

Colony structure and parentage in wild colonies of co-operatively breeding Damaraland mole-rats suggest incest avoidance alone may not maintain reproductive skew

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Abstract

Colonies of co-operatively breeding African mole-rats have traditionally been thought to be composed of a single breeding female, one or two breeding males, and their offspring. In the naked mole-rat (*Heterocephalus glaber*), the occurrence of facultative inbreeding means incest avoidance cannot prevent reproduction in subordinate group members, and physiological suppression of reproductive function by the breeding female occurs in both sexes. In contrast, previous studies of captive colonies of the Damaraland mole-rat (*Cryptomys damarensis*) suggest that breeding within a colony is restricted to a single breeding pair, simply because all other colony members are highly related (first- or second-order relatives) and this species is an obligate outbreeder. Using microsatellite markers, we investigated parentage and colony composition in 18 wild Damaraland mole-rat colonies to determine whether inbreeding avoidance alone can explain the high levels of reproductive skew in this species. Multiple and unidentified paternity was widespread within colonies and immigrants of both sexes were regularly identified. Unrelated, opposite-sex nonbreeders were found coexisting in two colonies. These results suggest that, in the wild, conditions exist where nonreproductive females can come into contact with unrelated males, even when they do not disperse from their natal colony. Inbreeding avoidance alone is therefore insufficient to maintain the high levels of reproductive skew identified in this species suggesting that the breeding female somehow suppresses the reproductive function in nonbreeding females.

Keywords: African mole-rat, Bathyergidae, colony structure, parentage, reproductive skew, reproductive suppression

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Introduction

Co-operative breeding strategies have evolved in a variety of animal taxa, and are characterized by social groups where nonbreeding individuals assist dominant breeding animals to successfully rear young (either directly or indirectly). The mechanisms underlying the evolution of co-operative breeding and its maintenance at a proximate level may be varied, but are dependent on the patterns of

genetic relatedness within groups. The role of kin selection and other factors such as mutualism have received much discussion (for review see Clutton-Brock 2002; Griffin & West 2003). By definition, co-operative breeding requires that some members of a social group refrain from breeding, resulting in a clear-cut reproductive division of labour. How this reproductive skew is achieved may be highly variable among species, or even populations (for review see Faulkes & Bennett 2001). In the cases of delayed dispersal from monogamous family groups (one breeder of each sex plus their offspring), reproduction may be limited simply as a result of incest avoidance. In other cases,

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physiological or behavioural suppression of nonbreeding colony members by breeders, may occur (Keller & Reeve 1994; Clutton-Brock 1998; Reeve *et al.* 1998).

Species of the African mole-rat family (Bathyergidae) display a wide range of co-operative breeding strategies (reviewed in Bennett & Faulkes 2000). Estimates of skew in individual lifetime reproductive success reach the highest levels observed in vertebrates, both in the Damaraland mole-rat (*Cryptomys damarensis*, < 8% individuals achieving direct reproductive success) and the naked mole-rat (*Heterocephalus glaber*, < 1% individuals). Wild colonies of both these species are traditionally thought to be composed of a single breeding female (the queen), together with one or two breeding males, and their offspring. In the naked mole-rat the occurrence of facultative inbreeding means that inbreeding avoidance is not sufficient to prevent reproduction among subordinate group members, and consequently the queen suppresses reproductive function in both males and females. It is thought that this suppression is mediated through behavioural interactions between the queen and the nonbreeders (Faulkes & Abbott 1997). In contrast to the naked mole-rat, field, laboratory and genetic studies suggest that the Damaraland mole-rat displays inbreeding avoidance (Jarvis & Bennett 1993; Burda 1995; Bennett *et al.* 1996; Cooney & Bennett 2000; Burland *et al.* 2002). In this instance, 'inbreeding' refers to breeding with related colony members, which, in the case of colonies comprising a single breeding female with breeding male(s) and offspring, would mean first- and second-order relatives.

