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UNIVERSITY OF ALBERTA

PROXIMATE MECHANISMS OF SOCIAL DISCRIMINATION BY JUVENILE COLUMBIAN GROUND SQUIRRELS.

BY

James F. Hare

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

DEPARTMENT OF ZOOLOGY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled PROXIMATE MECHANISMS OF SOCIAL DISCRIMINATION BY JUVENILE COLUMBIAN GROUND SQUIRRELS submitted by James F. Hare in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

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Abstract

Kin discrimination mechanisms allow the maximization of inclusive fitness via kin-differential behaviour. Previous studies of kin discrimination in ground squirrels (*Spermophilus* spp.) have concentrated on the proximate mechanisms by which kin are discriminated from non-kin. Few studies have examined the extent to which kin discrimination is expressed among free-living squirrels, or the extent to which squirrels also discriminate among non-kin.

To elucidate the level at which juvenile Columbian ground squirrels (*Spermophilus columbianus*) discriminate among conspecifics, dyadic interactions between otherwise free-living siblings, neighbours, non-neighbouring members of the same colony, and individuals from two different colonies were staged in a neutral arena. Similar levels of cohesive and agonistic behaviour were recorded for all intra-colony dyads while interactions between non-colony members were characterized by decreased cohesion and increased agonism. That result suggests that siblings are not discriminated from non-siblings, but that colony members are discriminated from non-colony members.

In a second experiment, behavioral data were obtained for non-neighbouring colony member and non-colony member dyads captured routinely with live-traps that were interchanged between the interactants natal areas every 3 days, or with traps that were not interchanged. As in the previous experiment, colony members were discriminated from non-colony members; however, trap-mixing significantly increased cohesion and decreased agonism both within and between colonies. Those results indicate that juvenile Columbian ground squirrels discriminate familiar from unfamiliar conspecifics, and can do so using olfactory cues acquired indirectly. Experimenter-induced indirect familiarity has ramifications for the present study of social discrimination, as well as to studies of dispersal, reproduction, and territoriality.

Data obtained by fostering pups among litters so that non-siblings were reared together demonstrated that rearing association overwhelms any effect of kinship on

subsequent social discrimination. Further, individual juveniles that interacted with both littermates and non-littermates in arena tests were no more cohesive nor less agonistic toward littermates than non-littermates. Thus virtually no evidence of discrimination based specifically upon kinship was apparent.

The results obtained unambiguously support the notion that juveniles in their natural setting establish familiarity with local members of their colony during the pre- and post-weaning period through direct association and/or indirect exposure to olfactory discriminator substances. That level of discrimination could function to facilitate optimal outbreeding, promote cooperation among neighbours, and on average may ensure that access to resources is restricted to related individuals. The results also support the hypothesis that Columbian ground squirrel sociality is maintained by, and perhaps evolved in the context of benefits of social grouping that accrue via direct selection rather than through kin selection per se.

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Chapter 1: General Introduction

The Random House Dictionary of the English Language (1967), defines discriminate as "to note or distinguish as different". All life, from single cells through multicellular plants and animals display some capacity to discriminate among the range of stimuli they are exposed to. We infer that an organism is capable of discriminating among levels of a given variable when the organism displays a response that can be observed or measured with changes in that variable. Although often regarded as synonymous with discrimination, recognition (the identification of something as having been previously seen, heard, known, etc.; *ibid*) is actually a derivative, requiring an as yet unobservable process by which present stimuli are compared to those from the past (i.e. memory). Thus in the most strict sense, we can infer that organisms discriminate from the measurement of physiological processes or observation of their behaviour, but cannot infer that recognition underlies the observed discrimination (Byers and Bekoff 1986). Further, a lack of response in any given instance does not necessarily imply that recognition did not occur, since other factors may preclude the organism from responding to the alteration of extrinsic stimuli (Blaustein et al. 1987, Waldman 1988). In spite of those caveats, we tend to assume that where animals consistently display differential behaviour toward other organisms, a process of recognition underlies that discrimination.

Discrimination mechanisms are of paramount importance to an animal's biological fitness. Animals must select mates of their own species, and of the appropriate gender, and accurately assess the breeding status of a prospective mate to reproduce successfully. Animals also discriminate among conspecifics across myriad levels (e.g. group, neighbour, ally or rival, caste etc.; see Colgan 1983 for a review), all of which presumably aid in survival and reproduction. A relatively recent addition to that list is the discrimination of conspecifics on the basis of kinship. Hamilton (1964) and Maynard Smith (1964) extended Darwin's (1859) theory of natural selection by suggesting that animals could not only be successful in propagating genes via the production of their own offspring, but could also propagate like copies of their own genes by aiding in the reproductive effort of related individuals (i.e. kin selection). Since the persistence of species required that ancestors of present day forms behaved in such a way as to propagate their genes, and accepting a link between genes and behaviour (Dawkins 1976), we have come to expect that on average, behaviour will be suited to that purpose (i.e. will be adaptive). This notion has been referred to as the "central principle of behavioural biology" and states that animals are expected to behave so as to maximize their inclusive fitness (Barash 1982). Inclusive fitness refers to the sum total of genes propagated through the production of ones own offspring (direct fitness) and like copies of genes propagated through effects on the reproductive effort of related individuals (indirect fitness). The ability to discriminate kin from non-kin, at least to some extent, is essential for the maximization of either of those fitness components. To maximize direct fitness, animals should choose a mate to whom they are neither too closely nor too distantly related (Shields 1982, Bateson 1983). Further, if the species in question provides parental care, such care should typically be directed towards one's own offspring. Maximization of indirect fitness requires that cooperative and altruistic behavior be directed towards related individuals. It has often been stated that all other things being equal, the expression of such behavior should be distributed in proportion to the extent to which the actor and recipients are related. However, Altmann (1979) argued that indirect fitness would in fact be maximized by directing all altruistic behaviour solely toward the actor's closest relative. Although rightfully criticized as the adaptationist or "Panglossian" paradigm (Gould and Lewontin 1979), the assumption of adaptive value does provide a useful framework for the generation of testable hypotheses (Mayr 1983).

This theoretical framework has triggered a proliferation of empirical studies on kin-differential behaviour (Waldman et al. 1988) and the ability to discriminate kin from non-kin has been demonstrated in a wide range of animal taxa (for reviews see Colgan 1983, Holmes and Sherman 1983, Fletcher and Michener 1987, Waldman 1988, Hepper 1991).

Among the most extensively studied species are group-living, semi-fossorial rodents referred to collectively as the ground-dwelling squirrels (marmots, prairie dogs, and ground squirrels). From a purely logistical standpoint, ground squirrels are species whose biology is readily amenable to study since they are diurnal, are large enough that each individual can be given a distinctive mark that is readily discernable, can be easily trapped for marking or manipulation, and habituate well to human observers (Barash 1974). Ground squirrels are appropriate for studies of kin recognition since close kin are temporally and spatially available (Sherman 1981, McLean 1982, Michener 1983, King & Murie 1985, Waterman 1986, King 1989a); litters are raised in isolation of others prior to weaning (McLean 1978), a context that may facilitate the evolution of discrimination mechanisms (Michener 1983, Waldman 1988), and yearlings often remain in their natal area, interacting with the young of the year (Michener 1983, King & Murie 1985, King 1989a). Data suggesting a role of kinship in mediating spatial relationships, the nature of social interactions, the likelihood of emitting alarm calls, and the likelihood of committing infanticide have been obtained from studies of ground squirrels (see references in Holmes 1984, King 1989b). Further, ground squirrels are perhaps the only group of vertebrates for which the adaptive significance of kin discrimination in the natural context is reasonably well documented (Blaustein et al. 1991).

The potential fitness payoffs derived from assisting relatives suggest that kin selection can be regarded as a driving force in the evolution of sociality (Wilson 1975). Ground squirrels range from virtually asocial to highly social animals cooperating in the detection of predators and in the defense of resources (see Armitage 1981 and Michener 1983 for reviews). Because kin selection is facilitated by kin discrimination (see above), the ground-squirrels represent an ideal group for which comparative data can be obtained on the extent to which the development of kin recognition mechanisms parallel the level of sociality. Prior to my work, no systematic study of the level of discrimination or proximate mechanism underlying the discrimination of conspecifics by Columbian ground squirrels (*Spermophilus columbianus*), one of the more social ground-squirrel species (Armitage 1981, Michener 1983), has been conducted. Holmes (1984) and Sherman and Holmes (1985) predicted that the extent to which kin-discrimination mechanisms depend on direct familiarity should decrease with increasing sociality as measured by spatial association and the frequency of social encounters. Data garnered from naturally occurring social interactions among Columbian ground squirrels (King 1984, 1989a, 1989b; Waterman 1985, 1986, 1988) suggest that squirrels of this species discriminate kin from non-kin (see Appendix B).

Previous studies have focused on the potential proximate mechanisms of kin recognition in several ground squirrel species (see Sherman & Holmes 1985, Table 2 & Chapter 3); however, those studies do not address kin discrimination in a natural context. With only one exception, (Sherman's work on *S. beldingi*; see Holmes and Sherman 1982), research has been conducted in the laboratory with highly constrained conditions of rearing association. Under natural conditions, post-weaning association with other juveniles could affect observed patterns of kin discrimination. On the one hand, the sibling bond may be constantly reinforced by preferential association of juvenile siblings (Michener 1983) and the influence of their mother and non-littermate siblings in keeping the siblings more or less isolated from other litters (i.e. by excluding non-siblings from the natal area). Alternatively, any innate kin recognition or influence of pre-weaning association may be overwhelmed by effects of familiarity via interactions with non-littermate neighbours after emergence from the natal burrow. In addition to discriminating between siblings and non-siblings, juvenile squirrels may discriminate neighbouring non-siblings from less familiar non-siblings within their own colony and may also discriminate colony members from non-colony members. No previous study has examined the extent to which ground squirrels discriminate among non-kin. An investigation of kin recognition (at least at the outset) should include a consideration of this hierarchy of levels of discrimination since the precise "level" at which discrimination occurs can provide valuable insight into the mechanism employed in discrimination amongst individuals (see Chapter 3 and Appendix C).

To broaden our understanding of the comparative development of kin recognition in the ground-dwelling squirrels, I undertook research on kin discrimination of Columbian ground squirrels (*S. columbianus*). By working with a free-ranging population, but assaying discrimination abilities by staging dyadic interactions in an arena, data were obtained that address both the level and proximate mechanism of intraspecific discrimination by Columbian ground squirrel juveniles.

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Chapter 2: General Methods

Research was initiated in 1988 and proceeded from the spring emergence of the first squirrel each April until all juveniles had immerged in August of 1988 and 1989, or until all experimental trials were completed in July of 1990 (see Appendix A). The principal study site was a meadow in the Sheep River Wildlife Sanctuary, approximately 2 km east of the junction of the Sheep River and Gorge Creek (50°38' N, 114°37' W; elevation 1470m) in southwestern Alberta. This site (subsequently referred to as the hay meadow or HM) was a 1.9 ha area selected on the basis of high burrow concentrations and large numbers of squirrels in previous years (P.J. Young pers. comm.). The area is contiguous with a larger 72 ha meadow where Columbian ground squirrels were studied by Boag and Murie (1981), and Young (1988). The site forms a natural bench bordered on the north and west by a small (approx. 1 m) south-facing ridge of suitable squirrel habitat and on the south by a sheer face of loose scree and rock dropping some 50m to the Sheep River below (see Figure 2.1).

The plant community on the site was dominated by grasses, particularly fescue (*Festuca*), wheatgrass (*Agropyron*), mountain timothy (*Phleum alpinum*), bent grass (*Agrostis*), and brome grass (*Bromus*). Several dicot species present on the site, including dandelions (*Taraxacum*), yarrow (*Achillea*), cinquefoil (*Potentilla*), and clover (*Trifolium*), likely represented the most important food plants for squirrels (Murie unpublished data, Elliott and Flinders 1991). Other species present included; gentian (*Gentiania*), anemone (*Anemone*), Solomon's seal (*Smilacina*), meadow rue (*Thalictrum*), goldenrod (*Solidago*), mouse-ear chickweed (*Cerastium*), milk vetch (*Astragalus*), bedstraw (*Gallium*), violets (*Viola*), and horsetail (*Equisetum*). In addition to Columbian ground squirrels, pocket gophers (*Thomomys talpoides*) and voles (*Microtus* spp.) occupied the site. Potential predators of ground squirrels included coyotes (*Canis latrans*), domestic dogs (*Canis familiaris*), weasels (*Mustela* spp.), badgers (*Taxidea taxus*), red-tailed hawks (*Buteo jamaicensis*), goshawks (*Accipiter* *gentilis*), Swainson's hawks (*Buteo swainsoni*), golden eagles (*Aquila chysaetos*) and ravens (*Corvus corax*) (see MacWhirter 1989 for further accounts of predators observed in the general area).

The entire 1.9 ha area was overlaid with a 10 x 10 m Cartesian grid of colored flags on wire pins to provide reference for the location of individual squirrels and their burrows. Observations of squirrels in the field were conducted from two 3m high observation stands using Bushnell 10x50 binoculars or a Bushnell 60mm spotting scope. Squirrels were trapped with Tomahawk and National live traps (41x14x14 cm, 1x2 cm mesh or 48x16x16 cm, 2x2 cm mesh respectively) baited with peanut-butter and placed near burrows, mounds or along well-traveled paths through tall grass. All traps were fitted with cardboard covers to provide shade for captured squirrels. During 1988 and 1989, traps were moved about the principal site as required but in 1990 a strict protocol of trap dispersion was adopted (see Chapter 4). Squirrels were removed from traps for handling by transferring them to handling bags (ca. pillow case size) of cotton/polyester cloth and then removing them from the bag by hand. I fitted all trapped squirrels with numbered metal ear tags (Monel #1, National Band & Tag Co.) for permanent identification and applied a unique mark to their dorsal pelage (using Clairol Nice 'n Easy™ Blue-Black #124 hair dye) for identification in the course of observations. New ear tags were fitted occasionally if squirrels lost one of their tags; dye-marks were re-applied as required (approximately 3 times /summer) after moulting or when a mark faded.

Squirrels were weighed to the nearest 5 g with 1.5 kg Pesola or Long Jim spring scales at each capture. Since squirrels were unmarked at the onset of my study, individuals were designated as either yearlings or adults based on a comparison of their mass to those of squirrels of known age from the same area (Boag and Murie 1981). In 1988, squirrels <270 g were considered to be yearlings while all others were considered to be adults (see Appendix A). In 1989 and 1990, age-class was known for all individuals on the site.

In each year (1988, 1989 and 1990) breeding dates were obtained for virtually all females on HM by extensive trapping subsequent to their emergence and visually examining changes in external genitalia associated with estrous and breeding (see Murie and Harris 1982). Females were assigned an ordinal score ranging from 1 to 4 describing the degree of swelling of the vulva (1 least to 4 most swollen) and were further described by the extent to which the vagina was open (ranging from closed to fully open), and the qualitative appearance of the vagina itself (ranging from flat to "flowered"). Females typically bred as their vulva advanced beyond a 3 in swelling and were most frequently fully open and "slightly flowered" on the day they bred. Breeding was confirmed in virtually all cases by trapping females on the day they bred and finding copulatory plugs (Murie and McLean 1980) in the vagina and/or finding the fur surrounding the genitals "caked" with hardened ejaculate. Where females were not trapped until the day after their predicted breeding date, breeding was confirmed by finding remains of copulatory plugs, some caking of ejaculate or fur groomed completely away around the genitals, slight "puckering" of the vagina, or by obtaining a vaginal smear (Murie and Harris 1982) containing sperm.

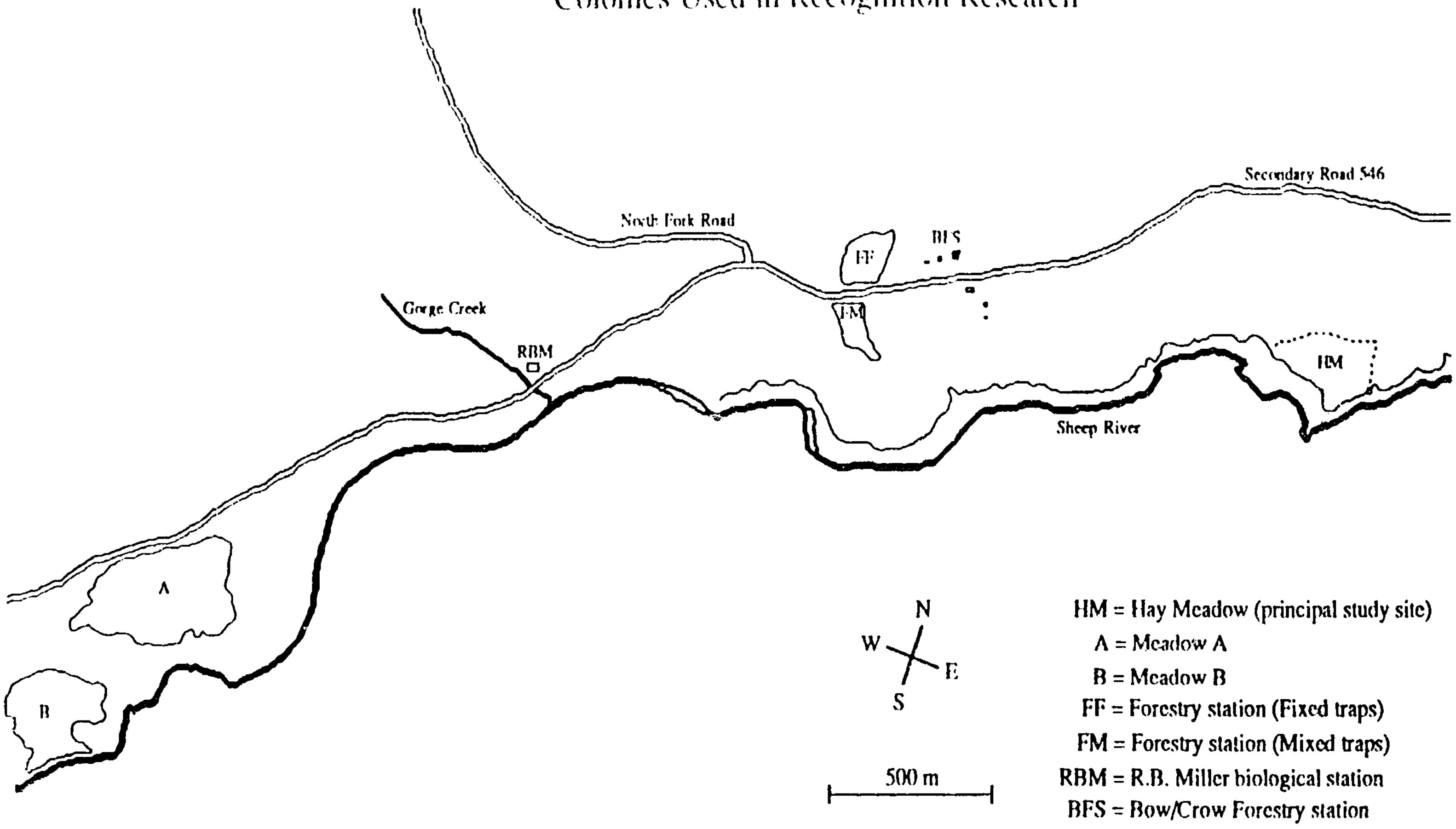
Breeding and the intensive trapping to obtain breeding dates continued from mid-April to early May each year (see Appendix A). Previous studies (Shaw 1925, Murie and Harris 1982) have estimated the length of gestation to be 24 days and thus the end of breeding roughly coincided with the onset of parturition each year. To obtain data on the length of gestation and to further improve my estimate of juvenile emergence (parturition + 30 days; Murie and Harris 1982), females were trapped each day for the 2 days prior, the day of, and 2 days following their predicted parturition date. Weight drops ranging from 20 to 160 g (see Appendix A) and a change in nipple appearance from swollen and black to swollen and pinkish with a red tip were regarded as indications that the female had delivered and was nursing a litter. I conducted observations (scan and focal animal sampling; Altmann 1974) during that time to locate nest burrows (McLean 1978) in which females were rearing their young, and manipulated litter size and composition in 1988 and 1989 (see Chapter 5 and Appendix I). Nipples were inspected each time a female was trapped subsequent to parturition. A change from swollen and pinkish nipples with red tips and fur matted in the surrounding area to flaccid and greying nipples with undisturbed fur suggested, but was not always reliably associated with the female losing her litter.

We scanned for the emergence of juveniles 2 days before and up to 3 days after their predicted emergence date by walking over the site (Dobson 1990) and watching for small, unmarked squirrels in the area of the nest burrow of a dam whose litter was due up. Juveniles were trapped using wire mesh multiple capture traps (1.0 x 0.5 x 0.3m), designed and constructed by J.M. Waterman, A.L. Steiner and J.O. Murie, that were placed over the nest burrow, or by saturating the area where juveniles were observed with live-traps. Juveniles that could not be trapped after 3 days using the above methods were hand-trapped by lying motionless in the grass next to a burrow the juvenile was using and grabbing the juvenile as it came above ground (Wiggett 1987). Upon first capture, the sex, weight, distinctive physical traits (e.g. missing toe-nails; see Chapter 5) and prospective dam of each juvenile were recorded. Juveniles were ear-tagged and given a dye-mark using the methods described for adults (above) with the exception that juveniles were given numbers rather than symbols. Numbers were assigned arbitrarily rather than in sequence to impose a "blind" on relatedness and rearing association in subsequent coding of behavioral interactions (see Chapters 3, 4 and 5). For the most part, the assignment of juveniles to a given dam was unambiguous given the precision of emergence date estimates and dispersion of nest burrows. Where there was any possibility of incorrectly assigning juveniles to dams (i.e. if 2 or more litters came up in the same general area within a few days), focal observations of the juvenile in question allowed its assignment to the appropriate dam. Observations of the behaviour of both prospective dams and the behaviour of prospective littermates toward that juvenile in addition to the burrow the juvenile immerged to in the evening and emerged from the next morning (i.e. which nest of the two or more prospective dams and litters it associated with) were used to that end.

Blood samples were collected from as many individual squirrels on the HM as possible in 1988 and 1989. Samples were obtained by clipping a toe-nail to the quick and collecting blood in an EDTA treated Vacutainer™ tube (not collected under vacuum). Those samples were treated for storage with a lysis solution of 100mM TRIS, 40mM EDTA, 1.0M NaCl and 0.5% SDS (unpublished protocol; Molecular Diagnostic Laboratory, Alberta Children's Hospital) and were kept refrigerated at 4 °C. Attempts were made in collaboration with Drs. Y. Plante and S. Schmutz of the Saskatchewan Research Council and University of Saskatoon respectively to obtain DNA "fingerprints" (Jeffreys et al. 1985, Burke 1989) to ascertain paternity of juveniles. Those attempts failed however since too many prospective sires copulated with dams relative to the amount of blood, and hence DNA, obtained for each adult male.

In 1988 and 1989, juveniles from an artificially established colony (meadow "B"; Wiggett and Boag 1986, MacWhirter 1989) 3 km west of the principal site were used in experiments (see Chapter 3 and Figure 2.1). Squirrels on that site were handled using similar methods to those outlined above as part of a study of vigilance and antipredator behaviour by *S. columbianus* (see MacWhirter 1989). In 1990 juveniles from a site approximately 1.5 km west of the hay meadow (referred to as FF and FM) were used in experiments (see Chapter 4 and Figure 2.1). Time constraints did not allow individual females to be followed through parturition on either FF or FM. Thus juveniles on those sites were marked with ear tags and dye marks as they became available without regard to their relationship to other squirrels within that site (see Chapter 4). All sites (HM, B, FF and FM) were similar with respect to potential predators and small mammal communities (see above), but B, FF and FM had plant communities which were richer in dicot diversity and abundance than HM (D.A. Boag pers. comm.).

Figure 2.1: Dispersion of Columbian Ground Squirrel Colonies Used in Recognition Research



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Chapter 3: The Level of Behavioral Discrimination Among Juvenile Columbian Ground Squirrels.

Introduction

The "central principle of behavioural biology" states that animals should behave so as to maximize their inclusive fitness (Hamilton 1964, Maynard Smith 1964). Inclusive fitness refers to the sum total of genes propagated through the production of ones own offspring (direct fitness) and like copies of genes propagated through effects on the reproductive effort of related individuals (indirect fitness). The ability to discriminate kin from non-kin is essential for the maximization of either of those fitness components. To maximize direct fitness, animals should choose a mate to whom they are neither too closely nor too distantly related (Shields 1982, Bateson 1983). Further, if the species in question provides parental care, such care should be directed towards one's own offspring. The maximization of indirect fitness requires that any cooperative or altruistic behaviour be directed towards kin (i.e. be nepotistic).

This theoretical framework has triggered a proliferation of empirical studies on kin-differential behaviour (Waldman et al. 1988), and the ability to discriminate kin from non-kin has been demonstrated in a wide range of animal taxa (see Fletcher and Michener 1987, Hepper 1991 for reviews). In the ground-dwelling squirrels, kinship is known to affect spatial relationships, the nature of social interactions, the likelihood of emitting alarm calls, and the likelihood of committing infanticide (see references in Holmes 1984). Ground squirrels represent the only vertebrate group for which the adaptive significance of kin discrimination has been documented in the natural context (Blaustein et al. 1991). Female offspring often show philopatry to their natal area (see Dobson 1982, Holekamp 1986 for reviews) sharing in resources (burrow systems, hibernacula) of their dams. Kin are thus available to cooperate in the detection of predators (Sherman 1977, 1980) and in excluding intruding conspecifics from their territories (Sherman 1980).

A variety of proximate mechanisms allow kin discrimination and ultimately facilitate kin selection in ground squirrels. In thirteen-lined ground squirrels (*Spermophilus* *tridecemlineatus*), association prior to weaning completely overrides any effect of genetic relatedness and indeed siblings reared in separate litters behave as if they were non-siblings (Holmes 1984). Thus familiarity is both sufficient and necessary to the normal ontogeny of "kin" recognition in that species. Conversely in Richardson's ground squirrels (*S. richardsonii*), pre-weaning association exerts a lesser influence on the development of "kin" differential behaviour, and siblings reared apart retain the ability to discriminate kin from non-kin (Davis 1982). The situation in Arctic (*S. parryii*) and Belding's ground squirrels (*S. beldingi*) is intermediate to those extremes with sibling females demonstrating the ability to recognize one another in the absence of rearing association, while non-siblings reared in the same litter behave as full siblings (Holmes and Sherman 1982). In Arctic and Belding's ground-squirrels then, familiarity is sufficient to induce animals to behave as "kin" but is not necessary for kin recognition proper among females. For Belding's ground squirrels, littermate full-sisters are also discriminated from maternal-half sisters (Holmes and Sherman 1982). Although Grafen (1990, 1991a, 1991b, 1991c) has argued that only those species that discriminate kin from non-kin "by genetic similarity detection" (e.g. settlement and fusion in *Botrylus schlosseri*; Grosberg & Quinn 1986) recognize kin in the strict sense, it is important to realize that kin-aligned behaviour, and the benefits that accrue via kin selection, can be brought about via indirect mechanisms (e.g. familiarity, spatial proximity) and may rely on cues that are not mediated genetically (e.g.environmental cues) but that are reliably correlated with kinship (Byers and Bekoff 1991, Stuart 1991).

Although earlier studies of ground squirrel social recognition identify the potential proximate mechanisms for kin-differential behaviour, they do not address the ontogeny of kin discrimination in the natural context. With only one exception, (Sherman's work on *S. beldingi*; see Holmes and Sherman 1982), those studies were conducted in the laboratory with highly constrained conditions of rearing association. Under natural conditions, post-weaning association with other juveniles could affect the pattern of kin discrimination. On the one hand, the sibling bond may be constantly reinforced by preferential association of juvenile siblings (Michener 1983) and the influence of their mother and non-littermate siblings in keeping the siblings more or less isolated from other litters (i.e. by excluding non-siblings from the natal area). Alternatively, any innate kin recognition or influence of pre-weaning association may become obscured by interactions with non-littermate neighbours after emergence from the natal burrow. Further, such studies may fail to resolve behavioral discrimination at levels that rely on environmentally-acquired discriminator substances. No previous study has directly examined the extent to which ground squirrels discriminate among non-kin. In addition to discriminating between siblings and non-siblings, juvenile squirrels may discriminate neighbouring non-siblings from less familiar non-siblings within their own colony and may also discriminate colony members from non-colony members. The precise "level" at which discrimination occurs can provide valuable insight into the mechanism employed in discrimination amongst individuals (see below and Appendix C).

Kin are both spatially and temporally available in Columbian ground squirrel (*S. columbianus*) colonies (Michener 1983, Murie and Harris 1984, King 1984, 1989a, King and Murie 1985). However, King (1984) reported that adult female kin formed aggregations rather than the dense "kin clusters" predicted by Michener (1983). In King's research, the only significant spatial attraction among female adults for close over more distant kin was detected between 24 and 60 days after juvenile emergence. She concluded that aggregation during that period may reflect a common attraction to natal areas and their resources (i.e. burrow systems, hibernaculae) rather than to matrilineal relatives proper. Unlike adult females, juvenile and non-breeding yearling sisters tended to associate closely throughout the active season (King 1984).

Studies examining interactions between free-living Columbian ground squirrels have documented kin-differential behaviour. Steiner (1973) reported that allogrooming (see Appendix B) occurs most frequently among "well-acquainted" members of the same social group with the highest frequencies between dams and their offspring and between littermate juveniles (Steiner 1970). King (1984, 1989b) reported that relationships between littermate sisters were typified by play (see Appendix B), and were less agonistic than interactions between non-littermate siblings and non-siblings. Waterman (1985, 1986, 1988) found that play was the most common social behaviour among juvenile Columbian ground squirrels, and that for the first 20 days subsequent to juvenile emergence, siblings played significantly more frequently than non-siblings. Further, sisters played most often, intersexual pairs played somewhat less frequently and brothers played the least (Waterman 1986).

Despite data suggesting kin-biased behaviour among dam-offspring pairs and littermate juveniles, recent studies of Columbian ground squirrels have failed to resolve any influence of kinship on behaviour nor any apparent fitness benefits accrued through associating with kin. MacWhirter (1989) found that the likelihood of emitting alarm calls in response to simulated terrestrial and avian predator attacks was not higher for squirrels with close nondescendent kin present than for those without kin. However, parous females were more likely to call in response to the terrestrial predator model than were nonparous females. MacWhirter (1989) correctly interpreted that finding as a component of maternal care rather than nepotism per se. King et al. (1991) found no difference in the reproductive success of females with adult matrilineal kin present in the same colony relative to those without matrilineal kin present. Murie and King (pers. comm.) found no influence of the mother's presence on the dispersal distance of yearling females and no effect of the presence of close kin (mother, daughter, sister) on the spatial dispersion of nest burrows. Further, Murie and King (*ibid*) reported no significant effect of the presence of close kin on current reproductive success; any trends apparent suggested weak negative effects. Given these recent findings, the level at which Columbian ground squirrels discriminate among conspecifics merits further examination.

In this study, behavioral data were collected from interactions between littermate sibling, neighbouring, non-neighbouring non-sibling colony members, and non-colony member pairs of juvenile Columbian ground squirrels. The extent of agonistic and cohesive behaviours were recorded to assay the level at which juveniles discriminate among conspecifics. All possible gender-pair combinations were examined to address any sex bias in the expression of nepotism.

Methods

Squirrels were studied on a 1.9 ha meadow in the Sheep River drainage of southwestern Alberta (50°38' N, 114°37' W; elevation 1470m; see Chapter 2, Figure 2.1). The area is contiguous with a larger 72 ha meadow where Columbian ground squirrels were studied by Boag and Murie (1981) and Young (1988). The entire 1.9 ha area was overlaid with a 10 x 10 m Cartesian grid of plastic flags on wire pins to provide reference for the location of individual squirrels and their burrows. Squirrels were trapped using Tomahawk and National live traps (41x14x14 cm, 1x2 cm mesh or 48x16x16 cm, 2x2 cm mesh respectively; 70 traps used as needed throughout the site) baited with peanut butter from the time of spring emergence of the first squirrel in 1988 and 1989 (see Appendix A). All individuals were ear-tagged for permanent identification with numbered fingerling fish tags (National Band & Tag Co., Monel #1) and were marked uniquely with hair dye (Clairol Nice n' Easy™, Blue Black #124) applied to their dorsal pelage for identification during observations. Breeding dates were established for females on the site each year following the techniques outlined in Murie and Harris (1982; see Chapter 2). The addition of 54 days to the breeding date (24 days for gestation and 30 days for lactation; Shaw 1925, Murie and Harris 1982; but see Appendix A) estimates the date of juvenile emergence. By focusing on nest burrows of dams with litters due to emerge, juveniles were trapped and marked as they emerged from their natal burrow. Thus in all cases juveniles were assigned to their dams unambiguously. Care was taken to avoid dye-marking juveniles within litters with sequential numbers, thus avoiding potential observer bias in subsequent coding of behavioral interactions (see below).

To ascertain the level at which juveniles discriminate among conspecifics and to shed light on the proximate mechanism underlying discrimination (see Appendix C), behavioural interactions among juveniles were examined. Juvenile Columbian ground squirrels rarely stray more than 10m from their natal burrow within the first 10 days post-emergence (Waterman 1985). Subsequent to that, they center their activities about the natal burrow, but expand their forays to a maximum mean distance of 33 m about that central area during their first season above ground (Waterman 1985, based on regression equation at 60 days post emergence in her Figure 2.1). *Ad libitum* sampling of juvenile activity from 2-3m high observation stands throughout that period confirmed that the range of juvenile movements in my population during the years of study were consistent with those described by Waterman (1985). Based upon relatedness, rearing association, and post-weaning familiarity via interactions with conspecifics (or their odours) over the course of their movements, 4 classes of relatedness/association were distinguished. Juveniles emerging from a common natal burrow were considered siblings reared together (SRT), although this class would also include maternal half siblings. Those juveniles are closely related and directly familiar both during the pre- and post-weaning period. Juveniles emerging from natal burrows within 40m were considered neighbours (non-siblings reared apart close; NSRAC), although this class also includes paternal half siblings since males and females of this species mate multiply (Murie and Harris 1978, Murie pers. comm., Hatfield pers. comm.). Those juveniles are at most related at the level of half-siblings and are directly familiar with one another during the post-weaning period. Juveniles originating from nests more than 70m apart on the same colony were regarded as non-siblings (but are at most distantly related) and would not be directly familiar with one another (non-siblings reared apart distant; NSRAD). Finally, juveniles originating from, and residing in a second colony (Meadow "B", 3 km west of the principal site; see Chapter 2, Figure 2.1; 60 traps used throughout site) were regarded as non-siblings that would not be closely related and that would not be familiar directly, indirectly, nor be exposed to common environmental odors (non-siblings reared apart in 2 different colonies; NSRA2).

Based on a review of the literature documenting behavioral interactions in Columbian ground squirrels, ground squirrels in general, and other rodents, the potential behaviours of squirrels in the arena were operationally defined prior to the collection of any data (Appendix B). Sixty behaviours were outlined and those were collapsed into 7 categories with certain behaviours in each category considered as "key" behaviours. Key behaviours were those that could be identified unambiguously and for which specific predictions regarding recognition-differential behaviour could be made based on the existing literature for Columbian ground squirrels or underlying theory. The categories and their associated key behaviours were:

Recognitive behaviour; the focal animal's nose in contact with any part of the object animal's body; no key behaviours distinguished.

Cohesive behaviour; prolonged physical contact or proximity without forced displacement of one animal by the other; allogroom, play fight, play mount, and follow were considered key behaviours.

Agonistic behaviour; overt aggression, threat or submission often involving displacement of one animal by the other; arch back, lateral display, tail bush, run at, lunge, ball fight, jump back, and bite were considered key behaviours.

Investigative and escape behaviour; behaviours directed towards the physical elements of the arena itself; no key behaviours distinguished.

Scent related behaviour; behaviours which in the field have been associated with the deposition of a scent mark or those which in the context of the arena may be associated with the reception of scent; no key behaviours distinguished. Since scent marking was rarely seen in the arena, data were categorized only for scent reception (Appendix B).

Miscellaneous vocalizations; any vocalization not immediately interpretable as being directed towards or in response to the action of the other animal; no key behaviours distinguished.

