The dissection of a despotic society: exploration, dominance and hormonal traits

Tshepiso Lesedi Majelantle, Andre Ganswindt, Daniel William Hart, Nicole Hagenah, Stefanie Birgit Ganswindt and Nigel Charles Bennett

Department of Zoology and Entomology, Mammal Research Institute, University of Pretoria, Pretoria, 400364, South Africa

Author for correspondence: Tshepiso Lesedi Majelantle
e-mail: tshepiso.majelantle@zoology.up.ac.za

Abstract

Naked mole-rats (*Heterocephalus glaber*) live in large colonies with one breeding female (queen), one to three breeding males (BMs) and the remainder are non-reproductive subordinates. The animals have a linear dominance rank with the breeders at the top of the hierarchy. We investigated how dominance rank in naked mole-rats differs with exploration (the propensity to explore a novel environment) and related endocrine markers. Exploration behaviour, faecal progestagen metabolite (fPM), faecal glucocorticoid metabolite (fGCM), faecal androgen metabolite (fAM) and plasma prolactin concentrations were quantified in breeding, high-, middle- and low-ranked females and males from five naked mole-rat colonies. There were no significant differences between the dominance rank and exploration behaviour. Interestingly, the queens and high-ranking females had higher fGCM and fAM concentrations compared with middle- and low-ranked females. The queens had significantly higher fPM concentrations than all other ranked females, since they are responsible for procreation. In the males, the BMs had higher fGCM concentrations compared with high- and low-ranked males. In addition, BMs and middle-ranking males had overall higher prolactin levels than all other ranked males, which could be linked to cooperative care. Overall, the results suggest that physiological reproductive suppression is linked to high dominance rank.

Keywords: dominance ranking; glucocorticoids; prolactin; progestagens; androgens; naked mole-rat

1. Introduction

Sociality, the non-random formation of groups, includes group living whereby asymmetry in aggression leads to a dominance ranking in which the highest position has priority access to resources such as reproduction [1]. The dominance hierarchy is established through antagonistic social interactions such as fighting but is further maintained by aggressive behaviour [1]. In societies with distinct dominance hierarchies, some individuals forfeit their right to reproduce and are likely to spend their entire life without reproducing [2–4]. In most cases, the non-reproductive individuals have the capacity to reproduce but are reproductively suppressed by the dominant members of the same sex of the group [5,6]. Thus, the reproductive individuals have additional energy costs and demands in reproductively suppressing the subordinates [7]. The reproductive suppression of subordinates by

dominants may result from social suppression and/or indirect physiological suppression. Social suppression may arise as a consequence of aggression directed towards subordinates from dominants such as through infanticide of subordinate female young, direct interference of mating or eviction from the group [8,9]. Whereas physiological suppression of reproduction causes impaired or inhibited fertility of the subordinate possibly by mechanisms such as stress-related impairment of the hypothalamic–pituitary–gonadal (HPG) axis (refer to [10,11] for review). Knowledge concerning the mechanisms of reproductive suppression in social species can help to understand if the formation of the social system in these species is potentially owing to the social niche theory [12]. The social niche theory posits that dominance ranks could be generated by the process of social interaction among group members [12]. Alternatively, the prior attributes hypothesis suggests that dominance rank is a consequence of heritable and/or predetermined characteristics [12,13].

The HPG axis is responsible for regulating a cascade of hormones linked to reproductive behaviour and its subsequent success. When stimulated, the HPG releases gonadotrophin-releasing hormone (GnRH) from the hypothalamus [14], which, in turn, stimulates the release of gonadotropins such as follicle-stimulating hormone (FSH), and luteinizing hormone (LH) [15]. These gonadotrophins activate the gonads of both sexes resulting in the production of steroid hormones, such as oestrogens and progestagens in females, and androgens, such as testosterone, in males [16,17]. Progestagens and oestrogens play a crucial role in female primary and secondary characteristics as well as the oestrous cycle [18]. Testosterone plays a crucial role in male physiology, and in females, it may function as a precursor in the pathway for sex hormones [17,19]. In addition, alterations in androgen concentrations have been linked to sexual behaviour as well as behavioural acts such as aggression [20].

There are intrinsic and extrinsic factors that can lead to the disruption of the HPG axis. When animals are confronted with a stressor, such as aggressive behaviour directed from the breeding male or female, the hypothalamic–pituitary–adrenal axis is activated, and this in turn results in an increase in the secretion of glucocorticoids by the adrenal glands [21,22]. In some species, glucocorticoids have previously been linked to HPG axis function as an inhibitor of GnRH secretion through a variety of pathways [23]. Conversely, a positive correlation between glucocorticoid and androgen concentrations during the mating season has been reported, possibly owing to intra-specific competition [24]. The protein-hormone prolactin is a regulatory hormone not only involved in the lactation of mammals but also has an inhibitory role in the secretion of FSH and LH [25]. In addition, prolactin plays an important role in expressing parental behaviours, as well as social and cooperative behaviours [26,27]. However, prolactin suppresses reproductive activity during the period of lactation and elevated circulating levels interfere with reproductive functions in males and non-lactating females [28].

African mole-rats (Bathyergidae) possess genera that exhibit a diversity of social systems ranging from solitary to eusocial [29]. Naked mole-rats (*Heterocephalus glaber*) and Damaraland mole-rats (*Fukomys damarensis*) are the only two mammalian species that can categorically be classified as eusocial [10,30]. These species are considered eusocial because they have a reproductive division of labour, overlapping generations, as well as cooperative care of young [30]. The naked mole-rats and Damaraland mole-rats occur in colonies composed of one breeding female or queen, one to three breeding males and the rest are

non-reproductive subordinates. Only 1% of non-reproductive subordinate individuals ever attain reproductive status in the naked mole-rat and less than 15% in the Damaraland mole-rat, as they are reproductively suppressed by the dominant breeding individuals while in a colony [10,31]. Naked mole-rat subordinates can be classified into three behavioural phenotypes: soldiers, workers and disperser morphs which are linked to differences in behaviour [32,33]. Soldiers are linked to aggressive behaviour towards foreign conspecifics, whereas workers are non-aggressive and non-explorative individuals [33,34], while dispersers are linked to exploration behaviour owing to them persistently exploring burrow openings to disperse [35]. Exploration is a behavioural trait that has been linked to social dominance in many group-living species [36]. For example, the faster adult male great tits (*Parus major*) explored novel environments, the higher their dominance rank position [37]. Thus, exploration behaviour could potentially contribute to dominance ranking position in naked mole-rat colonies.

Several studies have previously investigated the role of hormones and agonistic behaviours by the queen towards subordinates in both the naked mole-rat and Damaraland mole-rat reproductive suppression [20,34,38–42]. In naked mole-rats, the dominance hierarchy in the species is presumed to be established by shoving and fighting behaviour, as the queen is usually the most aggressive member of the colony [43]. In addition, the dominance hierarchy is maintained in naked mole-rats by passing over or under behaviour [44]. Endocrine data suggest queens have higher plasma and urinary concentrations of progestagens and oestrogens compared with subordinate females, whereas breeding males have higher testosterone concentrations compared with subordinate males [20]. In addition, there appears to be no difference in glucocorticoid concentrations between breeders and non-breeders [40,41]. However, one caveat in all these studies is that all non-breeders have been placed into one category that being subordinate and non-reproductive. However, there is probably a linear dominance hierarchy whereby individual *x* dominates over all, individual *y* dominates over all except *x* and so on until the last individual is dominated by all [44,45]. To our knowledge, Clarke & Faulkes [20,44] provide the only studies that compare naked mole-rat dominance rank with different correlates without grouping non-breeding subordinates into one group. The results thus far suggest there is correlation between male dominance position and age, body mass and urinary testosterone [20,44]. However, these studies did not incorporate exploration, stress-related hormone or progestagen concentrations. Thus, the aim of this study was to investigate the link between exploration behaviour, concentrations of androgens, progestagens, glucocorticoids and prolactin, in addition to the dominance rank in entire naked mole-rat colonies. We hypothesized that higher-ranked colony members would be classified as more explorative than lower-ranked members of the colony. In addition, there should be significant differences in endocrine markers between the different ranking positions, whereby high-ranked individuals would have comparatively higher androgen and glucocorticoid concentrations, possibly owing to competition with the breeders. Finally, we predicted that the lower-ranked colony members will have higher prolactin concentrations than the higher-ranked animals since the hormone is linked to infertility and cooperative care of young.

2. Material and methods

2.1. Study animals

Data were collected from healthy adult members (older than six months) from five captive-bred naked mole-rat colonies (total 96 animals, table 1). All colonies used did not have any suckling offspring, and thus no lactating queens were included in the study. All adults were previously implanted subcutaneously with a microchip (Identipet, Johannesburg, South Africa) as part of a long-term monitoring programme for the animals. The room that housed the animals was located at the Small Animal Physiological Research Facility at the University of Pretoria, South Africa, with the mole-rats maintained on a 12L:12D-light schedule and temperature in the room controlled to around 29°C (28–30°C). Every second day, the animals were fed cut fruits and vegetables ad libitum with no supply of water since naked mole-rats derive water from food [46]. All naked mole-rat colonies were kept in tunnel systems with several plastic chambers serving as food storage, toilet and sleeping areas and connected by acrylic glass tunnels. These tunnel systems contained sterilized wood shavings as nesting material. The systems ranged in size from 1 to 2.5 m and varied in their length depending on the size of the colony.

