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Cooperative behaviour in naked mole-rats (*Heterocephalus glaber*) - Specialized castes or social plasticity?

Master thesis

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submitted by

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1. Introduction

In some social insect societies, all individuals are morphologically differentiated in specialised reproductive and working castes (Wilson, 1971; Boomsma, 2009; 2013). In the most specialised species, workers often show divergent developmental trajectories that lead to functionally distinct groups of individuals that differ in behaviour, morphology and physiology and predispose them to certain tasks in the group (Bourke & Franks, 1995; Roisin & Korb, 2010). Other social insect species show temporary specialisation or no specialisation at all. In contrast, cooperatively breeding vertebrates have not evolved reproductive and sterile castes and mostly show behavioural plasticity rather than specialisation. Naked mole-rats (*Heterocephalus glaber*) are among the most specialised cooperatively breeding vertebrates. They live in groups of up to 300 individuals and cooperate in foraging, territory defence, and breeding. Some studies suggest that individuals in naked mole-rat groups may show permanent or temporal task specialisation (Jarvis, 1981; Jarvis et al., 1991; Lacey & Sherman, 1997). However, recent studies from closely related mole-rat species suggest that helping behaviour is plastic and that individuals do not specialise (Zöttl, Vullioud et al., 2016; Thorley et al., 2018; Van Daele, Desmet, Šumbera, & Adriaens, 2019). The general aim of this study is to clarify whether non-reproductive group members of naked mole-rats show behavioural specialisation similar to some social insects, or whether they show social plasticity comparable to other cooperatively breeding mammals.

1.1 Cooperative breeding in vertebrates and invertebrates

The eusocial insects such as ants, termites and certain species of bees and wasps show the most extreme forms of cooperative societies among animals (Wilson, 1971). These taxa exhibit reproductive division of labour with more or less sterile individuals working on behalf of the breeding individuals, leading to irreversible behavioural and morphological specialisation. They also show overlapping generations and alloparental care in their groups (Batra, 1966; Michener, 1969; Wilson 1971). Eusociality is therefore often considered the most complex form of social organisation on a continuum of cooperative social systems (Gadagkar, 1994; Sherman, Lacey, Reeve, & Keller, 1995).

Reproductive division of labour, or the restriction of reproduction to a small number of animals within a group, is a key feature of eusociality (Wilson, 1971; Crespi & Yanega, 1995).

Eusocial insect societies form behaviourally and often morphologically distinct groups (“castes”) of reproductive females and males (“queens” and “kings”) and non-reproductive individuals (“workers”) (Wilson, 1971). Most groups contain only a single queen and the workers are physiologically sterile, resulting in high reproductive skew (Sherman et al., 1995). Individuals follow divergent developmental trajectories from early life and become irreversibly fixed in their social roles (Oster & Wilson, 1978; Smith, Toth, Suarez, & Robinson, 2008). In some species, especially ants and termites, extensive morphological specialisation occurs, with queens developing enlarged ovaries and growing to several times the size of workers (Wilson, 1971; Eggleton, 2010). The functional role of the queen is exclusively limited to colony foundation and production of offspring, while workers perform all other cooperative tasks, including brood care (Oster & Wilson, 1978). The size of eusocial insect colonies ranges from a few hundred or thousand (hornets, e.g. *Vespa crabro*: Archer, 1993; honeybees, e.g. *Apis mellifera*: Seeley, 1985; wasps, e.g. *Vespula vulgaris*: Green, 1991) to several million individuals (ants, e.g. *Atta sexdens:* Hölldobler & Wilson, 1990).

Another form of complex sociality is cooperative breeding. Cooperative breeders are species with members of a social group that, rather than breeding, assist other group members in raising their offspring (Solomon & French, 1997). These *alloparents* or *helpers* perform various tasks that typically improve other’s reproductive success, such as feeding the young, building, cleaning and maintaining nests, guarding the territory, and defending against predators (reviewed in Brown, 1987; Heinsohn & Legge, 1999). Helpers show prolonged natal philopatry and temporarily forego direct reproduction for indirect fitness benefits through helping to raise related offspring or by increasing the probability of successful breeding later in life, either by dispersing or by attaining a breeder role in their native group. All helpers therefore retain reproductive totipotency (Solomon & French, 1997; Boomsma, 2013). Cooperative breeding has been reported in fish, birds and several mammalian taxa including rodents, canids, and primates (Lukas & Clutton-Brock, 2012; Koenig & Dickinson, 2016).

Groups of cooperatively breeding vertebrates are much smaller than eusocial insect colonies and typically composed of one breeding pair and several helpers (up to six in wolves, *Canis lupus*: Miklósi, 2015; 14 in cooperative cichlids, *Neolamprologuls pulcher:* Balshine et al., 2001; 18 in African wild dogs, *Lycaon pictus*: Creel & Creel, 1995; 28 in meerkats, *Suricata* *suricatta:* Clutton-Brock et al., 2001). Whereas the workers in eusocial societies are irreversibly sterile, helpers in cooperatively breeding species retain their reproductive potential even though they may be reproductively supressed (Crespi & Yanega, 1995). The helper role is therefore temporary rather than permanent, and the majority of subordinates will ultimately attempt to breed directly, either by dispersing and filling a vacant breeding position, founding a new group, replacing breeders in their natal group, or by mating while they are helpers (e.g. Mongolian gerbil, *Meriones unguiculatus*: Agren, Zhou, & Zhong, 1989; dwarf mongoose, *Helogale parvula:* Creel & Waser, 1997; meerkat: Griffin et al., 2003; wolf: Mech, 1987). There are very few morphological differences between breeders and helpers in cooperative breeders and all group members typically participate in all cooperative tasks (Brown, 1987; Solomon & French, 1997).

In addition to labour division between breeding and non-breeding group members, some eusocial systems are characterized by a secondary division of cooperative labour among the non-reproductive individuals. The allocation of cooperative tasks to different individuals improves efficiency of a group and the extent to which sets of individuals within a society specialise on selected tasks is therefore often considered an indicator for its complexity (Oster & Wilson, 1978; Bourke, 1999). In eusocial insect societies, the division among workers is so pronounced that they can be grouped into morphologically specialised castes. In their most extreme form, mostly restricted to higher ant and termite species, these physical castes represent permanent and irreversible phenotypes that are predisposed by divergent developmental trajectories during ontogeny (Bourke & Franks, 1995; Smith et al., 2008; Roisin & Korb, 2010). Morphological specialisation among non-breeders is most striking in the soldier caste of higher termites (Termitidae: Wilson, 1971) and leafcutter ants (*Atta*: Hölldobler & Wilson, 1990) whose members possess enlarged heads, elongated or scissor-like mandibles or the ability to spray secretions on aggressors. Along with their unique physical features these soldiers developed a specialised behavioural repertoire that in extreme cases prevents them from feeding themselves, so that they rely on workers for survival (Engel, Barden, Riccio, & Grimaldi, 2016).

Other eusocial invertebrates, among them bees and paper wasps, form temporal castes that are the product of age-related polyethism, defined as the “regular changing in labour roles by colony members as they age" (Wilson, 1971, p. 461). The sterile workers of the honeybee (*Apis mellifera)* for example occupy several roles during their lifetime: starting as cell cleaners, they go on to feed the young and care for the queen as nurses, later perform a variety of tasks related to nest building and food storage and eventually leave the nest to become foragers (Seeley, 1982). Such temporal worker sub-castes are not distinguishable by morphological traits (Wilson, 1971). There is some flexibility to revert to prior stages of specialisation in response to colony needs in later life, but early caste transitions are more rigidly fixed (Oster & Wilson, 1978; Johnson, 2010).

Cooperatively breeding vertebrates exhibit diverse forms of labour division among non-reproductive group members. They are typically monomorphic and display a higher degree of social plasticity than eusocial insects. In some species, evidence for the specialisation of individual group members is rare and restricted to specific, temporally limited behavioural contexts. African wild dogs for example engage in cooperative hunting where members of the group take on different roles for the duration of the activity (Creel & Dugatkin, 2001). Helpers of other cooperative vertebrate species such as meerkats (Clutton-Brock, Russell, & Sharpe, 2003) and white-winged choughs (*Corcorax melanorhamphos*: Heinsohn & Cockburn, 1994) do not specialise temporally or permanently in specific helping activities. Instead of qualitatively distinct behavioural repertoires, subordinates here display quantitative differences in their general investment in all cooperative tasks. The extent of their overall contribution is informed by the fitness costs and benefits of cooperating, which vary with sex, individual body condition as a consequence of age, weight and growth, and demographical or ecological conditions, all of which may influence an individual's chances to reproduce (McNamara & Houston, 1996; Cant & Field, 2001; Field, Cronin, & Bridge, 2006). Supplemental feeding, for example, increases helping behaviour in several species, showing the influence of body condition on cooperative investment (meerkats: Clutton-Brock et al., 2000; moorhens*, Gallinula chloropus*: Eden, 1987; white-winged choughs: Boland, Heinsohn, & Cockburn, 1997). In subordinate meerkats, the amount of cooperative investment is dependent on age and weight, with older and fast-growing animals contributing more to all cooperative tasks (Clutton-Brock et al., 2003), and similar relationships have been found in other cooperatively breeding mammals (Solomon & French, 1997), birds (Cockburn, 1998) and fish (Bruintjes & Taborsky, 2011).

1.2 Cooperative breeding in naked mole-rats – a special case

Naked mole-rats were the first mammals to be described as eusocial due to the unusual complexity of their social system (Jarvis, 1981). These small fossorial rodents live in colonies of

75-80 and occasionally up to 295 individuals with one single breeding female (Brett, 1991). The large group size and high reproductive skew make them unique among mammals and may have led to the formation of a highly specialised social organisation similar to that of eusocial insects. Jarvis (1981) hypothesized in her seminal paper that naked mole-rats exhibit all three attributes of eusociality found in social insects, i.e., reproductive division of labour, overlapping generations and alloparenting, as well as a system of distinct worker castes.

Naked mole-rats exhibit a clear division of reproductive labour: in most groups, free-living and laboratory-held, only one female (which, following the terminology of insect colonies, are referred to as “queens”) and one to three males breed (Jarvis, 1981; Brett, 1991). The occurrence of two simultaneously breeding females in one colony is rare, with Braude (1991a) reporting that only as many as 10% of free-living groups at most may have more than one queen at a time. Reproductive skew is therefore very high with only an estimated 1% of the population ever attaining breeding status (Bennett & Faulkes, 2000).

Breeding females are distinct from other female group members not only regarding their monopoly on reproduction and related behaviours (behavioural oestrus, mating, lactation), but also in their dominance status and frequent displays of aggression towards non-breeders, which are the primary mechanisms of reproductive suppression (Faulkes, Abbott, Liddell, George, & Jarvis, 1991; Reeve & Sherman, 1991). Despite the stress-induced reproductive inhibition, non-breeding naked mole-rats, unlike workers in eusocial invertebrate societies, are not permanently physiologically sterile (Faulkes & Abbott, 1991; 1996). Similar to eusocial insect queens, breeding individuals in the wild have long life spans compared to non-breeders and remain capable of reproduction throughout their lives (Buffenstein, 2008; Hochberg, Noble, & Braude, 2016; Ruby, Smith, & Buffenstein, 2018). However, once a breeding animal dies, the socially induced reproductive inhibition is removed and several non-breeders from within the colony compete to replace them. The succession process among females involves violent attacks on potential rivals and their allies and may be influenced by dominance hierarchies established among juvenile litter mates (Clarke & Faulkes, 1997; Jarvis, 1991). Once females attain breeder status, they develop unique morphological features such as elongated vertebrae, enlarged teats and a perforate vagina (Jarvis, 1991; Henry, Dengler-Crish, & Catania, 2007). Besides being among the largest members of a colony, male breeders are less easily identifiable by physical traits (Lacey & Sherman, 1997).

Typically, there are several overlapping generations in naked mole-rat colonies. Although dispersal for the formation of new colonies does occur in wild mole-rat groups (Braude, 2000), the majority of the offspring remain in their natal groups throughout their lives (Braude, 1991a; Brett, 1991). Recruitment arises almost exclusively from within groups and breeders are replaced by subordinates from the same colony. Consequently, colonies consist mostly of closely related animals (Braude, 1991a; Reeve, Westneat, Noon, Sherman, & Aquadro, 1990).

Non-breeders of both sexes frequently engage in alloparental care. All members of a colony contribute to pup care by grooming young, carrying them back to or between nests and huddling in the nest for warmth (Jarvis, 1991; Lacey et al., 1991; Lacey & Sherman, 1991; 1997). Non-breeders are the main providers of caecotrophes and thereby nutrients and endosymbiotic gut flora for the pups until they are fully weaned (Jarvis, 1991; Lacey & Sherman, 1991; 1997). They also indirectly help the reproductive success of the breeders by foraging and carrying food to the nest as well as maintaining and defending the colony’s burrow (Jarvis, 1981; Lacey & Sherman, 1991 Like eusocial insects, a breeder's contribution to most maintenance and foraging tasks is negligible. Male and female breeders do, however, play a substantial part in direct pup care (Lacey & Sherman, 1991). In contrast to many other cooperatively breeding mammals where allolactation is a common occurrence (e.g. African wild-dogs: Malcolm & Marten, 1982; meerkats: Clutton-Brock, Russell, & Sharpe, 2004; dwarf mongooses: Rood, 1980), queens are the only females in naked mole-rat colonies to lactate and nurse pups (Jarvis, 1991; Lacey & Sherman, 1991; 1997).

Naked mole-rats thus fit all the classic criteria of eusociality, except that they lack sterile workers, and they have become widely referred to as "eusocial mammals" in scientific literature. However, the claim that naked mole-rat colonies are organized in a caste system analogous to eusocial insects is still unclear and a matter of scientific debate.

1.3 Secondary division of labour in naked mole-rats

Some evidence suggests that naked mole-rats may show an insect-like caste organisation with discrete division of labour. Jarvis (1981) described the organisation of labour division among the non-breeding group members both as discrete physical castes and as a partial age polyethism. When looking at the frequency with which non-reproductive members of a colony performed certain tasks, she identified three castes of helpers: “frequent workers”, who performed mostly colony maintenance activities (including nest building, carrying food, digging, and transporting soil), “infrequent workers”, who worked at less than half the rate of frequent workers, but engaged in many of the same tasks, and “non-workers”, who were occasionally observed digging, but otherwise had no readily identifiable role in the colony. However, males of this group were most likely to eventually become breeders. Besides differences in behaviour, the proposed castes were morphologically distinguishable in terms of body mass, with non-workers being the heaviest animals in a group and infrequent workers weighing on average more than frequent workers. Additionally, Jarvis proposed a partially age-based mechanism by which all juvenile mole-rats first enter the frequent worker caste, and while slow-growing animals remain in this group indefinitely, some fast-growing individuals eventually reduce their working load and become “non-workers” or even breeders. This distinction meant that body size was not consistently indicative of age, and some of the oldest animals in her study colony were among the smallest.

