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Mother–offspring recognition in communally nesting degus, *Octodon degus*

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Evolutionary theory predicts that mothers will direct care preferentially to their own offspring. This may be difficult when mothers nest communally, as occurs with degus, a social South American rodent. We used an olfactory habituation–discrimination technique to determine whether co-nesting mothers could discriminate among the odours of young that differed with respect to relatedness (a mother's own offspring or not), familiarity (a mother and pup did or did not occupy the same cage) or both. We tested mothers that were housed with an unrelated female and her litter (experiment I) or a sister and her litter (experiment II) and conducted tests when pups were 2 weeks old (during lactation, experiments I and II) and 6 weeks old (after weaning, experiment I). In experiment I, lactating mothers discriminated between the odours of their own pups and their co-nesting partner's pups, and between the odours of their own pups and unfamiliar pups, but not between odours of their co-nesting partner's pups and unfamiliar pups. After weaning, however, mothers discriminated between odours based on familiarity, but not kinship. In experiment II, lactating mothers discriminated between the odours of their own offspring and their co-nesting sister's pups. Thus, degu mothers could distinguish between their own offspring and those of their co-nesting partner whether mothers were related or not. Mothers could therefore potentially use olfactory cues to care discriminatively for pups they encounter in their communal nest.

Evolutionary logic suggests that individuals should allocate parental investment so as to maximize their reproductive return, which means that parents should care discriminatively for their own genetic offspring under most conditions (Trivers 1972; Westneat & Sherman 1993). Discriminative care of own offspring is common in mammals (Gubernick & Klopfer 1981; Holmes 1990; Clutton-Brock 1991), which makes adaptive sense for mothers given the high cost of lactation (Hanwell & Peaker 1977; König 1997) and other forms of maternal care. For mothers to care discriminatively for their own young, they must be able to distinguish between their own and alien young (i.e. the genetic offspring of other females) assuming that mothers routinely encounter both

types of young. Prior association (Holmes & Sherman 1982) is the most common mechanism that mediates mother–offspring recognition in mammals when offspring are mobile or when mothers are temporarily separated from their offspring (Poindron & Le Neindre 1980; Insley 2000). As a result of prior association, which often occurs in a natal burrow or nest that is occupied by a single litter or brood, mothers become familiar with the phenotypes of their offspring and later distinguish between familiar and unfamiliar young (Bekoff 1981). However, prior association may not be a reliable correlate of mother–offspring relatedness when two or more mothers and their offspring occupy a common rearing environment, and under these conditions discriminative care may not occur.

We define communal nesting in mammals as occurring when two or more females and their dependent young occupy a common nest or burrow. Our definition does not

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imply communal care, which occurs when females that are nesting communally provide at least some care to both their own offspring and the alien offspring of their co-nesting partner(s), although communal care could occur. (For a discussion of terms like communal nesting, communal breeding and cooperative breeding in mammals, including alternate usages of these terms, see Jennions & Macdonald 1994; Solomon & French 1997; Hayes 2000.) For a mammalian mother, communal nesting may present a clear challenge: how to discriminate between her own and alien young, all of which occupy the same rearing environment. If co-nesting mothers are closely related (Hamilton 1964) or engage in reciprocal care (Trivers 1972), there may be no adaptive advantage for mother–offspring recognition, but there are circumstances in which such recognition abilities might be valuable (e.g. the avoidance of nursing alien young) Here we report the results of laboratory studies of mother–offspring recognition by olfaction in communally nesting degus, *Octodon degus*.

Degus are group-living, semifossorial, South American caviomorph rodents (Hystricognathi: Octodontidae) that live throughout Chile (Woods & Boraker 1975; Fulk 1976; Meserve et al. 1984). Degus breed once per year, and all females in a population give birth synchronously in underground burrows after a 90-day gestation period (Fulk 1976; Meserve et al. 1984). Young (3–10 pups/litter) are precocial and can locomote independently shortly after birth (Weir 1970). Weaning and emergence from the natal burrow do not occur until about 4 weeks of age (Reynolds & Wright 1979), when young are classified as juveniles. Adult females nest communally (i.e. two or more females share a common underground rearing environment; Fulk 1976; Ebensperger et al. 2004). Burrows typically possess more than one chamber (Fulk 1976), making it possible for females to separate their own offspring from other young in the burrow. This seems unlikely due to the observation that when given a choice of nesting sites, captive female degus tend to spontaneously nest together (Ebensperger et al. 2002), and radiotracking evidence suggests that free-living degu mothers share common underground nesting chambers (Ebensperger et al. 2004; Jesseau 2004). In addition, pups from multiple litters have been observed in large mixed groups, both in field and laboratory studies (Fulk 1976; Jesseau 2004). Since degu mothers live in close proximity with both their own and alien young prior to pups' natal emergence, offspring recognition and discriminative care may be difficult (e.g. Ebensperger et al. 2004, 2007).

