

## The development of littermate preferences in juvenile Belding's ground squirrels

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**Abstract.** Kin favouritism has been documented widely in free-living ground squirrels (genus *Spermophilus*), but the development of kin favouritism has not been explained. To investigate the development of littermate preferences in captive Belding's ground squirrels, *S. beldingi*, groups composed of four dams and 16 juveniles (young-of-the-year) were observed in outdoor enclosures for a period of 5–11 days, beginning when juveniles first emerged aboveground from natal burrows (buried nestboxes). Social play was the most frequently observed social interaction. Littermates played together about twice as often as non-littermates on a per pair basis and juveniles were also more likely to share a burrow at night with their littermates than non-littermates. Independently of relatedness, the sex of a pair also affected play-bout frequencies ( $m-m > m-f > f-f$ ), as did body-weight differences between juveniles. It was concluded that juveniles displayed social preferences for littermates shortly after they emerged from natal burrows, which suggests that interactions between littermates prior to or just after emergence affected the development of social preferences. Dams indirectly affected the development of social preferences: when dams were not placed in enclosures littermate preferences were not observed. The results of this study support the hypothesis that kin favouritism develops from early social interactions involving dams and littermates. However, it remains to be determined whether the preferences manifested by recently emerged juveniles are maintained throughout the summer and whether such preferences provide the foundation for kin favouritism in adults.

In a landmark paper, Hamilton (1964) provided a functional explanation for the preferential treatment of genetic relatives. As Hamilton noted, preferential treatment of kin is often mediated by an ability to discriminate among conspecifics based on direct or indirect correlates of genetic relatedness. It is important to note, however, that 'differential' treatment of kin is not always the same as 'preferential' treatment of kin (Hoogland 1985; Gamboa et al. 1991) even if preferential treatment is evidence of differential treatment (Holmes 1990). Although the ontogeny of kin discrimination has been pursued intensively in some taxa (see reviews by Blaustein & Waldman 1992: anuran amphibians; and Schwagmeyer 1988: ground squirrels), few studies have sought to elucidate the ontogeny of kin favouritism, the central topic of Hamilton's landmark paper.

Kin favouritism or nepotism (sensu Alexander 1974; Sherman 1980a) occurs between adults in several species of ground squirrels (genus *Spermophilus*; reviews in Murie & Michener 1984) and is hypothesized to develop from behavioural

interactions that occur during the first few weeks of juveniles' lives (Armitage 1981; Michener 1983). This suggests that to understand the ontogeny of nepotism in adult ground squirrels one must first understand the ontogeny of social relations in juveniles.

Free-living adult female Belding's ground squirrels, *S. beldingi*, behave nepotistically towards descendant and non-descendant kin in two contexts: when terrestrial predators appear (Sherman 1977, 1985) and during territorial defence of natal burrows (Sherman 1980b). Because females live among conspecifics that vary in genetic relatedness, nepotism depends on kin recognition, which is inferred from the differential treatment of conspecifics that correlates with genetic relatedness (see reviews in Sherman & Holmes 1985; Schwagmeyer 1988). The ontogeny of *S. beldingi* kin recognition is influenced by social interactions during early development, especially with littermates, as seems to be the case in many vertebrates (see review articles in Blaustein et al. 1988). The question remains, however, whether the early

social experience that influences littermate recognition also affects littermate favouritism in juveniles and kin favouritism in adults.

The following description of early social development in *S. beldingi* places my research in an ecological context. An adult ( $\geq 1$  year old) female produces one litter annually of about five young (Morton & Gallup 1975), which results from mating with multiple males (Hanken & Sherman 1981). Thus a female's litter is typically composed of full- and maternal half-siblings, which I refer to as littermates. Each female constructs an underground burrow (the natal burrow) after mating that she defends throughout gestation and lactation until her litter first comes aboveground (the natal emergence), which coincides approximately with weaning (Sherman 1976, 1981a). From birth to natal emergence, littermates are exposed only to each other and their dam. Natal emergence occurs when young reach 25–28 days of age and most litters in a population emerge during a 10-day period (Holekamp 1984).

During the first 2–3 days after their natal emergence, juveniles (young-of-the-year that have emerged) remain near their burrow and interact with their littermates and dam, although they may also encounter older juveniles that intrude from nearby burrows (Morton et al. 1974; Sherman 1980b). Juveniles routinely venture more than 30 m from their natal burrow after they have been aboveground about 4–7 days and regularly encounter other related and unrelated juveniles and adults, but they continue to use their own natal burrow as an activity centre and night-time refuge and interact much more often with littermates than non-littermates (Holekamp 1983, 1986). Besides nose-body contact, play is among the most frequently observed juvenile-juvenile social interaction that occurs during the first 7–10 days after natal emergence (Holekamp 1983). Two to 3 weeks after emerging, juveniles routinely travel 50–100 m from their natal burrow. A few weeks later, litters dissolve as juveniles establish their own burrow system, which for females is about 30 m from their natal burrow and for males is more than 200 m from their natal burrow (Holekamp & Sherman 1989).