Miscellaneous activities and postures; behaviours which don't readily fit into the above categories; no key behaviours distinguished. For the purpose of analysis, behaviours in this category were separated into 2 subcategories, active and resting (Appendix B).

Cohesive and agonistic behaviour are, *a priori*, the most suitable behavioural assays of recognition (Appendix B). In general, where animals recognize one another, I predicted that they would behave more cohesively and less agonistically than animals that did not recognize each other. Further, I predicted that animals recognizing one another would remain closer together over the course of trial than those that did not recognize each other.

Previous studies quantifying behavioral interactions of juvenile Columbian ground squirrels (King 1984, 1989b, Waterman 1985, 1986, 1988) have employed behaviour sampling (Altmann 1974) to examine discrimination in the field. In spite of the obvious virtue of their direct application to the natural context, those studies are plagued by problems that arise from the pooling fallacy (Machlis et al. 1985) since individual squirrels contribute multiple observations to the data set. Further, the collection of "naturalistic" data precludes balancing such factors as sex-of-pair, and the data themselves are confounded by the influence of other squirrels, or site-specific cues that render the area on which individuals interact non-neutral. Finally, individuals residing at distances over 40m within a colony rarely interact and those in different colonies never interact during their first year above ground. Therefore to document the behaviour of individuals in the 4 classes outlined above, dyadic interactions were staged in a neutral arena (see Davis 1982, Holmes and Sherman 1982, Holmes 1984).

The arena was 2.25 m^2 (1.5m square, 1m in height) and constructed from clear plexiglass. A removable opaque plexiglass partition divided the arena in half and allowed animals to be isolated from each other prior to testing. The substrate was a brown linoleum sheet with a 10 x 10 cm Cartesian grid applied with a permanent-ink marker to facilitate recording distances between interactants. Subsequent to each trial, any scats were removed and the substrate and walls of the arena were washed thoroughly with warm water and dried with disposable paper towels. The arena was situated in a tent platform at the R.B. Miller Biological Station, approximately equidistant between the meadows (HM and B; see Chapter 2, Figure 2.1) from which squirrels were obtained. The tent itself covered all but the northernmost side of the tent platform, thus eliminating most direct sunlight and reducing any distractions to individuals in the arena (e.g. birds flying overhead, trees moving, red squirrels (*Tamiasciuris hudsonicus*) or chipmunks (*Eutamius amoenus*) moving along overhanging branches). A single light fixture equipped with a 500 watt bulb (Sylvania B-2 superflood) was suspended 1.75m above the center of the arena and provided even illumination of the arena at an absolute minimum of 48 foot candles (readings taken 10cm from substrate on a moonless night with General Electric Light Meter Type 214). Direct sunlight at that elevation at 1200 h on 30 July 1990 registered 1000 foot candles while readings taken in the arena were even across the substrate at 72 foot candles on that day. All trials were videotaped using a Minolta V-1400 VHS camcorder suspended 4m above the arena floor and filming through the open, northern end of the tent. This arrangment provided a full screen image of the entire arena at an angle allowing maximum resolution of the animal's behaviour. Squirrels were also observed directly during trials through one of two viewing holes (10 x 15cm) cut in the eastern side of the tent. The observer noted any audible ambient noise and its time of occurrence, and also noted any behaviours among the interactants that might be difficult to discern from the videotape.

In 1988, 6 to 10 squirrels (enough for 3-5 trials) were trapped from the two sites each morning (0700 to 1200 h) and were transported to the field station in their live-traps covered with cloth bags in a 2 row pile (position arbitrary) on an automobile seat. Animals were then housed while awaiting trial inside their traps under a plywood cover with each individual separated by a fabric partition. Individuals were returned to their colony and released at the point of capture after all trials for that day were complete. Given the potential for olfactory and/or auditory communication among individuals in transit or awaiting trial, and to reduce any holding effect on the animals, that protocol was modified in 1989. In 1989, only 2 juveniles were returned for trial at any one time. Those individuals were also transported in their traps covered with cloth bags but were separated by the upright portion of the front automobile seat while in transit. They were not held for any length of time prior to trial. While that pair of individuals were being run in the arena, an assistant returned to the field site to trap the next pair of squirrels, and returned and released the pair of squirrels from the previous trial at their respective points of capture. The return of the next pair typically coincided with the completion of the previous trial so that trials were run concurrently with the subsequent pair being trapped.

For each trial, the videocamera was secured in position, the light above the arena was turned on, and recording was commenced. Juveniles were placed on opposite sides of the opaque partition and allowed 10 min to acclimate to the arena. After that time, the partition was removed and juveniles were allowed to interact freely for a period of 30 min (acclimation and trial length determined via pilot trials with juvenile squirrels from the station grounds). Trials were staged with respect to two principle independent variables from the perspective of the focal animal; relatedness/association (SRT, NSRAC, NSRAD, or NSRA2), and gender pair (female-female, female-male, male-female, or male-male). Physical factors were recorded for each trial including: the maximum and minimum temperature inside the tent platform, the wind speed (subjectively as an ordinal ranging from 1 to 6), the extent of cloud cover (a subjective ordinal ranging from 1 to 3), and precipitation during the trial (none, drizzle or after-rain). Factors intrinsic to the focal animal were also quantified in each trial including; the number of days post emergence (age), and the mass of each interactant subsequent to trial (using a Pesola spring scale). Other independent variables recorded included; the animal's site of origin, the time of trial, the order of the trial within day, and the number of times each individual had appeared in the arena. Due to the limited number of animals available for testing each year, individuals appeared in the arena up to 2 times as an "object" animal before data were collected on it as a focal individual. Wherever possible, focal data were collected on an animal's first appearance in the arena. Where individuals participated in trials more than once, their appearances in the arena were separated by at least 2 days and individuals were never run more than once in any one relatedness/association class.

Videotaped interactions were viewed using an Akai VS-35U video-cassette player and Electrohome 33CC11 14" colour television. Behavioral data were coded using a microcomputer event recorder program written in BASIC for the Macintosh™ computer. Because the records were long, fixed-interval point sampling (Martin and Bateson 1986) was employed using a 3 sec interval length (sampling method determined via pilot coding sessions and comparison of measures obtained to a continuous record; Appendix D). The program prompted the user to input 1 of 60 possible behaviours (Appendix B) on the sample point of each time interval (via clicking the "mouse" button after positioning the cursor over an on screen "button" corresponding to the appropriate behaviour) and at the end of the coding session produced scores (proportion of the 600 sample points on which each behaviour occurred) for those behaviours over the entire 30 min. Scores from the 60 individual behaviours were summed into categories for the purpose of analysis as outlined above (see Appendix B).

Spatial data were coded using a BASIC program that sampled the position of the two individuals at ten second intervals throughout the trial. On each sample point, the computer prompted the user to position the cursor and click the mouse button at points on an "on screen" representation of the 10 x 10 cm grid that corresponded to the squirrels position on the actual grid in the arena. The 180 distances were then combined to produce a mean and associated variance for the distance between individuals in each trial.

In total 42 of 80 trials conducted between 12 July and 15 August 1988, and 39 of 68 trials conducted between 5 July and 29 July form the data set of 81 cases used in subsequent analyses. The remaining trials from 1988 were excluded to avoid allowing one individual to contribute more than one observation to the data set (see Machlis et al. 1985). In 1989 trials for this experiment were staged so as not to commit the pooling fallacy (*ibid*), and the remaining 1989 trials provide data for other experiments (see Chapter 5).

Behavioral data (proportions) were transformed using an arcsine square root transformation to normalize their distribution prior to analysis (Zar 1974). All analyses used parametric statistical procedures where the underlying assumptions of normality and homoscedasticity were met or where sample sizes were approximately equal in the levels of the factor under consideration (Zar 1974, Sokal and Rohlf 1981). Otherwise the analagous nonparametric statistical technique was employed (Siegel 1956). Analyses were performed using Statview, Super ANOVA or Systat on a Macintosh computer. Results were considered significant where the probability of type I error (alpha) was less than 0.05. Post-hoc pairwise comparisons were performed using the sequential Bonferroni technique recommended by Rice (1989) for an overall experimentwise error rate (alpha) of 0.05. Results are presented as mean ± SE unless otherwise noted.

The influence of all potential independent variables was assessed by performing single-factor ANOVA (if a categorical variable) or simple linear regression (if a continuous variable) pooling across all other independent variables on each of the 60 dependent variables (behaviours). An independent variable was considered to have an "effect" if a significant between groups difference (or significant regression) was detected for any 1 "key" behaviour (see above) or if significant differences were detected for ≥ 10% (=6) of the behaviours overall. Although performing multiple univariate tests increases the probability of committing type 1 error (Zar 1974), it was my desire to be conservative in concluding a given independent variable had no effect. Where independent variables exerted an effect, parametric analysis of variance or the Kruskal-Wallis test were used to test whether the independent variable was balanced across the main factors of interest (relatedness/association, gender pair). Where independent variables were well balanced across the levels of the principal factors, they would not exert any systematic bias in elucidating the effects of relatedness/association or gender pair. Finally, a simultaneous multiple regression was performed, regressing all dependent variables on the entire suite of independent variables. Due to the limited number of cases (n=81), the results of those multivariate tests must be interpreted cautiously; however, those tests were performed only as a means of confirming the above analyses with respect to the simultaneous effect of all variables on behaviour. Where independent variables exerted an effect and were unbalanced across the principal factors of interest, analysis of covariance was performed to factor out the influence of those confounding variables (Cochran 1957).

Results

Which Independent Variables Influenced the Behaviour of Juveniles?

Of the independent variables (temperature, wind speed, cloud cover, precipitation, age, mass, trial within day, number of times run, site, year, gender pair, relatedness/association), only relatedness/association, the mass of the focal animal, the number of days post-emergence (age), the number of times animals were run, and the trial within day were found to have significant effects on behaviour (see Appendix E). The apparent effect of mass however disappears when NSRA2 are excluded from the analysis. This suggests that the mass effect is largely due to the high correlation between mass and relatedness/association groups (animals from the second site were heavier) and that mass per se does not exert a strong influence on behaviour (see Appendix E). Such was not the case for age which had an influence on 9 (mostly recognitive) of the 60 behaviours and was not balanced across relatedness/association groups (NSRA2 older on average than other groups). Aggression, recognitive behaviour, and one key cohesive behaviour increased with advancing age. Juveniles ranged between 9 and 61 days post-emergence at the time of testing (mean±SE; 30.7 ± 1.6). Given its potential confounding influence, age was used as a covariate in the analysis of relatedness/association effects on recognitive, cohesive, and agonistic behaviour.

Marginal effects of trial within day and the number of times animals were run were detected and those variables tended toward imbalance across relatedness/association groups (see Appendix E). Specifically, certain cohesive behaviours tended to increase in frequency with trial within day and number of appearances in the arena. Activity in general also increased with increasing number of appearances in the arena (Appendix E). Thus both trial within day and the number of times animals were run (for cohesive behaviour), and the number of times animals were run (for active behaviour) were treated as covariates in the analysis of relatedness/association data in those categories.

Most notably, no gender-pair differences were detected for any of the 60 individual behaviours, and significant gender pair x relatedness/association interactions were detected for only 3 of the 60 behaviours (see Appendix E). Thus gender pair levels (and levels of other factors that did not exert a significant influence on behaviour) were pooled in analysing the relatedness/association data.

Multiple regression analyses largely confirmed the role of independent variables outlined above. Relatedness/association emerged with the highest partial correlations with the majority of key behaviours (see Appendix E).

I used analysis of covariance to examine the potential effects of relatedness/association in isolation from the effects of age, trial within day, and number of times run. With the exception of agonistic behaviour, categorized data met the parametric assumptions of normality and homoscedasticity. Further, parametric tests were used (even for agonistic behaviour) since sample sizes were almost equivalent among relatedness/association groups (see below). The homogeneity of slopes assumption was met for all data subjected to ANCOVA (all $P > 0.05$).

Relative Frequency of Behaviour Categories in the Arena

Squirrels were inactive (resting) for the majority of the time (56.8%) they were in the arena (Figure 3.1). Social interactions accounted for only 14.1 % of the behaviours sampled, with recognitive contacts occurring on 10.5%, cohesive behaviour on 3.3%, and agonistic behaviour on 0.3% of the sample points (Figure 3.1). Investigative (escape related) behaviour occurred on 7.1%, behaviours associated with scent reception on 4.4% , and other active behaviours on 7.9% of the sample points coded. Miscellaneous vocalizations and other behaviours that were not included in the 7 major categories were pooled into the category "other", and accounted for 9.7% of the behaviours sampled.

Relatedness/Association Effects

Recognitive behaviour: When adjusted for the influence of age, relatedness/association groups differed significantly in the frequency of recognitive contacts ($P = 0.0485$). Non-siblings from separate colonies (NSRA2) displayed significantly fewer recognitive contacts than non-siblings reared more than 70m apart in the same colony (NSRAD; $P = 0.041$) and somewhat less recognitive behaviour than neighbours (NSRAC) or siblings (SRT), although the latter differences were not statistically significant (see pairwise comparisons, Figure 3.2). Neighbours and siblings showed virtually the same amount of recognitive behaviour, while distant non-siblings within colony showed the highest frequency of recognitive behaviour (Figure 3.2).

If recognitive data are not adjusted for the age of the focal animal, no difference is found among the 4 relatedness/association groups (*P* =0.6481; Appendix F, Figure F.1). Further, no difference among those groups were detected for any of the 8 individual behaviours comprising the recognitive behaviour category (Table 3.1).

Cohesive behaviour: A highly significant difference (*P* =0.0003) among the 4 relatedness/association groups was detected for cohesive behaviour when those data were adjusted for the effects of age, trial within day, and the number of times individuals had appeared in the arena. Juveniles from two different colonies displayed significantly fewer cohesive acts than non-neighbouring colony members, neighbours, or siblings (Figure 3.3). All intracolony groups had similar, relatively high levels of cohesion (Figure 3.3).

An overall significant difference (*P* =0.0387) persisted among the 4 groups without adjustment for the covariates, although a statistically significant pairwise difference remained only between non-colony members and siblings (see Appendix F, Figure F.2). Juveniles from separate colonies showed the least cohesive behaviour, non-neighbouring colony members and neighbours displayed an intermediate level (although not significantly different from siblings), while siblings had the highest frequency of cohesive behaviour.

Juveniles in all 4 groups allogroomed with approximately equal frequency (Table 3.2). A non-significant trend was apparent for following, with siblings, non-neighbouring colony members, and to some extent neighbours following somewhat more frequently than members of two different colonies (Table 3.2). Statistically significant differences were detected for play mounting and play fighting. Juveniles from two different colonies displayed the least amount of both play behaviours, neighbours and spatially distant colony members showed a higher but somewhat intermediate amount of play, while siblings played most frequently (Table 3.2). Post-hoc comparisons on those behaviours revealed that siblings play mounted and play fought significantly more frequently than juveniles from different colonies (*P* =0.025 and *P* =0.014 respectively); no other levels were significantly different (all *P* >0.05).

Agonistic behaviour: The frequency of agonistic behaviour differed significantly among the 4 relatedness/association groups when those data were adjusted for the age of the focal animal (*P* =0.0003). Juveniles from different colonies showed significantly more agonism than any of the intracolony conditions (Figure 3.4 pairwise contrasts). Non-neighbouring individuals from the same colony, neighbours, and siblings showed the same low levels of aggression (Figure 3.4). Those differences, remain unchanged if the agonistic data are not adjusted for age (Appendix F, Figure F.3).

Trends consistent with the categorical data are apparent for all 8 key agonistic behaviours, but not for the 3 non-key agonistic behaviours (Table 3.3). Differences among the 4 groups were statistically significant for tail bushing and lunging.

Investigative behaviour: Juveniles in all 4 relatedness/association groups engaged in similar amounts of behaviours associated with escape from the arena (*P* =0.524, Figure 3.5). All 5 individual behaviours comprising this category were consistent in that lack of difference (Table 3.4).

Scent Reception: No significant difference among the 4 relatedness/association groups was detected for the frequency of scent reception behaviour (*P* =0.488, Figure 3.6). Further, none of the 5 individual behaviours comprising this category gave any indication of a diffference among the 4 relatedness/association groups (Table 3.5).

Active: Whether adjusted for the number of appearances in the arena or not, no significant difference in general active behaviour of squirrels was detected among the 4 relatedness/association groups (*P* =0.479, Figure 3.7, and *P* =0.437, Appendix F, Figure F.4 respectively). Further, no differences were detected among groups for any of the 3 individual behaviours underlying this category (Table 3.6).

Resting: Juveniles in the 4 relatedness/association groups were inactive in the arena (resting or alert posture) for equivalent proportions of time (*P* =0.478, Figure 3.8). No differences among the 4 relatedness/association groups were detected for any of the 3 individual behaviours pooled to form this category (Table 3.7).

Spatial Relationships: When adjusted for the age of the focal animal, the average distance between interactants in the arena differed significantly among the 4 relatedness/association groups (P =0.003). Juveniles from separate colonies were significantly farther apart over the course of trials than non-neighbouring juveniles from the same colony (P =0.001) and tended to be farther apart on average than neighbours (P =0.098) or siblings (P =0.059) (Figure 3.9). Non-neighbouring animals from the same colony were closer together on average over trials than any other relatedness/association group, although the differences between that group and neighbours or siblings were not statistically significant (Figure 3.9, pairwise comparisons). If spatial data are not adjusted for age, any difference among the 4 relatedness/association groups is no longer apparent (P =0.693, Appendix F, Figure F.5).

Discussion

Social interactions among juvenile Columbian ground squirrels were rare in the arena relative to non-social behaviour. Further, too few of any individual social behaviours may be scored in any condition to resolve a statistically significant difference among groups for those behaviours individually (i.e. a floor effect; but note even a rare behaviour may have drastic effects on social interactions in their natural context). However, where several behaviours function in the same manner, they can be pooled into an operational category with a greater range in frequency that would allow the detection of differences among groups. There was a high degree of internal consistency among individual behaviours pooled to form the 3 categories of social behaviour; recognitive, cohesive, and agonistic. Thus the social behaviour categories present reliable assays of recognition (see Appendix B).

Differences among the 4 relatedness/association groups occurred only in the categories for which *a priori* predictions about recognition-differential behaviour were made. Sibling, neighbour, and non-neighbouring colony members showed similar high levels of cohesive behaviour and little aggression while juveniles from separate colonies showed significantly less cohesion and significantly more aggression than intracolony dyads. Further, spatial relationships among juveniles in the 4 relatedness/association groups generally supported those behavioral differences with intercolony pairs maintaining the greatest average distance relative to the lesser average interindividual distances among intracolony pairs. However, if spatial data are not adjusted for the age of the focal animal, no difference in average interindividual distance is apparent among the 4 groups. Given the drastic influence of the covariate on the spatial data, that apparent difference in spatial relationships must be interpreted cautiously.

Similarly, without adjusting for the effects of age, trial within day, and number of appearances, the cohesive data indicate that siblings show somewhat higher (but not statistically significant) levels of cohesion than other intracolony pairs. In spite of that, the overall trend remains unchanged with intracolony pairs behaving more cohesively than intercolony pairs. Any trend toward greater cohesion among littermates could result from kin recognition in the strict sense (i.e. genetic similarity detection, Grafen 1991a, 1991b, 1991c) but is most parsimoniously interpreted as resulting from greater familiarity among littermates who have shared a common uterine environment (but see vom Saal 1984) and have been isolated from other juveniles in a nest burrow during a 30 day lactation period. Further support for that contention derives from a comparison of the present findings to those of Waterman (1985). Waterman (1985; page 31, Figure 2.6) found significantly greater rates of play among siblings than among non-sibling neighbours for the first 20 days post-emergence. That difference was no longer significant after 20 days, and persistently waned with increasing age so that at 51 to 60 days post-emergence, play rates did not differ (although exceedingly low rates of play were measured for all pairs at that point). If data from the present experiment are restricted to the first 20 days post-emergence, 6 littermate pairs and 5 neighbour pairs remain for analysis and a strong but non-significant trend toward greater cohesion and lesser aggression among littermates relative to neighbours in those data are apparent (Appendix G). As with Waterman's data however, inclusion of older juveniles obscures that trend, and suggests that with time neighbours (and other colony members, see above) are incorporated into a class of familiar animals that are not discriminated from littermates. Waterman (1985, 1986) also reported that sibling (littermate) females played more frequently than intersexual pairs of littermates, and these in turn played more frequently than male littermate pairs. There is some indication of that pattern in my data, but again that difference falls out more in line with direct familiarity rather than relatedness per se. Both littermate and neighbour female pairs play-fought more frequently than female pairs in the other relatedness/association conditions, and that difference was most pronounced for female-female pairs, less so for intersexual pairs, and least for male-male pairs (see gender pair x relatedness interaction; Appendix E). The similarity between the results obtained in the arena and those of Waterman (1985) for unrestricted juveniles further suggests that arena interactions are representative of those that occur among free-living juveniles. For the majority of behaviours, littermates and neighbours were virtually indistinguishable.

Whether categorized or treated as individual behaviours, and regardless of adjustment for concomitant variables, juveniles from separate colonies behaved more aggressively than non-neighbouring colony members, neighbours, or littermates which showed similar, low frequencies of aggressive behaviour. Taken together with the greater cohesion among colony members relative to non-colony members, juvenile Columbian ground squirrels apparently come to discriminate colony members from non-colony members. That level of discrimination does not rely on site-specific cues present at the time of interaction since no such cues were available in the neutral arena. The mechanism underlying colony member discrimination could rely upon greater genetic similarity (and hence greater similarity in individually produced discriminator substances) within colony, environmentally acquired cues that differ among colonies, odour mixing among individuals so that a "gestalt" colony odour is formed (Crozier and Dix 1979, Crozier 1987, Stuart 1987), or a combination of both direct and indirect familiarity via which juveniles in the arena treated individuals as either familiar or unfamiliar.

Recognition based on genetic similarity detection seems unlikely given 1) the complexity of recognition alleles in general and their potential "outlaw" nature (Alexander and Borgia 1978; but see Blaustein 1983), 2) the fact that littermates should on average be more closely related than other intracolony classes and thus would be predicted to show a stronger indication of kin-differential behaviour than was documented, and 3) that the limited data on the ontogeny of discrimination suggest that neighbours and non-neighbouring colony members are incorporated into a class of "familiar" animals with advancing age (see above). However, the possibility of genetic similarity detection cannot be ruled out without direct manipulation of relatedness and association (see Chapter 5).

Similarly, environmentally acquired discriminator substances seem unlikely since there is little qualitative difference in forage materials among colonies (but see Chapter 2). Thus the use of such cues would not reliably provide discrimination of colony members from non-colony members. Again further experiments would be necessary, perhaps providing food impregnated with different odiferous substances (e.g. Porter et al. 1989) to different groups, to further elucidate the contribution of environmentally acquired cues to discrimination.

My data cannot distinguish between odour mixing producing a colony gestalt and familiarity allowing the classification of conspecific juveniles via individual recognition. However, interesting trends in both the recognitive and spatial data suggest that the latter mechanism may be the most likely. When adjusted for the age of focal individuals, non-neighbouring colony members had a significantly higher frequency of recognitive contacts than juveniles from separate colonies, and tended to show more recognitive behaviour than neighbours or siblings. Similarly, non-neighbouring colony members maintained the lowest interindividual average distance over the course of trials; significantly less than non-colony members, and somewhat lower than neighbours and siblings. Although both of those results depend on adjustment of the data for the effect of age, taken together they could indicate that non-neighbouring juveniles are reinforcing some modicum of familiarity (i.e. "passing familiarity") with animals perceived to be similar (or familiar) in some respect. Further research into the ontogeny of familiarization is necessary to examine that notion.

The significance of colony member discrimination may relate to optimal inbreeding/outbreeding (Shields 1982, Bateson 1983). On average colony members are likely to be more closely related than non-colony members (Zammuto and Millar 1985, MacNeil and Strobeck 1987, Dobson pers. comm.). By discriminating colony members from non-colony members, juveniles that remain philopatric to their natal area can avoid close inbreeding when they recruit to the breeding cohort. Further, dispersers that emigrate to the same area could avoid inbreeding (and cooperate in other important respects with former colony members to enhance inclusive fitness) if colony member discrimination persists among yearlings and adults. Unfortunately, high mortality of juveniles on my study site (Appendix A) precluded any quantification of the level of discrimination among older age classes.

It may also be adaptive to discriminate familiar colony members from other conspecifics to exclude non-kin from valuable resources (e.g. burrow systems, hibernaculae). Steiner (1975), in speculating about the possibility of group odours in Columbian ground squirrels suggested that the adaptive significance of "xenophobic" responses may relate to the defence of offspring from "marauding" infanticidal male intruders (although there is some doubt that male Columbian ground squirrels commit infanticide; Hare 1991, Appendix I). Finally, by cooperating with group members in the detection of predators, individuals may enhance their own fitness by increasing foraging efficiency and avoiding predation (Kildaw 1991).

One aspect of the experimental protocol potentially confounds the above results and their interpretation. In this experiment, a group of 70 live traps were used as necessary to trap juveniles on the main site without regard to their location. A second group of 60 live traps were used to trap individuals in the second colony. If individuals become familiar indirectly (as the results suggest since non-neighbouring colony members don't interact directly but aren't discriminated from other colony members), then the live-traps themselves could act as a vector for the exchange of discriminator substances. This presumes that those cues are olfactory in nature since visual, auditory, tactile, or electromagnetic cues could not (or are simply unlikely in the case of electromagnetic) be transferred via the traps. That presumption is reasonable since discrimination based on olfactory cues is common in mammals (see Eisenberg and Kleiman 1972 for a review). Further, Columbian ground squirrels are well equipped with glands that could function in the context of recognition (Steiner 1975, Kivett et al. 1976, Kivett 1978), show greeting behaviour that seems to imply mutual investigation of the oral angle (an area rich in glandular secretions) (*ibid*), and are known to discriminate among their own odours, the odours of neighbours, the odours of strangers, and unscented substrates (Harris and Murie 1982). In the course of trapping, the traps become soiled with feces and urine (personal observation), and undoubtedly would transfer other odours as animals rub their bodies over the wire mesh (personal observation). Thus non-neighbouring colony members may become incorporated into the class of familiar animals at an artefactually high level under my protocol. This possibility requires further investigation (see Chapter 4) but does not seriously confound the present interpretation. Even if indirect familiarity is facilitated by trap mixing, the results indicate that juveniles possess the capacity to become familiar using indirectly acquired cues. In nature, such cues would presumably be deposited on natural substrate (via simple scent marking, twist marking, ventral dragging, urinating or defecating; see Appendix B) and could promote familiarity as has been documented for odours deposited at Merriam's kangaroo rat (*Dipodomys merriami*) dust-bathing sites (Randall 1991).

Although benefits of group member recognition must ultimately be advantageous at the individual level to be selected for, the discrimination of familiar from unfamiliar individuals can enhance fitness. On average, familiar animals (particularly if non-neighbouring colony members are not as familiar in the absence of trap mixing; see above) will also be closely related maternally or paternally. Thus by cooperating with familiar individuals, squirrels would enjoy any benefits that accrue via kin selection (see above) in addition to other benefits from cooperating with familiars (e.g. "dear enemies"; Fisher 1954, Wilson 1975, Jaeger 1981).

Table 3.1: Relatedness/Association effects on individual behaviours comprising recognitive behaviour (NSRA2= non-siblings from two different colonies, NSRAD= non-siblings reared ≥ 70 m apart within the same colony, NSRAC= non siblings reared ≤ 40m apart within the same colony, SRT= siblings reared together: all values mean±SE arcsine square-root transformed proportions, unadjusted for concomitant variables).

Behaviour	NSRA2 (n=21)	NSRAD (n=21)	NSRAC (n=20)	SRT (n=19)	Single factor ANOVA F (3,77)	*P*
nose to nose	0.055±0.009	0.034±0.008	0.046±0.008	0.037±0.009	1.245	0.299
nose to mouth	0.064±0.009	0.058±0.007	0.059±0.006	0.057±0.006	0.160	0.923
nose to head	0.144±0.025	0.133±0.017	0.127±0.012	0.123±0.015	0.256	0.857
nose to body	0.205±0.027	0.257±0.041	0.194±0.027	0.207±0.023	0.856	0.468
nose to genitals	0.007±0.004	0.016±0.005	0.008±0.004	0.008±0.005	0.953	0.419
nose to anus	0.000±0.000	0.002±0.002	0.000±0.000	0.002±0.002	0.687	0.563
nose to tail	0.054±0.008	0.050±0.009	0.049±0.008	0.049±0.011	0.073	0.974
approach	0.062±0.009	0.084±0.008	0.068±0.008	0.080±0.010	1.274	0.289

Table 3.2: Relatedness/Association effects on individual behaviours comprising cohesive behaviour (group acronyms as defined in Table 3.1: all values mean±SE arcsine square-root transformed proportions, unadjusted for concomitant variables).

	NSRA2	NSRAD	NSRAC	SRT	Single factor ANOVA	
Key Behaviours	(n=21)	(n=21)	(n=20)	(n=19)	F (3,77)	*P*
allogroom	0.041±0.015	0.039±0.018	0.045±0.016	0.048±0.015	0.071	0.975
play mount	0.033±0.010	0.073±0.019	0.054±0.012	0.094±0.015	3.245	0.026*
play fight	0.018±0.009	0.051±0.021	0.079±0.026	0.119±0.031	3.538	0.019*
follow	0.019±0.007	0.046±0.014	0.027±0.008	0.049±0.011	1.920	0.133

Table 3.3: Relatedness/Association effects on individual behaviours comprising agonistic behaviour (group acronyms as defined in Table 3.1: all values mean±SE arcsine square-root transformed proportions, unadjusted for concomitant variables).

	NSRA2	NSRAD	NSRAC	SRT	Single factor ANOVA	
Key Behaviours	(n=21)	(n=21)	(n=20)	(n=19)	F (3,77)	*P*
arch back	0.007±0.004	0.003±0.003	0.000±0.000	0.000±0.000	1.884	0.139
lateral display	0.002±0.002	0.000±0.000	0.000±0.000	0.000±0.000	0.951	0.421
tail bush	0.038±0.011	0.005±0.005	0.000±0.000	0.000±0.000	8.460	0.000*
run at	0.006±0.004	0.000±0.000	0.000±0.000	0.000±0.000	1.786	0.157
lunge	0.016±0.006	0.002±0.002	0.002±0.002	0.000±0.000	4.689	0.005*
ball fight	0.005±0.004	0.000±0.000	0.000±0.000	0.000±0.000	1.859	0.144
jump back	0.006±0.003	0.006±0.005	0.000±0.000	0.000±0.000	1.428	0.241
bite	0.010±0.006	0.000±0.000	0.002±0.002	0.002±0.002	1.691	0.176
Non-key behaviours						
tail flick	0.005±0.003	0.003±0.003	0.002±0.002	0.004±0.003	0.202	0.895
growl	0.010±0.007	0.000±0.000	0.002±0.002	0.015±0.007	1.920	0.133
squeal	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	---	---

Table 3.4: Relatedness/Association effects on individual behaviours comprising investigative behaviour (group acronyms as defined in Table 3.1: all values mean±SE arcsine square-root transformed proportions, unadjusted for concomitant variables).

	NSRA2	NSRAD	NSRAC	SRT	Single factor ANOVA	
Behaviour	(n=21)	(n=21)	(n=20)	(n=19)	F (3,77)	*P*
scratch floor	0.011±0.005	0.020±0.009	0.021±0.007	0.023±0.009	0.472	0.703
jump up	0.013±0.006	0.005±0.004	0.010±0.004	0.004±0.003	0.834	0.479
scratch plexiglass	0.215±0.028	0.209±0.030	0.226±0.026	0.265±0.025	0.805	0.495
bite plexiglass	0.022±0.008	0.033±0.011	0.037±0.010	0.026±0.007	0.520	0.670
bump plexiglass	0.026±0.006	0.024±0.006	0.031±0.010	0.049±0.009	1.888	0.139

Table 3.5: Relatedness/Association effects on individual behaviours comprising scent reception (group acronyms as defined in Table 3.1: all values mean±SE arcsine square-root transformed proportions, unadjusted for concomitant variables).

	NSRA2	NSRAD	NSRAC	SRT	Single factor ANOVA	
Behaviour	(n=21)	(n=21)	(n=20)	(n=19)	F (3,77)	*P*
sniff plexiglass	0.173±0.012	0.141±0.014	0.138±0.015	0.154±0.012	1.567	0.204
lick plexiglass	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	----	-----
sniff floor	0.091±0.014	0.113±0.011	0.092±0.008	0.110±0.011	0.982	0.406
sniff urine	0.047±0.009	0.032±0.008	0.040±0.008	0.039±0.008	0.545	0.653
sniff scat	0.028±0.007	0.037±0.008	0.026±0.007	0.017±0.007	1.148	0.335

Table 3.6: Relatedness/Association effects on individual behaviours comprising active behaviour (group acronyms as defined in Table 3.1: all values mean±SE ar[illegible]ne square-root transformed proportions, unadjusted for concomitant variables).

	NSRA2	NSRAD	NSRAC	SRT	Single factor ANOVA	
Behaviour	(n=21)	(n=21)	(n=20)	(n=19)	F (3,77)	*P*
walk	0.241±0.020	0.228±0.019	0.243±0.020	0.278±0.019	1.187	0.320
lope	0.087±0.014	0.096±0.014	0.085±0.015	0.098±0.013	0.228	0.877
groom	0.213±0.023	0.245±0.028	0.260±0.037	0.297±0.026	1.403	0.248

Table 3.7: Relatedness/Association effects on individual behaviours comprising resting behaviour (group acronyms as defined in Table 3.1: all values mean±SE arcsine square-root transformed proportions, unadjusted for concomitant variables).

	NSRA2	NSRAD	NSRAC	SRT	Single factor ANOVA	
Behaviour	(n=21)	(n=21)	(n=20)	(n=19)	F (3,77)	*P*
S-4	0.651±0.066	0.660±0.076	0.624±0.066	0.587±0.049	0.242	0.867
slouch	0.473±0.042	0.400±0.042	0.494±0.055	0.439±0.040	0.837	0.478
alert	0.048±0.011	0.051±0.012	0.054±0.013	0.053±0.013	0.044	0.988

Figure 3.1: Relative percentage of sample points juvenile squirrels engaged in the 7 behaviour categories in the arena.

