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FACTORS INFLUENCING MATE CHOICE IN A EUSOCIAL MAMMAL, THE
DAMARALAND MOLE-RAT (*Fukomys damarensis*)

by

John Bowman Kelley

A Dissertation

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Preface

Chapter 1 of the manuscript has been published in the journal *Animal Behaviour*, issue 158, 2019. The title of the published article that comprises chapter 1 of this manuscript is “Mechanism for establishing and maintaining the reproductive hierarchy in a eusocial mammal, the Damaraland mole-rat.” Here, the title has been changed to “Mechanism for establishing and maintaining the reproductive hierarchy.” Chapter 1 thus follows the formatting specifications for the journal *Animal Behaviour*. Chapters 2 and 3 of this manuscript are formatted similarly for continuity.

Table of Contents

Chapter	Page
List of Figures	iv
Introduction	1
Chapter 1 – Mechanism for establishing and maintaining the reproductive hierarchy	4
Chapter 2 – Simulated dispersal: the occurrence of mating behavior between siblings following removal from the colony	36
Chapter 3 – Partner preference and mate fidelity	51
Conclusion	66

List of Figures

Figure	Page
1. Proportion of male mounting behavior split by treatment group	13
2. Proportion of female solicitation behavior split by treatment group	14
3. Proportion of female lordosis behavior split by treatment group	15
4. Average number of male mounts in each treatment group	17
5. Average number of female solicitations in each treatment group	20
6. Average number of female lordosis occurrences in each treatment group	21
7. Proportion of sibling pairs expressing mating behavior both inside and outside the colony	42
8. Proportion of sibling pairs that expressed mating behavior outside of the colony split by breeding status of the colony	43
9. Proportion of animals that expressed mating behavior split by sex	44
10. Amount of time spent by test animal in each chamber	58
11. Amount of time spent by test animal mating with either stimulus animal	59

Introduction

Eusociality is a social system in which animals live in colonies with overlapping generations of adults. Only a few individuals directly engage in reproduction, while the remaining members are non-reproductive and help to raise their siblings. Currently, only two mammalian species meet the criteria for eusociality, the Damaraland mole-rat (DMR; *Fukomys damarensis*) and the naked mole-rat (NMR; *Heterocephalus glaber*). In DMR colonies, only one female and one male within the colony reproduce, resulting in high reproductive skew (Holmes et al. 2009). DMR colonies are composed of the breeding female (often termed the “queen”), her breeding partner, and their offspring. The non-breeding offspring do not expend energy to increase their fitness directly, they contribute to the reproductive effort of the breeding pair by: helping to care for their siblings, foraging for food, defending the colony from predators, and maintaining and enlarging the burrow system. DMR represent a unique opportunity to gain insight into the behavioral and physiological mechanisms that regulate sexual behavior in a eusocial mammal.

DMR practice strict inbreeding avoidance within their natal colonies, possibly preventing the deleterious effects that can result from inbreeding and, as a result, DMR are considered obligate out-breeders (Cooney & Bennett 2000). Non-breeding females do not express sexual behavior (solicitation, lordosis) with colony mates under stable circumstances. These females do not undergo puberty while in their natal colony, but this developmental characteristic alone may not prevent sexual behaviors from occurring with familiar males. When paired with an unfamiliar male, prepubertal females express sexual behaviors (Cooney & Bennett 2000; Carter et al. 2014). The expression of sexual behaviors in DMR does not depend solely upon exposure to an unfamiliar conspecific, as

opposite sex siblings separated for a period of 5 weeks will express sexual behaviors towards one another (Carter et al. 2014). These findings indicate that identification of individuals is not based on genetic relatedness per se.

In the wild, DMR disperse when ecological conditions relax (following heavy rainfall). Individuals may then go above ground and attempt to locate an unfamiliar opposite-sex conspecific to form a new colony (Young et al. 2010). This process exposes DMR to novel stimuli, as well as the challenge of finding a mate and building a new burrow system; all of which may initiate a stress-response. The traditional generalization is that the activation of the hypothalamic-pituitary-adrenal axis under stress has an inhibitory effect on the hypothalamic-pituitary-gonadal axis (Wingfield & Sapolsky 2003). However, for a dispersing individual, the repression of the reproductive axis during this time would be maladaptive. Mating opportunities are essentially non-existent for non-breeding colony members unless they encounter unfamiliar individuals, which happens primarily through the process of dispersal. The expression of sexual behaviors has been observed rapidly (within 10 minutes) following removal from the colony between unfamiliar individuals. Moreover, siblings have been observed expressing sexual behavior with one another following concurrent removal from the colony and transport to a testing facility (personal observation).

A result of colonial living in a species with low dispersal rates is the formation of stable pair bonds. DMR individuals can live for 18 years or more in captivity and can successfully breed from less than 1 year of age, meaning that pairs have the potential to form bonds that last for years, possibly their entire lives. This situation leads to an apparent sexual monogamy, although the fidelity of the pair bonding is tested when

foreign individuals are introduced (Rickard & Bennett 1997; Jacobs et al. 1998; Cooney 2000; Cooney & Bennett 2002). The experiments that follow were designed to analyze the role that familiarity, both in breeders and non-breeders, has in mate choice in DMR, as well as the effect of disturbance and simulated dispersal on mating behavior.

Mechanism for Establishing and Maintaining the Reproductive Hierarchy

Introduction

Eusociality is a social system in which animals live in colonies with overlapping generations of adults. Only a few individuals reproduce, while the remaining colony members, the offspring of the breeding individuals, are non-reproductive. Eusociality is widespread in bees, wasps, ants and termites (Wilson & Holldobler 2009). Currently only two mammalian species are commonly considered to meet the criteria for eusociality: The Damaraland mole-rat (*Fukomys damarensis*) and the naked mole-rat (*Heterocephalus glaber*) (Bennett & Faulkes 2000).

The Damaraland mole-rat is a highly fossorial species native to arid habitats of southwest Africa, where they live in colonies that average about 16 individuals (ranging as high as 41) consisting of one breeding pair and their non-reproductive offspring from several litters. The non-breeders contribute to the reproductive effort of the breeding pair by maintaining and enlarging the burrow system, foraging, and defending the colony from predators. New colonies are formed when previously non-breeding individuals go above ground to disperse from their natal colonies and pair with other dispersers, most likely from a different colony. Dispersal of individuals from the natal burrow is thought to be the sole mechanism for the formation of new colonies (Bennett & Faulkes 2000).

Non-reproductive Damaraland mole-rat females, even after achieving adult body size, do not undergo puberty while in the natal colony and retain unstimulated ovaries and uteri (Holmes et al. 2009). However, these females frequently express sexual behaviors within minutes upon exposure to a foreign male, suggesting that appropriate social cues initiate the mating response without prior exposure to elevated concentrations

of gonad hormones (Cooney & Bennett 2000; Nice et al. 2010; Carter et al. 2014). This contrasts with the large majority of mammals in which gonadal steroid hormones are required for supporting female sexual behavior (Nelson 2005); in these species a linkage between preovulatory hormone secretions and sexual behaviors suggests that this hormone-dependent system evolved to ensure that females restrict sexual receptivity to times when conception is possible (Pfaff & Schwartz-Giblin 1988). Among the relatively few mammals in which ovarian hormones are not required for support of sexual behavior are humans and some non-human primates; in these animals, sexual behaviors have social functions in addition to direct reproduction, and the relative hormone-independence of sexual behaviors allows these behaviors to be expressed in the context of social functions throughout the ovulatory cycle (Baum et al., 1978; Wallen & Zehr, 2004). Observations that non-breeding female Damaraland mole-rats with unstimulated ovaries will readily mate with foreign males (Rickard & Bennett 1997) and that mating behavior can occur independent of gonadal hormones in both sexes (Carter et al. 2014), suggest that mating behavior may have some function in Damaraland mole-rats in addition to its function for direct reproduction. The present study was motivated, in part, by our desire to more fully understand the role of mating behavior in Damaraland mole-rats.

There is abundant evidence indicating that incest avoidance has a strong role in maintaining the reproductive hierarchy in Damaraland mole-rats, whereby only the breeding pair exhibit sexual behaviors under normal conditions. It has been suggested that the female Damaraland mole-rat plays a larger role in enforcing incest avoidance as compared to the male (Jacobs et al. 1998). However, it remains unclear how individual

mole-rats distinguish their close relatives (colony members) from foreign (unfamiliar) individuals with respect to the expression of sex behaviors. Familiarity alone cannot provide a full explanation for choice of mating partners, since the breeding pair are presumably as familiar with each other as are the nonbreeding colony members. It is also clear that genetics does not play a large role in establishing sexual partnerships (Carter et al. 2014; Rickard & Bennett 1997; Bennett et al. 1996). The present study was designed to further explore how mating partnerships form among Damaraland mole-rats. As a part of this study we also investigated whether there are conditions under which unrelated animals might treat each other, with respect to reproduction, as if they were kin. It seemed possible that a better understanding of these issues could reveal the mechanistic basis for the reproductive structure of Damaraland mole-rats eusociality and provide insights into how eusociality might have evolved in this species.

While Damaraland mole-rats have commonly been designated as eusocial, it must be recognized that for this species the label depends almost exclusively on the existence of a remarkably strict reproductive hierarchy within colonies. The more familiar cases of eusociality among insects not only exhibit a reproductive hierarchy but are also characterized by specializations among different groups of nonbreeding individuals, or so-called caste systems (Wilson & Holldobler 2009). A study of energetics provided some evidence for physiologically distinct castes within DMR colonies (Scantlebury et al. 2006). However, extensive longitudinal studies revealed an absence of individual differences in task specialization like those that are characteristic of eusocial insects (Thorley et al. 2018; Zottl et al. 2016). The absence of a caste system in Damaraland mole-rats might be taken to suggest that this species would be more appropriately

designated as a case of cooperative breeding rather than eusociality. In any event, the present study focused entirely on the mechanistic basis for the reproductive hierarchy in Damaraland mole-rats; as such, the results have potential implications for similar explorations in any group-living species that exhibits a strict reproductive hierarchy.

Methods

Animals

Damaraland mole-rats used in this study came from colonies housed at the University of Memphis (originally provided by Dr. Bruce Goldman at the University of Connecticut). Their diet consists of ad libitum sweet potatoes supplemented with dry rodent pellets (Harlan 2019, 19% protein diet). Each colony is housed within a complex constructed of two different sized cages (60x40x20cm and 48x25x20cm) connected by varying lengths of extruded polycarbonate tubing to roughly simulate natural burrow architecture. The number of cages and lengths of tube are dependent upon the size of the colony and all colonies contain a 1:1 mixture of corncob and pine bedding. The animals used in the study were adults ranging from 2 to 5 years old; DMR can live at least 15 years in captivity (Holmes et al. 2009). All experimental procedures and husbandry were approved by the University of Memphis Animal Care and Use Committee (Protocol # 797) and comply with the criteria established by NIH Guide for the Care and Use of Laboratory Animals.

Treatment Groups

One non-breeding male and one non-breeding female were selected from each of 8 colonies for this study, for a total of 16 animals. Each individual was randomly assigned three opposite-sex unfamiliar partners from the pool of sixteen so that, including sibling pairs, each Damaraland mole-rat was a part of four male/female dyads, and each dyad was assigned to one of the four different treatment groups created for the study. Thus, each treatment group included 8 dyads.

Familiarization

Two treatment groups included males and females that were “familiarized”. The apparatus used for familiarizing these groups consisted of an arena (28x52x38cm) constructed of alternating walls of plywood and acrylic sheets and divided in half by a wire mesh barrier.