Degus, like many other rodents (Halpin 1986), use scent as an important means of conspecific communication (Fischer & Meunier 1985). Adult degus scent-mark and saturate areas with urine and anal gland secretions (Kleiman 1975), and juveniles routinely sniff each other before interacting (Wilson & Kleiman 1974). Degus may also attend to Harderian gland secretions (Friedle & Fischer 1986), and information gained from urine or glandular secretions may mediate individual identification (Davis 1975). Given the prominence of odours in degu social communication, we used olfactory cues to examine mother–offspring recognition.

Using an olfactory habituation–discrimination technique, we performed two experiments to examine the discrimination abilities of degu mothers that were housed in pairs so that a mother was exposed to both her own offspring and those of her co-nesting partner during pup development. In experiment I, we sought to determine (1) whether unrelated degu mothers housed together would discriminate between the odours of pups that differed in relation to familiarity (a mother did or did not share a burrow with the pup), relatedness (the pup was or was not the mother's genetic offspring), or both, and (2) whether mothers' discrimination abilities would change between the lactation and postlactation period. In experiment II, we examined whether co-nesting mothers that were sisters would discriminate between the odours of their own young and odours of their sister's young during lactation. These experiments enhance our understanding of how kin recognition operates in a species when familiarity and relatedness are confounded during early ontogeny, which is typical in communally nesting species of mammals (Hayes 2000) and birds (Komdeur & Hatchwell 1999).

GENERAL METHODS

Habituation–Discrimination Technique

The olfactory habituation–discrimination technique has been used to examine discrimination abilities in many mammals (reviewed in Halpin 1986; Todrank & Heth 2003). With this technique, a test animal is presented one odour (the habituation odour) over a series of several trials. The amount of time the animal spends investigating (e.g. sniffing) the odour decreases over trials as the animal becomes familiar with and thus habituates to it. Then a different odour (the test odour) is presented. If the animal can differentiate between the two odours, the novelty of the test odour will cause investigation time to increase significantly compared with the last habituation trial (Johnston 1993; Houston-Price & Nakai 2004).

Since the habituation–discrimination technique had not previously been used to examine degus' discrimination abilities, we conducted pilot tests to determine whether females would distinguish between the odours of two unfamiliar, opposite-sexed adults, which research using a different technique verifies that females can do (Fischer & Meunier 1985). Females showed robust discrimination abilities (Jesseau 2004), which validated the habituation–discrimination technique for use in our experiments.

Experimental Design: Rationale and General Description

The rearing regimens and discrimination tests that we used in the laboratory were based on the mother–offspring recognition problems that communal nesting presents to free-living female degus. Following controlled breeding (details below), pregnant females were housed in pairs to mimic a two-female communal nesting situation. After co-nesting females gave birth, there were three types

of pups that existed in relation to a given mother based on different combinations of genetic relatedness and early rearing environment: (1) familiar own (FO), (2) familiar alien (FA), and (3) unfamiliar alien (UFA). 'Own' and 'alien' indicate whether a mother did or did not give birth to a pup, respectively, and 'familiar' and 'unfamiliar' indicate whether a mother did or did not occupy the cage in which a pup was born and reared. During a discrimination test, we presented a habituation odour from a pup four consecutive times to a mother, and then presented the test odour from a different pup (details below). Later, we compared the time mothers spent investigating the last habituation odour with the test odour (Johnston 1993).

Housing and Animals

To produce mothers for discrimination tests, female degus ($N = 70$) from a colony at the University of Michigan were paired with randomly chosen males for mating. Each day after pairing, females were vaginally swabbed and samples were examined under a microscope for sperm. Females that mated closest in time based on vaginal swabs were subsequently housed together in pairs (hereafter, co-nesting mothers), with the hope that they would give birth in close temporal proximity. Co-nesting mothers were mated by males unrelated to each other, and co-nesting mothers were either unrelated (experiment I) or sisters (experiment II). Once matched for expected parturition date, each pair of co-nesting females was housed in a 'duplex', which consisted of two plastic tub cages measuring $46 \times 26 \times 21$ cm connected by a PVC tunnel (16 cm long, 12 cm diameter), which allowed females access to both cages and to one another (Fig. 1). Five days prior to the estimated date of parturition, a screen divider was placed in the PVC tunnel at night when animals were not observed. This restricted each female to one side of the duplex, which ensured that we could accurately assign maternity if both females gave birth on the same night. After the first female of a co-nesting pair gave birth, females and pups remained separated by the screen at night until the first litter was 1 day old (mean interbirth interval $\pm$SE for co-nesting mothers $= 6.2 \pm 6.7$ days, $N = 16$ pairs), after which the screen divider was not used again. When pups were 1 day old, they were sexed and individually marked with a numbered eartag (National Band and Tag No. 1005-1). Sample sizes reported for tests reflect that some females either failed to get pregnant or give birth, and some litters failed to thrive, such that only a subset of the original 70 females could be used. All animals were given food (Prolab RMH 2000 5P06 Laboratory Animal Diet) and water ad libitum, and colony housing rooms were maintained at 20°C on a 12:12 h light/dark cycle.

