LH responses to single doses of exogenous GnRH by social Mashona mole-rats: a continuum of socially induced infertility in the family Bathyergidae

N. C. BENNETT^{1*}, C. G. FAULKES² AND A. C. SPINKS³

¹*Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, Republic of South Africa*

²*Institute of Zoology, Regent's Park, London NW1 4RY, UK*

³*Department of Zoology, University of Cape Town, Rondebosch 7700, Republic of South Africa*

SUMMARY

The Mashona mole-rat, *Cryptomys darlingi*, exhibits an extreme reproductive division of labour. Reproduction in the colony is restricted to a single breeding pair. The non-reproductive male and female colony members are restrained from sexual activity by being familiar and related to one another and the reproductive animals.

Circulating basal concentrations of luteinizing hormone (LH) as well as LH levels measured in response to a single exogenous gonadotropin releasing hormone (GnRH) challenge are not significantly different between the reproductive and non-reproductive groups of either sex. Socially induced infertility in both non-reproductive males and females does not result from a reduced pituitary secretion of LH or decreased sensitivity to hypothalamic GnRH, but rather appears to result from an inhibition of reproductive behaviour in these obligate outbreeders.

The African mole-rats exhibit a continuum of socially induced infertility with differing social species inhabiting regions of varying degrees of aridity. In this continuum a transition from a predominantly behavioural repression in a social mesic-adapted species through to complete physiological suppression lacking incest avoidance in an arid-adapted eusocial species occurs in this endemic African family of rodents.

1. INTRODUCTION

Reproductive suppression occurs to varying degrees amongst a wide range of mammalian species. Two major forms of reproductive suppression have been demonstrated. In many rodents, suppression results from increased population densities and is mediated by urinary semiochemicals (Brown & MacDonald 1984). Suppression also occurs in hierarchical groups of mammals where dominant individuals stress subordinates via behavioural interactions (Abbott 1987; Abbott *et al.* 1988).

The social species of the family Bathyergidae provide an ideal group for studying the degree of socially induced infertility and the different mediating mechanisms. The naked mole-rat, *Heterocephalus glaber* exhibits extreme socially induced infertility in that both sexes of non-breeding animals are physiologically suppressed from reproducing (Faulkes *et al.* 1990, 1991). The eusocial Damaraland mole-rat, *Cryptomys damarensis*, similarly shows physiological suppression and behavioural inhibition in the females, but males are behaviourally restrained (Bennett *et al.* 1994a, 1996; Bennett 1994).

Colonies of the Mashona mole-rat contain up to nine individuals. Inhibition of reproduction is such

that only one male and female are reproductively active (Bennett *et al.* 1994b), these being the founding members of the colony. The non-reproductive members of the colony are reproductively quiescent and do not form distinct groups based on the amount of burrow maintenance activity as has been found in the Damaraland mole-rat (Bennett & Jarvis 1988; Jacobs *et al.* 1991; Gabathuler *et al.* 1996).

The primary aim of this study was to investigate whether physiological suppression was apparent at the level of the pituitary by testing pituitary sensitivity to gonadotropin releasing hormone (GnRH) with the reproductive status of the males and females in the colony.

Mole-rats are entirely subterranean dwelling in an extensive gallery of dark labyrinths below the ground (Jarvis & Bennett 1991). The eyes of these animals are minute and show both structural and functional degeneration (Eloff 1958). Behavioural studies into dominance hierarchy formation and dyadic interactions show that the colony members are not shoved nor do they receive punishment as has been found in the naked mole-rat (Gabathuler *et al.* 1996; Jarvis 1991). Semiochemical control of reproduction is unlikely, since the reproductives lack any smearing or marking behavioural acts. One cannot exclude the possibility that a semiochemical is in the urine, but

* Author for correspondence.

