The nature and representation of individual recognition odours in Belding's ground squirrels

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In many taxonomic groups, odours provide cues to species identity, reproductive status, genetic related-ness and individual identity. These odour cues are often used to mark territories or other resources and to recognize individuals through direct or indirect olfactory investigation. Belding's ground squirrels, *Spermophilus beldingi*, frequently scent-mark their environment and they also investigate the scent glands of conspecifics, which suggests that odours play a modulating role in their social relationships. I conducted studies to determine what information is conveyed by various *S. beldingi* odours and whether this informa-tion is used by conspecifics for social recognition. *Spermophilus beldingi* produce a number of cues that are individually distinct, including odours from oral, dorsal, pedal and anal glands and from ears, but appar-ently not from urine, although it is unclear whether all of these odours are used for social recognition. This discrimination among odours of individuals does not require prior familiarity with the odour bearers. The volatile components of some odours are sufficient to permit individual discrimination, which may explain how animals appear to 'recognize' each other from a short distance. Finally, *S. beldingi* incorporate multiple odours into their memories of conspecifics as perception of one odour of an individual generalizes to a sec-ond odour from it, suggesting a mental representation of familiar individuals. The production of multiple unique odours may facilitate accurate discrimination of conspecifics along several social dimensions, and some of these odours also vary with relatedness. Together, these results indicate a rich olfactory milieu me-diating the social lives of *S. beldingi*.

Biologists have long been interested in the evolution of sociality and the factors promoting cooperation and competition. Given the importance of social relationships for understanding speciation, population dynamics, mat-ing systems and reproductive success, recent work has started to focus on how animals decide with whom they will form social bonds. Much theoretical work has focused on the advantages of helping kin (nepotism), avoiding inbreeding, and cooperating and competing with conspe-cifics (Hamilton 1964; Trivers 1971; Bateson 1982). Yet, for many species, we know little about whether or how they recognize each other, whether they use multiple cues for recognition or form mental representations of specific in-dividuals, or how this recognition influences their social behaviours (see Mateo 2003). In the present study, I

sought to determine whether Belding's ground squirrels, *Spermophilus beldingi*, produce individually distinct cues that could be used to identify conspecifics in a wide-range of social situations.

Recognition of conspecifics is mediated through olfac-tory cues in a variety of taxa (insects: Jaisson 1991; Gam-boa 1996; amphibians: Waldman 1991; mammals: Brown & MacDonald 1985; Halpin 1986; Johnston 1990; Swais-good et al. 1999; Beauchamp & Yamazaki 2003; fish: Olsén et al. 1998; Neff & Sherman 2003; perhaps birds: Zelano & Edwards 2002; Bonadonna et al. 2003), including sciurids (details in Kivett et al. 1976; Halpin 1984). Odour cues mediate recognition in *S. beldingi* and could be used to discriminate between individuals, kin classes or sexes (Holmes 1984a; Mateo 2002; see also below). Odours or other recognition cues can have multiple functions, par-ticularly in mammals when odours are complex mixtures of several to hundreds of compounds (Albone 1984). For example, in addition to indicating an animal's individual identity, sex or relatedness, odours may also reveal its

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genetic quality, its reproductive status, its age or even its location (Brown & MacDonald 1985; Penn & Potts 1998; Beauchamp & Yamazaki 2003).

Individually distinct cues would be useful when animals interact repeatedly over time and when discrimination among multiple familiar individuals is beneficial, such as in reciprocal altruism and dominance hierarchies (e.g. Trivers 1971; Colgan 1983; Bergman et al. 2003). Individual recognition is defined here as a cognitive process (without implying any level of processing or awareness) whereby an animal becomes familiar with a conspecific and later discriminates it (or its cues) from other familiar individuals. Individual recognition is based on unique features of animals learned through direct experience with those cues and associated with memories of prior interactions with those individuals, rather than based on simple differences in familiarity (see Mateo 2004). Note that individual recognition is not required for kin selection, mate choice, tolerance of neighbours or parental care; familiarity or spatial location may suffice for discrimination in these contexts. Yet such recognition can certainly play a role in these social situations and lead to behaviours directed towards particular individuals (see Mateo 2004). Individually distinct cues have been demonstrated in a variety of mammals (golden hamsters, *Mesocricetus auratus*: Johnston et al. 1993; guinea pigs, *Cavia aperea*: Martin & Beauchamp 1982; mongooses, *Herpestes auropunctatus*: Gorman 1976; Eurasian deer, *Cervus* spp.: Lawson et al. 2000; sheep, *Ovis aries*: Porter et al. 1991; rhesus macaques, *Macaca mulatta*: Rendall et al. 1996; and perhaps armadillos, *Dasypus novemcinctus*: Loughry & McDonough 1994; reviewed in Halpin 1986), in birds (swallows, *Hirundo* and *Riparia* spp.: Beecher et al. 1989), and in mantis shrimp, *Gonodactylus festae* (Caldwell 1985), although the functions of these distinct cues are not always clear.

Belding's ground squirrels are group-living, burrowing rodents found in alpine and subalpine regions of the western United States (Jenkins & Eshelman 1984). They are socially active above ground between April and August and hibernate the remainder of the year. Each adult female produces one litter annually of five to eight pups, which is reared for about a month in an underground burrow (the natal burrow). Young first come above ground (emerge) as nearly weaned, 4-week-old juveniles (Sherman 1976; Sherman & Morton 1984). Females live an average ± SE of 3.4 ± 0.3 years (up to 12 years); males live 2.1 ± 0.4 years (up to 7 years; Sherman & Morton 1984). Therefore, there is potential for adults, particularly philopatric females, to interact repeatedly within and between years, which might favour the evolution of an ability to recognize individuals.

