

Growing out of a caste – reproduction and the making of the queen mole-rat

Erin C. Henry¹, Christine M. Dengler-Crish² and Kenneth C. Catania^{1,*}

¹Department of Biological Sciences and ²Neuroscience Graduate Program, Vanderbilt University, Nashville, TN 37235, USA

*Author for correspondence (e-mail: ken.catania@vanderbilt.edu)

Accepted 7 November 2006

Summary

Naked mole-rats have a eusocial colony structure consisting of non-reproductive workers and a reproductively active caste where a single, dominant queen and 1–3 males produce all of the offspring. Well-established queens have elongated bodies that characterize their caste. Worker females retain the ability to transform into queens, however the trigger and time course for this physical transformation remain a mystery. Here, we show a direct link between periods of pregnancy and vertebral lengthening in nascent queens. Adult female mole-rats were paired with a male and radiographed weekly for two and a half years to track the growth of the lumbar vertebrae as the mole-rats became sexually mature and

experienced pregnancies. The lumbar vertebrae of breeding females grew at an increased rate during each pregnancy but growth rates returned to normal between pregnancies and during extended periods without reproduction. The rate of lumbar lengthening was reduced to normal rates in older, established queens experiencing pregnancies. Our results imply that the length of a new queen mole-rat is proportional to the number of pregnancies experienced and suggest that hormones related to pregnancy may play the critical role in bone growth associated with caste transformation.

Key words: eusocial, vertebra, lumbar, birth, bone.

Introduction

Naked mole-rats (*Heterocephalus glaber* Rüppell 1842) live in subterranean colonies of 70–80 members from overlapping generations (Brett, 1991). They are eusocial mammals with separate castes and a social structure based on division of labor (Jarvis, 1978; Jarvis, 1981; Burda, 1990; Alexander et al., 1991). Each colony contains a single, sexually active ‘queen’ that breeds with 1–3 males while other colony members help with pup rearing, colony maintenance, and defense (Brett, 1991; Jarvis, 1981; Jarvis, 1991; Lacey et al., 1991). When the queen dies or is removed from the colony, subordinate females can undergo rapid sexual maturation, and a violent competition often ensues until a victor emerges to become the new queen (Clarke and Faulkes, 1997). Mole-rats thus share a remarkable number of features in common with eusocial insects and these findings have generated considerable interest in both the evolutionary pressures resulting in eusociality and the mechanisms that allow different castes to develop in such disparate species (Thorne, 1997; Thorne et al., 2003).

An important finding in this regard was the demonstration that queen mole-rats and non-reproductives are dimorphic in their anatomy and thus represent separate morphological as well as behavioral castes. O’Riain and colleagues showed that established mole-rat queens have significantly longer lumbar vertebrae for their body size compared with all other

colony members (Jarvis et al., 1991; O’Riain et al., 2000). In addition to confirming that physical castes exist in mole-rats, these findings provided another obvious parallel between queens in insect and mole-rat colonies; both have elongated abdomens to accommodate the reproductive tract during gestation.

The findings raise a number of intriguing questions. For example, what triggers vertebral growth in formerly non-reproductive mole-rats that transforms them into the queen caste? Is this an irreversible step that will progress to completion once initiated? What is the time course for this transformation and how is it influenced by reproduction? What are the candidate physiological mechanisms that may contribute to this transformation? Here, we begin to address these questions by documenting changes in the morphology of newly established queens during their first 30 months of reproductive activity. We show that growth of the lumbar vertebrae is increased in new queen mole-rats during pregnancy but returns to normal rates during intervening periods without pregnancies. In addition, this growth is not uniform during the course of each pregnancy but instead appears to reach a maximum in the last few weeks of the 70-day gestation period. These results show a direct link between vertebral growth and pregnancy and suggest that hormones related to pregnancy may play the primary role in caste transformation.