The long-term goal of my research is to explain the development of nepotism in adult Belding's ground squirrels. As a first step towards this goal, in this study I observed juveniles in outdoor enclosures with three purposes in mind. First, I

sought to determine whether newly emerged juveniles exhibited social preferences for littermates. A social preference is an internal construct inferred from the frequency, duration, intensity, and/or form of social acts directed differentially among conspecifics that implies the existence of an amicable or cohesive relationship (Holmes 1988). Second, I sought to determine whether sex differences existed in the social preferences of recently emerged juveniles. A juvenile sex difference, if found, might parallel adult sex differences in nepotism, that is, adult females behave nepotistically, but adult males do not (Sherman 1981b, 1985). Finally, I wished to determine whether dams affected the development of social preferences in their offspring by treating their own and alien young differentially after natal emergence.

## METHODS

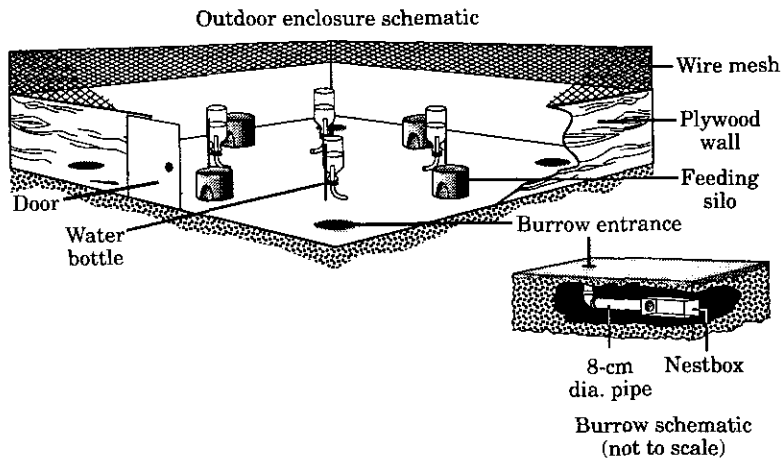
### Study Location and Animals

Research was conducted at the Sierra Nevada Aquatic Research Laboratory (SNARL), situated near Mammoth Lakes, Mono County, California, in the east-central Sierra Nevada. Study animals were field-mated adult females and the offspring they bore at SNARL. Pregnant females were live trapped from four populations in Mono County: near SNARL (2100 m elevation), near the town of Mammoth Lakes (2300 m elevation), along the upper reaches of Rock Creek Canyon (2900 m elevation), and in areas near Saddle Bag Lake (3000–3400 m elevation). At SNARL females were housed individually in a 'nursery' building in stainless steel cages (61 × 45 × 35 cm), which contained plywood nestboxes (28 × 20 × 20 cm). Each nestbox had a removable top, a 6-cm-diameter entry hole and was filled partially with wood-shavings. Food (Purina mouse breeder chow) and water were available ad libitum.

In May and June 1989, 31 of the 42 females bore litters. The day after they were born, pups were weighed, sexed, and their toenails were clipped for permanent identification. Pups were weighed every 4–5 days to ensure that all young later observed in the same outdoor enclosure grew at similar rates.

### Outdoor Enclosures for Behavioural Observations

Ground squirrels were observed in four open-air, outdoor enclosures measuring 9.7 × 9.7 × 1.6 m (Fig. 1). Near each of the four corners of an



**Figure 1.** Schematic diagram of one of the four outdoor enclosures measuring  $9.7 \times 9.7 \times 1.6$  m in which ground squirrels were observed. Besides water bottles and feeding silos, each enclosure included four buried nestboxes (see text). Poultry netting on the ground allowed vegetation to grow and prevented ground squirrels from digging.

enclosure, holes were dug where plywood nestboxes (see above) could be placed 10 cm below ground inside plywood frames measuring  $56 \times 56 \times 30$  cm, which were buried permanently in the ground. Hereafter, burrow refers to a tunnel entrance, which was connected by a 1.8-m tunnel of plastic pipe to a buried nestbox. Ground squirrels foraged on vegetation inside enclosures, but acquired most of their nutrients from food (Purina mouse chow No. 5015) placed in four silos. Food and water were available ad libitum except during observation sessions when food was removed to eliminate the possibility of food-related social interactions.

### Behavioural Observations in Outdoor Enclosures

#### *Methods and schedules of observation*

An enclosure was typically populated with four dams and 16 juveniles. Each animal was dye-marked for individual identification, and body weights were measured on the day animals were placed in and removed from an enclosure. A dam and her four offspring (hereafter a 'litter') were placed below ground in an enclosure in the litter's nestbox (Fig. 1). Each burrow entrance was covered by a removable  $76 \times 61 \times 46$  cm 'gazebo' constructed of plywood and hardware cloth with a hole in the plywood bottom that fitted over the burrow entrance. Gazebos allowed animals to

come aboveground (hereafter, to 'emerge'), but prevented them from moving away from their burrow until gazebos were removed.

Litters were placed in an enclosure on day 0 when weaned juveniles were about 32 days old (Table I). Before behavioural observations began on day 1, animals were familiarized with an enclosure on day 0 by removing one gazebo at a time for a period of 1–2 h. Animals in an enclosure were observed 4–5 h/day for 5–11 days, and 90% of all observation hours were accumulated when ground squirrels were most active (0630–1100 hours). Scan samples (see patterns of spatial use, below) revealed that during these hours  $93.5 \pm 2.0\%$  ( $\bar{X} \pm SE$ ) of juveniles were aboveground continuously and thus 'available' to interact. Gazebos were placed over burrows at night (2000 hours), which allowed me to identify each morning the animals that shared a burrow the previous night (hereafter, 'sleeping partners').