Among ground-dwelling squirrels (ground squirrels, prairie dogs and marmots), amicable and agonistic social interactions are typically preceded by nasal contacts involving investigation of oral-gland secretions from apocrine glands located in the mouth corner. These contacts suggest that oral-gland odours facilitate identification of conspecifics according to their social group, sex, status or identity (e.g. King 1955; Steiner 1970; Kivett et al. 1976; Harris & Murie 1982; Walro et al. 1983). Another source of chemical signals is the dorsal-gland field, which

is an area of small apocrine glands extending caudally from the scapular region. Across *Spermophilus*, dorsal-gland fields are larger in males than females, and their sizes increase with sociality (Kivett et al. 1976). In *S. beldingi*, oral and dorsal odours also vary with genetic relatedness, and are often used for social recognition (Mateo 2002, unpublished data). Secretions may be passively deposited as animals dustbathe (rolling the dorsum and ventrum in a dusty area), move through burrow systems or brush against rocks, or they may be actively transferred to the environment by scent marking (Steiner 1975; Kivett et al. 1976; Halpin 1984). Oral odours are deposited during cheek marking as the oral gland is quickly pressed against an object. Dorsal odours are deposited during twist marking as an animal twists its torso so that its shoulders and back are pressed against a surface (e.g. dirt mound, tree trunk, or burrow entrance).

Other body odours could also be sources of recognition cues among rodents. Anal glands are three-lobed apocrine glands, which are everted when animals are fearful or highly stressed or during anogenital olfactory inspection. The pungent secretions of the anal glands can elicit approach or avoidance (Barash 1974; Salmon & Marsh 1989; Manaf et al. 2003; personal observation). Eccrine pedal glands appear to be individually distinct in golden but not Djungarian hamsters, *Phodopus campbelli* (Johnston et al. 1993; Lai & Johnston 1994), and may also be distinct in ground-dwelling squirrels (Kivett 1978). In some sciurids, the area between the ear pinna and eye (hereafter 'supraorbital odour') changes secretory patterns during the mating period, and although it probably primarily reflects gonadal hormone levels, it may also provide information about identity (Steiner 1973). Urine is distinct in laboratory rodents and some primates (Johnston et al. 1993; Brown & Eklund 1994; Lai & Johnston 1994; Laska & Hudson 1995; Zenuto & Fanjul 2002), and despite being influenced by diet (Schellinck et al. 1997), urine may serve a recognitive purpose in *S. beldingi*, because animals often urinate above ground on dirt piles near burrow entrances, and conspecifics will pay particular attention to these marks (personal observation). Note that recognition odours do not need to be produced by structures specially constructed for this purpose.

To understand the processes of social recognition, one needs to understand the sources and distribution of phenotypic labels or cues, as well as how others perceive these cues. To determine what information is conveyed by various *S. beldingi* odours and whether this information is used in social recognition, I conducted the following five tests. (1) I examined whether oral and dorsal odours are individually distinct (having shown already that they are kin distinct; Mateo 2002), using habituation–discrimination tests. (2) I evaluated the distinctiveness of *S. beldingi* supraorbital, pedal and anal-gland odours as well as urine. (3) I tested whether familiarity with odour donors is required for discrimination of individual odours. (4) I tested whether *S. beldingi* recognize individuals, rather than their separate individual odours; in this case multiple cues would contribute to a higher-order representation of individuals (with memories of each odour all associated with the odour bearer itself), such that habituation to one

odour of an individual would generalize to its other odours (Johnston & Jernigan 1994). (5) I tested whether the unique qualities of *S. beldingi* odours used for individual recognition are volatile or nonvolatile and also whether they persist in the substrate after active scent marking.

METHODS

Animals and Housing

I studied Belding's ground squirrels at the Sierra Nevada Aquatic Research Laboratory (SNARL; near Mammoth Lakes, California, U.S.A.). Details of trapping, marking and housing animals are in Mateo & Holmes (1997). Pregnant females were live-trapped and housed in a laboratory building at SNARL where they gave birth and reared their young. Litters probably comprised full- and half-siblings because of multiple mating by females (Hanken & Sherman 1981). When young were 25–28 days of age, they and their mothers were transferred to outdoor enclosures at SNARL (3–4 litters/enclosure) to serve as subjects or donors for odour tests. Individuals within an enclosure moved about and interacted freely. Each open-air enclosure ($10 \times 10 \times 2$ m) included natural vegetation, laboratory food and water, and four buried nestboxes connected to the surface by plastic tunnels. Animals were maintained on a Purina diet (no. 5015) to minimize environmental influences on odours. Juveniles (young-of-the-year > 30 days old) and yearlings (~ 1 year old) served as subjects, and both juveniles and adults (>2 years old) served as odour donors (see below). Adult female donors had ceased lactating at least 2 weeks before all tests except the pedal-gland test, in which juveniles had emerged one week earlier and still attempted to nurse. At the end of the studies animals were released at the mother's point of capture (see Mateo & Johnston 2000 for details on releases).

Odour-testing Methods

Odour collection

Most odour tests involved presentation of one odour type collected from two individuals (exceptions noted below). We collected most odours from donors on 3-cm^3 polyethylene cubes within 15 min of presentation to other conspecifics ('subjects'). For tests with oral-angle gland secretions (hereafter 'oral odours'), we rubbed one surface of a cube anteroposteriorly eight times along each mouth corner. To collect dorsal odours, we rubbed a cube cephalocaudally along the back and shoulder region eight times. Anal-gland odours were first collected from a juvenile on cotton swabs by rubbing the swab four times along the glands, which are typically everted when animals of any age are picked up by hand. Each swab was then rubbed along the top of a cube. Urine was collected each day by placing the donor in a plastic cage ($38 \times 33 \times 18$ cm fitted with a wire lid) until she urinated (typically within 30 min). The urine was collected with a syringe, placed in a polypropylene microcentrifuge tube (Cole Parmer; Vernon Hills, Illinois, U.S.A.) and either used immediately (Fig. 2b) or frozen at $-15\,^\circ$C until use (Fig. 2a). A drop of urine approximately 1 cm in diameter was placed on the top of each cube to be scented. To collect odours for the pedal-gland test from adult females, one corner of a cube was wiped four times along a rear foot pad, and the opposite corner was wiped four times along a front foot. Supraorbital odours from the region between the eye and ear (Steiner 1973) were collected by rubbing a cube on this area eight times.