Mark-recapture studies of the Damaraland mole-rat show extremely low rates of natal dispersal and movement among colonies (Jarvis & Bennett 1993), strongly suggesting that inbreeding avoidance within an extended family situation is sufficient to explain the high levels of reproductive skew associated within the Damaraland mole-rat. There are no recorded cases of plural breeding among females of this species, either in captivity or in the wild, where colony sizes may exceed 40 individuals. Furthermore, physiological studies have demonstrated diminished reproductive function in nonbreeding females (reviewed in Faulkes & Bennett 2001). Such findings suggest either that nonbreeding individuals show 'self-restraint' from reproductive activity (which may result from a lack of available mates, and therefore be consistent with inbreeding avoidance, Clarke *et al.* 2001), or that a form of external, socially induced suppression of reproductive function (dominant control) is exerted over nonbreeding females, probably by the breeding female. Two, nonexclusive, hypotheses therefore exist to explain the manner by which reproductive skew is maintained in the Damaraland mole-rat: (i) that nonbreeding females do not breed, and show physiologically reduced reproductive function because of the costs of breeding with closely related males; (ii) that the breeding

female suppresses reproductive function in the nonbreeding females within her colony (dominant control).

Previous studies on captive colonies of the Damaraland mole-rat support the first hypothesis (Cooney & Bennett 2000; Clarke *et al.* 2001). However, despite the importance of these studies, their conclusions stem from the assumption that a socially and genetically monogamous family unit is the norm for wild colonies. A genetic investigation into the mating behaviour and composition of wild colonies is therefore essential to resolve this issue fully. Here we describe the use of microsatellite markers to investigate, in detail, parentage and colony structure in Damaraland mole-rat colonies from two geographically separate populations. The results of this study provide sufficient resolution to assess, for the first time, the relative importance of inbreeding avoidance and dominant control in the evolution and maintenance of reproductive skew in this species in the wild.

Materials and methods

Study sites and sample collection

Damaraland mole-rats were sampled at two sites, Dordabis (Namibia, 22°58' S, 17°41' E) and Hotazel (Northern Cape Province, South Africa, 27°17' S, 23°0' E). Individuals were live trapped and sampled using tissue biopsy as previously described in Burland *et al.* (2002). The population at Dordabis has been subject to an extensive mark–recapture study since 1988. For this study, individuals were sampled from 11 colonies between 1993 and 2002. The total number of individuals sampled from each colony (over the sampling period) ranged from 10 to 49. Extensive ecological data including sex, reproductive status (as detailed in Jarvis & Bennett 1993), mass and colony membership (including any movement between colonies) were available for each individual. Seven colonies were also sampled once at Hotazel in January 1996, with colony numbers ranging from five to 19; no mark–recapture data were available for this population. The composition of all colonies sampled from both populations are detailed in Table 1.

Genotyping and data analysis

DNA was extracted and individuals were genotyped at 10 autosomal and one X-linked microsatellite loci: DMR 1–5, 7, CH1–3, NCAM and LV25 as previously detailed in Burland *et al.* (2001, 2002). Allele frequencies were calculated separately for each population using the program RELATEDNESS 5.08 (<http://gsoft.smu.edu/GSoft.html>) and these frequencies were used in all the data analyses. This program allows for the inclusion of haploid data such as that generated for males at the X-linked locus DMR1. In addition, it allows for frequencies to be weighted by colony

Location colony name*	Colony sampling date(s)	Total sampled	Breeding females	Marked males from other colonies†	Other females	Other males
Dordabis						
A	01/94–10/95	13	0	0	7	6
B	07/93–06/96	22	1	0	8	13
C	02/93–07/96	16	1	1	8	6
D	01/94–07/99	50	1	0	27	22
E	07/99–09/01	27	1	0	12	14
F	07/98–07/99	18	1	1	9	7
G	07/98–07/00	20	1	1	5	13
H	07/00–04/01	12	1	1	5	5
I	02/98–07/99	10	1	0	3	6
J	02/98–02/99	20	1	1	15	3
K	02/98–09/01	42	1	2	15	24
L	01/97–09/01	45	1	0	20	24
M	01/92–06/96	14	0	0	6	8
N	11/92–06/96	13	0	0	5	8
Hotazel						
A	09/96	14	1	—	6	7
B	09/96	5	1	—	1	3
C	09/96	7	1	—	3	3
D	09/96	8	1	—	5	2
E	09/96	10	1	—	1	8
F	09/96	18	1	—	4	13
G	09/96	17	1	—	6	10

*Colonies Dordabis A–D and Hotazel A–G are the same colonies as identified using this nomenclature in Burland *et al.* (2002). In the cases of Dordabis B–D further individuals have been included for this study.

