MATING BEHAVIOR OF COLUMBIAN GROUND SQUIRRELS

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MATING BEHAVIOR OF COLUMBIAN GROUND SQUIRRELS

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MATING BEHAVIOR OF COLUMBIAN GROUND SQUIRRELS

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VITA

Theodore G. Manno was born in Trenton, New Jersey on December 4, 1981 and grew up in nearby Hamilton. He received a Bachelor of Science in Biology in 2004 from Rider University, and worked at Hersheypark in Pennsylvania as a musician and zoo docent. After several jobs as a field technician in Utah, Canada, and Costa Rica, Theo began his graduate research in Biological Sciences at Auburn University. Theo finished his degree requirements for a Doctor of Philosophy in August, 2008.

THESIS ABSTRACT

MATING BEHAVIOR OF COLUMBIAN GROUND SQUIRRELS

Theodore G. Manno

Doctor of Philosophy, August, 2008
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Directed by F. Stephen Dobson

Columbian ground squirrels (*Spermophilus columbianus*) are diurnal, herbivorous, colonial, and hibernatory rodents that reside in the Rocky Mountains of western North America. During April-July in 2005-2007, field assistants and I studied the mating behavior of Columbian ground squirrels at colony DOT in Sheep River Provincial Park, Alberta, Canada, by following a well-established protocol that consisted of various techniques that were applied in the following order of priority: (*i*) live-trapping of all individuals 1-2 days after emerging from hibernation in spring for weighing and sexual condition examinations, along with eartagging and painting a unique marker on the fur with black-dye for long-term identification; (*ii*) focal animal sampling for behavior and location of breeding females; (*iii*) all-occurrence sampling and digital recording of vocalizations emitted during courtship interactions of sexually mature males and females; (*iv*) all-occurrence sampling of amicable and hostile dyadic interactions involving all individuals; (*v*) scan sampling at 20-30 minute intervals for location and behavior of all

individuals in view; (*vi*) all-occurrence sampling of predation events, predatory attacks, and predator sightings that involved the study animals as victims or targets. My intention in obtaining these data was to decipher the consequences of six different behaviors on which natural selection would act to favor expression of the traits leading to the behaviors. My approach was to generate as many options as possible for the environmental context of a behavior, and then to derive a priori expectations from those hypotheses that I could quantify in the field, either under natural conditions or experimentally.

Columbian ground squirrels have a promiscuous mating system that exhibits a conflict of interest between males and females regarding the optimal number of mates per females. That is, territorial adult males become reproductively successful by monopolizing females, while females attempt to mate with multiple males. Thus, males and females have evolved auditory signals exchanged during mating interactions that are consistent with these interests. Males engaged in postcopulatory mate guarding, which included hostile encounters with other males after emerging from a copulatory burrow, violent herding of females to keep them close to the copulatory area as she attempted to court other males, and a repetitive cheeping vocalization which I deemed the "mating call". A postcopulatory "estrus call" emitted by females while they attempted to escape the guarding of their consort male appeared to assist females in finding additional mates during their estrus, as females that called were more likely to mate with another male than females that did not call.

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Because of collaborations with individuals mentioned above, I write in the plural first-person for chapters I, III, IV and V, although most of the work contributed to these chapters is mine.

Besides financial assistance, I am forever indebted to my parents (Theodore and Maria Manno) for their assistance, patience, and encouragement during the research for and writing of this dissertation.

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I. WHY ARE MALE COLUMBIAN GROUND SQUIRRELS TERRITORIAL?

ABSTRACT. Male territorial defense is a component of many vertebrate mating systems which is often regarded as a tactic for acquiring mates. Traditionally considered within the context of overt site-specific defense, territoriality actually may have several components which encompass varied behavioral tactics (e.g., post-copulatory mate-guarding, defense of resources that females need, defense of area around females) that underlie a mating system. The purpose of our study was to evaluate such influences on the territorial behavior of male Columbian ground squirrels in Southwestern Alberta, Canada. Males were "dominant" if they defended a core area of activity as a territory by chasing other males more within their core area than they were chased. Subordinate males had no territory because they exhibited the opposite trend, but they competed for mates by increasing chases in their core area when nearby females were in estrus. Dominant males tended to chase other dominant males from their territory when nearby females were in estrus, but traveled outside their core areas to chase subordinate males when females were not estrous. Although females mated first with a dominant male on whose territory they resided (and in order from oldest to youngest if several territories overlapped), mating pairs were not exclusive as females usually mated with additional males. Males also guarded females after copulation and defended females directly just

before estrus, rather than defending territory per se during those times. Thus, males possess a repertoire of behaviours that complement site-specific territoriality.

INTRODUCTION

Territorial defense by males, typically involving an area occupied exclusively or semi-exclusively via overt defense, is a component of many vertebrate mating systems that probably increases the access to potential mates (Wilson 1971; Jarman 1974; Clutton-Brock 1989; Ligon 1999). In Emlen & Oring's (1977) ecological and social framework of mating systems, the spatial distribution of resources or members of the limiting sex are factors that may influence differences in the intensity of sexual selection. Thus, male territoriality has been implicated in monopolization of a discrete portion of habitat that contains females (Dobson 1983; Desrochers & Hannon 1989), or resources that are crucial for mate attraction (e.g., food or hibernacula; Orians & Wittenberger 1991; Sato 1994; Dodson 1997) to increase copulatory success relative to other males.

Recent studies suggest that traditional views of fixed territories containing females or resources are not comprehensive (e.g., Rodrigues 1998; Lacey & Wieczorek 2001; Maher & Lott 1995). For example, male territoriality does not usually prevent extrapair copulation of female residents on the territory, although territory ownership may facilitate first copulations that are likely to yield offspring (Birkhead & Møller 1992; but see Hoogland 1995). Females may also widen their area of activity during periods of sexual receptivity (Boellstorff et al. 1994; Michener & McLean 1996), increasing the importance of males dominating agonistic interactions near females rather than on a particular portion of habitat. Thus, males may exhibit alternative competitive tactics such as moving directly to potential mates (Wells 1977; Barash 1981; Hogg 1984), increased male-female amicable interaction (Hoogland 1995), changes in territory size according to female spatial distribution (Tobias & Seddon 2000), or guarding females directly after copulation (Tamura 1995; McElligott & Hayden 2001; Grafe et al. 2004). Dominance relationships among specific males (e.g., old males versus young males) or reproductive interests of females (e.g., extrapair copulations) may therefore influence male copulatory success, even when males exhibit site-specific or resource-based territoriality per se.

The purpose of this study was to characterize territoriality in male Columbian ground squirrels (*Spermophilus columbianus*) by examining the potential causes and consequences of the various behavioural components underlying the mating system. Columbian ground squirrels are diurnal, herbivorous, and colonial rodents (Betts 1976). Females copulate with several males during a single annual day of estrus, which occurs 2-12 days after emergence from hibernation in April (Murie 1995; Manno et al. 2007). Although more than two-thirds of litters (20/29 = 69%) are sired by multiple fathers, males that are a female's first mate have sperm precedence and usually sire all or most of her litter (Betts 1976; Murie 1995; Murie & Harris 1978; Hare et al. 2004; Manno et al. 2007).

From a 2-year study of *S. columbianus*, Murie & Harris (1978) concluded that sexually mature males defend a distinct portion of habitat during a 3-week mating period when females come into estrus. After this period, males cease defense of their territories, and females begin defending a natal area (Murie & Harris 1982). Although males 2-3 years old are physically able to copulate, only males ≥4 years old are successful in their defense of these areas as territories. If copulatory success in *S. columbianus* hinges on spatially defined defense, then the outcomes of male-male interactions (e.g., chaser or chased) should be influenced mostly by the locations at which the encounters occur (i.e., on or off a male's territory), and territory owning males should have first access, perhaps exclusively, to females resident on their territory before and during sexual receptivity.

Earlier results (Murie & Harris 1978) might also indicate defense of mate-attractant resources on the territory, if an increase in agonistic interactions occurs when food is in short supply or is an important resource for females (i.e., gestation and lactation). While this hypothesis seems unlikely due to female dominance during gestation and lactation (Murie & Harris 1982), resources may be in short supply during emergence from hibernation. Thus, we examined evidence that males defend resources that are shared with their mates. We also examined the possibility that male-female spatial relationships or outcomes of inter-sexual and intra-sexual interactions change according to the sexual receptivity of nearby females, suggesting other competitive tactics besides site-specific defense that significantly affect copulatory success (Manno et al. 2007).

METHODS

Study Population

From April to July 2006-2007, we observed free-ranging Columbian ground squirrels at colony DOT in Sheep River Provincial Park, Alberta, Canada (50° 38′ N, 114° 38′ W, elev. 1500 m) with the help of trained field assistants. All squirrels were trapped 1-2 days after they emerged from hibernation, ushered into a cloth bag, weighed, and fitted with numbered metal fingerling eartags for long-term identification (National

Band and Tag Co., Newport, KY). For visual identification from a distance, we painted each animal with a unique symbol using black dye (Lady Clairol Hydrience; Proctor and Gamble, Stamford, CT). We considered males that exhibited a pigmented scrotum and large, descended testes at trapping to be sexually mature (all males ≥2 years old). Sexually immature males had no descended testes and gray or pink scrotums. Yearling males and females were also distinguishable from adults on the basis of their lower body weight (Murie & Harris 1978). With few exceptions ($N = 8$ males), squirrels were of known age and genealogy. Four of these exceptions were males that immigrated to the colony. The other four males were a minimum of 8 years old; we used their known minimum age for analyses.

During 2006-2007, the 2.5 ha site was inhabited by 14-17 adult males (≥2 years old), 41-48 adult females (≥2 years old), and 20-40 yearlings of both sexes, for a density of 32.8-39.2 individuals per hectare and 23.2-24.8 adults per hectare. Assisted by 10x42 binoculars and 4-m-high towers ($N = 3$), we used the methods of Sherman (1976) and Hoogland (1995) to observe marked individuals from dawn until dusk daily. Male emergence from hibernation was asynchronous within 6-7 days, so we began recording behavioural data after this period when most males were active aboveground every day. Males and females are sexually mature at 2-3 years of age, gestation is 23-25 days, and almost-weaned juveniles emerge from their natal burrows in late June after about 27 days of lactation (Betts 1976; Murie & Harris 1982).

Mating Behaviour

Each female was sexually receptive for a few hours on a single day. We trapped females several times during the 3-week breeding period and examined their vulvar condition to determine whether they were estrous (with fully opened vulva). Copulations occasionally occurred aboveground ($N = 9$ copulations) and were therefore observed easily. We also used well-established methods to infer underground copulations from aboveground diagnostic behaviours: (1) immergences of a male and female in the same burrow on the night before the female exhibited a fully opened vulva; (2) other immergences of both partners into the same burrow, where they remained for at least several minutes; (3) self-grooming of genitals upon later emergence, which was sometimes accompanied by dustbathing; (4) a postcopulatory "mating call" by the male; and (5) other behaviours that indicated male mate guarding, such as chasing the female into a burrow, sitting on or "herding" the female into that burrow as she attempted to flee the area, and fighting with other males (e.g., Sherman 1976; Hoogland 1995; Murie 1995; Lacey et al. 1997; Manno et al. 2007).

A female's first copulation usually occurred early in the morning (about 700 or 800 h Mountain Standard Time) and often before she and the consort male first emerged from the same burrow on her estrus day (Murie 1995; Manno et al. 2007). To infer the first copulation via diagnostic behaviour and to determine if males and females "switched" burrows after all other squirrels had immerged, we continued observations for 20-30 additional minutes after the last apparent daily submergence and timed our arrival at the colony in the morning to 20-30 minutes before the first emergence (Hoogland

1995; Hoogland et al. 2006). Estrus lasted another 5-10 hours to when the female's last copulation occurred (Murie 1995).

As for black-tailed prairie dogs (*Cynomys ludovicianus*) and Belding's (*S. beldingi*) and Richardson's ground squirrels (*S. richardsonii*) (Sherman 1976; Michener 1985; Hoogland 1995), vulvar examinations (open vulva, sometimes with blood stains) in combination with changes in body mass and aboveground diagnostic behaviour (early immergence or late emergence on or near the 24th day after estrus) pinpointed the day of parturition for females. Mothers reared offspring in separate nursery burrows, so we easily established dates of juvenile emergence from natal burrows for each litter.

Spatial Data

We used scan sampling (Altmann 1974) to delineate the areas of activity maintained by sexually mature males and females during the 3-week breeding period. Using the methods of Lacey & Wieczorek (2001), we divided the study site into 6 non-overlapping sections ($N = 2$ per observer) with boundaries determined by the shape of the meadow and recorded the locations of each animal visible to the nearest meter (ascertained from a 10m x 10m grid of flagging placed over the colony) every 30 min. We used data from days when no nearby females were estrous or one day before estrous to delineate core areas of males. This seemed appropriate because spatial relationships among males may be influenced by the presence of sexually receptive females, owing to increased male-female interaction during and just before estrus (Hoogland 1995; Michener & McLean 1996). Thus, for each section of the study site, we divided scan samples into those conducted on days when (1) all females in that section were anestrous, and (2) days when one or more females in that section were estrous or one day before estrous (hereafter, “anestrus” and “estrus” days).

We drew minimum convex polygons with 5% of outlying points eliminated to estimate a “core area” for each male using the Biotas 1.3 software package (Ecological Software Solutions LLC; Hegymagas, Hungary, 2004). These subsets of positional data reduced estimates of male core areas by up to 50%, so this method detected spatial overlap appropriately (Murie & Harris 1978; Lacey & Wieczorek 2001). This method also reconciled the data with the interdependence of successive data points (Hundertmark 1997). Results do not include 1 male in 2006 that was an apparent transient and seen on the study area for only several days ($N \leq 10$ scan samples).

Social Associations

We characterized social relationships with all-occurrence sampling (Altmann 1974) of interactions during the entire 3-month study period (Manno 2008). We recorded the identity of each interaction participant and the time and location of occurrence. For amicable interactions (e.g., male-female sniffing), we recorded the instigator and the recipient of the interaction. In addition, we recorded the participants in agonistic chases (e.g., male-male disputes, which sometimes escalated into fighting; Betts 1976) as “chased”, “chaser”, or “undetermined” using the criteria of Hoogland (1995) and Lacey & Wieczorek (2001). For example, we considered individuals that were chased from an encounter site or following a fight as chased, and individuals that initiated chasing were considered chasers. If an interaction had no discernable outcome or instigator, we scored the interaction accordingly.

We used the locations of residence burrows to assign females as resident in a particular male's core area. Although females interacted with several males, we observed that females always emerged and immerged from one of several burrows within 3-4 meters (Murie 1995; Manno et al. 2007; see also Hoogland 1995). We used scan samples and all-occurrence interaction data sampling on females and males to calculate the distance between male and female centers of activity (arithmetic mean *x* and *y* coordinates) and rates of male-female interaction during and following estrus. These data also allowed me to determine which core areas a female visited during the breeding period.

Mean number of days per male on which scan and interaction sampling were completed for anestrus days was 4.8 ± 1.2 (range = 3-9, both years combined); mean number of days for estrus days was 6.2 ± 1.3 (range = 4-10, both years combined).

Statistical Analysis

Because the dynamics of the colony were different each year and males sometimes did not occupy a core area in the same part of the colony in both years, we considered, like other investigators of ground squirrel behaviour before us (Hoogland 1995; Lacey et al. 2001) that territories from different years were independent statistically. In general, we considered dependence of data from the same individual in the same year and independence of data from the same individual in different years. Whereas data from estrous females represented independent samples of behaviour, males usually mated with several females. Thus, we were forced to assume that analyses of consortships were not biased by particular male identities.

Difficulties also arose in applying statistical tests to interaction data summed over all males, since each interaction between two males appeared twice in the dataset (once each for the instigator and recipient), violating the assumption of mutual exclusivity. Our goal in these analyses was to determine the likelihood of chasing versus being chased during interactions that were inside or outside a male's core area. We therefore follow the methods of Murie & Harris (1978) and Lacey & Wieczorek (2001) and present results from 2x2 chi-square tests for homogeneity. For these tests, the expected values were derived from the null hypothesis that occurrence was equally likely in both categories being compared (e.g., on or off a male's core area).

We used StatistiXL 2005 and Biotas 1.3 for statistical analyses and tested data for normality with Kolmogorov-Smirnov tests. When data did not meet the assumptions of a parametric analysis, we used the appropriate non-parametric test (in all of these cases, transformations did not yield a normal distribution). *N*'s show the number of individuals in the sample unless otherwise stated. Values are means ± 1 *SE*. All *P*-values result from two-tailed tests ($\alpha = 0.05$).

RESULTS

Spatial Parameters

Males commenced intrasexual agonistic behaviour (male-male chasing and fighting) a few days after emerging from hibernation. Using 2006 as an example, agonistic behaviour was frequent during the 3-week mating period, but decreased significantly after females gave birth (Fig. 1). Results from 2007 were similar.

Scan sampling indicated that the mean area occupied by a male was 1020.7 ± 159.9 m^2 (range = 101.3-2003.4 m^2) in 2006 ($N = 14$ males) and 1104.4 ± 203.4 m^2 (range = 200.0-3650.2 m^2) in 2007 ($N = 17$ males). Mean percent overlap between areas occupied by adjacent males was 78.2 ± 9.8 (range = 40.0-100.0%) in 2006 and 72.9 ± 5.0 (range = 25.0-100.0%) in 2007. Neither the yearly differences in mean area occupied ($t_{29} = 0.31$, $P = 0.76$) or mean percent overlap ($t_{29} = 0.74$, $P = 0.46$) were significant statistically (Fig. 2a-b).

Social Relationships Among Males

Yearling males ($N = 12$ in 2006 and 15 in 2007) were never scrotal. They were always chased during intra-sexual interactions (156/156 = 100%). We classed 22 scrotal males ($N = 9$ in 2006 and 13 in 2007) as “dominant” because they were chased within their core areas less than they chased others, although the trend was weaker on anestrous days (respectively; $G = 5.5$, *d.f.* = 1, $P = 0.02$; $G = 3.6$, *d.f.* = 1, $P = 0.06$). The other 9 scrotal males (5 in 2006 and 4 in 2007) were called subordinate, because they were chased within their core areas more than they chased others, but these males were also chased more on anestrous days (respectively; $G = 2.4$, *d.f.* = 1, $P = 0.13$; $G = 17.6$, *d.f.* = 1, $P < 0.001$; Fig. 3a-b).

Dominant and subordinate males were chased less in their core areas than outside during estrus days (dom: $G = 4.5$, *d.f.* = 1, $P = 0.04$; sub: $G = 6.5$, *d.f.* = 1, $P = 0.01$; Fig. 3a-b). This trend was not present for subordinates during anestrus days ($G = 0.32$, *d.f.* = 1, $P = 0.86$), and dominants were chased more in their core areas than outside during anestrus days ($G = 5.6$, *d.f.* = 1, $P < 0.02$). The former result occurred apparently because subordinates were chased in their core areas more during anestrus days than estrus days ($G = 5.0$, $d.f. = 1$, $P < 0.05$); these interactions were perpetrated mostly by dominant males (127/153 = 83.0%), and dominant males were more likely to interact with subordinate males on anestrus days than estrus days ($G = 4.9$, $d.f. = 1$, $P < 0.05$). Thus, when considering interactions of dominants with only other dominants on anestrus days, they were chased in 29% of the interactions within their core areas and in 67% of the interactions outside, a highly significant difference ($G = 18.3$, $d.f. = 1$, $P < 0.001$). Results for estrous days did not change significantly. In any case, core areas appeared to be defended and therefore approximated "territories" for dominants, but not subordinates.

Dominant males were older than subordinate males (6.6 ± 0.27 vs. 3.0 ± 0.21 yrs., $t_{27} = 9.2$, $P < 0.001$); all males ≥4 years of age were dominant, and all males ≤3 years old were subordinate. When a 3-year-old male in 2006 turned 4-years-old in 2007, that male switched from being subordinate to dominant. Dominants had slightly larger core areas than subordinates (1214.5 ± 178.3 vs. 832.5 ± 174.3 m^2, $t_{27} = 1.7$, $P = 0.10$). Percent overlap between core areas, however, did not differ significantly for dominant and subordinate males (76.6 ± 4.7% for dominants vs. 73.3 ± 5.6%, $t_{27} = 0.45$, $P = 0.66$). Territories of dominants overlapped those of other dominants less than subordinates (both years included: 32.5 ± 4.3% for dominants vs. 84.3 ± 5.6%, $t_{42} = 10.4$, $P < 0.001$).

Male-Female Associations

Most female burrow systems in 2006 (40/48 = 83.3%) and 2007 (40/41 = 97.6%) were located on male territories or core areas. Similarly, most male territories or core areas in 2006 (13/14 = 92.9%) and 2007 (15/17 = 88.2%) contained the burrow system of at least one female. Mean number of females per territory or core area in 2006 was 5.9 ± 1.1 (range: 1-14, $N = 14$ male areas), but most female burrow systems (25/48 = 52.1%) were overlapped by more than one male territory or core area. Mean number of females per core area in 2007 was 3.8 ± 0.77 (range: 0-10, $N = 17$ male areas); again, most female burrow systems (23/41 = 56.1%) were overlapped by multiple male territories or core areas (Fig. 2a-b). Yearly differences were not significant for females per male core area ($U = 155$, $N = 31$, $P = 0.16$) or burrow systems in overlap areas ($G = 0.14$, *d.f.* = 1, $P = 0.71$). Number of females per male core area was not correlated significantly with territory size in 2006 (Spearman's rank correlation: $r = 0.09$, $N = 14$, $P = 0.76$), or 2007 ($r = 0.13$, $N = 17$, $P = 0.62$), or with combined years ($r = 0.11$, $N = 31$, $P = 0.55$).

Mating Behaviour

Using females with complete mating data ($N = 27$ in 2006, 24 in 2007), the number of mates per female did not differ significantly between years (3.0 ± 0.24 for 2006 vs. 2.6 ± 0.25 males, $t_{49} = 1.1$, $P = 0.28$). Almost half (23/51 = 45.1%) of the monitored females resided in burrows that were contained within the territory of a single dominant male. All of these females (23/23 = 100%) mated first with the male on whose territory they resided. However, all of these females also copulated with additional males. A few females (5/51 = 9.8%) lived in burrows that were not on a male's territory or core area. All of these females (5/5 = 100%) mated first with the male whose center of activity was closest to their center of activity (avg. distance: 12.4 ± 0.9 m).

The other females (23/51 = 45.1%) resided in burrows that were contained within the territory or core area of 2-4 males. Most of these females (18/23 = 78.3%) copulated with all of these males in order of their age (oldest first to youngest last). Those females that deviated from this trend (3/23 = 13%) did so by copulating with a male one year younger than the oldest male. Some females resided in burrows that were contained within the territory of several males of unknown exact age (2/23 = 8.7%); the female copulated with all males that overlapped her burrow. All copulations involving males on which we had complete data (143/143 =100%) occurred on the territory or core area of the consort male.

On all occasions (51/51 = 100%), a female's first mate was a dominant male. However, some copulations involved subordinate males (26/152 = 17.1%), and almost half (23/51 = 45.1%) of the females copulated with subordinates. Copulations involving subordinates occurred after the estrous female entered their core area, and we saw no instances of displacement of a dominant male before these copulations.

Females interacted with several males during the days before their estrus (avg: 4.0 ± 0.25 in 2006 and 4.1 ± 0.43 males in 2007). On the evening before estrus, females appeared to be guarded by their eventual first mate. During the day before estrus, females interacted amicably with their eventual first mates and other males more than on anestrus days ($U = 986$, $N = 51$, $P < 0.01$ and $U = 1052$, $N = 51$, $P < 0.001$, respectively; Fig. 4). Almost all such courtships (50/51 = 98.0%) featured the immergence of the female and her eventual first mate into the female's burrow system (on the male's territory) together at around 1900 h, then emerging from a burrow 2-3 m away early the next morning.

Upon emerging on the morning of female estrus, the consort male usually guarded the female by either herding her towards the copulatory burrow or chasing other males from his territory (41/51 = 80.4%) for an average of 77.8 ± 12.9 mins. Most females

(43/51 = 84.3%) fled the mating area and copulated with additional males, thus reducing interaction with their first mate and increasing interaction with other males ($U = 887$, $N = 51$, $P < 0.01$; Fig. 4). Examination of the distance between the center of activities for estrous females and their first mates was similar; the distance decreased during the day before estrus and during estrus days, and increased after the first mate ceased guarding and when the female was anestrus (Wilcoxon matched-pairs signed-ranks test: $P < 0.01$ for these pair-wise comparisons; Fig. 5).

DISCUSSION

Results concerning the behaviour of male Columbian ground squirrels are generally consistent with the conclusions of Betts (1976) and Murie and Harris (1978). Dominant males exhibited a spatiotemporal territorial system as defined by Wilson (1971), occupying an exclusive or semi-exclusive site via repulsion of intrasexual competitors during the 3-week breeding period. Subordinate males did not show the same characteristics as dominants, since they did not defend territories and were typically chased during interactions both inside and outside their core areas. These dominant-subordinate male roles were associated with age and probably have equivalents in other sciurid species. For example, Arctic ground squirrels (*S. parryi*) have “floater” males that travel in between the territories of dominants (Carl 1971), some male yellow-bellied marmots (*Marmota flaviventris*) are peripheral or isolate (Armitage 1974), and sexually immature males that are 1-2 years old live in the territory of an older, dominant male in black-tailed, Gunnison’s, and Utah prairie dog colonies (*C. ludovicianus*, *C. gunnisoni*, *C. parvidens*) (Hoogland 1995; Manno 2007).

Is territoriality a form a reproductive competition that facilitates male access to potential mates? The answer appears affirmative, since females mated with the dominant male on whose territory they resided. However, unlike black-tailed prairie dog, dwarf mongoose, or ungulate males that maintain exclusive territories containing several females with rare multiple paternity for litters (Owen-Smith 1972; Hoogland 1995; Rood 1983), male Columbians were usually not rewarded with exclusive access to females. Considering the first male sperm precedence in *S. columbianus* (Hare et al. 2004), territoriality probably increases the fertilization success of a territory owner by facilitating the first mating with females that live on his territory, rather than providing exclusive access to females that have not yet mated. Nevertheless, multiple paternity is common in this species and accordingly, males seemed to copulate with as many females as possible, including females that entered their territory having already mated elsewhere. These results indicate that *S. columbianus* exhibits a polygynandrous rather than polygynous mating system per se.

Although our results in general supported a more or less conventional view that male territory ownership enhances access to female mates (Lacey et al. 2001), traditional views of spatially fixed territories that encompass female mates (e.g., Wilson 1971; Jarman 1974; Emlen & Oring 1977) did not appear to be applicable. Spatiotemporal territoriality was not, unto its own, a comprehensive strategy during reproductive competition, although site-specific defense was an underlying subtext for most agonistic and courtship behaviours. For example, territorial defense was not a prerequisite for copulation, although successful defense yielded the opportunity to mate. Subordinate males managed to copulate with females occasionally, despite their inability to defend territory.

Copulations always occurred on the core area of the consort male, but at least five other behaviours that we observed suggested that males defend females directly in conjunction with spatiotemporal dominance. First, dominant males were sometimes chased more in their territories than outside. This occurred when a dominant male interacted less with other dominants because all his nearby females were anestrus, and he traveled outside his territory in search of estrous females. In the process, such dominant males chased and fought with subordinate males that were also competing for mates, the dominant male often winning. Second, dominant and subordinate males increased dominance within core areas on estrus days versus anoestrus days. Third, like avian species where males deter extrapair copulations by females on their territory (Birkhead & Møller 1992; Tobias & Seddon 2000), males guarded females after copulation by attempting to prevent females from moving outside their defended area (Manno et al. 2007). Fourth, females immerged and emerged with their first mate on the evening preceding and morning of their estrus. Finally, males that copulated first with a female on their territory improved their familiarity with that female by increasing amicable interaction just before estrus. Thus, as for Arctic ground squirrels (Lacey & Wieczorek 2001), and several avian species (Birkhead & Møller 1992; Rodrigues 1998; Tobias & Seddon 2000), defense of a particular site probably represents only one aspect of male-male reproductive competition.

