



Retention of social recognition after hibernation in Belding's ground squirrels

JILL M. MATEO & ROBERT E. JOHNSTON

Department of Psychology, Cornell University

(Received 8 March 1999; initial acceptance 7 June 1999;
final acceptance 25 October 1999; MS. number: A8432)

The retention of social memory during long periods of separation, such as hibernation or migration, has not been well documented, despite evidence for long-term social relationships in migrating species or in long-lived sedentary species. We investigated the ability of captive Belding's ground squirrels, *Spermophilus beldingi*, to remember previously familiar individuals as well as littermates after 9 months of isolation. Before hibernation, young ground squirrels discriminated between odours of familiar and unfamiliar individuals, as shown by greater investigation of a novel individual's odour. The following spring, these yearlings did not respond differentially to odours of previously familiar and unfamiliar individuals, suggesting that memory for familiar conspecifics was lost during hibernation. In contrast, both female and male yearlings continued to discriminate between odours of littermates and previously familiar nonlittermates. Thus, recognition of close kin was maintained during prolonged social isolation, but recognition of familiar, unrelated individuals was not. If re-establishment of familiarity is not costly or if adults rarely interact with the same individuals in successive years, then selection may not favour retention of individual memories of particular conspecifics over the winter. Even though males rarely encounter kin after dispersal, yearling males did recognize their siblings, suggesting that the relative costs of maintaining kin-recognition abilities year-round may be low. Possible mechanisms underlying the formation and maintenance of individual and kin recognition are discussed.

© 2000 The Association for the Study of Animal Behaviour

Repeated social interactions can facilitate the formation and maintenance of long-term relationships, yet memory for individuals during extended periods of separation, such as migration or hibernation, is not well understood. For example, long-term pair bonds in migrating birds may be due to site fidelity (see references in Rowley 1983; Black 1996) rather than recognition of and preference for the same mate year after year. Male hooded warblers, *Wilsonia citrina*, however, can retain associations between neighbours' songs and their territories during 8 months of overwintering (Godard 1991), suggesting at least a long-term memory for individuals' songs and locations, if not the individuals themselves. Social paper wasps, *Polistes metricus*, continue to discriminate between nestmates and non-nestmates after an overwintering period of 26 weeks and an additional 99 days of social isolation (Ross & Gamboa 1981), but it is not known if they can also discriminate among individual nestmates in the spring. Likewise, Belding's ground squirrels, *Spermophilus beldingi*, are obligate hibernators that spend about 8 months of each year in social isolation, but it is not

Correspondence: J. M. Mateo, Department of Psychology, Uris Hall, Cornell University, Ithaca, NY 14853-7601, U.S.A. (jmm52@cornell.edu).

known whether animals continue to recognize previously familiar nonkin after hibernation or if they must re-establish familiarity each spring after emergence.

Despite extensive research on the physiology of hibernation (e.g. Lyman & Dawe 1960; Fisher et al. 1967; South et al. 1972), it is not clear how prolonged torpor influences memory. Short periods of hypothermia or hibernation are thought to minimize interference with memory formation and thus facilitate retention (Mihailovic 1972). For instance, retention of maze learning by ground squirrels is either unaffected (Mihailovic et al. 1968) or enhanced (McNamara & Riedesel 1973) by short periods (≤ 1 month) of hibernation. However, most investigations on the effects of hypothermia on memory have used nonsocial tasks or have focused on nonhibernating species (Mrosovsky 1967), thus limiting generalizations.

Belding's ground squirrels are ideal animals for studying the retention of social memory during hibernation. They are group-living, burrowing rodents that live in alpine and subalpine regions of the western United States (Jenkins & Eshelman 1984). They are active above ground between April and August and hibernate the remainder of the year. After hibernation, several females (but not

males) typically emerge from a burrow system, with about half of hibernation groups containing relatives (Sherman 1976). It is not known, however, whether females actually share a hibernaculum within a burrow system. Females mate within a week of emergence from hibernation and each female produces one litter of five to eight pups, which is reared 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); 2–3 weeks later, juvenile males begin to disperse permanently from their birthplace, whereas juvenile females establish their own burrow system within 25 m of their natal burrow (Holekamp 1983). Females are likely to interact repeatedly with particular kin and nonkin, both because they are long-lived (Sherman & Morton 1984) and because they are philopatric and typically use the same breeding territory in successive years. Although females nest in equal proximity to close and distant kin, nepotism among females (including territory sharing, defence against infanticide and alarm-call production) is limited to close kin such as mothers, daughters and sisters (Sherman 1976, 1980, 1985).

Research on social preferences in Belding's ground squirrels suggests that kin recognition may persist during hibernation. Captive yearlings of both sexes are less agonistic towards kin than either previously familiar (W. G. Holmes & J. M. Mateo, unpublished data) or unfamiliar (Holmes & Sherman 1982; Holmes 1986) nonkin. In addition, free-living yearling females are less agonistic towards females with which they were reared the previous year (whether related or in-fostered, unrelated females) than towards females with which they were not reared (Holmes & Sherman 1982). Thus *S. beldingi* appear to recognize kin after hibernation; it is unknown whether they also recognize particular individuals that they had been familiar with the previous summer.

The ability to remember specific individuals from year to year has a number of potential advantages. For example, it would allow ground squirrels to remember the competitive abilities or agonistic tendencies of previously familiar individuals (particularly males' reproductive rivals and females' neighbours). In addition, females prefer to mate with males that they have observed previously winning fights or successfully mating with other females (Sherman 1976). Thus given the nature and duration of social relationships among *S. beldingi*, selection may favour their ability to recognize and distinguish among conspecifics without recent exposure.