Odour presentation

One person collected the odours and coded the cubes while wearing latex gloves to prevent the transfer of other ground-squirrel odours or human odours to the equipment or to the animals. Thus, observers ($N = 2$) were blind to the odour donors' identities and which cubes were scented (with the exception of urine-scented cubes, which reflected sunlight). Pairs of cubes (e.g. scented and unscented) were placed by the odour collector 3 cm apart and 1 cm in front of each of four burrow entrances, anchored by 3-cm screws (inserted in the middle of each cube) for investigation by all animals in the enclosure. Although more than one animal could investigate a set of cubes at a given time, the presence of conspecifics does not make ground squirrels more or less likely to investigate cubes, nor does it influence their duration of investigation (unpublished data). In addition, animals always went below ground when we entered the enclosure to place the cubes, and typically re-emerged from burrows one by one after cube placement, with the majority of investigations occurring during this initial ~ 10-min re-emergence period. The total number of contacts each subject made with each cube (subject's nose within 1 cm of a cube) and the total duration of contact (time spent smelling an odour) were recorded for 30 min by observers blind to what was on the cubes. If a cube was licked, scent-marked or dislodged, data collection from that pair of cubes ceased. Cubes were washed with hot water and unscented soap after use and allowed to air dry.

Tests of Individually Distinct Odours

I used habituation–discrimination tasks to determine which odours are individually distinct (Schultze-Westrum 1969; Halpin 1986; Johnston et al. 1993; see Gheusi et al. 1997 for an alternate method). In this task, an animal is repeatedly presented with a particular stimulus (here, an individual's odour) until it habituates to it, and then the animal is presented with a novel stimulus (here, another individual's odour) to determine whether the animal dishabituates to it, indicating discrimination of the two stimuli. This task tests for true discrimination of individual conspecifics, because familiarity and relatedness were controlled (donors were familiar to subjects, with exceptions noted below, and donors were unrelated to each other) and could not be used as a basis for discrimination. Subjects were presented with an odour from an individual ('Individual 1') for three to four habituation trials, and then tested with odour from the same odour source collected from another individual ('Individual 2') of the same sex, age class and reproductive condition

('discrimination trials'). Donors were typically familiar individuals living in the enclosure with the subjects (exceptions noted below). Adult subjects were trapped from locations at least 100 m apart and so were unlikely to have been closely related to the odour donors (unpublished data). All trials were separated by 24 h. During habituation trials, an unscented cube was presented along with the cube containing Individual 1's odour, to verify that subjects habituated to the odour rather than the cubes. Subjects typically smelled the scented cube significantly longer than the unscented cubes during the first two to three habituation trials, and were considered habituated to the scent when they did not smell the cubes differentially; data on investigation of unscented cubes are not presented. After the habituation trials, Individual 2's odour was presented during the discrimination trial on one cube, with a second cube either unscented (tests conducted in 1996) or scented with Individual 1's odour (tests conducted 1997–1999, 2004; 'cross-scent habituation studies' also used two different odours during test trials; see below).

The perceived dissimilarity of the test stimuli, relative to the habituation odour, was reflected in the magnitude of the response differences to the test odours, because animals usually attend to novel stimuli more than familiar stimuli (Schultze-Westrum 1969; Johnston 1981; Halpin 1986; Stoddard 1996; Mateo & Johnston 2000; Mateo 2002). Thus, if ground squirrels produce distinct odours, then Individual 2's odour should be perceived as dissimilar to Individual 1's and be investigated longer than the final habituation odour. Because both odour donors were familiar to subjects and fresh exemplars of odours were collected for each of the trials (except urine, which was collected and frozen in advance for one of the studies), the only unique difference between the odour stimuli was the source of the odour (i.e. individual identity; familiarity, sex, age class and reproductive condition were controlled). A significant decrease in investigation across habituation trials indicated habituation to (and hence recognition of) Individual 1's odour, and a significant increase in investigation from the final habituation trial to the test trial indicated discrimination of Individual 2's odour as distinct from Individual 1's. Animals were included in an analysis if they investigated at least one cube during each of the habituation and discrimination trials. Data from donors' investigations of the cubes were omitted from the analysis. Responses of donors' offspring were included, because I found no statistical differences between their responses and those of unrelated subjects (Kruskal–Wallis one-way ANOVA using litter as the main effect; all $Ps > 0.10$). Some subjects in one group participated in two habituation–discrimination tasks (one with urine, one to test whether familiarity is important for discrimination of individual odours), and another group was used for two cross-scent habituation tasks (Fig. 7a, b).

To determine whether S. beldingi need to be familiar with conspecifics to be able to discriminate among their individual odours, I repeated the habituation–discrimination studies described above, this time presenting subjects with odours from unfamiliar adult females living in separate enclosures and trapped from different populations (>10 km from the subjects' population). Next, I used a variant of the habituation–discrimination task to determine whether S. beldingi form multiple representations of familiar individuals. That is, does knowledge of an odour from a familiar individual generalize to other odours of that individual? Subjects were habituated to one odour collected from Individual 1, and were then tested with odour from a second source collected from Individual 1 and the same odour source from Individual 2. If S. beldingi recognize individuals as a whole, rather than just remember their separate odours, they should generalize among several odours of an individual and dishabituate (show an increased response) only to odours of another individual. I also tested whether familiarity with individuals is necessary for this generalization; that is, whether generalization is simply due to common chemical characteristics shared among the odours, or due to learned associations among odours as a result of experience with an individual. To determine whether the cues important for individual recognition are volatile, I repeated the individual-discrimination studies with oral- and dorsal-gland odours using hardware-cloth 'covers'. These were made with 1-cm^2 wire and were designed to be placed over a pair of cubes in front of each burrow entrance, with the top and sides approximately 1.5 cm away from cube surfaces. Subjects could investigate volatile components of the odours, but not come into direct contact with the cubes or the substances on them.