Familiarized groups included: 1) male and female pairs from unrelated colonies that were paired for 20 min daily for 14 days, separated from each other by a wire mesh to prevent physical contact and allow only potential olfactory, auditory and visual interaction (“No Initial Physical Contact” group), and 2) male and female pairs from unrelated colonies that were allowed physical contact on Day 0 but on 14 subsequent days were paired with the wire mesh barrier between them (“Initial Physical Contact” group). Individuals from all pairs in both groups were separated by a wire mesh barrier for 14 consecutive days; the only difference between the two groups was whether they were allowed a 20 min period of physical contact prior to the 14 daily episodes of separation by wire mesh. All individuals were returned to their natal colonies following

each 20 min familiarization event. After 14 days of familiarization, with or without physical contact allowed on Day 0, all pairs were placed in a testing arena in order to assess the expression of sexual behavior (see below).

Control groups

The two control groups described below (sibling and unfamiliar) were stand-alone tests and were not subjected to 14 days of pre-exposure.

Sibling pairs

Since siblings rarely express sexual behavior with each other (Carter et al., 2014), male and female siblings from the same colony were paired a single time for 20 minutes to serve as negative controls with regard to the expression of sexual behavior.

Unfamiliar pairs

Male and female Damaraland mole-rats from unrelated colonies readily express sexual behaviors when paired (Carter et al. 2014). Thus, male and female Damaraland mole-rats from separate colonies were paired in a mating arena a single time for 20 minutes to serve as positive controls with regard to the expression of sexual behavior.

Timeline of pairings

Before any unrelated individuals were matched for testing, we placed sibling pairs into the testing arena in order to establish the frequency of sexual behaviors that occur between siblings. These pairs constituted our “sibling” experimental group. The

following day, each individual was randomly matched with an unrelated, opposite sex individual and the familiarization protocol began (14 days of familiarization separated by wire mesh = “No Initial Physical Contact” group). On the 15th day, half of these individuals were paired without restriction with their newly “familiarized” partner. The other half were paired randomly with another unrelated, opposite sex individual, these were the “unfamiliar” pairs. On day 16, these animals were paired with either their familiarized partner or an unfamiliar individual, whichever they did not experienced the previous day. Six weeks later, all individuals were subjected to the same familiarization protocol with a new partner, with the exception being that each individual was allowed to interact without restraint on their first day of meeting (Day 0 this constituted the “Initial Physical Contact” group).

Sexual behavior tests

We removed individuals from their natal colonies and paired them together in a separate testing arena (28x52x38cm) constructed of alternating walls of plywood and acrylic. This was the same arena used for separating dyads by wire mesh, but with the mesh removed. We recorded all instances of mating behavior, including female solicitation (backing the anogenital region toward the male's head) and lordosis (arching of the spine, deflection of the tail, and immobility), male mounting and thrusting. All tests were recorded using a JVC GZ-MG21U camcorder and behaviors of both males and females were scored from the recordings. Videos were scored using The Observer software (Version 6.1, Noldus Information Technology, Leesburg, VA). The data were normally distributed (Kolmogorov-Smirnov test for normality; $p > 0.09$ for each group).

The proportion of individuals in each treatment that exhibited sexual behaviors were analyzed via Fisher's exact test. Additionally, for each treatment group, the mean values for all sexual behaviors were analyzed by repeated-measures ANOVA. We followed this main test with pre-planned comparisons, thus, the experimental design included planned comparisons of the expression of sexual behaviors between groups of mole rats that were familiarized in the absence of physical contact, and those that were allowed one day of physical contact on Day 0 of the experiment. Because we hypothesized that physical interaction on Day 0 might result in the formation of a breeding relationship, we planned to compare sexual behavior of that group to unfamiliar animals that are known to readily exhibit sexual behavior (Carter et al. 2014). We planned to compare the expression of sexual behaviors between pairs that were familiarized in the absence of physical contact for 14 days pairs to that of sibling pairs, because we hypothesized that familiarization in the absence of mating results in a sibling-like relationship. Lastly, we planned to confirm previous results by comparing the expression of sexual behaviors between our two Control groups (i.e., unfamiliar opposite-sex individuals and sibling pairs). The planned comparisons were analyzed by paired *t*-tests using Bonferroni correction for each test. *Cohen's d* was calculated to determine effect sizes for pairwise comparisons.

Ethics Statement

Animal care personnel and researchers received special training from the university veterinarian to help minimize stress during handling of the animals. The mole-rats were always handled carefully to avoid undue noise or vibration to reduce stress. They were

transported to the testing room on a heavy-duty thermoplastic rubber cart to reduce vibration and noise, after transfer to the testing room they were allowed 15 minutes to acclimate prior to being placed in the testing arena. All pairs were monitored continuously and would be separated immediately if fighting or aggressive behavior was observed, though no such interventions were necessary.

Results

Analyses of categorical data

Male Mounting Behavior

The proportion of males that exhibited mounting behavior was significantly higher when paired with females they had been allowed physical contact with on Day 0 (Initial Physical Contact Group) as compared to being paired with females where physical contact was restricted (No Initial Physical Contact Group) on Day 0 (Fig. 1, 7/8 vs 2/8 respectively; Fisher's exact; $p = 0.04$). With regard to the Control groups, the proportion of males that exhibited mounting behaviors was greater when paired with unfamiliar females as compared to siblings (8/8 vs 3/8 respectively; Fisher's exact $p=0.03$). Interestingly, the proportion of males that exhibited mounting behavior did not differ between males paired with unfamiliar females vs females with which they were allowed physical contact on Day 0 (8/8 vs 7/8 respectively; Fisher's exact $p>0.99$). Additionally, the proportion of males that mounted siblings did not differ from the proportion of males that exhibited mounting behavior when paired with females they were familiarized with in the absence of physical contact (3/8 vs 2/8 respectively; Fisher's exact, $p>0.99$).

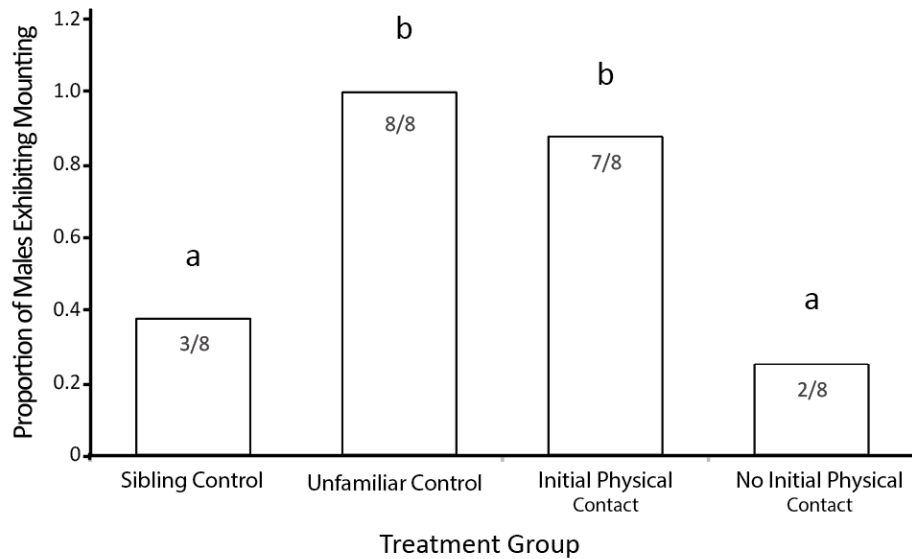


Figure 1. The proportion of male Damaraland mole-rats that exhibited mounting behavior when paired with female siblings, unfamiliar females, or females with which they were previously familiarized for 14 days with or without initial physical contact on Day 0. Groups with different letters differed significantly (Fisher’s Exact, $p < 0.05$) whereas those with the same letters did not (Fisher’s Exact; $p > 0.99$). The number of individuals out of the total that exhibited mounting are included in each bar.

Female Solicitation Behavior

The proportion of females that exhibited solicitation behavior when paired with males that they were allowed physical contact on Day 0 was significantly greater than when paired with males with which physical contact was restricted on Day 0 (Fig. 2, 7/8 vs 1/8, respectively; Fisher’s exact $p=0.01$). The two control groups also differed significantly, thus, a higher proportion of females that were paired with unfamiliar males exhibited solicitation behavior as compared to females paired with siblings (7/8 vs 1/8 respectively; Fisher’s exact $p=0.01$). Identical proportions (7/8 for each) of females exhibited solicitation behavior when paired with either unfamiliar males, or males that were allowed physical contact on Day 0. Similarly, identical proportions of females

exhibited solicitation (1/8 for each) when paired with either siblings, or males with which they were restricted from physical contact with on Day 0.

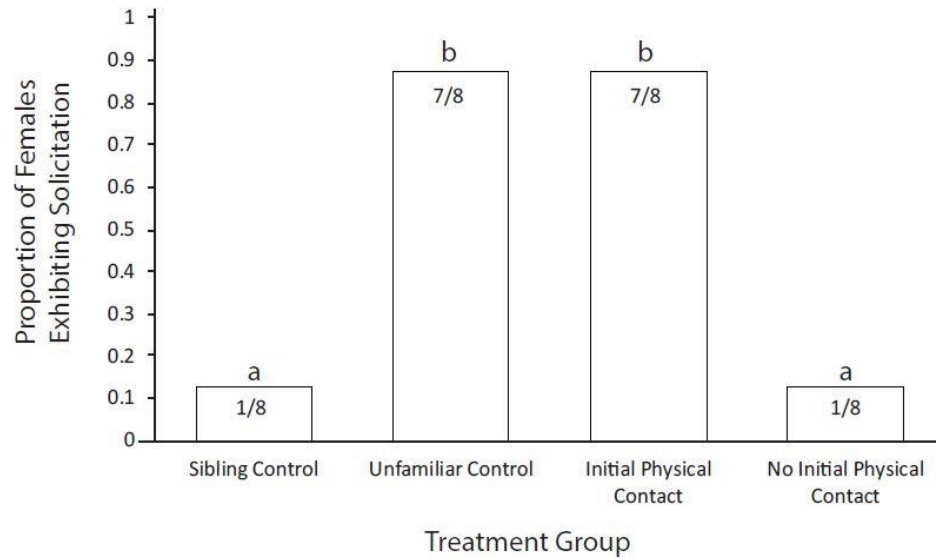


Figure 2. The proportion of female Damaraland mole-rats that exhibited solicitation behavior when paired with male siblings, unfamiliar males, or males with which they were previously familiarized for 14 days with or without initial physical contact on Day 0. Groups with different letters differed significantly (Fisher's Exact, $p < 0.05$) whereas those with the same letters did not (Fisher's Exact; $p > 0.99$). The number of individuals out of the total that exhibited solicitation behavior are included in each bar.

Female Lordosis Behavior

There was no significant difference in the proportion of females that exhibited lordosis among pairings. Thus, 3/8 exhibited lordosis when paired with males with which they were allowed physical contact on Day 0, 2/8 lordosed when paired with males that they were not allowed physical contact on Day 0; 1/8 females paired with a sibling exhibited lordosis whereas 2/8 lordosed when paired with unfamiliar males (Fig.3, Fisher's exact $p > 0.99$ for all analyses).

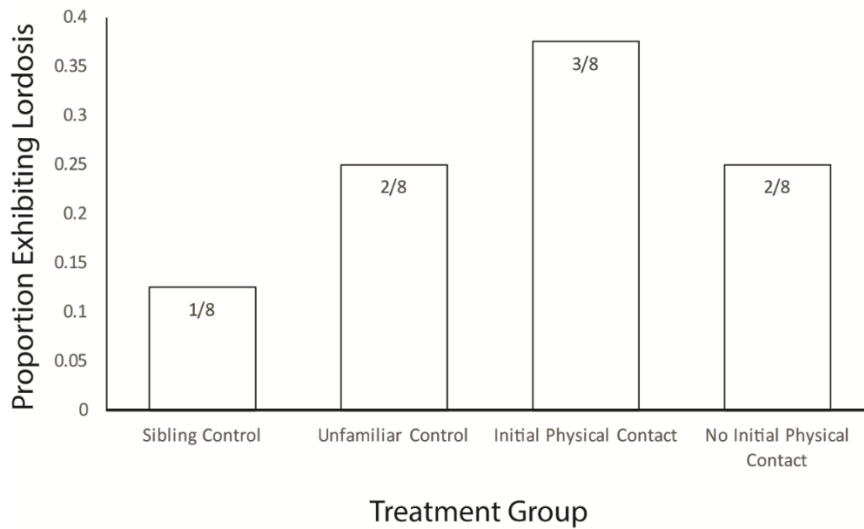


Figure 3. The proportion of female Damaraland mole-rats that exhibited lordosis behavior when paired with male siblings, unfamiliar males, or males with which they were previously familiarized for 14 days with or without initial physical contact on Day 0. The proportion of females exhibiting lordosis did not differ among the groups (Fisher's Exact, $p > 0.9$). The number of individuals out of the total that exhibited lordosis behavior are included in each bar.