Recognition of conspecifics is mediated through olfactory cues in a variety of taxa (e.g. references in Halpin 1986; Hepper 1991), including ground-dwelling squirrels (see Kivett et al. 1976; Halpin 1984; Holmes 1984a, b). Belding's ground squirrels produce at least four odours that are individually distinct (from oral, dorsal and anal glands and from urine), and both oral and dorsal gland odours vary with genetic relatedness (based on the behavioural responses of *S. beldingi* to odours; J. M. Mateo, unpublished data).

In this study we investigated whether yearling *S. beldingi* continue to recognize previously familiar

individuals (both kin and nonkin) after hibernation. Our research was conducted in four stages: (1) a test for discrimination of oral gland odours from familiar versus unfamiliar individuals prior to hibernation; (2) a second familiarity test just after arousal from hibernation; (3) a similar test 2 weeks after arousal; and (4) a test of kin recognition after hibernation, using odours of littermates and nonlittermates. *Spermophilus beldingi* discriminate between kin and nonkin during their yearling summer (Holmes & Sherman 1982; W. G. Holmes & J. M. Mateo, unpublished data), but we do not know whether yearlings can recognize kin just after arousal from torpor or if some social experience with conspecifics is necessary.

GENERAL METHODS

Study Sites and Animal Housing

We conducted our research on captive *S. beldingi* at the Sierra Nevada Aquatic Research Laboratory (SNARL; near Mammoth Lakes, California; juveniles and yearlings) and at the University of Michigan (Ann Arbor, Michigan; yearlings). These data were collected opportunistically during a long-term study of *S. beldingi* social preferences (W. G. Holmes & J. M. Mateo, unpublished data). We refer to *S. beldingi* as 'juveniles' during their first summer of life (≥ 25 -day-old young-of-the-year) and as 'yearlings' during their second year of life (after arousal from their first hibernation). Ground squirrels were tested for their ability to discriminate odours toward the end of their juvenile summer, housed individually for 9 months (including approximately 6 months of hibernation), and tested again at the beginning of their yearling summer (details below).

Prehibernation housing

Our subjects and odour donors were juveniles born in captivity in 1996. To obtain these juveniles, we live-trapped pregnant females (from Mono County Park, 1966 m elevation, Rock Creek Canyon, 2615 m elevation, and Mammoth Lakes Ranger Station, 2377 m elevation; Mono County, California) and housed them in a laboratory building at SNARL where they gave birth and reared their young (for about 4 weeks). Due to distances between females (>50 m), mothers of the subjects as well as mothers of the odour donors were unlikely to have been closely related or to have mated with the same males (J. M. Mateo, unpublished data; see also Sherman 1976). Because of multiple mating by females, litters were probably composed of full and half-siblings (Hanken & Sherman 1981). Mothers ($N=4$) and some of their juveniles (ca. 26 days old; $N=2$ males and 3 females/litter) were given unique combinations of hair dye and coloured ear tags for identification and were transferred on 18 June 1996 to an outdoor enclosure measuring $20 \times 10 \times 2$ m. The enclosure included natural vegetation (*Carex* spp.), provisioned food (Purina mouse chow no. 5015; ca. 15 g/animal per day) and water, and eight buried nestboxes connected to the surface by plastic tunnels (see Holmes

1994 for details). The prehibernation odour discrimination test was conducted in this enclosure, after animals had been living together for 5 weeks. One juvenile died prior to testing. Mothers were removed from the enclosure on 5 July 1996, before the first odour discrimination test, and released with their offspring that were not used in the study at the mother's site of capture.

Hibernation

On 24 July 1996, after the prehibernation familiarity test (see below), we shipped 19 juveniles from the enclosure to the University of Michigan, where they were housed individually in hibernation cages. Ground squirrels were provided with food and water until they displayed signs of torpor. Males and females entered hibernation at similar weights (males: $\bar{X} \pm SE = 218.9 \pm 5.0$ g; females: 211.8 ± 3.3 g; $t_{17} = 1.23$, NS). Each cage measured $25 \times 18 \times 17$ cm with wire-mesh front and bottom panels and solid side and back panels, with 2 cm between adjacent cages. Animals could hear the calls and probably smell the odours of other individuals, but could not see or touch them. After we gave animals burlap (ca. $1.5 \text{ m}^2/\text{animal}$) for nesting material, we placed them in a cold room and adjusted photoperiod and temperature in two steps between September and November (from a 12:12 h light:dark cycle and 20°C to LD 0:24 and $4\text{--}6^\circ\text{C}$). Humidity ranged from 30 to 85%, and ca. 0.57 m^3 of air was exhausted per hour. We checked animals periodically under red-light conditions for signs of torpor. All animals began to hibernate in November and aroused spontaneously between 1 and 25 April (terminal arousal was defined as 7 consecutive days of activity). They were provided with food and water beginning 7 April when lights were turned on and the temperature raised (LD 12:12, 8°C). Two animals died during the winter. Distance between pairs of hibernating *S. beldingi* was estimated by counting the number of cages separating animals.

Posthibernation housing

On 19 April, we transferred each animal from its hibernation cage to a standard plastic cage ($38 \times 33 \times 18$ cm; solid sides and bottom, wire top; animals and cages remained in the cold room) and provided it with pine shavings and shredded burlap for nesting materials as well as food and water. Yearlings gained weight quickly after arousal, and body weights of the two sexes were similar when posthibernation testing began (males: $\bar{X} \pm SE = 241.6 \pm 9.5$ g; females: 248.6 ± 6.8 g; $t_{15} = -0.62$, NS). Most yearling males appeared sexually mature with descended, pigmented testes (Bushberg & Holmes 1985), probably because we provisioned ground squirrels with food during their juvenile year; this contrasts with free-living males, which typically do not mature sexually until 2 years of age (Morton & Gallup 1975). Prior to all discrimination tests (see below) we removed food, water and burlap pieces. At the end of the study, animals were shipped to California and released at the site where their mother was collected.