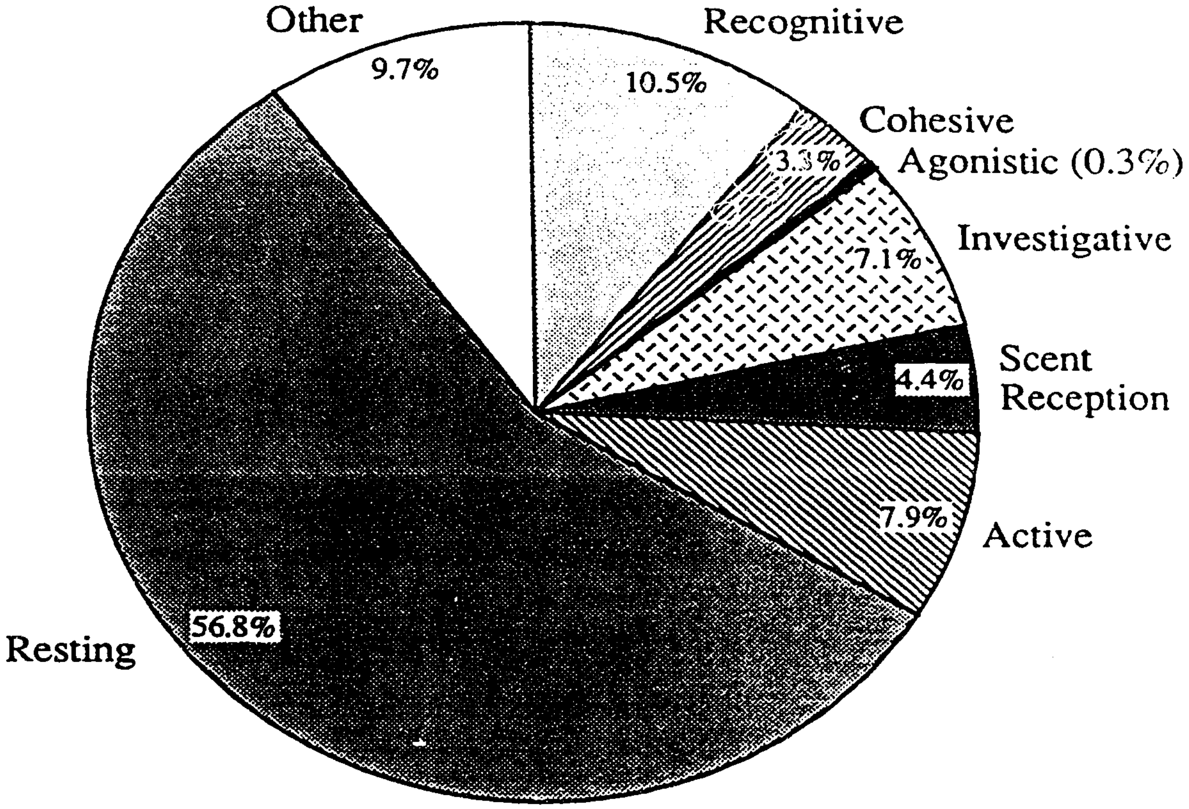
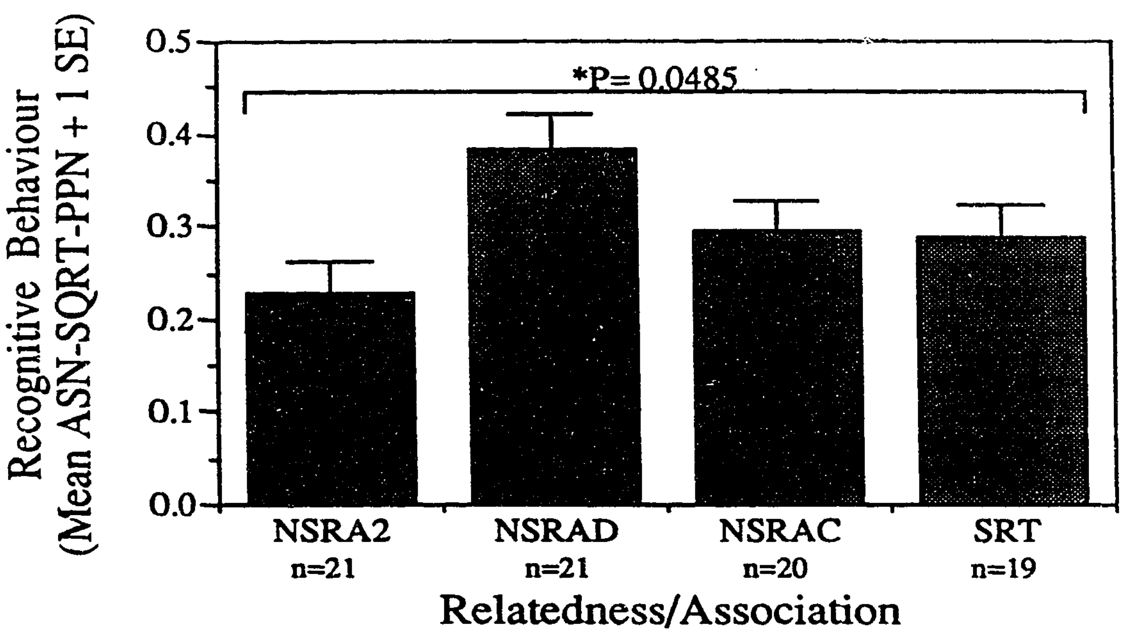


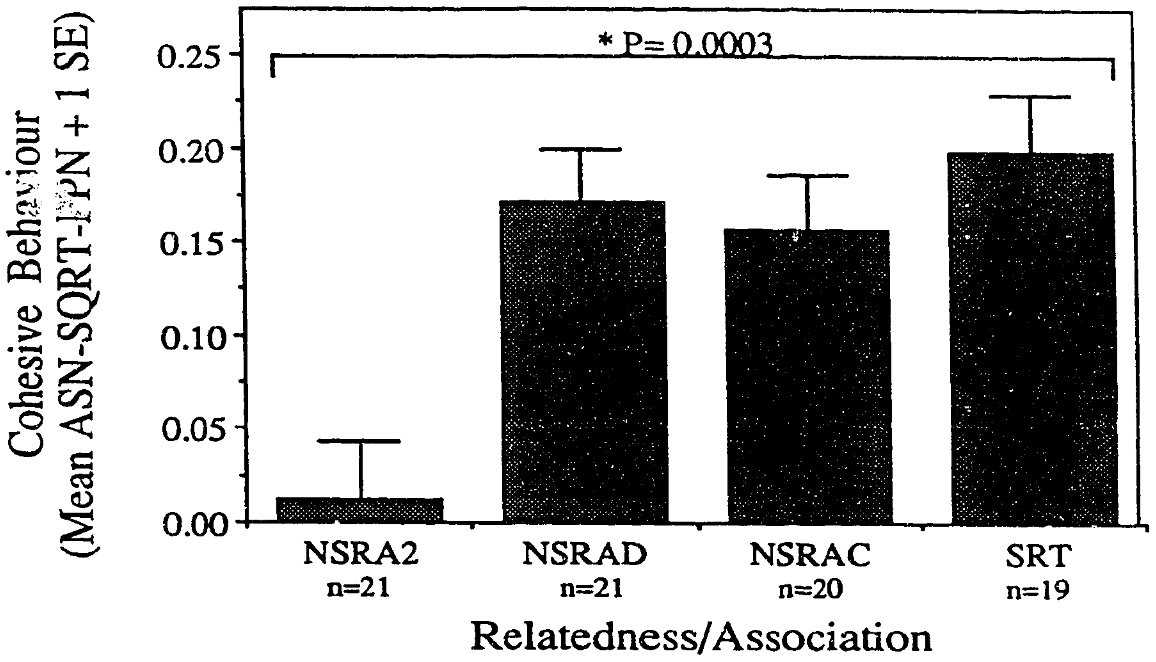
Figure 3.2: Recognitive behaviour displayed in interactions between siblings (SRT), neighbours (NSRAC), spatially distant non-siblings (NSRAD), and members of two different colonies (NSRA2) (adjusted for age); dependent variable is the mean of the arcsine square-root transformed proportions.



Pairwise Contrasts (sequential Bonferroni)

Contrast	p
NSRA2 vs. NSRAD	0.041*
NSRA2 vs. NSRAC	0.585
NSRA2 vs. SRT	0.585
NSRAD vs. NSRAC	0.265
NSRAD vs. SRT	0.265
NSRAC vs. SRT	0.897

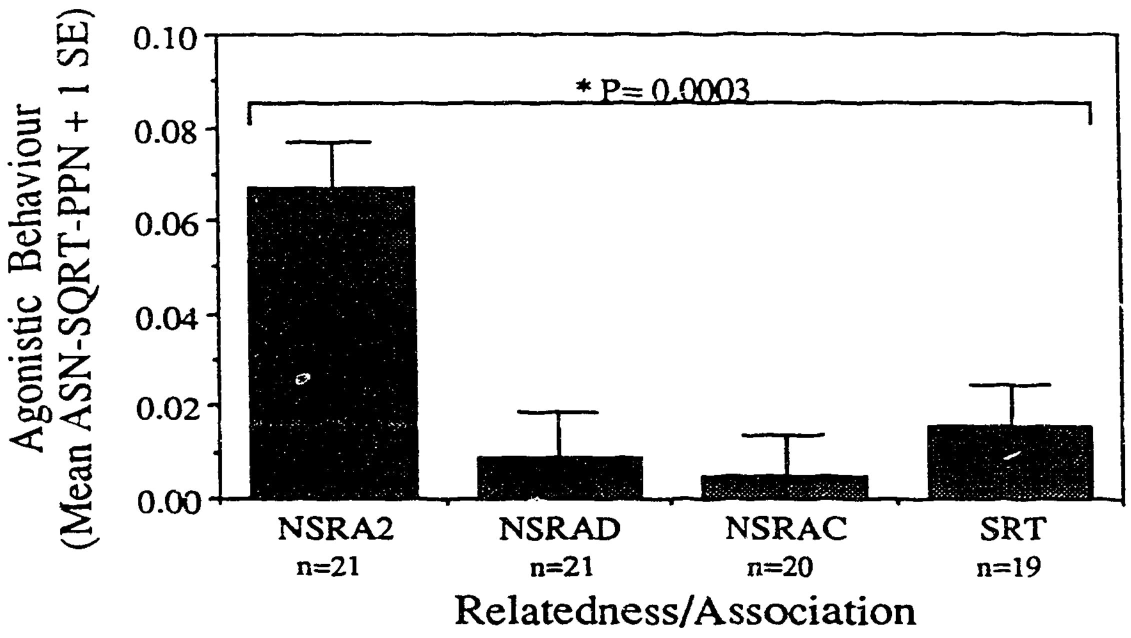
Figure 3.3: Cohesive behaviour displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (adjusted for age, trial within day, and number of times individuals run).



Pairwise Contrasts (sequential Bonferroni)

Contrast	p
NSRA2 vs. NSRAD	0.005*
NSRA2 vs. NSRAC	0.005*
NSRA2 vs. SRT	0.000*
NSRAD vs. NSRAC	0.984
NSRAD vs. SRT	0.984
NSRAC vs. SRT	0.816

Figure 3.4: Agonistic behaviour displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (adjusted for age).



Pairwise Contrasts (sequential Bonferroni)

Contrast	p
NSRA2 vs. NSRAD	0.002*
NSRA2 vs. NSRAC	0.001*
NSRA2 vs. SRT	0.002*
NSRAD vs. NSRAC	1.000
NSRAD vs. SRT	1.000
NSRAC vs. SRT	1.000

Figure 3.5: Investigative behaviour displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (no concomitant variables).

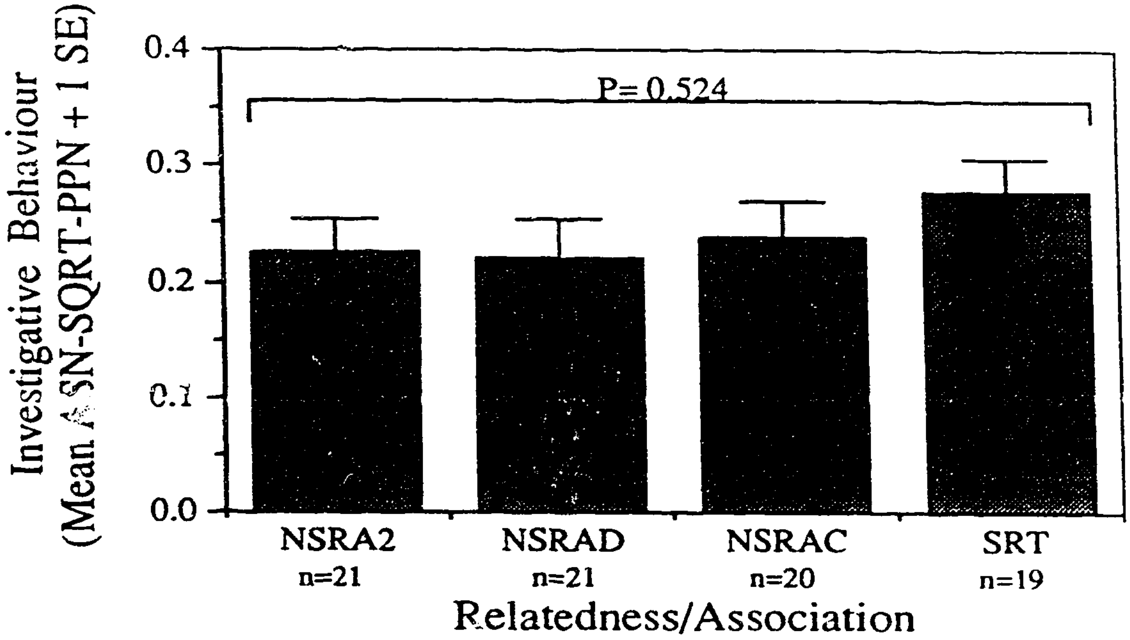


Figure 3.6: Scent reception displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (no concomitant variables).

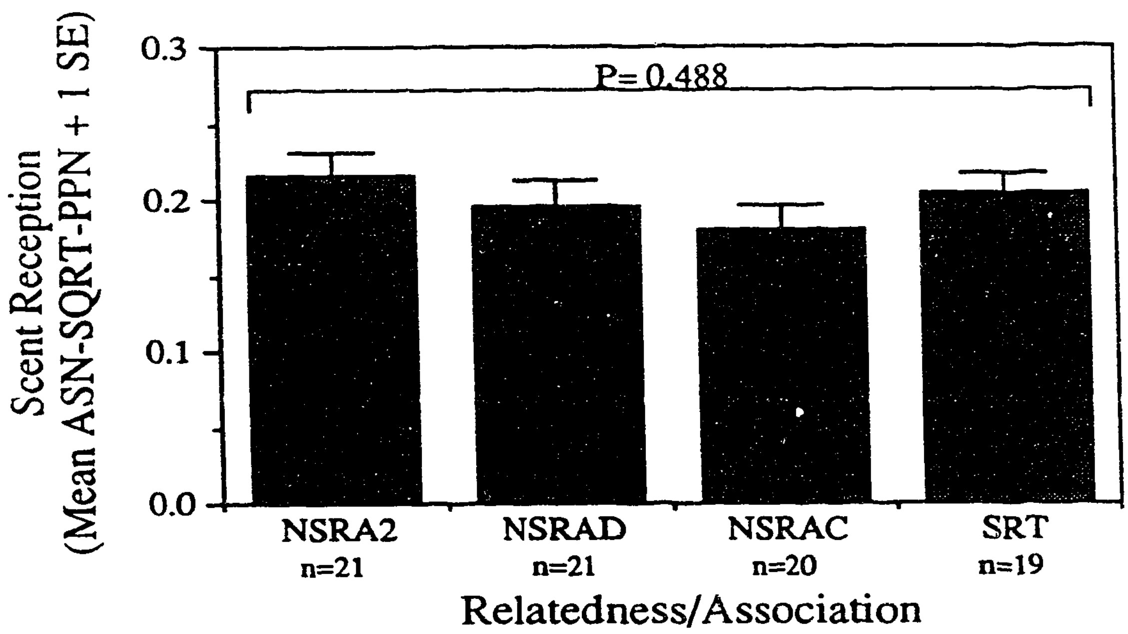


Figure 3.7: Active behaviour displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (adjusted for number of appearances)

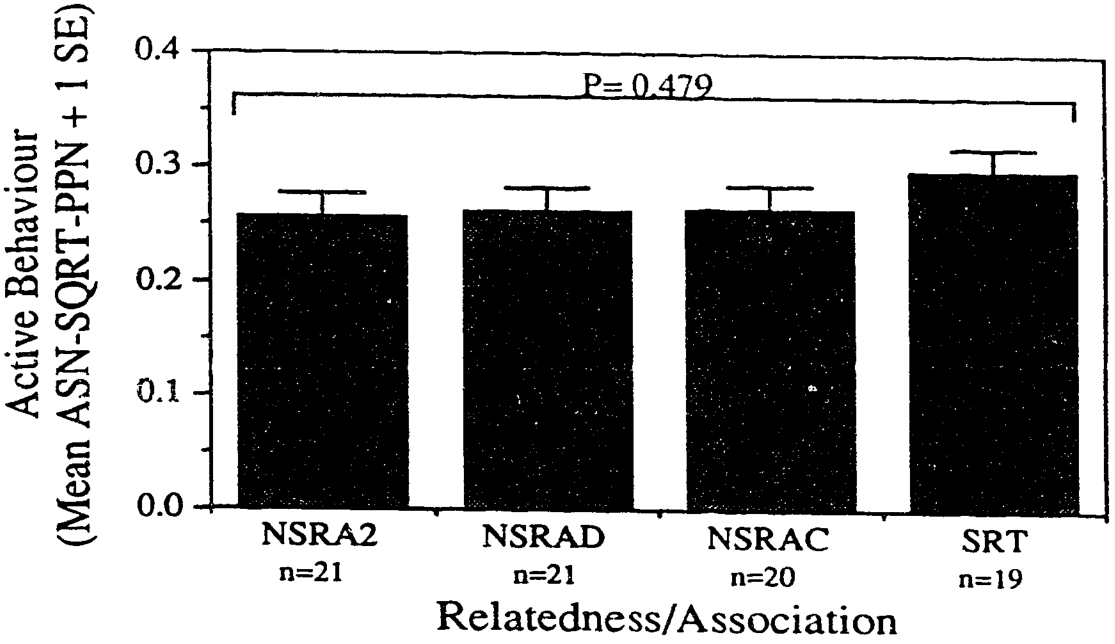


Figure 3.8: Resting behaviour displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (no concomitant variables).

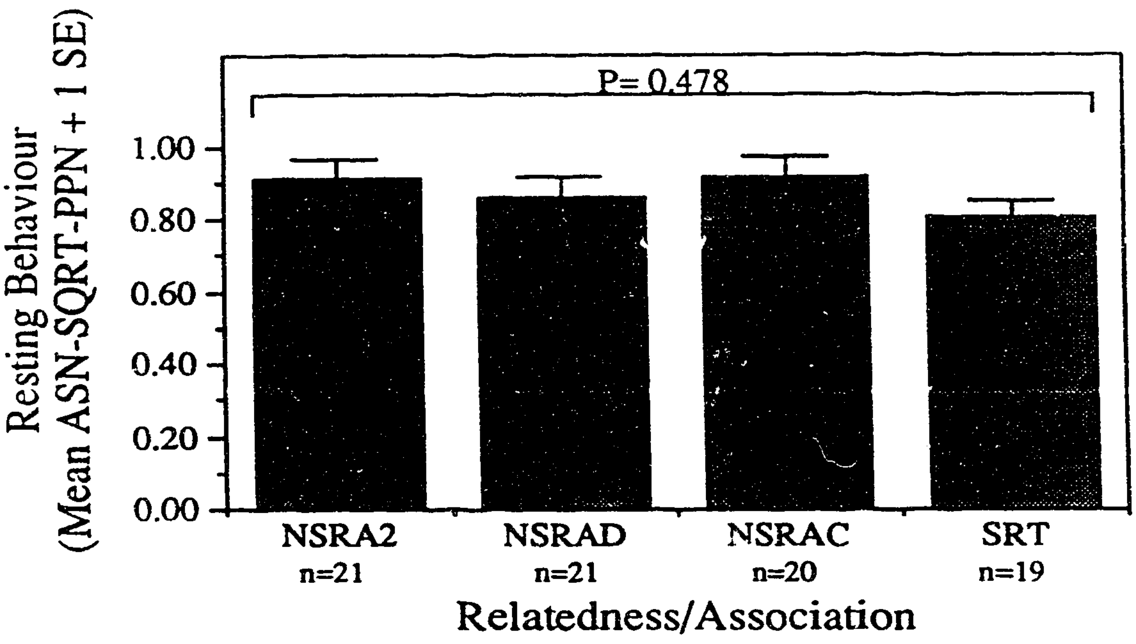
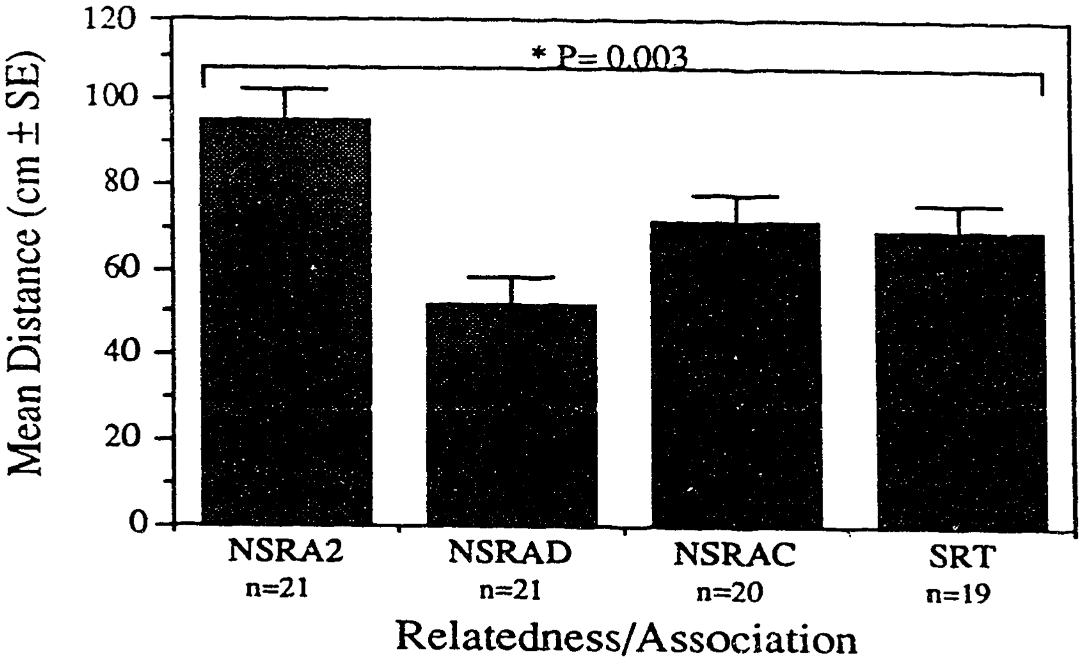


Figure 3.9: Average interindividual distance in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (adjusted for age).



Pairwise Contrasts (sequential Bonferroni)

Contrast	p
NSRA2 vs. NSRAD	0.001*
NSRA2 vs. NSRAC	0.098
NSRA2 vs. SRT	0.059
NSRAD vs. NSRAC	0.109
NSRAD vs. SRT	0.143
NSRAC vs. SRT	0.815

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Chapter 4: Indirect Familiarity and Group Member Discrimination by Columbian Ground Squirrels

Introduction

Several proximate mechanisms have been proposed to account for kin recognition in animals (see Holmes and Sherman 1983, Hepper 1986, Porter and Blaustein 1989 for reviews, and Chapter 5). Where close kin are reared in isolation from more distant relatives and non-kin, as is the case in Columbian ground squirrels (McLean 1978), social familiarization can produce kin discrimination (Bekoff 1981a, Holmes and Sherman 1982, Holmes 1984).

In addition to facilitating kin selection (Hamilton 1964, Maynard Smith 1964), such a mechanism can promote an optimal balance between inbreeding and outbreeding (Shields 1982, Bateson 1983) as implied by the results of Boyd and Blaustein (1985) for gray-tailed voles (*Microtus canicaudus*). However, benefits of familiarity extend beyond the level of kin as costs of aggression can be reduced through familiarity among territorial neighbours (i.e. the "dear enemy" phenomenon; Fisher 1954, Wilson 1975, Jaeger 1981).

Work by Hare (Chapter 3) examining the level of discrimination among juvenile Columbian ground squirrels (*Spermophilus columbianus*) suggests that juveniles become familiar with neighbouring and non-neighbouring colony members subsequent to emergence from their natal burrow. Hare's results indicate that juveniles come to discriminate colony members from non-colony members despite the fact that non-neighbouring juveniles rarely, if ever, interact directly during their first season above ground (Waterman 1985, unpubl. data).

Familiarity can be achieved by both direct association (Bekoff 1981a, Holmes 1984) and indirect exposure to stimuli produced by other individuals (e.g. song in some bird species, reviewed by Falls 1978; possibility of odour cues in mammals "mediated" by intermediaries so that individuals "recognize" each other if they have associated with a common individual, Holmes and Sherman 1983). Familiarity may also be promoted by cues deposited on natural substrates (Müller-Schwarze 1971) as reported by Randall (1991) for Merriam's kangaroo rats (*Dipodomys merriami*) that become familiar via scent deposited at sandbathing sites. Further, a commonly invoked mechanism of kin recognition, phenotype matching (Holmes and Sherman 1982, Lacy and Sherman 1983, Porter and Blaustein 1989), relies on what could be considered indirect familiarity since previously unencountered individuals are discriminated on the basis of perceived similarity to cues originating from known kin (theoretically including matching to self).

Colony (or group) member discrimination is best documented for social insects (see examples in Wilson 1971, Hölldobler and Wilson 1990) but has also been reported for vertebrates (Colgan 1983, Hepper 1986). Group members can be discriminated through 1) the acquisition of common environmental odours (e.g. nest odours in the wasp genus *Polistes* are "imprinted" shortly after emergence from the pupal cocoon, reviewed in Michener and Smith 1987; dietary odours in spiny mice (*Acomys cahrinus*), Porter et al. 1989; see further examples in Hepper 1986), 2) "branding" with the odour of a single individual (e.g. queen odours in carpenter ants, Carlin and Hölldobler 1987; maternal "labelling" of offspring in goats, Gubernick 1981; sugar gliders (*Petaurus breviceps*) mark group members with glandular secretions which may produce group odour, Schultze-Westrum 1969), 3) recognition of individual's odours within the colony (e.g. in the sweat bee *Lasioglossum zephyrum* guards learn the odours of their nestmates and admit bees to the hive based on similarity to those learned odours, Buckle and Greenberg 1981; but see Getz 1982; in rabbits (*Oryctolagus cuniculus*) inguinal gland secretions are suspected to lead to recognition of individuals as group members, Hesterman and Mykytowycz 1982a, 1982b), 4) odour mixing resulting in an emergent (gestalt) colony odour (Crozier and Dix 1979, Crozier 1987, e.g. in the ants *Leptothorax ambiguus* and *L. longispinosus*, Stuart 1987), or 5) combinations of the above mechanisms (e.g. in spiny mice (*Acomys cahrinus*), dietary cues, maternal labelling, and genetically mediated individual specific odours interact in producing familiarity and subsequent recognition, Porter et al. 1989).

For discrimination to occur, animals must possess certain attributes that vary among the classes discriminated. Those attributes are referred to as discriminator substances (or labels; Waldman et al. 1988) and can be visual, auditory, tactile, olfactory, or even electromagnetic in nature. Olfactory cues have often been implicated in the discrimination of kin from non-kin (and other classes; see Colgan 1983), and are common among vertebrates (see Eisenberg and Kleiman 1972, Brown 1985, Halpin 1986, 1991, Porter and Blaustein 1989 for reviews). Olfactory cues (or chemical cues of some form) are known to mediate sibling recognition in social insects (see Michener and Smith 1987, Breed and Bennett 1987, Hölldobler and Wilson 1990, and Jaisson 1991 for reviews), larval amphibians (see Blaustein et al. 1987a, and Waldman 1991 for reviews), rodents (see Porter 1988, and Blaustein et al. 1987a, 1987b), and humans (Porter and Moore 1981, also Wells 1987, and Porter 1991 for reviews). Experiments conducted by Holmes (1984) involving olfactory impairment using zinc sulfate (Alberts and Galef 1971, Alberts 1974) demonstrated the importance of olfactory cues in the discrimination of familiar from unfamiliar juvenile thirteen-lined ground squirrels (*S. tridecemlineatus*). Scent marking behaviour is widespread in the family Sciuridae (see Ralls 1971, Steiner 1974), and although most frequently interpreted in the context of territorial advertisement (Ralls 1971, Eisenberg and Kleiman 1972, Kivett 1975, Murie and Harris 1978, Müller-Schwarze and Heckman 1980, Festa-Bianchet and Boag 1982), the deposition of scent could also function in promoting the identification of individuals (Steiner 1975, Kivett et al. 1976, Kivett 1978, Harris and Murie 1982). Columbian ground squirrels possess an abundance of glands that could function in the context of recognition (Kivett et al. 1976, Kivett 1978) and show behaviour patterns that entail mutual investigation of scent-rich areas implying social discrimination (e.g. greeting ; Steiner 1975). Harris and Murie (1982) reported that Columbian ground squirrels spent the most time investigating acrylic cubes impregnated with odours of strangers, less time investigating odours of neighbours, less time still with their own odours, and the least time investigating cubes without odour. That result provides additional support for a potential role of olfactory cues in mediating social interactions in Columbian ground squirrels. Results from an experiment employing a similar protocol to that of Harris and Murie (1982) suggest that woodchucks (*Marmota monax*) also discriminate neighbours from strangers on the basis of scent (Meier 1991).

In the level of discrimination experiment (Chapter 3), juveniles within colony but not between colonies were presented with the opportunity to become familiar with the odours of individuals deposited on traps and handling bags. Traps and handling bags became soiled with feces and urine (Hare pers. obs.) and would undoubtedly act as the repository for odours from scent glands as individual's bodies came into contact with them (Hare pers. obs.). Salmon and Marsh (1989) found that male California ground squirrels (*Spermophilus beecheyi douglasii*) were more likely to be trapped when anal-gland scent from conspecific males was suspended on a cotton swab in the trap than in traps without scent. That result suggests that like other rodent species (see references in Salmon and Marsh 1989), ground squirrels attend to odours deposited in traps. Indirect familiarity via trap-borne odours in my previous experiment (Chapter 3) would clearly indicate that juvenile Columbian ground squirrels have the capacity to become familiar without direct contact. However, such indirect familiarization could potentially confound the results so that non-neighbouring colony members would be incorporated into the class of familiar individuals at an artificially high level.

Here I report results from an experiment that tests the validity and generality of "colony-member" discrimination by incorporating juveniles from a previously second colony into between colony trials while controlling trap mixing. Furth experiment tests whether animals become familiar indirectly via live-traps by including both mixed and unmixed traps within and between colonies. The relative strength of discrimination with and without trap mixing within colony examines the extent of naturally occurring indirect familiarity. Finally, since only olfactory cues are transferred reliably via traps, this experiment tests whether familiarity can be based on olfactory cues.

Methods

Squirrels were studied on a 1.9 ha meadow in the Sheep River drainage of southwestern Alberta (50°38' N, 114°37' W; elevation 1470m; see Chapter 2, Figure 2.1). The area is contiguous with a larger 72 ha meadow where Columbian ground squirrels were studied by Boag and Murie (1981) and Young (1988). To examine the relative contribution of colony membership proper and familiarity via traps to behavioral discrimination among juvenile Columbian ground squirrels, an experiment was conducted during the summer of 1990. One hundred and one juveniles on the hay meadow (HM) were marked with ear tags and hair dye within 2 days of emergence from their natal burrow (Chapter 2) and and all were assigned unambiguously to their dams at that time. To address the generality of colony member discrimination, juveniles from the vicinity of the Alberta Forest Service's Bow/Crow forestry station were used in intercolony trials rather than juveniles from meadow B as in the previous experiment (see Chapter 2, Figure 2.1). Seventeen juveniles on the forestry station "mix" site FM and 12 juveniles on the forestry station "fix" site (FF) were also marked with ear tags and hair dye (between 1 and 6 July) but could not be assigned to dams or associated with littermates since squirrels in those areas were not trapped or observed earlier. The experiment followed a factorial design with colony membership and trap manipulation as factors. Colony membership had 2 levels: both within (NSRAD), and between colony (NSRA2) trials were conducted as in the level of discrimination experiment (Chapter 3). Within each of those levels, trap manipulation involved either exchanging traps between sub-areas every third day (mixed) or ensuring that traps did not mix at all between the two sub-areas from which subjects were drawn (fixed) (Figure 4.1). Rather than using a unique set of traps on each site and mixing those within site indiscriminately as in 1988 and 1989, groups of

10 tomahawk live-traps were assigned and reserved for use within 8 circumscribed sub-areas (locales) of the two sites. With 6 locales on the hay meadow and 2 locales on the forestry station site, juveniles were tested in each of 4 treatment conditions including; non-siblings reared apart distant within site with traps exchanged between the participant's locales (NSRADmixed), non-siblings reared apart distant within site without trap exchange (NSRADfixed), non-siblings reared apart on separate colonies with trap exchange (NSRA2mixed), and non-siblings reared apart on separate colonies without trap exchange (NSRA2fixed) (Figure 4.1). Siblings reared together (SRT) were also run in 1990 to act as a reference against which the behaviour of the experimental groups could be compared, and to act as a standard of comparison between the data from this experiment and the level of discrimination experiment conducted in 1988 and 1989.

Locales were delineated by the sum of the partially overlapping areas frequented by at least 4 breeding females and their litters, and ranged in area from 414 m^2 to 2500 m^2 (Figure 4.1). Those areas were assigned initially from the movements of adult females and their litters in 1988 and 1989 (based on informal observations) but were adjusted to include the area of common use by juveniles and their dams in 1990. Locales were established as far apart as possible within site (Figure 4.1) to minimize direct and indirect contact between squirrels and to reduce the potentially confounding influence of paternal kinship. Traps were associated with locales by attaching colour-coded flagging tape to trap handles and were restricted to their respective sub-areas from 18 June (prior to the emergence of any juveniles in those locales) until the end of the experiment on 30 July, 1990. New, unsoiled traps were used in all fixed trap locales. Traps were baited and set in all locales at least once and often several times each day from 18 June to 6 July. Mixed group traps were exchanged between their locales every three days and on the days traps were mixed, all traps were baited and set at least 3 times in their new (mixed) locales to facilitate any transmission of odour. Fixed traps were also set at least three times on those days to balance exposure to traps and handling between fixed and mixed groups. Traps mixed between sites were transported in the trunk of a car while those mixed within site were carried to their new location. A ninth group of 20 live traps was used to trap individuals in the interstices of locales and in areas peripheral to the main grid but not within defined locales on the hay meadow. During the course of the experiment, I recorded all instances in which juveniles or their dams were trapped in an area outside their assigned locale.

Since the traps were not the only potential source of odour mixing, handling bags (see Chapter 2) were also kept isolated by storing a bag in a waterproof container within a trap in each set of fixed traps. Thus only the bag specific to each fixed-trap area was used for handling squirrels within that locale. Two common handling bags were used arbitrarily when handling squirrels for all mixed trap locales to further facilitate odour transfer among those groups. Juvenile squirrels were transported from the meadows to the arena inside the live-trap in which they were captured, covered with a new opaque plastic bag (Glad™ Kitchen Catchers™). Odour contamination of the transit bags themselves and of the vehicle in which the squirrels were transported was prevented by using each bag only once. To further decrease the probability that squirrels would become familiar while in transit (ca. 3 min), one squirrel was placed on the front seat of the car while the prospective trial partner was placed on the back seat (separated by a full bench seat).

Trials were conducted and videotaped as outlined in the previous experiment (see Chapter 3) and upon the completion of a trial, squirrels were returned to their respective locales and released at the point of capture. Traps used to transport squirrels were returned to the group of traps from which they came. The test arena was washed after each trial as outlined in Chapter 3. Between 10 and 30 July, 1990, 35 trials were conducted with 7 trials in each of the 5 treatment groups. The 35 trials are treated as being independent since data were coded from 32 of 35 focal individuals on their first appearance in the arena. Data were included from 3 individuals run previously (in 2 of 3 cases run with a sibling in the second trial after having been run as a focal or object animal in another treatment group and in the third case being run in the same treatment condition twice) to balance the design at a sample of 7 trials per treatment group. I coded behavioral data using an event recorder program and categorized data in the same manner as those from the level of discrimination experiment (Appendix B). Juveniles from the hay meadow were designated as the focal individual in each trial so that any intersite differences in behaviour could be attributed to discrimination and not simply to a difference in the behavioral propensities of squirrels from different sites. The limited number of juveniles in each locale and the assignment of traps to locales prior to the emergence of juveniles precluded balancing the number of trials with each possible gender pair across treatment groups. However, gender pair exerted little influence on discrimination in the previous experiment (see Chapter 3) nor in this experiment (see below).