Taken together, results indicated that female Columbian ground squirrels did not copulate randomly with scrotal males in the general vicinity. Like black-tailed prairie dogs (Hoogland 1995), females increased amicable interaction with the nearest territorial male (or the oldest of several males that owned a territory overlapping her burrow) on the day before estrus, having a center of activity close to their first mate during this time. Females also left the territory of their first mates to copulate with additional males, increasing the distance between the center of their activity and their first mate's. Therefore, even though female burrows were usually located on male territories, females typically did not restrict their activity to a site defended by a single male. These results suggest that despite male territoriality, females have opportunity to assess different prospective mates, and female choice may therefore play a large role in shaping reproductive competition among males.

The typical pattern for females was to mate first with the male on whose territory they reside, or the oldest of several males with territories that overlapped her burrow. This might seem to suggest the absence of female choice, but dominant males frequently faced challenges from intraspecific rivals inside their territories. When a female copulates first with the male in closest proximity, she "chooses" the male that has demonstrated prowess at repelling reproductive competitors to sire all or most of her offspring. The same applies for additional copulations, because males increased chasing of individuals inside their core areas more when nearby females are estrus, thus defending the opportunity to copulate with females that reside or approach their territory. The fact that females could eventually evade dominant males to mate with others suggests adequate female capability to choose their mates (Manno et al. 2007).

The spatiotemporal territoriality of male Columbian ground squirrels did not appear to allow pre-estrous females disproportionate access to resources within the territory (e.g., food or burrows). Under this hypothesis, an increase in agonistic interactions should occur when food or other important resources for females are in short supply (e.g., during lactation in late May or early June). While food resources may have been in short supply during the time of emergence from hibernation (shortly before mating), males attention towards females during this period was not hostile, but commonly was investigatory in the form of close contacts with sniffing that did not occur between the same males and females after the day of estrus. After the mating period, females became more aggressive and held territories from which most males were excluded (Murie & Harris 1982). Thus, there was little support for the hypothesis that male territoriality sequestered resources that could be used to attract mates (Emlen & Oring 1977).

Male Columbian ground squirrels appeared to be territorial in order to improve their chances of mating with females that lived within their territories. This territoriality was augmented with direct mate guarding during a female's day of estrus (Manno et al. 2007). Territoriality weakened considerably, however, when the females on a male's territory were not in estrus, and such males went in search of matings on the territories of other males. Thus, while the territories of males likely reflect intrasexual competition for mates, they should be viewed as only one of a complex of behavioral traits that enhance opportunities for copulations.

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FIGURES

Figure 1. Annual cycle of male-male agonistic behaviour (chases and fights) for Columbian ground squirrels at colony DOT using 2006 as a representative case (*N* = 14 males).

Figure 2. Minimum convex polygons estimating the core areas of activity maintained by male Columbian ground squirrels resident on colony DOT (*N* = 14 in 2006, 17 in 2007) during the 3-week mating period (17 April-6 May in 2006, 21 April-3 May in 2007). Mean number of visual fixes used to map these areas was 62.3 ± 5.8 (range = 25-93) in 2006 and 75.4 ± 9.8 (range 35-102) in 2007. Points represent the locations of female residence burrows during the mating period (*N* = 48 females in 2006, 41 in 2007).

Figure 3. Male-male chases on estrus and anestrus days for Columbian ground squirrels at colony DOT listed by location relative to areas of activity. Numbers above the bars represent the number of females followed by the number of interactions (data from the same individuals in different years are independent). Pairwise comparisons from 2x2 chi-square tests are in the text.

Figure 4. Behavioural interactions between sexually mature male(s) and females before, during, and after estrus (*N* = 51 females). Error bars are ± 1 *SE*.

Figure 5. Mean distance between the centers of activity of females and their first mate during different portions of the reproductive cycle (*N* = 51 estrous females). Error bars are ± 1 *SE*.

Fig. 1.

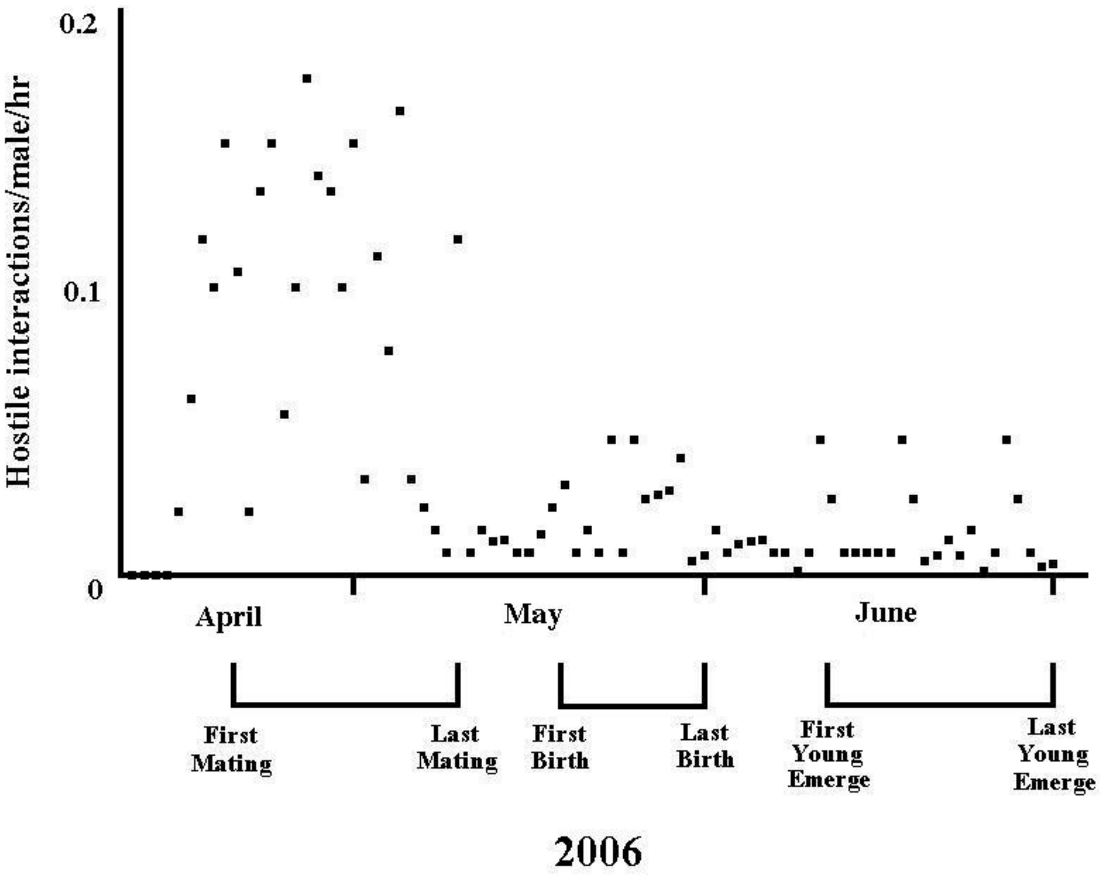
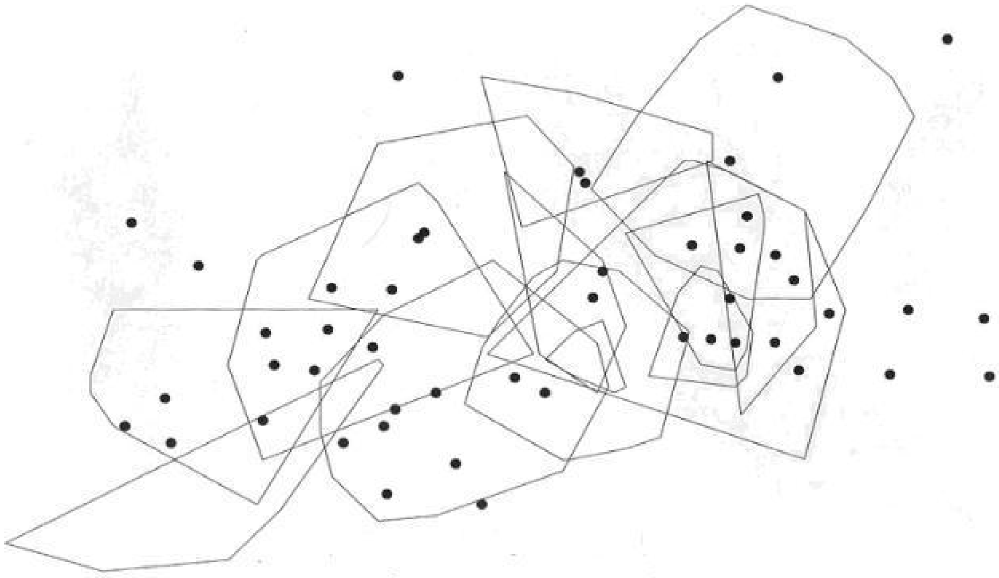

0.2
0.1
0
Hostile interactions/male/hr
April
May
June
First Mating
Last Mating
First Birth
Last Birth
First Young Emerge
Last Young Emerge
2006

Fig. 2a-b.

2006



2007

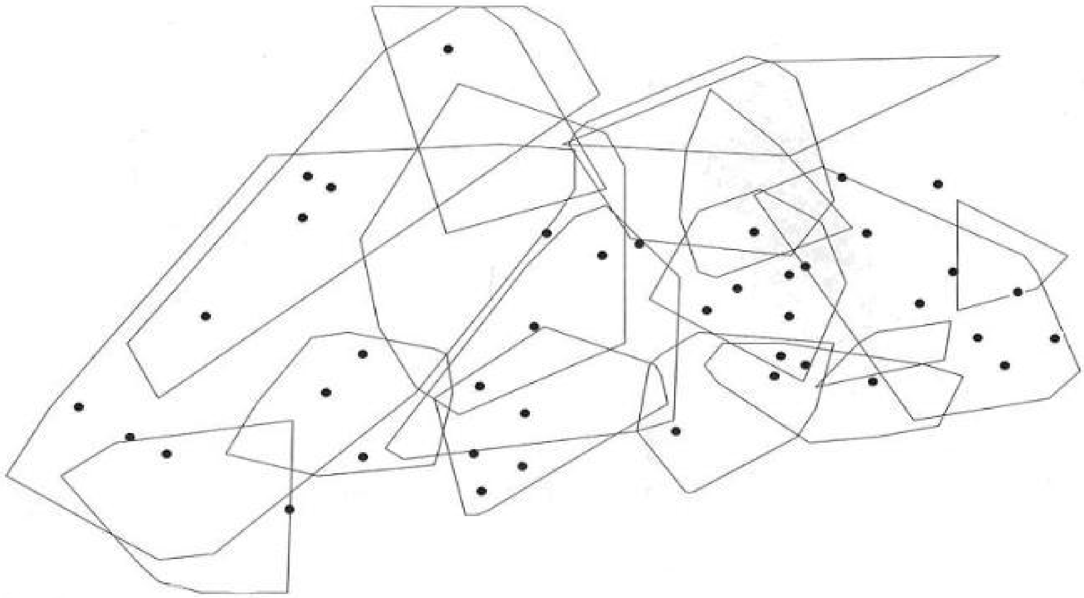


Fig. 3a-b.

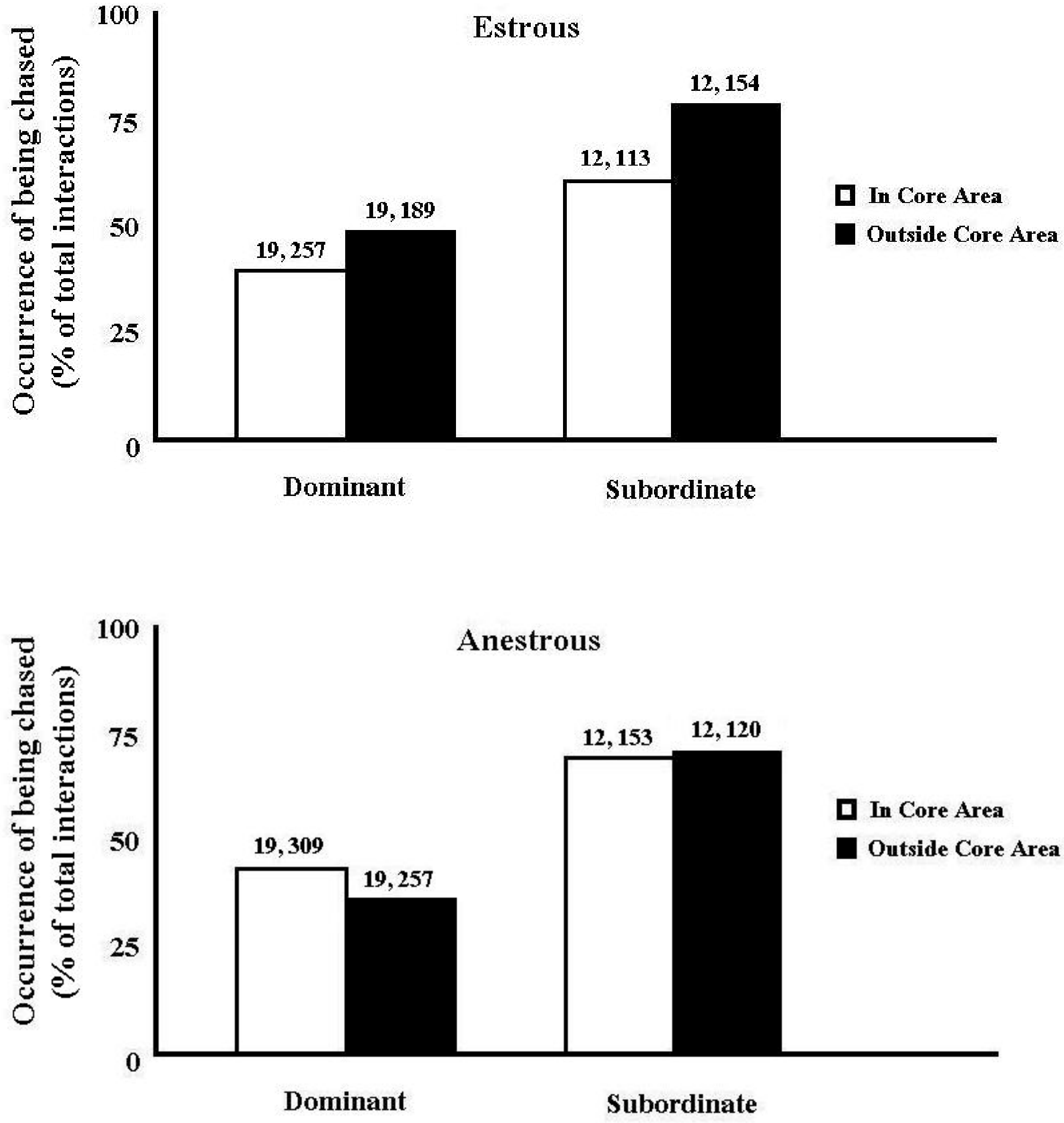

Estrous
Occurrence of being chased
(% of total interactions)
100
75
50
25
0
19, 257
19, 189
12, 113
12, 154
Dominant
Subordinate
☐ In Core Area
■ Outside Core Area
Anestrous
Occurrence of being chased
(% of total interactions)
100
75
50
25
0
19, 309
19, 257
12, 153
12, 120
Dominant
Subordinate
☐ In Core Area
■ Outside Core Area

Fig. 4.

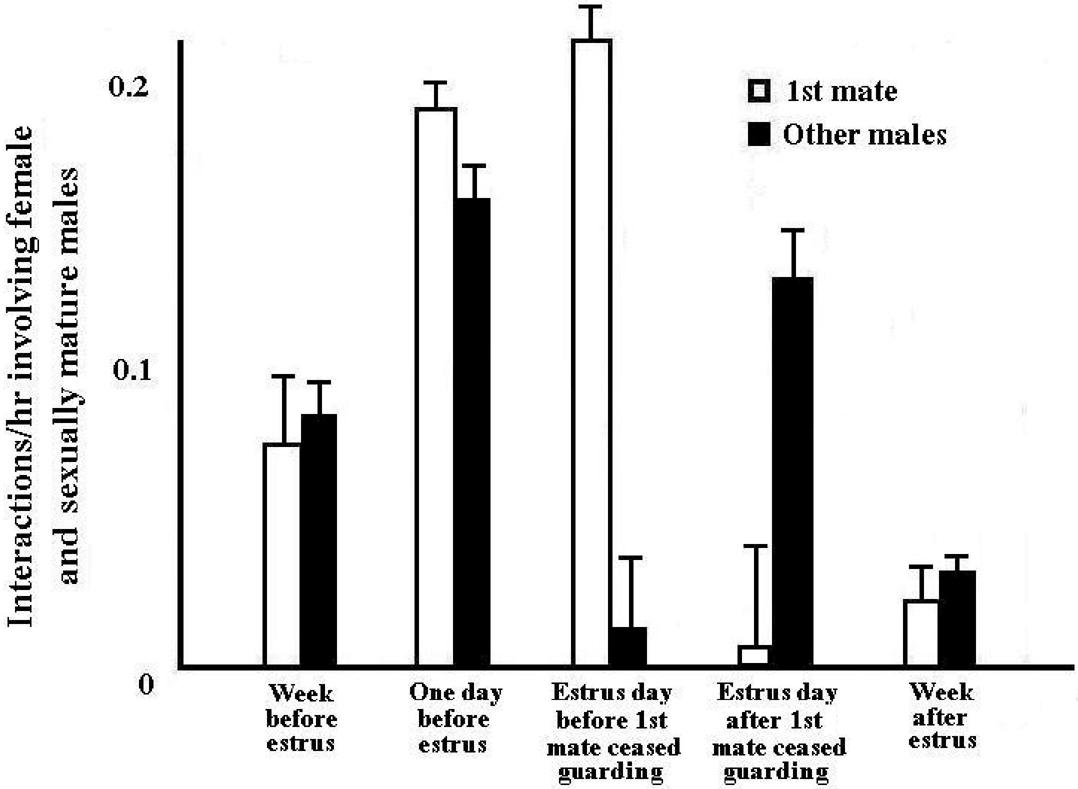
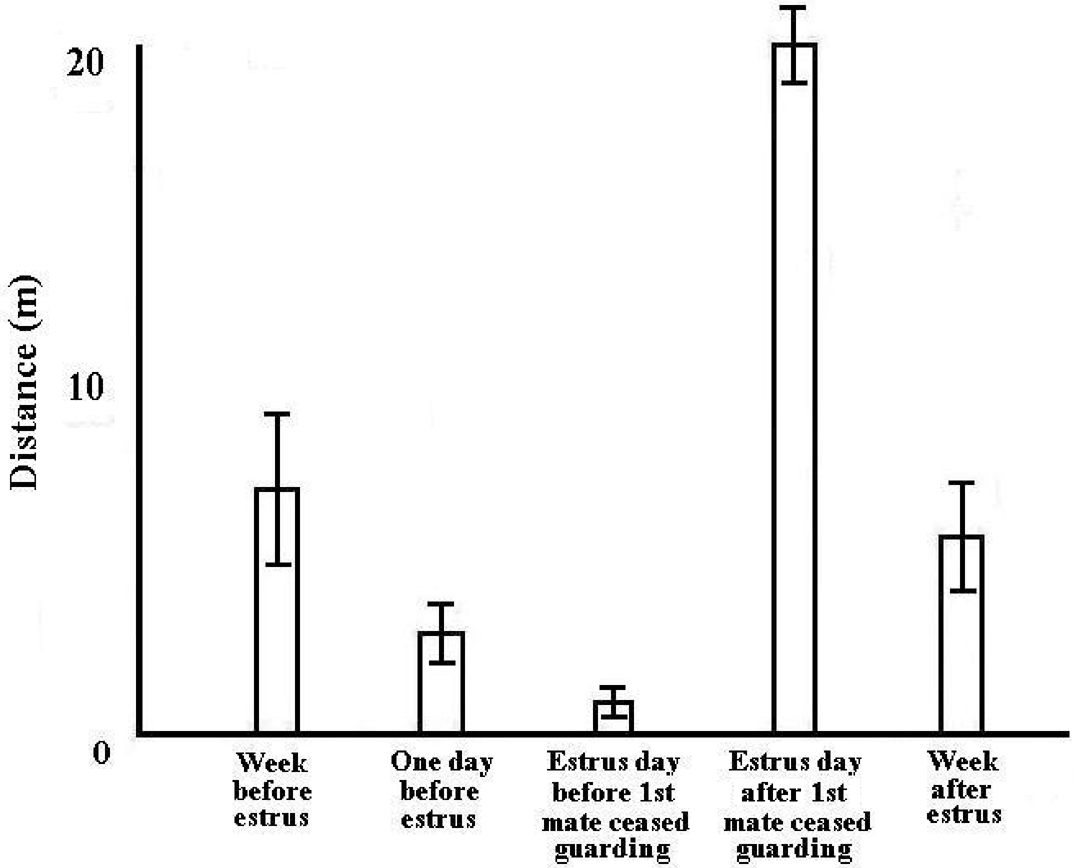

Interactions/hr involving female and sexually mature males
0.2
0.1
0
□ 1st mate
■ Other males
Week before estrus
One day before estrus
Estrus day before 1st mate ceased guarding
Estrus day after 1st mate ceased guarding
Week after estrus

Fig. 5.


20
10
0
Distance (m)
Week before estrus
One day before estrus
Estrus day before 1st mate ceased guarding
Estrus day after 1st mate ceased guarding
Week after estrus

II. SOCIAL NETWORKING IN THE COLUMBIAN GROUND SQUIRREL

ABSTRACT. Networks are collections of units that can potentially interact as a system. Electronic power grids, human societies, the Internet, food webs, and metabolic pathways are examples of networks that have emergent properties which allow all vertices (viz., individuals, components, species, etc.) to be linked by a short chain of intermediate vertices. My field observations on a colony of 65 free-ranging Columbian ground squirrels suggest that their society also exhibits these characteristics via social interaction. On average, any dyad of squirrels in the colony can be connected via amicable interaction with 3 intermediate individuals. The connectivity of individuals (viz., the number of individuals to which an individual is directly connected) decays following a scale-free power-law distribution. Individuals that have similar age, reproductive status, and number of associates (viz., the number of individuals to which the individual is connected via social interaction) interact amicably with each other more than other squirrels. The network is robust to the removal of random individuals. However, simulated removal of individuals that are connected to many other squirrels increases the number of intermediates between two random individuals, and fragments the network into smaller clusters when removals exceed 10% of the individuals in the colony. Thus, certain individuals appear to play more central roles than others in the cohesion of the network. My results reinforce previous studies showing that network

theory can be used to determine the roles played by individuals in the cohesion of animal societies, thus providing a framework for studying sociality across species.

INTRODUCTION

When animals aggregate, they may form complex social relationships and structure via amicable social interaction (e.g., Armitage 1986; Koenig & Mumme 1987). For at least two reasons, these social interactions are significant biologically. First, discrete social groups or coalitions may result from the association of individuals, providing basis for the evolution of cooperative behavior via kin selection (e.g., Goodall 1986; Hoogland 1995). Second, amicable interactions allow individuals to become familiar with and select appropriate breeding partners (e.g., Rood 1972; Sherman 1976; Barash 1981). By studying the structure of social relationships and interactions, we can better understand the causes and consequences of sociality and the role of interaction in shaping the evolution of sociality (Hinde 1976; Whitehead & Dufault 1999; Krause & Ruxton 2002).

Individuals play different roles in the cohesion and social organization of animal societies (Moore & Newman 2000; Abramson & Kuperman 2001). The consequences of removing individuals with different roles from the society (through natural mortality, or unnatural alterations such as sport-shooting, plague, etc.) should vary with the importance of the individual to group cohesion. Association of different sex and age animals can be an important mechanism in group formation with a few individuals holding structurally important positions in their society (Lusseau & Newman 2004; Croft et al. 2005). For instance, black-tailed prairie dog social groups may abruptly cease amicable interaction and fission and when a matriarch dies (Manno et al. 2007), and preferential poaching or trophy hunting of ungulates adjusts information transfer and reproductive success in male and female social groups (McComb et al. 2001; Coltman et al. 2003). Flack et al. (2005, 2006) also examined the effect of removing key individuals on social structure in primate societies, using both simulated and experimental removals, and found that these individuals can have disproportionately large effects on social group cohesion.

Columbian ground squirrels (*Spermophilus columbianus*) are diurnal, herbivorous, and colonial rodents that hibernate during winter. Females live in philopatric kin clusters with a few non-reproductive animals of both sexes that are overlapped by a territorial reproductive male (usually ≥3 years old) (Murie & Harris 1978; King & Murie 1985; Murie & Harris 1988; Murie 1995). Young subordinate males (2-3 years old) usually do not maintain a territory, but are physically able to reproduce and sometimes obtain copulations (Murie & Harris 1978; Manno et al. 2007). Females are highly promiscuous during their single annual day of estrus (which occurs sometime during a 3-week courtship and breeding period in late April and early May), and may solicit copulations from and interact with their territorial male, adjacent territory holders, and subordinate young males (Murie 1995; Manno et al. 2007). After this courtship and breeding period, reproductive males usually disperse away from the colony, while females begin to defend a natal burrow and therefore cease amicable interaction with other squirrels.

The role of different age-sex classes in the cohesion of rodent societies has not been studied extensively. Strong philopatry of female ground squirrels combined with extensive courtship suggests non-random colony structure and variable contributions of individuals to the local (social group) and global (colony) cohesion of the network (Croft et al. 2004, 2005; Lusseau 2003; Lusseau & Newman 2004; Lusseau et al. 2006). Burrowing rodents are susceptible to predation, sport shooting, and plague (Hoogland 1995; Hoogland et al. 2006), and selective removal of individuals with important roles in the society may effect society structure and cohesion differently than random removals.

I describe the social system of free-ranging Columbian ground squirrels in Alberta, Canada by presenting information on the amicable social interactions of 65 individuals. I then examine the role of different life-history characteristics in maintaining the cohesion of the "social network". A network models a system composed of individual components (*vertices*) connected by interactions (*edges*) (see Appendix for definitions of italicised terms). By providing information about individual group members and the entire group, as well as direct and indirect interactions, network analysis offers an alternate way to define animal social groups based on social interactions and associations (e.g., Wasserman & Faust 1994; Barabási 2003). Using a network approach, I test the hypothesis that individuals contribute differently to maintaining the cohesion of communities and the entire colony. From this hypothesis, I predict that (1) a *scale-free power-law* explains the likelihood that a vertex is linked with other vertices (Newman 2003), and (2) the network will fragment into small clusters after the targeted removal of individuals that interact amicably with many other individuals.

METHODS

From 4-m high observation towers, two trained assistants and I studied 65 wild, free-ranging Columbian ground squirrels at colony DOT in Sheep River Provincial Park, Alberta, Canada (50° 38′ N, 114° 38′ W, elev. 1500 m) during April-July 2006. Breeding at DOT occurs earlier than other colonies in the park, so squirrels began to emerge from hibernation during the first week of April (Murie & Harris 1982; Manno et al. 2007). The squirrels were of known age and matrilineal genealogy.

Squirrels were trapped 1-2 days after they emerged from hibernation, ushered into a cloth bag, restrained by hand, weighed, and fitted with numbered metal fingerling eartags for long-term identification (National Band & Tag Co., Newport, KY). For visual identification from a distance, I painted each squirrel with a unique symbol using black dye (Lady Clairol Hydrience; Proctor and Gamble, Stamford, CT). I determined whether males were reproductive or non-reproductive by the presence of a pigmented scrotum and large descended testes after hibernation. I also trapped females several additional times in the weeks following emergence from hibernation and examined their vulvar condition to determine whether they had been estrous (viz., with fully opened vulva; Hoogland 1995; Murie 1995).

During the 3-week breeding period, my assistants and I always arrived at the colony before the first squirrel had emerged for the day and remained in our towers, observing until the last squirrel had submerged for the night (each of us watched for 300 h, × 3 persons = 900 person hours of observation). Like black-tailed, Gunnison’s and Utah prairie dogs (*Cynomys spp.*; Hoogland 1995, 2007), Columbian ground squirrels rarely submerge into a burrow after first daily emergence, except during inclement weather, a predatory attack, while taking nesting material underground, or when retiring for the night. Columbian ground squirrels also copulate underground, (Murie 1995, Manno et al. 2007), so I used the methods of Hoogland (1995) and made a “missing squirrel list” every 20 minutes to alert myself to determine which squirrels were foraging aboveground. Besides helping me to identify squirrels that were possibly copulating for my long-term study on sexual selection (Manno et al. 2007), this method allowed me to estimate the amount of time each squirrel spent aboveground and under observation (the time a squirrel was “active”). All animals used in this study were present for >50% of the checks conducted each day.

