

## Familiarity and phenotypic similarity influence kin discrimination in the social rodent *Octodon degus*

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To evaluate the two most widely accepted mechanisms of kin recognition, prior association and phenotype matching, we studied kin discrimination in the South American hystricognath rodent *Octodon degus*. We first assessed differential treatment towards kin during paired encounters in an experimental arena (experiment 1). In addition, we used the habituation technique to investigate odour kin discrimination (experiment 2). In both experiments the relative influence of familiarity and phenotypic similarity were assessed using familiar and unfamiliar siblings, half siblings and genetically unrelated individuals. The results showed that *O. degus* treat conspecifics differentially as a function of familiarity; young reared apart were equally likely to explore each other independently of genetic relatedness. However, the exploratory behaviour of siblings reared apart and siblings reared together did not differ (experiment 1). On the other hand, degus did discriminate between odours of siblings, half siblings and unrelated individuals reared apart, and between odours of unrelated individuals reared together. Subjects did not discriminate between scents of their siblings reared together with them (experiment 2). These results suggest that familiarity is important in the interaction between animals and in the discrimination of conspecific scents. Nevertheless, our results suggest that phenotypic similarity may also influence social interactions and scent discrimination, although only between close kin, hence possibly influencing communal nesting. We propose that no single mechanism exclusively explains kin discrimination in degus. The prior association recognition mechanism mediated most instances of kin recognition, but phenotype matching is also used in close kin discrimination.

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Kin selection theory establishes that genetic relatedness influences the evolution of social behaviour (Hamilton 1964). Since its proposal, there has been great interest in kin recognition, which has been considered an important mediator of altruistic behaviour (Tang-Martínez 2001). In general, studies behaviourally infer kin recognition via the observation of differential treatment of conspecifics based on genetic relatedness (Hepper 1991). Nevertheless, at the proximate level of analysis this differential treatment can be due to familiarity (i.e. common living) and not to kinship (i.e. genetic relatedness; see Holmes & Sherman 1982). The factors controlling kin recognition, such as how individuals perceive, distinguish and express cues or labels that correlate with relatedness, has been the focus of much research (Holmes & Sherman 1982; Halpin 1991; Tang-Martínez 2001; Mateo 2003), and has resulted in the proposal of two widely accepted mechanisms: (1) recognition by prior association and (2) recognition by phenotype

matching (Holmes & Sherman 1982; Tang-Martínez 2001). The first mechanism predicts that discrimination is based on familiarity resulting from prior association where individuals learn and then recognize odours or other cues of specific individuals with whom they are familiar, but they cannot discriminate unfamiliar kin that have not already been encountered. In the second mechanism, individuals learn family-specific cues from their relative's phenotype, or based on their own cues (self-referent phenotype matching). These cues are then compared with other conspecifics and, if the correspondence between the cues is high, the conspecific is identified as kin. Via this mechanism, and unlike recognition by prior association, animals can discriminate unfamiliar kin (Mateo 2002). In addition, self-referent phenotype matching can also mediate discrimination of familiar but unequally related kin (e.g. full and maternal half sibs). Thus, phenotype matching depends on the correlation between genotypic and phenotypic similarity, such that, relatives share more similar cues than non-kin (Holmes & Sherman 1982; Todrank et al. 1998; Mateo & Johnston 2000).

The majority of kin recognition studies evaluating the production and discrimination of olfactory signals have almost exclusively been conducted in rodents belonging to the suborder Sciurognathi

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(e.g. Holmes & Mateo 2007). Studies on kin recognition mechanisms have been focused almost exclusively on two infraorders of Rodentia, namely Myomorpha and Sciuromorpha, with virtually no research on the third most abundant infraorder, Caviomorpha (see Tang-Martínez 2003). The New World Hystricognathi rodents (known as the infraorder Caviomorpha) has over 200 species, most of them living in South America, inhabiting diverse ecological conditions as well as showing different life forms and social organizations. Some caviomorph species are also highly social (see Ebensperger 1998), and perhaps more importantly, some species present cooperative breeding where kin recognition mechanisms could be involved (see Holmes & Mateo 2007). Research on caviomorph species could be valuable as some species display a drastically different biology compared to the widely studied Muridae and Sciuridae families. For example, the caviomorph rodent degu, *Octodon degus*, represents an interesting scenario to examine kin discrimination mechanisms because they use a communal nesting system where different adult females share a nest and care for young (Ebensperger et al. 2004). Therefore, rearing association and kinship can be confounded. Thus, it seems unlikely that only prior association mediates sibling discrimination and hence, phenotype matching might allow mothers and siblings to discriminate further between different categories of kin (Holmes 2004). Therefore, studies in a caviomorph species such as degus can offer new insights about kin recognition mechanisms.