†Marked individuals were only caught at Dordabis, where a long-term mark–recapture study has taken place.

size to ensure that they more closely resemble the frequency of alleles of the breeders within the population and are not distorted by colonies with a large number of nonbreeding individuals.

Parentage was investigated using CERVUS 2.0 (Marshall *et al.* 1998). This program calculates a likelihood score for each offspring–candidate parent and determines, by simulation, the difference in likelihood score required between the most likely and second most likely parent for parentage to be assigned at a given level of confidence (Delta criteria). The program does not allow the inclusion of haploid data. However, because allele frequencies had already been calculated for each population including the haploid data (see above), males were designated as diploid homozygotes for the X-linked haploid allele.

For each colony, the offspring of the colony breeding female (who was identified easily by the presence of prominent teats, Jarvis & Bennett 1993) were identified using the Delta criteria for 95% confidence and specifying 99% of loci typed and an error rate of 0.01 (Burland *et al.* 2002). Calculations of Delta criteria were performed separately for each population using the respective weighted allele frequencies. All colony members, except those in the Dordabis

Table 1 Details of colonies and individuals included in the study. For Dordabis colonies A, M and N tissue from the breeding female was not available. As such, individuals from these colonies were included only for the estimation of population allele frequencies

population known from mark–recapture data to originate from a different colony (all male, Table 1), were regarded as putative offspring of the breeding female. Once mother–offspring pairs had been identified, those males fathering offspring within each colony were identified. Any colony male either known to originate from a different colony (Dordabis only) or who had previously been excluded as offspring of the breeding female was regarded as a putative father. Male offspring of the breeding female were not considered to be putative fathers, as both behavioural (Jarvis & Bennett 1993; Burda 1995; Bennett *et al.* 1996; Cooney & Bennett 2000) and genetic evidence (Burland *et al.* 2002) strongly support the hypothesis that this species shows strict inbreeding avoidance. For example, mitochondrial DNA sequencing revealed that in each of six colonies that were studied, the breeding pairs were from different matrilines (Bennett & Faulkes 2000). Furthermore, average relatedness (R) among breeding pairs in 15 colonies is estimated at 0.02, indicating that the breeding pair are not related to one another (Burland *et al.* 2002). Simulations to obtain the Delta criteria were performed separately for each colony. The number of typed candidate fathers was assumed to be 90% of the total candidate males (Burland

et al. 2002). Where more than one typed male was included as a putative father, the mean pairwise relatedness value among these males (see below) was specified to prevent an over-estimation of parentage assignment confidence (Marshall *et al.* 1998). The critical likelihood of difference scores obtained for assigning parentage with 95% confidence ranged between one and two (except for two colonies; see Results). For the paternity analysis, male offspring were regarded as having a missing genotype for the X-linked locus, as fathers and male offspring are not expected to share an allele at this locus.

Pairwise and mean levels of relatedness were estimated with the 10 autosomal loci using RELATEDNESS 5.08, which uses the calculation of Queller & Goodnight (1989). A bias-corrected allele frequency value was incorporated into each calculation of relatedness (Queller & Goodnight 1989). Standard errors were calculated by jackknifing over loci.

Colony structure

Using the results of the parentage and relatedness analyses, in combination with mark-recapture data (where available), a summary of colony structure was compiled for each colony. The total number of pairwise relationships within each colony was calculated as $(n * n - 1)/2$, where n is the number sampled in a colony. Each pairwise relationship was assigned to one of the following categories: breeding pair; parent-offspring pair; sibling pair (subsets – full siblings, half siblings where both fathers were identified, half siblings where only one father was identified, other siblings where no father was identified); other pairings with no identified relationship (subsets – same sex pair, opposite sex pair – either breeding male vs. females excluded as his offspring or other opposite sex pairs).