Odour Discrimination Tests

Behavioural assay

We presented animals with odours from two individuals (e.g. a familiar and an unfamiliar conspecific; details provided below for each test) to assess discrimination of pairs ofagemates' odours in the absence of behavioural cues to identity. We presented two odours simultaneously (details on odour collection below), and recorded the total number of contacts made with each odour (subject's nose within 1 cm of an odour) and the total duration of investigation (time spent sniffing, licking or biting the odour). If an animal contacted only one odour, we recorded a zero for both frequency and duration of investigation of the other odour. We randomized the location of odours (e.g. familiar odour on right or left side) across ground squirrels, and observers were always blind as to which odour was on each side. We recorded the frequency and duration of investigation of the odours either by speaking into a tape recorder and later transcribing the data for analysis (prehibernation test) or with stopwatches and hand-held counters (posthibernation tests). In this type of discrimination task we have found that ground squirrels respond more strongly or more often to unfamiliar stimuli, as has been shown for other species (e.g. investigating unknown odours longer than familiar odours or reacting more strongly to calls of non-neighbours than neighbours; Johnston 1981; Halpin 1986; Stoddard 1996).

Odour collection and presentation

Oral gland odours (hereafter, 'oral odours') were collected from 'donors' on either 3-cm^3 polyethylene cubes or on glass plates (7.6×17.8 cm) 15 min or less before use. Subjects were presented with either two cubes (one odour on each cube) or one plate (one odour on each half of the plate). Secretions were collected by rubbing the cube or plate anterior-posteriorly 16 times along each mouth corner. Pilot data demonstrated that odours were not significantly degraded across repeated collections from an animal, and informal inspections of odour donors indicated that oral regions were not irritated by repeated collections. An assistant, who wore latex gloves to prevent the transfer of other ground squirrel odours or human odours to the equipment or to the animals, collected and coded the odours so that observers were blind to the identity of the odour donors. Cubes and glass plates were washed with hot water and unscented soap after use and allowed to air dry.

Analyses

Because the data were not normally distributed and traditional transformations were not successful, we used two-tailed normal scores tests for analyses of investigations of the two test odours. This test is similar conceptually to a paired *t* test because the difference between two matched data sets is normalized and compared against a null hypothesis that the mean difference is zero (Darlington 1990), and is more powerful than a Wilcoxon signed-ranks test for many data sets (Darlington 1996).

We used Mann–Whitney U tests to assess sex differences in investigation durations, and Spearman rank correlations to determine whether investigation durations were associated with either the number of days since an animal aroused from hibernation or with the distance between subject and donor animals in the cold room during hibernation. We present the data as unadjusted means \pm SE and consider results significant when $P < 0.05$.

Ethical Note

The animals we studied were born in captivity and released as yearlings at the end of this study near the site at which their mothers were originally captured. Since then we have recaptured several of these animals, although we have not trapped systematically to determine the percentage of animals that survived. To reach adult (≥ 2 years old) status, our released animals had to avoid predators and survive hibernation. The experiences of animals in captivity allowed them to acquire species-typical responses to antipredator alarm calls (Mateo & Holmes 1999) and the feeding regimen of captive animals resulted in higher body masses than those reached by same-aged, free-living animals (J. M. Mateo, unpublished data; Holmes 1994). The likelihood of recapturing captive-born animals released as 1- to 3-month-old juveniles appears to be similar to the likelihood of recapturing field-born animals (personal observations; W. G. Holmes, personal communication). This is because 60% of field-born juveniles die during their first hibernation (Sherman & Morton 1984), probably due to insufficient body-fat reserves. Thus, the *S. beldingi* that we held temporarily in captivity and released as yearlings likely survived as well as field-born animals.

PREHIBERNATION FAMILIARITY TEST

Methods

On 23 July 1996 we tested 14 juvenile ground squirrels living in an outdoor enclosure for their preference for oral odours of familiar versus unfamiliar juveniles. Subjects were about 60 days old when tested, and had been housed together in the enclosure for 35 days. Two cubes were placed simultaneously at each of the eight burrow entrances in the enclosure. On one cube we placed the odour of a same-aged, familiar female juvenile living in the same enclosure as the subjects. On the other we placed the odour of a same-aged, unfamiliar juvenile female living in a different enclosure. The same two donors were used for each of the eight pairs of cubes. Cubes were anchored by 3-cm screws (inserted in the middle of each cube) 1 cm in front of each burrow entrance and 3 cm apart.

Both odour donors were unrelated to the subjects. The donor of the familiar odour was in the enclosure during the discrimination test. Observations of her and her four littermates were not included in this data set. We collected data for 20 min, to give all animals in the enclosure

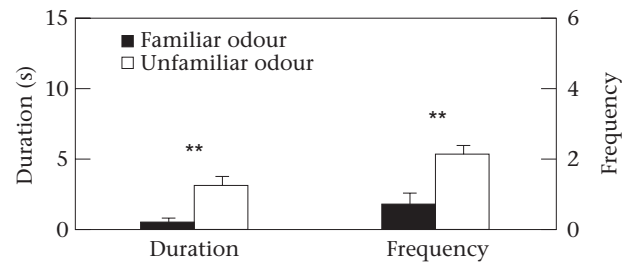


Figure 1. Mean \pm SE duration (s) of investigation of and frequency of contact with oral odours of familiar and unfamiliar juveniles by Belding's ground squirrel juveniles ($N=6$ males and 6 females) prior to hibernation. *Significant differences in responses to the two odour types (normal scores tests; $P < 0.01$).

ample opportunity to contact the stimuli. All subjects were observed simultaneously as they moved about the enclosure and investigated pairs of cubes freely. Although more than one juvenile could investigate a set of cubes at a given time, the presence of conspecifics did not make ground squirrels more or less likely to investigate cubes, nor did it influence their duration of investigation (J. M. Mateo, unpublished data).

