

Premating behavioral tactics of Columbian ground squirrels

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In polygynous and polygynandrous mating systems males possess a variety of behavioral tactics that increase their access to reproductive females. In addition to overt combat or defending resources that attract mates, males use premating tactics that provide them with subsequent opportunities to copulate with receptive females. For Columbian ground squirrels, *Urocitellus columbianus*, we report that co-occupation of a burrow system by a reproductive male and a female on the night before the female exhibits diurnal estrus is an example of such a tactic. Our hypothesis was that nocturnal underground association results in successful consortships and therefore constitutes a mating tactic that is complementary to other mating behaviors exhibited during a female's estrus. Under this hypothesis appropriate predictions are that: males co-occupying a burrow system with a female at night should mate first with that female; males co-occupying a burrow system with a female overnight should sire more of her offspring than her subsequent mates; and the reproductive success of males co-occupying a burrow system with females should be higher than the reproductive success of mates that do not. To test our predictions we used a combination of field observations on nocturnal underground consortships (NUCs) and microsatellite DNA analyses of paternity. Males copulated with females during NUCs, as evidenced by inseminations. These males sired more offspring than males that did not participate in NUCs. Males ≥ 3 years old participated in more NUCs than sexually mature 2-year-old males. Our results supported the hypothesis that entrance into NUCs with a female before she exhibits estrus was a premating tactic that increased male reproductive success when exhibited in concert with other mating tactics such as territorial defense.

Key words: Columbian ground squirrels, mate guarding, mating strategy, mating tactic, multiple mating, multiple paternity, *Urocitellus columbianus*

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In polygynous and polygynandrous mating systems males often compete for matings through direct contest with one another (Darwin 1871). Males may use a variety of behavioral strategies that increase their access to reproductive females, including behaviors that occur before the onset of estrus (Alfaro 2005; Armitage 1986). In myriad species male mating strategies involve overt combat between males to achieve proximity to females, exclusion of other males from receptive females, competitive mate searching, or sperm competition (Birkhead and Møller 1992; Clutton-Brock 1983; Le Boeuf 1974; Schwagmeyer and Parker 1990; Waterman 2007). Alternative mating

strategies also can be used by subordinate males that cannot defend females directly (Taborsky et al. 2008). Examples include monopolization of females in coalition with subordinate males, “sneaky” copulations, or establishment of “friendship” with a female and others (Birkhead and Moller 1995; Crockford et al. 2007; Formica and Tuttle 2009; Gross 1996; Mainguy et al. 2008; Rios-Cardenas et al. 2007; Smuts 1985; Widemo 1998).



Any mating strategy can consist of several tactics that maximize reproductive success (Waterman 2007). For example, mating success of competitively searching male 13-lined ground squirrels, *Ictidomys tridecemlineatus*, depends not only on their ability to locate females but also on the duration of the copulations and mate guarding (Schwagmeyer and Foltz 1990). Other examples are males that maintain a territory on which several females reside, combined with postcopulatory mate guarding or overt combat near an estrous female, exhibited in California ground squirrels, *Otospermophilus beecheyi* (Dobson 1983), and Arctic ground squirrels, *Urocitellus parryi* (Lacey and Wiczorek 2001).

We describe a premating tactic for Columbian ground squirrels, *Urocitellus* (formerly *Spermophilus*—Helgen et al. 2009) *columbianus*. Specifically, we document entrance into an overnight burrow system and its subsequent co-occupation by a male and a female preceding the female's day of estrus, how it can enhance other mating tactics of males (e.g., territoriality and mate guarding), and its potential to become a part of a mating strategy. Overnight co-occupancy of a burrow system can have costs and benefits that are somewhat distinct from those associated with actual mating, because female partners might not come into estrus and mate the following day, and males must choose among several potential females for nocturnal underground co-occupancy of a burrow system (choice of 1 partner could be associated with lost opportunities with other partners).

Columbian ground squirrels are burrowing rodents. They form colonies and are active during the day (Betts 1976; Elliott and Flinders 1991). When they emerge from hibernation in early spring, a 3–4-week mating period follows when both males and females mate multiply (Murie 1995; Raveh et al. 2010). Females have a single annual day of estrus with a continuous receptive period of 5–7 h, which intensifies male competition in their vicinity (Manno and Dobson 2008; Murie 1995). Most reproductive males establish territories and aggressively defend them from other males (from other territorial or subordinate, nonterritorial males), which increases the probability that females with home ranges that overlap the territory will mate with the territory holder (Manno and Dobson 2008; Murie and Harris 1978). Copulations usually take place underground early in the day and can be inferred from one or more diagnostic courtship behaviors that take place aboveground (Manno et al. 2007, 2008b; Murie 1995). In addition to the typical mating behaviors, we describe a complementary behavior—co-occupying a burrow system with a female overnight before her annual day of estrus—practiced by dominant territorial and subordinate males.

