The best of both: No apparent trade-off between immunity and reproduction in two group-living African mole-rat species

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Abstract

Co-operatively breeding mammals often exhibit a female reproductive skew and suppression of the subordinate non-breeding group members. According to evolutionary theory and the immunity-fertility axis, an inverse relationship between reproductive investment and survival (through immunocompetence) is expected. As such, this study investigates if a trade-off between immunocompetence and reproduction arises in two co-operatively breeding African mole-rat species, namely the Damaraland mole-rat (*Fukomys damarensis*) and common mole-rat (*Cryptomys hottentotus hottentotus*), who possess female reproductive division of labour. This study also attempted to investigate the relationship between the immune and endocrine systems in Damaraland mole-rats. There was no trade-off between reproduction and immunocompetence in co-operatively breeding African mole-rat species, and in the case of the Damaraland mole-rats, breeding females (BFs) possessed increased immunocompetence compared to non-breeding females (NBFs). Furthermore, the increased levels of progesterone possessed by Damaraland mole-rat BFs compared to NBFs appears to be correlated to increased immunocompetence. In comparison, BF and NBF common mole-rats possess similar immunocompetence. The species-specific differences in the immunity-fertility axis may be due to variations in the strengths of reproductive suppression in each species.

1. Introduction

Mammals have evolved both behavioural and physiological adaptations to increase their individual fitness (how well a mammal can survive and reproduce in its environment) in their habitat despite biotic and abiotic constraints [1]. One adaptation that has garnered significant interest is group living and its ultimate causes and consequences. Mammals may form groups ranging from small pair-bonded units to large organised groups with highly complex social interactions that have been compared to eusocial insects, such as bees, ants and termites [2–4]. Compared to so-called solitary mammalian species, that only come together to breed or when females have young, group living or social mammalian species have a number of benefits that increase the individual fitness of group members, including, but not limited to, enhanced protection against predators, increased energy and water savings (through increased foraging efficiency and resource accessibility), access to potential mates, the access to social information and possibly the benefit of alloparental care (offspring receive care not only from their parents but also from other group members) [2,5,6]. However, individuals of a species that partake in a group living strategy also face several consequences that are likely not experienced by their solitary living counterparts; these include increased risk of exposure to diseases and parasites through intra-group transmission and intraspecific competition for both resources and the opportunity to reproduce [7–9]. As a consequence of balancing the fitness benefits and costs of group living, a spectrum of sociality has evolved (see Clutton-Brock et al. [3] for review).

On the more extreme end of the spectrum of sociality is cooperative breeding, a social system whereby alloparental care is conducted; these groups of mammals often comprise of a monogamous breeding pair and their offspring, with a fluctuating number of immigrants and are often characterised by a reproductive skew (partitioning of reproduction among the same-sex individuals within social groups) [10]. The reproductive skew varies between species and can be associated with group size, with species forming larger groups often showing increased reproductive skew

[11,12]. Interestingly, a high reproductive skew within a group is often accompanied by reproductive suppression [defined as when group members beyond the age of sexual maturity fail to raise their own offspring successfully [10,13,14]]. Group members that are reproductively suppressed are often referred to as non-breeders or subordinates [10], and reproductive suppression is often more common in females in co-operatively breeding species. Reproductive suppression in females can range from infanticide of the subordinate's offspring, anovulation of female non-breeding subordinates and, in some extreme cases, a lack of follicular maturation in the ovaries of non-breeding female (NBF) subordinates, with NBF anovulation and lack of follicular maturation being more common in co-operatively breeding mammal species that show a high reproductive skew and suppression [15–18].

Reproduction in female mammals, including follicular development, ovulation, pregnancy and lactation, requires substantial energy and resource investment. Consequently, there is a disproportional investment into reproduction between breeding females (BFs) and NBFs in co-operatively breeding mammal species that show a high reproductive skew and suppression, with BFs investing more energy and resources into reproduction than NBFs. Investment into reproduction may divert energy and resources away from processes, pathways and systems that increase the health- and life-span of an individual, ultimately affecting an individual's fitness [19]; this includes the immunocompetence [an individual's ability to mount an immune response to pathogenic invasion to provide strong resistance to disease and infection [20,21]] of an individual [22].

Widespread evidence has indicated reduced fertility in individuals under pathogenic challenges, while an increase in reproduction induces a drop in immune strength [23]. Accordingly, the immunity-fertility axis infers an inverse relationship between reproductive investment and immunocompetence [22]. Therefore, higher fertility and reproductive investment and output come with the costs of lower immunocompetence. Immunocompetence relies on both innate and adaptive immunity (see Box 1). The innate, or non-specific, immune response [comprised of

