Genetic relatedness discrimination in eusocial Cryptomys anselli mole-rats, Bathyergidae, Rodentia

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Abstract. Previous research has demonstrated that, irrespective of prior familiarity, both social and solitary rodent species respond differentially to odours of other individuals based on the degree of genetic relatedness between them. To investigate whether eusocial species also make odour-based genetic relatedness discriminations, male and female Ansell’s mole-rats (Cryptomys anselli) were exposed to ano-genital odours of unfamiliar opposite-sex conspecifics (one unrelated individual and one “estranged” sibling, from whom they had been separated for 27 days). Habituation tests with the odours of “estranged” siblings and courtship tests confirmed that the “estranged” siblings and their odours were treated as unfamiliar. Subjects spent significantly more time investigating unrelated individuals and their odours, than their “estranged” siblings and their odours, indicating discriminative behaviour toward less genetically similar individuals. The findings suggest that eusocial mole-rats can respond differentially to odours based on their degree of relatedness to the odour donors.

Key words: recognition mechanisms, kin recognition, individual odours

Introduction

The importance of familiarity for individual recognition is readily demonstrable in many mammalian species with various types of social organization (e.g., solitary hamsters, Mesocricetus auratus (Todd et al. 1999); social voles, Microtus ochrogaster (Paz-y-Miño & Tang-Martinez 1999b); eusocial mole-rats, Cryptomys anselli (Burda 1995, Heth et al. 2002)). For species in which familiar individuals are likely to be relatives, individual recognition may also serve as a basis for discriminative responses to kin and non-kin. Strictly speaking, however, individual recognition should not be considered a mechanism for “kin recognition” (Todd & Heth 2003). With the evolution of sociality, individual recognition may have become the predominate mechanism in interactions among conspecifics, but discrimination based on degrees of genetic relatedness could still be adaptive even in social species. Such behaviour may not be strong enough to provide a means to avoid inbreeding but may be sufficient to enable an outbreeding preference.

Recent research indicates that, in addition to the traditional kin recognition mechanisms, rodents are able to discriminate their degree of genetic relatedness to other individuals by assessing the degree of similarity between another individual’s odour and their own odour (reviewed in Todd & Heth 2003). These responses are possible across a wide spectrum of relatedness from, for example, sibling versus half-sibling hamsters (Heth et al. 1998) to closer versus more distant heterospecific mice from the Mus species complex.

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(Heth et al. 2003). These findings raise the possibility that other mammalian species may demonstrate genetic relatedness discriminations as well. This may be true even in species that appear to rely exclusively on individual recognition to modulate their social interactions.

African mole-rats (Cryptomys, Bathyergidae) are among such species. They are blind subterranean rodents that live in families consisting of a single breeding pair and their non-breeding offspring, who remain with their parents and forage and maintain the burrow system (Bennett & Faulkes 1999, Burda et al. 2000). This eusocial system was first found in the naked mole-rat (Heterocephalus glaber), another bathyergid (Jarvis 1981, Sherman et al. 1991). Whereas in the naked mole-rat, the queen monopolizes breeding through behavioural suppression of her daughters (Reeve & Sherman 1991), in Zambian Ansell’s mole-rats (Cryptomys anselli), both daughters and sons refrain from mutual mating because of incest avoidance based on continued familiarity with each other. They readily initiate courtship when encountering unfamiliar conspecifics, including their own siblings, however, after separation of 16–18 days (Burda 1995). Although an outbreeding preference has been found in naked mole-rats (Ciszek 2000), odour-based genetic relatedness discrimination has not yet been tested in eusocial mole-rats. The assumption remains, however, that recognition in social mole-rats relies entirely on familiarity and “does not have a genetic basis” (Bennett et al. 1999, p. 106; see also Clarke & Faulkes 1999, Jacobs & Kuiper 2000).

In rodents, however, greater genetic similarity between two individuals is associated with greater similarity in the qualities of their individual odours (“odour-genes covariance”), thus enabling assessments of genetic relatedness to other individuals by comparing another individual’s odour with one’s own (Heth & Todrank 2000, Heth et al. 2001, Todrank & Heth 2003). Evidence of “odour-genes covariance” has also been found within colonies and across species of Zambian Cryptomys (C. mechi, C. anselli and C. kafuensis; Heth et al. 2002), demonstrating that odour similarities provide a reliable indication of degrees of genetic relatedness in these species. Furthermore, Zambian giant mole-rats (C. mechowi) can recognize particular individual colony members from their ano-genital odours, but do not discriminate between the odours of unfamiliar siblings from another colony (Heth et al. 2002). There is also evidence that naked mole-rats use odour to recognize colony members (Ó Ríain & Jarvis 1997). Thus odour-based recognition may be more important than previously suspected in eusocial mole-rats.