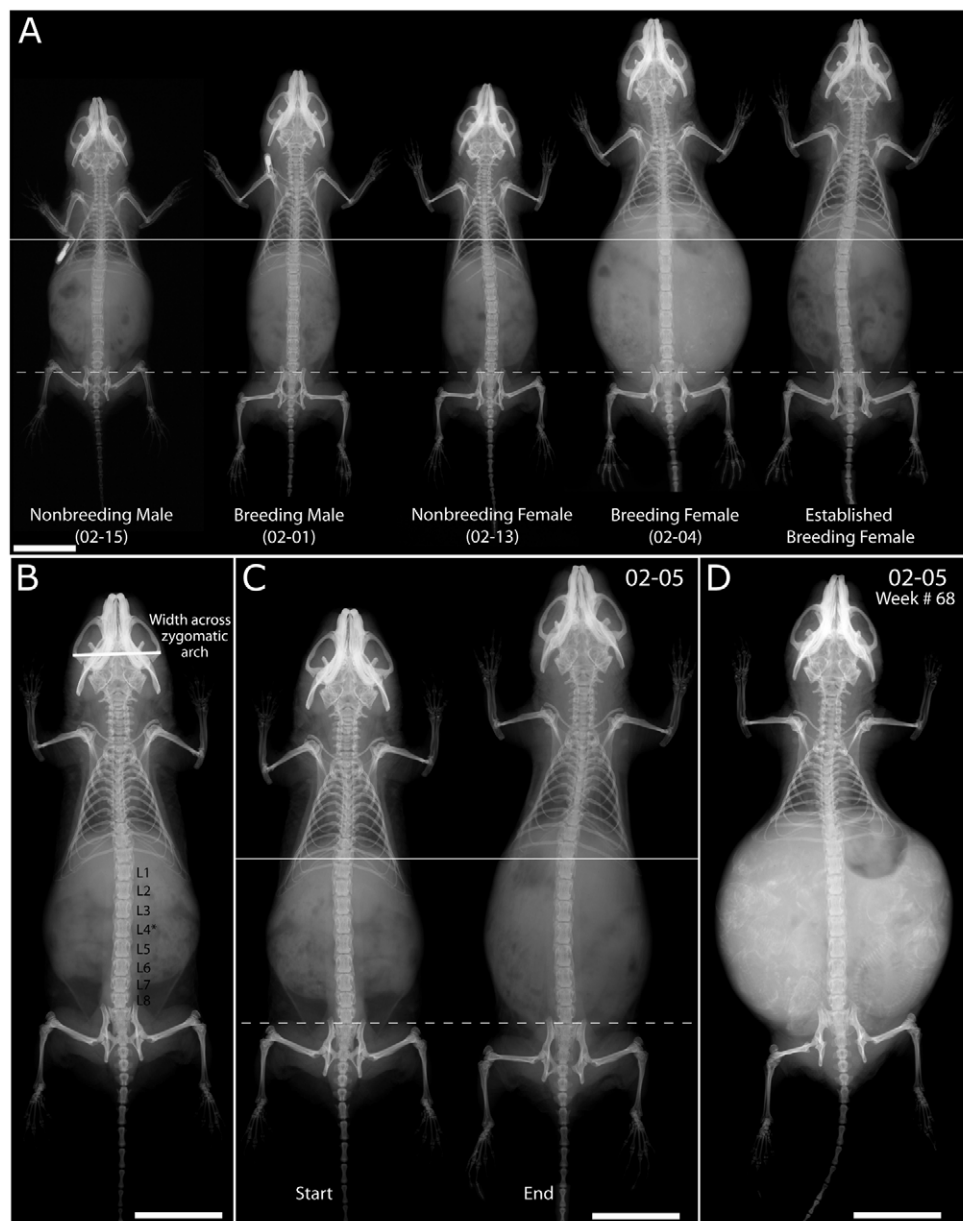


Fig. 1. Radiographs of the naked mole-rats. (A) Relative sizes of mole-rats examined in the study. All images are aligned at the base of the L8 vertebra (broken line). The solid white line is aligned to the top of the lumbar spinal column (anterior aspect of L1) in the breeding female (02-04). Only the established breeding female has a comparably long lumbar spinal column. (B) Diagram showing the width across the zygomatic arch (white line) and the labeling scheme for the lumbar vertebra, with L4 marked with an asterisk. (C) Two images of the same female mole-rat taken at the beginning and end of the study. (D) An image of a late-stage pregnant mole-rat. Scale bar for all panels=2 cm.

Materials and methods

Morphometric analysis of bone growth was used to assess changes during sexual maturation and reproduction. 1007 radiographs were taken over a period of 2.5 years.

Animal subjects

Fifteen naked mole-rats were used in this study. Procedures followed National Institutes of Health guidelines and standards set by the Animal Welfare Act and the Vanderbilt University Institutional Animal Care and Use Committee. Twelve mole-rats (six females, six males) were isolated from a single colony and pair-housed to form breeding pairs. Two females died and are not included in this report. Of the remaining paired females, three became reproductively active and were designated Breeder 02-02, Breeder 02-04 and Breeder 02-05. The three breeders displayed swollen, perforate vaginas, prominent teats

and reproduced multiple times (≥ 5 births). One pair-housed female mole-rat's vagina remained imperforate and she failed to reproduce. This animal served as a 'Nonbreeder 02-13' control. These paired females were closely observed during the study and underwent weekly radiographs. The paired males, along with additional mole-rats from established colonies, were also radiographed intermittently during the study for comparison to the pair-housed females.

Radiography

Before radiography, each mole-rat was anesthetized with isoflurane in an induction chamber. Anesthesia did not exceed 5 min. Mole-rats were placed in an MX-20 specimen x-ray cabinet (Faxitron X-ray; Wheeling, IL, USA). X-rays (34 kV at 0.3 μ A for 80 s) were taken in the dorsal and sagittal planes at a magnification of 1.5 $\times$ (Fig. 1). Female mole-rats and

