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BRIGGS, JESSICA, M.S. The Individual Context of Ultrasonic Vocalizations in Wild Monogamous California Mice (*Peromyscus californicus*). (2009) Directed by Matina Kalcounis-Rüppell. 54 pp.

Ultrasonic vocalizations (USVs) are commonly produced by the rodent species in the Super Family Muroidea. The bulk of USV research has been conducted on *Mus* and *Rattus* exclusively in a laboratory setting. There is variation in the production and function of USVs between *Mus* and *Rattus* as a result of contrasting social structures. *Peromyscus californicus* is an obligately monogamous species that regularly produces USVs. The objective of my study is to determine the context of USV production in free living *P. californicus* in California. I investigated motif use and spectral and temporal characteristics of USV as they related to sex, and estrous state. I determined when pairs produced USVs as well as the individuals present when USVs were produced. I looked at the production of USVs in response to sub-adult dispersal and I compared the variation between pairs with the variation within pairs. The most commonly recorded USV motifs were 1-4 syllable vocalizations. Sex and estrous state were independent of motif type and the spectral characteristics of USVs did not differ between males and females or estrous and non-estrous females. Pairs never vocalized when they were in contact and motif type was independent of individuals present on the focal area. The majority of USVs were recorded during the breeding season, however, USV production was highly positively correlated with sub-adult dispersal (Pearson's correlation 0.79). While I did not find a difference between pairs or individuals within a pair, my analysis suggests that the difference between pairs is more significant than the difference between individuals within a pair. My results suggest USVs reinforce pairbonds and advertise a pair's

territory. *Peromyscus californicus* is an excellent model for monogamy in mammals and USVs are an important component of *P. californicus* behavior in the wild.

THE INDIVIDUAL CONTEXT OF ULTRASONIC VOCALIZATIONS IN WILD MONOGAMOUS CALIFORNIA MICE (*PEROMYSCUS CALIFORNICUS)*

by

Jessica Briggs

A Thesis Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the requirements for the Degree
Master of Science

Greensboro
2009

Approved by

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APPROVAL PAGE

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ACKNOWLEDGMENTS

I thank Dr. Matina Kalcounis-Rüppell for being my advisor and guiding me throughout the project especially the writing process.

I am grateful to Radmila Petric, Eden Gonzalez, and Catherine Carney for the work and support in the field. I would also like to thank Hastings Natural History Reserve for allowing access to the field site and use of the facilities and also Eric Walters for assistance with the setup of equipment.

I greatly appreciate the many hours dedicated to helping with the organization and analysis of data by the undergraduates and high school students of the Kalcounis-Rüppell bat and mouse lab. I greatly appreciate the support and companionship from my fellow graduate students (in particular Radmila Petric).

My committee members: Dr. John Lepri, Dr. Cheryl Logan, and Dr. Matina Kalcounis-Rüppell, provided comments and suggestions to improve my thesis. Funding was provided by the University of North Carolina at Greensboro and the National Science Foundation.

Importantly, I thank my family for their support and encouragement through this process.

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CHAPTER I

INTRODUCTION

Animals use communication to relay information to members of their social group, rivals, potential mates, and predators [Bradbury, 1998]. Communication involves the transfer of information encoded in a signal from a sender to a receiver ultimately affecting the receiver's behavior [Bradbury, 1998]. Vocalizations are auditory signals used to transmit information between a sender and receiver [Bradbury, 1998]. Information that can be conveyed in spectral and temporal characters of vocalizations includes an individual's identity, status, and affective state [Geissler and Ehret, 2002].

Vocal signals often act as mate attraction signals which broadcast the caller's location and availability to the opposite sex [Bradbury, 1998]. This allows for a shorter time searching for mates which can have a large energetic cost and increase an individual's risk of predation. Mate attraction signals are usually broadcast when males and females are far apart and also include information on the caller such as species, age, or phenotype.

Once a potential mate is close by, courtship vocalizations are produced to coordinate reproductive behavior [Bradbury, 1998]. Males often produce courtship vocalizations which are short range and directed towards a particular female [Bradbury, 1998]. Courtship vocalizations indicate a willingness to mate and the male's intentions, which reduces aggression from the female [Bradbury, 1998, White et al., 1998]. Post-

copulatory calls of males may help to maintain proximity to the female and keep her arousal levels high in order to achieve additional copulations [Barfield and Geyer, 1975, Pomerantz and Clemens, 1981]. Females produce pre-copulatory and post-copulatory vocalizations to indicate sexual state and proceptivity [Thomas and Barfield, 1985, Poole et al., 1988, Semple et al., 2002].

For animals that develop pair bonds and defend territories, communication is also important for the coordination of territorial defense. Many bird species form pair bonds [Mock and Fujioka, 1990]. To facilitate the coordination of territorial defense, pairs duet in response to a territorial intrusion [Wickler, 1976]. Duetting is the joint acoustic display where two birds coordinated their song with a degree of temporal precision [Farabaugh, 1982]. For species in which duetting is common, pairs maintain a territory throughout the year and both sexes participated in territorial defense [Wickler, 1976]. Consistent with territorial calls and displays, duets are often loud, easily located, and performed from prominent places [Payne, 1971, Harcus, 1977]. For example, purple crowned fairy wrens (*Malurus coronatus*) show an intense duetting to a simulated territorial intrusion with duets being initiated, and intruders being approached, equally by both sexes, indicating joint defense of their shared territory [Hall and Peters, 2008].

When animals form long term pair or group bonds, vocalizations often converge in temporal and spectral characters [Tyack, 2008]. For example, the vocalizations of individual pygmy marmosets (*Cebuella pygmaea*) converge shortly after being paired with an unrelated individual and do not diverge for the length of the pairing [Snowdon and Elowson, 1999]. Convergence that coincides with the pairing of two individuals may

aid in the formation of the pair bond in pygmy marmosets [Snowdon and Elowson, 1999]. In addition, convergence in orca (*Orcinus orca*) vocalizations is responsible for pod specific dialects [Strager, 1995, Yurk et al., 2002]. Individuals in specific pods produce group distinctive calls which aid in maintaining group cohesion during foraging or interactions with other pods [Yurk et al., 2002]. Similar to orcas, greater spear-nosed bats (*Phyllostomus hastatus*) use group distinctive vocalizations that help maintain contact while outside the roost [Boughman, 1997].

Pairs that maintain a continuous social bond are considered behaviorally monogamous [Black 2001]. Monogamy is commonly seen in animals that produce altricial young and an important precondition to monogamy is biparental care [Mock and Fujioka, 1990]. In monogamous systems, males generally provision the young and act as sentinels [Hannon, 1984, Bart and Tornes, 1989]. Monogamy is maintained through hormones such as dopamine, vasopressin, and oxytocin [Curtis et al., 2007].

While behavioral monogamy is widely seen in birds (90% of all species), behavioral monogamy is rare among mammals (3-5% of all species) [Mock and Fujioka, 1990]. Rodents display varying degrees of monogamy. In obligate monogamous species paternal care is critical to the survival of the offspring. Obligate monogamy is seen in *Peromyscus californicus, Peromyscus polionotus, Mus spicilegus, Microtus ochogaster,* and *Castor canadensis* [Solomon and Keane, 2007, Waterman, 2007]. In facultative monogamous species, there is no paternal care and mating strategies vary with female density. Facultative monogamy is seen in *Peromyscus maniculatus, Peromyscus leucopus, Microtus californicus, Microtus montanus, Microtus townsendii, Marmota*

flaviventris, and *Marmota caligata* [Solomon and Keane, 2007, Waterman, 2007].

The California mouse (*Peromyscus californicus*) is an obligate behaviorally and genetically monogamous mouse [Ribble, 1991]. Individuals establish a pair bond with another individual and mate for life unless their mate dies or disappears [Ribble, 1992b]. All of a female's offspring are sired by her mate [Ribble, 1991]. Mated pairs nest together during breeding and non-breeding seasons and maintain an exclusive territory [Ribble and Salvioni, 1990]. The territory is protected year round by both the male and female who display aggression toward any intruder [Gubernick and Nordby, 1993]. Males and females are sexually monomorphic ranging in size from 35-60g with a 1:1 sex ratio [Merritt, 1978]. Individuals have a lifespan of 18-20 months [Ribble and Salvioni, 1990]. Pairs produce between two and three litters with an average of two pups per litter [Rood, 1966] with an overlap in litters occurring whereby older siblings assist in the care of the younger siblings [Gubernick and Laskin, 1994]. Sub-adults are older offspring who are about to disperse or recently dispersed. Dispersal of sub-adults occurs 60 days post-partum and is female biased; males disperse a distance equivalent to one home range (1161 m^2) or inherit their parent's home range whereas females disperse at least two home ranges from their natal home range [Ribble and Salvioni, 1990, Ribble, 1992a]. Sub-adults reach sexual maturity at 250 days postpartum [Gubernick, 1988]. Bi-parental care is displayed by *P. californicus* [Dudley, 1974] and males exhibit all components of parental care behavior except nursing. Male care is needed for offspring survival in the wild [Gubernick and Teferi, 2000] and is mediated by chemosignals in the female's urine and copulation [Gubernick, 1990, Gubernick et al., 1994].

Peromyscus californicus is known to produce vocalizations in the ultrasonic range [above 20 kHz; Sales and Pye, 1974] [Kalcounis-Rueppell et al., 2006]. Ultrasonic vocalizations (USVs) have been recorded in the lab and the wild [Kalcounis-Rueppell et al., manuscript submitted, Kalcounis- Rueppell et al., 2006]. Although there has never been a study to specifically examine USVs in adult *P. californicus*, Gubernick et al. (1993) observed that mated pairs remained quiet while they were in close proximity to each other.

Individual *P. californicus* frequently emit long multi-syllabic vocalizations and motifs are distinguishable by the number of syllables in each vocalization (**Figure 1**)[Kalcounis-Rueppell et al., 2006]. The common motifs consist of 1-4 ultrasonic long syllables that range in duration from 100ms to 200ms. Syllables are separated by an interval of approximately 200ms (**Figure 1)**.

The ultrasonic vocalizations that have been previously recorded from wild *P. californicus* [Kalcounis- Rueppell et al., 2006] were not attributed to particular individuals, but rather were passively recorded. Therefore the behavioral context of USVs production in wild *P. californicus* is not known. Based on evidence from laboratory *Mus* and *Rattus,* which are in the same Super Family Muroidea, USVs in *P. californicus* may be indicators of affective state, sexual receptivity, or individual rank/status [Thomas and Barfield, 1985, Moles et al., 2007, Burgdorf et al., 2008]. The Norway rat (*Rattus norvegicus*) produces USVs to establish dominant subordinate relationships and to convey an individual's affective state [McIntosh and Barfield, 1980, Knutson et al., 1998, 2002]. The house mouse (*Mus musculus*) produces USVs to

facilitate or inhibit social interactions [Whitney et al., 1973, Pomerantz et al., 1983, White et al., 1998]. However, although *R. norvegicus* and *M. musculus* are muroid rodents, neither is obligately monogamous. Because of the strong pair bond in *P. californicus* [Ribble and Salvioni, 1990], USVs may function in pair bond formation and maintenance as in other vocalizing animals that produce strong bonds between individuals [Ford, 1989, Sugiura, 1998, Kazial et al., 2001, Hall and Peters, 2008].