Table 1. *Range in body mass of groups of Cryptomys darlingi administered 1 µg GnRH or physiological saline*

body mass (g) (mean ± s.e.)	range in body mass (g)	sample size	reproductive status	sex	saline or GnRH
96 ± 7.2	76–118	6	reproductive	M	GnRH + saline
65 ± 4.0	57–80	6	reproductive	F	GnRH + saline
75 ± 6.3	52–115	9	non-reproductive	M	GnRH + saline
68 ± 3.3	62–80	6	non-reproductive	F	GnRH + saline

then one would ask how does the reproductive female prevents the chemical switching her off. In addition, removal of the reproductive female from the colony is not met by queen succession from within. Indeed, there is no sexual activity emanating in these reproductively quiescent colonies.

The results of this study are compared with suppressive mechanisms operating in two other species of highly social mole-rats. The concept of the continuum of socially induced infertility is proposed whereby a transition from a predominantly behavioural incest avoidance in which familiarity and relatedness is important in social mesic-adapted species of mole-rat through to complete physiological suppression in arid-adapted eusocial species occurs in this remarkable endemic African family of rodents.

2. MATERIALS AND METHODS

A colony of the Mashona mole-rat was trapped at Goromonzi (17° S 31° E) near Harare, Zimbabwe in June 1992. Two additional colonies formed from pairs of mole-rats from two different colonies that were captured at Goromonzi in July 1990, and a further two reproductive pairs derived from animals captured at Goromonzi were used in the study.

Blood samples were obtained from 11 females and 14 males. Of these nine females and 12 males were members of three colonies and the remaining animals came from two reproductive pairs.

The mean, s.e., range in body mass, and numbers of mole-rats used in the study are presented in table 1.

The mole-rats were housed in their natal colonies in large plastic containers (1 m × 0.5 m × 0.5 m) containing wood shavings and wood wool that served as nesting material. The room temperature ranged between 26 °C and 28 °C and the relative humidity was maintained at approximately 50% (Bennett *et al.* 1988). The room was maintained on a constant light regimen of 14L:10D.

The mole-rats were fed on a variety of chopped fruits, roots and green vegetables. The mole-rats drank no free water and obtained a positive water balance from the food source.

Reproductive females are characterized by prominent axillary and inguinal teats, in addition to a perforate vagina. In non-reproductive females the teats are rudimentary and the vagina imperforate. The imperforate vaginae in non-reproductives is a consequence of the lack of penetration by males. All males have abdominal testes of similar size. Reproductive males are distinguished by their observed copulatory activity, a darkly stained coating around the periphery of the mouth and bulging testes which project from abdominal inguinal pockets. Testes from non-reproductive males are difficult

to discern through the abdomen. Histological examination of the testes revealed that spermatogenesis and production of mature sperm occurred in both groups.

On pairing non-reproductive males with unrelated non-reproductive females, the coating around the mouth develops and the testes move into the inguinal pockets within three weeks of introduction.

(a) *Blood sampling*

Blood samples were obtained between 14:00 and 17:00 h. The animals were hand-held and blood samples were obtained from veins in the foot. Approximately 300–400 µl of whole blood was collected by capillary action using heparinized micro-haematocrit tubes. After collection, the samples were held on ice for 20 min before being centrifuged at 500g and the plasma was stored at –70 °C.

(b) *GnRH administration*

Ampoules of 1 µg GnRH in 200 µl sterile physiological saline were stored at –70 °C until required. The GnRH was synthesized in the laboratory of R. P. Millar, using solid phase methodology (the purity of GnRH was greater than 98% homogeneity) (Millar *et al.* 1989). In all experiments, 1.0 µg exogenous GnRH was administered s.c. as a bolus 200 µl injection. Blood samples were taken from reproductive and non-reproductive animals immediately before and 20 min after a single subcutaneous (s.c.) injection. Ampoules of 200 µl sterile physiological saline were administered s.c. as a single injection to the control animals.

