Inbreeding avoidance and reproductive skew in a cooperative mammal

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In animal social groups, socially subordinate individuals frequently show low reproductive success or completely fail to breed. This suppression of subordinate reproduction is currently typically attributed to control by dominant individuals. However, subordinates in cooperative groups often lack access to unrelated mates, and an alternative possibility is that their reproduction is limited by inbreeding avoidance. Using the eusocial Damaraland mole-rat *Cryptomys damarensis*, this paper provides the first experimental evidence, to our knowledge, for this explanation. Subordinate, non-breeding female mole-rats were given access to unrelated mates while remaining in the presence of dominant females, and many became reproductively active soon after unrelated males were introduced. Inbreeding avoidance and the availability of unrelated mates provides a plausible and untested explanation for variation in reproductive skew across animal societies.

Keywords: mole-rats; cooperative breeding; incest avoidance; reproductive skew

1. INTRODUCTION

Cooperatively breeding species vary from egalitarian to highly despotic in the distribution of reproduction among group members (Keller & Reeve 1994). Commonly observed failure to reproduce by subordinate individuals is typically interpreted as resulting from control at a behavioural or physiological level by dominant same-sex individuals (Emlen 1982; Vehrencamp 1983a,b; Creel & Waser 1991, 1997; Reeve & Ratnieks 1993; Reeve & Keller 1995; Doolan & Macdonald 1997; Jamieson 1997). However, among vertebrates, breeding between close relatives is frequently deleterious (Connor & Bellucci 1979; Ralls *et al.* 1979; Van Noordwijk & Scharloo 1981; Ballou & Ralls 1982; Haigh 1983; Keane 1990; Bensch *et al.* 1994; Jimenez *et al.* 1994; Kempenaers *et al.* 1996; Keller 1998) and close inbreeding ($r < 0.5$) is typically avoided (Ralls *et al.* 1986; Hoogland 1995; but see Reeve *et al.* 1990). If subordinates in closely related groups lack access to unrelated mates, high reproductive skew could result even in the absence of dominant control. While inbreeding avoidance is increasingly implicated as a major determinant of animal dispersal and mating patterns (Clutton-Brock 1989; Wolff 1992; Pusey & Wolf 1996; Koenig *et al.* 1998), its role in shaping patterns of reproductive skew is currently largely unexplored.

We tested whether a lack of unrelated males limits the reproduction of female subordinate Damaraland mole-rats. This subterranean hystricomorph rodent lives in large colonies (mean = 11, range 2–41, $n = 110$ colonies; J. U. M. Jarvis and N. C. Bennett, unpublished data) in the red Kalahari sands of southern Africa. Colonies are founded by an unrelated pair or small group fragment, and grow by recruitment (Jarvis & Bennett 1993). Field-based calculations show that reproduction is highly skewed: only a single breeding female in each colony has ever been found and over 90% of individuals do not

breed (Jarvis & Bennett 1993). Among vertebrates, this degree of reproductive skew is exceeded only by the naked mole-rat *Heterocephalus glaber* (Lacey & Sherman 1997). We removed breeding males from family colonies of mole-rats and replaced them with unrelated males, giving subordinate females access to unrelated males while remaining in the presence of the dominant female. We examined the effect of these introductions on aggression between females, levels of luteinizing hormone (LH) and on reproductive activity.

If the non-breeding of subordinate females is due to inbreeding avoidance rather than to control by the dominant female, the introduction of an unrelated male should have the following effects: (i) aggression between females should increase, as the presence of an unrelated male introduces a conflict over breeding status which was previously precluded by inbreeding avoidance; (ii) disruptions in the female dominance hierarchy, as access to an unrelated male gives subordinates an incentive to contest dominance; (iii) a rise in reproductive hormones of subordinates, as they are presented with a potential breeding opportunity; and (iv) an increase in sexual and reproductive activity of subordinates, as they are presented with a suitable mate.

2. METHODS

We studied ten family colonies of mole-rats (median size = 8; range 3–15), containing 23 subordinate females (median per colony = 2.5; range 1–4; excluding females < 50 g from all analyses) from December 1996 to July 1998. In these family colonies subordinate females were familiar with and related to all males. Family colonies were either bred from pairs or from a small group of related males to which a single unrelated female had been added. All subordinate females were the offspring of the breeding female. Mole-rats were housed in large Perspex burrow systems with *ca.* 9 m tubing and several nesting chambers for each colony, and provided with paper and wood

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shavings for bedding, chipboard 'gnawboards' for stimulation, and *ad libitum* food. Details of general husbandry are described elsewhere (Bennett 1990). Individuals were marked for recognition when necessary with gentian violet.