Peromyscus californicus is an excellent model for monogamy in mammals and USVs are an integral component of their behavior [Kalcounis-Rueppell et al., 2006]. The first step in understanding the function of USVs in *P. californicus* is to determine the context of the vocalizations produced by individuals in the wild. Therefore, the objective of my study was to determine the context of USV production in individual free-living *P. californicus* in the wild. My first hypothesis was that *P. californicus* would use USVs to maintain contact between members of a pair. I predicted that males and females would both produce USVs, that USVs would be produced when members of a pair were not in direct contact, and that USV production by females would be independent of estrous state. My second hypothesis is not mutually exclusive from my first hypothesis and was that *P. californicus* would use USVs to facilitate territorial defense by members of a pair. I predicted that USVs would be produced in the presence of non-mates and production of USVs would be concomitant with dispersing sub-adults due to the territorial behavior of pairs [Gubernick and Nordby, 1993]. My third hypothesis was that spectral characters of USVs would be more similar within than

between mated pairs of *P. californicus* indicating convergence of vocalizations. For all hypotheses I examined motif type and spectral characters.

CHAPTER II

METHODS

Field Methods

Study Site - Field work took place at The Hastings Natural History Reservation (HNHR) in the foothills of the Santa Lucia mountains in upper Carmel Valley, California (Monterey Co: 36º22'N, 121º33'W) between December 2007 and June 2008 and during January 2009. Mean annual rainfall is 53 cm, occurring mainly between November and April (peak in January and February) which corresponds to the breeding season of local *Peromyscus* species [McCabe and Blanchard, 1950]. The HNHR encompasses three narrow valleys with habitat types including riparian, oak-bay woodland, chaparral, and grassland [Griffin, 1977]. The site has long-term live-trapping grids (10m x 10m array) that were established for the study of *P. californicus*. My study occurred on the Lower Robertson Creek grid (Grid LRC) which consists of a 4 by 34 configuration of trap stations encompassing 2.2 ha.

Live Trapping- To determine densities of mice on the grid, areas throughout the entire trapping grid were consecutively trapped for three nights throughout the entire field season using standard live-trapping techniques. Mice were captured using Sherman and Longworth traps provisioned with oats and bedding. Two Sherman and one Longworth were set at each station at sunset and checked ~4 hours prior to sunrise. Upon capture of

a new individual, a single ear tag with a unique numeric code was attached. Mass, sex, age, and reproductive status were recorded for every individual upon capture. Age was determined based on 1) size, 2) stage of molt, and 3) reproductive condition [Merritt, 1978]. Sub-adults were non-reproductive and going through a molt from a grey juvenile coat to a brownish red adult coat. The molt began laterally and progressed dorsally. Sexual maturity is reached at 250 days post-partum therefore sub-adults were not pregnant or lactating. Mice were released at the site of capture. All capture data were uploaded into Microsoft Access after every trapping session. An individual that was captured at least 3 times during previous trapping sessions at a specific area was classified as a resident. Trapping data were uploaded into Arcview GIS 3.2 to map individual home ranges and examine density hot spots of *P. californicus* on the trapping grid.

Establishment of Focal Areas- Eleven approximately $10m^2$ sections of the grid were designated as focal areas for the purpose of recording USVs from individual mice. Focal areas were chosen sequentially because it was only possible to collect data from a single focal area at a time due to equipment and personnel limitations. Focal areas were chosen based on the presence of resident *P. californicus* and the site's suitability for deploying our remote sensing equipment. At each focal area, we set up a microphone array, a radio-telemetry system, and a thermal imaging camera to record USVs from individual mice. Briefly, the 12 microphones recorded broadband sound from all resident mice and were set out in a 4 by 3 configuration approximately 2 meters apart. The telemetry system surveyed all resident mice in the focal area so that we could localize individuals that were

producing USVs. The thermal camera system surveyed the focal area from the canopy of the forest and recorded all activity in the focal area to ensure that only known, resident mice were present when a particular USV was recorded. Specific details follow.

Sound Recording in the Focal Area- The microphone array consisted of 12 Emkay FG Series microphones capable of recording broad band sound (10-120 kHz). All microphones were plugged into an Avisoft UltraSoundGate system (Avisoft Bioacoustics) attached via a 2.0 USB interface to a laptop (DELL Latitude D410). The system was powered by a 12V dry cell battery using a 150W inverter. The microphones sampled at 250 kHz with a 16 bit resolution. Vocalizations were recorded using Avisoft RECORDER software (Avisoft Bioacoustics). The array was capable of localizing the position of vocalizations by calculating time-delay of arrival (TDOA) of sound intercepted by all 12 microphones. Prior to the start of recording, an ultrasonic whistle was used to verify proper function of all microphones. Recording began just before sunset and continued until sunrise. Every morning, sound files were reviewed and uploaded to a Drobo (Data Robotics Inc.) external hard drive.

Radio Telemetry of Resident Mice in the Focal Area- Captured residents at each focal area were fitted with a mouse-style 0.55g M1450 transmitter (Advanced Telemetry Systems, ATS). Transmitters were secured around the necks of the mice. When all resident mice were outfitted with collars, small antennae (Sigflex 15 cm omni-directional) were set in each corner of the focal area. The antennae were wired to a central receiver (4MHz R4000), an antenna switch box, and a data logger (DSU D50410; all from ATS). To facilitate identification of individual mice, each radio transmitter had

a unique frequency. The receiver was programmed to search for all frequencies in the focal area so that each mouse could be monitored. When a frequency was detected, the receiver recorded the signal strength at each antenna and then moved onto the next frequency.

Prior to putting the transmitters on the mice, each transmitter was tested on the focal area at each microphone site. The transmitters were placed at each microphone site for 3 minutes while the signal strength at each antenna was recorded. Testing the collars beforehand ensured that the system and collars were working properly and gave us a reference database to assign positions of an individual at a particular time in the focal area. The radio telemetry data recorded by the cataloguer were uploaded and reviewed each morning to determine if any radio-collars had been removed or were no longer working. The receiver was on continuously during recording in the focal area.

Thermal Imagery of the Focal Area- A thermal imaging camera (Photon 320 14.25 mm; Flir/Core by Indigo) was used to observe whether or not a mouse was at the location of the USV and that uncollared mice were not seen in the focal area. The camera was suspended on a rope and pulley system between two trees and was approximately10 meters above the focal area allowing us to film the entire microphone space. The thermal imaging camera fed real time images directly (via standard video cabling) to a ground-based digital video recorder (JVC Everio DVR). The DVR recorded up to 12hrs during each night and stored the images directly to a 30 GB hard-drive; the data were downloaded each morning to a Drobo (Data Robotics Inc.) external hard drive. The camera was turned on at the same time as the microphone array.

At the start of recording each evening, time on all equipment was synchronized. Thus, the 12 microphones recorded sound continuously (with the capability of localizing the origin of the sound), the receiver continuously scanned for the radio-transmitter frequencies of each radio-collared mouse constantly, and the thermal imaging camera documented all mice present.

Laboratory Analysis

Integration of Data Recorded from Focal Areas- To assign an individual to each USV recorded in a focal area, we integrated live-trapping data, the acoustic recordings, the radio telemetry data, and the thermal imagery. First, all sound files recorded were played back at 11.025 kHz to determine if the sound was a USV from a mouse. Second, sound files that contained mouse USVs were spectrographically examined in Avisoft SASLab Pro (Avisoft Bioacoustics) noting the time of arrival of the sound at each microphone in the array. The order in which the USV arrived at each microphone was used to determine where on the focal area the mouse was located during the production of the vocalization. Third, telemetry data were used to determine which individual mouse was at the position of the USV in the focal area when the USV occurred. The signal strength recorded at each of the four antennae was used to determine if any detected transmitter matched the location of the USV. Fourth, thermal images were used to determine: 1) if there was a mouse present during the production of the USV and 2) if there was another untransmittered mouse on the focal area that could be responsible for the production of the USV.

Integration of the data to assign individuals to a particular USV was done manually for every USV we recorded. Three researchers separately assigned an individual to each USV and any discrepancies were re-examined and either resolved or the USV was not included in further analyses.

Ultrasonic Vocalizations assigned to an individual were analyzed spectrographically using Avisoft SASLab Pro (Avisoft Bioacoustics). Spectrographs were recorded with an FFT length of 256 in a Hamming window, with a frame size was 100%, and a resolution overlap of 50%. A USV was analyzed from one microphone in the array and was chosen based on the amplitude of the waveform. The entire USV had to be clearly visible in the waveform window but not be too loud to cause artifacts (i.e., aliasing). Using the automated detection feature in SASLab pro, I measured the following parameters: group duration (duration of the entire USV), syllable duration (duration of each syllable), peak frequency, minimum frequency, maximum frequency, and bandwidth at the start, end, and peak amplitude within the vocalization. Each USV was characterized as one of five previously defined motifs.

To determine the individuals composed a mated pair, I used hand held telemetry during the day to track pairs to their nest sites. Pairs nest together throughout the year, therefore radio-tracking to nest sites is a reliable method for assigning pairs [Ribble and Salvioni, 1990]. In addition, trapping data were used in ArcView GIS 3.2 (ESRI, Redlands, CA) to map homeranges of all individuals (kernel estimates: 50% core and 95%, smoothing factor=5). Pairs maintain exclusive homeranges [Ribble and Salvioni, 1990] and overlapping homeranges were used to confirm pairs.

Statistical Analysis- For the analysis of spectral characters of USVs, the four unique but common motifs were examined separately [Kalcounis-Rueppell et al. 2006]. All USVs assigned to the same individual were averaged for each syllable of each motif to avoid pseudo-replication. Acoustic variables for each syllable of each motif were subjected to a principal components (PC) analysis in order to reduce the number of variables for analyses. To compare spectral characters between males and females, and between estrous and non-estrous females, two to three PC scores for each syllable were assessed in one-way analysis of variance tests (ANOVA).

To determine if USV production was influenced by sex or by estrous state of females, I used Chi Squared Tests of Independence. I also used Chi Squared Tests of Independence to examine whether USVs were more frequently produced when mates were in close proximity (<1m) or not in close proximity (>1m), and to examine if USVs were more frequently produced in the presence of a non-mate. I determined whether or not production of USVs was related to sub-adult dispersal by examining the correlation of USV production and capture rate of sub-adults by month.

To determine whether or not USV characteristics converge within pairs of mice, I examined spectral characters of 3SVs, as this was the only motif with multiple USVs for more than two pairs. To analyze differences among pairs, PC scores for each syllable were used in an ANOVA. To analyze the difference in acoustic variables between members in a pair of mice, I used the acoustic variables without averaging for each individual mouse. The acoustic variables for each syllable were subjected to a PC analysis and tested using the Mann-Whitney U test. All ANOVAs were completed using

SPSS Statistics 17.0 (SPSS Inc., Chicago, IL). Chi Squared tests were computed using a formula from *The Statistical Sleuth, Second Edition*, Ramsey and Schafer, 2002.