Odour Collection and Discrimination Test Procedures

To acquire odours for discrimination tests, we collected a 'whole-body' odour by briefly removing (<30 s) a pup from its home cage and swabbing it five times with a cotton cosmetic pad, measuring 5×5 cm, on its back, anogenital region, and along the side of the face where Harderian gland or oral secretions may be present. The pad was quickly sealed in a small plastic bag and used within an hour of being collected. We collected whole-body odours to ensure that at least some portion of the odour was biologically relevant for this kind of testing, and it is possible that a pup's odour profile may have included components acquired from its mother and nesting material. In each test, we conducted four habituation trials with odours from the same donor (e.g. a familiar own pup's odour) followed by a new odour in the test trial (e.g. a familiar alien pup's odour). Since the intensity of the odour obtained on the pads might decrease after each successive swab, we swabbed both the habituation-donor pup and the test-donor pup four times with four different pads. Habituation-trial pads were presented in
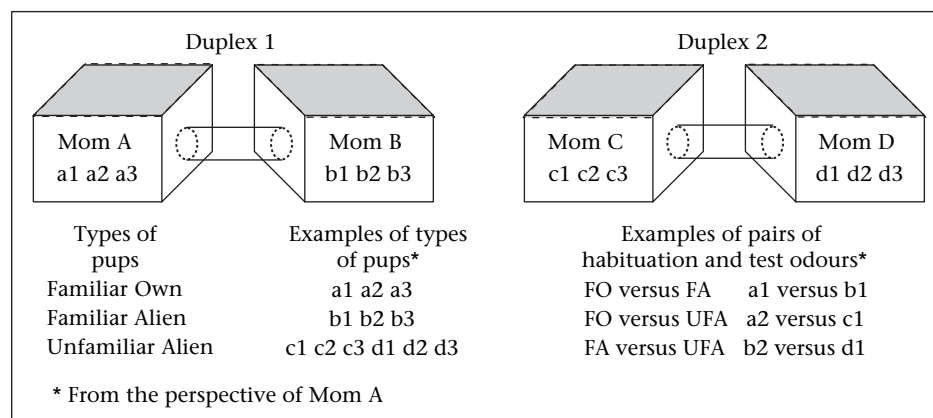

Duplex 1		Duplex 2	
Mom A a1 a2 a3	Mom B b1 b2 b3	Mom C c1 c2 c3	Mom D d1 d2 d3

Types of pups	Examples of types of pups*	Examples of pairs of habituation and test odours*	
Familiar Own	a1 a2 a3	FO versus FA	a1 versus b1
Familiar Alien	b1 b2 b3	FO versus UFA	a2 versus c1
Unfamiliar Alien	c1 c2 c3 d1 d2 d3	FA versus UFA	b2 versus d1

* From the perspective of Mom A

Figure 1. A schematic of two duplex cages (Duplex 1 and Duplex 2), in which degu mothers and pups were housed during pup rearing. Each duplex cage comprised two plastic tub cages connected to each other by a PVC tunnel, which allowed animals to move between cages (all dimensions are in the text). Each duplex housed two mothers (e.g. Moms A and B) and their offspring (e.g. pups a1, a2 and a3 and b1, b2 and b3, respectively), which are depicted in separate tub cages. However, mothers and their litters could move freely between the two tub cages and were typically observed in the same tub cage. 'Types of pups' is defined in the text.

random order, and one pad was randomly selected for the test trial.

Right after (<5 min) pup odours were collected, a mother was transferred to an empty room and tested in her home cage (all other animals were placed temporarily in a clean cage and remained in the colony room). We presented pup odours to mothers by sandwiching a cotton pad between two perforated (with 16 1-cm diameter holes) pieces of Plexiglas (15.5 × 15.5 cm), which we suspended with wire from the rear of the mother's cage. This allowed mothers to sniff pads without being able to lick or chew them. A mother was presented with the habituation odour during four consecutive 3-min trials, followed immediately by a 3-min test-odour trial. Approximately 5 s elapsed between all trials to allow for changing of the odour. For mothers that were tested in multiple conditions, they were tested only once on a given day, and the order in which tests were presented was randomized. Each trial was videotaped, and we later recorded with a stopwatch a mother's total investigation time for each odour. Two observers scored the trials and were unaware of which female, trial and condition they were scoring. A mother was scored as investigating an odour when her nose was less than 1 cm from the Plexiglas sandwich, and we refer to this as her 'investigation time'. Plexiglas pieces (five sets, one for each trial of a test) were washed with Alconox soap and dried with clean paper towels prior to being used. All equipment and animals were handled using latex gloves to prevent contamination of odours, and gloves were changed each time a different odour was handled.

