THE ROYAL SOCIETY

Kin discrimination and female mate choice in the naked mole-rat *Heterocephalus glaber*

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Naked mole-rats are fossorial, eusocial rodents that naturally exhibit high levels of inbreeding. Persistent inbreeding in animals often results in a substantial decline in fitness and, thus, dispersal and avoidance of kin as mates are two common inbreeding avoidance mechanisms. In the naked mole-rat evidence for the former has recently been found. Here we address the latter mechanism by investigating kin recognition and female mate choice using a series of choice tests in which the odour, social and mate preferences of females were determined. Discrimination by females appears to be dependent on their reproductive status. Reproductively active females prefer to associate with unfamiliar males, whereas reproductively inactive females do not discriminate. Females do not discriminate between kin and non-kin suggesting that the criterion for recognition is familiarity, not detection of genetic similarity *per se*. In the wild, naked mole-rats occupy discrete burrow systems and dispersal and mixing with non-kin is thought to be comparatively rare. Thus, recognition by familiarity may function as a highly efficient kin recognition mechanism in the naked mole-rat. A preference by reproductively active females for unfamiliar males is interpreted as inbreeding avoidance. These findings suggest that, despite an evolutionary history of close inbreeding, naked mole-rats may not be exempt from the effects of inbreeding depression and will attempt to outbreed should the opportunity arise.

Keywords: kin discrimination; kin recognition; mate choice; naked mole-rat; *Heterocephalus glaber*

1. INTRODUCTION

Discrimination mechanisms are of paramount importance to an animal's biological fitness. The ability to discriminate kin from non-kin is essential in the maximization of both fitness accrued through the production of one's own offspring (direct fitness) and that accrued indirectly (Hamilton 1964). To maximize indirect fitness, cooperative or altruistic behaviour must be directed towards kin. To maximize direct fitness, it has been suggested that animals should chose a mate to whom they are neither too closely (Shields 1982) nor too distantly related (Bateson 1982). That kin bias is a feature of social organization in many species (Fletcher & Michener 1986; Hepper 1991) and close inbreeding in nature is rare (Shields 1982; Blouin & Blouin 1988) suggests that mechanisms exist that allow discrimination between kin and non-kin. However, there is debate over the occurrence of kin recognition in animals (Grafen 1990; Blaustein *et al*. 1991). If one follows Grafen's (1990) narrow definition of true kin recognition as 'recognition of relatedness *per se* (through the perception of genetic similarity) with the object of biasing responses with respect to perceived relatedness (kin discrimination)' (pp. 52–53), then few examples are known (but see Grosberg & Quinn 1986). However, it is argued that, if in the natural environment mechanisms exist that allow for the successful differentiation of kin and non-kin, then these mechanisms should be regarded as examples of kin recognition (Blaustein *et al*. 1991; Hepper 1991).

Naked mole rats (*Heterocephalus glaber*) are small subterranean rodents endemic to eastern Africa. Along with the Damaraland mole-rat (*Cryptomys damarensis*) they are arguably the most social vertebrates known (Jarvis *et al*. 1994). Occupying discrete burrow systems, naked mole-rat colonies contain around 80 individuals (Brett 1991). Breeding is restricted to one dominant female and one to three males (Jarvis 1981; Lacey & Sherman 1991). Other subordinate colony members are infertile, but not sterile (Faulkes *et al*. 1990; Faulkes & Abbott 1991). Genetic studies have indicated that high levels of inbreeding and variation within colonies is extremely low (Honeycutt *et al*. 1991; Faulkes *et al*. 1997) with a mean coefficient of relatedness among colony members estimated at 0.81 (Reeve *et al*. 1990). Naked mole-rats appear to lack an incest avoidance mechanism and new breeders are typically recruited from within colonies (Braude 1991; Jarvis *et al*. 1994; Clarke & Faulkes 1997, 1998). Although genetic variation between colonies is low, genetic variation is higher between neighbouring colonies than within colonies. The naked mole-rat population genetic structure is indicative of colony genesis through fissioning of highly inbred colonies coupled with rare male dispersal events (Faulkes *et al*. 1997).