To explore whether odours are left in the substrate after ground squirrels stand, groom or scent-mark in a particular area, I compared responses of S. beldingi to soil from two sources. One was dirt collected from a burrow entrance at a long-term study site ('used dirt'; see Mateo 1996 for details on the site), where several adult males and females were observed dustbathing and scent marking within a 3-h period the morning of the odour test. The other soil ('clean dirt') was collected from a large dirt pile at SNARL approximately 2 m high. This dirt pile was situated well away from where ground squirrels were typically found, and because it had been sifted of rocks, it was unstable and would have been difficult for animals to climb. It had been undisturbed for at least 4 weeks. Thus, whatever animal odours had been in it had probably dissipated by the time of the odour test. The used dirt was collected in a plastic bag the morning of the test and placed in a household freezer for about 4 h. The clean dirt was collected upon return to SNARL that day and stored in a freezer for 1 h before testing, at which time both samples were brought to ambient temperature. Approximately 0.25 cup of dirt was placed in paper cups (5 cm bottom diameter, cut to 3 cm high) and secured in front of enclosure burrows by nails through the bottom of the cups. Thus, one used-dirt cup and one clean-dirt cup were placed at each of the four burrow entrances.

Durations of investigation of odours were not normally distributed and transformations were largely unsuccessful, so all data were analysed with Wilcoxon signed-ranks tests. I used one-tailed tests when analysing results of the habituation–discrimination tasks because repeated presentation of the habituation odour should lead to a decrease in investigation of that odour, and because subjects should investigate the novel odour longer than the

habituation odour if odours are individually distinct (e.g. Schultze-Westrum 1969; Harrington 1976; Halpin 1986; Johnston et al. 1993; Murdock & Randall 2001; Mateo 2002; Zenuto & Fanful 2002). A two-tailed test was used to analyse the results of the dirt test because the direction of the difference in investigation duration could not be predicted a priori. Analyses of frequency of contact with odours are not presented because they were less discriminating than investigation durations. Data are presented as unadjusted means + SE, and I considered results significant when $P < 0.05$. I tested each data set for litter effects, because each study involved more than one litter. In all cases, between-litter variation was not significantly greater than within-litter variation, and therefore I used individuals as the unit of analysis. No sex differences were found for any of the statistical comparisons (two-tailed Wilcoxon signed-rank tests; all $P > 0.10$).

RESULTS

Individually Distinct Odours

Oral-gland odours

I tested 19 *S. beldingi* juveniles ($N = 8$ males and 11 females from four litters, about 45 days old) for their ability to discriminate between the oral odours of two familiar adult females. Subjects' investigation durations declined across the four habituation trials (day 1 versus day 4: $Z = 1.98$, $P = 0.024$; Fig. 1a), indicating recognition of and habituation to Individual 1's odours. Investigation of Individual 2's oral odour during the test trial was significantly longer than investigation of the final habituation odour ($Z = 3.18$, $P = 0.0005$). These results demonstrate that *S. beldingi* can discriminate between two familiar conspecifics on the basis of oral odours alone.

Dorsal-gland odours

I also tested subjects ($N = 8$ male and 11 female juveniles from four litters, about 52 days old) for their ability to discriminate between dorsal-gland odours of familiar adult females. Subjects habituated to repeated exposure to Individual 1's dorsal odour ($Z = 2.91$, $P = 0.002$) and discriminated it from Individual 2's dorsal odour ($Z = 3.39$, $P = 0.0005$; Fig. 1b).

Urine

Two habituation–discrimination tests were conducted to determine whether urine is individually distinct. In the first, subjects ($N = 9$ male and 10 female juveniles from two litters, about 50 days old) habituated to urine from a familiar adult female ($Z = 1.82$, $P = 0.035$; Fig. 2a), but during the test phase did not investigate urine from Individual 2 longer than urine from Individual 1 ($Z = 1.481$, $P = 0.07$). In the second test, a separate group of subjects (1 adult female, 3 male and 6 female juveniles from two litters, about 60 days old) habituated to urine from an adult female (day 1 versus day 3: $Z = 2.43$, $P = 0.008$; Fig. 2b), but again subjects did not differentially investigate the novel urine from Individual 2 and the habituation urine from Individual 1 on the test day ($Z = 1.481$, $P = 0.07$). Although investigation patterns on the test day were in the predicted direction for the second test, the results of the two tests do not indicate that *S. beldingi* urine is individually distinct.
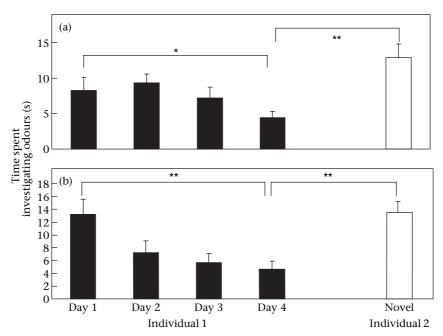

Figure 1. Mean + SE duration of investigation (s) of (a) oral-gland and (b) dorsal-gland odours by *S. beldingi* subjects in habituation–discrimination tasks. ■: investigation of the habituation odour from Individual 1; □: investigation of test odour from Individual 2. Horizontal lines and asterisks indicate significant differences in investigation of odours (*$P < 0.05$; **$P < 0.01$) based on Wilcoxon signed-ranks tests.
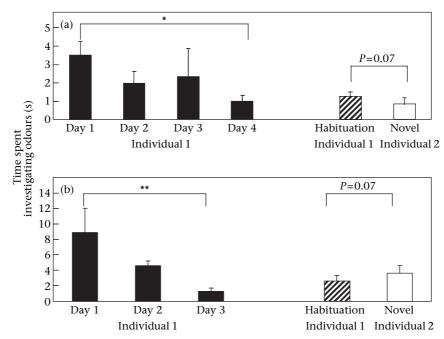
Figure 2. Mean + SE duration of investigation (s) of *S. beldingi* urine odours by subjects in two habituation–discrimination tasks (a, b). ■ (habituation trials) and ▨ (discrimination trials): investigation of the habituation odour from Individual 1; □: investigation of test odour from Individual 2 (discrimination trials). Horizontal lines and asterisks indicate significant differences in investigation of odours (*P < 0.05; **P < 0.01) based on Wilcoxon signed-ranks tests.