All results are presented as mean ± 1 SD unless otherwise noted. All data sets were tested for normality using a Levene's Test of Normality, when data were not normal a non-parametric test was used. A probability criterion of $\alpha=0.05$ was used for all statistical tests unless otherwise noted (i.e. Bonferroni corrections).

CHAPTER III

RESULTS

Data were successfully collected on 131nights from February 2008 through June 2008 and during January 2009. Equipment was placed at 11 different focal areas for an average of 13.6±4.76 days at each focal area. Forty-three transmitters were placed on resident *P. californicus*. Eleven mice received transmitters at more than one focal area. Overall, 28 individual mice (17 females, 11 males) received transmitters through the field season. Of these, 3 were sub-adults, 1 was a juvenile, and 24 were adults. On average 3.8±1.68 individuals carried transmitters at each focal area.

In total, I recorded 1416.5 hours of thermal imaging video, 1747.5 hours of telemetry data, 1392 hours of audio data, and 116,871 separate audio files. Most of the audio files were ultimately classified as being not from animals but from rain and wind and these were not assessed further. Of the audio files recorded,1090 were USVs from *Peromyscus californicus* or the syntopic *P. boylii* (as part of a companion study). Of the1090 *Peromyscus* USVs recorded, 1050 were 1-5 syllable vocalizations. Based on adequate verification from telemetry and imaging, I was able to assign 223 USVs to individual *P. californicus* from 18 residents (13 females, 5 males; 17 adults, 1 sub adult) recorded on 39 nights. Based on daily nest cohabiting and shared core home ranges from trapping data, I determined there were 4 unequivocal male-female mating pairs among

the sample. I recorded 204 USVs from pairs. Females from the pairs produced 145 USVs and males produced 59 USVs.

Across all evenings, USVs were first recorded no earlier than 18:00 with a peak at 21:00 and USVs always concluded prior to 06:00 **(Figure 2)**. The number of USVs was highest between February-April (11.56±28.43/night). The total number of USVs decreased after most individuals had finished breeding in May (0.89±0.94/night) and June (0.50±0.58).

Descriptive statistics of the original 14 spectral and temporal variables for each USV motif by sex are reported in Tables 1-4. The original 14 spectral and temporal variables were reduced to 2-3 PC variables using the Principle Component Analyses. The PC axes explained 76%-93% of the total variation in spectral and temporal characters. In general, frequency variables had high factor loadings on the first PC axis (PC1), bandwidth variables had high factor loadings on the second PC axis (PC2), and syllable duration had high factor loadings in the third PC axis (PC3; data not shown). Most USVs were multi-syllabic with low bandwidth (6.83±0.31 kHz across all motifs). One-syllable vocalizations (1SV, n=42), two-syllable vocalizations (2SV, n=80), three-syllable vocalizations (3SV, n=72), and Four-syllable vocalizations (4SV, n=29), were the most common motifs recorded from *P. californicus*. I recorded five-syllable vocalizations (5SV) from one female (not further analyzed due to rarity). The 1SV consisted of one long syllable with a mean frequency of 27.24±3.10 kHz and a mean duration 132±38.5 ms **(Figure 3)**. The 2SV consisted of two syllables with a mean frequency of 26.11±3.2

kHz separated by a 287±123 ms interval and a duration of 460±120 ms **(Figure 4)**. The 3SVs consisted of three syllables with a mean frequency of 24.76±3.31 kHz separated by a 249±103 ms interval and a duration of 720±240 ms **(Figure 5)**. The 4SVs consisted of four syllables with a mean frequency of 26.58±2.34 kHz separated by a 231±68 ms interval and a duration of 810±221 ms **(Figure 6)**. There were three different situational contexts in which USVs were produced **(Figure 7)**: **a)** when the caller was alone on the focal area, **b)** when a non-mate was present with the caller on the focal area, and **c)** when the caller's mate was present on the focal area but >1m away from the mate. However, when mates were within 1 m of one another there were no USVs produced **(d)**.

Both males and females produced all common motif types (**Figure 8**). To analyze the relationship between motif type and sex, the data from one female were removed from the data set because her nest site was on the focal area and I recorded 125 USVs from her over 3 nights, a comparable number of USVs was not recorded from any other individual and therefore I considered her vocalization behavior to be an outlier. The spectral characters of each individual were averaged for each syllable of each motif type to prevent the female from biasing the results. The type of USV motif produced was independent of sex (χ^2=7.21, p=0.13) **(Figure 8)**. There was no difference between males and females in spectral and temporal variables of USVs **(Table 5)**.

Due to sample size restrictions, I was only able to analyze 2SVs for differences between estrous and non-estrous females and they did not differ in acoustic structure **(Table 6).** In addition, the type of USV motif produced was independent of estrous state (χ^2=0.50, p=0.08).

I recorded 12 USVs from individuals with mate present in the focal area and 20 USVs from individuals with non-mates present in the focal area (see **Figure 7**). I never recorded a USV from an individual when its mate was in close proximity i.e. less than 1m apart from the caller; all 12 USVs recorded from individuals in the presence of a mate were recorded when that mate was greater than1 m away (see **Figure 7**). However, individuals were just as likely to vocalize in the presence of a non-mate as they were to vocalize when a mate was on the focal area (χ^2=2.0, p=0.16). There was no difference in the acoustic structure of 1-3SVs produced in the presence of a mate or a non-mate **(Table 7),** nor was there a difference in USV type produced in the presence of a mate or a non-mate (χ^2=3.87, p=0.72) **(Figure 9)**. Sub-adult dispersal and USV production by month were highly positively correlated (Pearson's Correlation = 0.79) **(Figure10).**

There was no significant difference in spectral and temporal characters among pairs that produced 3SVs when considering the Bonferroni corrected rejection criterion of p<0.006 (**Table 8)**. However, there was a trend toward statistically significance for PC1 of the second syllable (p=0.01) and PC2 of the third syllable (p=0.04; **Table 8**). Members of the same pair did not differ in spectral and temporal characteristics of USVs **(Table 9)** and there were no trends towards significance. Thus, although neither was significant, among-pair comparisons suggested larger differences in spectral and temporal characters of 3SVs than did within-pair comparisons.

CHAPTER IV

DISCUSSION

I found that free-ranging, adult male and female *P. californicus* in their native habitat produce ultrasonic vocalizations, with the most common motifs being 1, 2, 3, and 4 syllable vocalizations. Ultrasonic vocalizations were common during the breeding season and produced throughout the night with a peak around 9pm. I predicted that if males and females both produced USVs, if USVs were produced when members of a pair were not within 1m of one another, and if USV production by females was independent of estrous state, then USVs could serve to maintain contact between members of a pair. I found that both males and females produced USVs in the same proportion of common motifs and acoustic characters. Furthermore, USVs were produced when members of a pair were more than 1m apart from each other. Finally, estrous and non-estrous females did not differ in motif type or acoustic characteristics of USVs. All three results support the hypothesis that USVs could help maintain contact between members of breeding pairs.

I found that USVs were produced in the presence of non-mates and USV production was positively correlated with sub-adult dispersal, supporting my hypothesis that USVs could also help maintain territorial boundaries of breeding pairs. Both results support my hypothesis that USVs could facilitate territorial defense by members of a pair. My data suggests a statistical trend toward convergence of USVs in mated pairs which

suggests that USVs may serve two concurrent functions: 1) to maintain pairbonds and 2) to reinforce territorial boundaries.

Both male and female *P. californicus* produced equivalent proportions of the USV motifs. Similarly, male and female laboratory bred strains of rats also produce the same motifs during copulation, play, and aggressive interactions [Thomas and Barfield, 1985b]. In contrast, although both male and female laboratory bred strains of mice produce USVs, the number of USVs of particular motifs produced by each sex varies depending on the individual with whom they are interacting [Warburton et al., 1989]. During male-female interactions, the male produces the majority of USVs [Warburton et al., 1989]. During female-female interactions the resident females produce USVs [Moles et al., 2007]. The differences in USV production between laboratory bred strains of rats and mice can be explained by their different social and breeding systems. Laboratory bred strains of rats are more colonial than laboratory bred strains of mice and share extensive pair bonding. The difference between laboratory bred strains of mice and rats in USV patterns in males and females has been attributed to the different social structures of mice and rats [Costantini and D'Amato, 2006]. Laboratory bred strains of rats use USVs in a wider variety of social interactions than mice, reflecting the more complex social structure of rats [Costantini and D'Amato, 2006]. Like laboratory bred strains of rats, *P. californicus* have strong pairbonds and interact more often with another adult than laboratory bred strains of mice. Thus, I proposed that USV production in *P. californicus* is more likely similar to USV production in laboratory bred strains of rats because of the similar social context.

Spectral and temporal characteristics of USVs did not differ between male and female *P. californicus*, suggesting that sex is not being communicated through USVs. Laboratory bred strains of mice, which are polygamous or promiscuous, use vocalizations while searching for a mate and may therefore benefit by advertising their sex acoustically [Whitney et al., 1973]. Male laboratory bred strains of mice vocalize via USVs while approaching females, a behavior that reduces aggressive behavior from females [Whitney et al., 1973]. Because of their monogamous, long term pairbonds, *P. californicus* males and females are in regular contact with their mates and would be expected to advertise sex only when searching for a mate. In addition, captive *P. californicus* display aggression towards unknown conspecific individuals regardless of sex, therefore, USVs in *P. californicus* likely do not assist in mediating aggression between mates. Testing the hypothesis that USVs are independent of aggressive interactions between *P. californicus* mates would require an experimental approach whereby USVs were investigated when one mate was removed to initiate searching for a new mate, or when female sub-adults or newly scrotal territorial males search for a mate. If USVs are found to not mediate encounters between non-mates it would further support my hypothesis that USVs are produced to maintain contact between members of a pair.

Reducing aggressive behavior is not the sole reason individuals have for indicating sex through the production or acoustic characters of USVs. Males that compete for females vocalize to attract a female and advertise their genetic viability. If vocalizations are used for courtship behaviors, both males and females benefit by being able to identify the sex of the caller. If *P. californicus* produced USVs to attract a mate, I

would predict a divergence between the sexes in sub-adults since they most likely do not have a partner and would therefore more likely produce courtship vocalizations. Due to sample size, I did not have enough sub-adults to compare USVs between male and female sub-adults. If in fact there was a difference in the production of USVs of male and female *P. californicus* sub-adults and no difference between male and female adults, this would give evidence of a courtship function of USVs as well as convergence of USVs in mated pairs.

All vocalizations by *P. californicus* in this study were recorded while individuals were moving through their homerange. Although I found no difference in acoustic structure of USVs by males and females, there may be a sex difference in context (i.e. in the nest). For example, big brown bats (*Eptesicus fuscus*) displayed context dependent USVs in which vocalizations by males and females differed within the roost but not while foraging [Grilliot et al., 2009]. As described above, while foraging in the territory, the vocalizations of *P. californicus* may serve a territorial and contact function, however, in the nest, USVs may serve a different function which may lead to a divergence in acoustic characteristics between males and females in that context.

