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Large-Scale Surveillance of Captive Naked Mole-Rat Colonies Shows Caste Differences in Space Utilization

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Abstract

African naked mole-rats are eusocial mammals that provide unique opportunity to study complex mammalian social behavior and large-group dynamics in a controlled vivarium setting. Previous reports of captive and wild naked mole-rats have identified a division of labor among non-reproductive colony members along a size polyethism, with large animals specializing in defense behaviors, and small animals performing foraging, nest building, and caretaking functions. This study utilized radio frequency identification (RFID) and advanced computational approaches to monitor the activity patterns and place preferences of all members in two naked mole-rat colonies (N = 36 and 37 animals) for a period of 26 days. Results demonstrated colony differences in- and therefore suggested social regulation of- patterns of colony behavior. Mapped onto different colony rhythms were more universal rules for space preferences depending upon the size and role of the individual: the Queen/Male breeders and the large workers spent almost all of their time in and around (colony-defined) nest chamber, while the smaller workers were more likely to visit the (experimenter-defined) feeding chamber and other areas of the semi-natural habitat. Earlier claims of “lazy” large colony members, were not confirmed, as there were no differences in activity or measures of stationary hours between castes of workers. Animals in the breeder castes demonstrated among the fewest stationary hours of all colony members, with a high concentration of activity around the nest chamber. These findings provide a unique insight to patterns of activity that would be difficult to identify visually and allow for a better understanding of individual contributions to naked mole-rat colony behavior.

Keywords: Convergent Evolution, Habitat Utilization, Nesting, Colony Behavior, Eusociality, Heterocephalus Glaber

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Evolution, driven by global and niche conditions, selects morphology, as well as specific feeding, reproductive and social behaviors. In many separate cases, the emergence of harsh foraging conditions has directed multiple species across different taxa to enlist unique social systems towards eusocial behavior. Despite the diversity of species that select for eusociality, all have in common the cooperation of multiple overlapping generations to perform specialized tasks including labor intense foraging (i.e., work for sustenance), nurturing and protecting progeny, and maintaining and defending a durable *nest* (Nowak, Tarnita, & Wilson, 2010; Summers & Crespi, 2013).

Eusocial strategies have been recognized in *Hymenoptera* (ants, bees, and wasps) and *Isoptera* (termites) for some time, and even identified by Darwin as a challenge to natural selection (Darwin, 1859). Identification of eusociality in vertebrates, however, is relatively recent, with two species identified to date (Jarvis, 1981; Jarvis & Bennett, 1993, but see Wilson, 2013). As predicted by Alexander in 1975 (see Braude, 1998; Sherman, Jarvis & Alexander, 1991) both species are African fossorial rodents with a defensible, expandable nest (Nowak et al., 2010; Summers & Crespi, 2013). The African naked mole-rat (NMR) was the first mammal to evolve eusociality roughly 31 million years ago (Faulkes & Bennett, 2013), likely as a strategy to expand foraging capacity in an increasingly arid habitat (O’Riain & Faulkes, 2008; Schilima, et al., 2008). The study of the NMR therefore provides an opportunity to measure the behavioral and physiological outcomes when eusociality is mapped onto a mammalian genetic template.

NMRs have been maintained in captivity over the past fifty years, and have been studied not only for their unique reproductive qualities and complex social behavior, but also for their physiological capabilities to resist cancer (Tian, et al., 2011), live long (Buffenstein et al., 2008),

and tolerate extreme environmental conditions (Larson & Park, 2009; Park, et al., 2008).

Colony-housed captive NMRs demonstrate colony and individual physical characteristics similar to wild-caught animals (Brett, 1991), and innate, ethologically relevant cooperative behaviors even after generations of captive breeding (Jarvis, 1981; 1991). Examination of reproductive, foraging, nest building, caretaking and defense behaviors has demonstrated task specialization, which is related to morphology. A bifurcation of weight demonstrated that in captivity, the breeding couple(s) (1 queen and 1-3 breeding males) are larger (and with higher body fat) along with a relatively small group of larger non-breeding animals specializing almost exclusively in colony defense behaviors, while the smaller workers, the large majority of the colony are more involved in colony maintenance work behaviors. i.e., digging, carrying bedding, and pup care (Jarvis, 1981; Sherman, et al, 1991). Furthermore, these task specialization traits are stable over a period of months (Mooney et al. 2015). Thus, as animals of different size and reproductive status exhibit different behavioral repertoires, four castes of NMRs have been identified (breeding female or “queen”, breeding male, large workers, small workers). Due to their apparent lack of engagement in colony tasks other than colony defense, the large workers of captive colonies have been referred to as “lazy, infrequent workers, or non workers”, indicating a lower activity pattern than smaller colony members (Jarvis, 1981; Reeve, 1992; Mooney et al. 2015).