Pedal-gland odour

Subjects ($N = 10$ male and 7 female juveniles from three litters, about 36 days old) habituated to repeated presentations of pedal-gland odour from an adult female (day 1 versus day 4: $Z = 1.789$, $P = 0.037$; Fig. 3). Subjects investigated the novel pedal odour of the second female significantly longer than the habituation pedal odour on the test day ($Z = 2.107$, $P = 0.018$).

Anal-gland odour

I tested six juveniles (3 males and 3 females from one litter, about 35 days old) with anal-gland odours collected from a familiar female juvenile. After a strong initial response to the odour, juveniles habituated across trials (day 1 versus day 3: $Z = 2.023$, $P = 0.022$; Fig. 4) and on the test trial discriminated between the anal-gland odours

from Individuals 1 and 2 ($Z = 2.201$, $P = 0.014$). Subjects also investigated the anal odour from Individual 2 significantly longer than the final habituation odour on day 3 ($Z = 2.023$, $P = 0.021$), but there was no significant difference in investigation of Individual 1's odours on day 3 and the test day ($Z = 0.944$, $P = 0.345$). Although the sample was small, these within-subject results suggest that juvenile anal-gland odours are also individually distinct.

Supraorbital odour

Eight yearlings (5 male and 3 female) were tested with supraorbital odours from two familiar adult females. Subjects habituated to presentations of supraorbital odours from Individual 1 across 4 days (day 1 versus day 4: $Z = 2.197$, $P = 0.014$; Fig. 5). On the test day, they
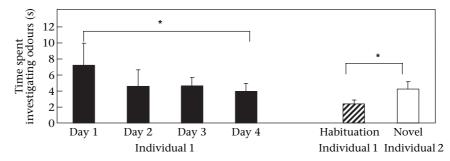

Figure 3. Mean + SE duration of investigation (s) of *S. beldingi* pedal-gland odours by subjects in habituation-discrimination tasks. ■ (habituation trials) and ▨ (discrimination trial): investigation of the habituation odour from Individual 1; □: investigation of test odour from Individual 2 (discrimination trial). Horizontal lines and asterisks indicate significant differences in investigation of odours (*P < 0.05) based on Wilcoxon signed-ranks tests.
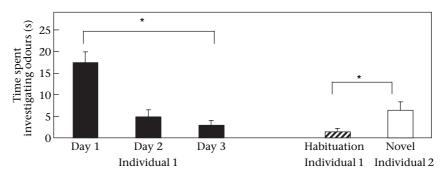
Figure 4. Mean + SE duration of investigation (s) of *S. beldingi* anal-gland odours by subjects in habituation–discrimination tasks. ■ (habituation trials) and ▨ (discrimination trial): investigation of the habituation odour from Individual 1; □: investigation of test odour from Individual 2 (discrimination trial). Horizontal lines and asterisks indicate significant differences in investigation of odours (*$P < 0.05$) based on Wilcoxon signed-ranks tests.

investigated supraorbital odours from Individual 2 longer than those from Individual 1 ($Z = 1.82$, $P = 0.035$), indicating that supraorbital odours are individually distinct.

Importance of Familiarity for Individual Discrimination

Subjects included six male and three female juveniles (about 49 days old) for the oral-gland odour test, and seven male and six female juveniles (about 42 days old) for the dorsal-gland odour test. Subjects habituated to the odours across the three habituation days (oral: $Z = 2.67$, $P = 0.004$ dorsal: $Z = 2.90$, $P = 0.002$; Fig. 6). On the test day, both groups tended to investigate odour from the novel individual longer than they investigated the habituation odour on day 3 (oral: $Z = 1.82$, $P = 0.035$; dorsal: $Z = 1.922$, $P = 0.028$), suggesting that the odours are individually distinct and *S. beldingi* do not require prior experience with them for discrimination.

Cross-scent Habituation–Discrimination Tests

Two series of 'cross-scent habituation–discrimination' studies were conducted to determine whether familiarity with one odour source of an individual generalizes to a second odour source from that individual. First, in two

replicates with two sets of donors that were familiar to the subjects, subjects ($N = 6$ female and 3 male yearlings) were habituated to oral-gland odour from Individual 1, and then tested with dorsal-gland odours from Individual 1 and Individual 2. In the first study, animals habituated within 3 days, and the second study required 4 days of habituation due to an unexplained increase in investigation of cubes on day 3. In both cases, subjects habituated to repeated presentation of Individual 1's oral odour (day 1 versus day 3: $Z = 2.19$, $P = 0.014$; day 1 versus day 4: $Z = 2.38$, $P = 0.009$; Fig. 7a, b). Both groups also investigated dorsal odour from Individual 2 significantly longer than dorsal odour from Individual 1 on the test day ($Z = 2.10$, $P = 0.018$ and $Z = 2.67$, $P = 0.004$, respectively).

For the other replicate, subjects ($N = 2$ adult females and 7 male and 9 female juveniles from three litters, about 36 days old) were habituated to dorsal odour from Individual 1 and tested with oral odours from Individuals 1 and 2 (subjects were equally familiar with both odour donors). Subjects habituated to repeated presentations of the habituation odour (dorsal odour from Individual 1; day 1 versus day 4: $Z = 3.18$, $P = 0.0005$; Fig. 7c), and investigated Individual 2's oral odour significantly more than the oral odour from Individual 1 on the test day ($Z = 3.23$, $P = 0.0005$). In all three tests, there was no significant difference in investigation of Individual 1's habituation
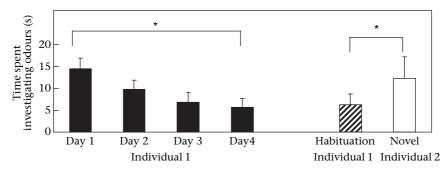