Statistical Analysis

We used Wilcoxon signed-ranks tests to compare females' investigation times during the final habituation trial and the test trial because investigation times did not meet the assumptions required to use parametric tests (e.g. mothers showed considerable interindividual variation in total investigation times). All tests were two tailed, and graphs show medians, quartiles, and 90th and 10th percentiles.

EXPERIMENT I: UNRELATED CO-NESTING MOTHERS

In experiment I, we chose to use unrelated co-nesting females as test subjects because we wanted to begin our exploration of mothers' discrimination abilities by using a test that would be most likely to reveal discrimination abilities if they existed. In various species of mammals, the odours of close kin are perceived by conspecifics as being more similar than those of nonkin (reviewed by Todrank & Heth 2003). Assuming that this generalization holds for degus and that degu pups' olfactory signatures are heritable, we presented to mothers the odours from two pups that were neither paternally nor maternally related and thus likely to produce disparate odours. We also began with unrelated females because field data based on

microsatellite analysis show that degus sometimes nest with unrelated females (Ebensperger et al. 2004; Jesseau 2004).

Methods

Pairs ($N = 10$) of co-nesting mothers consisted of two unrelated females which first had contact with each other when they were housed together after being mated by unrelated males. Pairs of females were therefore housed together for the approximately 90 days of gestation prior to parturition. We tested mothers' discrimination abilities when their young were 2 weeks old, which is about halfway through the lactation period, and again at 6 weeks old, by which time free-living degus are weaned and active aboveground (Fulk 1976; Reynolds & Wright 1979). We presented each mother three habituation–test odour combinations in randomized order: (1) familiar own versus familiar alien odours (FO versus FA), (2) familiar own versus unfamiliar alien odours (FO versus UFA) and (3) familiar alien versus unfamiliar alien odours (FA versus UFA). For a particular test, habituation odours and test odours came from same-sexed pups and about equal numbers of odours from males and females were used across all tests. One of the central issues in using the habituation–dishabituation paradigm is the number and length of the habituation trials that precede the test trial, because it is difficult to interpret subjects' test-trial responses when habituation trials are inadequate to ensure that subjects become thoroughly familiar with the stimulus presented during the habituation period (Houston-Price & Nakai 2004). To avoid this problem, we always used familiar-pup odours as a habituation odour when the test trial involved odours of familiar and unfamiliar pups, and we used the odour of a mother's own pup as the habituation odour for tests that involved odours from own and alien pups. By using the odours of familiar pups in all habituation trials, we sought to ensure that mothers habituated fully and thus that mothers were likely to display their ability to distinguish between the habituation and test odours if, indeed, mothers possessed such abilities. In tests with odours from familiar and unfamiliar pups, pups were matched for age within 2 days. Each mother was tested only one time in a given condition so, for example, if co-nesting mothers A and B both had three pups, then each mother was tested with only one of her pup's odours in the familiar own versus familiar alien discrimination condition.

Results

Mothers habituated during all tests with odours from 2-week-old pups, that is, investigation time decreased significantly between trails 1 and 4 (paired sign test, $P < 0.05$). When mothers were presented odours from their familiar own and familiar alien pups, mothers investigated the test odour significantly more than the last habituation odour (FO versus FA, $Z = -2.82$, $N = 14$, $P = 0.005$), which shows that mothers discriminated between odours of their familiar own and familiar alien
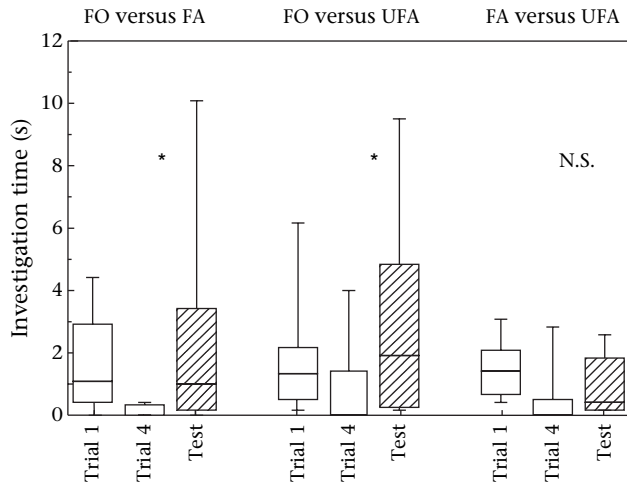
Figure 2. Box plots of the olfactory investigation times (in s) that degu mothers directed to odours of familiar own versus familiar alien (FO versus FA) pups, familiar own versus unfamiliar alien (FO versus UFA) pups, and familiar alien versus unfamiliar alien (FA versus UFA) pups when pups were 2 weeks old. See the text for definitions of terms like 'familiar', 'alien', etc. The graph shows only the final habituation trial (open bars) and the test trial (hatched bars) for each of the three different types of habituation—discrimination tests that were conducted. An asterisk indicates that the habituation time differed significantly ($P < 0.01$) from the test time. N.S., not significant.

