

Winter 12-2013

Determining parentage and the effects of relatedness on play partner preference in Belding's ground squirrels

Jessica Weidenbach

University of San Francisco, jweidenbach5204@comcast.net

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**Determining parentage and the effects of relatedness on
play partner preference in Belding's ground squirrels**

by

Jessica Weidenbach

Thesis

Submitted in partial Satisfaction of the Requirements
For the degree of

**Master of Science
In Biology**

In the
College of Arts and Sciences
University of San Francisco
San Francisco, California

Committee in Charge

Approved: _____
Faculty Advisor Date

Approved: _____
Committee Member Date

Approved: _____
Committee Member Date

Approved: _____
Dean, College of Arts and Sciences Date

Acknowledgements

Thank you to my advisor, Dr. Nunes and to my thesis committee members, Dr. Dever and Dr. Niles for your help and support throughout this research.

Thank you to Adelisa Legaspi for your much needed help in the field and the lab and to Alfonso for your help with the PCR samples.

Thank you to Jon Woo, manager of the Genomics Core Facility at the University of California, San Francisco, for processing the PCR samples and explaining the process to me.

Thank you to my family and friends for their love, prayers, and support through my education and through this research endeavor.

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Abstract

I investigated 1) multiple paternity in Belding's ground squirrels (*Urocitellus beldingi*) and 2) social partner preferences in juvenile *U. beldingi*. Prior work with blood allozymes demonstrated multiple paternity in *U. beldingi* litters. I evaluated paternity using microsatellite DNA analysis, which is more accurate than examining blood allozymes. My results indicate that multiple paternity in *U. beldingi* is more extensive than previously shown, occurring in about 90% of litters with more than one juvenile, and averaging 2.95 fathers in multiply sired litters. I also evaluated the hypothesis that play and other social behavior promotes bonding among juvenile female *U. beldingi*. All male *U. beldingi* emigrate from the natal area before reproducing, whereas most females remain in their natal areas throughout their lives and act cooperatively with close female relatives. Thus, it may be important for young females to establish bonds with sisters, and especially full sisters since kin selection favors cooperation with full sisters over half-sisters. However, my data did not support this hypothesis. Juvenile females demonstrated no preference for interacting with full versus half-sisters in play or other social behaviors. By contrast, juvenile male *U. beldingi* played and affiliated with full brothers for longer durations than they did with half-brothers. This result suggests that social behavior based on relatedness is important for juvenile male *U. beldingi*; however, how preferential interaction with full brothers might be important is not yet clear.

Introduction

Urocitellus beldingi, or Belding's ground squirrel, is a species whose range extends from central California to Washington, and east to Idaho and central Nevada. Belding's ground squirrels are ideal subjects for behavioral studies because they are group-living, large enough to be easily seen, easily handled when trapped, and are diurnal and above ground during the daytime (Sherman and Morton, 1979). A population of Belding's ground squirrels at Tioga pass, CA in the Sierra Nevada Mountains has been observed and studied since the late 1960s. (Sherman 1977, Sherman and Morton 1979, Nunes et al. 1999). This species is only active during the late spring and summer months and hibernates for the rest of the year. Dates of emergence from hibernation depend on the duration of the winter and melting of the winter snowpack, but sexually mature males typically emerge from their burrows from late April to early June. These males are reproductively viable for approximately one month. Sexually mature females emerge about one to two weeks after the males and are sexually receptive for only three to six hours for one day a year (Morton and Sherman 1978). During this time, females and males mate with many different partners. After mating, females dig and line maternal burrows below the ground in which to give birth to their litters. Gestation normally lasts 24-25 days and young are born in late June or early July. During the lactation period, all juveniles stay underground in the burrow and first emerge near the time of weaning, usually 25-28 days after birth in late July or early August (Holekamp et al., 1984).

Multiple paternity occurs in animals when a female is inseminated by more than one male during the same sexually receptive period and results in littermates being a mixture of full siblings and maternal half-siblings. This phenomenon occurs in natural populations of many

mammals, including Gunnison's prairie dogs (Haynie et al., 2003), alpine marmots (Goossens et al. 1998) and Belding's ground squirrels (Hanken and Sherman, 1981).

Although social monogamy, raising young with a single partner, may have many benefits to females, such as having paternal care for their young, many mammals including *U. beldingi* display reproductive polygamy and females have multiple mates. Many theories have arisen for the reason behind this mating pattern. Females may mate multiply to ensure that their offspring will have the best chance of survival. If offspring are genetically diverse, there is a better chance some will be suited to survive in different environmental conditions (Yasui, 1998). Genetic diversity in a population or within a litter can increase resistance to disease and parasites, and flexibility in dealing with environmental changes and challenges (Lacy, 1997). Many *U. beldingi* juveniles do not survive their first winter, and mating with multiple males may increase the chance of offspring survival if some offspring have genes better suited for hibernation. Females may also mate with subsequent males they encounter if these males are better, genetically, than previous mates. Females may also engage in copulation with males to prevent future infanticide of their offspring (Coulon et al. 1995). Infanticide of young has been shown in Belding's squirrels. Adult males or females may engage in infanticide of other young, with as much as 29% of juvenile deaths occurring as a result of intraspecific aggression (Jenkins and Eshelman 1984). Female mate choice occurs in this species, with females preferring larger, older males who win more fights during the mating period. When Belding's females mate multiply, the second and subsequent matings are typically with males that are more successful than the males they first mate with (Sherman 1976). Larger and more experienced males tend to have greater mating success (Sherman and Morton 1984).

