



## Why do male Columbian ground squirrels give a mating call?

THEODORE G. MANNO\*, ANNA P. NESTEROVA†, LILIANA M. DEBARBIERI‡,  
STUART E. KENNEDY§, KELSEY S. WRIGHT\* & F. STEPHEN DOBSON\*

\*Department of Biological Sciences, Auburn University

†Department of Biology, University of Pennsylvania

‡Department of Environmental Studies, Prescott College

§WSHU Public Radio Group, Fairfield

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Male Columbian ground squirrels, *Spermophilus columbianus*, give 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 oestrous females; and (4) whether mating calls advertise the caller to females that have not yet mated. Our approach was to observe mating behaviour 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 oestrous 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 given. 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.

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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 favour 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; Heth et al. 1988). Mated females are hindered from extrapair 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; Møller 1991; Kelley 2004; Velez & Brockmann 2006).

For species such as black-tailed prairie dogs, *Cynomys ludovicianus*, mating calls are acoustically unique vocalizations (Grady & Hoogland 1986; Hoogland 1995). But

Correspondence and present address: T. G. Manno, Department of Biological Sciences, 331 Funchess Hall, Auburn University, AL 36849, U.S.A. (email: [mannotg@auburn.edu](mailto:mannotg@auburn.edu)). A. P. Nesterova is at the Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, U.S.A. L. M. DeBarbieri is at the Department of Environmental Studies, 220 Grove Avenue, Prescott College, Prescott, AZ 86301, U.S.A. S. E. Kennedy is at the WSHU Public Radio Group, 5151 Park Avenue, Fairfield, CT 06432, U.S.A. K. S. Wright and F. S. Dobson are at the Department of Biological Sciences, 331 Funchess Hall, Auburn University, AL 36849, U.S.A.

other animals such as Formosan squirrels, *Callosciurus erythraeus thaiwanensis*, give 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). Furthermore, 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, 1991; Leger 1993).

Broadcasting the presence of an oestrous female might compromise a male's opportunity to sire progeny, owing to the increased possibility of subsequent copulations with the oestrous 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 nonreproductive animals of both sexes (King & Murie 1985). During a 3-week mating period, a territorial reproductive male (usually  $\geq 3$  years old) overlaps the ranges of one or a few females (Murie & Harris 1978, 1988). Young subordinate males (2–3 years old) usually do not maintain a territory, but are physically able to reproduce and sometimes obtain copulations (F. S. Dobson, T. G. Manno, P. H. Jones & A. P. Nesterova, unpublished data). Females are highly promiscuous during their annual day of oestrus, 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; J. O. Murie, personal communication).

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 oestrous females; and (4) whether mating calls advertise the caller to females that have not yet mated. Our approach was to observe mating behaviour 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

### Oestrus 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, elevation 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 ear tags for long-term identification (National Band & Tag Co., Newport, KY, U.S.A.). 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, U.S.A.). We considered males with 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 were in oestrus (viz., had a 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 behaviours: (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) behaviours 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 the breeding season during which they were territorial.

### Recording of Vocalizations and Vigilance

Males sometimes give a series of 'chirps' shortly after copulating with an oestrous female. During our observations of oestrus and copulation, we noted every time this occurred; each series of chirps was termed a 'mating call' (after Grady & Hoogland 1986; Hoogland 1995). In 2006, three of us (T.G.M., L.M.D., K.S.W.) 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 behaviours 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 forebody 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 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, U.S.A.) towards reproductive males that had previously given mating calls, 2 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 (T.G.M. and L.M.D.) retreated to our towers, which were located about 20 m from where the badger was placed, and one of us (K.S.W.) 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, K.S.W. pulled the badger from behind the blind at a constant rate of 25 cm/s, while T.G.M. and L.M.D. recorded subsequent calls, noting the same types of reaction data as for the mating calls from the observation towers. We conducted no more than two experimental runs per day on the same male ( $N = 24$  attacks total), and all were separated by more than 1 h 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, U.S.A.) with a 256GB Lexar Compact Flash Drive (Lexar Media, Inc., Fremont, CA, U.S.A.), a directional condenser microphone encased in a windscreen (Shure PG-81; Shure, Inc., Niles, IL, U.S.A.) and a parabolic reflector (Mineroff Electronics, Elmont, NY, U.S.A.). We generated spectrograms and oscillograms with Raven 1.2 (Cornell Lab of Ornithology, Ithaca, NY, U.S.A.) using 512-point short-time Fourier transformations with 50% overlap, and a Hamming window. We selected three notes (viz., separate sounds or 'chirps' given 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.