Figure 3. Box plots of the olfactory investigation times (in s) that degu mothers directed to odours of familiar own versus familiar alien (FO versus FA) pups, familiar own versus unfamiliar alien (FO versus UFA) pups, and familiar alien versus unfamiliar alien (FA versus UFA) pups when pups were 6 weeks old. See the text for definitions of terms like 'familiar', 'alien', etc. The graph shows only the final habituation trial (open bars) and the test trial (hatched bars) for each of the three different types of habituation—discrimination tests that were conducted. An asterisk indicates that the habituation time differed significantly ($P < 0.05$) from the test time. N.S., not significant.

pups (Fig. 2). Similarly, when mothers were presented odours from their familiar own and unfamiliar alien pups, mothers investigated the test odour significantly longer than the last habituation odour (FO versus UFA, $Z = -2.77$, $N = 17$, $P = 0.006$), which shows that mothers discriminated between familiar own and familiar alien pups (Fig. 2). Mothers did not, however, discriminate between the odours of familiar alien and unfamiliar alien pups (FA versus UFA, $Z = -0.94$, $N = 15$, $P = 0.349$; Fig. 2). In summary, mothers discriminated between odours from their own pups and alien pups (both FA and UFA), but they did not discriminate between odours from the two classes of alien pups (FA and UFA) during the 2-week tests.

Mothers habituated during all tests with odours from 6-week-old young, that is, investigation time decreased significantly between trials 1 and 4 (paired sign test, $P < 0.05$). When mothers were presented odours from their familiar own and familiar alien juveniles, mothers did not investigate the test odour significantly more than the last habituation odour (FO versus FA, $Z = -1.35$, $N = 15$, $P = 0.177$), which indicates that mothers did not discriminate between the odours of their familiar own and familiar alien juveniles (Fig. 3). However, when mothers were presented odours from their familiar own and unfamiliar alien juveniles, mothers investigated the test odour significantly longer than the last habituation odour (FO versus UFA, $Z = -2.77$, $N = 18$, $P = 0.006$), which suggests that mothers discriminated between familiar own and unfamiliar alien juvenile odours (Fig. 3). In addition, when mothers were presented odours from familiar alien and unfamiliar alien juveniles,
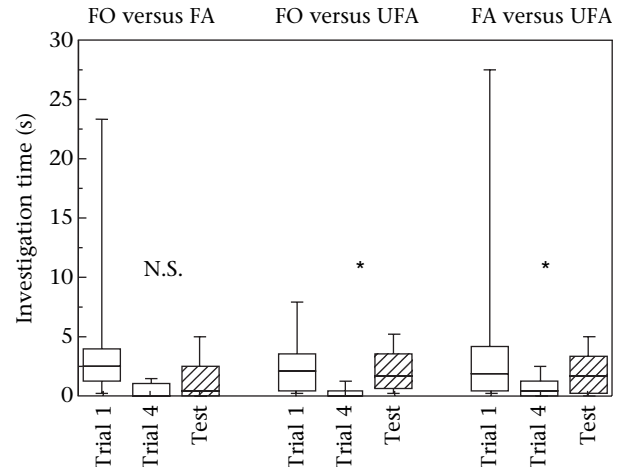
mothers investigated the test odour significantly longer than the last habituation odour (FA versus UFA, $Z = -1.99$, $N = 17$, $P = 0.047$), which means that mothers discriminated between familiar alien and unfamiliar alien juvenile odours (Fig. 3). In summary, mothers during 6-week tests discriminated between odours from familiar and unfamiliar juveniles, but not between odours from their own and familiar alien pups. The results of the 2- and 6-week tests are summarized in Table 1.

EXPERIMENT II: SISTERS AS CO-NESTING MOTHERS

Having learned in experiment I that degu mothers could discriminate between the odours of familiar categories of young during lactation (i.e. their own young and those of their co-nesting partner), in experiment II we sought to determine whether during lactation mothers could discriminate between the odours of familiar young that were related to both mothers. By establishing pairs of co-nesting sisters, we examined whether mothers would

Table 1. Experiment I results

	FO versus FA	FO versus UFA	FA versus UFA
2 weeks	0.005	0.006	N.S.
6 weeks	N.S.	0.006	0.0047

P values for discrimination tests of unrelated degu mothers for familiar own (FO) versus familiar alien (FA), familiar own (FO) versus unfamiliar alien (UFA), and familiar alien (FA) versus unfamiliar alien (UFA) pups when pups were 2 or 6 weeks old. N.S., not significant.