neutrophils, monocytes, eosinophils and basophils (Table 1)] is the first line of defense against non-self pathogens. The primary purpose of the innate immune response is to immediately prevent the spread and movement of foreign pathogens throughout the body [24]. An increased prevalence of innate immune system components would indicate increased ability to protect the body against foreign pathogens. The defense against non-self pathogens is called the adaptive immune response. [24]. The adaptive immune system comprises of namely T and B lymphocytes (Table 1), to name a few [25]. The B lymphocytes produce antibodies to attack invading bacteria, viruses, and toxins [25]. The T lymphocytes destroy the body's cells that viruses have taken over or become cancerous [25]. Thus, humoral immunity depends on the B lymphocytes, while cell immunity depends on the T lymphocytes [25]. Lymphocytes can be either memory and effector T or B lymphocytes, with the effector cells being short-lived and produced in high numbers in response to exposure to a specific pathogen, while the memory cells persist in lower number in the body for many years, providing lifelong protection against reinfection by the same pathogen [25]. An increased prevalence of adaptive immune system components, particularly memory cells, would indicate an increased ability to protect the body against specific foreign pathogens. Furthermore, memory T and B lymphocytes are not only essential to immunity against microbial pathogens, but are also involved in autoimmunity and maternal-fetal tolerance [26]. Ratios of these leukocytic components have proven invaluable as prognostic tools for diagnosing the degree of inflammation, disease severity, mortality and tumorigenesis [27–32]. Lower neutrophil-to-lymphocyte ratio (NLR) is usually associated with favourable prognostic factors in every field of application, mirroring a preserved immune balance [33–38]. While, a higher monocycte-to-lymphocyte ratio (MLR) is favourably associated with disease-free survival after infection or cancer [33–38].

Box 1: Functions of the various innate immune strength proxies used in this study with patterns indicating a strong innate immune systems

Immune strength proxies (Immune system)	Function
Neutrophils (Innate)	Neutrophils are the first cells of the immune system to respond to invaders such as bacteria or viruses. Neutrophils help prevent infections by blocking, disabling, digesting, or warding off invading particles and microorganisms. Neutrophils constantly search for signs of infection and quickly respond to trap and kill pathogens.
Lymphocyte (Adaptive)	Lymphocytes are responsible for antibody production (consequently destroying the pathogens), the direct cell-mediated killing of virus-infected and tumour cells, and regulation of the immune response.
Monocytes (Innate)	Monocytes surround and kill microorganisms (phagocytosis), ingest foreign material, remove dead cells, and boost immune response and the efficiency of other immune cell types.
Eosinophils (Innate)	Eosinophils move to inflamed areas, trap foreign substances, cause cell-mediated killing of virus-infected and tumour cells, anti-parasitic and bactericidal activity, participate in immediate allergic reactions, and modulate inflammatory responses.
Basophils (Innate)	Basophils release histamine to mount a non-specific immune response.
Neutrophil-to-lymphocyte ratio (NLR)	Informative in identifying immunocompromised patients due to infection and risk stratification uses.
Monocycte-to-lymphocyte ratio (MLR)	Represents a pro-inflammatory immune microenvironment.

Source material: [33–38].

Accordingly, it would be expected that a trade-off between reproductive investment and immunocompetence in co-operatively breeding mammal species would be observed, with the BF of a group expected to exhibit lower immunocompetence, which would be measured through the decreased bactericidal capacity of their blood and decreased proportions of the various components of the innate and adaptive immune system, compared to the NBFs that do not ovulate, fall pregnant or nurse young. To test this prediction, this study investigated the immunocompetence of BFs and NBFs of two co-operatively breeding African mole-rat species, namely the Damaraland mole-rat (*Fukomys damarensis*) and common mole-rat (*Cryptomys hottentotus hottentotus*). The Damaraland and common mole-rat, show an extreme reproductive skew and reproductive suppression that usually results in a single BF and breeding male in each group responsible for procreation, with the remaining colony members (often the offspring of the breeding pair) never achieving reproductive activation whilst in the confines of their natal colony [11]. Damaraland and common mole-rat NBFs show follicular growth, but are anovulatory in the presence of the breeding pair [11]. Furthermore, this study also attempted to investigate the relationship between the immune and endocrine systems using the endocrine profiles of BF and NBF Damaraland mole-rats. A comprehensive bidirectional relationship between the immune and endocrine systems has been well documented, with sex hormones, such as progesterone and testosterone (which have been recorded to be significantly higher in BFs compared to NBFs [39,40]), and hormones related to group maintenance, such as cortisol (which have been recorded to be higher in NBFs compared to BFs, but not significantly so [18,39,41]), being observed to possess both an immuno-enhancing and immuno-suppressive role [42–46].

2. Methods

2.1. Study species

Twenty-five adult female Damaraland mole-rat, comprising nine BFs (144±7.28g) and 16 NBFs (130±6.19g) (Damaraland mole-rat body mass BF vs NBF: $t = -1.34$, $p = 0.19$), from ten captive-bred colonies from the mole-rat laboratory at the University of Pretoria (25.7545° S, 28.2314° E), South Africa, were used in this study. In addition, a further 12, six BFs (87.7±4.10g) and six NBFs (66.3±6.65g) (common mole-rat body mass BF vs NBF: $t = 2.78$, $p = 0.02$), adult female common mole-rats, were captured using Hickman live traps, baited with a small piece of sweet potato [47] from Steinkopf (29.2602° S, 17.7340° E) and Kamieskroon (30.2195° S, 17.9236° E), in the Northern Cape, South Africa. The traps were positioned at the entrance of excavated burrows where tunnels were open. Traps were monitored for captures or blocking every 2-3 hours for the day and left overnight, being checked first thing in the morning. All animals were grouped and housed in large polyurethane crates (1m x 0.5m x 0.5m) with wood shavings and paper towelling for nesting material. Animals were maintained at ~25°C and 50% humidity with a 12 L:12D light cycle. Animals were fed fresh tubers and vegetables *ad libitum*.