The influence of uncontrolled physical factors and miscellaneous grouping factors including temperature, wind speed, cloud cover, precipitation, juvenile age, juvenile mass, trial within day, the number of times an individual was run, and gender pair of interactants were examined as outlined in Chapter 3. Multiple regression analyses were not performed to confirm the independent analysis of each factor (see Appendix E) since the number of cases relative to the number of independent variables was extremely limited (35 cases for 11 potential factors). The effect of colony membership and trap manipulation were evaluated for each of the seven behaviour categories (recognitive, cohesive, agonistic, investigative, scent reception, active and resting) using parametric two-factor ANOVA. Differences were considered significant where $P \leq 0.05$. Parametric statistical tests were used throughout because all categories of behaviour could have been drawn from normal underlying distributions (D'Augustino's D test, all $P >$ 0.05) and treatment group variances were homogeneous for all behaviour categories (F_{max} , all $P >0.05$) except cohesive and agonistic behaviour. Parametric analyses were used for those behaviour categories as well since departures from homogeneity were not severe ($0.025 < P < 0.05$), current nonparametric analogues to ANOVA do not quantify the interaction between the main factors, and sample sizes were equivalent for each treatment group (see Zar 1974). To determine which treatment groups differed significantly, single factor analysis of variance was performed comparing all treatment groups. All possible pairs of the 5 treatment groups were contrasted (SuperANOVA™ contrast routine) with the resultant *P* values adjusted to protect the experimentwise error rate (at alpha=0.05) using the sequential Bonferroni technique recommended by Rice (1989). Analysis of covariance was employed where other independent variables confounded the analysis of colony membership/trap manipulation effects. Differences among the 5 groups for each of the key behaviours (Appendix B, Chapter 3) were analysed using the Kruskal-Wallis test since those data were not likely drawn from an underlying normal distribution (D'Augustino's D test; all $P < 0.05$) and group variances were heterogeneous (F_{max}; all $P < 0.05$). Group means for those behaviours are not presented although the extent to which the behaviours are consistent within categories is described for each behaviour category. Spatial data were not coded for this experiment since those data did not show conclusive evidence of discrimination in the previous experiment (see Chapter 3) and represent a considerable investment of time in coding. All data are reported as mean±SE unless otherwise noted.

Results

I. Physical Factors and Miscellaneous Grouping Factors

Of the miscellaneous independent variables examined, significant effects on the behaviour of juveniles were detected only for cloud cover, juvenile mass, and the number of times individuals were run (Appendix H). That little or no effect of those variables was detected is not surprising since trials were conducted over a more circumscribed period of time than in the previous experiment. Thus juveniles were more similar in age and mass throughout, individuals appeared in the arena only once, and trials were conducted with similar physical factors throughout.

A significant difference among the 5 treatment groups was detected for cloud cover with decreased escape related behaviour (Appendix H, investigative) when overcast (0.13±0.02) relative to partially-overcast (0.31±0.16) or clear skies (0.25±0.03) prevailed. However, investigative behaviour did not decrease with increasing cloud from clear to partially-overcast skies. Given the small sample size in all but the clear-sky classes (n= 23, 3, and 9 for clear, partially-overcast, and overcast respectively), that effect may be an artifact of sample size. Further, no such effect was apparent from the larger data set in the previous experiment (Appendix E), and cloud cover was balanced across the 5 treatment groups in this experiment (Appendix H). Thus adjusting the behaviour of individuals in the arena for cloud cover was deemed inappropriate.

Significant effects of juvenile mass were detected for both recognitive and cohesive behaviour (Appendix H). Both recognitive and cohesive behaviour decreased with increasing mass ($y= -0.001x+ 0.417$, $r^2= 0.207$ and $y= -0.001x+ 0.261$, $r^2= 0.144$ respectively) and mass was not balanced across those colony membership/trap manipulation groups (Appendix H). Juveniles run in fixed group trials tended to be heavier (231±14 g and 213±26 g for NSRA2fixed and NSRADfixed respectively) than those in mixed group (174±6 g and 153±6 g for NSRA2mixed and NSRADmixed respectively) or sibling trials (184±9 g). To ascertain whether mass had an effect on recognitive and cohesive behaviour in isolation of trap manipulation, data were reanalysed excluding all fixed-trap cases. Statistically significant differences were no longer apparent for recognitive and cohesive behaviour (df=1,12; F=2.10, P =0.17 and F=1.86, P =0.20 respectively) using those data; however, trends toward decreasing recognitive and cohesive behaviour with increasing mass persisted ($y= -0.002x+ 0.500$, $r^2= 0.149$ and $y= -0.002x+ 0.419$, $r^2= 0.134$ respectively). Despite that, there was no indication of a mass effect on cohesive behaviour using the larger data set of the previous experiment ($y= -9.2 \times 10^{-6}x+ .124$, $r^2=5.03 \times 10^{-5}$) (see also Appendix E), and the apparent effect of mass on recognitive behaviour was the reverse of that detected here (recognitive behaviour increased with increasing mass, $y= 2.7 \times 10^{-4}x+ .207$, $r^2=0.035$; see also Appendix E). The dilemma of an intimate link between mass and one of the main factors of interest (trap manipulation) combined with the inconsistency between data sets in elucidating a mass effect argue that adjustment of behavioural data for juvenile mass is ill advised. To resolve that dilemma, both unadjusted data and data adjusted for mass are considered in examining treatment effects on recognitive and cohesive behaviour. Further, cautious interpretation of trap manipulation effects on recognitive and cohesive behaviour are warranted; fixed trap groups may be somewhat more cohesive and show more recognitive behaviour by virtue of a mass effect than the unadjusted data suggest.

The number of appearances in the arena (times run) also had apparent effects on recognitive and cohesive behaviour (Appendix H). More recognitive behaviour was recorded for an animal's second appearance in the arena than on their first appearance (0.41±0.11 and 0.17±0.11 respectively), and greater cohesion was recorded on the second relative to the first appearance (0.28±0.02 and 0.08±0.02 respectively). However, that effect must be interpreted cautiously since only 3 animals in this experiment were run in the arena more than once, and 2 of those individuals appeared in sibling trials on their second appearance while the third appeared in the intracolony mixed-trap group on its second appearance. Those treatment groups predispose an animal to show greater recognitive and cohesive behaviour (Chapter 3 and below). In fact, recognitive behaviour of the siblings (0.615 and 0.283) and the intracolony mixed individual (0.316) in their second trial exceeded the range of recognitive behaviour shown by siblings (0 to 0.338) in only one case, and barely exceeded the range for intracolony mixed groups (0.142 to 0.313) during their first appearance in the arena. Cohesion in the second trial shown by those siblings (0.316 and 0.240) and the one intracolony mixed individual (0.274) does not lie outside the range of cohesive behaviour recorded for squirrels in those groups in their first trial (0 to 0.356 and 0 to 0.284 for siblings and NSRADmixed respectively). Thus there is scant evidence that the number of times animals appeared had a real effect on behaviour in this experiment (but see Appendix E for effect in previous experiment) so adjustment is unwarranted.

II. How often were squirrels trapped outside their locale?

Although traps were restricted to circumscribed areas, squirrels were trapped occasionally outside their assigned locale. This was rare among juveniles as only 3 individuals were ever caught outside their locale. One juvenile from the intersite mix group on the hay meadow (Figure 4.1, locale 1) was trapped once in the northernmost intrasite fixed trap group (Figure 4.1, locale 7), one juvenile from the western intrasite mix group (Figure 4.1, locale 5) was also trapped in locale 7, and one juvenile from the intersite fixed trap group on the hay meadow (Figure 4.1, locale 3) was trapped 5 times in the southeastern intrasite fixed trap area (Figure 4.1, locale 8). Additionally, 2 juveniles from the interstices between locales were trapped once each inside the restricted traps. One juvenile was trapped twice in locale 7, and another was trapped once in locale 3 (Figure 4.1). Juveniles were never caught in the area of their prospective trial partners (i.e. in their reciprocal locale) and no mixing occurred within the forestry site. Relative to 363 recorded captures of juveniles within their assigned locale (the actual number is probably 4 to 6 times that as we recorded whether juveniles were trapped within their locale only once daily) over the period 5 to 29 July, only 10 captures occurred in an undesired area (< 3% of recorded captures and < 1% of likely number of captures).

Dams of juveniles involved in the experiment were trapped outside their assigned locale 37 times over the course of the experiment. Of those only one dam was caught once in a reciprocal locale and this was among the intrasite mixed trap groups (Figure 4.1, locales 5 and 6). Ten of the 37 "extra-locale" captures of dams occurred in the 20 mobile traps (see above) in a two day period when those traps were placed in an interstitial space equidistant from two non-reciprocal locales. In the remaining 26 captures, dams were caught in neighbouring (but non-reciprocal) locales. Since adult female squirrels are trapped at least 3 times as often as juveniles (Hare unpubl. data), the 37 undesired captures represent less than 1% of all adult female trapping during the experiment (estimate based on 37 + (3 X 1452) juvenile captures). No adults were trapped outside their assigned locale at the forestry site and no squirrels from either of the two sites (forestry station and hay meadow) were trapped on the other site throughout the summer.

III. Colony Membership and Trap Manipulation Effects

a) Behaviour of experimental groups relative to siblings

Cohesive behaviour differed significantly among the 5 groups in this experiment ($F_{4,30}$= 4.79, *P* =0.004). A posteriori comparisons revealed that siblings were significantly more cohesive than non-colony members where traps were fixed (*P* =0.011; Figure 4.2), and that non-sibling colony members where traps were mixed tended to behave more cohesively than non-colony members with fixed traps (*P* =0.068; Figure 4.2). Siblings and non-sibling colony members with trap mixing showed similar high levels of cohesion, non-sibling colony members without trap mixing and non-colony members with trap mixing showed a similar intermediate level of cohesion, while non-colony members without trap mixing displayed the least cohesive behaviour (Figure 4.2). The same trend is apparent for each of the 4 key behaviours pooled into the cohesive category, and those differences were statistically significant for allogroom (H_c= 11.34, *P* =0.023), play mount (H_c= 12.81, *P* =0.012), and play fight (H_c= 12.13, *P* =0.016) but not for follow.

An overall difference persists when cohesive behaviour is adjusted for juvenile mass ($F_{4,29}$= 3.33, *P* =0.023). Despite strong trends toward siblings behaving more cohesively than either inter-colony group, none of the pairwise comparisons resolve statistically significant differences (Figure 4.3). The trend remains unchanged from that described above with adjustment for mass (Figure 4.3).

A significant difference was also detected among the 5 groups in agonistic behaviour ($F_{4,30}$= 4.58, *P* =0.005). Both siblings and non-sibling colony members with trap mixing were significantly less agonistic than non-colony members without trap mixing (*P*

=0.005 and *P* =0.017 respectively, Figure 4.4). Non-colony members without trap mixing were the most agonistic (Figure 4.4). Among the other groups, agonism decreased in a practically linear fashion from non-colony members with trap mixing, to non-sibling colony members without trap mixing, to non-sibling colony members with trap mixing and finally to the least agonism amongst siblings (Figure 4.4). Jump back, ball fight, arch back, tail bush and lateral display followed that same trend although a statistically significant difference was detected only for arch back (H_c= 12.17, *P* =0.016). No difference was apparent for lunge, run at, or bite.

A strong but non-significant trend was detected for recognitive behaviour ($F_{4,30}$=2.66, *P* =0.052). Siblings and non-sibling colony members with trap mixing displayed a similar high level of recognitive behaviour while all other groups showed fewer recognitive contacts. A significant difference within that category occurred only for nose to head (H_c= 9.91, *P* =0.042), with siblings and both mixed trap groups showing somewhat higher levels of recognitive behaviour than fixed trap groups. Trends for the remaining 7 recognitive behaviours suggested no difference among groups (nose to genitals, nose to anus), or were in accord with the overall trend for that category (approach, nose to nose, nose to mouth, nose to body, nose to tail). When recognitive behaviour is adjusted for juvenile mass, the difference among groups is no longer apparent ($F_{4,30}$=1.31, *P* =0.291), although the same trends persist (Table 4.1).

No difference among the 5 groups was detected for investigative behaviour, scent reception, active behaviour, or resting (all *P* >0.05, Table 4.1). No significant difference among the 5 groups was detected for any of the remaining individual behaviours (Kruskal-Wallis test, all *P* >0.05).

b) Colony membership

When colony membership and trap manipulation were treated as separate factors, juveniles were significantly more cohesive and significantly less aggressive with non-siblings from their own colony than with non-siblings from the second colony (Figure

4.5). Juveniles from the same colony also displayed a significantly higher frequency of recognitive contacts than juveniles from different colonies (Table 4.2). No difference between colony members and non-colony members was apparent for investigative behaviour, scent reception, active behaviour, or resting (Table 4.2). Key behaviours followed the trends reported for their categories, with significant colony membership differences detected only for allogroom ($F_{1,24}$=9.30, *P* =0.006), and play mount ($F_{1,24}$=5.61, *P* =0.026). Statistically significant differences between colony members and non-colony members were not detected for any of the other individual behaviours.

c) Trap manipulation

Juveniles from mixed-trap groups were significantly more cohesive and showed a strong but non-significant trend toward less agonism than juveniles from fixed-trap groups (Figure 4.6). Significantly more recognitive contacts and investigative (escape related) behaviours were recorded among juveniles from mixed groups relative to fixed groups (Table 4.2). Juveniles from fixed-trap groups spent a significantly greater proportion of their time resting and tended to be less active than juveniles from mixed-trap groups (Table 4.2). No difference between trap manipulation groups was apparent for behaviours associated with scent reception (Table 4.2). When adjusted for juvenile mass, statistically significant differences were no longer apparent for cohesive or recognitive behaviour although the trends reported for those categories remained unaltered (Table 4.2). In general, the results for key behaviours followed their respective categories. Statistically significant trap manipulation differences were detected for tail bush (fixed>mixed, $F_{1,24}$=4.38, *P* =0.047), approach (fixed<mixed, $F_{1,24}$=8.26, *P* =0.008), play mount (fixed<mixed, $F_{1,24}$=4.61, *P* =0.042), play fight (fixed<mixed, $F_{1,24}$=6.42, *P* =0.018), S-4 (fixed>mixed, $F_{1,24}$=9.02, *P* =0.006), slouch (fixed<mixed, $F_{1,24}$=4.71, *P* =0.040), scratch plexiglass (fixed<mixed, $F_{1,24}$=4.81, *P* =0.038), nose to nose (fixed<mixed, $F_{1,24}$=4.77, *P* =0.039), nose to head (fixed<mixed, $F_{1,24}$=7.84, *P* =0.010), and nose to body (fixed<mixed, $F_{1,24}$=6.72, *P* =0.016).

d) Interactions between colony membership and trap manipulation

No significant interactions between colony membership and trap manipulation were detected for any of the 7 behaviour categories (all *P* >0.25), nor for any of the individual behaviours (all *P* >0.05).

Discussion

As in the previous experiment (Chapter 3), behavioral discrimination among juvenile Columbian ground squirrels was readily apparent for cohesive and agonistic behaviours, and somewhat so for recognitive behaviour. Average frequencies for those behaviour categories were similar in this and the previous experiment (recognitive, 0.26±0.08 versus 0.29±0.03; cohesive, 0.19±0.03 versus 0.19±0.03; and agonistic behaviour 0.006±0.006 versus 0.017±0.009 respectively). Frequencies in the remaining 4 behaviour categories were also similar. Further, non-colony members that did not have traps exchanged between their natal areas in this experiment showed similar frequencies of recognitive (0.14±0.05 versus 0.23±0.04), cohesive (0.04±0.04 versus 0.01±0.03), and agonistic behaviour (0.12±0.03 versus 0.07±0.01) to non-colony members in the previous experiment. That consistency in behaviour between experiments allows interpretation of overall trends in discriminative behavior to be based on results from both data sets.

Juveniles were significantly more cohesive and less aggressive in intra-colony trials than in inter-colony trials. Further, frequencies of recognitive contacts were greater among juveniles from the same colony than among juveniles from different colonies. Those trends hold when comparing non-sibling colony members to non-colony members where traps are not mixed (NSRADfix versus NSRA2fix), and suggest that in a natural context, colony members are discriminated from non-colony members. Thus colony member recognition is not restricted to the two colonies used in the prior experiment (Chapter 3) and may be universal for Columbian ground squirrels (but see below).

Trap mixing also contributed significantly to the discriminative behaviour of juveniles. Squirrels from mixed-trap groups were more cohesive than juveniles from fixed-trap groups. Further, there was a strong but non-significant trend toward greater agonism among juveniles from fixed relative to mixed-trap groups. Differences between trap manipulation groups were also apparent for other behaviour categories. Where traps had been fixed, juveniles showed less investigative behaviour, had fewer recognitive contacts, were less active overall, and spent more time in resting postures than juveniles that had traps mixed between their respective locales. Thus exposure to traps and/or handling materials can promote indirect familiarity among juveniles. Trap mixing exerted a like influence on the behaviour of both colony and non-colony members as no significant interaction between colony membership and trap manipulation was detected.

In the absence of trap mixing, non-neighbouring colony members tended to be less cohesive and were more agonistic than siblings. However, the behaviour of juveniles that had traps mixed between their natal areas was virtually indistinguishable from that of siblings. Thus familiarity acquired via traps and/or handling materials can result in a lack of discrimination between siblings and non-neighbouring juveniles. In the previous experiment (Chapter 3), all non-neighbouring colony members were essentially exposed to a mixed-trap regime. The apparent absence of discrimination of those non-neighbouring juveniles from neighbours and siblings can thus be interpreted as a product of indirect familiarity via traps and/or handling materials.

Despite the pronounced influence of indirect familiarization through trapping, such familiarity alone was not sufficient to induce non-colony members to behave in a similar fashion to siblings and "familiarized" colony members. Non-siblings from separate colonies that had traps exchanged between their natal areas tended to be more agonistic and less cohesive than either non-sibling colony members with mixed traps or siblings. Thus an additional component underlying recognition must operate within colonies. Colony members could be more similar genetically than non-colony members (Zammuto and Millar 1985, MacNeil and Strobeck 1987, Dobson pers. comm.) and thus produce more similar discriminator substances. Genetic mechanisms are known to influence odor cues used in recognition (see Boyse et al. 1991 for a review of MHC (major histocompatibility complex) influence on recognition, Greenberg 1979 for sweat bees, Porter et al. 1986 for spiny mice, Porter et al. 1985 for humans). Further, individuals might acquire those differences from environmental cues that vary among colonies. Given the capacity of juveniles to become familiar indirectly, as demonstrated by the trap manipulation, it is likely that at least some component of indirect familiarization occurs naturally. Columbian ground squirrels investigate acrylic cubes impregnated with scent from the oral angle (Harris and Murie 1982), and are known to deposit such scent cues on natural substrates (Steiner 1974, 1975, Kivett et al. 1976, Kivett 1978). Passive transfer of scent to the substrate may also occur as the dorsal gland field is brought into contact with burrow entrances, as feces are passed over the area of the anal gland, or via urine or secretory products of pedal glands (*ibid*). Thus juveniles are presented with ample opportunity to become familiar with cues emanating from conspecifics. That naturally occurring component of indirect familiarity (or discriminator substance similarity) exerts an influence equal in magnitude to indirect familiarization through trapping. Non-sibling colony members that did not experience mixed traps showed similar levels of all behaviours to non-colony members that had traps exchanged between their natal areas. Further research is necessary to clarify the contributions of genetic similarity detection, environmental odours, and naturally occurring indirect familiarity to discrimination. Such experiments could involve fostering pups between colonies (either by fostering the pups themselves (Chapter 5) or by transplanting pregnant females (Wiggett and Boag 1986)) to examine the contribution of genetic relatedness, and could use artificial diets treated with odoriferous substances (Porter et al. 1989) to examine the role of environmental odours.

Indirect familiarization produced by trap-mixing in this study must have relied on olfactory cues. Visual, auditory, or tactile cues can be dismissed from consideration since they could not have been transmitted via traps. Electromagnetic cues, although theoretically transferrable to metallic objects, are unlikely to be involved. By contrast, a plethora of olfactory cues are available in traps (see above), and indirect familiarity via substrate borne cues has been documented for rodents (Randall 1991). It is tempting to conclude that familiarity in general in this species relies on olfactory cues; however, there is no *a priori* reason to support the notion that visual, auditory, tactile, or even electromagnetic cues could not contribute to recognition. Further research is necessary, perhaps involving temporary olfactory impairment with zinc-sulfate (Alberts and Galef 1971, Alberts 1974) as employed by Holmes (1984) to demonstrate the necessity of olfactory cues to the discrimination of familiar from unfamiliar juvenile thirteen-lined ground squirrels.

The ontogeny and precise mechanism of indirect familiarization via trap mixing also require further examination. Since trap-mixing was commenced prior to juvenile emergence, it is conceivable that dams transmitted discriminator substances associated with juveniles in the reciprocal locale to their juveniles in the natal burrow. Such "mediated" familiarity (sensu Holmes and Sherman 1983) seems unlikely however since neighbouring juveniles in the previous experiment (Chapter 3) were discriminated from littermates during their first 20 days post emergence (Appendix G), but were incorporated into a class of familiar individuals with advancing age (Chapter 3). If familiarity were mediated by dams, that effect should be strongest among neighbours and I would predict that neighbours would not have been discriminated from littermates shortly after emergence. Thus indirect familiarity promoted by trap mixing most likely occurred in the post-emergence period. The extent to which juveniles simply become familiar with the odours of others and later regard them as familiar versus the possiblity that odours from others are adsorbed into a group badge (e.g. family badges in the terrestrial isopod *Hemilepistus reaumuri*, Linsenmair 1987) also requires further examination. Experiments testing indirectly familiar juveniles together and subsequently testing one of those indirectly familiar juveniles with familiars of the other individual from the first trial may be useful in discerning between those hypotheses. If discrimination proves not to be invariably transitive, the gestalt model can be ruled out (Crozier 1987).

Given that neighbouring and non-neighbouring juveniles become familiar either directly or indirectly and are not discriminated from siblings, the results can be extrapolated to elucidate the level of discrimination among juveniles in the absence of experimenter induced familiarization. Those apparent mechanisms would result in overlapping "spheres" of familiarity within colonies so that juveniles would discriminate more familiar group members from less familiar group members. Non-colony members in that light represent an extreme in the continuum of familiarity. Discrimination at the colony level proper can be regarded as an artefact in large colonies but could operate for all practical purposes among individuals occupying relatively small areas of suitable habitat. Further experiments are warranted incorporating individuals from a broader range of distance within colony to examine that hypothesis.

The same fitness benefits advanced for colony member discrimination in the previous experiment apply to the more limited "group" discrimination documented here and are more tenable when restricted to that level. By cooperating with group members in the detection of predators, individuals can enhance their own fitness by increasing foraging efficiency and avoiding predation. For black-tailed prairie dogs (*Cynomys ludovicianus*), Kildaw (1991) found that the influence of group size on vigilance diminishes when the group size considered encompasses individuals occurring at greater distances from the focal individual. Costs of territorial aggression can also be reduced by familiarity if it leads to tolerance among group members (Fisher 1954, Jaeger 1981). Since female kin in this species form aggregations (King 1984), and males inseminate multiple females in the same general area, juveniles in those sub-areas of a colony will share genes by both maternal and paternal descent. Thus by discriminating familiar from unfamiliar individuals, juveniles may be able to select mates who are neither too closely nor too distantly related upon recruitment to the breeding cohort (Bateson 1983). Further experiments are necessary to examine how behavioral discrimination among juveniles translates into discrimination in older age cohorts. It may also be adaptive to discriminate familiar individuals from other less closely related conspecifics to monopolize valuable resources (e.g. burrow systems, hibernaculae, King 1984). Finally, "xenophobic" responses to strangers (sensu Steiner 1975) may ultimately relate to the defence of offspring from infanticidal females (see Appendix I). Although no data are available, any of those functions could facilitate the evolution of a mechanism promoting indirect familiarization as a byproduct of selection for direct familiarity.

In addition to the implications of my results to the proximate mechanism of behavioral discrimination among juveniles, the demonstration of indirect familiarity via traps raises practical considerations for future studies of Columbian ground squirrels, and other species in general. Experimenters must exercise new caution in the use of live-traps. The use and dispersion of traps should be documented and depending on the nature of the research, traps should be restricted to circumscribed areas, or washed thoroughly after each use. At the very least, results should be interpreted in light of the potential influence of experimenter-induced familiarity. Those considerations are crucial to studies of social discrimination, but are also applicable to other research including but not limited to 1) studies of breeding, since familiarity influences both mate choice within species (Barnard and Fitzsimons 1988) or between species (D'Udine and Alleva 1983) and subsequent reproductive performance (Bruce 1959, Boyd and Blaustein 1985), 2) studies of dispersal, since the "coefficient of familiarity" (Bekoff 1981a, 1981b) within groups is affected and settlement may be influenced by familiarity since dispersing individuals would experience decreased aggression among familiar individuals, and 3) studies of territoriality, since familiarity determines which individuals will be regarded as "dear enemies" (Fisher 1954).

Table 4.1: Summary of the behaviour of the colony membership/trap manipulation groups relative to littermate juveniles (NSRA2= non-siblings from two different colonies, NSRAD= non-siblings reared ≥ 70 m apart within the same colony, SRT= siblings reared together; mixed= traps shared by groups, fixed= traps restricted to one locale only; data are mean arcsine/square root/proportion ± SE, n=7).

Behaviour Category	Treatment Group					Single Factor ANOVA	
	SRT	NSRAD mixed	NSRAD fixed	NSRA2 mixed	NSRA2 fixed	F (4,30)	*P*
Recognitive	0.26±0.08	0.26±0.03	0.15±0.03	0.17±0.03	0.11±0.03	2.56	0.052
Recognitive[a]	0.26±0.04	0.22±0.05	0.17±0.04	0.15±0.04	0.14±0.05	1.31	0.291
Investigative	0.21±0.05	0.25±0.04	0.20±0.06	0.30±0.07	0.14±0.02	1.39	0.262
Scent Reception	0.18±0.04	0.18±0.02	0.16±0.04	0.18±0.03	0.16±0.02	0.19	0.944
Active	0.24±0.05	0.33±0.02	0.24±0.05	0.30±0.04	0.24±0.02	1.11	0.368
Resting	0.92±0.12	0.91±0.05	1.10±0.11	0.95±0.11	1.14±0.03	1.30	0.291

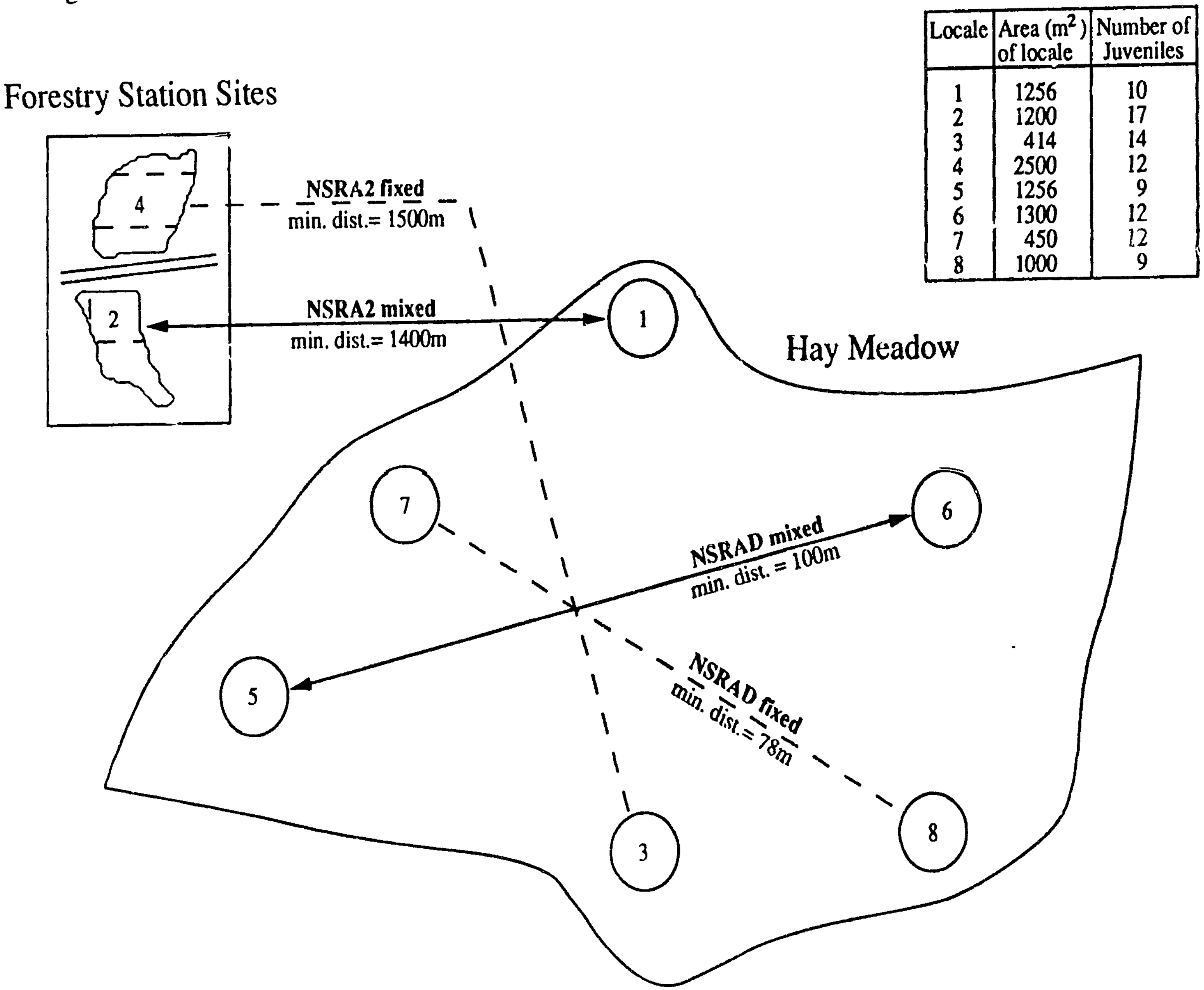
[a] behaviour adjusted for juvenile mass using ANCOVA; df=4,1,29

Table 4.2: Summary of 2-factor ANOVA results evaluating the effects of colony membership and trap manipulation on the behaviour of juvenile Columbian ground squirrels (data are mean arcsine/square root/proportion ± SE, $n=14$)

Behaviour	Colony Membership				Trap Manipulation			
Category	mean±SE intra-site	mean±SE inter-site	ANOVA F (1,26)	*P*	mean±SE mixed	mean±SE fixed	ANOVA F (1,26)	*P*
Recognitive	0.20±0.02	0.14±0.02	5.037	0.034*	0.21±0.02	0.13±0.02	8.769	0.007*
Investigative	0.23±0.04	0.22±0.04	0.006	[illegible]	0.28±0.04	0.17±0.03	4.456	0.045*
Scent Reception	0.17±0.02	0.17±0.02	0.000	0.989	0.18±0.02	0.16±0.02	0.556	0.463
Active	0.29±0.03	0.27±0.02	0.191	0.666	0.31±0.02	0.24±0.03	3.996	0.057
Resting	1.01±0.06	1.04±0.06	0.185	0.671	0.93±0.06	1.12±0.06	4.889	0.037*
Recognitive[a]	---	---	---	---	0.20±0.02	0.15±0.02	1.846	0.186
Cohesive[a]	---	---	---	---	0.09±0.02	0.05±0.02	1.359	0.255

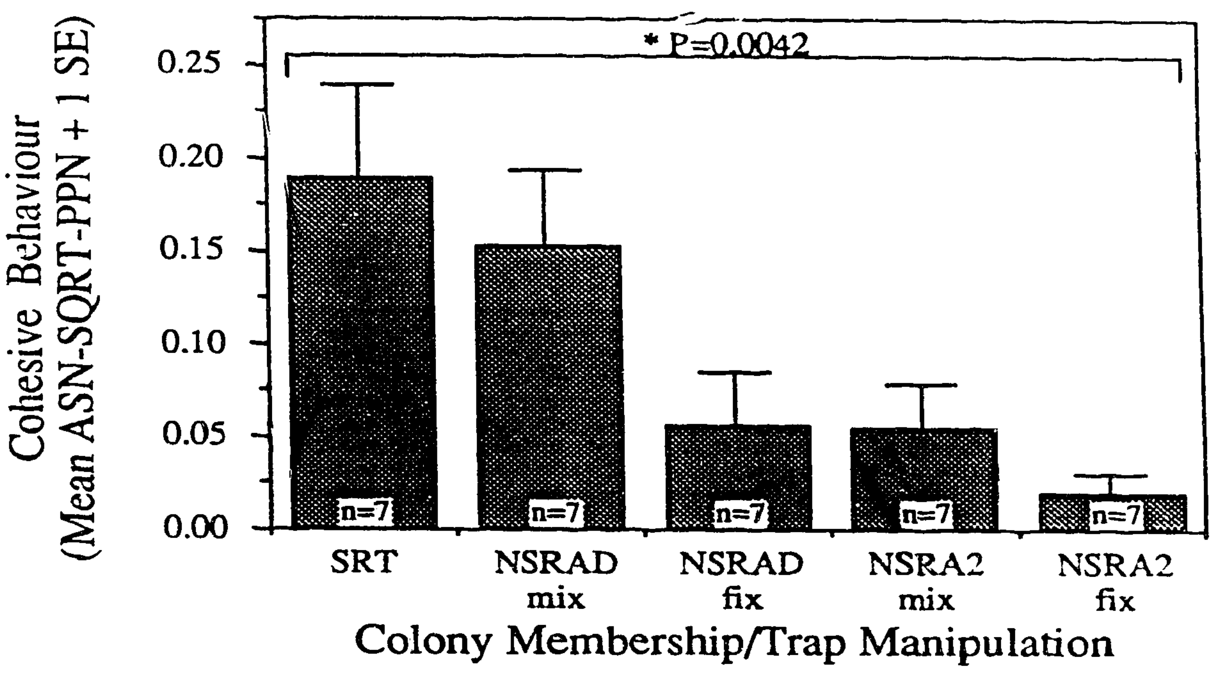
[a] behaviour in trap manipulation groups adjusted for juvenile mass using ANCOVA; df=1,1,25

Figure 4.1: Schematic Representation of Trap Mixing Experiment (not to scale)



Locale	Area (m^2) of locale	Number of Juveniles
1	1256	10
2	1200	17
3	414	14
4	2500	12
5	1256	9
6	1300	12
7	450	12
8	1000	9

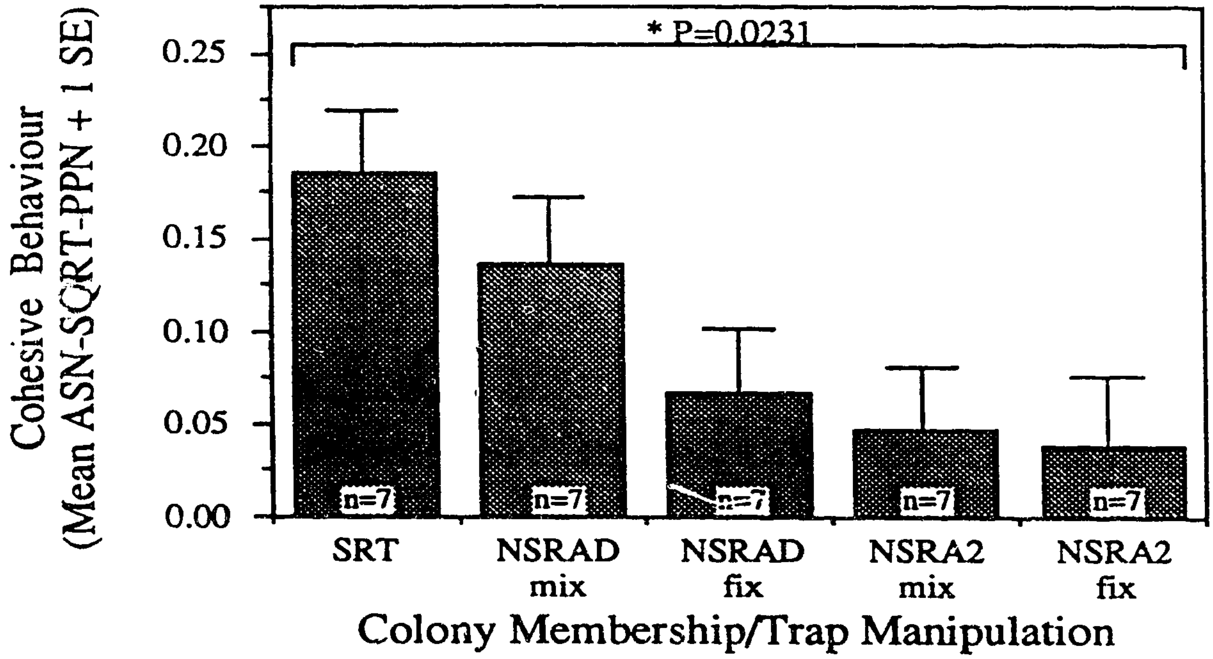
Figure 4.2: Cohesive behaviour displayed in interactions between siblings (SRT), spatially distant non-siblings (NSRAD), and members of two different colonies (NSRA2) with and without trap mixing.