(c) *LH bioassay*

Luteinizing hormone (LH) was measured using an *in vitro* bioassay based on the production of testosterone by dispersed mouse Leydig cells (Van Damme *et al.* 1974) as previously described and validated in the naked mole-rat by (Faulkes *et al.* 1990, 1991) and Damaraland mole-rat (Bennett *et al.* 1993). Details of the assay have been described by Harlow *et al.* (1984), Hodges *et al.* (1987) and Abbott *et al.* (1988). Plasma samples were assayed in duplicate in two dilutions (1:20 and 1:40), as a routine check for parallelism and compared with the rat LH standard (rLH antigen preparation: rLH-1-7 from NIDDK, Baltimore) over the range 0.0625–2 mIU^{–1}. The testosterone produced was measured by radioimmunoassay as described by Hodges *et al.* (1987).

Checks for parallelism were carried out to validate the LH bioassay for plasma taken from animals after GnRH treatment. Dilutions of the Mashona mole-rat plasma taken before and after the GnRH treatment were parallel to and not significantly different from the reference preparation.

The sensitivity of the assay (determined at the 90%

Figure 1. Concentrations of plasma bioactive LH (means $\pm$ s.e.) in reproductive (RM) and non-reproductive (NRM) male *C. darlingi*, before (0) and 20 min after (20) a single s.c. injection of GnRH or saline. * $P < 0.05$. Pre- and post-saline challenges within male categories were non-significant. Magnitude of GnRH response between male classes was non-significant, $p > 0.05$.

Figure 2. Concentrations of plasma bioactive LH (means $\pm$ s.e.) in reproductive (RF) and non-reproductive (NRF) female *C. darlingi*, before (0) and 20 min after (20) a single s.c. injection of GnRH or saline. * $P < 0.05$. Pre- and post-saline challenges within female categories were non-significant. Magnitude of GnRH response between female classes was non-significant, $p > 0.05$.

binding) was 0.1 miu per tube. Intra- and inter-assay mean coefficient of variation for repeated determinations of LH quality control (1.53 miu ml^{-1}) were 10% and 16%, respectively.

Results from the plasma LH determinations were analysed using non-parametric Mann Whitney U-tests. Means are presented $\pm$ s.e. (Siegel 1956).

(d) Behavioural experiments

Reproductive females were removed from two colonies containing seven and nine mole-rats, respectively, each raised from initial pairs of reproductive animals. The colonies were left for a period of 11 and 16 months, respectively. During this time the non-reproductive females were monitored weekly for the presence of perforate vaginae and sexual activity.

Four pairs of unfamiliar non-kin mole-rats obtained from the above two colonies were put in separate molearia. Likewise two pairs of related and familiar animals (siblings) were housed separately in individual plastic molearia. Again the females were examined for perforate vaginae, sexual activity and the final production of young.

3. RESULTS

(a) Endocrine experiments

Basal LH concentrations were not significantly different between reproductive males ($3.50 \pm 0.55 \text{ miu ml}^{-1}$, $n = 9$) and non-reproductive males ($3.83 \pm 0.42 \text{ miu ml}^{-1}$, $n = 17$) $U = 51$, $n_1 = 9$, $n_2 = 17$, $p > 0.05$. Likewise, basal LH concentrations in reproductive females ($4.8 \pm 1 \text{ miu ml}^{-1}$, $n = 9$) were not significantly different from those of non-reproductive females ($3.0 \pm 0.4 \text{ miu ml}^{-1}$, $n = 13$) $U = 30$, $n_1 = 9$, $n_2 = 13$, $p > 0.05$.

There was no significant difference in pre- and post-LH concentrations to a challenge of placebo

physiological saline in reproductive males ($U = 4$, $n_1 = 3$, $n_2 = 3$, $p > 0.5$) and in non-reproductive males ($U = 33$, $n_1 = 9$, $n_2 = 9$, $p > 0.05$) (figure 1). Likewise, there was no significant difference to a challenge of placebo physiological saline in reproductive females ($U = 6$, $n_1 = 3$, $n_2 = 3$, $p > 0.05$) and non-reproductive females ($U = 21$, $n_1 = 7$, $n_2 = 7$, $p = 0.36$) (figure 2).