All Damaraland mole-rat BFs had produced at least five litters prior to the onset of sampling. Furthermore, a NBF had not bred and was still housed within their natal colony prior to the start of the experiment. All BFs showed no visible signs of pregnancy during sampling. All common mole-rats were in captivity for approximately one month before the start of this study. For more details, please see the supplementary material.

Experimental procedures were approved by the animal ethics committee of the University of Pretoria (NAS016/2021, NAS017-2021 and NAS022/2021).

2.2. Urine collection and hormonal analysis

Urine samples were collected from all 25 female Damaraland mole-rats and urine samples were analysed for testosterone, progesterone and cortisol using a coat-a-

count kit (Diagnostic Products Corporation, Los Angeles, California, USA). See supplementary material for more details.

2.3. Blood collection

Whole blood was collected from all 25 female Damaraland mole-rats, three days after urine collection, and all 12 common mole-rats. Before blood collection, a sterile bleed site was ensured by thoroughly swabbing the female hindfoot with Biotaine (0.5% Chlorhexidine gluconate, 70% ethanol; Braun Medical PTY Ltd, Reg. no. 33/13.1/0526) and allowing it to dry. Next, the dorsal tarsal vein was pierced using a sterile 23G needle. Finally, an EDTA-coated microhematocrit capillary tube was used to collect the whole blood into a sterile EDTA tube.

2.3.1. Complete blood count

Complete blood counts were performed on all 25 Damaraland mole-rats and 12 common mole-rats whole blood samples in an automated haematological analyser (ADVIA 21209(i) Siemens), designed for *in vitro* use at the Clinical Pathology unit at the Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria. Quantification of white cell count ($x10^9$/L) and leukocyte cell type count [segmented neutrophil ($x10^9$/L), lymphocyte ($x10^9$/L), eosinophil ($x10^9$/L), basophil ($x10^9$/L) and monocyte ($x10^9$/L)] was completed. Breeding females and NBFs of both species possessed similar white blood cell counts ($t < 0.11$, $p > 0.91$, for both species). As such, the quantification of the leukocyte percentage [(leukocyte cell type count ($x10^9$/L)/ white cell count ($x10^9$/L))*100] was calculated [segmented neutrophil (%), lymphocyte (%), eosinophil (%), basophil (%) and monocyte (%)]and used for further analysis. From the leukocyte percentage, the ratios of neutrophil-to-lymphocyte (NLR) and monocyte-to-lymphocyte (MLR) were determined.

2.3.2. Bacterial killing assay (BKA)

The bacterial killing assay (BKA) was used to directly measure the ability of blood from a subset of 16 female Damaraland mole-rats, eight BFs and eight NBFs, and all 12 female common mole-rats to kill or prevent the growth of a laboratory strain of bacteria, in this case, *Escherichia coli.* The procedure used in this study followed the protocol demonstrated by Millet et al. [48] and DeRogatis et al. [49], with modifications (See supplementary material for more details).

Data is presented as percentage (%) *E. coli* killed or prevented from growing.

2.4. Data analysis

Statistical analyses were performed with R 2022.02.0 and Graphpad Prism 8.4.3. Statistical significance was denoted by $p \leq 0.05$, and data are presented as mean ± standard error (SE).

Body mass was observed to not affect any measured biological parameters of Damaraland or common mole-rats (Supplementary Table 1). Likewise, age did not affect any biological parameter of non-breeding female Damaraland mole-rats (Supplementary Table 2). Therefore, age and body mass were not included in any subsequent statistical analysis. The distribution of each biological parameter was assessed, and the required model distribution was utilised (Supplementary Table 3). Normality of each biological parameter was tested visually using QQ plots, Shapiro-Wilk tests, and Levene's tests on model residuals. Data that were not normally distributed were log transformed and tested for normality. General linear models were used to analyse normally distributed variables, while generalised linear models were used to analyse non-normal variables. The authors want to highlight the complication of a two-species comparison, such as speciation between species, no genetic exchange and different environmental conditions (captive vs wild) [50].

These factors can all increase the likelihood of a type 1 error [50]. In light of this problem the response of each species is discussed and not directly compared. However, a multiple species (6 or more) comparison would circumvent this problem and should be attempted in the future [50].