Figure 5. Mean + SE duration of investigation (s) of *S. beldingi* supraorbital odours by subjects in habituation–discrimination tasks. ■ (habituation trials) and ▨ (discrimination trial): investigation of the habituation odour from Individual 1; □: investigation of test odour from Individual 2 (discrimination trial). Horizontal lines and asterisks indicate significant differences in investigation of odours (*$P < 0.05$) based on Wilcoxon signed-ranks tests.
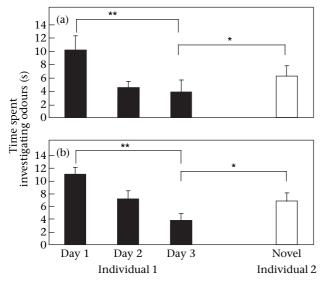
Figure 6. Mean + SE duration of investigation (s) of unfamiliar (a) oral-gland and (b) dorsal-gland odours by *S. beldingi* subjects in habituation–discrimination tasks. ■: investigation of the habituation odour from Individual 1; □: investigation of odour from Individual 2 during the discrimination trial. Horizontal lines and asterisks indicate significant differences in investigation of odours (*$P < 0.05$; **$P < 0.01$) based on Wilcoxon signed-ranks tests.

odour on the final habituation day and her novel odour on the test day ($P = 0.242$, $P = 0.201$ and $P = 0.069$, respectively), indicating that subjects generalized among the two odours from one individual.

The second set of cross-scent habituation tests was conducted to determine whether *S. beldingi* can generalize among an individual's odours without being familiar with it. In the first test, subjects ($N = 2$ adult females, 7 male and 7 female juveniles from four litters, about 45 days old) habituated to repeated presentations of the habituation odour (unfamiliar oral odour from Individual 1; day 1 versus day 4: $Z = 1.86$, $P = 0.032$; Fig. 8a). Subjects investigated unfamiliar Individual 2's dorsal odour significantly longer than that from Individual 1 ($Z = 3.154$, $P = 0.001$), indicating discrimination of odours from the two individuals as distinct. Subjects investigated dorsal odour from Individual 1 significantly less than its oral-gland odour on day 4 ($Z = 1.965$, $P = 0.0245$) but not less than on day 3 ($Z = 0.724$, $P = 0.234$). Day 4 was a rainy, cool day and subjects tended to remain near the burrow entrances, and thus near the cubes, which may explain the increase in investigation durations that day. If subjects did not recognize the novel odour from Individual 1 as belonging to the same individual as the habituation odour, I would expect subjects to have smelled the novel odour longer, not for less time as they did here. Thus the data are difficult to interpret, but given the lack of difference in investigation between the novel odour of Individual 1 and the habituation odour on day 3, the results suggest that subjects did generalize among Individual 1's odours.

In the second study, subjects ($N = 4$ male and 8 female juveniles from four litters, about 55 days old) were habituated to dorsal odour from Individual 1 and tested with

oral odours from Individuals 1 and 2 (subjects were unfamiliar with both odour donors). Subjects habituated to repeated presentations of the habituation odour (dorsal odour from Individual 1; day 1 versus day 4: $Z = 2.134$, $P = 0.0165$; Fig. 8b), but showed no difference in their response to oral odours from Individuals 1 and 2 on the test day ($Z = 1.156$, $P = 0.124$). Subjects investigated both oral-gland odours longer than Individual 1's dorsal-gland odour on the final habituation day (Individual 1's oral: $Z = 2.045$, $P = 0.02$; Individual 2's oral: $Z = 2.223$, $P = 0.013$). The results of the two cross-habituation studies with unfamiliar donors are inconclusive as to whether familiarity is necessary for *S. beldingi* to generalize among the various odours an individual produces.

Importance of Nonvolatile Cues to Recognition

For both the oral- and the dorsal-gland odour tests (oral: 9 male and 7 female juveniles from three litters, about 39 days old; dorsal: 11 male and 6 female juveniles from three litters, about 38 days old), subjects investigated the scented cube longer than the unscented cube on day 1 ($P = 0.002$ and $P = 0.04$, respectively), indicating that the hardware-cloth cover did not prevent the animals from perceiving the odour on the cubes. Subjects habituated to repeated presentations of odour from Individual 1 (oral: $Z = 3.24$, $P = 0.0005$; dorsal: $Z = 2.62$, $P = 0.005$; Fig. 9a, b). When presented with odour from Individual 2, both groups of subjects investigated it significantly longer than the final habituation odour (oral: $Z = 2.17$, $P = 0.015$; dorsal: $Z = 2.86$, $P = 0.002$). These results indicate that at least some of the odour cues signalling individual identity are volatile and can be detected and differentiated from a short distance.

Transfer of Body Odours to the Substrate

Subjects included 11 male and 9 female juveniles about 39 days old. During testing, some *S. beldingi* dug in the dirt samples, and some ate the used dirt. The used dirt elicited significantly more investigation than did the clean dirt ($Z = 2.837$, $P = 0.0025$; Fig. 10).

DISCUSSION

These results of the odour-discrimination tests extend my previous work on social recognition in Belding's ground squirrels (Mateo & Johnston 2000; Mateo 2002, 2003) by identifying several secretions that can by used by conspecifics to discriminate among individuals. The habituation–discrimination tests used here revealed that secretions from oral, dorsal, anal and pedal-gland odours are individually distinct, as are odours from the supraorbital area (Figs 1, 3–5), but *S. beldingi* urine does not appear to be distinct (Fig. 2). Belding's ground squirrels are thus similar to other mammals in which odours from several sources are unique and can be used for individual recognition of social partners (Albone 1984; Brown & MacDonald 1985).
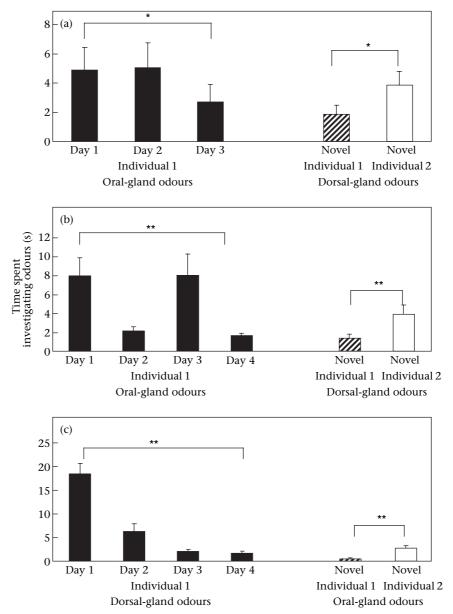
Figure 7. Mean + SE duration of investigation (s) of *S. beldingi* odours by subjects in cross-habituation–discrimination tasks with odours from familiar donors. (a, b) ■: investigation of the habituation odour from Individual 1 (oral-gland odours); ▨ and □: investigation of dorsal-gland odours from Individuals 1 and 2, respectively, during discrimination trials. (c) ■: investigation of the habituation odour from Individual 1 (dorsal-gland odours); ▨ and □: investigation of oral-gland odours from Individuals 1 and 2, respectively, during discrimination trials. Horizontal lines and asterisks indicate significant differences in investigation of odours (*$P < 0.05$; **$P < 0.01$) based on Wilcoxon signed-ranks tests.