Table 1. The number of adult naked mole-rat (H. glaber) individuals for each dominance rank in each of the studied naked mole-rat colonies. Bold indicates the colony where only faecal samples were collected, and italics indicates colonies where only blood samples were collected from the breeders.

	dominance ranking								
	breeders		high-ranking		mid-ranking		low-ranking		
colony name	F	M	F	M	F	M	F	M	total
Babirwa	1	1	2	2	3	1	0	3	13
Bakalaka	1	1	5	5	7	1	3	4	27
Bakgatla	**1**	**1**	**0**	**0**	**1**	**0**	**1**	**1**	**5**
Bangwato	1	1	5	7	3	3	7	3	30
Batokwe	1	1	4	4	6	0	2	1	17
Balobedu	*1*	*1*	*0*	*0*	*0*	*0*	*0*	*0*	*2*
Barolong	*1*	*1*	*0*	*0*	*0*	*0*	*0*	*0*	*2*

2.2. Experimental design

Blood sample collection and behavioural observations were conducted on adult naked mole-rats; first, one blood sample was collected from each adult individual in the colony (except for the Bakgatla colony). After a minimum of 28 days, behavioural observations were conducted on the same colonies. However, in the case of the Bangwato colony, the behavioural observations were conducted first, followed by blood sampling one week later. It is unlikely that the difference in the order of procedure affected the overall behaviour and stress-related endocrine correlates of naked mole-rats in our study. For example, after adrenocorticotrophic hormone and saline administration to the species, stress-related hormones for males returned to baseline 96 h post-administration, and females showed decreases in stress-related hormones 120 h post-administration [47].

2.3. Blood sample collection

All individuals in a colony were removed from their natal tunnel system, and each adult individual was weighed. Venous blood samples within 1% of the body mass of an individual were collected from the tail for each adult naked mole-rat (total 92 samples) using heparinized micro-haematocrit tubes and collected into Eppendorf tubes. In addition, based on breeding behaviour, previously identified queens and breeding males from two additional colonies were included in the blood sample collection. Blood samples were centrifuged at 500*g* immediately after collection and the resulting plasma was decanted and subsequently kept frozen at −80°C for plasma prolactin measurement.

2.4. Behavioural observations

Two sets of behavioural observations were undertaken to quantify exploration behaviour and dominance hierarchy. The first set of observations was performed to quantify the explorative behaviour of each adult member in a colony, and the second set of behavioural observations was done to establish the dominance hierarchy for all individuals within each colony. To quantify explorative behaviour, all individuals within the colony were removed from their natal tunnel system. Once removed, the animal's tag ID was recorded, and each individual was uniquely marked using a permanent marker pen (from here on referred to as markings) and weighed. In addition, faecal samples from known individuals were collected opportunistically (a total of 91 samples from 59 individuals). The tunnel system was washed and cleaned to remove the familiar colony scent and reassembled to have an introduction pod with a 1 m tunnel connecting it to the rest of the tunnel system. The rest of the tunnel system size and structure was dependent on the colony size. Thereafter, an adult individual was randomly selected and added to the now novel tunnel system through the introduction pod. All movement behaviours of each individual in the colony were recorded using a video camera for 2.5 min. This time period was selected because naked mole-rat colony dynamics are sensitive, thus the individuals cannot be separated for long periods of time [34,39–41]. After the recordings for the first individual were completed, the introduction pod was wiped down with 70% ethanol to remove the familiar colony scent. The next randomly selected individual was added to the tunnel system, and again all movement behaviours were recorded. The same procedure was followed until all individuals were added to the tunnel system. Thereafter, all behavioural acts performed by the mole-rats were measured as described by Lacey *et al.* [48] and Majelantle *et al.* [49] and recorded using MS Excel™ by the principal investigator (electronic supplementary material, table S1). Of these, seven behaviours were recently identified as reliable and biologically relevant [49]. Movement speed was calculated using the 100 cm mark of the novel tunnel system.

After all the colony members were returned to the tunnel system, the animals were given a rest period between 12 and 16 h. Thereafter, to determine the social hierarchy, focal animal observations were used to record the frequency of passing behaviour (moving over, under or beside behaviour). Passing behaviour is a useful tool to establish the dominance hierarchy position of individuals in a colony [44,50]. Passing behaviour that occurred when at least one individual was performing a task, such as digging or retreating [49], was not included in the dataset. Each individual within a colony was identified by its unique marking, was observed for three 10 min time periods, and its above-mentioned passing behaviour and the ID of the

individual which was passed were recorded. When the individual was asleep or resting, the nearest active colony member was observed. Each colony was observed, and data on passing-over behaviours were collected until individual ID markings were not visible (approx. 48 h). Owing to the light cycle in the housing, focal observations were made between 9.00 and 17.00.

2.5. Faecal steroid extraction

Faecal samples were freeze dried and crushed into powder [51]. Samples weighing 0.0150–0.0249, 0.0250–0.0366 and 0.0370–0.055 g were extracted using 0.5, 1 and 1.5 ml of 80% ethanol, respectively [52]. Thereafter, suspensions were vortexed for 15 min, centrifuged at 1500*g* for 10 min and the supernatant then transferred into microcentrifuge tubes [53]. Faecal extracts were stored at −20°C for further analysis.

2.6. Enzyme immunoassays

Faecal glucocorticoid metabolite (fGCM), faecal androgen metabolite (fAM) and faecal progestagen metabolite (fPM) concentrations were measured using a 11-oxoaetiocholanolone enzyme immunoassay (EIA) [54], an epiandrosterone EIA [55] and 5α-tetrahydroprogesterone EIA [56], respectively. These EIAs were previously identified as suitable for quantifying fGCMs [47], fAMs and fPMs [57] in naked mole-rats. Quantifications were conducted following Ganswindt *et al*. [58]. Details on the EIA sensitivities, antibodies, labels, standards and intra- and inter-assay coefficients of variation of high- and low-quality controls are provided in the electronic supplementary material, table S2. Serial dilutions of faecal extracts resulted in displacement curves parallel to respective standard curves and had variation slopes of respective trend lines of less than 2% for the 11-oxoaetiocholanolone EIA, less than 5% for the epiandrosterone EIA and less than 4% for the 5α- tetrahydroprogesterone EIA.

Prolactin (PRL) concentrations were measured in blood plasma using a commercial enzyme-linked immunosorbent assay (Elabscience© guinea pig prolactin ELISA kit, catalogue no. E-EL-GP0358) according to manufacturer's instructions. This procedure was previously validated and applied by Bennett *et al.* [38]. The intra-assay coefficient of variation of the Elabscience guinea pig PRL assay was less than 10%, and the EIA sensitivity was 0.09 ng ml^{-1}.

2.7. Data analysis

All statistical analyses were carried out using RStudio (version 3.6.1; [59]), with the level of significance set at $p \leq 0.05$, and results are reported as mean ± standard error. To quantify exploration behaviour, first, all recorded behavioural acts were converted to a proportion of the total time of the test for each individual (150 s), and movement speed for all individuals in all colonies was included in a principal component analysis (PCA; table 2) using the *stats* package [59]. The final exploration score values were from the first PCA. A linear mixed effects model using *lme4* package [60] with 'colony' as a random effect was used to test if other individuals present in the colony, therefore order of addition to the colony, affected the overall PCA score.

Table 2. First PCA for each behaviour during the exploration test. Bold indicates avoidant behavioural acts, and italics indicates loadings less than 0.1.

behaviour	PCA loading
climbing	**−0.311**
gnawing	**−0.259**
rotation	**−0.636**
speed	***−0.074***
crouch advancing	0.507
peeking	0.274
freezing	*−0.003*
retreat	0.308
standard deviation	1.253
proportion of variance	0.196

To investigate the dominance hierarchy for each colony, the Clutton–Brock index (CBI [61]) was applied to the passing behaviour data of the mole-rats over the observation period using the DomiCalc program on MS Excel™ [62]. Specifically, the individual naked mole-rat which went over (win) was considered the more dominant individual, whereas the individual which went under (loss) was the subordinate. When animals passed side by side, the interactions were considered a tie. The CBI formula uses the number of individuals an animal won against added to the total number of times they won, divided by the number of individuals they lost to add to the number of times they lost. For each colony, one female was classified as queen based on mating observation, prominent axillary and inguinal teats and a perforated vagina. The breeding male (BM) was assigned on observations of him mating with the fecund queen. Females and males with CBI values greater than 1 were classified as high-ranking non-breeding females (HRF) or high-ranking non-breeding males (HRMs) because their number of wins was greater than their number of losses (figure 1). Females and males with CBI values between 1 and 0.5 were classified as middle-ranking non-breeding females (MRFs) and middle-ranking non-breeding males (MRMs; figure 1). Finally, CBI values less than 0.5 classified low-ranking non-breeding females (LRFs) and males (LRMs) since they lost against the majority of the colony (figure 1).