Multiple paternity in *U. beldingi* has been shown to occur in 78% of litters in a given year, with most multiple matings typically resulting in multiple paternity (Hanken and Sherman 1981). Analysis of blood allozymes indicated an average of 2.3 fathers per litter (Hanken and Sherman 1981).

Many mammals show different patterns of philopatry, remaining in their birth areas throughout life, and natal dispersal, emigrating from the birth area to a new home range prior to reproducing. In the majority of mammalian species, females are philopatric and males disperse. Individuals that disperse are vulnerable to predation and social resistance from animals already occupying their new home range (Metzgar 1967, Wolff 1994). There are many different hypotheses proposing an explanation to natal dispersal, including avoidance of inbreeding, competition for mates, and competition for environmental resources. Some individuals may leave their natal sites to find mates that are unrelated to themselves. Dispersal is generally sex biased when animals disperse to avoid inbreeding, with males typically dispersing more often or farther than females in mammals. Many species avoid inbreeding because it can reduce heterozygosity and increase the expression of recessive lethal alleles (Nunes, 2007). Competition for mates or environmental resources can also drive natal dispersal in some mammals, with competition for mates more commonly driving dispersal in males and competition for environmental resources more commonly driving dispersal in young females. Researchers also believe that natal dispersal may be caused by a combination of causes, which may vary among different species (Nunes 2007).

Philopatry is typically sex biased, with the females remaining in their natal sites throughout life among most mammalian species. Males can increase their reproductive success by mating with multiple females whereas females optimize their reproductive success by

investing time and energy in care of their offspring to ensure the juveniles reach reproductive maturity. Females may remain in their natal areas to help them acquire food and territories in which to raise their young. Residing near female relatives also allows for cooperation in defense of young against predation and infanticide by conspecifics (Sherman 1977). Close relatives have been shown to share portions of their territories and chase away trespassers from burrows of close kin (Sherman and Morton, 1979).

In *U. beldingi*, males emigrate from the natal area, while females are philopatric. All young males leave their natal area regardless of environmental resources or social conditions (Holekamp, 1986). Nunes et al. (1999) found that treating young female squirrels with androgens when born causes them to disperse more than control females. This suggests that hormones may play a developmental role in dispersal of Belding's ground squirrel males by acting on neural substrates underlying dispersal behavior. Female ground squirrels remain in their natal area and cooperate with family members. Mother and daughter squirrels set up territories near one another and defend them together against conspecifics. Cooperation among female family members is important in the survival of their offspring (Nunes 2007).

Kin selection is an evolutionary process in which individuals favor the reproductive success of related individuals. Kin selection has also been shown in a variety of species including house mice, deer mice, rats, swallows, fur seals, gulls, and *U. beldingi* (Mateo 2003; Holmes and Sherman 1983; Sherman 1977). Kin selection can have many benefits to ground squirrels. Alarm calls can be used to warn relatives of a predator or to be a diversion of a predator's attention. Kin recognition and nepotism in Belding's ground squirrels are important for survival of each kin group. It is beneficial to have kin nearby to warn of incoming predators or to divert a predator away from the rest of the group.

Male *U. beldingi* disperse from the natal area before mating, and most males disperse as juveniles beginning at about 7 weeks of age. By contrast, most female *U. beldingi* remain in their natal areas throughout their lives (Holekamp 1984). Therefore, it is more beneficial for female ground squirrels to develop a close kinship bond early in life. The closer bond of the females, the more likely they are to protect their kin in the future from predators and infanticide (Sherman and Morton 1979).

Preferential treatment of kin is mediated by an ability to discriminate among conspecifics. Kin recognition has been shown in *U. beldingi* (Holmes, 1994). This recognition is hypothesized to develop from interaction during the first few weeks in the life of a juvenile, when littermates spend all their time with each other (Armitage, 1981). However, individual ground squirrels are able to distinguish between littermates and non-littermates by self-referential phenotype matching, using one's own cues as a referent for recognizing kin (Mateo, 2010). This means Belding ground squirrels can discriminate equally between familiar maternal full siblings and half-siblings and between unfamiliar paternal half-siblings and unfamiliar non-kin (Holmes and Sherman, 1982). Belding's ground squirrels have been shown to be able to discriminate between maternal full and half siblings. Maternal half-siblings show more aggressive behavior toward one another than full sisters, showing ground squirrels can discriminate based on relatedness, even though all individuals were reared together (Sherman, 1980). This ability to discriminate between full and half siblings is partially by self-referent phenotype matching, in which individuals use their own odors to compare to the odor cues of another individual, with the degree of cue matching correlating to the degree of relatedness (Mateo 2010).

Play is a characteristic commonly found among young mammals. The prevalence of this play behavior suggests that it is important to mammalian development. Juvenile mammals engage in play for a variety of reasons including the development of social, cognitive, and motor skills and strengthening of social bonding in play partners (Bekoff 1972). Strengthening of social bonds can promote kin recognition and kin selection in the future. In *U. beldingi* juveniles, play has been shown to improve motor skills; however, it is not known whether play promotes social development or social bonding (Nunes et al. 2002, 2004).