We studied kin discrimination in the caviomorph rodent *O. degus*. The degu is a diurnal, semifossorial and highly social rodent, endemic to central Chile. This species constructs underground burrows in which nights are spent and young are raised, although most of their daytime activities are conducted on the surface (Fulk 1976; Kenagy et al. 2002a, b, 2004; Vásquez et al. 2002; Quispe et al. 2009). Degus live in groups typically consisting of one or two males and one to four females and their young (Fulk 1976; Ebensperger et al. 2004; Soto-Gamboa 2004). Within a colony, different groups live adjacent to each other, so individuals of one group routinely encounter members of other groups. Females that form stable associations are genetically related with a kinship coefficient  $r = 0.25$  (Ebensperger et al. 2004). Cooperative behaviours within degus include communal nesting and nursing (Ebensperger et al. 2004; Jesseau 2004), cooperation in burrow construction (Ebensperger & Bozinovic 2000), social vigilance (Vásquez 1997; R. A. Vásquez, unpublished data) and predator detection (Ebensperger & Wallem 2002). In addition, studies have shown that chemical communication is very important in the social life of this rodent, such as the use of urine signals in social relationships (Kleiman 1975) and sex discrimination (Fisher & Meunier 1985), as well as, in dustbathing marks (Ebensperger & Caiozzi 2002). Furthermore, familiarity influences agonistic interactions among male degus (Davis 1975; R. A. Vásquez, unpublished data). The high level of social cooperation, including communal breeding, the high levels of relatedness within groups, and the use of chemical communication all suggest that kin recognition may be important in the life history of *O. degus*.

The aim of this study was to evaluate the influence of the two most widely accepted mechanisms of kin discrimination, prior association and phenotype matching, in the South American rodent *O. degus*. For this, we conducted observations of differential treatment towards kin and nonkin during encounters between paired subjects, in an experimental arena (experiment 1). Experimental subjects were full sibs that either remained with their natal family or were cross-fostered to a genetically unrelated family, half sibs that were reared in separate environments and thus had never encountered each other, or nonrelatives that were reared together or apart (see Methods for details). In addition, we implemented the olfactory habituation technique to evaluate both, odour-based kin discrimination abilities and the perception of odour differences

between animals with different degrees of genetic relatedness (family cues) (Mateo 2002; Todrank & Heth 2003). If chemical cues are related to species, sex and individual information, they could also transmit information on relatedness if odours vary with kinship (Todrank et al. 1998; Mateo 2003). Thus, odour cues may be similar between relatives (Heth & Todrank 2000). Following the habituation/dishabituation paradigm where animals can discriminate between two odours (Johnston 1993), we assessed whether degus discriminate between odours of individuals with different degrees of kinship and familiarity (experiment 2). If degus discriminate their relatives solely via prior association, we predicted that they would display differential treatment towards conspecifics and discriminate their odours based only on familiarity rather than kinship. In contrast, if degus use phenotype matching, we predicted that they would discriminate between conspecifics and their odours based on kinship, thus providing evidence about the correlation between genotypic and phenotypic similarity. Thus, in experiment 2, we predicted that degus would not discriminate between odours of relatives advertising familiar cues. Therefore, by conducting two types of experiments, we sought to obtain a complete view of degu behaviour in relation to kin discrimination, assessing both the discrimination of individuals and the discrimination of odours.

## METHODS

### Experiment 1

In this experiment we assessed behavioural discrimination during paired-encounter tests by quantifying differential treatment between individuals that varied in familiarity and genetic relatedness.

### Subjects and cross-fostering

Degus were captured from three populations in central Chile with similar habitats and elevation: La Campana (32°55'S, 71°05'W), Lampa (33°17'S, 70°53'W), and Rinconada de Maipú (33°29'S, 70°53'W). In each population, adults were caught with Sherman live traps. Animals were housed in standard metal cages 50 × 40 × 35 cm with a layer of wood shaving, under natural photoperiod, in an air-conditioned animal room at the Universidad de Chile. They were fed with rabbit pellet and alfalfa and provided water ad libitum during the entire experimental period. We produced 16 mating groups during the reproductive period (May–June 2004), each consisting of three unrelated females and one unrelated male from the same population (to obtain paternal half sibs). Before the females were mated, they were kept for 4 months in isolation without any males to ensure that they were not impregnated before our experiments. The offspring of these females were the subjects of both experiments. All procedures of capture, transportation, maintenance and experimentation followed the recommendations of the ethics committee of the Faculty of Sciences of the Universidad de Chile, and complied with Chilean regulations (see Acknowledgments) as well as recommendations by the Animal Behavior Society.

After a 3-month gestation period we carried out daily inspections to detect parturition. When a female in a mating group gave birth, she was separated from her group and reared her litter alone. This regimen ensured that pups were correctly assigned to their genetic mothers and it allowed us to create various experimental groups (see below). In total, 24 females gave birth to 155 young (83 females, 72 males).

When two unrelated females from different experimental mating groups from the same population gave birth on the same day or within a day of each other, we cross-fostered them to create litters that included both related and unrelated pups. To assure that these

females were not relatives, they were captured at least 400 m apart (see Ebensperger et al. 2004). No female living in the same cage gave birth on the same day; therefore, there was no litter mixing. Cross-fostering was done reciprocally on the day that pups were born or 1 day later; exchanges were made male by male, and female by female. When the litter consisted of an even number of individuals, we exchanged half the litter, while half plus one of the individuals were exchanged for litters with an odd number of individuals. Each pup was weighed and marked with eartags (National Band & Tag Co., Newport, KY, U.S.A.) for permanent identification. In total we cross-fostered 47 pups (24 males and 23 females) from 14 adult females. Observations revealed that all females with one exception accepted their foster offspring, and no difference was observed regarding the breeding and mortality of cross-fostered and non-cross-fostered offspring. The mentioned exception occurred when two foster pups that were housed with the same adult female died, from unknown causes. Each litter was housed alone with its genetic or fostered mother until the time of the experiments, 4–5 months after birth (i.e. until January–February 2005). Although subjects are mostly reproductively mature at this age (see Hummer et al. 2007), they are not reproductively active, allowing for the possibility of focusing our experiments on the social interactions between animals of the same sex. The experiments took place in the mornings between 0900 and 1100 hours, when this species is active (see Kenagy et al. 2002b).

