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Phenotypic plasticity in female naked mole-rats after removal from reproductive suppression

Christine M. Dengler-Crish[1,*] and Kenneth C. Catania[2]

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

*Author for correspondence (e-mail: christine.crish@vanderbilt.edu)

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Summary

Naked mole-rats are fossorial African rodents that live in large, eusocial groups. Adult subordinate female mole-rats are reproductively suppressed by the dominant breeding female in their colonies. As a result, subordinate females remain reproductively quiescent for their entire lives unless they are removed from the suppressive presence of the dominant female. This makes subordinate female mole-rats a tractable model for studying phenotypic plasticity. We measured skeletal growth of subordinate, suppressed females as they changed reproductive status. After housing subordinate female mole-rats separately from their home colonies, these animals experienced a growth surge that dramatically increased their body mass and length. After removal from reproductive suppression, females showed an 82% increase in body mass and a 37% increase in the length of their lumbar spines. The lumbar vertebrae were the only skeletal structures that exhibited this puberty-like growth. After colony separation, body mass and lumbar vertebrae growth rates peaked and remained elevated for several weeks before returning to control levels – suggestive of a puberty-like 'growth spurt'. Although previous studies have characterized pregnancy-induced lumbar spine elongation in female mole-rats, we demonstrate a significant change in the body morphology of female mole-rats after removal from reproductive suppression but before the first pregnancy.

Key words: eusocial, vertebrae, bone, social status, puberty, reproduction.

Introduction

Morphological differences between castes of animals are seen in highly cooperative communal groups such as those of some social insects [i.e. termites, ants and wasps (O'Donnell, 1998)] and naked mole-rats (Jarvis, 1981; Holmes et al., 2007; Seney et al., 2006). Members of reproductive castes can differ in size but also in shape (Keeping, 2002) and these differences can be genetically predetermined or can be triggered by environmental changes (Keeping, 2002; Keller, 2003; O'Donnell, 1998). When body morphology is altered by a change in environment or status, animals are said to exhibit 'phenotypic plasticity' (Keller, 2003). In the present investigation, we examined the changes that occur to the spinal column of female naked mole-rats when they are released from the reproductive suppression of the colony queen.

Naked mole-rats (*Heterocephalus glaber* Rüppell 1842) are small, blind fossorial rodents that live in large underground colonies. The animals are eusocial, and a single reproductively active female, or queen, produces offspring while the remaining colony members assist with pup rearing, foraging, nest defense and other colony maintenance (Jarvis, 1981). The queen mole-rat mates with one or several males of her choosing and behaviorally suppresses reproduction in the remaining subordinate mole-rats (Smith et al., 1997; Smith et al., 2007). Studies have shown that subordinate female mole-rats lack both circulating sex hormones and mature gonads due to the inactivity of gonadotropin-releasing hormone (GnRH) and the quiescence of the hypothalamic–pituitary–gonadal (hpg) axis (Faulkes et al., 1990; Jarvis, 1991). In most other mammals, puberty begins with the release of GnRH and activation of the hpg axis (Terasawa and Fernandez, 2001).

In the laboratory we can transform subordinate female mole-rats into reproductively viable animals by removing them from the colony and housing them with a male. Within about a week, these females typically exhibit cyclic changes in luteinizing hormone and sex steroids and display perforate vaginas (Faulkes et al., 1990), suggesting the onset of puberty (Ojeda et al., 1976; Delemarre-van der Waal et al., 2002).

Previous studies demonstrate that female mole-rats that successfully breed experience an elongation of the body caused by the expansion of the lumbar vertebrae (O'Riain et al., 2000; Jarvis et al., 1991; Buffenstein, 1996; Henry et al., 2007). Additional data collected by Henry et al. have shown that lumbar spine growth increases during pregnancy and is attenuated in the period after or between pregnancies (Henry et al., 2007). Lumbar expansion occurs during the first pregnancy experienced by a female mole-rat (Henry et al., 2007), and growth rates of the lumbar spine increase with each subsequent

pregnancy until a maximal length is obtained (C.M.D.-C., unpublished).

In the present investigation, we examined a growth phase in female mole-rats that occurs after separation from the colony queen in the absence of pregnancy and corresponding to a puberty-like phase. Puberty is a period where peak bone mass is obtained and body mass surges are seen in rodents (Sengupta et al., 2005; Hu et al., 1993), and it is possible that female mole-rats make a significant physical transformation after removal from reproductive suppression: a time when puberty-like hormonal changes may be occurring. The goal of the current study was to determine if the female mole-rat skeleton grew significantly after removal from reproductive suppression but before the first pregnancy. We conducted a longitudinal study using radiographs to track body mass and bone changes in adult female mole-rats as they transformed from subordinate animals into reproductively viable females.