Persistent inbreeding in animals often results in a substantial decline in fitness due to inbreeding depression

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(Charlesworth & Charlesworth 1987). Dispersal and avoidance of kin as mates are common inbreeding avoidance mechanisms. However, strong ecological constraints severely limit dispersal and make incestuous breeding a relatively secure fitness option for naked mole-rats (Jarvis *et al*. 1994). Only recently has evidence for a disperser phenotype in naked mole-rat colonies come to light (O'Riain *et al*. 1996; S. H. Braude, personal communication). Invariably males, they are sexually primed before dispersal and prefer unfamiliar unrelated conspecifics over familiar related conspecifics in choice tests (O'Riain *et al*. 1996). These findings have been interpreted as evidence for outbreeding in naked mole-rats. However, theoretical and empirical evidence indicates that examining female mate choice would be a more convincing test of outbreeding. Females are typically the choosier sex with regard to mates, as females maximize male quality and quantity (Shields 1982). In naked mole-rat colonies, queens are the most aggressive and dominant animals and ultimately determine which males breed by soliciting and mating with some and suppressing or killing others (Clarke & Faulkes 1997, 1998). Therefore, it is of particular importance to investigate recognition mechanisms and female mate choice. Using a series of choice tests, we addressed three main questions. Do female naked mole-rats discriminate between conspecifics or their odours on the basis of familiarity or relatedness? What is the effect of female reproductive status on discrimination? Finally, do reproductively activated females outbreed or inbreed when given the choice?

2. MATERIAL AND METHODS

(a) *Females*

Eight reproductively activated and reproductively inactive females were used in odour and social preference trials. All females were selected from colony 800 and were matched for both age and weight, as confirmed by statistical comparison between the groups (*t*-test for independent samples: age, $t = 0.67$ and $p > 0.50$ and weight, $t = 0.51$ and $p > 0.50$). The reproductively inactive female group was simply non-breeding females removed from their natal colony for the duration of the experimental trials (< 20 min). Previous studies have shown that non-breeding subordinate females are reproductively suppressed with an altered secretion of hypothalamic gonadotrophin releasing hormone (GnRH) and reduced concentrations of plasma luteinizing hormone (LH), which ultimately result in a state of anovulation with their reproductive tracts and ovaries remaining in a prepubescent state (for a review, see Faulkes & Abbott 1997).

Reproductively activated females (queens) were obtained by removing adult females (over two years) from their natal colony 14 days before the start of trials and housing them singly. Previous studies have shown that reproductive suppression is readily reversible if the social cues maintaining reproductive suppression are removed (Faulkes & Abbott 1997). If non-breeding females are removed from their colony and housed singly, their plasma LH concentrations increase and, within approximately eight days, their urinary progesterone concentrations rise for the first time to levels indicative of a luteal phase of an ovarian cycle. In this study it was not logistically possible to monitor urinary progesterone to ascertain that ovulation in the separated females had actually taken place. Nevertheless, to confirm the reproductive status of both colony-housed and singly housed females, three to four blood samples were collected from each female to determine their LH levels.

(b) *Males*

Eight 'familiar, related', 'unfamiliar, related' and 'unfamiliar, unrelated' males were used in odour and social preference trials. Familiar, related males were obtained from colony 800 (i.e. the same colony as the test females). This colony was originally formed by pairing a female of Lerata 4 wild/genetic stock (northern Kenya) and a male of Tsavo wild/genetic stock (southern Kenya). Unfamiliar, related males were obtained by selecting males from colony N2. This colony was formed eight years previously by pairing a male and female from colony 800. Males from colony N2 are therefore assumed to be closely related to the females used in the trials, although unfamiliar to them. Unfamiliar, unrelated males were obtained from colony Lerata 3 (Lerata 3 wild/genetic stock, northern Kenya), which is known to be genetically divergent from colony 800 (Faulkes *et al*. 1997). Lerata 3 males can therefore be regarded as both unfamiliar and unrelated to the females used in the trials. All males were matched for age and weight, as confirmed by statistical comparison between the groups (one-way ANOVA: age, $F_{2,21} = 2.96$ and $p > 0.05$ and weight, $F_{2,21} = 0.18$ and $p > 0.05$). Males were removed from their natal colony ten days before the start of trials and housed singly throughout. The concentrations of plasma LH and urinary testosterone have been shown to increase significantly in such singly housed, non-breeding males, with urinary testosterone reaching levels comparable to breeding males after around five days (Faulkes & Abbott 1991). Therefore, the separated males in this study were all assumed to be reproductively activated.