Task specialization of captively held NMRs would likely result in different patterns of behavior of NMR castes, which would manifest as differences in activity patterns and space utilization in the colony space. This is true for other eusocial societies, such as bees, which show differences in circadian pattern based on caste (Yerushalmi, Bodenheimer, & Bloch, 2006), and there is evidence that caste differences may also affect circadian patterns in captive NMRs

(Riccio & Goldman, 2000). Using a Radio Frequency Identification (RFID) sensor network and computational analysis, the current study measured activity, stationary time, circadian entrainment and habitat utilization for each colony member in a complex housing environment over an extended period. Analysis was conducted on two different colonies over a continuous period of 26 days that included four 60-hr segments with little or no human presence near the colony habitat. Using individual identification and computational models, we attempted to quantify and qualify the nature of NMR *colony behavior*.

Methods

Participants

African naked mole-rats (*Heterocephalus Glaber*) were maintained as captive colonies at the College of Staten Island, CUNY, in accordance with IACUC and USDA regulations. Two colonies, bred in-house from offspring of colonies generously donated by Bruce Goldman, were studied in detail. The TT-2 Colony (N = 36; male = 19, female = 17) weight M = 37.26g, SD = 17.12g. The colony was formed from captive NMR pair approximately 6-yr 4-mo old at the time of the study. Twenty-one workers were at least 4-yr old and the remaining 12 workers were aged M = 1-yr 9-mo. The L-4 colony (N =37; male=25 female=12), weight M = 35.08g, SD = 18.71. This colony was formed from a pair 4 years and 5 months prior to the observation and the remaining NMR were at least 2 years old. Although naked mole-rat colonies may have multiple breeding males, breeding males for this analysis were considered only as the males of initial pairing with the eventual queen, as no additional breeding males have been identified for either colony. **Figure 1** shows each colony from heaviest to lightest weight, indicated by gender and caste.

Materials

Animals were housed in a 12h:12h low-light (50 Lux): dark (<1 Lux) environment (lights on: 7AM) maintained in a temperature ($29.2 \pm 1.4^\circ \text{C}$) and humidity (~ 20 %) controlled room (5m X 7m). The housing environment was comprised of a series of 5 cm inner diameter clear polycarbonate tubes connecting square (27 x 32.5 x 10 cm) or rectangular (53 x 32.5 x 10 cm) chambers (polycarbonate steam table pans purchased from a restaurant supply store). Extra heat sources, reptile heat cables, were used under the cages to provide additional warmth to segments of each cage. A few centimeters of corncob bedding, supplemented with pelleted rolled paper bedding, was placed in the bottom of each chamber. The size of the habitat was determined by the size of the colony such that there is roughly one chamber for every 5-10 animals. Animals were fed *ad libitum* on a mixed diet of tubers, squash, fruits and Teklad Global 2019 lab chow (Harlan). Food was provided to the colony Monday, Wednesday, and Friday between noon and 3 p.m. by the care staff. The colony habitat was maintained in a room with one to two other colonies in a facility dedicated to the well being of these captive NMR colonies. The entire colony was disassembled and cleaned approximately every two weeks and individual toilet chambers were checked and cleaned daily on weekdays.

Colony Environmental Design

Two colonies were housed in semi-naturalistic environments equipped with 20 RFID antennae to track the movements of each animal over extended periods of time. Each animal in the colony was implanted with a Trovan Unique (Dorset) radio frequency identification transponder (transponder size 11.5 x 2.2mm) injected under the skin. RFID antennae, circular in shape (100 mm inner diameter), and were placed around the tubing in the colony. When an animal passed a RFID antenna, a record of this action was entered into a text file by the Trovan software. Events recorded from each antenna were passed to a computer at a maximum rate of 10

events per second. Events were entered into a single text file, with each event containing the read RFID tag, the antenna where the reading occurred, and the date and time of the reading.