Results

Two juveniles did not contact either cube, reducing the sample size to 12 ($N=6$ males and 8 females from three litters). Subjects investigated the unfamiliar odour longer (normal scores test: $t_{11}=3.55$, $P < 0.01$) and more often ($t_{11}=3.81$, $P < 0.01$) than the familiar odour (Fig. 1), thus demonstrating that they discriminated between odours of a familiar and an unfamiliar individual. Presumably ground squirrels investigated the familiar scent less because it was recognized as a known odour in the enclosure, whereas the novel individual's scent was not familiar and therefore was investigated more. We found no sex differences in duration of investigation of familiar and unfamiliar odours (Table 1). Nor did we find significant differences in duration of investigation or frequency of contact among the three litters (Kruskal–Wallis ANOVAs: all $H_2 \leq 2.30$, NS).

POSTHIBERNATION FAMILIARITY TESTS

Methods

After yearlings aroused from hibernation in the spring, we tested them in their individual cages for discrimination of odours from previously familiar and unfamiliar conspecifics. Thirteen yearlings (eight females and five males; data from nine of these yearlings were included in the prehibernation test) were tested on 26 April 1997; at this time animals had been active for $\bar{X} \pm \text{SE} = 12.1 \pm 3.0$ days (range 1–25 days) but had not interacted directly (via tactile or visual contact) with other *S. beldingi*. Subjects were presented with oral odour from an individual that was in their enclosure the previous summer and an odour from an individual that lived in a different enclosure that summer (and thus was unfamiliar). Both odour donors

Table 1. Mean±SE time (s) females and males spent investigating odours of previously familiar and unfamiliar conspecifics and odours of littermates and nonlittermates during 20-min (prehibernation) or 5-min (posthibernation) olfactory discrimination tests

	Females	Males	U^*	Females	Males	U^*
	Familiar			Unfamiliar		
Prehibernation familiarity test (6,6)†	0.80±0.54	0.38±0.19	18.0	2.02±0.31	4.20±1.08	11.0
Posthibernation familiarity test 1 (8,5)§	4.59±1.27	3.23±0.95	21.0	6.49±1.36	3.85±1.16	28.0
Posthibernation familiarity test 2 (10,7)	6.35±1.09	17.99±2.89	5.0‡	5.57±1.26	13.29±2.52	10.0‡
	Littermate			Nonlittermate		
Posthibernation littermate test (10,6)	6.83±0.61	10.16±2.01	16.0	9.84±2.28	14.00±3.37	19.5

*Mann–Whitney U tests, all NS unless otherwise noted.

†Numbers in parentheses refer to the number of females and males, respectively, included in each analysis.

‡ $P < 0.05$.

§Yearlings were tested twice for their discrimination of unfamiliar and previously familiar odours (test 1: 26 April; test 2: 9 May); see text for details.

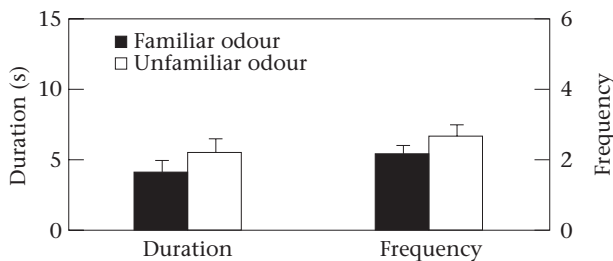


Figure 2. Mean±SE duration (s) of investigation of and frequency of contact with odours of familiar and unfamiliar yearlings by male ($N=5$) and female ($N=8$) Belding's ground squirrel yearlings after hibernation.

were female yearlings unrelated to the subjects, and had been aroused for 16 days. These two odour donors were different from the two used for the prehibernation test. We presented odours on a glass plate placed on the floor of the cage. We tested subjects singly, recording their responses to odours during a 5-min period.

Results

Yearlings did not differentially investigate odours of unfamiliar and previously familiar individuals (normal scores test: duration of investigation: $t_{12}=1.33$, $P=0.21$; contact frequency: $t_{12}=1.39$, $P=0.19$; Fig. 2). The duration of investigation and contact frequency were not directly comparable to those in the prehibernation test (Fig. 1) because yearlings were tested singly in their cages, whereas juveniles were tested as a group in a large outdoor enclosure. There were no significant sex differences in frequency or duration of investigation (see Table 1 for durations). The duration of investigation was not significantly correlated with the number of days since termination of hibernation (Spearman rank correlation; familiar odours: $r_s=0.04$, $N=13$, NS; unfamiliar odours: $r_s=-0.37$; $N=13$, NS) or with the distance between each subject and the odour donor during hibernation (distance to familiar donor: $r_s=-0.01$, $N=13$, NS; distance to unfamiliar donor: $r_s=-0.46$, $N=13$, NS). The lack of a difference

in odour investigation suggests that yearlings do not remember odours of individuals they had been familiar with prior to hibernation.

