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Reproductive suppression in female Damaraland mole-rats *Cryptomys damarensis*: dominant control or self-restraint?

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Colonies of Damaraland mole-rats *Cryptomys damarensis* exhibit a high reproductive skew. Typically one female breeds and the others are anovulatory. Two models, the dominant control model (DCM) and the self-restraint model (SRM), have been proposed to account for this reproductive suppression. The DCM proposes that suppression is under the control of the dominant breeder and is imposed by mechanisms such as aggression, pheromones and interference with copulation, whereas the SRM does not involve aggression directed towards non-breeders and may function in order to minimize inbreeding. We investigated potential proximate mechanisms involved in the suppression of females in a series of experiments. Socially induced stress through aggression did not appear to be responsible for anovulation. Nor did breeders actively interfere with subordinate copulation. Females were physiologically suppressed when housed in intact colonies. However, as predicted by the DCM, they did not become reproductively active when removed from the presence of breeders. We found no evidence that pheromonal cues block ovulation. We suggest that the SRM is the basic model found in the Damaraland mole-rat and that self-restraint functions in order to minimize inbreeding by restricting reproduction until an unrelated male is present. This would explain the rapid onset of reproductive activation in females when paired with an unrelated male, as demonstrated in this study.

Keywords: *Cryptomys damarensis*; mole-rat; reproductive suppression; dominant control; self-restraint; incest avoidance

1. INTRODUCTION

Reproductive skew is the distribution of direct reproduction among same-sex members of a social group (Keller & Reeve 1994). Skew ranges from egalitarian, in which all individuals breed, to despotic, where reproduction is restricted to a single individual and is suppressed in others. Snowdon (1996) proposed that there are essentially two models that explain reproductive suppression in cooperatively breeding animals. The dominant control model (DCM) proposes that reproductive suppression is under the control of the dominant breeding animal. The breeder benefits from recruiting non-breeding animals for assisting in infant care and must prevent reproductive competition from these helpers. This mechanism requires that reproductive suppression be imposed by aggression and related stress effects, active interference with copulation, pheromonal cues that block ovulation and infanticide. For example, in some cooperatively breeding monkeys (cotton-top tamarin *Saguinus oedipus* (Savage *et al*. 1988) and common marmoset *Callithrix jacchus* (Barret *et al*. 1990)), both behavioural and olfactory signals appear to be implicated in reproductive suppression. In contrast, the self-restraint model (SRM) does not involve aggression directed towards non-breeding females. The function of self-restraint is to minimize inbreeding by restricting reproduction until an unrelated male is present, sufficient helpers or resources are available or adequate parental skills can be acquired (Snowdon 1996). According to Snowdon (1996) the mechanisms of this model are unlikely to involve aggression and stress as a subordinate female can control her interactions with the dominant female. While olfactory cues may be important for a subordinate female in identifying the presence of a dominant female, olfactory cues are unlikely to function as a pheromone that automatically suppresses reproduction.

Proximate cues mediating reproductive suppression have been investigated in the naked mole-rat *Heterocephalus glaber*. In colonies, which average *ca*. 80 animals, reproduction is confined to one dominant female, i.e. 'the queen' and up to three males (Jarvis *et al*. 1994). Ovulation is physiologically suppressed in female subordinates as a result of reduced pituitary luteinizing hormone (LH) that arises from disrupted secretion of hypothalamic gonadotrophin releasing hormone (GnRH) (Faulkes & Abbott 1997). Primer pheromones from urine do not play a major role in the suppression of reproduction (Faulkes & Abbott 1993) nor do behavioural (tactile) cues from other non-breeding colony members (Smith *et al*. 1997). Nevertheless, the queen is by far the most aggressive colony member. This led Faulkes & Abbott (1997) to propose that the DCM is operational, with agonistic interactions between the queen and non-breeders resulting in an inhibition of gonadal function in the latter. The cooperatively breeding Damaraland mole-rat *Cryptomys damarensis* has a comparably high skew. Colonies range in size from pairs of animals up to families of 41 individuals (median of 11 animals) and are typically composed of a single unrelated breeding pair and their

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Table 1. *Compositions of the three colonies and experimental groups of female Damaraland mole-rats*

(Six females from colony A and two females each from colonies B and C were removed from their parental burrow system and divided into groups for receiving differing treatments.)

	intact colonies			experimental group		
colony	total	males	females	control	bedding	social
A	21	10	11	A6 and A7	A8 and A9	A10 and A11
B	14	6	8	B5	B6	B7
C	7	2	5	C2	C3	C4
total	—	—	—	4	4	4

surviving offspring from several litters (Jarvis & Bennett 1993). Estimates of the number of colony members that never attain breeding status are 92% for the Damaraland mole-rat ($n > 400$ animals) and 99.9% for the naked mole-rat ($n > 4000$ animals) (Jarvis *et al*. 1994). As with the naked mole-rat, non-breeding female Damaraland mole-rats have lower concentrations of basal LH and reduced pituitary sensitivity to exogenous GnRH when compared to breeding females (Bennett *et al*. 1996). However, while studies of the proximate factors involved in reproductive suppression in naked mole-rats are fairly well advanced, little is known about what cues may be involved in the physiological suppression of subordinate female Damaraland mole-rats. An increase in the plasma LH of non-breeding females in colonies that had been 'queenless' for several years, but which did not breed until an unrelated male was available, led Bennett *et al*. (1996), in their socially induced infertility hypothesis, to suggest two components to suppression, namely inhibition by breeders and incest avoidance. Further to this, Molteno & Bennett (2000) demonstrated spontaneous ovulation in non-breeding females separated from the queen for more than six months. However, the long periods between queen removal and measurement of reproductive status in non-breeders are a major caveat in these studies when it comes to inferring the importance of queen control. Recently, Cooney & Bennett (2000) tested the role of incest avoidance in explaining skew by introducing an unrelated male into intact colonies.

Here we test the relative importance of both components of Bennett *et al*.'s (1996) socially induced infertility hypothesis in one experiment. Specifically, we investigated evidence for dominant control by (i) recording aggressive and sexual behaviours and assaying steroid hormones in urine in order to monitor reproductive activity and 'stress' levels in intact colonies of Damaraland mole-rats, and (ii) then separating non-breeding females from breeders and continuing to monitor the above parameters. Reproductive activity was monitored by determining the urinary progesterone profile of females, which is a far more reliable indicator of ovarian activity than pituitary LH, immediately prior to and following separation from breeders. Furthermore, in order to examine whether dominant control, if operational, is mediated through pheromones and/or behaviour, separated females were exposed to semiochemicals in soiled bedding or to non-breeding siblings from their parent colony. Finally, the role of incest avoidance in maintaining reproductive suppression and skew was investigated by allowing separated females access to unrelated males.