#### Experimental groups

More than one female got pregnant in 10 of the 16 mating groups, resulting in 35 half paternal sibs. Using the cross-fostering individuals and these half sibs, we were able to create five experimental groups differing in rearing conditions (i.e. reared together or apart) and genetic relatedness (i.e. related or unrelated): (1) sibs reared together (SRT), (2) sibs reared apart (SRA), (3) nonsibs reared together (NSRT), (4) half sibs reared apart (HSRA), (5) nonsibs reared apart (NSRA). From this point on, we use the word 'familiar' to refer to the pups that were reared together, regardless of their genetic relatedness.

#### Experimental procedures

Discrimination abilities were evaluated by observing the behaviour of pairs of rodents interacting for a 5 min period in an experimental arena. The pairs were of the same sex, and 14 pairs were used in each experimental group (i.e. 7 male pairs, 7 female pairs). Although under natural conditions degus of different sex frequently interact with each other, we selected to study, as a first step, only same-sex pairs because of logistic constraints. During the experiments, animals were individually carried in plastic cages to the test room housing the arenas. Two arenas (80 × 80 × 50 cm) were used independently; each arena had two opaque partitions, so the degus could not see each other whilst inside, and each was illuminated with two 100 W light bulbs. The floor of each arena was an extractable metallic plate that facilitated its cleaning. As the subject left urine and faeces on the plate, as well as most of their signal odours, the plate was cleaned with detergent between tests to remove odours from previously tested individuals. Before starting the test, one animal of the pair was marked with nontoxic paint, so the observer could distinguish between the two degus. The mark did not cause differences in exploratory behaviour between marked and unmarked subjects (paired  $t$  test:  $t_{1,69} = 1.383$ ,  $P = 0.171$ ), and previous studies have not observed a change in locomotion and vigilance behaviour due to marking (see e.g. Vásquez et al. 2002). The degus were placed in the arena with the opaque division during a 10 min acclimation period, after which the division was removed; social interactions between the degus subjects were then quantified over a 5 min period. We concluded that a 5 min period was sufficient to determine discriminative behaviour by degus, after

observing that animals showed similar levels of exploratory behaviour of their environment and of other degus during 5 min compared to longer periods (20 min) (paired  $t$  test:  $t_{1,139} = -1.384$ ,  $P = 0.168$ ). The test period commenced when the two animals first touched each other. If the animals did not touch each other (14.3% of subjects), the test was repeated on a different day. All animals were weighed after the experiments and all were tested only once, except for animals that did not make contact with each other during the first trial. Each session was videorecorded with a camera located above each arena (colour CCTV camera connected to a Sony video recorder), and was subsequently analysed with the JWatcher software (Dan Blumstein, University of California, Los Angeles, U.S.A.). We recorded the following behaviours during the experiments: (1) olfactory exploratory behaviour, by quantifying the time that subjects spent exploring the mouth, flanks and anus zones of the companion. This behaviour was defined as when the nose of one of the individuals was less than 1 cm from the mouth, flanks or the anus zones of its partner; (2) cohesive behaviour, measured via scoring when the degus were in close proximity and either side by side, on a right angle to each other, one over the other, or grooming; and (3) antagonistic behaviour. During the trials, antagonistic behaviours were almost never recorded, so we did not consider them further. Videos were scored by one observer who was blind regarding the relatedness, familiarity, sex and treatment of the subjects. All of the above behaviours have previously been observed in natural and captive populations of degus (see Davis 1975; Fulk 1976). We created a total exploratory score that we called 'exploratory behaviour' by adding the time that animals spent exploring each zone. A similar procedure was done for cohesive behaviours. These scores were used for the statistical analysis. Differences in behaviours between treatment groups (in exploratory encounters) were used to infer differences in kin discrimination and familiarity (e.g. see Holmes & Sherman 1982; Paz y Miño & Tang-Martínez 1999).

#### Statistics

The exploratory encounters were analysed with a two-way ANCOVA after data were square-root transformed. The dependent variables were familiarity and kinship. Given that large differences in body mass might bias social encounters, we used body mass of all animals tested as a covariable. We used paired  $t$  tests for comparisons between paired groups followed by sequential Bonferroni corrections. The data on cohesive behaviours were analysed using a Kruskal–Wallis test, because the data did not meet the parametric requirements. Antagonistic behaviours could not be analysed statistically because of the few cases recorded. For all analyses we used the software Statistica 7 (Statsoft, Tulsa, OK, U.S.A.).

#### Experiment 2

In this experiment we investigated whether *O. degus* individuals discriminate between odours of individuals with different degrees of familiarity and kinship, via the habituation/dishabituation paradigm (see Johnston 1993).

#### Animals and experimental groups

We used the same animals in this experiment as in experiment 1, but without repeating the same encounter patterns between subjects as in experiment 1. We used 30 males and 30 females as odour donors, whose odorants were subsequently presented to the experimental subjects. We used 90 individuals as subjects (45 males, 45 females), consisting of 18 subjects (9 males, 9 females) per habituation/discrimination test. Subjects were tested only once but some ( $N = 17$ ) were subsequently also used as odour donors.