Once weekly, all wood shavings (bedding and litter) were removed from each male's cage, mixed thoroughly, divided into six 5 g samples (wet weight) and stored in plastic bags at −20 °C. Cages were then cleaned with a bactericidal detergent (32G Nonidet, Teepol Products, Surrey, UK), rinsed in water and 40 g of fresh shavings added. Odour samples were stored for less than one week before use. Before use, samples were allowed to thaw at 27 °C for 30 min.

(c) *Blood sampling and luteinizing hormone assay*

Animals were hand held, the tip of the tail was cut with a sterile blade and blood (*ca.* 200 µl) was collected by capillary action using microhaematocrit tubes. Blood samples were collected within 2–4 min of animal capture and afterwards the wound was treated with an antibiotic powder (Aureomycin) and the animal returned to its cage. The samples were stored on ice for a maximum of 2 h before being centrifuged at 2400 r.p.m. for 15 min and the plasma stored at −20 °C before LH determination. A total of three to four blood samples were collected from each female, with a three week interval between the collection of each sample. LH was measured using an *in vitro* bioassay based on the production of testosterone by dispersed mouse Leydig cells (Van Damme *et al*. 1974). The method and validation for the naked mole-rat has been described previously in a number of studies (e.g. Faulkes *et al*. 1990). The intra- and inter-assay coefficients of variation were 11 ($n = 8$) and 15% ($n = 2$), respectively.

(d) *Preference trials*

Y-maze apparatus was constructed using clear Perspex and consisted of two long tunnel sections (arms) radiating from a

short central tunnel section. The section of the Y-maze where the three tunnel sections were connected (5 cm × 5 cm × 5 cm) is referred to as the decision zone. An entrance chamber was attached to the end of the central tunnel and a choice chamber to the end of each Y-maze arm. To prevent females directly accessing odour samples or males, choice chambers were separated from the Y-maze arms by a steel partition. A 12 V DC fan (40 mm × 40 mm × 10 mm; R. S. Components, Corby, UK) run at 4 V served to circulate odours down each arm of the Y-maze.

During testing, odour samples (odour preference trials) or live males (social preference trials) were placed in the choice chambers of the Y-maze apparatus. The placing of odour samples or males in each of the choice chambers was randomized to control for any directional bias. Each female was placed in the entrance chamber 10 min after starting the fan to allow time for the male odours to circulate through the Y-maze. Trials lasted 10 min, beginning when the female entered the decision zone. It is assumed that females were equally exposed to odours from both samples or live males at the decision zone. Typically, females would stop at the decision zone and sniff the air before advancing up one arm, implying a choice had been made. Very rarely (17 out of 512 trials), females would initially move no more than 2 cm into one arm but then selected the other arm. Females visited both arms of the Y-maze in every trial, typically visiting each arm several times (mean = 5). Each of the 16 females were tested in eight familiarity and eight relatedness trials with odour samples and eight familiarity and eight relatedness trials with live males in order to determine individual female preferences. Females were tested with the same odour sample or male only once, so that familiarity and learning effects did not influence their choice. Between trials the entire apparatus was cleaned with Nonidet and then rinsed with water to remove residual odours.

The trials were filmed using a video camera positioned directly above the Y-maze. In order to write a time-code for the video tape, the video recorder was connected to the video camera via a VITC time-code generator (Adrienne Electronics Corporation, Las Vegas, USA). A 33 MHz 486 desktop PC installed with The Observer/VTA^{TM} v. 3.0 for Windows (Noldus Information Technology, Wageningen, Netherlands) served as an event recorder to retrieve the following behaviours: (i) the frequency and duration females spent in the decision zone, entrance chamber and each arm of the Y-maze and, for each location, and (ii) the frequency and duration of gnawing and digging by females. In pre-experimental trials, females were often observed attempting to access the chamber containing the odour sample or male by gnawing and digging at the partition.