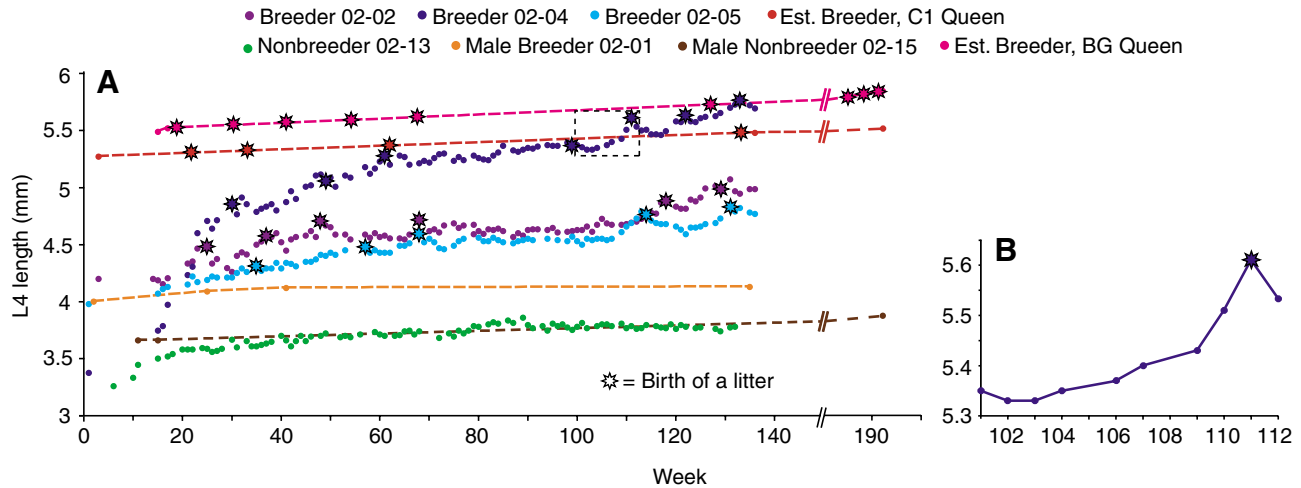


Fig. 2. Lumbar vertebra growth over time. (A) Each dot on the graph represents the length of the L4 vertebra at the time of radiography. The three breeders (02-02, 02-04 and 02-05) show increased growth of L4 over time as compared with nonbreeders and the breeding male. Starred dots indicate the L4 measurement just prior to parturition (parturition <7 days from recorded radiograph). (B) An expanded view of pregnancy 5 for Breeder 02-04 (enlarged version of the boxed area in A). Growth occurs throughout the 10-week pregnancy, ending at parturition (starred dot).

their offspring showed no obvious effects from the anesthesia and imaging. The breeding male (02-01) lost mass over the course of the study, however breeding males typically lose body mass with sustained breeding responsibilities (Jarvis et al., 1991).

The following measurements were taken from the X-ray films using digital calipers (accurate to 0.01 mm): length of each lumbar vertebra, total lumbar vertebrae length (L1–L8) including intervertebral spaces, and width across the zygomatic arch (ZA) (Fig. 1B). L4 was used as the representative vertebra as it provides a reliable index of vertebral lengthening independent of changes in the size of intervertebral spaces (O’Riain et al., 2000). L4 also corresponds to the vertebra examined in O’Riain et al. (O’Riain et al., 2000), identified as L5 (nomenclature variation is due to differing labeling schemes – see Fig. 1B for our labeling). ZA width measurement provides a control for general bone growth (O’Riain et al., 2000) and is not part of reproduction-induced morphological changes in queens. All measurements were corrected for magnification of the radiograph images. Two experimenters measured radiographs independently and inter-rater reliability for this study was $r=0.92$.

Data analysis

To determine differences in L4 lengthening between animals, we plotted the L4 length over time (Fig. 2). We also used the ratio of L4 length divided by zygomatic arch width (L4/ZA) to control for non-specific body growth (Fig. 3A). In addition, we compared our results to the L4 lengths and ZA widths of nonbreeders and of breeding males reported in O’Riain et al. (O’Riain et al., 2000) by using data from their plots of L4 (called L5) and ZA in their fig. 2. Mann–Whitney tests were used to assess significance in all analyses.

Growth changes for all eight individual lumbar vertebra

were investigated by calculating percent change between the beginning length to the ending length (Fig. 4). To determine changes in L4 length over specific time periods, we used growth rate and linear regression analyses to establish whether variations existed. For growth rate comparisons between pregnancy and nonpregnancy periods (Fig. 5), the duration of each pregnancy was determined by recording birth dates for each litter and then counting backwards 10 weeks from parturition (mean gestation length, 72–77 days) (Jarvis, 1991). Any time period between pregnancies that was greater than 7 days was considered a non-pregnancy period (see Table 2 for examples). Growth rate was calculated for pregnancy and nonpregnancy time periods by dividing the change in L4 length (mm) by the time period (weeks). For the linear regression graph (Fig. 5D), the L4 length values for each week during the pregnancy and non-pregnancy periods for the three new queens were standardized to baseline before being averaged to illustrate growth independent of initial differences in L4 length.

Results

Female mole-rats (Breeders 02-02, 02-04 and 02-05) were radiographed weekly from the time they were paired with a male through a minimum of five pregnancies (see Table 1). We present the results in three different sections, concentrating on changes in the length of the L4 vertebra as an index of bone growth in the lumbar spinal column. First, we show the raw data for how L4 length changed over time in the different cases (Fig. 2). Next, we show how L4 and the other lumbar vertebra changed in relation to body size (Fig. 3) using ZA width as an index for overall skeletal growth (O’Riain et al., 2000). Finally, we compare growth rates during pregnancies and intervening periods without pregnancies (Fig. 4).