Figure 2 provides a schematic of the two colony habitats, including the RFID antennae, in this study. The colonies were maintained in two separate rooms and cleaning and feeding were provided by a different caregiver for each room. The Feeding chamber (**F**) of each colony was determined by the caregiver (the cage closest to the room entrance). Toilet chambers (**T**) were determined by the colony and varied within each colony, but were always single entry, “dead end” small chambers of the colony structure. These chambers were easily identifiable as they were devoid of corncob bedding and were the only chambers that contained excrement. Observation indicated that certain chambers were identifiable as gathering areas, as they contained the unrolled paper bedding, and often had a mass of animals huddled in them; these nest locations were determined by the colony members. A data set for each colony was selected as a 25.58 day (614-hr) series for L-4 (collected November 4-30, 2016), and a 26.67 day (640-hr) series for TT-2 (collected April 1-27, 2015). Each collection period included four 60-hr periods, with minimal staff activity for a second detailed analysis. Periodic caregiver notes taken at cleanings and feedings confirmed RFID data.

Data Analysis

The RFID data was preprocessed to correct misreads and eliminate duplicate reads created when animals hovered under a reader. A state matrix, compiled in MATLAB using custom code, was populated by the last identified location for each animal and updated for every new entry in the RFID data file. Activity was measured by moves, which were defined as a reading at a different antenna location from the last known antenna, or at the same antenna location after the animal has moved away from the antenna range (which was approximately

4cm). Although the antennae were positioned at the tunnels near entryways to chambers, our observation demonstrated that moves primarily represented activity between or within chambers, as prolonged stationary positioning in tunnels was rare. A stringent metric for stationary behavior was developed wherein not moving or remaining stationary was defined in units of an entire hour passing in which an individual animal was not read at a new antenna location from the last known antennae location. Nest location was determined through statistical analysis (see below). Time in the nest, as well as time in the feeding chamber was calculated separately for the reproductive pair, the large workers (L-4 & TT-2 n= 6) and the small workers (L-4: n= 29 & TT-2: n= 28)

Activity patterns were calculated for each colony member and the whole colony over a period of 26 days and are reported for >600h of continuous monitoring, with an emphasis on four periods of 60 hours when the animal laboratory has less staff activity, usually weekends. Rhythmic analysis for the movement was conducted using chi square periodogram (Enright, 1965; Refinetti, 1993 & 2004; Sokolove & Bushell, 1978). Feeding behavior was defined as the duration of time spent each hour in the feeding chamber designated by activity at the two antennae leading to it for each animal and by counting the number of trips. Each feeding session was initiated when an animal was identified at either of the antennae leading to the food cage and was terminated when the animal crossed an antenna away from the food chamber. The total time of feeding session was aggregated per hour.

Results

Colony weight distribution dimorphism

Colony weight distribution for both the L-4 and TT-2 colonies exhibited clear size differences associated to reproductive status and a bimodal weight distribution as noted

previously (Jarvis, 1981; Sherman, et al., 1991; Mooney et al., 2015). Overall, colony weights were similar L-4: $M = 35.08$ $SD = 18.71$ g; TT-2: $M = 37.17$ $SD = 17.21$, which is similar to the distribution reported in the field (Brett, 1991). Breeding animals were in the top quartile of colony weights. **Figure 1** shows the distribution of weight for each of the colonies. The designation of large and small workers was determined prior to analysis of RFID data by a 45g cutoff weight. This criterion was based upon a natural break point in the colony distributions, and is similar to criterion used in other studies to distinguish large and small non-breeders (40g: Hathaway et al., 2016; 49g: Brett, 1991). The large and small worker castes for L-4 and TT-2 weighed $M = 59.67$, $SD = 15.88$; $M = 69.83$, $SD = 17.21$; and $M = 27.66$ $SD = 1.67$ g; $M = 29.39$ $SD = 5.84$, respectively.

Activity, Rest, Circadian Entrainment and Status

Raw RFID data produced 3,674,543 observations for the L-4 colony and 3,108,137 observations for the TT-2 colony over the entire recording period. Colony-wide moves per hour over the recording period showed a consistent level of overall colony activity, $M = 34.84$, $SD = 17.60$ moves per hour in the L-4 Colony and $M = 34.66$, $SD = 20.28$ moves per hour in the TT-2 Colony. **Figure 3** shows the mean moves for each colony plotted for each hour over the recording period. Peaks in colony-wide activity often correlated with periods of human intervention (e.g. colony chamber cleaning). The colony-wide mean moves per hour and the mean moves of each individual over those hours was submitted to a simple light versus dark analysis of the variance. The colony-wide moves for both colonies was subjected to t-tests for Light:Dark: L-4, $t(622) = 8.82$, $p > 0.001$ and TT-2, $t(586) = 0.19$, $p > 0.05$; L-4: Light $M = 41.10$, $SD = 20.84$, Dark $M = 28.65$, $SD = 10.50$, TT-2 Light $M = 34.81$, $SD = 24.30$, Dark $M = 34.51$, $SD = 15.22$.