Social interactions were recorded on preconstructed data sheets by observers who were not told the sex or relatedness of animals they observed. When a dyadic interaction occurred, an observer recorded the dye marks of the two interactants, their location on a grid system (see below), and the type of interaction (definitions below). Initiators and recipients were not distinguished and only dyadic interactions were recorded.

**Table 1.** Characteristics of four groups of ground squirrels observed in outdoor enclosures to record juvenile social interactions

	Groups observed			
	1	2	3	4
Group composition	Standard*	Standard*	No dams	2 litters
Juvenile age (days)†	31.8 ± 0.3	30.4 ± 0.1	37.0 ± 0.5	32.1 ± 0.3
Juvenile body weight (g)†	68.6 ± 6.5	64.5 ± 6.5	82.6 ± 8.2	74.2 ± 9.4
Days observed‡	5–11	1–11	1–8	1–5
Total hours of observation	24.2	39.6	20.3	16.2
Total play bouts observed	297	639	251	84
Total pairs observed	45	105	91	28
Littermates	12	21	18	12
Non-littermates	33	84	73	16

\*The standard group was composed of four dams and 16 juveniles with two males and two females per dam.

†Mean ± SE when juveniles were placed in an enclosure.

‡Animals were placed in an enclosure on day 0 and familiarized with it. Behavioural observations typically began on day 1.

#### *Social interactions recorded*

Because the purpose of my study was to investigate the development of social preferences, I sought behavioural measures indicative of affiliative or cohesive relations often characteristic of social preferences (Holmes 1988). I report play behaviour as the primary measure of social preference for three reasons. First, although agonistic interactions have often been used in studies of ground squirrel social relations (references in Murie & Michener 1984), agonism rarely occurred between the recently emerged juveniles I studied (<5% of all social interactions). Second, pilot observations in 1988 indicated that besides nose-body contact, social play was the most frequent kind of juvenile social interaction (>80% of all interactions). Third, although the function of ground squirrel play is unknown, it often reflects an amicable or cohesive relationship in many mammals (Bekoff & Byers 1981, 1985; Fagen 1981; Martin & Caro 1985), including ground squirrels (Michener 1981; Waterman 1988).

Social play in ground squirrels is composed of a series of motor patterns directed at a partner that may include (1) nudging, usually with the nose, (2) broad-side or lateral postures, (3) rushing at, pouncing on, or chasing, (4) biting or chewing on, (5) swiping or boxing with the forepaws, (6) clasping, hugging, and mounting, or (7) wrestling with the interactants grasping or clinging to each other (detailed descriptions and drawings are in

Steiner 1971; see also Waterman 1988). Juveniles also engaged in 'tense play' that included three specific motor patterns: active chasing, vigorous wrestling, and fleeing by one participant, which terminated the interaction.

Observers recorded 'play bouts' composed of one or more motor patterns (1–7 above) or tense play bouts (above) rather than individual motor patterns. In 1988 I videotaped and analysed a random sample of 45 play bouts and found no specific motor pattern that reliably distinguished between pairs based on relatedness and sex. I reasoned that if an analysis of individual motor patterns did not consistently distinguish differences in play as a function of relatedness and sex and if recording individual motor patterns required an observer to focus exclusively on one pair when another pair was playing simultaneously, which happened often, then it was sensible to record bouts rather than individual motor patterns.

A play bout had to last more than 3 s to be recorded, and two bouts involving the 'same' two juveniles were not recorded unless at least 1 min had passed between the end of the first bout and beginning of the second bout. A bout was considered to start when any one of the above motor patterns began and ended when play partners separated by more than 1 m or failed to interact for at least 3 s. These subjective criteria were derived from pilot observations of more than 275 play bouts in 1988.

### *Patterns of spatial use*

I used a grid system composed of 64 1.2-m<sup>2</sup> squares to assess the spatial distribution of play bouts by recording the location of two juveniles when a bout began. I also used the grid to assess nearest-neighbour distances with a scan-sampling technique (Altmann 1974) using a 2-min scan interval, during which juveniles typically moved through at least two grid squares.

### *Training and assessment of observers*

Observers ( $N=4$ ) were trained by watching a practice group of 16 juveniles for 5 days (4 h/day). Following training, I estimated the percentage of all play bouts occurring during a 1-h period that a single observer could record, given that more than one bout often occurred simultaneously. The average observer, who was responsible for watching the entire enclosure, recorded  $92.4 \pm 3.7\%$  ( $\bar{X} \pm \text{SE}$ ) as many bouts as did three observers combined when each of the three intensively watched only one-third of the enclosure. I also determined inter-observer reliability for play-bout frequencies by a correlation method (Caro et al. 1979, page 306) and found that correlation coefficients ranged between 0.88 and 0.97.

### **Groups Observed in Enclosures**

Data from four groups are reported (Table I). A group refers to the set of dams and juveniles placed together in the 'same' enclosure. Litters that were placed together in the same group satisfied three criteria: (1) each litter had to include at least two males and two females so that each dam could be placed in an enclosure with two sons and two daughters, which were chosen randomly when litter size exceeded four juveniles; (2) litters had to be born within 3 days of each other; and (3) among litters that satisfied (1) and (2), juveniles were chosen so as to minimize body-weight differences when litters were placed in an enclosure.