Mole-rats were monitored over a pre-manipulation period in which subordinate females had no access to unrelated males. Dominant males were then removed. Two weeks after this removal, unrelated, unfamiliar males were introduced to colonies, and colonies were then observed over a post-manipulation period.

(a) Aggression

We observed each colony for 15–25 h over two months in each of the pre-manipulation and post-manipulation periods, sampling only when at least one individual was active. Observation sessions lasted 20–60 min. Each colony was observed for at least 90 min per week in at least three separate sessions per week. We recorded the absolute incidence of two measures of aggression within colonies and the identities of individuals involved. 'Bites' were sudden, fast, and did not break the skin. These were the only aggressive acts mole-rats were observed to carry out. 'Vocalized hops' (Jacobs *et al.* 1991) are a stereotyped submissive display, involving a lowered head, hunched posture, one or several hops in which all four feet leave the ground and one or a series of high-pitched squeaks. These were given by victims of biting, and sometimes also by animals encountering a dominant individual in a tunnel. For analysis, we calculated for each group a value for bites per hour per female and vocalized hops per hour per female. Values for the pre-manipulation period and post-manipulation period were compared using Wilcoxon matched-pair comparisons.

(b) Luteinizing hormone sampling and assay

To assess the reproductive state of female mole-rats we examined basal levels of LH, and levels of LH after injection of gonadotrophin releasing hormone (GnRH) in pre-manipulation colonies and 18–22 days after introduction of an unrelated male. GnRH releases stored LH from the pituitary, allowing assessment of the activity of the pituitary for this hormone (Bennett *et al.* 1993). Seven colonies, containing 14 subordinate females (median = 2; range 1–4) were sampled. Mole-rats were warmed in an incubator at 36°C for *ca.* 20 min before sampling, and blood was sampled immediately before and 20 min after GnRH injection. GnRH was synthesized in the laboratory of R. P. Millar, using solid-phase methodology. Purity of GnRH was greater than 98% homogeneity (Millar *et al.* 1989). A solution of 1 µg GnRH in 200 µg sterile physiological saline was prepared and stored at –70°C. One microgram GnRH was administered subcutaneously as a 200 µg bolus injection. Mole-rats were hand-held by an assistant, and a needle was used to prick a vein on the upper surface of the foot. Blood was drawn off in heparinized micro-haematocrit tubes, with *ca.* 300–400 µl of blood taken from each animal. Blood was stored on ice for 2–30 min before being centrifuged at 500g. Plasma was then drawn off and stored at –70°C. 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 this species (Bennett *et al.* 1993). The testosterone produced was measured by radio-immunoassay as described by Hodges *et al.* (1987). 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, MD,

USA) over the range 0.0625–2.0 mIU^{–1} (mIU, milli international units). The sensitivity of the assay (determined at 95% binding) was 12.7 (IU per tube or 2.5 mIU ml^{–1}). The validation curve was parallel to and not significantly different from the reference preparation. Intra- and inter-assay mean coefficients of variation for repeated determinations of LH quality control (1.53 mIU^{–1}) were 10% and 16%, respectively. Pre-manipulation values and post-manipulation values were compared for each female using Wilcoxon signed-rank tests for paired samples.

(c) Sexual activity

We recorded all observed solicitations, copulations and pregnancies over the pre-manipulation and post-manipulation period. For this analysis the pre-manipulation period was defined as two months before the introduction of an unrelated male and the post-manipulation period as two months from the date of male introduction. Solicitation and copulation were recorded *ad libitum* during observation periods and pregnancies were diagnosed by enlarged nipples, distended abdomens and weight gain. The proportion of subordinate females in each group showing sexual behaviour was compared for the pre-manipulation period and the post-manipulation period using Wilcoxon signed-rank tests.

3. RESULTS

(a) Aggression

In family colonies, in which subordinate females were related to all males present, bites and vocalized hops between females were very rare (figure 1). However, after an unrelated male was introduced, the level of aggression between females increased sharply (figure 1). The introduction of an unrelated male significantly increased both the level of biting (figure 1; $n = 9$ groups, $T = 1$, $p = 0.031$) and of submissive displays ($n = 9$ groups, $T = 0$, $p = 0.004$). These figures represent a conservative estimate, as one group was excluded from analysis because the only subordinate female was removed soon after manipulation due to high levels of received aggression. In six out of ten groups, most aggression occurred primarily between a mother and high-ranking daughter, while in the remaining groups aggression was more evenly distributed across female–female dyads. Aggression could be severe: during the course of the study five females were injured and were removed from their colonies to recover. Analysis of participation in aggression showed that the incidence of submissive displays increased between mothers and daughters ($n = 9$ groups, $T = 9$, $p = 0.008$), and between daughters ($n = 9$ groups, $T = 6$, $p = 0.04$). The incidence of biting increased between mothers and daughters and between daughters but these increases were not significant when analysed separately (mothers and daughters: $n = 9$ groups, $T = 4$, $p = 0.08$; daughters: $n = 9$ groups, $T = 6$, $p = 0.12$).