Analysis of Continuous variables:

The pattern of expression of sexual behaviors differed among treatment groups in both males and females. Thus, there was a significant difference among groups with regard to the number of male mounts (Repeated-measures ANOVA, $F_{(3,21)}=7.17$, $p=0.002$, Fig. 4). In females, there was a significant effect of treatment on the number of solicitations (Repeated-measures ANOVA $F_{(3,21)}=6.67$, $p=0.003$, Fig. 5), but not the number of lordoses (Repeated-measures ANOVA $F_{(3,21)}=2.39$, $p=0.09$, Fig 6).

Planned Comparisons between the two familiarized groups

The expression of sexual behavior for both males and females of the two familiarized groups (*Initial Physical Contact* group and *No Initial Physical Contact* group) varied as a function of whether the pairs were allowed physical contact during their initial pairing of the experiment. Thus, males allowed initial physical contact with females on Day 0 exhibited significantly more mounting behavior following familiarization as compared to males in pairs that were physically separated for the entire familiarization period (Fig. 4; male mounting, paired *t*-test; $t_7 = 2.281$, $p = 0.04$; Cohen's $d = 1.28$). Females in pairs that were allowed physical contact with males on Day 0 exhibited significantly more solicitation but not lordosis behavior following familiarization than females in pairs that were not allowed physical contact during the first interaction with their male partner (Fig 5; female solicitation, paired *t*-test; $t_7 = 3.658$, $p = 0.008$, Cohen's $d = 1.63$; Fig. 6; female lordosis, paired *t*-test; $t_7 = 1.836$, $p = 0.11$, Cohen's $d = 0.83$).

Planned Comparisons between sibling and unfamiliar control groups

The expression of sexual behavior differed significantly between the two control groups. Thus, males exhibited more mounts when paired with an unfamiliar female than with a sibling (Fig. 4; paired *t*-test; $t_7 = -3.737$, $p = 0.007$; Cohen's $d = 1.75$). Similarly, females paired with unfamiliar males exhibited more solicitations than when paired with a sibling (Fig. 5; paired *t*-test; $t_7 = 2.849$, $p = 0.03$, Cohen's $d = 1.44$). The expression of lordosis did not differ between females paired with a sibling as compared to an unfamiliar male (Fig. 6; paired *t*-test; $t_7 = -1.00$, $p = 0.4$, Cohen's $d = 0.494$).

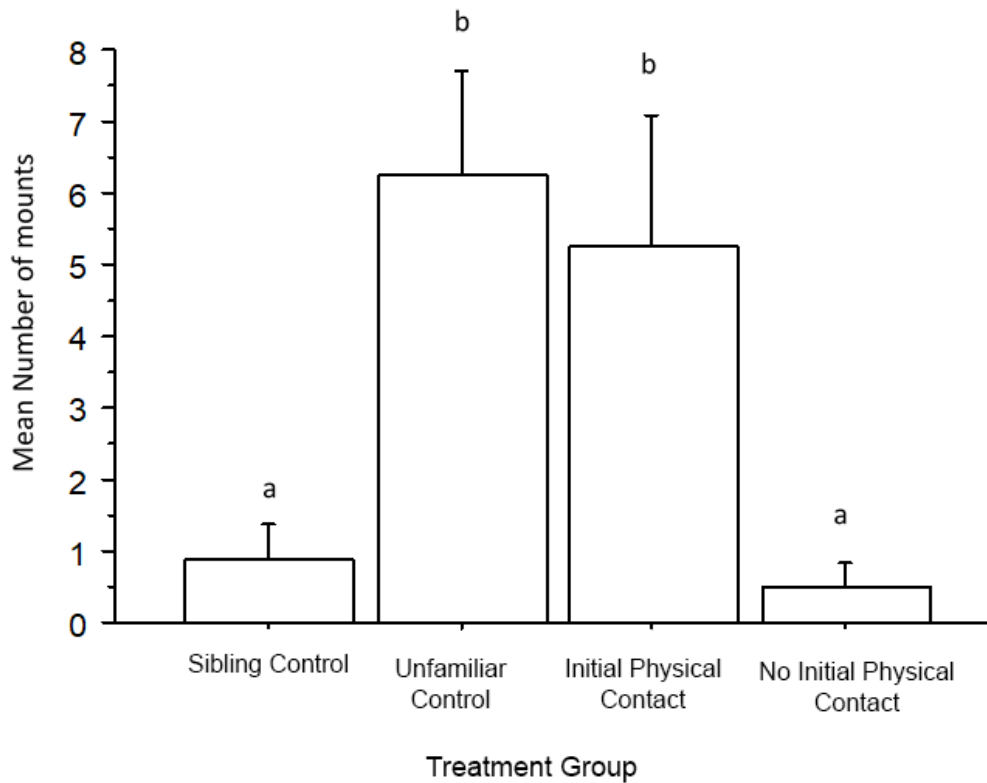


Figure 4. Mean (\pm s.e.m.) number of times male Damaraland mole-rats exhibited mounting behavior when paired with females that were siblings, unfamiliar, or had been familiarized for two weeks either with or without initial physical contact on day 0 of the experiment. Bars with different letters differ significantly (planned comparisons; paired *t*-tests; $p < 0.05$). Mounting behavior of males in Damaraland mole-rat pairs allowed physical contact at initial meeting did not differ from males paired with unfamiliar females (Unfamiliar Controls), whereas the mounting behavior of males not allowed physical contact at initial meeting did not differ from males paired with female siblings (Sibling Controls). The number of mounts exhibited by the Unfamiliar and Sibling Control groups differed significantly from each other, as did the number of mounts exhibited by the experimental groups with or without initial physical contact.

Sexual behavior in the Initial Physical Contact and the No Physical Contact groups

Initial pairings

On the first day of pairing (Day 0), 6 of the 8 males that were allowed physical contact with an unfamiliar female in the Initial Physical Contact group exhibited mounting behavior, 8 of the 8 females solicited the males, and 4 of the 8 females exhibited lordosis. None of the animals in the No Physical Contact group exhibited any sexual behavior on day 0, when separated by the wire mesh (0/8 males; 0/8 females for all behaviors).

Planned Comparisons between familiarized and control groups

To assess whether 14 days of familiarization in the absence of physical contact at initial meeting resulted in a sibling-like relationship or a breeding relationship, we compared the expression of sexual behavior between Damaraland mole-rats that were familiarized in the absence of physical contact (No Physical Contact) with those that were paired with a sibling and with those paired with unfamiliar individuals. The mounting behavior exhibited by males from the group with no initial physical contact on Day 0 paired with females after 14 days of familiarization did not differ from the same males paired with siblings (Fig 4; paired t -test; $t_7 = 0.704$, $p = 0.5$, $p=0.8$, Cohen's $d = 0.307$), but the mean number of mounts for the same group (No Initial Physical Contact) was significantly lower as compared to these males paired with unfamiliar females (Fig. 4; paired t -test; $t_7 = 3.670$, $p = 0.008$, Cohen's $d = 1.936$). Similarly, solicitation behavior did not differ between females paired with males following 14 days of familiarization without physical contact and those paired with siblings (Fig. 5; paired t -test; $t_7 = -0.314$,

$p=0.076$, Cohen's $d = 0.172$), but females familiarized for 14 days in the absence of physical contact exhibited significantly fewer solicitations than females paired with unfamiliar males (Fig. 5; paired t -test; $t_7 = 3.044$, $p=0.02$, Cohen's $d = 1.465$). Lordosis behavior did not differ between females paired with males after 14 days of familiarization in the absence of physical contact and females paired with siblings (Fig. 6; paired t -test; $t_7 = 0.552$, $p = 0.6$, Cohen's $d = 0.303$), nor did it differ from females paired with unfamiliar males (Fig. 6; paired t -test; $t_7 = 0.935$, $p = 0.4$, Cohen's $d = 0.390$).

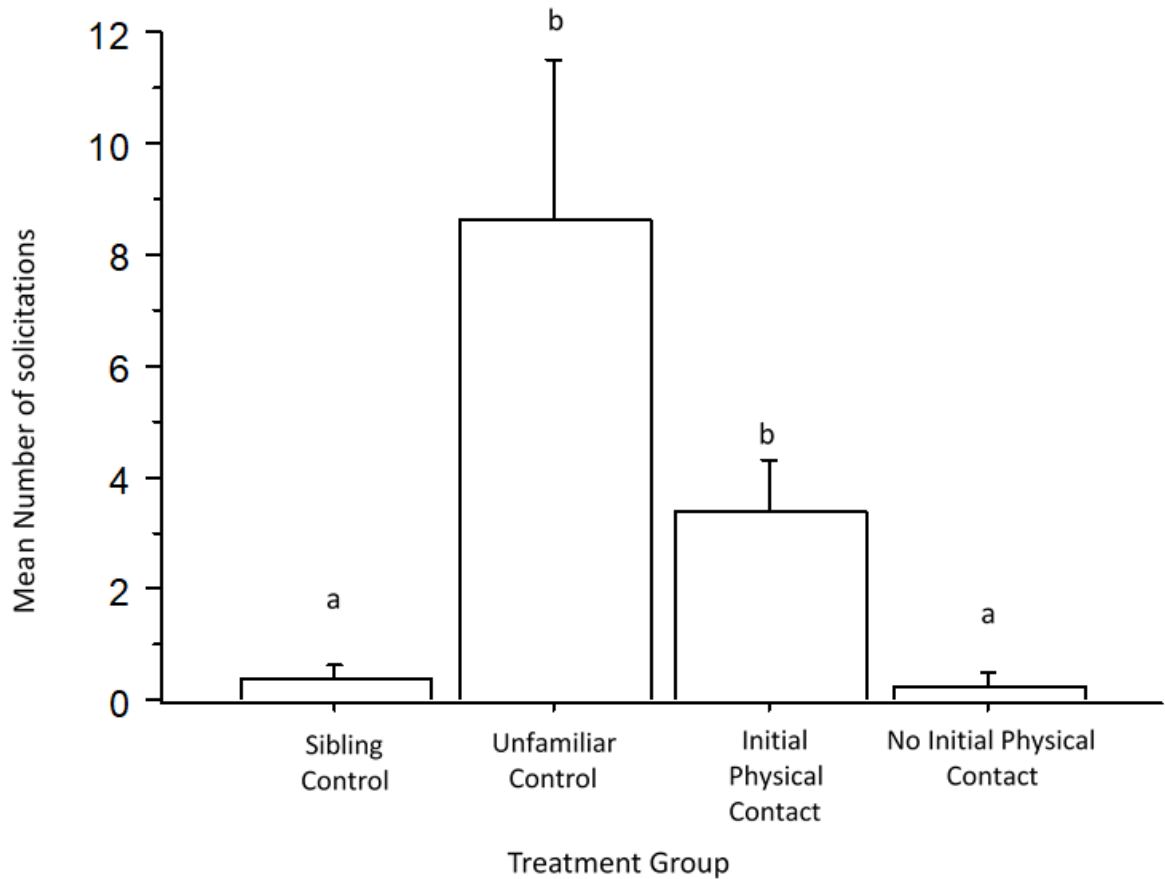


Figure 5. Mean (\pm s.e.m.) number of times female Damaraland mole-rats exhibited solicitation behavior when paired with males that were siblings, unfamiliar, or had been familiarized for two weeks either with or without initial physical contact on day 0 of the experiment ($n=8$ /group). Bars with different letters differ significantly (planned comparisons; paired t -tests; $p < 0.05$). Solicitation behavior of females in Damaraland mole-rats pairs allowed physical contact at initial meeting did not differ from females paired with unfamiliar males but was significantly greater than that of the Sibling Control group. The solicitation behavior of females not allowed physical contact at initial meeting did not differ from females paired with male siblings but was significantly lower as compared to the Unfamiliar Control group. The solicitation behavior of the Unfamiliar and Sibling Control groups differed significantly from each other, as did the solicitation behavior expressed by the experimental groups with or without initial physical contact.