Males and females might occupy a common burrow system for a variety of reasons, including thermoregulation during the cold spring and a shortage of available burrow systems during the period just after emergence from hibernation (e.g., Richardson's ground squirrels, *Urocitellus richardsonii*—Michener 2002). However, we hypothesized that the co-occupation of a burrow system by a reproductive male and pre-estrous female is a mating tactic that facilitates copulation

and improves the reproductive success of males, on the basis of the proximity of the co-occupation to the female's day of estrus and on the absence of co-occupation at other times (e.g., during gestation and lactation—Festa-Bianchet and Boag 1982; A. P. Nesterova, pers. obs.). We attempted to test this hypothesis because co-occupation of a burrow system around time of estrus is a relatively rare occurrence in the life of adult ground squirrels, because co-occupancy has not previously been described in detail, and because co-occupancy might represent a premating tactic of males.

If entrance into a burrow system and its co-occupation by a reproductive male and pre-estrous female is diagnostic of copulation, and if such behavior is a form of reproductive competition that facilitates male access to females, then we can make the following predictions. First, a male that co-occupies a burrow system with a female at night usually should mate first with that female when she is in estrus, as indicated by males having no subsequent consortships (behavioral patterns indicative of copulation) with the same female during the day, but fathering offspring in the female's litter. Second, males that co-occupy a burrow system with a female overnight should sire more of her offspring than her subsequent mates because female Columbian ground squirrels, like other ground-dwelling sciurids (Foltz and Schwagmeyer 1989; Hanken and Sherman 1981; Hare et al. 2004; Hoogland 1995; Lacey et al. 1997), mate with multiple males, and their litters commonly exhibit a pattern of first male sperm precedence (Murie 1995; Raveh et al. 2010). Co-occupation of a burrow system with a female just before estrus therefore might assist a male in acquiring the first mating. Finally, we predicted that the reproductive success of males that co-occupied burrow systems with females should be higher than the reproductive success of males that did not co-occupy burrow systems with females on the night before estrus.

We tested our predictions with field observations of male–female consortships and microsatellite DNA analyses of paternity. We observed complete sequences of consortships between individual females in estrus and males and then compared those sequences with success in offspring production. These results allowed us to examine our expectations of reproductive success for males that were co-occupying burrow systems with females on the night before estrus.

MATERIALS AND METHODS

Study animals and site.—As part of a long-term study (Engen et al. 2009; Raveh et al. 2011; Viblanc et al. 2010) we observed Columbian ground squirrels of known age and matrilineal genealogy on two subalpine meadows (B and Dot) in the Sheep River Provincial Park of Alberta, Canada (50°N, 118°W; elevation 1,500 m) during April to July in 2004–2007. All squirrels were trapped 1–2 days after they emerged from hibernation, prodded into a cloth bag, weighed with a spring balance (Pesola Co., Baar, Switzerland), and identified on the basis of numbered metal fingerling ear tags (National Band and Tag Co., Newport, Kentucky). For visual identification

from a distance each animal was painted on the dorsal pelage with a unique symbol using black hair dye (Lady Clairol Hydrience; Proctor and Gamble, Stamford, Connecticut). As for animals such as black-tailed prairie dogs (Hoogland 1995), such dye marking has been practiced for >15 years in the study populations without any detectable effect on survival of the animals. 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 abdominal testes and a gray or pink scrotum. Females usually reached sexual maturity at 2 or 3 years of age (Murie and Harris 1982).

Female Columbian ground squirrels usually remain in the natal colony for life, whereas most males disperse when they are 1 or 2 years old, before reaching sexual maturity (Festa-Bianchet and Boag 1982; Wiggett and Boag 1992). All animals on meadow B were of known age and genealogy, except 1 male that immigrated from an unknown area during the study. On meadow Dot the exact age and genealogy were known for animals aged ≤ 5 years. On the basis of their appearance (descended testes, dark scrotum), all of the immigrant males we observed ($n = 1$ on B, 8 on DOT) were estimated to be 3 years old. We used the known minimum age of animals for analyses (maximum age was 9 years for males and females). The age of 3 males and 5 females on meadow Dot could not be estimated, and therefore they were excluded from age-related analyses.

Data collection.—Assisted by 10×42 binoculars and 2- or 3-m-high observation towers, we conducted behavioral observations of all marked individuals from morning to evening every day during the time between emergence from hibernation and the last day that any female was in estrus. We used all-occurrence sampling (Altmann 1974) to record the time, location, and individuals involved for all social interactions, including where (in what burrow system) and with whom males and females immersed at night and emerged in the morning.

Locations were recorded to the nearest 1 m on a $10 \text{ m} \times 10 \text{ m}$ Cartesian coordinate system delineated with colored flagging. To increase the possibility that we would detect nocturnal cohabitation of the same burrow system by a male and female and any switching of burrow systems, we began observations 20–30 min before the first emergence in the morning and ended 20 min after the last sexually mature animal immersed for the night. Our priority for observations of night immersion and morning emergence was females that had not yet mated.