Belding's ground squirrels were chosen for the study because of the extensive background available from past studies and because they spend most of their time above ground and are readily observable. Information acquired from observation of a population of social rodents can be applied to other species that may not be as readily observable or as easy to study.

The objectives of this research are to describe the extent of multiple paternity in this species using microsatellite DNA analysis, which is more accurate than the blood allozyme analysis used in the past, and to elucidate the role of play and other affiliative behaviors in helping to establish social bonds between juveniles.

Chapter 1 - Parentage Analysis of *Urocitellus beldingi*

Introduction

Urocitellus beldingi hibernate during the winter months and come out of hibernation in the spring. Sexually mature males come out of hibernation from late April to early June and are reproductively viable for about one month. Females emerge from their burrows about two weeks after males and are only sexually receptive for a few hours, one day each year (Morton and Sherman 1978). During this time, females and males mate multiply with many different partners. Multiple paternity occurs in animals when a female is inseminated by more than one male during the same sexually receptive period and results in littermates being a mixture of full siblings and maternal half-siblings. This phenomenon occurs in natural populations of Belding's ground squirrels (Hanken and Sherman, 1981) and in many other small mammals.

There are many hypotheses that attempt to explain why females may mate multiply. Genetic diversity of offspring may lead to a greater chance of maternal gene survival if some genes are better suited for hibernation or other environmental challenges (Lacy, 1997). Females may also mate with subsequent males they encounter if these males are better, genetically, than previous mates so their offspring will have a greater chance at reproducing and passing down maternal genes. These subsequent mating choices may also be based on male size with larger and more experienced males having greater mating success in the mating period (Sherman and Morton 1984). Females may also engage in copulation with males to prevent future infanticide of their offspring (Coulon et al. 1995). Adult Belding's males or females may engage in infanticide of other young, with as much as 29% of juvenile deaths occurring as a result of intraspecific aggression (Jenkins and Eshelman 1984).

Multiple paternity in *U. beldingi* has been shown to occur in 78% of litters in a given year and multiple matings usually result in multiple paternity in this species (Hanken and Sherman 1981). A study by Hanken and Sherman (1981) used loci in allozymes to determine the occurrence of multiple paternity in this species. The study found that the average number of fathers per liter was 2.3. These blood allozyme markers, which are highly conserved, may not be sensitive enough to estimate relatedness between individuals accurately. Many allozyme loci may be similar between even unrelated individuals and are therefore a less accurate way to determine relatedness (Queller et al. 1993). Microsatellite DNA analysis is a more accurate method for evaluating multiple paternity in *U. beldingi* than the allozyme analysis used by Hanken and Sherman (1981). Microsatellites are non-coding regions of the genome consisting of repeats of certain base pairs. The repeated unit can be between two and nine base pairs long and it can be repeated from a few to a few hundred times. A unique fingerprint of an individual can be established using many polymorphic microsatellite loci (Wright et al. 1994). Microsatellite data has been used to determine parentage in a variety of species, including humans (Litt and Luty 1989) and squirrels (Garner et al. 2005).

Polymerase Chain Reaction (PCR) is a technique used to amplify specific microsatellite loci within a genome (Mullis et al. 1986). Each DNA sample is run using PCR with each different primer used in the experiment. Reaction numbers can rise quickly using this method, and therefore, multiplex PCR may be used to reduce the number of total reactions. This method is a technique in which multiple reactions take place in one tube in order to reduce the amount of reagents and time spent doing PCR (Eggelston et al. 2002, Markoulatos et al. 2002). The products of different primers are identified by using different fluorescent labels that can be recognized by a DNA analyzer (Ziegle et al. 1992).

Our objective was to determine the extent of multiple paternity in Belding's ground squirrels using microsatellites. We believed the occurrence of multiple paternity is much greater than previously shown by the studies using allozyme markers because the previous study used only six polymorphic loci and many litters in this study were unresolved or lacked complete data.

Methods

Squirrels were sampled from 21 May to 26 July 2012 in a meadow near Tioga Pass in Mono Co., California. Adult male and female squirrels were trapped using Tomahawk chipmunk live traps baited with peanut butter. These squirrels were marked with permanent monel metal ear tags (National Band and Tag Co., Newport, Kentucky, USA), dyed with black hair dye (Clairol, Stamford, Connecticut, USA) and weighed to the nearest 1g with spring balance scales (Avinet, Dryden, New York, USA). Squirrels also had a 1mm – 2mm clipping of their ear tissue cut off for a DNA sample. The tissue sample was collected from the medial edge of the ear using surgical scissors. This method of tissue extraction usually causes no bleeding and little discomfort to the squirrels. Samples were labeled and kept on ice temporarily until they could be transferred to -20°C for storage.

Beginning on 1 July 2012, the maternal burrows of lactating females were observed daily from elevated posts such as rocks or hilltops to determine the date on which their young first emerged from the natal burrow. Young were trapped within two days of their first appearance above ground during which time they remain close to the natal burrow and can be unambiguously assigned to litters (Holekamp, 1984). Juvenile squirrels were trapped and marked in the same fashion as adult squirrels.