An alternative explanation for these results is that because the donors had been out of torpor for less than 2 weeks, their odours may not have been distinguishable. That is, *S. beldingi* may not produce individually distinctive odours until several weeks after emergence from hibernation, when their metabolism, activity and food intake return to normal (McKeever 1963; Hudson & Deavers 1972; Morton 1975). Oral glands of Columbian ground squirrels, *S. columbianus*, for example, undergo changes in morphology and secretory activity during the active season (Kivett 1975), although it is not known whether these changes in secretions are correlated with changes in their information content. Another possibility is that olfactory systems (e.g. sensitivity, perceptual acuity) change during the initial weeks following emergence. Therefore, we retested the same yearling subjects 2 weeks after the first posthibernation test (9 May 1997; $\bar{X} \pm SE=25.1 \pm 3.0$ days since arousal; range 14–38 days). If time after hibernation affects either odour production or perception, yearlings' ability to discriminate odours would be expected to improve over the first few weeks after arousal. Subjects ($N=10$ females and 7 males) were presented with oral odours from previously familiar and unfamiliar opposite-sex yearlings (different donors from the previous tests). We presented females with male odours for this test because females were tested at about the time they would be mating, and therefore may be more interested in opposite-sex odours. Within each sex all subjects were presented with odour from the same unfamiliar odour donor, whereas the familiar odour was collected from one of two donors (for a total of six different donors). For this test, odours were presented on cubes suspended from the tops of cages (by clamping each cube's screw to the wire cage lid; cubes were about 5 cm apart). We switched to cubes because some animals walked on the glass plates and thus contaminated the stimulus odours.

Again, yearling ground squirrels did not investigate the two classes of odours differentially. There was no significant difference in their duration of investigation

of unfamiliar odours and previously familiar odours ($\bar{X} \pm \text{SE} = 8.75 \pm 1.55$ s and 11.15 ± 1.93 s, respectively; normal scores test: $t_{16} = -1.79$, $P = 0.09$), nor was there a difference in the frequency of contact with these two odours (4.88 ± 0.73 and 5.29 ± 0.73 contacts, respectively; $t_{16} = -0.60$, $P = 0.56$). Duration of investigation was not correlated with the number of days since arousal (Spearman rank correlation: familiar odour: $r_s = 0.40$, $N = 17$, NS; unfamiliar odour: $r_s = 0.46$, $N = 17$, NS). We did find sex differences in duration of investigation, although sample sizes are small: males investigated both familiar and unfamiliar odours longer than did females (Table 1). In addition, males appeared to investigate both familiar and unfamiliar female odours longer during the second test than they did just after arousal from hibernation (normal scores test: familiar odour: $t_4 = 3.01$, $P < 0.05$; unfamiliar odour: $t_4 = 3.01$, $P < 0.05$; compare male data in rows 2 and 3 in Table 1). We do not know whether this increase was due to changes in male responsiveness to odours, to changes in female odour production, or both. In contrast, investigation by females did not differ significantly across the two tests (female odours for first test, male odours for second test; familiar odours: $t_7 = 0.93$, $P = 0.39$; unfamiliar odours: $t_7 = 0.38$, $P = 0.72$).

It is possible that the posthibernation testing environment (odours placed inside individual home cages) hampered yearlings' ability to differentiate classes of odours. However, we have since tested a second group of enclosure-housed juveniles for their ability to discriminate between familiar and unfamiliar odours in the laboratory setting. After a short (ca. 24 h) acclimatization period to this environment and to their individual cages, juveniles investigated odours of unfamiliar juveniles longer than those of familiar juveniles (subjects and familiar donors had lived in same enclosure for 23 days; $t_9 = 3.41$, $P < 0.05$), indicating that tests in this environment are sensitive enough to reveal discrimination (see also below).

POSTHIBERNATION LITTERMATE DISCRIMINATION TEST

Because yearling *S. beldingi* did not appear to recognize odours of previously familiar individuals after hibernation (e.g. Fig. 2), we examined their responses to odours of littermates and nonlittermates. Juvenile Belding's ground squirrels, including those studied here, are more amicable and less agonistic towards littermates than nonlittermates (Holmes 1994; W. G. Holmes & J. M. Mateo, unpublished data). Because littermates are full or half-siblings, these findings demonstrate recognition of close kin. Yearlings and adult females continue to interact differentially with kin and nonkin after a species-typical period of hibernation (Holmes & Sherman 1982; Holmes 1986; W. G. Holmes & J. M. Mateo, unpublished data), but this discrimination may require brief interactions to reinstate it after hibernation; recognition has not been tested immediately following spring emergence and before any social interactions occur.

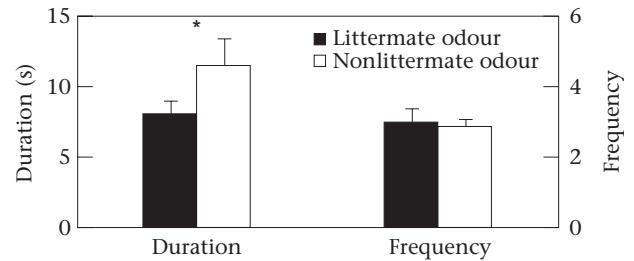


Figure 3. Mean \pm SE duration (s) of investigation of and frequency of contact with odours of littermates and nonlittermates by male ($N = 6$) and female ($N = 10$) Belding's ground squirrel yearlings after hibernation. *Significant difference in responses to the two odour types (normal scores test; $P < 0.05$).

Methods

On 27 April 1997, 24 h after the first posthibernation familiarity test, we presented 16 yearlings ($N = 10$ females, 6 males) with oral odours from a same-sex littermate and a same-sex nonlittermate. Each subject was used as an odour donor at least once. Subjects had lived in an outdoor enclosure with both donors the previous summer for at least 4 weeks, and thus at that time were familiar with both individuals and their odours. Each 5-min test was conducted in a subject's home cage, and odours were presented on pairs of cubes suspended from cage lids. Note that this test was designed to examine *S. beldingi*'s ability to discriminate between classes of relatedness (kin and nonkin), rather than between classes of familiarity (familiar and unfamiliar).