Every year of the study during April–May we trapped females that recently emerged from hibernation every 1–3 days until they mated and examined the condition of their vulva to determine whether they were in estrus (with fully opened vulva—Murie 1995). To reduce interference with mating activities females were trapped in the late afternoon. Animals were processed almost immediately after they were trapped, because traps were monitored from the observational towers.

Field observations of mating.—During April to July in 2004–2007 we observed 41 different males ($n_{\text{Dot}} = 29$, $n_{\text{B}} = 12$) and 86

different females ($n_{\text{Dot}} = 57$, $n_{\text{B}} = 29$) in two colonies. We collected information on mating and offspring birth-related activities (e.g., identification of nest burrow entrance, offspring emergence after weaning). Some males and females were present over multiple years. Overall, we observed 75 breeding male-years ($n_{\text{Dot}} = 53$, $n_{\text{B}} = 22$) and 174 ($n_{\text{Dot}} = 118$, $n_{\text{B}} = 56$) breeding female-years in the colonies.

Each female was sexually receptive for several hours on a single day of the year. Copulations occasionally occurred aboveground ($n = 9$ copulations), and these were observed easily (Manno et al. 2008a). Several types of aboveground behaviors common to ground-dwelling sciurids were used to diagnose underground consortships (Hoogland 1995; Lacey et al. 1997; Manno and Dobson 2008; Manno et al. 2007, 2008b; Murie 1995). First, males sniff a female's genital area and exhibit body contact with females on the day before she has a fully opened vulva. Second, one or both participants self-groom genitals upon emergence from a burrow system on the day of estrus, sometimes accompanied by dust bathing. Third, males often give a postcopulatory mating call. Fourth, females sometimes remove a copulatory plug that is deposited by the male during copulation. Fifth, females give an estrus call on some occasions after copulations. Sixth, a sexually mature male follows a female into the same burrow entrance when no alarm calls are given. Finally, other behaviors indicate male mate guarding, such as chasing the female into a burrow system, sitting on or herding the female into that burrow system as she attempts to flee the area, and fighting with other males in the vicinity of a female. Our observations were supported further by the finding that recorded dates of parturition in the lab correlated closely with the dates of inferred consortships (Pearson $r = 0.920$, $n = 217$ litters born in the lab, $P < 0.0001$).

A female's first consortship usually occurred early in the morning (0700 or 0800 h). Morning and subsequent consortships during estrus were characterized by a male and female first immersing into the same burrow entrance and then emerging from the same burrow system. Males entered a burrow system immediately after females. We concluded that a male and a female entered the same burrow system only if they both immersed into the same burrow entrance. In addition to these typical diurnal underground consortships, we also observed males and females emerging from the same burrow entrance in the morning after co-occupation of the burrow system overnight and then exhibiting the classic diagnostic postcopulatory behaviors (e.g., mate guarding or genital grooming). When immersing into a burrow entrance for the night, males entered immediately after females. On several occasions we observed males and females co-occupying a burrow system for 2 or 3 nights before the female was in estrus. The overnight burrow entrances were located on the basis of grid flagging and natural landmarks present on the meadow.

We focused on burrow system co-occupation only during the last night before estrus. For our analysis we included data from all females ($n = 142$) for which (a) we could record the

submergence for the females and all males in her vicinity on the last night before the day of estrus, and (b) we could track the female throughout the entire day of her sexual receptivity. We excluded data from females ($n = 32$) for which we did not have accurate data on either (a) or (b).

Henceforth, we use the term nocturnal underground consortship (NUC) for a male and a female that we saw immersing in the same burrow entrance in the evening or emerging from the same burrow entrance in the morning. Pre-estrous females are females on the night before diurnal estrus.

Paternity analyses.—After mating occurred we observed the burrow entrance to which each female that copulated collected and brought nesting material (e.g., grass, leaves) and deemed that location to be her nursery burrow system. Approximately 2 days before a female was predicted to undergo parturition, as per the average gestation period of 24 days (Murie and Harris 1982), she was captured, removed from the field, and temporarily housed in a laboratory at the field site until she gave birth.

Using the protocol of Murie and Harris (1982), these females were harbored in polycarbonate cages ($48 \times 27 \times 20$ cm) with wood shavings and newspaper for nesting material. We provided females with horse feed (EQuisine Sweet Show Horse Ration, Unifeed, Okotoks, Alberta, Canada), lettuce, and apples ad libitum. Shortly after its birth we sexed and weighed each neonate. We also removed a small (1 mm^2) sample of skin from the outer toe of the hind foot or the tail of each neonate for DNA analyses. Such toe biopsy usually resulted in a claw not growing on an outer hind toe. We chose hind toes so as not to influence feeding and digging, and no adverse effects of the procedure were observed. Skin was removed with sharp, sterile scissors. No septic powder was used because the procedure resulted in very little bleeding. We stored tissue samples in 95% ethyl alcohol at -20°C . These tissue samples were collected as a part of an ongoing long-term study.