A Qiagen DNeasyTM Blood & Tissue kit (Qiagen, Hilden, Germany) was used to isolate DNA from each sample of ear tissue clipping. Extractions were performed according to the

purification protocol of total DNA from animal tissues with two elutions in the final step. DNA was isolated from 98 adults and 123 juveniles and stored at -80 degrees.

Ten microsatellite primers designed by Genetic Identification Services specifically for *U. beldingi*, were used to analyze each sample of DNA collected to determine paternity. The microsatellites are two to four repeats in length and markers used were A116, A120, B6, B12, B108, C4, D4, D106, D107, and D108 (Table 1). The conditions of these primers for Polymerase Chain Reaction have also been determined (Lafler 2011). The reverse primers for A116 and D107 were PIGtailed with GTGTCTT to avoid band stuttering (Brownstein et al. 1996). The Qiagen Type-it kit was used to multiplex the PCR reactions. All concentrations were prepared according to the instructions for the kit, with 1µl of each DNA sample added to the final mix. Forward and reverse primers were both used in 0.2µM concentrations. The PCR protocol utilized for the samples was as follows: 95°C hot start for 5 minutes, 35 cycles of 95°C for 30 seconds melting, X°C annealing, where X is the annealing temperature of the particular primer (Table 1), 72°C for 30 seconds extension, followed by a 60°C 30 minute final extension period.

All samples were analyzed via capillary gel electrophoresis at the University of California, San Francisco (UCSF) on the ABI 3730xl DNA Analyzer. This analysis allows for better fragment size resolution and assisted in determining paternity for each of the juvenile ground squirrels. Information was analyzed by Gene Mapper (Applied Biosystems) and output was given from in the form of fragment lengths of each allele, two numbers per tissue sample. Maternity of juvenile squirrels was determined based on observation of burrows. Paternity was determined by using the allele fragment lengths of each primer sample and analyzed by hand. Alleles from each juvenile were matched to the mother and the remaining allele was used to match each juvenile to a father, based on the pool of possible sires which included all adult males

trapped during the year (two years and older) since yearling males do not reproduce. The analysis was done by two individuals and compared for accuracy. In total, 23 litters were analyzed.

Results

Out of 23 litters of juveniles, two litters consisted of only one juvenile, and the average number of juveniles per litter was 5.2. The results of the paternity analysis showed that 19 out of 21 litters (95%) with more than one juvenile had multiple fathers. Litters examined had a minimum of 1 father and a maximum of 5 fathers with the average number of 2.76 fathers per litter. Multiply sired litters had an average of 2.95 fathers per litter. There were only 2 litters (with more than one juvenile) that had only one father; and male 9810 sired the most pups, 20 out of 120 total (16.7%). The rate of fathers per juvenile was 1 father per 7.06 juveniles.

Discussion

Urocitellus beldingi exhibits promiscuous mating in both sexes and multiple paternity occurs as a result of this mating behavior. The data collected confirm previous studies of multiple paternity in this species using allozyme proteins (Hanken and Sherman 1981). These previous studies showed an occurrence of multiple paternity in only 78% of litters while our research showed a much higher occurrence of 95% of litters being multiply sired. The study using allozyme proteins also showed an average of 2.3 fathers per multiply sired litter while our research showed an average of 2.95 fathers per litter. Studies using microsatellites are more reliable than allozyme proteins and may show a more complete picture of multiple paternity in Belding's ground squirrels. Larger sample sizes and analysis of data by computer program instead of by hand should be done to support the data concerning the occurrence of multiple paternity in this species.

Table 1. Characterization of the primers used, including fluorescent labels and multiplex information.

Locus Name	Primer Sequence	Repeat Motif	No. Alleles	Fragment Size	Allelic Range	Multiplex Group	Annealing T °C
A116	F: FAM-TCTGTCTCACCTCCTGTGTC R: GCAAACCTCACCTCTAAGATGG	CA	12	300-316	16	2	57.0
A120	F: FAM-TTCCTCTGAGCCTCCCTAAC R: AACCCAAGTGAGAAGTAACGG	CA	13	143-182	39	3	57.0
B6	F: FAM-CACCCTCCACCTTTTAGAAG R: TCCAATGAACTTTTCCATCTC	AAC	4	129-147	18	1	55.0
B12	F: HEX-CCAGCCTACTTTGTTGTTCC R: CACCAGGACAGCACACATAC	AAC	4	213-225	12	3	57.0
B108	F: HEX-GGAGCGTCAATGGAGAGG R: GGCAGAAGGCAGAACTGG	AAC	7	279-298	19	5	58.5
C4	F: FAM-ACTTGCATCCTTCTAGCTCTG R: TCCCATTTCTTTGTAAACTACCC	AAAG	15	225-281	56	4	56.0
C11	F: HEX-CCTGTTCTGGTGACTTCTG R: AGGTCCACTGTAATGCTTG	AAAG	12	206-229	23	1	55.0
D4	F: HEX-AGCAAGACCCTAAGCAAC R: AGCACCTGTTACAAAGG	TAGA	14	271-343	72	4	56.0
D106	F: FAM-GGACCAGAGTGGTACTTCTGTG R: AGCACCCAGAGACTGTGACTTA	TAGA	4	164-176	12	5	58.5
D107	F: NED-CAACTCTGAATCCCTCACAG R: TCCAAGCTGAATCCTCTACTAG	TAGA	20	169-207	38	4	56.0
D108	F: HEX-CACCAACTGTAAACCTGTTCTG R: CAACGTCAGTGAGACTGTGTC	TAGA	7	199-223	24	2	57.0