Results

Genotypes were generated for a total of 401 individuals from 21 colonies across the two study sites (Table 1), and included the animals used in the study of Burland *et al.* (2002). Morphological examination identified a single breeding female in each colony. Although previously captured, tissue samples were missing for breeding females from three colonies sampled in Dordabis (A, M and N; these individuals had disappeared from colonies before the tissue-sampling program began), and so these colonies were included for the estimation of population allele frequencies only.

Parentage analyses

Just three of 171 nonbreeding females tested were excluded as offspring of their respective colony breeding female (1.8%). Measures of relatedness between these excluded

females and their respective breeding female suggest that they were not closely related (Table 2). The number of males excluded as offspring of the breeding female was higher; 19 of 205 tested (with a further seven in Dordabis already known to originate from outside the colony, giving a total of 12.7% of males excluded). Mean levels of relatedness between the excluded males and the breeding female were generally low, although in a few colonies, it is possible that they were second- or third-order relatives (Table 2).

At least one breeding male (defined as a male for which offspring could be assigned with 95% confidence) was identified in 16 of the 17 colonies investigated (Table 2). For one colony (Dordabis I), all males were assigned as offspring of the breeding females, and so this colony was not included in the paternity analysis. More than one breeding male was directly identified in just two colonies (Dordabis K and Hotazel A). In both cases, these males had been captured together within the colony and values of relatedness between the pairs of males were consistent with these males being first-order relatives (Table 2). A number of offspring in these two colonies could only be assigned a father with 80% confidence (Table 2). This is considered to be a result of the high relatedness among the candidate males, which meant a much higher likelihood of difference value (5.6–5.7) was necessary to distinguish between them with 95% confidence. Including these offspring, the ratios of offspring fathered for each pair was 1.3 : 1 for Dordabis K ($n = 39$) and 7 : 1 for Hotazel A ($n = 8$). In the case of Dordabis K, both males fathered offspring born over a period of more than 3 years, and mixed paternity within a single litter was indicated when two juveniles, caught weighing just 18 g, were assigned different fathers with 95% confidence – this weight suggests that the juveniles were less than 1 month old (Bennett *et al.* 1994), while only two or three litters are born each year (Bennett & Faulkes 2000).

For nine of the 17 colonies investigated, no father could be assigned to at least one offspring (Table 2). The minimum number of males achieving paternities with the colony is therefore one more than the number of breeding males identified within these colonies. This occurred more frequently in Hotazel, where six of the seven colonies contained offspring with no father assigned, in contrast to half of the colonies in Dordabis (excluding colony Dordabis I). It was not possible to determine whether offspring where no father was assigned were full or half siblings: when a likelihood approach (Goodnight & Queller 1999) was taken, a Type I error rate of 5% was associated with a Type II error rate of 50%.

Not all males excluded as offspring of the breeding female were breeders (Table 2). The mean level of relatedness among the breeding female and males who did not father her offspring was -0.01 ± 0.14 , -0.11 ± 0.1 and -0.2 ± 0.1 (colonies Dordabis J, Dordabis L and Hotazel A, respectively).

