

Belding's Ground Squirrels (*Uroditellus beldingi*) are more acoustically similar to Relatives than to Unrelated Individuals

Christina R. Kastely

ABSTRACT

Belding's ground squirrel (*Uroditellus beldingi*) alarm calls encode information about the age, sex and identity of the caller, but little is known about whether alarm calls encode information about relatedness. I investigated the potential correlation between acoustic and genetic similarity in *U. beldingi* from four populations. I extracted DNA from tissue samples and performed PCR for 12 microsatellites. I analyzed this data in MLRelate to find coefficients of relatedness for each pair. I also calculated the genetic distances between populations in GenePop. I analyzed the acoustic samples in Praat, an acoustic analysis program, which measured fifteen variables. I performed a discriminant function analysis (DFA) with the variables, which calculated the acoustic dissimilarity (Mahalanobis distance) between each sample pair. I used a Mantel test to compare the acoustic and genetic data and found that there is a negative correlation between genetic similarity and acoustic dissimilarity (Mantel's $r = -0.172$, $p < 0.001$). This relationship indicates that related individuals sound more similar than unrelated individuals, and *U. beldingi* could use alarm calls for kin recognition. The DFA also sorted calls to population. Calls from three of the four populations were correctly classified significantly more than expected by chance. This suggests that populations have a distinct acoustic structure. Using acoustic information as a proxy for genetic information could have conservation implications: acoustic sampling is quicker, cheaper and less invasive, so the acoustic structure of a population could be used as an efficient tool to diagnose the genetic health of a population and the species at large.

KEYWORDS

Alarm call, acoustic variability, kin recognition, microsatellite analysis, population call structure

INTRODUCTION

Alarm calls are an important form of communication for social animal species because they alert conspecifics to the presence of a predator and allow for the chance to escape predation. This type of vocalization is a widespread behavior that has been observed in avian, amphibian and mammalian species (Tunner and Hoedl 1978, Cooper 2011, Owens and Goss-Custard 1976, Sherman 1977, Long et al 1998). Alarm calling is thought to be evolutionarily important because it is hypothesized that the caller will increase their inclusive fitness by alerting their relatives to the presence of danger and thereby insuring that a percentage of the caller's genes will be passed on by several surviving relatives (Hamilton 1964, Sherman 1977). Further understanding the function, development, and diversity of alarm calls could illuminate on the communication of social species.

Researchers have discovered that many species show individually distinctive calls or "acoustic fingerprints." Individual vocal patterns have been observed in primates including spider monkeys (Chapman and Weary 1990) and chimpanzees (Mitani et al. 1992) as well as non-primate mammalian species such as giant pandas (Charlton et al. 2009) and ground squirrels (Matrosova et al. 2010, McCowan and Hooper 2002, Schneiderova and Politch 2010, Volodina et al. 2010). Vocal variation has also been observed at the population level. Parrots have been found to have regional variety in their calls (Wright 1996) and mammalian species as different as prairie dogs and meerkats have also been shown to have regional dialects (Slobodchikoff and Coast 1980, Schibler and Manser 2007).

Recently, researchers have focused on understanding what information about the caller can be extracted from the distinct alarm calls. In many avian and several mammalian species, researchers found that these calls can encode information about the age, sex and even the identity of the caller. Within some avian species, calls encode information about relatedness (McDonald and Wright 2011, Sharp and Hatchwell 2006, Searby and Jouventin 2004), but less is known about whether mammalian calls could communicate genetic information.

To address this knowledge gap, I studied the relationship between genetic and vocal variation in Belding's ground squirrels, *Urocitellus beldingi*. In addition, I compared the acoustic structures between four geographically distinct populations of Belding's ground squirrels. Ground squirrel alarm calls are of particular interest in communication research because they are

easily provoked and readily recordable (Sherman 1977, Mateo and Holmes 1999). Moreover, there is evidence that ground squirrel alarm calls communicate the caller's individuality, age and sex (McCowan and Hooper 2002, Matrosova et al. 2010). However, there is little information about whether acoustic variability encodes relatedness in ground squirrels or even whether acoustic structure differs within and between populations.

Alarm call structure is either learned or inherited (Hollen and Radford 2009). Belding's ground squirrels spend much of their developmental stage with closely related individuals and they are exposed to alarm calls before they emerge from their natal burrow (Mateo and Holmes 1999). Therefore, I hypothesized that Belding's ground squirrels are more acoustically similar to relatives than to unrelated individuals either because they are inheriting call structure from relatives or they are being exposed to and learning from relatives' alarm calls. I also predicted that populations would be acoustically distinct and that calls could be correctly assigned to population. Populations with more gene flow may have similar vocal structures, whereas genetically isolated populations will be more distinct. In addition, if there is a relationship between vocal similarity and genetic relatedness, the vocal variation within each population could reflect that population's genetic diversity and therefore populations with less genetic diversity will also have less diverse acoustic patterns.

METHODS

Study Species

Belding's ground squirrels, *Urocitellus beldingi*, are social burrowing rodents that vocalize or "alarm call" to alert their relatives when a predator is near. The females live in close proximity to relatives, and are capable of recognizing closely related individuals (Holekamp 1984). Belding's ground squirrels produce several types of alarm calls including whistles, chirps and trills to alert conspecifics to the presence of different types of predators. They are able to distinguish between different types of alarm calls and react differently depending on the call type (Mateo 2010).