Chapter 2 - Partner Preferences in Play and Social Affiliation

Introduction

Play is a characteristic commonly found among young mammals. The prevalence of play behavior suggests that it is important to mammalian development. Juvenile mammals engage in play for a variety of reasons including the development of social, cognitive, and motor skills and strengthening of social bonding (Bekoff 1972). In *U. beldingi* juvenile play improves motor coordination; however, it is still not known whether play promotes social and cognitive development or social bonding (Nunes et al. 2004). Most juvenile social play (~97% of interactions) in Belding's ground squirrels occurs between littermates. Most play occurs during juveniles first two weeks above ground, and declines to near zero thereafter as juveniles start making exploratory excursions far from their natal areas.

Play behavior increases motor coordination among juvenile *U. beldingi* and appears to be more important in increasing coordination among males than females, possibly to help prepare them physically for natal dispersal during the juvenile summer. Males disperse from the natal burrow in order to avoid inbreeding. Female squirrels may engage in play to forge social bonds with sisters; however, this possibility has not yet been examined. Females act cooperatively with close female relatives in defending maternal territories during the reproductive cycle. Females are also more likely to alarm call when close female relatives are nearby and therefore must forge strong bonds with female relatives. Thus, it might be important for females to establish strong social bonds with their sisters. This forging of strong social bonds is probably less important for male juveniles, as they disperse from the natal burrow and will likely have little or no interaction with kin in the future (Sherman and Morton 1979).

Preferential treatment of kin is mediated by an ability to discriminate among conspecifics (Hamilton, 1964). Kin recognition has been shown in *U. beldingi* (Holmes, 1994). This recognition is hypothesized to develop from interaction during the first few weeks in the life of a juvenile, when littermates spend all their time with each other (Armitage, 1981). However, individual ground squirrels are able to distinguish between littermates and non-littermates by self-referential phenotype matching, using one's own cues as a referent for recognizing kin, which has been shown by using juveniles raised with biological and foster siblings (Mateo 2010). This means Belding ground squirrels can discriminate equally between familiar maternal full siblings and half-siblings and between unfamiliar paternal half-siblings and unfamiliar non-kin (Holmes and Sherman 1982). Belding's ground squirrels have been shown to be able to discriminate between maternal full and half siblings. Maternal half-siblings show more aggressive behavior toward one another than full sisters, showing ground squirrels can discriminate based on relatedness, even though all individuals were reared together (Sherman 1980).

We examined the hypothesis that social play behavior promotes bonding among juvenile female *U. beldingi* in support of cooperative behavior later in life. If this is the case, then juvenile females should engage in social play more frequently with full than half sisters, and play interactions should last longer between full than half-sisters. By contrast, the frequency and duration of play interactions between brothers or between brothers and sisters should not vary with degree of relatedness. Moreover, other affiliative interactions should occur at higher frequency and be longer in duration between full sisters than half sisters, but should not differ with relatedness between brothers or brothers and sisters.

Methods

Squirrels were observed from 22 May to 26 July 2012 in a meadow near Tioga Pass in Mono County, California. Upon first trapping, squirrels were fitted with ear tags and marked with unique symbols using hair dye as described above. Juvenile ear-tags were painted different colors with nail polish to help with identification during observation. Ear-tagging and dye-marking are common and minimally invasive ways to allow for identification of squirrels both up close and from a distance during observation within the field season and between seasons (Sherman 1976, Sherman and Morton 1984).

Juvenile squirrels from 17 litters were observed for affiliative interactions with littermates including play and investigatory behavior, and remaining in proximity of littermates. Observations were made during juveniles first two weeks above ground. Each litter was observed on an average of eight different days for an average of 538 total minutes. Behavioral observation began at juveniles' first emergence from the natal burrow when they were approximately four weeks old, and continued for an additional two weeks. Rates of play among juveniles begin to decline sharply at approximately six weeks of age, as they begin to roam further from their natal burrow and encounter littermates less frequently (Nunes et al. 1999). Play, affiliative, and investigative behaviors were recorded using methods described by Altmann (1974). These behaviors are described below and were used to assess relationships between littermates.

Play Behavior:

Wrestling—The juvenile faces a littermate, typically in a ventrum to ventrum clench or embrace, and pecks at the littermate's neck, throat, cheeks, or abdomen with inflicting bite wounds.

Tackling—The juvenile jumps or pounces on the littermate, usually from a running start.

Boxing—The juvenile bats with the forepaws at a littermate, who usually reciprocates.

Chasing—The juvenile follows and pursues the littermate while both are running.

Mounting and Play Copulation—The juvenile climbs on the back of a littermate and places the forepaws around the littermate's chest or abdomen, grasping in the mouth the skin of the littermate's neck, cheek, or back, and aligning the pelvis with the littermate's pelvis; the juvenile and littermate typically both lie on their sides on the ground; no intromission occurs.