Social networks are context-based (Flack et al. 2006), so compiling behavioural information from different contexts may result in an inaccurate representation of social interactions between individuals. Because my hypotheses concerned patterns of amicability and affiliation (as opposed to dominance hierarchies, for example), I used only amicable interactions (excluding inferred copulations) from the courtship and breeding period to visualize a social network based on affiliative social ties between individuals with a *sociomatrix* in UCINET 6 (Borgatti et al. 2002). My assistants and I used all-occurrence sampling (Altmann 1974) to record the time, location, and individuals involved for all social interactions. I defined an amicable interaction as involving a “kiss” (i.e., oral contact), anal sniff, play, or allogrooming without any subsequent aggression (King 1955; Hoogland 1981; Hoogland 1995). Hostile interactions (which were not used in the analysis), were easily distinguished from amicable interactions because they began with a kiss or anal sniff that escalated into a fight, chase, or a physical territorial display (King 1955; Hoogland 1981; Hoogland 1995; see Betts 1976 for a further description of these interactions). I scored combinations of amicable (e.g., kiss + kiss or kiss + allogrooming) or hostile (e.g., fight + chase or chase +

territorial display) interactions that were not interrupted by one or more of the actors engaging in feeding activity as single interactions.

Networks may exhibit temporal dynamics (Jain & Krishna 2002). Like other investigators of ground squirrel behaviour (Lacey & Wieczorek 2001), I used the courtship and breeding period to quantify and examine associations between individuals for two reasons. First, males stabilize their territories 1-2 weeks after hibernation. Second, after this courtship and breeding period, reproductive males usually disperse away from the colony, while females begin to defend a natal burrow and therefore cease amicable interaction with male and female squirrels (Murie & Harris 1978; King & Murie 1985; Murie & Harris 1988; Murie 1995). Only one individual (a non-reproductive male) in the social network died during the period of data recording, and this individual was still included in the dataset because he survived the first two weeks of the breeding season.

For each dyad, I calculated a half-weight index from a matrix of 65x65 squirrels, based on the whether the squirrels had engaged in amicable interaction:

$$\mathrm{HWI} = X/(X + Y_{\mathrm{A}} + Y_{\mathrm{B}})/2, \qquad (1)$$

where X is the number of times A and B were present for the same 20-minute check, Y_A is the number of times individual A was present without B, and Y_B is the number of times individual B was present without A. The social network of the 65 squirrels was then visualized using preferred companionship. I defined preferred companionships as individuals that interacted amicably more often than one would expect from random association, i.e., dyads with HWI higher than $\mathrm{HWI}_{\mathrm{null}}$ (Whitehead 1995). The null HWI was determined from the average number of amicable interaction partners a squirrel had and the number or squirrels which were available.

To detect communities within the network, I used the algorithm of Girvan & Newman (2002). This method finds natural divisions of networks by looking for edges that run between groups and identifying them with a *betweenness centrality* measure (Freeman 1977). I used the commands in UCINET to calculate the *diameter*, *clustering coefficient*, and *degree* of the network, and compared these values to those calculated from the average of 20 random networks that contained the same number of links and vertices. I tested for association of different age and sex animals by calculating Newman's (2003) assortativity coefficient or Pearson's correlation coefficient (r), as appropriate.

Using the techniques of Lusseau (2003), I used UCINET to test the resiliency of the Columbian ground squirrel network to simulated targeted attacks (viz., removal of vertices with high betweenness) and simulated random attacks (viz., removal of random individuals) by observing the size (S) of the largest cluster (group of connected individuals) in the network and the size (s) of any clusters that became isolated. I also observed changes in the diameter of the network after both types of attacks. Thus, I tested the likelihood that the network would break down into isolated clusters after targeted and random removals. I repeated targeted and random attacks 10 times. I used Excel 2005 (with Pop-tools and StatistXL add-ins) for non-network statistics. All values are ± 1 *SE*, and P-values result from two-tailed tests ($\alpha = 0.05$).

RESULTS

My colleagues and I observed 2200 amicable interactions between 345 dyads of individuals during the 3-week courtship and breeding period. Of these dyads, 240 were retained in the social network as preferred companionships. The algorithm detected 2 communities, which were further divided into 2 and 4 sub-communities, respectively (Fig. 1). Each sub-community consisted of 2-6 reproductive females (where genealogies were known, these females always belonged to the same matriline), 1-2 non-reproductive individuals of both sexes, and 1-2 reproductive males. The 240 edges were not mixed assortatively by sex (Newman's assortativity: $r = 0.08$, $P = 0.35$), but there was evidence of assortative interaction by degree (Pearson's correlation: $r = 0.82$, $P < 0.001$; Table 1). When individuals were split into two age categories (viz., reproductive and non-reproductive individuals), there was also evidence of assortative interaction by age (Newman's assortativity: $r = 0.24$, $P < 0.001$; Table 1).

The squirrel network was sparse, containing 240 edges out of a possible 2080 that could have existed, but was highly structured. The average degree of the network (k) was 5.6. Whereas the random networks were homogeneous, and the number of edges per vertex followed a Poisson distribution ($P > 0.2$ for all, Goodness-of-fit test), the number of edges possessed by each vertex (k) in the squirrel network differed significantly from a Poisson distribution ($\chi^2 = 515.2$, $P < 0.001$). Instead, the distribution of k resembled that of a scale-free network, and decayed following a power-law with $\gamma_{\text{squirrel}} = -0.85 \pm 0.10$ (Fig. 2a). When compared with the random networks, the squirrel network had a somewhat higher diameter ($d_{squirrel} = 2.92$ vs. $d_{random} = 2.74 \pm 0.03$), and a much higher level of clustering ($C_{squirrel} = 0.81$ vs. $C_{random} = 0.14 \pm 0.02$).

Individuals with the highest betweenness fell on the boundary between the communities and sub-communities found within the network, and individuals with high betweenness were usually reproductive males. Sub-communities contained 1-2 reproductive males of high betweenness, along with a few reproductive females per sub-community that possessed high betweenness (Fig. 1). As with degree, the betweenness of individuals decayed following a power-law distribution with $\gamma_{\text{squirrel}} = -1.1 \pm 0.1$ (Fig. 2b).

The network was more resilient to simulated random attacks than to simulated targeted attacks. Whereas the diameter of the network increased by only 0.13 with the removal of 20% of vertices randomly, targeted attacks on the same percentage of individuals with high betweenness increased the diameter of the network by 1.01 (Fig. 3a). The integrity of the network was affected more by targeted than random attacks. When a few individuals were removed either randomly or selectively, the network maintained a large cluster which encompassed the vast majority of individuals left after the removal, complemented by mostly single individuals without any associates. However, after the removal of about 10% of the high-betweenness vertices, the network fragmented more than when removal occurred at random (Fig. 3b).

DISCUSSION

For a social network based on the amicable interactions of 65 Columbian ground squirrels living in Canada under natural conditions, a scale-free power-law explains the likelihood that a squirrel is linked with other squirrels, and a small percentage of individuals serve as “activity centers” (viz., have high degree). Furthermore, most individuals seem to have marginal influence over others with respect to betweenness (ergo, the power-law distribution of *n*), but all reproductive males and some reproductive females are individuals that help maintain a short diameter between individuals. This cohesion is maintained at least partly by assortative interactions between animals of similar age, reproductive status, and degree. Taken together with the high level of clustering, these patterns are similar to other animal societies (McComb et al. 2001; Mitani et al. 2002; Lusseau 2003; Newman 2003).

A breakdown of interaction between certain "key" components can cause a network to disintegrate (Chepko-Sade et al. 1989; Albert et al. 2000). Such individuals or components can therefore be attacked specifically to more effectively stop the spread of a disease, for example, or maintained via wildlife management decisions to continue the cohesiveness of an animal society (Ortiz-Pelaez et al. 2006; Tarlow & Blumstein 2007). Indeed, the squirrel social network was vulnerable to attacks that target individuals with high betweenness and degree values. While the network remains united if removals of such individuals do not exceed 10% of the colony, there is strong evidence that certain individuals (viz., reproductive males and a few reproductive females) have more crucial roles than others in maintaining a short path between individuals. These may be important properties of ground squirrel social networks during breeding season that should be considered when designing management plans. For instance, if breeding males are likely to be eaten during the breeding period in their distraction of looking for mates (Hoogland et al. 2006; Manno 2007), then the network may be vulnerable and adjustment of the population via shooting or otherwise may not be appropriate. Likewise, relocation programs for ground-dwelling rodents that move individuals of different sexes separately or any other management procedures that treat all individuals generically probably do not provide the situation needed for the squirrels to maintain their social connections (Williams & Lusseau 2006). Although the network seems robust to removal of only one-tenth of the individuals in the colony, procedures that remove large numbers of individuals seem to fragment the social network, particularly if those individuals hold important positions in the cohesion of the network via their connections to many other individuals.

Further studies of animal social networks are likely to increase our understanding of sociality. With regard to the roles that individuals play in social cohesion of the colony, this network exhibits statistical properties that are consistent with most other evolving networks. However, the generality of these properties remains unknown pending the visualization of networks from other species. Quantifying standard measures such as diameter, clustering, degree, and betweenness for varied species can also facilitate robust interspecific comparisons of sociality by determining the roles played by individuals in the cohesion of societies (Faust & Skvoretz 2002). This approach to defining sociality measures association and integrates social interactions locally and globally, rather than focusing on the number of age-sex roles in a social group (Blumstein & Armitage 1997), the spatial distributions of different age and sex animals (Michener 1983) or dyadic interactions (Whitehead & Dufault 1999).

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TABLES

Table 1. Glossary of network analysis terms used in this study

Basic terms

Edge: a relationship between two components of a network, where the two related components are vertices in the graph model representing the network; in a social network, these can be any sort of social relationship, such as social interactions or information transfer; also called a *tie* or *link*

Sociomatrix: for a group with *n* members, an *n* x *n* matrix with each group member along the vertical and horizontal axes and each entry in the grid as the weight of the social relationship, if any, between the two intersecting individuals

Vertex: a component of a network with known relationships to others in the graph model representing the network; in a social network, this can be an individual animal or group; also called a *node* or *point*

Egocentric or individual properties

Betweenness: centrality based on the number of shortest paths between every pair of other group members on which the focal individual lies

Centrality: a measure of an individual's structural importance in a group based on its network position

Degree (k): the number of edges a focal animal has; in an un-weighted network, this is the number of other animals with which the focal individual interacts; in a weighted network, this will reflect the strength or frequency of interactions; also called *connectivity*

Properties of the local network

Clustering coefficient (C): the density of the sub-network of a focal individual's neighbours; the number of edges between neighbours is divided by the maximal possible number of edges between them

Properties of the global network

Diameter (d): the largest distance between any two vertices in the network

Scale-free power-law: a degree distribution described by $p(k) \approx k^{-\gamma}$; demonstrated by a straight line on a log-log plot

Table 2. Number of edges between dyads of different age and sex Columbian ground squirrels in Sheep River Provincial Park, Alberta during a three-week courtship and breeding period in 2006. RM: reproductive male (*N* = 18); RF: reproductive female (*N* = 40); NRM: non-reproductive male (*N* = 6); NRF: non-reproductive female (*N* =1).

Interacting individuals	Total number of edges
RM – RM	4
RM – NRM	9
RM – RF	143
RM – NRF	1
NRM – NRM	4
NRM – RF	30
NRM – NRF	0
RF – RF	49
RF – NRF	0
NRF – NRF	0
	240

FIGURES

Figure 1. The communities (viz., 1 & 2) and sub-communities within the Columbian ground squirrel colony DOT identified by the algorithm of Girvan & Newman (2002). Vertex shading indicates sub-community membership. Males are represented by squares, females are represented by circles; a line through the vertex denotes a non-reproductive individual, and all other individuals are reproductive. The size of the symbol increases with the betweenness of the vertex as indicated by the legend. Sample sizes for individuals of different sex and reproductive state are the same as for Table 1 ($N = 65$ individuals).

Figure 2. (a) Log-log plot of the cumulative distribution function of the number of edges (k) for the 65 free-ranging Columbian ground squirrels in Sheep River Provincial Park, Canada, that comprised the social network. The line fits a power-law distribution and has the slope $\gamma_{\mathrm{squirrel}} = -0.85 \pm 0.10$; (b) Log-log plot of the cumulative distribution function of the betweenness scores of all squirrels. The line fits a power-law distribution and has the slope $\gamma_{\mathrm{squirrel}} = -1.1 \pm 0.1$.

Figure 3. (a) Changes in the diameter of the Columbian ground squirrel network with different fractions of removed vertices. The circles denote random attacks, and the squares denote attacks where individuals with high betweenness were removed (viz., “targeted” attacks). Both symbols represent the average of 10 attacks; (b) Fragmentation of the ground squirrel network under random and targeted attacks. The size (S) of the largest cluster in the network is relative to the total number of squirrels in the network

(viz., varies from 0 to 1). The average size (s) of the isolated clusters (viz., clusters other than the largest) is 1 if they all contain single squirrels, and more than 1 if some contain multiple squirrels.

Fig. 1.

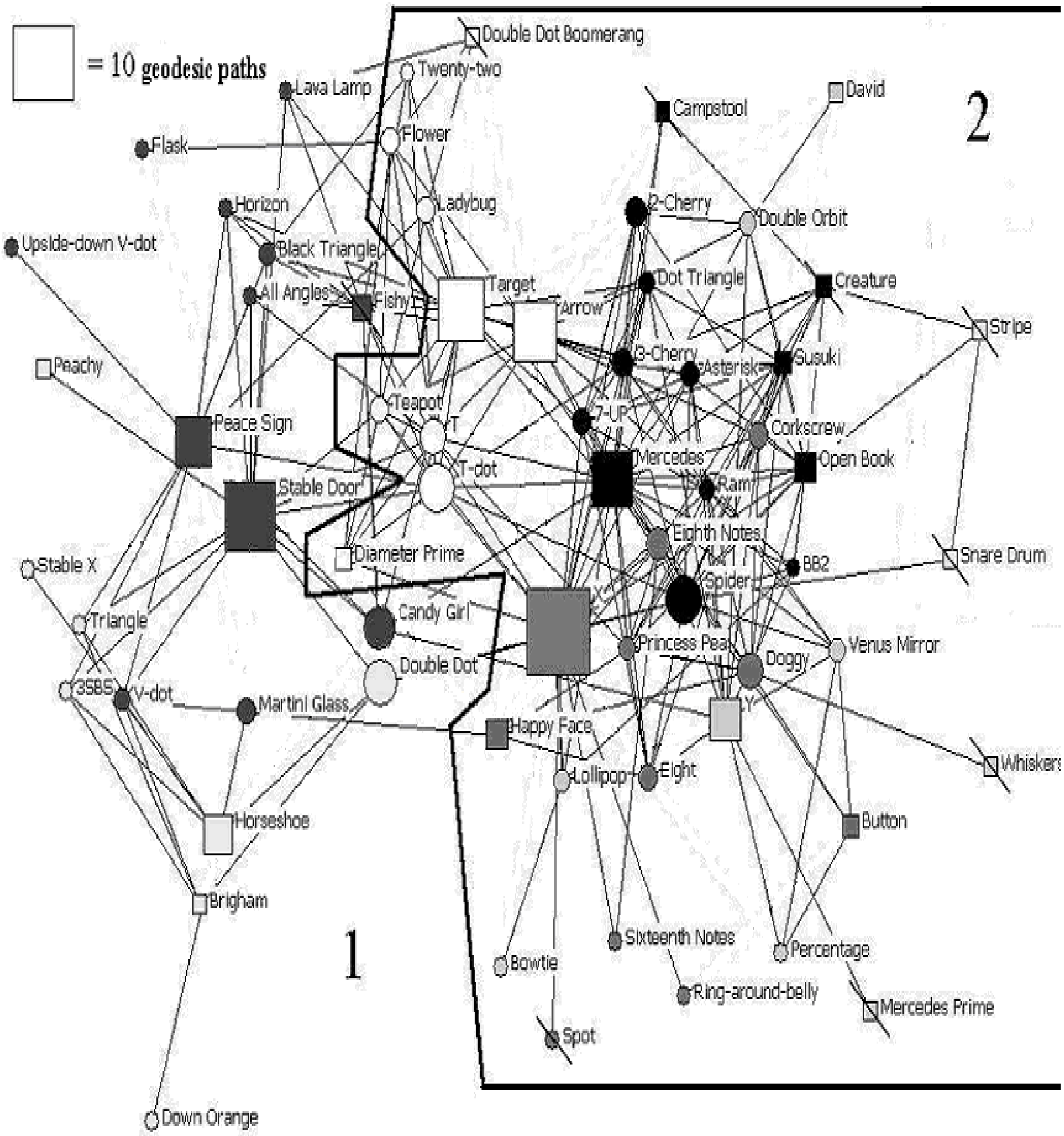

= 10 geodesic paths
1
2
Double Dot Boomerang
Twenty-two
Lava Lamp
Flask
Flower
Campstool
David
Horizon
Ladybug
2-Cherry
Double Orbit
Upside-down V-dot
Black Triangle
All Angles
Fishy
Target
Arrow
Dot Triangle
Creature
Stripe
Peachy
3-Cherry
Asterisk
Gusuki
Teapot
T
Peace Sign
7-UP
Corkscrew
T-dot
Mercedes
Open Book
Stable Door
Ram
Eighth Notes
Diameter Prime
Stable X
BB2
Snare Drum
Spider
Triangle
Candy Girl
Princess Peak
Venus Mirror
Doggy
Double Dot
3SBS
V-dot
Martini Glass
Y
Happy Face
Whiskers
Lollipop
Eight
Horseshoe
Button
Brigham
Sixteenth Notes
Percentage
Bowtie
Ring-around-belly
Mercedes Prime
Spot
Down Orange

Fig. 2a-b.

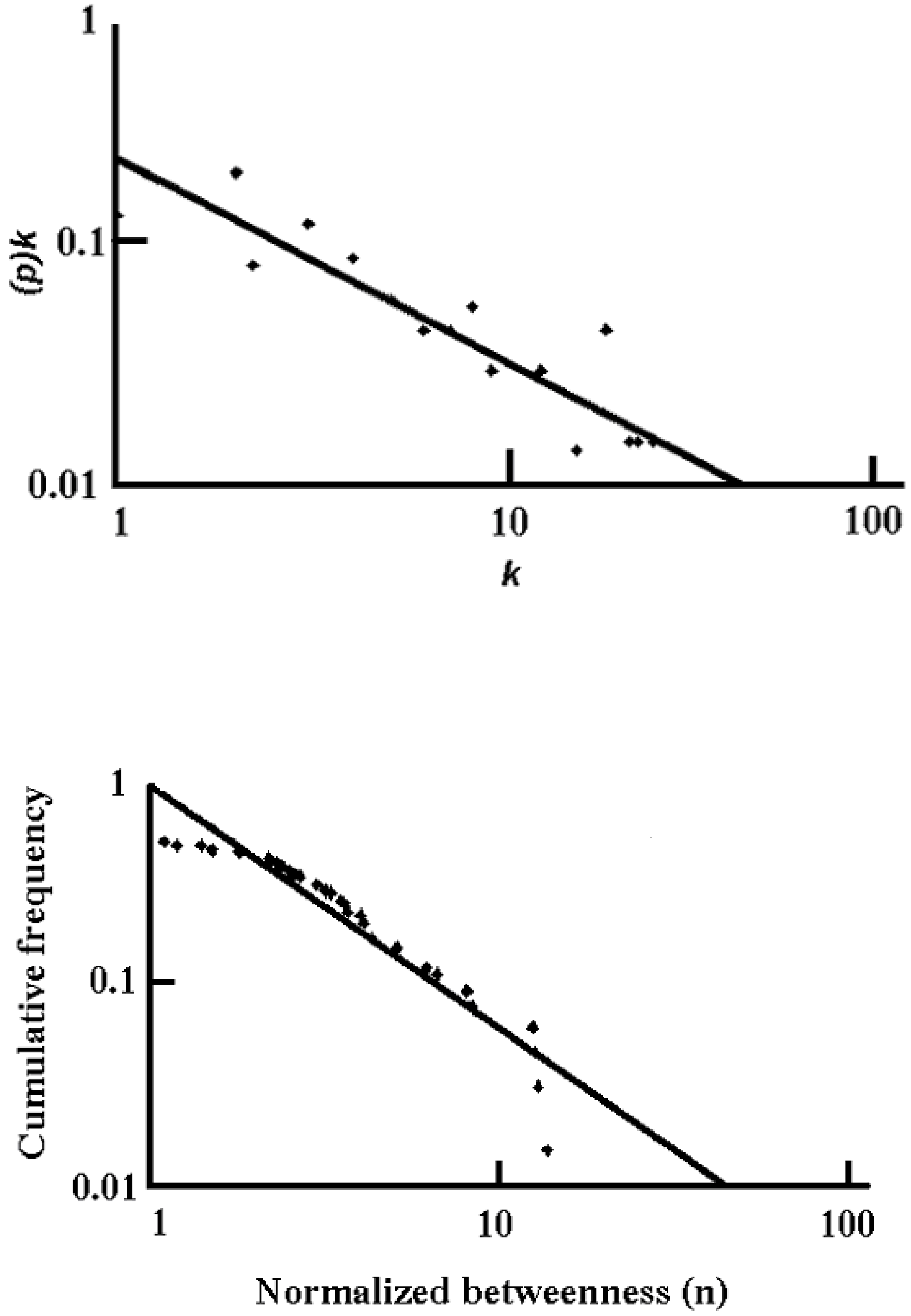
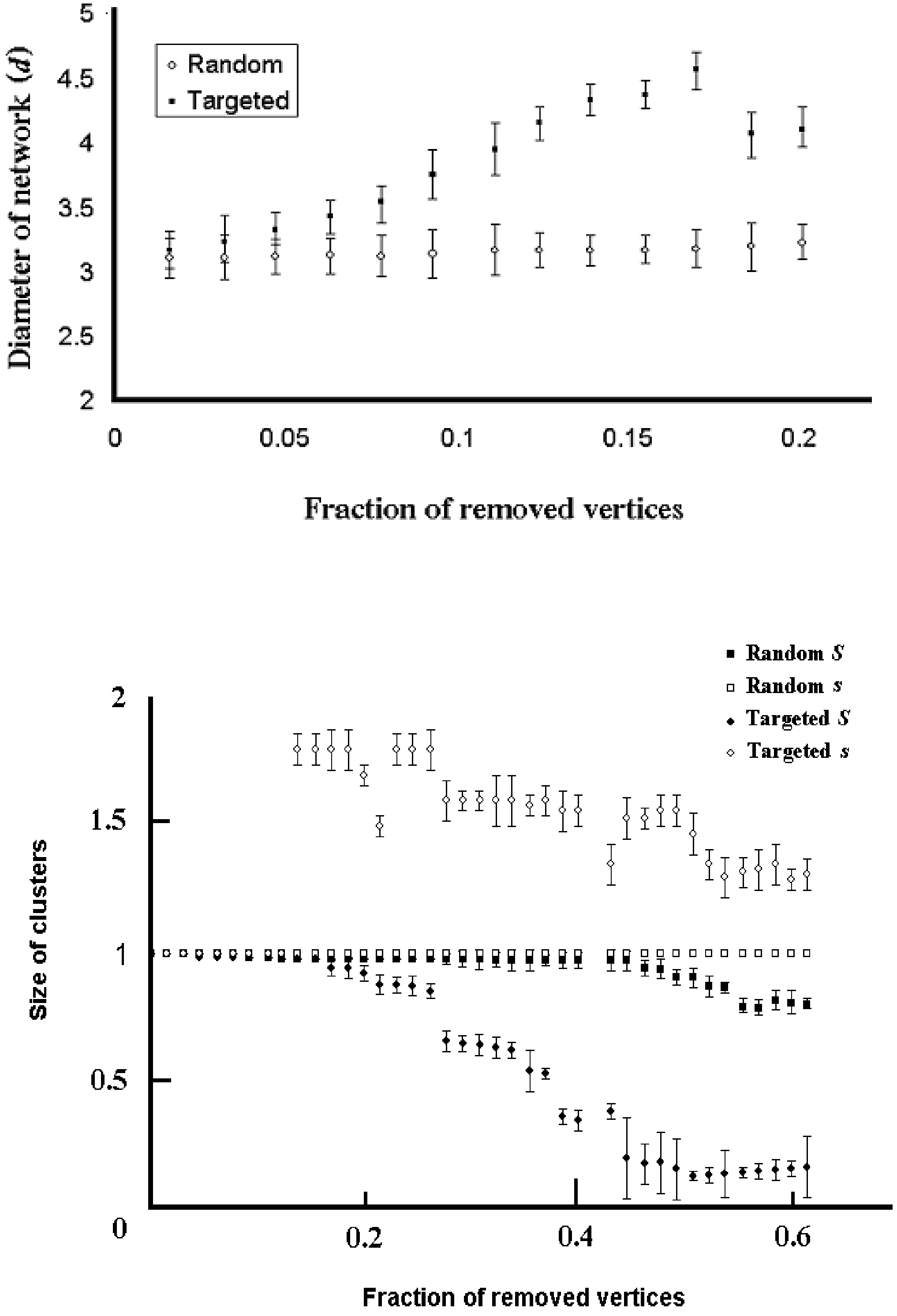

1
(p)k
0.1
0.01
1
10
100
k
1
Cumulative frequency
0.1
0.01
1
10
100
Normalized betweenness (n)

Fig. 3a-b.



Diameter of network (d)
5
4.5
4
3.5
3
2.5
2
0
0.05
0.1
0.15
0.2
Fraction of removed vertices
◇ Random
▪ Targeted
Size of clusters
2
1.5
1
0.5
0
0.2
0.4
0.6
Fraction of removed vertices
■ Random S
□ Random s
◆ Targeted S
◇ Targeted s

III. WHY DO COLUMBIAN GROUND SQUIRRELS COPULATE UNDERGROUND?

ABSTRACT. Columbian ground squirrels usually copulate underground in a burrow. Underground copulations might be associated with two non-exclusive benefits: reducing probability of predation during copulation and reducing interference by conspecific males. We tested whether either of these benefits was involved in determining if the copulation site was underground or aboveground. In 2006 and 2007, we obtained detailed information on the copulatory behavior and social interactions of free-ranging individuals in Southwestern Alberta, Canada. During the 3-week annual breeding period, we also recorded the activity of predators of Columbian ground squirrels such as ravens, foxes, and hawks. Squirrels that lived on the periphery of the population were more susceptible to predation than squirrels in the center. Despite this risk, aboveground copulations usually occurred on peripheral territories. In addition, aboveground copulations were not further removed in time from predator attacks or sightings than underground copulations. When copulations occurred aboveground, they were sometimes disrupted by prior mates of the estrous female. Probability that copulation would occur aboveground increased when the density of reproductive males around an estrous female was low. Taken together, our results suggest that although underground copulations protect individuals from predation, male-male competition for

females and interference with copulations have been more important than predation in determining copulatory sites for Columbian ground squirrels in our study population.

INTRODUCTION

The choice of a copulation site may have a major impact on fitness, since it influences whether mating is successful and production of young occurs (Tryjanowski et al. 2007). Perhaps because of the environmental and social context surrounding matings, different copulatory sites are appropriate for mating pairs at different times. For example, sand-bubbler crabs (*Scopimera globosa*) copulate either in burrows or on the beach surface (Koga and Murai 1997), auklets (*Aethia spp.*) copulate either on land or aquatically (Hunter and Jones 1999), and garter snakes (*Thamnophis sirtalis parietalis*) mate either in or away from their dens (Shine et al. 2000). Ground squirrels (*Spermophilus spp.*) and prairie dogs (*Cynomys spp.*) also exhibit this variation, as many species copulate either aboveground or underground. Examples include black-tailed, Utah, and Gunnison's prairie dogs (*C. ludovicianus, C. parvidens,* and *C. gunnisoni*; Erpino 1968; Hoogland 1995, 2001; Manno 2007), as well as Richardson's (*S. richardsonii*; Davis 1982; Michener 1985) and thirteen-lined ground squirrels (*S. tridecemlineatus*; Schwagmeyer 1984; Schwagmeyer and Parker 1987).

Two factors have been invoked to explain this variation in copulation site (e.g., Hoogland 1995; Hunter and Jones 1999). First, predation risk is a concern for animals such as birds, water striders (*Gerris buenoi*), garter snakes, and Utah prairie dogs, in which sexually receptive individuals are reduced in their ability to escape predator attacks during copulation (Rowe 1994; Wesolowski 1999; Shine et al. 2000; Hoogland et al.