I found that USVs did not occur when members of the pair were within 1m of one another but were more often produced when the members of a pair were more than 1m away from each other. In the latter scenario, one member either moved outside the field of view for the imaging system or moved to a different part of the focal area. In the laboratory, when members of the pair of *P. californicus* were in contact, neither partner vocalized [Gubernick and Nordby, 1993]. In the wild, nocturnally active *P. californicus*

live in habitat that has dense understory. Therefore, mates may easily lose visual contact when one member moves away from the other and USVs may help reestablish contact.

Estrous and non-estrous female *P. californicus* produced the same motif types and did not vary in the acoustic structure of USVs. While both male and female laboratory bred strains of rats produce an equal number of USVs, estrous females produce more vocalizations than non-estrous females which facilitates reproductive coordination and advertises receptivity [Thomas and Barfield, 1985]. My data suggest that *P. californicus* do not use motif type, number of calls, or spectral and temporal characters of USVs to communicate reproductive state. Male *P. californicus* only copulate with their mate on her first day postpartum estrous [Gubernick, 1988]. The male is present for the birth and parturition and the subsequent estrous is likely a sufficient cue to communicate copulatory readiness. Therefore, male *P. californicus* likely do not need auditory cues as an indication of reproductive state. Members of *P. californicus* pairs are extremely familiar with one another; therefore coordination of reproductive behavior through a mechanism such as USVs is not as likely as in laboratory bred strains of mice and rats that do not exhibit the same level of monogamous, life-long pair bonding.

Ultrasonic vocalizations were produced across the breeding season and peaked in February-April. In addition, dispersal of the first litters of the breeding season coincides with the peak breeding season. During this time sub-adults are seeking mates and breeding pairs are defending territories. While sub-adults are no more of a threat than adults, more sub-adults are emerging from their nest to forage and disperse, ultimately increasing the threat to territorial pairs. My data show a positive correlation between

sub-adult dispersal and USV production. Pairs maintain and defend exclusive territories and display aggression toward any individual that is not their mate regardless of reproductive condition. In the laboratory, *P. californicus* males and females have been shown to actively defend territories against intruders, presumably reducing infanticide and cuckoldry [Gubernick and Nordby, 1993]. Laboratory results suggest that in the wild, there should be a shift to vigilance with active mate guarding and nest guarding during times of high levels of sub-adult dispersal to prevent usurpation and infanticide. An increase in USV production during sub-adult dispersal may indicate a behavioral shift towards increased vigilance. Besides USV production being correlated with sub-adult dispersal in the field, I also found that USVs were produced in the presence of a non-mate. This suggests USVs are not solely directed toward an individual's mate but may also be used to advertise a pair's territory to a potential intruder.

Until experiments are conducted, it is difficult to distinguish territorial defense from aggression. If USVs are produced in defense of a pair's territory USVs should be produced at the edge of a pair's territory. USVs produced in aggressive encounters should occur in a neutral area between two territories or within the core of a pair's homerange. Pair's maintain a homerange that is larger than each focal area. Based on homerange data half the focal areas were setup on the border of a pair's territory while the other half were in or near a pair's core homerange. Experiments can be conducted to determine the difference between USV production during aggression and territorial defense. Once a pair's homerange has been determined, a non-mate should be introduced at the border of the territory, in the core, and in the neutral area between two territories.

While USV production may be used to advertise a pair's territory, USVs may also be produced not in defense of their territory but to coordinate territorial defense between members of a pair. It is hypothesized that birds produce duets to coordinate territorial defense and duets allow the partners to determine where each other is on the pair's homerange [Hall, 2004]. When *P. californicus* pairs are foraging together, they remain close to each other and may not need vocalizations to coordinate territorial defenses. Also, there is a possibility that contact vocalizations could be misinterpreted as vocalizations to coordinate territorial defense or vise versa since both types of vocalizations would be produced when a pair is no longer in close proximity. Therefore, the true function of USVs may be difficult to determine through observational studies alone.

Pairs of *P. californicus* are territorial and form long term pairbonds, therefore I predicted that USVs would be more similar within pairs than between pairs. I did not find a difference in USVs between pairs or within pairs. However, my results suggest that there is more of a difference between pairs than within a pair because p-values were generally smaller in the between pair comparisons than with the within pair comparisons. These results tentatively suggest that USV structure might converge within pairs. It is likely that with a larger sample size the difference in USVs among pairs may become significant. Birds that are behaviorally monogamous and protect a territory throughout the year produce matching songs they sing in duets [Wickler, 1976]. Although *P. californicus* are not known to produce duets, their matching vocalizations may serve a similar function to duets in birds. Like birds that duet, *P. californicus* are monogamous,

and defend territory throughout the year. Because USVs are produced in the presence of non-mates and my results show a trend toward USV convergence within pairs, a territorial function for USVs is likely. Convergence or similarity in USVs within pairs would not only reinforce territorial boundaries but also reaffirm a pair's bond [Hall, 2000]. My results suggest that *P. californicus* USVs function not only as territorial vocalizations but also contact vocalizations that reaffirm bonding between pair members.

Another observation that could support the production of USVs as contact calls comes from the female that was removed from the data set when I examined the independence of motif type and sex. A high number of USVs were recorded from the female because a microphone happened to be near her nest. The USVs were produced over a 3 night period at the start of a new focal area. The female and her mate were both outfitted with transmitters and during the time the USVs were produced, her partner's transmitter was not recorded by the telemetry system. Pairs usually forage together except the first 15 days post partum when the pups are incapable of thermoregulation and one parent is needed in the nest [Gubernick and Teferi, 2000]. The female was lactating which indicates there was a litter in the nest. The female may have been producing USVs in order to establish contact with her mate while he was not near the nest.

Collectively, my results suggest a dual function of ultrasonic vocalizations in *P. californicus*. It seems likely that USVs maintain contact between members of bonded breeding pairs while at the same time reinforcing strong territorial behavior by the pairs. Being able to distinguish between a contact function and territorial function may be difficult. If an individual vocalizes in response to a non-mate, the vocalization could

potentially be directed to a mate to signal commitment instead of advertising or protecting a territory. This could deter a partner from deserting while warning intruders[Hall, 2000]. However, controlled experiments in the field are needed. For example, a test of the territorial function of USVs would be to introduce a strange individual into a mated pair's territory. If USVs serve a territorial function, both male and female residents should vocalize in response to an intruder relative to a control (for example an introduction of a mate or an offspring). A test of a contact function would be to remove a member of a pair from the pair's territory. Pre-recorded vocalizations from the removed individual could be played back while the partner is active on the territory. If the vocalizations function as contact calls, each partner tested would respond more to the playback of the missing partner's vocalizations (relative to a control) by vocalizing and/or approaching the playback speaker. My results open up exciting potential for experimental tests of USV function in wild *P. californicus*.

In summary, this is the first analysis of USVs from known individual *P. californicus* in the wild. I found that both males and females produced the same common motifs and males and females do not differ in the acoustic characteristics of USVs. Similarly, estrous state has no effect on the motif type or acoustic structure of USVs. Members of a pair only produced USVs when they were not in contact with their mate or when they were in the presence of a non-mate. On a monthly scale, production of USVs was positively correlated with sub-adult dispersal. My results suggest that USVs not only serve to reinforce pairbonds but also serve to reinforce a pair's territorial behavior.

This is the first and only study of USV production by individual wild *P. californicus*. The production of USVs as contact or territorial vocalizations is not mutually exclusive. I have shown that for both male and female *P. californicus* USVs are an important component in their behavioral repertoire in different situational contexts. Combined with the rare monogamous mating system of *P. californicus,* further studies of *P. californicus* USVs in the wild will allow us to determine the role USVs play in maintaining long term pairbonds, mate guarding, territorial defense, and bi-parental care.

REFERENCES

Barfield, R. J., and Geyer, L. A. (**1975**). The ultrasonic post-ejaculatory vocalization and the post-ejaculatory refractory period of the male rat, Journal of Comparative and Physiological Psychology 88, 723-734.

Bart, J., and Tornes, A. (**1989**). Importance of monogamous male birds in determining reproductive success: evidence for house wrens and a review of male removal studies, Behavioral Ecology and Sociobiology 24, 109-116.

Boughman, J. W. (**1997**). Greater spear-nosed bats give group-distinctive calls," Behavioral Ecology and Sociobiology 40, 61-70.

Bradbury, S. V. a. J. (**1998**). *Principles of Animal Communication* (Sinauer Associates, Inc., Sunderland).

Burgdorf, J., Kroes, R. A., Moskal, J. R., Pfaus, J. G., Brudzynski, S. M., and Panksepp, J. (**2008**). Ultrasonic vocalizations of rats (*Rattus norvegicus*) during mating, play, and aggression: behavioral concomitants, relationship to reward, and self-administration of playback, Journal of Comparative Psychology 122, 357-367.

Costantini, F., and D'Amato, F. (**2006**). Ultrasonic vocalizations in mice and rats: social contexts and functions, Acta Zoologica Sinica 52, 619-633.

Curtis, J. T., Liu, Y., Aragona, B. J., and Wang, Z. (**2007**). Neural regulation of social behavior in rodents, in *Rodent Societies: An Ecological and Evolutionary Perspective*, edited by J. O. Wolff, and P. W. Sherman (The University of Chicago Press, Chicago), pp. 185-194.

Dudley, D. (**1974**). Paternal behavior in the California mouse *Peromyscus californicus parasiticus*," Behavioral Biology 11, 247-252.

Farabaugh, S. M. (**1982**). The ecological and social significance of duetting, in *Acoustic Communication in Birds*, edited by D. E. Kroodsma, and E. H. Miller (Academic Press, New York), pp. 85-124.

Ford, J. K. B. (**1989**). Acoustic behavior of resident killer whales (*Orcinus orca*) off Vancouver Island, British Columbia, Canadian Journal of Zoology-Revue Canadienne De Zoologie 67, 727-745.

Geissler, D. B., and Ehret, G. (**2002**). Time-critical integration of formants for perception of communication calls in mice, Proceedings of the National Academy of Sciences of the United States of America 99, 9021-9025.

Griffin, J. R. (**1977**). Oak Woodland, in *Terrestrial Vegetation of California*, edited by M. G. Barbour, and J. Major (Wiley-Interscience, New York), pp. 383-414.

Grilliot, M. E., Burnett, S. C., and Mendonca, M. T. (**2009**). Sexual dimorphism in big brown bat (*Eptesicus fuscus*) ultrasonic vocalizations is context dependent, Journal of Mammalogy 90, 203-209.

Gubernick, D. J. (**1988**). Reproduction in the California mouse, *Peromyscus californicus*, Journal of Mammalogy 69, 857-860.

Gubernick, D. J. (**1990**). A maternal chemosignal maintains paternal behavior in the biparental California mouse, *Peromyscus californicus*, Animal Behaviour 39, 936-942.

Gubernick, D. J., and Laskin, B. (**1994**). Mechanisms influencing sibling care in the monogamous biparental California mouse, *Peromyscus californicus*," Animal Behaviour 48, 1235-1237.