We constructed five experimental groups that were divided into two broader classes: familiar and unfamiliar subjects. In the familiar group, we investigated whether the subjects discriminated between (1) the odour of their own sibs that were reared together with them (SRT, for sib reared together) and (2) the odour of nonsibs (genetically unrelated subjects) that were reared together with them (NSRT). In the unfamiliar group (subjects and odour donors were genetically unrelated and unfamiliar), we investigated whether the subjects discriminate between (3) the odour of sibs reared apart (SRA), (4) the odour of paternal half sibs reared apart (HSRA) and (5) the odour of nonsibs reared apart (NSRA). This design allowed us to determine the importance of rearing association and genetic relatedness with the donor odour discrimination.

#### *Experimental procedures*

We conducted three habituation trials and one test trial (see Johnston 1993), each of 5 min in duration, with 3 min between trials. In each habituation trial, the subject was exposed to two plates (see below), one of them with an odour (referent odour) and another plate with no odour (as control). Subsequent to the third trial, we conducted a fourth trial, in which subjects were tested by simultaneously presenting a novel odour (test odour, the identity of which depended on the treatment; see below) and the habituation odour (referent odour). When an animal is habituated to a referent's odour, it will spend less time investigating the referent's odour across habituation trials (Halpin 1986; Johnston 1993). In addition, if the subject discriminates the novel odour, by perceiving the difference between the two odours, it should spend more time investigating the novel odour than the habituated odour (see e.g. Johnston 1993).

We collected the odour by rubbing round, plain and unscented cosmetic cotton balls (5 cm diameter) 15–20 times around three zones of the body: the mouth, the flanks and the anus–genital zone. We also rubbed the cotton in urine. To obtain the urine, the odour donors were left in individual cages for one night, and at the time of the experiment, after having rubbed the cotton in the body, we soaked a quarter of the cotton in the urine. We could therefore obtain a 'complete-body-odour'. This was important as the exact source of odours used during discrimination is unknown. Immediately after we collected the odour, we put the cotton in plastic hermetic bags for less than 1 h to avoid loss of scent. The odours were presented to the subjects by placing the cotton between two acrylic plates (10 × 10 cm), one of them with 30 holes of 0.5 cm in diameter, so the subjects could smell the scent (Jesseau 2004). Because the intensity of the scent may diminish if the cotton is repeatedly rubbed on the body and presented to the subjects, we collected four cotton samples for each odour donor. The scented cotton samples were kept separated by subject in a hermetic bag until we used them in the test. We used different cotton for each habituation trial (the same odour donor) and test trial (different odour donor) at random. At the completion of the tests, the plates were washed with neutral detergent (Virginia Neutral Detergent, Virginia S.A., Santiago, Chile) and allowed to air dry. Disposable gloves were used in the manipulation of the cotton and the plates to prevent contamination with human scent.

Two days before the tests, the subjects were housed in their home cages in mixed groups of three (one male and two females, or one female and two males), and taken to the experimental room, so they could become accustomed to the new environment. Subjects were tested in groups of three to reduce the stress of the test, as degus live naturally in groups. In addition, previous experiments have shown that degus tested in solitary tend to be very inactive and to show little, if any, exploratory behaviour (R. A. Vásquez, personal observation). The size of the plates permitted simultaneous exploration by all subjects. In cases where subjects were also used as odour donors, individuals were always used first as subjects

and subsequently, as donors. The subjects were marked with a small amount of nontoxic paint to aid in individual identification by the observer. We detected no perceptible change in the behaviour of animals as a result of marking. During the habituation trials the plate with the referent scent and a plate without odour (control plate) were placed in one half of the cage. In the test trial the control plate was replaced by the test scent. The positions of the two plates were determined at random to avoid bias in the results due to animal preference for a particular side of the cage. The donors of the habituation and the test odour were of the same sex for each test, but both sexes were tested. Because the subjects were in mixed groups, the sex of the subjects did not always match the sex of the odour donors. Each session was videorecorded (colour CCTV camera) from above for subsequent analysis with the software JWatcher. Videos were scored by one observer who was blind to the treatment of the subjects. We measured the time that subjects spent exploring the plates. The subject was considered to be investigating when its nose was less than 1 cm from the plate.

#### *Statistics*

The results were analysed with Wilcoxon signed-ranks test, because data did not meet parametric assumptions. We compared the time that subjects spent exploring the habituation and the test plates in the test trial for each test group. In addition, we compared exploration of the first and the last trial of the habituation sessions for each test group.