2006). Second, interference with copulation—when a male tries to separate a copulatory pair—commonly occurs in group-living animals such as Belding's ground squirrels (*S. beldingi*), black-tailed prairie dogs, yellow-toothed cavies (*Galea musteloides*), primates, earthworms (*Lumbricus terrestris*), and several species of birds (Rood 1972; Hanken and Sherman 1981; Davies 1985; Chardine 1986; Gratson et al. 1991; Bruce and Estep 1992; Hoogland 1995; Nuutinen and Butt 1997; Alfaro 2005; Tryjanowski et al. 2007). If predation or copulation interference becomes prevalent, then avoiding either or both may affect where copulation occurs (Davis 1982; Møller and Birkhead 1989; Schwagmeyer 1990; Gratson et al. 1991; Tryjanowski et al. 2007). These hypotheses are not mutually exclusive and testing them requires detailed information on both mating behavior and predator risk as breeding occurs.

Foraging aboveground from dawn until dusk, Columbian ground squirrels (*Spermophilus columbianus*) are burrowing, colonial rodents (e.g., Betts 1976; Murie 1995). Like other Marmotine ground squirrels (Tribe: Marmotini), *S. columbianus* usually copulates underground in a burrow, but occasionally copulates aboveground (Murie 1995; Manno et al. 2007). During a three-week mating period, females live in philopatric kin clusters that are overlapped by a territorial reproductive male (usually ≥4 years old) (King and Murie 1985; Manno 2008). Younger, subordinate males (2-3 years old) usually do not maintain a territory, but are physically able to reproduce and sometimes obtain copulations (Murie & Harris 1978; Manno et al. 2007). Females mate with multiple males during their single annual day of estrus, which occurs 2-12 days after emergence from hibernation in April (Betts 1976; Murie 1995); estrous females may solicit copulations with their territorial male, adjacent territory holders, and subordinate non-territorial males (Murie 1995; Manno et al. 2007). The first male to mate with a female (usually the nearest territorial male) has sperm precedence and may mate guard via postcopulatory vocalizations, fighting with approaching males, and hostile behavior towards the female as she attempts to flee the copulatory site (Murie 1995; Manno et al. 2007).

Columbian ground squirrels are prey for myriad terrestrial and aerial predators during April-May (e.g., mustelids, accipiter hawks, and ravens; reviewed by: Elliott and Flinders 1991; see also Murie 1992). Thus, predation pressure may potentially influence mating location. Individuals on the periphery of a population are expected to be especially vulnerable to predation because this is where predators first appear, because fewer squirrels are available on the periphery to detect predators, and because alarm call warnings increase as a predator moves toward the center of the population (Hamilton 1971; Hoogland 1981; Brown and Brown 1987; Hoogland et al. 2006; Manno 2007). If risk of predation is the primary factor determining the site of copulation, then the likelihood that copulation will occur aboveground should decrease when a mating pair is on the periphery of the population rather than the center, particularly during periods of heightened predator activity.

In contrast, if male-male competition for females and interference with copulations are the primary factors influencing mating site, then the likelihood that copulation will occur aboveground should decrease in areas where conspecific presence and competition for mates are heightened. Increased competition should occur when the mating pair is in the center of the population rather than on the periphery, because more reproductive males should be present near the estrous females in central areas. At the periphery of the site, however, the density of individuals is usually decreased, potentially leading to decreased competition and reduced pressure to mate underground. Thus, the hypotheses considered here lead to distinct predictions that can be used to assess the relative impacts of predation and intraspecific competition on the locations of copulations by *S. columbianus*.

METHODS

Study Population

From April to July in 2006 and 2007, we observed free-ranging Columbian ground squirrels of known age and matrilineal genealogy at a hill called DOT in Sheep River Provincial Park, Alberta, Canada (50° 38′ N, 114° 38′ W, elev. 1500 m). All squirrels were trapped 1-2 days after they emerged from hibernation, ushered into a cloth bag, weighed, and fitted with numbered metal fingerling eartags for long-term identification (National Band and Tag Co., Newport, KY). For visual identification from a distance, we painted each animal with a unique symbol using black dye (Lady Clairol Hydrience; Proctor and Gamble, Stamford, CT). During 2006-2007, the 2.5 ha site was inhabited by 14-17 adult males (≥2 years old), 41-48 adult females (≥2 years old), and 20-40 yearlings of both sexes, for a density of 32.8-39.2 individuals per hectare and 23.2-24.8 adults per hectare.

We considered males to be reproductive if they exhibited a pigmented scrotum and large, descended testes at the time of capture. We trapped females several additional times during the 3-week breeding period and examined their vulvar condition (i.e., fully opened) to determine whether they would be estrous (Schwagmeyer and Brown 1983;

Michener 1984). Our methods followed American Society of Mammalogists guidelines for animal care and use (Animal Care and Use Committee 1998), and field methods were approved by the Institutional Animal Care and Use Committees at Auburn University and the University of Calgary.

Behavioral Observations

The breeding period extended from the third week of April to the first week of May but, as for other ground-dwelling sciurids (e.g., Davis 1982; Sherman 1976, 1989; Schwagmeyer 1990; Hoogland 1995; Lacey et al. 1997; Manno 2007), each female was sexually receptive for only a few hours on a single day. When a social interaction occurred (e.g., chasing, fighting, sniffing, allogrooming, playing, females "leading" males), we used all-occurrence sampling (Altmann 1974) to record the identities of the animals involved. We then scored individuals that chased conspecifics or remained at the location of a fight as victorious in the interaction (Hoogland 1995; Lacey and Wieczorek 2001), and recorded the time and location of the interaction (ascertained from a 10m x 10m grid placed with flags on the ground) (Manno, 2008).

Copulations occasionally occurred aboveground and were therefore observed directly (Murie 1995). We used established methods to infer underground copulations from the following aboveground diagnostic behaviors: (1) female movements to elicit social interaction with males and to "lead" them into prospective copulatory burrows; (2) immergence of a male and female in the same burrow on the night before the female exhibited a fully opened vulva; (3) other immergences of both partners into the same burrow, where they remained for at least several minutes; (4) self-grooming of genitals by both partners upon later emergence, which was sometimes accompanied by dustbathing; (5) a postcopulatory “mating call” by the male; and (6) other behaviors indicating that males were mate guarding, such as chasing the female into a burrow, sitting on or “herding” the female into that burrow as she attempted to flee the area, and fighting with other males (Hoogland 1995; Murie 1995; Lacey et al. 1997, 2001; Manno et al. 2007). Aboveground copulations also featured all or most of these behaviors.

Detecting Predators

Using 10x42 binoculars and 4-m-high towers, we followed the methods of Sherman (1976) and Hoogland (1995) to observe marked individuals from dawn until dusk every day. Thus, we documented the frequency of sightings for predators of Columbian ground squirrels and the number of predator attacks at the study site. An attack occurred when a predator moved to capture a particular individual, via either a “swoop” close to the ground (including landing on the ground) for aerial predators or a “pounce” towards a squirrel by terrestrial predators. Predation events occurred when the predator was successful in killing a squirrel during an attack. When a predator of ground squirrels was seen at the site but no attack occurred, we recorded our sighting of the predator. We also checked the colony daily for signs of subterranean predation events by animals such as badgers (*Taxidea taxus*) and weasels (*Mustela* spp.) (e.g., fresh diggings or enlargement of burrows, predators emerging from burrows, suddenly absent individuals; Sherman 1976; Murie 1992; Hoogland 1995; Hoogland et al. 2006). We recorded the location, time of day, and an anecdotal description for all predator sightings and attacks (Altmann 1974; Sherman 1976; Hoogland et al. 2006). Thus, we were able to measure predation in terms of number of predation events, attacks, or sightings per day, as well as the time elapsed since each of these types of events last occurred (Sherman 1976). Unfortunately, we usually could not determine whether predator sightings resulted from the observations of different individuals or the same individual at different times (Hoogland et al. 2006). We therefore considered all predator sightings to be independent.

Estimating Reproductive Competition

The operational sex ratio (OSR) is the number of breeding males per estrous female. During the breeding period, 1-6 females per day were estrous. Using this variation, we calculated a daily OSR for our study population that served as a measure of intraspecific competition for mates. We also calculated two measures of local competition around an estrous female. First, if a male maintained a territory that either contained the burrow from which the female emerged daily or abutted the territory that contained her emergence burrow, we considered that male to be “neighboring” to the estrous female (Manno et al. 2007). Second, we considered males to be “familiar” with an estrous female if they engaged in any social interaction (e.g., kissing, sniffing, chasing, playing, allogrooming) before estrus (Armitage 1986; Koenig and Mumme 1987; Manno 2008). We classified a male territory as central if >50% of its boundary was contiguous with the boundaries of other territories, and as peripheral if <50% of its boundary abutted other territories (Hoogland et al. 2006).

Statistical Analysis

For univariate procedures, we assumed dependence of data from the same individual in the same year and independence of data from the same individual in different years (Machlis et al. 1985). Thus, for individuals included in our data set more than once during the same field season, we used the within-season average for each animal in our analyses. We tested data for normality with Kolmogorov-Smirnov tests. When the data did not meet the assumptions of a parametric analysis, we used the appropriate non-parametric test (in these cases, transformations did not yield a normal distribution). The expected values in statistical tests of frequency data result from the assumption that occurrence was equally likely in both categories being compared.

Using the copulations ($N = 142$) for which we had complete data, we conducted a multivariate logistic regression analysis with Statistical Analysis Software (SAS 1999; SAS Institute Inc., Cary, NC) to determine which variables were related significantly to the site of copulation. For this analysis, the dependent variable was the occurrence of an underground or aboveground copulation. We considered the following independent variables that reflected the short-term risk of predation: time elapsed since the last predator sighting or attack, number of predator sightings or attacks that occurred on the day of copulation, and position of the copulation (peripheral or central territory). We also included the following independent variables related to mate-competition: order of copulation in males, weight and age of the consort male and estrous female, OSR, and number of males that were neighboring to or familiar with the estrous female. Male weight and age were included because young or small males may be unable to monopolize heavy females with breeding experience (Hoogland 1998), leading to an increased chance of interference by male competitors or the inability to bring a female underground where she will be easier to guard after copulation. Copulation order was included because, given the pattern of first male sperm precedence in this species (Murie 1995), males that copulate early in a female's series of matings may be more likely to pursue unmolested copulations underground (but see Tryjankowski et al. 2007).

Because our study yielded multiple observations of the same individuals in the same or different years, we used a mixed model regression that treated the identity of individuals as a random variable, along with the date and year of the copulation. We examined our dataset for significant interactions (i.e., co-linearity) among independent variables and tested for such influences via interaction terms. We then generated all possible models and determined the best-fit model by minimizing Akaike's information criterion (AIC_c) (Burnham and Anderson 1998), removing any interactions or variables that impacted negatively the fit of the data to the model. Thus, our multivariate approach augments our univariate analyses by allowing us to determine the relative influence of independent variables on the dependent variable.

For seemingly similar analyses, sample sizes sometimes varied because we did not have complete data on every individual in the sample required for a particular comparison. *N*s indicate the number of individuals in the sample. Values are presented as means ± 1 *SE*. All *P*-values result from two-tailed tests ($\alpha = 0.05$).

RESULTS

We observed the complete series of matings for 56 females (28 in each year). These females copulated with an average of 2.8 ± 0.2 males ($N = 151$ copulations). About

6% (9/151) of the copulations took place aboveground, with the rest occurring underground in a burrow. No female copulated aboveground more than once.

We routinely observed predator attacks and male interference with copulations during the 3-week breeding period, with these events sometimes occurring in rapid succession. For example, on 1 May 2007 at 900 h (Mountain Standard Time), a reproductive male on a peripheral territory copulated aboveground with an estrous female, the male having been attacked and nearly killed by a raven only 19 minutes prior to the copulation. A minute or so into the copulation, a male that had mated with the estrous female an hour earlier attacked the consort male and interrupted the mating pair. The female left the area during the ensuing agonistic interactions and, hence, the copulation with the focal male was not completed.

During the breeding periods of 2006-2007, we observed predators on 120 occasions. Across years, ravens were both the most commonly observed (64/120 sightings = 53%) and most successful predators (6/20 attacks yielded prey = 30%). Red-tailed hawks were the only predator to attack ground squirrels during breeding in 2007 (8/15 sightings involved attacks = 53%), although none of these attacks were successful. The 2006 breeding season featured significantly more predator sightings ($G = 34.9$, $N = 120$, $d.f. = 1$, $P < 0.001$), attacks ($G = 19.1$, $N = 43$, $d.f. = 1$, $P < 0.005$), and successful predation events ($G = 13.8$, $N = 8$, $d.f. = 1$, $P < 0.01$) than the 2007 breeding season. We never found evidence of underground predation.

Individuals living on peripheral territories were more vulnerable to predation than individuals living in central territories (Fig. 1). Specifically, all 6 individuals captured by ravens as well as the single individuals captured by a lynx and red fox lived on peripheral territories ($G = 13.8$, $N = 8$, $d.f. = 1$, $P < 0.01$). The red fox victim was a 2-year-old scrotal male; the lynx victim was a non-reproductive yearling female. Other victims were killed before we were able to capture them and hence they were of unknown age and sex. We suspect, however, that these individuals may have been breeding males that had recently immigrated to the population because they did not appear from our observations to have eartags.

Over two-thirds of the aboveground copulations (7/9 = 77.8%) occurred on peripheral territories. Indeed, the likelihood that copulation would occur aboveground was significantly higher on the periphery than in the center of the population ($G = 16.6$, $N = 151$, $d.f. = 1$, $P < 0.01$; Fig. 2). The percentage of copulations that occurred aboveground was also significantly related to the local density of males around an estrous female (the number of reproductive males that neighbored the estrous female). Overall, 14.0 ± 8.6% of copulations occurred aboveground when females had ≤3 neighboring males versus 0.0 ± 0.0% of copulations with >3 neighboring males ($G = 33.1$, $N = 151$, $d.f. = 1$, $P < 0.001$; Fig. 3). Thus, females that copulated aboveground had fewer neighboring males than females that never copulated aboveground ($U = 791$, $d.f. = 54$, $P < 0.05$).

With regard to predation, aboveground copulations were not significantly further removed in time than underground copulations from the most recent predator sighting (451.3 ± 153.8 mins when aboveground vs. 481.9 ± 26.4 mins, $U = 697$, $d.f. = 149$, $P = 0.66$) or attack (1102.8 ± 330.6 mins when aboveground vs. 1029.7 ± 99.3 mins, $U = 737$, $d.f. = 149$, $P = 0.45$). Number of predator sightings or predator attacks did not differ significantly between days when an aboveground copulation occurred and days when no aboveground copulations occurred ($U = 682$, *d.f.* = 149, $P = 0.86$ and $U = 749$, *d.f.* = 149, $P = 0.40$, respectively). Although the 2006 breeding period featured 3 times as many predator sightings and 4 times as many predator attacks as the 2007 breeding period, two-thirds of the aboveground copulations that we witnessed (6/9 = 67%) occurred in 2006. None of the individuals that copulated aboveground were attacked by predators while mating (0/9 = 0%).

With regard to interference by conspecifics, we never observed a non-consort male enter the burrow where a pair was believed to be copulating (0/142 = 0%). In contrast, one-third (3/9 = 33%) of aboveground copulations were disrupted by males that had mated previously with the estrous female; this difference was significant ($G = 36.0$, $N = 151$, *d.f.* = 1, $P < 0.001$). In all instances of male interference, the previous sexual partner of the estrous female harassed the consort male by instigating a hostile interaction as the consort male mounted the female. The mating pair then split as the males fought, after which the female left the area.

Multivariate logistic regression also revealed that position in the population (central vs. peripheral) and number of neighboring reproductive males were significantly related to where copulation occurred (Table 1a). *AIC* analyses yielded similar results; the best-fit model included position in the population and number of neighboring males, both of which had a significant relationship with the dependent variable (Table 1b). The next two best-fit models that were within 5 points of the *AIC* for the best-fit model included only position in the population or number of neighboring males. None of the interaction effects ($N = 5$) had a significant relationship with the dependent variable (all $P > 0.05$).

DISCUSSION

Our results indicated that (1) individuals living on peripheral territories were more likely to be preyed upon than individuals living on central territories; (2) aboveground copulations were more likely to occur at the periphery of the population; (3) aboveground copulations were sometimes disrupted by prior mates of the estrous female, and (4) the probability that a mating would occur underground increased when the local density of reproductive males was high. These data suggest that predation and interference by conspecifics were potentially important influences on the location of copulations by members of the study population.

If risk of predation is the primary factor determining the site of copulation, then the likelihood that copulation will occur aboveground should decrease when predation risk is heightened. Our results did not support this prediction. Under the predation hypothesis, aboveground copulations should have occurred on central territories, where predation is less frequent. Aboveground copulations, however, were more common on the periphery of the population. Further, if predation is important, squirrels should have avoided aboveground copulations during periods of high predator presence, including just after a predator attack or sighting. However, aboveground copulations appeared to be no further removed temporally from such events than underground copulations. Further, underground and aboveground copulations were equally common in both years of our study, even though 2006 featured a much higher rate of predator activity. We acknowledge that predation may be a factor in determining the overall prevalence of underground copulations and that predation-related variation in the frequency of underground copulations may be evident among populations. Nevertheless, our results do not support the hypothesis that immediate risk of predation is the primary determinant of mating location.

In contrast, if male-male competition for females and interference with copulations are the primary factors determining the site of copulation for Columbian ground squirrels, then the likelihood that copulation will occur aboveground should decrease when risk for either is heightened. Our results supported this prediction. While no mating pairs that copulated aboveground were attacked by predators, three such pairs had their copulations disrupted by a prior mate of the estrous female. Indeed, aboveground copulations were significantly more likely to be disrupted than underground copulations. Estrous females never copulated aboveground when the local density of reproductive males (and presumably, the male-male competition) was high, but sometimes copulated aboveground when few neighboring males were present. Peripheral territories where aboveground copulations typically occurred featured decreased conspecific presence and, presumably, decreased competition for mates because they were not surrounded by other territories. Thus, the local density of males around an estrous female was related negatively to the likelihood that copulation would occur aboveground.

We emphasize that efforts to test these hypotheses in additional populations of Columbian ground squirrels and other species with varied frequencies of predation and copulation interference are important. In addition to assessing the generality of the relationships identified here, such comparative studies can exploit naturally occurring variation in population structure to explore determinants of mating location in greater detail. Our results may therefore be applicable to other animals, particularly other mammals (Davis 1982; Møller and Birkhead 1989; Schwagmeyer 1990), but future research will be necessary to increase our understanding of why animals vary the locations of their copulations.

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Table 1. Summary of multivariate logistic regression analyses. In (a), the variables included in the general model are shown, as is their relationship with the location of copulations. The overall model included all independent variables considered (AIC_c = 114.6). In (b), the parameters of the best-fit model (AIC_c = 5.7) are shown. Data are from Columbian ground squirrels observed in Sheep River Provincial Park, Alberta, during 2006-2007 (N = 142 copulations, 56 estrous females). For all comparisons, *d.f.* = 1.

Overall model

Variable	Wald's statistic (χ^2)	*P*-value
Order of male copulation	2.3	0.13
Body weight of the consort male (during breeding)	0.7	0.42
Number of estrous females in colony	0.0	0.99
Number of familiar males	1.1	0.30
Number of neighboring males	6.1	0.01

Number of reproductive males in colony	1.3	0.26
OSR	0.0	0.99
Number of predator sightings on day of estrous	0.6	0.45
Number of predatory attacks on day of estrous	0.3	0.59
Position on the colony during copulation (central vs. peripheral)	11.4	0.0007
Body weight of the estrous female	1.1	0.30
Age of the estrous female	0.6	0.46
Age of the consort male	0.1	0.82

Time elapsed since last predator sighting	0.0	0.96
Time elapsed since last predatory attack	0.3	0.57

Best-fit Model

Variable	Wald’s statistic (χ^2)	*P*-value
Position on colony (central or peripheral)	12.6	0.0007
Number of neighboring males	5.7	0.019

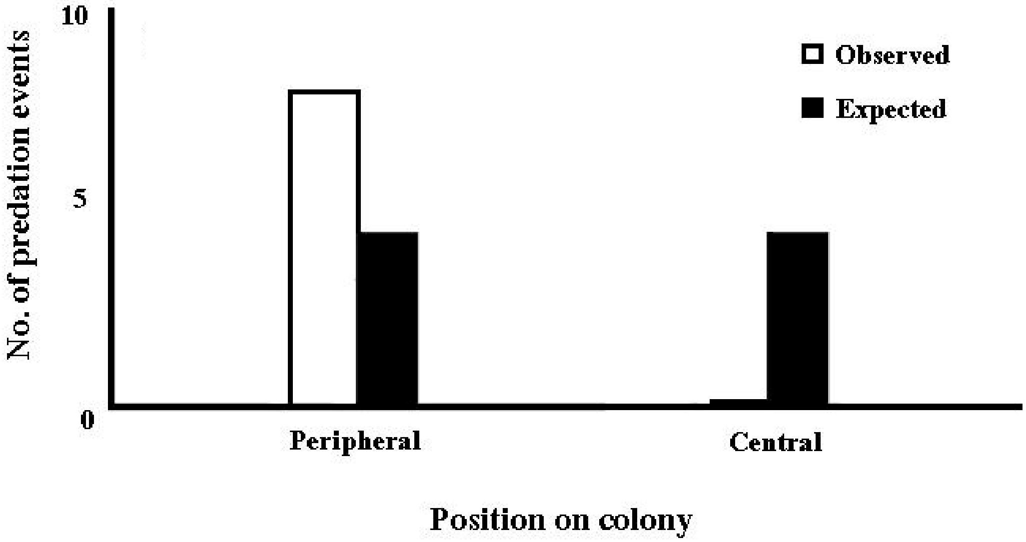
FIGURES

Figure 1. Observed versus expected frequencies of predation events on Columbian ground squirrels living in peripheral territories. Data are from 8 predation events observed during the breeding seasons of 2006 and 2007; expected numbers are based on the assumption that predation was equally likely for individuals on central and peripheral territories. Peripheral territories were located at the edge of the study colony and were not surrounded by other territories.

Figure 2. Percentage of copulations that occurred aboveground on peripheral and central territories belonging to male Columbian ground squirrels. Data are from 151 copulatons observed during the breeding seasons of 2006 and 2007. The number above each bar represents the number of copulations occurring on that territory type.

Figure 3. Percentage of copulations that occurred aboveground versus the local density of males (i.e., number of neighboring males) around an estrous female. Values shown are means ± 1 *SE*; the number above each bar represents the number of females subject to that local density. Data are from 56 females monitored throughout estrus during 2006 and 2007. Data from females with ≥ 6 neighboring males were pooled due to small sample sizes for females with 7 or 8 neighbors ($N = 2$ for both).

Fig. 1.



10
5
0
No. of predation events
□ Observed
■ Expected
Peripheral
Central
Position on colony

Fig. 2.

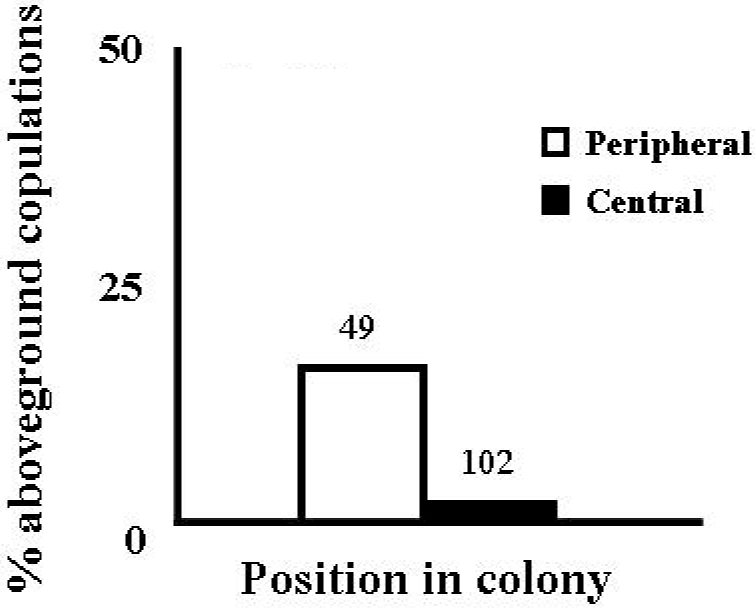
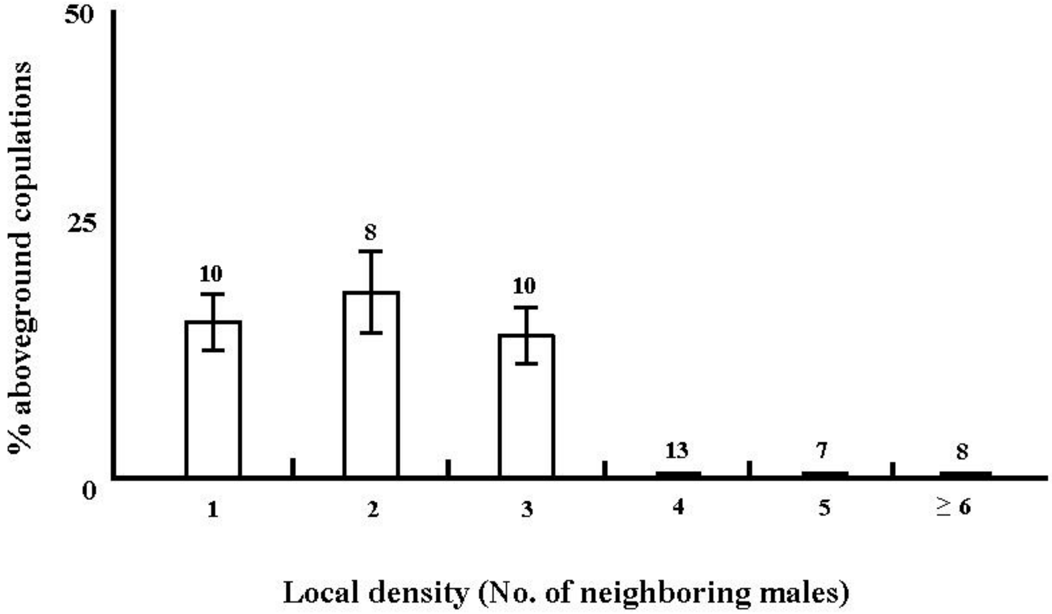

% aboveground copulations
50
25
0
□ Peripheral
■ Central
49
102
Position in colony

Fig. 3.



% aboveground copulations
50
25
0
10
8
10
13
7
8
1
2
3
4
5
≥ 6
Local density (No. of neighboring males)

IV. WHY DO COLUMBIAN GROUND SQUIRRELS GIVE A MATING CALL?

ABSTRACT. Male Columbian ground squirrels (*Spermophilus columbianus*) emit a repetitive vocalization after copulation. This “mating call” sounds similar to certain alarm calls that are given during attacks by terrestrial predators. During 2005-2006, we investigated (1) the acoustic structure and similarity of mating calls and alarm calls; (2) the environmental context when mating calls occur; (3) whether males that are likely to benefit from mate guarding (viz., males that have sperm precedence because they are the first to copulate in a female’s series of matings) give mating calls to guard estrous females; and (4) whether mating calls advertise the caller to females that have not yet mated. Our approach was to observe mating behavior in the field and quantify reactions of squirrels to mating calls and alarm calls, both in and out of their normal context, with playback experiments. Males that were the first to copulate with an estrous female called during mate guarding, and guarded females experienced delays before subsequent copulations. Although sound characteristics of mating calls and alarm calls did not differ, squirrels became vigilant and sought protection upon hearing alarm calls, but continued feeding during mating calls. However, when we played recorded mating calls to squirrels after breeding season, they usually reacted as if an alarm call had been emitted. We conclude that (1) the male mating call is an intrasexual or intersexual signal

that announces postcopulatory mate guarding, and (2) contextual information is important for assessing the message of mating calls.