Despite colony-wide differences in apparent photo-entrainment, colony-wide rhythmic analysis of moves of both colonies during the recording period showed a significant circadian rhythm with a period of 24 hours $Qp(23) = 128$, $p < 0.001$ for L-4 and 25 hours $Qp(24) = 57$, $p < 0.001$ for TT-2 using the chi square periodogram. Tests for rhythmic behavior of the breeding pair showed non-significant rhythmic behavior for both L-4 and TT-2 with a $Qp(23) = 39$, $p > 0.05$ and $Qp(24) = 34$, $p > 0.05$, respectively. Both the large and small workers showed predominant 25- or 24-hour cycles. The large workers showed a significant circadian rhythm with period 24 hours $Qp(23) = 49$, $p = 0.009$ and $Qp(23) = 45$, $p = 0.044$ for L-4 and TT-2, respectively, as did the small workers of 24-hours $Qp(23) = 147$, $p < 0.001$ and 25-hours $Qp(24) = 60$, $p < 0.001$, respectively.

Figure 4 shows the activity of individual colony members over the recording period in relation to the 12hr light/dark cycle of the environment. The panels represent the mean moves per hour displayed by individuals. The tests on differences in individual animal moves for either colony was: L-4, $t(72) = 3.58$, $p > 0.001$ while TT-2 produced $t(70) = 0.13$, $p > 0.05$; L-4: Light $M = 41.09$, $SD = 17.06$, Dark $M = 10.50$, $SD = 12.94$, TT-2 Light $M = 34.81$, $SD = 9.43$, Dark $M = 34.51$, $SD = 10.39$. Therefore, despite having a similar colony size, weight distribution, and chamber organization, there was evidence for differences in light entrainment between colonies.

Stationary time was defined as a time interval in which the individual made no moves or stayed at the same reader. Our analysis focused on entire hours wherein animals met these criteria, and remained at a single antenna location for more than 60 minutes without registering on any other antennae. Colony L-4 had a $M = 0.20$, $SD = 0.15$ and TT-2 a $M = 0.38$, $SD = 0.20$ stationary hours over time. **Figure 5** presents the percentage of animals in a colony that are stationary each hour showing light and dark cycles over the period. L-4, $t(622) = 4.46$, $p < 0.001$

and TT-2, $t(586) = 0.62$, $p > 0.05$; L-4: Light $M = 0.18$, $SD = 0.17$, Dark $M = 0.23$, $SD = 0.14$, TT-2 Light $M = 0.39$, $SD = 0.21$, Dark $M = 0.38$, $SD = 0.18$. The results indicated that the individuals in the colony spent significant amounts of time in a single location although the number of stationary hours appeared to be closely related to the presence of a centralized nest (see below). Stationary hours were also counted as the basis of circadian patterns within the colony. Colony wide, rhythmic analysis for stationary hours for the entire recording period showed a significant circadian rhythm with a period of hours $Qp(23) = 59$, $p < 0.001$ for L-4 and none for TT-2 $Qp(22) = 30$, $p > 0.05$ using the chi square periodogram. In both colonies, unlike activity, there was no concurrence of circadian periodicity for the Breeding Pair, the Large- or Small- colony members.

Figure 6 presents stationary behavior by individual. Based on colony observations, stationary hours predominantly represent communal resting in the nest chamber. Tests revealed L-4, $t(72) = 3.72$, $p < 0.001$ and TT-2, $t(70) = 0.49$, $p > 0.05$; L-4: Light $M = 0.173$, $SD = 0.05$, Dark $M = 0.23$, $SD = 0.08$, TT-2 Light $M = 0.39$, $SD = 0.08$, Dark $M = 0.38$, $SD = 0.08$. Remarkably, the breeding pair in the TT-2 colony had the least number of stationary hours of all colony members, with the Queen being the lowest. To measure togetherness we found the chamber with the modal number of individuals present for each hour. To identify the nest chamber and track activity around it, clusters of antennae identified chamber locations, and analysis was conducted whereby the mean time of all colony members for each chamber for each hour was calculated. In this way we were able to identify and show the nest locations based on the RFID surveillance.