The objective of this study was to assess whether Ansell’s mole-rats, which rely heavily on familiarity in differential responses to conspecifics, can also discriminate genetic relatedness.

**Material and Methods**

**Animals**

Families of presumably unrelated mole-rats, Cryptomys anselli (Burda et al. 1999) (wild-captured parents and their captivity-born offspring) were housed in large glass terrariums on a layer of horticultural peat and kept in an animal room under natural light regime (see Begall & Burda 1998 for more detail on housing conditions). All animals were fed the same vegetable and fruit diet *ad libitum*.
Adult non-breeding mole-rats (17 females and 16 males) were subjects in this investigation. Some served in more than one phase of the investigation (as described in experimental procedures below). Twenty-five (13 females and 12 males) of these were “estranged” (see below) siblings. Five females and 4 males were subjects in the “estranged” sibling habituation tests; 8 females and 8 males were subjects in the “estranged” sibling differential investigation of odours and individuals tests. These same 25 “estranged” sibling mole-rats also were subjects in courtship tests. Four mole-rats (2 males and 2 females) from the “estranged” sibling habituation tests also served as subjects in the familiar siblings “courtship” control test. The 8 mole-rats (4 females and 4 males) that were not “estranged” siblings served only as subjects in the familiar siblings “courtship” control test. None of the subjects had previous experience in experiments involving chemosensory stimuli. An additional 18 mole-rats (9 females and 9 males) served as odour donors and/or were presented as stimulus animals in differential investigation and/or courtship tests.

Experimental procedures

To enable mole-rats that would be subjects in the experiment to become unfamiliar with (or in our terminology “estranged” from) two of their opposite-sex siblings but maintain their familiarity with other members of their home colonies, we removed pairs of brothers and pairs of sisters from their home colonies to separate terrariums on an alternating schedule. Thus, while the two sisters were in the separate terrariums for one to three days, the brothers remained in their colony and then the two brothers were removed from the colony just before the sisters were returned, and vice versa. This schedule was continued for 27 days prior to testing.

Odour presentation

For the tests involving presentation of odours, we transferred ano-genital secretions to glass plates (9 x 11 x 1 cm) by holding the donor and gently rubbing the ano-genital area against the plate for 5 sec (see H e t h et al. 2002). We exposed each subject to ano-genital odours on these plates in a clean glass cage (21 x 40 x 25 cm) in a separate room from the housing facility. During the tests we measured the amount of time subjects spent investigating the odours (when the subject’s nose was within 0.5 cm of the stimulus area). Ano-genital secretions were used as odorous stimuli because our previous observations had shown that investigation of this area is involved in greeting behaviour, and hence may play a role in individual recognition.

Habituation (discrimination or generalization)

The habituation (discrimination or generalization) test (T o d r a n k et al. 1998) was designed to assess whether rodents spontaneously discriminate between (i.e., treat as different) or generalize between (i.e., treat as similar) odours from other individuals. In the habituation phase, the subject is presented with an “habituation odour” over repeated trials, during which the subject’s investigation of the odour decreases through the process of habituation. In the test phase, which follows the habituation phase, a second odour, the “test odour”, is introduced. If the subject perceives the test odour as different from the habituation odour, it spends more time investigating the test odour. When the odours are sufficiently similar, subjects do not discriminate between them, sometimes even spending less time investigating the test odour than the habituation odour on the last habituation trial. When there is no statistically significant increase in the investigation time, this indicates that subjects generalized between
the habituation odour and the test odour. Discrimination between odours of familiar siblings indicates recognition of known individuals whereas failure to discriminate between odours of unfamiliar siblings indicates that differential responses depend on familiarity with the donors and their odours (e.g., Todrank et al. 1998, 1999). The habituation (discrimination or generalization) test was conducted in order to determine whether Ansell’s mole-rats discriminate between the odours of their familiar siblings and generalize between the odours of unfamiliar / estranged siblings, as is the case in another Zambian mole-rat, *C. mechowi* (Heth et al. 2002) and in other rodent species (reviewed in Todrank & Heth 2003).