In golden hamsters, for example, five odours are individually distinct but six others are not (Johnston et al. 1993). Although odours may not have evolved to serve a communicative function, perceivers can use the unique information contained in various odours to identify conspecifics or to detect their recent presence in a particular area (Johnson 1973; Kivett et al. 1976; Thiessen & Rice 1976; Gosling & Roberts 2001).

If individual discrimination among odours or their bearers serves a social purpose, such as for mate choice, cooperation or competition, then discrimination might be expected to occur rapidly without extensive prior social interactions. This would allow an animal to avoid mating with a previous partner, or to re-acquire a partner if copulation is interrupted. In the habituation–discrimination tasks I used to determine whether odours are individually distinct (Figs 1–5), subjects and odour donors were familiar because of common housing in large outdoor enclosures for at least one week. However, discrimination of individual *S. beldingi* odours does not require previous experience with those individuals, because subjects unfamiliar with the odour donors investigated novel odours during the discrimination phase longer than they did odours from the habituation donor (Fig. 6). Thus, individual discrimination of odours is possible without prior direct familiarity with animals bearing the odours.
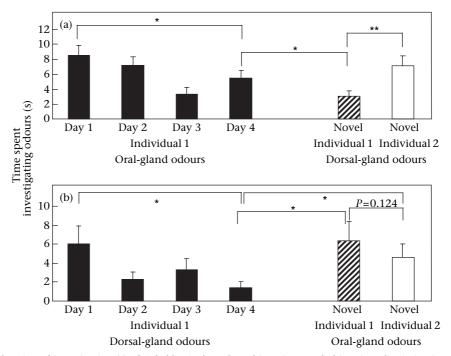
Figure 8. Mean + SE duration of investigation (s) of *S. beldingi* odours by subjects in cross-habituation–discrimination tasks with odours from unfamiliar donors. (a) ■: investigation of the habituation odour from Individual 1 (oral-gland odours); ▨ and □ investigation of dorsal-gland odours from Individuals 1 and 2, respectively, during discrimination trials. (b) ■: investigation of the habituation odour from Individual 1 (dorsal-gland odours); ▨ and □: investigation of oral-gland odours from Individuals 1 and 2, respectively, during discrimination trials. Horizontal lines and asterisks indicate significant differences in investigation of odours (*$P < 0.05$; **$P < 0.01$) based on Wilcoxon signed-ranks tests.

Although *S. beldingi* can discriminate among odours without prior familiarity with their bearers, long-term social relationships may facilitate, or be facilitated by, more complex recognition abilities. Representations of familiar individuals may be required for the evolution of reciprocal altruism or dominance hierarchies. The production of multiple unique odours can facilitate accurate discrimination of conspecifics (Beecher 1982; Mateo 2002). Indeed, my cross-scent habituation tests indicated that *S. beldingi* not only learn the various odours that an individual produces, but they also integrate these odours to form a higher-order representation of that individual (Fig. 7). That is, when *S. beldingi* become familiar with an individual, they learn its odours and associate those odours with one another, as if they form multiple-component representations of familiar individuals. This concept of 'individual' goes beyond responding differentially to familiar and unfamiliar cues, or associating a cue with a previous social encounter. Instead, the various odours acquire meaning, referring to a particular individual regardless of the odour being perceived. I only tested cross-scent habituation between two odour types (oral and dorsal), but it is possible that other *S. beldingi* odours are also incorporated into representations of individuals (see also Johnston & Bullock 2001), perhaps with other traits such as vocalizations for multimodal recognition.

Cross-scent generalization may simply be due to chemical similarities in odours, if dorsal and oral odours contain redundant compounds. If this is the case, then *S. beldingi* should be able to generalize among an individual's odours even without prior familiarity with that individual. The

results of the cross-scent habituation tests with unfamiliar odour donors suggest that *S. beldingi* may require experience with individuals for this representation to form, as is true for golden hamsters (Johnston & Jernigan 1994). One of the cross-scent tests with unfamiliar odour donors resulted in discrimination between two odours from an individual (Fig. 8b), but another test resulted in generalization (Fig. 8a). Thus whether multiple-component representations form because odours are structurally similar or because chemically distinct odours come to represent that individual remains unclear, although the lack of clear generalization among odours when donors are unfamiliar is consistent with the latter explanation. It would be adaptive if the development of individual representations depended on direct experience, because it would prevent animals from incorrectly associating together the odours of several individuals encountered in one location.