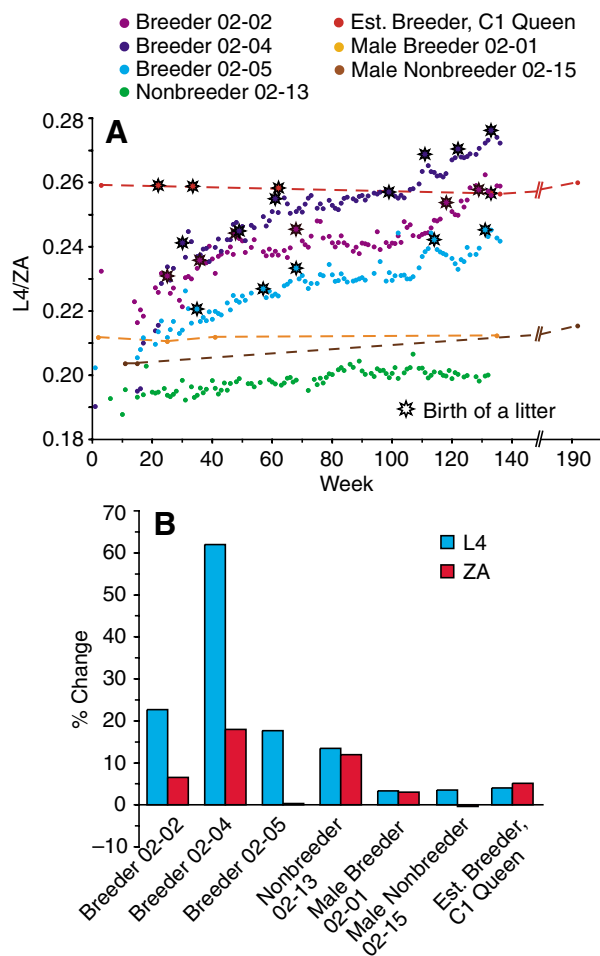


Fig. 3. Lumbar vertebra lengthening exceeds non-specific body growth. (A) The ratio of L4 length to zygomatic arch (ZA) width over time reveals that lumbar growth is occurring at a higher rate than ZA growth (a marker for general growth) in the new queens. Starred dots indicate births. (B) Bar graph plots the percentage change in L4 length and ZA width over the course of the study. The new queens (02-02, 02-04 and 02-05) all have greater increases in L4 length compared with ZA width. The other mole-rats show minimal differences in the percentage change of the two measurements.

Changes in L4 length

Fig. 2 shows L4 length over time for three female mole-rats (Breeders 02-02, 02-04, 02-05) as they transition from virgins to multiparous breeders. The length of L4 in these new queens was compared to L4 lengths of a nonbreeding female (02-13), established female breeders (C1 and BG Queens) and male mole-rats (02-01 and 02-15). The mole-rats initially had a wide range of L4 lengths and body masses, but clear trends developed through the course of the study.

First, the new queens showed growth of their L4 vertebra over time (Fig. 2). Initially, the lengths of their L4 vertebrae were not significantly different from the other no-pregnancy animals (Nonbreeder 02-13 and Males 02-01, 02-15) (Mann-Whitney test; $z=-1.091$, $P=0.275$), but by the end of

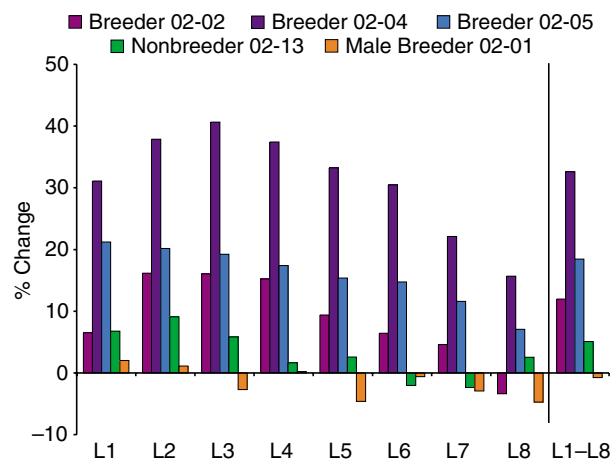


Fig. 4. Growth changes for each lumbar vertebra were normalized for general body enlargement using the ratio of L#/ZA for the eight vertebrae ($\# = 1-8$) and (L1-L8)/ZA for the total lumbar spinal column, which includes intervertebral spaces. The ratios were calculated using the beginning and ending vertebrae and zygomatic arch (ZA) values to derive the percentage change over time. The results are reported for the three breeding females (02-02, 02-04 and 02-05), the nonbreeding female (02-13) and the breeding male (02-01).

the study the L4 of the new breeders were significantly longer than the L4 of the no-pregnancy animals (Mann-Whitney test; $z=-1.964$, $P=0.050$, $N=6$).

Second, by the end of the study, the L4 vertebrae in the new queens had increased by an average of 34% (5.20 ± 0.29 mm, mean $\pm$ s.e.m.), making them nearly as long as the L4 in established queens (5.66 ± 0.18 mm). By contrast, L4 in the no-pregnancy mole-rats increased by an average of 7%, with an L4 mean length of 3.95 ± 0.09 mm (details in Table 1). The vertebral growth in the new queens did not level off, suggesting that L4 will continue to grow for some time in these mole-rats. However, L4 growth in older queens that had been breeding for several years (Fig. 2, Est. Breeders C1 and BG Queens) appeared minimal, suggesting that L4 (and the rest of the lumbar vertebrae) eventually stops growing in established queens.

Third, close examination of L4 length as a function of time during pregnancies revealed that growth of L4 in the new queens was most rapid in the second half of each pregnancy and reached a maximum just before parturition. This is shown in detail in Fig. 2B.

L4 length compared with body size

To control for nonspecific growth due to skeletal maturation, we used the width across the ZA as an index of body size. O'Riain et al. demonstrated that ZA width provides a convenient index of body size that can be used to identify caste-specific differences in the relative length of the lumbar vertebrae (O'Riain et al., 2000). Using this measure as a comparison, we found that growth of L4 in the new queens was not accounted for by changes in body size. A plot of the ratio