We released mothers with offspring near their presumed nest burrow system after processing. After the mother entered the burrow system she either retrieved her neonates or they were placed inside an entrance to that burrow system to facilitate retrieval (Murie et al. 1998). Females immediately reclaimed their territory once released in the field.

Samples for paternity analysis were available only for 2005–2007 for meadow B. In the paternity assignment analysis we used litters from 47 female-years (birth events) that accounted for 147 offspring at birth. Litter sizes ranged from 1 to 7 offspring, with an average of $3.13 \pm 1.87 \text{ SE}$ offspring, which is typical for Columbian ground squirrels (Broussard et al. 2008; Dobson and Murie 1987). Some females and their litters were not included in our paternity data analysis because they either did not give birth ($n = 2$) or offspring DNA samples were unavailable ($n = 1$). Male reproductive success was analyzed on the basis of the information from 144 offspring. One litter ($n = 3$ offspring) was eliminated because of incomplete observations of consortship activities for the mother.

We collected tissue samples from adult males and females by clipping a small (1 mm^2) piece of the skin from the ear with sterile sharp scissors. No septic powder was used because the procedure resulted in very little bleeding. We stored the samples in 95% ethyl alcohol at -20°C .

Microsatellite DNA analysis took place in the Colman Laboratory, Department of Biological Sciences, University of Alberta. DNA was extracted from tissues by means of DNeasy Tissue extraction kits (Qiagen, Venlo, Netherlands). Thirteen microsatellite loci were amplified using polymerase chain reaction. We used primer pairs developed for *U. columbianus* (loci GS12, GS14, GS17, GS20, GS22, GS25 and GS26—Stevens et al. 1997), *Marmota marmota* (loci BIBL18—Goossens et al. 1998; and loci MS41 and MS53—Hanslik and Kruckenhauser 2000), and *M. caligata* (2g4, 2h6—Kyle et al. 2004); and 2h4 GenBank accession no. GQ294553. At each locus deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium between pairs of loci within cohorts were tested. Simulation paternity analyses were conducted using CERVUS 3.0 (Kalinowski et al. 2007; Marshall et al. 1998) with 95% and 99% trio-confidence. We performed 10,000 cycles using the allele frequencies of all genotyped ground squirrels, with 90% of the population sampled (a very conservative assumption, as virtually 100% of individuals were known). We analyzed each year separately. All offspring were assigned to their respective parents. A comprehensive description of paternity analyses can be found in Raveh et al. (2010).

Statistical analysis.—Analyses were performed with SPSS 17 (SPSS Inc., Chicago, Illinois) and SAS 9.1 (SAS Institute Inc., Cary, North Carolina). To assess the likelihood of male consortships with females we calculated an operational sex ratio (OSR) for both colonies in each field season. OSR was defined as the number of reproductively active males per estrous female.

We used Pearson correlation, r , to test whether the number of NUCs during a mating season correlated with the total number of consortships obtained during the season. To establish whether male age was related significantly to the number of NUCs we used mixed-model analyses, Z , F . Because our study yielded multiple observations from the same individuals in different years, we treated the identity of individuals as a random variable, and the year of study and male age were our covariates. To reduce the possibility of young reproductive males (2 years old) accounting solely for observed dependence we analyzed the data with and without these males and obtained similar results. We used this technique because 2-year-old males differ from older males in not having previous mating experience (Betts 1976; Murie and Harris 1982).

Small sample sizes and colinearity among variables precluded us from using mixed models for other analyses. Therefore, to account for repeated observations of individuals in different years we ran repeatability analyses (Sokal and Rohlf 1995) for dependent variables and found these to be not significant (number of NUCs [males]: $n = 75$, $R^2 = 0.58$,

TABLE 1.—Summary data for each Columbian ground squirrel mating season of the study on meadows Dot and B. Operational sex ratio (OSR) is the number of reproductively active males per estrous female.

Year	Mating season	Total number of consortship days	Minimum number of estrous females	Maximum number of estrous females	Total number of males	Mean OSR \pm SE
Meadow DOT						
2004	11–23 April	10	1	6	11	5.1 \pm 1.09
2005	17 April–5 May	15	1	4	14	9.8 \pm 1.09
2006	21 April–6 May	12	2	5	12	12.0 \pm 0.37
2007	17 April–1 May	12	2	7	16	5.4 \pm 0.52
Meadow B						
2005	25 April–13 May	14	2	3	6	2.6 \pm 0.13
2006	23 April–7 May	9	2	6	8	2.7 \pm 0.22
2007	26 April–13 May	11	1	3	8	5.7 \pm 0.67

$F_{41,33} = 1.10$, $P = 0.389$; reproductive success [males]: $n = 22$, $R^2 = 0.56$, $F_{11,10} = 1.15$, $P = 0.414$; presence/absence of NUCs [females]: $n = 134$, $R^2 = 0.48$, $F_{85,48} = 0.53$, $P = 0.995$; partner switching [females]: $n = 97$, $R^2 = 0.78$, $F_{70,26} = 1.30$, $P = 0.230$). Thus we considered individual years as our sampling units.