2. MATERIAL AND METHODS

(a) *Study colonies*

Three intact colonies of Damaraland mole-rats were studied. Two large colonies (A and B) were collected one month prior to the start of the experiment at Hotazel, Northern Cape Province, South Africa (27°17′ S, 22°58′ E). Another colony (C) was formed in the laboratory by pairing an unrelated male and female from Dordabis, Namibia (22°58′ S, 17°41′ E) three years before the start of the experiment. An intact colony refers to a colony that is functionally complete, consisting of one breeding male and female and a number of adult non-breeders of both sexes. It was assumed that the wild-caught colonies were composed of an unrelated breeding pair and their offspring, though we cannot be certain that unrelated immigrants were not present. The compositions of the colonies at the start of the experiment are given in table 1. Each colony was housed in plastic crates (1 m × 0.5 m × 0.5 m) with nesting boxes interconnected via plastic Perspex tubes. Separated animals were housed in smaller plastic crates (0.6 m × 0.3 m × 0.3 m). Animals were provided with wood shavings and shredded paper towelling for nesting material. Food, in the form of sweet potato, apple, carrot and gem squash, was available *ad libitum*. *Cryptomys damarensis* is an aseasonal breeder and colonies were maintained in a constant temperature room at 25 °C under a cycle of 10 L:14 D.

(b) *Experimental protocol*

Behavioural observations were undertaken in all three colonies in order to record agonism and urine samples were collected from each female in order to monitor reproductive activation and steroid hormone levels for 30 days (control phase). Six non-reproductive females from colony A and three non-reproductive females each from colonies B and C (adults of more than 60 g body mass) were then removed from their parental burrow system, housed separately for 30 days and then paired with an unfamiliar, unrelated male for 40 days (table 1). Separately housed females A8, A9, B6 and C3 received daily transfer of soiled litter and bedding from the parent colony (bedding transfer group) ($n = 4$), females A10, A11, B7 and C4 received social contact with non-breeders of both sexes from their parent colonies (social contact group) ($n = 4$) and females A6, A7, B5 and C2 were subjected to a once-daily disturbance of the bedding and litter in their nest, food and toilet chamber that was equivalent to that of the other groups (control group) ($n = 4$).

Rotation of non-breeders and bedding transfer started on the day of separation (designated day 0) and continued for 30 and 70 days, respectively. Due to ethical considerations, rotation of non-breeders was stopped immediately prior to the introduction of the unrelated male (day 30). Separated females had attacked transferred, non-breeding females and it was thought that the introduction of the unrelated male would cause further fatalities.

A constant volume (*ca.* 500 ml) of material was transferred from each colony for the bedding transfer group. For the social contact group, contact was maintained by the rotation of predetermined, fixed groups of non-reproductive animals between the parent colony and the burrow system of the separated female. Half of the non-breeders in colonies B and C were rotated between the parent colony and the separated female in the social contact group every second day. In this way, separated females in the social contact group were exposed to all colony members except the breeding male and female every four days. For colony A, one-quarter of the non-breeders were rotated between the parent colony and each of the two separated females in the social contact group every second day. Thus, separated females in the social contact group were exposed to all colony members except the breeding male and female every eight days. In this way, as in colonies B and C, transferred non-breeders in colony A spent 50% of the time in their parent colony and 50% of the time with the separately housed female(s). It was assumed that, in the social contact group, non-volatile chemical cues present in the parent colony were also transferred into the cage of the separated females (on the bodies of rotated non-breeders). The body weight of non-breeders was recorded every 30 days, from 30 days before to 60 days after separation from their parental burrow system.

(c) *Behavioural observations*

Individual colony members were identifiable by a unique, white occipital head patch. However, in order to facilitate rapid identification of individuals during behavioural observations, different coloured vegetable dyes, which are used commercially as food colorants, were applied to each animal's head patch. Focal animal sampling (Martin & Bateson 1986) was used for quantifying behavioural interactions within the three study colonies and also between separated females kept in social contact with non-breeders. Individual mole-rats were observed for a period of 10 min between 8.00 and 17.00 when more than 50% of the colony members were active. The frequencies of agonistic interactions (biting, incisor fencing, shoving or shunting, and chasing) were then recorded on check-sheets, together with the identities of the animals involved. Incisor fencing is where two animals stand face to face with their mouths at right angles and their incisors locked together. The mole-rats may then shove back and forth and rock their heads from side to side with their incisors locked together. Shoving or shunting is when one mole-rat presses its muzzle onto the face or body of another animal, then moves forward pushing the other animal violently backward. A mean of 24 focal samples (range 18–28) was carried out on each breeding male and female and non-breeding females before colony manipulation (control phase). In total, mole-rats in colonies A, B and C were observed for 30, 23 and 18 h of focal observation, respectively. After manipulation, females in the social contact group were observed for a minimum of 30 min each day corresponding to 16 h focal observation. Finally, all separated females were observed for a minimum of 30 min each day corresponding to 21 h focal observation. Sexual and agonistic behaviour were recorded whenever observed.

(d) *Urine collection*

Urine samples were collected from all singly housed females in order to monitor reproductive activity. Urine was chosen in preference to blood due to ethical considerations and to allow repeated sampling over several months with minimum disturbance to the animals. On average, urine was collected from each female every three days, from 30 days before (control phase) to 70 days after separation from their parental colonies. During this period, urine was also collected from the reproductive female in all three colonies in order to confirm reproductive status and determine the length of the ovarian cycle in *C. damarensis*.

Urine collection involved placing each animal individually into a toilet chamber for no more than 2 h. Toilet chambers were cylindrically shaped aluminium mesh cages (0.2 m wide × 0.5 m high) fitted with a plastic tray under the mesh floor in order to trap deposited urine. During urine collection each toilet chamber with the mole-rat inside was kept in the animal's cage, allowing odour and (limited) tactile contact between the animal from which urine was to be collected and other mole-rats. Individuals were removed from the toilet chamber immediately after urination. If the mole-rat had not urinated within the 2-h period, it was then returned to its cage and urine collection resumed the following day. The deposited urine was collected in a Pasteur pipette immediately after each urination and the toilet chamber wiped clean with damp tissue paper. Urine samples were stored at $-20\,^{\circ}\mathrm{C}$ until hormone determination. Urine samples were assayed for progesterone as an indicator of ovarian cyclicity. Before progesterone determination, urine samples were subjected to a determination of creatinine as described by Bonney *et al.* (1982) in order to correct for differing dilutions. All urinary progesterone concentrations were expressed as mass per milligram of creatinine (mg^{-1} Cr).