INTRODUCTION

Mating interactions often involve an exchange of auditory signals. The pioneering description of a postejaculatory vocalization given by male brown rats (*Rattus norvegicus*) (Barfield & Geyer 1972) led to studies that considered some consequences of male "mating calls" on which sexual selection could act to favor call expression. Precopulatory calling can expedite ovulation (McComb 1987), coordinate mating interactions (Lobel 1992; Palombit et al. 1999), or increase female sexual receptivity by reducing female aggression (e.g., Nyby & Whitney 1978; Pomerantz et al. 1983; Herth et al. 1988). Mated females are hindered from extra-pair copulations if males give intrasexual or intersexual threat calls that announce postcopulatory mate guarding (Tamura 1995; McElligott & Hayden 2001; Grafe et al. 2004). Calling males can also attract the attention of prospective mates or entice nearby females to copy the mate choice of earlier-copulating females (e.g., Mobley et al. 1988; Clutton-Brock et al. 1989; Hoglund et al. 1990; Gibson et al. 1991; Moller 1991; Kelley 2004; Velez & Brockmann 2006).

For species such as black-tailed prairie dogs (*Cynomys ludovicianus*), mating calls are acoustically unique vocalizations (Hoogland 1995; Grady & Hoogland 1986). But other animals such as Formosan squirrels (*Callosciurus erythraeus thaiwanensis*) emit mating calls that are acoustically similar to antipredator alarm calls, thus manipulating listeners through a dishonest "cry of wolf" (Tamura 1995). These deceptive calls may occupy the attention of male rivals or prolong lordosis of a mated female, thereby ensuring that the caller's sperm reach the female's uterus (Barfield & Geyer 1972; Cherry 1989; Tamura 1995). However, listeners can sometimes differentiate between similar calls by extracting information from subtle changes in the rate and duration of the calls (Leger et al. 1984; Cherry 1989), or individual differences in the acoustic characteristics (Shipley et al. 1981; Gyger & Marler 1988). Further, incidental contextual cues may be available from the circumstances surrounding calls, rather than from the structure or pattern of the calls per se (Smith 1977; Smith 1991; Leger 1993).

Broadcasting the presence of an estrous female might compromise a male's opportunity to sire progeny, owing to the increased possibility of subsequent copulations with the estrous female by nearby males. So, why give a mating call? And what sources of information do listeners use to interpret mating calls? To address these questions, we report a postcopulatory vocalization given by male Columbian ground squirrels (*Spermophilus columbianus*) that has not been described previously. Columbian ground squirrels are diurnal, herbivorous, and colonial rodents (Betts 1976; Elliott & Flinders 1991). When a predator attacks, they run to a burrow and assume a vigilant posture, and may give an alarm call at any time (Harris et al. 1983; Lickley 1984; MacWhirter 1992). Females live adjacently in philopatric kin clusters and with a few non-reproductive animals of both sexes (King & Murie 1985). During a three week mating period, a territorial reproductive male (usually ≥3 years old) overlaps the ranges of one or a few females (Murie & Harris 1978; Murie & Harris 1988). Young subordinate males (2-3 years old) usually do not maintain a territory, but are physically able to reproduce and sometimes obtain copulations (Murie 1995; Murie & Harris 1978; Manno et al. 2007).

Females are highly promiscuous during their annual day of estrus, which occurs 2-12 days after emergence from hibernation in April (Betts 1976; Murie 1995). Females may solicit courtship or copulate with their territorial male, adjacent territory holders, and subordinate young males. Litter size is 2-4, and males that copulate first in a female's series of matings (viz., usually the nearest territorial male) have sperm precedence (Murie 1995).

We examined (1) the acoustic structure and similarity of mating calls and alarm calls; (2) the environmental context when mating calls occur; (3) whether males that are likely to benefit from mate guarding (viz., males that copulate first in a female's series of matings) give mating calls to guard estrous females; and (4) whether mating calls advertise the caller to females that have not yet mated. Our approach was to observe mating behavior in the field and quantify reactions of squirrels to mating calls and alarm calls, both in and out of their normal context, with playback experiments (after Hoogland 1995 & Tamura 1995).

METHODS

Estrus and Copulation

From April to July in 2005 and 2006, we observed wild, free-ranging Columbian ground squirrels of known age and matrilineal genealogy at two colonies (Meadow B and DOT) in Sheep River Provincial Park, Alberta, Canada (50° 38′ N, 114° 38′ W, elev. 1500 m) from 4-m high observation towers. Squirrels were trapped 1-2 days after they emerged from hibernation, ushered into a cloth bag, restrained by hand, weighed, and fitted with numbered metal fingerling eartags for long-term identification (National Band

& Tag Co., Newport, KY). For visual identification from a distance, we painted each animal with a unique symbol using black dye (Lady Clairol Hydrience; Proctor and Gamble, Stamford, CT). We considered males that exhibited a pigmented scrotum and large descended testes at the time of trapping to be reproductive. We also trapped females several additional times during the 3-week breeding period and examined their vulvar condition to determine whether they had been estrous (viz., with fully opened vulva).

We watched squirrels at both colonies from dawn until dusk every day during breeding. This observation period extended from the third week of April to the first or second week of May. Each reproductive female was sexually receptive for a few hours on a single day during this period, and we easily observed the copulations that occasionally occurred aboveground. We also used the methods of Hoogland (1995) and Murie (1995) to infer underground copulations of individuals from aboveground diagnostic behaviors: (1) submergence of both partners into the same burrow, where they remained for at least several minutes; (2) self-licking of genitals by both partners upon later emergence, which was sometimes accompanied by dustbathing; and (3) behaviors indicating that males were mate guarding, such as chasing the female into a burrow, sitting on that burrow, and fighting with other males. We considered males to be territorial if there was an established area in which they were victorious in hostile interactions with other males (other males were considered subordinate; Dobson 1983). We scored the territoriality level of males based on the proportion of breeding season during which they were territorial.

Recording of Vocalizations and Vigilance

Males sometimes emitted a series of “chirps” shortly after copulating with an estrous female. During our observations of estrus and copulation, we noted every time this occurred; each series of chirps was termed a “mating call” (after Hoogland 1995 & Grady & Hoogland 1986). In 2006, three of us (TGM, LMD, and KSW) also made audio recordings of mating calls as they occurred during breeding at colony DOT ($N = 33$ calls). We recorded the calls from our towers, which were about 20-30 m away from the calling males, so as to not interfere with courtships. During the recordings, we simultaneously noted the behaviors of individuals that were within 10 m of calling males in four ways. First, we noted the maximum vigilant posture that occurred during the calling bout, classified according to Harris et al. (1983): 0 = not vigilant; 1 = head up, with four feet remaining on ground; 2 = slouch (sitting with fore-body slouched on hindquarters); 3 = vertical (sitting on hindquarters with back held straight); and 4 = stretch (standing on toes and propped by tail, with back straight). Second, we noted the length of time that individuals spent in one or more of these vigilant postures (viz., postures 1-4) after the start of the calling bout. Third, we noted whether or not the individuals ran to a burrow (presumably to facilitate escape from a would-be predator) at any time during the calling bout. Lastly, we noted if any individuals looked in the direction of the caller.

To make audio recordings of alarm calls from males for acoustic comparison with mating calls, we used the protocol of Hoogland (1995) and deliberately pulled a previously concealed mounted badger (Fowl First Taxidermy, North Platte, NE) towards reproductive males that had previously given mating calls, two weeks after the 2006 breeding period at DOT. We picked focal males randomly, and placed the badger (attached to a cable behind a blind) in areas where the males were known to forage. Afterwards, two of us (TGM and LMD) retreated to our towers, which were located about 20 m from where the badger was placed, and one of us (KSW) went behind the blind, which was located on the ground about 50 m from where the badger was placed. We then waited until the focal male and individuals of the nearby female kin-cluster were foraging aboveground and unalarmed (the only individuals to be considered in simulated danger). When this occurred, KSW pulled the badger from behind the blind at a constant rate of 25 cm per second, while TGM and LMD recorded subsequent calls, noting the same types of reaction data as for the mating calls from the observation towers. We conducted no more than 2 experimental runs per day on the same male ($N = 24$ attacks total), and all were separated by more than 1 hr to prevent habituation of the squirrels to the badger. By simulating danger, we were able to detect subtle movements of individuals, control which individuals were threatened, and promote large sample sizes that we could not acquire from encounters with natural predators.

For all recordings, we used a digital recorder (Marantz PMD-660; Marantz America, Inc., Mahwah, NJ) with a 256 GB Lexar Compact Flash Drive (Lexar Media Inc., Fremont, CA), a directional condenser microphone encased in a windscreen (Shure PG-81; Shure Inc., Niles, IL) and a parabolic reflector (Mineroff Electronics, Elmont, NY). We generated spectrograms and oscillograms with Raven 1.2 (Cornell Lab of Ornithology, Ithaca, NY) using 512-point short-time Fourier transformations with 50% overlap, and a Hamming window. We selected 3 notes (viz., separate sounds or “chirps” emitted during the calling bout, as defined in Tamura 1995) at random for every 2 min in each calling bout for acoustic analysis. We omitted indistinct notes from the analyses, and replaced them with another note selected at random from the 2 min section. According to specifications, all equipment covered a frequency range of at least 20-20,000 Hz and had a flat frequency response.

Playback Experiments

To observe reactions of squirrels to the mating calls outside of their normal context, we used the protocol of Tamura (1995) and played the recorded mating calls and alarm calls to squirrels along a nearby road during June 2006 (viz., the recordings used for playbacks reflected the differences between mating calls and alarm calls; see Results). We used a continuous white noise track with the same amplitude as the playback calls (90 dB at 5 m from the sound origin) for a control sound. We picked five areas along the road where squirrels resided, and used one of these areas per day (on a rotating basis) to establish a feeding area where squirrels were attracted by small amounts of supplemental food (a high-protein horse feed) for playbacks. After establishing the feeding area, one of us (TGM) waited behind a blind about 20 m from the feeding area until the squirrels were feeding and unalarmed, and then played one of the calls or the control sound for either 3, 5, or 10 min (selection determined at random, and taken from 5 exemplars of each stimulus). TGM then noted the reactions of individuals as described above. We conducted 5 playbacks (1 in each area) for each combination of type of call (viz., mating, alarm, control) and length of time (viz., 3, 5, or 10 min). We conducted no more than 2 experimental runs per day in the same area, and all were separated by more than 1 hr to prevent habituation of the squirrels to the stimuli.

Statistical Analysis

We digitized 9 coordinates from the spectrograms to calculate dependent variables used in a stepwise discriminant function analysis (MINITAB 13.32; Minitab Inc., State College, PA) to determine if mating calls and alarm calls differed acoustically (Fig. 1a-d). We used the method of minimizing Wilks' lambda as the stepping criterion. We then selected the mean standardized measurements of all of the notes for each individual, and analyzed variation in mating calls among individuals by performing a cluster analysis. We used the centroid method to determine the pattern of clustering.

We conducted a multivariate logistic regression analysis using Statistical Analysis Software (SAS 1999; SAS Institute Inc., Cary, NC) to determine if certain variables increased the likelihood that a mating call would be given after copulation. For this analysis, the dependent variable was the presence or absence of a mating call. Because our study routinely yielded multiple observations from the same individuals in the same or different years or colonies, we used a mixed model regression that treated the identity of individuals as a random variable, along with their colony of residence, and the date and year of the copulation. To control for pseudoreplication of data regarding the behavior of listening squirrels, we considered samples to be independent if from different mating calls or alarm calls but not if they came from the same individual in the same year. In these cases, we consolidated the data and used the average for each individual in the calculation of the average for all observations (Machlis et al. 1985). For reactions during playbacks, we never obtained repeated samples on the same individual.

We tested for normality using Kolmogorov-Smirnov tests. *N*'s show the number of individuals in the sample. Values are presented as means ± 1 standard error. All probability levels are for two-tailed tests.

RESULTS

Observations of Mating Calls

We observed the complete series of matings for 63 females. These females copulated with an average of 3.3 ± 0.12 males. About one-third of the copulations (80/216) were followed by a mating call, and over 95% (60/63) of the estrous females elicited at least one mating call during their series of matings. The mean duration of the mating calls was 21.9 ± 3.7 min (range: 1-83 min), and mating calls consisted of 200-2,000 individual notes. During all 80 mating calls (*N* = 27 males), we observed male mate guarding behaviors (described above); after a guarded female left the area of mating, the mating call of her previous sexual partner (Fig. 1d) became louder to our ears. Upon examination of the spectrograms, the separate sounds emitted during mating calls resembled the antipredator "soft chirps" of Betts (1976) and Koeppl et al. (1978) (Fig. 1c-d). Mating calls always followed an inferred copulation. We have no evidence that males gave precopulatory calls.

Recordings of Elicited Alarm Calls

We recorded 24 alarm calls from 13 squirrels, including 5 of the 8 reproductive males at DOT that emitted mating calls during the 2006 breeding period. Spectrograms showed that the notes in immediate response to the badger were either the pure or harsh "shrill chirps" of Betts (1976), Koeppl et al. (1978), Harris et al. (1983), and Lickley (1984) (Fig. 1a-b). After the mounted badger was no longer visible (usually 2-5 min from when it was first pulled), the squirrels continued vocalizing and emitted "soft chirps" (Fig. 1c). The mean duration of the calling bouts (including shrill and soft chirps) was 16.5 ± 2.1 min (range: 3-45 min).

Reactions During Mating Calls and Alarm Calls

The mean duration of vigilance for squirrels that were in simulated danger (regardless of whether they gave an alarm call during the badger presentation) was 15.4 ± 0.3 min ($N = 53$ individuals). The mean for maximum vigilant postures was 3.5 ± 0.7 (range: 0-4), and squirrels ran to a burrow during 66% (35/53) of the observations. When the average length of vigilance for all individuals was compared to the length of the calls, the relationship was highly significant ($r^2 = 0.92$, $N = 24$, $P < 0.001$).

The mean duration of vigilance during mating calls ($N = 33$ calls from colony DOT in 2006) was 1.3 ± 0.3 min ($N = 40$ individuals). The mean for maximum vigilant postures was 2.4 ± 0.5 (range: 0-4), and squirrels ran to a burrow during 4.8% (17/353) of the observations. The differences between reactions to mating calls and alarm calls were highly significant for running to a burrow ($\chi^2_1 = 149.2$, $P < 0.001$) and mean duration of vigilance ($t_{91} = 14.2$, $P < 0.001$) but not for mean maximum posture ($t_{91} = 1.3$, $P = 0.44$). Whereas all individuals that were exposed to simulated badger attacks became vigilant at some point during the alarm call (53/53), only 11.3% (40/353) became vigilant during mating calls; this difference was highly significant ($\chi^2_1 = 200.2$, $P < 0.001$). Apparent listeners to mating calls and alarm calls did not orient towards the caller.

Analysis of Sound Characteristics

Our ears could not distinguish between soft chirps from mating calls and alarm calls in the field, and the total duration of calling was not significantly different between mating calls and alarm calls (21.9 ± 3.7 vs. 16.5 ± 2.1 min; $t_{55} = 1.3$, $P = 0.43$). The mating call consisted of only one type of sound, the soft chirp (Fig. 1d), while the alarm calls started with pure shrill chirps and changed to soft chirps (Fig. 1a-c) after an average of 4.2 ± 0.5 min. ANOVA showed that all sound characteristics except internote interval (INI) and note duration (DUR) changed with time for alarm calls, so we chose two series of analysis—one each for the notes before and after the change of sounds (viz., the change from "pure shrills" to "soft chirps"; Tamura 1995). For mating calls, we accumulated notes from different times for analysis (ANOVA: $P > 0.2$ for all). Discriminant function analysis of the 9 variables (Table 1) distinguished between the mating calls ($N = 822$ notes) and alarm calls ($N = 594$ notes) before the change in sounds ($D^2 = 204$, 100% correct discrimination rate for both; binomial test: $P < 0.001$), but not after ($D^2 = 1.2$, 62% for mating calls and 54% for alarm calls; binomial test: $P = 0.29$). Cluster analysis did not suggest considerable individual variation in the sound characteristics of mating calls (mean squared Euclidean distance = 0.26; range: 0.12-0.35; $N = 5$ males, 10 dyads).

Playback Experiments

Squirrels occasionally assumed an upright posture (mean for maximum vigilant postures: 1.2 ± 0.3; range: 0-3) and looked into the distance for a few seconds upon playback of the control stimulus, but resumed foraging directly afterwards. When the mating calls (viz., only soft chirps) and alarm calls (viz., beginning with shrill chirps and progressing to soft chirps) were played, however, squirrels always ran to the nearest burrow and assumed a vigilant posture about 10-30 seconds after the playbacks started (mean for maximum vigilant postures: 3.2 ± 0.7 and 3.4 ± 0.8 respectively, Table 2); no squirrel entered a burrow. Squirrels ran to a burrow more often during mating calls and alarm calls than the control ($\chi^2_1 = 26.2$, $P < 0.001$ and $\chi^2_1 = 29.4$, $P < 0.001$ respectively), but reactions were not significantly different between mating calls and alarm calls with respect to running to a burrow ($\chi^2_1 = 0.83$, $P = 0.32$) or maximum vigilant postures ($t_{62} = 0.97$, $P = 0.52$). Squirrels kept their vigilance for all or most of both the mating calls and alarm call playbacks (Table 2). Indeed, the mean duration of vigilance was slightly (but not significantly) higher for the mating call playback than the alarm call (3 min: $t_{24} = 0.46$; 5 min: $t_{18} = 0.31$; 10 min: $t_{18} = 0.76$; $P > 0.20$ for all). For both calls, the duration of vigilance correlated with the duration of the playback. Squirrels seemed to resume their normal activity (i.e., feeding, socializing, chasing) after the mating calls and alarm call playbacks were completed. Many stayed at the feeding station and continued eating the food supplement.

Probability of Calling

Males that were the first to copulate with a particular estrous female (viz., the most likely to sire all or part of her litter) were more likely to call after copulation (ANOVA: $F_{5,73} = 21.7$, $P < 0.001$; Fig. 2) and mate guard (5x2 chi-square test: $\chi^2 = 24.9$, $P < 0.001$) than males that copulated later in the female's series of matings. Old males were more likely to call than young males ($r^2 = 0.76$, $F_{8,24} = 19.1$, $P < 0.01$), but the age of the estrous female did not significantly affect calling likelihood ($r^2 = 0.001$, $F_{8, 62} = 0.05$, $P = 0.83$). Territorial males were slightly (though not significantly) more likely to call than subordinate males (39.7 ± 6.2% vs. 23.5 ± 6.2%; $t_{24} = 1.8$, $P = 0.08$). Females took longer to copulate with another male after copulations that were followed by calls when compared with copulations that were not followed by calls (1.2 ± 0.2 vs. 0.63 ± 0.09 hrs.; $F_{5, 57} = 3.7$, $P < 0.05$).

A multivariate logistic regression using the copulations for which we had complete data ($N = 199$ copulations) showed that the order of copulation in males ($P < 0.001$) and the age of the copulating male ($P < 0.001$) were the only variables that influenced the likelihood of calling significantly (Table 3). The percentage of females in the colony that were pre-estrous during the time of copulation (and had emerged from hibernation) affected the likelihood of calling slightly, but not significantly ($P = 0.09$). A correlation matrix of all variables showed that co-linearity between independent variables was unlikely to affect these results (all $r < 0.70$).

DISCUSSION

Consecutive copulation by intrasexual rivals may compromise the opportunity of a male to sire offspring with his sexual partner (Schwagmeyer & Foltz 1990). So, why risk the advertisement of an estrous female by giving a mating call? Our results suggest a payoff for male Columbian ground squirrels living under natural conditions. Mating calls were always postcopulatory, and males that gave mating calls were usually (1) territory holding, older males that were mate guarding, and (2) males that copulated first in a female's series of matings and were therefore likely to sire progeny (these males, however, were not necessarily of higher body mass than other males). Females were also delayed from copulation with additional males when their sexual partners gave a mating call. Taken together, these results support the hypothesis that mating calls transmit an intrasexual or intersexual signal that is part of guarding an estrous female after copulation. Thus, even though the mating call may seem risky, a viable strategy may be to guard the estrous female with vocalization, particularly if other males can detect the presence of an estrous female without hearing a mating call (e.g., via observation, olfaction, or female vocalization; see Koprowski 1992).

Since mating calls are given after copulation rather than before, they probably do not reduce female aggression or increase female sexual receptivity. But the male could be calling to the estrous female to keep her proximate to the copulation site so that she will not copulate with other males. This explanation may be applicable because we heard the calls intensify after the female left the area of copulation. However, we never saw a calling male engage in multiple copulations with the estrous female after she escaped from his attempts to guard her, indicating that females were not retained nearby to increase the opportunity for repeated copulation. Furthermore, over 96% (61/63) of the estrous females we observed copulated with more than one male (see also Murie 1995). Since callers fought with other reproductive males while calling, it also seems possible that male mating calls were intrasexual threat signals. In any case, the mating call is part of guarding an estrous female, and this result addresses the lack of explanation for sciurid mating calls in previous studies (e.g., Farentinos 1972; Davis 1982; Koford 1982; Lishak 1982; Grady & Hoogland 1986).

Mating calls had an effect of delaying the female from further copulation. But mating calls also might have attracted other males to the vicinity of the estrous female. Thus, the mating calls may have had both costs and benefits, with the outweighing benefit being greater assurance of paternity due to delaying estrous females from mating again (see Schwagmeyer & Foltz 1990). Because litter size is only 2-4 and males that are the first to copulate in a female's series of matings have sperm precedence, the most likely males to benefit from mating calls and mate guarding are those that are the first to copulate with a female. Our results confirmed this expectation by showing that most mating calls were emitted by a female's first mate.

The percentage of females that had emerged from hibernation and were pre-estrous during the time of copulation affected the probability of calling slightly, but not significantly. Are copulating males therefore advertising themselves to females that have not yet mated by giving a mating call? Perhaps this is a secondary benefit of calling in certain instances. Calling by older territorial males is consistent with an advertising context, but the likelihood of giving a mating call was not significantly related to the number of females with which a male copulated. Thus, we have no evidence that the mating call attracted females. The complete absence of precopulatory calls is also not consistent with the hypothesis that mating calls are sexual displays that attract females. Contrary to the popular notion that vocalizations emitted during courtship serve to attract potential mates, our results suggest that male Columbian ground squirrels emit mating calls primarily in the context of deterring a recent sexual partner from subsequent copulations with conspecifics.

Might the mating call, like roaring in red deer (McComb 1987), induce or expedite the ovulation of the estrous female or females that have not yet mated? Most physiological details of ovulation in Columbian ground squirrels are unknown (Elliott & Flinders 1991), so we do not have data to investigate this intriguing possibility. However, two lines of indirect evidence make this option seem unlikely—again, that there were no precopulatory calls and the percentage of post-hibernatory females that were pre-estrous during the time of copulation affected the probability of calling after copulation only vaguely.

Mating calls contained only “soft chirps”. Since alarm calls contained a changing pattern between “pure shrills” and “soft chirps”, it is likely that squirrels were able to differentiate between mating calls and alarm calls, but only within the context of breeding. Indeed, reactions to playbacks of mating calls outside of their normal context were different than reactions during the breeding season, but were not significantly different than those during the later alarm calls. Furthermore, since males and females were not alert during mating calls, they were probably not manipulated by a “cry of wolf”; in fact, the estrous female was often either underground or “lost” to the guarding male (viz., left the area to court other males) during the call. We also found no individual differences in the acoustic characteristics of mating calls, so listeners probably did not disregard the possibility of a predation in favor of an “honest” message (Gyger & Marler 1988). For Columbian ground squirrels, it therefore seems that the similarity of the sounds has been facilitated by the consistent difference in contextual cues associated with these two different situations (viz., mate guarding and predator warning) (Smith 1986).

Columbian ground squirrels seem to have a conflict regarding the optimal number of sexual partners for each female—males increase reproductive success by monopolizing females to prevent their solicitation of copulations with additional males. Sexual selection therefore favors males that emit a call as part of mate guarding, but selection apparently acts on the vocal repertoire already present in the species, rather than favoring a new call. Perhaps different reactions to mating calls and alarm calls may be retained in this mating system because of the necessary preoccupation of breeding ground squirrels with finding mates, sometimes in lieu of self-preservation (Hoogland et al. 2006). The type of information or mechanism behind this context-dependent reaction to mating calls (e.g., a subtle contextual clue given by the caller, or the ability to process cognitively, catalog information, or retain event sequences) remains unclear and deserves future study.

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TABLES

Table 1. Data for 9 measured characteristics of duration and frequency in alarm calls (before and after the change in sound) and mating calls for Columbian ground squirrels living along the Gorge Creek trail in Sheep River Provincial Park, Alberta during 2006; the characteristics are labeled as in Fig. (1a-d), and the numbers in parentheses represent the number of notes that were measured for analysis.

Variables	Alarm Calls (before change in sound)	Alarm Calls (after change in sound)	Mating Calls
INI (s)	0.52 ± 0.08 (46)	0.64 ± 0.12 (164)	0.58 ± 0.14 (233)
DUR (s)	0.60 ± 0.06 (151)	0.73 ± 0.09 (443)	0.65 ± 0.10 (822)
MF (kHz)	11.2 ± 0.02 (151)	22.5 ± 0.03 (443)	22.3 ± 0.05 (822)
FPA (kHz)	10.6 ± 0.01 (151)	22.8 ± 0.03 (443)	23.1 ± 0.04 (822)
MFL (%)	27.4 ± 0.02 (151)	33.2 ± 0.07 (443)	33.0 ± 0.06 (822)
MinF (kHz)	4.2 ± 0.05 (151)	1.6 ± 0.3 (443)	2.2 ± 0.16 (822)
LPA (%)	43.2 ± 0.2 (151)	13.4 ± 0.07 (443)	13.3 ± 0.05 (822)
HFB (kHz)	5.8 ± 0.1 (151)	3.5 ± 0.3 (443)	3.0 ± 0.6 (822)
LFH (kHz)	9.5 ± 0.1 (151)	4.2 ± 0.07 (443)	4.7 ± 0.02 (822)

Table 2. Duration and type of reaction elicited by the playbacks of mating calls, alarm calls, and the control sound to free-ranging Columbian ground squirrels living along the Gorge Creek Trail in 2006.

Type of playback	Duration of playback	Number of individuals sampled	Number of individuals that ran to a burrow	Number of individuals that assumed a vigilant posture	Duration of vigilance (mean ± SE)
Control	3 min	17	0	2	Less than 10 s
	5 min	17	1	2	Less than 10 s
	10 min	14	1	2	Less than 10 s
Mating call	3 min	16	16	16	3.9 ± 0.4 min
	5 min	12	12	12	5.6 ± 1.2 min
	10 min	4	4	4	11.3 ± 0.7 min
Alarm call	3 min	8	8	8	4.2 ± 0.6 min
	5 min	8	8	8	6.1 ± 1.0 min
	10 min	16	16	16	12.4 ± 1.0 min

Table 3. Significance of 9 variables on the likelihood of a male emitting a postcopulatory mating call for Columbian ground squirrels from two colonies (Meadow B and DOT) at Sheep River Provincial Park, Alberta, during 2005-2006 ($N = 199$ copulations, 63 estrous females).

Variable	Wald's statistic (χ^2)	*P*-value
Male body weight (during breeding)	0.43	0.51
Female body weight (during breeding)	0.04	0.84
Male copulatory success (number of mates)	1.05	0.31
Order of copulation in males	30.30	<0.001
Time of year (date)	0.70	0.40
Age of copulating male	16.82	<0.001

Age of estrous female	1.64	0.20
Male status (level of territoriality)	1.03	0.31
Percentage of females in colony that were pre-estrous during copulation	2.95	0.09

FIGURES

Figure 1a-d. Spectrograms of (a) the pure shrill and (b) the harsh shrill given during the simulated attacks (with oscillogram on top showing amplitude vs. time); (c) the soft chirp given during the simulated attacks; and (d) the soft chirp from the mating calls. The variables used in the analysis are abbreviated in the spectrograms as follows: INI: internote interval (s); DUR: note duration (s); MF: maximum frequency attained by note (kHz); FPA: frequency of first harmonic at peak amplitude (kHz); MFL: maximum frequency location (% of note); MinF: minimum frequency attained by note (kHz); LPA: location of peak amplitude (% of note); FF: fundamental frequency (kHz); HFB: highest frequency of first harmonic; and LFH: lowest frequency of first harmonic (kHz).

Figure 2. Probability of giving a mating call versus the order in which a calling male mated with an estrous female (mean ± SE). The number above each bar indicates the number of individual males that called (viz., we assumed dependence of data for multiple observations on the same individual in the same year). The *P*-value from the overall analysis is significant (ANOVA: $P < 0.001$). The following 2 x 2 pairwise analyses are also significant: first vs. second, third, fourth, and fifth and others, second vs. fifth and others, third vs. fifth and others, and fourth vs. fifth and others.