## **RESULTS**

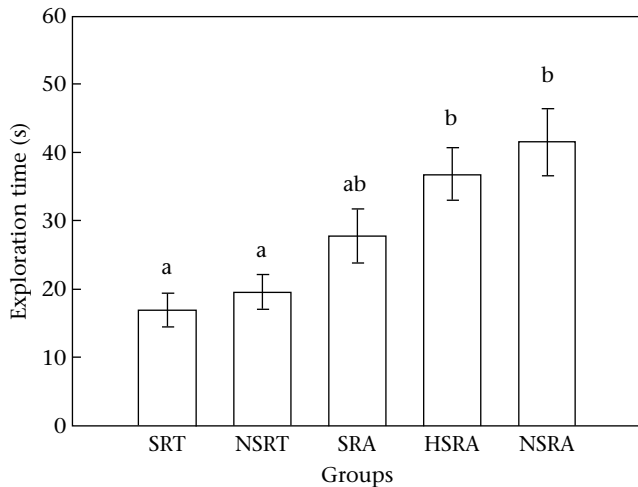
### *Experiment 1*

On average, degus were  $155.8 \pm 0.8$  days of age (mean  $\pm$  SE,  $N = 150$ ) and weighed  $176.4 \pm 1.9$  g at the time of the experiments. We found a significant difference in weight between sexes. Males weighed  $185.9 \pm 2.8$  g and females weighed  $167.3 \pm 1.9$  g (unpaired  $t$  test:  $t_{1,150} = -5.49$ ,  $P < 0.001$ ). However, body weight did not affect the exploratory behaviour of degus (ANCOVA:  $F_{1,135} = 0.43$ ,  $P = 0.52$ , power  $> 0.4$ ). There were no sex effects ( $F_{1,131} = 0.46$ ,  $P = 0.50$ , power  $> 0.5$ ) in the exploration time between individuals in different treatments. Likewise, we found no effect of kinship ( $r = 0.5, 0.25$  and  $0$ ) on exploratory behaviour (ANCOVA:  $F_{2,135} = 2.26$ ,  $P = 0.11$ , power  $> 0.3$ ). In contrast, significant differences for familiarity were detected (ANCOVA:  $F_{1,135} = 14.42$ ,  $P = 0.0002$ ), where individuals explored familiar individuals less than unfamiliar ones regardless of genetic relatedness. Given the significant effect of familiarity, we contrasted each pair of groups to determine which groups differed from each other. These analyses showed that familiarity accounted for differences between certain groups ( $t$  tests using sequential Bonferroni corrections for comparisons between groups:  $t > -3.12$ ,  $P < 0.0004$ ) for the following paired comparisons: SRT versus NSRA, SRT versus HSRA, NSRT versus NSRA and NSRT versus HSRA. The remaining comparisons were not significantly different ( $t = -2.360$ – $0.703$ ,  $P > 0.05$ ; Fig. 1). SRT and NSRT did not differ significantly in exploration time but they were significantly lower than HSRA and NSRA, which also did not differ in exploration times (Fig. 1). SRA did not differ in exploration time to either of the groups (Fig. 1). There were no significant differences across groups for cohesive behaviour (Kruskal–Wallis test:  $H_4 = 4.52$ ,  $P = 0.34$ ).

### *Experiment 2*

#### *Familiar group*

*Siblings reared together.* Across the three habituation trials, subjects significantly decreased the time they spent investigating the scent



**Figure 1.** Exploration time (mean  $\pm$  SE) observed in arena tests of paired encounters of subadult *Octodon degus*. Using the cross-fostering subjects and the half sibs, we created five experimental groups differing in rearing condition and genetic relatedness: sibs reared together (SRT), nonsibs reared together (NSRT), sibs reared apart (SRA), half sibs reared apart (HSRA) and nonsibs reared apart (NSRA). There were 14 pairs per experimental group. Different letters represent statistically significant differences between groups ( $t$  tests between pairs of groups using sequential Bonferroni corrections for multiple comparisons; see text for  $t$  values).

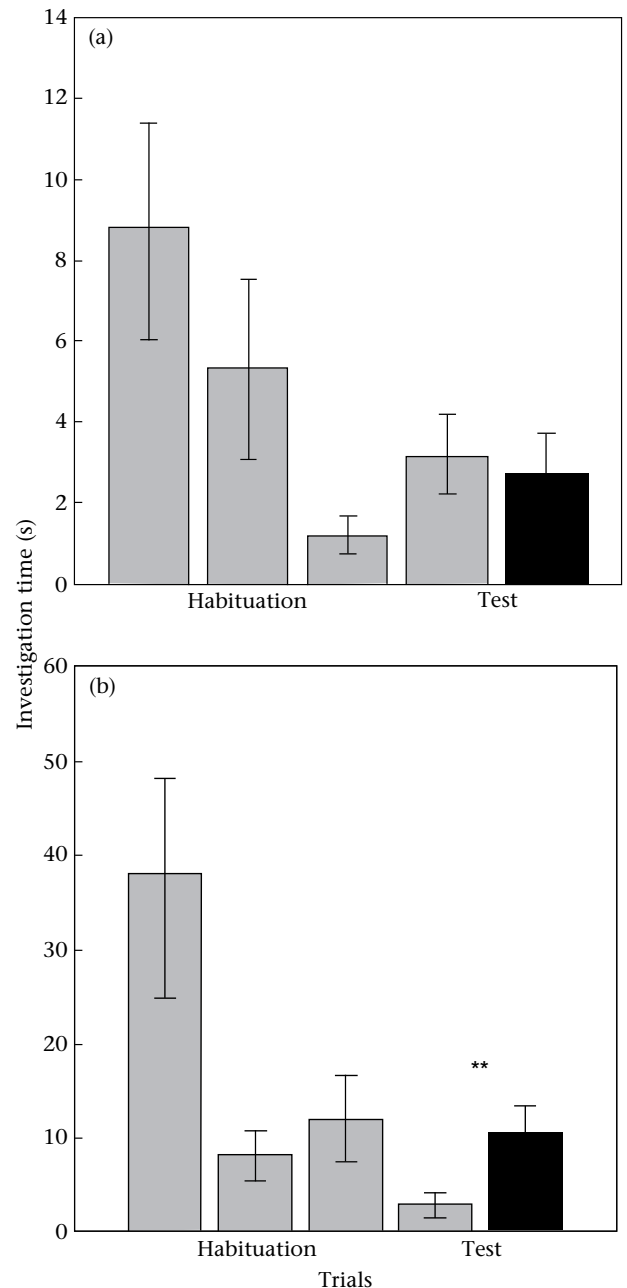
plate (Wilcoxon signed-ranks test:  $Z = 2.97$ ,  $N = 18$ ,  $P = 0.003$ ), showing that they habituated to the referent odour (i.e. their own familiar sibling). During the test trial there were no significant differences in the time that subjects spent exploring the referent and the test odours (i.e. another sibling) ( $Z = 0.22$ ,  $N = 18$ ,  $P = 0.83$ ; Fig. 2a). In addition, there was no significant difference between the last habituation and the test odour either ( $Z = 1.69$ ,  $N = 18$ ,  $P = 0.09$ ), which indicates that subjects did not discriminate between odours from two of their familiar siblings.