Recognition cues may evolve specifically for recognition purposes, or they could be artefacts of some other unrelated mechanism(s). For instance, sexually selected traits such as distinct plumage patterns or songs might be coopted for social recognition among neighbouring male birds (e.g. Beecher et al. 1989). Oral odours of *S. beldingi* are individually distinct (Fig. 1) and kin distinct (Mateo 2002), suggesting a generalized mechanism by which some glandular secretions reflect genetic variation among individuals as well as among kin classes (e.g. Todrank & Heth 2003). That is, the mechanism that makes pedal-gland odours, for example, unique at the level of kin or individual may be the same mechanism that makes dorsal-gland odours unique. However, these odours do not
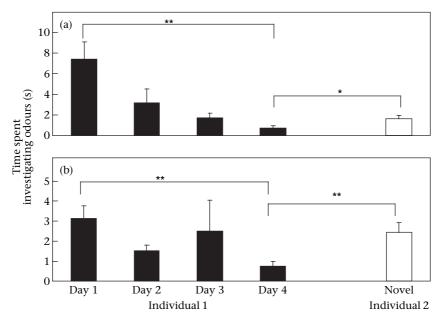
Figure 9. Mean + SE duration of investigation (s) of (a) oral-gland and (b) dorsal-gland odours by *S. beldingi* subjects in habituation–discrimination tasks that were prevented from direct contact with the odours. ■: investigation of the habituation odour from Individual 1; □: investigation of test odour from Individual 2. Horizontal lines and asterisks indicate significant differences in investigation of odours (*$P < 0.05$; **$P < 0.01$) based on Wilcoxon signed-ranks tests.

appear to simply be redundant sources of one odour type, since *S. beldingi* did not consistently generalize between two odours of an unfamiliar individual (Fig. 8). How genes influence the uniqueness of odours is unclear, although recognition of conspecifics may be facilitated by the major histocompatibility complex (MHC). This group of highly variable genes is involved in vertebrate immune function, and because of the large number of alleles involved, the likelihood of two random conspecifics having the same MHC haplotypes is very low, but since relatives by definition share many genes in common, the MHC can serve as an accurate indicator of relatedness (Brown & Eklund 1994). The MHC also influences odour production, probably through an interaction between MHC by-products and bacteria on gland surfaces (leading to unique secretions; e.g. Wobst et al. 1999) or in gastro-intestinal tracts (creating unique urine and faecal odours; e.g. Schellinck et al. 1995). Although odour recognition cues probably did
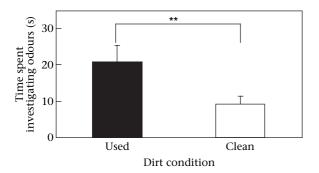
not evolve for recognition purposes per se, use of MHC-mediated cues to discriminate individuals and kin classes would provide a parsimonious mechanism for social recognition (see also Mateo 2004). I am currently investigating the influence of MHC haplotypes on *S. beldingi* odours and social behaviours.

Odour donors were maintained on similar diets of mouse chow and ad libitum grasses (*Carex* spp.), and although diet cues may be important for social recognition (Gamboa et al. 1991; Schellinck et al. 1997), *S. beldingi* odours retained their distinctive properties, again indicating that the important chemical components of *S. beldingi* odours have a genetic basis. Although urine is individually distinct in captive rats and golden hamsters (Brown et al. 1987; Johnston et al. 1993), it is not in *S. beldingi* (Fig. 2) or in giant pandas, *Ailuropoda melanoleuca* (Swaisgood et al. 1999). Diet effects may explain why *S. beldingi* urine was not as distinct as other odour sources, since excreted molecules unique to an individual may be masked by food metabolites (even if animals are on similar diets). For example, anal-gland odours of North American beavers, *Castor canadensis* are individually distinct, but odours from castoreum are diet derived, and therefore too variable to code for individuality (see Sun & Müller-Schwarze 1999).

The volatile components of *S. beldingi* odours appear to be sufficient for recognition, because subjects could discriminate among individual odours even when prevented from direct contact with the odours (Fig. 9). This would allow animals to make accurate assessments of identity while in close proximity but without direct contact. Although individuals typically engage in nasal investigations upon meeting or before social interactions, often with extensive investigation of oral-gland odours, pairs are more likely to investigate each other if they are unfamiliar or distantly related compared with familiar or

Figure 10. Mean + SE duration of investigation (s) of 'clean' and 'used' dirt by *S. beldingi* juveniles in an odour preference task. Horizontal line and asterisk indicate a significant difference in investigation of odours (**$P < 0.01$) based on Wilcoxon signed-ranks tests.

closely related individuals (e.g. Figure 3 in Mateo 2003; see also Holmes 1984b). This suggests that some initial discrimination occurs prior to contact, presumably through the volatile cues of social odours. Nonvolatile components of odours, perhaps including major urinary proteins, could provide additional information about identity in addition to increasing the longevity of odours left on the substrate (e.g. Hurst et al. 2001; Nevison et al. 2003).

The discrimination tasks presented here indicate that *S. beldingi*, like other species (see references in the Introduction), produce a variety of distinctive odours. These cues permit recognition during direct social interactions and can also allow identification of animals that had been in an area previously, through passive and active deposition of odours (e.g. pedal-gland odours from walking, oral and dorsal odours from scent marking). Persistence of odours could be especially important for territorial animals, allowing them to be 'present' throughout their range without the energetic expenses of continually patrolling it or displaying to defend it (see also Gosling & Roberts 2001). *Spermophilus beldingi* odours can remain in the environment, and scented substrate elicits more intense investigation than areas that have not been used recently (Fig. 10). Kangaroo rats, *Dipodomys merriami*, leave dorsal-gland odours in the substrate after sandbathing, and these areas are often attractive to conspecifics, especially to opposite-sex kangaroo rats (Randall 1991). *Spermophilus beldingi* might be able to identify territory owners even in their absence, or identify trespassers of their own territories or burrows. Finally, other animals can 'eavesdrop' on odours, for example, when conspecifics use foot trails to locate foraging sites (e.g. Galef & Buckley 1996), or when venomous snakes use foot odour to follow their prey after strikes (e.g. Lavín-Murcio et al. 1993).

My results demonstrate that *S. beldingi* can use several distinct odour cues to discriminate among conspecifics, but additional research is needed to determine the role of each cue in social interactions, and whether some odour sources are more salient or weighted more heavily for recognition purposes. In addition, future work could use gas chromatography/mass spectrometry analyses of the odours to determine whether the same compound(s) cause each gland odour to vary with genetic similarity, to examine how odours decay across time, and to explore how sex and reproductive status influence secretions (e.g. Lawson et al. 2000; Smith et al. 2001; e.g. Buesching et al. 2002; Safi & Kerth 2003). These results add to a growing body of literature on how recognition cues from a variety of modalities are developed and perceived by others, and to what extent these cues mediate cooperative, competitive, reproductive and parental social interactions.

Acknowledgments

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