Investigative Behavior: The juvenile engages in olfactory examination of the littermate with head extended and nose within 2 cm of the conspecific, or walks around the littermate with the head oriented toward the littermate.

Affiliative Behavior: The juvenile remains within 0.5 m of the littermate for > 10 sec without engaging in other social interactions.

I calculated the frequencies of play, affiliative, and investigative behavior, among dyadic pairings that would be expected if juveniles were selecting social partners at random. Dyadic interactions were categorized as female-female, female-male, or male-male. All possible dyadic associations in a litter were determined, and the number of possibilities for each category of dyad was expressed as a proportion of all possible dyads. Proportions were multiplied by the total number of interactions observed within a litter to determine the expected number for each category of dyadic pairing. Sibling determinations were made using parentage data (see previous

chapter). Juveniles within the same litter and sired by the same father were said to be full siblings while juveniles within the same litter but sired by different fathers were said to be half siblings. *Statistical Analysis*—Data in the study did not follow the normal distribution and so were analyzed with nonparametric tests. Comparisons between observed and expected frequencies of play, affiliative, and investigative interactions were made with Friedman sign tests for paired samples. Durations of play and affiliative interactions among juveniles overall were analyzed with Kruskal-Wallis tests, and post-hoc pairwise comparisons were made with Conover-Inman tests. Comparisons of the duration of play and affiliative interactions between full and half siblings were made with Mann-Whitney *U*-tests. Statistical tests were performed with Systat 13 software (Chicago, Illinois, USA). Mean values are presented ± 1 SEM. Differences between groups were considered significant when $P \leq 0.05$.

Results

Frequency of Interactions among Juveniles—Dyadic pairings in play and affiliative behavior did not differ from what would be expected by random association of juveniles within litters (Figs. 1A-B). However, female-male pairings in investigative interactions occurred more frequently than would be expected by random association (Fig. 1C, Wilcoxon, $z_1 = 2.50$, $P = 0.013$), and male-male pairings occurred less frequently (Fig. 1C, Wilcoxon, $z_1 = 2.19$, $P = 0.028$).

Associations between full siblings did not differ from what would be expected by random pairing in either play or affiliative behavior (Figs. 2A-B). However, investigative interactions between full siblings occurred at a higher frequency than expected in female-male dyads (Fig. 2C, Wilcoxon, $z_1 = 2.84$, $P = 0.005$).

Duration of Interactions among Juveniles—The duration of play interactions did not differ overall among dyads (Fig. 3A). However, there was a significant trend for play interactions to

last longer between full brothers than between half-brothers (Fig. 3A, Mann-Whitney, $U_I = 3160.5$, $P = 0.036$).

There was significant variation in the duration affiliative associations among dyads overall (Fig. 3B, Kruskal-Wallis, test statistic = 14.71, $df = 1$, $P < 0.001$), with affiliative pairings having the shortest duration among female dyads, intermediate duration among mixed-sex dyads, and lasting longest among male dyads (Conover-Inman, $P < 0.05$ for all pairwise comparisons). I also observed a significant trend for affiliative associations to last longer between full brothers than between half-brothers (Fig. 3B, Mann Whitney, $U_I = 2520.5$, $P = 0.046$).