Materials and methods

Subjects

Twenty-seven naked mole-rats originating from four separate colonies were used for this study. All mole-rats were adult subordinate animals between 12 and 18 months old at the beginning of the study. Mole-rats were grouped into experimental cohorts (CC1, CC2, CC3, CC6, CC8, CC9, CC10, CC11 and CC12) that consisted of three animals per cohort. The three animals in each cohort originated from the same litter to control for differences in growth rate that occur between naked mole-rat litters (O'Riain and Jarvis, 1998). Within each cohort, litter- and size-matched animals were assigned to one of three conditions: paired females, paired males or in-colony controls. Between cohorts, animals were carefully selected for comparable age and size to minimize differences in growth or growth rate at the beginning of the study so that data could be pooled across cohorts for analysis (O'Connor et al., 2002). Mole-rats were identified with microchip implants (AVID; Norco, CA, USA). At the beginning of the study, none of the mole-rats displayed any reproductive activity, nor did any of the females show perforate vaginal openings – an indication of reproductive maturation (Yingling and Khaneja, 2006; Ojeda et al., 1976; Delemarre-van der Waal et al., 2002). After a 5-week baseline measurement period, females were removed from their natal colony and paired with a male from the same cohort. This time point was defined as 'removal from reproductive suppression' and is also synonymous with 'colony separation', and 'status change'. Paired male mole-rats served as controls for the environmental and status changes of being removed from the home colony. Unlike subordinate females, male mole-rats are thought to have mature gonads and are not subjected to the same form of reproductive suppression as females (Clark and Faulkes, 1998). The in-colony controls consisted of males and female littermates of the paired mole-rats, but these animals remained reproductively suppressed within their natal colony and, thus, did not change status. All procedures involving mole-rats were approved by the Vanderbilt University Institutional Animal Care and Use Committee and complied with the National Institutes of Health guidelines.

Measurements

Mole-rat growth measurements in cohorts CC1, CC2, CC3 and CC6 were taken twice weekly for the duration of 50 weeks. The remaining cohorts were added later as a replication study and were measured twice weekly for only 20 weeks. The paired female in CC3 died three weeks into the study so data from that cohort is not included in this study. Body mass was recorded from each animal and bone measurements were derived from radiographs of the subjects. A Faxitron MX-20 specimen x-ray cabinet (Wheeling, IL, USA) was used to obtain radiographs of each mole-rat. During the measurement period, mole-rats were removed from their cages, weighed and lightly anesthetized with isoflurane to provide immobilization for the x-ray procedure. Individual mole-rats were placed in the x-ray cabinet and dorsal radiographs were taken at a magnification of 1.5× at 35 kV and 0.3 mA for 80 s. Once the x-ray was taken, animals were placed into a holding chamber until recovery from anesthesia and then were returned to their housing facility. These radiography methods have not shown any deleterious effects on mole-rats or their reproductive activity (Henry et al., 2007; O'Riain et al., 2000).

X-ray measurements began with a 5-week baseline period where all mole-rats were housed in their original natal colonies and were still reproductively suppressed. After the 5-week baseline period, paired females and paired males were separated from their home colonies and pair-housed in their own cage units. Measurements were continued as described previously. Paired female mole-rats were also examined for the presence of perforate vaginal openings.

Radiograph analysis

Digital calipers (accurate to 0.01 mm) were used to measure bones from the radiographic images. Although all lumbar vertebrae have been shown to increase in size in queen mole-rats (Henry et al., 2007), the length of one lumbar vertebra, L4, was used as the main index of lumbar growth. Measurement of this vertebra is an accurate index of bone growth because it is not confounded by the angle of the spine or changes in the size of intervertebral spaces (Henry et al., 2007; O'Riain et al., 2000). This method of measurement is a standard of the field and allows for cross-comparison with other studies (Henry et al., 2007; O'Riain et al., 2000). The entire length of the lumbar spine (L1–L8 vertebrae) was also measured, as well as the combined cervical/thoracic spine. Other control bone measurements taken were the lengths of the femur and pelvis and the width of the zygomatic arch of the skull. Femur and pelvis growth have not been linked to reproduction-related growth in female mole-rats, and the width of the zygomatic arch of the skull provides a reliable index of general skeletal growth that occurs with time (O'Riain et al., 2000). Radiograph measurements were corrected for magnification before data analysis.

Analysis

Data were first individually examined by cohort and then were pooled across cohorts for each of the experimental conditions. Measurements over the 20- or 50-week study periods were grouped into 5-week blocks for ease of analysis. Mean values for each of these 5-week blocks were compared

across conditions. The first block represented the 5-week baseline period and the remaining blocks were designated as post-colony separation data. A mixed-design factorial analysis of variance (ANOVA) with experimental condition (paired female, paired male and in-colony controls) as the between-subjects factor and week block as the within-subjects factor was used to test for differences between conditions. *Post-hoc* ANOVAs were used to follow up any significant effects. SPSS software (SPSS Inc., Chicago, IL, USA) was used to perform the data analyses. For figure presentation, measurement data were standardized to zero at baseline to allow comparison across different cohorts and to demonstrate relative growth of each of the experimental conditions as time progressed.

Results

One paired female mole-rat became pregnant rapidly and produced a litter before the end of the study, so data from that cohort (CC6) could not be used for statistical analysis but are presented in Fig. 1. It is notable that this female demonstrated an increase in L4 length after separation from the colony but before the onset of her first pregnancy (Fig. 1). No evidence of pregnancy was seen in the remaining paired females. In the weeks after separation from their home colonies, all paired females exhibited perforate vaginal opening – a classical sign of puberty onset in other rodents (Terasawa and Fernandez, 2001; Yingling and Khaneja, 2006; Ojeda et al., 1976). Perforate vaginas were not seen in any of the in-colony control females.