There was a significant response in both reproductive males and females to a single challenge of $1 \mu\text{g}$ GnRH, ($U = 0$, $n_1 = 6$, $n_2 = 6$, $p < 0.001$; $U = 0$, $n_1 = 6$, $n_2 = 6$, $p < 0.001$, respectively) (figures 1 and 2). There was also a highly significant response in both non-reproductive males and females to a single challenge of $1 \mu\text{g}$ exogenous GnRH, ($U = 0$, $n_1 = 8$, $n_2 = 8$, $p < 0.0001$) and ($U = 0$, $n_1 = 6$, $n_2 = 6$, $p < 0.0001$, respectively) (figures 1 and 2).

There was no significant difference in the magnitude of the LH response to the administration of $1 \mu\text{g}$ GnRH between reproductive and non-reproductive males, $U = 20$, $n_1 = 7$, $n_2 = 7$, $p = 0.33$ or between reproductive and non-reproductive females, $U = 11$, $n_1 = 6$, $n_2 = 6$, $p = 0.155$ (figures 1 and 2).

(b) Behavioural experiments

Four pairs of unfamiliar and unrelated mole-rats produced young within 115 days of pairing. The mole-rats copulated immediately on pairing and were routinely seen copulating between the time of pairing and eventual conception. Two pairs of familiar and related mole-rats (siblings) did not exhibit any copulatory or sexual activity and consequently failed to produce young, even though they were removed from the presence of the reproductive pair (table 2).

Eleven and 16 months after removal of the reproductive females from the two colonies, siblings had not exhibited any courtship or copulation and did not

Table 2. *The breeding success of four couples of non-kin Mashona mole-rats*

pair	courtship and copulation	days to birth of litter	litter size and sex	
1	+	107	1	1M
2	+	99	2	2F
3	+	108	2	1M 1F
4	+	115	1	1F

produce offspring within the respective colonies and effectively remained reproductively quiescent. The females remained imperforate and the teats rudimentary.

4. DISCUSSION

The African mole-rats of the family Bathyergidae provide a remarkable example of a continuum of socially induced infertility operating on differing social species inhabiting regions of varying degrees of aridity.

In the eusocial naked mole-rat, *H. glaber*, a socially induced suppression of reproductive physiology is evident in non-reproductive animals of both sexes. Non-reproductive females are anovulatory, whereas non-reproductive males have reduced concentrations of testosterone in their urine and many produce sperm that are few in number and non-motile (Faulkes *et al.* 1990, 1991, 1994).

In the Damaraland mole-rat, *C. damarensis*, previous studies have indicated that, like *H. glaber*, non-reproductive females have a block to ovulation (Bennett *et al.* 1994b; Bennett 1994). However, unlike non-breeding female *H. glaber*, the ovaries of non-reproductive *C. damarensis* show some follicular development and contain many luteinized secondary and tertiary follicles (Bennett *et al.* 1994b). Non-reproductive females have measurable concentrations of progesterone attributable to the luteinized unruptured follicles, whereas concentrations of oestrogen are low or undetectable (Bennett *et al.* 1994b). However, in contrast to the naked mole-rat, there are no measurable hormonal differences between reproductive and non-reproductive males of the Damaraland mole-rat (Bennett 1994). All males undergo spermatogenesis, can produce motile spermatozoa and have similar urinary testosterone concentrations (Bennett *et al.* 1988; Bennett 1994; Faulkes *et al.* 1994). Recent work by Bennett *et al.* (1996) and Rickard & Bennett (1997) shows that reproductive suppression in females results from a two fold control, namely: (i) a reduced pituitary synthesis and/or secretion of LH leading to a block to ovulation; and (ii) a strong inhibition to breed with familiar kin. It would appear that non-reproductive females and males are only freed from sexual restraint during favourable environmental conditions (soft workable soils following good rains). It is then that mate selection can actively favour non-kin and non-familiar

mates from neighbouring colonies in the population deme.