Several studies have since reported the existence of different functional and physical groups in naked mole-rats. A dispersive morph with distinctive morphological, physiological and behavioural characteristics was discovered in captive (O'Riain, Jarvis, & Faulkes, 1996) as well as in free-living colonies (Braude, 2000). Dispersers are mole-rats of both sexes with high amounts of body fat and elevated levels of plasma luteinizing hormone, rendering their endocrine profile similar to that of reproductively active animals. They were also shown to solicit mating only with foreign and unrelated mole-rats and engaged less in cooperative activities than non-dispersive workers (O'Riain et al., 1996). These characteristics suggest a specialised phenotype adapted to dispersal and the founding of new colonies reminiscent of alates, the winged reproductive forms of termites that store excess fat to provide energy during swarming and initial colony foundation (Myles & Nutting, 1988). Further polymorphisms among non-breeders include slight variations in incisor width and jaw musculature that have been associated with the role of colony defence, suggesting the existence of a defender phenotype distantly comparable to the soldier caste found in many ant and termite species (O'Riain, 1996).

Studies following up on Jarvis’ initial suggestions found that the investment in cooperative labour among non-breeding mole-rats varied in accordance with body mass, with small animals participating more frequently in maintenance tasks (Faulkes et al., 1991; Jarvis, O'Riain, & McDaid, 1991). Further studies investigated the full cooperative task repertoire of naked mole-rats and showed that in addition to group maintenance, small non-breeders performed more alloparental pup care, while larger non-breeders were primarily engaged in colony defence, including threatening and attacking predators and foreign conspecifics and “guarding the nest” by lying near and facing out of its entrances (Lacey & Sherman 1991, 1997; O'Riain & Jarvis, 1997; Mooney, Filice, Douglas, & Holmes, 2015). Animals with a higher body mass are also more likely to perform “volcanoing”, the expulsion of soil from tunnels, which opens up the burrow and exposes these mole-rats to considerable predation risk (Braude, 1991b). Brett (1991) found a significant negative correlation between capture order and body weight in five of six wild colonies (but only in four out of 12 colonies trapped by others, reviewed by Brett, 1991), suggesting that heavier animals are the first to appear at tunnel breaches and to respond to potential danger. However, the cited studies aiming to replicate Jarvis’ findings failed to identify permanent physical or functional worker castes in non-reproductive mole-rats (Faulkes et al., 1991; Jarvis et al., 1991; Lacey & Sherman, 1991). While they reported substantial differences in body mass as well as cooperative investment amongst non-breeders, the variations appeared continuous rather than discrete, thereby refuting the categorisation of workers into distinct castes (O’Riain & Faulkes, 2008).

Despite several studies investigating division of labour in naked mole-rats it is still unclear how age and body mass affect investment in different cooperative tasks. Lacey and Sherman (1991; 1997) proposed a polyethism based on size and age, where all non-breeders gradually switch roles from maintenance worker to defender as they become older and larger. In contrast, Jarvis et al. (1991) reported that in their study colonies, both body size and cooperative behaviour varied widely within litters and age therefore did not predict working behaviour. Recently, Mooney et al. (2015) showed that non-breeding individuals not only consistently differed in their relative contribution to different cooperative tasks over several months but also traded-off investment in pup care against maintenance and defence activities. However, when colony composition was disturbed by the removal of task specialists, mole-rats showed the ability to switch tasks depending on demand, indicating a degree of behavioural flexibility that is incompatible with rigid caste organisation and irreversible individual specialisations.

Moreover, a number of recent studies challenge the existence of a discrete caste system in several closely related species of social mole-rats. Damaraland mole-rats (*Fukomys* *damarensis*) have also been described as eusocial and their colonies, although smaller (averaging 18 animals), show considerable resemblance to those of naked mole-rats (Bennett & Jarvis, 1988; Jarvis & Bennett, 1993; Jarvis, O'Riain, Bennett, & Sherman, 1994). While subordinate Damaraland mole-rats exhibit differences in overall investment in cooperation, individuals do not specialise on specific tasks and behavioural variation appears to be a consequence of differences in age and growth among non-breeders (Zöttl, Vullioud et al., 2016; Thorley et al., 2018). Similarly, the first study of social organisation in the cooperatively breeding Micklem's mole-rat (*Fukomys micklemi*) showed that non-breeding individuals could not be categorized into discrete castes based on physical characteristics or amount of working behaviour and lacked task specialisation (Van Daele et al., 2019). A radio-tracking study on wild Ansell's mole-rats (*Fukomys anselli*) found no discontinuity in their space-use pattern indicative of discrete behavioural castes. Instead, any variability in the data was likely to be the result of age-related polyethism (Šklíba, Lövy, Burda, & Šumbera, 2016). No distinct morphological castes based on body mass were found in captive (Zöttl, Thorley, Gaynor, Bennett, & Clutton-Brock, 2016) and free-living Damaraland mole-rat colonies (Voigt, ter Maat, & Bennett, 2019) and further field studies did not provide evidence for the existence of dispersal morphs (Torrents-Ticó, Bennett, Jarvis, & Zöttl, 2018) or a relationship between body mass and capture order and therefore a size-related specialisation on defence activities (Voigt et al., 2019). In sum, there seems to be little evidence for an insect-like caste system with irreversible physical or behavioural specialisation in most of the social African mole-rats. Although eusociality has evolved independently in Damaraland mole-rats and naked mole-rats (Jarvis & Bennett, 1993) and it is therefore possible that the social structures of their colonies differ from one another, evidence for age-related polyethism and functional flexibility in this closely related species suggests that similar mechanisms might be responsible for behavioural variation in naked mole-rats.

Most studies in naked mole-rats so far have focussed on inter-individual differences in the investment in each cooperative task in isolation and failed to investigate trade-offs between different cooperative tasks within individuals. Yet, it is still unclear whether individuals engage in certain behaviours while simultaneously neglecting others in order to infer task specialisation. Additionally, a majority of studies did not record longitudinal data for individuals, therefore failing to show whether or not differences in behaviour are temporally stable, which would support the notion of permanent caste allocation as found in eusocial insects. Conversely, low repeatability of cooperative behaviour over time would suggest that naked mole-rats maintain behavioural plasticity similarly to other cooperatively breeding vertebrates. Unlike previously mentioned studies, Mooney et al. (2015) used longitudinal data to demonstrate behavioural consistency of individuals over the span of several months. They also found trade-offs between pup care and maintenance and defence activities but based their evidence on aggregated data from behavioural observations conducted within one week, a period too brief to show long-term specialisation. Moreover, considering the unpredictable and highly individual activity patterns of naked mole-rats, observation periods of 30-60 minutes as used in earlier studies appear too short to adequately reflect the time budgets of individual animals (Riccio & Goldman, 2000).

1.4 Aim and predictions

The aim of this study is to clarify whether non-reproductive colony members of naked mole-rats are organised in a caste system of behavioural specialisation similar to eusocial insects or whether they show social plasticity comparable to other cooperatively breeding mammals. While the formation of distinct castes is not an explicit criterion in most definitions of eusociality (but see Crespi & Yanega, 1995; reviewed in Costa & Fitzgerald, 2005), it is a typical feature of eusocial insect societies. The existence of a caste system analogous to the social organisation of insect species in naked mole-rats would be unique among mammals. A better understanding of the social organisation of naked mole-rats will help highlight similarities and contrasts between evolutionary independent pathways to the development of complex sociality and especially caste systems in different animal societies.

To distinguish between these two alternative behavioural strategies, we tested five predictions concerning cooperative behaviour and body mass using observational data from laboratory colonies (Tab. 1). Social organisation in a caste system would predict that (i) individuals specialise permanently or temporarily in certain tasks, which results in negative correlations of some forms of cooperation within individuals; (ii) maximum body mass is indicative of caste membership; (iii) repeatability of cooperative behaviour through ontogeny is high; (iv) behavioural phenotypes show a bi- or multimodal distribution; (v) maximum body mass is multimodally distributed. Alternatively, social plasticity in colony organisation would predict that (i) individuals do not specialise in certain activities, which results in positive correlations of different cooperative tasks within individuals; (ii) maximum body mass may not be related to cooperative behaviour;

(iii) repeatability of cooperative behaviour through ontogeny is low; (iv) cooperative behaviour is continuously distributed; (v) maximum body mass is unimodally distributed.

Tab. 1. Predictions discriminating between behavioural specialisation and plasticity (cf. Zöttl, Vullioud et al., 2016)

		Specialisation in castes (eusocial insects)	**Behavioural plasticity (cooperative vertebrates)**
i	Specialisation in cooperative tasks	yes	no
ii	Body mass predicts behavioural phenotype	yes	no
iii	Repeatability of cooperative behaviour	high	low
iv	Frequency distribution of cooperative behaviour	bi- or multimodal	unimodal, continuous
v	Frequency distribution of max. body mass	bi- or multimodal	unimodal, continuous

In order to analyse long-term differences among non-breeders, we collected behavioural data over several months with individual observation periods lasting six hours. We used multilevel, multinomial logistic regression models to investigate task specialisation and the relationship between body mass and cooperative behaviour. These models treat behaviour as a multinomial response and therefore avoid the need to aggregate data across different behaviours or across observation sessions, as has been the case in previous studies (e.g. Jarvis et al., 1991; Lacey & Sherman, 1991; Mooney et al., 2015). It is therefore possible to assess variance in behaviour at the level of the individual animal as well as the co-occurrence of different behaviours within individuals. Negative correlations between individual random effects would hereby indicate a trade-off, meaning the animal allocates relatively more of their time to one behaviour (e.g. nest building) than to another (e.g. food carrying). In addition to examining behavioural patterns of subordinate naked mole-rats, we looked at differences in cooperative behaviour between female breeders and non-breeders.

2. Material and Methods

2.1 Animals and housing

This study includes data from five groups of naked mole-rats maintained at Zoo Schönbrunn, Vienna. The five groups comprised 78 animals in total with 12 to 21 individuals per group. All animals were born and raised in captivity and housed in tunnel systems made of either transparent PVC or glass. Each group occupied a self-contained pipe system including at least one nest box and one waste box, but length of pipes (3.20–6.90 m) and number of boxes (2–8) varied according to group size (Fig.1). To simulate the mole-rats' natural underground environment, three groups (C, D, H) were kept in constant darkness, while two groups (A, B) were kept in another room with dimmed overhead lighting and lamps with red bulbs aimed at the nesting box of each colony as an additional heat source. Temperatures in the housing facilities were maintained close to natural burrow conditions at 28°–30°C. Animals were fed *ad libitum* once daily on a diet of root vegetables (*Beta vulgaris* subsp. *vulgaris, Daucus carota* subsp. *sativus, Ipomoea batatas, Pastinaca sativa*) and celery (*Apium graveolens*) and provided with wood wool as nesting material. The boxes were cleaned of used nesting material and food waste once a day. During observations, a standardised amount of digging substrate (wood shavings) was inserted into the tunnel system every 2 h through several openings in the pipe ceilings to induce the expression of "working behaviours" such as digging and sweeping. A radio constantly played on low volume in both rooms to habituate the animals to noises outside the tunnel system and mask sounds made during observations.

All individuals were identifiable by a passive transponder tag that was implanted at the beginning of the study. Additionally, each mole-rat of a group received a unique coloured mark applied with permanent marker on the day before each observation, so as not to disturb the colony immediately prior to data collection. Age of the animals was unknown except for a litter of eight individuals that were born in April 2018. Since the external genitalia of naked mole-rats show only few differences between the sexes (Jarvis & Sherman, 2002), we determined the sex of the animals via molecular sexing using buccal mucosa samples (see below). We identified 53 males and 25 females. This number includes 6 breeding females, with one group containing two simultaneously breeding females. It was not possible to identify the breeding males, as no sexual behaviour was observed over the course of this study. We therefore included all remaining 72 individuals in the behavioural analysis as non-breeders.

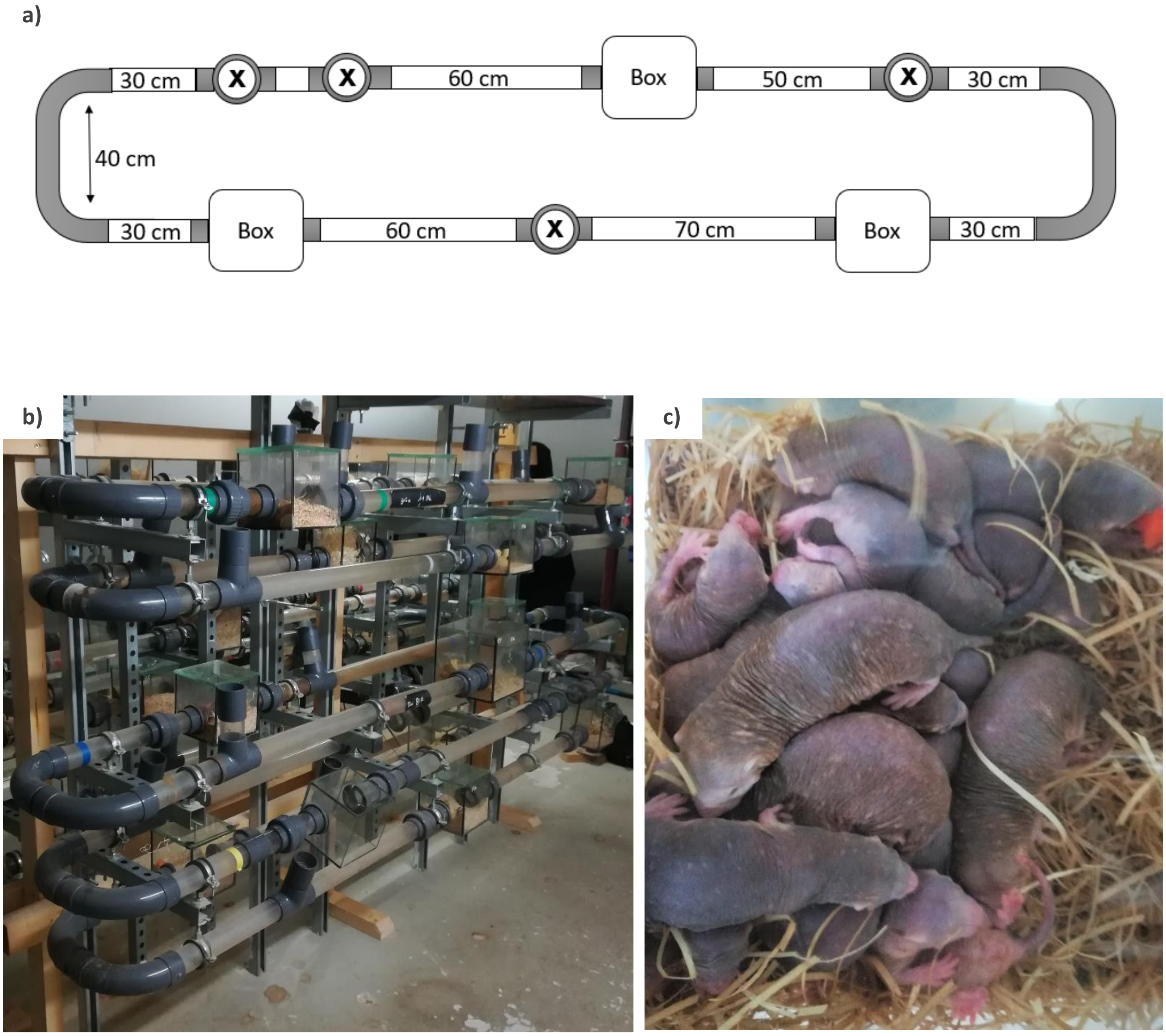

Fig. 1. Animal housing**. a)** Schematic diagram of a tunnel system for 12 animals (top tier of tunnels depicted in Fig 1b). Dimensions of the boxes are 15 x 15 x 15 cm and diameter of the tubes is 5 cm. Xs mark openings for the insertion of digging substrate. **b)** Tunnel systems for groups C, D, H. **c)** Naked mole-rats huddle in a nesting chamber with nesting material (wood wool).