Nesting and Feeding Behavior

The four representative cycles from each colony of 60-hr when there was little or no human caregiving are shown in **Figures 3 and 5**. These periods were selected because no areas of the colony were cleaned, and no new food was introduced. Therefore, the behavior patterns are independent of human intervention. For both nesting and feeding behavior, we considered the whole colony, as well as behavior differences between individuals of the four castes. **Figure 7** shows the mean time spent by colony members in each chamber over each of the 4 quiet cycles. Both colonies showed changes in nest *patterns* over the cycles. Colony L-4 had one fully-nested cycle (Chamber M during Cycle 1), and the three different gathering/nesting chambers for subsequent cycles (Chambers M, E, & J during Cycles 2-4). Colony TT-2 was less dynamic and fully nested on all four cycles in two locations (Chamber I for Cycles 1 & 2, Chamber E for Cycles 3 & 4). The presence of a clear, centralized nest attended by all colony members (i.e. a “fully nested” colony) appeared to be an important predictor of the time spent in the nest. More detailed analysis of quiet periods demonstrated that the patterns were consistent across the cycle. In other words, a multi-nest colony represents multiple nests areas that exist simultaneously, not a rapid transition between nesting sites. **Table 1** presents the percentage of time in the nest. The breeding animals spent over 90% of the time in the nest when fully nested. On multi-nested cycles in Colony L-4 (Cycles 2-4) the proportions were smaller, and analysis showed breeders repeatedly transitioning between multiple nest sites within a single hour. Consistently, Large workers spent more time in the nest chamber than Small workers, regardless of colony.

Feeding trips were quantified as excursions to the feeding chamber based on activation of one of the two RFID antennae next to the feeding chamber, and feeding time was quantified as the time between initially triggering a feeding chamber antenna, and a first trigger of a non-

feeding chamber antenna. Food was introduced as intact, or large pieces, which were too large to carry through colony tunnels. Observation showed that all animals ate primarily in the feeding chamber, and only rarely were smaller pieces moved to other parts of the colony. **Figure 8** shows the *frequency distribution* of the fundamental frequencies (periods/cycle hr) of the feeding behavior of all colony members over the four cycles. The data for the L-4 colony shows a principal fundamental frequency of four, which represents approximately 15 feeding bouts per 60-hour cycle, with a distribution of similar short times around it. The TT-2 colony produced a bimodal distribution of fundamental frequencies of feeding bouts. Once again, a four-hour feeding cycle was apparent, but more animals demonstrated a 12-hour feeding cycle.

Table 1 presents the percentage of time feeding by caste for each of the 60-hr cycles. The queen and breeding male consistently spent the lowest percent of time in the feeding chamber regardless of colony or cycle. Also, large workers in each colony consistently spent more time than breeders but less time than small workers in the feeding chamber. When nest behavior and feeding behavior are combined, the L-4 queen spent on average 66.01 percent of the 60 hour cycle in the nest and feeding chambers, but in Cycle 1, when the colony was fully nested, the queen spent 98.25% of time between these two chambers. The TT-2 queen spent 94.93% percent of time in the nest and feeding chambers across the four cycles.

Discussion

This study reported on the behavior of two colonies of chronically-housed African NMRs over an extended period. The maintenance of a clean and fecund colony in captivity requires frequent human care intervention to provide food and reduce waste accumulation. The sensitivity of naked mole-rats to airflow and vibration (Artwohl, et al., 2002; Jarvis, 1991) results in a rapid change in behavior throughout the housing environment any time one of the chamber

lids are opened. The analysis of the colony behavior over a long time period (~26 days) reflected human-care intervention, while the examination of the four quiet periods, with little or no human intervention permitted more detailed analyses of colony wide and individual behavior. The use of an RFID antenna network allowed for continuous monitoring of dozens of animals dispersed throughout a naturalistic laboratory housing habitat. This approach allowed for the simultaneous measurement of whole-colony and individual behavior, and provides new insight into the patterns of the ethogram of colony-behavior, activity and rest. So deeply studied for their unique reproductive behaviors, the enactment of the emergence of a dynamic process of colony-wide nesting behavior is vital to proceed to clearly identify other individual NMR patterns such as foraging, nest building and maintenance, pup caring (and carrying), and colony defense.