discriminate between odours of their own offspring and odours of their nieces or nephews when pups were 2 weeks old. This discrimination question may be more ecologically relevant than the one we posed in experiment I because in nature, co-nesting females are more likely to be related than unrelated (Ebensperger et al. 2004) and are often full sisters (Jesseau 2004). Inclusive fitness theory predicts that this nesting situation could provide mothers with opportunities to affect both their direct and indirect fitness, depending, in part, on females' discrimination abilities (Hamilton 1964). At the proximate level, however, discriminating between familiar own young and familiar nieces/nephews may be a more difficult discrimination task than the one presented in experiment I because the odours of close kin are perceived by conspecifics as being more similar than those of nonkin in various rodents (e.g. Sun & Müller-Schwarze 1997; Mateo 2002).

Methods

Mated females were housed together in pairs ($N = 6$ pairs), as described above (see General Methods), and each pair comprised two full sisters that had been reared together since birth. We conducted habituation–discrimination tests with odours from 2-week-old young, but because of time constraints we only presented to mothers the odours of familiar own and familiar alien young (FO versus FA). All other methods and analyses were identical to those in experiment I.

Results

Mothers habituated during tests with odours from 2-week-old young as shown by the significant decrease in investigation time between trials 1 and 4 (paired sign test, $P \leq 0.05$). Mothers investigated the test odour significantly
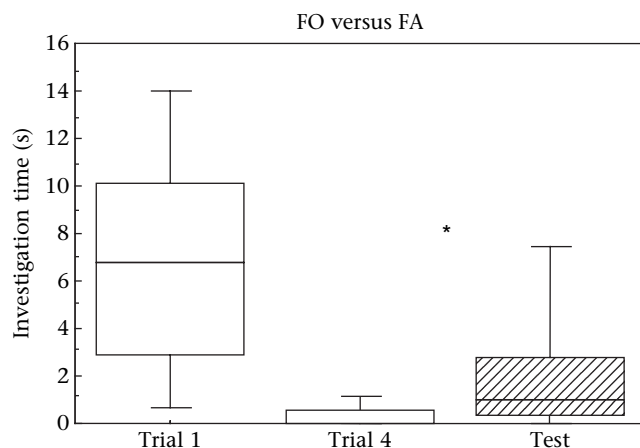

Figure 4. Box plots of the olfactory investigation times that degu mothers directed to the odours from familiar own (FO) versus familiar alien (FA) pups when pups were 2 weeks old. Co-nesting mothers were sisters, so the odours from alien pups were from a female's niece or nephew. An asterisk indicates that the habituation time differed significantly ($P < 0.05$) from the test time.

longer than the last habituation odour when mothers were presented odours from their familiar own and familiar alien pups (FO versus FA, $Z = -2.58$, $N = 12$ mothers, $P = 0.01$). These results (Fig. 4) show that mothers could discriminate between the odours of their own offspring and those of their nieces/nephews despite having lived with both categories of pups since pups were born.

DISCUSSION

Among other discriminations, degu mothers were able to distinguish between the odours of their own offspring and those of their co-nesting partner in our laboratory tests. Before discussing our results, however, we consider three aspects of our research design. First, we do not believe that the screen dividers, which we used only at night to keep co-nesting mothers apart, were important to the development of maternal discrimination abilities. In experiment I, in only 3 of 10 duplexes were litters born on the same night, which meant that for only 6 of 20 litters were pups and mothers separated longer than they would have been had dividers not been used. In experiment II, none of the litters was born on the same night, meaning that no pairs of mothers and litters were separated longer than if screens had not been used. Thus, we do not think that dividers made it possible for mothers to learn the identities of their own pups' odours before they encountered their co-nesting partner's pups.

Second, the length of interval between the births of co-nesting mothers' litters did not appear to influence the development of maternal discrimination between familiar own and familiar alien pups. For example, in the 2-week tests, the mean interbirth interval for the three mothers whose investigation times did not increase when presented the test odours (i.e. they did not discriminate) was about three times longer ($\overline{X} \pm \text{SE} = 5.3 \pm 5.1$ days) than the mean interbirth interval for the 11 females whose investigation time did increase ($\overline{X} \pm \text{SE} = 1.8 \pm 3.3$ days). If the length of the interbirth interval had had an effect, then mothers with longer intervals would have had more time to learn pups' odours, yet these mothers did not appear to distinguish between familiar own and familiar alien pups' odours. In addition, all females with an interbirth interval of zero ($N = 3$ pairs) discriminated (i.e. they increased their investigation time between the last habituation trial and the test trial), again indicating that a short interbirth interval did not disrupt mother–offspring recognition.