**Nonsiblings reared together.** The odour of the referent (i.e. their own familiar sibling) was explored less by the subjects on the third trial than during the first trial ( $Z = 2.59$ ,  $N = 18$ ,  $P = 0.001$ ), thus showing habituation. During the test trial, subjects explored the test odour (i.e. nonsibling reared together with them) significantly more than the odour of the referent ( $Z = 2.59$ ,  $N = 18$ ,  $P = 0.01$ ; Fig. 2b), which indicates that subjects could discriminate between the odours of their familiar sibling and their familiar nonsibling.

#### Unfamiliar group

**Sibling reared apart.** Subjects tended to spend less time investigating the scent plate with the referent odour (i.e. unrelated and unfamiliar to the subject) across habituation trials, but the difference was not significant ( $Z = 1.60$ ,  $N = 18$ ,  $P = 0.11$ ). Nevertheless, we found significant differences in the investigation time of the referent odour in the first trial and the odour of the referent in the test trial ( $Z = 2.13$ ,  $N = 18$ ,  $P < 0.05$ ). This finding suggests that the subjects became habituated to that odour. During the test trial, subjects investigated the test odour (i.e. sibling of the referent, reared apart) more than the referent odour ( $Z = 2.69$ ,  $N = 18$ ,  $P < 0.007$ ; Fig. 3a), which indicates that subjects could discriminate between the odours of an unfamiliar and unrelated conspecific, as well as those of its sibling.

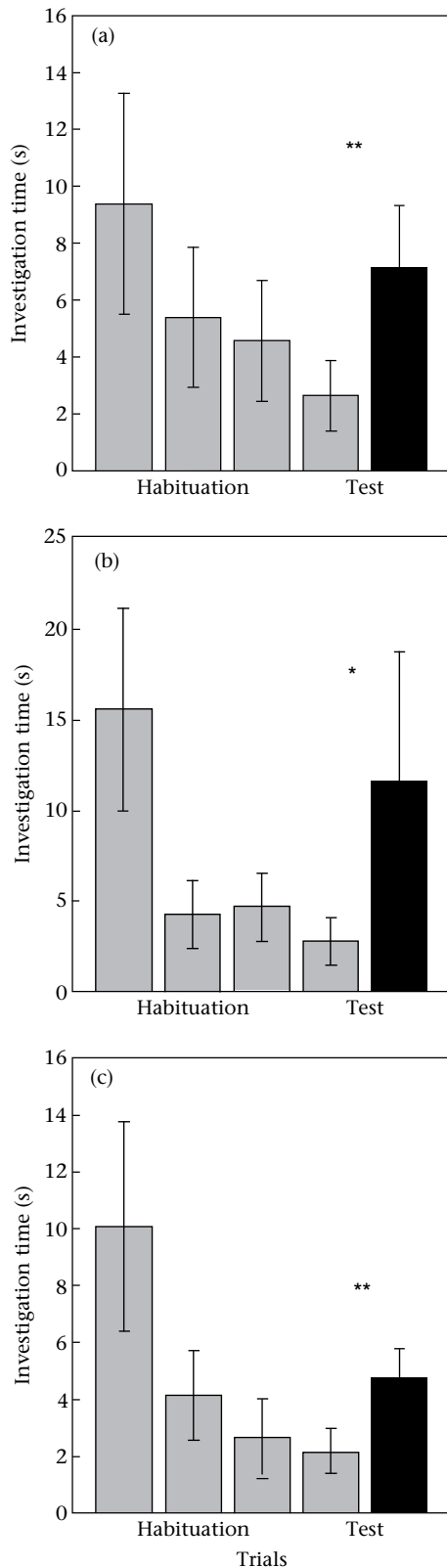
**Half-sibling reared apart.** The subjects explored the odour of the referent (i.e. unrelated and unfamiliar to the subject) significantly less on the third trial than during the first trial ( $Z = 2.51$ ,  $N = 18$ ,  $P = 0.01$ ), thus showing habituation to the scent. During the test



**Figure 2.** Investigation time (mean  $\pm$  SE) of the scent plates in the habituation-discrimination experiments of the familiar group.  $\square$ : time that the subjects spent investigating the referent odour (i.e. their own sibling);  $\blacksquare$ : time that the subjects spent investigating the test odour. In (a), the test odour corresponds to another sibling (SRT). In (b), the test odour corresponds to a nonsibling reared together with the focal subject (NSRT). Asterisks represent significant differences in the investigation time between the referent and the test scent in the test trial: \*\* $P < 0.01$  ( $t$  test).

trial, subjects significantly increased the investigation time of the referent odour (i.e. half sib of the referent, reared apart), compared to the odour of the referent ( $Z = 2.43$ ,  $N = 18$ ,  $P = 0.02$ ; Fig. 3b), which indicates that subjects could discriminate between the odours of an unfamiliar and unrelated conspecific as well as those of its half sib.