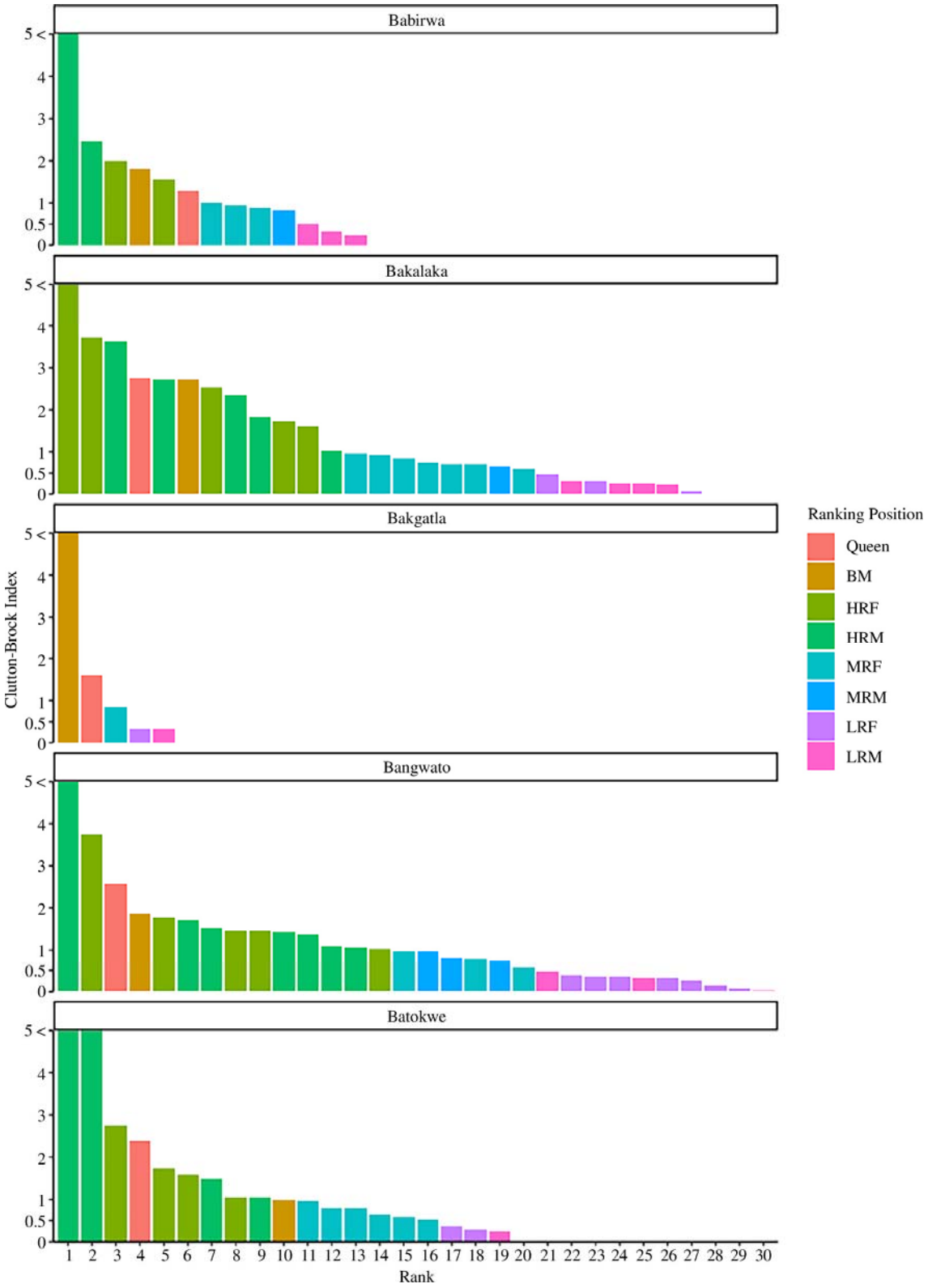

Figure 1. Bar plots showing CBI [61] for each dominance ranking in each colony: queen, BM, HRFs, HRMs, MRFs, MRMs, LRFs and LRMs.

Further analyses were conducted on the sexes separately. A linear model using the *car* package [63] with body mass, and dominance ranking as predictor variables was used in relation to each response variable (exploration score, plasma prolactin, fGCM, fAM and fPM concentrations). Individual values for each response variable in relation to dominance rank are available in electronic supplementary material, figures S1–S5. During analysis for fGCM, fAM and fPM, the middle-ranking males were excluded because no faecal samples could be collected from the dominance rank. Pairwise *t*-tests with a Holm correction were conducted *post hoc* to determine the difference between dominance rankings for each response variable. Normality was tested on model residuals visually using quantile comparison plots, a Shapiro–Wilk test of normality, and a Levene's test of homogeneity using *car* and *stats* packages [59,63]. The female fGCM, fPM and prolactin data were positively skewed in distribution and were consequently $\log_{10}$ transformed prior to statistical analysis.

Pearson's correlation was used to test if there was a significant correlation between the exploration score, fGCM, fAM, fPM and plasma prolactin concentrations per colony using the *stats* package [59]. A Bonferroni correction was applied to all *p*-values from correlation tests to reduce the possibility of a type-II error.

3. Results

Overall, the variance explained by the exploration test was 19.6% (table 2). Avoidance behaviours were positively loaded, whereas explorative behaviours were negatively loaded (table 2). Therefore, naked mole-rat individuals with PCA values less than 0 were considered explorative individuals (table 2). The linear mixed effects model with colony and order of test as predictor and the exploration score as response explained 14.3% (conditional R^2) of the variation, and the order of the test explained 1.84% (marginal R^2) of the variation. In addition, there was no significant relationship between the exploration score and the order of the test ($F_1 = 1.647$, $p = 0.199$). Thus, it is unlikely that having other individuals in the colony during the exploration test affected overall exploration test results. Finally, there was no significant difference between the exploration score and the dominance rankings for both females ($F_{3,56} = 3.346$, $p = 0.363$, figure 2) and males ($F_{3,49} = 1.322$, $p = 0.283$, figure 2). In addition, there was no significant relationship between the exploration score and body mass for both females ($F_{1,56} = 0.002$, $p = 0.971$) and males ($F_{1,49} = 0.361$, $p = 0.552$).

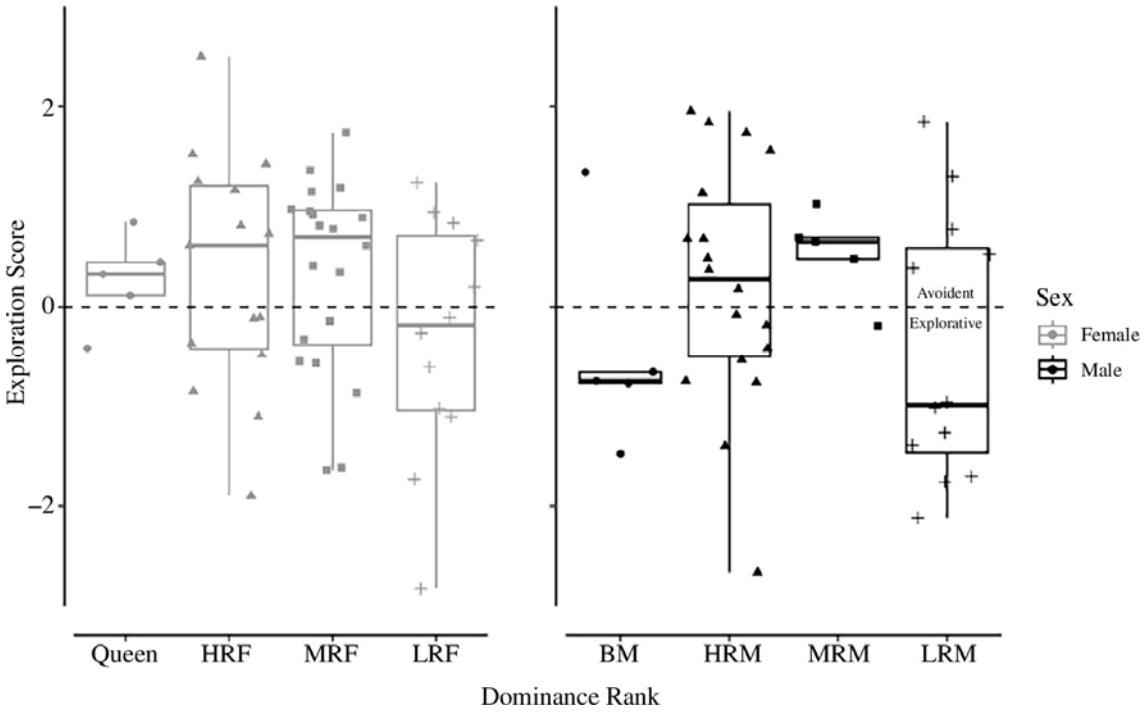

Figure 2. Box plots showing exploration score for naked mole-rat (*H. glaber*) by dominance ranking: queen (*n* = 5), HRF (*n* = 15), MRF (*n* = 20), LRF (*n* = 12), BM (*n* = 5), HRM (*n* = 18), MRM (*n* = 5) and LRM (*n* = 12). Thick lines represent the median, edges of the box represent the upper (third) and lower (first) quartiles, the vertical lines above and below represent the maximum and minimum values and symbols indicate individual data points.