2.2 Molecular sexing

We obtained cells for DNA samples from buccal mucosa by twirling Celltip cytobrushes (Servoprax, Wesel, Germany) on the animals' inner cheeks for 5-10 seconds. Heads of the brushes were stored in 1.5 ml Eppendorf tubes at 4°C for subsequent DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer’s protocol. We used multiplex PCR to simultaneously amplify two sets of primers: GAPDH (NMR_GADPH_F: 5′-CAG CCT TGG GCA GGT TTG TG-3′ and NMR_GADPH_R: 5′-CAC AAA CAT GGG GGC ATC CG3′), which generates a PCR product in both sexes and was used as an internal control for PCR failure and ZFY (NMR_ZFY_F: 5′-TTT TCC GTG TAG AAA GGG TTT-3′ and NMR_ZFY_R: 5′-TTG TGA CTC GCC ACC CTT TC-3′), which is located on the Y-chromosome and thus present only in males. PCR amplification therefore resulted in one band for female samples and two bands for male samples (Fig. 2).

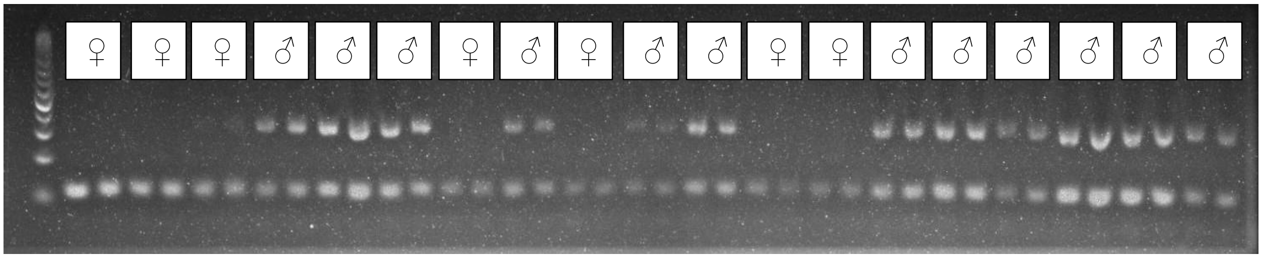

Fig. 2. Molecular sexing pattern of colony D. Samples were amplified in duplicates to ensure reliability. One band indicates a female (♀), two bands indicate a male (♂). The left lane shows a 100 bp DNA ladder as a molecular weight size standard.

2.3 Data collection

Data were collected between July 2018 and July 2019. Body mass was recorded a mean of 7.0 ± 1.5 times from every animal by placing them on an electronic scale. The number and the time between measurements varied between animals, since body weight was not recorded on a fixed schedule but opportunistically whenever the animals were removed from the tunnel system (e.g. before observation sessions or when taking mucosa samples).

Behavioural data was collected using instantaneous scan sampling (Altmann, 1974). The behaviour of every animal in a group was recorded in 6-10-min intervals, depending on colony size. The ethogram was conceived following the comprehensive description of non-vocal behaviours by Lacey et al. (1991) and reduced to 16 relevant behaviours during preliminary observations (Tab. 2). Behaviours were recorded on a handheld device using software Animal Behaviour Pro version 1.2 (University of Kent, UK). Since observations were conducted in darkness, observers wore headlamps to identify individual dye marks. Unless alerted mole-rats move in their tunnel system with closed eyes and do not show a behaviour response to the headlamp.

Tab. 2. Naked mole-rat ethogram

Response category		**Subcategories**	**Description**
Active non-help		Locomotion	Moving through the tunnel system, not engaged in obvious work.
		Other	Behaviours that cannot be assigned to other categories or the subject but not its behaviour can be identified.
		Self-groom	Self-directed grooming, e.g. scratching, wiping, cleaning own body with incisors and feet.
		Sniff	Sniffing objects, the tubes, the air or other individuals.
		Social interaction	“Incisor fencing” (locking incisors, shoving each other back and forth), biting, nuzzling each other’s’ bodies and genitals.
Eat		Eat	Eating food.
Food carry	(cooperation)	Food carry	Transporting food by pushing or dragging it through the tubes.
Nest build	(cooperation)	Nest build	Engaging with nest material (wood wool) by either dragging it in the direction of the nest or trying to pull it out of a certain location, but not sweeping it.
Pup carry	(cooperation)	Pup carry	Carrying or dragging pups through the tunnels with the incisors.
Work	(cooperation)	Dig	Using incisors and forelegs to dig in the litter or attempting to dig at the tube.
		Gnaw	Scratching, biting, chewing on the tunnel walls with the incisors.
		Kick	Kicking litter upwards out of the tubes or boxes with the hindlegs. In contrast to sweeping, material is moved vertically, and the animal does not move backward between successive kicks.
		Locomotion between work	Moving between bouts of sweeping.
		Sweep	Moving backwards while pushing litter with the hind legs.
Rest		Rest	Subject is immobile in a tunnel, head is down.
		Sleep	Subject lies in the nest box with its eyes closed.

Observation sessions lasted 6 h (10:00 to 16:00) and were carried out by two observers in alternating 30-min shifts. The observational time span was chosen since naked mole-rats show unpredictable activity patterns with considerable inter-individual variation (Riccio & Goldman, 2000). Each of the five groups was observed five times, although two sessions were cut short after 3 h due to technical complications, resulting in a total of 144 observation hours. Over all sessions, a mean of 219 ± 67 sampling events was recorded per individual (range 78-300). The variation in sampling events resulted due to the varying sampling intervals and because three animals died after three sessions and eight individuals lost their transponder tags over the course of the study. Groups were observed over a mean time span of 216 ± 61 d, with a mean time of 54 ± 41 d between sessions.

The study was discussed and approved by the ethics and animal welfare committee of the University of Veterinary Medicine Vienna in accordance with Good Scientific Practice guidelines and national legislation (Ref. ETK-02/05/2018).

2.4 Statistical analyses

2.4.1 Analysis of within-individual trade-offs, body mass and group size effects

Individual correlations between types of cooperative behaviour and the general effect of body mass on cooperative behaviour were analysed with the use of three multilevel, multinomial behaviour models that increased in complexity due to the successive inclusion of fixed effect covariates and higher-level random effects (Koster & McElreath, 2017). All three models were calculated separately for males and females so that behavioural variance estimates were specific to each sex and in order to draw qualitatively informed conclusions about sex differences in mole-rat behaviour.

For analysis with the multinomial models, the 16 recorded behaviours were grouped into six categories: three types of cooperative behaviour, carrying food or nest material and working (which aggregates all activities related to burrow maintenance such as gnawing at the tunnel walls, digging in or kicking and sweeping substrate), and three non-cooperative behaviour types, resting, eating and active non-help, which summarizes all other active behaviours not related to cooperation, so that distinctions can be made between investment in cooperation and other activities. Very few instances of pup carrying were observed, since pups (animals less than 40 days old) were only present for 3 of the 25 sessions, and these records were subsequently not included in the analysis as a separate category of cooperative behaviour.

The Widely Applicable Information Criterion (WAIC) was calculated to evaluate relative model fit, but due to their varying predictors and random effects structure, each of the three models and the comparison of their output provided information relevant to different aspects of our analysis of cooperative behaviour. The WAIC score was therefore not used for model selection, but rather as a general indicator of model quality.

Model 1 included only intercepts and random effects for individuals and therefore showed the extent of individual-level variance for each behavioural category as well as the within-individual correlations between the five non-resting behaviours. Since we were interested in individual trade-offs between active behaviours, resting was set as the reference category. This meant that coefficients of the intercepts indicated how much time individuals allocated to the respective behaviours relative to resting. Consequently, variance of the reference category or correlations between the other categories and resting were not calculated.

In addition to the individual-level random effects, Model 2 included predictor variables that may be related to the expression of behavioural phenotypes in naked mole-rats. Body mass was added as a fixed effect to test the assumption that max. body mass influences the cooperative investment of non-breeders. As another well-established predictor of behavioural contributions in cooperative societies, group size was also incorporated as a fixed covariate (Balshine et al., 2001). Both continuous predictors were z-score transformed before model fitting and specified as first- and second-order polynomials. The comparison of individual-level variances between Models 1 and 2 gave some indication of the proportion of variance in the behavioural categories that could be explained by the fixed effects. However, the inclusion of predictor variables can increase the higher-level variance estimates in multilevel models, which is why the variances in Model 2 should be interpreted with caution (Koster & McElreath, 2017). The within-individual correlations between the behavioural responses are not sensitive to this issue and the changes in correlation estimates relative to Model 1 reflected the impact of the predictor variables on the random effects.

The structure of Model 3 was further expanded to include random effects at the level of observation session and group while maintaining the set of fixed effects from the previous model.

Random effects for sessions were incorporated to account for temporal pseudo-replication created by recording the same individuals repeatedly throughout one session. Group-level random effects were introduced to adjust for clustering of the data by group, but since the number of observations per group was relatively small (especially for the female sample), they were estimated with low confidence. Variances at group-level and their correlations therefore offered only limited insight and were not considered further in this study. The complex random effects structure of this model affects the interpretation of the individual random effects and their correlations: individual-level variance estimates do no longer reflect variation across all the individuals of the population, but within-group variations and as a result, individual-level correlations in this model do not represent individual trade-offs between behavioural responses. However, including higher-level random effects improved the overall model fit and allowed a more precise estimation of the fixed effects. As a result, Model 3 was particularly suited for analysing the effects of group size and body mass on cooperative investment.

Additionally, we applied Model 3Q, a variant of Model 3 that retained its random and fixed effects structure but incorporated the categorical predictor of reproductive status (breeder/non-breeder) to a dataset of all female individuals to investigate differences in cooperative behaviour between breeding and non-breeding female mole-rats.

Models were fitted and analysed in a Bayesian framework with the R packages *rstan* vers. 2.19.2 and *rethinking* vers. 1.59 (Stan Development Team, 2019; McElreath, 2015). Instead of the conventional Markov chain Monte Carlo algorithms, *rstan* employs Hamiltonian Monte Carlo chains, which are more efficient at achieving sufficiently mixed posterior distributions (Monnahan, Thorson, & Branch, 2017). We used three chains of 2000 iterations for model fitting, half of which were devoted to the warm-up. To ensure adequate mixing of the chains, a non-centred parameterization of the varying effects was realized with a Cholesky decomposition of the variance-covariance matrices (Koster & McElreath, 2017). Additionally, we assigned weakly informative priors to the fixed effect parameters and variance-covariance matrices that prevent overfitting while influencing the posterior distribution as little as possible (Koster & McElreath, 2017). To diagnose potential problems with chain mixing and convergence, we examined the trace plots of the chains as well as the effective number of samples and the Gelman-Rubin convergence diagnostic ($\hat{R} < 1.1$) (McElreath, 2015).

The correlations between random effects and the fixed effects coefficients were considered significant if the 95% credible intervals of their posterior distributions did not include zero. In multinomial logistic regression, the interpretation of the coefficients of fixed effects are complicated by the fact that they are conditional on the other parameters and therefore do not represent the direct effect of the predictor on the dependent variable (Retherford & Choe, 1993, 153). Following the advice of Koster & McElreath (2017), we instead calculated the predicted probabilities and their credible intervals in order to visualize the impact of body mass, group size and reproductive status on mole-rat behaviours. Probabilities were based on fixed effects only while averaging over random effects. Prediction intervals cannot be used to test categorical predictor variables for significance, because they contain uncertainty from all covariates, so in order to examine differences in behaviour between female breeders and non-breeders, we calculated the contrasts between the predicted probabilities for the two groups and examined the distribution of these differences (Koster & McElreath, 2017). Statistical significance was inferred if the 95% credible intervals of the predicted differences did not span zero.

2.4.2 Analysis of repeatability of cooperative behaviour

For the analysis of the repeatability and distribution of cooperative behaviour, a measure of total cooperation was derived, which describes the proportion of sampling events that an individual was engaged in any type of cooperative behaviour (including pup carrying) during an observation session. Repeatability of total cooperation for individuals across observation sessions was calculated using the rpt-function for proportion data from the R package *rptR* vers. 0.9.22 (Stoffel, Nakagawa, & Schielzeth, 2017), which is based on a generalized linear mixed-effects model with binomial error distribution (logit link function). The repeatability estimate was adjusted for the effects of sex and body mass by including them in the model as fixed effects. Confidence intervals were calculated via parametric bootstrapping (1000 iterations) and significance was estimated using likelihood ratio tests.

2.4.3 Analysis of frequency distributions of body mass and cooperation

Maximum body mass was calculated as the mean of all weight measurements for each individual, since most animals had reached their asymptotic body mass and showed little variation over the study period (Fig. S1). The still-growing litter of eight was excluded from the analysis of max. body mass, as was the heaviest male of each colony. These were likely to be breeding males (Brett, 1991) and including them may have led to overestimation of sex differences in body mass among non-breeders. Sex differences in body mass and total cooperation were analysed by fitting linear mixed models with sex as fixed effect, group identity as random effect, and max. body mass (log-transformed) or proportion of mean total cooperation across all observations as the respective response. The effect of sex was tested for significance with Satterthwaite's degrees of freedom method implemented in the R package *lmerTest* vers. 3.1-0 (Kuznetsova, Brockhoff & Christensen, 2017). The frequency distributions of max. body mass and cooperative behaviour for all non-breeders and separately for each sex were tested for normality using the Shapiro-Wilk test and body mass distributions were additionally tested for unimodality/multimodality with Hartigan's dip test using the R package *diptest* vers. 0.75-7 (Maechler, 2016). All statistical analysis was performed in R vers. 3.6.1 (R Core Team, 2019).