One primary goal of the current paper was to measure the circadian patterns and photo-entrainment of colony housed animals. The use of RFID to track diurnal activity patterns of captive NMRs was first established by Riccio and Goldman (2000), who measured the movement of colony-housed members near an open cage. With the exception a few nocturnal animals proposed to be preparing for departure from the colony, most animals in this earlier study showed a lack of photo-entrainment to a 12:12 L:D cycle in colony-housed animals, with a robust photo-entrainment of isolated animals. Our results suggest that photo-entrainment may be colony dependent, with the L-4 colony showing significant photo-entrainment, but the TT-2 colony showing a lack of photo-entrainment. As these colonies were recorded in different rooms, with different care staff at different times of year, it is difficult to determine whether external zeitgebers may account for these differences, but the differences in the ability for the colony to establish and maintain a well-defined nest may be an important factor. Activity in the L-4 colony was greater during the light period than the dark period. Previous reports show that

focussed bright light, but not low ambient light, causes animals to disperse from the nest chamber (Hetling et al., 2005). However, low ambient light during the light period may cause an uptick in activity as animals seek out more preferable nesting environments. Regardless of photo-entrainment, both colonies had a significant circadian rhythm of moves. One explanation for the similarity of a circadian pattern, but differences in photo-entrainment is that social cues from influential colony members drive the colony-wide behavior pattern. This may be especially true in a crowded, centralized nest. Breeding pairs did not seem to drive the colony-wide rhythm, however, because they showed the weakest circadian pattern in both colonies, and had among the fewest hours of presumed rest (stationary hours). It is important to note, also, that over the ~26 day recording periods, there were no hours where all colony members are completely stationary or even remained within the confines of the nest environment. This may reflect “shift-work” of colony members who are out patrolling or foraging all hours of the day as a dynamic pulse of the colony behavior pattern.

One important predictor of activity in the current study was the presence of a clear centralized nesting chamber. When four quiet periods were analyzed, the TT-2 colony consistently showed a centralized nest chamber, the location of which shifted between the second and third quiet period. Colony L-4, however was “fully-nested” only during the first quiet period, with multiple nest locations utilized by all colony members in subsequent periods. When a colony was fully nested, much of the daily behavior of animals (> 80 %) could be accounted for by monitoring activity at the nest and feeding chambers. Large workers spent a minimum of 84% of their time between these two chambers, and breeders spent between 91% and 98% of their time between these two locations. It is noted that this activity was measured with food provided *ad libitum*. Activity outside of the nest chamber might increase by some castes if food

became scarcer, which has been demonstrated to trigger work behaviors in this species (Reeve, 1992). However, it is intriguing that although heat and bedding were provided throughout the colony, all animals in both colonies showed a clear preference to conduct the majority of their behavior in the nest chamber, a location where some resources (i.e. space and oxygen) would be more limited than isolated areas of the colony habitat. This pattern is different than primates, for example (Reinhardt, 1992), where only 9.5% of the animals, on average, are huddled in the same area of the housing environment. One explanation is that millions of years of adaptation to tolerate hypoxia (Johansen, et al., 1976, Larson & Park, 2009), which is likely achieved through retention of neonatal characteristics (Penz, et al. 2015), results in a situation where captive NMRs congregate in the nest chamber because it best resembles the microhabitat for which they have adapted.

The large proportion of the overall time spent in the nest should not be misinterpreted as a large proportion of time sleeping. Although sleep was not directly studied in this analysis, stationary hours in the nest are the most likely measures to represent sleep behavior. For example, over the entire recording period, animals in both colonies spent an average of 65.4% of their time in the nest chamber, but only 12.8% of their overall hours were *entirely* stationary. Therefore, behavior in the nest chamber likely represents a variety of behaviors rather than just sleep. Perhaps the strongest demonstration of non-sleeping nest behavior came from TT-2 breeders, who had the greatest percentage of time in the nest chamber; they had more moves per hour than the colony average and the fewest stationary hours overall. This combination of behaviors likely represents activity in and around the nest, perhaps including inducement of other colony members to do work (Reeve, 1992). A more detailed analysis of what types of behaviors

take place in the nest when animals are moving around may further identify task specialization among castes.