With respect to the females, fGCM concentrations differed significantly between dominance ranks ($F_{3,29} = 5.689$, $p = 0.003$, figure 3*a*). Queens (55.114 ± 19.662 ng g^{-1} dry weight (DW), *n* = 4, figure 3*a*) had significantly higher fGCM concentration compared with HRFs (119%; 25.200 ± 4.015 ng g^{-1} DW, *n* = 8, *p* = 0.044), MRFs (278%; 14.593 ± 1.918 ng g^{-1} DW, *n* = 14, *p* < 0.001), and LRFs (283%; 14.370 ± 1.347 ng g^{-1} DW, *n* = 8, *p* < 0.001). The higher-ranked females had significantly higher fGCM concentrations compared with MRFs (73%; *p* = 0.044). Furthermore, the HRFs had overall 75% higher fGCM concentrations compared with LRFs (*p* = 0.072), although this was not significantly different. Finally, there was no significant relationship between fGCM concentrations and female body mass ($F_{1,29} = 1.281$, $p = 0.267$). Similarly, fGCM concentrations differed significantly between male dominance rank ($F_{3,12} = 6.033$, $p = 0.015$, figure 3*a*). The BMs' fGCM concentrations (34.612 ± 10.179 ng g^{-1} DW) were significantly higher than LRMs fGCM concentrations (13.381 ± 1.300 ng g^{-1} DW, 159%, *n* = 7, *p* = 0.013). In addition, although not significantly different, BM fGCM concentrations were higher than the HRMs (19.275 ± 3.383 ng g^{-1} DW, 80%, *n* = 6, *p* = 0.061). Finally, there was no significant relationship between fGCM concentrations and male body mass ($F_{1,12} = 0.579$, $p = 0.461$).

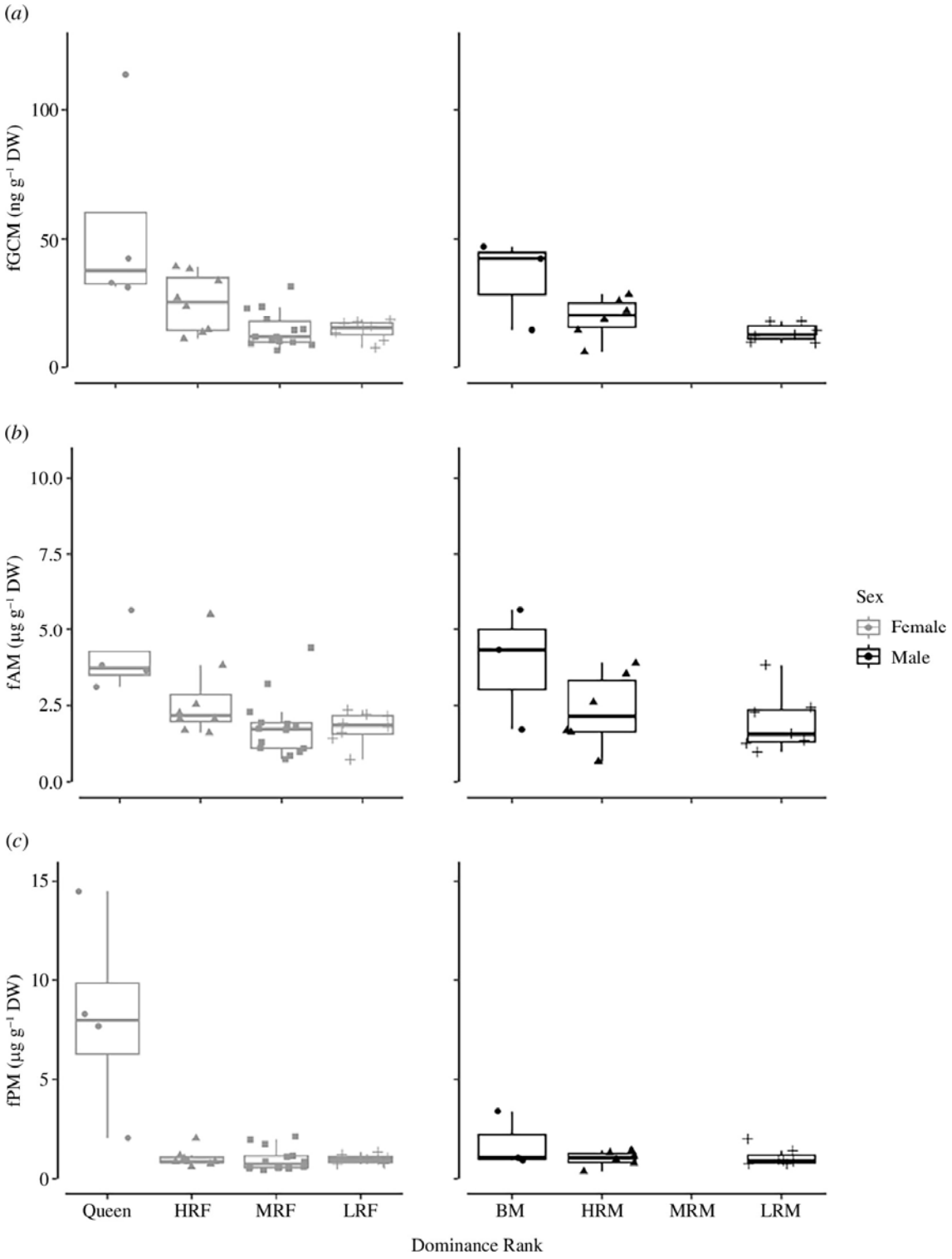

Figure 3. Box plots (*a*) fGCM (ng g^{-1} DW), (*b*) fAM (µg g^{-1} DW) and (*c*) fPM (µg g^{-1} DW) for naked mole-rat (*H. glaber*) by dominance ranking for five colonies: queen (*n* = 4), HRFs (*n* = 8), MRFs (*n* = 14), LRFs (*n* = 8), BMs (*n* = 3), HRMs (*n* = 6), MRMs (*n* = 0) and LRMs (*n* = 7). Thick lines represent the median, edges of the box represent the upper (third) and lower (first) quartiles, the vertical lines above and below represent the maximum and minimum values, and symbols indicate individual data points.

The fAM concentrations differed significantly with female dominance rank ($F_{3,29} = 3.661$, $p = 0.024$, figure 3*b*). Queens (4.056 ± 0.554 µg g^{-1} DW, $n = 4$), had significantly higher fAM concentrations compared with the MRFs (126%, 1.794 ± 0.266 µg g^{-1} DW, $n = 14$, $p = 0.005$) and LRFs (107%, 1.957 ± 0.370 µg g^{-1} DW, $n = 7$, $p = 0.016$), respectively. However, there were no significant differences between HRFs (2.70 ± 0.471 µg g^{-1} DW, $n = 8$) and the queens (50%, $p = 0.260$). There was also no significant relationship between fAM concentrations and body mass ($F_{1,29} = 0.042$, $p = 0.840$). Conversely, fAM concentrations did not differ significantly between the male dominance ranking ($F_{2,12} = 2.229$, $p = 0.150$, figure 3*b*). In addition, there was no significant relationship between fAM concentrations and male body mass ($F_{1,12} = 0.041$, $p = 0.843$).

In females, fPM concentrations differed significantly between dominance rankings ($F_{3,29} = 16.904$, $p < 0.001$). Queens (8.128 ± 2.546 µg g^{-1} DW, $n = 4$, figure 3*c*) had significantly higher fPM concentrations compared with HRFs (693%, 1.025 ± 0.157 µg g^{-1} DW, $n = 8$, $p < 0.001$), MRFs (731%, 0.978 ± 0.153 µg g^{-1} DW, $n = 14$, $p < 0.001$) and LRFs (722%, 0.989 ± 0.076 µg g^{-1} DW, $n = 8$, $p < 0.001$). There was no significant relationship between fPM concentrations and body mass ($F_{1,29} = 1.317$, $p = 0.261$). Conversely, there was no significant difference between dominance ranking in relation to fPM concentrations in males ($F_{2,12} = 0.996$, $p = 0.398$, figure 3*c*). In addition, there was a significant positive relationship between fPM concentrations and male body mass ($ß = 0.031$, $F_{1,12} = 5.308$, $p = 0.040$).

From the 93 individuals tested for plasma prolactin concentrations, eight individuals had plasma prolactin concentrations below the detection limit (0.09 ng ml^{-1}; table 3) and seven individuals had prolactin concentrations considered as hyperprolactinaemia (25.00 ng ml^{-1} in females, 20.00 ng ml^{-1} in males; table 3). There was no significant difference between dominance rankings in plasma prolactin concentrations in females ($F_{3,42} = 1.554$, $p = 0.215$, figure 4). Overall, the queens had plasma prolactin concentrations (13.262 ± 5.651 ng ml^{-1}, $n = 5$) that were higher than that of HRFs (53%, 8.655 ± 1.355 ng ml^{-1}, $n = 15$) and MRFs (75%, 7.597± 1.560 ng ml^{-1}, $n = 17$). However, the queens overall had similar plasma prolactin concentrations to LRFs (2% difference, 13.487 ± 5.885 ng ml^{-1}, $n = 11$). Interestingly, there was a significant negative relationship between plasma prolactin concentrations and female body mass ($ß = -0.026$, $F_{1,42} = 7.025$, $p = 0.011$). There was a significant difference between dominance rankings in relation to plasma prolactin concentrations in males ($F_{3,27} = 3.692$, $p = 0.0240$, figure 4). The BMs (23.353 ± 7.183 ng ml^{-1}, $n = 6$) were significantly higher compared with the HRMs (135%, 9.919 ± 1.482 ng ml^{-1}, $n = 15$, $p = 0.019$) and LRMs (277%, 6.197 ± 1.461 ng ml^{-1}, $n = 9$, $p = 0.006$). Although not significantly different, BMs were overall higher in plasma prolactin than MRMs (48%, 15.693 ± 4.095 ng ml^{-1}, $n = 3$, $p = 0.690$, figure 4). Furthermore, there was no significant relationship between plasma prolactin concentrations and body mass ($F_{1,27} = 0.579$, $p = 0.453$).