The absence of differing basal concentrations of bioactive LH in plasma or LH responses to an exogenous GnRH challenge between both reproductive and non-reproductive males and females implies that in the Mashona mole-rat, physiological suppression does not appear to be operating to the degree that has been elegantly illustrated in the Damaraland and naked mole-rat. In these latter species, there are marked differences in basal LH concentrations between reproductive and non-reproductive females, and the response of the pituitary to a GnRH challenge (Faulkes *et al.* 1990, 1991; Bennett *et al.* 1993).

The most thoroughly documented mechanism of suppression occurring at the hypothalamic and hence pituitary level has come from studies on endogenous opioid production. Natural endogenous opioid peptides have been implicated in the suppression of hypothalamic GnRH (Grossman *et al.* 1981). The use of naloxone (an opiate receptor blocker) stimulates the secretion of LH in women that have previously experienced hypothalamic amenorrhea. These findings have suggested that opioid peptides may cause GnRH and hence LH infertility (Quigley & Yen 1980; Yen 1986). The lack of a difference between reproductive and non-reproductive animals in both basal LH and the response to a single exogenous challenge of GnRH in both sexes suggests that this neuroendocrine mechanism of suppression is absent in the Mashona mole-rat. Similarly, it would appear that the bioactivity of LH secreted by non-reproductive animals has not been altered. In humans, GnRH can change the biological activity of LH by altering the carbohydrate content of the molecule (Montanini *et al.* 1984).

Two colonies of the Mashona mole-rat from which the queen had been removed failed to show any sexual activity and remained reproductively quiescent for periods of 11 and 16 months, respectively. However, on pairing unrelated animals from the two colonies, sexual activity readily occurred. Four pairings of unrelated animals from these two colonies, in which the opposite sex was unrelated genetically and was also unfamiliar, resulted in copulatory activity, conception and eventual parturition in these previously sexually inactive animals. Hence we suggest that in the Mashona mole-rat reproductive suppression does not involve physiological infertility at the pituitary level and probably not at the hypothalamic level either. It appears that an inhibition to mate with familiar or kin is important in reproductive inactivity since reproductive and non-reproductive animals of both sexes exhibit similar responses to GnRH challenges as well as basal levels of LH. This is markedly different from *C. damarensis*, where both incest avoidance and physiological suppression appear to operate simultaneously (Bennett *et al.* 1996).

The disparate hormone responses of non-reproductive female and male colony members in *C. damarensis* indicate that suppression in the females is more profound than in the males since physiological suppression and behavioural restraint

(in the form of incest avoidance) is operating (Bennett *et al.* 1993, 1996). Non-reproductive males are restrained from sexual activity by being subordinate to the reproductive male(s) as well as by being excluded from the pair bond and being the progeny of the reproductive animals (Bennett & Jarvis 1988; Jacobs *et al.* 1991; Jarvis & Bennett 1993).