(e) *Progesterone assay*

Progesterone assays were performed using a coat-a-count progesterone kit (Diagnostic Products Corporation, Los Angeles, CA, USA) as described by Bennett *et al.* (1994). The antiserum is highly specific for all naturally occurring steroids with a typical cross-reactivity of less than 0.5%. The assay was validated for *C. damarensis*' urine by using a test for parallelism in which the slope of the curve produced using serial doubling dilutions of unextracted mole-rat urine obtained from a pregnant female (over the range 1:1 to 1:64) is compared against that of the standard curve. Following logit-log transformation of the data, the slopes of the lines were compared and found not to differ significantly (ANCOVA, $F_{1-6} = 4.9$ and $p > 0.05$). The sensitivity limit of the assay (determined as 90% binding) was 0.1 ng ml^{-1}. The inter- and intra-assay coefficients of variation, expressed as the coefficient of variation for repeated determinations of a quality control, were 3.5% ($n = 4$) and 2.2% ($n = 6$), respectively.

(f) *Testosterone assay*

A testosterone assay was carried out using a coat-a-count testosterone kit (Diagnostic Products Corporation). The assay was validated for this species' urine by testing for parallelism using serial doubling dilutions of unextracted mole-rat urine obtained from a individual with high testosterone levels (range 1:1 to 1:64). The slopes of the lines were compared and found not to differ significantly (ANCOVA, $F_{1-6} = 4.3$ and $p > 0.05$). Six urine samples from breeding and non-breeding females were assayed for testosterone in intact colonies (pre-manipulation).

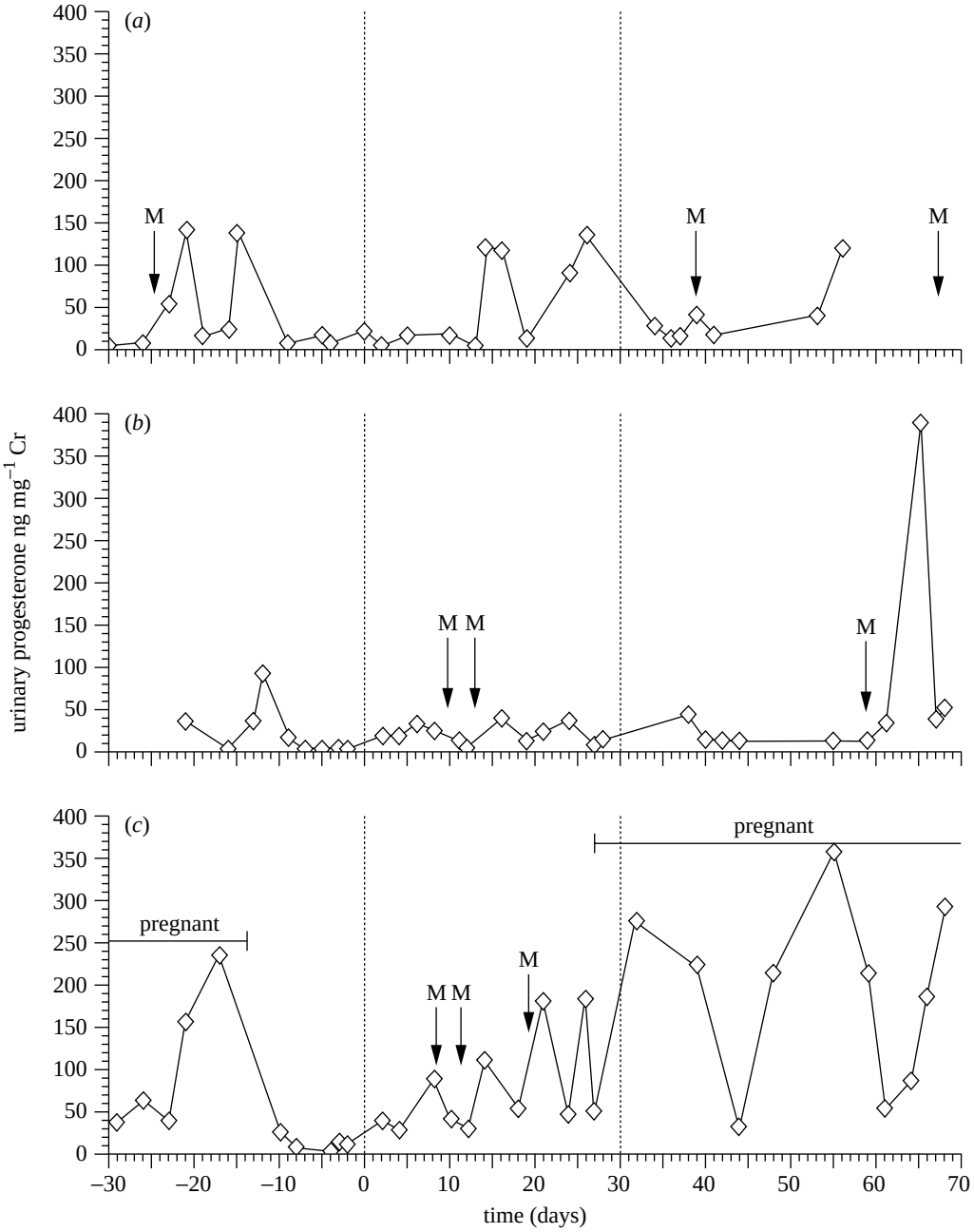

Figure 1. Urinary progesterone profiles for the single reproductively active females (queen) in each of the three colonies: (*a*) colony A queen, (*b*) colony B queen and (*c*) colony C queen. Day 0 refers to the day on which non-reproductive females were experimentally removed from their parental burrow system and housed singly. Arrows indicate the day(s) that mating (M) was observed.

The sensitivity limit of the assay (90% binding) was 0.1 ng ml^{-1}. The intra-assay coefficient of variation was 2.6% ($n = 6$).

(g) *Cortisol assay*

Cortisol assays were performed using a coat-a-count cortisol kit (Diagnostic Products Corporation). The assay was validated for urine by testing for parallelism using serial doubling dilutions of mole-rat urine obtained from an individual with high cortisol levels (range 1:1 to 1:64). The slopes of the lines did not differ significantly (ANCOVA, $F_{1-6} = 4.7$ and $p > 0.05$). Six urine samples from breeding and non-breeding females were assayed for cortisol in intact colonies and also in non-breeding females when separated from their parental burrow system. The sensitivity limit of the assay (90% binding) was 6.1 ng ml^{-1}. The inter- and intra-assay coefficients of variation were 2.8% ($n = 3$) and 2.1% ($n = 6$), respectively.