Within one day to two weeks of the introduction of an unrelated male, sudden female dominance reversals occurred in 50% of colonies (five out of ten). These involved intense aggression directed by a daughter against the previous dominant, her mother. Mothers in each case became extremely submissive within several days, despite being consistently substantially larger (mass of females involved in reversals—mothers: mean = 138.5 g, s.d. = 16.6; daughters: mean = 101.8 g, s.d. = 21.3; $n = 5$, paired *t*-test,

Figure 1. Effect of introduction of unrelated male on aggression between females. Median rate of biting (hatched bars) and median rate of submissive displays (solid bars) per hour per adult female in nine colonies are shown pre- and post-manipulation. Error bars show interquartile ranges.

$t = 5.13$, $p = 0.007$). Determinants of the incidence of takeover attempts remain unclear: mothers who retained dominance were not larger than those who were usurped (retained: mean = 144.4, s.d. = 35; usurped: mean = 138.5, s.d. = 16.6; unpaired t -test, $t = 0.337$, $p = 0.75$). Daughters who took over were not significantly larger than those which did not (usurping daughters: $n = 5$, mean = 101.8, s.d. = 21.3; remaining daughters: $n = 18$, mean = 94.5, s.d. = 25.3; $t = 0.582$, $p = 0.57$), and the size difference between mothers and successful usurpers was not smaller than the size difference of mothers who were not usurped and their largest daughters (usurped: $n = 5$, mean difference = 36.8, s.d. = 16; not usurped: $n = 5$, mean difference = 33, s.d. = 52.3; unpaired t -test, $t = 0.155$, $p = 0.88$).

(b) Reproductive hormones

Levels of LH post-GnRH challenge were significantly higher among subordinate females after the introduction of an unrelated male (figure 2; $n = 13$, $T = 13$, $p = 0.021$). Levels of basal LH did not rise (figure 2; $n = 13$, $T = 43$, $p = 0.75$). This rise in post-challenge LH is unlikely to have been an effect of weight increase during the manipulation: although weight did increase between pre-manipulation and post-manipulation sampling (pre-manipulation: $n = 13$, mean = 104.7; post-manipulation: $n = 13$, mean = 112.75; paired t -test, $t = 3.104$, $p = 0.01$), post-challenge LH and weight were not correlated either before or after manipulation (pre-manipulation: $n = 13$, $r^2 = 0.06$, $p = 0.42$; post-manipulation: $n = 13$, $r^2 = 0.06$, $p = 0.44$) and there was no correlation between the increase in weight and increase in LH ($n = 13$, $r^2 = 0.011$, $p = 0.73$).

(c) Sexual activity and reproductive status

In family colonies, containing only related males, only a single subordinate female was observed soliciting on a single occasion. No subordinate females were observed copulating and none became pregnant in these colonies. In the post-manipulation period, after introduction of an unrelated male, subordinate females were significantly more likely to solicit (figure 3; $n = 10$ groups, $T = 0$,

Figure 2. Effect of introduction of an unrelated male on LH levels of subordinate female mole-rats. Median level of basal LH (hatched bars) and LH after GnRH challenge (solid bars) are shown pre- and post-manipulation. Error bars show interquartile ranges.

$p = 0.012$), and to copulate (figure 3; $n = 10$ groups, $T = 0$, $p = 0.043$). All sexual activity involved the introduced unrelated male. After a variable period of time (one to three weeks) only a single female still engaged in sexual activity. There was a greater likelihood of a change in the identity of the female breeder (defined as attaining unambiguous behavioural dominance and becoming the sole female to regularly solicit and mate) after the introduction of an unrelated male (pre-manipulation: female breeder changed in zero out of ten groups; post-manipulation: in five out of ten groups; Fisher's exact test, $p = 0.016$). All these females who took over the breeding position became pregnant except one, who died of unrelated causes (respiratory infection) several weeks after the manipulation. In one colony, male introduction led to two females breeding, giving birth within three days of each other. Both females (mother and daughter) had mated repeatedly with the unrelated male when he was first introduced, but after less than a week the daughter had unambiguously attained dominance and the mother had ceased to show sexual activity. This is the first reported instance of multiple female breeding in this species.