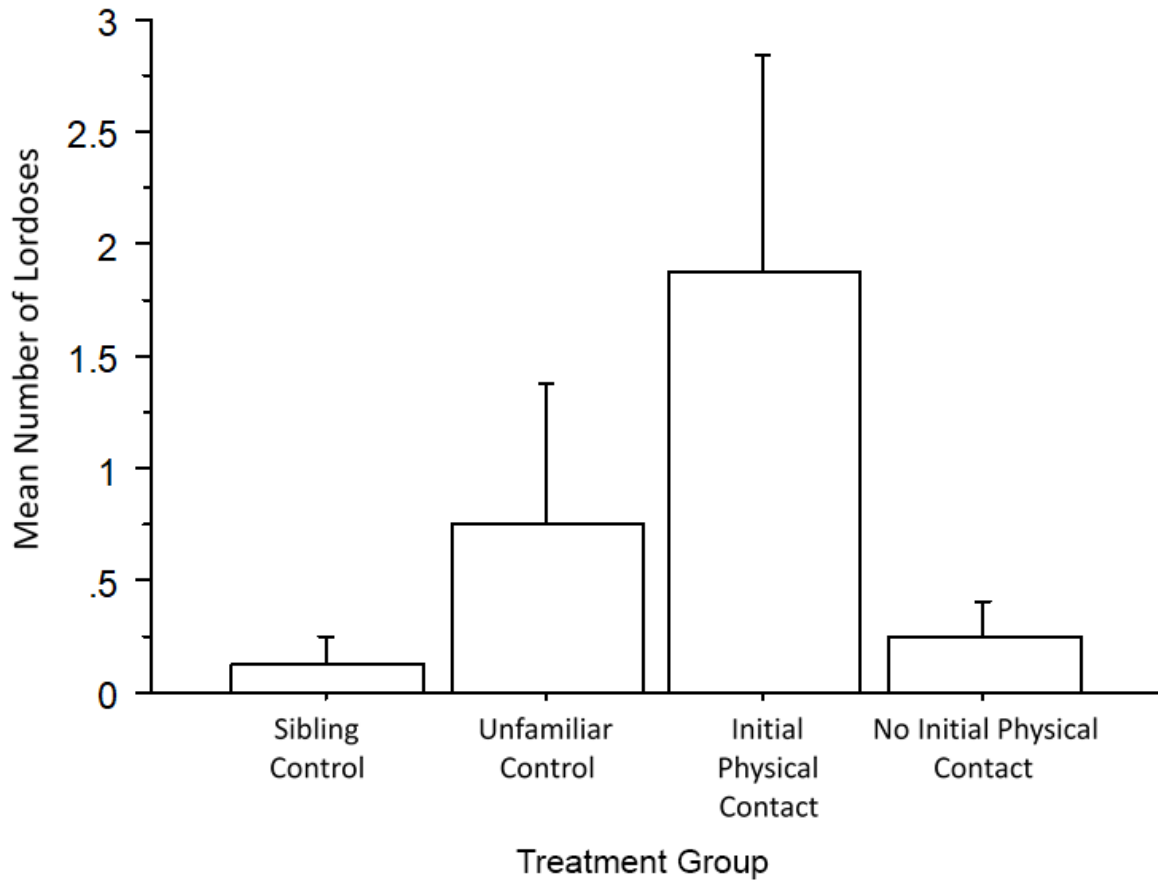


Figure 6. Mean (\pm s.e.m.) number of times female Damaraland mole-rats exhibited lordosis behavior when paired with males that were siblings, unfamiliar, or had been familiarized for two weeks either with or without initial physical contact on day 0 of the experiment. Lordosis behavior did not differ significantly among any of the groups ($n=8$ /group; paired t -tests; $p > 0.1$ for each planned comparison).

To determine whether 20 minutes of physical contact on Day 0 followed by 14 days of familiarization without physical contact resulted in sibling-like or breeder-like relationships, we compared the behavior of animals in the Initial Physical Contact group with both the unfamiliar and sibling control groups. Male mounting behavior did not differ between pairs that were allowed physical contact on day 0 and unfamiliar controls (Fig. 1; paired t -test; $t_7 = -0.632$, $p = 0.55$; Cohen's $d = 0.214$), and these males exhibited significantly more mounting behavior

than males paired with siblings (Fig. 1; paired *t*-test; $t_7 = 2.455$, $p = 0.04$; Cohen's $d = 1.151$). The mean number of solicitations in females from pairs that were allowed physical contact on Day 0 was significantly greater than females paired with siblings (Fig. 2; paired *t*-test; $t_7 = 2.763$, $p=0.03$, Cohen's $d = 1.561$), and did not differ from that of females paired with unfamiliar males (Fig. 2; paired *t*-test; $t_7 = 1.688$, $p=0.14$, Cohen's $d = 0.877$). The mean number of lordoses exhibited by females in pairs that were allowed physical contact on day 0 was not different than females paired with siblings (Fig.3; paired *t*-test; $t_7 = 1.727$, $p = 0.13$; Cohen's $d = 0.893$), and did not differ significantly from females paired with unfamiliar males (Fig.3; paired *t*-test; $t_7 = 1.386$, $p = 0.21$; Cohen's $d = 0.488$).

Discussion

A large number of highly social mammals exhibit reproductive hierarchies. The species investigated range from cooperative breeders like meerkats (Clutton-Brock et al. 2003), dwarf mongooses (Rood 1990) and African wild dogs (Gusset & Macdonald 2009) to two species of mole rats that have frequently been labeled as eusocial with comparisons to some of the eusocial insects, particularly with respect to the rigidity of the reproductive hierarchy and the genetic relationships between individuals within colonies (Jarvis et al. 1994). Recently, careful examination of one of the purported eusocial species, the Damaraland mole-rat, failed to reveal evidence of a caste system among nonbreeders in colonies, a major feature that distinguishes between clearly eusocial species such as ants, termites, and some bees and cooperative breeders such as meerkats (Thorley et al. 2018; Zottl et al. 2016). The basis for establishing and maintaining the

reproductive hierarchy has not been clearly established for any of the various highly social mammals. The present investigation sought to elucidate the mechanism underlying the rigid reproductive hierarchy that is present in colonies of Damaraland mole-rats.

Prior to the discovery that naked mole-rats colonies include a strict reproductive hierarchy, Richard Alexander, who had studied eusocial insects, suggested that if a eusocial mammal existed it would likely be a burrowing species, since eusociality in insects is generally associated with colonies that inhabit relatively enclosed and protected nests; among mammals, these conditions are most frequently met for subterranean species (Alexander et al. 1991). By contrast with naked mole-rats, Damaraland mole-rats and several other mole rat species (see below), all the other mammalian cooperative breeders live above ground. This may be of significance for social structure and especially for the frequency of emigration or dispersal from colonies. Though dispersal or emigration in colonial mammals always involves movement above ground (Bennett & Faulkes 2000), the process may be less dangerous for species that live above ground as individuals are adapted to that lifestyle. Dangers associated with dispersal from subterranean colonies may be one factor leading to delayed and less frequent dispersal in mole rat social groups. It should be noted that while naked mole-rats and Damaraland mole-rats are the only mammals commonly labeled as eusocial, these two species are both members of the family Bathyergidae; several other bathyergid species are cooperative breeders but with smaller colony sizes than the eusocial species. All bathyergids are native to Africa, and many inhabit arid or semi-arid regions (Bennett & Faulkes 2000). Another factor that likely has been of predominant importance in the evolution of communal lifestyle in the bathyergid mole-rats is the scattered distribution of

underground food sources and the subsequent uncertainty of finding food that confronts a solitary individual; this is particularly true in the arid habitats often associated with the social mole rats. Group-living with more extensive burrow systems is thought to confer an advantage in foraging in such habitats (Bennett & Faulkes 2000).

The basis for establishing and maintaining the reproductive hierarchy has not been clearly established for any of the various highly social mammals. The present investigation sought to elucidate the mechanism underlying the rigid reproductive hierarchy that is present in colonies of Damaraland mole-rats. Individual recognition is a key element in the organization of the strict reproductive hierarchy that is central to eusociality in Damaraland mole-rats: siblings generally do not mate with each other, but opposite-sex individuals derived from two different colonies will readily mate (Carter et al. 2014). Discrimination between individuals does not appear to be based on genetic relatedness *per se*; siblings separated from each other for 5 weeks will treat one another as unfamiliar (i.e., as non-siblings), as shown by an increased probability of expressing sexual behavior when they are reunited (Carter et al. 2014; Jacobs & Kuiper 2000). Similarly, in another social species from the same genus, Ansell's mole-rat (*Fukomys anselli*), siblings separated for a period of 27 days did not differ from unrelated pairs in the expression of mutual sexual behaviors (Heth et al. 2004).

Familiarity *per se* is insufficient to explain regulation of Damaraland mole-rat sexual behavior, as both non-breeders (generally siblings) and breeders can be assumed to be familiar with one another; one factor that differs between the two types is the conditions under which the individuals of any given dyad first met. Specifically, the breeding pair is composed of individuals that probably dispersed from two separate

colonies (Burland et al. 2002), and they most likely expressed mating behaviors at first meeting, as suggested by our laboratory observations showing that unfamiliar individuals will express sexual behaviors within minutes of their first encounter (Carter et al. 2014). By contrast, non-breeders are the offspring of the breeding pair and are in frequent contact with all other colony members from the time of birth.

There were two novel and closely related observations in the present study, and both may be fundamental for understanding the mechanistic aspects of the reproductive hierarchy in Damaraland mole-rats: (1) First, we observed that if a pair of Damaraland mole-rats, each taken from a different colony, are allowed restricted access (separated by a mesh barrier) to one another over a 2-week period, the two individuals show little sex behavior when subsequently placed together with unrestricted access (No Initial Physical Contact Group). This contrasts with the robust display of sex behaviors that is typically exhibited by a pair of unrelated Damaraland mole-rats that are placed together without any previous form of mutual exposure. This observation suggests that a given pair of Damaraland mole-rats can achieve familiarity without physical contact, most likely via olfactory recognition, and that familiarity achieved in this manner dramatically decreases the likelihood of subsequent mutual mating behavior. (2) Second, when a pair of Damaraland mole-rats were allowed 20 min of unrestricted access to each other (and thus allowed to mate) *before* the 2-week period of separation by a mesh barrier, the pair exhibited robust mating behaviors when reunited with unrestricted access (Initial Physical Contact Group). We suggest that familiarity per se is achieved in both paradigms (1) and (2). However, the order in which familiarity and physical contact occurs is all important for determining whether a pair of Damaraland mole-rats will establish a sexual bond. *If*

mating behavior occurs simultaneously with the initial stages of familiarity, then a sexual bond is formed, but if familiarity is achieved in the absence of mating there will be no sexual bond. Note that familiarity is a potentially reversible state, as non-breeders from a given colony will treat one another as foreigners following experimental isolation from one another for several weeks (Carter et al. 2014) ---a paradigm that would presumably not occur under field conditions.