Table 2 Summary of parentage analysis for each colony

Colony	Candidate offspring	Maternity analysis				Paternity analysis			
		Female offspring of breeding female	Females excluded (R ± SE)	Male offspring of breeding female	Males excluded (R ± SE)	Candidate males (R ± SE)	Father assigned	No father assigned (m,f)	Breeding males identified in colony (R ± SE)
Dordabis									
B	21	8	0	12	1 (-0.02 ± 0.11)	1	20	0	1
C	14	8	0	6	0	1	14	0	1
D	49	27	0	21	1 (0.16 ± 0.16)	1	47	(1.0)	1
E	26	12	0	13	1 (0.21 ± 0.18)	1	24	(0.1)	1
F	16	9	0	7	0	1	16	0	1
G	18	5	0	13	0	1	18	0	1
H	10	5	0	5	0	1	9	(0.1)	1
I	9	3	0	6	0	—	—	—	1
J	18	13	2 (0.04 ± 0.19)	2	1 (0.03 ± 0.22)	2 (0.34 ± 0.10)	12	(0.3)	2
K	39	15	0	24	0	2 (0.37 ± 0.18)	39*	0	2 (0.37 ± 0.18)
L	44	19	1 (-0.02 ± 0.2)	20	4 (-0.1 ± 0.09)	4 (0.02 ± 0.08)	36	(0.3)	1
Hotazel									
A	13	6	0	3	4 (-0.05 ± 0.15)	4 (0.51 ± 0.16)	8*	(0.1)	2 (0.5 ± 0.18)
B	4	1	0	2	1 (0.34 ± 0.13)	1	0	(2.1)	3
C	6	3	0	2	1 (-0.24 ± 0.14)	1	5	0	1
D	7	5	0	1	1 (-0.06 ± 0.14)	1	4	(0.2)	2
E	9	1	0	7	1 (0.3 ± 0.14)	1	6	(2.0)	2
F	17	4	0	12	1 (0.23 ± 0.14)	1	2	(10.4)	2
G	16	6	0	9	1 (-0.1 ± 0.16)	1	14	(1.0)	2

For the maternity analysis, the number of individuals assigned and excluded as offspring of the breeding female are detailed separately for each sex, while the relatedness (R) values are mean values calculated between the breeding female and the excluded individuals. For the paternity analysis, candidate males are those males considered as putative fathers; where more than one candidate male was present within a colony, the mean R-value among these males is given. Breeding males are defined as those males to whom offspring could be assigned; where more than one was identified within a colony, the mean R-value among breeding males is given. The number of colony offspring for which no father could be identified is given separately for each sex, with the values for males given first.

*For colonies Dordabis K and Hotazel A, nine and two offspring, respectively, were assigned fathers with only 80% confidence (see text).

Table 3 Summary of the structure of the wild mole-rat colonies studied, detailing the mean relatedness (\pm SE, number of pairs) of each category of relationship identified within each colony