We used Mann–Whitney U -tests, U (Siegel and Castellan 1988), to compare the number of nocturnal consortships obtained by 2-year-old males and older males; the number of offspring sired by males during nocturnal consortships and diurnal consortships; the age of females that engaged or did not engage in NUCs; and the age of females that switched or did not switch their partners overnight. We considered that a female switched her partners if she was observed going into a burrow entrance with 1 male in the evening but emerging from a burrow entrance of another male the next morning. We used Wilcoxon matched-pairs signed-ranks tests, Z (Siegel and Castellan 1988), to compare the number of offspring sired by males during NUCs and the first consortship that took place after morning emergence (1st-day consortships). We reported median values and interquartile range, IR . To be conservative, all P -values are from two-tailed tests ($\alpha = 0.05$).

Ethical note.—This research conformed to guidelines of the American Society of Mammalogists for the use of mammals in research (Gannon et al. 2007). It also adhered to the legal requirements of the Alberta Sustainable Resource Development Organization (Canada), the Institutional Animal Care and Use Committees of Auburn University (USA), and the Life and Environmental Sciences Animal Care Committee of the University of Calgary (Canada).

RESULTS

During the mating season we observed a distinct and repetitive pattern. In the evening Columbian ground squirrel reproductive males immersed for the night in the same burrow entrance as females just before their estrous day, then emerged with these females from the same burrow entrance the following morning.

Columbian ground squirrel males and nocturnal consortships.—Mean daily OSR was strongly male biased (Table 1). Reproductively active males always outnumbered estrous

females. Regardless of the number of estrous females on any particular day, males engaged in NUCs. Most males (57/75 = 76.0%) spent a night in the same burrow system with at least 1 pre-estrous female during a mating season (Table 2). Number of females with whom a male had NUCs showed significant variation across individual males (median = 1.0 number of females, $IR = 2.0$; $n = 75$; Fig. 1). We never observed ≥ 2 reproductively active males retiring for the night into the same burrow entrance.

For males the number of NUCs during a mating season was correlated significantly with the total number of consortships obtained during the season ($r = 0.60$, $n = 75$, $P < 0.001$). All males that had NUCs also engaged in diurnal consortships with other females. Males sometimes had nocturnal and diurnal underground consortships with the same female (29/75 = 38.7%). Half of these males ($n = 14$) were the first mates of the estrous female in the diurnal sequence and the other males ($n = 15$) were later in the sequence (2nd, 3rd, or 4th mate). When considering only diurnal mating sequences, repeated diurnal consortships with the same female were rare (14/142 = 9.9%). For example, the consortship sequence of male A on a particular day included female 1, female 2, female 3, and female 1 again. Specifically, 12 males engaged in 2 diurnal consortships with the same female on the same day; the other 2 males engaged in 3 diurnal consortships with the same female.

Number of nocturnal underground copulations for males during a mating season increased with their age irrespective of male identity (ID) or the year of the study ($n = 72$; male ID, $Z = 0.38$, $P = 0.351$; year, $F_{3,22} = 0.24$, $P = 0.870$; male age, $F_{1,22} = 21.07$, $P < 0.001$; Fig. 2). Similar results were obtained when 2-year-old males were excluded ($n = 65$; male ID, $Z = 0.57$, $P = 0.284$; year, $F_{3,18} = 0.38$, $P = 0.767$; male age, $F_{1,18} = 10.87$, $P < 0.004$). Two-year-old males had fewer NUCs than older males ($n_{2yo} = 7$, $n_{>2yo} = 65$, $U = 65.0$, $P = 0.002$, median_{2yo} = 0.0 NUC, $IR = 1.0$; median_{>2yo} = 2.0 NUC, $IR = 2.0$). We observed three 2-year-old males engaging in courtships during the day, but they did not spend a night with any females. When these 3 males became 3 years old, they spent a night with 2 or 3 females.

Paternity assignments.—We successfully genotyped 12 males, 24 females, and 147 offspring at meadow B, resulting in 183 unique genotypes (5 female offspring became

TABLE 2.—Summary data for each Columbian ground squirrel mating season of the study on meadows Dot and B. NUC signifies nocturnal underground consortship(s).

Year	Total no. of females	No. of females with NUC	No. of females with no NUC	No. of females with unknown night activities	No. of females with NUC with one male	No. of female switchers ^a	No. of females with unknown switching ^b	Total no. of sexually mature males	No. of males with NUC	No. of males with no NUC
Meadow DOT										
2004	19	17	2	0	7	4	6	11	8	3
2005	19	17	1	1	7	6	5	14	12	2
2006	28	28	0	0	25	0	3	12	8	4
2007	26	25	0	1	19	0	7	16	11	5
Meadow B										
2005	16	15	0	1	8	0	8	6	5	1
2006	18	12	1	5	9	1	7	8	6	2
2007	16	16	0	0	11	0	5	8	7	1
Total	142	130	4	8	86	11	41	75	57	18

^a Female switchers were observed going into a burrow entrance with 1 male in the evening but emerging from a burrow entrance of another male the next morning. Only females were seen to switch burrow systems.