From the observations related above, we can formulate a hypothesis to explain the establishment and maintenance of the strict reproductive hierarchy in Damaraland mole-rat colonies: When a pair of opposite-sex Damaraland mole-rats, dispersing from separate colonies, meet in the field they are likely to engage in sexual behaviors as did our pairs of unrelated animals (the unfamiliar pairs in the current experiment). Mating behavior probably will not lead to conception immediately, especially if the female has unstimulated ovaries, as appears to be the case for most dispersers (Hazell et al. 2000). However, a sexual bond will be established so that if the animals remain together, subsequent matings can eventually lead to pregnancy once the female's ovaries become activated (Snyman et al. 2006). When the first litter is born it seems likely that the littermates become familiar with each other and that familiarity is also established between these newborns and their parents *before* the pups/juveniles reach an age where mating is possible. Similarly, as future offspring are produced, they will become familiarized with each other and with all the older colony members *before* they are old enough to express mating behaviors.

It is important to note that there are two untested assumptions implicit in the hypothesis outlined above: First, we have suggested that some aspect of mating behavior

itself is crucial to establishing sexual bonding. The data presented in this study establish that physical contact is crucial (i.e., absence of the mesh barrier); while the mating activity that is expressed in this situation seems most likely to be the primary factor in the formation of a sexual bond, it remains possible that some other aspect of physical contact is involved. Second, our hypothesis assumes that young Damaraland mole-rats can achieve adequate familiarity with each other and with older colony members before they reach an age at which they can express mating behaviors. Both these assumptions are subject to experimental test.

In the present experiment, pairs that were familiarized without any physical contact (i.e., separated by wire mesh barrier for 20 min/day for 14 days) behaved in all respects like sibling pairs in subsequent mating tests. This result clearly supports our hypothesis that animals that become familiarized in the absence of mating interactions will continue to avoid sexual behaviors with each other, as do all the non-breeders within a colony. Males in the group that were allowed 20 min physical contact prior to 14 days of exposure across the mesh barrier behaved like males of unfamiliar (non-sibling) pairs in mating tests. Females in the 20 min physical contact group showed solicitation and lordosis behaviors that were not statistically different from those expressed by unfamiliar pairs, but the absolute amount of solicitation responses was somewhat less than for the unfamiliar controls. Our experimental design was adequate to demonstrate the importance of an initial 20 min period of unrestricted physical interaction for the development of a sexual bond; however, it does not preclude the possibility that a sexual bond is further strengthened by the presumably much longer period of physical interaction that would occur between disperser pairs in the field.

The eusociality exhibited by Damaraland mole-rats may have arisen through refinement of phenomena that are seen in other mammals: First, dispersal from the natal nest occurs in most species that are born in a confined situation, and dispersal usually occurs at approximately the time of weaning in species that do not live in groups (Hamilton & May 1977). In the social mole rats, dispersal is delayed, sometimes by a period of years, and it is not yet clear what circumstances might determine when an individual leaves its natal colony (Braude 2000). Delayed dispersal from the natal nest appears to be an early step in the evolution of insect eusociality as well (Wilson & Hölldobler 2009), and is common among group-living animals in general (Kingma 2018). Second, Damaraland mole-rats appear to exhibit an intensification of the mechanisms that lead to inbreeding avoidance based on familiarity achieved early in life in other species, and this may be sufficient to account for the absence of sexual activity among the non-breeders within a colony. Finally, it is tempting to speculate that the relative independence of female sexual behaviors from ovarian hormones may have evolved in conjunction with the advantage that would accrue to dispersing females (often with undeveloped ovaries) to rapidly bond with dispersing males.

The most widely studied eusocial mammal is the naked mole-rat, a fossorial species that lives in colonies that typically include 60-80 individuals (Bennett & Faulkes 2000). Based on phylogenetic criteria, it has been proposed that eusociality evolved at least twice in the various genera of African mole-rats (family Bathyergidae) and, specifically, that naked mole-rats and Damaraland mole-rats represent separate lines in the evolution of eusociality (Allard & Honeycutt 1992; Jarvis & Bennett 1993). There are a few clear differences between the reproductive hierarchies of these two species: (1)

Naked mole-rat colonies often include 2-3 breeding males, whereas Damaraland mole-rat colonies have not been reported to include more than a single breeding male at any given time (Faulkes & Bennett 2000). (2) When male and female naked mole-rats from different colonies are paired in a neutral arena, sexual behavior is not observed immediately, as it is in Damaraland mole-rats. However, if a pair of naked mole-rats, either from the same or different colonies, are left together, they often reproduce, but this generally requires at least 5-6 months of cohabitation (B Goldman, personal observations). Additionally, a laboratory study revealed that when nonbreeding naked mole-rats are removed from their natal colonies and housed with both siblings and individuals from a foreign colony, they are more likely to breed with foreign animals, suggesting a mechanism that favors outbreeding (Ciszek 2000). (3) For naked mole-rats, it has been proposed that the breeding female enforces a reproductive inhibition over the nonbreeding members of her colony. This inhibition does not appear to be exerted via a urinary pheromone (Smith et al. 1997; Faulkes & Abbott 1993). It has been suggested, but not directly demonstrated, that the breeding female might inhibit reproductive development in other colony members through physical interactions with them. In view of the findings in Damaraland mole-rats, it seems that it would be worthwhile to carefully re-evaluate the generally accepted hypothesis that the breeding female naked mole-rat is directly responsible for the inhibition of sexual activity among the non-breeders.

There is one intriguing exception to the observations that naked mole-rats differ from Damaraland mole-rats in that the former fail to express sexual behaviors during first encounters with foreign individuals of opposite sex. In a laboratory study, it was reported that naked mole-rats that developed a morphology and behavior suggested to be

indicative of transformation to disperser status expressed sexual behavior when paired with foreign naked mole-rats, but not when paired with animals taken from their own colony. All but one of the 19 disperser morphs discovered in that laboratory study were males (O’Riain et al. 1997), though dispersing naked mole-rats identified in a field study consisted of approximately equal numbers of both sexes (Braude 2000). In any event, the observations from the single laboratory study suggest that male disperser naked mole-rats exhibit similar mating activity as did all non-breeding Damaraland mole-rats in the current study. Perhaps the triggering of a propensity to express sexual behaviors is a part of the changes involved in preparation for dispersal in naked mole-rats, whereas in Damaraland mole-rats a similar inclination for sex behavior appears to be present in most or all the non-breeders, awaiting only contact with a foreign (unfamiliar) Damaraland mole-rats to trigger expression, as observed in the present study.

Damaraland mole-rats are not sexually monogamous in the strict sense, since breeders will mate with foreign individuals introduced into the colony (Jacobs et al. 1998; Cooney & Bennett 2000). Indeed, when given a choice between their mate and a breeding individual from a different colony, there was no preference for the familiar breeding partner with respect to duration of sexual interaction with the stimulus animals (Cho and Freeman, unpublished data).

The data from our studies indicate that it is the absence of a foreign individual, rather than the presence of inhibitory stimuli, that accounts for the total inhibition of sexual behaviors among non-breeders in colonies of Damaraland mole-rats. This observation is consistent with earlier reports in this species (Bennett et al. 1996; Rickard & Bennett 1997). Whereas this appears clear for the expression of reproductive

behaviors, it remains possible that the failure of nonreproductive females to exhibit stimulation of the reproductive tract is related to inhibition exerted by the breeding animals. Alternatively, exposure to a foreign individual might be important for stimulation of the ovaries of nonbreeding females just as it is for evoking sexual behaviors. Indeed, there is some indication that exposure to a foreign DMR may lead to ovarian stimulation. In one study, only 1 of 6 non-breeding female Damaraland mole-rats housed in isolation showed evidence of ovulation after 11 weeks, whereas 5 of 6 females ovulated when housed with foreign vasectomized males (Snyman et al. 2006). There is evidence that a parallel phenomenon may occur for non-breeding male Damaraland mole-rats, as exposure to unrelated females resulted in a rapid change in testis morphology and an increased level of follicle-stimulating hormone receptors in the testis (Nice et al. 2010).

The present results suggest that the strict reproductive hierarchy that is a basis for Damaraland mole-rat eusociality may result from conditions in place during the earliest interactions via which any pair of opposite-sex Damaraland mole-rats become familiar with each other. Namely, if familiarity is achieved and sustained in the absence of sexual behaviors, then the pair will continue to refrain from mutual sexual behavior. But if the initial stage of familiarization is coincident with the display of mutual sexual behavior, a long-lasting sexual bond is formed. With these conditions in place, the presence of the breeding pair does not appear to be required for inhibition of reproductive activity among the non-breeding members of the colony. The information gleaned from the present study provide for the first data-based paradigm to explain how the reproductive hierarchy is established and maintained in colonies of a eusocial mammal. In broader context, these

observations suggest that a strict reproductive hierarchy can be established and maintained in a social species by a relatively simple mechanism, based on avoidance of mating with familiar individuals while retaining a propensity to mate with unfamiliar individuals.

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Simulated Dispersal: The Occurrence of Mating Behavior Between Siblings Following Removal from the Colony

Introduction

Eusociality is a social system in which animals live in colonies with overlapping generations of adults. Only a few individuals directly engage in reproduction, while the remaining members are non-reproductive and help to raise their siblings. Eusociality is widespread in bees, wasps, ants and termites (Wilson & Holldobler 2009). In DMR, only one female and one male within the colony reproduce, resulting in high reproductive skew. Currently, only two mammalian species meet the criteria for eusociality, the Damaraland mole-rat (DMR; *Fukomys damarensis*) and the naked mole-rat (NMR; *Heterocephalus glaber*) (Bennett & Faulkes 2000). DMR colonies are composed of the breeding female (often termed the “queen”), her breeding partner, and their offspring. Average colonies consist of roughly 16 individuals, ranging from 2 to 41. The non-breeding offspring do not expend energy to increase their fitness directly; they contribute to the reproductive effort of the breeding pair by helping to care for their siblings, forage, defend the colony from predators, and maintain and enlarge the burrow system (Bennett & Faulkes 2000).

Mating naturally occurs between unfamiliar individuals from separate colonies (Jacobs et al. 1998). However, DMR practice strict inbreeding avoidance within their natal colonies, possibly preventing the deleterious effects that can result from inbreeding and as a result, DMR are considered obligate out-breeders (Cooney & Bennett 2000). For example, non-breeding females do not express sexual behavior (solicitation, lordosis) with colony mates under normal circumstances. These females do not undergo puberty

while in their natal colony, retaining unstimulated ovaries and uteri even after reaching adult body size (Holmes et al. 2009). This developmental characteristic alone may not prevent sexual behaviors from occurring with familiar males, as pairing with an unfamiliar male results in expression of sexual behaviors within minutes, without prior exposure to gonadal hormones (Cooney & Bennett 2000; Nice et al. 2010; Carter et al. 2014). Among the relatively few mammals in which ovarian hormones are not required for support of sexual behavior are humans and some non-human primates; in these animals, sexual behaviors have social functions in addition to direct reproduction, and the relative hormone-independence of sexual behaviors allows these behaviors to be expressed in the context of social functions throughout the ovulatory cycle (Baum et al., 1978; Wallen & Zehr 2004). Observations that non-breeding female DMR with unstimulated ovaries will readily mate with foreign males (Rickard & Bennett 1997) and that mating behavior can occur independent of gonadal hormones in both sexes (Carter et al. 2014), suggest that mating behavior may have some function in DMR in addition to its function for direct reproduction.

The expression of sexual behaviors in DMR does not depend solely upon exposure to an unrelated conspecific. In one study, opposite sex siblings expressed mating behavior with one another when separated for a period of 5 weeks (Carter et al. 2014). DMR appear capable of discriminating individuals based upon familiarity and not genetic relatedness. While familiarity appears necessary to maintain a sibling-like relationship among siblings within a colony, familiarity does not preclude mating between members of a breeding pair, who can be considered just as familiar as non-breeding colony mates. Thus, familiarity per se is insufficient to explain mate choice in

DMR. The difference in the expression of mating behavior between siblings and breeding pairs might depend upon what behaviors occur the first time two individuals meet. Newborns, incapable of physically engaging in mating behavior, become familiar with older siblings during the first days of life resulting in a long-term sibling-like relationship. Conversely, when unfamiliar opposite-sex individuals are paired, they typically exhibit mating behavior within minutes of meeting (Carter et al. 2014). Both pairs become “familiar” over time. Manipulating the behavior expressed during the initial interaction of two individuals affects the presence or absence of mating behaviors in subsequent, unrestricted interactions. If mating is prevented in the first meeting, mating frequency is significantly lower in future unrestricted interactions when compared to pairs who experience unrestricted physical contact in their first meeting (Kelley et al. 2019).