Groups 1 and 2 were both composed of four dams and 16 juveniles. In group 3, dams reared their offspring in the nursery like dams in other groups, but only 16 juveniles were placed in an enclosure to examine whether juvenile interactions would be affected by the absence of dams. Finally, group 4 was composed of only two dams and

eight juveniles to examine whether social interactions would be affected by lower density.

### **Statistical Methods and Data Analysis**

In all analyses, multiple observations on an individual or pair were averaged before statistical tests were performed (Machlis et al. 1985), except as explained below for play-bout frequencies. Comparisons between various categories of juveniles such as littermates versus non-littermates were weighted by the number of pairs in each category. Some statistical comparisons could not be done on all four groups because of limited sample sizes. Means  $\pm 1 \text{ SE}$  are reported.

Play data were analysed to understand the independent effects of three factors on play-bout frequency: relatedness (littermates versus non-littermates), sex of a pair (female-female versus male-male versus female-male), and weight difference of a pair. Relatedness is a descriptive label to distinguish littermates, which were reared together, from non-littermates, which were reared apart. Littermates presumably included full- and maternal half-siblings because free-living females typically mate with multiple males (Hanken & Sherman 1981). Weight difference is the absolute difference in mean body weight between two juveniles that played together. (For each juvenile, a mean body weight was calculated based on weights when observations began and ended on a group.) Play-bout frequency is the number of times a pair of juveniles played together summed across all days of observation. I calculated these frequencies for each pair of juveniles in a group, and analysed data from each group separately because several factors differed among groups (Table I).

Play-bout frequencies within each group most closely followed a Poisson distribution. Log-linear models (Aitkin et al. 1989; McCullagh & Nelder 1989) using GLIM software were employed to model play-bout frequencies as a function of relatedness, sex and weight difference. A log-linear analysis, which is conceptually similar to an analysis of covariance, models the log-transformed response variable, play bouts, as a linear combination of the factors of interest, but assumes a Poisson distribution for play-bout frequencies (the response variable) rather than a normal distribution (details in Appendix). The 'independent'

**Table II.** *P*-values resulting from quasi-likelihood-ratio testing based on log-linear modelling of the frequency of play bouts between juvenile ground squirrels

Test group observed	Relatedness of pair	Sex of pair	Weight difference of pair	Number of pairs
1	<0.01	<0.01	<0.01	45
2	NA	NA	>0.50	105
3 (no dams)	>0.50	<0.01	<0.01	91
4 (0.50 density)	<0.025	<0.05	>0.10	28

NA: Tests for significant main effects were not applicable due to a significant ( $P \leq 0.025$ ) relatedness by sex interaction.

effects of relatedness, sex and weight difference on play-bout frequencies were summarized as play ratios, the amount by which the expected play-bout frequency of one type of pair exceeded that of another type of pair when the other two factors were held constant. For example, the ratio of littermate to non-littermate play-bout frequencies could be evaluated statistically when the effects of sex and weight difference were controlled.

## RESULTS

### Play-bout Frequencies by Pairs of Juveniles

To facilitate statistical procedures, I included a juvenile or pair of juveniles in an analysis only if it or the pair was present every day the group was observed, which is one reason why sample sizes differ among groups. For example, two juveniles died in group 1 when a ground-squirrel dug burrow collapsed, and one juvenile was removed following an injury caused by a dam.

Sampling information for log-linear models is shown in Table I. With one exception considered below, log-linear models did not reveal significant two-factor interactions and so main effects are reported. Play-bout frequencies varied significantly as a function of pairs' relatedness in groups 1, 2 and 3, but not in group 4 in which dams were never present in the enclosure (Table II). Pairs' sex had a significant effect on play in all four groups. Pairs' weight difference had a significant effect on play in groups 1 and 3, but not in groups 2 and 4.

In all four groups, I quantified the direction and magnitude of the main effects of relatedness, sex and weight difference using the estimated coefficients from log-linear models. Coefficients were used to calculate play-frequency ratios, which are presented in Table III with 95% confidence

intervals. In group 1, for instance, models estimated that littermate pairs played 2.21 times as often as non-littermate pairs of the same sex and weight difference (Table III). Overall, littermate pairs played about twice as often as non-littermate pairs (of the same sex and weight difference) when dams were present in an enclosure (groups 1, 2 and 4), but littermate and non-littermate pairs played about equally often when dams were absent (group 3). Note also that for pairs in group 4, which lived at a density that more closely approximated field conditions compared with groups 1 and 2, littermate play also occurred about twice as often as non-littermate play.

Considering sex, in all four groups male-male pairs played much more often than male-female pairs, which, in turn, played more often than female-female pairs, when relatedness and weight difference were held constant (Table III). For instance, male pairs in group 1 were estimated to play 3.53 times as often as female pairs and 1.95 times as often as male-female pairs independently of relatedness and weight difference.

Weight difference affected play frequencies much less than relatedness or sex, as indicated by the play ratios which were close to 1.0 (Table III). That weight-difference ratios in Table III were all less than 1.0 means that as pairs' weight differences increased their play frequencies decreased. In group 1, for instance, for every 1 g increase in a pair's weight difference there was a 0.96 decrease in the pair's play-bout frequency.