4. DISCUSSION

(a) Reproductive skew in Damaraland mole-rats

Evidence from long-term field data indicates that Damaraland mole-rat colonies typically consist of family groups (Jarvis & Bennett 1993), in which subordinate females lack access to unrelated males. In the wild, reproduction is always restricted to the founding female (Jarvis & Bennett 1993). In this study the introduction of an unrelated male into family groups in the continued presence of the dominant female led to a dramatic increase in female–female aggression, female dominance reversals, a rise in the LH response to GnRH challenge in subordinate females, an increased probability that subordinate females engage in sexual activity and attain breeding status and, in one case, multiple female breeding. It is possible that in the field situation,

Figure 3. Effect of male introduction on sexual behaviour of subordinate female mole-rats. Median proportion of subordinate females in each group soliciting the dominant male (hatched bars) and copulating with the dominant male (solid bars) are shown pre- and post-manipulation. Error bars show interquartile ranges.

reproductive skew in mole-rats is affected by unknown factors which are not accurately reflected in this captive situation. However, these data all indicate that in family groups of Damaraland mole-rats, the reproduction of subordinate females is limited by inbreeding avoidance.

(b) *Dominant control and inbreeding avoidance in family groups*

Is subordinate reproduction in family colonies also limited by dominant control? This experiment alone does not exclude the operation of dominant control among females in family colonies, and it is plausible that both inbreeding avoidance and dominant control could operate together to determine patterns of subordinate reproduction (Emlen 1995; Koenig *et al.* 1998; Reeve *et al.* 1998). The introduction of an unrelated male increases the potential fitness pay-off to a subordinate for attempting to breed by eliminating inbreeding costs (Wright 1977), so the optimum effort a subordinate female should be willing to expend on resisting control and attempting to breed increases. This explanation could provide an account for the dominance reversals and heightened aggression between females observed in this study. However, other evidence makes this explanation unlikely. Previous experimental evidence shows that subordinate female mole-rats who have access only to related males continue to fail to breed even after the removal of the dominant female, indicating their lack of reproduction is not due to her presence (Jarvis & Bennett 1993; Bennett *et al.* 1996; Rickard & Bennett 1997). In addition, in family colonies aggression between females was rare and mild, suggesting a lack of aggressive enforcement of reproductive monopoly. In conjunction with the observed abrupt takeovers of reproductive status by subordinates when given access to a suitable mate, this evidence suggests that dominant control by breeding females is of little importance in limiting the reproduction of subordinate females in family colonies, although it cannot entirely exclude its operation.

(c) *Reproductive skew in colonies containing unrelated males*

While reproductive skew in family groups appears to be due to inbreeding avoidance rather than dominant control, in groups containing an unrelated male aggression was common and severe, aggressive contests over dominance took place and several females frequently attempted to mate before a single female took over unambiguous breeding status. This strongly suggests that dominant control is important in determining reproductive skew in colonies which contain unrelated males. In the field, however, Damaraland mole-rats typically live in family groups (Jarvis *et al.* 1994). While unrelated males have on rare occasions been observed in breeding colonies in the field (J. U. M. Jarvis and N. C. Bennett, unpublished data), laboratory studies show intruding males are typically aggressively ousted by the resident breeding male (Jacobs & Reid 1998; R. Cooney, unpublished data). The observed high reproductive skew in Damaraland mole-rats will thus rarely be due to this form of aggressive suppression.

(d) *Inbreeding avoidance and reproductive skew*

Optimal skew models (OSM) of reproductive skew (Emlen 1982; Vehrencamp 1983a,b; Reeve & Ratnieks 1993; Keller & Reeve 1994; Reeve & Keller 1995; Johnstone *et al.* 1999) build on the assumption of dominant control to predict the level of reproduction dominants that will concede to subordinates to induce them to peacefully cooperate within the group. A central prediction of OSM is that there should be a positive correlation between relatedness within cooperative groups and the degree of reproductive skew they exhibit. This correlation appears widespread in vertebrates and invertebrates and has consistently been interpreted as support for OSM (Keller & Reeve 1994; Reeve & Keller 1995; Bourke *et al.* 1997; Jamieson 1997; Reeve *et al.* 1998; but see Field *et al.* 1998). However, the same correlation is predicted by inbreeding avoidance: in more closely related groups, subordinates are more likely to lack unrelated partners. Cooperative groups in vertebrates consist primarily of multigenerational family groups (88% of birds; 95% of mammals; Emlen 1995), and no study has yet controlled for access of subordinates in these groups to unrelated mates. It remains possible that optimal skew theories can explain the distribution of reproduction in groups after controlling for inbreeding avoidance. However, current evidence fails to exclude the alternative, and arguably more parsimonious possibility, that variation in reproductive skew in cooperative groups is caused by variation in opportunities for subordinates to breed with unrelated partners.

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