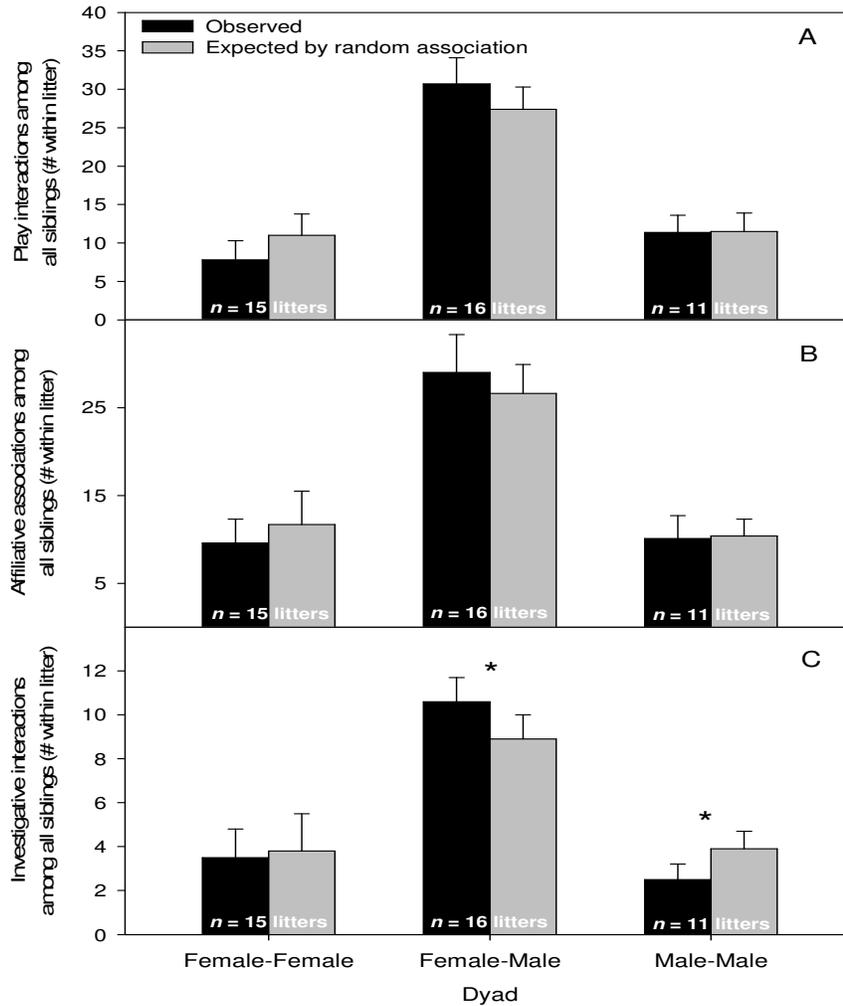


Figure 1. Frequencies of A) play, B) affiliative, and C) investigative behavior among juvenile *U. beldingi*. Data reflect pairings among all siblings within litters. Asterisks indicate a significant difference between observed frequencies and frequencies expected by random pairings of juveniles within litters. Sample sizes are indicated on the graph.

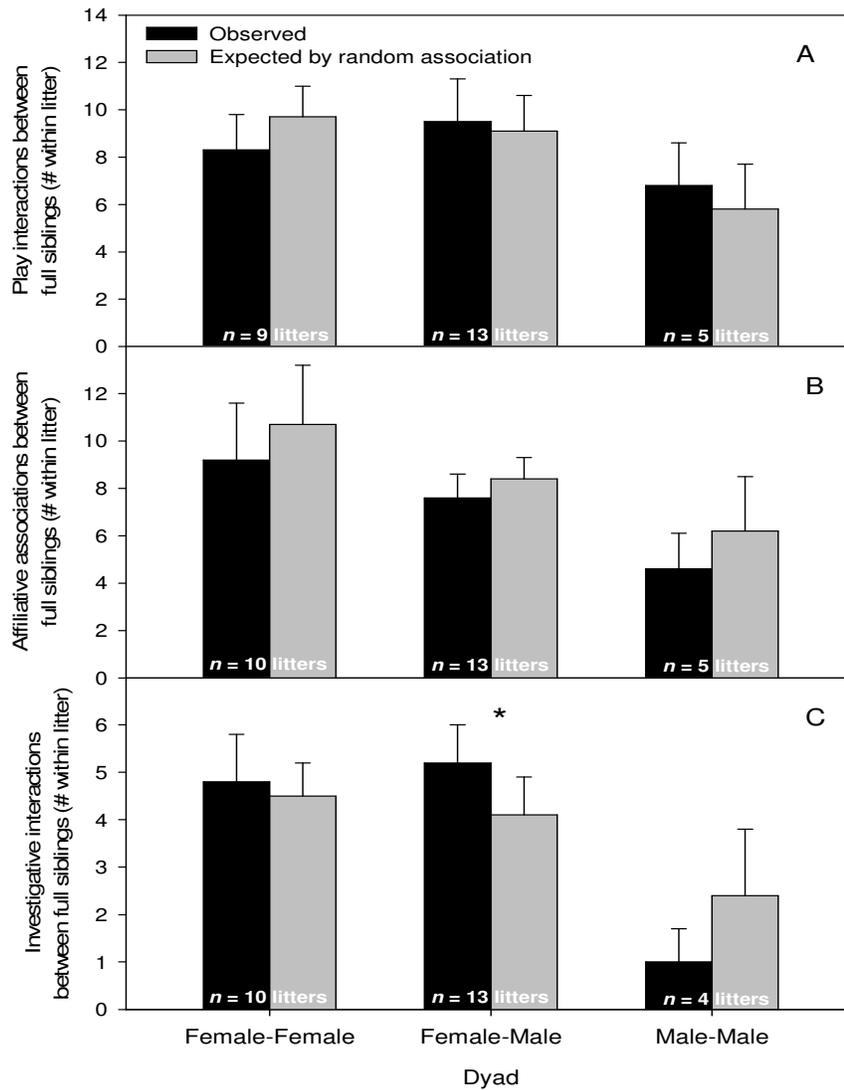


Figure 2. Frequencies of A) play, B) affiliative, and C) investigative behavior among juvenile *U. beldingi*. Data reflect pairings of full siblings. The asterisk indicates a significant difference between the observed frequency and the frequency expected by random pairings of juveniles within litters. Sample sizes are indicated on the graph.