Due to a significant sex  $\times$  relatedness interaction in group 2, I separated pairs by sex to compare littermate with non-littermate play and separated pairs by relatedness to examine a sex effect. Such separations produced the same basic picture in group 2 as was seen in groups 1 and 4. For the three sex combinations, littermates played from 1.5 times as often (male-female

**Table III.** Play-bout ratios† and (95% confidence intervals) estimated by log-linear modelling for pairs of ground squirrels that differed in relatedness, sex, and weight

Test group observed	Relatedness		Sex of the pair		Weight difference‡
	Littermate	Male-male	Male-male	Male-female	Weight difference increase (1 g)
	Non-littermate	Female-female	Male-female	Female-female	
1 (standard)	2.21* (1.7, 2.8)	3.53* (2.4, 5.2)	1.95* (1.4, 2.7)	1.81* (1.3, 2.4)	0.96* (0.9, 1.0)
3 (no dams)	1.05 (0.7, 1.6)	8.22* (2.9, 23.3)	1.64* (1.1, 2.3)	5.01* (1.8, 14.2)	0.97* (0.98, 1.01)
4 (0.50 density)	2.22* (1.2, 4.2)	2.34 (0.6, 9.2)	2.39* (1.2, 4.7)	0.98 (0.2, 3.9)	0.99 (0.9, 1.0)

\*Statistically significant ratios ( $P \leq 0.05$ ).

†Ratios are the frequency of play bouts for pairs in the numerator divided by the frequency of play bouts for pairs in the denominator while the other two variables are held constant. For example, in group 1 littermate pairs played 2.21 times as often as non-littermate pairs of the same sex and weight difference.

‡The reduction in the number of play bouts between two juveniles for every 1 g increase in the absolute weight difference between the two juveniles.

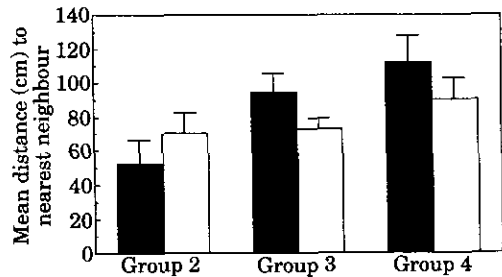
littermates:male-female non-littermates) up to 3.7 times as often (female-female littermates: female-female non-littermates) as non-littermates. For both littermates and non-littermates, play ratios for each sex-of-pair combination ranged between 1.1 and 4.1 and were rank ordered (high to low) male-male > male-female > female-female.

#### Patterns of Spatial Use of Enclosures

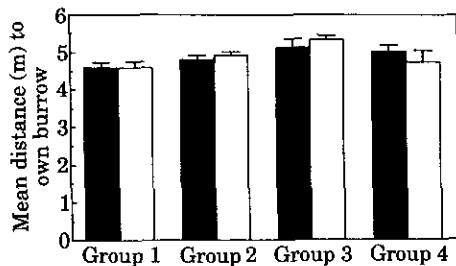
If juveniles restricted their movements to particular parts of an enclosure then play frequencies may have reflected differences in the spatial availability of social partners. I examined patterns of spatial use in two ways. First, I analysed scan-sample data and found that a juvenile was typically about 1 m from both its nearest littermate and nearest non-littermate neighbour ( $P \geq 0.1$ ; Fig. 2), which indicates that both types of juveniles were equally available for social interactions. Second, for each juvenile I partitioned play bouts into those with a littermate and those with a non-littermate and then determined how far the focal juvenile was from its own burrow during the two types of bouts. In none of the four groups did the mean distance from a juvenile's own burrow differ significantly ( $P \geq 0.1$ ) for littermate versus non-littermate play (Fig. 3). Note also that juveniles did not restrict their play to a small region around their own burrow but typically played about 4 m away (Fig. 3).

#### Play by Day in an Enclosure

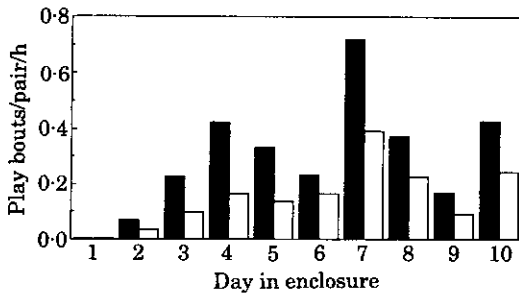
To examine patterns of juvenile play across days in an enclosure, I calculated rates of play



**Figure 2.** Mean (+SE) distances to nearest littermate (■) and nearest non-littermate (□) neighbours based on scan-sample data from the three groups for which adequate sample sizes were available.



**Figure 3.** Mean (+SE) distances to a focal juvenile's home burrow when the juvenile played with a littermate (■) and with a non-littermate (□) in outdoor enclosures.



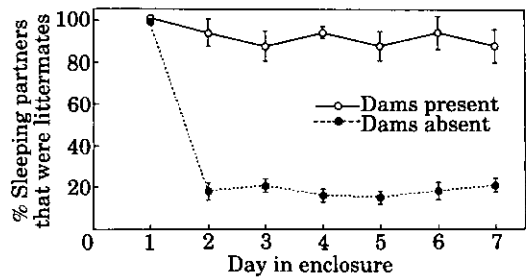
**Figure 4.** Rates of play bouts between littermates (■) and between non-littermates (□) during the 10 days juveniles from group 2 were observed in outdoor enclosures.