Gubernick, D. J., and Nordby, J. C. (**1993**). Mechanisms of sexual fidelity in the monogamous California mouse, *Peromyscus californicus*," Behavioral Ecology and Sociobiology 32, 211-219.

Gubernick, D. J., Schneider, K. A., and Jeannotte, L. A. (**1994**). Individual differences in the mechanisms underlying the onset and maintenance of paternal behavior and

the inhibition of infanticide in the monogamous biparental California mouse, *Peromyscus californicus*, Behavioral Ecology and Sociobiology 34, 225-231.

Gubernick, D. J., and Teferi, T. (**2000**). Adaptive significance of male parental care in a monogamous mammal, Proceedings of the Royal Society of London Series B-Biological Sciences 267, 147-150.

Hall, M. L. (**2000**). The function of duetting in magpie-larks: conflict, cooperation, or commitment?, Animal Behaviour 60, 667-677.

Hall, M. L., and Peters, A. (**2008**). Coordination between the sexes for territorial defense in a duetting fairy-wren, Animal Behaviour 76, 65-73.

Hannon, S. J. (**1984**). Factors limiting polygyny in the willow ptarmigan, Animal Behaviour 32, 153-161.

Harcus, J. L. (**1977**). Functions of vocal duetting in some African birds, Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology 43, 23-45.

Kalcounis-Rueppell, M. C., Metheny, J. D., and Vonhof, M. J. (**2006**). Production of ultrasonic vocalization by *Peromyscus* mice in the wild. Frontiers in Zoology 3, http://www.frontiersinzoology.com/content/3/1/3/ (open access).

Kazial, K. A., Burnett, S. C., and Masters, W. C. (**2001**). Individual and group variation in echolocation calls of big brown bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae), Journal of Mammalogy 82, 339-351.

Knutson, B., Burgdorf, J., and Panksepp, J. (**1998**). Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats, Journal of Comparative Psychology 112, 65-73.

Knutson, B., Burgdorf, J., and Panksepp, J. (**2002**). Ultrasonic vocalizations as indices of affective states in rats, Psychological Bulletin 128, 961-977.

McCabe, T. T., and Blanchard, B. D. (**1950**). *Three species of Peromyscus.* (Rood and Associates, Santa Barbara).

McIntosh, T. K., and Barfield, R. J. (**1980**). The temporal patterning of 40-60 kHz ultrasonic vocalizations and copulation in the rat (*Rattus norvegicus*), Behavioral and Neural Biology 29, 349-358.

Merritt, J. F. (**1978**). *Peromyscus californicus*, Mammalian Species, 1-6.

Mock, D. W., and Fujioka, M. (**1990**). Monogamy and Long-Term Pair Bonding in Vertebrates, Trends in Ecology & Evolution 5, 39-43.

Moles, A., Costantini, F., Garbugino, L., Zanettini, C., and D'Arnato, F. R. (**2007**). Ultrasonic vocalizations emitted during dyadic interactions in female mice: A possible index of sociability?, Behavioral Brain Research 182, 223-230.

Payne, R. B. (**1971**). Duetting and chorus singing in African birds, Ostrich Supplement, 125-146.

Pomerantz, S., and Clemens, L. (**1981**). Ultrasonic vocalizations in male deer mice (*Peromyscus maniculatus bairdii*): their role in male sexual behavior. Physiology and Behavior 27, 869-872.

Pomerantz, S. M., Nunez, A. A., and Bean, N. J. (**1983**). Female behavior is affected by male ultrasonic vocalizations in house mice, Physiology and Behavior 31, 91-96.

Poole, J. H., Payne, K., Langbauer, W. R., and Moss, C. J. (**1988**). The social contexts of some very low-frequency calls of African elephants, Behavioral Ecology and Sociobiology 22, 385-392.

Ramsey, F. L. and Schafer, D. W. (**1997**), *The Statistical Sleuth: A Course in Methods of Data Analysis*, Belmont, CA: Duxbury Press.

Ribble, D. O. (**1991**). The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting, Behavioral Ecology and Sociobiology 29, 161-166.

Ribble, D. O. (**1992a**). Dispersal in a monogamous rodent, *Peromyscus californicus*. Ecology 73, 859-866.

Ribble, D. O. (**1992b**). Lifetime reproductive success and its correlates in the monogamous rodent, *Peromyscus californicus*, Journal of Animal Ecology 61, 457-468.

Ribble, D. O., and Salvioni, M. (**1990**). Social organization and nest co-occupancy in *Peromyscus californicus* a monogamous rodent," Behavioral Ecology and Sociobiology 26, 9-16.

Rood, J. P. (**1966**). Observations on the reproduction of *Peromyscus* in captivity, American Midland Naturalist 76, 496-503.

Semple, S., McComb, K., Alberts, S., and Altmann, J. (**2002**). Information content of female copulation calls in yellow baboons, American Journal of Primatology 56, 43-56.

Snowdon, C. T., and Elowson, A. M. (**1999**). Pygmy marmosets modify call structure when paired, Ethology 105, 893-908.

Solomon, N. G., and Keane, B. (**2007**). Reproductive strategies in female rodents, in *Rodent Societies: An Ecological and Evolutionary Perspective*, edited by J. O. Wolff, and W. Sherman (The University of Chicago Press, Chicago), pp. 42-56.

Strager, H. (**1995**). Pod-specific call repertoires and compound calls of killer whales, *Orcinus orca* (Linnaeus 1758), in the waters of northern Norway," Canadian Journal of Zoology-Revue Canadienne De Zoologie 73, 1037-1047.

Sugiura, H. (**1998**). Matching of acoustic features during the vocal exchange of coo calls by Japanese macaques, Animal Behaviour 55, 673-687.

Thomas, D. A., and Barfield, R. J. (**1985**). Ultrasonic vocalization of the female rat *Rattus norvegicus* during mating, Animal Behaviour 33, 720-725.

Tyack, P. L. (**2008**). Convergence of calls as animals form social bonds, active compensation for noisy communication channels, and the evolution of vocal learning in mammals, Journal of Comparative Psychology 122, 319-331.

Warburton, V. L., Sales, G. D., and Milligan, S. R. (**1989**). The emission and elicitation of mouse ultrasonic vocalizations: the effects of age, sex and gonadal status, Physiology and Behavior 45, 41-47.

Waterman, J. (**2007**). Male mating strategies in rodents, in *Rodent Societies: an ecological and evolutionary perspective*, edited by J. O. Wolff, and P. W. Sherman (The University of Chicago Press, Chicago), pp. 27-41.

White, N. R., Prasad, M., Barfield, R. J., and Nyby, J. G. (**1998**). 40 and 70 kHz vocalizations of mice (*Mus musculus*) during copulation, Physiology and Behavior 63, 467-473.

Whitney, G., Coble, J. R., Stockton, M. D., and Tilson, E. F. (**1973**). Ultrasonic emissions: do they facilitate courtship of mice?, Journal of Comparative and Physiological Psychology 84, 445-452.

Wickler, W. (**1976**). Duetting songs in birds: biological significance of stationary and non-stationary processes, Journal of Theoretical Biology 60, 493-497.

Yurk, H. L., Barrett-Lennard, J. K., Ford, B., and Matkin, C. O. (**2002**). Cultural transmission within maternal lineages: vocal clans in resident killer whales in southern Alaska, Animal Behaviour 63, 1103-1119.

APPENDIX A. TABLES

Table 1. Descriptive Statistics for Measured Variables within 1 Syllable Vocalizations

The means, plus or minus standard deviation, for the descriptive statistics for 1 syllable vocalizations produced my female and male *Peromyscus californicus*. The original acoustic variables are used instead of PC scores to allow for an easier interpretation of the results. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Duration is in milliseconds and all frequency and bandwidth variables are in kilohertz.

Acoustic Variable	Females (n=13)	Males (n=5)
Duration	136±54	128±23
Peak Freq (Start)	26.69±5.10	28.27±4.28
Min Freq (Start)	24.12±5.60	25.72±4.45
Max Freq (Start)	30.54±5.13	32.33±4.29
Bandwidth (Start)	6.37±1.01	6.56±0.70
Peak Freq (End)	24.35±5.67	26.59±4.98
Min Freq (End)	21.6±5.87	23.78±4.95
Max Freq (End)	28.37±5.58	30.78±4.68
Bandwidth (End)	6.71±0.78	6.99±0.38
Peak Freq (Max)	26.04±6.24	28.79±4.89
Min Freq (Max)	23.44±6.25	25.83±4.94
Max Freq (Max)	30.27±6.73	32.79±4.98
Bandwidth (Max)	6.8±0.75	6.93±0.48

Table 2. Descriptive Statistics for Measured Variables within 2 Syllable Vocalizations

The means, plus or minus standard deviation, for the descriptive statistics for 2 syllable vocalizations produced by female and male *Peromyscus californicus*. The original acoustic variables are used instead of PC scores to allow for an easier interpretation of the results. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Duration is in milliseconds and all frequency and bandwidth variables are in kilohertz.

	Females (n=13)		**Males (n=5)**	
Acoustic Variable	**Syllable 1**	**Syllable 2**	**Syllable 1**	**Syllable 2**
Duration	174±43	124±53	173±40	173±61
Peak Freq (Start)	24.41±5.81	24.96±3.95	27.77±5.21	27.17±6.00
Min Freq (Start)	21.64±6.22	22.12±3.99	24.68±5.09	24.35±6.12
Max Freq (Start)	28.47±6.30	28.99±4.40	31.46±5.04	31.08±6.82
Bandwidth (Start)	6.80±.63	6.84±.84	6.76±.50	6.70±1.22
Peak Freq (End)	21.88±5.05	24.16±5.84	24.98±4.73	27.30±4.66
Min Freq (End)	18.92±5.67	20.89±5.20	21.92±4.93	24.43±4.78
Max Freq (End)	25.79±5.59	27.85±5.79	28.70±4.64	31.22±4.66
Bandwidth (End)	6.84±.74	6.91±1.10	6.72±.40	6.75±.65
Peak Freq (Max)	24.04±6.60	26.13±5.96	27.17±5.56	28.79±6.79
Min Freq (Max)	21.47±6.20	23.47±5.66	24.31±5.50	25.88±7.58
Max Freq (Max)	28.29±6.44	30.58±5.67	31.33±5.46	33.28±6.82
Bandwidth (Max)	6.80±.41	7.09±0	7.01±.21	7.37±.95

Table 3. Descriptive Statistics for Measured Variables within 3 Syllable Vocalizations

The means, plus or minus standard deviation, for the descriptive statistics for 3 syllable vocalizations produced by female and male *Peromyscus californicus*. The original acoustic variables are used instead of PC scores to allow for an easier interpretation of the results. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Duration is in milliseconds and all frequency and bandwidth variables are in kilohertz.