Differences between innate immune strength proxies (BKA, prevalence of neutrophils, lymphocytes, eosinophils, basophils and monocytes and NLR and MLR) of BFs and NBFs of Damaraland mole-rat and common mole-rat, respectively, were investigated using either linear models or Generalised linear models (Supplementary Table 3). Furthermore, linear models were used to investigate differences between steroid hormones (testosterone, cortisol and progesterone) of BFs and NBFs of Damaraland mole-rats (Supplementary Table 3). Generalised linear models, with beta logit-link (BKA and neutrophil and lymphocyte prevalence and NLR and MLR) or negative binominal (monocyte, eosinophil and basophil prevalence) distributions (Supplementary Table 3) were used to investigate if the steroid hormones (testosterone, cortisol and progesterone) significantly affected any innate immune strength proxy (BKA, prevalence of neutrophils, lymphocytes, eosinophils, basophils and monocytes and NLR and MLR) in female Damaraland mole-rats (BFs and NBFs combined).

3. Results

3.1. Reproductive status effects on immunocompetence

Breeding female and NBF common mole-rats showed a similar bactericidal capacity of their blood ($z = -1.07$, $p = 0.28$, Fig. 1), suggesting BFs and NBFs have similar innate immune strengths. Furthermore, the similar NLR ($z = -0.93$, $p = 0.40$) and MLR ($t = -0.13$, $p = 0.90$) values, as well as the similar prevalence of neutrophils ($z = -125$, $p = 0.21$), lymphocytes ($z = 0.90$, $p = 0.37$), monocytes ($z = 0.05$, $p = 0.96$), eosinophils ($z = 0.29$, $p = 0.77$) and basophils ($z = 0.32$, $p = 0.75$) between common mole-rat BFs and NBFs (Fig. 2) further support this.

In contrast, a clear effect of reproductive status on immunocompetence was observable in Damaraland mole-rats, with BFs possessing a higher blood bactericidal capacity ($z = -3.31$, $p = 0.001$), increased monocyte ($z = -2.86$, $p = 0.004$), lymphocyte (not significant: $z = -1.86$, $p = 0.06$) and eosinophil (not significant: $z = -0.90$, $p = 0.37$) prevalence and MLR value ($t = -2.25$, $p = 0.03$), and decreased neutrophil ($z = 2.10$, $p = 0.04$) and basophil (not significant: $z = 1.87$, $p = 0.06$) prevalence and NLR value ($z = 2.63$, $p = 0.02$) (Fig. 1 & 2).