^b Includes females with unknown night activities.

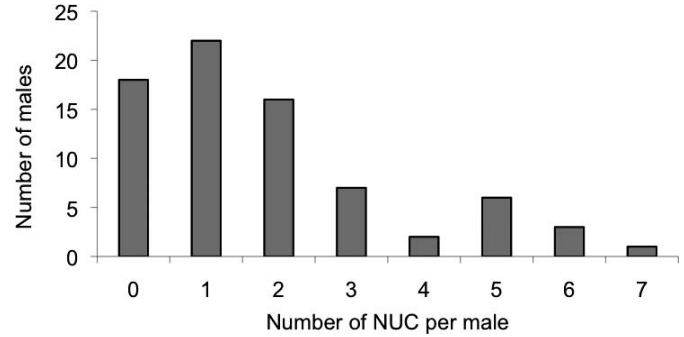


FIG. 1.—Variation in the number of nocturnal underground consortships (NUC) among Columbian ground squirrel males during 2004–2007 mating seasons on meadows Dot and B. Bars indicate the number of males that had night associations with 0–7 females. $n = 75$ males.

reproductively active in subsequent years, for a total of $n = 29$ meadow B females). Genotyping success rate was 97% with a genotyping error rate of 3% ($n = 183$). We retained all 13 loci in our analyses, as deviation from HWE or linkage disequilibrium was not observed. Paternity analyses assigned all 147 offspring successfully to both parents; 98% of the offspring had 99% trio-confidence, and the remaining 2% were assigned with 95% trio-confidence.

Columbian ground squirrel male reproductive success and nocturnal consortships (meadow B).—Males that had NUCs sometimes sired offspring. Over half of the NUCs (that were or were not followed by diurnal consortships with the same female) resulted in at least 1 offspring sired ($27/41 = 65.9\%$). Males were able to sire offspring even if they had only nocturnal underground copulations with females ($19/29 = 65.5\%$).

A majority of offspring was sired by males during NUCs ($48/144 = 33.3\%$) and first consortships of the day ($41/144 = 28.5\%$; Fig. 3). To investigate whether number of offspring sired due to NUCs and 1st-day consortships differed, we selected females that engaged in these 2 activities with different males. Number of offspring sired by males during NUCs (NUC offspring) was not significantly different from the number of offspring sired during 1st-day consortships (1st DC offspring; $n = 30$, $Z = -1.687$, $P = 0.092$; median_{NUC offspring} = 2.0 NUC offspring, $IR = 3.0$; median_{1st DC offspring} = 1.0 1st DC offspring, $IR = 2.0$). Overall, males that had NUCs with females (NUC males) sired more offspring than males that did not (no-NUC males; $n_{no-NUC males} = 4$, $n_{NUC males} = 18$, $U = 2.0$, $P = 0.003$; median_{no-NUC males} = 0.0 offspring, $IR = 0.0$, median_{NUC males} = 7.0 offspring, $IR = 5.0$).

Columbian ground squirrel females and nocturnal consortships.—Females usually had consortships (nocturnal and diurnal) with several males (median = 3.0 number of consortships, $IR = 1.0$). The vast majority of pre-estrous females ($130/134 = 97.0\%$) had NUCs with a male in addition to diurnal consortships (Table 2). A few females ($10/134 = 7.5\%$) had only nocturnal consortships and no diurnal consortships. Paternity data were available for 2 of these females. One female sired 2 offspring, and another did not

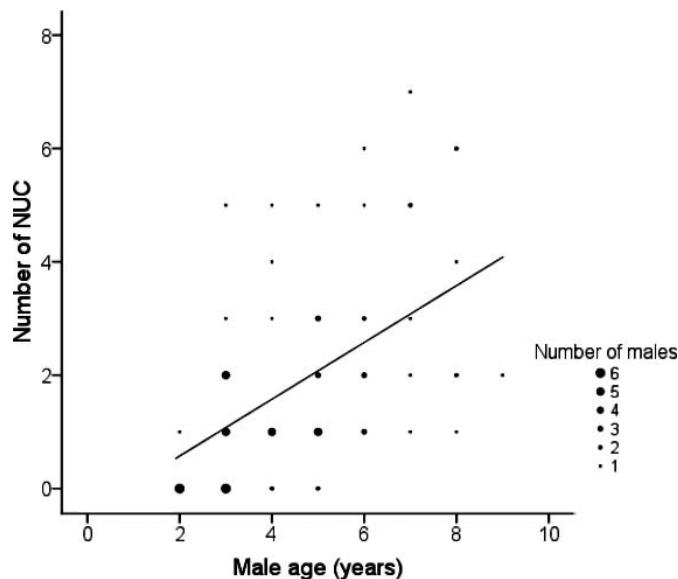


FIG. 2.—Relationship between the number of nocturnal underground consortships (NUC) of males with females and age of Columbian ground squirrel males during 2004–2007 mating seasons on meadows Dot and B. Dot size in the figure indicates the number of cases in each category. $n = 72$ males.

produce any offspring. Overall, younger females were as likely to have NUCs as older females ($n_{\text{no-NUC females}} = 4$, $n_{\text{NUC females}} = 125$, $U = 171.0$, $P = 0.274$; median $_{\text{no-NUC females}} = 3.0$ years old, $IR = 2.0$; median $_{\text{NUC females}} = 4.0$ years old, $IR = 3.0$).