(e) *Mate choice*

Using odour samples or males in Y-maze arenas does not test mate choice directly, only odour or social preference. To examine whether preferences correlate with mate choice, once the social preference trials were completed, all eight reproductively active females were each housed long term with one familiar, related male and one unfamiliar, unrelated male. Two additional mate choice colonies were formed by housing two females each with one familiar, related male and one unfamiliar, unrelated male. These males and females were not used in the odour and social preference trials and were housed singly for 14 days before being housed together to ensure they were reproductively active. This mate choice design was chosen because (i) it simplifies yet reflects the mate choice females face under natural circumstances (familiar, related, colony males versus unfamiliar, unrelated, disperser males), (ii) it provides a sufficient genetic contrast between males for future paternity determination by DNA fingerprinting, and (iii) the preference trials indicated relatedness is not a confounding variable in female choice. Males were matched for age and weight, as confirmed by statistical comparison using *t*-tests for independent samples (age, $t = 0.21$ and $p > 0.05$ and weight, $t = 0.18$ and $p > 0.05$). Animals were caught once weekly and their unique toe-clip number drawn on their back with an indelible marker pen to facilitate easy identification of individuals. Colonies were monitored for *ca.* 10 h each week for the first two months and observations of agonistic and sexual behaviours recorded.

(f) *Data analysis*

Female preference is defined as the odour or male with which she spent most of her time. The strength of female preference for familiarity was estimated by calculating the amount of time females spent in the Y-maze arm of the familiar odour/male divided by the amount of time they spent in the Y-maze arm of the unfamiliar odour/male. Similarly, female preferences for relatedness were estimated by calculating the amount of time females spent in the Y-maze arm of the related odour/male divided by the amount of time they spent in the Y-maze arm of the unrelated odour/male. Statistical analysis was carried out on a natural log transformation of this ratio, to correct for both skew and heteroscedasticity. χ^2 goodness-of-fit tests were carried out to see whether the transformed data fitted a normal distribution. A positive ratio in the log-transformed data indicates a preference for the familiar or related odour/male, whereas a negative ratio indicates a preference for the unfamiliar or unrelated odour/male. For each female the natural log of preferences in each trial ($n = 8$) was compared against zero (random) using one-sample *t*-tests (d.f. = 7). For reproductively active or reproductively inactive females the mean natural log of preferences of each female ($n = 8$) was compared against zero (random) using one-sample *t*-tests (d.f. = 7). The amounts of time that mole-rats spent attempting to access odours or males (duration of gnawing and digging) were compared using Wilcoxon matched-pairs tests (Z). Unless otherwise stated, all statistical analysis was two-tailed and the cut-off point for statistical significance was $p = 0.05$.

3. RESULTS

(a) *Female reproductive status*

The reproductive status of singly and colony-housed females was determined by measuring their plasma LH concentrations. As expected, the plasma LH concentrations of singly housed females were significantly higher than females remaining in their natal colony (Student's *t*-test $t = 4.64$ and $p < 0.001$). The plasma LH concentrations of singly housed females (6.0 ± 2.2 mi.u. ml^{-1}, where mi.u. are milli-International units) were higher than the concentrations previously reported for breeding females (3.0 ± 0.2 mi.u. ml^{-1}) by Faulkes *et al.* (1990), suggesting that these females were indeed reproductively activated. The plasma LH concentrations of females remaining in their natal colony (2.1 ± 1.0 mi.u. ml^{-1}) were comparable to those demonstrated by Faulkes *et al.* (1990) for non-breeding females (1.6 ± 0.6 mi.u. ml^{-1}), confirming that they were reproductively inactivated.

(b) *Odour preference for familiarity*

When given the choice between the odour of a familiar and an unfamiliar, related male three reproductively

Table 1. *Means (s.d.) of log ratios of preference by reproductively active (A–H) and reproductively inactive (I–P) females tested with the urinary odours of familiar and unfamiliar, related males (n = 8 trials per female)*

(The numerical values for all females represent the means (±s.d.) of female preferences ($n = 8$ females). t indicates the one-sample t-test statistic.)

reproductively activated			reproductively inactive		
female	mean (s.d.)	t	female	mean (s.d.)	t
A	0.020 (0.71)	0.86	I	−0.06 (0.47)	0.32
B	0.640 (0.65)	2.60[a]	J	0.46 (0.65)	2.00
C	0.200 (0.49)	1.17	K	0.46 (0.18)	7.30[b]
D	0.360 (0.57)	1.79	L	0.56 (0.52)	3.04[a]
E	0.800 (0.66)	3.34[a]	M	−0.56 (0.39)	4.11[b]
F	−0.003 (0.47)	0.02	N	0.61 (0.55)	2.90[a]
G	−0.260 (0.47)	1.56	O	−0.02 (0.25)	0.18
H	−0.480 (0.50)	2.55[a]	P	−0.63 (0.63)	2.84[a]
all females	0.160 (0.67)	1.04	all females	0.10 (0.50)	0.58

[a] Preference is significantly different from zero at $p < 0.05$, d.f. = 7.
[b] Preference is significantly different from zero at $p < 0.01$ mH, d.f. = 7.