Fig. 1a-d.

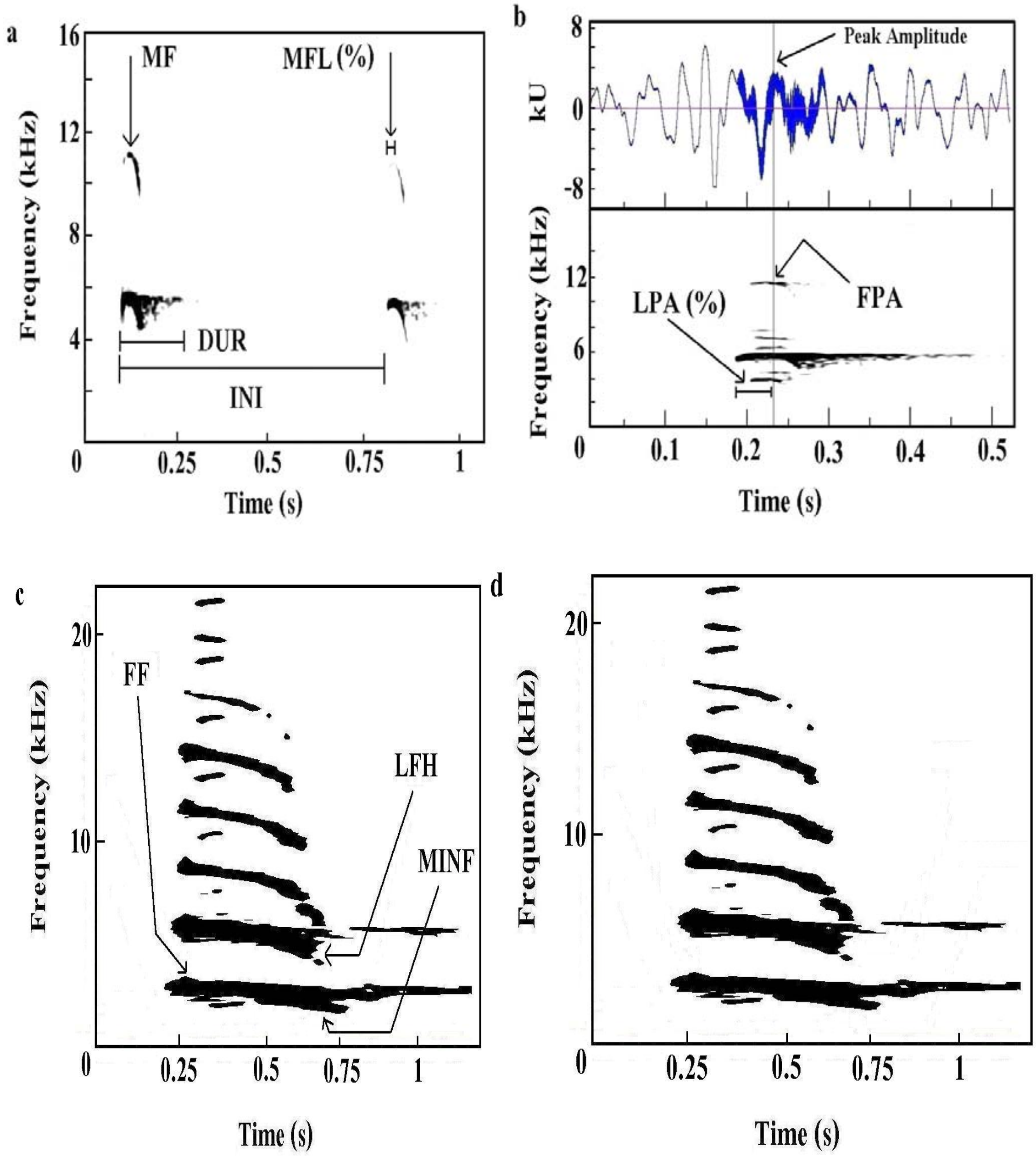
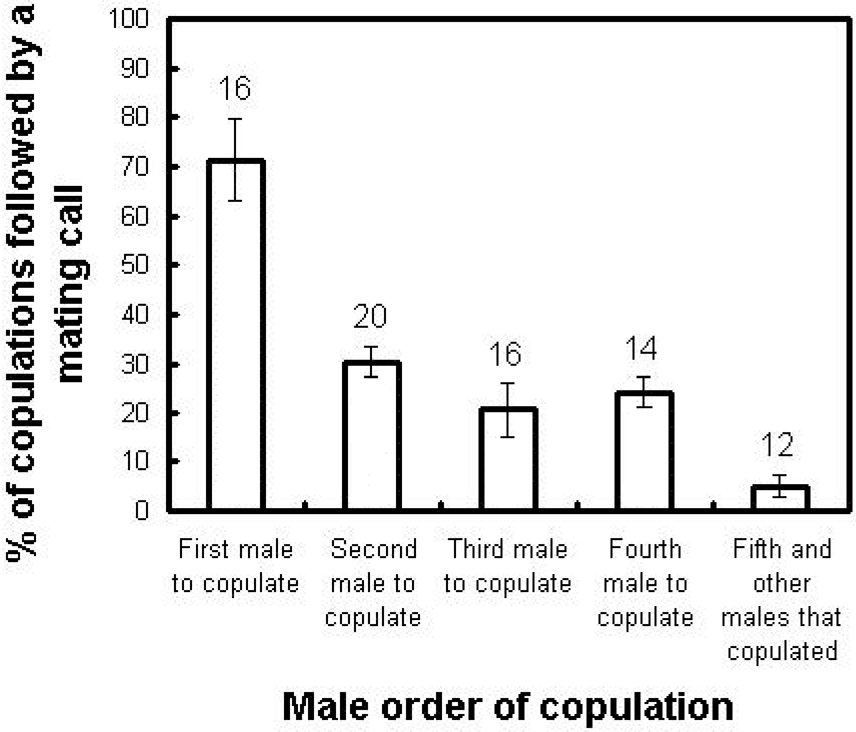

a
16
12
8
4
0
0.25
0.5
0.75
1
Frequency (kHz)
Time (s)
MF
MFL (%)
DUR
INI
b
8
0
-8
kU
Peak Amplitude
12
6
Frequency (kHz)
LPA (%)
FPA
0
0.1
0.2
0.3
0.4
0.5
Time (s)
c
20
10
0
0.25
0.5
0.75
1
Frequency (kHz)
Time (s)
FF
LFH
MINF
d
20
10
0
0.25
0.5
0.75
1
Frequency (kHz)
Time (s)

Fig. 2.


% of copulations followed by a mating call
100
90
80
70
60
50
40
30
20
10
0
16
20
16
14
12
First male to copulate
Second male to copulate
Third male to copulate
Fourth male to copulate
Fifth and other males that copulated
Male order of copulation

V. WHY DO FEMALE COLUMBIAN GROUND SQUIRRELS GIVE AN ESTRUS CALL?

ABSTRACT. Female Columbian ground squirrels (*Spermophilus columbianus*) sometimes emit a repetitive vocalization after copulation. We examined two possible explanations for why sexual selection would act to favor expression of these “estrus calls”: to encourage mating with additional males and to increase mate guarding by the consort male as a mechanism of indirect mate choice. During three annual mating periods, we observed mating behaviour, estrus calls, and postcopulatory behavioural interactions of free-ranging individuals. Age of the consort male increased linearly with the likelihood that an estrus call would follow copulation. After emitting an estrus call, females typically solicited courtship interactions with non-consort males. Females that emitted an estrus call were more likely to acquire additional matings than non-calling females if calls were emitted after the female’s first mating. Thus, we suggest that estrus advertisement is the most likely social context of female postcopulatory calling.

INTRODUCTION

Mating interactions often involve an exchange of auditory signals. While male vocalizations may be part of attracting or guarding mates (McComb 1987; Mobley et al. 1988; Gibson et al. 1991; Tamura 1995; McElligott & Hayden 2001; Kelley 2004; Velez

& Brockmann 2006; Manno et al. 2007), the majority of studies involving mating vocalizations examine rhythmic sounds given by sexually receptive females before, during, and after copulation (reviewed by: Pradhan et al. 2006). The timing of occurrence relative to copulation for some of these female vocalizations (viz., during the last stage of intercourse or after copulation) suggests that they probably play a role in postcopulatory sexual selection (Birkhead & Pizzari 2002), although this possibility has been investigated only recently (e.g., Dixson 1998; Maestripieri et al. 2005).

Postcopulatory sexual selection can operate via two mechanisms—female choice and sperm competition. Therefore, there are two consequences of female postcopulatory calling on which sexual selection may act to favor call expression. First, calls could be a mechanism of indirect mate choice for a robust male as a sexual partner (Maestripieri et al. 2005). Under this hypothesis, a calling female should initiate mate guarding by the consort male, minimize likelihood of subsequent copulations with other males, and hinder sperm competition (Maestripieri & Roney 2005). Second, calls may “advertise” estrous females and promote mating with multiple males (Hamilton & Arrowood 1978; Semple 1998). This hypothesis predicts that calling females should not initiate mate guarding and should solicit and acquire additional matings more than females that do not call. Via either multiple paternity or intra-uterine sperm competition, the female could then reap any benefits that would result from extra-pair copulations, including the birth of a robust litter (Hoogland 1998) or reducing likelihood of losing offspring to infanticide via paternity confusion (O’Connell & Cowlishaw 1994; Lacey et al. 2001).

Female postcopulatory calling has been studied primarily in primates (e.g., Dixson 1998; Nikitopoulos 2004; Pradhan et al. 2006), with only a few notable exceptions (birds: Montgomerie & Thornhill 1989; Sheldon 1994; Pizzari & Birkhead 2001; pinnipeds: Cox & LeBoeuf 1977; rodents: Taylor 1966; Callahan 1981; Blake 1992). Even for groups of species such as Marmotine ground squirrels that have well-studied vocal repertoires (e.g., Leger et al. 1984; Blumstein 2003; Hoogland 2007), calls that follow mating are relatively undocumented and poorly understood (Hoogland 1995; Lacey et al. 1997). We report a repetitive "estrus call" given by some female Columbian ground squirrels (*Spermophilus columbianus*) 1-5 minutes after copulation, and examine the social context in which estrus calls occur.

Columbian ground squirrels are colonial, diurnal, herbivorous, and burrowing rodents (e.g., Betts 1976; Murie 1995). Females live adjacently in philopatric kin clusters and with a few non-reproductive individuals of both sexes (King & Murie 1985). During a 3-week mating period, a territorial reproductive male (usually ≥4 years old) overlaps the ranges of one or a few females (Murie & Harris 1978; Murie & Harris 1988; Manno 2008). Young subordinate males (2-3 years old) usually do not maintain a territory, but are physically able to reproduce and sometimes obtain copulations (Murie 1995; Murie & Harris 1978; Manno et al. 2007). Females are highly promiscuous during their single annual day of estrus, which occurs 2-12 days after emergence from hibernation in April (Betts 1976; Murie 1995). Females move short and long distances (10-100 m) to solicit copulations via courtship interactions with their territorial male, adjacent territory holders, and subordinate young males. Males and females conduct amicable sniffing and females "leading" males into burrows (where they may then copulate; Manno et al. 2007). Males that are first to copulate in a female's series of matings (viz., usually the nearest territorial male) have sperm precedence and mate guard via postejaculatory vocalization, fighting with approaching males, and hostile behaviour towards the female as she attempts to flee the copulatory site (Murie 1995; Manno et al. 2007). About two-thirds of litters (20/29 = 69%) are sired by multiple males (Murie 1995). Almost-weaned juveniles emerge from their natal burrows in late June after 27-days of lactation, and males invest little paternally other than sperm (Murie & Harris 1982, 1988). Infanticide is occasionally committed by female marauders 1-5 days after juveniles have appeared aboveground; infanticidal attacks by males are documented but rare (Dobson 1990; Stevens 1998).

We examined (1) the acoustic structure of estrus calls; (2) the relationship of several characteristics of consort males and estrous females and the presence or absence of a postcopulatory estrus call; (3) interactions of males and females after estrus calls (viz., occurrence of male mate guarding or female solicitation of mates); and (4) the social context when estrus calls occur, particularly with respect to the opportunity for sperm competition. Since copulation with multiple males is common among female *Spermophilus columbianus* and other ground squirrel species (e.g., Hanken & Sherman 1981; Sherman 1989; Schwagmeyer & Foltz 1990; Hoogland 1995, 1998; Murie 1995; Lacey et al. 1997), we hypothesized that estrus calls may assist females in finding or soliciting additional mates.

METHODS

Mating Behaviour

From April to July in 2005-2007, we observed wild, free-ranging Columbian ground squirrels of known age and matrilineal genealogy at two colonies (Meadow B and

DOT) in Sheep River Provincial Park, Alberta, Canada (50° 38′ N, 114° 38′ W, elev. 1500 m) from 4-m high observation towers. Squirrels were trapped 1-2 days after they emerged from hibernation (for juveniles, 1-2 days after emergence from their natal burrow), ushered into a cloth bag, restrained by hand, weighed, and fitted with numbered metal fingerling eartags for long-term identification (National Band & Tag Co., Newport, KY). For visual identification from a distance, we painted each animal with a unique symbol using black dye (Lady Clairol Hydrience; Proctor and Gamble, Stamford, CT). We considered males that exhibited a pigmented scrotum and large descended testes at trapping to be reproductive. We also trapped females several additional times during the 3-week breeding period and examined their vulvar condition to determine whether they had been estrous (viz., with fully opened vulva).

We used the methods of Hoogland (1995) and Hoogland et al. (2006) to watch squirrels at both colonies from dawn until dusk every day during the breeding period, which extended from the third week of April to the first or second week of May. Each female was sexually receptive for a few hours on a single day. When a social interaction occurred (viz., chasing, fighting, sniffing, allogrooming, playing, females "leading" males), we used all-occurrence sampling (Altmann 1974) to record the individuals involved, which individual instigated the interaction, and the time and location of occurrence (ascertained from a 10m x 10m grid of flagging placed over the colony). Copulations occasionally occurred aboveground ($N = 9$ copulations) and were therefore observed easily. We also used well-established methods to infer underground copulations of marked individuals from aboveground diagnostic behaviours: (1) females moving short and long distances (to about 100 m) to elicit social interaction with males and to "lead" them into prospective copulatory burrows; (2) late final submergences of a male and female in the same burrow on the night before the female exhibited a fully opened vulva; (3) other submergences of both partners into the same burrow, where they remained for at least several minutes; (4) self-grooming of genitals by both partners upon later emergence, which was sometimes accompanied by dustbathing; (5) a postejaculatory "mating call" by the male; and (6) other behaviours that indicated male mate guarding, such as chasing the female into a burrow, sitting on or "herding" the female into that burrow as she attempted to flee the area, and fighting with other males (e.g., Hoogland 1995; Murie 1995; Lacey et al. 1997; Manno et al. 2007). Occasional aboveground copulations also featured all or most of these behaviours, and the dates of juvenile emergence for each female correlated strongly with our inferred dates of estrus for both colonies ($R^2 > 0.90$, $P < 0.001$ for both comparisons).

Although these criteria allowed us to discern when copulations occurred, they did not enable us to determine the precise number or duration of copulations, nor the interval between consecutive copulations. We therefore use the term "copulation" to refer to behavioral evidence that mating occurred. A "consortship" occurred during the period of time that a male and female spent together in a burrow (Lacey et al. 1997), and the consort male was the male associated with the consortship. We used the term "non-consort male" to refer to all other males that were aboveground in the colony.

We considered males to be territorial if there was an established area in which they were victorious in hostile interactions with other males (other males were considered subordinate or non-territorial; Murie & Harris 1978; Dobson 1983). We then scored the territoriality level of males based on the proportion of mating season during which they were territorial (a 0-1 scale). When we observed the entire series of matings for each estrous female, we noted the number and identity of males that guarded the estrous female and their duration of guarding. We considered that the duration of postcopulatory guarding ended when the male no longer emitted a mating call, no longer responded aggressively to advances by other males, and made no attempt to prevent the estrous female from leaving the area of copulation (Lacey et al. 1997).

We considered males to be familiar with an estrous female if they engaged in any social interaction (e.g., sniffing, chasing, playing, allogrooming) before the estrus. When a male maintained a territory that either contained the burrow from which the female emerged daily, or abutted the territory that contained her emergence burrow, we considered the male to be neighboring to the estrous female. Operational sex ratio (OSR) is the number of breeding males per estrous female. On different days during the 3-week breeding period, 1-6 females on a colony were estrous. Using this variation, we calculated a daily OSR for both colonies to use as an indicator of the possibility for multiple matings by females.

Observation and Recording of Vocalizations

When we heard a vocalization from a consort male or estrous female during the female's series of matings, we noted the location of the caller, the direction of calling, and the duration of the call (Manno et al. 2007). We also noted the reactions of all conspecifics within 10 m of the caller, in three ways. First, we noted the maximum vigilant posture that occurred during the calling bout, classified according to Harris et al. (1983): 0 = not vigilant; 1 = head up, with four feet remaining on ground; 2 = slouch (sitting with fore-body slouched on hindquarters); 3 = vertical (sitting on hindquarters with back held straight); and 4 = stretch (standing on toes and propped by tail, with back straight). Second, we noted the duration that individuals spent in one or more of these vigilant postures (viz., postures 1-4) after the start of the calling bout. Finally, we noted if any individuals looked in the direction of the caller.

From our observation towers, which were about 20-50 m from vocalizing females, we made audio recordings of female estrus calls using a digital recorder (Marantz PMD-660; Marantz America, Inc., Mahwah, NJ) with a 256 GB Lexar Compact Flash Drive (Lexar Media Inc., Fremont, CA), a directional condenser microphone encased in a windscreen (Shure PG-81; Shure Inc., Niles, IL) and a parabolic reflector (Mineroff Electronics, Elmont, NY). We generated spectrograms and oscillograms with Raven 1.2 (Cornell Lab of Ornithology, Ithaca, NY) using 512-point short-time Fourier transformations with 50% overlap, and a Hamming window. According to specifications, all equipment covered a frequency range of at least 20-20,000 Hz and had a flat frequency response.

Whereas the mating calls of male Columbian ground squirrels usually occurred in an area where a male and female had submerged in a burrow together on the night before the day of estrus (and we therefore expected them to copulate; Manno et al. 2007), estrus calls often occurred far away from our observation towers in less predictable places and times. Therefore, despite our best efforts, we were able to record only a few short calls in their entirety, and usually obtained recordings of partial calls. Our data therefore preclude a robust statistical test for individual uniqueness of estrus calls.

Statistical Analysis

Using the copulations for which we had complete data on all variables (*N* = 396 copulations), we conducted a multivariate logistic regression analysis using Statistical Analysis Software (SAS 1999; SAS Institute Inc., Cary, NC) to determine the variables which were related significantly to whether or not an estrus call would be emitted after copulation. For this analysis, the dependent variable was the presence or absence of an estrus call. We examined the body mass, age, and copulatory success (viz., number of sexual partners) of the consort male and estrous female as independent variables. We also considered the relationships of level of territoriality for males, OSR, the number of familiar and neighboring males to the estrous female, whether or not the consort male guarded, and the order of copulation of the consort male (for a specific female's series of matings) with the dependent variable.

Because our study yielded multiple observations from the same individuals in the same or different years or colonies, we used a mixed model regression that treated the identity of individuals as a random variable, along with their colony of residence, and the date and year of the copulation. We also examined our dataset for significant interactions (viz., co-linearity) among independent variables, and tested for such influences via interaction terms. We then generated all possible models and determined the best-fit models using the method of minimizing Akaike's information criterion (AIC_c) (Burnham & Anderson 1998). Thus, we removed any interactions or variables that were a burden to the fit of the data to the model.

For univariate procedures, we assumed dependence of data from the same individual in the same year and independence of data from the same individual in different years (Machlis et al. 1985). Thus, we consolidated the data and used the average for each individual in the calculation of the average for all observations. We tested data for normality with Kolmogorov-Smirnov tests. When the data did not meet the assumptions of a parametric analysis, we used the appropriate non-parametric test (in all of these cases, transformations did not yield a normal distribution). *N*'s show the number of individuals in the sample. Values are means ± 1 *SE*. All *P*-values result from two-tailed tests ($\alpha = 0.05$).

RESULTS

Observations of Estrus Calls

We observed the complete series of matings for 124 estrous females. These females copulated with an average of 3.5 ± 0.05 males (range: 1-6). One-third (41/124 = 33%) of the females emitted at least one estrus call during their series of matings, and about one-sixth of the copulations (60/428 = 14%) were followed by an estrus call. Estrus calls always followed an inferred copulation by about 1-5 mins. The mean duration of estrus calls was 7.4 ± 1.8 mins, but duration varied widely across calls (range: 0.5-57 mins). Estrus calls consisted of 2-200 notes (viz., separate sounds or "chirps") that were emitted during a single continuous calling bout (53/60 = 88%) or 2-3 distinct bouts that were separated by about 2 mins (7/60 = 12%).

Upon examination of the spectrograms, the notes emitted during estrus calls resembled the antipredator "soft chirps" of Betts (1976) and Koeppl et al. (1978), which also constitute the postcopulatory male "mating call" of Manno et al. (2007). However, further inspection revealed that estrus calls contained two types of unique notes, not previously reported for Columbian ground squirrels, usually emitted within the same calling bout. Whereas type 1 notes (Fig. 1a) had a fundamental frequency at 2.5 kHz with 4 harmonics ranging from 3.5-9.5 kHz, type 2 notes (Fig.1b) had the same fundamental with 2 harmonics at 6 kHz and 9.5 kHz. Thus, we found stacked harmonics within the sounds emitted during estrus calls, combined with lack of the higher frequencies occurring in the aforementioned soft chirps and mating calls. These characteristics probably gave estrus calls the very soft sound we heard, as we were able to distinguish them easily from various antipredator and male mating calls (Koeppl et al. 1978; Manno et al. 2007).

Calling behaviour varied widely across individuals, in three ways. First, most females never called after copulation, and others called after copulation with certain males but not others (Fig. 2). Indeed, almost half of the females that were breeding ≥2 years during the study and for which we had complete data called in some years but not in others (13/29 = 45%). Second, 8 estrous females called 4 times during their series of matings. Finally, a few females that emitted estrus calls with many “chirps” after copulation with certain males gave short calls (≤10 sec) following consortships with other males (4/41 = 9.8%).

Probability of Calling

Whereas the males that elicited female estrous calls after copulating with a female were older than males that did not elicit calls (5.7 ± 0.2 yrs. when female calls vs. 5.0 ± 0.1 yrs., $t_{394} = 2.7$, $P < 0.01$), the age of the estrous female did not affect calling likelihood (3.8 ± 0.2 yrs. for callers vs. 4.0 ± 0.1 yrs., $t_{394} = 0.46$, $P = 0.65$). Indeed, the percentage of copulations followed by an estrus call increased linearly with the age of the consort male (Fig. 3). While copulations usually occurred on the territory of the consort male (338/401 = 84.3% when the male maintained a territory), estrus calls were more likely to occur when the female was located on (and had copulated at) the consort male's territory than when elsewhere (56/60 = 93.3% on male's territory when female called vs. 282/341 = 82.6%, $G = 10.2$, *d.f.* = 1, $P < 0.05$).

Likelihood of giving an estrus call also varied with the number of mates for the estrous female ($P < 0.001$; Fig. 4). Females with 2 or 3 mates called slightly more than females with single mates ($U = 210$, $P = 0.09$ and $U = 306$, $P = 0.01$, respectively). Excluding the 12 females with single mates from the comparison, females with 2-3 mates called slightly more often than females with 4-5 mates ($U = 1945$, $P = 0.09$; Fig. 4). When we compared the number of mates for females that gave no estrus calls and females that emitted at least one estrus call during their series of matings, the results were not significant (3.4 ± 0.15 for callers vs. 3.1 ± 0.14, $t_{122} = 1.3$, $P = 0.20$).

We found no other variables that significantly increased the likelihood that an estrus call would be given after copulation with univariate analyses. For instance, weight of the consort male (535.9 ± 8.3 when female calls vs. 534.5 ± 3.5 g, $t_{394} = 0.14$, $P = 0.89$) and the estrous female (398.8 ± 6.8 for callers vs. 405.2 ± 3.1 g, $t_{394} = 0.78$, $P = 0.43$) did not affect calling likelihood significantly. Likelihood of giving an estrus call did not vary according to the order of the copulating male in the female's series of matings (Kruskal-Wallis: $H = 2.2$, *d.f.* = 4, $P = 0.69$). Nor did variables that illustrated the amount of competition in colonies affect calling likelihood significantly, such as the number of neighboring males to the estrous female (3.9 ± 0.3 for callers vs. 3.6 ± 0.1, $t_{394} = 1.1$, $P = 0.27$).

A multivariate logistic regression analysis using the copulations for which we had complete data ($N = 396$ copulations, 124 estrous females) yielded similar results. We report the best fit model and all models within 10 AIC_c points (Table 1a-b). The best fit (1^{st}) model ($AIC_c = 355.2$) featured a significant positive relationship between the age of the consort male and the likelihood of estrus calling ($P < 0.005$). In the second model ($AIC_c = 359.1$), females with different numbers of sexual partners had a significantly different likelihood of calling ($P < 0.05$). These were also the only variables to have a significant relationship with calling likelihood in the overall model (which integrated all of the independent variables; $AIC_c = 3668.8$) and 2 other models ($AIC_c < 500$). Occurrence of male postcopulatory guarding and level of territoriality of the consort male were included in both models that fit our data well, but neither had a significant relationship with calling likelihood ($P > 0.10$ for both).

Estrus call frequency varied across years, was higher for some females than others (although male identity did not influence calling likelihood), and was higher on colony DOT than colony B (Table 1a-b). Estrus calls also happened during earlier rather than later dates, probably because breeding occurs 1-2 weeks earlier on DOT (Table 1a-b). Nevertheless, male age and female success were likely not biased because of interactions with other variables, as they remained highly significant predictors of calling likelihood in the models that fit our data well. Likewise, it is unlikely that our results were influenced by co-linearity between independent variables. None of our independent variables were significantly co-linear with female mating success (number of mates).

Level of male territoriality was the only variable associated with male age, and this correlation was not related significantly with calling likelihood in any of the models we generated.

Postcopulatory Behaviour

Over half of copulations that were followed by estrus calls (33/60 = 55%), were also followed by the consort male emitting a postejaculatory mating call and exhibiting mate guarding behaviours (see also Manno et al. 2007). Thus, males and females sometimes "duetted" after emerging from the burrow of copulation. The relationship of the duration of mate guarding and the presence or absence of an estrus call was not significant (Table 1a-b).

Estrus calling usually coincided with the estrous female soliciting males other than the consort male. Most estrus calls were followed by the female initiating amicable interaction and copulating with another male within 10 mins of ceasing her estrus call, regardless of whether the female was guarded (50/60 = 83%). When a female emitted an estrus call after her first mating, she was more likely to acquire additional copulations than females that did not call ($G = 9.2$, *d.f.* = 1, $P < 0.05$; Fig. 5). This trend was not significant for other copulations when we considered the order of male copulation (viz., 2-5) or whether copulations were first, middle, or last copulations in a female's series of matings ($P > 0.4$ for all); indeed, calling females were significantly less likely to obtain an additional mate if they called after their second mate ($G = 13.4$, *d.f.* = 1, $P = 0.01$; Fig. 5).

Postcopulatory male-female contact and interactions appeared to be male initiated. When the male and female “duetted”, the male started calling before the female in every instance (33/33 = 100%). The female always stopped calling as soon as the male ceased his guarding (33/33 = 100%); the female never seemed to initiate further contact with the consort male by calling after he stopped guarding, and the female then fled the consort male’s area. Every interaction we observed between a consort male and an estrous female after copulations that were followed by an estrus call was initiated by the male (120/120 = 100%; avg: 2.0 ± 0.3 postcopulatory interactions per consortship). Most of these interactions consisted of the male herding the female into a burrow and sitting on that burrow while she was emitting the estrous call and attempting to leave the area of copulation (110/120 = 92%). The other interactions ($N = 10$) were the consort male sniffing the posterior region of the estrous female.