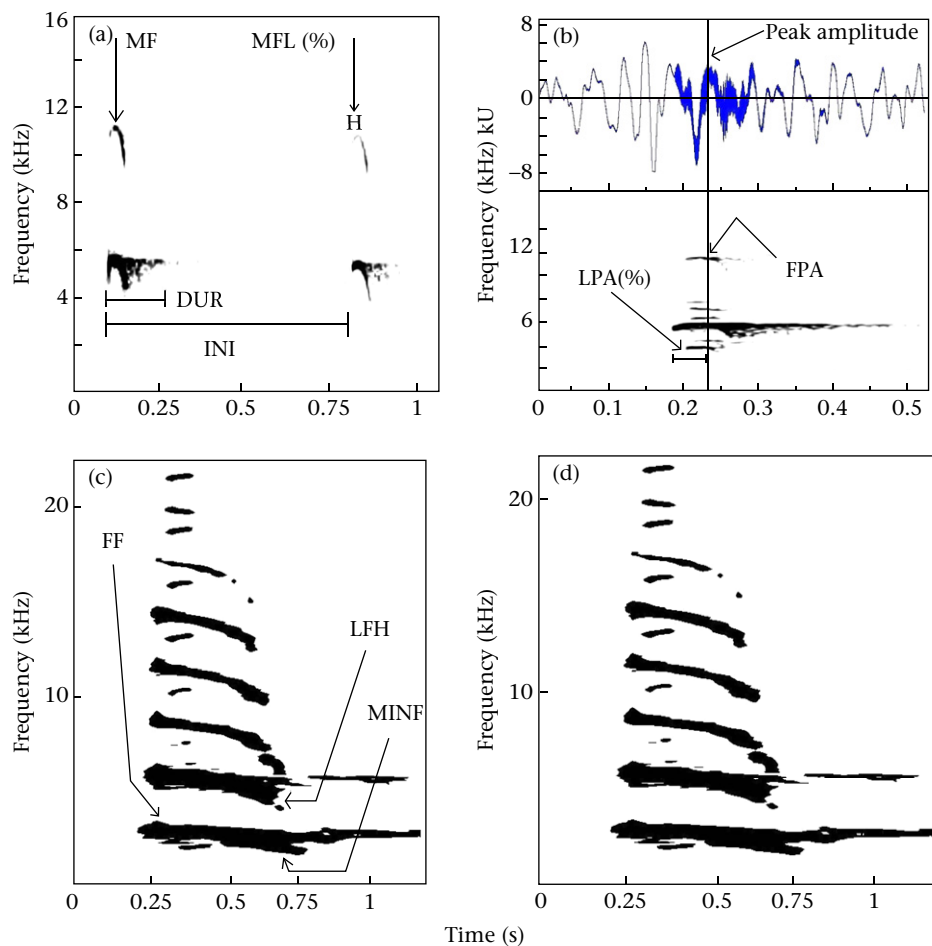
## Playback Experiments

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 (T.G.M.) 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 five exemplars of each stimulus). T.G.M. then noted the reactions of individuals as described above. We conducted five playbacks (one 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 two experimental runs per day in the same area, and all were separated by more than 1 h to prevent habituation of the squirrels to the stimuli.

## Statistical Analysis

We digitized nine coordinates from the spectrograms to calculate dependent variables used in a stepwise discriminant function analysis (MINITAB 13.32; Minitab, Inc., State College, PA, U.S.A.) to determine whether 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 analysed 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 Institute, Inc., Cary, NC, U.S.A.) to determine whether 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 individual in the same and 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 behaviour of listening squirrels, we considered samples to be independent if they were 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



**Figure 1.** (a–d) Spectrograms of (a) the pure shrill and (b) the harsh shrill given during the simulated attacks (with oscillogram on top showing amplitude versus 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 (ms); DUR, note duration (ms); 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); and LFH, lowest frequency of first harmonic (kHz).

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* values show the number of individuals in the sample. Values are presented as means  $\pm$  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  $3.3 \pm 0.12$  males. About one-third of the copulations (80/216) were followed by a mating call, and over 95% (60/63) of the oestrous females elicited at least one mating call during their series of matings. The mean duration of the mating calls was  $21.9 \pm 3.7$  min (range: 1–83 min), and mating calls consisted of 200–2000 individual notes.

During all 80 mating calls ( $N = 27$  males), we observed male mate-guarding behaviours (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 given during mating calls resembled the antipredator ‘soft chirps’ of Koepl et al. (1978) and Betts (1976) (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 five of the eight reproductive males at DOT that gave 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), Koepl 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 gave ‘soft chirps’ (Fig. 1c). The mean duration of the calling bouts (including shrill and soft chirps) was  $16.5 \pm 2.1$  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  $15.4 \pm 0.3$  min ( $N = 53$  individuals). The mean for maximum vigilant postures was  $3.5 \pm 0.7$  (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 with 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  $1.3 \pm 0.3$  min ( $N = 40$  individuals). The mean for maximum vigilant postures was  $2.4 \pm 0.5$  (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 ( $21.9 \pm 3.7$  versus  $16.5 \pm 2.1$  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  $4.2 \pm 0.5$  min. Analysis of variance (ANOVA) showed that all sound characteristics except internote interval and note duration 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 nine 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 Euclidean distance squared = 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:  $1.2 \pm 0.3$ ; 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 s after the playbacks started (mean for maximum vigilant postures:  $3.2 \pm 0.7$  and  $3.4 \pm 0.8$  respectively, Table 2); no squirrel entered a burrow. Squirrels ran to a burrow more often during mating calls and alarm calls than during 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