**Nonsibling reared apart.** During the habituation trials, the subjects significantly decreased the time they spent investigating the scent plate with the referent odour (i.e. unrelated and unfamiliar with the subject) ( $Z = 2.67$ ,  $N = 18$ ,  $P = 0.008$ ), showing habituation. During the test trial, subjects investigated the odour of the test odour



**Figure 3.** Investigation time (mean  $\pm$  SE) of the scent plates in the habituation-discrimination experiments of the nonfamiliar group.  $\square$ : time that the subjects spent investigating the referent odour (i.e. unrelated and unfamiliar to the subject);  $\blacksquare$ : time that the subjects spent investigating the test odour. In (a), the test odour corresponds to a sib of the referent reared apart (SRA); in (b), the test odour corresponds to a half sib reared apart (HSRA); and in (c), the test odour corresponds to a nonsib reared apart (NSRA). Asterisks represent significant differences in the investigation time between the referent and the test scent in the test trial: \* $P < 0.05$ ; \*\* $P < 0.01$  ( $t$  tests).

(i.e. unrelated and unfamiliar to the referent) more than the odour of the referent ( $Z = 2.90$ ,  $N = 18$ ,  $P = 0.004$ ; Fig. 3c), which indicates that subjects could discriminate between the odours of two unfamiliar, unrelated conspecifics.

In all treatments, animals showed habituation to the control plate in the three habituation trials, with only one exception. There were no significant differences in the exploration time between the control plate and the scent plate in each habituation trial (Wilcoxon signed-ranks test:  $Z = 0.00$ – $1.88$ ,  $N = 18$ ,  $P = 1.0$ – $0.06$ ). These times are not shown in the figures (see e.g. Johnston 1993). There were no differences in the exploration time of the habituated and test plates between sexes in each test trial (Mann-Whitney  $U$  test:  $Z = -0.04$ – $0.88$ ,  $N_1 = N_2 = 18$ ,  $P = 0.96$ – $0.38$ ).

## DISCUSSION

### *Effects of Familiarity and Phenotype Similarity on Conspecific Discrimination*

The first experiment revealed that degus distinguish individuals based largely on familiarity, because familiar individuals explored each other significantly less than did unfamiliar ones (half sibs and nonsibs). Furthermore, degus reared together explored each other in the same way regardless of genetic relatedness, such that NSRT responded similarly to nonsiblings and siblings. Likewise, degus reared apart explored each other equally, independent of whether they were sibs, half sibs or nonsibs. Therefore, we found that degus at 4–5 months of age display differential treatment based mainly on familiarity. Nevertheless, our finding that exploration time of SRA did not differ from that of SRT indicates that factors other than familiarity influence full sib discrimination. This could be explained by phenotype matching, whereby animals learn family-specific cues shared between full sibs that allow further discrimination, even though they have not previously encountered each other. On the other hand, an intrauterine effect could also exist, in the sense that there is a familiarity effect obtained inside the uterus during pregnancy (Hepper 1987). Thus, according to experiment 1, familiarity plays a fundamental role in sibling discrimination in degus, but other factors also influence full sib discrimination, such as phenotype similarity or intrauterine familiarization.

The tendency of rodents to explore nonfamiliar conspecifics more thoroughly than they do familiar ones has been described in other species (e.g. Holmes 1984; Johnston 1993). Individuals are expected to decrease investigation effort when the exploratory signal (odour) is already familiar, for instance, if individuals have been previously imprinted to that odour. On the other hand, if the odorant is unrecognized, individuals are expected to explore it with more effort. After repeated encounters with a signal, habituation to that smell occurs, after which it is considered a familiar signal (Holmes 1984; Halpin 1986; Johnston 1993). Thus, in degus, memory can be maintained with repeated exposures to another individual, as has been described in other rodents, such as rats and prairie voles (Popik & Van Ree 1998; Paz y Miño & Tang-Martínez 1999; see also Vásquez et al. 2006 for a study on degu memory capabilities). In the current investigation, degus lived with the same individuals and so became familiar with each other. They could therefore reinforce their memory by living together, and then discriminate familiar individuals in the experiments. Although we cannot rule out the possibility that an imprinting-like process might mediate the learning of familiar signals, this would indicate that rodents require little time for learning the identities of their rearing mates, which could be adaptive in communal nesting because nonsiblings would be encountered soon after birth. Nevertheless, the design of our study did not allow us to determine the length of stimulus exposure required to augment discrimination abilities.

In experiment 2, in which we evaluated discrimination of the odours of individuals with different degrees of kinship and familiarity, we found that degus can discriminate between odours from two individuals that were unrelated to each other and to the subject, as has been demonstrated for other rodent families (e.g. Halpin 1986; Johnston 1993; Todrank et al. 1998). In our study, degus discriminated between individuals that were genetically unrelated and unfamiliar. In addition, they discriminated between familiar siblings and unrelated individuals with whom they had been familiar. Recently, Jesseau et al. (2008) used communal rearing (pairs of mothers and their litters lived together) and olfactory habituation (analogous to our experiment 2) and found that degu mothers discriminate between 2-week-old familiar offspring and familiar alien pups, but they did not discriminate between them when they were 6 weeks old. In contrast with their last result, our study shows that 4–5-month-old degus can discriminate between familiar siblings and familiar nonsibling odours, indicating that a mechanism other than prior association is controlling sibling discrimination. Interestingly, and in contrast with rodents such as hamsters or *Cryptomys* mole-rats (Todrank et al. 1998; Heth et al. 2002), but similar to the coruro, *Spalacopus cyanus*, another caviomorph rodent belonging to the Octodontidae family (Hagemeyer & Begall 2006), degus can also discriminate between odours from unfamiliar sibs and half sibs. The results of our experiments suggest that the odours of relatives are different enough for individuals to be discriminated, although they share a great percentage of their genes. However, degus did not discriminate between the odours of their own siblings with whom they were familiar. This lack of distinction can be interpreted in two ways. First, it suggests that odours of relatives are considered similar, and this similarity can explain the lack of discrimination among sibs reared together. Possibly, when individuals share a similar resource (e.g. external diet or maternal milk), they are more likely to express or perceive similar odours when they are genetic relatives. This possibility should be investigated in detail in further studies, for example, by assessing the role of diet in the production of odours (see Halpin 1991; Columbelli-Negrel & Gouat 2006). Second, animals may have been unmotivated at the time of the test. In this regard, the habituation paradigm can involve some interpretation problems, because a lack of response to a familiar and novel stimulus does not necessarily mean that there is lack of discrimination. Therefore, degus might still be able to discriminate between familiar siblings despite our inability to detect such discrimination (see Gheusi et al. 1997; Johnston 2003).