20-week data

As expected, all mole-rats experienced general skeletal growth over the course of the 20-week study, and no differences in growth were seen between experimental conditions in cervical/thoracic spine, femur, pelvis or zygomatic arch. However, significant effects were revealed for the variables of L4 length, total lumbar length and body mass. Results from a mixed-design factorial ANOVA showed a significant main effect for L4 length ($F_{3,45}$=22.92, P<0.01, η^2=0.60) and a significant condition by block interaction ($F_{6,45}$=3.33, P<0.01, η^2=0.31). *Post-hoc* contrasts indicated that the L4 of paired females grew significantly longer than that of paired males ($F_{3,30}$=21.97, P<0.05, η^2=0.35) and colony controls ($F_{3,30}$=5.34, P<0.01, η^2=0.35). Males and colony controls did not differ. These data are presented in Fig. 2. Significant differences between the paired females and other two groups appeared approximately 6–10 weeks post colony-separation.

As anticipated from the L4 length results, paired females' total lumbar spine length differed from those of males and controls (Fig. 3). A significant main effect of total lumbar length ($F_{3,45}$=26.96, P<0.01, η^2=0.64) was shown, as well as a significant condition by time block interaction ($F_{3,45}$=4.12, P<0.01, η^2=0.36). Paired females' lumbar spines grew significantly longer over time than those of paired males ($F_{3,30}$=3.94, P<0.05, η^2=0.28) and colony controls ($F_{3,30}$=6.46, P<0.01, η^2=0.39). Differences in lumbar spine growth between the paired females and the other two groups emerged 6–10 weeks after removal from reproductive suppression. By 15 weeks post-colony separation, paired females had experienced a 9.4% increase in the length of their lumbar spines compared with a 6.3% increase in paired males and 3.6% increase in colony controls.

Both paired females and males attained greater body mass after colony separation than did controls (Fig. 4), as confirmed by a significant main effect of block on mass ($F_{3,45}$=22.31, P<0.01, η^2=0.60) and a condition by block interaction ($F_{6,45}$=4.777, P<0.01, η^2=0.39). Paired females gained more mass than controls ($F_{3,30}$=8.00, P<0.01, η^2=0.44), as did males ($F_{3,30}$=6.68, P<0.01, η^2=0.40). This effect also emerged 6–10 weeks post-colony separation. Paired females and males did not differ. Fifteen weeks after they were removed from their home colonies, the body masses of paired females had increased by 29.4%, and paired males increased by 20.1%. Controls that remained within their home colonies only experienced a 1.3% weight gain over the entire 20-week period.

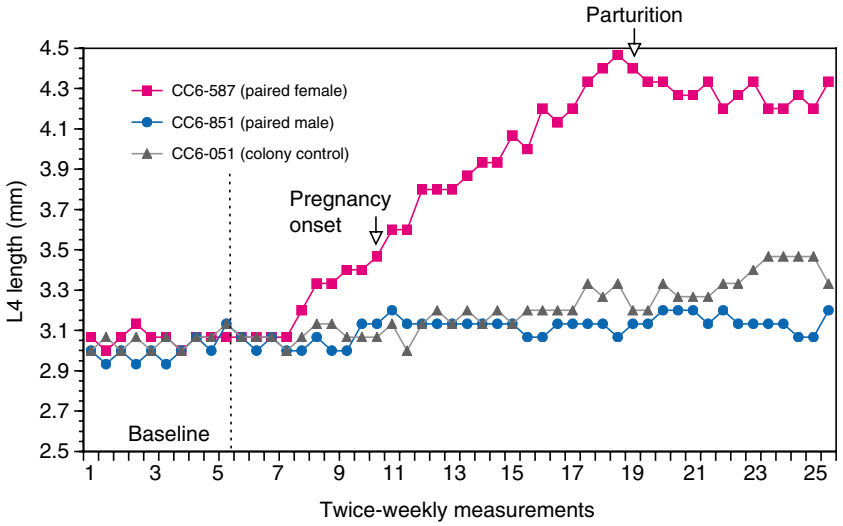

Fig. 1. Measurements (twice weekly) of L4 length in cohort CC6. The paired female in this cohort (CC6-587) became pregnant during the 10th week of the study and produced a litter during week 19. The dotted line indicates when the paired female and male were separated from the home colony. Note the increase in L4 length that occurs after colony separation but before pregnancy onset in the paired female.

50-week data

While the 20-week data set established the timing of bone growth after removal from reproductive suppression, data from the extended 50-week study were used to assess the duration of these growth periods and determine bone growth rates. Since general skeletal growth over time could artificially elevate L4 values, a correction factor was applied to the L4 length data to confirm its validity. L4 values were divided by the corresponding weekly measurements of the zygomatic arch (ZA), which is a measurement of general skeletal growth over time (O'Riain et al., 2000). This made an index of L4 growth (L4/ZA) that was specific to reproduction and not affected by non-specific skeletal growth. When replacing the L4 length variable with L4/ZA, a significant main effect of block was found ($F_{9,27}$=48.83, P<0.01,