On some occasions females were observed going into a burrow entrance with 1 male in the evening but emerging from a burrow entrance of another male the next morning, which indicated that females switched burrow systems overnight. We observed such switching during the pre-estrous night for 11.3% of females (11/97, considering females with completely known night activities). Only females and not males were seen to switch burrow systems. Female age had no significant effect on whether they switched partners overnight ($n_{\text{switched}} = 11$, $n_{\text{no switch}} = 83$, $U = 411.5$, $P = 0.589$; median $_{\text{switched}} = 5.0$ years old, $IR = 3.0$; median $_{\text{no switch}} = 3.0$ years old, $IR = 3.0$).

DISCUSSION

Our results indicate that 76% of Columbian ground squirrel males and 97% of females spent a night co-occupying the same burrow system just before the female was in estrus. Paternity data show that some males copulated with females during these nights, and 66% of nocturnal consortships resulted in offspring. We also found that males engaging in NUCs sired more offspring than males that did not. Older males engaged in more NUCs than younger males. Whether females engaged in nocturnal consortships was independent of age.

We hypothesized that in Columbian ground squirrels coincident immergence into a common burrow system by a male and a pre-estrous female was a pre-mating male mating tactic that improved reproductive success. Our study provided

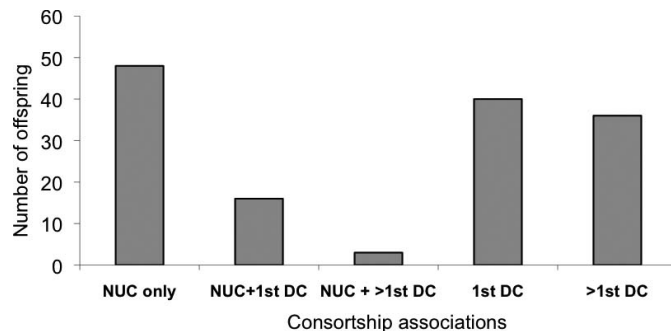


FIG. 3.—Number of offspring sired by Columbian ground squirrel males in different consortship associations during 2005–2007 mating seasons on meadow B. Bars represent the number of offspring that were sired by males due to nocturnal underground consortships (NUC only); nocturnal underground consortships and 1st day consortships (NUC + 1st DC); nocturnal underground consortships and 2nd, 3rd, and 4th day consortships (NUC + >1st DC); 1st day consortships (1st DC); and 2nd, 3rd, and 4th day consortships (>1st DC). $n = 144$ total offspring.

support for the 3 predictions appropriate for this hypothesis. First, we expected that nocturnal burrow system co-occupation with a female should provide a male with mating opportunities. On numerous occasions males sired offspring with females that were sleeping partners regardless of whether they copulated the next day. However, not all NUCs produced offspring for the male. One explanation is that some females do not come into estrus until later the following morning. Another explanation is that a male and female might not have copulated, despite immerging into the same burrow entrance before nightfall, owing to the extensive tunnel network underground (Nesterova and Hansen 2009). A female potentially can avoid a male underground if she chooses not to mate with him. Finally, some copulations might not produce offspring because of sperm selection (Newcomer et al. 1999; Simmons et al. 2006; Simmons and Emlen 2006; Zeh and Zeh 2006). For instance, Columbian ground squirrel males that were a female's first mate (nocturnal and diurnal) did not sire offspring in 15.5% of consortships (Raveh et al. 2010).

The second prediction that Columbian ground squirrel males with NUCs should have higher reproductive success than subsequent mates during the estrus day was supported by some, but not all, of the results. Litters of Columbian ground squirrels can have several fathers, and 57.5% of offspring are sired during first consortships (nocturnal and diurnal—Murie 1995; Raveh et al. 2010). Therefore, it is important for males to be early in a female's sequence of copulations. Nocturnal burrow system co-habitation can provide a male with an opportunity to be the female's first mate.

Paternity analyses revealed that the highest proportion of Columbian ground squirrel offspring was sired during NUCs and during the first diurnal consortship when compared with other consortship positions. This suggests that males co-occupy the same burrow system with a female for direct mating benefits, specifically the higher likelihood of siring offspring. Only 37% of males that engaged in NUCs were also

the 1st, 2nd, 3rd, or 4th diurnal mates of the same female. This is not surprising given that males apparently can sire offspring overnight. It therefore might behoove a male to look for other mating opportunities if he has already copulated.