#### *Kin Recognition Mechanisms*

Our results suggest that degus discriminate between kin and nonkin largely, but not exclusively, using a prior association mechanism in which direct familiarization is required for subsequent discrimination. This conclusion arises from our findings in experiment 1 in which degus showed differential behaviours based on familiarity. Nevertheless, degus in our study also used phenotype matching for discrimination (see Mateo & Johnston 2000), because SRA and SRT did not differ in their exploration time in experiment 1, and degus discriminated between odours of familiar sibs and familiar unrelated individuals in experiment 2.

Therefore, taken together, the two experiments indicate that social discrimination is based on both prior association and phenotype matching. Degus discriminated between odours of familiar siblings and individuals with no genetic relationship, indicating that they discriminated based on kinship cues because both odours were familiar. In addition, we found a familiarity–genetic relatedness interaction, because degus discriminated between unfamiliar sibling odours, but they did not discriminate between familiar sibling odours (experiment 2). Nevertheless, degus treated

genetically unrelated familiar individuals and their own familiar sibs similarly (experiment 1). Therefore, although degus have the ability to discriminate kin, they may not necessarily use this ability in behavioural contexts, as do other rodents (Mateo 2003).

The two mechanisms mentioned above are not mutually exclusive or necessarily exhaustive (Holmes & Sherman 1982; Tang-Martínez 2001; Mateo 2003), and may therefore operate in a combined way or even simultaneously to allow discrimination between conspecifics during social encounters (Todrank & Heth 2003; Mateo 2003). For example, an individual might use familiarity to discriminate between a familiar sib and an unfamiliar nonsib, and use phenotype matching to discriminate between familiar sibs and nonsibs, or between sibs and half sibs.

#### *Implications of Discrimination on Degu Ecology*

In the wild, during the summer period (December–March), degu groups consist of pups and subadults, and their mothers. Pups remain together until the reproductive season approaches, when males have dispersed in the attempt of conform their territory (Soto-Gamboa 2004). Evidence suggests that females use communal nesting (Ebensperger et al. 2004; Jesseau 2004), such that the pups become familiar with sibs and nonsibs from an early age (Soto-Gamboa 2004). In communal-nesting species, such as degu, kin recognition can be complicated because differentially related young share a common environment. Thus, prior association and genetic relatedness could be confounded because young can readily learn all labels they encounter during early development even if they maintain different litter-specific labels, or because young may acquire similar phenotypic labels during their early life. Therefore, a mechanism, such as phenotype matching, that can allow discrimination between communally nesting juveniles could be used for kin-selected behaviours. Results of our study suggest that under natural conditions degus may be able to discriminate between siblings and nonsiblings living together with them, because in experiment 2, degus discriminated between odours of their siblings and unrelated individuals familiar to them. This type of recognition could be mediated specifically by self-referent phenotype matching (Mateo & Johnston 2000). As already mentioned, Jesseau et al. (2008) found that degu mothers discriminate between familiar offspring and familiar alien pups of 2 weeks old, suggesting that self-referent phenotype matching may be involved in kin discrimination. Our results support this mechanism as one of the explanations for kin discrimination in degus, and hence it could play a role in communal nesting in this species. However, degus may still not treat familiar unrelated individuals differently than their own siblings, as observed in experiment 1, where familiar siblings and familiar nonsiblings were treated in the same way (see Ebensperger et al. 2006 for a similar result in pup retrieval by degu mothers).

It has been described that under natural conditions females form associations with two to four females, where every member of the association share the same sleeping site and each association represents a discrete social unit (i.e. a nesting group using a common burrow system). The degree of kinship between female members of a given association is equivalent to that corresponding to half sibs, and it is significantly higher than the kinship degree of randomly selected females from the same population (Ebensperger et al. 2004). On the other hand, social interactions in the field are higher between nest companions than between randomly selected females (Jesseau 2004), and it has been reported that familiarity is very important in the social life of this rodent, influencing the expression of agonistic behaviours in males (Davis 1975; Jesseau et al. 2008). According to our results, familiarity is fundamental for social discrimination, particularly at the colony level, such that in

free-living degus, the degree of kinship among members of a common burrow system (nesting group), where social experiences and cooperative behaviours are more frequent, may be higher than that between other individuals in the population. However, in order to conclude that kin recognition is mediating these behaviours following kin selection theory, it is necessary to assess whether cooperative behaviour actually increases fitness in degus (Mateo 2003).

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