Table 2. *Means (±s.d.) of log ratios of preference by reproductively active (A–H) and reproductively inactive (I–P) females tested with the urinary odours of related and unrelated, unfamiliar males (n = 8 trials per female)*

(The numerical values for all females represent the means (±s.d.) of female preferences ($n = 8$ females). t indicates the one-sample t-test statistic.)

reproductively activated			reproductively inactive		
female	mean (s.d.)	t	female	mean (s.d.)	t
A	−0.280 (0.62)	1.26	I	−0.12 (0.42)	0.80
B	0.160 (0.61)	0.73	J	0.15 (0.65)	0.67
C	−0.160 (0.65)	0.51	K	0.80 (0.65)	3.46[a]
D	0.260 (0.41)	1.67	L	0.24 (0.54)	1.27
E	0.007 (0.84)	0.02	M	−0.69 (0.26)	7.00[b]
F	0.620 (0.63)	2.60[a]	N	0.31 (0.74)	1.20
G	0.160 (0.55)	0.81	O	−0.04 (0.32)	0.41
H	−0.520 (0.44)	3.30[a]	P	−0.35 (0.84)	1.02
all females	0.030 (0.35)	0.25	all females	0.04 (0.45)	0.24

[a] Preference is significantly different from zero at $p < 0.05$, d.f. = 7.
[b] Preference is significantly different from zero at $p < 0.01$ mH, d.f. = 7.

activated females showed discrimination (table 1). Two preferred the odour of familiar males and the other preferred the odour of unfamiliar males. When female preferences were combined, no significant preference by reproductively activated females was found. Five reproductively inactive females showed discrimination, with three preferring the odour of familiar males and two preferring the odour of unfamiliar males (table 1). When female preferences were combined, no significant preference by reproductively inactive females was evident.

(c) *Odour preference for relatedness*

When given the choice between the odour of a related male and an unrelated, unfamiliar male, two reproductively activated and two reproductively inactive females showed discrimination (table 2). Within each reproductive group, one female showed a preference for the odour of related males and the other for the odour of unrelated males. When female preferences were combined, no significant preference by reproductively activated nor reproductively inactive females was found.

(d) *Social preference for familiarity*

When given the choice between a familiar and an unfamiliar, related male, two reproductively activated females showed discrimination, showing a significant preference for unfamiliar males (table 3). However, in addition there was also a clear trend in the results: seven out of the eight reproductively activated females spent more time in the 'unfamiliar' Y-maze arm. Combining female preferences, reproductively activated females exhibited a significant preference for unfamiliar males (one-sample t-test, $t = 3.20$, $p < 0.02$ and d.f. = 7). The duration females spent digging at the partition separating odours from each arm of the Y-maze gives a further indication of female preference. Reproductively activated females spent significantly more time attempting to access unfamiliar males than familiar males (unfamiliar mean s.d. = 218.8 ± 152.3 s and familiar mean s.d. = 91.5 ± 95.0 s; $\chi = 4.64$, $p < 0.001$ and $n = 64$ trials). In contrast, six out of the eight reproductively inactive females spent most of their time in the 'familiar' Y-maze arm. Only two females showed discrimination with both showing a preference for familiar males (table 3). However when female preferences were combined, no significant preference by reproductively inactive females was found.

(e) *Social preference for relatedness*

When given the choice between a related and an unrelated, unfamiliar male, one reproductively activated and one reproductively inactive female showed discrimination (table 4). Both females preferred to associate with related males. When female preferences were combined, no significant preference by either reproductively activated females or reproductively inactive females was evident.