Colony (total pairings)	Breeding pair(s)	Parent– offspring pairs	Sibling pairs			Other pairs (no identified relationship)			
			Full	Half: both fathers identified	Half: one father identified	Other: no father identified	Same sex	Breeding male/ excluded females*	Opposite sex (other)†
Dordabis									
B (231)	-0.13 (\pm 0.15, 1)	0.44 (\pm 0.08, 40)	0.49 (\pm 0.07, 190)	—	—	—	—	—	—
C (120)	0.38 (\pm 0.22, 1)	0.70 (\pm 0.08, 28)	0.72 (\pm 0.09, 91)	—	—	—	—	—	—
D (1225)	0.14 (\pm 0.17, 1)	0.58 (\pm 0.02, 95)	0.58 (\pm 0.02, 1081)	—	0.38 (\pm 0.16, 47)	—	0.24 (\pm 0.17, 1)	—	—
E (351)	0.19 (\pm 0.18, 1)	0.60 (\pm 0.08, 49)	0.60 (\pm 0.09, 276)	—	0.23 (\pm 0.18, 24)	—	—	0.34 (\pm 0.21, 1)	—
F (153)	-0.19 (\pm 0.17, 1)	0.43 (\pm 0.09, 32)	0.45 (\pm 0.11, 120)	—	—	—	—	—	—
G (190)	-0.18 (\pm 0.23, 1)	0.45 (\pm 0.08, 36)	0.40 (\pm 0.07, 153)	—	—	—	—	—	—
H (66)	-0.10 (\pm 0.19, 1)	0.58 (\pm 0.08, 19)	0.63 (\pm 0.09, 36)	—	0.57 (\pm 0.15, 9)	—	—	0.40 (\pm 0.18, 1)	—
I (45)	—	0.73 (\pm 0.10, 9)	—	—	—	0.68 (\pm 0.09, 36)	—	—	—
J (190)	-0.18 (\pm 0.16, 1)	0.35 (\pm 0.06, 27)	0.36 (\pm 0.07, 66)	—	0.25 (\pm 0.07, 36)	0.33 (\pm 0.10, 3)	0.22 (\pm 0.06, 32)	0.08 (\pm 0.11, 5)	0.16 (\pm 0.09, 25)
K (861)	0.19 (\pm 0.16, 2)	0.5 (\pm 0.07, 78)	0.5 (\pm 0.07, 367)	0.34 (\pm 0.06, 374)	—	—	0.19 (\pm 0.07, 25)	0.21 (\pm 0.08, 15)	—
L (990)	-0.11 (\pm 0.24, 1)	0.54 (\pm 0.11, 75)	0.54 (\pm 0.11, 630)	—	0.44 (\pm 0.14, 108)	0.65 (\pm 0.11, 3)	0.01 (\pm 0.07, 86)	0.22 (\pm 0.17, 4)	0.01 (\pm 0.17, 83)
Hotazel									
A (91)	-0.02 (\pm 0.14, 2)	0.46 (\pm 0.07, 17)	0.45 (\pm 0.10, 21)	0.38 (\pm 0.07, 7)	0.28 (\pm 0.11, 8)	—	0.35 (\pm 0.17, 15)	0.27 (\pm 0.12, 7)	0.18 (\pm 0.14, 14)
B (10)	—	0.42 (\pm 0.06, 3)	—	—	—	0.41 (\pm 0.09, 3)	0.12 (\pm 0.14, 2)	—	0.25 (\pm 0.14, 2)
C (21)	-0.24 (\pm 0.14, 1)	0.37 (\pm 0.05, 10)	0.33 (\pm 0.06, 10)	—	—	—	—	—	—
D (28)	-0.06 (\pm 0.14, 1)	0.47 (\pm 0.06, 10)	0.34 (\pm 0.07, 6)	—	0.27 (\pm 0.08, 8)	0.59 (\pm 0.11, 1)	—	0.14 (\pm 0.15, 2)	—
E (45)	0.3 (\pm 0.14, 1)	0.57 (\pm 0.05, 14)	0.67 (\pm 0.04, 15)	—	0.39 (\pm 0.02, 12)	0.39 (\pm 0.21, 1)	0.26 (\pm 0.14, 2)	—	—
F (153)	0.23 (\pm 0.14, 1)	0.56 (\pm 0.08, 18)	0.82 (\pm 0.09, 1)	—	0.64 (\pm 0.07, 28)	0.50 (\pm 0.05, 91)	0.23 (\pm 0.04, 10)	0.25 (\pm 0.08, 4)	—
G (136)	-0.1 (\pm 0.16, 1)	0.37 (\pm 0.05, 29)	0.36 (\pm 0.06, 91)	—	0.16 (\pm 0.11, 14)	—	0.04 (\pm 0.11, 1)	—	—

*Breeding males/excluded female pairs are composed of any male identified as a breeding male within the colony and any female within the colony who was excluded as offspring of the breeding male in question (whether or not the female is an offspring of the breeding female).

†Opposite sex (other) pairs are composed of the following: immigrant females/colony males (colonies Dordabis J, L); colony females/nonbreeding immigrant males (colonies Dordabis J, L, Hotazel A, B); nonbreeding immigrant males/immigrant females (colonies Dordabis J and L).

Colony structure

Table 3 summarizes the categories of pairwise relationships identified within each colony. First-order relatives are the

most numerous, represented by both parent–offspring and full sibling pairs. The mean level of relatedness observed for first-order relatives is highly variable among colonies, reflecting the variation in relatedness among the breeding

pairs. Half siblings are expected to show relatedness levels of approximately half that of first-order relatives within the same colony, if their fathers are unrelated. However, in the colonies containing half siblings where both fathers have been typed (Dordabis K, Hotazel A) the relatedness values of these half siblings are greater than half that of the first-order relatives. This is probably a result of the high levels of relatedness among the breeding males (Table 2). Interestingly, in most other colonies containing half sibling pairs, mean relatedness values were also found to be more than half that of first-order relatives, again suggesting that their fathers may be related (in these cases only one father had been identified). Exceptions to this were observed in colonies Dordabis E and Hotazel G, where mean levels of relatedness among half siblings were less than half those of the full siblings within the same colony (Table 3). Where no father was identified for either sibling, the levels of relatedness suggested that, in most cases, these pairs were full siblings (Table 3).