	Females (n=13)			**Males (n=5)**		
Acoustic Variable	**Syllable 1**	**Syllable 2**	**Syllable 3**	**Syllable 1**	**Syllable 2**	**Syllable 3**
Duration	141±64	173±75	95±50	163±50	189±74	100±28
Peak Freq (Start)	22.95±5.81	23.78±4.66	23.01±5.42	24.26±8.27	26.16±5.76	26.94±3.93
Min Freq (Start)	20.05±5.79	21.03±4.29	20.17±5.33	22.04±8.50	23.37±5.81	24.50±4.26
Max Freq (Start)	27.02±5.95	27.74±4.82	26.81±5.46	28.14±8.16	29.91±5.98	30.55±3.95
Bandwidth (Start)	6.96±.38	6.68±.60	6.60±.31	6.05±1.20	6.49±.24	5.99±.69
Peak Freq (End)	20.86±4.25	22.69±5.76	22.88±6.14	22.83±7.73	24.36±6.33	24.63±6.17
Min Freq (End)	17.84±4.17	19.88±5.61	20.04±5.95	18.80±8.29	21.45±6.32	22.21±6.68
Max Freq (End)	24.67±4.14	26.67±5.89	26.89±6.12	25.40±8.35	28.45±6.38	28.55±5.98
Bandwidth (End)	6.79±.69	6.77±.68	6.83±.65	6.59±.66	6.96±.45	6.31±.80
Peak Freq (Max)	22.51±5.48	24.43±6.10	24.63±5.85	24.71±8.30	28.65±5.48	28.42±4.91
Min Freq (Max)	19.62±5.44	21.84±6.27	21.74±5.88	21.75±8.30	26.13±5.78	25.68±4.79
Max Freq (Max)	26.70±5.52	28.70±5.95	28.99±5.89	29.08±8.31	33.33±5.94	32.54±5.02
Bandwidth (Max)	7.07±.23	6.81±.39	7.20±.20	7.30±0	7.20±.20	6.84±.43

Table 4. Descriptive Statistics for Measured Variables within 4 Syllable Vocalizations

The means, plus or minus standard deviation, for the descriptive statistics for 4 syllable vocalizations produced by female and male *Peromyscus californicus*. The original acoustic variables are used instead of PC scores to allow for an easier interpretation of the results. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Duration is in milliseconds and all frequency and bandwidth variables are in kilohertz.

	Females (n=13)				Males (n=5)			
Acoustic Variable	**Syllable 1**	**Syllable 2**	**Syllable 3**	**Syllable 4**	**Syllable 1**	**Syllable 2**	**Syllable 3**	**Syllable 4**
Duration	134±97	164±53	145±46	90±42	101±53	142±26	136±10	97±36
Peak Freq (Start)	22.02±4.49	24.25±4.94	25.25±6.02	25.48±7.47	25.58±6.83	29.23±1.95	27.28±3.99	28.53±3.68
Min Freq (Start)	19.18±4.99	21.87±5.09	22.48±5.79	22.39±7.22	23.15±6.88	26.45±2.05	24.98±4.36	25.23±3.38
Max Freq (Start)	25.58±5.00	28.24±5.35	29.02±5.80	29.15±7.37	29.98±6.83	33.38±1.84	32.05±4.30	32.55±3.33
Bandwidth (Start)	6.36±.47	6.31±.69	6.49±.66	6.70±.56	6.80±.58	6.93±.48	7.05±.65	7.30±.41
Peak Freq (End)	19.93±3.00	24.24±6.54	25.09±6.70	25.78±7.27	22.18±6.82	27.53±3.77	26.05±3.22	26.80±4.82
Min Freq (End)	16.82±3.64	21.97±6.43	22.41±6.60	23.11±7.16	19.25±6.76	24.60±2.45	24.00±3.21	24.23±5.17
Max Freq (End)	23.28±3.55	28.03±6.75	28.96±7.36	29.82±7.28	26.68±5.22	32.08±2.48	30.85±2.97	30.70±4.98
Bandwidth (End)	6.40±.62	6.02±.89	6.53±.93	6.68±.65	7.40±1.61	7.43±.25	6.80±.71	6.43±.95
Peak Freq (Max)	21.94±3.53	27.21±6.42	27.48±7.23	26.86±7.41	24.63±5.44	31.20±.82	31.70±1.29	30.95±1.26
Min Freq (Max)	19.05±3.53	24.31±6.37	24.60±7.31	23.64±7.82	21.43±5.55	28.28±.78	28.75±1.24	27.90±1.21
Max Freq (Max)	26.28±3.59	31.60±6.41	31.63±7.56	31.14±7.43	29.03±5.44	35.35±.96	35.85±.96	35.10±1.29
Bandwidth (Max)	7.23±.16	7.26±.10	7.00±.41	7.47±.73	7.55±.50	7.05±.50	7.05±.50	7.18±.25

Table 5. Analysis of the Spectral and Temporal Characteristics of Male and Female USVs.

The results of 28 One-Way Analysis of Variance tests using PC scores for the spectral characteristics of vocalizations between the sexes of *Peromyscus californicus*. PC1 is correlated with the frequency axis, PC2 is correlated with the bandwidth axis, and PC3 is correlated with the duration axis. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. The rejection criterion of 0.002 has been adjusted for multiple comparisons.

Motif	Syllable 1			Syllable 2			Syllable 3			Syllable 4		
	PC Factor	F Stat	p value	PC Factor	F Stat	p value	PC Factor	F Stat	p value	PC Factor	F Stat	p value
1-Syllable vocalization n=42		$F_{1,9}$										
	1	0.35	**0.57**									
	2	0.00	**0.97**									
	3	0.08	**0.79**									
2-Syllable Vocalization n=80		$F_{1,12}$			$F_{1,12}$							
	1	0.87	**0.37**	1	0.71	**0.42**						
	2	0.00	**0.99**	2	0.04	**0.84**						
	3	0.13	**0.72**	3	4.49	**0.06**						
3-Syllable Vocalization n=72		$F_{1,9}$			$F_{1,9}$			$F_{1,9}$				
	1	0.15	**0.71**	1	0.30	**0.60**	1	1.14	**0.31**			
	2	0.04	**0.85**	2	6.88	**0.03**	2	2.13	**0.18**			
	3	4.11	**0.07**				3	0.15	**0.71**			
4-Syllable Vocalization n=29		$F_{1,7}$			$F_{1,7}$			$F_{1,7}$			$F_{1,7}$	
	1	0.73	**0.42**	1	2.38	**0.17**	1	0.63	**0.46**	1	0.55	**0.48**
	2	3.29	**0.11**	2	0.26	**0.63**	2	0.00	**1.00**	2	1.12	**0.33**
	3	0.00	**0.98**				3	1.26	**0.30**	3	0.02	**0.89**

Table 6. Analysis of the Spectral and Temporal Characteristics of 2-SVs for Estrous State

The results of 6 One-Way Analysis of Variance tests of a 2-syllable vocalization (n=54) using PC scores for the spectral characteristics of vocalizations between estrous and non-estrous female *Peromyscus californicus*. PC1 is correlated with the frequency axis, PC2 is correlated with the bandwidth axis, and PC3 is correlated with the duration axis. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. The rejection criterion of 0.008 has been adjusted for multiple comparisons.

	PC Factor	$F_{1,8}$	p Value
Syllable 1	1	0.15	**0.71**
	2	0.10	**0.76**
	3	1.29	**0.29**
Syllable 2	1	1.01	**0.35**
	2	1.17	**0.31**
	3	2.20	**0.18**

Table 7. Mean ±SD Values of the PC Scores of USVs Produced in the Presence of Mates and Non-Mates of *Peromyscus californicus*.

Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Significant values are indicated with a *.

Vocalization	PC Factor	Mate	Non-Mate	t Stat	p Value
1-Syllable Vocalization		n=3	n=2		
	PC1	0.41±0.99	0.65±0.06	-1.43	**0.25**
	PC2	0.98±2.27	1.13±0.12	-1.24	**0.30**
	PC3	1.19±1.01	0.68±0.97	0.56	**0.61**
2-Syllable Vocalization		n=3	n=7		
	PC1	0.46±1.07	0.36±0.95	0.15	**0.89**
	PC2	0.47±0.83	0.58±0.96	-0.17	**0.87**
	PC3	0.19±.60	0.15±1.65	-0.04	**0.97**
3-Syllable Vocalization		n=6	n=7		
	PC1	0.73±1.06	0.30±1.16	0.70	**0.50**
	PC2	1.05±0.53	1.13±0.65	-0.25	**0.81**
	PC3	.16±0.59	0.12±0.57	-0.12	**0.91**

Table 8. An Analysis of the Spectral and Temporal Characteristics of 3-SVs Produced by *Peromyscus californicus* Pairs.

The results of 8 One-Way Analysis of Variance tests for 3 syllable vocalizations (n=78) using PC scores for the spectral characteristics of vocalizations between *Peromyscus californicus* pairs. PC1 is correlated with the frequency axis, PC2 is correlated with the bandwidth axis, and PC3 is correlated with the duration axis. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. The rejection criterion of 0.006 has been adjusted for multiple comparisons.

	PC Factor	$F_{2,3}$	p value
Syllable 1	1	9.93	**0.05**
	2	1.50	**0.35**
	3	1.74	**0.32**
Syllable 2	1	48.58	**0.01**
	2	1.21	**0.41**
Syllable 3	1	1.97	**0.28**
	2	10.70	**0.04**
	3	6.07	**0.09**

Table 9. An Analysis of the Spectral and Temporal Characteristics of 3-SVs Produced by *Peromyscus californicus* within a Pair.

PC1 is correlated with the frequency axis, PC2 is correlated with the bandwidth axis, and PC3 is correlated with the duration axis. PC scores of individuals belonging to the same group were analyzed using a Mann-Whitney U test. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Significant values are indicated with a *.

Group	Syllable 1		Syllable 2		Syllable 3	
	PC Factor	p value	PC Factor	p value	PC Factor	p value
1131 &1229	1	**0.60**	1	0.35	1	**0.47**
n=20	2	**0.18**	2	0.92	2	**0.75**
	3	**0.92**			3	**0.05**
3936 & 1131	1	**0.62**	1	0.32	1	**0.13**
n=7	2	**0.32**	2	0.13	2	**0.32**
	3	**0.13**			3	**0.13**
1206 & 1065	1	**0.09**	1	0.10	1	**0.10**
n=51	2	**0.69**	2	0.12	2	**0.23**
	3	**0.54**			3	**0.75**

APPENDIX B. FIGURES

Figure 1. An example of a spectrograph of a 3-SV USV produced by *Peromyscus californicus.*

Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Parameters of the spectrogram included: FFT length of 256, and a 100% Frame size with a Hamming window. Window overlap was 50%. Frequency is measured in kHz on the y axis and time is measured in seconds on the x axis.