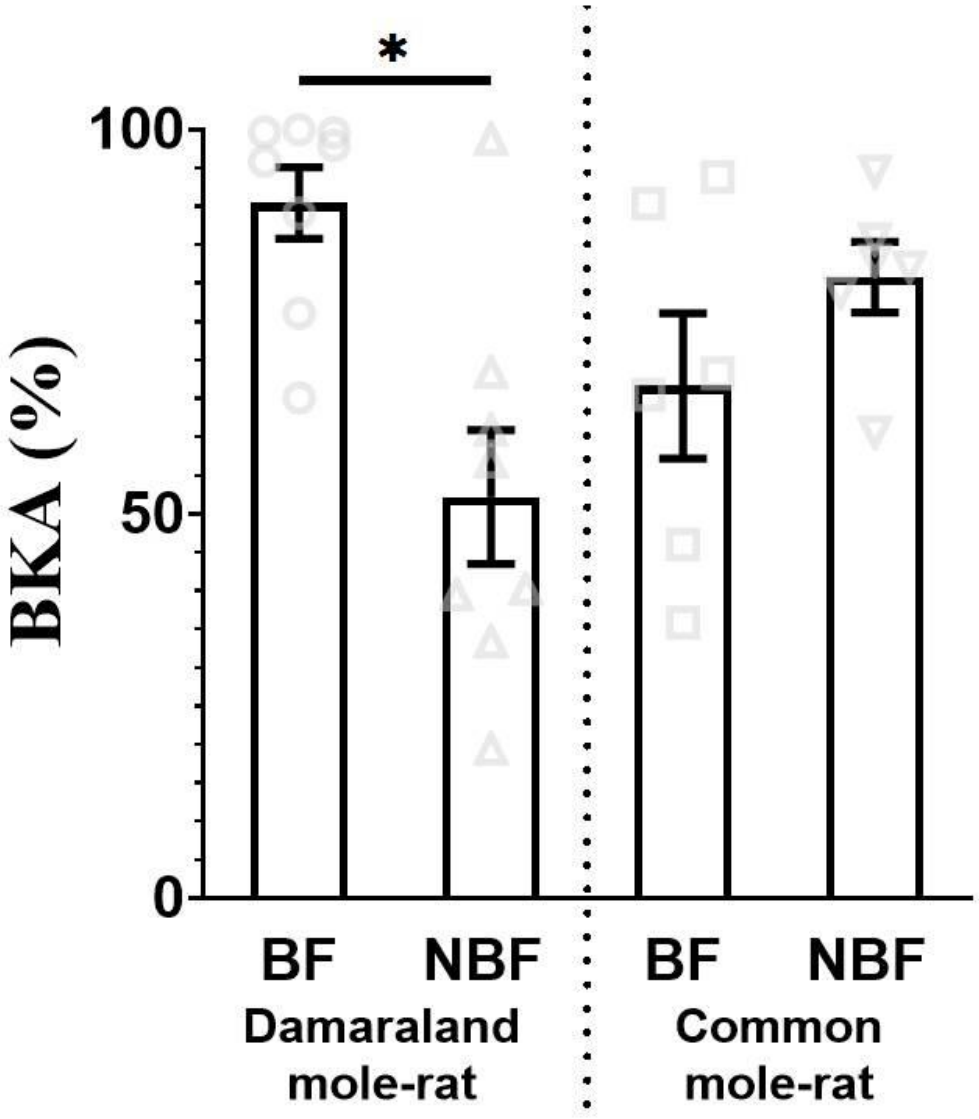
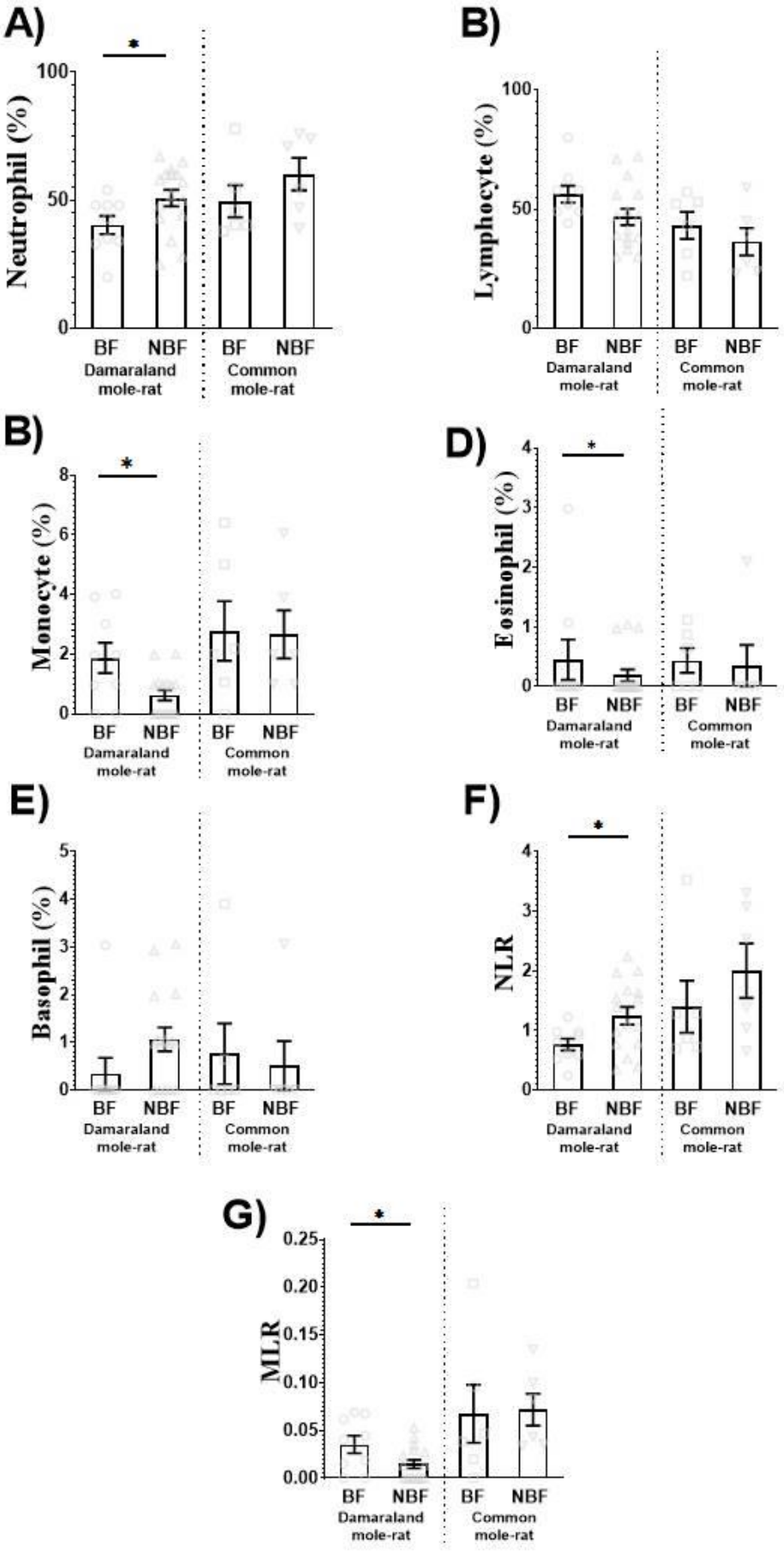


Fig. 1: The variation of the blood bactericidal capacity [bacterial killing assay (BKA) score (% of *E. coli* killed or prevented from growing)] of Damaraland

mole rat (*Fukomys damarensis*) and common mole-rat (*Cryptomys hottentotus hottentotus*) breeding females ('BFs' denoted by open circles (○) in Damaraland mole-rats and open square (□) in common mole-rats) and non-breeding female ('NBFs' denoted by open triangle (Δ) in Damaraland mole-rats and open upside-down triangle (▽) in common mole-rats)). Statistical significance was denoted by astriks (*) $p \leq 0.05$, and data are presented as mean ± standard error (SE).