(h) *Statistical analyses*

We calculated values for bites h^{-1} and incisor fencing h^{-1} for the breeding and non-breeding females within each colony for analysis of behaviour. Due to small sample sizes, statistical analyses were carried out using non-parametric tests. Values were log transformed for statistical analyses in order to correct for heteroscedacity. Changes in the body weight and urinary cortisol concentrations of non-breeding females between treatments were compared using Wilcoxon matched-pair tests (Z). Comparisons of individual female urinary progesterone concentrations

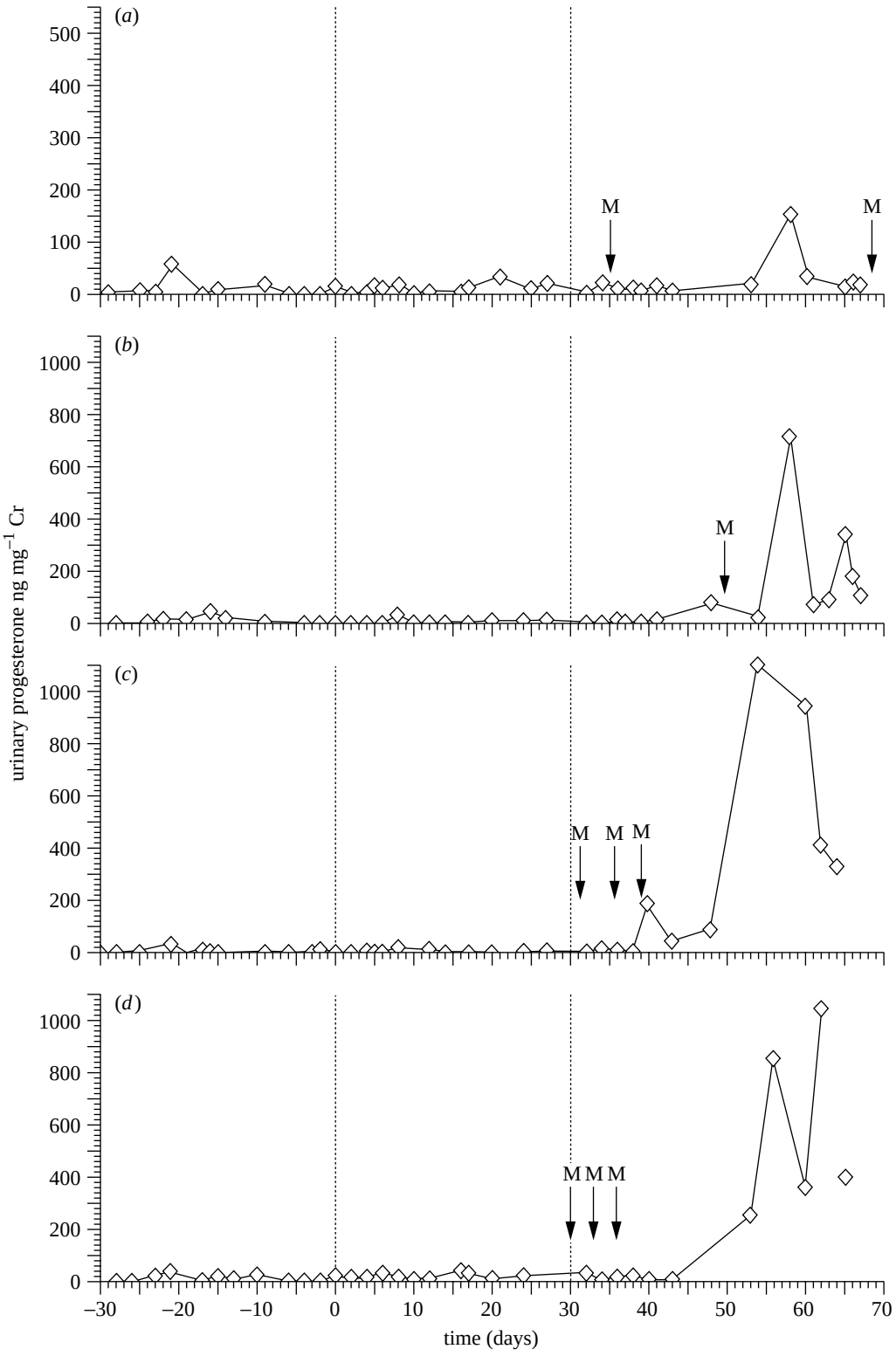

Figure 2. Urinary progesterone profiles for females while non-breeders in their parental colony (days −30 to −1), when removed and housed singly (days 0–29) and when paired with an unfamiliar unrelated male (days 30–70). These females represent the control group. Arrows indicate the day(s) that mating (M) was observed. (*a*) Female A6, (*b*) female A7, (*c*) female B5 and (*d*) female C2.

between treatments were made using Friedman ANOVA tests. Comparisons of urinary progesterone between non-breeding female groups were undertaken using Kruskal–Wallis tests. All tests were two-tailed and the level of statistical significance was taken as $p < 0.05$. All analyses were carried out using the Statistica computer package (Statsoft, Tulsa, OK, USA).

3. RESULTS

(a) *Pre-manipulation*

One male and one female in each colony were observed soliciting and mating indicating a single breeding male and female pair in each colony. Urinary progesterone profiles