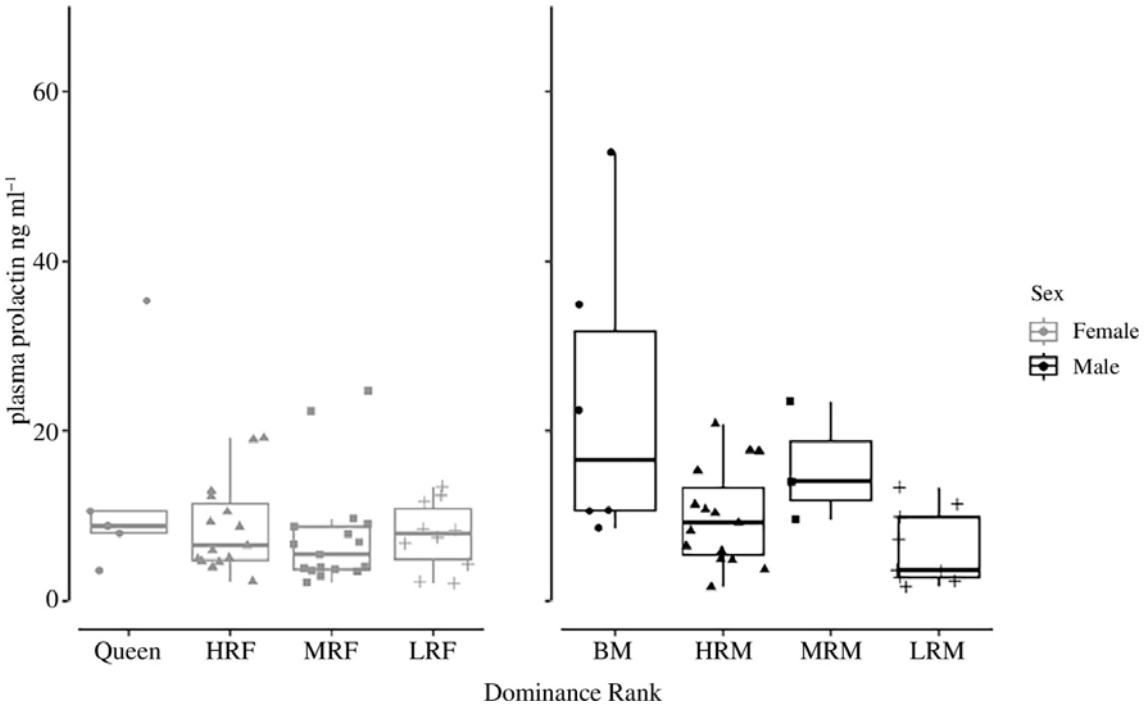

Figure 4. Box plots showing plasma prolactin (ng ml^{-1}) for naked mole-rat (*H. glaber*) by dominance ranking: queen (*n* = 5), BMs (*n* = 6), HRFs (*n* = 15), HRMs (*n* = 15), MRFs (*n* = 17), MRMs (*n* = 3), LRFs (*n* = 11) and LRMs (*n* = 9). Thick lines represent the median, edges of the box represent the upper (third) and lower (first) quartiles, the vertical lines above and below represent the maximum and minimum values and symbols indicate individual data points.

Table 3. Summary of the number of individual naked mole-rats (H. glaber) within each dominance rank which had plasma prolactin concentrations below detection (less than 0.09 ng ml−1) and above hyperprolactinaemia (greater than 25.00 ng ml−1 females and greater than 20.00 ng ml−1 males).

dominance rank position	below detection	hyperprolactinaemia
queen	1	1
breeding male	0	3
high-ranking female	0	0
high-ranking male	2	1
middle-ranking female	1	0
middle-ranking male	1	1
low-ranking female	1	1
low-ranking male	2	0

For both females and males, there was no significant correlation (p = 1.00) between exploration behaviour and concentrations of fGCM (correlation coefficient = −0.009), fAM (correlation coefficient = −0.067), fPM (correlation coefficient = −0.052) or plasma prolactin (correlation coefficient = 0.127) concentrations. In addition, there was no significant correlation (p = 1.00) between plasma prolactin and fGCM (correlation coefficient = −0.102), fAM (−0.028) and fPM (−0.072) concentrations. There was a significant correlation between fPM and fGCM concentrations (p = 0.002, correlation coefficient = 0.497), as well as fAM concentrations (p = 0.048, correlation coefficient = 0.393). Finally, there was a significant

correlation between fGCM and fAM concentrations (p < 0.001, correlation coefficient = 0.764).

4. Discussion

This study evaluated how differences in naked mole-rat exploratory behaviour, fGCM, fAM, fPM and plasma prolactin concentrations varied between their dominance rank positions in functionally intact colonies. There do not appear to be any significant differences between the dominance rank and an animal's exploration behaviour. Queens and higher-ranking females had higher fGCM concentrations and fAM concentrations, respectively, compared with all other ranked females. BMs had the highest fGCM concentrations compared with all other ranked males. In agreement with previous literature, the queen within a colony had the highest fPM concentrations compared with all other ranked females. Interestingly, BMs and MRMs had the overall highest plasma prolactin concentrations compared with all other male-ranking positions, and there was no difference in plasma prolactin concentrations between all the female-ranking positions.

Complex animal societies often have a strict dominance hierarchy where there is structure to the individuals that occur in the group. In naked mole-rats, the queen is usually at the top of the hierarchy [20]. In contrast, in the Damaraland mole-rat, it is usually the BM who is at the apex of the hierarchy, with a number of non-BMs below him [64]. The queen is not at the top of the hierarchy but is the most dominant of the female colony members [64]. In the naked mole-rats, classically dominance has been ascertained by monitoring the frequency of pushing of individuals (shoving) by the dominant queen [44]. However, using this observation to determine a dominance hierarchy for subordinates may not be useful as the mid- to low-ranked animals, rarely, if ever, are shoved [44]. Using these calculations, the queen was frequently found to be the highest-ranked colony member, with dominance positions being established by the rest of the colony. Alternatively, when two mole-rats meet in a tunnel face to face, after a very brief period of mutual sniffing of the facial area, one dominant individual passes over the top of the other (except for rare side-to-side passes), which may be used to determine dominance hierarchy [20,44,50]. Interestingly, using passing behaviour, our results suggest the queen is not the highest-ranking member of the colony which is in agreement with Clarke & Faulkes [44]. However, if the passing over behaviour is used in combination with the agonistic behaviour of shoving, then the queen is likely to be the highest-ranking member of the colony.

Exploration behaviour is an animal personality trait (consistent differences in behaviour across time, situation or both) which is used in behavioural observations to identify disperser morphs, since they are known for exploration of colony openings [34,35]. In addition, the personality trait was previously quantified as continuous in naked mole-rats disperser morphs [49]. The behaviour could be distributed along dominance ranks, and not linked to a specific dominance rank position, but rather linked to a behavioural phenotype, disperser morphs [50]. Likewise, we found no difference between the dominance rank and exploration score. This finding supports the literature, since disperser morphs do not occupy a specific dominance ranking range or position in the colony [50].

The role of stress-related glucocorticoids in naked mole-rat colonies and reproductive suppression has been under much debate [65–67]. However, stress responses are of considerable importance in the suppression of reproduction in social mole-rat species [67]. To date, there is no link between stress-related glucocorticoids and dominance hierarchy in breeders versus non-breeders [39–41]. In addition, in the eusocial Damaraland mole-rat, there were no significant differences in circulating glucocorticoids between breeding and non-breeding females [42,68]. However, this study showed that after the separation of the non-breeders into different dominance ranks, queens and high-ranking naked mole-rat females had higher stress-related hormone (glucocorticoid) concentrations compared with the lower female dominance ranking classes. One possible reason could be that the high-ranking females pose a greater threat to the queen compared with the other females, thus these individuals might well be subject to more agonistic behaviours from the queen. By analysing the hormonal status in relation to dominance position our results suggest a finding which may have been overlooked when comparing queens with subordinates as a cohort of non-breeding animals. However, Majelantle *et al*. [47] showed that females showed no increase in fGCM concentrations after low-dose adrenocorticotrophic (ACTH) administration, but had an increase in fGCM after high-dose ACTH administration suggesting that females only respond to more distinct stressful events. Thus, the high-ranking females are probably subjected to targeted high stressors from the queen in the form of shoving events [44]. In addition, although the middle-ranking and low-ranking females are probably not completely free from stressors, they may have fewer stressful interactions with the queen. Furthermore, the results suggest that the BMs had the highest fGCM concentrations compared with all other male-ranking positions. Interestingly, during their breeding tenure, the BMs become emaciated and thin, which could arise from the heightened chronic levels of stress hormones. In summary, the results presented here illustrate the potential that higher-ranking positions in both females and males are more stressed than subordinate-ranking positions. However, increases in glucocorticoids are associated with increases in energetic demands, such as external demands (daily energy expenditure) and internal demands (metabolic rate) [69]. As such, in this study, fGCMs difference between dominance ranks in this study may be owing to differences in energetic demands. Unfortunately, to date, the variation in energetic demands between naked mole-rat dominance ranks has not been studied.