A)
Neutrophil (%)
100
50
0
*
BF
NBF
BF
NBF
Damaraland mole-rat
Common mole-rat
B)
Lymphocyte (%)
100
50
0
BF
NBF
BF
NBF
Damaraland mole-rat
Common mole-rat
B)
Monocyte (%)
8
6
4
2
0
*
BF
NBF
BF
NBF
Damaraland mole-rat
Common mole-rat
D)
Eosinophil (%)
4
3
2
1
0
*
BF
NBF
BF
NBF
Damaraland mole-rat
Common mole-rat
E)
Basophil (%)
5
4
3
2
1
0
BF
NBF
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NBF
Damaraland mole-rat
Common mole-rat
F)
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4
3
2
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*
BF
NBF
BF
NBF
Damaraland mole-rat
Common mole-rat
G)
MLR
0.25
0.20
0.15
0.10
0.05
0.00
*
BF
NBF
BF
NBF
Damaraland mole-rat
Common mole-rat

Fig. 2: The variation of the prevalence of leukocytes [A) neutrophils (%), B)lymphocytes (%), C) monocytes (%), D) eosinophils (%), E) basophils (%) and F) neutrophil:lymphocyte ratio (NLR) and G) monocycte:lymphocyte ratio (MLR)] of Damaraland mole rat (*Fukomys damarensis*) and common mole-rat (*Cryptomys hottentotus hottentotus*) breeding females ('BFs' denoted by open circles (○) in Damaraland mole-rats and open square (□) in common mole-rats) and non-breeding female ('NBFs' denoted by open triangle (Δ) in Damaraland mole-rats and open upside-down triangle (▽) in common mole-rats). Statistical significance was denoted by astriks (*) $p \leq 0.05$, and data are presented as mean ± standard error (SE).

3.2. Hormonal parameters

3.2.1. *Reproductive phenotype in hormonal parameters*

Damaraland mole-rat BFs possessed significantly higher levels of progesterone ($t = -5.54$, $p = 0.00001$) than their NBF counterparts (Fig. 3). In contrast, there were no differences in testosterone ($t = -1.97$, $p = 0.06$) or cortisol ($t = 0.44$, $p = 0.67$) concentrations between Damaraland mole-rats BFs and female NBFs.

3.2.2. Effect on innate immune strength proxies

All three steroid hormones (testosterone, progesterone and cortisol) were observed not to affect the prevalence of neutrophils, lymphocytes and basophils and the BKA and NLR (Supplementary table 4) in female Damaraland mole-rats. Similarly, the testosterone and cortisol profiles of female Damaraland mole-rats did not affect the prevalence of monocytes and eosinophils and NLR, but in contrast, progesterone profiles did in fact, affect the prevalence of monocytes and eosinophils and MLR (Supplementary table 4). In female Damaraland mole-rats with high progesterone

levels, the prevalence of monocytes and MLR was high, while the eosinophils prevalence was low (Fig. 3).