Similarly, in tests when young were 6 weeks old, the mean interbirth interval ($\overline{X} \pm \text{SE} = 6.0 \pm 8.1$ days) for the six mothers whose investigation times did not increase when presented with the test odour was statistically indistinguishable ($t = 0.0389$; $P = 0.70$) from the mean ($\overline{X} \pm \text{SE} = 7.8 \pm 8.6$ days) for the 10 mothers whose investigation times did increase. And similarly in experiment II, the interbirth interval (mean $= 4.5 \pm 2.9$ days) also did not appear to play a role in mothers' discriminations. Of the two mothers that did not discriminate, one had a short interbirth interval with her partner (1 day), and one had a long interbirth interval with her partner (8 days). If

the duration of the interbirth interval had played a role in discrimination, then we would have expected that only short interbirth intervals would have been associated with the absence of discrimination. Thus, our results from both experiments suggest that neither the screen dividers nor the length of the interbirth interval was pivotal to the development of mothers' abilities to discriminate between the odours of their own offspring and those of their co-nesting partner.

In addition, it does not appear that birth order influenced whether females discriminated their own from familiar alien young. When the young in experiment I were 2 weeks old, in the instances where we knew the birth order (i.e. in pairs where females did not give birth on the same night), females that gave birth first discriminated one out of four times, and females that gave birth second discriminated four out of four times. However, when young were 6 weeks old, females that gave birth first discriminated five out of five times, while females that gave birth second discriminated only three out of six times. In experiment II (adult females were sisters, young were 2 weeks old), females that gave birth first discriminated six out of six times, while females that gave birth second discriminated four out of six times. Although birth order can influence maternal behaviour in species that nest communally (e.g. Mennella et al. 1990), we found no evidence of a birth-order effect on recognition behaviour in degus.

We now consider the implications of our results. In experiment I, degu mothers distinguished between the odours of their own pups and alien pups produced by their co-nesting partner during lactation (2-week tests), and mothers also distinguished between the odours of their own pups and alien pups produced by a mother in a separate cage. These discrimination abilities could be important for mothers by allowing them to care discriminatively for their own offspring despite nesting communally with alien young. However, the question of discriminative care requires direct study because an ability to distinguish between own and alien young does not necessarily ensure discriminative care (König 1997). In lions, *Panthera leo*, for example, females can distinguish between their own and alien cubs, yet females routinely nurse aliens (Pusey & Packer 1994). Similarly, allosuckling often occurs in wild fallow deer, *Dama dama*, yet mothers' behaviour clearly shows that they can distinguish between their own and alien fawns (Ekvall 1998). Female Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, also nurse alien young (McCracken 1984) despite being able to recognize their own offspring in large maternity colonies (McCracken & Gustin 1991). In retrieval tests, lactating degus do not discriminate between their own pups and unfamiliar aliens (Ebensperger et al. 2006), although our results suggest that mothers could distinguish between these two categories of young.

Prior association (Holmes & Sherman 1982), which results in learned 'familiarity', may explain how degu mothers discriminated between the odours of their familiar own and unfamiliar alien young (Figs 2 and 3). However, prior association would appear inadequate to explain how mothers discriminated between the odours

of their own offspring and those of their co-nesting partner (Fig. 2) because mothers had lived with both types of young since pups were born. Perhaps mothers learned the olfactory signatures of their own offspring quickly, within a few hours of birth and before they spent time with the offspring of their co-nesting partner. In domestic sheep and goats, for example, it takes a mother just a few hours immediately after birth to learn her newborn's odour, which she then uses to restrict care to her own offspring and reject aliens (Poindron & Le Neindre 1980; Poindron et al. 1993). Degu mothers may also have labelled their newborns by licking (e.g. Gubernick 1980) or nursing (Doane & Porter 1978) them immediately after birth and used the already-familiar olfactory cues that they transferred to discriminate between their own offspring and their co-nesting partner's (Gubernick 1981; but see Romeyer et al. 1993).

Our finding that mothers did discriminate between the odours of their own and alien young when pups were 2 weeks old, whether aliens were familiar or unfamiliar, and yet did not discriminate between the odours of familiar and unfamiliar alien young, calls attention to our rather modest understanding of how prior association mediates kin recognition (Hepper 1991; Holmes 2004). For example, using the term 'familiarity' as a synonym for the prior association mechanism (Bekoff 1981) assumes that animals housed together will inevitably learn to recognize their co-residents' phenotypes, regardless of relatedness, and later distinguish between 'familiar' and 'unfamiliar' individuals while not distinguishing among equally 'familiar' individuals. This reasoning indicates that degu mothers should not have been able to discriminate between odours of their own young and those of their co-nesting partner's, and yet they did (Fig. 2). In addition, mothers should have been able to discriminate between the odours of familiar and unfamiliar aliens, and yet they did not. Much remains to be learned about the operation of the prior association mechanism, including when and how familiarity is established and maintained (e.g. Insley 2000; Porter et al. 2001).