(f) *Mate choice*

Immediately following each social preference trial, females were allowed to access males for a 10 min period in order to interpret the motivational context of their choice (e.g. xenophobia, nepotism or mate choice). No agonistic or sexual interactions were observed. On completion of all trials, mate choice colonies were formed by housing each female long term with one familiar, related male and one unfamiliar, unrelated male. After 47 days one female escaped and died, ruling this colony out of further analysis. Although mating was not observed in any of the remaining mate choice colonies, in six colonies females gave birth and in another colony the female's vaginal closure membrane was perforate, indicating that mating had occurred in at least seven out of the nine colonies. No serious female–male agonistic interactions such as biting were observed in any of the colonies. Towards the end of the period during which they were housed together, females were sometimes observed shoving males, although this occurred too infrequently to

Table 3. *Means (±s.d.) of log ratios of preference by reproductively activated (A–H) and reproductively inactive (I–P) females tested with familiar and unfamiliar, related males (n = 8 trials per female)*

(The numerical values for all females represents the means (±s.d.) of female preferences ($n = 8$ females). t indicates the one-sample t-test statistic.)

reproductively activated			reproductively inactive		
female	mean (s.d.)	t	female	mean (s.d.)	t
A	0.05 (0.52)	0.28	I	0.007 (0.16)	0.15
B	−0.20 (0.32)	1.72	J	0.060 (0.51)	0.32
C	−0.25 (0.50)	1.41	K	0.280 (0.25)	3.10[a]
D	−0.14 (0.63)	0.61	L	−0.230 (0.65)	0.99
E	−0.35 (0.50)	2.00	M	0.330 (0.26)	3.60[a]
F	−0.14 (0.32)	1.23	N	0.006 (0.39)	0.04
G	−0.36 (0.38)	2.67[a]	O	−0.180 (0.60)	0.85
H	−0.76 (0.59)	3.70[a]	P	0.120 (0.25)	1.41
all females	−0.27 (0.24)	3.20[a]	all females	0.050 (0.20)	0.71

[a] Preference is significantly different from zero at $p < 0.05$, d.f. = 7.

Table 4. *Means (±s.d.) of log ratios of preference by reproductively activated (A–H) and reproductively inactive (I–P) females tested with related and unrelated, unfamiliar males (n = 8 trials per female)*

(The numerical values for all females represent the means (±s.d.) of female preferences ($n = 8$ females). t indicates the one-sample t-test statistic.)

reproductively activated			reproductively inactive		
female	mean (s.d.)	t	female	mean (s.d.)	t
A	0.26 (0.51)	1.45	I	0.21 (0.21)	2.92[a]
B	−0.36 (0.61)	1.69	J	−0.12 (0.66)	0.49
C	0.14 (0.72)	0.53	K	−0.17 (0.45)	1.00
D	0.44 (0.27)	4.52[b]	L	0.15 (0.69)	0.64
E	0.13 (0.68)	0.55	M	0.17 (0.40)	1.15
F	−0.19 (0.58)	0.93	N	0.05 (0.61)	0.25
G	0.06 (0.44)	0.37	O	0.12 (0.62)	0.51
H	0.19 (0.43)	1.17	P	−0.09 (0.67)	0.34
all females	0.08 (0.25)	0.94	all females	0.04 (0.15)	0.77

[a] Preference is significantly different from zero at $p < 0.05$, d.f. = 7.
[b] Preference is significantly different from zero at $p < 0.01$ mH, d.f. = 7.

analyse quantitatively. However, in colonies where females did shove, both males appeared to be equal recipients. Male–male agonism was observed in only one colony where, after six months, both males began shoving each other. The familiar, related male shoved more frequently and intensely. The following day the unfamiliar, unrelated male was found severely injured and was euthanased. In three other colonies one of the males died. Two were unfamiliar, unrelated males and the other a familiar, related male. No evidence of fighting such as scarring was found and in all instances post-mortem examination failed to reveal the cause of death.

4. DISCUSSION

Controlling for the effects of familiarity, no kin bias was evident in this study when female preferences were combined, suggesting that the criterion for recognition is not through recognition of relatedness *per se*. Nevertheless, the absence of a detectable preference does not logically imply an absence of recognition and this study does not rule out the possibility that naked mole-rats can recognize relatedness through the perception of genetic similarity (i.e. 'true' kin recognition; Grafen 1990). Unfortunately, it was not logistically possible to produce familiar, but unrelated males to test against familiar, related males. However, reproductively activated females did exhibit a social preference for unfamiliar over familiar, related males, spending significantly more time in the Y-maze arm(s) adjoining the 'unfamiliar' male(s) and attempting to access unfamiliar males. A mechanism based on familiarity is often proposed to be the most common mechanism used in the recognition of kin and depends upon the ability of animals to treat as kin those conspecifics with whom they have associated during certain periods of their lives (Holmes & Sherman 1982; Hepper 1991). Correct identification of kin occurs because, under natural conditions, there is normally a reliable correlation between genetic relatedness and the spatio-temporal component of association.