Queens and high-ranking females similarly had higher fAM concentrations than those of the middle- and low-ranking females. Interestingly, Toor *et al*. [50] found no difference in concentrations of the androgen precursor dehydroepiandrosterone and testosterone between naked mole-rat soldier, worker and disperser behavioural non-breeder phenotypes. In the Damaraland mole-rat, there was no significant difference between BMs and breeding females and non-BMs and non-breeding females with respect to fAM concentrations [68]. In terms of males, the results here are in agreement with what was found in Damaraland mole-rats, that although not significant, BMs had slightly higher fAM concentrations compared with non-BMs [68]. The Damaraland male reproductive suppression is primarily behavioural since non-BMs have active spermatogenesis with motile sperm [70,71]. However, in the naked mole-rat, it is possible that the lower fAM concentrations could be owing to a lower sensitivity in the HPG axis in non-BMs, and thus evidence suggests that reproductive suppression is physiological [70]. As with fGCMs, naked mole-rat queens and high-ranking females had higher fAM concentrations compared with all other social ranking positions. The high concentrations of fAMs in the high-ranking females may possibly be linked to their higher

fGCM concentrations. The hormone testosterone is linked to the expression of aggressive behaviours [72]. Thus, possibly, the queen might have higher faecal androgen metabolites concentrations as the most aggressive member of the colony. On the other hand, the high-ranking females could have relatively high faecal androgen metabolites concentrations as they frequently challenge the queen for her position in the colony. Notably, Clarke & Faulkes [44] found that high-ranking females with high urinary testosterone metabolite concentrations often succeeded as new queens. This supports the suggestion that the high-ranking females with high fAM metabolite concentrations may be more competitive towards the queen for reproductive supremacy in the colony.

Interestingly, there was a significant correlation between fGCM and fAM concentrations. This could be owing to cross-reactivity in EIAs since some glucocorticoids and androgens have structural similarities [73,74]. However, this is unlikely because studies which used similar enzyme immunoassays detected no significant cross-reactivity [73,74]. Future studies should investigate the cross-reactivity of the EIAs used in this study for quantifying glucocorticoid and androgen metabolites in naked mole-rat faecal samples. A further possible explanation for the relationship is that there is a positive association between glucocorticoids and androgens during intraspecific mating competition [24]. The results show the highest fGCM concentrations and fAM concentrations are in the breeders and high-ranking individuals of both sexes. Thus, there is possibly intra-sexual competition for mating between the groups, and this might be reflected in fGCM and fAM concentrations. If so, then high-ranking females possibly have higher fGCM concentrations not because of the agonistic behaviours directed from the queen, but rather as a consequence of competition for mating opportunities in the presence of the queen.

As the only reproductively active female members of the colony, it is unsurprising that the queen had overall higher fPM concentrations compared with all other female dominance ranks. Furthermore, the variation of fPM levels among the queens may reflect pregnant females or cycling breeding females. Finally, there was no difference between high-ranking females and all other female ranking positions. This supports earlier work which found that pregnant females had the highest urinary progestagen concentrations, followed by cyclic breeding females, and finally non-breeding females were below assay detection limits [75]. The naked mole-rat is a spontaneous ovulator. The non-breeders, including high-ranking females are reproductively suppressed and fail to ovulate. Interestingly, the higher androgen concentrations of high-ranking females may potentially indicate a functional HPG axis. The degree of reproductive suppression orchestrated at the level of the pituitary on various members of entire naked mole-rat colonies was found to be unequal, with some colony members being more suppressed than others [76]. Consequently, reproductive suppression in naked mole-rats may operate on a two-tier system: (i) through neuro-endocrine mechanisms acting on middle- to low-ranking females differentially and (ii) as a consequence of socially orientated behaviours by the queen towards high-ranking females.

The majority of naked mole-rats had detectable prolactin concentrations as was originally reported by Bennett *et al.* [38]. This is in direct contrast to the Damaraland mole-rats, where all non-breeding individuals had plasma prolactin concentrations which were below the detection limit [38,42]. Furthermore, Mulugeta *et al.* [77] and Bennett *et al.* [38] reported that the non-breeders had plasma prolactin concentrations comparable to the queens. In

both studies, some non-breeders had plasma prolactin concentrations higher than pregnant or lactating females [38,77]. This finding may provide additional evidence to support the notion that plasma prolactin plays a role in non-breeder reproductive suppression, especially since the protein hormone inhibits the release of GnRH. Interestingly, the BMs and MRMs had significantly higher plasma prolactin concentrations than other male dominance rankings. Furthermore, more than half of the BMs had plasma prolactin concentrations which are considered clinical hyperprolactinaemia (20 ng ml^{-1}) in male humans [78]. However, these results are preliminary since BMs and MRMs had the lowest sample sizes compared with other male dominance ranks. In all, the high plasma prolactin concentrations in the BMs and MRMs could aid in the expression of cooperative and social behaviours.

There are a few limitations of this study, which constrain how conclusive the findings are. First, aggressive behaviour was not evaluated owing to the potential of aggressive behaviour observations disrupting colony cohesion. However, this would be an important facet, which could further support the dominance hierarchies and explain some trends found in the results. The sample sizes are relatively small for all dominance-ranking classes, especially the middle-ranking males. The limited sample size in middle-ranking males was mainly owing to opportunistic faecal sampling during blood collection and behavioural observations. In the future, opportunistic sampling could incorporate longer-term sampling from the colony. However, the study highlights the usefulness of opportunistic faecal collection for non-invasive hormone monitoring in small mammals such as naked mole-rats.

5. Conclusion

Reproductive suppression in naked mole-rat colonies appears to be unequally distributed, with some members being more inhibited than others. The queens and high-ranking females exhibited comparatively high faecal glucocorticoid and androgen metabolite concentrations, possibly resulting from the queen trying to maintain her despotic society and the high-ranking female challenging the queen for mating acquisitions. Furthermore, there is possibly a mutually promoting effect between glucocorticoid and androgen concentrations, which may be a function of these agonistic interactions. As expected, queen naked mole-rats exhibited higher concentrations of progestagens compared with all other females in the colony. Interestingly, breeding males and middle-ranking males possess high levels of plasma prolactin that is potentially linked to the cooperative care of the young. Overall, the results of this study suggest that physiological reproductive suppression is potentially linked to dominance ranks.

Ethics

Experimental procedures were approved by the animal ethics committee of the University of 511 Pretoria (NAS199-2020) and DAFF section 20 approval (SDAH-Epi-20072707041).

Data accessibility

Data are available from [79].

Declaration of AI use

We have not used AI-assisted technologies in creating this article.

Authors' contributions

T.L.M.: conceptualization, formal analysis, investigation, methodology, validation, writing—original draft, writing—review and editing; A.G.: conceptualization, formal analysis, methodology, supervision, writing—review and editing; D.W.H.: conceptualization, methodology, supervision, writing—review and editing; N.H.: formal analysis, methodology, writing—review and editing; S.B.G.: formal analysis, methodology, writing—review and editing; N.C.B.: conceptualization, formal analysis, funding acquisition, methodology, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration

We declare we have no competing interests.

Funding

The project was funded by the SARChI Chair of Mammalian Behavioural Ecology and Physiology from the DST–NRF South Africa, the National Research Foundation (grant no. 64756), and the University of Pretoria to N.C.B.

Acknowledgements

We are grateful for funding from the SARChI Chair of Mammalian Behavioural Ecology and Physiology from the DST–NRF South Africa, the National Research Foundation (grant no. 64756), and the University of Pretoria to N.C.B. We would like to thank S. Brandt, K. Grenfell and K. du Plessis for the assistance with sample collection.

References

1. Tibbetts EA, Pardo-Sanchez J, Weise C. 2022 The establishment and maintenance of dominance hierarchies. *Phil. Trans. R. Soc. B.* 377, 20200450. (doi:10.1098/rstb.2020.0450)
2. Creel S, Creel NM. 2002 The African wild dog. In *The African wild dog: behavior, ecology, and conservation* (eds S Creel, NM Creel), pp. 201–222. Princeton, NJ: Princeton University Press. (doi: 10.1515/9780691207001)
3. Spong GF, Hodge SJ, Young AJ, Clutton-Brock TH. 2008 Factors affecting the reproductive success of dominant male meerkats. *Mol. Ecol.* 17, 2287–2299. (doi:10.1111/j.1365-294X.2008.03734.x)
4. Wong M, Balshine S. 2011 The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. *Biol. Rev.* 86, 511–530. (doi:10.1111/j.1469-185X.2010.00158.x)
5. Lukas D, Clutton-Brock T. 2012 Cooperative breeding and monogamy in mammalian societies. *Proc. R. Soc. B* 279, 2151–2156. (doi:10.1098/rspb.2011.2468)