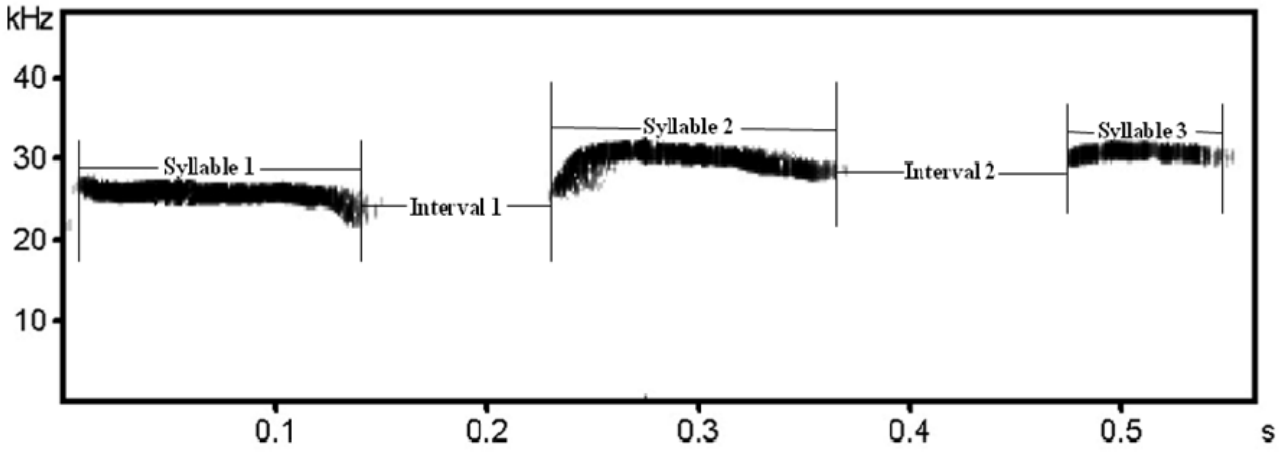

Figure 2. Frequency Histogram of Vocalizations by Time of Night throughout Field Season

The mean number of all *Peromyscus californicus* vocalizations (n=507), including unassigned USVs (data from Carney, 2009), produced each hour throughout 39 nights. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009.

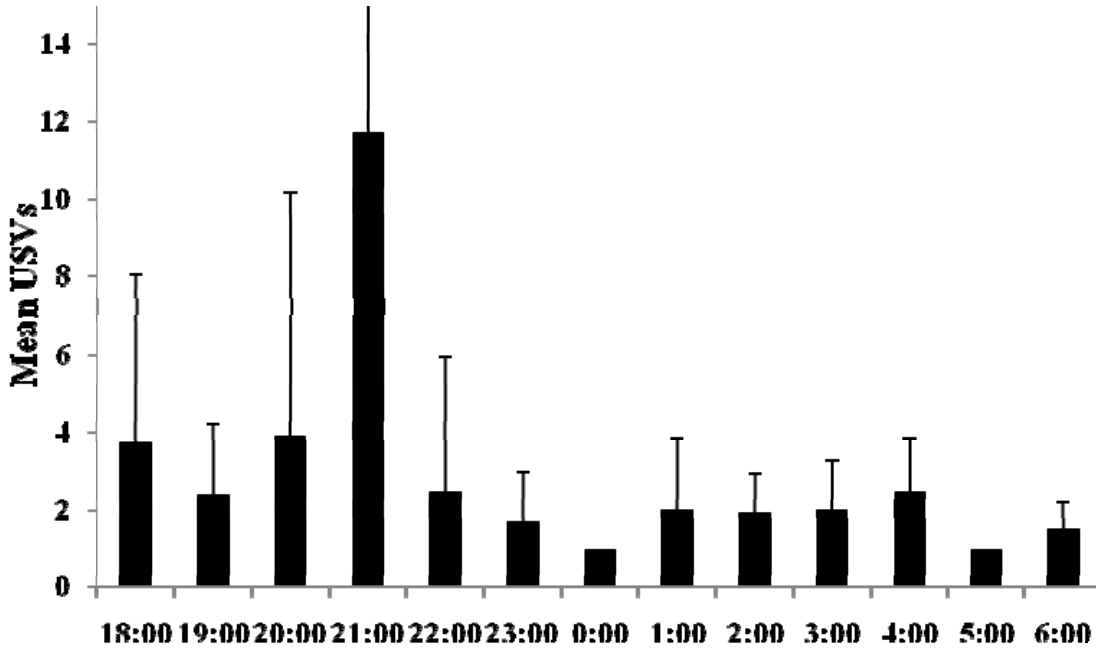

Figure 3. Spectrograph of 1-SV USVs produced by *Peromyscus californicus.*

Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Parameters of the spectrograph included: FFT length of 256, and a 100% Frame size with a Hamming window. Window overlap was 50%. Frequency is measured in kHz on the y axis and time is measured in seconds on the x axis.

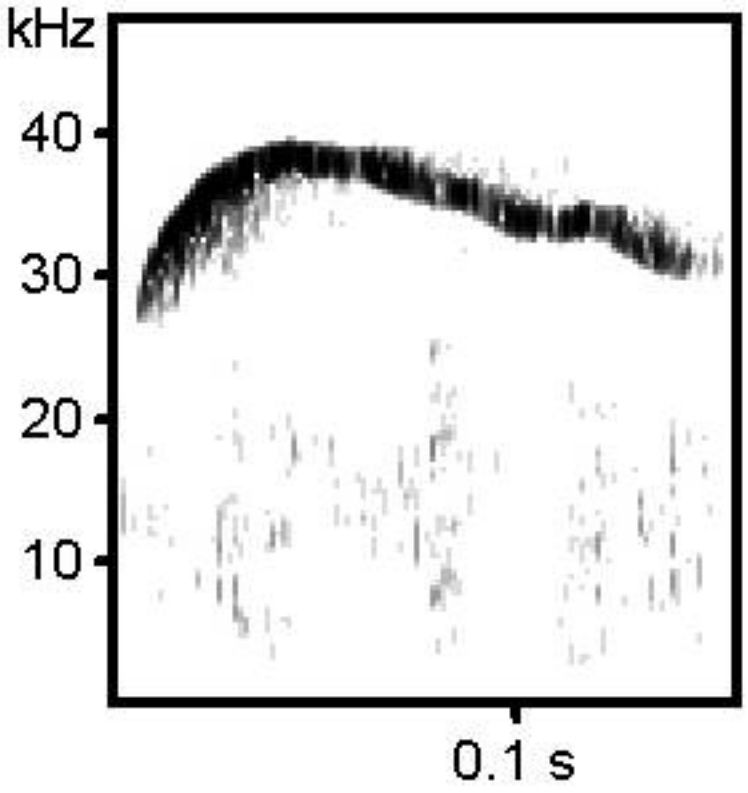

Figure 4. Spectrograph of 2-SV USVs produced by *Peromyscus californicus*.

Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Parameters of the spectrograph included: FFT length of 256, and a 100% Frame size with a Hamming window. Window overlap was 50%. Frequency is measured in kilohertz on the y axis and time is measured in seconds on the x axis.

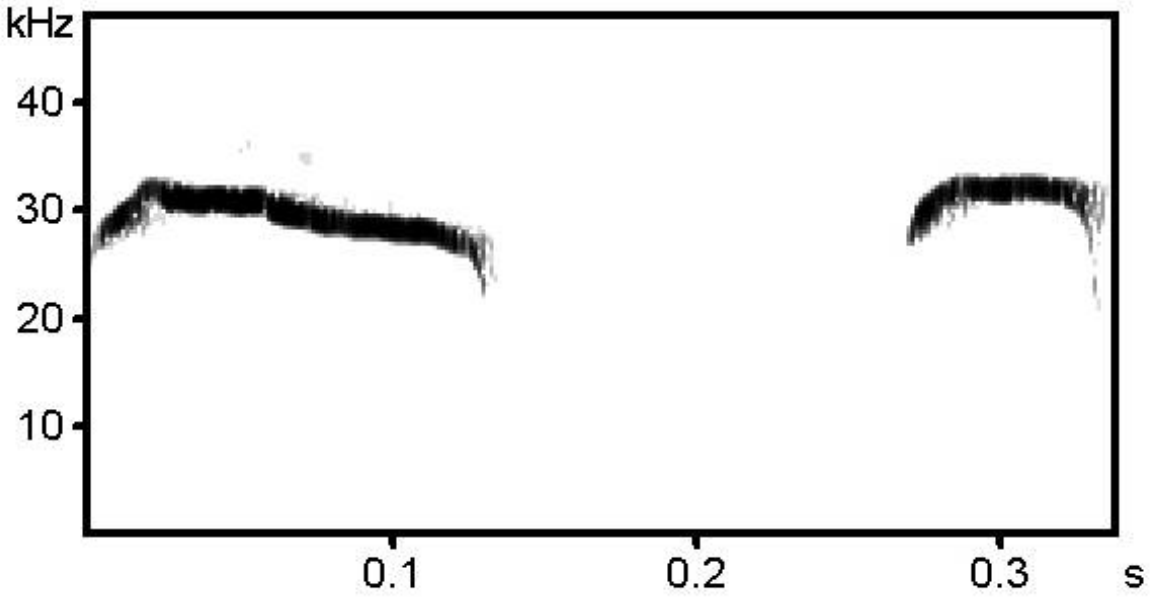

Figure 5. Spectrograph of 3-SV USVs produced by *Peromyscus californicus*.

Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Parameters of the spectrograph included: FFT length of 256, and a 100% Frame size with a Hamming window. Window overlap was 50%. Frequency is measured in kilohertz on the y axis and time is measured in seconds on the x axis.

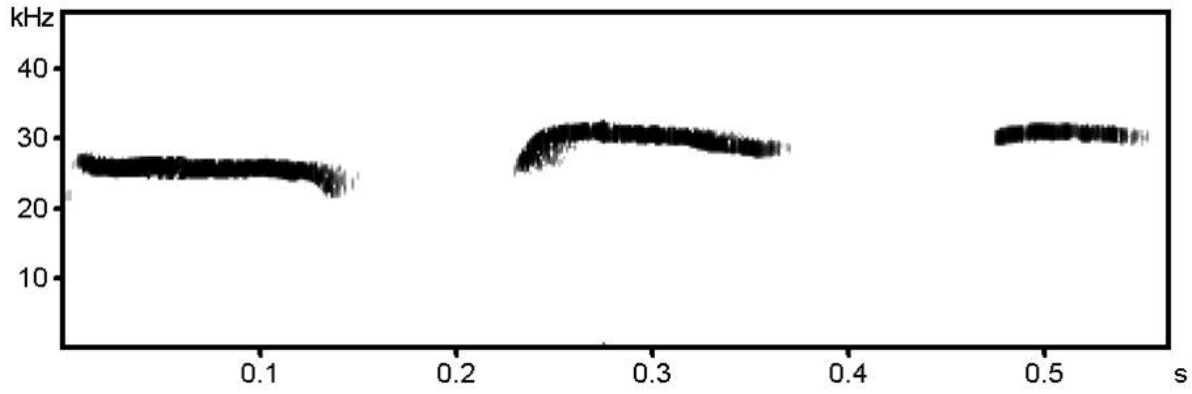

Figure 6. Spectrograph of 4-SV USVs produced by *Peromyscus californicus*.

Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Parameters of the spectrograph included: FFT length of 256, and a 100% Frame size with a Hamming window. Window overlap was 50%. Frequency is measured in kilohertz on the y axis and time is measured in seconds on the x axis.