(bouts/pair/h of observation) for group 2, the most extensively observed group. Twenty-five nasal investigations (nose-head, nose-body contact) were recorded during 175 min of observation on day 1, the first day juveniles had unrestricted access to the enclosure, but play bouts were not observed until day 2. On each of days 2–10, play rates for littermates were about twice as high as they were for non-littermates (Fig. 4). Comparisons of play rates across days must be made cautiously because weather conditions, which were variable, affected daily rates.

### Sleeping Partners

Sleeping partners, juveniles that shared the same burrow on a given night, were identified each morning before gazebos were removed from burrows. For each juvenile in group 2 (dams present) and group 3 (dams absent), I calculated the percentage of its partners on day 1 that were the juvenile's littermates and then calculated means based on all juveniles in a group. I repeated this calculation for days 2–7.

Typically, three to five juveniles shared each burrow each night along with one dam, and when dams were present (group 2)  $93.7 \pm 2.6\%$  of juveniles shared a burrow with their own dam based on sleeping patterns for days 2–7. Very rarely (<5% of all nights) did two dams use the same burrow on a given night. As shown in Fig. 5, all juveniles in both groups 2 and 3 slept only with littermates on day 1, but for the average juvenile in group 2, more than 85% of its sleeping partners were littermates on days 2–7. In contrast, for the average juvenile in group 3 about 20% of



**Figure 5.** The percentage of a juvenile's sleeping partners on a per capita basis that were littermates (rather than non-littermates) during the first 7 days juveniles lived in outdoor enclosures. Data are shown for group 2 juveniles (dams present) and group 3 juveniles (dams absent).

its sleeping partners were littermates each day even though for each juvenile non-littermates outnumbered littermates 4:1.

I also examined sleeping burrow use. Based on the first 7 days ground squirrels were in an enclosure, I calculated the mean percentage of juveniles that slept in their own burrow, the one into which they were placed when litters were put in an enclosure on day 0. In group 2,  $81.3 \pm 0.1\%$  of juveniles slept in their own burrow on days 1–7, whereas in group 3 the comparable mean was only  $42.0 \pm 0.3\%$ .

## DISCUSSION

### Play Bouts and Littermate Preferences

I observed juvenile Belding's ground squirrels and their dams in outdoor enclosures for a period of 5–11 days, beginning when juveniles first emerged aboveground from natal burrows, and found that juveniles interacted preferentially with their littermates during this time period. Play was the most frequent type of social interaction between juveniles and analyses of play-bout frequencies revealed that when dams were present in enclosures, littermates played together about twice as often as non-littermates on a per pair basis (Table III). In addition, juveniles were much more likely to share a burrow at night with littermates than non-littermates despite the presence of four non-littermates for every littermate in an enclosure for each juvenile (Fig. 5). Thus, when juvenile *S. beldingi* had their first opportunities to interact with related and unrelated age-mates, which coincided with their initial appearance



aboveground, juveniles interacted preferentially with their littermates by playing and sleeping with them more often than with non-littermates, that is, juveniles displayed a social preference for their littermates.

The functional significance of mammalian social play remains enigmatic (Caro 1988), but play often reflects amicable or cohesive relations between juveniles (Bekoff & Byers 1981, 1985; Martin & Caro 1985). In the genus *Spermophilus* (ground squirrels), play is among the most common social interactions between newly emerged, free-living juveniles and, although juvenile play may include a competitive component (Yeaton 1972; Waterman 1986), littermate play is more common than non-littermate play in several species (Michener 1981: *S. richardsonii*; Waterman 1988: *S. columbianus*; Ferron 1985: *S. lateralis*), including *S. beldingi* (Holekamp 1983).

The play-partner preferences of juvenile *S. beldingi* did not result from a simple spatial-availability rule: play with age-mates that are consistently nearest you or nearest your natal burrow. Nearest-neighbour analyses revealed that mean distances to the nearest littermate versus non-littermate did not differ significantly (Fig. 2), and whether a juvenile was playing with a littermate or non-littermate it was equally distant from its home burrow (Fig. 3). Borrowing from a discussion of kin recognition (Waldman et al. 1988), juvenile *S. beldingi* displayed direct social preferences for littermates (preferences based on individuals' phenotypic attributes) rather than indirect preferences (those based on contextual cues like locations in space).

The most likely proximate explanation for the development of littermate preferences in my study is prior association (Holmes & Sherman 1982) or familiarity (Bekoff 1981) because littermates associated during early development, whereas non-littermates did not. Because juveniles began playing preferentially with littermates the day after they emerged (day 2 in Fig. 4), littermate preferences may have developed from interactions prior to or coincident with natal emergence, as discussed below. Field data on cross-fostered *S. beldingi* also suggest that interactions around the time of natal emergence are important to the development of littermate preferences (Holmes & Sherman 1982).

In earlier work, I determined that association affected the development of *S. beldingi* littermate

recognition (Holmes & Sherman 1982), but links between that work and the current study must be made cautiously for three reasons. First, littermate 'recognition' and littermate 'favouritism' are not synonymous (Gamboa et al. 1991) and one should not assume that a proximate explanation for kin recognition is also a proximate explanation for kin favouritism even though kin favouritism demonstrates kin recognition (Holmes 1988; Waldman et al. 1988). Second, I previously studied yearlings that as juveniles were housed with littermates for 3 months before hibernating alone for 8 months, whereas here I report on juveniles that shared a natal burrow for less than 1 month. Finally, when studying yearlings, I used 5-min paired encounter tests in a 1-m<sup>2</sup> arena and recorded agonistic interactions, but here I observed groups of eight to 16 juveniles for a period of 5–11 days in a 9.7-m<sup>2</sup> enclosure and recorded amicable interactions.