A spectrum of social organization occurs within the Bathyergidae ranging from solitary through to truly eusocial species. The naked mole-rat and Damaraland mole-rat exhibit all the criteria for eusociality (Michener 1969; Wilson 1971), namely: (i) a reproductive division of labour; (ii) cooperative care of the young; and (iii) an overlap of at least two generations. They, however, vary in the strength as well as the duration of the reproductive division of labour. Sherman *et al.* (1995) have proposed a quantitative means of comparing the social systems of cooperatively breeding species which essentially assesses the probability of an individual in a social group ever attaining a breeding status or its 'lifetime reproductive success' is used to define the degree of reproductive division of labour. In this paper we further show that within the family Bathyergidae, there is a 'continuum of socially induced infertility' occurring amongst different species inhabiting regions of varying degrees of aridity. The lack of a marked physiological component to LH suppression in the Mashona mole-rat may be correlated with environmental factors, since these mole-rats occur in more mesic regions where rainfall is predictable and consequently dispersal events from the natal colony are probably more frequent than that of the Damaraland mole-rat, which inhabits semi-arid to arid regions in which rainfall patterns are highly unpredictable and infrequent. Hence, in mesic habitats where opportunities for dispersal and of becoming reproductives in new colonies are great—there is a predominant incest avoidance component to social suppression and colony members are obligate outbreeders. In marked contrast, the naked mole-rat, an obligate inbreeder, exhibits physiological suppression in both sexes of non-reproductives. The Damaraland mole-rat, an obligate outbreeder, lies between these two extremes in that while non-reproductive males are behaviourally suppressed, the non-reproductive females have both behavioural and physiological suppression operating upon them (Bennett *et al.* 1996). Thus, in the Damaraland mole-rat, while colony members opt to outbreed, reproductive suppression is still imposed upon non-reproductive female colony members (Jarvis *et al.* 1994; Bennett *et al.* 1996).

Long term field studies on naked mole-rats have shown that less than 0.1% of non-reproductives eventually attain reproductive status (Jarvis *et al.* 1994). Field studies on the Damaraland mole-rat (Jarvis & Bennett 1993) have shown that ecological constraints limit the opportunities for dispersion and $\pm 10\%$ of all non-reproductives in colonies attain reproductive status (Bennett *et al.* 1993, 1994b). Comparative field data are unavailable for the Mashona mole-rat, but we would predict from the small colony numbers and less stable social organization that opportunities

for dispersing and eventually becoming reproductives and founding new colonies should be greater for this species, with greater than 10% becoming reproductively active.

Currently, the available evidence suggests that in the Mashona mole-rat, the non-reproductive males and females refrain from sexual activity by being subordinate to the reproductive pair (Gabathuler *et al.* 1996) and as a result of their genetic relatedness are excluded from the pair bond of the reproductive animals that are characteristically the parents of the non-reproductive colony members. In conclusion, socially induced suppression of reproductive activity in both non-reproductive males and females of the Mashona mole-rat does not result from a decreased pituitary secretion of LH. The physiological suppression so markedly seen in the sister taxon *C. damarensis* is absent in *C. darlingi* and incest avoidance and the role of familiarity appears more pronounced than in the former species. The fact that siblings will not breed with one another and yet non-kin and unfamiliar individuals will readily breed if paired, suggests that in the Mashona mole-rat, incest avoidance appears to be important in the prevention of reproduction in non-reproductive members whilst in the confines of the colony. It would appear that in the Mashona mole-rat, the absence of reproduction in both sexes of non-reproductive animals results from sexual inactivity from a strong incest avoidance and mate choice actively favours non-kin or an unfamiliar animal. Initiation of sexual activity in non-reproductive animals of either sex can be brought about by pairing unrelated animals from differing colonies where mate choice favours non-kin. Removal of the queen from a colony results in sexual quiescence, a condition that has been reported in the closely related Damaraland mole-rat (Rickard & Bennett 1997).

The authors thank Mr and Mrs A. Douie for permission to trap mole-rats on their farm in Goromonzi; the Department of National Parks and Wildlife Services, Zimbabwe for providing the necessary collection and export permits; NIDDK, Baltimore, USA, and the National Hormone and Pituitary Program at the University of Maryland School of Medicine, USA, for the rat LH reference preparation (R-LH-1-7). We also thank R. P. Millar for the gift of the mammalian GnRH and W. Ferguson, T. Richter, R. Cooney, J. M. Greeff and A. J. Molteno for reading earlier drafts of the manuscript. This work was supported by research grants from the Foundation for Research Development (to N.C.B.) and the Wellcome Trust (to C.G.F.). Finally we acknowledge a travel grant to J. U. M. Jarvis from the National Geographic Society to collect the animals in Zimbabwe.