The long-term relationship may be determined early, but the pair needs consistent interactions to maintain the relationship, as shown by sibling pairs who will mate after a period of separation (Carter et al. 2014). Interactions occur frequently in the confines of the burrow system. However, upon removal from the colony, mating behavior manifests within sibling pairs within a period of five minutes, an occurrence typically limited to unfamiliar, opposite –sex pairs (personal observation). It is unlikely that familiarity is lost in that amount of time. The act of being removed from their natal colony may initiate the expression of mating behaviors. The aim of our present study is to understand under what conditions mating behavior may manifest between sibling pairs, without introducing long periods of separation between individuals.

Methods

Animals

DMR used in this study came from colonies housed at the University of Memphis (originally provided by Dr. Bruce Goldman at the University of Connecticut). Their diet consists of ad libitum sweet potatoes supplemented with dry rodent pellets (Harlan 2019, 19% protein diet). Each colony is housed within a complex constructed of two different sized cages (60x40x20cm and 48x25x20cm) connected by varying lengths of extruded polycarbonate tubing to roughly simulate natural burrow architecture. The number of cages and lengths of tube are dependent upon the size of the colony and all colonies contain a 1:1 mixture of corncob and pine bedding. The animals used in the study were adults ranging from 2 to 5 years old; DMR can live at least 15 years in captivity (Holmes et al. 2009). All experimental procedures and husbandry were approved by the University of Memphis Animal Care and Use Committee (Protocol #797) and comply with the criteria established by NIH Guide for the Care and Use of Laboratory Animals.

24 pairs of opposite sex siblings were selected from colonies that either did or did not contain an active breeding pair at the time of the study. Thus, we used 12 pairs from colonies without a breeder, and 12 pairs from colonies with active breeders. Opposite-sex sibling pairs were randomly assigned from a pool of individuals within the colony that met our criteria (individuals within 2-5 years of age).

Treatment

The sibling pairs were housed in their colonies prior to experimentation, with no disturbance except for routine care and maintenance (daily checks and feeding). On days

of testing 4 pairs, chosen at random from our pool of 24, were removed and transported from the animal care facility to the laboratory, a process that included: removal by hand, placement into a cage, and transportation on a cart via elevator. The entire process is completed in approximately 10 minutes. We transported siblings together in a single cage (48x25x20cm) consisting of fresh bedding material. To maintain consistency, all pairs experienced the same amount of time outside of their natal colony (~90 minutes) regardless of behavior expressed, with the exception of aggression and fighting, which would have resulted in separation of the siblings and return to the colony one at a time, but no instances of aggression were observed. Following transport to the lab, we observed each pair and recorded all instances of mating behavior, including female solicitation (backing the anogenital region toward the male's head) and lordosis (arching of the spine, deflection of the tail, and immobility), male mounting and thrusting. All tests were recorded using a JVC GZ-MG21U camcorder and behaviors of both males and females were scored from the recordings. Videos were scored using The Observer software (Version 6.1, Noldus Information Technology, Leesburg, VA). Following testing, sibling pairs were returned to their natal colonies and observed for a period of 20 minutes to assess the effect of their removal on the colony as a whole.

Control

We used the same sibling pairs for both the control and the treatment group. We observed the pairs both prior to and after the treatment described above. We spaced all tests and observations two weeks apart to analyze if: 1) mating behavior is present between siblings within the colony prior to removal, and 2) treatment in affects the

previously established sibling relationship. Animals in the control situation experienced no perturbation and sibling pairs were allowed to function as usual within the confines of their colony. Under this condition, we visited the animal care facility and observed the siblings within their colony. Male and female subjects were marked to aid in rapid identification, as there is little morphological variance in this species. We were looking specifically at mating behavior, so we only recorded instances of female solicitation (backing the anogenital region toward the male's head) and lordosis (arching of the spine, deflection of the tail, and immobility), male mounting and thrusting, as we did within the experimental group.

Results

Effects of Removal from the Colony

The proportion of sibling pairs that expressed mating behavior after removal from their natal colony was significantly greater than the proportion of pairs that expressed mating behavior while in the colony (Fig. 1; 9/24 vs 0/24, respectively; Fischer's exact; $p=.0016$).



Figure 1. The proportion of sibling pairs that expressed mating behavior either is the treatment group that involved removal from the colony, or undisturbed within their natal colony. Different letters indicate significantly different groups (Fischer’s $p < .05$). For “Outside of Colony” condition, the proportion is 0.375, or 9/24 pairs who exhibited mating behavior. For “Within Colony, the proportion is 0.0, or 0/24 pairs who exhibited mating behavior.

Effects of Active Breeders within Colony

The proportion of sibling pairs, from colonies without an active breeding pair, that expressed mating behavior after removal from the colony was significantly higher than the proportion of mating behavior expressed within siblings pairs from colonies with an active breeding pair when the siblings were removed from their natal colony and paired in the lab (Fig. 2; 7/12 vs 2/12, respectively; Chi-Square; $p = .035$). However, analysis with Fischer’s Exact does not reveal significance ($p = .089$).

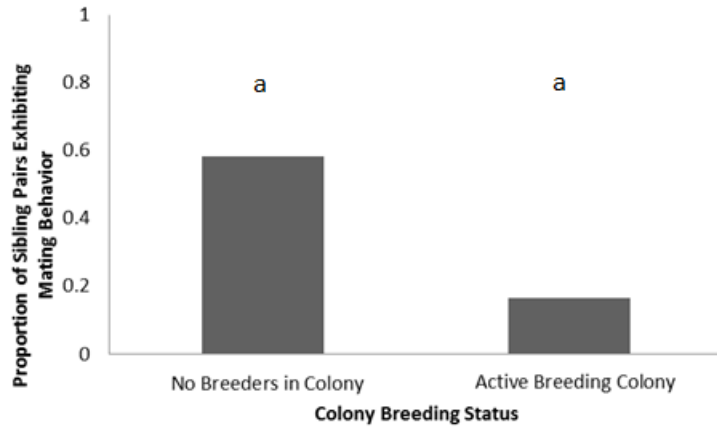


Figure 2. The proportion of sibling pairs that exhibited mating behavior split by the status of their natal colony in terms of breeding activity. Sibling pairs from a colony with an active breeding pair are sorted into the “Active Breeding Colony” group, while pairs from colonies without a breeding pair are sorted into the “No Breeders in Colony” group. Different letters indicate significantly different groups (Fischer’s $p < .05$). For “No Breeders in Colony,” the proportion is .583, or 7/12 pairs who exhibited mating behavior. For “Active Breeding Colony,” the proportion is .167, or 2/12 pairs who exhibited mating behavior.

Sex Differences in Frequency of Mating Behavior Expression

The proportion of animals that exhibited mating behavior did not differ significantly between males and females in siblings that were removed and paired outside of the natal colony (Fig. 3; Chi-square; $p = .873$). More males than females expressed mating behavior (9/24 vs. 6/24, respectively), however there was no significant difference. In 4 cases, males were the only sibling to express mating behavior towards their unresponsive female siblings, in contrast to only 1 case where the female was the only sibling to express mating behavior (data not shown).

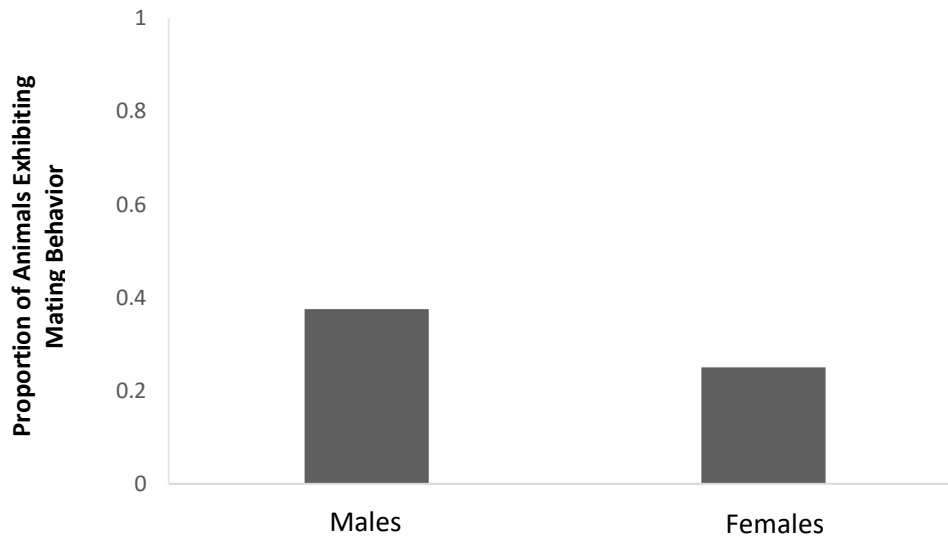


Figure 3. The proportion of animals that exhibited mating behavior, split by sex. All pairs consisted of siblings from the same colony and were paired outside of their natal colony in the testing arena. Different letters indicate significantly different groups (Fischer's $p < .05$). The proportion of males that exhibited mating behavior was 9/24 (0.375), while the proportion of females who exhibited mating behavior was 6/24 (0.25).

Discussion

The DMR is considered fossorial, living underground in Southwest Africa (Namibia), an area with low annual rainfall (~16") and thus, dry desert soil. Excavation of this soil requires a lot of energy and some individuals will increase their energetic output following heavy rainfall, as excavation becomes easier during this time (Scantlebury et al. 2006). Correlated with these periods of heavy rainfall is the dispersal of adult nonbreeding individuals. Dispersal is the natural equivalent to artificial removal from the colony, as we have done in this experiment. In nature, new Damaraland mole-rat colonies are formed when non-breeding individuals go above ground to disperse from their natal colonies and pair with other dispersers, most likely from a different colony. Dispersal of individuals from the natal burrow is thought to be the sole mechanism for the formation of new colonies (Bennett & Faulkes 2000). Dispersal, in order to find a

potential mate, occurs when ecological constraints have been relaxed (e.g. the period after heavy rainfall). Individuals may then go above ground and attempt to locate an unfamiliar opposite-sex conspecific to form a new colony (Young et al. 2010). Dispersal is not a consistent life history event, as fluctuations in rainfall influence when dispersal can occur. With such limited dispersion and sporadic distribution of colonies, less than 8% of individuals achieve reproductive success (Burland et al. 2004).