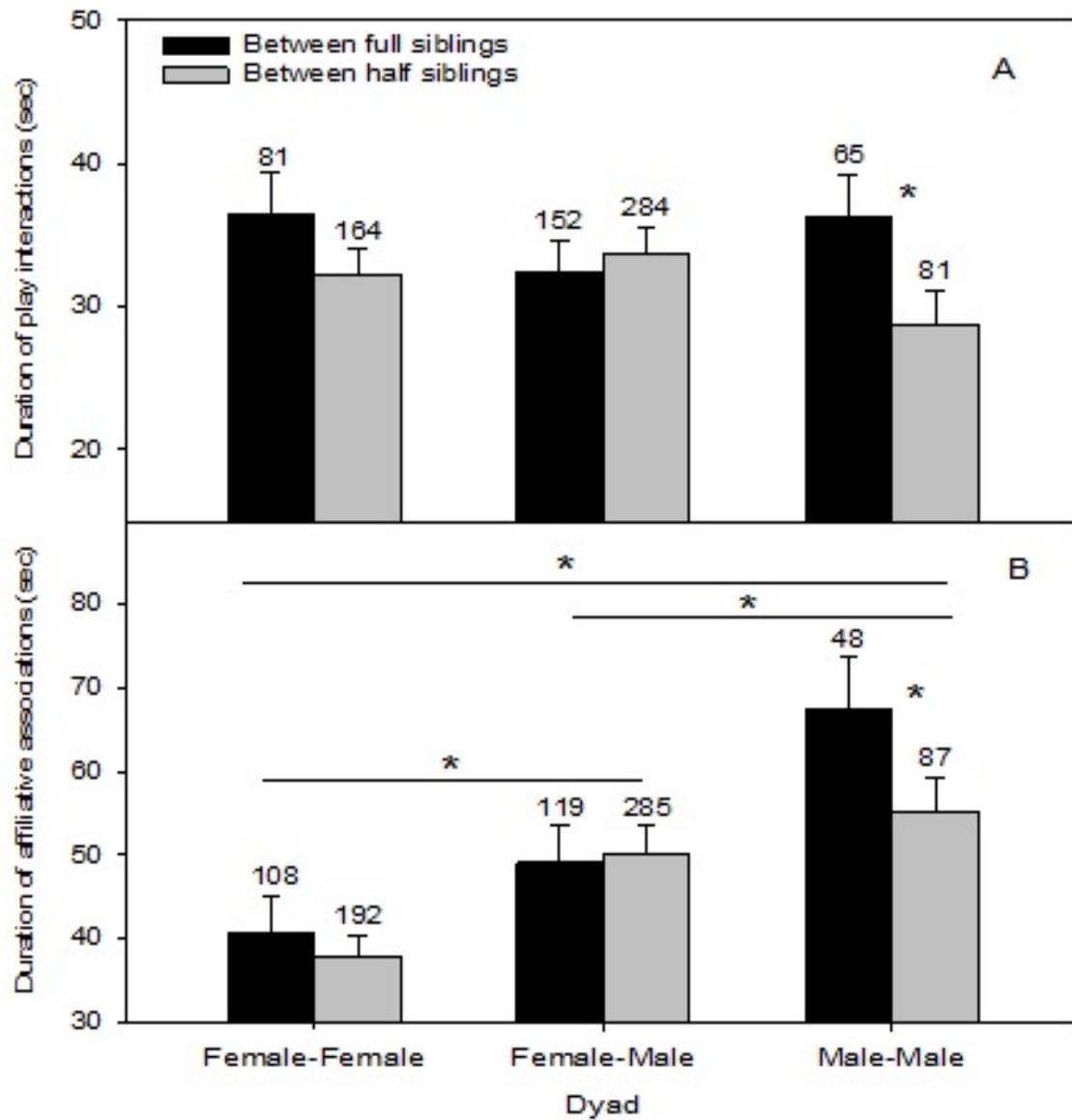


Figure 3. Duration of A) play interactions and B) affiliative associations among juvenile *U.*

beldingi. Asterisks above lines indicate significant differences between dyadic pairings.

Asterisks between bars indicate a significant difference between full and half siblings. Sample sizes indicate the number of interactions or associations.

Discussion

Data collected were not consistent with the hypothesis that play promotes social bonding in juvenile female *U. beldingi* to strengthen future cooperation among sisters. Females did not play more with full siblings versus half siblings. Females may not be playing to strengthen social bonds. There may be other factors that are helping to strengthen bonds between full sisters such as time spent in the natal burrow before first emergence. Another possibility is that juvenile females are playing with all female siblings equally because they are equally likely to have either a full sibling or a half sibling still around after the winter. Many juveniles are lost during hibernation, and females may want to bond with all female siblings, regardless of relatedness to increase their chances of a good bond if full siblings do not survive the winter. Relatedness was important for male juvenile interactions as males played and affiliated with full brothers for longer durations than with half-brothers. Duration of affiliative interactions was also longest between male-male pairings and shortest between female-female pairings. This interaction between males may strengthen social bonds and result in less fighting with full-siblings in future mating seasons, but the data do not clearly show a reason for the importance of relatedness in social interactions between male juveniles.

There are many implications for future research. A larger sample size may be beneficial in showing how females forge bonds with siblings or why females do not prefer full sibling play over half sibling play. Observing ground squirrels during their mating period can help identify a smaller number of possible fathers per litter to make paternity determination simpler. Since our research has shown that males show some preference for full brothers over half-brothers during play and affiliative interaction, future studies might focus on the significance of these preferences.

Conclusion

Belding's ground squirrels serve a model species for studying small social rodents. I hypothesized that females may establish strong social bonds with their sisters, especially full sisters, since females remain near their natal burrow and often cooperate in raising and protecting young. However, the data did not support this hypothesis. Juvenile female squirrels had no preference for playing with or having other social interaction with full sisters over half-sisters. The data did show that juvenile males played with full brothers longer than half-brothers. This data suggests that social behavior is important for male juveniles, but it is not understood how these social bonds among related males may be important. Future research would benefit from using a larger sample size, observing mating of ground squirrels, and studying why males have a preference for full brothers over half-brothers during play and affiliative interactions.

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