Pairwise Contrasts (sequential Bonferroni)

Contrast	p	Contrast	p
SRT vs. NSRA2fix	0.011*	NSRADmix vs. NSRA2mix	0.266
SRT vs. NSRA2mix	0.068	NSRADmix vs. NSRADfix	0.266
SRT vs. NSRADfix	0.068	NSRADfix vs. NSRA2fix	1.000
SRT vs. NSRADmix	1.000	NSRADfix vs. NSRA2mix	1.000
NSRADmix vs. NSRA2fix	0.068	NSRA2mix vs. NSRA2fix	1.000

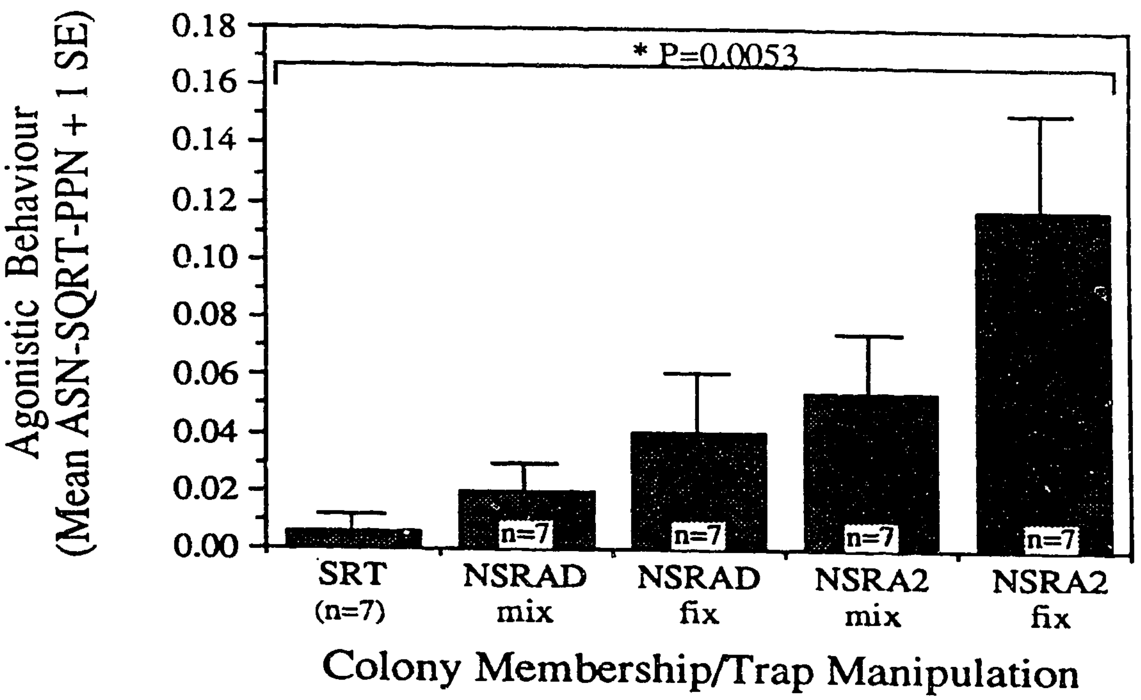
Figure 4.3: Cohesive behaviour displayed in interactions between siblings, spatially distant non-siblings, and members of two different colonies with and without trap mixing (adjusted for juvenile mass).



Pairwise Contrasts (sequential Bonferroni)

Contrast	p	Contrast	p
SRT vs. NSRA2fix	0.061	NSRADmix vs. NSRA2mix	0.505
SRT vs. NSRA2mix	0.061	NSRADmix vs. NSRADfix	0.972
SRT vs. NSRADfix	0.147	NSRADfix vs. NSRA2fix	1.000
SRT vs. NSRADmix	1.000	NSRADfix vs. NSRA2mix	1.000
NSRADmix vs. NSRA2fix	0.575	NSRA2mix vs. NSRA2fix	1.000

Figure 4.4: Agonistic behaviour displayed in interactions between siblings, spatially distant non-siblings, and members of two different colonies with and without trap mixing



Pairwise Contrasts (sequential Bonferroni)

Contrast	p	Contrast	p
SRT vs. NSRA2fix	0.005*	NSRADmix vs. NSRA2mix	1.000
SRT vs. NSRA2mix	0.608	NSRADmix vs. NSRADfix	1.000
SRT vs. NSRADfix	1.000	NSRADfix vs. NSRA2fix	0.095
SRT vs. NSRADmix	1.000	NSRADfix vs. NSRA2mix	1.000
NSRADmix vs. NSRA2fix	0.017*	NSRA2mix vs. NSRA2fix	0.241

Figure 4.5: Cohesive and agonistic behaviour of squirrels with colony members and non-colony members.

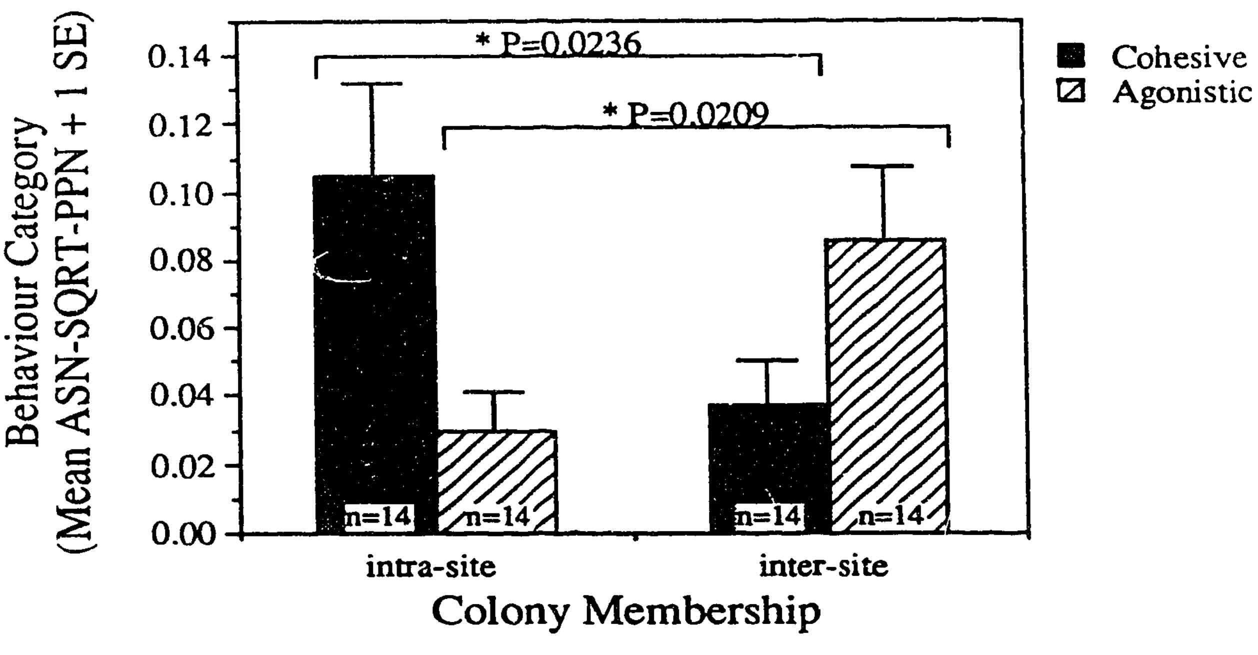
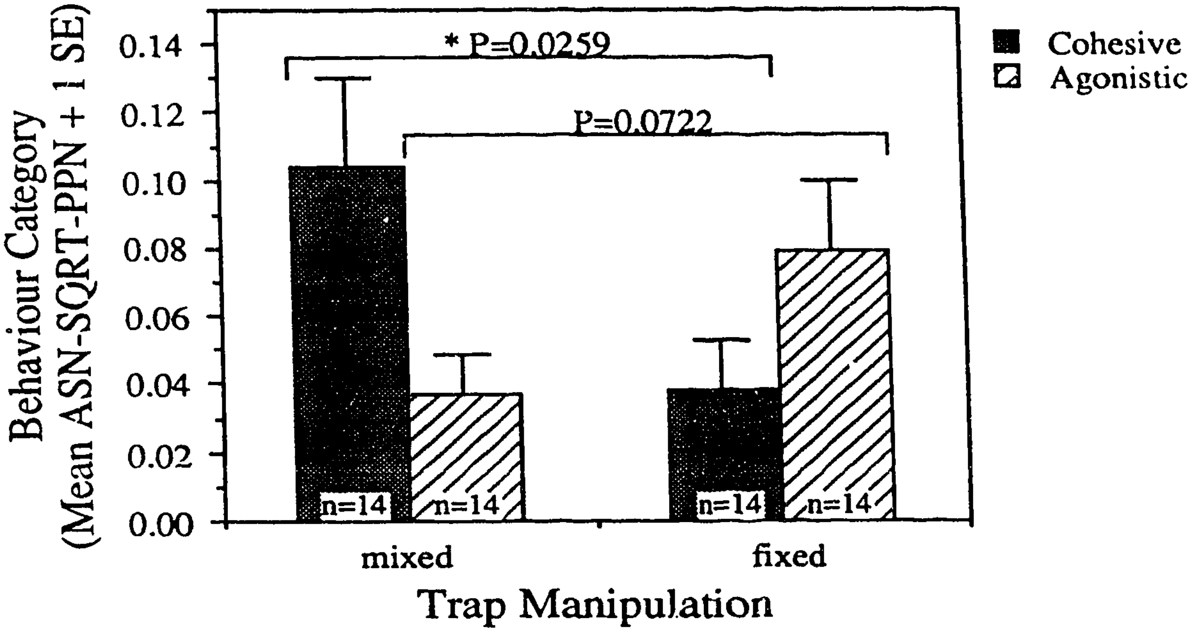


Figure 4.6: Cohesive and agonistic behaviour of squirrels exposed to mixed or fixed traps.



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Chapter 5: The quest for the "holy grail": does kinship influence behavioural discrimination by juvenile Columbian ground squirrels?

Introduction

Kin selection has often been invoked as a mechanism through which social behaviour might evolve (Wilson 1975, Barash 1982). To lend credence to the kin selection hypothesis, many studies have sought to document kin recognition (Waldman et al. 1988). Kin recognition by genetic similarity detection has been demonstrated unambiguously only for a limited number of species. Although Grafen (1991a, 1991b) maintains that the only convincing evidence of kin recognition in that strict sense has been in the colonial ascidian *Botrylus schlosseri* where individuals preferentially fuse with others of similar genotype (Grosberg and Quinn 1986), mating preferences of Japanese quail (*Coturnix coturnix*) for first cousins over full siblings (Bateson 1982), the discrimination of littermate maternal-half siblings from littermate full siblings in Belding's ground squirrels (Holmes and Sherman 1982), the mating preference of mice (*Mus musculus*) for individuals with non-self MHC (major histocompatibility complex) types (Yamazaki et al. 1976), and discrimination of paternal-half siblings from non-siblings in cascade frog (*Rana cascadae*) tadpoles (Blaustein and O'Hara 1982) are all consistent with kin discrimination by genetic similarity detection (but see Blaustein et al. 1991, Grafen 1991a, 1991b).

Perhaps owing to the seductive nature of the notion that kin selection can explain apparent altruism, studies have focussed on kinship at the expense of examining the influence of familiarity on social behaviour (Bekoff 1981). In studies of social discrimination where the effects of both kinship and familiarity are examined simultaneously, familiarity often proves the sole mechanism by which "kin" are discriminated in a natural context (e.g. Holmes 1984), modifies observed discriminatory behaviour (Davis 1982, Holmes and Sherman 1982), or completely overrides any apparent affect of genetic relatedness (Kareem and Barnard 1982). A reliance on early learning mechanisms does not necessarily preclude the discrimination of kin from non-kin

(Bekoff 1981), particularly where young are reared in isolation from distant kin and non-kin (Holmes and Sherman 1982). In those cases fitness benefits can still be accrued via kin selection (Stuart 1991).

The most frequently applied protocol to partition the effects of kinship and familiarity on subsequent social discrimination in small mammals involves cross-fostering newborn individuals among litters of females so that the behaviour of related-familiar, related-unfamiliar, unrelated-familiar, and unrelated-unfamiliar individuals can be assayed (e.g. Porter et al. 1981, Davis 1982, Holmes and Sherman 1982, Kareem and Barnard 1982, Holmes 1984, Fuller and Blaustein 1990). For instance, by comparing the behaviour of siblings reared together (SRT) to non-siblings reared apart (NSRA), one can examine the natural extent of discrimination between non-littermates. A comparison of the behaviour of SRT to non-siblings reared together (NSRT) allows an assessment of whether pre-weaning association is sufficient to account for any discrimination of littermates from non-littermates. Finally, comparing the behaviour of SRT to siblings reared apart (SRA) examines the extent to which rearing association is necessary for the discrimination of littermates from non-littermates (i.e. the extent to which kin may be discriminated in the absence of rearing association).

Use of the cross-fostering protocol with juvenile ground squirrels (*Spermophilus* spp.) has revealed variation in the ability of juveniles to discriminate kin in the absence of rearing association. In thirteen-lined ground squirrels, association prior to weaning completely overrides any effect of genetic relatedness and indeed siblings reared in separate litters behave as if they were non-siblings (Holmes 1984). Thus familiarity is both sufficient and necessary for the normal ontogeny of "kin recognition" in that species. Conversely in Richardson's ground squirrels, pre-weaning association exerts a lesser effect on the development of kin-differential behaviour, and siblings reared apart retain the ability to discriminate kin from non-kin (Davis 1982). The situation in Arctic ground squirrels (*S. parryii*) and Belding's ground squirrels (*S. beldingi*) is intermediate to those extremes with sibling females demonstrating the ability to recognize one another in the absence of rearing association, while non-siblings reared in the same litter behave as full siblings (Holmes and Sherman 1982). In Arctic and Belding's ground-squirrels then, familiarity is sufficient to induce animals to behave as "kin" but is not necessary for kin recognition proper among females.

Recent studies of Columbian ground squirrels (*Spermophilus columbianus*) have failed to resolve any influence of kinship on behaviour (MacWhirter 1989) nor any fitness benefits accrued through associating with kin (King et al. 1991, Murie and King pers. comm.). Hare (Chapter 3, Chapter 4) found that juvenile Columbian ground squirrels come to discriminate familiar from unfamiliar individuals using both direct association and olfactory cues acquired indirectly. However, there is evidence that littermates are discriminated from even neighbouring juveniles in the 20 day period subsequent to juvenile emergence (Appendix G, Waterman 1985) and that kin-differential behaviour occurs among adult females (King 1989a). Thus the ontogeny of social discrimination in Columbian ground squirrels merits further examination.

Holmes (1984) noted that an increasing reliance on direct mechanisms of kin discrimination (i.e. decreasing reliance on familiarity) paralleled increasing social complexity as measured by spatial association and frequency of social encounters in ground-dwelling squirrels. Columbian ground squirrels have been classified as highly social by both Armitage (1981) and Michener (1983). Thus the extent to which the discrimination of kin relies on familiarity in Columbian ground squirrels is pertinent to an understanding of the comparative sociality of ground squirrels in general.

The ability of juveniles to discriminate kin from non-kin in the absence of rearing association, and the extent to which rearing association is sufficient to account for subsequent discriminative behaviour are essential to a complete understanding of the ontogeny of social discrimination in Columbian ground squirrels. Further, such data are useful in re-evaluating the comparative social classification of the ground-dwelling squirrels. In this experiment, juveniles were fostered among litters of Columbian ground squirrels and data were collected to assess the extent to which rearing association allows littermate discrimination. Data collected while studying the level of discrimination among juveniles (Chapter 3) are also examined further to test for any apparent kin bias in behaviour.

Methods

Juvenile Columbian ground squirrels from a 1.9 ha section of a large (ca. 70 ha) meadow in the Sheep River Wildlife Sanctuary (ca. 2 km east of the junction of the Sheep River and Gorge Creek, 50°38' N, 114°37' W; elevation 1470m) in southwestern Alberta were used in this study. Squirrels on that area were live-trapped using Tomahawk or National live traps baited with peanut butter and were marked with ear tags (Monel #1 National Band & Tag Co.) for permanent identification and with human hair dye applied to their dorsal pelage (Clairol Nice n' Easy ™ #124) for identification during observations (see Chapter 2). Research activities commenced with the spring emergence of the first squirrel in mid-April (Appendix A), and breeding dates of all females on that site were established following the techniques outlined by Murie and Harris (1982; see Chapter 2). General techniques used in handling squirrels were as outlined in Chapter 2 except where noted below.

Since attempts to cross-foster juveniles among litters using field exclosures proved unsuccessful (Appendix I), I attempted to foster pups from "donor" females to "target" dams in their nest burrows in order to obtain four levels of relatedness/rearing association; siblings reared together (SRT), siblings reared apart (SRA), non-siblings reared together (NSRT), and non-siblings reared apart (NSRA). Pups for fostering were obtained from 18 "donor" females that were live-trapped 2 days prior to their predicted parturition date and housed in polycarbonate cages (48 by 27 by 20 cm) within a heated building at the field station. Donor females were provided with cedar chips and paper tissue for bedding and given Purina rodent blocks, lettuce, and water ad libitum [illegible]se females were checked each morning at 0800 h. If newborn pups were present, their gender was noted, mass was recorded to the nearest 1 g with a Pesola spring balance, and they were marked by clipping one toe-nail bud from a hind toe. Donor females were released at the original point of capture the morning their litter was delivered (n=14), or two days after their predicted parturition date if no litter was delivered (n=4). The 35 pups produced by the 14 donor females were fostered into litters of 22 dams that had given birth to young within the preceding 24 h (based on weight and nipple condition). Pups were handled with rubber gloves to avoid the transfer of human odours that might induce infanticide (Hare 1991; Appendix I). Pups were transported to the field site in a plastic container lined with paper tissue and carried inside a styrofoam container (to keep pups warm) for fostering to recently parturient dams. One or two pups (n=9 and n=13 respectively; depending on number of pups and number of recipient dams available on a given day) were fostered to each "target" dam by dropping the pup(s) down the entrance of the nest burrow after the dam had emerged to forage. Pups originating from the same litter were fostered into litters at least 70 m apart to avoid the confounding influence of direct post-weaning association. I scanned for the emergence of juveniles 2 days prior and up to 3 days following predicted emergence dates by walking over the site (Dobson 1990) and watching for small, unmarked squirrels in the area of the nest burrow of a dam whose litter was due up. Juveniles were trapped using wire mesh multiple capture traps (1 by 0.5 by 0.3 m), designed and constructed by J.M. Waterman, A.L. Steiner and J.O. Murie, that were placed over the nest burrow, or by saturating the area where juveniles were observed with live traps. Juveniles that could not be trapped after 3 days using the above methods were hand-trapped by lying motionless in the grass next to a burrow the juvenile was using and grabbing the juvenile as it came above ground (Wiggett 1987). Upon first capture, the gender, mass, toe-nail mark (see above), and prospective dam of each juvenile were recorded. Juveniles were ear-tagged and given a dye-mark using numbers assigned arbitrarily (rather than in sequence) to impose a "blind" on relatedness and rearing association in subsequent coding of behavioral interactions.

Interactions between juveniles in the relatedness/rearing association groups were staged in a 1.5 by 1.5 m plexiglass arena at the field station and followed the testing protocol outlined in Chapter 3. Those dyadic interactions were videotaped and behavioural data were coded from the tapes using a microcomputer event recorder program (see Chapter 3). Because prior research (Chapter 3 and Chapter 4) indicated that discrimination among juvenile Columbian ground squirrels was apparent for cohesive and agonistic behaviours (Appendix B), those behaviour categories were used as assays of recognition. Since discrimination between littermates and non-littermates is diminished by post-weaning association (Chapter 3, Appendix G), those data were analysed both for juveniles of all ages tested and with cases limited to juveniles less than 21 days post-emergence. For the full data set, agonistic behaviour was adjusted for the age of the focal animal and cohesive behaviour was adjusted for age, trial within day, and number of times run (see Appendix E) using analysis of covariance. For the data limited to individuals less than 21 days post-emergence, those potential confounding factors were balanced across the 5 levels of relatedness/association (single factor ANOVA, all $P > 0.75$) and thus overall differences among groups were examined using parametric analysis of variance. Post hoc comparisons between groups were achieved using pairwise contrasts (SuperANOVA™ contrast routine) with the resultant statistical significance (P) of those contrasts adjusted using the sequential Bonferroni technique (Rice 1989) to protect the experimentwise error rate.

In addition to data obtained by experimental manipulation of litter composition, data were compiled from 21 juvenile squirrels that interacted with both littermates (full or half-siblings) and non-littermates in the arena in 1988. By treating those juveniles as focal individuals, paired-sample data are available to address the degree to which individuals distinguish between kin and non-kin. The mean (±SE) age at trial with a sibling was 34

(±2.5) days post-emergence and at trial with a non-sibling 30 (±2.2) days post emergence. Because of the paired-sample nature of those data, restriction of cases to juveniles less than 21 days post-emergence was impossible. Differences in cohesive and agonistic behaviour were examined using paired-sample t-tests. Differences were considered significant where the probability of committing a type I error did not exceed 0.05. Data are presented as mean±SE unless otherwise noted.

Results

Fostering Success

Of the 35 pups fostered into the litters of 22 dams, six emerged as juveniles in four litters. Those litters were composed of one biological offspring and one foster offspring in one case, three biological offspring and one foster offspring in another, and three biological offspring and two foster offspring in two cases. The marking technique for pups likely failed to resolve all fostered individuals, since one "marked" individual showed slight regrowth of the clipped toe-nail, and two target dams that had no definite foster offspring produced litters of five juveniles at emergence, one pup above the maximum litter size observed on this site in 3 years of study (Appendix A). Unfortunately, no definite siblings reared apart survived to juvenile emergence. In staging behavioural interactions, only the 6 definite foster individuals (and their littermates) were used in the non-siblings reared together group, while for other groups (e.g. SRT), only juveniles from litters where no fostering was attempted were used to ensure appropriate assignment of relatedness.

Based on an average production of 2.5 pups per female with a litter (35 pups produced by 14 enclosed dams), we would predict that the remaining 36 females that had litters in 1989 would have produced 90 pups. In that year, 64 non-foster juveniles were trapped at emergence. Thus the survivorship of fostered pups (6/35=0.17) was low compared to the estimated survivorship of non-fostered offspring (64/90=0.71). Note however that the difference estimate represents a maximum since some of those emerging juveniles may have been fostered pups that regrew their clipped toe-nail.

Behaviour of Non-siblings Reared Together

The 6 fostered pups were used in 9 trials of non-siblings reared together. Although allowing a single individual to contribute multiple observations to the data set increases the probability of committing type I error (Machlis et al. 1985), it was my desire to have a reasonable sample size to examine the sufficiency of rearing association to subsequent discrimination, and to be conservative in supporting the null hypothesis. In committing the pooling fallacy one can be virtually certain that no difference exists where none is detected since the test is biased toward detecting a difference due to decreased within group variance (Machlis et al. 1985). Further, post-hoc contrasts were limited to comparisons between non-siblings reared together and siblings or non-colony members. Those 2 contrasts examine the hypothesis of interest, but not as much power is sacrificed as performing all possible pairwise contrasts.

When the NSRT cases are included in the analysis of the level of discrimination data (Chapter 3), an overall significant difference is detected in agonistic behaviour (*P* =0.0002) among the 5 groups. Non-siblings reared together showed the least aggression of any group (Figure 5.1) but showed similar levels of aggression to other intra-colony dyads (Figure 5.1). In a posteriori contrasts, non-siblings reared together were significantly less agonistic than non-colony members (*P* =0.0004), but were not significantly different from siblings (*P* =0.22). An overall difference among the 5 groups was also detected for cohesive behaviour (*P* =0.0005). Contrasts revealed that non-siblings reared together did not differ from siblings in cohesive behaviour (*P* =0.94), but were significantly more cohesive than non-colony members (*P* =0.002) (Figure 5.2).

Contrasts parallel to those outlined above but limiting the cases to juveniles ≤ 20 days post-emergence revealed no difference in agonistic behaviour between siblings and non-siblings reared together (*P* =1.0). Neither the 7 NSRT nor the 6 SRT pairs that met that age criterion showed any agonistic behaviour in those trials. Similarly, no difference between siblings and non-siblings of that age class was detected for cohesive behaviour (*P* =0.59). Siblings were slightly less cohesive (0.12±0.04) than were non-siblings reared together (0.15±0.06). Contrasts of non-siblings reared together to non-colony members were not possible for these data since only one non-colony member pair met the age criterion.

Behaviour of Focal Individuals Toward Littermates and Non-littermates

Twenty-one individual juveniles had arena trials with both a littermate and non-littermate colony member in 1988. Of those 21, eight juveniles were run with a littermate in their first trial and a non-littermate in the second trial while the remaining 13 paired trials were staged in the reverse order. Juveniles were not significantly less aggressive (*P* =0.49) nor significantly more cohesive (*P* =0.52) when in the arena with littermates than with non-littermates although for both slight trends toward greater cohesion and less aggression with littermates relative to non-littermates were apparent (Figure 5.3).

Discussion

Siblings reared together were not more cohesive nor less aggressive than non-siblings reared together. Further, individual juveniles were no more cohesive nor less agonistic in encounters with littermates than with non-littermates. Thus no evidence of kin-differential behaviour was apparent. However, the latter data could not be adjusted for the age of interactants, and given progressive familiarity with non-littermates (Chapter 2, Chapter 3) that result is not unexpected. The extent to which kin are discriminated in the absence of rearing association could not be assessed in this study because no siblings reared apart survived to juvenile emergence. Despite the importance of that group in examining social discrimination, the present data are consistent with those from prior research (Chapter 2, Chapter 3) and suggest that familiarity obscures any influence of kinship that might exist.

Holmes and Sherman (1982) suggested that recognition by association is "appropriately expected" when dams accept foster pups. The relatively low survivorship of foster offspring in this study may suggest kin discrimination proper (or maternal labelling) on the part of dams. However, that difference in survivorship is a maximum estimate since some surviving foster offspring may not have been detected at juvenile emergence due to regrowth of their toe-nail, and reduced survivorship of foster offspring is more readily interpreted as an artifact of the fostering technique. The fact that any foster pups survived to juvenile emergence suggests that the recognition abilities of dams are at best limited. In fostering pups among enclosed females in 1988 (Appendix I), there was scant evidence that females preferentially cannibalized foster pups over their own pups (for 6 dams that cannibalized pups while enclosed, 5 of 5 foster offspring and 7 of 11 biological offspring were consumed; Fisher's exact test, *P* =0.24). That suggests that dams did not discriminate between their own pups and those of other dams at least within the first 48 h post-partum. A similar lack of dam-offspring recognition within that time period (and essentially up to weaning) was reported by Holmes and Sherman (1982) for Belding's ground squirrels. Further research is necessary to unravel the intricacies of dam-offspring recognition in this species; however, these preliminary results suggest that dams do not discriminate among offspring by genetic similarity detection.

Overall, the data obtained are consistent with an emerging pattern of a lack of any kinship effects among Columbian ground squirrels. Armitage (1987) has argued that the evolution of sociality in ground squirrels is best explained by direct selection without recourse to kin selection. Field studies of Columbian ground squirrels support that contention since alarm-calling is consistent with maternal investment rather than kin selection per se (MacWhirter 1989), the presence of close kin has no apparent effect on reproductive success (King et al. 1991, Murie and King pers. comm.), the mother's presence does not influence the dispersal distance of yearling females (Murie and King pers. comm.), and the presence of close kin (mother, daughter, sister) does not affect the spatial dispersion of nest burrows (*ibid*).

Given the inverse correlation between sociality (in terms of spatial association and frequency of social encounters) and reliance on familiarity in members of the genus *Spermophilus* (Holmes 1984), the mechanisms of social discrimination documented for Columbian ground squirrels seem to place this species among the less social ground squirrels classified by Armitage (1981) and Michener (1983). Columbian ground squirrels would parallel either *S. tridecemlineatus* in which rearing association is both sufficient and necessary for kin discrimination (Holmes 1984) or *S. beldingi* and *S. parryii* in which rearing association is sufficient but not necessary among females (Holmes and Sherman 1982).

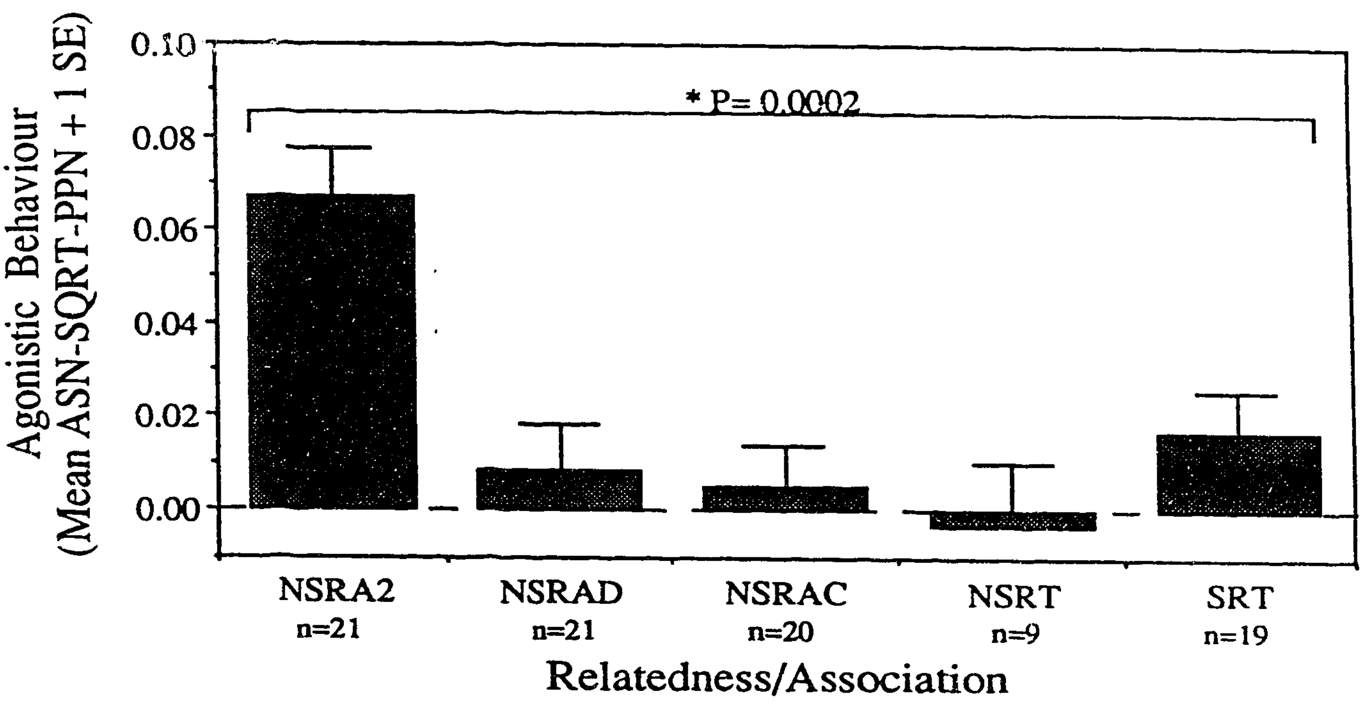
The application of the mechanism of social discrimination to sociality is somewhat misleading. Sociality by definition implies a high degree of cooperation among individuals. Kin selection (and hence kin discrimination) can be of paramount importance to the evolution of cooperative behaviour. Advanced sociality in the colonial invertebrates and haplodiploid social insects (2 of Wilson's (1975) 4 "pinnacles" of social evolution) almost certainly results from benefits that accrue via kin selection (but see Alexander et al. 1991). In colonial invertebrates, a coefficient of relatedness of unity among clonal individuals selects for specialization of individual zooids since the efficiency of fused assemblages of individuals functioning essentially as multicellular organisms is higher than could be achieved by free-living individuals (Wilson 1975). In the eusocial hymenopterans (ants, bees, wasps), the haplodiploid system of sex determination results in sisters sharing a maximum coefficient of relatedness of 0.75, thus selecting for cooperation of workers with their queen in producing sisters (Wilson 1971). Sociality in non-human mammals and man (Wilson's (1975) remaining 2 "pinnacles") is less constrained by kinship; however, both the operation of kin selection and direct selection facilitated by individual recognition in the formation of "selfish subgroups" (*sensu* Wilson 1975) promote sociality. Thus to presume that kin selection is operating and thus that kin recognition occurs is unnecessary for the evolution of vertebrate sociality.

Sociality could be selected for at the individual level via a myriad of "grouping" benefits (see Barash 1982 for a review) essentially unrelated to kin selection. Despite the positive relationship between an increasing ability to discriminate "kin" in the absence of rearing association and sociality in the genus *Spermophilus* (Holmes 1984), the use of that correlation as a prediction for ground squirrels in general is premature since it presumes that sociality evolved and is maintained via kin selection. It remains noteworthy that where kin selection is a prime force in shaping sociality and where close kin are not isolated spatially or temporally from non-kin, some direct means of kin discrimination seems likely. The level and mechanisms underlying social discrimination by juvenile Columbian ground squirrels (Chapter 2, Chapter 3) leading to familiar individuals interacting amicably is consistent with the maintenance (and perhaps evolution) of sociality through benefits of cooperation with group members that accrue via direct selection. This does not *de facto* imply that Columbian ground squirrels are any less social than congeners that employ more direct mechanisms of social discrimination (e.g. kin recognition). If sociality is defined using the general criterion of the nature of social interactions, Columbian ground squirrels can be regarded as more social than congeners that restrict the expression of amicable behaviour to kin. The above arguments do not discount any selective advantage accruing via kin selection where a correlation between familiarity and genetic relatedness exists (Gamboa et al. 1991).

Sociality in any comparative classification seems best defined in terms independent of the mechanisms promoting its existence. Armitage (1981) categorized species of the tribe Marmotini into 5 levels of sociality based on female burrow sharing and mating system. The social levels he distinguished correlated best with measures of delayed maturity (breeding and dispersal). He included Columbian ground squirrels with Olympic marmots (*Marmota olympus*) and yellow-bellied marmots (*M. flaviventris*), 2 species in which females share burrow systems associated with a territorial male (i.e. harem polygyny). Since yearling breeding (Festa-Bianchet 1981, Murie pers. comm.) and dispersal (Elliott and Flinders 1980, Boag and Murie 1981, Festa-Bianchet and King 1984, Wiggett and Boag 1989) have been documented for Columbian ground squirrels, and females defend territories and live individually rather than in "harems" (Festa-Bianchet and Boag 1982), this species should be included with the somewhat less social "group 3" species in Armitage's classification scheme (i.e. with white-tailed prairie dogs, *Cynomys leucurus*, and Gunnison's prairie dogs, *C. gunnisoni*). Michener (1983) placed Columbian ground squirrels with Arctic ground squirrels in her classification of sociality based on spatial overlap of females, male territoriality, and interactions of juveniles with non littermates. Although I generally agree with Michener's schema, Columbian ground squirrels should perhaps be considered less social than Arctic ground squirrels where young of closely related females are "clumped" into a common burrow system after emergence from their natal burrows (McLean 1982). Such cooperative care of young can be regarded as a highly social trait (Wilson 1971), and has not been observed in Columbian ground squirrels.

Further research is warranted on the extent to which Columbian ground squirrels discriminate among unfamiliar kin, and the ramifications of early social familiarity on mature individuals. However, kin discrimination proper is clearly secondary to the influence of social familiarization in this species. The results obtained to date suggest that sociality in Columbian ground squirrels may have evolved, and is most likely maintained, in the context of direct selection rather than kin selection per se.