REFERENCES

- Abbott, D. H. 1987 Behaviourally mediated suppression of reproduction in female primates. *J. Zool.* **213**, 455–470.
- Abbott, D. H., Hodges, J. K. & George, L. M. 1988 Social status controls LH secretion and ovulation in marmoset monkeys (*Callithrix jacchus*). *J. Endocrinol.* **117**, 329–339.

- Bennett, N. C. 1994 Reproductive suppression in social *Cryptomys damarensis* colonies: a lifetime of socially induced sterility in males and females (Rodentia: Bathyergidae). *J. Zool.* **234**, 25–39.
- Bennett, N. C. & Jarvis, J. U. M. 1988 The social structure and reproductive biology of colonies of the mole-rat *Cryptomys damarensis* (Rodentia, Bathyergidae). *J. Mammal.* **69**, 293–302.
- Bennett, N. C., Jarvis, J. U. M. & Davies, K. C. 1988 Daily and seasonal temperatures in the burrows of African rodent moles. *S. Afr. J. Zool.* **23**, 189–195.
- Bennett, N. C., Jarvis, J. U. M., Faulkes, C. G. & Millar, R. P. 1993 LH responses to single doses of exogenous GnRH by freshly captured Damaraland mole-rats, *Cryptomys damarensis*. *J. Reprod. Fert.* **99**, 81–86.
- Bennett, N. C., Jarvis, J. U. M. & Cotterill, F. P. D. 1994a The colony structure and reproductive biology of the afro-tropical Mashona mole-rat, *Cryptomys darlingi*. *J. Zool.* **234**, 477–487.
- Bennett, N. C., Jarvis, J. U. M., Millar, R. P., Sasano, H. & Ntshinga, K. V. 1994b Reproductive suppression in eusocial *Cryptomys damarensis* colonies: socially induced infertility in females. *J. Zool.* **233**, 617–630.
- Bennett, N. C., Faulkes, C. G. & Molteno, A. J. 1996 Reproductive suppression in subordinate, non-breeding female Damaraland mole-rats: two components to a lifetime of socially induced infertility. *Proc. R. Soc. Lond. B* **263**, 1599–1603.
- Brown, R. E. & MacDonald, D. W. 1984 *Mammalian social odours*. Oxford University Press.
- Eloff, G. 1958 Functional and structural degeneration of the eye of the South African rodent mole, *Cryptomys bigalkei* and *Bathyergus maritimus*. *S. Afr. J. Sci.* **54**, 293–302.
- Faulkes, C. G., Abbott, D. H. & Jarvis, J. U. M. 1990 Social suppression of ovarian cyclicity in captive and wild colonies of naked mole-rats, *Heterocephalus glaber*. *J. Reprod. Fert.* **88**, 559–568.
- Faulkes, C. G., Abbott, D. H. & Jarvis, J. U. M. 1991 Social suppression of reproduction in male naked mole-rats, *Heterocephalus glaber*. *J. Reprod. Fert.* **91**, 593–604.
- Faulkes, C. G., Trowell, S. N., Jarvis, J. U. M. & Bennett, N. C. 1994 Investigation of numbers and motility of spermatozoa in reproductively active and socially suppressed males of two eusocial African mole-rats, the naked mole-rat (*Heterocephalus glaber*) and the Damaraland mole-rat (*Cryptomys damarensis*). *J. Reprod. Fert.* **100**, 411–416.
- Gabathuler, U., Bennett, N. C. & Jarvis, J. U. M. 1996 The social structure and dominance hierarchy of the Mashona mole-rat, *Cryptomys darlingi* (Rodentia: Bathyergidae) from Zimbabwe. *J. Zool.* **240**, 221–231.
- Grossman, A., Moul, P. G. A., Gallard, R. C., Delitalia, G., Toff, W. D., Rees, L. H. & Besser, G. M. 1981 The opioid control of LH and FSH release: effects of met-enkephalin analogue and naloxone. *Clin. Endocrinol.* **14**, 41–47.