We also predicted that Columbian ground squirrel males should have better success at producing offspring when they have NUCs with females than when they do not. A majority of males in the population had night associations with females. Paternity data on meadow B suggest that males with no NUCs were less successful at producing offspring. However, this result should be viewed with caution, given the small sample size of males with no NUCs. Lack of night association with females is probably not a comprehensive explanation. Other factors such as a male being of young age, which leads to subordination in territorial interactions, could contribute to the lack of night associations (Manno and Dobson 2008).

Nocturnal underground consortships might be an important mating tactic when several females are in estrus on the same day, which was often the case. In this situation a male might mate with 1 female overnight and also be the first diurnal consort male of another. Columbian ground squirrel males sire a similar number of offspring during NUCs and 1st diurnal consortships (with the females who already engaged in NUC). Moreover, NUCs have other advantages. For instance, OSR can be relatively high in Columbian ground squirrels—as many as 12 males could be competing on a given day to mate with a couple of females. Having NUCs can minimize agonistic interactions with other males. In addition, at night males might find it easier to monopolize females, who usually try to mate with multiple males (Manno et al. 2008b; Murie 1995). Females might not leave a burrow system at night because of high predation risk or because they do not know where other reproductively active males are.

One result that begs interpretation is the significant relationship between night associations with females and male age. Even though they were reproductive, young Columbian ground squirrel males (2 years old) were less likely to spend a night with a female. Perhaps young males are not able to detect the onset of estrus early enough to associate. Overall, the number of nights that males spent with females during a mating season increased with male age. This pattern is different from what was observed for the number of offspring (at weaning) sired by males, because middle-aged males were the most successful, whereas young and old males sired fewer offspring (Raveh et al. 2010). It appears that males continue to have overnight associations with females even at the age of 8 or 9 years, perhaps as a result of experience or competence in male–male competition (Manno and Dobson 2008). Male senescence is also one possibility that might explain the lack of offspring sired during night associations of the oldest males (Hamilton 1966; Rose 1991; Williams 1957). Finally, female choice is a possible explanation for the observed relationship between NUCs and male age—females might specifically avoid young males.

A majority of Columbian ground squirrel females engaged in NUCs with males. We detected no differences in age between groups of females that engaged in NUCs and those that did not. However, this result should be interpreted with caution given

the small sample size of females that did not engage in NUCs. Some females also switched their partners overnight. Whether males or females are responsible for this behavior, or what the benefits might be, remain to be investigated. Paternity data were available for 1 of the females that switched males overnight, and both males sired offspring with her.

Several other species of ground squirrels exhibit multiple paternity with a first mate sperm precedence and therefore might be good candidates for observations to reveal night associations (Foltz and Schwagmeyer 1989; Hanken and Sherman 1981; Hare et al. 2004; Lacey et al. 1997). However, observations of burrow system co-occupation by males and pre-estrous females in these species have not been reported. This might be due to the time of the onset of estrus. In Arctic ground squirrels, *U. parryii plesius*, the signs of behavioral estrus usually appear in mid-afternoon (1500 h), and copulations take place in the evening (1700–2100 h—Lacey et al. 1997). Because females become receptive only in the evening, males have the whole day to monitor the onset of estrus. In this case cohabiting a burrow system with a female overnight might not provide additional mating opportunities. Similarly, Belding ground squirrels, *U. beldingi*, are sexually receptive for several hours in the afternoon (Hanken and Sherman 1981). Thirteen-lined ground squirrels, *I. tridecemlineatus*, are another species that have first male sperm precedence. They are solitary and have a scramble-competitive mating system (Schwagmeyer and Parker 1987). Therefore, males might not always have an opportunity to locate females before the onset of the estrus.

Close associations between a male and a pre-estrous female have been observed in some species. Male Richardson's ground squirrels increase their proximity to females who are entering estrus (Michener and McLean 1996). In several species of prairie dogs males have a strong tendency to occupy the same burrow system with a female on the day before she becomes sexually receptive (Hoogland 1995, 1998a, 1998b). Close attention from a male directed to a female shortly before her estrus also occurs in Idaho ground squirrels, *U. brunneus* (Sherman 1989). In Southern flying squirrels, *Glaucomys volans*, scrotal males tend to nest communally with adult females during the mating period, but after mating females disperse and live solitarily (Layne and Raymond 1994). In many species of arthropods males often have close associations with females before copulation takes place (Bel-Venner and Venner 2006; Durbaum 1997; Jormalainen 1998; Mathews 2003).

Male Columbian ground squirrels use territorial defense (Manno and Dobson 2008) and mate guarding (Manno et al. 2007; Raveh et al. 2011) to secure mating opportunities. In addition, NUCs appeared to enhance reproductive success by ensuring that a consorting male had an increased chance of being the consorting female's first mate. We encourage researchers working on other species to look closely at the behaviors before the onset of the typical mating activities.

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