basis of organisation in ant colonies. *Trends Ecol. Evol.*, 3:65-68.

Winston, M.L. and Punnett, E.N. 1982. Factors determining temporal division of labour in honeybees. *Can. J. Zool.*, 60:2947-2952.

Withers, P.C. and Jarvis, J.U.M. 1980. The effect of huddling on thermoregulation and oxygen consumption for the naked mole-rat. *Comp. Biochem. Physiol. A, Comp. Physiol.*, 66:215-219.

Woolfenden, G.E. and Fitzpatrick, J.W. 1978. The inheritance of territory in group breeding birds. *BioScience*, 28:104-108.

Woolfenden, G.E. and Fitzpatrick, J.W. 1990. Florida scrub jays: a synopsis after 18 years of study. In: *Cooperative breeding in birds* (Ed. by P.B. Stacey and W.D. Koenig), pp.240-266. Cambridge University Press, Cambridge.

Zahavi, A. 1990. Arabian babblers: the quest for social status in a cooperative breeder. In: *Cooperative breeding in birds* (Ed. by P.B. Stacey and W.D. Koenig), pp.105-130. Cambridge University Press, Cambridge.

Zar, J.H. 1977. *Biostatistical Analysis*. Prentice-Hall, New Jersey.

Zimen, E. 1976. On the regulation of pack size in wolves. *Z.Tierpsychol.* 40:300-341.

Zullinger, E.M., Ricklefs, R.E., Redford, K.H. and Mace, M.M. 1984. Fitting sigmoidal equations to mammalian growth curves. *J. Mamm.*, 65:607-636.

APPENDIX I

An alphabetical list of the key behaviours recorded during scan and ad-lib sampling. Were applicable a brief description of the behaviour is provided.

Allocoprophage (donor): a mole-rat allows a colony member to eat faecal matter from its anus.

Allocoprophage (recipient): a mole-rat consumes faecal matter from another mole-rats anus.

Allogroom anogenital region (donor): a mole-rat grooms the genital region of another mole-rat.

Allogroom anogenital region (recipient): a mole-rat has its genitals groomed by another mole-rat.

Allogroom pup: grooming (primarily of the genitalia) a pup.

Autocoprophage: a mole-rat consumes its own faeces.

Autogroom anogenital region: a mole-rat grooms its own genitalia.

Bask: an animal stands with its body (usually the back) pressed against an externally heated section of the burrow system.

Bite: the jaws of one animal close over the body of another animal without breaking the skin of that individual.

Cannibalise pup: consuming either a dead or alive pup.

Carry food: food items are held in the incisors and transported within the burrow system.

Carry nest material: includes both the carrying (with incisors) and manipulation of nest material.

Carry pup: carrying a pup (with the incisors) in either the nest or the tunnels.

Clean teeth: grooming the incisors with the forefeet.

Defecate.

Dig: Includes both gnawing, where the incisors are scraped along a digging face, and foreleg digging, where the forefeet alternate in scraping the digging face.

Feed.

Hindleg Scratch: scratching the body or face with the hindfeet.

Mate: copulation with direct contact between the male and female genitalia.

Mount: a mole-rat mounts another mole-rat (head or rump) and makes repeated thrusts with his pelvic region.

Mutual naso-anal groom: two mole-rat lie head to tail with one another and groom each others genitals.

Nose-rub: a mole-rat rubs its nose vigorously against the bodies of other mole-rats and its forefeet.

Pup-push: a mole-rat (juvenile or adult) either places its nose against the body of a pup and then shoves or rams the pup from a short distance.

Run.

Shove (donor): a mole-rat shoves another mole-rat following a face to face encounter.

Shove (recipient): a mole-rat is shoved following a face to face encounter with another mole-rat.

Sleep.

Steal food: a mole-rat removes a food item from a colony member that is actively feeding.

Suck: the pups place their mouth directly over a nipple and make sucking motions.

Suckle: the breeding female nurses her pups.

Sweep: the animal kicks loose material in the tunnels behind itself while moving backwards. The back legs are used synchronously with the hind feet rotated inwards.

Teeth groom: grooming the body with the incisors.

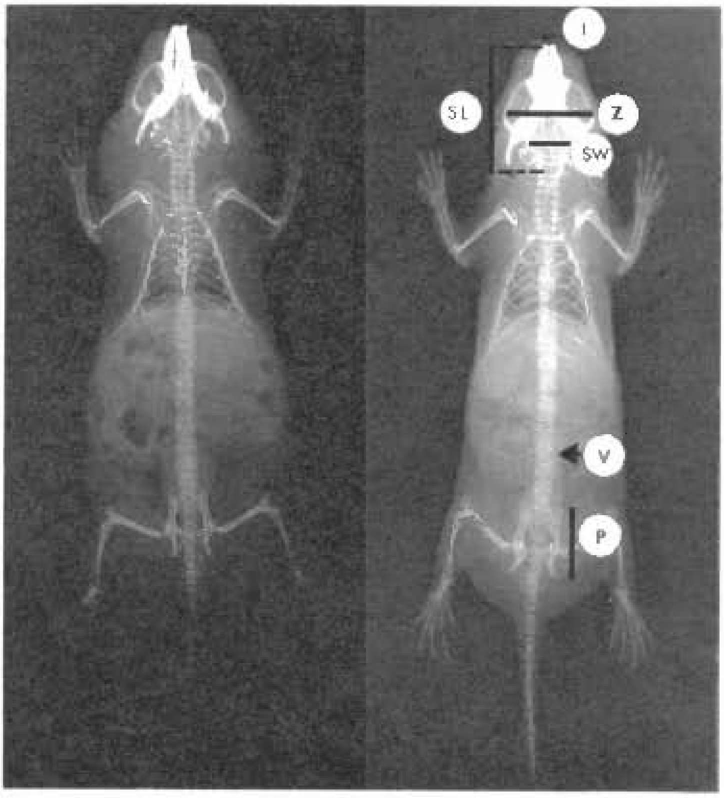
Tooth chisel: a mole-rat rubs its lower and upper incisors together.

Tooth-fence: at least two mole-rats stand face to face and fend one another off using their incisors. The incisors may lock briefly.

Urinate.

Walk.

APPENDIX II



Measurements taken from X-ray images of anaesthetised mole-rats for morphometric analyses. **I** (incisor width) = width across upper incisors; **SL** (skull length) = length of skull from the tip of the upper incisors to the posterior end of the basisphenoid; **Z** (zygomatic arch width) = widest point between the zygomatic arches; **SW** (skull width) = width across the base of the skull, immediately anterior to the auditory meatus; **V** (vertebral length) = length of the 5th lumbar vertebrae counting anteriorly from the sacral region; **P** (pelvis length) = length of the pelvis including the innominate and ischium bones.