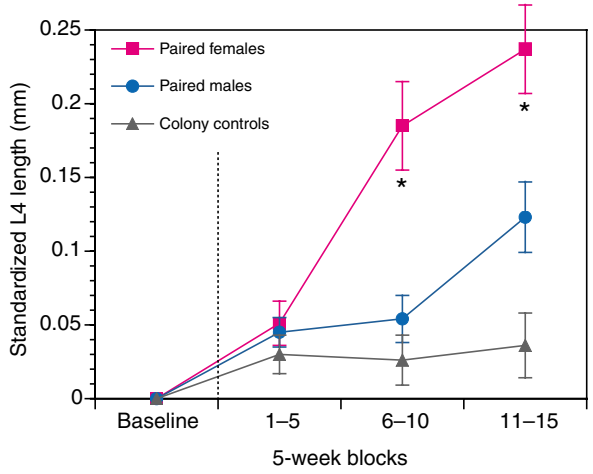

Fig. 2. Standardized measurements of L4 length over the course of the 20-week study. Means (± s.e.m.) for each of the 5-week blocks of the study are plotted on the *x*-axis. Baseline corresponds to the 5-week period prior to colony separation, and these data have been standardized to zero to show relative gains in L4 between the groups. The dotted line indicates the time of colony separation, and the remaining data blocks correspond to 1–15 weeks after removal from reproductive suppression. Significant differences between the paired females (N=6) and males (N=6) or controls (N=6) are noted with an asterisk (P<0.05). Data from cohorts CC1, CC2, CC9, CC10, CC11 and CC12 are included.

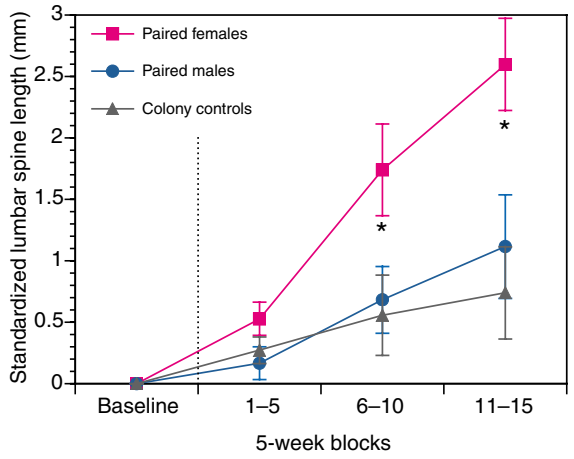

Fig. 3. Standardized measurements of total lumbar spine length over the course of the 20-week study. Means (± s.e.m.) for each of the 5-week blocks of the study are plotted on the *x*-axis. Baseline corresponds to the 5-week period prior to colony separation and these data have been standardized to zero to show relative gains in lumbar spine length between the groups. The dotted line indicates the time of colony separation, and the remaining data blocks correspond to 1–15 weeks after removal from reproductive suppression. Significant differences between the paired females (N=6) and males (N=6) or controls (N=6) are noted with an asterisk (P<0.05). Data from cohorts CC1, CC2, CC9, CC10, CC11 and CC12 are included.

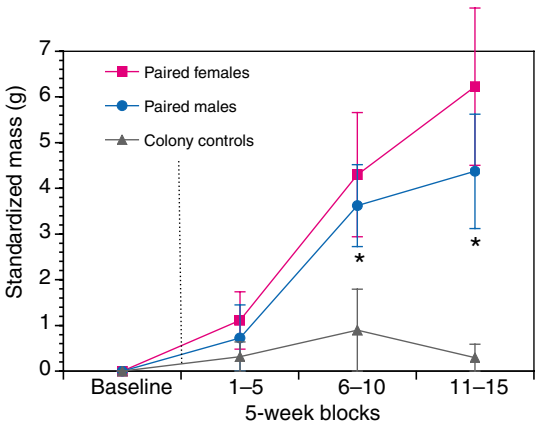

Fig. 4. Standardized measurements of body mass over the course of the 20-week study. Means (± s.e.m.) for each of the 5-week blocks of the study are plotted on the *x*-axis. Baseline corresponds to the 5-week period prior to colony separation and these data have been standardized to zero to show relative gains in weight between the groups. The dotted line indicates the time of colony separation, and the remaining data blocks correspond to 1–15 weeks after removal from reproductive suppression. Significant differences between the paired females (N=6) and males (N=6) or controls (N=6) are noted with an asterisk (P<0.05). Data from cohorts CC1, CC2, CC9, CC10, CC11 and CC12 are included.

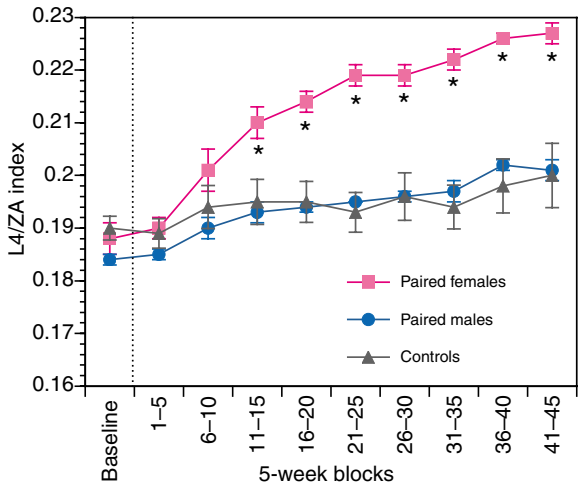

Fig. 5. L4/ZA index as a control for general growth of the skeleton over time not related to reproduction. Means (± s.e.m.) for each of the 5-week blocks of the 50-week study are plotted on the *x*-axis. Baseline corresponds to the 5-week period prior to colony separation; the dotted line indicates the time of colony separation, and the remaining data blocks correspond to 1–45 weeks after removal from reproductive suppression. Paired females (N=2) exhibited greater L4/ZA indices over the course of the study than paired males (N=2) or controls (N=2), P<0.01 (as noted by asterisks). Data from cohorts CC1 and CC2 are included.