O'Riain & Jarvis (1997) demonstrated discrimination at the level of colony member or non-colony member in the naked mole-rat and suggested that recognition cues are the distinct colony odour labels (an admixture of individual odours) acquired and learned by all colony members. Interestingly, mole-rats experimentally removed from their natal colonies, housed in groups for several months and then reintroduced into their former colonies were aggressively rejected (O'Riain & Jarvis 1997). Even mole-rats removed from colonies for as little as 24 h may be harassed when reintroduced (C. G. Faulkes and F. M. Clarke, personal observation). Although these findings suggest that recognition cues need to be continually relearned and updated for effective discrimination, the reintroduction of individuals into colonies may be problematical, not because they are recognized as foreign, but due to other factors. For example, males and females removed from the social suppressive influences of the colony rapidly become reproductively active (Faulkes *et al.* 1990; Faulkes & Abbott 1991). In addition, males separated from their parental colonies often show a significant increase in their testosterone levels (Faulkes & Abbott 1991) and become noticeably more aggressive (C. G. Faulkes and F. M. Clarke, personal observation). Such physiological changes may lead to conflict over dominance and reproductive status when reintroduction into colonies is attempted.

In this study environmental cues were controlled for by housing males singly in uniform conditions and, therefore, it is assumed that the recognition cues evaluated by females were individual-specific cues of genetic origin and produced endogenously. Nevertheless, our results do not rule out the involvement of colony labels in recognition. Although our study does not address imprinting, recognition through familiarization suggests social learning of recognition cues. That reproductively activated

females discriminated between males they had been separated from for over 16 weeks and males they had never encountered suggests that cues may be remembered over relatively long periods. In the naked mole-rat the possible genetic basis of recognition cues is unknown. However, major histocompatibility complex (MHC) loci remain prime candidates for involvement in recognition and mate choice (Bruford & Jordan 1998). The exceptionally high levels of polymorphism at MHC loci provide the variability required for a genetically based recognition system and plausible hypotheses exist for mechanisms by which MHC molecules might generate individual odours (Zavazava & Eggert 1997). Although naked mole-rats also have well-developed olfactory capabilities (Jarvis & Bennett 1991), the females in this study typically did not discriminate between odours. However, when the odour samples were replaced by males, reproductively activated females discriminated on the basis of familiarity. This does not rule out the possibility that odours are used as recognition cues but may merely reflect the stronger behavioural response elicited in females by live males.

Recognition mechanisms have been examined in only one other species of mole-rat. As with the naked mole-rat, a species of Zambian mole-rat (*Cryptomys* genus) discriminates between kin and non-kin on the basis on familiarity, rather than through genetic recognition *per se* (Burda 1995). Holmes & Sherman (1982) suggested that species displaying kin recognition through familiarization (social learning) are those with a low probability of mixing with relatives of varying degrees of relatedness or non-relatives during development. All species of mole-rats occupy discrete burrow systems. Although taken to its extreme in the naked mole-rat, colonies of all other social mole-rats (*Cryptomys* sp.) are composed of close relatives (Jarvis & Bennett 1991; Jarvis *et al*. 1994). Furthermore, low levels of dispersal in the naked mole-rat and many *Cryptomys* sp. means that unrelated conspecifics are rarely encountered by colony members. Thus, recognition by familiarity would appear to function as a simple yet highly efficient kin recognition mechanism in mole-rats.