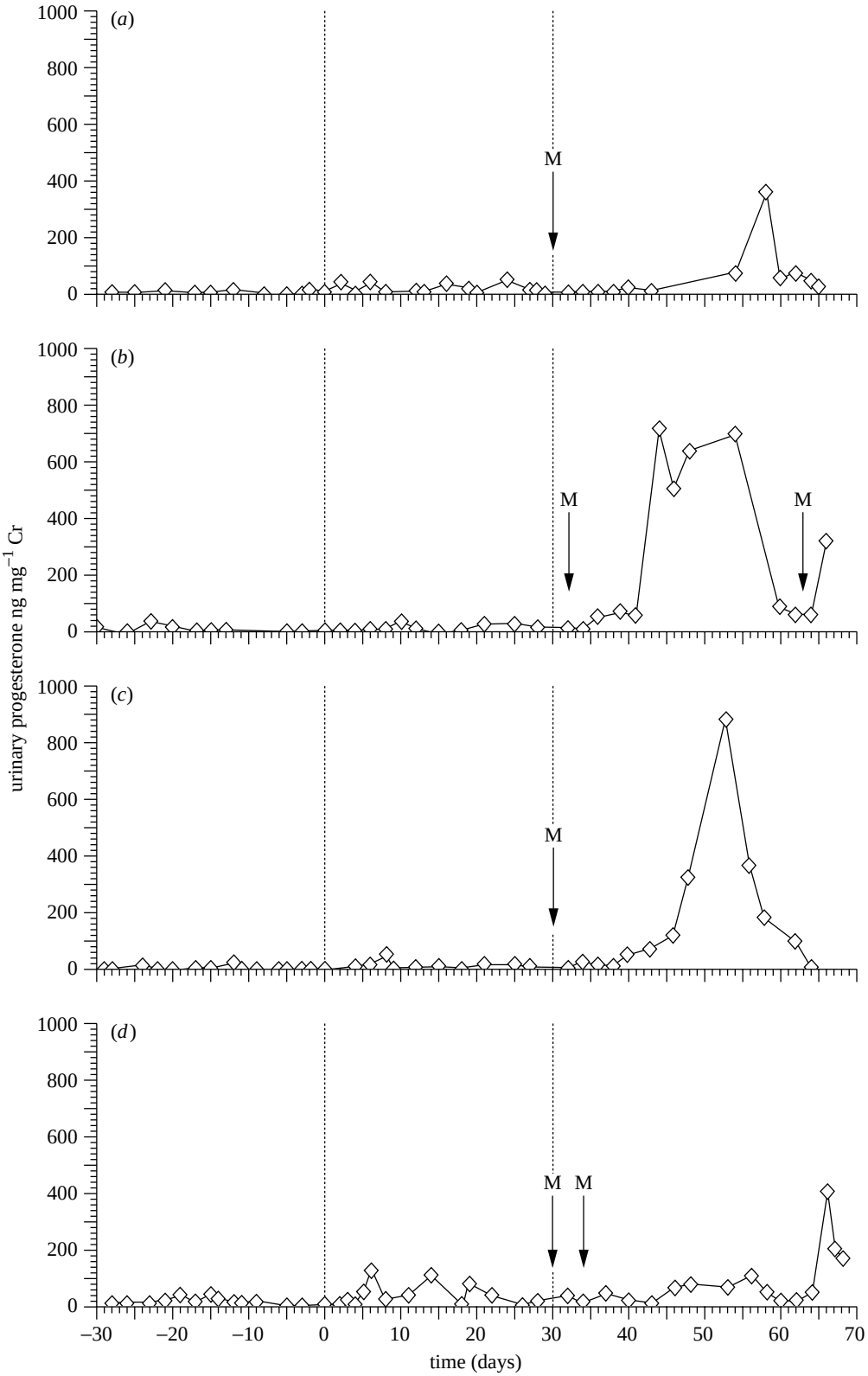

Figure 3. Urinary progesterone profiles for females while non-breeders in their parental colony (days −30 to −1), when removed and housed singly and subjected to daily transfer of soiled litter and bedding from the parent colony (days 0–29) and when paired with an unfamiliar unrelated male (days 30–70). These females represent the bedding transfer group. Arrows indicate the day(s) that mating (M) was observed. (*a*) Female A8, (*b*) female A9, (*c*) female B6 and (*d*) female C3.

also indicated only one reproductively active female per colony, as evidenced by ovarian cyclicity (figure 1*a*–*c*). The urinary progesterone levels of other colony females, although detectable, showed no sign of an ovarian cycle (figures 2–4). Furthermore, the urinary progesterone levels of these females were low in comparison to queens, the latter exhibiting the highest mean progesterone levels of colony females (figure 5*a*). The queen in colony C was the only female to breed in colonies during this study, becoming pregnant twice and giving birth to two litters (figure 1*c*).