η^2=0.94), as well as a significant L4/ZA block interaction ($F_{18,27}$=10.07, P<0.01, η^2=0.87). This analysis confirmed that paired females demonstrated increased L4 indices compared with paired males ($F_{9,18}$=14.654, P<0.01, η^2=0.88) and controls ($F_{9,18}$=14.652, P<0.01, η^2=0.88), therefore validating raw L4 length as an accurate variable (Fig. 5).

Data for lumbar spine growth over the 50-week period are shown in Fig. 6A. Over the course of this extended observation period, paired females experienced a 37% increase in the length

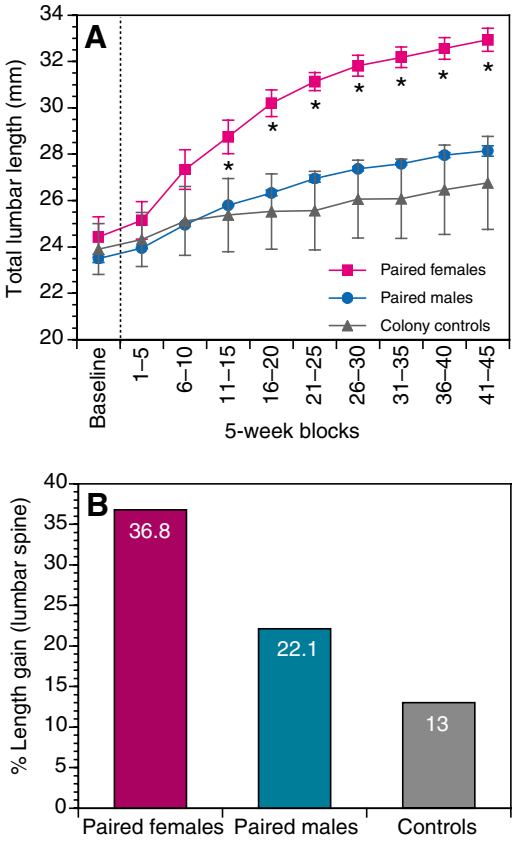

Fig. 6. (A) Measurements of total lumbar length over the course of the 50-week study. Means (± s.e.m.) for each of the 5-week blocks of the study are plotted on the *x*-axis. Baseline corresponds to the 5-week period prior to colony separation; the dotted line indicates the time of colony separation, and the remaining data blocks correspond to 1-45 weeks after removal from reproductive suppression. Significant differences between the paired females (N=2) and males (N=2) or controls (N=2) are noted with an asterisk (P<0.05). (B) Percent mean gain in total lumbar spine length over the course of the 50-week study. Inset numbers on bars indicate the exact percent gained in lumbar spine from baseline to 45 weeks after colony separation for each of the experimental conditions. Data from cohorts CC1 and CC2 are included.

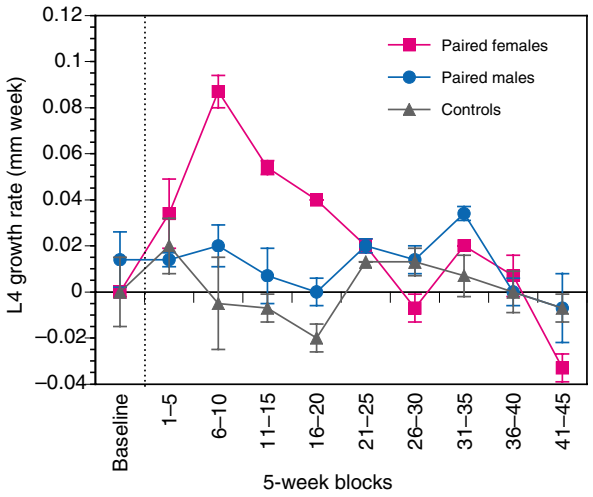

Fig. 7. Growth rate of L4. Mean weekly growth rate for each of the 5-week blocks of the study is plotted on the *x*-axis. Baseline corresponds to the period prior to colony separation; the dotted line indicates the time of colony separation, and the remaining data blocks correspond to 1–45 weeks after colony separation (N=2 per group).

of their lumbar spine (Fig. 6B), which is still significantly greater than for males ($F_{9,18}$=8.492, P<0.01, η^2=0.81) and controls ($F_{3,6}$=8.414, P<0.05, η^2=0.79). While males showed a 22% increase in lumbar length (control increases were 13%), no statistically significant differences were detected between males and colony controls. Bone growth rates (millimeters of bone accrued weekly) increased around the time of removal from the home colony and peaked approximately 6–10 weeks after colony separation (Fig. 7). This elevated growth rate remained high until about 25 weeks post-colony separation, when it decreased to control levels. This finding shows that increased bone growth rates coincided with removal from reproductive suppression although net gains in lumbar length were not seen for several weeks after this status change. The rate data showed a temporary 20-week surge in bone growth that eventually declined to baseline levels, suggestive of a puberty-like growth spurt.