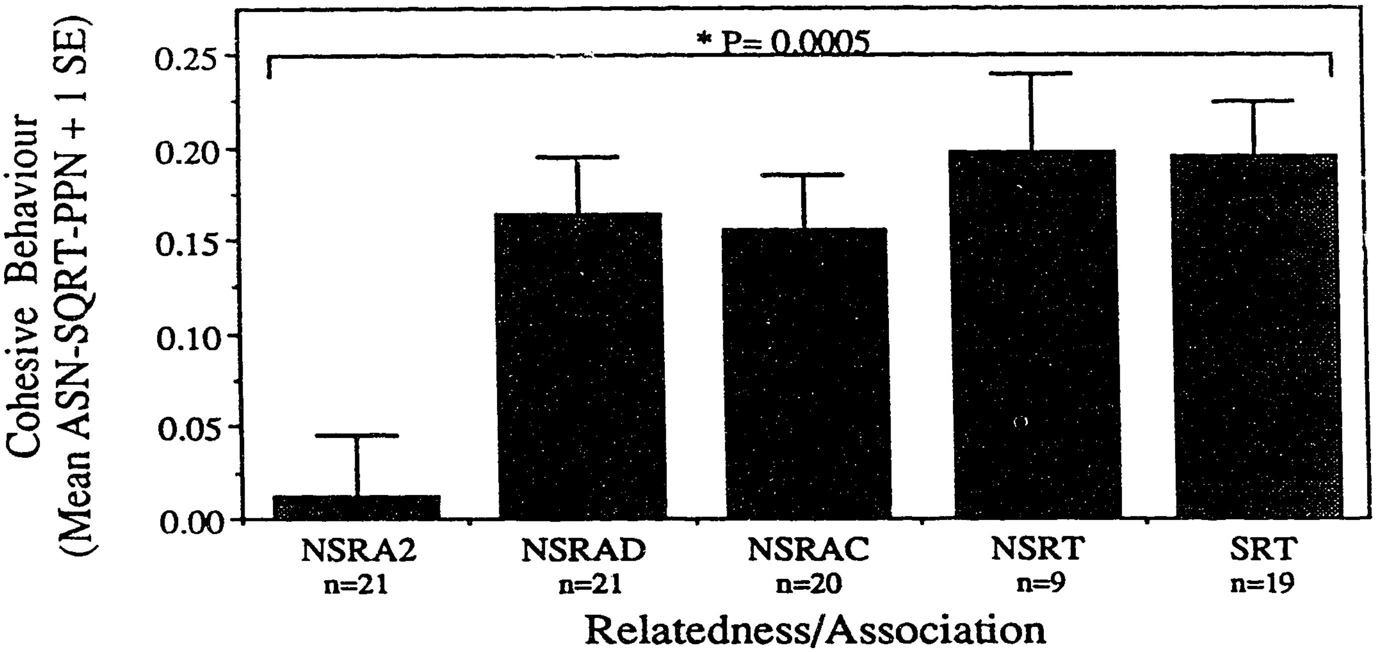
Figure 5.1: Agonistic behaviour displayed in interactions between siblings (SRT), non-siblings reared together (NSRT), neighbours (NSRAC), spatially distant non-siblings (NSRAD), and members of two different colonies (NSRA2) (adjusted for age).



Pairwise Contrasts (sequential Bonferroni)

Contrast	p
NSRT vs. SRT	0.2181
NSRT vs. NSRA2	0.0004*

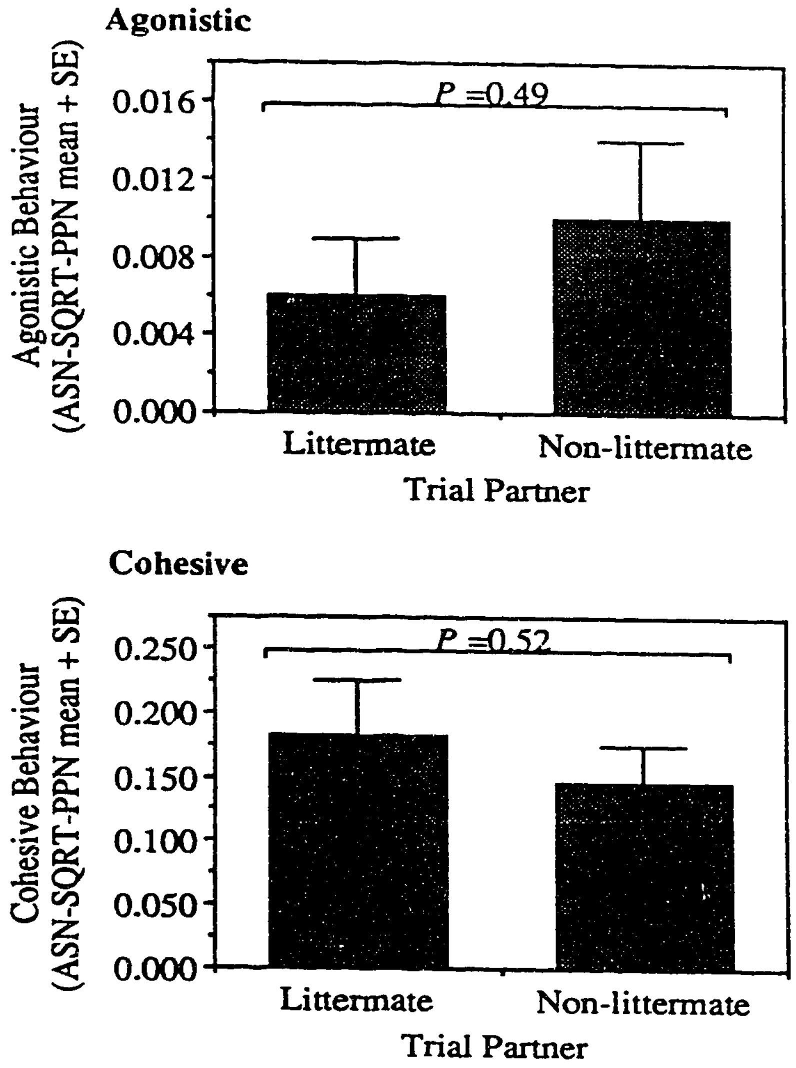
Figure 5.2: Cohesive behaviour displayed in interactions between siblings, non-siblings reared together, neighbours, spatially distant non-siblings and members of two different colonies (adjusted for age, trial within day, and number of times individuals run).



Pairwise Contrasts (sequential Bonferroni)

Contrast	p
NSRT vs. SRT	0.938
NSRT vs. NSRA2	0.002*

Figure 5.3: Agonistic and cohesive behaviour of 21 juvenile Columbian ground squirrels with both littermates and non-littermates (*P* values from paired t-test).



Agonistic

Cohesive

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Chapter 6: Concluding Discussion

Juvenile Columbian ground squirrels discriminated colony members from non-colony members in terms of both cohesive and agonistic behaviour but did not discriminate among colony member siblings, neighbours, or non-neighbours in the first experiment (Chapter 3). However, those results may have been confounded by the unrestricted mixing of live-traps within the site from which intra-colony dyads were obtained, and a lack of mixing of live-traps between sites used for inter-colony trials.

Results of the second experiment demonstrated that juveniles become familiar with conspecifics via olfactory cues present in live-traps (or handling materials) and subsequently show differential behaviour toward those "indirectly" familiar individuals relative to unfamiliar individuals (Chapter 4). That result was apparent both within and between colonies. Despite that effect, colony member pairs were significantly more cohesive and significantly less agonistic than non-colony member pairs exposed to the same trap-mixing protocol. Thus indirect familiarity via traps contributes to the observed pattern of discrimination but does not fully account for the differential treatment of colony members relative to non-colony members. That "residual" component of colony member discrimination could result from direct familiarity, naturally occurring indirect familiarity, odour mixing resulting in an emergent ("gestalt") colony odour, or greater genetic similarity among colony members so that individuals produce and perceive similar discriminator substances.

No evidence was obtained to suggest that juveniles discriminate among individuals on the basis of kinship alone (Chapter 5). A trend toward the discrimination of littermates from non-littermate neighbours within the first 20 days post-emergence waned with increasing age and thus is most parsimoniously interpreted as direct familiarization. Familiarity (pre-weaning, direct post-weaning, and indirect post-weaning) emerges as the pre-eminent factor underlying social discrimination among juvenile Columbian ground squirrels.

In the absence of experimenter-induced familiarity, the mechanisms documented would result in the discrimination of colony members within a certain range of one's natal burrow from other colony members and the discrimination of both those "classes" of individuals from non-colony members. That level of discrimination promotes cooperation among neighbours, may facilitate optimal inbreeding/outbreeding, and may ensure that valuable resources are retained by kin. Reduced costs associated with territorial aggression and fitness payoffs of selecting an appropriate mate function to enhance individual fitness; however, since neighbours are also more likely to be genetically related, some fitness payoffs can accrue via kin selection.

Although kin-differential behaviour has been documented for Columbian ground squirrels (King 1989, Waterman 1985), the results of those studies are consistent with familiarity effects, and recent studies have failed to document kin-differential behaviour (MacWhirter 1989) or any benefit of associating with kin (King et al. 1991, Murie and King pers. comm.). Those findings, in combination with my data that demonstrate mechanisms promoting the discrimination of familiar from unfamiliar individuals, suggest that sociality in this species has evolved and is maintained via direct selection rather than kin selection (Chapter 5).

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Appendix A: Significant Dates and "Colony" Demographics of squirrels in the Hay Meadow[1]

Significant Dates in Squirrel Activity	1988	1989	1990
Arrival date (experimentor)	5 April	19 April	10 April
Date 1st squirrel emerged	~8 April	< 20 April	14 April
Date 1st breeding	20 April	24 April	22 April
Median date breeding	28 April	3 May	4 May
Date last breeding	7 May	21 May	14 May
Date parturition start	14 May	18 May	16 May
Median date parturition	23 May	26 May	28 May
Date last parturition	31 May	5 June	7 June
Date first juveniles emerged	15 June	21 June	15 June
Median date juvenile emergence	22 June	23 June	26 June
Date last juveniles emerged	29 June	2 July	5 July
Gestation length in days (mean±SE; n)[2]	24.3±0.1; 29	24.5±0.1; 41	24.3±0.1; 53
Lactation length in days (mean±SE; n)[1]	27.8±0.5; 2	28.6±0.3; 19	28.4±0.2; 43
Breeding to juvenile emergence in days (mean±SE; n)[1]	52.2±0.6; 12	52.8±0.3; 22	52.7±0.1; 45
Departure date	18 August	19 August	6 August
Descriptors of Squirrel Numbers & Survival			
Adult males[3]	n=46	n=34	n=33
Adult females[2]	n=61	n=58	n=53
Ratio of adult males: adult females	1:1.33	1:1.71	1:1.61

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[1] sample sizes vary as not all pertinent data obtained for all individuals

[2] estimate includes squirrels living peripheral to 1.9 ha main grid

[3] inferred by weight at spring emergence in 1988; known in 1989 and 1990

(Numbers and Survival continued)	1988	1989	1990
Yearling males[2]	n=1	n=6	n=2
Yearling females[2]	n=11	n=3	n=2
Ratio of yearling males: yearling females	1:11	2:1	1:1
Adult male weight at emergence (mean±SE; n)	509±14; 36	490±14; 30	513±14; 30
Adult female weight at emergence (mean±SE; n)	402±8; 51	403±8; 55	405±7; 48
Yearling male weight at emergence (mean±SE; n)	210; 1	210±10; 6	190±10; 2
Yearling female weight at emergence (mean±SE; n)	190±9; 11	193±13; 3	160±-; 1
Juveniles remaining by 1 August	35/53=0.66	45/70=0.64	27/101=0.27
Juveniles returning in subsequent year as yearlings[4]	unknown	9/53=0.17	4/70=0.06
Yearlings returning in subsequent year[5]	unknown	6/12=0.5	7/9=0.78
Adults returning in subsequent year[4]	unknown	85/107=0.79	74/92=0.80
Dispersers settling on site	n=0	n=3	n=1
Measures of Reproduction			
Minimum number of breeding females[6]	n=41	n=52	n=50
Minimum number of breeding males[7]	n=21	n=27	n=27
Weight drop at parturition (mean ±SE (g); n)[8]	50±4; 13	67±7; 27	53±3; 41
Minimum litters by females (weight drop, lactation or litter)	n=35	n=36	n=45
Nests located (of all known with litters)	22/35=0.63	33/36=0.92	43/45=0.96
Minimum litters at emergence	n=19	n=22	n=39

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[4] losses due to mortality both prior to and during hibernation

[5] losses due to mortality and dispersal

[6] based on vaginal smears, observed breeding behaviour or litter

[7] based on direct observation of breeding behaviour or "caked" scrotum

[8] 1990 includes dams manipulated as part of cost of reproduction experiment (see Appendix 3)

(Reproduction continued)	1988	1989	1990
Litters lost prior to juvenile emergence (natural; based on nipples)	n=2	n=11	n=5
Litters lost prior to juvenile emergence (due to cross-fostering)	n=15	N/A	N/A
Male juveniles emerged	n=32	n=40	n=42
Female juveniles emerged	n=21	n=30	n=42
Overall ratio of juvenile males: juvenile females	1.56:1	1.33:1	1:1
Juvenile weight at emergence (males), (mean±SE; n)	142±8; 30	106±5; 36	101±4; 42
Juvenile weight at emergence (females), (mean±SE; n)	143±10; 21	98±4; 28	97±4; 42

Litter size at emergence (Mean ± SE, n, range)

1988:	Unmanipulated;	2.28 ± 0.19, n=18, range=1 - 4
1989:	Unmanipulated;	2.00 ± 0.33, n=8 , range=1 - 3
	Potential foster;	3.10 ± 0.43, n=10, range=1 - 5
	Definite foster;	4.00 ± 0.71, n=4 , range=2 - 5
	Pooled;	2.86 ± 0.30, n=22, range=1 - 5
1990:	Unmanipulated;	1.50 ± 0.43, n=6 , range=1 - 3
	Reduced in 1989;	2.25 ± 0.37, n=8 , range=2 - 3
	Augmented in 1989;	2.75 ± 0.48, n=4 , range=2 - 4
	Probable Augmented in 19[illegible];	2.33 ± 0.33, n=3 , range=2 - 3
	Pooled (includes foster attempts);	2.26 ± 0.12, n=46, range=1 - 4

Appendix B: Behavioural Assays of Recognition

(Modified from: Sheppard & Yoshida 1971[1], Michener & Sheppard 1972[2], Steiner 1973[3], Michener 1974[2], Dunford 1977[1], Davis 1982[1], Harris & Murie 1982[4], Holmes & Sherman 1982[1], McLean 1982[2], Holmes 1984[1], King 1984[3], Waterman 1985[3], Waterman 1986[3], Caley & Boutin 1987[5] , Wiggett 1987[2], Waterman 1988[3])

*note: behaviours which appear in bold were defined as "key" behaviours, since a priori predictions were made about differences in those behaviours that imply recognition. Other behaviours denoted by the following codes were not included in final analyses since they; were not seen (NS), could not be identified reliably (NR), are coded with the spatial data (S), or are ambiguous with respect to category (A).

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[1] applies to juveniles of other *Spermophilus* spp.

[2] applies to intra-adult or adult-juvenile *Spermophilus* spp.

[3] applies to juvenile Columbian ground squirrels directly

[4] applies only to inferences about scent related behaviours

[5] applies to juveniles in Order Rodentia other than *Spermophilus* sp.

Recognitive Behaviours (also termed exploratory encounters)-usually brief contact (< 5 sec.) involving any part of the body.

These include:

nose to nose- any contact in which two animals touch noses.
nose to mouth- nose of one animal contacts mouth of another, includes "greeting"
nose to head- nose of one animal touches head of another animal in area other than nose or mouth.
nose to body- nose of one animal contacts torso of another.
nose to genitals- nose of one animal contacts genital region of another
nose to anus- nose of one animal contacts anal region of another
nose to tail- nose of one animal contacts tail of another
approach- animal orients and walks towards other animal culminating in contact or approach to within a few cms.

Prior Findings and Predictions:

Field work on juvenile Columbian ground squirrels suggests that there is no significant difference in the frequency of recognitive behaviours between sibling and non-sibling pairs (King 1984, Waterman 1985, 1988). However, among Richardson's (*S. richardsonii*) juveniles (Sheppard & Yoshida 1971, Davis 1982), round-tailed (*S. tereticaudus*) juveniles (Dunford 1977) and Arctic (*S. parryii*) ground squirrels (McLean 1982: but note allogrooming pooled with the standard recognitive behaviours in that study), and among juvenile muskrats (*Ondatra zibethicus* ; Caley and Boutin 1987), recognitive behaviours occurred more frequently between kin than non-kin. Further, recognitive behaviours were recorded less frequently among familiar juvenile thirteen-lined (*S. tridecemlineatus*) ground squirrels than unfamiliar juveniles of that species (Holmes 1984).

No specific prediction arises from any single behaviour within this category, and given the weight of existing evidence, tests for sibling/non-sibling (or familiar/unfamiliar) differences must be two-tailed. Based on the only work on naturally occurring interactions between juvenile Columbian ground squirrels, I expect to find no significant difference in the frequency of these behaviours between sibling and non-sibling (or familiar/unfamiliar) pairs.

Cohesive Behaviours- prolonged physical contact or close proximity without forced displacement of one animal by another.

These include:

allogroom- grooming or licking of one animal by another

side by side (S)- one animal sitting or lying in physical contact with another animal

play mount- animal stands over and clasps other with forelimbs

play fight- wrestling or grappling without injury or displacement (includes "rearing" *sensu* Steiner 1971)

stand over (A)- one animal stands on or over top of other animal (often seen in context of play and has been interpreted as play solicitation among juveniles but may also be dominance posture)

roll on back (A)- animal rolls over and rests on back (submissive posture but often seen in context of play)

follow- one animal follows another so as to remain in close (<40cm) physical proximity

Prior Findings and Predictions:

Field studies examining interactions between juvenile Columbian ground squirrels have found a significantly higher frequency of play fighting among male-male sibling pairs and trends toward more play-mounting and more following among male-male sibling pairs than in non-sibling pairs (Waterman 1985, 1988). Waterman (1986) also reported that the most common social behaviour among juvenile Columbian ground squirrels was play and that for the first 20 days after juvenile emergence, siblings played more than non-siblings (with female-female > male-female > male-male pairs in terms of play frequency in that period). Play (including all play behaviours) is also reported to be significantly more frequent among female-female siblings than female-female non-siblings (King 1984). Steiner's 1973 study of allogrooming in Columbian ground squirrels suggests that allogrooming occurs most frequently among "well-acquainted" members of the same social group. Among juvenile Richardson's ground squirrels, Sheppard & Yoshida (1973) reported that sibling male-female pairs allogroomed and play mounted significantly more often than non-sibling pairs; a similar strong trend was reported for intra-sexual (male-male and female-female) pairs. The results of Michener & Sheppard (1972) and Michener (1974) support that finding with the ratio of cohesive behaviours (including allogrooming) to agonistic behaviours being higher in related versus unrelated individuals. McLean (1982) also found that the ratio of allogrooming (+ recognitive behaviours) to agonistic behaviours was higher for related than unrelated Arctic ground squirrels. Juvenile round-tailed ground squirrels showed strong trends for siblings to play fight (+ tiff), and play mount (+ nose to body) more than non-siblings (Dunford 1977). Finally, Caley & Boutin (1987) reported that the combined frequency of following, allogrooming and the recognitive behaviours listed above was greater for sibling than non-sibling pairs of juvenile muskrats. They also reported a weak negative correlation between that frequency and the distance interactants reside from one another in nature, a finding which may suggest a familiarity effect (but see Caley and Boutin 1987).

Based on the preceding findings, I would expect that for this category in general, a higher frequency of cohesive interactions should occur among siblings (or familiar) relative to non-siblings (or unfamiliar) if siblings are recognized in the context of the arena. Specifically, I expect play to be more frequent in sibling (or familiar) dyads than non-sibling (or unfamiliar) dyads. It thus seems reasonable to apply one-tailed tests to behaviours in this category and to use the combined frequency of all cohesive behaviours when comparing siblings to non-siblings and/or familiar to unfamiliar animals. It is also desirable to look for gender-pair differences in frequency for behaviours in this category.

Agonistic Behaviours- overt aggression, threat or submission often involving displacement of one animal by another.

These include:

arch back- animal has all four paws planted firmly on ground but back is arched upwards
lateral display- animals side to side within a few cms., both with back arched as above
open mouth (NR)- animal holds mouth open typically while facing other
circling (NS)- animals walking with heads and fanned tails directed towards each other in decreasing spiral movement
tail flick- rapid up and down motion of tail
tail bush- piloerection of hair on tail
tiff (A)- one or both animals sitting on hinds and strike out with forepaws at other
is run at (A)- other animal runs toward subject but does not result in displacement of subject
run at- one animal runs toward other resulting in displacement of sedentary animal
lunge- one animal pounces on other with forefeet
ball fight- grappling or wrestling usually with injurious bite and often accompanying growling or squealing vocalizations
jump back- animal leaps away from other that has approached or is in contact with it
turn & face (A)- animal turns head rapidly to face other that is in contact with it (often precedes nip or bite and often accompanied by growl)
nip (A)- animal bites at other but doesn't result in displacement or squeal on part of recipient (i.e. a "soft" bite)
bite- animal bites at other usually resulting in displacement or squeal on part of recipient
growl- a low, rasping vocalization
squeal- an abrupt, loud shriek usually in response to bite from other animal

Prior Findings and Predictions:

King (1984) reported no significant difference between dyads of female-female sibling juveniles and female-female non-sibling juveniles in terms of the proportion of agonistic encounters (=fight+chase+threat; note however that her sample size was quite small; n_{sib}=24 interactions, $n_{non\text{-}sib}$=17). However, when all age classes were considered, the proportion of agonistic behaviours was lower (but not significantly) among uterine than non-uterine kin. However, Waterman's (1985, 1986, 1988) work suggests several differences in the frequency of certain agonistic behaviours. Ball fighting was significantly more frequent between female-female non-siblings than among siblings and there was a trend towards this for male-male pairs as well. Tail bushing was significantly more frequent among intra-sexual non-siblings than among siblings and arch back was significantly more frequent among female-female non-sibling pairs than among female-female sibling pairs. There was also a trend toward more arch back in non-sibling pairs of the other gender combinations relative to sibling pairs. Waterman's work also points to several behaviours which may confound a clear interpretation of the results in this category and which should therefore be regarded as ambiguous when pooling behaviours. Significant or at least strong trends toward greater frequencies of biting + nipping, tiff, pounce (=lunge), and is run at were seen among sibling relative to non-sibling interactions. Waterman's findings also suggest that discrimination within this category may be most common amongst intra-sexual pairs of juveniles. In their study of juvenile Richardson's ground squirrels, Sheppard & Yoshida (1971) reported that the combined frequency of arch back, tail bush, open mouth, lateral display, ward, tiff, lunge, ball fight and chase was significantly higher (male-female and female-female) and approached significance (male-male) for non-siblings relative to sibling pairs. Similarly, Michener (1974) reported that the ratio of arch back, lateral display, lunge, tiff, ball fight and roll on back to cohesive behaviour was significantly greater for non-sibling than sibling pairs. For juvenile Arctic and yearling Belding's ground squirrels, Holmes & Sherman (1982) reported that the combined frequency of threat vocalization, withdraw, open mouth, tiff, lateral display, lunge, chase, squeal, bite, roll on back and ball fight was significantly greater in non-siblings than siblings and significantly greater in animals reared apart relative to those reared together. For adult female Arctic ground squirrels, the ratio of the combined frequency of ward, arch back, chase, fight, tail bush, tail flare and lunge to amicable behaviours was higher for unrelated than related squirrels (McLean 1982). Dunford (1977) found no significant differences in the frequency of agonistic behaviours among juvenile round-tailed ground squirrels but trends were apparent toward more lunging, arch back, tiff (+ball fight +play fight?) between siblings relative to non-sibling pairs as the juveniles grew older. No significant difference was found between sibling and non-sibling pairs of juvenile muskrats in terms of the combined frequency of ward, lunge, nip, bite and chase by Caley & Boutin (1987).

Once potentially ambiguous behaviours are eliminated from consideration, I would expect that overall, siblings should be less agonistic than non-siblings and neighbours should be less agonistic than animals residing more remote to one another. Based on prior work, one might expect siblings (or familiar?) animals to tail bush, run at, ball fight, jump back, bite, growl and squeal less than non-sibling (or unfamiliar?) juveniles. One-tailed significance tests seem appropriate within this category.

Investigative and Escape Behaviours- behaviours directed towards the physical elements of the arena itself

scratch floor- animal scratches floor of arena with forepaws
jump up- animal springs up propelled by hindlegs
scratch plexi- any contact of animals forepaws with plexiglass walls
bite plexi- animal opens and closes mouth on plexiglass walls
bump plexi- animal collides with plexiglass (often while running)

Prior Findings and Predictions:

No prior studies report findings from which predictions can be drawn directly for this category. However, it is conceivable that siblings (or familiar) animals may engage in less escape related behaviour since spatial attraction for close over distant kin has been reported for Columbian ground squirrels (King 1984; also for Richardson's ground squirrel, Davis 1982). It is also possible that siblings (or familiar animals) may spend more time in escape related behaviours when in these unfamiliar (and presumably "stressful") surroundings due to reduced social inhibition. Within this category significance tests must be two-tailed.

Scent Related Behaviours- behaviours which in the field have been associated with the deposition of a scent mark or those which in the context of the arena may be associated with the reception of scent

Reception

sniff plexi- nose of animal in very close proximity or contacting plexiglass
lick plexi- (rarely seen); tongue brought into contact with plexiglass
sniff floor- nose of animal in very close proximity (ca 1 cm) or contacting floor
sniff urine- nose of animal in very close proximity or contacting urine
sniff scat- nose of animal in very close proximity or contacting scat

Deposition

ventral drag- (rarely seen); animal stretches and pulls itself forward with forepaws
scent mark- (rarely seen); animal rubs oral angle on part of arena

twist mark- (rarely seen); animal rubs oral angle on part of arena but with full twisting motion of head

Prior Findings and Predictions:

There are no specific predictions that can be drawn from past work. However, Harris and Murie (1982) reported that adult Columbian ground squirrels spent more time investigating acrylic cubes with the scent of stranger's versus neighbour's and resident's, and more time with neighbour's than resident's scent. Thus we may see a greater frequency of behaviours in this category which could be involved in the reception of scent (i.e sniffing and licking) in non-siblings (or unfamiliar animals) relative to siblings (or familiar animals); however, given the scant background data available, tests within this category must be two-tailed.

Miscellaneous Activities and Postures- behaviours which don't readily fit into the above categories

walk- animal locomotes with alternating contra-lateral extension and flexion of foreleg and hindleg as pairs
lope- animal locomotes in bounding motion by alternating extension and flexion of forelegs as pair and hindlegs as pair
groom- any licking or rapid repeated nipping at an animal's own body
S-4- animal at rest with all four paws touching substrate
slouch- animal at rest on hind quarters, front paws not contacting substrate
alert- animal standing on hindlegs, back completely straightened
stretch- animal extends forelimbs outward while resting on belly
lick floor- (rare) tongue contacts and "sweeps" floor of arena
lick urine- tongue contacts and "sweeps" urine or dried urine
bite scat- (rare) animal opens and closes mouth on scat
urinate (NR)- animal urinates (usually hidden by animals body)
defecate (NR)- animal defecates (usually hidden by animals body)

Prior Findings and Predictions:

In terms of the ratio of activity (walk + lope + groom) to resting postures (S-4 + slouch + alert), it would seem reasonable to predict that siblings (or familiar individuals) may spend a greater proportion of a trial active since they are likely to be in transition between play bouts (often loping, personal observation) and may be less "inhibited" in the company of related and/or familiar animals. However, without any empirical data on which to base predictions, tests within this category should be two-tailed.

Miscellaneous Vocalizations- any vocalization not immediately interpretable as being directed towards or in response to action of other animal

chirp- a single, short duration (< 1 sec), high pitched but low intensity vocalization
alarm call- a single, short duration, high pitched and loud vocalization
repeated call- a series of chirps or alarm calls of varying duration with inter-call latency not exceeding 5 seconds

Prior Findings and Predictions:

The bulk of evidence from studies quantifying alarm-calling in the ground dwelling squirrels (*Spermophilus* and *Cynomys*) suggests that individual squirrels are more likely to call in response to a threat when kin are present. The extent to which this would apply to juveniles in the context of the arena is unknown and thus tests within this category should be two-tailed.

Other Behavioural Comparisons- it will also be interesting to code and compare the category of the first behaviour subsequent to contact across the treatment groups. In general I would expect the initial reaction to be more cohesive and less agonistic among siblings and/or familiar animals than among non-siblings or unfamiliar animals.

Spatial Patterns- data coded by recording location of each squirrel in arena at ten second intervals throughout trial.

Prior Findings and Predictions:

Both King (1984) and Waterman (1985) reported results that suggest a spatial attraction for close over distant kin among Columbian ground squirrel juveniles in the field. Davis (1982) reported that in Richardson's, juvenile siblings remained closer together in an arena than non-siblings (mean distance). Further, there were more contacts among siblings than non-siblings and siblings remained in contact for a significantly longer period of time than non-siblings (Davis 1982). However, in similar arena trials Holmes & Sherman (1982) found no significant difference in the mean distance between sibling (or familiar) versus non-sibling (or unfamiliar) squirrels.

Based primarily on the findings of King (1984) and Waterman (1985) I would expect that sibling (or familiar) squirrels will on average remain closer together than non-sibling (or unfamiliar) squirrels. I also expect more contacts between sibling (or familiar) relative to non-sibling (or unfamiliar) squirrels and hence one-tailed tests seem appropriate.

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Appendix C: Levels of Discrimination and Their Corresponding Mechanisms

1) At what level do juvenile Columbian ground squirrels discriminate between other juveniles they encounter?

SRT= siblings (also abbreviated as "sibs") reared together
(full sibs and maternal-half sibs, familiar pre & post weaning)
NSRAC= non-siblings reared apart close
(paternal half-sibs, cousins or unrelated, familiar post weaning)
NSRAD= non-siblings reared apart distant
(not closely related, familiar only via intermediary)
NSRA2= non-siblings reared apart in separate colonies
(essentially unrelated, completely unfamiliar)

<u>Reasonable Possibilities:</u>

	If H_A true, expect 1) cohesive 2) agonistic	potential mechanism(s)
a) no discrimination	1) SRT=NSRAC=NSRAD=NSRA2 2) SRT=NSRAC=NSRAD=NSRA2	H_0
b) sibs discriminated from non-sibs of all other types only	1) SRT>NSRAC=NSRAD=NSRA2 2) SRT<NSRAC=NSRAD=NSRA2	innate or pre-emerg assn.
c) sibs discriminated from famil. non-sibs which are in turn discrim. from all unfamil (both col.)	1) SRT>NSRAC>NSRAD=NSRA2 2) SRT<NSRAC<NSRAD=NSRA2	innate + assn. or pure assn.
d) colony members discriminated from non-members only	1) SRT=NSRAC=NSRAD>NSRA2 2) SRT=NSRAC=NSRAD<NSRA2	gene simil. env. cues indir. famil. odor mixing (gestalt)
e) sibs discriminated from colony members which in turn discrim. from non-members	1) SRT>NSRAC=NSRAD>NSRA2 2) SRT<NSRAC=NSRAD<NSRA2	innate &/or pre-em. assn. + indir.famil.or + col. odor
f) sibs and familiar non-sibs not discrim. but sibs and familiar discriminated from unfamiliar & other colony	1) SRT=NSRAC>NSRAD=NSRA2 2) SRT=NSRAC>NSRAD=NSRA2	pure assn

g) sibs and familiar not discrim. but discrim. from unfamil. which in turn discrim. from other colony	1) SRT=NSRAC>NSRAD>NSRA2 2) SRT=NSRAC<NSRAD<NSRA2	pure assn. or assn. + indir.famil.or + col. odor
h) sibs discr. from fam. non-sibs which in turn discrim. from unfamiliar same colony which in turn discrim. from members of other colony	1) SRT>NSRAC>NSRAD>NSRA2 2) SRT>NSRAC>NSRAD>NSRA2	innate + assn or innate + assn. + indir.famil.or + col. odor

Note: For purposes of above alternatives, candidate mechanisms are:
- i) innate (includes familiarization *in utero*)
- ii) pre-emergence association
- iii) post-emergence association
- iv) indirect familiarity or environmental and/or individually produced cues resulting in colony odor

Appendix D: Comparison of Coding Methods for Arena Behaviour Data

Due to the length of the behaviour records, coding all data from the videotapes using a continuous record was impractical. In order to choose an effective sampling method, data from a single trial were coded for 13 arbitrarily selected behaviours using a continuous record, one-zero sampling, or fixed interval point sampling(Table D.1).

The scores obtained using fixed interval point sampling more closely approximated the actual proportion of time the individual engaged in those behaviours than did the scores obtained using one-zero sampling (Table D.1). One-zero sampling consistently overestimated the proportion of time a behaviour occurred. This result is consistent with that reported by others in comparing the two time-sampling methods (see Altmann 1974, Martin and Bateson 1986). Further, a 3 second time interval provided a more accurate representation of the actual proportion of time spent in a given behaviour than did a 5 second time interval when using fixed interval point sampling (Table D.1). Time intervals that are short relative to the average duration of the behaviour coded typically provide records that approximate a continuous record (Martin and Bateson 1986). A shorter time interval was not used since 3 seconds was deemed the minimum amount of time necessary for the observer to input the behaviour using the event recorder program, and any shorter time interval would sacrifice the practical benefits of using time sampling (*ibid*).

In coding data, fixed interval point sampling was also deemed preferable since it was far less hectic than one-zero sampling (i.e. only one behaviour is input on each sample point with fixed interval point sampling while all behaviours within each sample interval are input when one-zero sampling). The harried pace required by one-zero sampling would likely lead to imprecision in data coding. Further discussion of the relative merits of the two time-sampling methods are in Altmann (1974) and Martin and Bateson (1986). Based on these pilot coding sessions, all behavioral data were coded using fixed interval point sampling with a 3 second interval length.

Table D.1: Behavioral data obtained for squirrel "11" in trial number 2 (1988) using continuous recording of behaviour, one-zero sampling, or fixed interval point sampling.

	Continuous Record		One-Zero Sampling		Fixed Interval Point Sampling			
	(actual freq. & time)		(5 sec. Interval; n=3)		(5 sec. Interval)		(3 sec. Interval)	
Behaviour	freq.	ppn. time	$\bar{x}$ freq.	$\bar{x}$ score	freq.	score	freq.	score
turn & face	2	0.11	1.67	0.47	2	0.56	1	0.17
approach	23	1.39	20.67	5.74	10	2.78	9	1.50
follow	6	1.44	11.67	3.24	11	3.06	7	1.17
play mount	5	0.39	3.33	0.93	3	0.83	3	0.50
play fight	5	0.78	9.00	2.50	8	2.22	10	1.67
groom	11	2.67	20.67	5.74	13	3.61	18	3.00
alert	15	2.22	16.67	4.63	13	3.61	19	3.17
nose-nose	8	0.50	8.67	2.41	3	0.83	4	0.67
nose-mouth	4	0.22	3.00	0.83	2	0.56	3	0.50
nose-head	4	0.22	4.67	1.30	0	0.00	1	0.17
nose-body	8	0.56	10.33	2.87	8	2.22	9	1.50
nose-tail	4	0.22	6.66	1.85	3	0.83	2	0.33
sniff urine	5	0.44	5.00	1.39	2	0.56	4	0.67

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Appendix E: The influence of independent variables including physical factors, miscellaneous grouping variables, and factors intrinsic to the animals, on the behaviour of juvenile Columbian ground squirrels in the "level of discrimination" experiment (Chapter 3).

Autocorrelation of Independent Variables

Autocorrelations were examined by constructing a correlation matrix of the 12 independent variables to be examined (see Table E.I). Correlations are described where the correlation coefficient exceeds 0.30 (designated arbitrarily). Relatedness/association was strongly correlated (-0.471) with site since the second site contributes only NSRA2 and a few SRT and was correlated with mass (-0.395) as NSRA2 individuals were typically heavier (see below). Gender pair was not strongly correlated with any other independent variable. Site of origin was strongly correlated with age (0.798) and mass (0.863) as individuals from the second site were typically older and heavier than those from the principal site. Site is also negatively correlated with year (-0.664) as more focal animals from the second site were used in 1988 than 1989. Trial within day is positively correlated with temperature (0.460) as temperature generally increased as the day progressed and is positively correlated with wind (0.507) since wind also increased during the day. The number of times animals were run was strongly correlated with site since animals from the hay meadow appeared in the arena more often than animals from meadow B. Age and mass show a high positive correlation (0.728) since juveniles gain weight as they age. Age is negatively correlated with year (-0.907) since trials were commenced and completed earlier in the season in 1989 than 1988. As would be expected given the correlation of mass and age, mass is also negatively correlated with year; animals in 1989 were lighter than those in 1988. Weather variables were correlated in a predictable fashion. As cloud increased, temperature decreased (-0.531); as precipitation increased, temperature decreased (-0.303); as temperature increased, wind increased (0.392); and as cloud increased, precipitation increased (0.471). None of these correlations seriously confound interpretation of the effects of relatedness/association or gender pair.