Results

Yearlings investigated nonlittermate odours significantly longer than littermate odours (normal scores test: $t_{15} = 2.25$, $P < 0.05$; Fig. 3). The number of contacts with the two odours did not differ ($t_{15} = 0.40$, $P = 0.70$), nor was there a significant difference in responses by males and females (Table 1). These data suggest that yearlings of both sexes can discriminate between kin and nonkin, both of which were familiar before hibernation. The mechanism for this recognition remains unknown, however, because yearlings could have discriminated based on differential familiarity during early development (e.g. Holmes 1997) or based on a self-referential, phenotype-matching mechanism (see Discussion).

The duration of investigation was not significantly correlated with the number of days since arousal for either littermate odours (Spearman rank correlation: $r_s = 0.11$, $N = 16$, NS) or nonlittermate odours ($r_s = 0.47$, $N = 16$, NS). The duration of investigation of littermate odours was not associated with the number of littermates housed immediately adjacent to (next to, above or below) each subject during hibernation ($r_s = -0.21$, $N = 16$; NS); investigation of nonlittermate odours was similarly unaffected by the number of nonlittermates in adjacent cages ($r_s = 0.42$; $N = 16$, NS). Finally, the duration of investigation was not significantly correlated with the distance between subjects and odour donors during hibernation

(littermate donor: $r_s = -0.48$; nonlittermate donor: $r_s = 0.48$; both $N = 16$, both NS).

To examine further the disparity in yearlings' abilities to discriminate between classes of odours, we compared the difference between investigation of littermate versus nonlittermate odours with the difference between investigation of familiar versus unfamiliar odours. We predicted greater difference scores for kinship classes than for familiarity classes, and therefore used one-tailed normal scores tests. Yearlings were more discriminating between classes of kinship than classes of familiarity (using data from the first posthibernation familiarity test: $t_{12} = 1.79$, $P < 0.05$; using data from the second posthibernation familiarity test: $t_{15} = 3.13$, $P < 0.01$). These comparisons further support our conclusion that after arousal from hibernation, yearlings were unable to recognize odours of previously familiar individuals.

DISCUSSION

In the summer, captive juvenile Belding's ground squirrels investigated the odours of unfamiliar animals more than those of familiar animals (Fig. 1), showing discrimination of conspecifics based on familiarity. Memory for familiar conspecifics was not maintained throughout the winter, however, because after 9 months of social isolation yearlings did not respond differentially to odours of previously familiar and unfamiliar individuals (Fig. 2). In contrast, yearlings did discriminate between littermate and nonlittermate odours (Fig. 3), indicating that kin recognition is maintained over the winter and does not require repeated social interactions or recent social experience.

There are at least three possible explanations for the lack of discrimination shown by yearlings to previously familiar and unfamiliar odours. First, if odours change during hibernation, yearlings may not produce individually distinct odours immediately after arousal from torpor. We know of no evidence, however, that the distinctiveness of individual odours correlates with changes in physiological state or diet (e.g. Brown 1988; Schellinck et al. 1992; but see Schellinck et al. 1997). Furthermore, yearlings did distinguish between oral odours of kin and nonkin (Fig. 3), indicating that odours secreted by yearlings were sufficient for recognition. A second potential reason is that yearlings simply did not remember the characteristic features of their enclosure-mates. We believe this is the most plausible explanation, because there are a number of reasons why *S. beldingi* might investigate familiar odours differently than novel odours if they could remember the differences. For instance, prior to mating, females may be more interested in the odours of potential, but unfamiliar, mates, and males would be expected to show heightened interest in unfamiliar females' odours in an attempt to assess their reproductive status (Sherman 1976; J. M. Mateo, unpublished data). A third possibility is that although *S. beldingi* could remember the characteristics of familiar conspecifics, this knowledge did not affect their investigation of odours. Although we cannot rule out this interpretation, it seems unlikely, both because unfamiliar stimuli

are usually investigated more than familiar ones (see above) and because the ground squirrels in our study did investigate odours differentially as juveniles (Fig. 1).

After hibernation, both male and female yearlings appeared to recognize their close kin (Table 1). Yet males are not nepotistic, nor are they targets of kin-directed behaviours (Sherman 1976), presumably because juvenile males disperse from their natal area and rarely encounter female kin as adults (Sherman 1976; Holekamp 1983). Our results show that despite no known selective benefit for doing so (if dispersal precludes inbreeding), adult males continue to produce kin-specific odours and continue to recognize kin odours. This suggests a dissociation in the evolution of the production (odour), perception (discrimination) and action (differential treatment of conspecifics as a function of relatedness) components of *S. beldingi* kin recognition (see Beecher 1982; Holmes & Sherman 1982; Reeve 1989 for discussions of recognition components).

What processes mediate *S. beldingi* recognition abilities before and after hibernation? Our studies suggest that they use at least two different mechanisms for recognizing specific individuals and for recognizing classes of relatives (Beecher 1982; Holmes & Sherman 1982). First, ground squirrels may learn the traits (e.g. odours) of an individual as it becomes familiar, and store a representation of these traits in memory (familiarity mechanism). Second, *S. beldingi* can use a phenotype-matching mechanism to recognize unfamiliar kin or to discriminate among classes of familiar kin. For this mechanism, animals learn their own odours or those of their familiar close kin, and later compare or match the phenotypes of unknown animals to this learned kin template, with the degree of similarity between the template and the new phenotype(s) indicating the degree of relatedness. Thus *S. beldingi* can recognize individuals during the active season using a familiarity mechanism, but even if memory for familiar individuals is lost during hibernation, they can still recognize kin the following spring through phenotype matching. Although the development of kin templates may involve direct experience with relatives, our data suggest that continued familiarity with or memory for these kin is not important for the maintenance of templates. One interpretation of our results is that there is less of a cost (or more of a benefit) for maintaining a kin template than for maintaining separate memories of specific individuals (required for familiarity-based recognition). In addition, males can recognize their littermates at an age when they are unlikely to encounter kin (Table 1), which further supports the notion that maintenance of kin discrimination via phenotype matching is not costly. Alternatively, if kin templates are not maintained throughout hibernation, *S. beldingi* may use a self-referential phenotype-matching mechanism (compare unfamiliar odours to their own; Holmes & Sherman 1982; Mateo & Johnston, in press) to recognize kin in the spring.