3. Results

3.1 Behavioural differences between breeding and non-breeding females

Female mole-rats differed in their behavioural expression according to reproductive status (Fig. 3). Model 3Q showed that female breeders engaged more in active non-helping than female non-breeders. Within this behavioural category, locomotion made up 89% and 72% of the recorded events for breeders and non-breeders, respectively. Conversely, non-breeders invested more in the cooperative behaviours carrying food, nest building and working. Contrasts between each posterior sample of the two groups revealed that among these differences, only active non-help and working reached significance at the 5% level (Fig. 4).

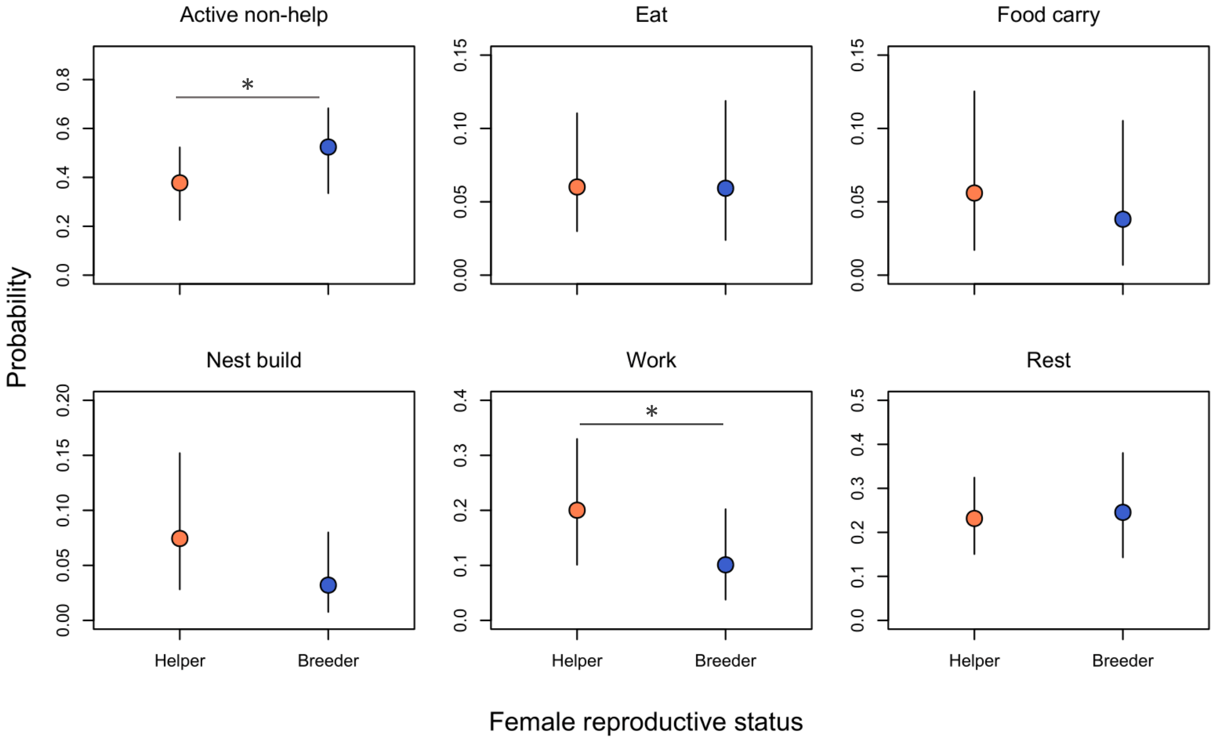

Fig. 3. Model predictions of response behaviours as a function of reproductive status in females (n = 25, consisting of 19 helpers and 6 breeders). All other fixed covariates are held constant at the sample mean. The confidence intervals are the 89% percentile intervals as calculated from the posterior samples of Model 3Q.

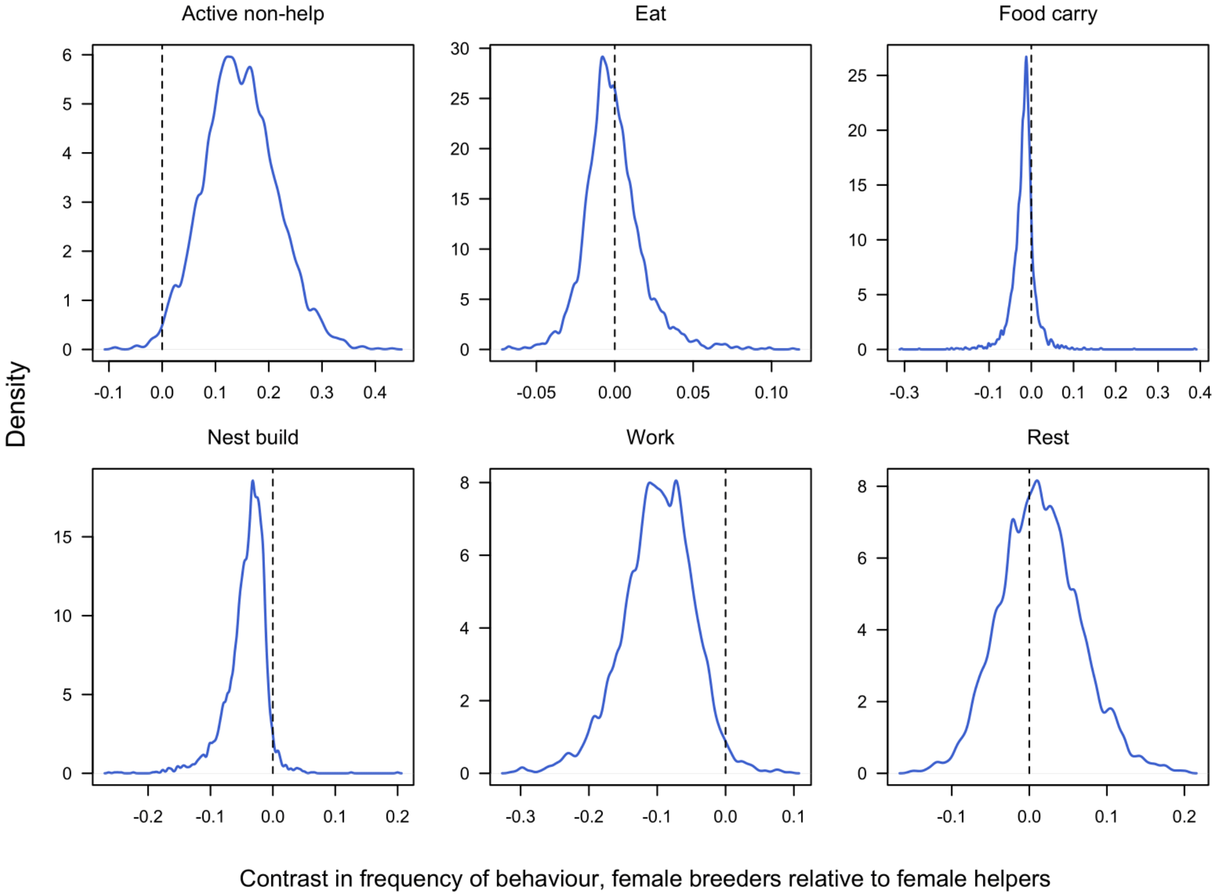

Fig. 4. Distribution of predicted differences in the probability of the respective response behaviours between female breeders and non-breeders.

3.2 Model comparison with WAIC

Comparison with the WAIC showed that model fit was increased by adding variables to the model structure for both sexes, with more complex models scoring lower WAIC values, indicating higher predictive accuracy. Model 3, which includes all random and fixed effects, also received the full Aikake weight for males and females, making it the best approximating model relative to the others (Tab. 3; Fig. 5). Effects of the predictor variables were therefore examined for Model 3 only, but because the complex structure changes the interpretation of individual random effects as previously mentioned, within-individual correlations were taken from Model 2 despite its lower accuracy.

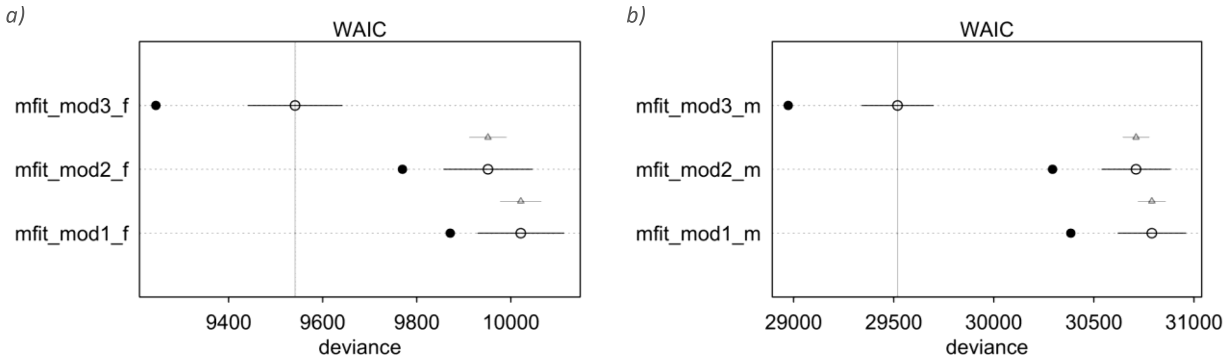

Fig. 5. Visualization of WAIC values from Tab. 3, comparing Models 1,2 and 3 for **a)** females and **b)** males. The open points are the WAIC score, while the lines through these points represent the standard deviation of the WAIC. The grey triangles above each WAIC show the model's difference to the top-ranking model and the standard deviation of this difference. The filled points are the in-sample deviance of each model.

Tab. 3. Comparison of Models 1, 2 and 3 for both sexes with WAIC (widely applicable information criterion)

Sex	Model	Random effects	Fixed effects	WAIC (SE)	ΔWAIC(SE)	Akaike weight
Female						
	1	Individual	-	10021.0(91.63)	479.7(43.70)	0
	2	Individual	Group size[1], body mass[1]	9951.3(94.13)	410.0(39.74)	0
	3	Individual, scan, group	Group size[1], body mass[1]	9541.3(100.33)	-	1
Male						
	1	Individual	-	30789.4(169.59)	1270.2(69.28)	0
	2	Individual	Group size[1], body mass[1]	30710.4(171.01)	1191.2(171.01)	0
	3	Individual, scan, group	Group size[1], body mass[1]	29519.2(178.03)	-	1

[1] Included as first- and second-order polynomials

3.3 General time budgets and individual variance

Both female and male mole-rats spent more time performing active non-helping behaviours and working than resting but invested less time in eating and cooperative behaviours food carrying and nest building (positive and negative coefficients of the intercepts from Model 1, respectively; Tab. 4). Variances of the individual random effects likewise show similar trends in females and males. The categories 'active non-help' and 'eating' exhibited relatively lower variances when compared to the 'helping' behaviours, which indicates that these non-cooperative activities are more commonly performed by all members of a group, whereas 'food carrying', 'working' and 'nest building' represent more specialised behaviours that are less evenly expressed across the population (Tab. 5; see Tab. S1 for higher-level variances). Notably, 'food carrying' shows the highest variance among these cooperative behaviours in both sexes and is therefore only performed by a few animals per group.

Tab. 4. Posterior means (SD in parenthesis) of the intercepts for Models 1-3 of each sex (i.e. expression of behaviours relative to the reference category resting)

Behaviour	**Female**			**Male**		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Active non-help	**0.47(0.15)**	-0.15(0.34)	0.52(0.46)	**0.62(0.12)**	**0.32(0.16)**	0.75(0.40)
Eat	**-1.55(0.17)**	**-1.64(0.37)**	**-1.11(0.52)**	**-1.80(0.12)**	**-1.70(0.19)**	**-1.13(0.47)**
Food carry	**-2.43(0.33)**	**-2.35(0.49)**	**-1.78(0.70)**	**-2.77(0.20)**	**-2.89(0.27)**	**-1.91(0.60)**
Nest build	**-1.37(0.24)**	**-1.21(0.46)**	-0.98(0.61)	**-1.83(0.16)**	**-1.96(0.23)**	**-1.37(0.40)**
Work	0.23(0.22)	-0.43(0.34)	-0.08(0.49)	0.21(0.14)	0.13(0.18)	0.56(0.36)

Parameters in bold indicate estimates where the 95% credible intervals do not span zero.

Tab. 5. Variance estimates of the individual-level random effects for Model 1, 2 and 3 of each sex

Behaviour	**Female**			**Male**		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Active non-help	0.62(0.12)	1.26(0.28)	0.58(0.13)	0.82(0.08)	0.88(0.10)	0.57(0.06)
Eat	0.58(0.13)	0.76(0.22)	0.27(0.16)	0.76(0.10)	0.79(0.11)	0.53(0.09)
Food carry	1.35(0.31)	1.27(0.32)	1.03(0.33)	1.33(0.16)	1.20(0.16)	0.77(0.13)
Nest build	0.99(0.20)	1.39(0.39)	1.01(0.26)	1.12(0.12)	1.15(0.14)	0.99(0.12)
Work	0.92(0.15)	1.20(0.27)	0.88(0.16)	0.99(0.09)	1.04(0.11)	0.84(0.09)

Reported estimates are the standard deviations of the random effects while the values in parentheses are the standard deviations of these estimates in the posterior samples.

3.4 Within-individual trade-offs

We found no evidence of task specialisation in naked mole-rats. Individual-level random effects correlations between any two of the observed behaviours were positive across both sexes, indicating there were no trade-offs between different cooperative behaviours within individuals (see Tab. S2 and S3 for random effects correlations on all levels from Models 1, 2 and 3 for females and males, respectively) On the contrary, individuals that performed one of the non-resting behaviours were also more likely to engage in other non-resting behaviours. Resting behaviour is excluded here, as no correlations were calculated for the reference category. Focussing on cooperative behaviours, females that worked more than the population mean were also significantly more frequently recorded nest building ($\rho_{4,5} = 0.60 \pm 0.16$, values from Model 1, Tab. 6, upper half of each matrix), whereas males exhibited strong positive correlations between all cooperative behaviours: individuals who were more frequently observed carrying food engaged more often in nest building ($\rho_{3,4} = 0.60 \pm 0.11$) and working ($\rho_{3,5} = 0.63 \pm 0.10$) and males who worked relatively more also allocated more of their time to nest building ($\rho_{4,5} = 0.75 \pm 0.07$). Most of the correlations exhibited even stronger effects after controlling for the influence of the fixed effects on behaviour in Model 2 (Fig. 6; Tab. 6, lower half of each matrix).