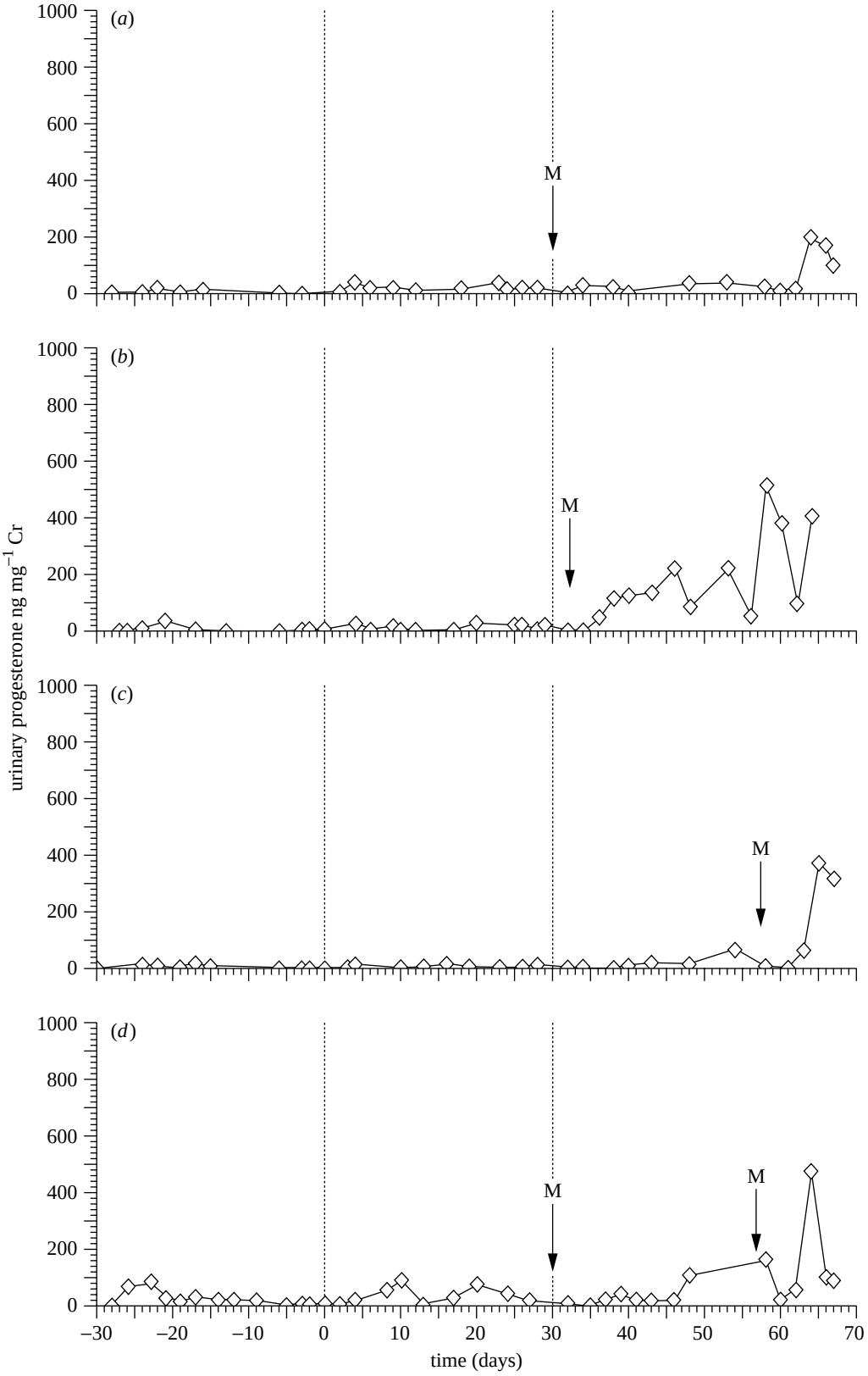

Figure 4. Urinary progesterone profiles for females while non-breeders in their parental colony (days −30 to −1), when removed from their parental colonies but kept in social contact with non-breeders from their parental colonies (days 0–29) and when paired with an unfamiliar unrelated male (days 30–70). These females represent the social contact group. Arrows indicate the day(s) that mating (M) was observed. (*a*) Female A10, (*b*) female A11, (*c*) female B7 and (*d*) female C4.

Despite a number of different agonistic behaviours described for this species, biting and incisor fencing were the only two observed during this study. The agonistic behaviour in colony A (30 recorded acts) was confined to two individuals, the breeding female (body mass 126 g) and her daughter, female A6, the largest colony female (body mass 141 g). Agonism between these two females took the form of bouts of incisor fencing, occasionally

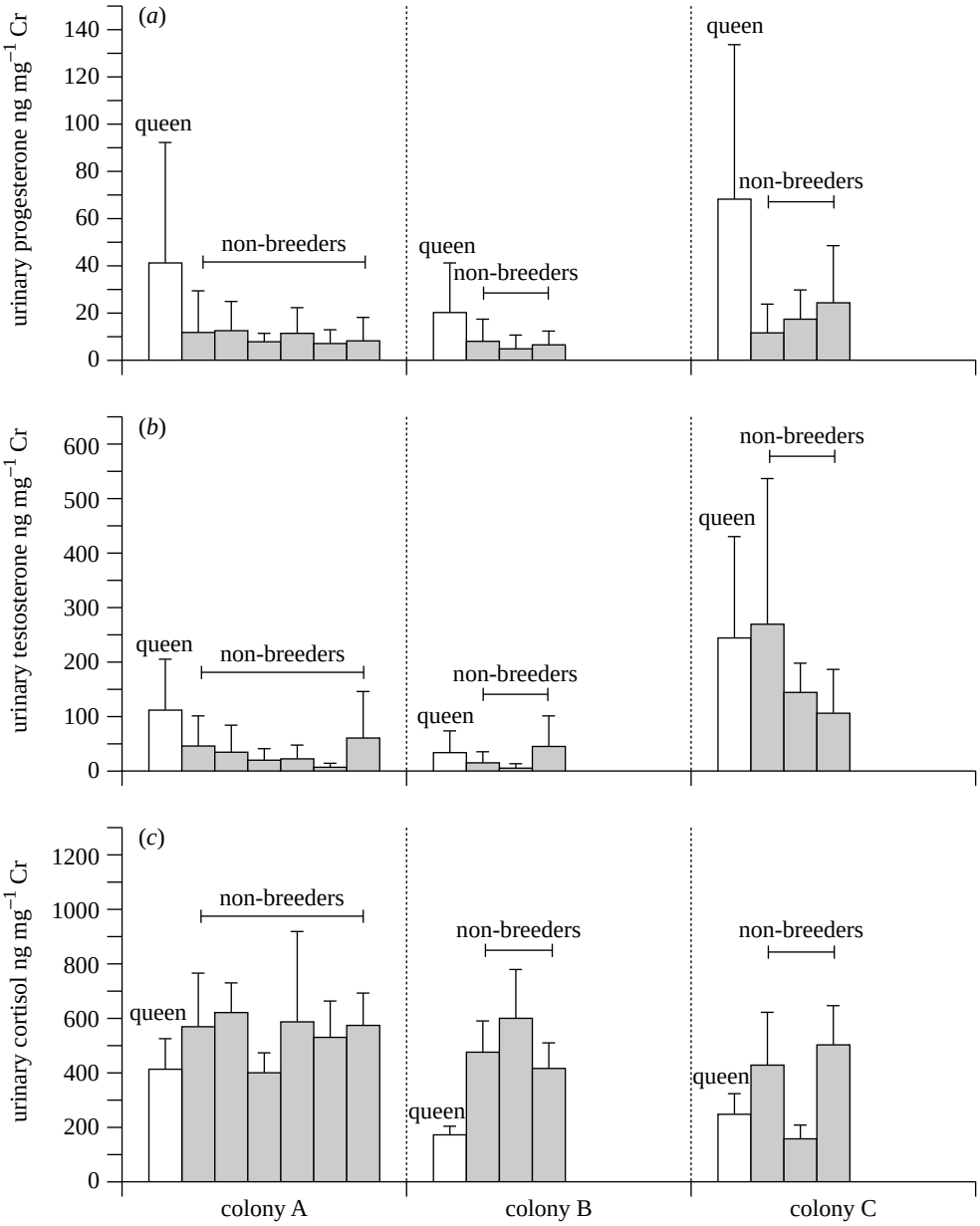

Figure 5. Urinary (*a*) progesterone, (*b*) testosterone and (*c*) cortisol concentrations (mean ± s.d.) of the breeding male and female and non-breeding females in colonies A, B and C before manipulation of colonies.

followed by one female biting the other (20% of occasions). The breeding female initiated agonistic encounters (1.3 bites h^{-1} and 6 bouts of incisor fencing h^{-1}) at approximately twice the rate of the non-breeding female A6 (0.67 bites h^{-1} and 2 bouts of incisor fencing h^{-1}). After 17 days of observation the breeding female was found with facial scars indicative of bites and after 28 days she was discovered badly bitten, presumably by female A6. As predetermined, female A6 was removed from the colony two days later and housed singly as one of the control group of females. Following removal of this female aggression stopped in the colony and the breeding female made a rapid recovery from her injuries. No agonistic behaviours were observed in colony B. The only agonism that was observed in colony C was incisor fencing between three non-breeding females. In contrast to the bouts of incisor fencing observed in colony A, the incisor fencing in colony C appeared to be play, which was less prolonged and intense and never followed by biting. The rates of incisor fencing for these females were 1.8, 1.7 and 2.6 acts h^{-1}. In colonies B and C, where there was no aggression between the breeding and non-breeding females, the former were heavier than their daughters (colony C breeding female 142 g and largest daughter 101 g) or of similar body mass (colony B breeding female 140 g and largest daughter 139 g).