In her review of sociality in ground-dwelling sciurids, Michener (1983) noted that adult social structure was based on female kinship and proposed that female kin favouritism developed from social interactions during the first 4–6 weeks of life. My results (Table III) are consistent with the early-social-interactions portion of Michener's proposal because young that were reared together (littermates) played preferentially with each other at emergence (Fig. 4). What is unspecified in Michener's (1983) hypothesis is the relative importance of pre-emergence and post-emergence interactions because Michener's window of 4–6 weeks includes the entire pre-emergence and early post-emergence periods.

I propose that the development of littermate preferences in *Spermophilus* could (1) depend exclusively on pre-emergence, natal-burrow interactions, (2) depend critically on pre-emergence interactions but require some amount of post-emergence interactions to crystallize, or (3) depend exclusively on post-emergence interactions. For free-living *S. beldingi*, littermate preferences could develop reliably if they depended only on pre-emergence interactions because dams rear litters isolated in underground burrows until natal emergence (Sherman 1981a). However, post-emergence, aboveground interactions could also be responsible for littermate preferences because juveniles remain close to their natal burrow and interact frequently with each other (Holekamp 1983). On the other hand, older more mobile

juveniles from neighbouring burrows sometimes intrude into the otherwise exclusive social world of newly emerged littermates (Sherman 1980b; unpublished data), which could confound the development of littermate preferences if they depended solely on aboveground interactions. In ongoing studies, I seek to clarify the precise effects or pre- and post-emergence interactions on the development of littermate preferences.

### Effects of Dams on Juvenile Preferences

Log-linear models revealed that dams affected their offsprings' social preferences because when dams were not placed in enclosures littermate preferences failed to materialize (Table III). Unfortunately, however, I could detect no simple, direct link between the behaviour of dams and the development of littermate preferences. For example, unlike the situation in some species of primates (Berman 1982; de Waal 1990), when *S. beldingi* dams were present in enclosures there was no evidence that they directly influenced social interactions by controlling movements of their offspring or intervening in juveniles' social interactions. On the other hand, dams may have affected juveniles' social interactions by two indirect routes. First, although dams did not actively pursue aliens, 85.4% of all dam-juvenile agonism (chases, bites and lateral displays combined) occurred when a juvenile came within 1.5 m of an alien dam (combined data for groups 1, 2 and 4). Such agonism typically elicited flight in juveniles. As a consequence, juveniles were unlikely to interact with non-littermates if juveniles encountered non-littermates in the presence of a non-littermate's dam. Second, the percentage of a juvenile's sleeping partners that were non-littermates was three to four times higher when dams were absent from an enclosure than when they were present (Fig. 5). Sharing a nestbox each night could influence play-partner preferences. For example, a juvenile played 1.9 times as often with a given non-littermate the day after the two shared a sleeping burrow compared with days after which the two did not sleep together. In ongoing experiments, I am manipulating the time when dams are present and absent from enclosures and dams' movements in enclosures to clarify their role in the development of juveniles' social preferences.

As was the case for enclosure-held ground squirrels, there is no obvious causal link between

the behaviour of dams and the development of littermate preferences in free-living *S. beldingi* (see Wiggett & Boag 1992 on how *S. columbianus* dams might indirectly affect littermate preferences). In the field, dams protect their unweaned, dependent offspring from infanticidal adults by defending an area around the natal burrow (Sherman 1981a), but once a dam's litter emerges aboveground she ceases territorial defence (Sherman 1976). In addition, dams routinely forage more than 50 m from their natal burrow and are often absent when their litter first emerges (unpublished data). Thus, as in enclosures, free-living dams do not intervene directly in juveniles' social interactions after natal emergence.

### Sex Differences

A hallmark of nepotism in *Spermophilus* is its sex-limited nature: nepotism occurs primarily between females and rarely involves males (see references in Murie & Michener 1984; but see Davis 1984: *S. richardsonii*). If adult social favouritism develops from interactions between littermates during the first few weeks of life (Michener 1983), one might expect juveniles to display sex differences in social behaviour that later influence sex differences in adult favouritism. Free-living juveniles do display sex differences in social behaviour (Michener 1981: *S. richardsonii*; Waterman 1986, 1988: *S. columbianus*; Pfeifer 1982: *S. elegans*; Holekamp 1983: *S. beldingi*) and so I sought to determine whether captive *S. beldingi* juveniles displayed sex differences in social preferences that paralleled adult sex differences in nepotism. In all four groups, the sex of a pair affected play-bout frequencies significantly (male-male > male-female > female-female), but for each sex-of-pair combination, littermate play was more common than non-littermate play (Table III). Thus, littermate play-partner preferences were not restricted to juvenile female littermates like nepotism is restricted to adult female kin (Sherman 1981b, 1985).