Within many colonies there were also pairs of individuals for whom no relationship could be established through the parentage analysis (Table 3). Opposite sex pairs with no identified relationship were identified in half of the colonies studied. Many of these pairings fell within the 'breeding male/excluded female' subcategory (Table 3), of which most appear to be related to one another at the level of a second-order relative. In colonies Dordabis K and Hotazel A these pairs may have an 'uncle-niece' relationship, given the high relatedness between the two colony breeding males. The high mean levels of relatedness among half-sibs in other colonies suggests that this may also be the case elsewhere. However, one exception to this is colony Dordabis J where the mean relatedness between the breeding male and five females is close to zero (Table 3).

Other mixed-sex pairings which did not include the breeding male were identified within four colonies (see note 2, Table 3). Within the two Hotazel colonies (Hotazel A and B), mean levels of relatedness suggest that these pairings may be related. By contrast, mean levels of relatedness within the two Dordabis colonies (Dordabis J and L) were low. Moreover, when only those pairs actually captured together in the colony are considered, mean relatedness is further reduced (Dordabis J $R = 0.1 \pm 0.1$, $n = 14$, Dordabis L $R = -0.08 \pm 0.1$, $n = 35$).

Discussion

The results of this study unambiguously show that wild colonies of Damaraland mole-rats frequently deviate from a simple family structure, and therefore provide important new insights into the control of reproductive skew in this species. There is little doubt that inbreeding avoidance plays an important role, as removal of the breeding female

in both captive and wild colonies leads to reproductive quiescence until an unrelated individual is introduced (Rickard & Bennett 1997). However, a number of studies have proposed that inbreeding avoidance can, on its own, explain both the high levels of reproductive skew and the reduced reproductive function observed (Cooney & Bennett 2000; Clarke *et al.* 2001). These studies have assumed that wild colonies typically conform to the monogamous family unit, with low levels of dispersal and little or no contact with individuals from other colonies. By contrast, this study demonstrates that the mating behaviour and structure of wild colonies is much more variable; just five of the 18 colonies conformed to such a model. Furthermore, the opportunity for nonbreeding colony females to come into contact with unrelated, reproductively active males while resident within a colony appears much greater than previously thought.

While more than one breeding male was identified as present at the same time in only two colonies, more than half of the colonies contained offspring for whom no father could be identified. Date, body mass at first capture (J. U. M. Jarvis & N. C. Bennett unpublished data) indicate that many of these offspring were conceived during the tenure of the colony breeding male. In many colonies the fathers of these offspring may be related to the breeding male although this is not always the case. The breeding female therefore appears able to have contact with unrelated males who are not permanent members of the colony. This could be achieved either by transient males entering the colony or by the female briefly leaving the colony. A similar situation appears to exist in the social common mole-rat (*Cryptomys hottentotus*), where colony-breeding males have been shown to father offspring in neighbouring colonies at the same time as breeding in their own colonies (Bishop 2002; Bishop *et al.* in press). Given that one female member of a colony is able to have contact with external males, it is probable that other female colony members could also be exposed in the same manner.

As well as this 'indirect' evidence for contact between unrelated individuals, our study also provides direct evidence of unrelated, opposite sex pairs coexisting within colonies with no evidence of reproduction (Table 3). Maswanganye *et al.* (1999) have demonstrated that nonbreeding males may show reduced reproductive function and hence some of these pairs may have difficulty in breeding, even if the females were not reproductively suppressed. However, five females from colony Dordabis J were identified alongside the unrelated colony breeding male (Table 3). Furthermore, field studies have shown that in newly founded colonies there can be a high turn-over of male breeders (J. U. M. Jarvis and N. C. Bennett, unpublished data) because when colony size is small breeders also perform the high-risk job of burrowing. In such instances, the male breeder will be in contact with the

female offspring of the previous breeder and a form of dominant control would be necessary to prevent reproduction between these unrelated pairs.