There was no relationship between female reproductive status and urinary testosterone or cortisol concentrations (figure 5*b*,*c*). Queens displayed similar levels to non-breeding females, except in colony B where the queen's urinary cortisol level was considerably lower. Testosterone levels showed noticeable intercolony variation, with colony C females possessing the highest concentrations. Finally, non-breeding females showed no significant changes in body weight over time when housed in their parental colonies ($Z = 1.2$, $p > 0.05$ and $n = 12$).

(b) *Separated from parental burrow system*

Females exhibited no changes in body weight when separated from their parental burrow system (control group $\chi = 1.07$ and $p > 0.05$, bedding transfer group $\chi = 0.18$ and $p > 0.05$, and social contact group $\chi = 0.37$ and $p > 0.05$). Compared to pre-manipulation levels, there was no change in the urinary progesterone concentrations of females in the control group (mean $\pm$ s.d. pre-manipulation $= 11.8 \pm 2.3$ ng mg^{-1}Cr and separated $= 10.6 \pm 4.8 \pm$ ng mg^{-1}Cr), bedding transfer group (mean $\pm$ s.d. pre-manipulation $= 10.8$ 5.5 ng mg^{-1}Cr and separated $= 19.2 \pm 11.2$ ng mg^{-1}Cr) or social contact group (mean $\pm$ s.d. pre-manipulation $= 12.2 \pm 8.7$ ng mg^{-1}Cr and separated $= 17.8 \pm 10.5$ ng mg^{-1}Cr). There was no significant difference in urinary progesterone concentrations between the groups following separation (Kruskal–Wallis, $H_2 = 1.85$, $p > 0.05$ and $n = 12$). The urinary cortisol levels of all females after separation from their parental burrow system decreased in the control group (mean $\pm$ s.d. pre-manipulation $= 525.0 \pm 88.2$ ng mg^{-1}Cr and separated $= 327.0 \pm 81.1$ ng mg^{-1}Cr), whereas they increased in all females in the social contact group (mean $\pm$ s.d. pre-manipulation $= 507.8 \pm 66.2$ ng mg^{-1}Cr and separated $= 792.7 \pm 258.5$ ng mg^{-1}Cr). These findings were only just above the cut-off point for statistical significance (both control and social contact groups $\chi = 1.83$ and $p = 0.06$). Females in the bedding transfer group showed little change in urinary cortisol concentrations (mean $\pm$ s.d. pre-manipulation $= 536.4 \pm 206.9$ ng mg^{-1}Cr and separated $= 688.4 \pm 373.2$ ng mg^{-1}Cr) ($\chi = 1.46$ and $p = 0.14$).

Some aggression was noted in two females in the social contact group. Fourteen days after female B7 was separated from her parental burrow system and the breeders, she attacked and killed one of the transferred non-breeding females. The other transferred female was also attacked, as evidenced by facial scars. On the next transfer day female B7 attacked both introduced females and solicited one of the males, although the latter showed no interest. Twenty-nine days after separation, another female in the social contact group, female A11, also attacked and injured a transferred female and unsuccessfully solicited a male sibling. The other two females in the social contact group exhibited no aggression and displayed no interest in males.

(c) *Paired with an unrelated male after 30 days separation*

Females paired with an unrelated male showed no changes in body weight (control group $\chi = 0.73$ and $p > 0.05$, bedding transfer group $\chi = 1.46$ and $p > 0.05$, and social contact group $\chi = 0.73$ and $p > 0.05$). There was a significant increase in the urinary progesterone concentrations of all females in the control group (mean $\pm$ s.d. unrelated male absent $= 10.6 \pm 4.8$ ng mg^{-1}Cr and male present $= 201.4 \pm 156.8$ ng mg^{-1}Cr) (Friedman ANOVA, $\chi^2 = 6.0$, d.f. $= 2$, $p < 0.05$ and $n = 4$), bedding transfer group (mean $\pm$ s.d. unrelated male absent $= 19.2 \pm 11.2$ ng mg^{-1}Cr and male present $= 143.3 \pm 94.3$ ng mg^{-1}Cr) (Friedman ANOVA, $\chi^2 = 8.0$, d.f. $= 2$, $p < 0.02$ and $n = 4$) and social contact group (mean $\pm$ s.d. unrelated male absent $= 17.8 \pm 10.5$ ng mg^{-1}Cr and male present $= 96.1 \pm 52.1$ ng mg^{-1}Cr) (Friedman ANOVA, $\chi^2 = 6.5$, d.f. $= 2$, $p < 0.05$ and $n = 4$) following the introduction of the unrelated male. There was no significant difference in urinary progesterone between the groups following the introduction of an unrelated male (Kruskal–Wallis, $H_2 = 1.19$, $p > 0.05$ and $n = 12$). The progesterone profiles of all females showed an onset of reproductive activity and evidence of ovarian cycles (figures 2–4). Upon introduction to the unrelated male six females, one from the control group, two from the social contact group and three from the bedding transfer group, mated immediately. All females had mated by 17 days, the mean time to mating being four days.

4. DISCUSSSION

An important assumption of most skew models is that dominant members of a social group control reproduction in subordinates (Verhencamp 1983; Keller & Reeve 1994; Clutton-Brock 1998). The mechanisms of the DCM that appear to produce the reproductive suppression of subordinates are aggression and related stress effects (e.g. female dwarf mongoose *Helogale parvula* (Creel *et al*. 1992) and common marmoset *C. jacchus* (Abbott *et al*. 1998)), active interference with copulation (e.g. wolves *Canis lupus* (Mech 1970) and male dwarf mongoose (Creel & Waser 1997)), pheromonal cues that block ovulation (see Vandenbergh 1988) and infanticide. The demonstrable negative effects of 'stress' on fertility (Dunbar 1985) make stress imposed by the breeding female through aggression a prime candidate for suppression of fertility in non-breeding female Damaraland mole-rats. However, the behavioural and hormonal results from this study suggest that socially induced stress through aggression is not responsible for inhibition of the reproductive axis. This and other studies have found that aggressive behaviour is rare in intact colonies (Rickard & Bennett 1997; Cooney & Bennett 2000). Breeding males never exhibited aggression and aggression between breeding and non-breeding females was only observed in one colony where the queen and her daughter engaged in serious fighting. Testosterone mediates male aggressive behaviour (Wingfield *et al*. 1994) and, in some female rodents at least, testosterone appears to form the foundation for hormone-dependent aggression (Albert *et al*. 1991). However, no relationship between breeding status and female urinary testosterone concentrations was found in this study, further supporting the observation that queens are no more aggressive than non-breeding females.