Tab. 6. Correlations of individual-level random effects across responses from Model 1 and 2 for both sexes

Sex	Behaviour	Behaviour				
		Active non-help	Eat	Food carry	Nest build	Work
Female	Active non-help	–	**0.47(0.20)**	**0.39(0.20)**	0.12(0.20)	**0.41(0.17)**
	Eat	**0.48(0.23)**	–	0.52(0.21)	0.30(0.21)	**0.49(0.19)**
	Food carry	0.21(0.25)	0.42(0.23)	–	0.26(0.21)	0.36(0.20)
	Nest build	0.42(0.21)	0.37(0.24)	0.20(0.25)	–	**0.60(0.16)**
	Work	**0.62(0.15)**	0.20(0.25)	**0.47(0.21)**	**0.67(0.15)**	–
Male	Active non-help	–	**0.49(0.12)**	**0.65(0.10)**	**0.72(0.08)**	**0.70(0.07)**
	Eat	**0.47(0.21)**	–	**0.75(0.09)**	**0.52(0.12)**	**0.58(0.11)**
	Food carry	**0.72(0.10)**	**0.69(0.12)**	–	**0.60(0.11)**	**0.63(0.10)**
	Nest build	**0.71(0.09)**	**0.53(0.13)**	**0.69(0.11)**	–	**0.75(0.07)**
	Work	**0.70(0.08)**	**0.69(0.09)**	**0.78(0.08)**	**0.82(0.06)**	–

The upper half of the matrix lists correlations from Model 1, the lower half correlations from Model 2. Reported values are means from the posterior samples (SD in parenthesis); parameters in bold indicate estimates where the 95% credible intervals do not span zero.

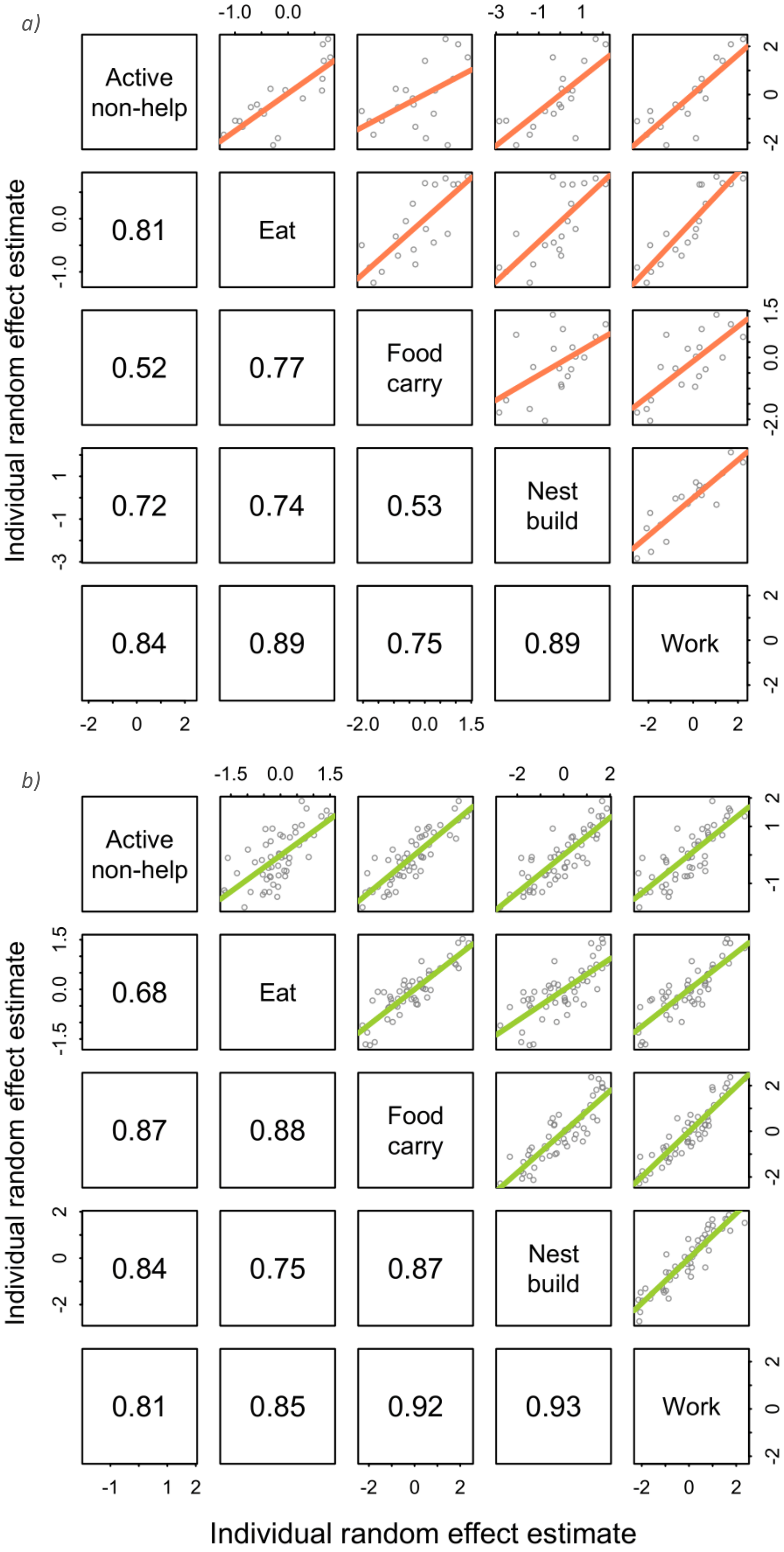

Fig. 6. Within-individual random effects correlations from Model 2 for **a)** females and **b)** males.
Values in the lower half of the matrix represent the correlations between the median individual level intercept in the posterior samples for each behaviour. They are therefore larger than the correlations presented in Tab. 6 which are taken directly from the variance-covariance matrices of the posterior samples.

3.5 Effects of body mass and group size on cooperative behaviour

Neither body mass nor group size significantly influenced the expression of cooperative behaviours in non-breeding naked mole-rats of either sex, with the exception of working behaviours in males declining with increasing body mass (Tab. 7). Although the fixed effects estimates did not meet conventional significance thresholds, some general trends were notable in the visualisation of the predicted probabilities (Figs. 7 and 8). Males and females were similarly influenced by changes in body mass. The probability of food carrying increased with larger body mass, while the opposite trend was apparent in regard to working behaviours. Nest building displayed an inverse quadratic relationship with body mass, predicting a decrease in nest building activity for the smallest and largest animals. With respect to group size, investment in food carrying and nest building was higher towards extreme group sizes in both sexes, while working showed the inverse tendency for females and a steady decline with larger group sizes in males. Most behaviours followed quadratic trends that manifested at the minimum and maximum group sizes, where most data points were generated during the study. Only a small fraction of sampling events was recorded for groups of 15-18 animals (10% for females, 7% for males) after three animals of a larger group had died, weakening confidence in the estimates in this group size range. Overall, uncertainty around the predictions concerning both fixed effects was large for most behaviours due to the small sample size, a trend even more apparent in females.

Tab. 7: Posterior means (SD in parentheses) of fixed effects in Model 3 for each sex

Model	Fixed effect	Active non-help	Eat	Food carry	Nest build	Work
3, females	Group size	0.06(0.30)	0.26(0.32)	0.88(0.47)	0.39(0.43)	0.04(0.32)
	Group size^2	-0.03(0.35)	-0.26(0.37)	-0.81(0.49)	-0.23(0.44)	0.34(0.34)
	Body mass	-0.12(0.17)	-0.09(0.16)	0.11(0.31)	-0.03(0.28)	-0.30(0.22)
	Body mass^2	0.11(0.12)	-0.05(0.13)	0.10(0.19)	-0.21(0.22)	0.10(0.14)
3, males	Group size	0.10(0.33)	0.25(0.41)	0.67(0.50)	0.34(0.36)	-0.19(0.32)
	Group size^2	-0.27(0.27)	-0.57(0.30)	-0.82(0.41)	-0.37(0.28)	-0.26(0.25)
	Body mass	**-0.30(0.09)**	-0.13(0.11)	-0.01(0.14)	-0.24(0.16)	**-0.26(0.12)**
	Body mass^2	**0.18(0.06)**	0.13(0.09)	0.14(0.10)	-0.01(0.10)	0.07(0.07)

Parameters in bold indicate estimates whose 95% credible intervals do not span zero.

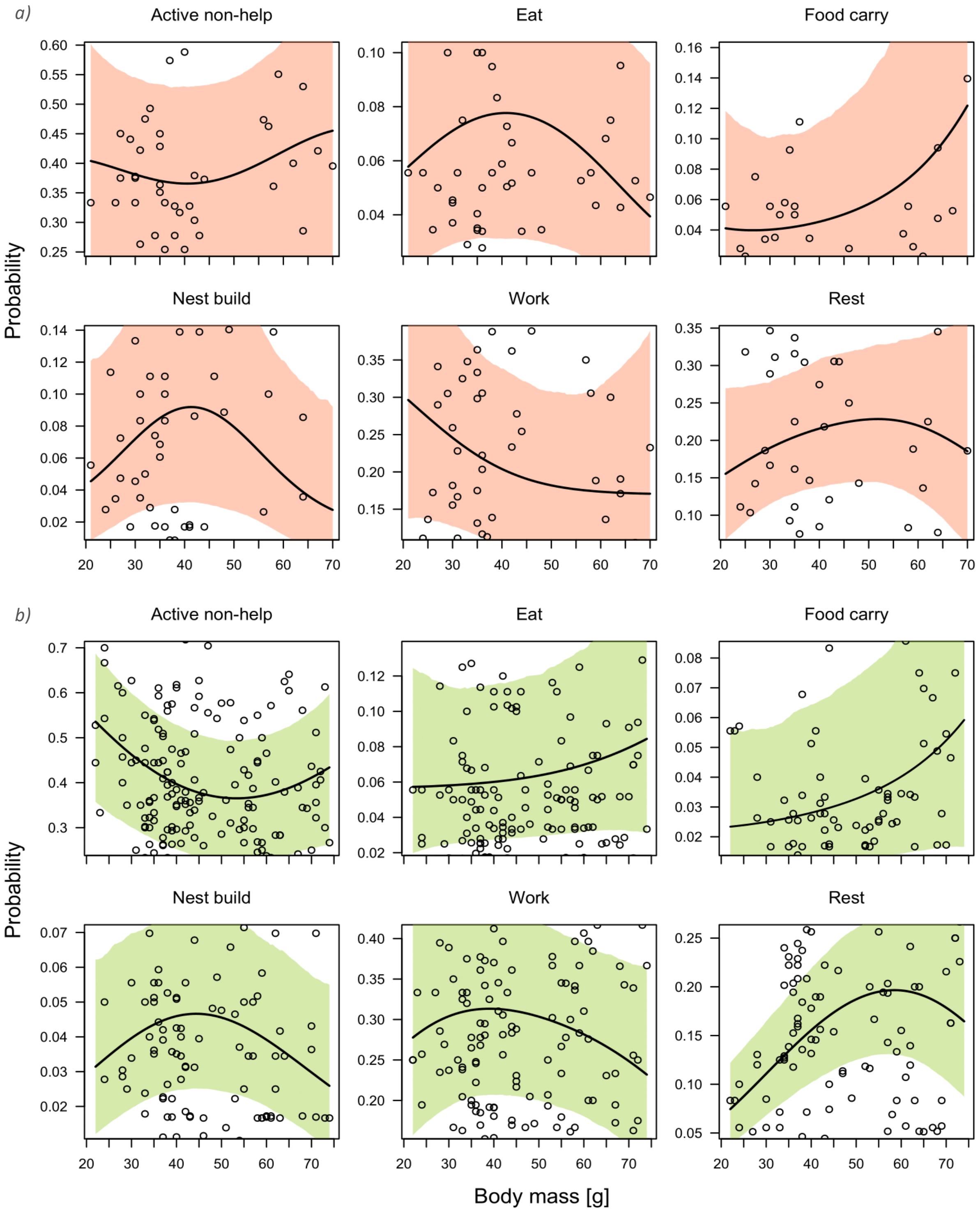

Fig. 7. Model predictions of response behaviours as a function of body mass for **a)** females and **b)** males. All other fixed covariates are held at the sample mean. Shaded regions show the 89% percentile intervals calculated from the posterior samples of Model 3 for each sex.

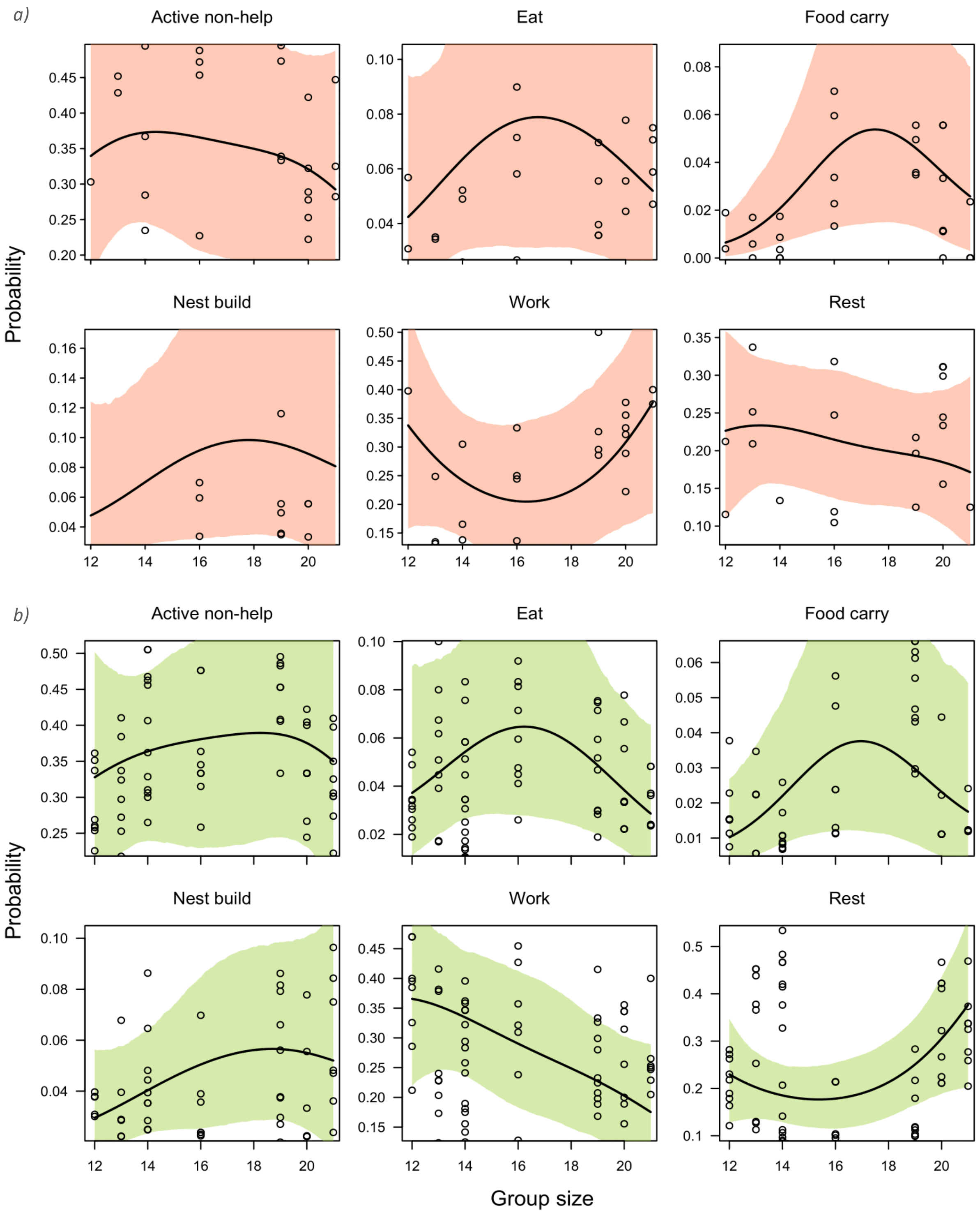

Fig. 8. Model predictions of response behaviours as a function of group size for **a)** females and **b)** males. All other fixed covariates are held at the sample mean. Shaded regions show the 89% percentile intervals calculated from the posterior samples of Model 3 for each sex.