During the test, we exposed each subject to ano-genital odours of their siblings on 4 consecutive 3-min trials. The first 3 trials were habituation trials, in which the ano-genital odour came from the same donor, either a familiar or an “estranged” sibling. The fourth trial was a test trial, in which the ano-genital odour came from a different familiar or “estranged” sibling of the same sex as the first donor. We used paired t-tests to assess whether the investigation of the odour during the test trial was significantly greater than during the last habituation trial.

Differential investigation of odours and individuals

During the 3-min differential investigation of odours test, we exposed each subject to ano-genital odours of two unfamiliar opposite-sex conspecifics (one unrelated; one “estranged” sibling). (Because we had separated pairs of brothers and pairs of sisters, subjects could be tested with one opposite-sex sibling and act as an odour donor and stimulus animal for the other opposite-sex sibling, e.g., brother 1 was tested with sister 1; sister 1 was then tested with brother 2; brother 2 was then tested with sister 2; and then sister 2 was tested with brother 1.) We used paired t-tests to analyse the differences in the investigation of the two test odours.

During the 5-min differential investigation of individuals test, we exposed subjects to two unfamiliar opposite-sex conspecifics: one was an unrelated individual; the other was an “estranged” sibling. Subjects were exposed to the same individuals that had been the odour donors in the odours test. The stimulus animals were confined behind wire racks in diagonally opposite corners of the glass cage. Then, the subject was placed in the central compartment of the cage, between the compartments holding the two stimulus animals. We measured the amount of time subjects spent investigating each of these animals (when the subject’s nose was touching the wire rack). One experimenter who was familiar with the individual identities of the animals brought the animals for the tests; two experimenters who were not familiar with the individual animals measured the investigation times. We used paired t-tests to analyse the differences in the investigation of the two animals.

Courtship test

To confirm that the separation time was sufficient for subjects to treat their “estranged” siblings as unfamiliar and also to demonstrate comparable sexual interest in “estranged” siblings and unrelated conspecifics, we conducted courtship tests. These tests were conducted following the habituation and differential investigation tests. These tests were not designed to assess mating preferences or to imply that any differential investigation in the previous tests was suggestive of mating preferences. They were simply intended to demonstrate that all the donors were being treated as unfamiliar individuals. Opposite-sex pairs were placed in clean terrariums containing nesting material. Half the subjects were paired with an “estranged”
sibling and the other half were paired with an unrelated individual. We observed the pairs for 30 min watching for evidence of courtship behaviour (including courtship vocalization, ano-genital licking, and attempted mounting). Levels of intensity of courtship are variable across pairs whether the partners are “estranged” siblings or unrelated individuals, thus we treated courtship behaviour as a categorical rather than as a quantitative activity. (In mating tests with pairs of “estranged” siblings and pairs of unrelated individuals, only half the pairs in each group completed copulations during 24 hours together (Het h, Tod r a n k, B e g a l l, and B u r d a, unpublished data).)

The familiar siblings “courtship” control test

To compare behaviours toward “estranged” opposite-sex siblings with behaviours toward familiar siblings, mole-rats were separated from their colonies for 1–4 days and then placed with a familiar opposite-sex sibling in a clean terrarium for 30 min while we observed their behaviour, looking for any evidence of courtship.

Results

Differential investigation of odours: Male and female mole-rats spent significantly more time investigating the odour of the unrelated individual than the odour of the “estranged” sibling ($t_{15} = 3.540, P < .003$) during the 3-min test (Fig. 1a). Differential investigation of individuals: Subjects spent significantly more time investigating the unrelated individual than the “estranged” sibling ($t_{15} = 3.409, P < .004$) during the 5-min test (Fig. 1b). Thus, in both types of test, the unrelated individuals and their ano-genital odours elicited stronger responses from the subjects than the “estranged” siblings and their odours. There were no noticeable sex differences in responses to the donors or their odours.

![Fig. 1.](image-url)
Habituation (discrimination or generalization) tests: All the subjects habituated to the odour of their sibling over the 3 habituation trials, as evidenced by a significant decrease in the investigation time during the third as compared with the first habituation trial (“estranged” siblings: \( t_8 = 4.866, P < .001 \), Fig. 2a; familiar siblings: \( t_3 = 7.790, P < .004 \), Fig. 2b). Subjects did not discriminate between the odours of their “estranged” siblings: the investigation time was not significantly longer during the test trial than during the last habituation trial (\( t_8 = .788, P = .453 \), Fig. 2a). Subjects did discriminate, however, between the odours of their familiar siblings: the investigation time was significantly longer during the test trial than during the last habituation trial (\( t_3 = 3.849, P < .031 \), Fig. 2b). Thus subjects recognized the individually distinctive qualities in the odours of their familiar siblings but did not distinguish between the individually distinctive qualities in the odours of their “estranged” siblings.