Physical Factors

Temperature

The effect of temperature was analysed by independently regressing each of the 60 dependent variables on the median range of ambient temperature (Maximum + Minimum/2). Of those 60 behaviours, significant effects were detected for 4 of the key behaviours (arch back, P =0.005, r^2=0.083; tail bush P =0.006, r^2=0.079; lunge P =0.03, r^2=0.049; and allogroom P =0.045, r^2=0.038) and 1 other behaviour (roll on back P =0.003, r^2=0.093). All 5 behaviours affected showed slight increases with increasing temperature. Thus there was a tendency for aggression (and allogrooming) to increase with increasing temperature. However, there was a correlation between temperature and relatedness/association with NSRA2 run at higher average temperature than other relatedness/association groups (but not significant, see below). To address the effect of temperature in isolation of relatedness/association, NSRA2 were excluded and the regression analyses were re-calculated. Only allogroom (P =0.045, r^2=0.038) and roll on back (P =0.003, r^2=0.093) retained statistical significance. Thus any increase in aggression with increasing temperature appears intimately linked to relatedness/association and renders control of temperature in analysis of relatedness/association effects ill advised. Further, no significant difference was detected in temperature across the 4 relatedness/association groups (df=3,77, F=2.03, P =0.12) nor across the 4 gender pair levels (df=3,77, F=1.957, P =0.13). Thus the potential confounding influence of temperature was balanced across groups.

Wind Speed

Wind was categorized as an increasing ordinal ranging from 1 to 6. Its effect was analysed using single factor analysis of variance treating wind class as the independent variable and the 60 behaviours as dependent variables in univariate analyses. No significant effect on any key behaviour was detected although significant differences were detected for 7 other variables (df=5,75; nip, *P* =0.046; groom, *P* =0.02; S-4, *P* =.03; sniff plexiglass, *P* =0.03; nose to nose, *P* =0.02; nose to head, *P* =0.049; nose to tail, *P* =0.01). In general, increasing wind tended to decrease the amount of time spent sitting (S-4) and increased the number of recognitive contacts. However, wind was well balanced both across rearing/association (Kruskal-Wallis test; H=0.10, *P* >0.99) and gender pair (H=0.91, *P* >0.75).

Cloud Cover

Cloud was categorized as an increasing ordinal from 1 to 3. Its effect was analyzed using single factor analysis of variance treating those ordinals as levels of the independent variable and treating the 60 behaviours as dependent variables in separate univariate analyses. Cloud cover had a significant effect on one key behaviour (follow, df=2,78, F=3.809,*P* =0.03) and on three other behaviours (df=2,78; is run at, F=4.459, *P* =0.015; chirp, F=3.132, *P* =0.049; and lick floor, F=3.792, *P* =0.027). None of those behaviours revealed consistent trends with increases in cloud cover across the 3 levels. Further, cloud cover was balanced across relatedness/association (H=3.02, *P* >0.25) and gender pair (H=1.69, *P* >0.50).

Precipitation

Precipitation was classified as an increasing ordinal from 1 to 3 (none, light drizzle, after rain). Its effect was analysed using single factor analysis of variance treating precipitation as the independent variable and the 60 behaviours as dependent variables in separate univariate analyses. Precipitation had significant effects on 3 key behaviours (df=2,78; follow, F=4.01, *P* =0.02; play mount, F=3.295, *P* =0.04; play fight, F=12.89, *P* =0.0001) and one other behaviour (is run at, df=2,78, F=4.58, *P* =0.01). Play behaviours were most frequent after rain, while is run at and following were most frequent during drizzle. Note that these effects may be artifactual since only 6 trials were run during drizzle and 1 trial after rain. Precipitation was balanced across both relatedness/association (H=3.02, $P > 0.25$) and gender pair (H=1.33, $P > 0.50$).

Factors Intrinsic to the Animals

Age

The influence of age on behaviour was assessed by regressing each of the 60 behaviours (dependent variables) on the number of days juveniles had been above ground. Age had a significant effect on 1 key behaviour (allogroom, $P = 0.01$, $r^2 = 0.066$) and on 8 other behaviours (turn & face, $P = 0.003$, $r^2 = 0.095$; nip, $P = 0.0001$, $r^2 = 0.25$; bite, $P = 0.035$, $r^2 = 0.043$; growl, $P = 0.01$, $r^2 = 0.07$; roll on back, $P = 0.001$, $r^2 = 0.12$; nose to nose, $P = 0.014$, $r^2 = 0.062$; nose to head, $P = 0.003$, $r^2 = 0.092$; nose to tail, $P = 0.02$, $r^2 = 0.053$). Aggressive behaviours (and allogrooming) increased with increasing age, as did the majority of recognitive contacts. The age of interactants was not balanced across relatedness/association (df=3,77, F=15.809, $P = 0.0001$) with the age of intercolony interactants (NSRA2) being greater than that in all other groups. Age was balanced across gender pair (df=3,77, F=0.897, $P = 0.45$). The trends reported (and statistical significance for 7 of 9 behaviours) persist even if the older NSRA2 individuals are excluded from the analysis of age effects. Since aggression, recognitive behaviour and one key cohesive behaviour increase with advancing age, analysis of relatedness/association effects for those categories should control for age.

Mass

Individuals were matched as closely as possible for size in trials. Thus the influence of mass on behaviour was analysed by regressing the 60 behaviours on the masses of focal individuals. Mass had a significant effect on one key behaviour (tail bush, $P = 0.03$, $r^2 = 0.062$) and significantly affected 4 other behaviours (turn & face, $P = 0.02$, $r^2 = 0.079$; nip, $P = 0.002$, $r^2 = 0.145$; nose to nose, $P = 0.03$, $r^2 = 0.068$; nose to tail, $P = 0.04$, $r^2 = 0.059$). Thus aggression and recognitive behaviour tended to increase with increasing mass. Mass was not balanced across relatedness/association (df=3,77, F=17.15, P

=0.0001; NSRA2 heavier than all others) but was balanced across gender pair (df=3,77, F=0.929, *P* = 0.43). If NSRA2 are excluded from the analysis, trends toward increasing aggression and recognitive behaviour with increasing mass disappear completely in all but 2 minor variables (nip, turn & face). Thus the effect of mass may result from an autocorrelation of mass with age (see above), or is only expressed among NSRA2 individuals, in which case it is a bona fide effect and should not be corrected for in the analysis of relatedness/association (i.e. it is intimately linked to relatedness/association).

Miscellaneous Grouping Factors

Trial Within Day

The number of trials run on any given day ranged from 1 to 5. To quantify the effect of trial order on behaviour, the trial number within day was treated as the independent variable in single factor analysis of variance on each of the 60 behaviours. Trial order had a significant effect on 2 key behaviours (df=4,76; follow, F=3.26, *P* =0.02; play mount, F=4.286, *P* =0.004) and on 5 other behaviours (df=4,76; nip, F=5.103, *P* =0.001; bite, F=4.338, *P* =0.003; growl, F=2.835, *P* =0.03; nose to mouth, F=3.694, *P* =0.008; and lick floor, F=3.861, *P* =0.007). For nip, bite, growl, nose to mouth, and lick floor, the fifth trial within day contributed inordinately to the difference detected (i.e. had a significantly higher frequency of the behaviours than all other trials). That effect may be an artefact of sample size since only 3 of the 81 trials were run fifth within day. A consistent trend was observed only for play mount, which increased with increasing trial within day. This was not simply the product of a time of day effect since regression of that behaviour on trial start time did not resolve a significant effect (P =0.473, r^2=0.007). An obvious question is whether elimination of the holding effect in 1989 rendered trial within day a "different" variable in 1989. If so, then interactions between year and trial within day should be significant for those behaviours affected by trial within day. 2-factor analysis of variance detected significant year x trial within day interactions for only 2 (nip, play mount) of the 7 behaviours and those interactions disappeared when the small sample (n=3) of the 5th trial within day group was excluded. Since squirrels were held prior to trial only in 1988, but the effect was unchanged in 1989, the effect of trial within day was not due to a holding effect. The effect could result from higher order interactions with other variables (e.g. trial within day x temperature x wind) but is beyond the scope of further examination with the present data. Trial within day was balanced across relatedness/association (H=5.819, $P > 0.10$) but shows a consistent decreasing trend with increasing relatedness/association (i.e. NSRA2>NSRAD>NSRAC>SRT). Given that trend and the effects of trial within day on 2 key behaviours (follow, play mount) in the same critical category (cohesive behaviour), its effect should be controlled for in analysis of relatedness/association effects. Trial within day was balanced across gender pair (H=0.917, $P > 0.75$).

Number of Appearances in the Arena

Individuals were restricted to a maximum of 3 appearances in the arena. The effect of the number of times an animal was run was quantified by treating the number of appearances in the arena as the independent variable in single factor analysis of variance on the 60 behaviours. Number of times run had a significant effect on 2 key behaviours (df=2,78; follow, F=3.56, $P = 0.03$; play mount, F=3.64, $P = 0.03$) and had significant effects on 4 other behaviours (df=2,78; is run at, F=7.492, $P = 0.001$; tail flick, F=4.94, $P = 0.01$; walk, F=3.361, $P = 0.04$; repeated call, F=5.336, $P = 0.007$). Following increased consistently with increasing number of times an animal appeared in the arena, and for the other behaviours, higher frequencies were recorded the third time an animal was run. The number of times animals were run was balanced across relatedness/association (H=6.076, $P > 0.10$) and gender pair (H=5.081, $P > 0.10$). However there was a trend toward increasing times run with decreasing relatedness/association within colony (i.e. SRT<NSRAC<NSRAD). Given its effect on 2 key behaviours (follow and play mount) in the same critical behaviour category (cohesive), and the effect on walking (a major component of the "active" category), this

factor should be controlled for in analysing the relatedness/association effects on those categories.

Site

The only data available to examine the effect of site of origin per se on the behavioural propensities of juvenile squirrels are those from 5 female-female sibling pairs from the principal site (HM) and 4 of those pairs from the secondary site (B) run in 1988 (note these were the only intracolony trials run using animals from the second site and were conducted due to the limited number of female-female littermates on the main site). Comparisons of littermate females (SRT) for all 60 behaviours using Mann-Whitney U tests found no significant difference in any behaviour (all $P > 0.05$). Thus no effect of site of origin was apparent.

Year

Trials contributing data to this experiment were conducted in both 1988 and 1989. As noted previously (Chapter 3), certain changes in protocol were made between years. The effect of year on behaviour was examined with single factor analysis of variance treating year as the independent variable and performing univariate analyses for each of the 60 possible behaviours. Year had a significant effect on 3 key behaviours (df=1,79; arch back, F=4.593, P =0.035; follow, F=4.451, P =0.038; play fight, F=10.02, P =0.002) and on 15 other behaviours (df=1,79; turn & face, F=19.43, P =0.0001; nip, F=17.05, P =0.0001; bite, F=4.18, P =0.044; walk, F=4.47, P =0.04; stand over, F=4.29, P =0.04; growl, F=4.05, P =0.048; roll on back, F=5.81, P =0.02; jump up, F=5.51, P =0.02; nose to nose, F=10.17, P =0.002; nose to head, F=13.92, P =0.0004; nose to body, F=18.78, P =0.0001; nose to genitals, F=4.26, P =0.04; nose to tail, F=14.43, P =0.0003; sniff urine, F=6.92, P =0.01; sniff scat, F=6.37, P =0.01). For all of the above behaviours except arch back and walk, frequencies were higher in 1988 than 1989. This could be due to the alteration of protocol so that animals weren't held prior to trial in 1989, could reflect the autocorrelation of age, mass and year (see above), or could reflect subtle biases in data coding. It is important to note that despite those differences, no systematic bias on the analysis of relatedness/association nor gender pair is incurred since year is well balanced across both those factors (H=0.99, $P > 0.75$ and H=0.33, $P > 0.95$ for relatedness/association and gender pair respectively).

Principal Factors of Interest

Gender Pair

Analyses of variance treating gender pair as the independent variable and pooling across all other variables found no significant effect of gender pair on any of the 60 behaviours (all $P > 0.05$). Gender pair is balanced across the 4 relatedness/association levels (H=3.201, $P = 0.362$). Significant gender pair x relatedness/association interactions were detected for only 3 of the 60 behaviours (2-way ANOVA, df=9, 65; follow, F=3.01, $P = 0.005$; play fight, F=2.123, $P = 0.04$; slouch, F=2.518, $P = 0.02$). For following, that interaction is the result of non-neighbouring colony member (NSRAD) female focal juveniles following males more frequently than in any other relatedness/association class. Non-littermate non-neighbouring females with male trial mates from the same colony (NSRAD) also play fought more than other relatedness/association groups while female-female littermates (SRT) and neighbours (NSRAD) play fought more than other relatedness/association groups of that gender pair. The interaction between relatedness/association and gender pair on slouching was not readily interpretable as the frequency of slouching fluctuated greatly across gender pairs within relatedness/association. Thus gender exerted only a minor effect on behaviour with some indication that littermate and neighbouring females may play fight more often than female pairs of other relatedness/association levels (similar to results of Waterman 1985). Since gender effects were minimal and balanced across relatedness/association levels, gender pairs were pooled in subsequent analyses of relatedness/association effects.

Relatedness/Association

Significant effects of relatedness/association were detected for 2 key agonistic behaviours (df=3,77; lunge, F=4.689, *P* =0.005; tail bush, F=8.460, *P* =0.0001) and 2 key cohesive behaviours (df=3,77; play mount, F=3.245, *P* =0.03; play fight, F=3.538, *P* =0.02). For the aggressive behaviours, NSRA2 showed higher frequencies than the other groups, while cohesive behaviours increased with colony membership and with littermate status (i.e. NSRA2<NSRAD=NSRAC<SRT). Significant differences were detected for 4 of 12 key behaviours, but not for any of the other individual behaviours.

Simultaneous Effect of All Variables

To assess the relative contributions of the 12 independent variables to behaviour in the arena, multiple regression analyses were performed regressing the 60 behaviours on the entire suite of independent variables. Although the application of a multivariate technique to examine the influence of so many factors (12) with so few cases (81) renders the interpretation of factor effects suspect, these analyses were performed largely to confirm (or refute) the above analyses that examined each factor separately. In these analyses, factors are described as having an effect where they have a high partial correlation, explaining at least 25% of the regression. The value of the partial correlations and their statistical significance are not reported due to the exploratory nature of the analyses.

Relatedness/association had the highest partial correlation with behaviour in 8 of 60 cases (lunge, jump back, tail bush, play fight, groom, nose to anus, sniff scat, lick urine) and had the second largest contribution in an additional 2 cases (follow, play mount). Perhaps more importantly, relatedness/association was the most significant factor for 3 of the 8 key agonistic behaviours (lunge, jump back, tail bush) and was the most significant or second most significant in 3 of 4 key cohesive behaviours (play fight, follow, play mount). Thus relatedness/association had tangible effects on 6 of the 12 key behaviours.

Gender pair had the highest partial correlation with only 1 behaviour (allogroom) and had the second highest partial correlation with 1 other behaviour (turn & face). The high partial correlation with only 1 key cohesive behaviour (allogroom), but with very few other behaviours supports the interpretation (see above) that sex-of-pair exerts a relatively minor influence on the behavioral interactions of juvenile squirrels.

Trial within day had the highest partial correlation with behaviour in 8 of 60 cases (nip, bite, lope, growl, S-4, bump plexiglass, nose to mouth, sniff urine) and had the second largest contribution in an additional 3 cases (allogroom, stand over, nose to head). It was the most significant factor for only 1 key aggressive behaviour (bite) and was second most important in only one key cohesive behaviour (allogroom). Thus, relative to relatedness/association, the effects of trial within day were minor, yet still powerful enough to warrant control when analysing the principal factors (see above).

Year also had high partial correlations with certain behaviours, appearing 7 times as the most significant factor (turn & face, follow, stand over, roll on back, jump up, nose to head, nose to body) and an additional 5 times as the second most significant factor (nip, play fight, alarm call, slouch, nose to tail). Year had no high partial correlations with key aggressive behaviours but was most significant or second most significant for 2 key cohesive behaviours (follow, play fight). In total, year apparently influenced 2 of the 12 key behaviours, although this could result from correlations with other variables (see above). Although year had an apparent effect, it was balanced across the principal factors of interest so does not confound interpretation of relatedness/association or gender pair effects.

Temperature had the highest partial correlation with 2 behaviours (scratch plexiglass, sniff floor) and had the second highest partial correlation with 4 other behaviours (bite, arch back, sniff plexiglass, sniff scat). Thus 2 key aggressive behaviours were influenced by temperature (bite, arch back). This result is consistent with that suggested by the analysis of temperature effects when all other factors are pooled (see above).

The number of appearances in the arena had the highest partial correlation with only 1 behaviour (nose to nose) and had the second highest partial correlation with 6 other behaviours (is run at, tail flick, walk, growl, repeated call, nose to mouth). None of those behaviours were designated *a priori* as key behaviours. Thus the number of times an individual was run did not have a great effect relative to other variables.

Cloud cover had the highest partial correlation with 1 behaviour (play mount) and ranked second for 2 other behaviours (scratch plexiglass, bump plexiglass). Thus cloud cover strongly influenced 1 key cohesive behaviour (play mount) but had little other effect overall. This result is consistent with the earlier analysis of cloud cover effects suggesting some slight increase in cohesion with increasing cloud.

Wind had the highest partial correlation with 2 behaviours (tail flick, nose to tail) and the second highest partial correlation with 2 other behaviours (lope, sniff urine). None of the key behaviours were strongly influenced by wind. This result is consistent with the earlier analysis of wind effects that suggested some increase in activity and recognitive behaviour with increasing wind speed.

Precipitation had the highest partial correlation with 3 behaviours (is run at, sniff plexiglass, nose to genitals). No key behaviours were strongly influenced by precipitation. Thus it appears that precipitation exerts no major confounding influence on the interpretation of the main factor effects and lends support to the notion that the apparent effect of precipitation on play behaviours (see above) was an artefact of sample size.

Age (number of days post-emergence) had the highest partial correlations with 3 behaviours (arch back, alarm call, slouch) but had no high partial correlations with any other behaviours. The high partial correlation with 1 key agonistic behaviour (arch back) offers some support for the finding that aggression increased with age (see above).

Mass had the highest partial correlations with 2 behaviours (walk, repeated call) but no high partial correlations with key or any other behaviours. This is consistent with the earlier contention that apparent mass effects may be due to autocorrelations with other variables (see above).

Site of origin had no high partial correlation with any behaviour. This supports the analysis of site effects (above) which indicated that site of origin does not contribute significantly to differences in behaviour.

These multivariate tests generally confirm the analyses of the independent variables conducted by examining each independent variable separately. Relatedness/association clearly exerted the strongest influence on the key behaviours of squirrels in the arena although lesser effects of the 11 other potential independent variables were also apparent. The analyses suggest that age, trial within day and the number of times animals were run could confound the interpretation of relatedness/association results for certain behaviours given their effects and strong or significant trends toward imbalance across the relatedness/association groups. Thus analyses of relatedness/association in certain categories were controlled for those effects by treating age, trial within day, and/or times run as covariates.

Table E.1: Autocorrelation of independent variables quantified in the "level of discrimination" experiment.

Variable	Relatedness/ Association	Gender Pair	Site	Trial/ Day	Times Run	Age = days up	Mass (g)	Temp. (Mr, °c)	Cloud	Precip	Wind	Year
Rel/Assoc	1.000											
Gndr Pair	.085	1.000										
Site	-.471	.245	1.000									
Trial/Day	-.169	-.162	.213	1.000								
Times Run	.199	.059	-.426	-.019	1.000							
Age (days up)	-.296	.173	.798	.175	-.130	1.000						
Mass (g)	-.395	.108	.863	.132	-.326	.728	1.000					
Temp (°c)	-.157	.148	.325	.460	-.029	.255	.294	1.000				
Cloud	-.023	-.133	-.070	.015	-.019	-.028	-.081	-.531	1.000			
Precip	.268	-.090	-.200	-.084	.177	-.165	-.157	-.303	.471	1.000		
Wind	-.108	.059	.207	.507	.161	.325	.238	.392	-.066	-.064	1.000	
Year	.164	-.084	-.664	-.167	.095	-.907	-.565	-.211	-.052	.051	-.288	1.000

Figure F.1: Recognitive behaviour displayed in interactions between siblings (SRT), neighbours (NSRAC), spatially distant non-siblings (NSRAD), and members of two different colonies (NSRA2) (no concomitant variables).

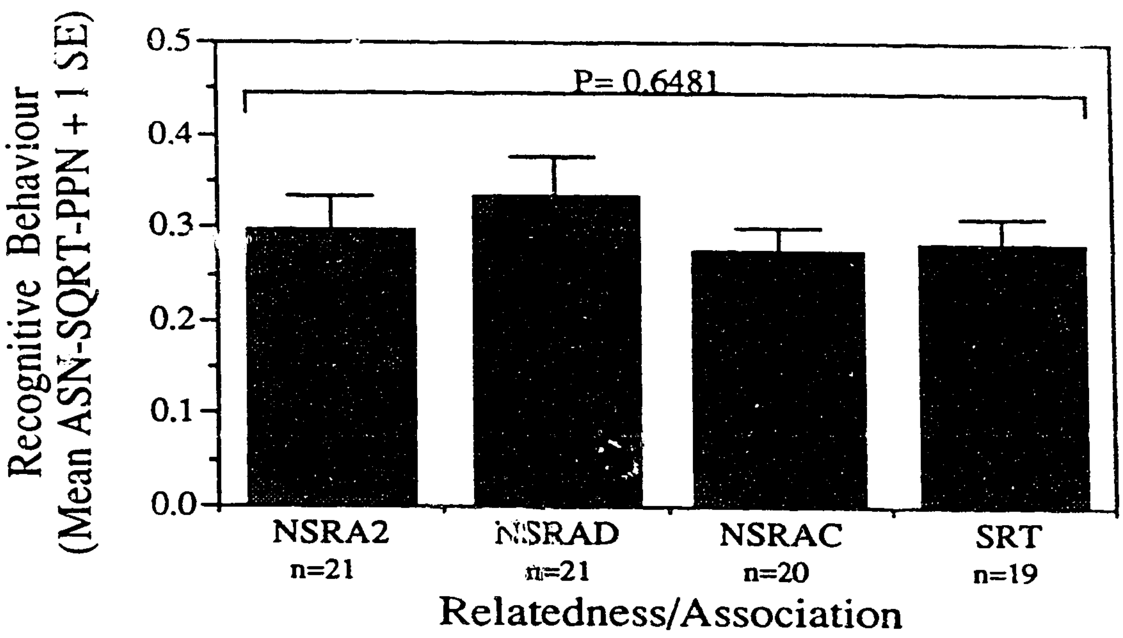
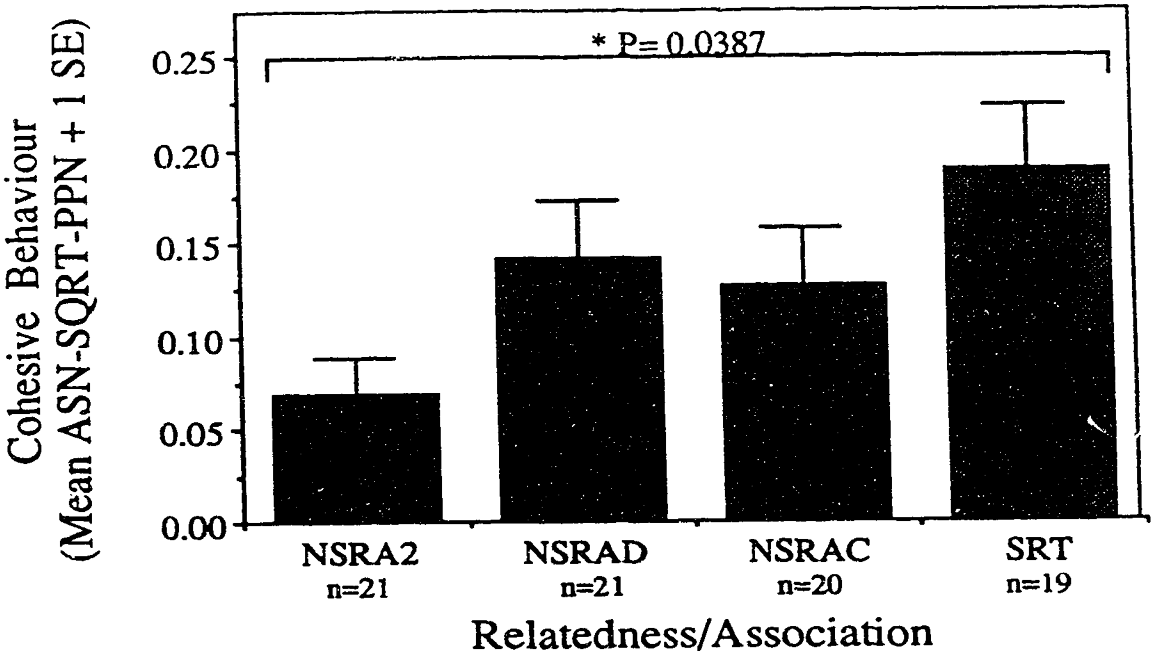


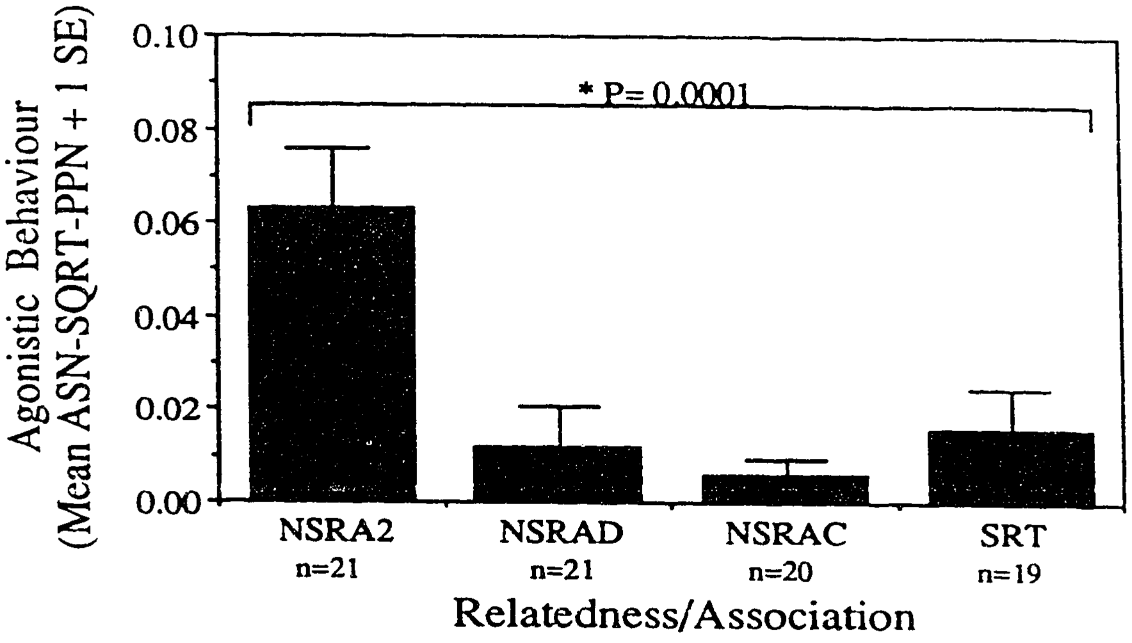
Figure F.2: Cohesive behaviour displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (no concomitant variables).



Pairwise Contrasts (sequential Bonferroni)

Contrast	p
NSRA2 vs. NSRAD	0.369
NSRA2 vs. NSRAC	0.569
NSRA2 vs. SRT	0.026*
NSRAD vs. NSRAC	0.734
NSRAD vs. SRT	0.569
NSRAC vs. SRT	0.569

Figure F.3: Agonistic behaviour displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (no concomitant variables).



Pairwise Contrasts (sequential Bonferroni)

Contrast	p
NSRA2 vs. NSRAD	0.001*
NSRA2 vs. NSRAC	0.001*
NSRA2 vs. SRT	0.002*
NSRAD vs. NSRAC	1.000
NSRAD vs. SRT	1.000
NSRAC vs. SRT	1.000

Figure F.4: Active behaviour displayed in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (no concomitant variables).

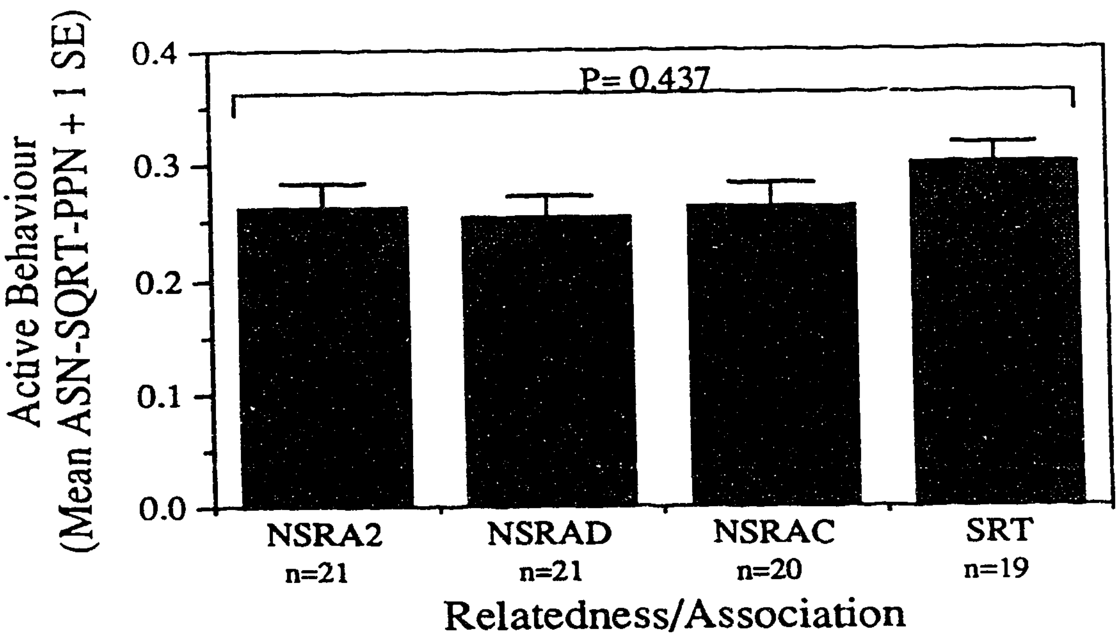
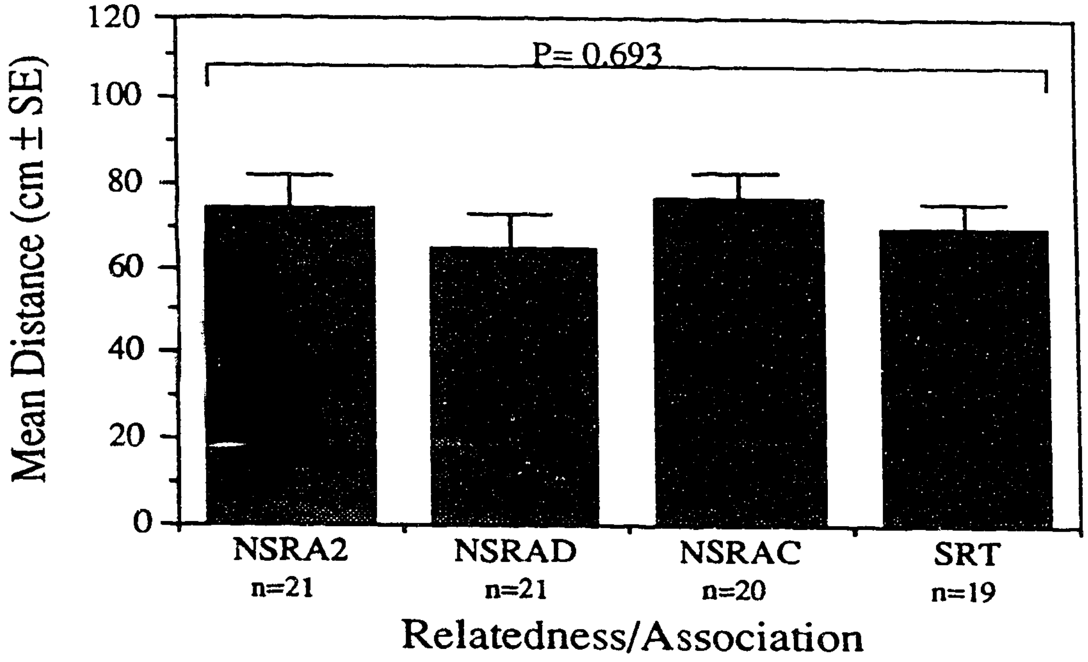


Figure F.5: Average interindividual distance in interactions between siblings, neighbours, spatially distant non-siblings and members of two different colonies (no concomitant variables).



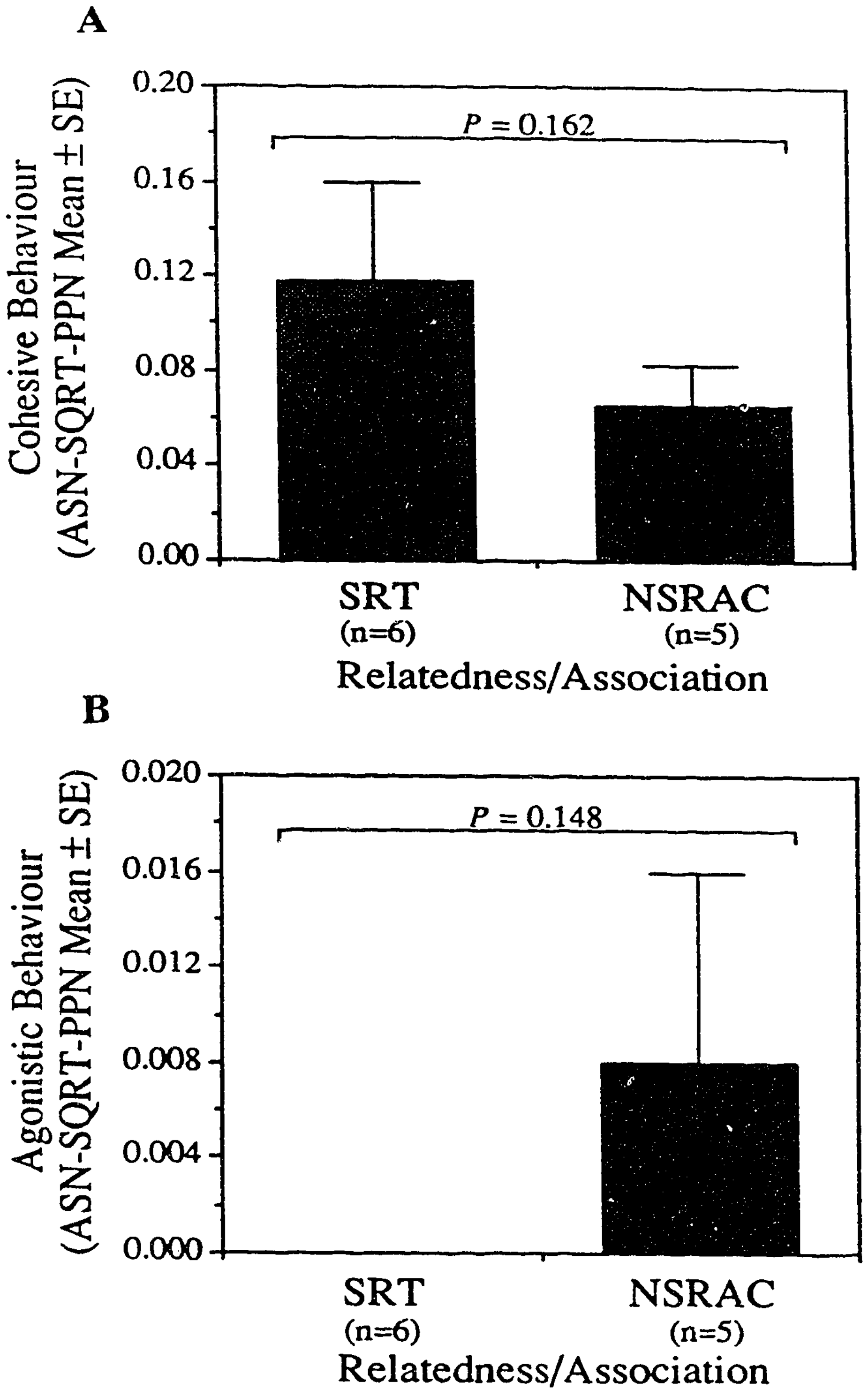
Appendix G: Analyses of cohesive and agonistic behaviour of neighbouring and littermate juveniles less than 21 days post-emergence (for comparison to Waterman 1985)

Waterman's data (1985; her Figure 2.6, page 31) on play in Columbian ground squirrels suggest that sibling (littermate) juveniles are discriminated from neighbouring juveniles for the first 20 days post-emergence. After that time, any difference between littermates and neighbours wanes until at 51 to 60 days post-emergence, no difference is apparent between littermates and neighbours. To further examine the ontogeny of behavioural discrimination among juveniles, data from the "levels of discrimination" experiment (Chapter 3) for littermates and neighbours were restricted to include only focal animals that had been above ground for less than 21 days. Six sibling pairs and 5 neighbour pairs were available for analysis given that criterion. Data were analysed using 1-tailed Student's t-tests with differences considered significant where the probability of type I error did not exceed 0.05. Non-significant trends suggest that siblings indeed play more frequently and are less aggressive than neighbours within those age limits (see Figure 1).