Nest location was dynamic and shifted between chambers for both colonies during the course of the recording period. The observations from this study and additional unpublished observations of chamber contents and RFID data suggest this to be a common occurrence for the species in captivity. Maps of naked mole-rat burrows in the field indicate multiple nest-locations within a single burrow (Brett, 1991). It would be interesting to know whether the “fully nested” and dispersed nest approaches we have observed here, even within a single colony, are both adopted by NMRs in their natural habitat. Alexander identified an expandable nest as important for eusociality (see Braude, 1998), however the polycarbonate chambers used in this study do not allow for expansion. Perhaps cycling nest locations represents an attempt at colony expansion. At most, animals in this study used 3 out of 15 chambers, or 20% of the colony space for nesting. It is unclear why naked mole-rats choose certain chambers and not others for a nest, or what factors determine when a nest movement should occur. Measurement of chamber lid temperatures show that chamber M in colony L-4 (the site of a clear nest during the first quiet period) is warmer (35.5°C) than the room temperature ($29.2 \pm 1.4^{\circ}\text{C}$), as it is positioned near the ceiling mounted heater. However, the nest locations in colony TT-2 (chambers E and I) are furthest from the room heater, and are cooler (25°C) than other cages. Some nest movements occurred around the time of large-scale cleanings, when the animal care staff replaced the bedding throughout the colony, while other nest movements did not correlate with any known human intervention. We have observed cases of attempted movement, where a few animals in a colony began moving paper nesting material to a new chamber, only to move it back again

moments later. The dynamic movement of the nest may therefore require shared decision making with agreement among multiple colony members.

Regardless of the nest location, our results demonstrate that the colony nest in a laboratory setting remains an important focus of animal activity, with a majority of time for all animals spent in the nest chamber vicinity. Regardless of age, size or gender, each individual spent, on average, more than 50% of each hour in the vicinity of the nest chamber. When colony members were subdivided into castes, we found that small workers (< 45g) spent significantly less time in the nest chamber than the other three castes. While this difference may support the previous suggestion that large workers are more “lazy” than smaller animals, examination of overall activity patterns did not support this.

Taken together, the results of this study indicate that members of the large worker caste are not less active than smaller animals, but their activity is more concentrated in the nest vicinity. As this morphological caste has repeatedly been demonstrated to have a clear role in colony defense behaviors, we propose that large workers stay close to the nest to keep it defensible and protect the colony’s most precious resources (breeders and pups). Dimorphism, given that the activity levels of the large workers and the breeders were similar to other animals, but that the queen, breeding male, and large workers had a higher proportion of time spent at the nest locations, we conclude that breeders and especially the large workers perform as much work as the small workers, in and around the nest location and should be considered “Consorts”, not “lazy” nor singularly “aggressive ones.” Finally we hypothesize that these consorts would be equally or more likely to engage in nest-centric work (e.g. nest building and brood care) as smaller animals. Previous examination of task specialization in this species may have examined behaviors out of colony context, in isolation and often separated from the colony nest, or on the

visible outskirts of the colony-housing environment, which would naturally favor observation of small workers performing work and nest-centric large workers exhibiting defensive behaviors.

The present study utilized technology to unobtrusively track individual NMR behavior in a colony setting without relying on human observation, therefore avoiding potential errors inherent in such designs, and the influence of the presence of humans on the NMRs (Jarvis, 1991). Large number computational power allowed us to capture trends and patterns of movement, periods of being stationary and nest-centric gathering. Overall, our results confirmed that social behaviors in captive colonies were similar to those in field studies. In addition, the technology allowed us to discover new patterns that would be virtually impossible to assess in a natural underground setting by giving us real-time access to surveil each colony member's activity. For example, while often in the nest chamber, the queen rarely is stationary and that while the larger consorts do spend more time in the nest, they move as frequently as smaller individuals. This methodology can now be used to investigate specific questions about the social behavior of this species, beyond the traditional focus of reproductive/aggressive behaviors. Researchers studying other captive colonies, as well as zoos, may now map and better understand the social dynamics of their animals. Uninterrupted real time access into the social world of NMR, a unique example of convergent evolution, will help us better anchor the myriad of species-specific biological differences they imbue with eusociality in a mammalian genomic foundation.