In the courtship test, all subjects that were paired with unfamiliar individuals showed courtship behaviour, such as courtship-appropriate vocalization, ano-genital and body licking and attempted mounts by the males and receptivity often coupled with lordosis by the females. The behaviour was the same whether they were paired with an “estranged” sibling or an unrelated animal, indicating that all the partner animals were treated as unfamiliar. None of the pairs of familiar siblings showed any signs of courtship behaviour, but did show amiable greeting behaviours, such as ano-genital sniffing and facial nuzzling, typical of greeting a familiar colony member.

**Discussion**

In Zambian *Cryptomys* mole-rats, litters are small and are temporally unpredictable (see Burda 1989, 1990, Begall & Burda 1998), thus it is impossible to synchronize breeding and to conduct a systematic cross-fostering study. Years of observation has consistently demonstrated, however, that mole-rats do not show courtship behaviour and do
not copulate with their familiar siblings, but that courtship behaviour consistently occurs, sometimes followed by copulation, between siblings that have been separated for 16–18 days (Burd 1995). Thus, one way to distinguish between familiar and unfamiliar siblings is by observing their behaviour when placed together in opposite-sex pairs. In addition, evidence from odour tests using the habituation technique (Todrank et al. 1998) has demonstrated in several rodent species, including Cryptomys mole-rats, that discrimination between odours of siblings is indicative of individual recognition and generalization across odours of siblings is indicative of unfamiliarity (see Todrank et al. 1999, Todrank & Heath 2003). Thus a second way to determine whether siblings are being treated as familiar or unfamiliar is to observe their behaviour in habituation tests.

We use the term “estranged” siblings to refer to siblings that have been separated from the subject for a sufficient period of time that they are treated as unfamiliar individuals. Because it is not possible to know whether there are residual memory traces and if the separated siblings have actually completely forgotten each other, it is necessary to infer from the behaviour that the stimulus elicits whether they are treating the stimulus odour or animal as familiar or not. If mole-rats treat these “estranged” siblings as unfamiliar individuals but at the same time show a consistent pattern of discriminative responses to “estranged” siblings and unrelated conspecifics, this would suggest differential responses based on genetic relatedness.

In the differential investigation tests, mole-rats spent more time investigating the odours of unfamiliar, unrelated opposite-sex conspecifics from different colonies than the odours of their biological siblings that they had not been in contact with for 27 days. These mole-rats also spent more time investigating the unrelated individuals than their “estranged” siblings. These findings indicate that genetically dissimilar (unrelated) individuals and their odours elicited stronger responses than the subjects’ own siblings and their odours, but drawing any conclusion from these findings depends on establishing that the “estranged” siblings were in fact treated as unfamiliar.

Two types of test were conducted to explore the effectiveness of separating mole-rats for 27 days in making these “estranged” siblings “unfamiliar”. In the habituation tests, Ansell’s mole-rats discriminated between the odours of familiar siblings, indicating that they recognized them. They failed to discriminate, however, between the odours of “estranged” siblings, indicating that they did not recognize them and that they could be considered “unfamiliar”. These findings are consistent with evidence from other rodent species (see Todrank & Heath 2003) and confirm that 27 days was sufficient for subjects to become “unfamiliar” with their siblings and to treat their odours as belonging to unfamiliar donors.

All pairs of unfamiliar individuals in the courtship test exhibited courtship, whether they had been paired with an unrelated member of a different colony or with an “estranged” sibling. Although the “estranged” siblings may not have been completely unfamiliar after 27 days of separation, this period of time was certainly sufficient to disrupt the incest avoidance. There was no evidence of courtship behaviour among familiar sibling pairs either by subjects from the familiar siblings habituation test or by subjects that only participated in the familiar siblings courtship test.