For the 38 estrus calls that occurred during 2006-2007 at one of the colonies, we examined the reactions of other squirrels that were within 10 m of the estrus calls. Whereas nearby females seemed to continue feeding during calls, 7 individual reproductive males assumed an upright posture, quickly looked towards the calling female and ran in her direction (108/108 = 100%). With the exception of those females that did not solicit matings after an estrus call ($N = 10$), females always emitted their estrus calls in close proximity to reproductive males other than the consort male (viz., within 10 m). After an estrus call was emitted, the consort male either continued guarding as before ($N = 33$) or had already left the area of copulation and did not return ($N = 27$). Thus, previous consort behaviour seemed unaffected by the estrus calls.

DISCUSSION

We examined an uncommon postcopulatory call emitted by female Columbian ground squirrels during their estrus by observing mating behaviour under natural conditions. Our results indicated that (1) estrous females that called after the first mate in their series of matings were more likely to acquire additional mates than females that did not call; (2) females typically attempted to leave the site of copulation while giving estrus calls; (3) females often emitted estrus calls after copulations with males of advanced age; and (4) the likelihood of giving an estrus call varied with the number of mates for the estrous female. We evaluated the meaning of these results in the context of the indirect female choice and estrus advertisement hypotheses. Estrus calls could be used selectively with robust males to encourage mate guarding, minimize the likelihood of mating with multiple partners, and possibly prevent sperm competition. Alternatively, the calls may "advertise" the estrous female to non-consort males and promote copulation with additional mates.

If estrus calls assist females in finding or soliciting additional mates, then females that call should be more likely to acquire additional matings than females that do not call. Some of our results supported this prediction. When a female called after her first mating, she always copulated with a second male. As for the 2nd through 5th mates in a female's series of matings that did not show this pattern, this may have occurred because females do not usually engage in multiple consortships with the same males during their estrus (Manno et al. 2007). Thus, there were fewer males to attract as a female's estrus progressed. The results were best explained by the estrus advertisement hypothesis, and suggest that at least some estrus calls assist females in finding or soliciting additional mates.

If estrus calls play a role in indirect female-choice, then calling females should attempt to initiate social contact with the consort male and stay proximate to the copulatory site after copulation. Our results did not support this prediction. Postcopulatory contact between the consort male and estrous female seemed to be hindered by the female and initiated by the male. Under the female-choice hypothesis, females should have continued calling after the consort male ceased his guarding or directly after emergence from the copulatory burrow, before the male commenced guarding. These were the times when encouragement of guarding would have been most crucial, but females that emitted an estrus call after copulation often did so while the consort male was guarding, and stopped calling once they were able to flee the area. Like the postcopulatory calls of female macaques and baboons (Semple 1998; Maestripieri et al. 2005), ground squirrel estrus calls also elicited approaches to the female by non-consort males, but there were no behavioural changes apparent in the consort male. These observations argue against the notion that estrus calls promote mate guarding by the consort male.

The weight and order of copulation of the consort male did not have a significant relationship with calling likelihood in any of our logistic models, but male age was significant in a model that fit our data well and in a univariate analysis. Might female Columbian ground squirrels exhibit an indirect mechanism of mate choice by calling after males of advanced age? The answer here is probably no, because calling females tried to flee the site of copulation for all males, regardless of age. Furthermore, the first male in a female's series of matings, and not necessarily the oldest male in the series, is most likely to sire all or most of the offspring (Murie 1995). The first mate would therefore be the male that was more likely "selected" by the female according to the female-choice hypothesis. Indeed, the relationship with age was significant and order of copulation was not in our multivariate regression, and we did not find significant co-linearity between these independent variables. The order of copulation in males was also not a significant influence in either of the best-fit models. Thus, the positive linear trend between the age of the consort male and calling likelihood does not appear to be explained well by the female-choice hypothesis. One explanation for this trend is that males of advanced age are more experienced breeders and perhaps more skillful mate guarders than younger males, which could make females entice additional mates more vigorously. This idea may be supported by our data showing that estrus calls were more likely to occur when the female was located on the consort male's territory than when elsewhere.

Our results indicated that the likelihood of giving an estrus call varied with the number of mates for the estrous female. One might predict that an association between estrus calls and mating with fewer partners is consistent with female choice hypothesis. On the other hand, calling females having only a few sexual partners can also be consistent with the notion that females give estrus calls to solicit matings with non-consort males, under the premise that females call when there is a greater need to encourage sperm competition, paternity confusion, or other possible benefits of copulating with several males (Maestripieri et al. 2005). Another complication in interpreting this result is that we can not determine if the number of mates for non-calling females would have increased had they called. For reasons that remain unclear, females with a total of 3 copulatory partners during the day of estrus (the approximate mean for our sample) were the mostly likely to emit an estrus call. We also observed 12 females, varied in age and breeding experience, that gave no estrus call and mated with only one male. Thus, although the likelihood of giving an estrus call varied significantly with the number of mates for the estrous female, the relationship is not cleanly positive or negative and does not seem to provide support for either the female choice or estrus advertisement hypotheses.

Might there be other explanations for the estrus calls of female Columbian ground squirrels (Pradhan et al. 2006)? We considered whether estrus calls could reflect an orgasm-like reaction (Hamilton & Arrowood 1978) or self-stimulate the occurrence of ovulation (Cheng 1992), but these reasons would not explain the exclusive postcopulatory occurrence of estrus calls (n.b., we never heard the calls during aboveground copulations or during any other observations). Likewise, the estrus call is probably not a vestigial phenomenon or non-adaptive byproduct of sexual intercourse (Henzi 1996) because calling females sometimes increased their chances of acquiring an additional mating. Strengthening of the pair bond between copulating individuals (Hamilton & Arrowood 1978) does not seem like a feasible explanation either, as Columbian ground squirrels are highly promiscuous and males do not invest paternally after mating (Murie 1995).

In contrast to some female primates that emit postcopulatory calls to encourage mate guarding by preferred mating partners (Maestripieri & Roney 2005), our results provide basis for rejection of the female-choice hypothesis. For certain situations, particularly after the first consortship, females that give an estrus call are more likely to acquire an additional mate than females that do not call. Thus, we suggest that estrus advertisement is the most likely social context of female postcopulatory calling in Columbian ground squirrels.

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TABLES

Table 1a-b. Multivariate logistic regression models showing the variables included in the model and their relationship with the likelihood of a female emitting a postcopulatory estrus call. The first model shown is the best-fit model ($AIC_c = 355.2$). The second model shown also fit the data well ($AIC_c = 359.1$). Columbian ground squirrels were observed from two colonies (B and DOT) at Sheep River Provincial Park, Alberta, during 2005-2007 ($N = 396$ copulations, 124 estrous females)

1st Model (Best fit)

Variable	Wald's statistic (χ^2)	Degrees of Freedom	*P*-value
Age of copulating male	9.2	1	<0.005
Male body weight (during breeding)	0.44	1	0.51
Male status (level of territoriality)	2.0	1	0.16

Occurrence of male mate guarding	0.76	1	0.38
Date of copulation	53.1	24	<0.001
Year of copulation	13.9	2	<0.001
Colony of copulation	11.6	1	<0.001
Identity of the consort male	30.6	30	0.43
Identity of the estrous female	106.3	69	<0.005

2nd Model			
Copulatory success of the estrous female (number of mates)	4.24	1	<0.05

Male status (level of territoriality)	0.95	1	0.33
Occurrence of male mate guarding	0.35	1	0.55
Date of copulation	53.9	24	<0.001
Year of copulation	5.32	2	0.07
Colony of copulation	4.21	1	<0.05
Identity of the consort male	21.1	30	0.89
Identity of the estrous female	110.4	69	<0.005

FIGURES

Figure 1a-b. Spectrograms of (a) type 1 notes with 4 harmonics, and (b) type 2 notes with 2 harmonics that were emitted during estrus calls by female Columbian ground squirrels at colony DOT in Alberta during the 2007 breeding period (late April to early May).

Figure 2. Percentage of female's copulations after which she gave an estrus call. The mean ± *SE* number of copulations for each female shown here is 3.5 ± 0.05. The most common percentage is 0% (*N* = 81 estrous females), followed by 50% (*N* = 10 estrous females).

Figure 3. Linear relationship between the age of the consort male and the percentage of copulations followed by estrus call. Y-values are the averages for all males in the sample (± 1 *SE*). Numbers above each bar represent the number of males in the corresponding age group, and numbers below each bar represent the number of copulations in the sample. We combined data from 8-9 year-old males for this analysis because of small sample size for 9-year-olds (*N* = 1 male, 7 copulations).

Figure 4. Percentage of copulations that were followed by an estrus call versus number of mates. Values are the averages for all females in the sample (± 1 *SE*). Numbers above each bar represent the number of females in the corresponding mating success group, and numbers below each bar represent the number of copulations in the sample. We combined data from females with 5-6 mates for this analysis because of small sample size

for females with 6 mates (*N* = 1 female). The significant *P*-value is from the Kruskal-Wallis analysis of variance. The following pairwise comparisons (from Mann-Whitney *U*-tests) are also significant or approach significance: 1 vs. 2 ($P = 0.09$); 1 vs. 3 ($P = 0.01$); 1 vs. 5 ($P = 0.09$); and 3 vs. 4 ($P = 0.07$).

Figure 5. Probability of acquiring an additional mating with the presence or absence of an estrus call for different males in a female's series of matings. Numbers above each bar represent the number of females in the sample. Asterisks denote significance for pairwise comparisons at $\alpha = 0.05$ from *G*-tests.

Fig. 1.

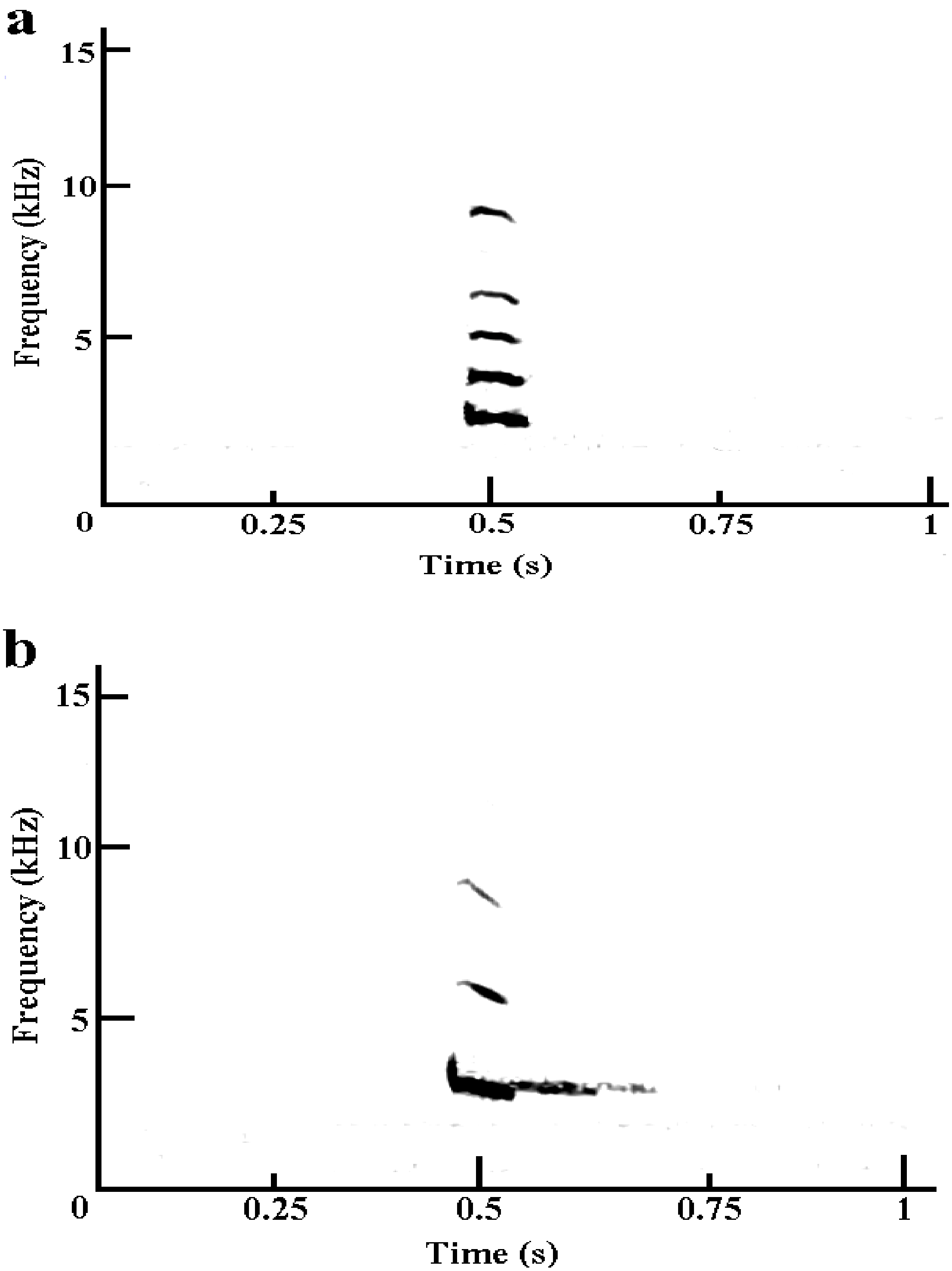

a
15
10
5
0
Frequency (kHz)
0.25
0.5
0.75
1
Time (s)
b
15
10
5
0
Frequency (kHz)
0.25
0.5
0.75
1
Time (s)

Fig. 2.

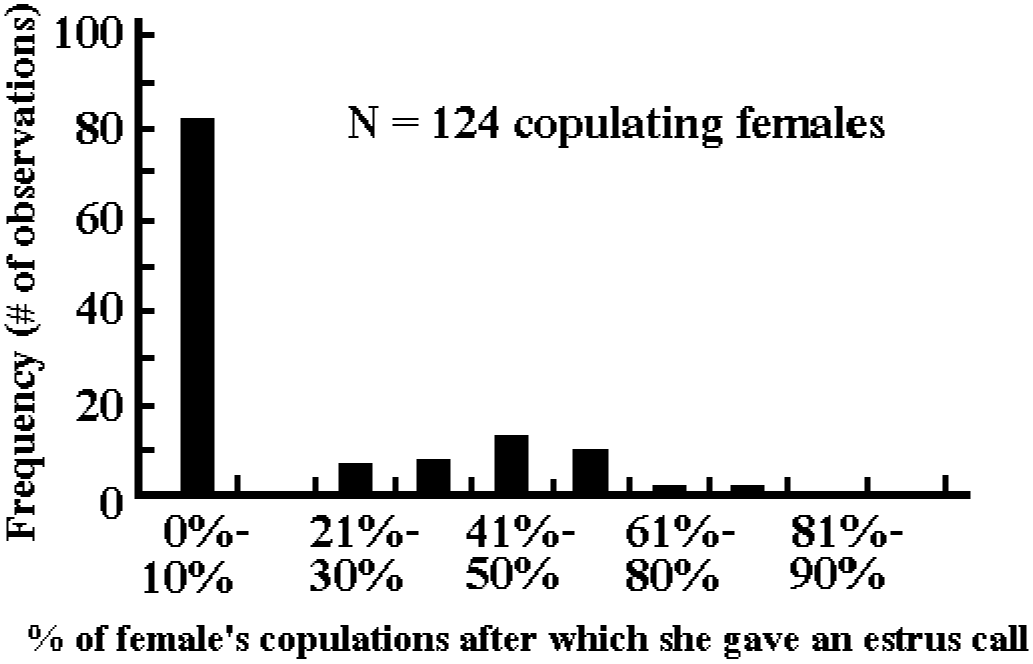

Frequency (# of observations)
100
80
60
40
20
0
N = 124 copulating females
0%-
10%
21%-
30%
41%-
50%
61%-
80%
81%-
90%
% of female's copulations after which she gave an estrus call

Fig. 3.

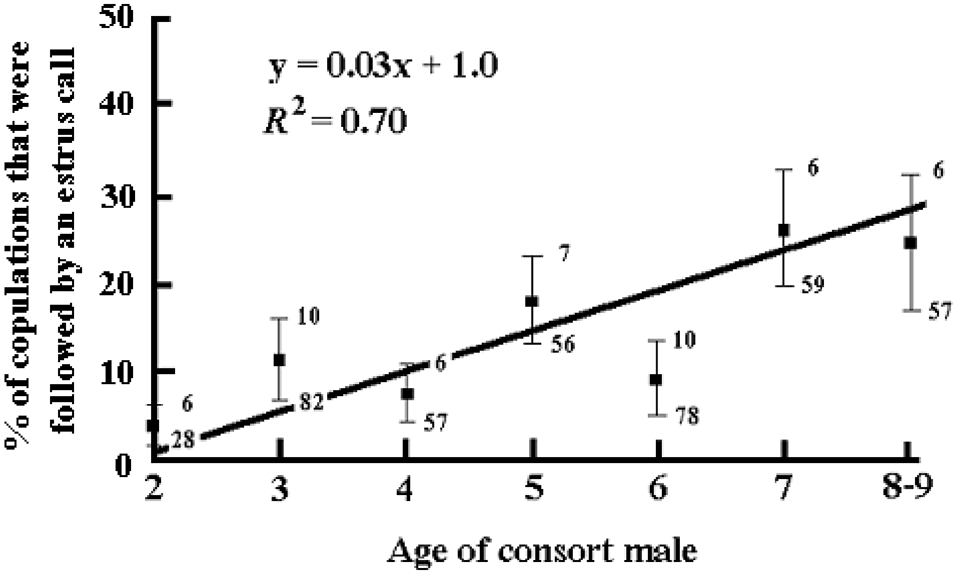

y = 0.03x + 1.0
R2 = 0.70
% of copulations that were followed by an estrus call
Age of consort male
0
10
20
30
40
50
2
3
4
5
6
7
8-9
6
28
10
82
6
57
7
56
10
78
6
59
6
57

Fig. 4.

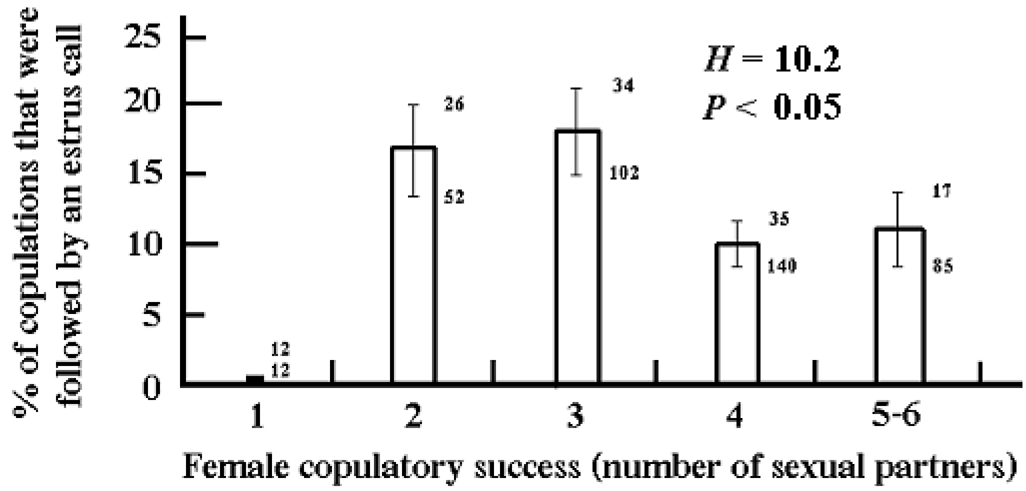
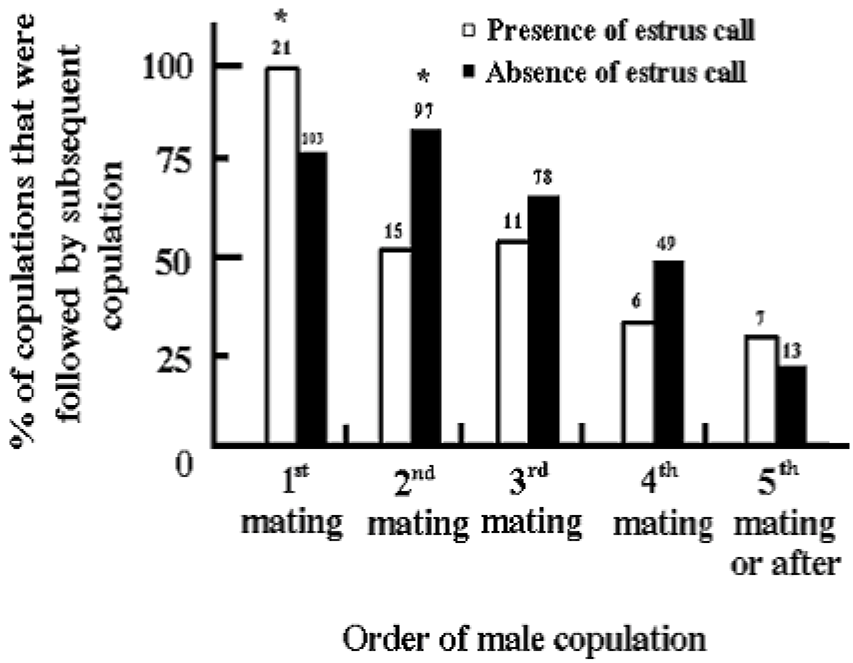

% of copulations that were followed by an estrus call
0
5
10
15
20
25
H = 10.2
P < 0.05
12
12
26
52
34
102
35
140
17
85
1
2
3
4
5-6
Female copulatory success (number of sexual partners)

Fig. 5.


% of copulations that were followed by subsequent copulation
100
75
50
25
0
*
21
103
*
97
15
11
78
6
49
7
13
□ Presence of estrus call
■ Absence of estrus call
1st mating
2nd mating
3rd mating
4th mating
5th mating or after
Order of male copulation

VI. DEATH SCREAM: RESPONSES OF FEMALE COLUMBIAN GROUND SQUIRRELS TO ANTI-INFANTICIDE PUP DISTRESS CALLS

ABSTRACT. Parents sometimes discriminate offspring from non-offspring while responding to vocalizations, ensuring that parental care is directed toward their progeny exclusively. This discrimination may result from differences in acoustic characteristics across individuals. However, recognition capabilities specific to individuals may not occur when parents differentiate offspring via location because litters are segregated spatially. Offspring discrimination is important during “distress calls” emitted by young-of-the-year for imminent danger because mothers that respond to non-offspring reduce the time available for protecting their litter, and failure to respond may result in offspring mortality. Columbian ground squirrel (*Spermophilus columbianus*) pups sometimes emit a scream-like distress call near their mothers when attacked by marauding conspecifics. With field observations of infanticide and playback experiments that manipulated the identity and location of the caller, I examined whether a location-based rule or vocal recognition per se mediate female responses to distress calls. Mothers became vigilant and approached the speaker in response to playbacks of distress calls broadcast on their territory, regardless of whether the recording was an offspring or non-offspring. However, mothers approached distress call recordings originating on their territory more often than those occurring on adjacent territories. Thus, female Columbian ground

squirrels respond to distress calls based on a location-based rule (viz., on or off their territory) rather than only true vocal recognition. This strategy seems appropriate, since lactating females are territorial and pups rarely mix between litters during the 1-5 day period just after emergence from their natal burrow when they are susceptible to infanticide.

INTRODUCTION

Individuals may increase their fitness by providing potentially expensive parental investment to kin exclusively. Thus, parents sometimes discriminate their offspring from non-offspring while responding to vocal signals in avian (e.g., Beecher 1981; Dobson & Jouventin 2003; Searby et al. 2004) and mammalian (e.g., Insley 2001; Illmann et al. 2002) systems. Offspring discrimination may result from differences in acoustic characteristics across individuals, but recognition capabilities specific to individuals may not occur when offspring are differentiated via location because litters or clutches are segregated spatially (Medvin & Beecher 1986; Beecher 1991). While prior studies of parent-offspring recognition have been relegated mostly to begging vocalizations (e.g., McArthur 1982; Barg & Mumme 1994; Lefevre et al. 1998; Draganoiu et al. 2006) and contact calls (e.g., Torriani et al. 2006; Kober 2007), offspring recognition may also extend to “distress calls”, emitted by young that are in imminent danger of predation or being killed by a conspecific (Chaiken 1992; Benedict 2007; Lingle et al. 2007). Distress calls usually induce an approach response by one or both of the caller’s parents, who may then chase a predator or marauder away from their offspring (Rohwer et al. 1976; Hogstedt 1983). Parents that respond to distress calls emitted by non-offspring may therefore place themselves under unnecessary predation risk or reduce the time available for protecting their young. However, a delay or failure to respond to an offspring distress call may result in offspring mortality. Thus, parental-offspring recognition of distress calls may play a role in maximizing fitness.

Responses to distress calls may be mediated by either of two behaviors related to offspring discrimination. First, recognition of individual offspring vocalizations per se could induce approach responses by parents (Beecher et al. 1981; Lefevre et al. 1998). This explanation usually applies to young from different mothers that are mixed together, as for the contact calls of animals such as penguins and bats (Van Parijs & Corkeron 2002; Searby et al. 2004). Second, parents may use a location-based rule for response (Beecher et al. 1981; Stoddard & Beecher 1983; Sherman et al. 1997). That is, when individuals are spatially segregated and mixing of young is unlikely, sophisticated recognition abilities such as individual recognition are unnecessary if parents can distinguish offspring from non-offspring via location. A location-based response pattern would put parents at unnecessary predation risk if responding to the calls of non-kin, but such a response rule may be adequate if individuals maintain territories on which they raise young, and if non-offspring would rarely emit distress calls in the vicinity of offspring.

Columbian ground squirrels (*Spermophilus columbianus*) are diurnal, herbivorous, and colonial rodents (Betts 1976; King & Murie 1985; Manno 2008). During a 3-week mating period that occurs a few days after emergence from hibernation in mid-April, females live in philopatric kin clusters that are overlapped by a territorial reproductive male (Murie & Harris 1978; King & Murie 1985). Males, who do not invest paternally, abandon their territories after this period. Meanwhile, females start defending territories of 10-30 m^2, rearing their offspring in separate nursery burrows (Festa-Bianchet & Boag 1982). Gestation averages 24 days, litter size is 2-4, and weaned pups emerge from their nursery burrows in late June after 27 days of lactation (Murie & Harris 1982). Like the pups of Richardson's ground squirrels (*Spermophilus richardsonii*) and black-tailed prairie dogs (*Cynomys ludovicianus*) (Hoogland 1995; Hare 1998), Columbian pups forage on their natal territory for 1-5 days following their emergence (Raynor & Armitage 1991). During this period, pups are susceptible to infanticide, mostly by female conspecifics (Balfour 1993; Waterman 1984; Dobson 1990; Stevens 1998; Manno 2007a). Infanticide risk and parental care ceases afterwards, as pups disperse to adjacent territories or other parts of the colony and mingle with members of other litters.

Columbian ground squirrel pups handled shortly after their first capture sometimes emit loud, piercing, scream-like vocalizations for 3-10 seconds. During these screams, the mother of the pup usually approaches to within 1 m, waiting until the conclusion of the vocalization to run off. The same "distress calls" and reaction occur during infanticidal attacks by female conspecifics under natural conditions (Waterman 1984). In the pages that follow, I examine field observations of attempted maraudings, and detail playback experiments intended to determine whether mothers use recognition of individual offspring vocalizations or a location-based pattern when responding to distress calls. If mothers respond to distress calls based on recognition of their own offspring, then mothers should approach the distress calls of their offspring preferentially over the distress calls of non-offspring. On the other hand, if mothers respond to distress calls based on location cues, then mothers should approach calls that occur on their territory indiscriminately, with an equal tendency to approach offspring and non-offspring distress calls. Under the location hypothesis, mothers should respond only to distress calls that originate on their territories near their offspring. Since these hypotheses are not mutually exclusive, elements of both of these trends may be present if offspring recognition and location cues are important.

METHODS

Fieldwork

From April to July in 2006 and 2007, field assistants and I studied wild, free-ranging Columbian ground squirrels of known age and matrilineal genealogy at colony DOT in Sheep River Provincial Park, Alberta, Canada (50° 38′ N, 114° 38′ W, elev. 1500 m). The study colony occupied 2.8 ha of meadowland surround mostly by coniferous woodland and measured about 140 m (north-south) by 200 m (east-west). For permanent identification of individuals, I used fingerling eartags (National Band & Tag Co., Newport, KY). For visual identification from a distance, I painted each animal with a unique symbol using black dye (Lady Clairol Hydrience; Proctor & Gamble, Stamford, CT). Assisted by 4-m-high observation towers and 10x42 binoculars, my assistants and I used the methods of Hoogland (1995) and Hoogland et al. (2006) to observe marked individuals every day from dawn until dusk.