Dispersal exposes DMR to novel stimuli, as well as the challenge of finding a mate and building a new burrow system, all of which may initiate endocrine activity through the hypothalamic-pituitary-adrenal (HPA) axis and a general “stress response.” Cortisol and corticosterone (glucocorticoids) are associated with HPA activation and are functional in an organism’s response to acute and chronic stressors. Common measures used to determine the activation of the stress response in animals including: increased serum concentrations of glucocorticoids (Sheriff et al. 2011), increased CRH secretion (Wiersma et al. 1995), and increased catecholamine signaling (De Boer et al. 1989). Endocrine activity, much like ecological challenges, can be deleterious to a dispersing individual. Juvenile male arctic ground squirrels (*Spermophilus parryii plesius*) experience chronic stress as a result of dispersal, indicated by poor immune response to antigen challenge coupled with high cortisol concentration. These males can lose up to 18% of their body mass during the time of dispersal (Boonstra et al. 2001). In the western screech-owls, a non-migratory species, plasma corticosterone concentration elevates to peak around 8 weeks post-fledging, when the juveniles are dispersing from their natal nesting territory (Belthoff & Dufty 1998). In most rodent species, “stress” inhibits the hypothalamic-pituitary-gonadal (HPG) axis and expression of sexual behaviors (Rivier &

Rivest 1991). Traditionally, elevated glucocorticoid concentration as a result of stress alternatively means that the individual has reduced sexually reproductive capability through decreased responsiveness of the gonads to luteinizing hormone (LH). This situation creates the classic “trade-off” between survival and reproduction, in terms of energy allocation (Wingfield & Sapolsky 2003). Stress may inhibit reproduction via actions of catecholamines, activation of the hypothalamo-pituitary-adrenal (HPA) axis involving hypothalamic corticotrophin-releasing hormone (CRH), glucocorticoid actions on the HPG axis, or a combination of these factors (Romero & Butler 2007). However, exceptions exist where reproductive function is not negatively correlated to the stress response. In the side-blotched lizard (*Uta stansburiana*), males with corticosterone implants have greater locomotor performance (higher stamina) than those implanted with saline shams. Increased stamina is adaptive in predator avoidance, territory acquisition, and location of potential mates. Males therefore have increased fitness as a result of elevated corticosterone (Miles et al. 2007). Bonobos (*Pan paniscus*), a highly social species, exhibit socio-sexual behaviors, presumably as a means to decrease social tension following aggressive encounters (Hohmann et al. 2008). Whether or not removal from the colony leads to elevated plasma corticosterone in Damaraland mole-rats was outside the scope of the present study. However, the act of removal from the colony, either through artificial means or natural dispersal, may initiate a similar response that ultimately leads to the expression of mating behavior.

The dispersing Damaraland mole-rat may be subject to multiple sources of HPA axis stimulation. Highly social species have been studied on their response to stressful stimuli both while in a social environment as well as when isolated. Isolated individuals

display a more dramatic physiological response to stressful stimuli, termed “isolation syndrome” (Kikusui et al. 2006). Isolated male rats reflect this paradigm, as their response to stressful stimulation was greater than males in social groups, despite no difference in baseline plasma corticosterone concentration (Weiss et al. 2004). Urinary cortisol concentration increased for adult non-reproductive Damaraland mole-rat females during periods of heavy rainfall, when dispersal is most likely to occur. Interestingly, these females also showed a stronger response to a GnRH challenge during the rainy season, evidenced by higher plasma LH concentration following the challenge than seen during the dry period. Apparent up-regulation of reproductive physiology, through increased sensitivity to GnRH accompanied increased cortisol concentrations (Young et al. 2010). The Damaraland mole-rat may represent an interesting and uncommon example of reproductive activity functioning in concert with stimulation of the HPA axis. Non-breeding individuals rarely experience reproductive opportunities with unfamiliar conspecifics, if ever, so it is vital to the fitness of individuals to be physiologically prepared for reproduction, even in the stressful environment that accompanies dispersal. Unfamiliar individuals will engage in sex behaviors with one another upon introduction (Carter et al. 2014). Transporting colonies from the animal care facilities to the laboratory, as we have done, results in the presence of sex behaviors between opposite-sex siblings, a situation that does not occur in an undisturbed colony.

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Mate Choice and Partner Fidelity

Introduction

The Damaraland mole-rat (DMR; *Fukomys damarensis*) exhibits eusociality, a degree of sociality defined by animals living in colonies with overlapping adult generations. Only a few individuals reproduce, while the remaining colony members, the offspring of the breeding individuals, are non-reproductive. Typically, in cooperatively breeding species, non-breeding individuals (helpers) contribute to the reproductive success of a breeding pair, eliminating competition by foregoing their own reproductive efforts (Krebs et al. 1993). Eusociality is widespread in bees, wasps, ants and termites (Wilson & Holldobler 2005). Only two mammalian species are traditionally considered to satisfy the criteria for eusociality: The Damaraland mole-rat and the naked mole-rat (*Heterocephalus glaber*) (Bennett & Faulkes 2000). However, a greater number of social mammals are considered to present eusociality, including the Ansell's mole-rat (*Fukomys anselli*), other species within the *Fukomys* genus, and many social vole species (Burda et al. 2000; Wilson & Holldobler 2005; Patzenhauerova et al. 2013).

Reproductive skew is high within DMR colonies and reproduction is typically limited to one breeding female and her mate (Holmes et al. 2009). This characteristic suggests a monogamous mating system. The presence of high reproductive skew across the entire species (~92% of DMR never achieve breeder status; Burland et al. 2004) indicates that mating opportunities are rare and thought to arise solely through dispersal of individuals from their natal colonies. Dispersal is not a function of age for DMR; dispersal rates appear to correlate with periods of heavy rainfall, when soil conditions become much more favorable for excavation. Individuals may then go above ground and

attempt to locate an unfamiliar opposite-sex conspecific to form a new colony (Hazell et al. 2000; Young et al. 2010), although dispersal may occur below ground as well (Jarvis & Bennett 1993). Given the conditions in which they live (arid deserts of southwest Africa), rainfall, and therefore dispersal, are infrequent events. Monogamy may appear to be the system of mating simply due to lack of available mating opportunities. In fact, a breeding female may mate with more than one male, as parentage analysis indicates that multiple males sire offspring within colonies, although infrequent (Holmes et al. 2009; Burland et al. 2002; Burland et al. 2004). This is most likely a result of dispersal events leading to the introduction of foreign males into a colony, as DMR practice strict incest avoidance, possessing mean colony relatedness values of ~ 0.46 (Rickard & Bennett 1997; Burland et al. 2002). Indeed, DMR colonies that have had their breeding pair removed will remain reproductively quiescent, up to five years in captivity, until a foreign animal is introduced or the colony disbands, as would most likely occur in the wild (Jarvis et al. 1994). Any mixed parentage within a colony may then be due to immigration of foreign individuals and not through the practice of inbreeding.

DMR appear capable of “kin discrimination” as a function of familiarity and not genetic relatedness, *per se* (Carter et al. 2014; Kelley et al. 2019). Non-breeding DMR females exhibit sex behaviour immediately upon introduction to an unfamiliar male, even though sex behaviour is absent within the colony between siblings (Jacobs et al. 1998). Carter et al. (2014) demonstrated that siblings separated for a period of 5 weeks express mating behaviour with the same frequency as unfamiliar pairs, while Kelley et al. (2019) demonstrated that unfamiliar opposite-sex pairs can be made to treat one another as siblings if they are familiarized without the opportunity to express mating behaviours,

highlighting the importance of mating early to establish a long-term breeding relationship. These studies not only reveal plasticity in relationship status among DMR, they also highlight the tendency for opposite-sex individuals to express mating behaviour with one another if they are perceived as unfamiliar. However, breeding pairs can remain together for years, consistently mating and reproducing. These animals can be considered familiar, through consistent spatial-temporal interaction.

The aim of our current study is to examine if breeder pairs, considered familiar, express mate fidelity, when there appears to be a preference to mate with unfamiliar individuals throughout DMR. Prairie voles (*Microtus ochrogaster*), are considered monogamous with high mate fidelity based on several characteristics, including: 1) long-term male and female association across breeding and non-breeding seasons; 2) aggressive xenophobia towards unfamiliar conspecifics; and 3) biparental care of offspring (Carter et al. 1995). In preference tests, in which females are given a choice between spending time with their mate, in isolation, or with an unfamiliar animal, female prairie voles spend significantly more time with and mating with familiar mates (Carter & Getz 1993). We look to replicate this approach in DMR to test the fidelity of social monogamy.

Methods

Animals

Damaraland mole-rats used in this study came from colonies housed at the University of Memphis (originally provided by Dr Bruce Goldman at the University of Connecticut). Their diet consisted of ad libitum sweet potatoes supplemented with dry rodent pellets (Harlan 2019, 19% protein diet). DMR were maintained on a 24-hour light

cycle. Each colony was housed within a complex constructed of two differently sized cages ($60 \times 40 \times 20$ cm and $48 \times 25 \times 20$ cm) connected by varying lengths of extruded polycarbonate tubing to roughly simulate natural burrow architecture. The number of cages and lengths of tube were dependent upon the size of the colony and all colonies contained a 1:1 mixture of corncob and pine bedding. The animals used in the study were adults ranging from 2 to 5 years old; Damaraland mole-rats can live at least 15 years in captivity (Holmes et al. 2009). All experimental procedures and husbandry were approved by the University of Memphis Animal Care and Use Committee (Protocol no. 797) and complied with the criteria established by U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Testing Arena

All animals selected for this study were breeders for their colony at the time of the study, to eliminate any variables that might be present when matching breeders and non-breeders (Holmes et al 2009 explore neuroendocrine differences between breeder and non-breeder brains). Behaviour tests were conducted in a tri-chambered apparatus, where a central chamber contained doorways leading to each of the other two chambers. The animal being measured for preference began in the central chamber with unrestricted access to each chamber. The two side chambers contained stimulus animals for the preference test and were both the opposite sex of the animal in the central chamber. The side chambers were separated by a dark wall so that the animals within would not encounter one another. To ensure that each stimulus animal stayed within their chambers, metal snapping keyring “collars” were placed around the necks of the stimulus animals

and were loosely tied to a mounted bracket on the wall of the chamber. No bedding was used during testing and the entire apparatus was washed thoroughly and dried between tests with 70% ethanol.

Behaviour Tests

Animals were removed from their colonies and placed within the testing arena in the appropriate chambers. If the behaviour test was analysing female preference, a female breeder was placed in the central chamber and two breeding males were placed in each of the other two chambers and vice versa for male preference. Animals were given five minutes to acclimate to their new environment before the behaviour test was conducted. In either preference test, one of the stimulus animals was the breeding partner of the animal in the central chamber (they came from the same colony and were that colony's breeding pair). The other stimulus animal was another breeder from a separate colony. Once each animal was in the appropriate chamber and acclimated, wire screens covering the two small doorways were removed and the animal in the central chamber could explore anywhere in the testing arena for 20 minutes, after which time testing ceased and the animals were removed from the arena. This protocol was followed for each animal in the study and served as our "preference test." If males were stimulus animals and the female was free to roam, the test was considered "female choice". The reverse was true for "male choice" tests.

Behavioural Scoring and Statistical Analysis

We recorded the total amount of time each test animal spent in each of the three chambers (in isolation; with partner; with stranger). Furthermore, we recorded all instances of mating, including female solicitation (backing the anogenital region toward the male's head) and lordosis (arching of the spine, deflection of the tail and immobility), male mounting and thrusting. The duration of any sexual behaviour described above, expressed by the test animal with each of the stimulus animals, was recorded. All tests were recorded using a JVC GZ-MG21U camcorder, and behaviours of all test animals were scored from the test recordings by a researcher blind to the experiment. Taken together, the two variables (time investigating and time mating) defined "preference" for our procedure. Data was analysed in StatView using repeated measures ANOVA with Fisher's PLSD post-hoc analysis. Significant levels were set at $p < 0.05$.

Ethics Statement

Animal care personnel and researchers received special training from the university veterinarian to help minimize stress during handling of the animals. The experimental design allowed for the number of individuals used to be kept to a minimum (N=16; 8 males, 8 females). All colonies were housed in caging systems that are designed to mimic their natural burrow system (see "Animals" above). To reduce stress, mole-rats were always handled carefully to avoid undue noise or vibration. They were transported to the testing room on a heavy-duty thermoplastic rubber cart to reduce vibration and noise, and after transfer to the testing room, they were allowed 15 min to acclimate prior to all individuals simultaneously being placed in the testing arena. Once placed in the

arena, animals were given an additional 15 minutes to acclimate. All interactions were monitored continuously, and individuals would be separated immediately if fighting or aggressive behaviour was observed, although no such interventions were necessary.

Results

Time Spent in Testing Arena

In our preference test, female breeders did not show any preference for amount of time spent in isolation, with their partner, or with an unfamiliar male breeder (Fig. 1; $p=.563$). For males, there was a significant difference between amount of time spent across the testing arena, with males showing a preference for the chamber containing the unfamiliar female (Fig. 1; $p=.0318$). There was no difference in the amount of time spent in isolation and time spent with their breeding partner for males ($p=.753$) or females ($p=.881$). Comparing the time spent by the test animal between the two stimulus animals, we did not find a significant difference for females ($p=.402$). However, we saw a significant difference for males in time spent between their breeding partner and an unfamiliar female, with a preference for the unfamiliar female ($p=.0303$).