Becoming unfamiliar with the “estranged” siblings disrupted both the typical pattern of incest avoidance for familiar siblings and the typical pattern of discriminating between the odours of familiar individuals in habituation tests. Any residual memory of the “estranged” sibling was not sufficient to enable discrimination between the odours in the habituation tests.
or to maintain incest avoidance. Thus it was probably not a significant factor in determining the strength of the response that the stimulus individuals or their odours elicited. The absence of courtship behaviour among familiar siblings supports previous findings (Burd 1995) and reaffirms what is consistently found in the laboratory, i.e., that familiar siblings refrain from courtship and mating. The presence of courtship behaviour among “estranged” siblings provides further confirmation that “estranged” siblings are treated as “unfamiliar” individuals. Having substantiated (by habituation as well as courtship behaviour tests) that 27 days was sufficient for subjects to treat their siblings as unfamiliar individuals, the findings suggest that mole-rats are not only able to discriminate between individuals and between their odours based on degrees of genetic relatedness, but they also respond more strongly to the less genetically similar individuals.

During the period of separation, subjects may have been exposed, while in the colony, to the lingering odours of the siblings from whom they were becoming “estranged”. Intentional exposure to siblings’ odours as a means of maintaining familiarity with the sibling has not been tested in mole-rats, but it is interesting to note that in prairie voles, Microtus ochrogaster, periodic exposure to a sibling’s odour in lieu of the sibling itself is sufficient to maintain recognition or “familiarity” with that sibling beyond the time that the animal would usually remember its sibling (Paz-y-Miño & Tang-Martinez 1999a). This does not appear to be true for mole-rats at least from the amount of accidental exposure to the odours of their “estranged” siblings in the home colony.

Subjects that engaged in courtship with their “estranged” siblings did not engage in courtship with any of the remaining familiar opposite-sex colony members either in the courtship test or when they were returned to their colony during the period of separation and after the experiment. This is another demonstration of recognition of particular familiar individuals and the failure to recognize “estranged” siblings. At the same time, the differential investigation of genetically dissimilar individuals (as opposed to “estranged” siblings) and their odours demonstrates that Ansell’s mole-rats can discriminate between conspecifics based on genetic relatedness.

The evidence from odour-genes covariance experiments (Heth & Todrank 2000, Todrank & Heth 2003), including assessments of individual odour similarities within and across species of Zambian Cryptomys (Heth et al. 2002a), indicates that degrees of odour similarity are reliable indicators of genetic similarity. The cumulative evidence from multiple species of rodents (e.g., Heth & Todrank 2000, Heth et al. 1999, 2001, 2002) provides a strong basis for the use of self-referent matching as an effective mechanism for assessing degrees of genetic relatedness. The evidence presented here is consistent with the hypothesis that Ansell’s mole-rats may also be using a self-referencing mechanism in differential responses to conspecifics.

Because subjects in this laboratory study showed comparable courtship behaviour when paired with an unfamiliar, i.e. “estranged”, sibling or with an unfamiliar, unrelated conspecific, there is no way to infer from these results whether mole-rats actually use their ability to discriminate between individuals based on genetic relatedness in nature. Clearly odour-based assessments of degrees of genetic relatedness are not sufficient to prevent inbreeding with “estranged” siblings. On the other hand, this discriminative ability does seem to be indicative of an outbreeding preference, which may or may not be used in nature. Although nothing is known about the mode of breeding dispersal and about how partners meet (Burd 1999), there is some evidence that families in Cryptomys mole-
rats are consistently founded by outbreeding, i.e., the originators are genetically unrelated (Bennett & Faulkes 1999, Burland et al. 2002). Interestingly, and contrary to previous assumptions, new evidence suggests that the same system is also the rule in the foundation of new colonies of naked mole-rats (Braudé 2000, Ciszek 2000).

There is laboratory evidence that prairie voles (M. ochrogaster) forget their siblings after 20 days and thus they probably forget their siblings after dispersal in nature. Yet field evidence indicates that they do not form breeding pairs with siblings in the field; this indicates that some mechanism in addition to individual recognition is being used to minimize their inbreeding in nature (see Paz-y-Miño & Tang-Martínez 1999b) and raises the intriguing possibility of concurrent individual recognition and genetic relatedness assessment mechanisms in this species. Concurrent individual recognition and differential responses by self-referent matching have already been demonstrated in hamsters (Heth et al. 1998, Todrank et al. 1999). The evidence presented here supports the idea of concurrent mechanisms in Ansell’s mole-rats. Individual recognition of familiar siblings is evident from the habituation tests and discrimination based on genetic relatedness is evident from the differential investigation tests. In future studies it will be important to investigate the possible existence of concurrent mechanisms (see Todrank & Heth 2001, 2003) and their relative importance not only in terms of which predominates but also in terms of the social structure of the tested species.

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