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Table 1

		<i>Percent of Time per Behavior by Colony and Caste During Four 60-Hour Cycles</i>									
		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Mean	
		N	F	N	F	N	F	N	F	N	F
Colony L-4											
Breeders	Queen	95.4	2.8	49.1	4.4	55.1	3.0	50.6	3.6	62.6	3.4
	Male	95.0	2.8	38.4	3.3	40.8	2.2	57.0	2.5	57.8	2.7
Workers	Large	81.0	4.3	39.2	5.5	49.4	4.9	41.6	3.6	52.8	4.6
	Small	80.2	5.3	28.2	9.4	36.6	7.9	41.4	5.6	46.6	7.0
	Mean	87.9	3.8	38.7	5.7	45.5	4.5	47.7	3.8	55.0	4.4
Colony TT-2											
Breeders	Queen	94.6	3.1	84.8	6.4	90.8	4.9	90.2	4.9	90.1	4.8
	Male	95.6	3.3	79.1	11.0	91.8	4.6	87.9	4.7	88.6	5.9
Workers	Large	86.1	6.5	75.3	11.4	83.1	9.9	78.2	11.9	80.7	9.9
	Small	75.8	10.8	79.3	13.3	79.3	12.0	66.7	13.6	75.3	12.4
	Mean	88.0	5.9	79.6	10.5	86.3	7.9	80.8	8.8	83.7	8.3

Note N= Nesting; F= Feeding

Figure Captions

Figure 1. Weight distribution of L-4 and TT-2 colonies. Animals in each colony organized by weight (highest to lowest) and identified by sex. Original breeding pair (queen and breeding male) are identified. The gray dotted line represents the weight cutoff used to distinguish large and small workers for analysis of caste.

Figure 2. Organization of chambers, tunnels and RFID antennae for the L-4 and TT-2 colonies. Circular RFID antennae were placed around tubing between cages to ensure close proximity to implanted RFID transponders as animals moved from chamber to chamber. The feeding location (F) was determined by the animal caretakers while the toilet (T) locations were determined by colony members. Colony chambers had the same general orientation in neighboring rooms.

Figure 3. Average moves by hour for each colony over the 26 day recording period. Bars represent the average number of moves for all colony members for each hour. Gray shaded areas indicate hours when room lighting was turned off (19:00 - 07:00 hours). The break in continuity in colony L-4 on day 3 represents an adjustment for daylight savings time. Boxes indicate 60 hour periods where human intervention in the colony was minimal (typically weekends), which were the focus of more intensive data analysis.

Figure 4. Average moves per hour in the light and dark periods over the 26 day recording period for each of the animals in the L-4 (top) and TT-2 (bottom) colonies. Colony L-4 showed a significantly greater amount of activity during the low light period (07:00 - 19:00 hours), which includes the periods of human intervention and feeding, compared to the dark period (19:00 - 07:00 hours). Colony TT-2 showed no significant difference in activity in the low light or dark periods. Q: Queen, B: Breeding male. Dotted line shows the division between the larger and smaller non-breeding animals.

Figure 5. Percent of stationary animals for each hour over the 26 day recording period. Stationary animals were defined as animals not moving from an antenna location for an entire hour. Gray shaded areas indicate hours when room lighting was turned off (19:00 - 07:00 hours). Boxes indicate 60 hour periods where human intervention in the colony was minimal (typically weekends), which were the focus

of more intensive data analysis. A higher percent of TT-2 animals remained stationary through the recording period, with the exception of the first recorded 60 hour weekend cycle for colony L-4, when the nest chamber was well-defined (see below).

Figure 6. Percent of stationary hours for each animal in the L-4 (top) and TT-2 (bottom) colonies.

Animals in the L-4 colony had fewer stationary hours than animals in the TT-2 colony. Note the queen (Q) and breeding male (B) in the TT-2 colony had the lowest and second lowest percentages of stationary hours, respectively.

Figure 7. Spatial distribution of animals in the L-4 (top) and TT-2 (bottom) colonies for each of the four 60 hour cycle periods. Grayscale values represent the percentage of time spent in each chamber by colony members across the cycle. Thus, the sum of all chamber values for each cycle equals 100. Areas of highest concentration (dark chambers) represent the nest locations. Note the lack of a clear nest chamber in the L-4 colony during cycles 2, 3 and 4.

Figure 8. Feeding frequency for animals in the L-4 (top) and TT-2 (bottom) colonies. Analysis of trips to the food chamber by all colony members during all four of the 60 hour cycle periods showed a predominant feeding frequency of 4 hours for animals in the L-4 colony and predominant frequencies at 4 and 12 hours for the TT-2 colony. Table 1 shows the feeding frequencies according to caste. Und: undetermined frequency.