Discrimination has been shown to be dependent on social context and the motivational state of the individual. For example, female house mice prefer males with dissimilar MHC genotypes as mates (Potts *et al*. 1991), apparently to avoid inbreeding (Potts *et al*. 1994). However, when females are pregnant or lactating, they form communal nesting and nursing associations with females that have similar MHC genotypes, which are usually close kin (Manning *et al*. 1992). Human females also prefer the odours of males with dissimilar MHC genotypes, except when they are taking birth control pills; in that case, they prefer the the odours of males with similar genotypes (Wedekind *et al*. 1995; Wedekind & Füri 1997). Wedekind *et al*. (1995) suggested that the basis of the former choice is optimal outbreeding, whereas the latter indicates nepotistic kin recognition by (pseudo-) pregnant females. The social preferences of female naked mole-rats appears to be dependent on their reproductive status. Reproductively activated females prefer unfamiliar males over familiar males, whereas reproductively inactive adult females show no preference. Interestingly, O'Riain *et al*. (1996) found that disperser males prefer to associate with unfamiliar, unrelated conspecifics, whereas, non-disperser males, who are also reproductively active, prefer familiar, related conspecifics. It appears that, for males, reproductive status *per se* does not determine preferences and other factors are involved.

Despite the fact that colonies of naked mole-rats are highly inbred (Reeve *et al*. 1990; Honeycutt *et al*. 1991; Faulkes *et al*. 1997), it is not clear to what extent if any they are affected by inbreeding depression. The latter results mainly from the unmasking of deleterious recessive alleles and, to some extent, the loss of heterosis (Charlesworth & Charlesworth 1987; Pusey & Wolf 1996). It could be argued that extant colonies of naked mole-rats do not suffer greatly from the effects of inbreeding depression, since the more a population inbreeds the less costly inbreeding is likely to become (Pusey & Wolf 1996). This is because most deleterious recessive alleles are likely to have been lost over the course of their evolutionary history. However, it is unlikely that purging can be so complete that inbreeding depression is only a temporary phenomenon. Purging of slightly deleterious mutations under moderate selection through inbreeding is less effective and fixation of such alleles is likely to occur through Müller's ratchet (Charlesworth & Charlesworth 1987; Barrett & Charlesworth 1991). Indeed, the most parsimonious explanation for inbreeding in naked mole-rat colonies is that it is attributable to the prohibitive costs associated with dispersal rather than to any benefits associated with inbreeding (Jarvis *et al*. 1994). As ecological constraints on the option of independent breeding become increasingly severe, a point may be reached where it is better to breed with kin than to risk not breeding at all.

Inbreeding depression is a strong and pervasive phenomenon in living systems (Charlesworth & Charlesworth 1987) and many specific mechanisms have evolved throughout the animal kingdom to prevent inbreeding (Blouin & Blouin 1988). Dispersal and a preference for unfamiliar conspecifics as mates are two commonly occurring inbreeding avoidance mechanisms in animals (Blouin & Blouin 1988). That in this study only reproductively activated female naked mole-rats exhibited a preference, in this case for unfamiliar males, suggests that the motivation underlying such a preference is mate choice. Similarly, O'Riain *et al*. (1996) found that disperser males preferred to associate with unfamiliar, unrelated conspecifics over familiar, related conspecifics. Although a mechanism which prevents incestuous matings within colonies appears absent, dispersal and a preference for unfamiliar conspecifics can be seen as two mechanisms which promote outbreeding in naked mole-rats. Their discovery suggests that naked mole-rats are not exempt from the effects of inbreeding depression.

In the wild, dispersing mole-rats, although rare, are known to form colonies with opposite-sex animals (S. H. Braude, personal communication). However, the rates of successful colony formation and survival of nascent colonies are extremely low. The observation that dispersal in naked mole-rats is strongly male biased (O'Riain *et al*. 1996) suggests that the more common strategy employed may be to join neighbouring colonies. The problems facing dispersers initially appear insurmountable. Apart from an increased risk of mortality from dispersing, these males need to be accepted into the new colony and breed

with the queen. Although colony members typically behave xenophobically towards foreign mole-rats (Lacey & Sherman 1991), there are instances of males being accepted into foreign colonies (Clarke 1999; J. U. M. Jarvis, personal communication). The demonstration in this study of a consistent preference for unfamiliar males by reproductively activated females (queens) suggests that, despite the problems of being accepted into foreign colonies and the large number of potential reproductive competitors for a few breeding vacancies, disperser males have a high probability of breeding. Indeed, there are at least two instances recorded where foreign males have not only been accepted into captive colonies but have become the breeding male (J. U. M. Jarvis, personal communication). Furthermore, in one mark–recapture study, S. H. Braude (personal communication) found a marked naked mole-rat moving between different wild colonies. Given that reproduction is highly skewed in colonies, even a small amount of gene flow through rare dispersal events may have a large impact on the genetic structure of naked mole-rat colonies.

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