3.6 Repeatability of cooperative behaviour

Adjusted repeatability of cooperative behaviour within individuals across observation sessions was low (R = 0.092, 95% CI = 0.055–0.12, p < 0.0001).

3.7 Frequency distributions of body mass and cooperation

Body mass varied widely between individuals, with a mean max. body mass of 45.44 g (SD = 11.12, range 28.29–70.00 g) for adult males and 39.82 g (SD = 11.94 g, range 26.40–65.50 g) for adult females. Male non-breeders were significantly larger than females (LMM: estimate ± SE = 0.13 ± 0.05, t = 2.82, p < 0.01, n = 59), but there was no difference in the mean proportion of total cooperation between the sexes (LMM: estimate ± SE = -0.04 ± 0.03, t = -1.10, p = 0.28, n = 72) (Fig. 9). There was no evidence for bi- or multimodality in cooperation frequency, as mean total cooperation followed a normal distribution for females (Shapiro-Wilk: W = 0.98, p = 0.88, n = 19), males (W = 0.98, p = 0.66, n = 59) and across all non-breeding animals (W = 0.99, p = 0.59, n = 72). Conversely, max. body mass distributions of both sexes (Shapiro-Wilk: females, W = 0.88, p = 0.04, n = 17; males, W = 0.92, p < 0.01, n = 42) and the total population (W = 0.93, p = < 0,01, n = 59) differed significantly from normality. The max. body mass distributions depicted in Fig. 10

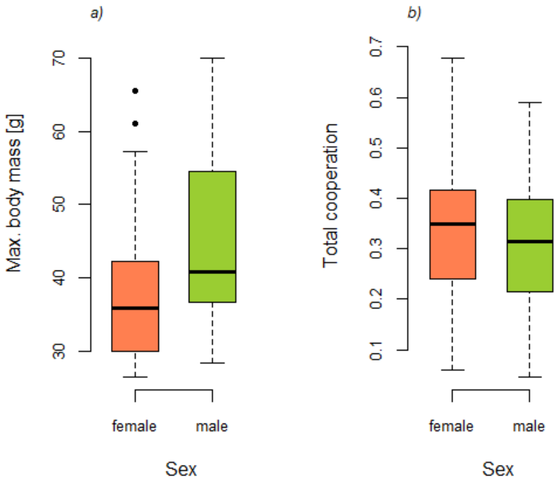

Fig. 9. a) Mean max. body mass in adult female (n = 17) and male (n = 42) non-breeders. **b)** Mean total cooperation over all observation sessions for female (n = 19) and male (n = 53) non-breeders.

show a moderate skew to the right that is most pronounced in females (skewness = 0.85 for females, 0.61 for males, 0.62 for all non-breeders), indicating that a large proportion of the non-breeding population consist of smaller animals, with only a few individuals attaining high body weights. Hartigan's dip test failed to identify significant bimodalities in the distributions of body mass (Hartigan's dip test: females, D = 0.08, p = 0.54, n = 17; males, D = 0.03, p = 0.99, n = 42; all non-breeders, D = 0.03, p = 0.99, n = 59).

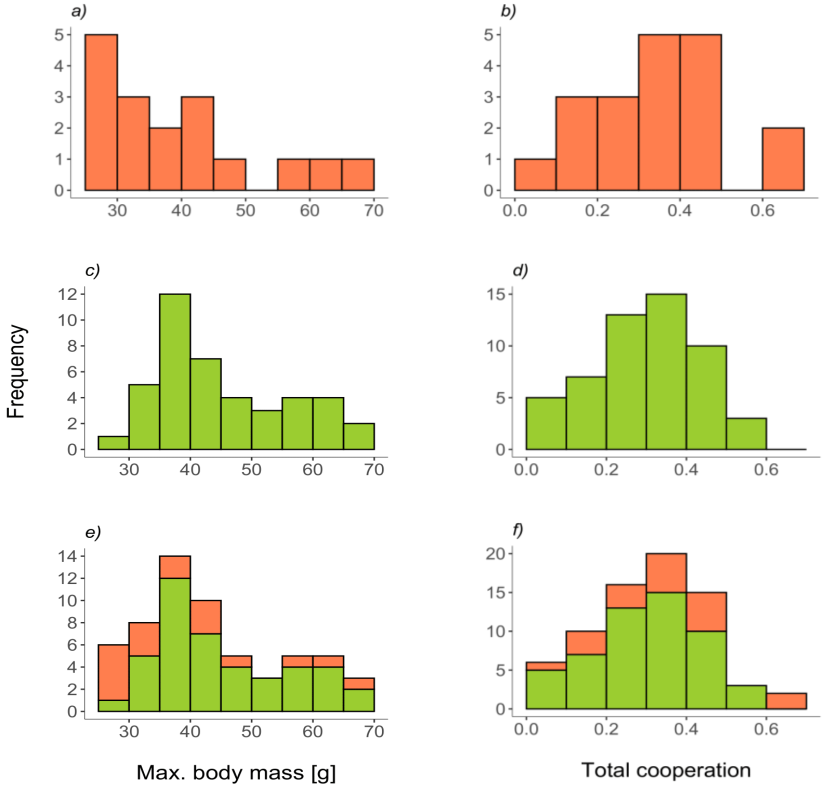

Fig. 10. Left: frequency distribution of max. body mass for **a)** adult female subordinates (n = 17), **c)** adult male subordinates (n = 42) and **e)** all adult non-breeders combined (n = 59). Right: frequency distribution of mean total cooperative investment for **b)** female subordinates (n = 19), **d)** male subordinates (n = 53) and **f)** all non-breeders combined (n = 72).

4. Discussion

Our observations provided no evidence that naked mole-rats are organized in a caste system with permanently non-breeding helpers having morphological or behavioural specialisations like eusocial insects. On the other hand, we confirmed that subordinate mole-rats showed phenotypic plasticity in their helping behaviours, as do other cooperatively breeding mammals. Below we address each of the other main findings of this study in detail.

In this study, we observed 78 individuals from five groups for a total of 144 hours over a mean period of seven months and during this time also periodically recorded their body mass. Most previous studies on naked mole-rat behaviour included data from 1-3 colonies with sample sizes of 40 (Jarvis, 1981) to 99 (Jarvis et al., 1991) animals collected in under 40 h of live observations spread out over 3-4 months (Faulkes et al., 1991; Jarvis et al., 1991; Lacey & Sherman, 1991). Mooney et al. (2015) investigated long-term behavioural consistency over six months and short-term task specialisation over the course of seven days for a combined observation time of approximately 10 h. Moreover, we recorded behaviours continuously for six hours during each observation session, which allowed us to account for the unpredictable activity patterns of naked mole-rats, whereas individual observations in previous studies typically lasted 30-60 minutes (Faulkes et al., 1991; Jarvis et al., 1991; Lacey & Sherman, 1991; Mooney et al., 2015). Unlike previous studies, we investigated individual trade-offs between different cooperative behaviours over several months, which is necessary to show long-term behavioural task specialisation.

Individual mole-rats did not specialise in specific cooperative behaviours. Task specialisation, as found in the castes of eusocial insects, results in within-individual trade-offs between functionally different behaviours (e.g. foraging, nest building, burrow maintenance). Instead, we found strong positive correlations among all of the observed cooperative behaviours and even among all non-resting behaviours within individuals. This result indicates that animals who are generally more active and spend less time resting also engage more in all types of cooperative tasks, namely food carrying, nest building and working. In contrast to our findings, Mooney et al. (2015) reported that non-breeding naked mole-rats do specialise in cooperative tasks, since they observed that individuals who participated more in pup care at the same time invested less in defence and working behaviours over several days. However, it is not at all clear that these short-term trade-offs represent the long-term task specialisation that is characteristic for physical castes, where individuals follow divergent developmental paths from early ontogeny and specialisations are permanent and irreversible (Wilson, 1971; Smith et al., 2008). We found no trade-offs between cooperative behaviours over a period of several months and therefore suggest that subordinate mole-rats do not specialise in certain behaviours in the long term. Future studies should measure cooperative trade-offs in the same individuals throughout ontogeny to conclusively show that there is no task specialisation in naked mole-rats. We did not record defence behaviours in this study, and pup carrying was too rarely observed to include it in the analysis of potential trade-offs. The three cooperative behaviours considered here, nest building, food carrying and working (digging, sweeping, gnawing), have often been grouped together in the past as maintenance tasks and contrasted with defence activities and sometimes pup care (Faulkes et al., 1991; Lacey & Sherman, 1991; 1997; Mooney et al., 2015). The possibility remains that trade-offs exist between these broader behavioural clusters. However, Mooney et al. (2015) also reported that there was a large overlap between participation in working and defence behaviours within individuals across long-term observational data. Digging behaviour has alternatively been considered maintenance (Jarvis, 1981; Faulkes et al., 1991; Jarvis et al., 1991; Mooney et al., 2015) or, for reasons not entirely clear, as a defence behaviour (Lacey & Sherman, 1991; 1997). Nevertheless, future research on specialisation in naked mole-rats should include the full repertoire of cooperative behaviours of naked mole-rats to avoid overlooking potential trade-offs.

Long-term task specialisation has not been conclusively demonstrated in any other cooperatively breeding mammal (Thorley et al., 2018). Damaraland mole-rats, which have also been described as forming frequent and infrequent worker castes (Bennett & Jarvis, 1988; Bennett, 1990; Scantlebury, Speakman, Oosthuizen, Roper, & Bennett, 2006), showed a lack of trade-offs between cooperative behaviours in two recent studies (Zöttl, Vullioud et al., 2016; Thorley et al., 2018). Meerkats likewise do not specialise in particular tasks but differ in their overall contribution to cooperation (Clutton-Brock et al., 2003). Besides these examples, few studies have investigated long-term trade-offs between cooperative behaviours in any vertebrate species. Behavioural specialisation in vertebrates seems to be mostly limited to short-term activities like group hunting (e.g. Stander, 1992; Creel & Dugatkin, 2001; Boesch, 2002), whereby, in contrast to insect castes, the functional capacity of a group relies on repeated interactions and mutual recognition among its members, and individuals are not readily replaceable (Anderson & Franks, 2001).

Jarvis (1981) proposed that subordinate naked mole-rats are organised in three morphologically distinct castes that are clearly distinguishable by body mass and frequency of cooperative behaviour. While we found large variation in cooperation frequency among non-breeders, these differences were not discrete and could not be reduced to two or three distinct behavioural phenotypes. Instead, participation in overall cooperation (food carrying, nest building, working and pup carrying combined) was continuously distributed across all non-breeders. This result contradicts the existence of frequent and infrequent worker castes as suggested by Jarvis, as do several other studies that found similar patterns of behaviour distribution in naked mole-rats (Faulkes et al., 1991; Jarvis et al., 1991; Lacey & Sherman, 1991).

Repeatability of total cooperation within individuals across several months was low, which further demonstrates that there is no discrete division of labour over workload in naked mole-rats. Instead, individuals maintained a high degree of behavioural flexibility. Our repeatability estimate for overall cooperation (R = 0.09) was comparable to or below repeatability levels reported for various helping behaviours in other cooperative breeders: total cooperation (R = 0.15) in Damaraland mole-rats (Zöttl, Vullioud et al., 2016); babysitting (R = 0.12) and escorting (R = 0.25) in male banded mongooses (*Mungos mungo*: Sanderson et al., 2015); babysitting (R = 0.22), provisioning (R = 0.51), raised guarding (R = 0.16) in meerkats (Carter, English, & Clutton-Brock, 2014; English, Nakagawa, & Clutton-Brock, 2010) and cooperative nest construction (R = 0.11) in sociable weavers (*Philetairus socius*: Leighton & Meiden, 2016).

We also found no evidence of bi- or multimodality in the body mass distributions of adult non-breeders and individuals could not be classified into morphologically distinct castes based on body mass. By contrast, workers in eusocial insect colonies are morphologically specialised for certain tasks, which among other physical modifications often manifests in large differences in body size between castes (Wilson, 1953; Oster & Wilson, 1978; Grüter et al., 2017). The small peak at the heavy end of the size distribution may point to an indistinct division into smaller and larger workers, however, it is likely that these few heavy animals are male breeders that were not excluded from the analysis. Brett (1991) found similar patterns in several of his wild-caught colonies and argued they might represent subdivisions of breeders and non-breeders or age classes. Continuous rather than discrete body mass distributions have been reported from other captive and free-ranging naked mole-rat colonies (Brett, 1991; Faulkes et al., 1991; Lacey & Sherman, 1991), as well as in related social mole-rats (Damaraland mole-rats: Zöttl, Vullioud et al., 2016; Voigt et al., 2019; Ansell's mole-rat: Šklíba et al., 2012).

In addition, body mass did not predict the expression of cooperative behaviours in non-breeders, with the exception that larger males worked less than smaller individuals. Most of the animals in our study colonies (except for one litter) had presumably reached asymptotic body mass, as little to no growth was apparent over the study period (Fig. S1). This result is not consistent with the existence of morphologically specialised castes in non-breeding naked mole-rats, which would predict that an individual's maximum body mass was indicative of its caste membership. On the contrary, the data seems more in line with patterns found in other cooperatively breeding mammals: in Damaraland mole-rats, fast-growing subordinates invest more in cooperation than slow-growing individuals, but smaller and larger Damaraland mole-rats do not differ in their contribution to cooperative behaviours once they reach their asymptotic body mass (Zöttl, Vullioud et al., 2016). Likewise, differences in cooperative behaviour are dependent on body weight in juvenile meerkat helpers but vary with foraging success in adults (Clutton-Brock et al., 2003). Individuals that are heavy for their age may be able to contribute more because their body condition allows them to better tolerate the energetic costs of cooperative tasks (McNamara & Houston, 1992). It is however possible that our models failed to detect significant effects of body mass due to the small sample sizes, a concern especially relevant for the female sample that comprised only 19 individuals.