Sex differences in mammalian play are common for energetic or aggressive types of play like play-fighting (Meaney & Stewart 1985). In *S. beldingi* juveniles, the most energetic or aggressive play occurred in tense play bouts (see Methods), which represented  $5.4 \pm 0.8\%$  of all play bouts based on data from four groups

combined. The frequency of tense play bouts was too low for meaningful statistical comparisons, but the sex-by-relatedness distribution of such play is interesting vis-à-vis sex differences in juvenile play and adult nepotism. Of the 55 tense play bouts recorded, 51 (92.7%) involved males interacting with non-littermates and all involved juveniles that were 40 days old or older. To determine whether tense play in juveniles is important to kin favouritism in adults, it will be necessary to observe juveniles for longer time periods to increase instances of tense play and to study older juveniles that engage in tense play.

### Body-weight Effects

Preferred play partners are often similar in body weight (Byers 1980) and if body-weight similarity is one factor that attracts young to play together, then in polytocous species, littermates might be attractive play partners in part because of their greater similarity in body weight compared with non-littermates. In my study, a pair's weight difference, the absolute difference in body weight between members of a pair, affected play-bout frequencies independently of relatedness or sex (Table II). As weight difference increased, play frequency decreased. However, the effect of weight differences was significant in only two of four groups (Table II) and Spearman correlation coefficients between weight differences and play-bout frequencies ranged from  $-0.28$  to  $-0.48$  among the four groups, but these coefficients were significant ( $P \leq 0.05$ ) in only two groups. I conclude that, compared with relatedness and sex, weight difference had a modest effect on play-partner preferences. However, this modest weight-difference effect may have resulted because juveniles of 'similar' body weight were placed together in an enclosure such that body-weight differences were minimized (see Methods). In nature, body weight may have a greater impact on the development of play-partner preferences if body weights of neighbouring juveniles (as well as their ages) vary more than they did in my study.

### Limitations of the Study

That captive juvenile *S. beldingi* played preferentially with their littermates is consistent with the

hypothesized developmental link between early social interactions and subsequent social preferences, but four caveats warrant consideration. First, the density of ground squirrels in enclosures was much greater than in nature, although when enclosure density was halved (group 4) littermate preferences were still manifested (Table III). Nevertheless, the density of animals in group 4 was still about three times that of free-living *S. beldingi* (Sherman 1976).

Second, natal burrows, which are typically 30 m apart in the field (Sherman 1981b), were about 5 m apart in enclosures, which enhanced the likelihood that juveniles would encounter non-littermates on the first day they emerged. Although day-1 encounters with non-littermates occur infrequently in free-living juveniles (unpublished data), the tendency of captive juveniles to play preferentially with littermates shortly after emergence (Fig. 4) suggests that littermate preferences in free-living juveniles would not be due simply to the absence of non-littermate partners.

Third, I suggested that littermate preferences were influenced by interactions prior to or shortly after emergence in enclosures, but littermates often engaged in play-like behaviour (e.g. fur chewing, clasping with forepaws, pushing with nose) outside nestboxes in cages in the nursery building 2–3 days before they were transferred outdoors. These interactions might have influenced play-partner preferences in outdoor enclosures.

Finally, the preferences of captive juveniles to play with littermates is consistent with Michener's (1983) early-social-interactions hypothesis, but my observations were limited to the first 5–11 days after natal emergence so it remains to be seen whether littermate preferences in *S. beldingi* are maintained throughout the summer, as they are in free-living *S. richardsonii* (Michener 1981) and *S. columbianus* (Waterman 1988). In addition, a developmental link must be forged between juvenile sex differences in play and adult sex differences in nepotism.

## APPENDIX

To analyse juvenile–juvenile play, a log-linear model of play-bout frequencies was written separately for each group as

$$\log_e(Y_{ijk}) = a + B_{(i)} + G_{(j)} + H(PW_{ijk}) + \text{interactions}$$

where  $Y_{ijk}$  was the play-bout frequency for the ( $ijk$ )th pair;  $B_{(i)}$  is the coefficient corresponding to each ( $i$ )th level of relatedness for the pair ( $i$ : littermates, non-littermates);  $G_{(j)}$  is the coefficient for each ( $j$ )th level of sex of the pair ( $j$ : f-f, m-m, m-f); and  $H$  is the coefficient for the pair's weight difference.

The importance of each of the three factors in a model was evaluated with model-based likelihood ratio statistics. This statistic determines the significance (via a  $P$ -value) of a factor by comparing a model containing the factor with the same model 'excluding' the factor and holding constant the other factors. First, all possible two-factor interactions between the three factors were evaluated. Then, in the absence of significant ( $P \leq 0.05$ ) interactions, the main effect of each factor was tested, given that the other two factors were held constant.

By design of the study, pairs in a group were not independent units because each juvenile was included in a pair with every other juvenile in a group. Standard model-based likelihood equations (above) assume the correlation structure of the units of observation (pairs' play frequencies) is one of independence. This assumption, however, misspecifies the true correlation structure of pairs in this study because, as noted, pairs were not independent units. Moreover, the variances of the coefficients from the model are underestimated when independence is falsely assumed. To help correct the independence problem, a quasi-likelihood approach to log-linear modelling (Aitkin et al. 1989; McCullagh & Nelder 1989) was employed because '... the quasi-likelihood estimates of [the coefficients] is often consistent even if the covariance matrix [the correlations among the observations] is misspecified' (McCullagh & Nelder 1989, page 333). Briefly, the quasi-likelihood method estimates a constant scale factor, which inflates the variance. By using this scale factor estimated from the play-frequency data, model-based likelihood ratio methods could be used and adjusted coefficients could be estimated for play ratios.

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