Another mechanism that mothers could have used to discriminate between the odours of their own offspring and those of their co-nesting partner is phenotype matching, in which individuals learn the phenotypes of self or kin and later match the acquired 'kin template' to individuals of unknown relatedness (Holmes & Sherman 1982). However, our experiments were designed to uncover some of the offspring recognition abilities that communally nesting degu mothers might have (Figs 2–4), so we do not know what specific recognition mechanism mothers used in our tests.

In 6-week tests, an age by which degus are fully weaned (Reynolds & Wright 1979), mothers only discriminated between the odours of familiar and unfamiliar juveniles, regardless of relatedness. These results differed in interesting ways from our 2-week tests (Table 1). For example, mothers did not discriminate between odours of their familiar own juveniles and familiar alien juveniles, a discrimination that mothers made in the 2-week tests. Changes in mother–offspring interactions with the age of young are not uncommon in mammals. For instance,

female pipistrelle bats, *Pipistrellus pipistrellus*, which nurse their young in large maternity colonies, recognize their own 0- to 7-day-old offspring (by vocalizations), but mothers' recognition abilities seem to decrease as infants age and appear absent by the time young are 15 days old (de Fanis & Jones 1996). In contrast, female prairie voles, *Microtus ochrogaster*, which sometimes nest communally, do not discriminate between their own and unrelated, unfamiliar newborns, but later discriminate between their own and unrelated, unfamiliar weanlings (Hayes et al. 2004). In a proximate sense, the odours of degu young may have changed during development, making it impossible for mothers to differentiate familiar own from familiar alien odours when offspring become juveniles (Mateo 2006). For example, if mothers labelled their newborns shortly after birth (see above), allowing them to differentiate early on between their own pups and their nestmate's, then these olfactory markers may dissipate as pups develop and increasingly mix, and may no longer be present at weaning.

If nursing degu mothers gain fitness benefits from discriminative maternal care, then once their young are weaned, mothers may no longer need to distinguish between their own offspring and their co-nesting partner's. However, mothers might still benefit from being able to distinguish between familiar and unfamiliar juveniles, which mothers did in 6-week tests. Because co-nesting degu mothers are usually closely related (Ebensperger et al. 2004) and because juveniles continue to use their natal burrow system as an activity centre for at least several weeks after weaning (Jesseau 2004), mothers routinely interact with their own offspring and those of their co-nesting partner, which would be mothers' nieces and nephews if co-nesting mothers were sisters. Mothers might therefore benefit from being able to distinguish between general categories of related (e.g. their own offspring and those of their sister) and unrelated juveniles for purposes of group defence, uttering alarm calls or social grooming, which are presumably less energetically expensive than lactation, and would not require an ability to distinguish between familiar own and familiar alien juveniles. Thus, by the time that the high cost of lactation is behind them, mothers might adaptively use the categories 'familiar' and 'unfamiliar' as proxies for 'related' and 'unrelated', respectively, which could facilitate gains in inclusive fitness.

Some of the potential costs of communal nesting, including misdirected maternal care, may be mitigated by indirect-fitness benefits if females preferentially nest with closely related partners (e.g. Wilkinson & Baker 1988; Packer et al. 1992, Dobson et al. 2002). Such benefits would accrue, at least to a degree, even if mothers could not discriminate between their own young and those of their related nest partner (e.g. Hoogland et al. 1989; Manning et al. 1995). This argument, however, may not apply to degus because lactating mothers can discriminate between the odours of their own young and those of their related nest partner (Fig. 4), which could mediate discriminative maternal care among young that share a common nest. In a laboratory study of communally nesting degus, one member of each co-nesting pair weaned fewer and lower-quality offspring than her partner, which shows a reproductive cost to communal nesting (Ebensperger et al. 2007). Our results (Figs 2 and 4) suggest that degu mothers may attempt to minimize such costs by using their olfactory recognition abilities to care preferentially for their own offspring within a communal nest. However, further study on degu maternal behaviour is required to determine how mothers' recognition abilities affect their maternal behaviour.

We propose that understanding mother–offspring recognition in communally nesting mammals will benefit from studies that separate maternal care from maternal recognition. Studies that use a recognition assay like pup retrieval (Beach & Jaynes 1956; Solomon 1993; Manning et al. 1995) or some other form of direct maternal care (e.g. Hayes et al. 2004) can reveal something about mothers' recognition abilities in a given context, but they may not reveal what mothers are actually capable of doing. In degus, for example, mothers do not retrieve their own offspring preferentially when presented with their own pup and an unfamiliar, unrelated pup (Ebensperger et al. 2006), although we know from our results that mothers can discriminate between these two types of pups. We believe that further insights into the costs and benefits of communal nesting and communal care, including the role of maternal recognition abilities in these behaviours, will come from studies that are designed to differentiate between maternal care and maternal recognition.

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