Taken together, the evidence presented above strongly suggests that non-breeding naked mole-rats do not form physical castes, wherein groups of individuals are permanently morphologically specialized for different behavioural roles. Moreover, the existence of dispersers as a separate caste has been questioned by Burda (1999), who cited the lack of experimental evidence showing the adaptive value of increased fat storage in naked-mole rats and proposed that instead of representing a divergent developmental pathway, physiological changes like fat storing in individuals may be dependent on colony composition or ecological conditions and one of the factors triggering dispersal, similar to mechanisms observed in other mammal species. A recent study on free-ranging Damaraland mole-rats showed that fast-growing individuals who are more likely to disperse are high-quality individuals rather than a specialised phenotype for early dispersal (Torrents-Ticó et al., 2018). To discriminate between these two alternatives in naked mole-rats, long-term field studies are needed that follow individuals from early life to establish whether fast-growing animals follow a divergent developmental trajectory by dispersing earlier and investing less in cooperation than slow-growing individuals (Torrents-Ticó et al., 2018).

But is it possible that naked mole-rats organize in a transient caste system similarly to the workers in honeybee colonies? It has repeatedly been argued that age is one of the main determinants of behaviour in naked mole-rats (Jarvis, 1981; Lacey & Sherman, 1991; 1997), and members of different temporal castes are not distinguishable by morphological traits, which is consistent with our data. However, caste formation would still require individuals to specialize in certain tasks and to change their behavioural specialisations at more or less fixed ages throughout ontogeny. Since we found no evidence for trade-offs between different cooperative behaviours, there is no indication that insect-like castes with or without morphological specialisation exist in naked mole-rats.

On the contrary, it seems that similarities to the social organisation of eusocial insects have been overestimated in the past and naked mole-rats may lack preconditions for the formation of permanently specialized groups. In comparison to insect colonies, especially the most complex societies with millions of group members, naked mole-rat groups are still relatively small. Rather than forming permanently specialized groups of workers, it may therefore be more advantageous for naked mole-rats to maintain behavioural plasticity and the ability to switch roles in order to adapt to changing ecological conditions or the loss of colony members, as has been shown by Mooney et al. (2015). Additionally, an individual's probability to directly breed if the current breeder dies is greater in smaller groups and may be too high in mole-rats to promote the development of a sterile worker caste (Alexander, Noonan, & Crespi, 1991; Sherman et al., 1995). Social insect species with smaller group sizes also often show less pronounced differences between worker castes (Wilson, 1971). Moreover, the chances of dispersal and subsequent successful breeding may be higher in naked mole-rats than in insect species. However, there is only very limited data available on dispersal rates in naked mole-rats and it is difficult to investigate rates of successful dispersal in the wild (Braude, 2000).

In many cooperatively breeding vertebrates, inter-individual differences in the extent of helping are mainly the consequence of age, and the varying fitness costs that are associated with helping and performing certain tasks at different ages and life-history stages (Clutton-Brock, 2016). However, this age-related variation does not represent a strict succession of social roles such as the age-dependent polyethism found in insects. Instead, age here mainly affects the overall amount of cooperative contribution and individuals are also able to adjust their cooperative investment strategically in response to dispersing or breeding opportunities (Field et al., 2006; Zöttl, Chapuis, Freiburghaus, & Taborsky, 2013; Heinsohn & Cockburn 1994). Such age-related mechanisms have been found in Damaraland mole-rats (Zöttl, Vullioud et al., 2016; Thorley et al., 2018), meerkats (Clutton-Brock et al., 2003), cooperatively breeding fish (Taborsky, 1985, 1994) and birds (Cockburn, 1998). Since the findings of the present study suggest that naked mole-rats are not organized in castes, but rather behave like other cooperatively breeding vertebrates, age-related variation may be a plausible alternative to explain the observed inter-individual variation in cooperative behaviour. Lacey & Sherman (1991) proposed an age/size polyethism for naked mole-rats, but lacked longitudinal data from known-aged individuals, which is why the relationships between body size, age, and behaviour remained unclear and conflicted with other research (Jarvis et al., 1991; Mooney et al., 2015). We too were unable to investigate the effects of age on behaviour or its relationship to body mass as the majority of our study animals were of unknown age. Further studies including growth and behavioural data from known-aged individuals are needed in order to determine if age-related variation may explain the expression of cooperative behaviour observed in naked mole-rat workers.

Generally, all individuals spent more time actively moving and working than resting during observation periods. This is uncommon for social mole-rats, as typically only a few individuals are active in the tunnels outside while most of the colony is found lying in the nest (Hickman, 1980; Lacey & Sherman, 1991; Schielke, Begall, & Burda, 2012). We assume that the heightened activity in our colonies stems from the fact that we provided new digging substrate every two hours during observations, which disrupted the huddle of animals in the nesting chamber. Usually most or all group members became active, and while not every individual engaged with the digging substrate, all animals moved around the burrow for a few minutes after the disturbance and some colonies never settled down completely during observation hours. We noticed no difference in this pattern or overall activity between the colonies housed in different rooms. While the higher activity levels represent a deviation from the undisturbed behaviour of naked mole-rats, they allowed us to more efficiently record active cooperative behaviours.

Sex differences in cooperative investment were minimal among non-breeders. Females and males overall performed the same amount of cooperative labour and neither sex showed signs of task specialisation or functional caste formation. The absence of sex-specific helping strategies in subordinate naked mole-rats has been reported previously, not only for maintenance tasks but also regarding group defence and pup care (Brett, 1991; Jarvis et al., 1991; Lacey & Sherman, 1991; Mooney et al., 2015). This lack of sex bias is in stark contrast to most other cooperatively breeding vertebrates, where sex is a major component of behavioural variation among individuals (Cockburn, 1998; Clutton-Brock, 2016). Helping is usually biased towards the philopatric sex that gains more fitness benefits from assisting breeders raise additional group members in their natal group than the dispersing sex (Clutton-Brock et al., 2002). In meerkats and brown hyenas for example, females are more likely to breed in their group of origin and contribute more to rearing young than males (Clutton-Brock et al., 2002; Owens & Owens, 1984). Conversely, in most cooperatively breeding birds and African wild dogs, males are the more philopatric sex and consequently help more (Cockburn, 1998; Malcolm & Marten, 1982). In naked mole-rats, dispersal is rare and not biased toward either sex (Braude, 2000). Since most non-breeders remain in their natal colony throughout life and opportunities to breed directly are similarly limited for both sexes, male and female mole-rats benefit equally from helping, which may explain the lack of sex differences in cooperative investment (Holmes, Goldman, Goldman, Seney, & Forger, 2009).

In Damaraland mole-rats, male non-breeders reduce their overall cooperation in larger groups, whereas females work more, and rest less when more helpers are present (Thorley et al., 2018). We found similar sex-dependent trends in naked mole-rats, but the effects of group size on cooperative behaviour were not significant. However, considering the small spectrum of group sizes included in this analysis in a species where colony size can range anywhere from a single breeding pair to almost 300 animals, differences in group size may simply have been too small to have a detectable impact on behaviour. In contrast to other social species, where larger group sizes are correlated with lower survival rates and breeding success, cooperative breeders benefit from the addition of helpers to the group (Clutton-Brock et al., 1999). Typically, bigger group sizes in cooperative breeders are associated with reduced workloads for reproductive and non-reproductive group members (Brown, Dow, Brown & Brown, 1978; Mumme & de Querioz, 1985; Farabaugh, Brown & Hughes, 1992; Heinsohn & Cockburn, 1994). In meerkats for example, subordinates in smaller groups compensate for the lack of helpers by babysitting more often, which entails substantial energetic cost and weight loss (Clutton-Brock et al., 1998). Helpers of the cooperatively breeding cichlid *N. pulcher* do not adjust their working efforts to group size but are able to feed more in larger groups (Balshine et al., 2001).

Queens' behaviour patterns differed from those of non-breeding females in our colonies. In line with previous studies, breeding females invested less in cooperative maintenance and foraging tasks than non-breeders (Jarvis, 1981; Faulkes, 1991; Lacey & Sherman, 1991; O'Riain, 1996). The difference was especially notable in working behaviours, which include digging and clearing the tunnels of substrate and protrusions. Direct pup-care was not observed frequently enough in our colonies to infer possible differences; however, it has been shown that queens, besides being the only females to nurse pups, participate more in grooming and pushing their offspring, while non-breeders of both sexes carry pups and provide them with caecotrophs (Lacey & Sherman, 1997). Since we were unable to conclusively identify male breeders, the same comparisons could not be made for male mole-rats. Lacey and Sherman (1991) reported that male and female breeders engage equally rarely in colony maintenance or defence, while others have found male breeders to behave more similarly to non-breeders, emphasizing the relatively greater divergence of breeding females from non-breeders that is also evident in their unique morphological features which are not shared by male breeders (Jarvis, 1981; Faulkes, 1991; O'Riain, 1996). Queens also invested more time in active non-helping than female non-breeders. Locomotion was by far the activity recorded most frequently for this behavioural category in both groups, indicating that queens spent substantially more time moving around the tunnel system without pursuing a cooperative task. This is most likely due to a behaviour termed "patrolling", where queens periodically leave the nest to move through the burrow and frequently shove workers (Faulkes et al., 1991; Reeve, 1992). A shove is a nose-to-nose push that may cause the receiving animal to move backwards up to one meter (Lacey et al., 1991). Reeve (1992) recorded patrols to occur as often as every 16 minutes in one colony. Patrolling and especially the accompanying shoves may play an important part in the socially induced reproductive suppression of the workers (Reeve & Sherman, 1991). Alternatively, Reeve (1992) suggested that by shoving them, queens may activate lazy workers to increase their workload, but subsequent studies found no evidence to support this hypothesis (Jacobs & Jarvis, 1996; Clarke & Faulkes, 2001).

5. Conclusion

The findings of this study show that non-breeding colony members of naked mole-rats are not organized in a caste system with morphological or behavioural specialization. This study was the first to investigate long-term individual trade-offs between different cooperative tasks in naked mole-rats. We found positive correlations between investments in different tasks, which means that individuals did not specialise in specific behavioural roles. Body mass did not predict behavioural phenotype, and maximum body mass as well as cooperative behaviour were continuously distributed, showing no signs of discrete functional or morphological castes. Repeatability of cooperative behaviour was low, suggesting behavioural plasticity similarly to that of other cooperatively breeding vertebrates. Data presented here as well as findings from closely related mole-rat species and other cooperative breeders indicate that the patterns of cooperative behaviour observed in naked mole-rats may be the consequence of age-related variation.

Further research is needed to collect behavioural and body mass data from individuals of known age throughout ontogeny to investigate the effects of age on cooperative behaviour and the much-debated relationship between age and growth in naked mole-rats. Additionally, the whole spectrum of cooperative tasks of naked mole-rats, including pup care and defence behaviours, should be included in future analyses of behavioural specialisation. As this study was conducted in a laboratory setting, the findings should be validated by testing the hypotheses in a wild population of naked mole-rats in the future.

6. Abstract

In cooperatively breeding vertebrates, non-reproductive group members assist in rearing offspring that is not their own and perform a variety of cooperative tasks such as foraging, nest building, and group defence. Investment in these activities varies between individuals according to state-dependent changes in fitness costs and benefits. The most extreme form of cooperative breeding is found in eusocial insects, where cooperative labour is divided among non-breeders via task specialisation, which leads to the formation of morphologically distinct permanent castes or temporal (age-based) castes. Naked-mole rats (*Heterocephalus glaber*) were the first mammals to be described as eusocial. Large group size and high reproductive skew make them unique among mammals and may have led to the development of a highly specialised social organisation similar to that of eusocial insects. The aim of the study was to clarify whether non-reproductive colony members of naked mole-rats are organised in a rigid caste system of behavioural specialisation or whether they show social plasticity similar to other cooperatively breeding mammals. We collected observational and body mass data from five naked mole-rat colonies over several months and employed multilevel, multinomial regression models to investigate task specialisation and the distribution of body mass and cooperative behaviour. Non-breeders did not specialise on specific tasks and there were no trade-offs between different cooperative behaviours within individuals. Body mass did not predict cooperative behaviour expression and behavioural phenotypes as well as maximum body mass were continuously distributed, showing no signs of discrete functional or morphological castes. Repeatability of cooperative behaviour was low, suggesting behavioural plasticity similarly to other cooperatively breeding vertebrates. In sum, we found no evidence of a distinct caste system in subordinate naked mole-rats. To investigate the mechanisms responsible for the observed variation in behaviour, future research requires longitudinal data from individuals of known age throughout ontogeny as well as data from free-ranging mole-rats.

7. Zusammenfassung

Nicht-reproduktive Mitglieder von Gruppen kooperativ brütender Wirbeltiere helfen bei der Aufzucht von Jungen, die nicht ihre eigenen sind, und verrichten eine Reihe an kooperativen Aufgaben wie Futtersuche, Nestbau und Gruppenverteidigung. Das Investment in diese Aktivitäten variiert zwischen den einzelnen Tieren mit vom individuellen Zustand abhängigen Veränderungen in Fitnesskosten und -vorteilen. Die extremste Form des kooperativen Brütens findet man in eusozialen Insekten, in deren Kolonien die kooperative Arbeit mittels Spezialisierung auf bestimmte Aufgaben zwischen den Nicht-Brütern aufgeteilt ist, was zur Bildung von morphologisch abgegrenzten, dauerhaften Kasten oder temporären, altersabhängigen Kasten führt. Der Nacktmull (*Heterocephalus glaber*) war die erste Säugetierart, die als eusozial beschrieben wurde. Die hohe Anzahl an Gruppenmitgliedern sowie der äußerst ungleiche Reproduktionserfolg in ihren Kolonien (*reproductive skew*) mögen zur Entwicklung einer hochspezialisierten sozialen Ordnung beigetragen haben, die der von eusozialen Insekten ähnelt. Das Ziel der Studie war es herauszufinden, ob die nicht-reproduktiven Mitglieder von Nacktmullkolonien in einem strikten Kastensystem mit Verhaltensspezialisierung organisiert sind oder ob sie sozial flexibel sind wie andere kooperativ brütende Säugetiere. Wir sammelten Beobachtungs- und Körpergewichtsdaten von fünf Nacktmullkolonien über mehrere Monate und verwendeten multilevel, multinomiale Regressionsmodelle, um Aufgabenspezialisierung und die Verteilungen von Körpergewicht und kooperativem Verhalten zu untersuchen. Nicht-Brüter spezialisierten sich nicht auf bestimmte Aufgaben und es gab keine Trade-offs zwischen verschiedenen kooperativen Verhaltensweisen innerhalb der Individuen. Verhaltensexpression war nicht abhängig vom Körpergewicht und Verhaltensphänotyp sowie maximales Körpergewicht waren stetig verteilt und ließen keine klar abgegrenzten funktionalen oder morphologischen Kasten erkennen. Die Wiederholbarkeit kooperativen Verhaltens war gering, was eine Verhaltensflexibilität ähnlich derer von kooperativ brütenden Vertebraten nahelegt. Zusammenfassend fanden wir keine Anzeichen für die Existenz eines strikten Kastensystems in nicht-reproduktiven Nacktmullen. Um die Mechanismen zu erforschen, die den beobachteten Verhaltensunterschieden zugrunde liegen, bedarf es weiterer Studien mit longitudinalen Daten von Tieren bekannten Alters sowie von freilebenden Nacktmullen.