6. Lukas D, Clutton-Brock T. 2017 Climate and the distribution of cooperative breeding in mammals. *R. Soc. Open Sci*. 4, 160897. (doi:10.1098/rsos.160897)
7. Bell MBV, Cant MA, Borgeaud C, Thavarajah N, Samson J, Clutton-Brock TH. 2014 Suppressing subordinate reproduction provides benefits to dominants in cooperative societies of meerkats. *Nat. Commun*. 5, 4499. (doi:10.1038/ncomms5499)
8. Beehner JC, Lu A. 2013 Reproductive suppression in female primates: a review. *Evol. Anthropol.* 22, 226–238. (doi:10.1002/evan.21369)
9. O'Riain MJ, Bennett NC, Brotherton PNM, McIlrath G, Clutton-Brock TH. 2000 Reproductive suppression and inbreeding avoidance in wild populations of co-operatively breeding meerkats (*Suricata suricatta*). *Behav. Ecol. Sociobiol*. 48, 471–477. (doi:10.1007/s002650000249)
10. Bennett NC, Jarvis JUM. 1988 The social structure and reproductive biology of colonies of the mole-rat, *Cryptomys damarensis* (Rodentia, Bathyergidae). *J. Mammal*. 69, 293–302. (doi:10.2307/1381379)
11. Bennett NC, Faulkes CG, Jarvis JUM. 1999 Socially-induced infertility incest avoidance and the monopoly of reproduction in cooperatively breeding African mole-rats. *Adv. Study Behav*. 28, 75–113.
12. Chase ID, Tovey C, Spangler-Martin D, Manfredonia M. 2002 Individual differences versus social dynamics in the formation of animal dominance hierarchies. *Proc. Natl Acad. Sci. USA* 99, 5744–5749. (doi:10.1073/pnas.082104199)
13. Correa LA, Zapata B, Samaniego H, Soto-Gamboa M. 2013 Social structure in a family group of Guanaco (Lama guanicoe, Ungulate): is female hierarchy based on 'prior attributes' or 'social dynamics'? *Behav. Processes* 98, 92–97. (doi:10.1016/j.beproc.2013.05.003)
14. Kriegsfeld LJ, Mei DF, Bentley GE, Ubuka T, Mason AO, Inoue K, Ukena K, Tsutsui K, Silver R. 2006 Identification and characterization of a gonadotropin-inhibitory system in the brains of mammals. *Proc. Natl Acad. Sci. USA* 103, 2410–2415. (doi:10.1073/pnas.0511003103)
15. Brüggemann M, Licht O, Fetter É, Teigeler M, Schäfers C, Eilebrecht E. 2018 Knotting nets: molecular junctions of interconnecting endocrine axes identified by application of the adverse outcome pathway concept. *Environ. Toxicol. Chem*. 37, 318–328. (doi:10.1002/etc.3995)
16. Wintermantel TM et al. 2006 Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility. *Neuron* 52, 271–280. (doi:10.1016/j.neuron.2006.07.023)
17. Jin JM, Yang WX. 2014 Molecular regulation of hypothalamus–pituitary–gonads axis in males. *Gene* 551, 15–25. (doi:10.1016/j.gene.2014.08.048)
18. Edson MA, Nagaraja AK, Matzuk MM. 2009 The mammalian ovary from genesis to revelation. *Endocr. Rev.* 30, 624–712. (doi:10.1210/er.2009-0012)
19. Gore AC. 2012 Neuroendocrine systems. In *Fundamental neuroscience* (eds L Squire, D Berg, FE Bloom, S du Lac, A Ghosh, NC Spitzer), pp. 799–817. 4th edn. Amsterdam, The Netherlands: Elsevier Inc.
20. Clarke FM, Faulkes CG. 1998 Hormonal and behavioural correlates of male dominance and reproductive status in captive colonies of the naked mole-rat, *Heterocephalus glaber. Proc. R. Soc. Lond. B* 265, 1391–1399. (doi:10.1098/rspb.1998.0447)
21. Gavrilovic L, Dronjak S. 2005 Activation of rat pituitary-adrenocortical and sympatho-adrenomedullary system in response to different stressors. *Neuro Endocrinol. Lett*. 26, 515–520.
22. Palme R. 2019 Non-invasive measurement of glucocorticoids: advances and problems. *Physiol. Behav*. 199, 229–243. (doi: 10.1016/j.physbeh.2018.11.021)
23. Son YL, Ubuka T, Tsutsui K. 2022 Regulation of stress response on the hypothalamic-pituitary-gonadal axis via gonadotropin-inhibitory hormone. *Front. Neuroendocrinol*. 64,100953. (doi: 10.1016/j.yfrne.2021.100953)

24. de la Peña E, Barja I, Carranza J. 2021 Social environment with high intrasexual competition enhances the positive relationship between faecal testosterone and cortisol metabolite levels in red deer. *Mamm. Biol*. 101, 207–215. (doi:10.1007/s42991-021-00100-x)
25. Grattan DR, Kokay IC. 2008 Prolactin: a pleiotropic neuroendocrine hormone. *J. Neuroendocrinol*. 20, 752–763. (doi:10.1111/j.1365-2826.2008.01736.x)
26. Carlson AA, Russell AF, Young AJ, Jordan NR, McNeilly AS, Parlow AF, Clutton-Brock T. 2006 Elevated prolactin levels immediately precede decisions to babysit by male meerkat helpers. *Horm. Behav*. 50, 94–100. (doi: 10.1016/j.yhbeh.2006.01.009)
27. Angelier F, Chastel O. 2009 Stress, prolactin and parental investment in birds: a review. *Gen. Comp. Endocrino*l. 163, 142–148. (doi: 10.1016/j.ygcen.2009.03.028)
28. Brown RSE, Herbison AE, Grattan DR. 2014 Prolactin regulation of kisspeptin neurones in the mouse brain and its role in the lactation-induced suppression of kisspeptin expression. *J. Neuroendocrinol*. 26, 898–908. (doi:10.1111/jne.12223)
29. Bennett NC, Faulkes CG. 2000 *African mole-rats: ecology and eusociality*. Cambridge, UK: Cambridge University Press.
30. Jarvis JU. 1981 Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. *Science* 212, 571–573. (doi:10.1126/science.7209555)
31. Jarvis JU, O'Riain MJ, Bennett NC, Sherman PW. 1994 Mammalian eusociality: a family affair. *Trends Ecol. Evol*. 9, 47–51. (doi:10.1016/0169-5347(94)90267-4)
32. Holmes MM, Goldman BD. 2021 Social behavior in naked mole-rats: individual differences in phenotype and proximate mechanisms of mammalian Eusociality. In *The extraordinary biology of the naked mole-rat* (eds R Buffenstein, TJ Park, MM Holmes), pp. 35–58. New York, NY: Springer.
33. Toor I, Maynard R, Peng X, Beery AK, Holmes MM. 2022 Naked mole-rat social phenotypes vary in investigative and aggressive behavior in a laboratory partner preference paradigm. *Front. Ecol. Evol.* 10, 379. (doi:10.3389/fevo.2022.860885)
34. Toor I, Edwards PD, Kaka N, Whitney R, Ziolkowski J, Monks DA, Holmes MM. 2020 Aggression and motivation to disperse in eusocial naked mole-rats, *Heterocephalus glaber. Anim. Behav.* 168, 45–58. (doi:10.1016/j.anbehav.2020.07.022)
35. O'Riain MJ, Jarvis JU, Faulkes CG. 1996 A dispersive morph in the naked mole-rat. *Nature* 380, 619–621. (doi:10.1038/380619a0)
36. Favati A, Leimar O, Løvlie H. 2014 Personality predicts social dominance in male domestic fowl. *PLoS One* 9, e103535. (doi:10.1371/journal.pone.0103535)
37. Dingemanse NJ, de Goede P. 2004 The relation between dominance and exploratory behavior is context-dependent in wild great tits*. Behav. Ecol*. 15, 1023–1030. (doi:10.1093/beheco/arh115)
38. Bennett NC, Ganswindt A, Ganswindt SB, Jarvis JUM, Zöttl M, Faulkes CG. 2018 Evidence for contrasting roles for prolactin in eusocial naked mole-rats, *Heterocephalus glaber* and Damaraland mole-rats, *Fukomys damarensis. Biol. Lett*. 14, 20180150. (doi:10.1098/rsbl.2018.0150)
39. Medger K, Bennett NC, Ganswindt SB, Ganswindt A, Hart DW. 2019 Changes in prolactin, cortisol and testosterone concentrations during queen succession in a colony of naked mole-rats (*Heterocephalus glaber*): a case study*. Sci. Nat*. 106, 5–6. (doi:10.1007/s00114-019-1621-1)
40. Blecher AS, Bennett NC, Medger K, Hagenah N, Ganswindt A, Oosthuizen MK. 2020 Effect of colony disruption and social isolation on naked mole-rat endocrine correlates*. Gen. Comp. Endocrinol*. 295, 113520. (doi:10.1016/j.ygcen.2020.113520)
41. Edwards PD, Mooney SJ, Bosson CO, Toor I, Palme R, Holmes MM, Boonstra R. 2020 The stress of being alone: removal from the colony, but not social subordination, increases fecal cortisol metabolite levels in eusocial naked mole-rats. *Horm. Behav*. 121, 104720. (doi:10.1016/j.yhbeh.2020.104720)