Social stress may result in an increase in the release of glucocortcoids from the adrenal cortex, in essence preparing the body for 'fight or flight' (Kaplan 1986; Wingfield *et al*. 1994) and, therefore, determining glucocorticoid concentrations can often prove a useful measure of the level of stress experienced by an individual. Furthermore, increased glucocorticoid release has been associated with decreased gonadotrophin and sex steroid secretion (Dunbar 1985; Wingfield *et al*. 1994). The major secreted glucocorticoid in mole-rats is cortisol (G. G. Ganem and N. C. Bennett, unpublished data). The urinary cortisol concentrations of breeding and non-breeding females in this study did not differ. Similarly, Molteno & Bennett (2000) found no differences in plasma cortisol between breeders and non-breeders perhaps suggesting that non-breeding females are no

more stressed than breeders. Furthermore, both studies appeared to rule out a causal link between elevated levels of cortisol and a decrease in reproductive function. However, care must be taken in interpretation of these results. Elevated cortisol titres generally occur in response to short-term stress, whereas we determined the cortisol levels of individuals in intact colonies approximately every five days. It is possible that, by not collecting urine samples specifically at times of aggression, we may have missed elevated cortisol levels in some individuals.

We found no evidence that pheromonal cues block ovulation. The addition of soiled bedding to an experimental group of females paired with an unrelated male failed to prevent or delay reproductive activation, as evidenced by the progesterone profiles and three out of the four females in the bedding transfer group mated within three days of being paired with an unrelated male. Faulkes & Abbott (1993) showed that primer pheromones or other secretions contained in soiled bedding or litter do not play a major role in the suppression of reproduction in non-breeding naked mole-rats. Indeed, Keller & Nonacs (1993) argued that pheromonal queen control has never been conclusively demonstrated and is evolutionary difficult to justify. For example, a queen-produced pheromone aimed at inhibiting other females' reproduction is also likely to inhibit queen reproduction as well. Although this study does not conclusively rule out a pheromonal effect, rather than controlling reproduction in subordinate mole-rats, it is more likely that pheromones from breeders, if operational, may instead act as honest signals of queen presence and fecundity.

There was no evidence that breeders actively interfere with subordinate copulation or practice infanticide. Non-breeding females in intact colonies showed little or no sexual interest in male colony members. Only two instances were observed in which non-breeding females solicited males and males did not reciprocate. A behavioural aversion to mate with familiar individuals (Bennett *et al*. 1996; Rickard & Bennett 1997), namely incest avoidance, precludes a role for active interference of subordinate mating by dominants in intact colonies of Damaraland mole-rats. However, even when an unrelated male is introduced into colonies, the breeding female does not appear to interfere to prevent solicitations and copulation between the male and other females, although an increase in female–female aggression may occur (Cooney & Bennett 2000). The rarity of multiple breeding females again precludes a role for infanticide in controlling reproduction in intact colonies of Damaraland mole-rats.

The DCM predicts that, in the presence of a dominant female (or her olfactory cues), no other female will attempt to breed. This study and others have demonstrated a clear physiological block to reproduction in non-breeding females housed in the presence of the breeders (Bennett *et al*. 1993, 1996; Molteno & Bennett 2000). Non-breeding females showed no sign of ovarian cycles and their urinary progesterone levels were significantly lower than those of queens. The DCM also predicts that, in the absence of a dominant female or her cues, a previously subordinate female should attempt to breed immediately. Indeed, the observed effect of removing the queen from mole-rat colonies on the reproductive physiology of non-breeding females has led to the concept of 'queen control' (Bennett *et al*. 1996; Faulkes & Abbott 1997). In colonies of Damaraland mole-rats that were 'queenless' for more than three years, the plasma LH concentrations of non-breeding females increased significantly, although not to the level of queens (Bennett *et al*. 1996). In addition, non-breeding females housed singly for longer than six months show elevated plasma LH levels and ovulated, in comparison to non-breeding females in intact colonies (Molteno & Bennett 2000). Although we found evidence for a physiological block to reproduction in non-breeding females housed in intact colonies, there was no onset of reproductive activity when they were removed from the presence of breeders and housed singly for 30 days. The fact that the physiological block to reproduction in non-breeding females is eventually lifted in the absence of the queen indicates that the queen has some effect on the reproduction of other colony females. However, we suggest that the long period before reproductive activation indicates that it is not a controlling signal.

The existence of proximate effects in Damaraland mole-rats due to the presence of a queen does not necessarily demonstrate control and these examples can be interpreted as increasing not only the queen's fitness, but also that of other colony members. Here we suggest that the SRM is the basic model found in the Damaraland mole-rat. Self-restraint in Damaraland mole-rats may function to minimize inbreeding by restricting reproduction until an unrelated male is present or until dispersal and independent breeding is anticipated following the death of a breeder and/or to restrict reproduction until sufficient helpers or resources are available. This would explain the rapid onset of reproductive activation following the introduction of an unrelated male and a more gradual onset following breeder removal. The SRM does not involve aggression directed towards non-reproductive females and, indeed, control by dominant breeders of reproduction in non-breeding females is unnecessary given that colonies consist entirely of siblings and their parents since incest avoidance alone should suffice. Indeed, despite an increase in the LH of females in queenless colonies, they showed no/little interest in colony males and never bred (Bennett *et al*. 1996).

The fact that the reproductive physiology of female non-breeding Damaraland mole-rats is suppressed, whereas in other species non-breeding females display similar circulating basal LH levels to breeding females (*Cryptomys darlingi* (Bennett *et al*. 1997) and *Cryptomys hottentotus hottentotus* (Spinks *et al*. 2000)), may merely occur in anticipation of dispersal and independent breeding. Ecological constraints against successful dispersal among social cryptomid species appear to be greatest for Damaraland mole-rats, whereas other species have higher rates of dispersal and spend a shorter time in the colony and have a higher chance of breeding at any particular time, i.e. they are 'reproductively ready'.

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