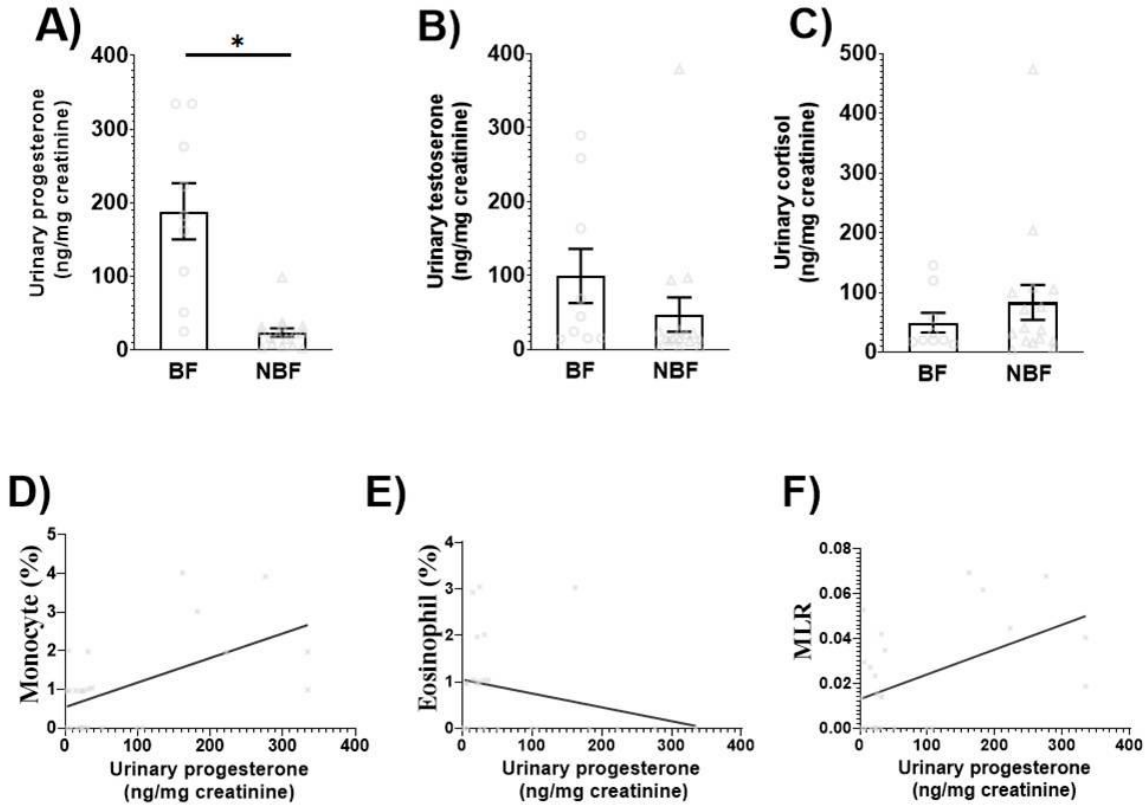

Fig. 3: The variation of the hormonal steroid profiles [a) urninary testosterone (ng/mg creatinine), urinary corisol (μg/mg creatinine) and urinary progesterone (ng/mg creatinine)] of Damaraland mole rat (*Fukomys damarensis*) a breeding females ('BFs' denoted by open circles (○)) and non-breeding female ('NBFs' denoted by open triangle (Δ)). The significant relationship between urinary progesterone (ng/mg creatinine) and the prevalence of c) monocytes (%), d) eosinophils (%) and e) monocycte:lymphocyte ratio (MLR) in female Damaraland mole-rats (BFs and NBFs combined) are also presented. Statistical significance was denoted by astriks (*) $p \leq 0.05$, and data are presented as mean ± standard error (SE).

4. Discussion

This study aimed to answer two fundamental, but crucial questions: 1) Is there a trade-off between reproduction and immunocompetence in co-operatively breeding African mole-rat species, and 2) what is the relationship between immunocompetence and the endocrine systems in a co-operatively breeding African mole-rat species? This study successfully answered these questions; firstly, there was no trade-off between reproduction and immunocompetence in co-operatively breeding African mole-rat species and in the case of the Damaraland mole-rats BFs actually possessed increased immunocompetence. Furthermore, the increased levels of progesterone possessed by Damaraland mole-rat BFs compared to NBFs appear to be correlated to increased immunocompetence, namely the prevalence of lymphocytes and monocytes and the MLR value.

Damaraland mole-rat BFs showed an increase in the bactericidal capacity of their blood (BKA scores) compared to NBFs indicating BFs possess an increased ability to fight a bacterial infection and/or NBFs possess a reduced ability to fight a bacterial infection. Damaraland mole-rat NBFs showed below average (2-8%), even compared to other African mole-rat species [53], prevalence of monocytes which could be a factor in the lowered bactericidal properties of their blood despite showing higher levels of neutrophils. Monocytes destroy foreign substances by phagocytosis and enhance the efficiency of the other immune cells (Box 1). Furthermore, there is evidence that BFs possess a more robust adaptive immunity through higher lymphocyte (likely in the form of memory cells) prevalence, similar to other African mole-rat species [53]. An increased prevalence of adaptive immune system components, particularly memory cells, would indicate an increased ability to protect the body against specific foreign pathogens, such as *E. coli* a common pathogen found in soils and plant roots (mole-rats main food source). Interestingly, the Damaraland mole-rat, particularly BFs, immune system displays similarities to the naked mole-rat (*Heterocephalus glaber*) and humans (*Homo sapiens*) than to that of other rodents [53,54], including increased prevalence of lymphocytes and monocytes

in the peripheral blood. Reduced NLR and raised MLR (within the range of healthy [55]) of BFs compared to NBFs indicate that BFs possess a more balanced immune system compared to NBFs (Box 1).

Increased levels of progesterone in BF Damaraland mole-rats compared to those of NBFs are correlated to increased immunocompetence. While, testosterone and cortisol did not affect the immunocompetence of female Damaraland mole-rats. As BFs ovulate and fall pregnant, progesterone levels are higher in BFs than NBFs [18,39]. Human and animal studies' data demonstrate that progesterone influences most components of innate and adaptive immunity [56]. Evidence of these effects is found in the differences in immune responses between females and males, with females mounting a more vigorous B and T lymphocyte response than males [56]. Further, in many females, the hormone changes associated with pregnancy, including increases in progesterone, lessen the severity of disease [56]. In addition, progesterone positively affects human naïve (or immature) T lymphocytes differentiation into memory and effector T lymphocytes [57], which ultimately increases the efficiency of the adaptive immune system. Increased progesterone has also been observed to increase the prevalence and function of monocytes [58,59]. While some studies have indicated progesterone's immunosuppressive role, particularly on macrophage-, antibody- and NK cell activity, our studies suggest an immuno-enhancing role of progesterone [43,60–64].