42. Wallace KME, Hart DW, Hagenah N, Ganswindt A, Bennett NC. 2023 A comprehensive profile of reproductive hormones in eusocial Damaraland mole-rats (*Fukomys damarensis*). *Gen. Comp. Endocrinol*. 333, 114194. (doi:10.1016/j.ygcen.2022.114194)
43. Reeve H, Sherman P. 1991 Intracolonial aggression and nepotism by the breeding female naked mole-rat. In *The biology of the naked mole-rat* (eds PW Sherman, JUM Jarvis, RD Alexander), pp. 337–357, Princeton, NJ: Princeton University Press. (doi:10.1515/9781400887132)
44. Clarke FM, Faulkes CG. 1997 Dominance and queen succession in captive colonies of the eusocial naked mole-rat*, Heterocephalus glaber. Proc. R. Soc. Lond. B* 264, 993–1000. (doi: 10.1098/rspb.1997.0137)
45. Cooney R, Bennett NC. 2000 Inbreeding avoidance and reproductive skew in a cooperative mammal. *Proc. R. Soc. Lond. B* 267, 801–806. (doi:10.1098/rspb.2000.1074)
46. Bennett NC, Jarvis JUM. 1995 Coefficients of digestibility and nutritional values of geophytes and tubers eaten by southern African mole-rats (Rodentia: Bathyergidae). *J. Zool*. 236, 189–198. (doi:10.1111/j.1469-7998.1995.tb04487.x)
47. Majelantle TL, Bennett NC, Ganswindt SB, Hart DW, Ganswindt A. 2023 Validation of enzyme immunoassays via an adrenocorticotrophic stimulation test for the non-invasive quantification of stress-related hormone metabolites in naked mole-rats. *Animals (Basel)* 13, 1424. (doi:10.3390/ani13081424)
48. Lacey E, Alexandra RD, Braude SH, Sherman PW, Jarvis JUM. 1991 An ethogram for the naked mole-rat: nonvocal behaviors. In *The biology of the naked mole-rat* (eds P Sherman, J Jarvis, R Alexander), pp. 209–242, Princeton, NJ: Princeton University Press. (doi: 10.1515/9781400887132)
49. Majelantle TL, Ganswindt A, Pirk CWW, Bennett NC, Hart DW. 2022 Aggression, boldness, and exploration personality traits in the subterranean naked mole-rat (*Heterocephalus glaber*) disperser morphs. *Animals (Basel)* 12, 22. (doi:10.3390/ani12223083)
50. Toor I, Faykoo-Martinez M, Edwards PD, Boonstra R, Holmes MM. 2022 Hormones do not maketh the mole-rat: no steroid hormone signatures of subordinate behavioral phenotypes. *Horm. Behav*. 145, 105236. (doi: 10.1016/j.yhbeh.2022.105236)
51. Fraňková M, Palme R, Frynta D. 2012 Family affairs and experimental male replacement affect fecal glucocorticoid metabolites levels in the Egyptian spiny mouse *Acomys cahirinus. Zool. Stud*. 51, 277–287.
52. Palme R, Touma C, Arias N, Dominchin MF, Lepschy M. 2013 Steroid extraction: get the best out of faecal samples. *Wiener Tierärztliche Monatsschrift* 100, 238–246.
53. Ganswindt A, Münscher S, Henley M, Palme R, Thompson P, Bertschinger H. 2010 Concentrations of faecal glucocorticoid metabolites in physically injured free-ranging African elephants *Loxodonta africana. Wildlife Biol*. 16, 323–332. (doi:10.2981/09-081)
54. Palme R, Mostl E. 1997 Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Mammal. Biol.* II, 192–197.
55. Palme R, Mostl E. 1993 Biotin–Streptavidin enzyme immunoassay for the determination of oestrogen and androgens in boar Faeces. In *Advances of steroid analysis* (ed. ES Görög), pp. 111–117, Budapest, Hungary: Akadémiai Kiadó.
56. Dehnhard M, Fanson K, Frank A, Naidenko SV, Vargas A, Jewgenow K. 2010 Comparative metabolism of gestagens and estrogens in the four lynx species, the Eurasian (*Lynx lynx*), the Iberian (*L. pardinus*), the Canada lynx (*L. canadensis*) and the bobcat (*L. rufus*). *Gen. Comp. Endocrinol.* 167, 287–296. (doi: 10.1016/j.ygcen.2010.03.023)
57. Majelantle TL, Ganswindt A, Ganswindt SB, Hagenah N, Hart DW, Bennett NC. 2023 Non-invasive quantification of faecal and urine reproductive hormone metabolites in the naked mole-rat (*Heterocephalus glaber*). *Animals (Basel)* 13, 19. (doi:10.3390/ani13193039)

58. Ganswindt A, Heistermann M, Borragan S, Hodges JK. 2002 Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. *Zoo Biol.* 21, 27–36. (doi:10.1002/zoo.10034)
59. R Core Team. 2022 R: *A language and environment for statistical computing* Vienna, Austria: R Foundation for Statistical Computing. See http://www. R-project. org/
60. Bates DM, Maechler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw*. 67, 1–48. (doi:10.18637/jss.v067.i01)
61. Clutton-Brock TH, Albon SD, Gibson RM, Guinness FE. 1979 The logical stag: adaptive aspects of fighting in red deer (*Cervus elaphus L*.). *Anim. Behav*. 27, 211–225. (doi:10.1016/0003-3472(79)90141-6)
62. Schmid VS, de Vries H. 2013 Finding a dominance order most consistent with a linear hierarchy: an improved algorithm for the I&SI method. *Anim. Behav.* 86, 1097–1105. (doi:10.1016/j.anbehav.2013.08.019)
63. Fox J, Weisberg S. 2011 *An R companion to applied regression*. New York, NY: Sage Publications.
64. Jacobs DS, Bennett NC, Jarvis JUM, Crowe TM. 1991 The colony structure and dominance hierarchy of the Damaraland mole-rat, *Cryptomys damarensis* (Rodentia: Bathyergidae), from Namibia. *J. Zoo.* 224, 553–576. (doi:10.1111/j.1469-7998.1991.tb03785.x)
65. Medger K. 2022 Stress in an underground empire. *Biol. Lett*. 18, 20220012. (doi:10.1098/rsbl.2022.0012)
66. Edwards PD. 2022 For the physiology of cooperative breeding, it's time to move beyond stress: a comment on: 'stress in an underground empire' (2022) by Medger. *Biol. Lett.* 18, 20220375. (doi:10.1098/rsbl.2022.0375)
67. Hart DW, Bennett NC, Voigt C. 2022 Social stress is unlikely to play a major role in reproductive suppression of female subordinate naked mole-rats and Damaraland mole-rats. *Biol. Lett.* 18, 20220292. (doi:10.1098/rsbl.2022.0292)
68. Medger K, Bennett NC, Lutermann H, Ganswindt A. 2018 Non-invasive assessment of glucocorticoid and androgen metabolite levels in cooperatively breeding Damaraland mole-rats (*Fukomys damarensis*). *Gen. Comp. Endocrinol*. 266, 202–210. (doi:10.1016/j.ygcen.2018.05.018)
69. Blanca J, Simon V. 2023 Do glucocorticoids indicate stress? Meta-analysis reveals a tight association between glucocorticoids and metabolic rate. *Elife* 12, RP88205. (doi:10.7554/eLife.88205.2)
70. Faulkes CG, Abbott DH, Jarvis JUM. 1991 Social suppression of reproduction in male naked mole-rats, *Heterocephalus glaber. J. Reprod. Fertil*. 91, 593–604. (doi:10.1530/jrf.0.0910593)
71. Bennett NC. 1994 Reproductive suppression in social *Cryptomys damarensis* colonies—a lifetime of socially-induced sterility in males and females (Rodentia: Bathyergidae). *J. Zool*. 234, 25–39. (doi:10.1111/j.1469-7998.1994.tb06054.x)
72. Sandell MI. 2007 Exogenous testosterone increases female aggression in the European starling (*Sturnus vulgaris*). *Behav. Ecol. Sociobiol*. 62, 255–262. (doi:10.1007/s00265-007-0460-9)
73. Ganswindt A, Palme R, Heistermann M, Borragan S, Hodges JK. 2003 Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to musth. *Gen. Comp. Endocrinol*. 134, 156–166. (doi:10.1016/s0016-6480(03)00251-x)
74. Pribbenow S, Wachter B, Ludwig C, Weigold A, Dehnhard M. 2016 Validation of an enzyme-immunoassay for the non-invasive monitoring of faecal testosterone metabolites in male cheetahs (*Acinonyx jubatus*). *Gen. Comp. Endocrinol*. 228, 40–47. (doi:10.1016/j.ygcen.2016.01.015)

75. Faulkes CG, Abbott DH, Jarvis JUM. 1990 Social suppression of ovarian cyclicity in captive and wild colonies of naked mole-rats, *Heterocephalus glaber. J. Reprod. Fertil*. 88, 559–568. (doi:10.1530/jrf.0.0880559)
76. van der Westhuizen LA, Bennett NC, Jarvis JUM. 2002 Behavioural interactions, basal plasma luteinizing hormone concentrations and the differential pituitary responsiveness to exogenous gonadotrophin-releasing hormone in entire colonies of the naked mole-rat (*Heterocephalus glaber*). *J. Zool.* 256, 25–33. (doi:10.1017/S0952836902000043)
77. Mulugeta E, Marion-Poll L, Gentien D, Ganswindt SB, Ganswindt A, Bennett NC, Blackburn EH, Faulkes CG, Heard E. 2017 Molecular insights into the pathways underlying naked mole-rat Eusociality. BioRxiv. (doi:10.1101/209932)
78. Majumdar A, Mangal NS. 2013 Hyperprolactinemia. *J. Hum. Reprod. Sci.* 6, 168–175. (doi:10.4103/0974-1208.121400)
79. Majelantle TL, et al. 2024 The dissection of a despotic society: exploration, dominance and hormonal traits [Dataset]. Dryad. (doi:10.5061/dryad.18931zd37)
80. Majelantle TL, Ganswindt A, Hagenah N, Hart DW, Ganswindt S, Bennett N. 2024 Supplementary Material from: The dissection of a despotic society: exploration, dominance and hormonal traits. Figshare (doi:10.6084/m9.figshare.c.7162535)