Literature Cited

Waterman, J.M. 1985. The behavioural ontogeny of the Columbian ground squirrel. M.Sc. Thesis, University of Alberta, Edmonton, Alberta: 80 pp.

Figure G.1: Cohesive (A) and agonistic (B) behaviour of littermate and neighbouring juveniles less than 21 days post-emergence.



Appendix H: The influence of independent variables, including physical factors, miscellaneous grouping variables, and factors intrinsic to the animals on the behaviour of juvenile Columbian ground squirrels in the "trap-mixing" experiment (Chapter 4).

Temperature (linear regression)

Behaviour	Difference among the 5 treatment groups		
Category	df	F	*P*
Recognitive	1,33	1.54	0.22
Cohesive	1,33	3.60	0.07
Agonistic	1,33	1.11	0.30
Investigative	1,33	0.06	0.80
Scent Reception	1,33	0.06	0.80
Active	1,33	0.17	0.69
Resting	1,33	0.94	0.34

Balance across	df	F	*P*
5 treatment groups (single factor ANOVA)	4,30	0.15	0.96

Wind Speed (single factor ANOVA)

Behaviour	Difference among the 5 treatment groups		
Category	df	F	*P*
Recognitive	4,30	0.49	0.75
Cohesive	4,30	0.62	0.65
Agonistic	4,30	0.42	0.79
Investigative	4,30	0.16	0.96
Scent Reception	4,30	0.30	0.88
Active	4,30	0.36	0.84
Resting	4,30	0.04	0.99

Balance across	df	H_c	*P*
5 treatment groups (Kruskal-Wallis)	4	0.15	0.80

Cloud Cover (single factor ANOVA)

Behaviour	Difference among the 5 treatment groups		
Category	df	F	*P*
Recognitive	2,32	0.14	0.87
Cohesive	2,32	0.50	0.61
Agonistic	2,32	2.18	0.13
Investigative	2,32	3.56	0.04*
Scent Reception	2,32	1.64	0.21
Active	2,32	2.45	0.10
Resting	2,32	0.80	0.46

Balance across	df	H_c	*P*
5 treatment groups (Kruskal-Wallis)	4	2.64	0.62

Precipitation (single factor ANOVA; no trials run during drizzle in this experiment)

Behaviour Category	Difference among the 5 treatment groups		
	df	F	*P*
Recognitive	1,33	0.80	0.38
Cohesive	1,33	0.08	0.78
Agonistic	1,33	0.00	0.97
Investigative	1,33	1.51	0.23
Scent Reception	1,33	0.90	0.35
Active	1,33	0.06	0.81
Resting	1,33	0.73	0.40

Balance across	df	H_c	*P*
5 treatment groups (Kruskal-Wallis)	4	3.09	0.54

Juvenile Age (linear regression)

Behaviour Category	Difference among the 5 treatment groups		
	df	F	*P*
Recognitive	1,33	0.04	0.84
Cohesive	1,33	0.33	0.57
Agonistic	1,33	0.08	0.77
Investigative	1,33	0.07	0.80
Scent Reception	1,33	0.10	0.76
Active	1,33	0.06	0.81
Resting	1,33	0.04	0.84

Balance across	df	F	*P*
5 treatment groups (Single factor ANOVA)	4,30	0.92	0.47

Juvenile Mass (linear regression)

Behaviour Category	Difference among the 5 treatment groups		
	df	F	*P*
Recognitive	1,33	8.62	0.01*
Cohesive	1,33	5.53	0.02*
Agonistic	1,33	0.62	0.44
Investigative	1,33	1.06	0.31
Scent Reception	1,33	0.41	0.53
Active	1,33	0.79	0.38
Resting	1,33	2.65	0.11

Balance across	df	F	*P*
5 treatment groups (Single factor ANOVA)	4,30	4.59	0.01*

Trial Within Day (Single factor ANOVA)

Behaviour Category	Difference among the 5 treatment groups		
	d f	F	*P*
Recognitive	4,30	1.15	0.35
Cohesive	4,30	1.05	0.40
Agonistic	4,30	0.33	0.85
Investigative	4,30	1.19	0.34
Scent Reception	4,30	0.70	0.60
Active	4,30	1.00	0.42
Resting	4,30	1.17	0.34

Balance across 5 treatment groups (Kruskal-Wallis)	d f	H_c	*P*
	4	0.65	0.96

Times Run (Single factor ANOVA; note only 3 individuals appeared twice)

Behaviour Category	Difference among the 5 treatment groups		
	d f	F	*P*
Recognitive	1,33	14.8	0.00*
Cohesive	1,33	13.3	0.00*
Agonistic	1,33	1.89	0.18
Investigative	1,33	0.06	0.81
Scent Reception	1,33	0.08	0.78
Active	1,33	0.00	0.98
Resting	1,33	2.76	0.11

Balance across 5 treatment groups (Kruskal-Wallis)	d f	H_c	*P*
	4	5.67	0.23

Gender Pair (Single factor ANOVA)

Behaviour Category	Difference among the 5 treatment groups		
	d f	F	*P*
Recognitive	3,31	0.49	0.69
Cohesive	3,31	0.53	0.66
Agonistic	3,31	1.20	0.32
Investigative	3,31	1.38	0.27
Scent Reception	3,31	0.33	0.80
Active	3,31	0.61	0.61
Resting	3,31	0.29	0.83

Balance across 5 treatment groups (Kruskal-Wallis)	d f	H_c	*P*
	4	2.71	0.61

Appendix I: Intraspecific Killing of Pre-weaned Young in the Columbian Ground Squirrel, *Spermophilus columbianus*.[1]

Abstract

The abandonment of Columbian ground squirrel neonates (*S. columbianus*) by their dams while releasing dams and litters from field enclosures resulted in the availability of newborn pups above ground to male and female conspecifics. Four incidents of infanticide were observed and, without exception, perpetrators of infanticide were female, although male conspecifics had equal access to pups. Contrary to an earlier conjecture that male Columbian ground squirrels are the more likely perpetrators of infanticide, this finding is consistent with the majority of incidents documented for this species.

Introduction

After reviewing accounts of intraspecific killing of pre-weaned and newly emerged young in ground squirrels, Sherman (1981, 1982) contended that infanticide may occur widely throughout the genus *Spermophilus*. Although this conclusion was considered premature by Michener (1982), because many cited instances were either inferred from circumstantial evidence or interpretable as territorial aggression, accounts of infanticide in this genus continue to accumulate (e.g. McLean 1983, Trulio et al. 1986).

For Columbian ground squirrels (*Spermophilus columbianus*), both the killing of pre-weaned young (Balfour 1983, Wiggett and Boag 1986) and newly emerged juveniles (Waterman 1984, Dobson 1990) have been reported. The infrequency of reported incidents of infanticide by Columbian ground squirrels (Dobson 1990) may lead to the conclusion that these squirrels rarely exhibit infanticidal behaviour. However, maternal behaviours such as increased territorial aggression during lactation (Festa-Bianchet and Boag 1982) and the plugging of nest burrows (McLean 1978), which are most readily interpretable in the context of thwarting infanticide, suggests a propensity to commit

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infanticide that may not be realized. Infanticide is difficult to detect in the field (Sherman 1981, Hoogland 1985, Dobson 1990). For some rodent species a propensity to commit infanticide has been demonstrated when pups are made available via experimental manipulation (see Labov et al. 1985 for examples). Here I report data obtained when pups were abandoned by *S. columbianus* dams released from field enclosures. Although the presentation of pups above ground without maternal defence places pups out of the natural context, my observations document the relative infanticidal tendencies of male and female squirrels.

Methods

Research was initiated in April 1988 on a 1.9-ha sub-section of a large meadow area (approximately 70 ha, elevation 1470 m; previously studied by Boag and Murie 1981a, 1981b) in southwestern Alberta (50°38'N, 114°37'W). Population density on the site was relatively high (15.8 lactating females/ha) compared to that reported from other studies of this species (see Dobson 1990, Table 1; Mean ± SE 8.4 ± 1.6). Upon first capture, squirrels were weighed and assigned to age classes (yearling or adult) by comparison to weights of known-age squirrels from the same area in previous years (Boag and Murie 1981a). Squirrels were marked for permanent identification with numbered metal ear tags (Monel #1 fish fingerling tags) and with hair dye (Clairol Nice & Easy™ #124) for identification of individuals during observations. Breeding dates were established for all reproductive females on the site following the procedures in Murie and Harris (1982).

To render neonates accessible for cross-fostering, seven pregnant females were enclosed within their own territory 2 days prior to their expected parturition date in wire mesh enclosures (1 by 1 by .5 m) containing a wooden nest box (0.3 m in all dimensions). Dry grass raked from the meadow was provided in both the nest box and enclosure and females were given lettuce and sunflower seeds *ad lib*. One dam cannibalized her own litter prior to cross-fostering; the remaining six were involved in cross-fostering.

Nest boxes were checked for newborn infants daily and one or two of the two to four pups were reciprocally fostered between litters delivered within 48 h of each other. The distal end of one toe (the toe-nail "bud") was clipped from each foster pup at the time of transfer so that fostered individuals could be distinguished from the dam's biological offspring. Pups were handled with rubber gloves to prevent the transfer of human odours that might have precipitated pup mortality. Subsequent to cross-fostering, daily litter checks continued until release of a given dam and her litter was attempted (24 to 72 h after manipulation).

In addition to fostering in the field, four dams were enclosed and involved in fostering as described above, with the exception that their enclosures were situated in a tent platform at the field station (lab enclosures) rather than on the study site. Of these four, one dam cannibalized the entire litter subsequent to cross-fostering, leaving three dams and their litters for release on the site.

My goal was for litters to be retrieved by their dam and sequestered below ground in a nest burrow (McLean 1978). To this end, a "release" involved either opening one side of the enclosure (for field enclosures) or taking the dam and litter to the site in the nest box (lab enclosures) then tipping the nest box on its side with the lid removed so that the dam would have ready access to the pups within. An observer atop a 3 m high stand recorded the behaviour of the dam as well as the identity and behaviour of other animals in the vicinity of the enclosure. A second observer remained on the ground (but at least 30 m from the enclosure) to terminate the release and recover surviving pups if necessary. Except in the case of the first release where two pups were killed, releases were terminated and surviving pups rescued after a single incident of infanticide if another enclosed dam was available to receive the pups. Releases were initiated either in mid-morning while other squirrels were active (one release), early morning prior to the emergence of other squirrels (seven releases, however other squirrels emerged during each of these), or in the evening after the immergence of other squirrels (one release).

Results

Of nine releases of dams and their litters, four dams retrieved all their offspring following release (two field fostered and two lab fostered). Two dams abandoned their pups; no other squirrels investigated the nest boxes within 2 h (20 May, evening release of field enclosed with 2 pups; 29 May, morning release of lab enclosed with 1 pup). Infanticide occurred following release of the remaining three dams.

Incidents of infanticide were observed then in three of eight releases attempted while squirrels other than the dam being released were active above ground. Infanticide was only observed during release of dams that had been held in field enclosures. Detailed descriptions of the behaviour of squirrels during releases are provided only for those three releases in which incidents of infanticide were observed.

During the first release (15 May; two male and two female pups), one pup was killed and consumed by each of two females. The enclosure was opened at 1155 h (Mountain Daylight Time) and the dam immediately ran into a burrow approximately 1.5 m northeast of the enclosure. A pregnant adult female was the first squirrel to enter the enclosure (1217 h) and immediately investigated the nest box and grasped a pup in her mouth. That female exited the enclosure and consumed the pup completely (distance moved from enclosure not recorded). The dam re-emerged and approached the enclosure for the first time at 1227 h and engaged in a brief fight (Murie and Harris 1988) with an adult male just outside the enclosure. At 1235 h after the dam had entered a burrow a few meters northeast of the enclosure, a non-breeding yearling female investigated the nest box, grasped a pup in her mouth, and ran approximately 8 m north before eating the pup. Subsequent to this, the adult male (same as above) entered the enclosure and approached the nest box but did not take either of the two remaining pups (one male and one female). The release was terminated and the remaining pups "rescued" at 1355 h since the dam showed no indication of retrieving the remaining pups, and no other squirrels had entered the enclosure since 1235 h. During this release then, two females and a male were in close proximity (≤ 1 m) to the pups but only the females exhibited infanticide.

Infanticide was next observed during the third release (19 May; one female pup). The enclosure was opened at 0615 h and upon release, the dam immediately ran into a burrow 6 m north of the enclosure. The dam remained below ground for at least 150 min. A pregnant adult female was the first to enter the enclosure at 0715 h but did not contact the single pup. At 0820 h, a second pregnant adult female entered the enclosure, grasped the pup in her mouth, and consumed it without leaving the enclosure. Observations were terminated at 0845 h, as the dam was not seen above ground after her initial release. No males entered the enclosure during this release, while of two females in close proximity to the pup, one female exhibited infanticide.

During the fourth release, (19 May; concurrent with third release; one male and one female pup), two pups were killed and consumed by a single adult female. The dam was released at 0605 h and immediately ran 5 m north of the enclosure and into a burrow. She did not re-emerge until 0642 h and did not approach the enclosure until 0825 h, 140 min after she had been released. She did not enter the enclosure until 0840 h and upon so doing consumed sunflower seeds but did not retrieve her litter. The dam was observed collecting nest material from outside the enclosure and stocking a burrow with same on 11 occasions between 0907 and 1048 h (including one occasion when she collected nest material from the nest box), but failed to retrieve either pup. An adult male was the first squirrel to enter the enclosure at 0800 h, but did not enter the nest box. That male ate sunflower seeds and lettuce until 0820 h when a pregnant adult female (not the dam) ran at and displaced the male from the enclosure. That female fed on sunflower seeds and chased the dam four times and one other squirrel (identity unknown) once from the enclosure (0835 to 0905 h). At 0920 h, the same female entered the nest box, grasped a pup in her mouth, and loped approximately 7 m east before resting on her haunches and devouring the pup. At 09:25 h the dam returned to the enclosure and fed on lettuce; 2 min later she was joined by the male that was displaced earlier by the infanticidal female. Over the next 65 min, both the dam and the adult male fed on seeds in the enclosure, with the dam displacing the male from the enclosure twice during that period. By 1030 h both the dam and male had left the enclosure. At 1050 h while the dam was collecting nest material 10 m north of the enclosure, the adult female that had previously consumed a pup returned to the enclosure. After 5 min of feeding on sunflower seeds, the female entered the next box, grasped the remaining pup in her mouth, and ran approximately 15 m northeast before eating the pup. Unlike other infanticide victims, this pup was not devoured completely; the tail and hindquarters were left unconsumed by the perpetrator. During this release then, an adult male and an adult female were observed in close proximity to the pups. The adult male did not contact the pups while the pregnant adult female committed infanticide twice.

In the four releases in which litters were successfully retrieved (17, 20, 30 May and 1 June; 4, 2, 2, and 3 pups respectively), dams did not retrieve the first pup until 106.5 ± 28.3 min (Mean ± SE) after release (range 65-190 min). After retrieving the first pup, they retrieved the remainder of their litter in 14.8 ± 6.0 min (Mean ± SE; range 4-32 min), or at a mean rate of 8.6 ± 2.8 min/pup retrieved (Mean ± SE; range 4-16 min/pup). During the 30 May and 1 June releases, two and one adult males respectively were observed in close proximity to pups prior to their retrieval by the dams. None of these males committed infanticide.

Without exception the perpetrators of infanticide were female; of the four females that committed infanticide, one was a non-breeding yearling and three were pregnant adults. Of the five pups that fell victim to infanticidal females, four were completely devoured and the fifth devoured except for the tail, hind legs, and posterior torso. The two male and three female pups were between 1 and 3 days old when killed.

During the releases, male and female squirrels were observed in close proximity (≤ 1 m) to the nest boxes containing pups with equal frequency. None of the five adult males killed pups. By contrast, four of the five females killed and consumed at least one pup. This gender difference has a probability of occurrence by chance of 0.024 (Fisher's exact test; Zar 1974).

Discussion

This experiment rendered pups available to conspecifics, allowing an assessment of the disposition of male versus female Columbian ground squirrels toward newborn pups. Contrary to Sherman's (1981) conjecture that male Columbian ground squirrels are more likely to commit infanticide than females, my data are consistent with accounts of infanticide for this species which document more killings by female than male squirrels (see Dobson 1990).

At the most proximate level, males may not practice infanticide and cannibalism because they do not endure the energetic costs of gestation and lactation and thus may not experience resource limitation akin to that of females (Trulio et al. 1986; but see Michener 1984, Michener and Locklear 1990). However, males should benefit by consuming high quality food to increase their foraging efficiency and so reduce their vulnerability to predators by reducing foraging time (MacWhirter 1989). All five males observed in close proximity to the nest boxes had copulated with females during the breeding season. Further, dams were released within the confines of their breeding territories where they had overlapped spatially with the males in question. In this context, a male unable to discriminate related versus unrelated infants (Michener 1983), would risk a substantial reduction in his own fitness by killing pups which he may have sired (Labov et al. 1985). This interpretation is consistent with the fact that the only case of infanticide known to be committed by a male in this species involved a non-breeding individual (Dobson 1990).

The females that committed infanticide were not lactating (although four of five were pregnant) at the time of the incidents. By killing pups prior to parturition, these females do not risk a reduction in direct fitness but could conceivably reduce their inclusive fitness by killing offspring of related individuals (Hamilton 1964, Hoogland 1985). Because genetic relationships between perpetrators and victims were unknown, this potential cost cannot be assessed. Costs to inclusive fitness may be outweighed if infanticide provides increased access to limiting resources such as food and burrow systems that favour the survival of a female's own offspring (i.e. resource competition; Sherman 1981, Hausfater and Hrdy 1984, Hoogland 1985). By consuming pups, infanticidal females may also improve their body condition prior to parturition (i.e. resource exploitation; Hausfater and Hrdy 1984). However, there is no indication from my data that dams losing their litters to infanticidal females emigrate, thereby creating vacancies, nor that infanticidal females enjoyed greater than average reproductive success (unpublished data).

Because females consumed pups, the present data support the resource exploitation hypothesis but cannot rule out the resource competition hypothesis. The propensity for female squirrels to kill and consume pre-weaned pups suggests that ground squirrels may indeed provide ideal subjects for research on the proximate causes and potential adaptive significance of infanticidal behaviour (Sherman 1981, Labov et al. 1985). The technique of placing pups in nest boxes above ground could be employed in more comprehensive studies examining these questions [illegible] leaving pups in the nest burrow while restraining dams (Sherman 1981) would more closely reflect the natural context.

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Appendix J: Manipulation of Litter Size Reveals No Cost of Reproduction in Columbian Ground Squirrels.[1]

Abstract

Survival and reproductive effort of female Columbian ground squirrels (*Spermophilus columbianus*) were examined for a year following experimental manipulation of litter size that either increased, decreased, or left their lactational cost of reproduction unmanipulated. Except for a trend towards females that incurred decreased costs in year 1 being heavier than other females at spring emergence in year 2, no short-term cost of reproduction was detected. The results fail to support the assumption of reproductive costs implicit in most life-history models, and are in accord with an earlier correlational study of reproduction of Columbian ground squirrels that found no trade off between current and future reproductive success.

Introduction

The evolution of life-history characteristics, particularly reproductive parameters, is of considerable interest to evolutionary biologists. Williams (1966a, 1966b) expounded the notion that natural selection could only act to optimize offspring number if a trade off existed between current and future reproductive success. That trade off is referred to as the "cost of reproduction" hypothesis, which states that an increased investment in current reproduction incurs costs such as reduced lifespan or reduced fecundity later in life (Reznick et al., 1986). Most models of the evolution of life-history patterns assume that reproduction imposes a cost (Bell, 1980; Gadgil and Bossert, 1970; Hirshfield and Tinkle, 1975; Lack, 1966; Stearns, 1976, Williams, 1966a: but see Tuomi et al., 1983).

Empirical data bearing on the validity and generality of the cost hypothesis have accumulated in recent years, and have produced contradictory results (Bell, 1984; Reznick, 1985). Data for small mammals are few as most analyses of the cost hypothesis deal with invertebrate and avian populations (Nur, 1984). Murie and Dobson (1987)

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[1] A version of this manuscript is in press. Hare, J.F. and Murie, J.O. *Journal of Mammalogy*.

examined the proximate costs of reproduction in Columbian ground squirrels (*Spermophilus columbianus*). Females of this group-living species mate shortly after emergence from hibernation and produce only one litter of young each year. Considerable phenotypic plasticity in survival and reproduction of Columbian ground squirrels (Dobson and Kjelgaard, 1985) suggests that short-term costs of reproduction may be expressed in terms of those variables (Murie and Dobson, 1987). However, no evidence of a negative correlation between reproductive effort and subsequent survivorship or fecundity was detected by Murie and Dobson (1987). Similar results (no apparent survivorship or fecundity cost) were obtained by Michener and Locklear (1990) for Richardson's ground squirrels (*Spermophilus richardsonii*).

Although correlational studies have detected costs of reproduction in some ungulates (Clutton-Brock et al., 1982, 1983; Festa-Bianchet, 1989), most results do not support the cost hypothesis (Reznick, 1985). The correlation between current reproduction and subsequent survival or reproduction may fail to reveal costs if individuals regulate their level of reproductive effort to avoid excessive costs (Michener and Locklear, 1990; Murie and Dobson, 1987; Reznick, 1985). Further, the existence of a negative correlation between current and future reproductive success does not provide conclusive evidence for costs since any correlation could be the result of a common correlation between those and a third variable (Partridge and Harvey, 1985).

In an attempt to address the contribution of rearing association to sibling recognition, we fostered pups among litters of female Columbian ground squirrels. In so doing, we produced augmented, reduced, and unmanipulated litters in 1989. By comparing the survival and reproductive effort of females in these groups in the subsequent year we circumvent the problems inherent to the correlative approach in evaluating short-term reproductive costs. This "manipulative" approach has been advocated by several authors (Bell, 1984; Nur, 1984; Partridge and Harvey, 1985), and although it fails to address the genetic basis of costs, it does provide a qualitative index of cost and information on how costs might be expressed among animals in a natural context (Reznick, 1985).

Methods

Squirrels were studied on a 1.9-ha section of a large meadow (ca. 70 ha, elevation 1470 m; Boag and Murie, 1981a, 1981b) in southwestern Alberta (50°38'N, 114°37'W). Individuals were marked with numbered metal ear tags (Monel #1 fish fingerling tags) for permanent identification and with hair dye (Clairol Nice & Easy™ #124) for identification during observations.

To reduce reproductive investment, 18 pregnant females were designated arbitrarily as "donor" females and had their pups removed post partum in 1989. Although this manipulation did not reduce gestational costs of reproduction, it did remove any energetic outlay for lactation, the most "expensive" component of reproductive investment for ground squirrels (Kenagy et al., 1989; Michener, 1989; Michener and Locklear 1990). Donors were live-trapped 2 days before their predicted parturition date and housed in polycarbonate cages (48 by 27 by 20 cm) within a heated building with windows allowing a natural photoperiod. Donor females were provided with cedar chips and paper tissue for bedding and given Purina rodent blocks, lettuce, and water ad lib. Dams were checked each morning at 0800 h. If newborn pups were present, their sex was noted, weight was recorded to the nearest 1 g with a Pesola spring balance, and they were marked by clipping one toe-nail bud from a hind toe. Donor females were released at the original point of capture the morning their litter was delivered (n=14), or two days after the predicted parturition date if no litter was delivered (n=4). Only the 14 dams that had their litters removed were included in this group for the purpose of analyses. Eleven of the 14 dams occupied areas peripheral to the main grid of 1.9 ha; these females were also surrounded by neighbouring squirrels and expanses of meadow, similar to females within the principal grid.

To increase reproductive investment, we attempted to foster the 35 pups produced by the 14 donor females into litters of 22 dams that had given birth to young within the preceding 24 h (based on weight and nipple condition). Pups were handled with rubber gloves to avoid the transfer of human odors that might induce infanticide (Hare, 1991). Pups were transported to the field site in a plastic container lined with paper tissue and carried inside a styrofoam container (to keep pups warm) for fostering to recently parturient dams. One or two pups (n=9 and n=13 respectively; depending on number of pups and number of recipient dams available on a given day) were fostered to each "target" dam by dropping the pup(s) down the entrance of the nest burrow after the dam had emerged to forage. Previous attempts to augment litters of dams in captivity resulted in an unacceptable level of infanticide (Hare, 1991). Of the 35 pups fostered, six emerged as juveniles in four litters (two litters of own plus two foster pups and two litters of own plus one foster pup). The marking technique for pups likely failed to resolve all fostered individuals, since one "marked" individual showed slight regrowth of the clipped toe-nail, and two target dams produced litters of five juveniles at emergence, one pup above the maximum litter size observed on this site in 3 years of study. Only dams rearing litters with definite foster offspring were considered to have incurred increased lactational energy demands (n=4) in this study.

A third group of six dams served as a reference group; they experienced no manipulation of litter size in 1989. Exclusion of those dams where fostering was attempted without apparent success from this group avoids confounding the reference data with dams that actually incurred increased reproductive costs (e.g. reared a foster pup but the toe-nail regrew). Exclusion of those that did not rear a litter to juvenile emergence avoids confounding the reference data with females that experienced a reduced cost in 1989.

In 1990, females from the 3 groups were trapped within 2 days of spring emergence and weighed to the nearest 10 g with a Pesola spring balance. Animals that did not have flakes of skin throughout their pelage had likely emerged earlier than 2 days before (Murie and Harris, 1982) and were excluded from analysis of emergence dates. Breeding and parturition dates were estimated and confirmed by back-dating from juvenile emergence as described in Murie and Harris (1982). Juveniles were live-trapped within 2 days of their first emergence from their natal burrow using Tomahawk live traps (41 by 14 by 14 cm, 1 by 2 cm mesh) baited with peanut butter or in wire-mesh multiple-capture traps (112 by 20 by 28 cm, 1 cm mesh; Waterman, 1986). All juveniles were weighed, ear-tagged, and given a distinctive dye mark at first capture. The survivorship of all juveniles on the site was estimated by scan sampling and intensive trapping until adults began autumn immergence (31 July 1990).

If a short-term cost of reproduction resulted from the 1989 manipulation, we predicted that in 1990 survivorship to spring emergence, mass at spring emergence, litter size, litter mass, average pup mass, reproductive effort (mass of newly emerged juveniles/dam mass *sensu* Michener, 1989), and survival of juveniles in that year (Nur, 1988) would be lowest for litter-augmented females, intermediate for unmanipulated females, and highest for litter-removed females. Further, if the hypothesis of a short-term cost of reproduction was supported spring emergence dates and breeding dates in 1990 would be later for litter-augmented females, intermediate for unmanipulated females, and earliest for litter-removed females (Murie and Harris, 1982).

Where the assumptions of normality and homogeneity of variance were met, parametric analysis of variance was employed; otherwise nonparametric techniques were used to test for differences among the three groups. Differences were considered significant where $P < 0.05$. Unless otherwise indicated, data are presented as mean ± SE.

Results

A significant difference among the number of juveniles reared to emergence by the three groups in 1989 ($P < 0.0001$; Kruskal-Wallis test) suggests the manipulation was effective in altering reproductive investment in the first summer. Dams that had pups fostered to them (n = 4) had 4.0 ± 0.7 offspring at juvenile emergence, those that were unmanipulated (n = 6) produced 2.2 ± 0.4 juveniles, while those having pups removed (n = 14) brought no juveniles above ground. As a reference to gauge the pertinence of the litter size groups, unmanipulated females in 1988 (n = 18) produced 2.3 ± 0.2 juveniles.

All dams in both the augmented and unmanipulated groups and 12 of 14 dams that had their litter removed survived to emerge the next spring. Thus, the three groups did not differ in the probability of overwinter survival (Table J.1). The timing of spring emergence was also similar among the three groups with less than 4 days separating the average date of emergence (Table J.1), and extensive overlap in the range of emergence dates among the three groups.

In spite of these similarities, litter-removed dams tended to be heavier at emergence in 1990 than either litter-augmented or unmanipulated dams (Table J.1). Further, litter-removed dams showed a slight tendency to breed earlier than other dams, although this trend falls short of statistical significance (Table J.1). As was the case for emergence dates, the range of breeding dates overlapped extensively among groups.

Measures of reproductive output in 1990 did not differ significantly among groups. The number of offspring produced by dams was similar in all three groups; if anything, dams that raised augmented litters in 1989 tended to produce the largest litters in 1990 (Table J.1), although no significant correlation between 1989 and 1990 litter size was detected (r = 0.083). Dams in the litter-augmented group also tended to produce the greatest total mass of offspring at juvenile emergence, approximately the weight of one average juvenile above that of the unmanipulated dams, whereas dams having pups removed in 1989 produced litters of an intermediate mass (Table J.1). Average juvenile mass at emergence was similar among groups with only a 12-g range in mean pup mass among the three groups (Table J.1). Reproductive effort did not differ significantly among groups, although a trend for litter-augmented females to show the greatest reproductive effort in 1990 was apparent (Table J.1).

No significant difference was detected in the proportion of juveniles brought above ground (emerged) that survived to the end of the 1990 observation period. However, juveniles raised by the litter-augmented dams of 1989 tended to survive in greater proportions than those raised by unmanipulated dams. In turn, juveniles of unmanipulated dams showed somewhat higher survival than juveniles raised by litter-removed dams (Table J.1).

Further analyses of these data treating the categorical independent variable as the number of pups added or removed were in complete concordance with the trends and conclusions reported above. Further, the inclusion of data from the two dams that reared litters of five juveniles with the litter-augmented group did not alter our results.

Discussion

Of the predicted responses of dams that would support the hypothesis of a short-term cost of reproduction for female Columbian ground squirrels, only mass of females at spring emergence provided support for that hypothesis. However, the greater mass of dams with pups removed did not translate into a discernable effect on the probability of overwinter mortality nor on the reproductive performance of dams in 1990. That no difference in hibernation mortality occurred is not surprising because even females in the augmented group emerged at weights similar to those reported by Young (1988; page 40, 389 ± 14 g) for unmanipulated adult females on the same 70-ha meadow. Further, the weight difference at emergence was no longer apparent among the three groups at parturition (495 ± 17g, 476 ± 15g, 496 ± 12g for augmented, unmanipulated and removed respectively; $P > 0.50$, parametric analysis of variance). Thus, females in the augmented and unmanipulated groups recouped their weight deficit by the onset of lactation in 1990.

Several authors have suggested that costs of reproduction may only be expressed under conditions of environmental or social stress (e.g. resource scarcity--Bell, 1986; outbreak of pneumonia epizootic--Festa-Bianchet, 1989; high population density--Clutton-Brock et al., 1982, 1983). The population density on our study site was relatively high (15.8 lactating females/ha) compared to an average of 8.4 ± 1.6 reported by Dobson (1990; page 9) for studies of Columbian ground squirrels. Further, the habitat quality of the site was poor relative to other areas inhabited by these squirrels (Festa-Bianchet, 1982). If Columbian ground squirrels incur a cost of reproduction, that cost should have been detectable in this population.

Bell (1984) suggested that the only measure of direct relevance to the cost hypothesis is fecundity. In this study we found no evidence of an inverse relationship between reproductive effort in one year and that in the next. Further, there was no evidence of decreased offspring quality in 1990 with increased cost incurred in 1989. Juveniles emerged at similar masses in all groups and any trend in the survival of those offspring was in the opposite direction to that predicted by the cost hypothesis. Thus the assumption of a short-term cost of reproduction remains unwarranted for this species (Murie and Dobson, 1987), although we cannot dismiss the possibility that costs might be expressed in terms of reduced survivorship or fecundity over a longer time period.

By arbitrarily assigning females in 1989 to the three groups, consistent individual differences in reproductive performance should not have confounded our attempt to measure cost (Clutton-Brock et al., 1983; Nur, 1984). However, it is possible that litter-augmented females essentially selected themselves for inclusion in that group by virtue of their ability to rear foster pups. Support for this notion is provided by the trends towards larger litter size, greater litter mass, and higher offspring survival in the augmented group in 1990. One might also argue that the lack of difference between squirrels having pups removed in 1989 and unmanipulated females could be explained by females adjusting their reproductive effort according to cues of resource availability in the spring of 1990

(Reznick, 1985). That Columbian ground squirrels show reductions in litter size both pre- and post-parturition is well documented (Murie et al., 1980). If these arguments are correct, we must still conclude that short-term reproductive costs are unimportant to survivorship and fecundity relative to variation owing to individual quality and individual responsiveness to habitat variables.

Table J.1: Reproduction and survival of female Columbian ground squirrels in southwestern Alberta whose litters were augmented, unmanipulated, or removed in the previous year. Cost hypothesis is supported if values for emergence and breeding dates decrease and other variables increase left to right (mean ± SE; sample sizes shown in parentheses).

	Litter manipulation in 1989			
Variable of interest in 1990	Augmented	Unmanipulated	Removed	P
Proportion of dams surviving to spring emergence	1.0 (4)	1.0 (6)	0.857 (14)	0.319[a]
Emergence date	28 April ± 3.4 (4)	1 May ± 1.8 (6)	27 April ± 2.0 (11)	0.517[b]
Mass of dams at spring emergence (g)	390 ± 17 (4)	393 ± 18 (6)	433 ± 9 (11)	0.054[b]
Breeding date	3 May ± 2.6 (4)	4 May ± 1.2 (6)	30 April ± 1.8 (12)	0.248[b]
Litter size	2.75 ± 0.48 (4)	1.50 ± 0.43 (6)	2.25 ± 0.37 (8)	0.181[b]
Total litter mass (g)	292 ± 45 (4)	176 ± 34 (5)	237 ± 15 (7)	0.061[b]
Mass of juveniles within litter (g)	108 ± 8 (4)	102 ± 15 (5)	96 ± 10 (7)	0.759[b]
Reproductive effort (litter mass/dam mass)	0.58 ± 0.07 (4)	0.37 ± 0.07 (5)	0.49 ± 0.03 (7)	0.073[b]
Proportion of litter surviving to 31 July	0.54 ± 0.21(4)	0.27 ± 0.19 (5)	0.14 ± 0.10 (7)	0.246[b]

[a] G-test

[b] parametric single factor analysis of variance

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