Data for mole-rat weight gain over the 50-week study are show in Fig. 8A. Paired females experienced a striking 82% gain in mass over the course of the study, almost doubling their body mass ($F_{9,18}$=2.963, P<0.05, η^2=0.60) (Fig. 8B). Males also gained mass after removal from their home colonies, although this only appeared as a trend in the 50-week dataset. Over the 50-week study duration, males showed a 56% gain in mass compared with the 40% gain seen in non-reproductive controls. The rate of weight gain in paired females also showed a characteristic growth surge around the time of colony separation but recovered to control levels after a 10-week increase (Fig. 9). Note that some growth rate values are negative numbers: bone and body mass are dynamic variables that exhibit both gains and losses over time, especially when they are not in an anabolic phase.

Discussion

Reproductive suppression by the dominant queen mole-rat prevents initiation of reproductive activity in subordinate females and this suppression may last their entire lifespan. However, the reproductive viability of these females can be rescued if they are separated from the queen since reproductive activity begins quite rapidly after removal from a suppressive environment (Faulkes et al., 1990). In the present study, we show that spine growth occurs in female mole-rats after removal from reproductive suppression but before the first pregnancy. Previous studies suggested that pregnancy alone was responsible for the phenotypic plasticity of queen mole-rats (O'Riain et al., 2000; Henry et al., 2007), but here we provide evidence suggesting that a puberty-like growth period may also contribute to the elongated morphology of breeding female mole-rats. In the current study, adult female mole-rats that were removed from reproductive suppression showed obvious increases in the length of their lumbar vertebrae compared with their male counterparts and colony controls. Fig. 10 illustrates a particularly clear example of spine lengthening between mole-rats of different experimental conditions over the duration of a 50-week observation period.

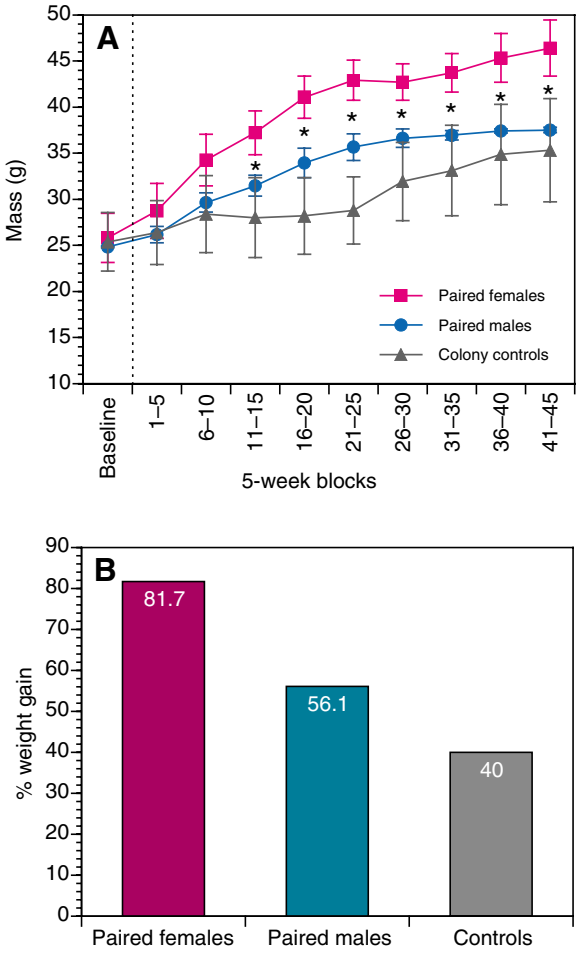

Fig. 8. (A) Measurements of body mass over the course of the 50-week study. Means (± s.e.m.) for each of the 5-week blocks of the study are plotted on the *x*-axis. Baseline corresponds to the 5-week period prior to colony separation; the dotted line indicates the time of colony separation, and the remaining data blocks correspond to 1–45 weeks after removal from reproductive suppression. Significant differences between the paired females (*N*=2) and males (*N*=2) or controls (*N*=2) are noted with an asterisk ($P<0.05$). (B) Percent mean weight gain over the course of the 50-week study. Inset numbers on bars indicate the exact percent weight gain from baseline to 45 weeks after colony separation for each of the experimental conditions. Data from CC1 and CC2 are included.