I identified a male as reproductive if I observed him copulate or if he exhibited a pigmented scrotum and large descended testes at the time of trapping. Nonbreeding males had gray or pink scrotums with undescended testes (Murie 1995). I identified a female as reproductive if I observed her copulate or if she was lactating (viz., with long, turgid nipples) in May or June. I also trapped females several additional times during the 3-week breeding period and examined their vulvar condition to determine whether they had been estrous (viz., with fully opened vulva). A "mother" is a female that weaned a litter, and was therefore defending territory when her pups first emerged from the natal burrow.

Like the females of black-tailed prairie dogs (*Cynomys ludovicianus*) and Belding's (*Spermophilus beldingi*) and round-tailed (*Spermophilus tereticaudus*) ground squirrels (Dunford 1977; Sherman 1980; Hoogland 1995), Columbian ground squirrels reared their offspring in separate nursery burrows (Festa-Bianchet & Boag 1982). Maternity was therefore easy to establish. Pups first emerged from their natal burrow in June when they were about 4 weeks old. By surrounding natal burrows with traps a few minutes after pups first appeared aboveground, I captured, eartagged, and marked all littermate siblings before they dispersed and mixed with other pups (Hoogland 1995).

When a social interaction occurred (viz., chasing, fighting, sniffing, allogrooming, playing, females "leading" males), I used all-occurrence sampling (Altmann 1974) to record the individuals involved, which individual instigated the interaction, and the time and location of occurrence (ascertained from a 10m x 10m grid placed with flags on the colony). These methods allowed me to record hostile interactions between females during gestation and lactation, including interaction with and infanticidal attacks on pups. Using these samples, I defined a territory for females as the established area in which they were victorious in hostile interactions with other females (Dobson 1983).

Playback experiments

I conducted playback experiments during late June and early July 2007, during the period when litters were emerging from their natal burrows for the first time. The manipulations employed playbacks of distress calls recorded in June 2007 from 10 pups from separate litters that were 1 day old and in my hand shortly following their first capture. I recorded the distress calls with a digital recorder (Marantz PMD-660; Marantz America, Inc., Mahwah, NJ) with a 256 GB Lexar Compact Flash Drive (Lexar Media Inc., Fremont, CA), and a directional condenser microphone encased in a windscreen (Shure PG-81; Shure Inc., Niles, IL). I then created 10 separate playback tracks (one per pup) using at least 5 exemplars of the vocalizations with Raven 1.2 (Cornell Lab of Ornithology, Ithaca, NY) and a COMPAQ Presario 2100 computer using 512-point short-time Fourier transformations with 50% overlap, and a Hamming window (see also Manno et al. 2007). Playback tracks contained a series of the 5-10 distress calls (3-10 seconds per series of calls) emitted from a single pup. The spacing of these calls was not adjusted, so as to reflect natural vocalization patterns, and the time between calls ranged from <1 sec to 2 sec (Benedict 2007). According to specifications, all equipment covered a frequency range of at least 20-20,000 Hz and had a flat frequency response.

I always conducted playbacks in the absence of other disturbances (e.g., predators), removed 30 minutes from other playbacks (no more than 5 per day; Hoogland 1995), and within 5 days of the litter's first emergence from the natal burrow (viz., during the period a mother's pups were at risk for infanticide). These procedures aimed to mimic distress call occurrences under natural conditions (described in results). I chose female subjects that occupied non-overlapping territories. For each trial, I placed speakers just above the ground in vegetation, concealing them in a stack of 6 single door traps (Tomahawk, 13 x 13 x 40 cm) (the squirrels were habituated to having traps on their territory). As an extra precaution, this equipment was set in place 1-2 hours before the playback. I broadcasted the playbacks of distress calls at a sound pressure level of 90 dB at 5 m from the sound origin, and monitored playback trials from the observation towers and recorded the identity of the responder, occurrence of an approach to the speaker (defined as movement to within 3 m of the sound), time spent within 3 m of the speaker, and time spent vigilant (viz., standing on both rear legs, *after* Manno 2007b). I scored these data during playback and up to 2 min thereafter (also see below). As a control, I also observed behavior during and following the playback of a 2 min continuous white noise track with the same amplitude as the playback calls.

To examine the role of pup identity in responses to distress calls, I gave 10 mothers a preference test in which they were presented on consecutive days with two recordings: (1) a series of distress calls recorded from their own offspring, preceded by 2 min of pre-playback silence and followed by 2 min of post-playback silence; and (2) a series of distress calls recorded from non-offspring (which was recorded on a territory ≥2 intervening territories away from the focal territory) from another speaker on the opposite side of the natal burrow, also preceded and followed by 2 min of silence. The presentation of playbacks at the same time on consecutive days controlled for location effects. I waited until after the mother either continued to feed or returned to feeding for 30 sec after being vigilant to play the second recording. On the first day, I randomized the order of presentation for offspring versus non-offspring distress calls. The next day, I played the recordings in the opposite order. I placed the speakers on the focal territory 2 m from the natal burrow, and each audio track was used twice as an offspring stimulus on the focal territory and a non-offspring stimulus on another territory. I conducted half of the playbacks between 800 and 1100 hours and the other half between 1600 and 1900 hours.

To examine the role of pup location in responses to distress calls, I gave 8 mothers a preference test in which they were presented with a series of distress calls recorded from a pup that was not their offspring. On three successive days, I presented mothers with the same recording either 2 m from their natal burrow, 2 m inside their territory boundary, and 2 m from the natal burrow of an adjacent territory. I randomly selected the playback location order, using each audio track for no more than two mothers. These recordings also consisted of the 3-10 sec distress calls, preceded by 2 min of pre-playback silence and followed by 2 min of post-playback silence. Ideally, I would have also ran treatments where mothers were presented with distress calls from their own offspring, but the amount of time for experiments was short because infanticide occurs only 1-5 days after young emerge from their natal burrow, and habituation of the squirrels to the distress calls was also a concern.

Because of the short length of distress vocalizations, my results preclude a robust statistical test for individual, sex, or age differences. Superficially, however, there do not appear to be any such differences. Five distress vocalizations given by a female in my hand were similar in note length and fundamental frequency to those of a male. There were also no significant difference in length and fundamental frequency between 10 distress vocalizations from each of three different individuals at 1, 2, and 3 days old ($P > 0.30$ for both length and frequency; Kruskal-Wallis: $H = 0.07$ and 0.12, respectively).

Values are means ± 1 *SE*. All *P*-values result from two-tailed tests, and paired analyses when appropriate.

RESULTS

Pups handled on their natal territory shortly after their first capture sometimes emitted 5-10 screaming vocalizations in succession, which I deemed "distress calls". I identified two different sounds, both having stacked frequencies that reached 18-22 kHz (Fig. 1a-b). I took no data on the frequency of or responses to these calls during trapping in 2006, nor did I record the calls digitally. In the following year, about one-third (29/79 = 36.7%) of captured pups emitted distress calls as I handled them on their natal territory. On almost three-fourths of these occasions (21/29 = 72.4%) the mother of the vocalizing pup approached to within 2 m of where I was sitting and assumed an upright position facing me. No other individuals ever approached me. Pups that emitted distress calls vocalized for 3-10 seconds.

In June 2006, I witnessed an infanticide and 5 unsuccessful attacks on pups. All of these maraudings were perpetrated by reproductive females on the natal territory of the pup, and all occurred 1-2 days after the pup had emerged from its natal burrow. The infanticide victim emitted distress calls for a few seconds and her mother approached the spot of attack. Despite the response, the marauder killed the pup quickly and threw the body into her burrow after pounding its corpse repeatedly. The other attacks also featured short distress calls by the endangered pup, and the mothers of the pups quickly approached the site of attack and fought off the marauders. In all of these cases, the mother of the attacked pup was the only individual to approach the site of the marauding. I saw no infanticidal attacks in 2007.

Playback experiments on the natal territories of 10 females showed that the responses of mothers to the distress calls of their offspring did not differ significantly from responses to distress calls of non-offspring (Fig. 2a-b). Specifically, mothers did not spend significantly more time vigilant (58.9 ± 3.2 min for offspring vs. 57.8 ± 4.6 min, $t = 0.20$, *d.f.* = 8, $P = 0.85$) or in the vicinity of the speaker (41.1 ± 2.4 min for offspring vs. 44.0 ± 2.7 min, $t = 0.81$, *d.f.* = 8, $P = 0.43$) during and following offspring playbacks when compared with non-offspring playbacks. Responses to offspring and non-offspring playbacks were greater than responses to the control sound with respect to time spent vigilant ($t = 16.4$, *d.f.* = 8, $P < 0.001$ and $t = 11.4$, *d.f.* = 8, $P < 0.001$, respectively) or in the vicinity of the speaker ($t = 14.1$, *d.f.* = 8, $P < 0.001$ and $t = 13.8$, *d.f.* = 8, $P < 0.001$, respectively). Furthermore, all 20 playbacks of distress calls (10 offspring, 10 non-offspring) elicited an approach by the mother to within 3 m of the playback speaker, while 3/20 (15%) of the control sounds elicited approaches.

Playback experiments that varied the location of the sound showed that mothers were more likely to respond to distress calls that originated near their offspring than away from their natal burrow (Fig. 3a-b). Specifically, time spent vigilant was significantly lower during and following playbacks on an adjacent territory of the mother when compared to playbacks near her natal burrow (3.9 ± 1.0 min when adjacent vs. 60.4 ± 5.2 min, $t = 10.6$, *d.f.* = 8, $P < 0.001$), and the edge of the territory (27.7 ± 2.4 min on edge, $t = 11.2$, *d.f.* = 8, $P < 0.001$). This was also the case for time spent in the vicinity of the sound, which was reduced for playbacks on adjacent territories when compared to playbacks near the natal burrow (3.4 ± 0.5 min when adjacent vs. 52.6 ± 3.5 min, t = 13.9, *d.f.* = 8, $P < 0.001$), and the edge of the territory (55.6 ± 6.5 min on edge, t = 8.0, *d.f.* = 8, $P < 0.001$). Responses to playbacks near the natal burrow and the edge of the territory did not differ significantly with respect to time spent vigilant (t = 0.22, *d.f.* = 8, P = 0.82), or in the vicinity of the speaker (t = 0.41, *d.f.* = 8, P = 0.69), but both were significantly greater than responses to their respective control sounds. Furthermore, all 16 trials that occurred near the natal burrow or on the edge of the mother's territory elicited an approach by the mother to within 3 m of the playback speaker, while only 3/8 (37.5%) of adjacent territory trials and 3/8 (37.5%) control trials elicited approaches.

Respondents always approached a few seconds after the distress calls were played and appeared agitated as they rushed around the speaker. Responses to playbacks did not differ significantly between first, second, and (if applicable) third trials with respect to time spent vigilant or in the vicinity of the speaker. Only the mother that occupied the territory on which the playback occurred responded.

DISCUSSION

Like investigators before me (Waterman 1984), I noticed that Columbian ground squirrel pups occasionally emitted distress calls upon being handled after first capture and when attacked by conspecifics. My field observations further indicated that mothers approached the area of the disturbance when her offspring emitted a distress call. I examined whether mothers use recognition of individual offspring vocalizations or a location-based pattern when responding to distress calls with playback experiments that varied the identity and the location of the caller. Mothers could approach the distress calls of their own offspring preferentially, but respond to location cues (viz., on their natal territory) rather than distinguishing between offspring and non-offspring per se. While other studies on individual discrimination and location variation address distress calls emitted during predation or becoming “lost” from a group, my study examined distress calls by pups emitted during infanticidal attacks, the only instance during which I observed such vocalizations.

European Starlings (*Sturnus vulgaris*) discriminate between the distress calls of offspring and unrelated chicks (Chaiken 1992). If Columbian ground squirrel mothers recognize individual offspring distress calls, then mothers should approach the distress calls of their own offspring preferentially over the distress calls of foreign young. My results did not support this prediction. Consistent with previous studies that failed to demonstrate individual characteristics in various vocalizations for Columbians (Betts 1976; Harris et al. 1983; Manno et al. 2007), mothers showed no evidence of individual distress call recognition. All playback trials featured approaches to the speaker and vigilance for 1-2 minutes, regardless of whether the recording was an offspring or non-offspring distress call. These results argue against the notion that mothers recognize individual offspring distress calls, although the rare nature of the call precluded a test of whether sufficient information was present in the calls to allow for discrimination of individuals (*sensu* Placer & Slobodchikoff 2004; Manno et al. 2007). In any case, even if individual recognition occurs, mothers do not seem to respond to distress calls using these criteria. Whereas the starling fledglings from multiple broods frequently mix and emit distress calls to deter predation while mixing, female ground squirrels maintain exclusive territories during a 1-5 day period after pups emerge from their natal burrow and may give distress calls if attacked. Since mixing of litters during the period of susceptibility for pups is rare, recognition of individual distress calls on acoustic qualities alone may be unnecessary for Columbian ground squirrels.

California Towhee (*Pipilo crissalis*) parents have a location-based decision rule for responding to the antipredator distress calls of chicks (Benedict 2007). If Columbian ground squirrel mothers approach distress calls based on location cues, then mothers should respond only to distress calls that originate on their territory near their offspring. My results supported this prediction. Indeed, mothers approached distress calls on their territory, but not on adjacent territories. These responses occurred even though the distress calls were from non-offspring. Distress call playbacks that were broadcasted near a mother's natal burrow elicited more vigilance than playbacks on the edge of her territory, so females may have responded to calls that were close to their offspring with more vigor than calls that were farther from the natal burrow (though on their territory nevertheless). The responses did not occur because the playbacks were inaudible, since Columbian ground squirrels respond to playbacks of the same amplitude at much greater distances (Manno et al. 2007). Thus, my results are best explained by the location-based decision hypothesis. While the location discrimination seems less refined than in California Towhees that respond only to distress calls in a small area around their chicks (Benedict 2007), pup location appears to determine maternal behavior nevertheless.

During playbacks that manipulated call location, female Columbian ground squirrels routinely responded to broadcasts of non-offspring distress calls that occurred either near the natal burrow of their offspring or on the edge of their territory. Why do female Columbian ground squirrels respond to all distress calls on their territory?

Probably because, like California ground squirrels that defend their offspring against snakes (Swaisgood et al. 1999), females are territorial and the likelihood that effort expended towards defense of the natal burrow would be wasted on unrelated pups is unlikely. Like the pups of black-tailed and Utah prairie dogs (*C. ludovicianus* and *C. parvidens*; Hoogland 1995) and several other ground-dwelling sciurids (Raynor & Armitage 1991; Hare 1998), Columbian ground squirrel pups rarely disperse more than 1-3 m from their natal burrow during the few days directly following their first emergence, and this is the period during which pups could be attacked by marauders and emit distress calls (Waterman 1984). Even if parents did occasionally react to non-offspring pups, they may still become aware of infanticidal threats on their territory that may affect their litter, and would usually be responding appropriately. Though colonial, Columbian ground squirrel pups rarely mix when they are susceptible to infanticide, so a location-based response for responding to distress calls is apparently sufficient for females.

Might the location-based rule simply result from females being hesitant to approach the playback speaker closely, since that would necessitate invading another female's territory? The answer is probably negative, for one major reason. If females were simply hesitant to invade a neighbour's territory, they would still be expected to remain vigilant from the edge of the territory. My results did not support this prediction, and therefore, support for the location-based hypothesis probably does not result from the setup of the experiments.

My results are consistent with theoretical studies that predict a location-based rule for discrimination of offspring distress calls when litters or clutches are segregated spatially (Medvin & Beecher 1986; Beecher 1991). Though these studies were originally intended to examine this trait in the context of bird species, my results suggest that mammalian societies may provide an exciting avenue for future research that examines offspring vocal recognition with respect to individual characteristics and location-based patterns.

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FIGURES

Figure 1a-b. Spectrograms of the two sounds (a and b) emitted during distress calling juvenile Columbian ground squirrels from colony DOT at Sheep River Provincial Park, Alberta, during 2006-2007.

Figure 2a-b. Time spent vigilant (a) and within 3 m of the playback speaker (b) for female Columbian ground squirrels during playbacks that manipulated pup identity. I conducted trials on colony DOT at Sheep River Provincial Park, Alberta, in June 2007. I recorded “own” distress calls from a pup of the experimental territory and “foreign” distress calls were recorded from a pup that was resident on a territory at least one intervening territory away from the experimental territory. Boxplots indicate median values and interquartile ranges. The numbers above the bars represent the number of females in the sample.

Figure 3a-b. Time spent vigilant (a) and within 3 m of the playback speaker (b) for female Columbian ground squirrels during playbacks that manipulated caller location. I conducted trials on colony DOT at Sheep River Provincial Park, Alberta, in June 2007. I presented calls of non-offspring to mothers 2 m from their natal burrow, 2 m inside their territory boundary, and 2 m from the natal burrow of an adjacent territory. Boxplots indicate median values and interquartile ranges. The numbers above the bars represent the number of females in the sample.

Fig. 1a-b.

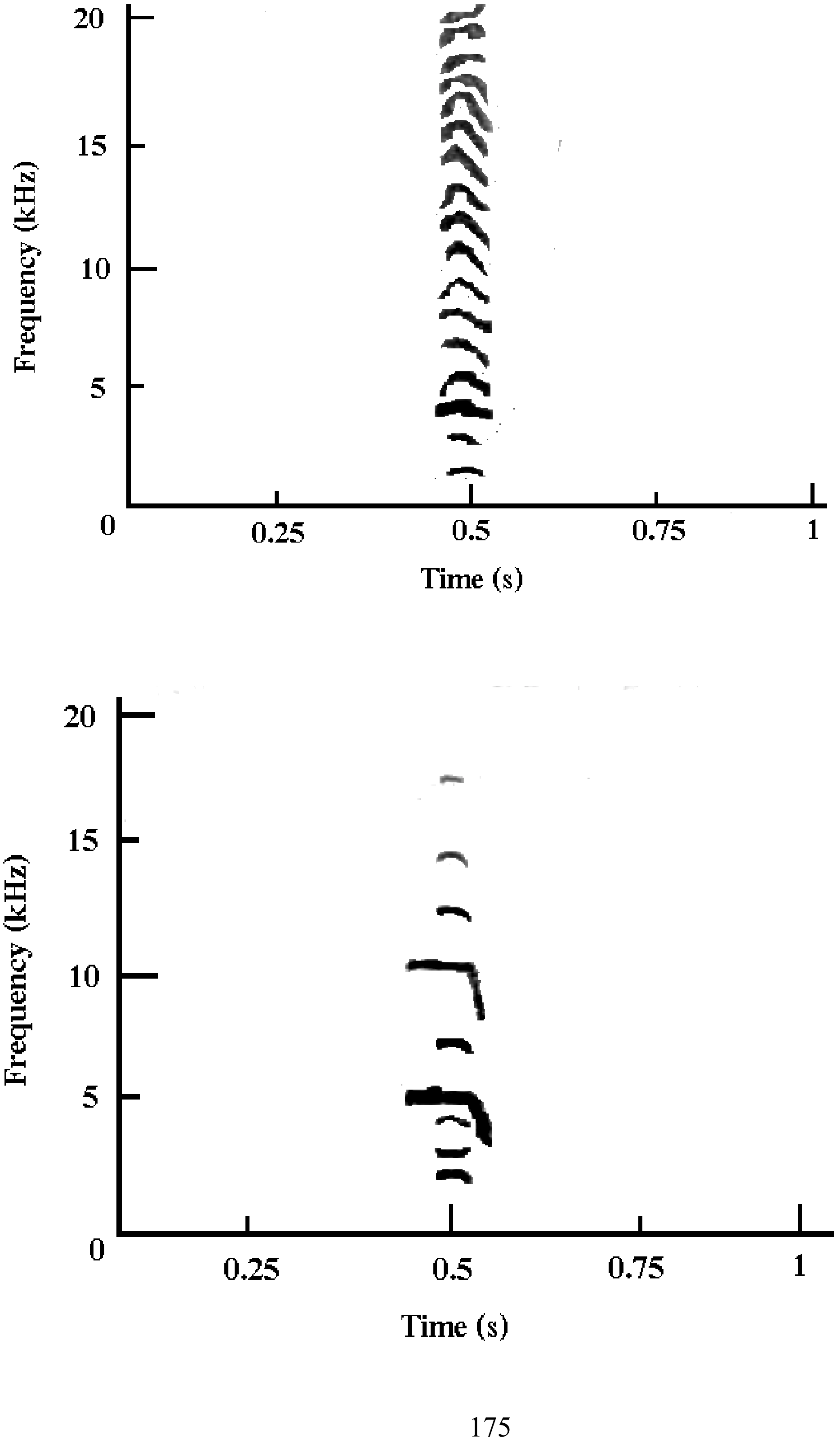

20
15
10
5
0
Frequency (kHz)
0.25
0.5
0.75
1
Time (s)
20
15
10
5
0
Frequency (kHz)
0.25
0.5
0.75
1
Time (s)

Fig. 2a-b.

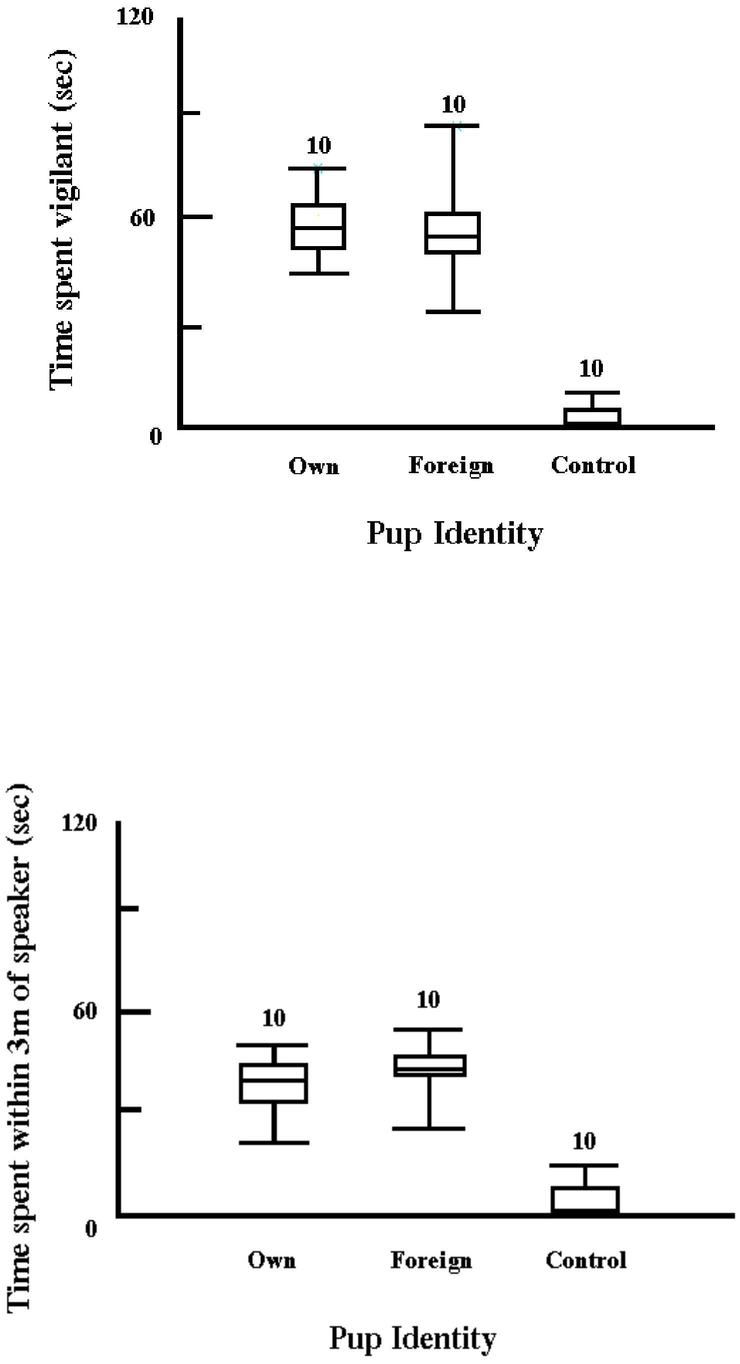
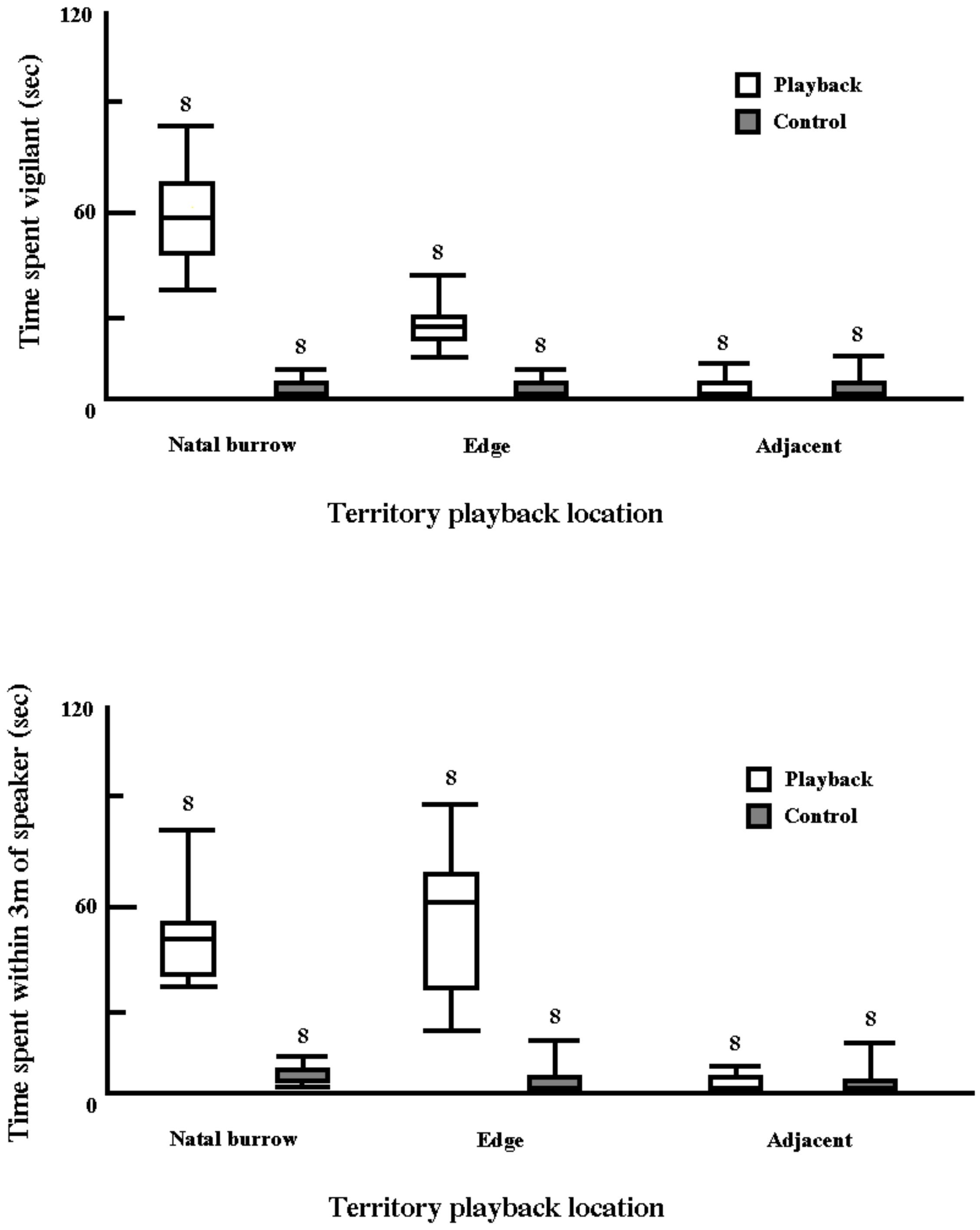

120
60
0
Time spent vigilant (sec)
10
10
10
Own
Foreign
Control
Pup Identity
120
60
0
Time spent within 3m of speaker (sec)
10
10
10
Own
Foreign
Control
Pup Identity

Fig. 3a-b.



120
60
0
Time spent vigilant (sec)
8
8
8
8
8
8
Playback
Control
Natal burrow
Edge
Adjacent
Territory playback location
120
60
0
Time spent within 3m of speaker (sec)
8
8
8
8
8
8
Playback
Control
Natal burrow
Edge
Adjacent
Territory playback location