**Table 1.** Data for nine 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

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

Characteristics are labelled as in Fig. 1a–d, and the numbers in parentheses represent the number of notes that were measured for analysis. INI, internote interval; DUR, note duration; MF, maximum frequency attained by note; FPA, frequency of first harmonic at peak amplitude; MFL, maximum frequency location; MinF, minimum frequency attained by note; LPA, location of peak amplitude; HFB, highest frequency of first harmonic; LFH, lowest frequency of first harmonic.

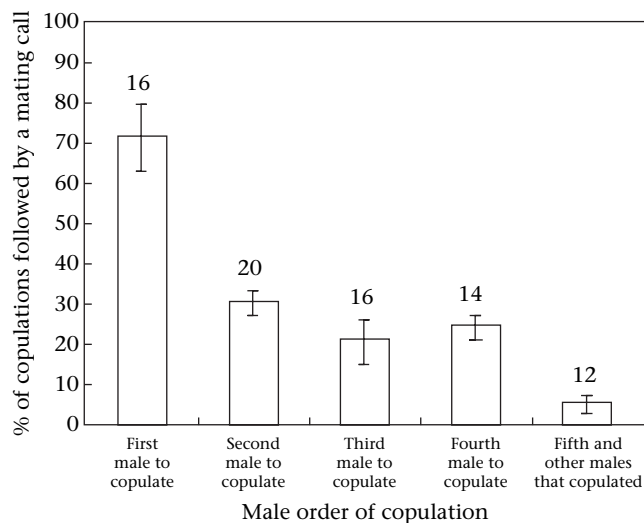
**Table 2.** Duration and type of reaction elicited by the playbacks of mating calls, alarm calls, and the control sound to free-ranging Columbian ground squirrels living along the Gorge Creek Trail in 2006

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

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 oestrous 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 ( $5 \times 2$



**Figure 2.** Probability of giving a mating call versus the order in which a calling male mated with an oestrous female (mean  $\pm$  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 was significant (analysis of variance:  $P < 0.001$ ). The following  $2 \times 2$  pairwise analyses were also significant: first versus second, third, fourth, and fifth and others, second versus fifth and others, third versus fifth and others, and fourth versus fifth and others.

chi-squared 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 oestrous 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 ( $39.7 \pm 6.2\%$  versus  $23.5 \pm 6.2\%$ ;  $t_{24} = 1.8$ ,  $P = 0.08$ ). 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 ( $1.2 \pm 0.2$  versus  $0.63 \pm 0.09$  h;  $F_{5,57} = 3.7$ ,  $P < 0.05$ ).

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 preoestrus 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 collinearity between independent variables was unlikely to affect these results (all  $r < 0.70$ ).

**Table 3.** Significance of nine variables on the likelihood of a male giving 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 oestrous females)

Variable	Wald's statistic ( $\chi^2$ )	$P$ 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 oestrous female	1.64	0.20
Male status (level of territoriality)	1.03	0.31
Percentage of females in colony that were preoestrous during copulation	2.95	0.09

## 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 oestrous 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 oestrous female after copulation. Thus, even though the mating call may seem risky, a viable strategy may be to guard the oestrous female with vocalization, particularly if other males can detect the presence of an oestrous 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 oestrous 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 oestrous 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 oestrous females that 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 oestrous 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 oestrous female. Thus, the mating calls may have had both costs and benefits, with the outweighing benefit being greater assurance of paternity because of oestrous females delaying from mating again (see Schwagmeyer & Foltz 1990). Because litter size is only two to four 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 given by a female's first mate.

The percentage of females that had emerged from hibernation and were preoestrus 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 given during courtship serve to attract potential mates, our results suggest that male Columbian ground squirrels give mating calls primarily in the context of deterring a recent sexual partner from subsequent copulations with other male conspecifics.

Might the mating call, like roaring in red deer (McComb 1987), induce or expedite the ovulation of the oestrous 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 posthibernatory females that were preoestrus 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 oestrous 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 favour 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, as males increase reproductive success by monopolizing females to prevent their solicitation of copulations with additional males. Sexual selection therefore favours males that give a call as part of mate guarding, but selection apparently acts on the vocal repertoire already present in the species, rather than favouring 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, catalogue information, or retain event sequences) remains unclear and deserves future study.

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