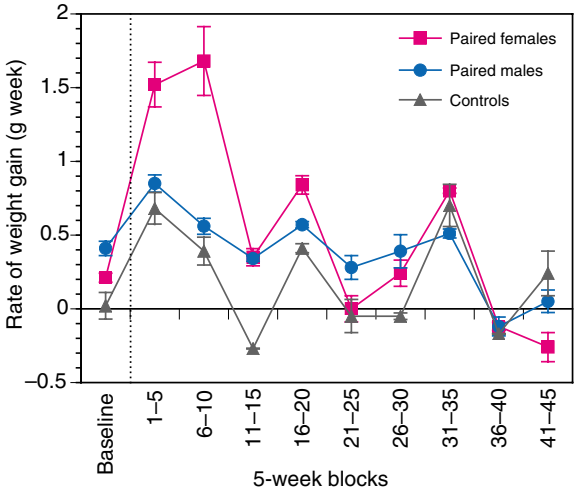

Fig. 9. Rate of weight gain. Mean weekly weight gain for each of the 5-week blocks of the study are plotted on the *x*-axis. Baseline corresponds to the period prior to colony separation; the dotted line indicates the time of colony separation, and the remaining data blocks correspond to 1–45 weeks after colony separation (*N*=2 per group).

In their study, O'Riain and colleagues (O'Riain et al., 2000) did not detect spine length differences in female mole-rats after removal from reproductive suppression compared with non-reproductive controls. These particular females were removed from reproductive suppression in a similar fashion to those mole-rats in the current study but had not been paired with males. While it is possible that copulation with males may be necessary to initiate growth-altering hormonal changes, it is more likely that the between-subjects design used by O'Riain et al. (O'Riain et al., 2000) was insensitive to subtle differences in spine length between groups due to the high individual variability in body mass and size seen in subordinate mole-rat populations (O'Riain and Jarvis, 1998).

Although we did not use hormone sampling or inspection of the internal gonads to confirm reproductive maturation in the paired female mole-rats, vaginal opening, a classical indicator of puberty in rodents (Delemarre-van de Waal et al., 2002; Ojeda et al., 1976), was observed in these animals. At the beginning of the study, female mole-rats did not exhibit vaginal opening, but by 1–2 weeks post colony separation, all of the paired females displayed perforate vaginal openings, suggesting that these animals became sexually mature. None of the colony control females exhibited vaginal opening through the duration of the 20- or 50-week studies. Vaginal opening is a common assessment for the timing of puberty onset because it coincides with increasing estrogen levels that result from folliculogenesis during the estrus cycle (Ojeda et al., 1976; Nathan et al., 2006; Delemarre-van de Waal et al., 2002). Evidence for activation of the hpg axis after removal from reproductive suppression (Faulkes et al., 1990) and the presence of vaginal opening suggest that female mole-rats may be experiencing a puberty-like event after separation from their home colonies.

Increased weight gain was also seen in paired female mole-rats and paired males compared with the controls (Figs 4 and 8), and this finding is consistent with other studies that show weight gain in mole-rats after they are removed from their home colonies and/or separated from the breeding female (Faulkes et al., 1994; Jarvis et al., 1991). Weight gain seen in the paired males may indicate some overall body growth that resulted from a change in environment and/or status. Males that were separated from their home colonies and paired with a female had less competition for food due to this reduction in colony size. Also, these formerly subordinate males were elevated to 'breeding male' status since their cage-mate was a reproductively viable female and it is possible this change caused weight gain, even though they are not under the same kind of suppression as the subordinate females (Clark and Faulkes, 1998; Faulkes and Abbott, 1991). Although weight gain is seen in the nascent reproductive males of this study, established breeding males lose 17–30% of their body mass over time and become distinguishable from conspecifics by an

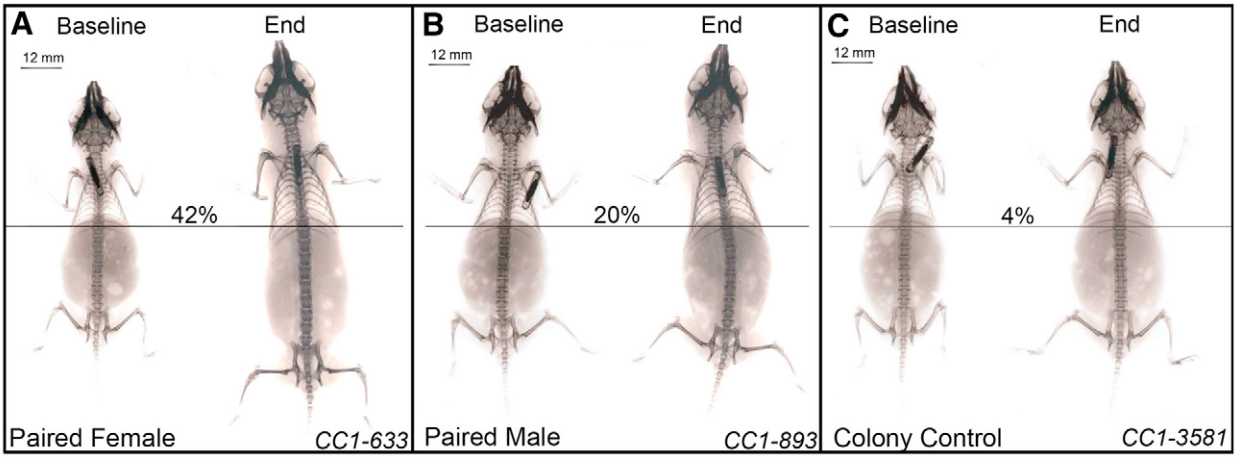

Fig. 10. Summary figure of polarized radiographs focused on the spine from one cohort (CC1) of experimental animals. (A) Paired female CC1-633; (B) paired male CC1-893; (C) colony control CC1-3581. In each panel, the first radiograph was taken during the baseline period; the second radiograph was taken at the end of the study (45 weeks after removal from reproductive suppression). The horizontal line in the center of each radiograph is aligned with the top of the vertebral body of L1. The diagram illustrates contrasts in lumbar lengthening from the beginning to the end of the study across experimental groups. The number on the center of the horizontal line indicates the percent of length gained in the lumbar spine by that animal over the duration of the 50-week study. The radiopaque cylindrical objects seen near the cervical spine are identification microchip implants.