From a functional perspective, natural selection may not favour long-lasting social memories if adults rarely interact with the same individuals year after year. The likelihood that two neighbouring adult females will both

survive hibernation and nest in the same area the following year is only about 23–36% (Sherman 1976; Sherman & Morton 1984). In addition, adult males are socially and spatially peripheralized during the active season, and some emigrate to new areas after mating (Sherman 1976; J. M. Mateo, unpublished data). Thus patterns of over-winter survival and active-season spatial use reduce the need for long-term (>4 months) memory for unrelated conspecifics. In addition, relearning of individuals' cues in the spring to re-establish familiarity may be beneficial if the nature of social relationships changes (e.g. a neighbour becomes more competitive). In such cases, memories for such individuals as well as the significance associated with them must be updated each year.

In summary, Belding's ground squirrels appear to lose their memories for familiar individuals during hibernation, whereas their kin recognition abilities are not affected by dormancy. Additional work is needed to determine how well ground squirrels can discriminate among more distantly related kin after hibernation (e.g. half-siblings or cousins), or among kin that animals were not as familiar with the previous summer (e.g. older sisters). Our finding that dormancy differentially affected *S. beldingi* discrimination abilities suggests that future studies on the persistence of social recognition in migrating or hibernating species need to consider multiple classes of recognition.

Acknowledgments

This research was funded in part by an NIH grant (MH 54146-O1A1) to R.E.J. J.M.M. was supported by an NIH Multidisciplinary Training Grant in Developmental Psychology (DHHS T3219389). We thank N. McAuliffe for assistance in collecting the data, W. G. Holmes and S. Yang for caring for the animals in Michigan and W. G. Holmes for helpful discussions during all phases of the research. B. G. Galef, Jr, D. J. Mayeaux, H. K. Reeve, P. W. Sherman and two anonymous referees provided stimulating comments on the manuscript. Our research was approved by the Cornell University Center for Research Animal Resources (No. 96-87; 11 November 1996), by the University of Michigan University Committee on Use and Care of Animals (No. 6282-6776A; 2 May 1996) and by the University of California at Santa Barbara Animal Care Council (No. 05-96-513-1; 5 May 1996).

References

- Beecher, M. D. 1982. Signature systems and kin recognition. *American Zoologist*, **22**, 477–490.
- Black, J. M. (Ed.) 1996. *Partnerships in Birds: The Study of Monogamy*. New York: Oxford University Press.
- Brown, R. E. 1988. Individual odors of rats are discriminable independently of changes in gonadal hormone levels. *Physiology and Behavior*, **43**, 359–363.
- Bushberg, D. M. & Holmes, W. G. 1985. Sexual maturation in male Belding's ground squirrels: influence of body weight. *Biology of Reproduction*, **33**, 302–308.
- Darlington, R. B. 1990. *Regression and Linear Models*. New York: McGraw Hill.
- Darlington, R. B. 1996. A normal-scores alternative to the Wilcoxon test. <http://comp9.psych.cornell.edu/Darlington/normscor.htm>. Read: 12 March 1998.
- Fisher, K. C., Dawe, A. R., Lyman, C. P., Schonbaum, E. & South, F. E. (Eds) 1967. *Mammalian Hibernation III*. New York: Elsevier.
- Godard, R. 1991. Long-term memory of individual neighbours in a migratory songbird. *Nature*, **350**, 228–229.
- Halpin, Z. T. 1984. The role of olfactory communication in the social systems of ground-dwelling sciurids. In: *The Biology of Ground-dwelling Squirrels* (Ed. by J. O. Murie & G. R. Michener), pp. 201–225. Lincoln: University of Nebraska Press.
- Halpin, Z. T. 1986. Individual odors among mammals: origins and functions. *Advances in the Study of Behavior*, **16**, 39–70.
- Hanken, J. & Sherman, P. W. 1981. Multiple paternity in Belding's ground squirrel litters. *Science*, **212**, 351–353.
- Hepper, P. G. (Ed.) 1991. *Kin Recognition*. Cambridge: Cambridge University Press.
- Holekamp, K. E. 1983. Proximal mechanisms of natal dispersal in Belding's ground squirrels (*Spermophilus beldingi*). Ph.D. thesis, University of California, Berkeley.
- Holmes, W. G. 1984a. Ontogeny of dam-young recognition in captive Belding's ground squirrels. *Journal of Comparative Psychology*, **98**, 246–256.
- Holmes, W. G. 1984b. Sibling recognition in thirteen-lined ground squirrels: effects of genetic relatedness, rearing association and olfaction. *Behavioral Ecology and Sociobiology*, **14**, 225–233.
- Holmes, W. G. 1986. Identification of paternal half-siblings by captive Belding's ground squirrels. *Animal Behaviour*, **34**, 321–327.
- Holmes, W. G. 1994. The development of littermate preferences in juvenile Belding's ground squirrels. *Animal Behaviour*, **48**, 1071–1084.
- Holmes, W. G. 1997. Temporal aspects in the development of Belding's ground squirrels' litter-mate preferences. *Animal Behaviour*, **53**, 1323–1336.
- Holmes, W. G. & Sherman, P. W. 1982. The ontogeny of kin recognition in two species of ground squirrels. *American Zoologist*, **22**, 491–517.
- Hudson, J. W. & Deavers, D. R. 1972. Thermoregulation of ground squirrels from different habitats. *Proceedings of the International Congress of Zoology*, **17**, 1–35.
- Jenkins, S. H. & Eshelman, B. D. 1984. *Spermophilus beldingi*. *Mammalian Species*, **221**, 1–8.
- Johnston, R. E. 1981. Attraction to odors in hamsters: an evaluation of methods. *Journal of Comparative and Physiological Psychology*, **95**, 951–960.
- Kivett, V. K. 1975. Variations in integumentary gland activity and scent marking in Columbian ground squirrels (*Spermophilus c. columbianus*). Ph.D. thesis, University of Alberta.
- Kivett, V. K., Murie, J. O. & Steiner, A. L. 1976. A comparative study of scent-gland location and related behavior in some northwestern nearctic ground squirrel species (Sciuridae): an evolutionary approach. *Canadian Journal of Zoology*, **54**, 1294–1306.
- Lyman, C. P. & Dawe, A. R. 1960. Mammalian hibernation. *Bulletin of the Museum of Comparative Zoology*, **124**, 1–549.
- McKeever, S. 1963. Seasonal changes in body weight, reproductive organs, pituitary, adrenal glands, thyroid glands and spleen of the Belding ground squirrel (*Citellus beldingi*). *American Journal of Anatomy*, **113**, 153–173.
- McNamara, M. C. & Riedesel, M. L. 1973. Memory and hibernation in *Citellus lateralis*. *Science*, **179**, 92–94.
- Mateo, J. M. & Holmes, W. G. 1999. Plasticity of alarm-call response development in Belding's ground squirrels (*Spermophilus beldingi*, Sciuridae). *Ethology*, **105**, 193–206.
- Mateo, J. M. & Johnston, R. E. In press. Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. *Proceedings of the Royal Society of London, Series B*.