Our results support the suggestion that a dual system maintains reproductive skew (Bennett *et al.* 1996), where both inbreeding avoidance and the suppression of reproductive function in females through dominant control by the breeding female both play an important role. Rickard & Bennett (1997) and Cooney & Bennett (2000) both describe an increase in aggression among colony females in the presence of an unrelated male, particularly between the breeding female and other high-ranking females (Cooney & Bennett 2000). When an unrelated male is introduced mating between this male and a number of the nonbreeding females can initially occur (Jacobs *et al.* 1998; Cooney & Bennett 2000). However, after a period of time, sexual activity becomes restricted to a single, dominant female, which, in some cases, is not the original breeding female (Cooney & Bennett 2000). In all but one case, pregnancy occurred in this female only, despite the fact that many females were in contact with the unrelated male.

Given these observations in captive colonies, it appears likely that the breeding female maintains reproductive control through aggression, but at what stage (ovulation, copulation, or implantation) such suppression occurs remains to be determined. To date, there have been no incidences identified of breeding female turnover identified in the wild (Jarvis & Bennett 1993; J. U. M. Jarvis and N. C. Bennett, unpublished data). It is therefore possible that take-overs, such as that described by Cooney & Bennett (2000), do not occur outside captive colonies. When exposed to an unrelated male, a nonbreeding female may instead, where ecological conditions permit, disperse with this male and found a new colony.

The results of this study also provide insight into colony dynamics in *Cryptomys damarensis*, in particular dispersal patterns and the sharing of paternity among relatives. On the two occasions where two breeding males were identified together, they were closely related (Table 2). In the case of Hotazel A, two nonbreeding males excluded as offspring of the breeding female were also closely related to the breeding males (Table 2). Moreover, as discussed above, there is evidence that the fathers of half siblings are frequently related (Table 3). The suggestion that related males may found colonies and/or disperse into existing colonies together is confirmed by field studies, where cohorts of brothers dispersing together have been reported, usually when a colony fragments (Jarvis & Bennett 1993). When joined by an unrelated female, in most instances one of these males remains as the breeder while the others disperse again. This study also suggests female immigration into colonies is relatively rare (two of 18 colonies studied) supporting previous suggestions by Hazell *et al.* (2000) and Burland *et al.* (2002).

The pattern of colony structure and proposed mechanism of maintenance of reproductive skew identified in this species show remarkable resemblance to those identified in co-operatively breeding common marmoset monkeys, *Callithrix jacchus*. In the wild, individuals live in mixed relatedness groups (Faulkes *et al.* 2003), where reproduction is typically monopolized by a single dominant female. Studies of captive family groups suggest a mix of dominant control and incest avoidance operates, where half of the nonbreeding females have ovulated, although none became pregnant because unrelated males were absent (Saltzman *et al.* 1997). By contrast, among unrelated captive individuals, behavioural and pheromonal cues from the breeding female bring about anovulation in subordinate females (reviewed in Abbott *et al.* 1997).

Conclusions

Our study highlights the necessity of unambiguously establishing kin structure and mating system in the wild to understand how reproductive skew is maintained at a proximate level in cooperatively breeding species. Various models of optimal reproductive skew, including 'concession' (Reeve 2000), 'incomplete control by dominants' (Clutton-Brock 1998) and 'threat of eviction' (Johnson & Cant 1999), generally fail to account for how the physiological and behavioural determinants of mating and reproductive suppression operating within a particular individual impinge on the underlying assumptions and predictions of the model. In a simple family group living in an environment with high constraints on dispersal, reproductive skew may be maintained simply by a lack of unrelated mates for nonbreeding group members and the need for dominant control of reproduction becomes redundant. However, in mixed kin groups, or in situations where dispersal is more common, then more complex models derived from empirical data, such as that collected in this study, may be required to describe the reproductive division of labour characteristic of cooperative breeders.

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This paper forms part of a long-term investigation of Damaraland mole-rat behavioural ecology and genetics by the authors, and is part of a broader programme studying social evolution in African mole-rats. Following their discovery of eusociality in this species of mole-rat, J.U.M. Jarvis and N.C. Bennett initiated intensive field studies to gain insight into the ecological constraints that may influence this behaviour, while C.G. Faulkes and T.M. Burland contributed to this and developed the genetic markers for mole-rats. During this study, T.M. Burland was a Leverhulme Trust funded postdoc, having previously worked on the molecular ecology of bats.