- Harlow, C. R., Gems, S., Hodges, J. K. & Hearn, J. P. 1984 The relationship between plasma progesterone and the timing of ovulation and early embryonic development in the marmoset monkey (*Callithrix jacchus*). *J. Zool.* **201**, 272–282.
- Hodges, J. K., Cottingham, P., Summers, P. M. & Yingnan, L. 1987 Controlled ovulation in the marmoset monkey (*Callithrix jacchus*), with human chorionic gonadotrophin following prostaglandin induced luteal regression. *Fert. Steril.* **48**, 299–305.
- Jacobs, D. S., Bennett, N. C., Jarvis, J. U. M. & Crowe, T. M. 1991 The colony structure and dominance hierarchy of the Damaraland mole-rat, *Cryptomys damarensis* (Rodentia: Bathyergidae). *J. Zool.* **224**, 553–576.
- 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 & R. D. Alexander), ch. 13. Princeton University Press.
- Jarvis, J. U. M. & Bennett, N. C. 1991 Ecology and behaviour of the family Bathyergidae. In *The biology of the naked mole-rat* (ed. P. W. Sherman, J. U. M. Jarvis & R. D. Alexander). Princeton University Press.
- Jarvis, J. U. M. & 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., Bennett, N. C. & Sherman, P. W. 1994 Mammalian eusociality: a family affair. *Trends Ecol. Evol.* **9**, 47–51.
- Michener, C. D. 1969 Comparative social behaviour of bees. *A. Rev. Entomol.* **14**, 277–342.
- Millar, R. P., Flanagan, C. A., de Milton, R. C. & King, J. A. 1989 Chimeric analogues of vertebrate gonadotrophin releasing hormones comprising substitutions of the variant amino acids in positions 5, 7 and 8. *J. Biol. Chem.* **264**, 21 007–21 013.
- Montanini, V., Celani, M. F., Baraaghini, G. F., Carani, C. & Marrama, P. 1984 Effects of acute stimulation with luteinizing hormone-releasing hormone (LHRH) on biologically active and immunoreactive serum luteinizing hormone (LH) in pubertal boys. *Acta Endocrinol.* **107**, 289–294.
- Quigley, M. E. & Yen, S. S. C. 1980 The role of endogenous opiates on LH secretion during the menstrual cycle. *J. Clin. Endocrinol. Metab.* **51**, 179–181.
- Rickard, C. A. & Bennett, N. C. 1997 Recrudescence of sexual activity in a reproductively quiescent colony of the Damaraland mole-rat, by the introduction of an unfamiliar and genetically unrelated male—a case of incest avoidance in ‘queenless’ colonies. *J. Zool.* **241**. (In the press.)
- Sherman, P. W., Lacey, E. A., Reeve, H. K. & Keller, L. 1995 The eusociality continuum. *Behav. Ecol.* **6**, 102–108.
- Siegel, S. 1956 *Non-parametric statistics for the behavioural sciences*. McGraw Hill.
- Van Damme, M. P., Robertson, D. M. & Diczfalusy, E. 1974 An improved in vitro bioassay method for measuring luteinising hormone (LH) activity using mouse Leydig cell preparations. *Acta Endocrinol.* **77**, 655–671.
- Wilson, E. O. 1971 *The insect societies*. Cambridge, MA: Harvard University Press.
- Yen, S. S. C. 1986 Chronic anovulation due to CNS-hypothalamic-pituitary dysfunction. In *Reproductive endocrinology, physiology, pathophysiology and clinical management* (ed. S. S. C. Yen & R. B. Jaffe), 2nd edn, pp. 500–545. Philadelphia, PA: W. B. Saunders.

Received 25 February 1997; accepted 4 April 1997