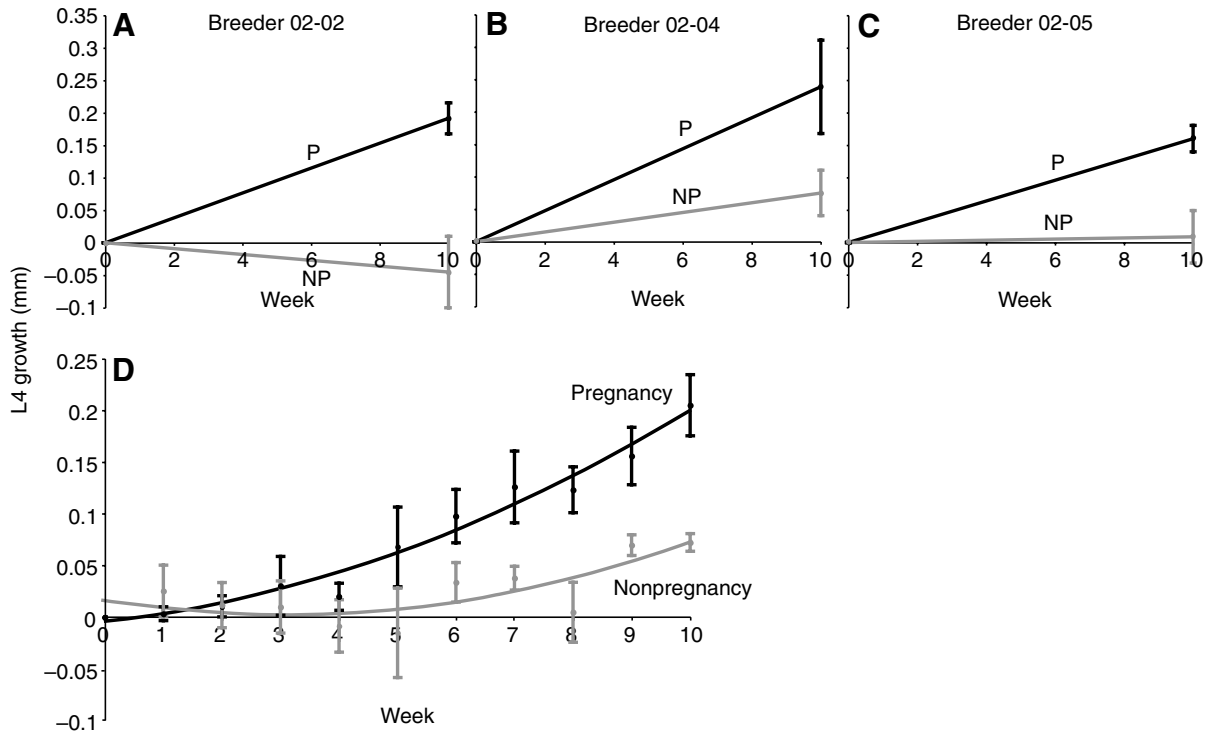


Fig. 5. Pregnancy periods (P) have higher growth rates compared with nonpregnancy periods (NP). (A–C) The mean growth rates for pregnancy and nonpregnancy periods, plotted on an arbitrary 10-week time scale. (A) Breeder 02-02 mean growth rates for pregnancy ($N=6$) and nonpregnancy ($N=2$) periods. (B) Breeder 02-04 mean growth rates for pregnancy ($N=7$) and nonpregnancy ($N=2$) periods. (C) Breeder 02-05 mean growth rates for pregnancy ($N=5$) and nonpregnancy ($N=3$) periods. (D) The mean L4 growth over time for the three breeders during pregnancy (black dots; $N=18$) and non-pregnancy (gray dots; $N=7$) time periods. The data from the three animals were standardized to baseline before they were averaged. The raw data used to generate the means varied in number between data points because some X-rays were excluded due to poor image quality (criteria for exclusion included images where the bones were not in focus or at an angle, which often happened if the mole-rat had moved or shifted during the X-ray exposure). Further, some of the nonpregnancy periods were less than 10 weeks in length, which also affected the number of data points. The N values for each data point used to generate this panel are listed in Table 3. Regression lines were fitted to both sets of data. Error bars in panels A–D represent $\pm$ s.e.m.

of L4 length divided by ZA width (L4/ZA) over time revealed the lumbar-specific growth in nascent queens (Fig. 3). The percentage change in L4 length from the beginning of the study to its conclusion for Breeders 02-02, 02-04 and 02-05 was always greater than the equivalent percent change for the ZA width (Fig. 3B; Table 1). However, this was not true for nonbreeding mole-rats (02-15 and 02-13) or the breeding male (02-01).

Based on their L4/ZA index, established queen mole-rats form a distinctly separate population from non-breeders in the colony (O’Riain et al., 2000). Fig. 3 shows the progressive increase in the L4/ZA index of new queens in our study as they join this morphological class (note that Breeder 02-04 reached an L4/ZA index that exceeded an established queen) whereas the non-breeding female and the males showed no such trend. Although the difference in the L4/ZA index between the paired females and males at the beginning of the study was not statistically significant (Mann–Whitney test; $z=-0.218$, $P=0.827$), the females that went on to breed successfully had significantly larger L4/ZA indexes than the nonbreeders and

breeding male by the end of the study (Mann–Whitney test; $z=-1.964$, $P=0.050$, $N=6$). As would be expected, the increased growth rate of L4 during late pregnancy (e.g. increased growth close to parturition) was still apparent after controlling for body size (Fig. 3).

To compare our results in breeding females with a larger control population, we examined the L4 and ZA measurements of nonbreeding females and breeding males in the previous study by O’Riain et al. (O’Riain et al., 2000) (data derived from their fig. 2). We found that the L4/ZA index for our nonbreeding female and males was not statistically different (0.210 ± 0.003 , mean $\pm$ s.e.m.) from the L4/ZA index of the nonbreeders and breeding males in the O’Riain et al. study (0.203 ± 0.002 , $N=16$) (Mann–Whitney test; $z=-1.006$, $P=0.314$). However, our new queens showed a statistically significant increase in lumbar-specific length over time when compared with the O’Riain female nonbreeders ($N=10$). Initially, the L4/ZA values of our females (02-02, 02-04, 02-05) were not different (0.205 ± 0.009) from the O’Riain nonbreeding females (0.205 ± 0.002 , $N=10$) (Mann–Whitney test;

Table 1. *Beginning and ending body masses, L4 vertebra lengths and zygomatic arch (ZA) widths*