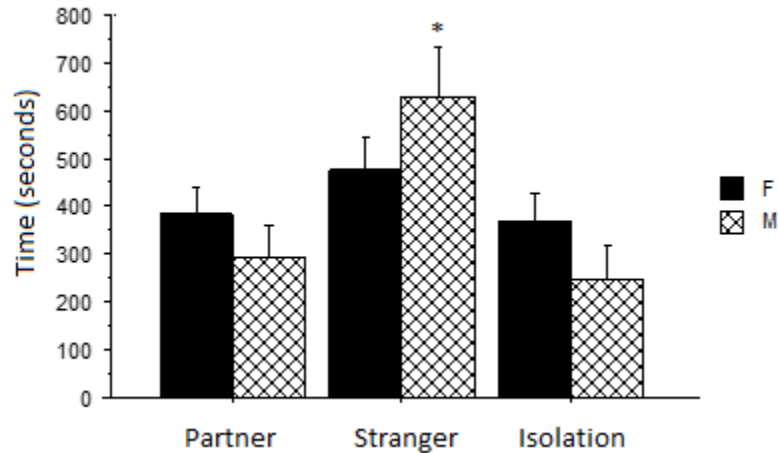


Figure 1. Amount of time in seconds spent by the test animal in each of the three testing arena chambers. Female test animals do not show a preference for any of the three chambers ($p=.563$). Males show a preference for the chamber containing an unfamiliar female breeder ($p=.0318$). Significant differences are indicated by “*” above the column.

Duration of Mating Behaviour

Mating behaviours were expressed to some extent by all but three of the test animals ($n=18$). Male and female test animals expressed mating behavior with either their partner alone (1/18); the unfamiliar breeder alone (3/18); or with both their partner and the unfamiliar animal (11/18) within the 20-minute testing period. The duration spent mating with each stimulus animal is represented in Figure 2. Females did not show a statistically significant preference for neither their breeding partner nor the unfamiliar breeding male in duration of mating (Fig. 2; $p=.298$). Male test animals did not show a difference in duration mating between their breeding partner and an unfamiliar breeding female either (Fig. 2; $p=.153$).

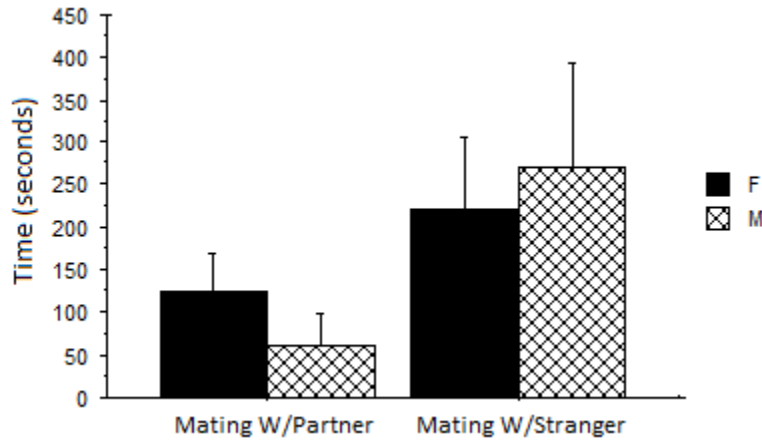


Figure 2. Amount of time in seconds spent by the test animal mating with either their breeding partner or the unfamiliar breeding conspecific. Females do not show a preference for either their partner or an unfamiliar breeding male ($p=.298$). Males do not show a preference for either their partner or an unfamiliar breeding female ($p=.153$).

Discussion

Damaraland mole-rats form colonies that can remain together for years, with the breeding pair mating frequently and producing up to three litters a year (gestational length is almost three months long). Following parturition, the male and female will mate frequently for about two weeks, until the female becomes pregnant again (Bennett & Jarvis 2004). This system can apparently continue until one of the breeders dies or is removed, at which point the colony would most likely disband or continue with suspended reproductive activity. If a dispersing unfamiliar individual enters the colony, reproductive activity will be rekindled, with multiple colony members attempting to mate with the foreign individual (Rickard & Bennett 1997; Jacobs et al. 1998). Burland et al. (2004) explored the presence of immigrants within colonies that contained mixed-parentage offspring, concluding that dispersal events bring new individuals into colonies and that immigrants are typically males. Dispersal is not a consistent life history event, as

fluctuations in rainfall influence when dispersal can occur. With limited dispersal and immigration into colonies, DMR might be considered monogamous due to the unavailability of extra-pair copulations.

Consistent with previous studies, test animals mated with unfamiliar stimulus animals (Fig. 2). However, this behavior still occurred when the test animals were given equal access to their familiar breeding partner. Although not statistically significant, males were scored mating 3 times longer, on average, unfamiliar females compared to mating with their familiar breeding partner (Fig. 2). Males tended to either express mating behavior for a large amount of time with strangers (half of the males spent 6 or more out of 20 minutes mating with the stranger; range: 5-17 minutes), or little time at all (half the males mated for 1 minute total or less; range 0-1 minute), with 3 males expressing no mating behavior at all with the unfamiliar female, such that males mated with the unfamiliar stimulus female in 6 out of the 8 tests. In contrast, only 3 out of 8 males expressed mating behavior with their breeding partner, and all 3 tests totaled 5 minutes or less (range: 1-5 minutes). Results appear to indicate that the presentation of an unfamiliar female to a male who is in a successful breeding relationship evokes an elevated mating response (commonly called the “Coolidge effect”), a phenomenon that has been described in numerous species (Dewsbury 1981). The lack of statistical significance in our results could be explained by the limited sample size and high within-group variance, although the trend appears to indicate that males preferred unfamiliar females over their familiar breeding partners.

Neither males nor females show preference for their breeding partner, measured by the amount of time spent investigating or mating with each other, indicating a lack of

partner preference and therefore a system that cannot be considered monogamous (Fig. 1; Fig. 2). Prairie voles express antagonistic behaviors to a significantly higher degree in the presence of unfamiliar, opposite-sex conspecifics, than they would in the presence of their breeding partner (Carter & Getz 1993). Xenophobic behaviour towards unfamiliar individuals is a characteristic of the prairie vole model of monogamy clearly absent in DMR individuals at most times; unfamiliar males and females attempt to mate when paired (Fig. 2). None of the tests we performed in this study had to be terminated prematurely due to aggressive behaviors between unfamiliar pairs, indicating a lack of xenophobia in opposite-sex unfamiliar dyads. In special circumstances, female DMR do begin displaying aggressive behaviour towards unfamiliar males, although these instances appear to be related to the reproductive status of the breeding female. If the breeding female is nearing parturition, resident females attack foreign males with significantly greater frequency than if the breeding female is not pregnant (Jacobs et al. 1998). The most common instance of xenophobia in DMR occurs in the presence of immigrating individuals, who receive aggressive behavior from resident individuals of the same sex (Jacobs et al. 1998). Xenophobia may be a misnomer in most cases of aggression, as Cooney (2002) described that the majority of aggressive acts are displayed by the resident breeder against immigrants of the same sex, and non-breeding colony members participate rarely in the aggression. This highly specific form of xenophobia might be an adaptation of mate guarding.

We have seen in our results that DMR readily mate with unfamiliar opposite-sex conspecifics (Fig. 2). This response could be an adaptation to their life history and relatively low mating opportunities DMR experience in the wild. Perhaps the default

response for DMR is to engage in the mating opportunity if the social situation is appropriate (colony is not actively breeding).

This might be true even for DMR that achieved reproductive status, as our tests shows a lack of partner preference (Fig. 1; Fig. 2). What inhibits this response might be the presence of the breeding partner, who engages in a competitive manner with the immigrant, leading to a mating system functioning around mate guarding, not necessarily genetic monogamy. If present at the time of introduction, resident breeders display aggression towards same-sex immigrants (Cooney 2002). If they are absent, multiple opposite-sex colony mates attempt to mate, and same-sex animals may or may not show express aggressive behaviors (Jacobs et al. 1998). Prior to introduction of foreign males into a colony in laboratory settings, dominant breeding males are removed, as their presence alone would result in intense and potentially fatal aggression towards the foreign male (Cooney & Bennett 2000). Resident females, however, may begin to engage in aggressive acts towards one another; aggression that was absent prior to the introduction of the foreign male. This may be a result of multiple females attempting to mate with the male, leading to aggressive competition as a new hierarchy is established. The foreign male may eventually take over the role of breeder, as any other colony male would be related to and familiar with the breeding female. “Mate guarding” appears early on in these instances, before a breeding relationship is established, and is directed towards familiar colony mates (Rickard & Bennett 1997; Cooney & Bennett 2000). Few studies introduce foreign females to colonies, as most dispersing DMR are thought to be male (Burland et al. 2004). However, in cases where foreign females have been introduced, in both reproductively active and inactive colonies, resident females attack

the foreign female aggressively, with a stronger aggressive response than non-breeding males when faced with a foreign male (Rickard & Bennett 1997; Jacobs et al. 1998; Cooney & Bennett 2002; Cooney 2000). In extreme cases, the foreign female will kill resident females once she has achieved dominant breeding status (Jacobs et al. 1998). This is perhaps a degree of mate guarding and exclusion of mating opportunities, both present and future. In the case of a foreign male entering the colony, non-breeding daughters of the breeding female that have successfully mated with the foreign male usurped the role of breeder from their mothers (Cooney & Bennett 2000). Protecting mating opportunities, especially when they are so rare, appear to generate the social monogamy observed in DMR colonies.

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Conclusion

The research on the Damaraland mole-rat mating system described here has provided significant insight on one particular aspect of eusociality: high reproductive skew across the species. Within a colony, mating is limited to one breeding pair. Based on our results in chapter 1, we see that this may in part be a function of siblings familiarizing with one another in the absence of mating. Two unfamiliar individuals that mate during their first interaction are more likely to form a mating relationship, evidenced by subsequent pairings where sex behavior is expressed, than two unfamiliar individuals that do not mate with one another. Indeed, newborn offspring are unfamiliar with each new individual they encounter, yet they are incapable of expressing mating behavior, setting the foundation for a sibling-like relationship. With this occurring for each opposite-sex sibling dyad throughout the colony, high reproductive skew is generated, and mating is limited to a single pair that have previously formed a breeding relationship. Even when the breeding pair are removed, mating does not arise between siblings, as shown in chapter 2. Only when siblings are removed from the colony and paired does mating behavior manifest within the dyad. Chapter 2 examined the difference between siblings from colonies with active breeders and those without in the frequency of this spontaneous mating behavior as a function of being removed from the colony. From these two studies we drew conclusions that mate choice is influenced by familiarity but is also context specific to the point of superseding familiarity under certain conditions.

Chapters 1 and 2 examined mate choice in non-breeding individuals, yet breeder mate choice had been dismissed in these studies. Chapter 3 examined the apparent monogamy on display in Damaraland mole-rat colonies, by testing mate fidelity in simple

preference tests for breeding pairs. Our results indicate that neither male nor female breeders show a preference for their breeding partner when given a choice between them and another opposite-sex unfamiliar breeder. Low rates of dispersal and immigration into the colony may contribute to the appearance of monogamy in this species but our results, along with those of others', describe a system functioning under mate guarding more so than fidelity. Supporting this conclusion are the situation-specific displays of xenophobia in Damaraland mole-rats described in chapter 3.

The benefit of the Damaraland mole-rat as an animal model lies in its unique social system. Eusociality is exceedingly rare among mammals (2 out of over 6000). All studies described have helped contribute to the understanding of how eusociality is maintained. Additionally, results have provided insight on how eusociality could have evolved within the Damaraland mole-rat.