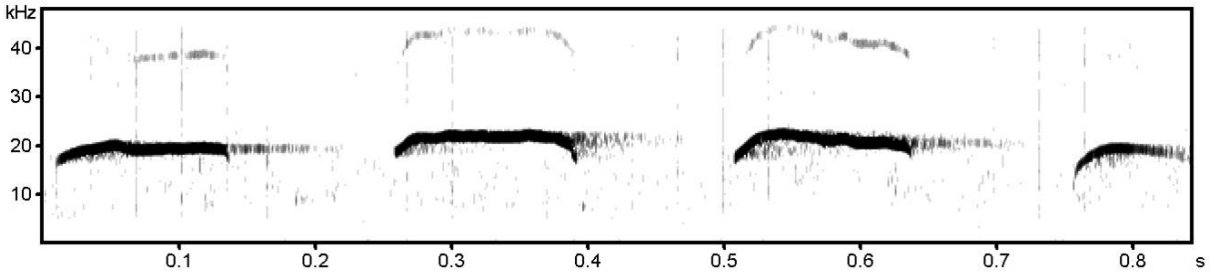

Figure 7. Thermal images of the situational contexts and corresponding ultrasonic vocalization of *Peromyscus californicus vocalizations.*

Vocalizations and images were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. a) Shows when the caller was alone on the focal area, b) shows when a non-mate was on the focal area with the caller, c) show when the caller's mate was present on the focal area, and d) shows when mates were not in contact, no USVs were produced. Parameters of the spectrograph included: FFT length of 256, and a 100% Frame size with a Hamming window. Window overlap was 50%. Frequency is measured in kilohertz on the y axis and time is measured in seconds on the x axis. A circle indicates the caller, a square indicates the non-mate, and a triangle indicates a mate.

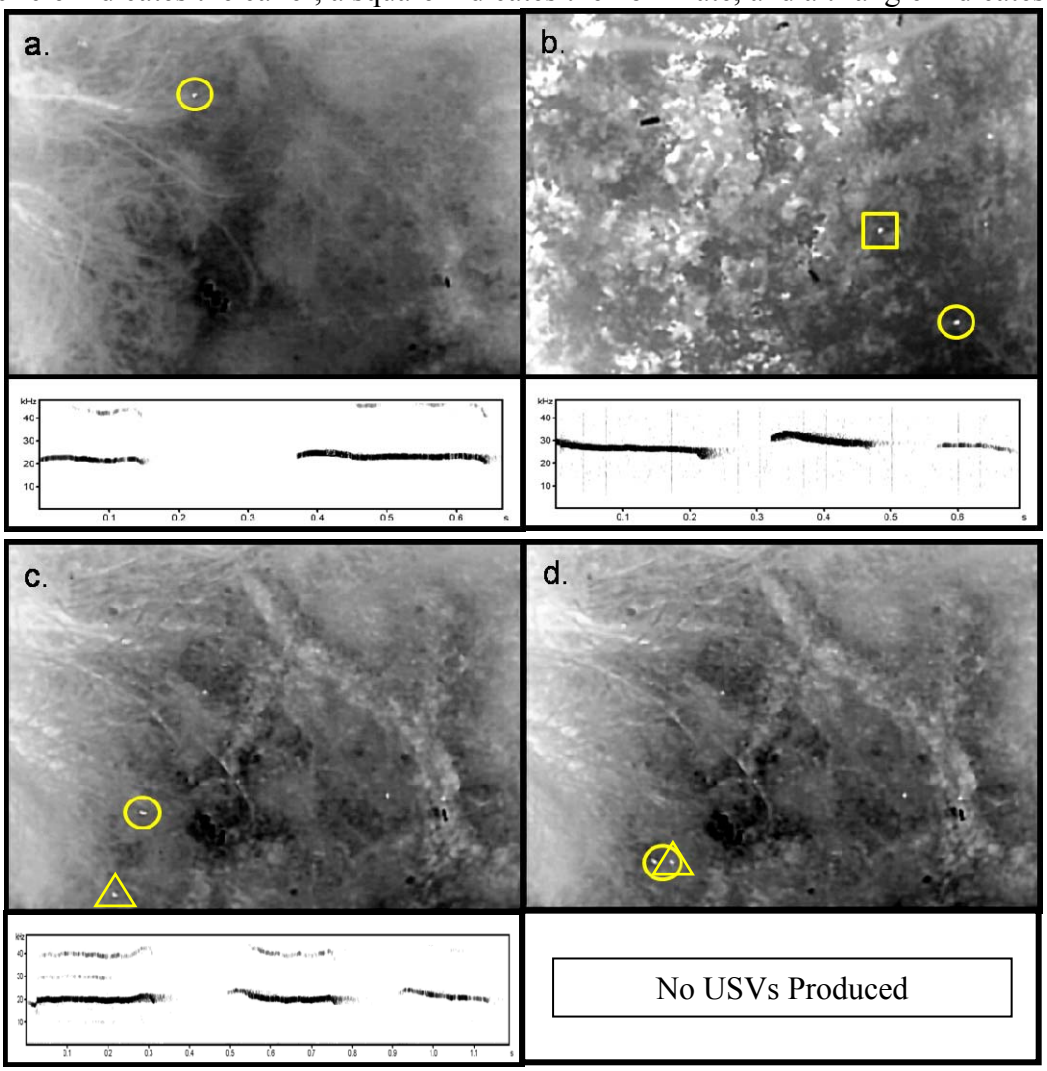

Figure 8. Motif Distribution by Sex

Total number of vocalizations produced by each sex of *Peromyscus californicus*. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. Males are represented as ■ and females are□. Call type is on the x axis.

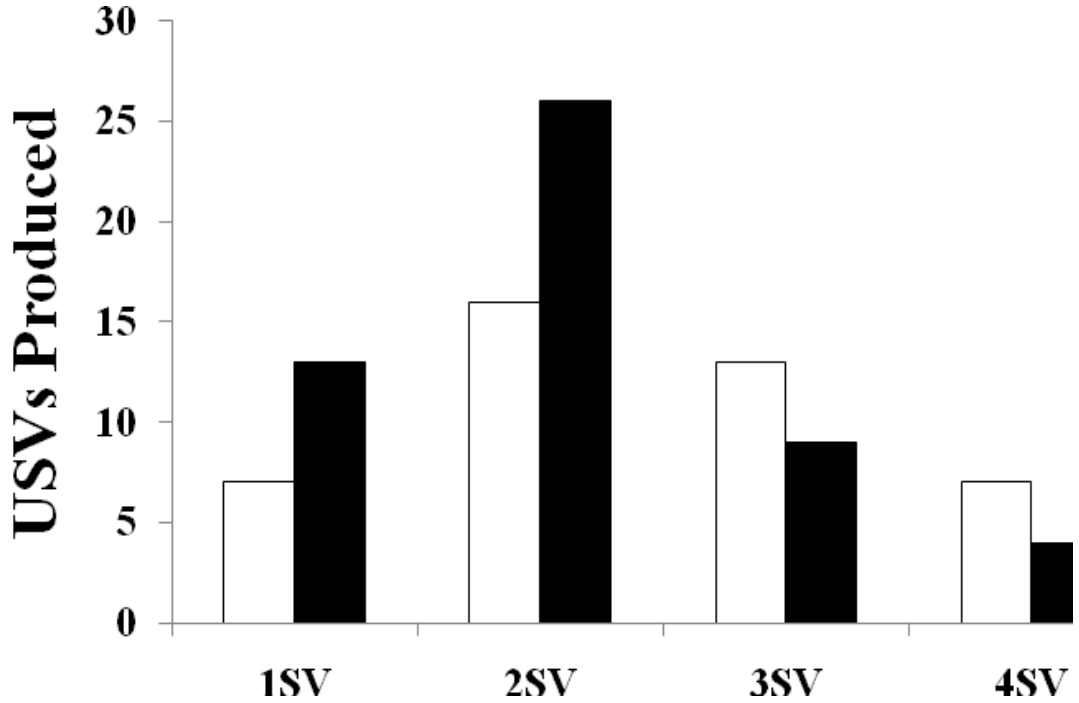

Figure 9. Motif Distribution in the Presence of Mates and Non-Mates

The total number of vocalizations produced during the presence of mates and non-mates of *Peromyscus californicus*. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008 and January 2009. USVs produced in the presence of a mate are represented as □ and USVs produced in the presence of a non-mate are ■. Call type is on the x axis.

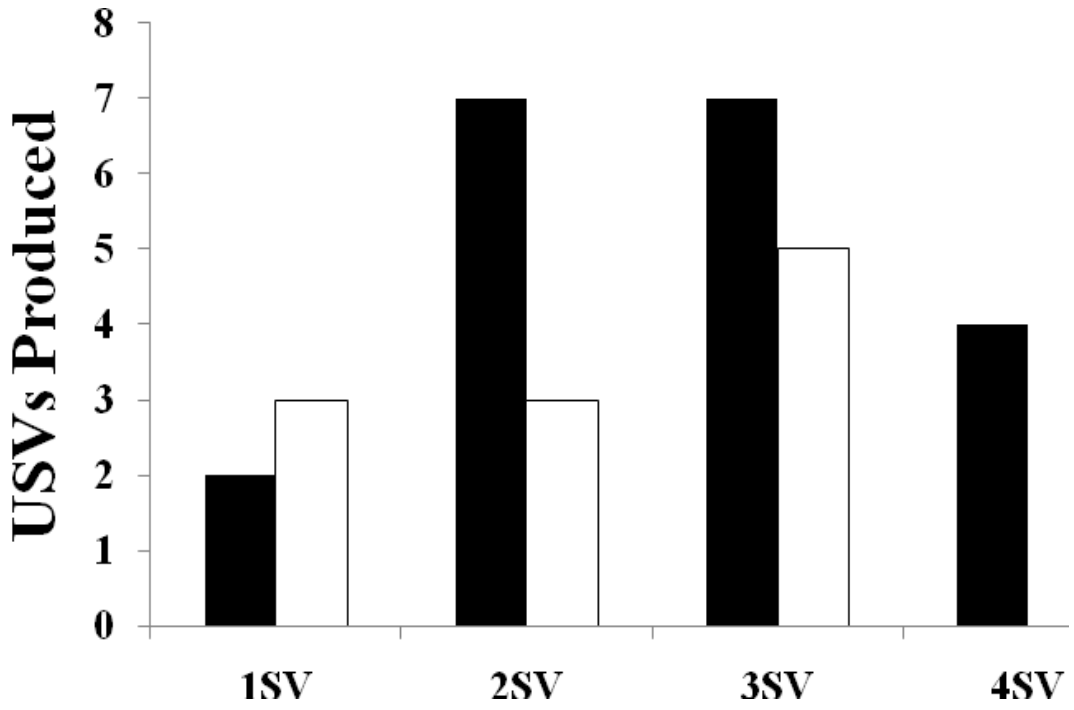

Figure 10. A plot of the total number of sub-adults captured and ultrasonic vocalizations produced each month by *Peromyscus californicus*.

The total number of sub-adults captured and ultrasonic vocalizations (n=111) produced by known individuals of *Peromyscus californicus* (n=17) each month. Vocalizations were recorded and sub-adults were captured from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, from February 2008-June 2008.