Litters	Mass (g)		L4 length (mm)			ZA width (mm)			Total
	Begin	End	Begin	End	% Change*	Begin	End	% Change*	
Breeder 02-02	45.0	47.5	4.13	5.07	22.7	18.08	19.26	6.5	6
Breeder 02-04	33.5	59.7	3.55	5.75	62.0	17.74	20.91	17.9	7
Breeder 02-05	45.5	56.3	4.07	4.79	17.7	19.68	19.73	0.3	5
Nonbreeder 02-13	30.0	39.6	3.40	3.86	13.5	16.90	18.89	11.8	0
Male Breeder 02-01	41.5	37.9	4.00	4.13	3.3	18.89	19.46	3.0	–
Male Nonbreeder 02-15	24.3	36.9	3.74	3.87	3.5	18.04	17.97	–0.4	–
Est. Breeder, BG Queen	42.9	51.7	5.49	5.84	6.4	–	–	12	–
Est. Breeder, C1 Queen	59.7	55.8	5.27	5.48	4.0	20.34	21.37	5.1	6 [†]

*Percentage increase over length of study.

[†]C1 Queen was an established (Est.) breeder when we acquired her. The number listed does not include litters in previous facilities.

Table 2. *L4 growth rate for pregnancy and nonpregnancy periods in female breeders*

Time period	No. of weeks	Growth rate (mm week ⁻¹)
Breeder 02-02		
Period prior to 1st pregnancy	14	0
Pregnancy 1	10	0.029
Pregnancy 2	10	0.020
Pregnancy 3	10	0.013
Period between P3 and P4	8	–0.011
Pregnancy 4	10	0.015
Period between P4 and P5	38	0.001
Pregnancy 5	10	0.022
Pregnancy 6	10	0.016
Mean during pregnancy	60	0.019
Mean between pregnancies	46	–0.005
Breeder 02-04		
Period prior to 1st pregnancy	19	0.037
Pregnancy 1	10	0.063
Period between P1 and P2	7	0.011
Pregnancy 2	10	0.026
Pregnancy 3	10	0.019
Period between P3 and P4	26	0.004
Pregnancy 4	10	0.004
Pregnancy 5	10	0.026
Pregnancy 6	10	0.010
Pregnancy 7	10	0.019
Mean during pregnancy	70	0.024
Mean between pregnancies	33	0.008
Breeder 02-05		
Period prior to 1st pregnancy	24	0.009
Pregnancy 1	10	0.012
Period between P1 and P2	10	0.008
Pregnancy 2	10	0.011
Pregnancy 3	10	0.016
Period between P3 and P4	34	0.0006
Pregnancy 4	10	0.021
Period between P4 and P5	5	–0.006
Pregnancy 5	10	0.020
Mean during pregnancy	50	0.016
Mean between pregnancies	39	0.0009

$z = -0.676$, $P = 0.499$) but, by the end of the study, the new queens had L4/ZA values (0.260 ± 0.009) that were significantly larger than those of the O'Riain nonbreeding females (Mann–Whitney test; $z = -2.535$, $P = 0.011$).

Although our analyses focus on L4 length as a representative vertebra for changes occurring throughout the lumbar spinal column, we did find subtle variations in the growth patterns within the lumbar region in the breeding females. This can be appreciated by examining the change that occurred over time in the vertebra to ZA index (L#/ZA) for each of the eight vertebra. Fig. 4 illustrates this as a percentage change of the L#/ZA index for each vertebra in each mole-rat over the duration of the study. Overall, lumbar growth seemed to peak around the L3 region. The right side of Fig. 4 shows the percentage change in this index over time for the entire lumbar spinal column [i.e. (L1–L8)/ZA], including intervertebral spaces. The (L1–L8)/ZA index for Breeders 02-02 and 02-05 increased by 15% whereas this index for Breeder 02-04 increased by 33%. The nonbreeding female showed little increase in this index, whereas the male breeder had no overall increase.

L4 growth rate compared during and between pregnancies

In the course of our experiment, there was a relatively long period during which the breeding females ceased reproduction. There were no births and no obvious signs of pregnancy. Mole-rat breeding is sensitive to environmental variables, and we suspect that a 2.2°C drop in ambient temperature in our animal facility during this time caused stress to the animals, which are poikilothermic and derive body heat from their environment (Jarvis and Bennett, 1993; Woodley and Buffenstein, 2002). When the temperature was readjusted, the animals returned to their regular breeding cycles. This unexpected change in environmental conditions provided an unpredicted insight: that increased L4 growth in female breeders was specific to pregnancy periods.

This difference in growth rate is most dramatic when comparing bone growth during pregnancies (0.020 ± 0.012 mm week⁻¹, $N = 18$) to the bone growth during the

Table 3. The number of data used to generate the mean L4 growth values plotted in Fig. 5D

Week	N (pregnancy)	N (nonpregnancy)
0	15	6
1	15	6
2	18	7
3	16	5
4	11	6
5	12	4
6	16	5
7	13	5
8	14	4
9	11	2
10	18	4

Variability in the X-rays prevented some measurements from being included in the data set, creating different numbers of data for each week. Some nonpregnancy periods were also <10 weeks, which is reflected in changing *N* values.

long non-pregnancy period (0.002 ± 0.001 mm week⁻¹, *N*=3; see Fig. 2, approximately weeks 70–110). There is a 10-fold increase in growth rate during the pregnancy periods. This variation in growth rate is also apparent in the shorter non-pregnancy periods experienced by each animal (Table 2). These shorter periods of slower L4 growth (or L4 reduction possibly related to lactation – see Discussion) between pregnancies suggest that temperature *per se* was not the cause of the decreased L4 growth during the longer non-pregnancy period.