- Mihailovic, L. J.** 1972. Cortical and subcortical electrical activity in hibernation and hypothermia. In: *Hibernation and Hypothermia, Perspectives and Challenges* (Ed. by F. E. South, J. P. Hannon, J. R. Willis, E. T. Pongelley & N. R. Alpert), pp. 487–534. New York: Elsevier.
- Mihailovic, L. J., Petrovic-Minic, B., Protic, S. & Divac, I.** 1968. Effects of hibernation on learning and retention. *Nature*, **218**, 191–192.
- Morton, M. L.** 1975. Seasonal cycles of body weights and lipids in Belding ground squirrels. *Bulletin of the Southern California Academy of Science*, **74**, 128–143.
- Morton, M. L. & Gallup, J. S.** 1975. Reproductive cycle of the Belding ground squirrel (*Spermophilus beldingi beldingi*): seasonal and age differences. *Great Basin Naturalist*, **35**, 427–433.
- Mrosovsky, N.** 1967. Lowered body temperature, learning and behaviour. In: *Mammalian Hibernation III* (Ed. by K. C. Fisher, A. R. Dawe, C. P. Lyman, E. Schonbaum & F. E. South, Jr), pp. 152–174. New York: Elsevier.
- Reeve, H. K.** 1989. The evolution of conspecific acceptance thresholds. *American Naturalist*, **133**, 407–435.
- Ross, N. M. & Gamboa, G. J.** 1981. Nestmate discrimination in social wasps (*Polistes metricus*, Hymenoptera: Vespidae). *Behavioral Ecology and Sociobiology*, **9**, 163–165.
- Rowley, I.** 1983. Re-mating in birds. In: *Mate Choice* (Ed. by P. Bateson), pp. 331–360. New York: Cambridge University Press.
- Schellinck, H. M., West, A. M. & Brown, R. E.** 1992. Rats can discriminate between the urine odors of genetically identical mice maintained on different diets. *Physiology and Behavior*, **51**, 1079–1082.
- Schellinck, H. M., Slotnick, B. M. & Brown, R. E.** 1997. Odors of individuality originating from the major histocompatibility complex are masked by diet cues in the urine of rats. *Animal Learning and Behavior*, **25**, 193–199.
- Sherman, P. W.** 1976. Natural selection among some group-living organisms. Ph.D. thesis, University of Michigan, Ann Arbor.
- Sherman, P. W.** 1980. The limits of ground squirrel nepotism. In: *Sociobiology: Beyond Nature/Nurture?* (Ed. by G. W. Barlow & J. Silverberg), pp. 505–544. Boulder, Colorado: Westview Press.
- Sherman, P. W.** 1985. Alarm calls of Belding's ground squirrels to aerial predators: nepotism or self-preservation? *Behavioral Ecology and Sociobiology*, **17**, 313–323.
- Sherman, P. W. & Morton, M. L.** 1984. Demography of Belding's ground squirrels. *Ecology*, **65**, 1617–1628.
- South, F. E., Hannon, J. P., Willis, J. R., Pongelley, E. T. & Alpert, N. R.** (Eds) 1972. *Hibernation and Hypothermia, Perspectives and Challenges*. New York: Elsevier.
- Stoddard, P. K.** 1996. Vocal recognition of neighbors by territorial passerines. In: *Ecology and Evolution of Acoustic Communication in Birds* (Ed. by D. E. Kroodsma & E. H. Miller), pp. 356–376. Ithaca, New York: Comstock.