8. References

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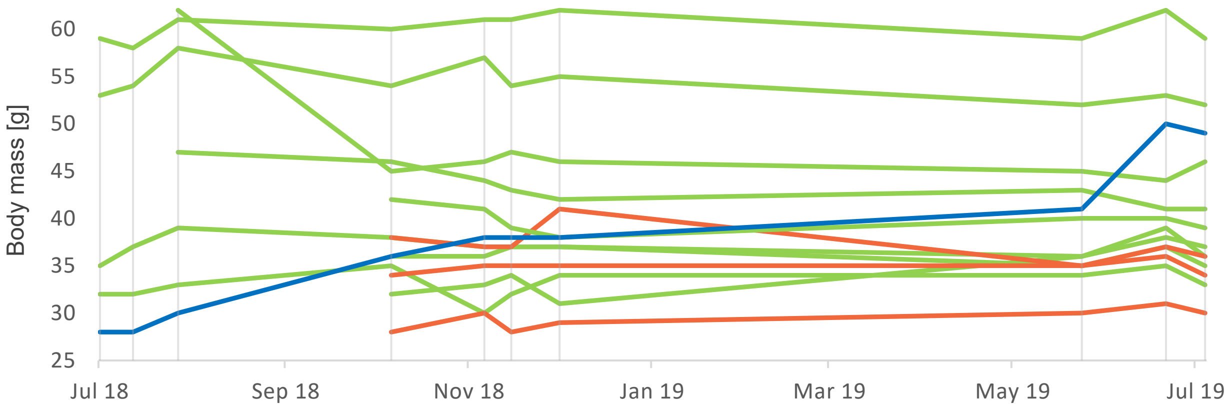
9. List of figures and tables

10. Supplementary Material

Group A



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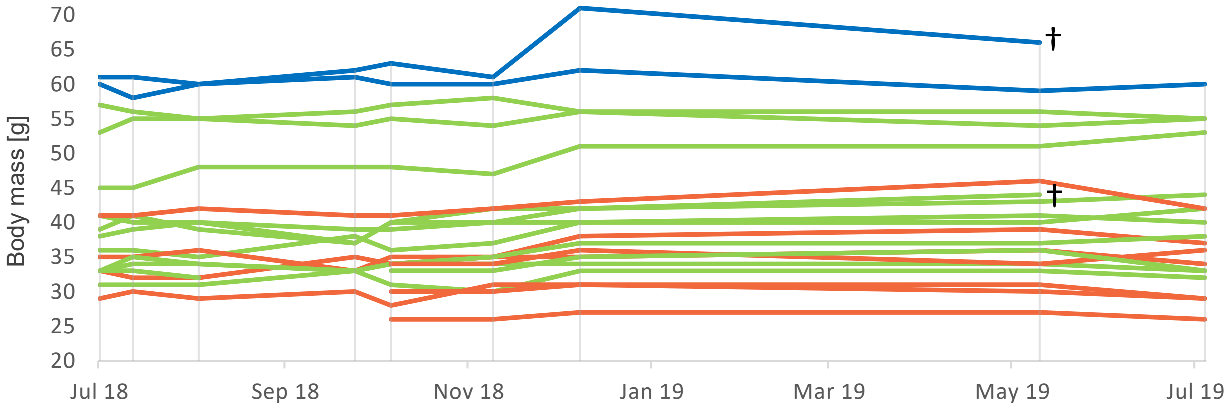

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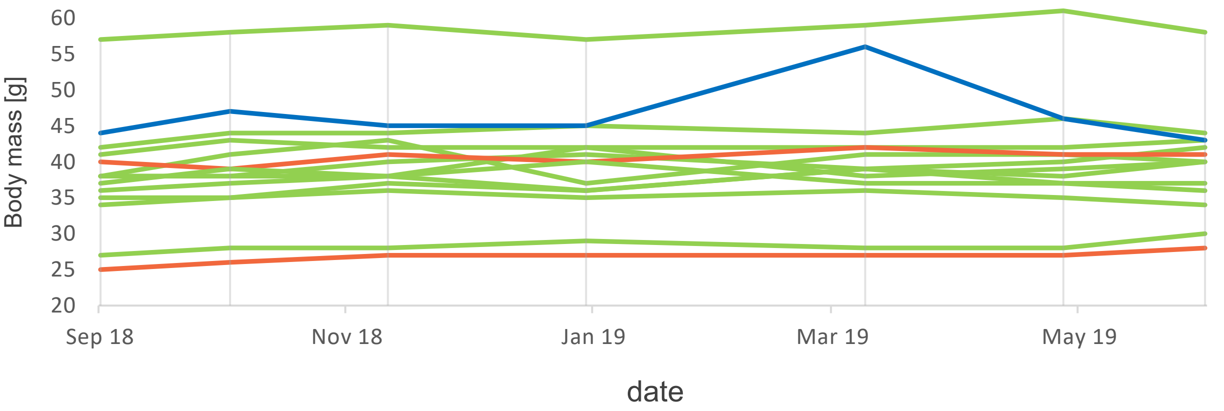

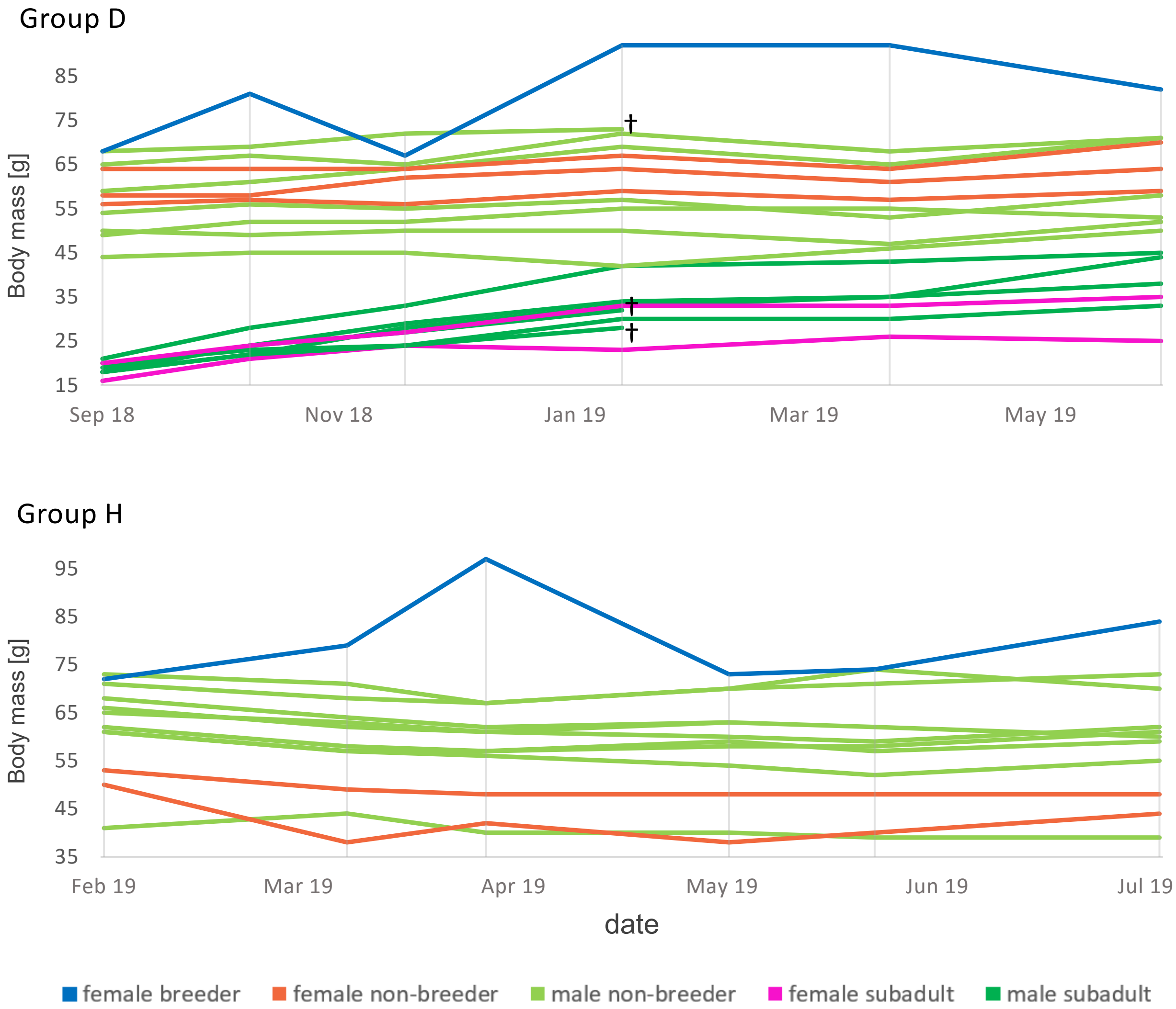

Fig. S1: Body mass measurements over the study period for five mole-rat groups.

Tab. S1: Variance estimates of the random effects for Model 1, 2 and 3 of each sex

Random effect	Female			Male		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Individual level						
Active non-help	0.62(0.12)	1.26(0.28)	0.58(0.13)	0.82(0.08)	0.88(0.10)	0.57(0.06)
Eat	0.58(0.13)	0.76(0.22)	0.27(0.16)	0.76(0.10)	0.79(0.11)	0.53(0.09)
Food carry	1.35(0.31)	1.27(0.32)	1.03(0.33)	1.33(0.16)	1.20(0.16)	0.77(0.13)
Nest build	0.99(0.20)	1.39(0.39)	1.01(0.26)	1.12(0.12)	1.15(0.14)	0.99(0.12)
Work	0.92(0.15)	1.20(0.27)	0.88(0.16)	0.99(0.09)	1.04(0.11)	0.84(0.09)
Scan level						
Active non-help			0.84(0.12)			0.78(0.10)
Eat			0.83(0.17)			0.79(0.15)
Food carry			1.14(0.28)			1.28(0.24)
Nest build			1.08(0.19)			0.90(0.13)
Work			0.76(0.12)			0.72(0.10)
Group level						
Active non-help			0.34(0.95)			0.50(0.35)
Eat			0.57(0.44)			0.68(0.46)
Food carry			0.86(0.74)			0.87(0.63)
Nest build			0.57(0.52)			0.36(0.34)
Work			0.49(0.40)			0.39(0.32)

The reported quantities are the standard deviations of the random effects while the values in parentheses are the standard deviations of these quantities in the posterior samples.

Tab. S2: Correlations of random effects across the behavioural responses in Models 1-3 for females

Sex	Model, random effect	Behaviour	Behaviour				
			Active non-help	Eat	Food carry	Nest build	Work
Female	1, Individual level	Active non-help		**0.47(0.20)**	**0.39(0.20)**	0.12(0.20)	**0.41(0.17)**
		Eat	-		0.52(0.21)	0.30(0.21)	**0.49(0.19)**
		Food carry	-	-		0.26(0.21)	0.36(0.20)
		Nest build	-	-	-		**0.60(0.16)**
		Work	-	-	-	-	
Female	2, individual level	Active non-help		**0.48(0.23)**	0.21(0.25)	0.42(0.21)	**0.62(0.15)**
		Eat	-		0.42(0.23)	0.37(0.24)	**0.57(0.19)**
		Food carry	-	-		0.20(0.25)	**0.47(0.21)**
		Nest build	-	-	-		**0.67(0.15)**
		Work	-	-	-	-	
Female	3, individual level	Active non-help		0.30(0.31)	0.27(0.25)	0.18(0.23)	**0.52(0.18)**
		Eat	-		0.23(0.32)	0.18(0.30)	0.32(0.30)
		Food carry	-	-		0.01(0.26)	0.30(0.24)
		Nest build	-	-	-		**0.52(0.19)**
		Work	-	-	-	-	
Female	3, scan level	Active non-help		**0.51(0.18)**	**0.54(0.19)**	**0.63(0.15)**	**0.70(0.13)**
		Eat	-		**0.61(0.19)**	**0.53(0.18)**	**0.49(0.18)**
		Food carry	-	-		**0.53(0.19)**	**0.48(0.19)**
		Nest build	-	-	-		**0.66(0.15)**
		Work	-	-	-	-	
Female	3, group level	Active non-help		0.01(0.35)	0.03(0.34)	0.02(0.35)	0.01(0.36)
		Eat	-		0.11(0.36)	0.05(0.36)	0.10(0.36)
		Food carry	-	-		0.03(0.36)	0.08(0.34)
		Nest build	-	-	-		0.05(0.35)
		Work	-	-	-	-	

Estimates represent the means from the posterior samples (SD in parentheses). Parameters in bold indicate estimates where the 95% credible intervals do not span zero.

Tab. S3: Correlations of random effects across the behavioural responses in Models 1-3 for males

Sex	Model, random effect	Behaviour	Behaviour				
			Active non-help	Eat	Food carry	Nest build	Work
Male	1, Individual level	Active non-help		**0.49(0.12)**	**0.65(0.10)**	**0.72(0.08)**	**0.70(0.07)**
		Eat	-		**0.75(0.09)**	**0.52(0.12)**	**0.58(0.11)**
		Food carry	-	-		**0.60(0.11)**	**0.63(0.10)**
		Nest build	-	-	-		**0.75(0.07)**
		Work	-	-	-	-	
Male	2, individual level	Active non-help		**0.49(0.13)**	**0.72(0.10)**	**0.71(0.09)**	**0.70(0.08)**
		Eat	-		**0.69(0.12)**	**0.53(0.13)**	**0.69(0.09)**
		Food carry	-	-		**0.69(0.11)**	**0.78(0.08)**
		Nest build	-	-	-		**0.82(0.06)**
		Work	-	-	-	-	
Male	3, individual level	Active non-help		**0.58(0.13)**	**0.66(0.14)**	**0.58(0.11)**	**0.63(0.10)**
		Eat	-		**0.51(0.18)**	**0.53(0.15)**	**0.66(0.13)**
		Food carry	-	-		**0.63(0.14)**	**0.74(0.11)**
		Nest build	-	-	-		**0.77(0.08)**
		Work	-	-	-	-	
Male	3, scan level	Active non-help		**0.55(0.17)**	**0.65(0.16)**	**0.68(0.13)**	**0.81(0.08)**
		Eat	-		**0.61(0.17)**	**0.60(0.16)**	**0.46(0.17)**
		Food carry	-	-		**0.63(0.16)**	**0.54(0.17)**
		Nest build	-	-	-		**0.69(0.13)**
		Work	-	-	-	-	
Male	3, group level	Active non-help		0.10(0.33)	0.03(0.34)	0.08(0.36)	0.06(0.35)
		Eat	-		0.17(0.34)	0.02(0.35)	0.07(0.34)
		Food carry	-	-		0.03(0.35)	0.08(0.35)
		Nest build	-	-	-		0.06(0.36)
		Work	-	-	-	-	

Estimates represent the means from the posterior samples (SD in parentheses). Parameters in bold indicate estimates where the 95% credible intervals do not span zero.