To illustrate these differences, we compared mean L4 growth rates for each new queen during all pregnancy periods and all non-pregnancy periods (Table 2). The difference in L4 growth rate between pregnancy and nonpregnancy periods is also illustrated graphically in Fig. 5. Fig. 5A–C shows mean pregnancy and between-pregnancy growth rates for each new queen. The growth rates are plotted over an arbitrary 10-week time scale. Fig. 5D combines the data from the three mole-rats, fitting regression lines to the mean pregnancy and non-pregnancy growth during a 10-week period. The values were standardized to baseline prior to averaging so that they could be combined. Note that both lines indicate an initial period of slow growth, which may reflect the mineral demands associated with lactation, as the early period of pregnancy (or post-parturition non-pregnancy) is generally when young from the previous litter are nursed. However, by week 5, more length was added to L4 during pregnancy than during nonpregnancy times, and this trend became more pronounced through the remaining five weeks up to parturition. The growth rates during the nonpregnancy periods were similar to the growth rates of the other, non-queen mole-rats in the study. The lengths of the nonpregnancy periods varied (resulting in varying *N* values for each time point) (see Table 3), but for comparison to the pregnancy periods, they are displayed to a maximum length of 10 weeks.

Table 4. The number of pups born per litter for each of the breeding females

Litter	Breeder 02-02		Breeder 02-04		Breeder 02-05	
	Week	No. of pups	Week	No. of pups	Week	No. of pups
1	25	11	30	9	35	4
2	37	11	50	10	57	10
3	48	12	61	15	68	12
4	68	5	99	6	114	13
5	118	2*	112	12	131	11
6	129	20	123	16	–	–
7	–	–	133	15	–	–

*Litter was counted more than 24 h post-birth. Total pup number may be larger than indicated.

Discussion

Naked mole-rats share a remarkable number of features in common with many eusocial insects. These include a colony structure with a single breeding female, division of labor and the existence of discrete morphological castes. Here, we investigated the ability of adult female workers to transform into the queen caste with the associated elongation of the lumbar vertebrae. We were particularly interested in testing the hypothesis that lumbar lengthening is directly related to reproduction (see O’Riain et al., 2000). We found that pregnancy was the critical factor for upregulating lumbar-specific bone growth in female mole-rats. During periods of pregnancy, nascent queens experienced increased bone growth, but this growth tapered off between pregnancies. The lumbar spinal column continued to grow at an increased rate with each subsequent pregnancy, and after 136 weeks and 5–7 pregnancies, the vertebrae in each new queen continued their disproportionate expansion. These findings suggest that hormones associated with pregnancy play an important role in the increased bone growth specific to the lumbar region.

Interestingly, one of the smallest new queens (02-04) at the beginning of the study experienced the greatest amount of lumbar growth and grew the longest over the monitored time period. Her final L4 length after seven litters rivaled our most established queen (BG) after 12 litters. The rapid growth of breeder 02-04 was reduced but not eliminated when normalized to general skeletal growth. It is likely that breeder 02-04 was low on the dominance hierarchy in the colony and had remained small as a result of her worker status. Jarvis and colleagues have found that body size is labile and correlated with social status in the colony (Jarvis, 1981; Jarvis et al., 1994). The largest subordinate females in the colony tend to have a greater likelihood of becoming the next queen if the colony is removed from reproductive suppression. Without competition for queen status, as in our pair-housing conditions, the size or former status of the female becomes unimportant, providing a unique chance for a small female to become the breeder. This could explain her rapid growth, both general and lumbar specific.

A pregnancy-induced mechanism for lumbar expansion, which accommodates a larger reproductive tract, probably arose as an extension of the less dramatic reproduction-related bone growth that prepares many mammals for lactation. Investigators have shown increased bone growth or density changes during pregnancy in rats, sheep, monkeys and humans, as an adaptation for concentrating mineral reserves that can be used for fetal skeletal development and milk production (Miller and Bowman, 1998; Miller and Bowman, 2004; Bowman and Miller, 1999). However, most gains are abruptly lost after parturition and subsequent lactation (Bowman and Miller, 1999; Redd et al., 1984).

A number of hormonal changes occurring during the reproductive cycle may support such skeletal modifications. For example, sex steroids such as estrogen, androgens and progesterone, which are up-regulated during pregnancy, have been shown to contribute to bone formation and remodeling (Syed and Khosla, 2005). Mole-rat queens differ from other mammals in that a net gain in bone length is achieved throughout each breeding cycle. In this sense, more general pregnancy-related variations in bone density that prepare rodents for lactation may have been an exaptation (Gould and Vrba, 1982) for the elongation of the lumbar region that accommodates large litters in mole-rats.

In this regard, queen mole-rats are uniquely challenged. They have the sole responsibility for reproduction in a colony that requires a large workforce and there has clearly been selection for large litter sizes. Well-established queens may have 15 or more pups in a litter (with a record of 28 recorded in the wild; Table 4 shows the number of pups born per litter for each of the breeding females in the current study). Yet the queen's girth is limited by the size of the colony tunnels (tunnel diameters average 35–45 mm) (Jarvis, 1991), which she must traverse to impose reproductive suppression on her colony members (Jarvis, 1991; Faulkes and Abbott, 1991; Faulkes et al., 1991). The need to interact with members of the colony prevents the queen from remaining isolated in her nest chamber during the later stages of pregnancy. Female mole-rats had to adapt to these constraints, and selection seems to have favored increased lumbar length as a mechanism for achieving large litter sizes without overly increasing girth. This scenario is supported by the observation that litter size increases over time in new queens (O'Riain et al., 2000; Jarvis and Bennett, 1991). The alternative, that mole-rats make wider tunnels to accommodate the pregnant queen, would be metabolically exorbitant.

The authors thank Dr M. S. Dietrich for help with the statistics. We also thank Dr E. Donnelly and G. E. Holburn for help with radiography techniques. In addition, we are indebted to Dr M. J. O'Riain for supplying us with our original mole-rat colonies and for his frequent advice on their husbandry. This research was supported by a NSF Career Award to K.C.C.