'emaciated' appearance (Jarvis et al., 1991). This weight loss is thought to result from immune suppression caused by years of sustained testosterone levels necessary for breeding (Clarke and Faulkes, 1998; Jarvis et al., 1991).

It is interesting to note that all bone and weight gains seen in paired female mole-rats appeared several weeks after removal from reproductive suppression and became statistically discernible from males or controls approximately 6–10 weeks after colony separation. This is most likely because small increases in growth need to accumulate to be observable. This explanation was confirmed by the growth rate data, which can be a more sensitive index of subtle changes that are occurring in bone turnover. Although it took weeks to see the net increase in bone length, increased bone growth rates in paired females occurred during the 5-week block immediately after colony separation, and this growth rate peaked 10 weeks after colony separation. Therefore, growth mechanisms appeared to accelerate immediately after colony separation. The growth rate data demonstrated a pattern that consisted of an initial sharp increase and asymptote in growth, with elevated growth rates continuing for several weeks before they returned to baseline/control levels (Fig. 7). This temporary surge in growth rate exemplifies a 'pubertal growth spurt' – a debated phenomenon in rodents (Nilsson and Baron, 2004; Sengupta et al., 2005).

All of the bones measured in paired females, paired males and controls grew over the 20- and 50-week study periods, and subtle sustained growth over time was expected for these relatively young adult animals. However, the highly anabolic skeletal growth shown in the paired females was specific to the lumbar region of the spine. Other skeletal structures we measured (femur, pelvis, skull and non-lumbar vertebrae) did not exhibit a highly anabolic growth phase after removal from reproductive suppression. This indicates that reproduction-related growth specifically affects certain aspects of the skeleton, suggesting specialized roles for these structures in the reproductive process. It is possible that the increased weight gain seen in paired female mole-rats after removal from reproductive suppression plays a role in spine growth (Eastell, 2005; Wertz et al., 2006), but in our current data it is hard to determine a causal relationship between weight gain and bone growth because they appear concurrently.

Why is it that phenotypic plasticity and anatomical distinction are so important to reproductively viable female naked mole-rats? Pregnancy and lactation place great demands on maternal bone, particularly for primiparous female rodents, requiring significant preparation of the skeleton before the first pregnancy (Kunkele and Kenagy, 1997). Other studies have shown that female rats acquire excess skeletal mass prior to their first pregnancy (Bowman and Miller, 1999; Redd et al., 1984). Larger bones are stronger bones, and the post-pubertal lengthening of the spine could provide the mechanical support necessary for carrying the increased load of an abdomen full of pups (Schoenau, 2006; Specker and Binkley, 2005). Jarvis et al. (Jarvis et al., 1991) previously suggested that the environmental constraints of living in small-diameter underground tunnels favor a process that involves extending the abdomen to optimize pup carrying capability; increases in abdominal girth would be counterproductive for navigating such a restrictive terrain. In other species, such as meerkats, increased maternal body length has been correlated with larger litter sizes (Russell et al., 2004). Also, the lactation period reduces bone mineral, often in the lumbar spine (Bowman and Miller, 1999; Tojo et al., 1998). Supplementation of maternal calcium stores would also be needed to accommodate nursing large litters, and bone accumulation could provide these mineral reservoirs (Black et al., 2000; Bowman and Miller, 1999; Miller and Bowman, 2004; Sengupta et al., 2005). The expansion of one local region of the skeleton, the lumbar vertebrae, could accommodate all of these needs and help refine the metabolically costly act of reproduction.

This study has demonstrated that reproductively viable nulliparous female mole-rats exhibited substantial elongation of their lumbar vertebrae – a characteristic previously relegated to

multiparous queen mole-rats. However, pregnancy-related growth still contributes most to phenotypic changes as spine length increases with each pregnancy until an asymptote is reached (O'Riain et al., 2000; Henry et al., 2007) (C.M.D.-C., unpublished). Since both removal from reproductive suppression and pregnancy cause lumbar spine expansion, it is plausible that both events rely on similar hormonal mechanisms – possibly estrogens – to facilitate growth (Buffenstein, 1996; Bowman and Miller, 1997; Eastell, 2005), but the exact endocrine mechanisms involved need to be elucidated. This puberty-like growth is eventually attenuated, as shown by the growth spurt pattern in the rate of spine elongation. Therefore, pregnancy is not simply a continuation of puberty-like growth but a new catalyst for growth. This is also supported by the fact that lumbar vertebrae growth rate in multiparous queen mole-rats is attenuated during non-pregnancy periods (Henry et al., 2007), and some bone loss even occurs during lactation (C.M.D.-C., unpublished). Future work will involve investigating the role that specific hormones play in augmenting bone growth in adult mole-rats.

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