The common mole-rat's immune system displays similarities to the Damaraland mole-rat, particularly NBFs, than to that of other rodents [53]. But, unlike the Damaraland mole-rat, common mole-rat BFs and NBFs show similar immunocompetence properties, including the bactericidal capacity of their blood and similar prevalence of neutrophils, lymphocyte and monocyte and similar MLR and NLR. As with the Damaraland mole-rat NBFs, common mole-rat NBFs are anovulatory in the presence of the breeding pair, and as such, it would be expected that sex hormones, such as progesterone, would be lower in NBFs compared to those of the BFs. Unfortunately, no study to date has compared progesterone

concentrations of BF and NBF common mole-rats; however, it is speculated that apart from periods of pregnancy or ovulation, common mole-rat BFs and NBFs may have similar levels of progesterone [51]. However, we refrain from further speculation as our current study did not compare progesterone concentrations of BF and NBF common mole-rats.

The difference in patterns of the relationship between immunocompetence and reproduction in the two co-operatively breeding African mole-rat species may be due to Damaraland mole-rats being captive-bred and the common mole-rats being wild-caught, even after longer than a month in captivity [65–68]. However, captive-bred and wild-caught Norway rats (*Rattus norvegicus*) showed similar prevalence levels of neutrophils and lymphocytes, but differing levels in prevalence of monocytes, eosinophils and basophils [68]. As such the source of the animals (captive vs. wild) may not be the primary reason for species-specific differences between immunocompetence in this study; but, it cannot be discounted.

The Damaraland and common mole-rat share many similarities, but there are significant divergences in the social behaviour and reproductive systems between Damaraland and common mole-rats. The common mole-rat forms groups of up to 12 individuals with the female reproductive suppression strategy comprising primarily of incest avoidance and/or aggressive behaviour from BFs directed towards NBFs, including BFs blocking mating attempts of their NBFs and/or the NBFs being the object of aggression (behavioural in nature) [12,69]. Whereas, the Damaraland mole-rats form much larger groups, of up to 41 individuals, than the common mole-rat. Consequently, the level of reproductive skew is likely greater in Damaraland mole-rats compared to the common mole-rat [70], resulting in the need for additional, possibly more potent, mechanisms of reproductive suppression (physiological in nature) to be required. In the Damaraland mole-rat NBFs are both physiologically and behaviorally suppressed while in the natal group. Physiologically reproductive suppression in the NBFs presents itself in the form of NBFs exhibiting low circulating basal luteinising hormone (LH) concentrations and a much-reduced

response to an exogenous gonadotropin-releasing hormone (GnRH) challenge in comparison to BFs [71,72]. In addition, the varied expression of potent regulators of gonadotropin release, namely the RFamide peptides kisspeptin (Kiss1) and RFamide-related peptide-3 (RFRP-3), have been implicated in the reduced fertility and exhibiting of sexual behaviours of NBFs compared to BFs [73–76]. This ensures that if unrelated males enter a stable group (consisting of an established and breeding BF and breeding male pair) when ecological constraints are relaxed (such as good rainfall events) that multiple BFs do not arise [77,78]. In marked contrast, common mole-rat NBFs have similar basal LH concentrations and responses to an exogenous GnRH challenge as their BF counterparts [69]. On occasion, multiple BFs can be found in the field in stable groups of the common mole-rat due to the immigration of unrelated males into the group as there is only a behavioural inhibition of reproduction [79]. This pattern is not observed in stable and unmanipulated Damaraland groups in the wild [78,80]. Despite the disparate mechanisms of social suppression, the BFs in groups of both species exhibit induced ovulation [51,81]. Similarly, both species show an aversion to inbreeding and avoid this at all costs (behavioural inhibition of reproduction) [11].

Interestingly several differences in behavioural and physiological traits between those species that possess larger groups sizes, likely increased reproductive skew and additional mechanisms of reproductive suppression, such as that found in the Damaraland mole-rat [71,72], while those species that possess smaller groups sizes, likely reduced reproductive skew and did not develop physiological regulation of reproductive suppression, as observed in the common mole-rat. For example, in Damaraland mole-rats, the BFs are 50% less active than NBFs, which likely results in Damaraland mole-rat BFs possessing higher body conditions and increased fat reserves compared to their NBFs [82,83]. In contrast, in Natal mole-rat (*C h. natalensis*), with similar colony sizes and mechanisms of female reproductive suppression [84], BFs and NBFs have similar activity levels [85], body conditions [86] and body fat [D.M. Scantlebury unpublished results]. Unsurprisingly, and similar

to the patterns observed in this common mole-rats, there was no difference in immunocompetence between breeding and non-breeding Natal mole-rats [87].

Our study has uncovered the natural inequality that arises in ecology and evolution. According to evolutionary theory, a balance must be struck between reproductive investment by the breeding individual and the breeding individual's survival. However, this study suggests that this is not always the case. Under condition of extreme reproductive skew the breeders have the best of both worlds: increased reproductive investment and survival (as a result of increased immunocompetence). The physiological underpinning of how social living could cause inequality of evolutionary success, via reproduction and immunity, is currently not fully understood in mammals. But studying social African mole-rat species may be critical to address this dearth of knowledge of such naturally occurring inequalities.

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