References

- Alexander, R. D., Noonan, K. M. and Crespi, B. J. (1991). The evolution of eusociality. In *The Biology of the Naked Mole-Rat* (ed. P. W. Sherman, J. U. M. Jarvis and R. D. Alexander), pp. 3–44. Oxford: Princeton University Press.
- Bowman, B. M. and Miller, S. C. (1999). Skeletal mass, chemistry, and growth during and after multiple reproductive cycles in the rat. *Bone* **25**, 553–559.
- Brett, R. A. (1991). The ecology of naked mole-rat colonies: burrowing, food, and limiting factors. In *The Biology of the Naked Mole-Rat* (ed. P. W. Sherman, J. U. M. Jarvis and R. D. Alexander), pp. 97–136. Oxford: Princeton University Press.
- Burda, H. (1990). Constraints of pregnancy and evolution of sociality in mole-rats. *Z. Zool. Syst. Evol.* **28**, 26–39.
- Clarke, F. M. and Faulkes, C. G. (1997). Dominance and queen succession in captive colonies of the eusocial naked mole-rat, *Heterocephalus glaber*. *Proc. Biol. Sci.* **264**, 993–1000.
- Gould, S. J. and Vrba, E. S. (1982). Exaptation; a missing term in the science of form. *Paleobiology* **8**, 4–15.
- Faulkes, C. G. and Abbott, D. H. (1991). Social control of reproduction in breeding and non-breeding male naked mole-rats (*Heterocephalus glaber*). *J. Reprod. Fertil.* **93**, 427–435.
- Faulkes, C. G., Abbott, D. H. and Jarvis, J. U. M. (1991). Social suppression of reproduction in male naked mole-rats, *Heterocephalus glaber*. *J. Reprod. Fertil.* **91**, 593–604.
- Jarvis, J. U. M. (1978). Energetics of survival in *Heterocephalus glaber* (Rüppel), the naked mole-rat (Rodentia, Bathyergidae). *Bull. Carnegie Mus. Nat. Hist.* **6**, 81–87.
- Jarvis, J. U. M. (1981). Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. *Science* **212**, 571–573.
- Jarvis, J. U. M. (1991). Reproduction of naked mole-rats. In *The Biology of the Naked Mole-Rat* (ed. P. W. Sherman, J. U. M. Jarvis and R. D. Alexander), pp. 384–425. Oxford: Princeton University Press.
- Jarvis, J. U. M. and Bennett, N. C. (1991). Ecology and behavior of the family Bathyergidae. In *The Biology of the Naked Mole-Rat* (ed. P. W. Sherman, J. U. M. Jarvis and R. D. Alexander), pp. 66–96. Oxford: Princeton University Press.
- Jarvis, J. U. M. and Bennett, N. C. (1993). Eusociality has evolved independently in two genera of bathyergid mole-rats – but occurs in no other subterranean mammal. *Behav. Ecol. Sociobiol.* **33**, 253–260.
- Jarvis, J. U. M., O'Riain, M. J. and McDaid, E. (1991). Growth and factors affecting body size in naked mole-rats. In *The Biology of the Naked Mole-Rat* (ed. P. W. Sherman, J. U. M. Jarvis and R. D. Alexander), pp. 358–383. Oxford: Princeton University Press.
- Jarvis, J. U. M., O'Riain, M. J., Bennett, N. C. and Sherman, P. W. (1994). Mammalian eusociality: a family affair. *Trends Ecol. Evol.* **9**, 47–51.
- Lacey, E. A., Alexander, R. D., Braude, S. H., Sherman, P. W. and Jarvis, J. U. M. (1991). An ethogram for the naked mole-rat: nonvocal behaviors. In *The Biology of the Naked Mole-Rat* (ed. P. W. Sherman, J. U. M. Jarvis and R. D. Alexander), pp. 209–242. Oxford: Princeton University Press.
- Miller, S. C. and Bowman, B. M. (1998). Comparison of bone loss during normal lactation with estrogen deficiency osteopenia and immobilization osteopenia in the rat. *Anat. Rec.* **251**, 265–274.
- Miller, S. C. and Bowman, B. M. (2004). Rapid improvements in cortical bone dynamics and structure after lactation in established breeder rats. *Anat. Rec. Part A Discov. Mol. Cell. Evol. Biol.* **276**, 143–149.
- O'Riain, M. J., Jarvis, J. U. M., Alexander, R., Buffenstein, R. and Peeters, C. (2000). Morphological castes in a vertebrate. *Proc. Natl. Acad. Sci. USA* **97**, 13194–13197.
- Redd, E. H., Miller, S. C. and Jee, W. S. S. (1984). Changes in endochondral bone elongation rates during pregnancy and lactation in rats. *Calcif. Tissue Int.* **36**, 697–701.
- Syed, F. and Khosla, S. (2005). Mechanisms of sex steroid effects on bone. *Biochem. Biophys. Res. Commun.* **328**, 688–696.
- Thorne, B. L. (1997). Evolution of eusociality in termites. *Annu. Rev. Ecol. Syst.* **28**, 27–54.
- Thorne, B. L., Breisch, N. L. and Muscedere, M. L. (2003). Evolution of eusociality and the soldier caste in termites: influence of intraspecific competition and accelerated inheritance. *Proc. Natl. Acad. Sci. USA* **100**, 12808–12813.
- Woodley, R. and Buffenstein, R. (2002). Thermogenic changes with chronic cold exposure in the naked mole-rat (*Heterocephalus glaber*). *Comp. Biochem. Physiol.* **133A**, 827–834.