Data Collection

I collected genetic and acoustic samples from *U. beldingi* at four sites in California. Three sites (Lundy Lake, Mono Lake and Silver Lake) were located in Mono County and the fourth was at Alturas in Modoc County in northern California. All four sites were modified by humans in some manner. The Mono Lake site was a county park, the Lundy Lake and the Silver Lake sites were campgrounds, and the Alturas site was an alfalfa field.

At each site, I worked with Dr. Toni Lyn Morelli to lay Tomahawk traps baited with peanut butter and oats. I used a Sony Linear PCM-D50 Recorder and a Sennheiser microphone with a wind guard to record calls from trapped ground squirrels. I recorded over 50 calls from each individual, and then Dr. Morelli clipped a piece of ear tissue from each squirrel for genetic analysis. We also recorded the sex of the squirrel and estimated whether the squirrel was an adult or juvenile based on its size. I collected acoustic and genetic data from 17 individuals in Alturas, 20 individuals from Mono Lake County Park, 15 individuals from Lundy Lake Campground, and 10 individuals from Silver Lake Campground. Each squirrel was released near the area that it was captured.

Analysis

DNA Extraction, Amplification, and Analysis

I extracted DNA from the tissue samples and amplified microsatellite markers from each sample by performing polymerase chain reaction (PCR) with 12 polymorphic primers optimized for *U. beldingi* (full list of primers found in Appendix A). The PCR was performed in Bio-Rad cyclers with a 12.5 μL mix of 9.1 μL of distilled water, 1.25 μL of buffer, 1 μL of magnesium chloride, 0.2 μL of both the forward and reverse primers, 0.25 μL of dNTPs, and 0.2 μL of taq polymerase. Microsatellites were electrophoresed on the ABI 3730 48-capillary automated sequencer in the Evolutionary Genetics Lab at the Museum of Vertebrate Zoology at Berkeley. I measured microsatellite lengths using the GeneMapper version 4.0 software (Applied Biosystems).

I entered this microsatellite data into MLRelate (Kalinowski et al. 2006) to find the coefficient of relatedness for each pair of *U. beldingi*. In addition, I used the web version of a population genetics analysis program, GenePop 4.0.10 (Raymond and Rousset 1995, Rousset

2008), to calculate the F_{ST} between each population. F_{ST} is a population level measure of genetic distance. A large F_{ST} indicates that populations are genetically dissimilar.

Measuring acoustic parameters, calculating acoustic distances, and sorting calls by population

I processed acoustic samples to measure 15 vocal parameters, and used these parameters to measure dissimilarity between calls of different squirrels. I used Cool Edit Pro to sort acoustic samples from each individual into separate files. Then, I imported the call files into Praat, an acoustic analysis software (Boersma 2001), and extracted each separate call from the file using a script originally created by Dr. Michael Owren and modified by Dr. Brenda McCowan. Then I used another script designed by Dr. Michael Owren and modified by Dr. Brenda McCowan to measure 15 call parameters that have been found to be statistically independent in Belding's ground squirrel alarm calls (McCowan and Hooper 2002, Appendix B).

I ran a discriminant function analysis with these 15 acoustic parameters and calculated a measurement of acoustic dissimilarity between each sample pair called a Mahalanobis distance. Small Mahalanobis distances indicate that pairs or populations are vocally similar while large Mahalanobis distances indicate vocal dissimilarity. I also calculated the Mahalanobis distance between each population by performing a discriminant function analysis on the population average of each of the 15 acoustic parameters.

Comparing genetic similarity to acoustic dissimilarity

I compared the coefficients of relatedness to the Mahalanobis distances by performing a Mantel test and two partial Mantel Tests (Mantel 1967) to correct for age (juvenile or adult) and sex in R (R Development Core Team 2011). The Mantel Test is part of the "ecodist" package in R (Goslee and Urban 2007). The Mantel Test compares two matrices, in this case coefficients of relatedness and Mahalanobis distances, to find the correlation between the two variables. I analyzed all of the samples from the four populations together, and then performed Mantel Tests and partial Mantel Tests for each population separately. I also performed a Mantel test to

compare the genetic distances between populations (F_{st} values) to the Mahalanobis distances between populations.

Finally, I compared the Mahalanobis distances between related and unrelated individuals. I could not assume that the data were independent or normally distributed. Therefore, I used a nonparametric test, the Wilcoxon rank sum test, to see if the Mahalanobis distances for related individuals were significantly lower than the Mahalanobis distances for unrelated individuals.

Examining Acoustic Distinctness between Populations

To examine whether or not each population was acoustically distinct, I evaluated the cross-validation table produced in the discriminant function analysis. This table summarizes the percent of calls correctly sorted to each population. Then, I compared the observed percentage of correctly sorted calls to the percent of correctly sorted calls expected to due chance using a Chi-square goodness of fit test. In this case, the expected percent of correctly sorted calls was 25% as there were four possible populations between which the discriminant function analysis could sort the calls.

RESULTS

Genetic relatedness compared to acoustic distance

Correlation between genetic relatedness and acoustic distance for all samples

There is a negative correlation between the coefficient of relatedness and the Mahalanobis distances ($r = -0.172$, $p = 0.001$) (Fig. 1). This result is slightly counterintuitive because the negative correlation actually indicates that related individuals are more acoustically similar. The correlation is negative because coefficients of relatedness measure the genetic similarity whereas Mahalanobis distances measure acoustic dissimilarity. When I performed the partial Mantel test to correct for sex the correlation between the Mahalanobis distances and the coefficients of relatedness became slightly larger ($r = -0.173$, $p = 0.001$), and the correlation stayed the same when corrected for age ($r = -0.172$, $p = 0.001$).

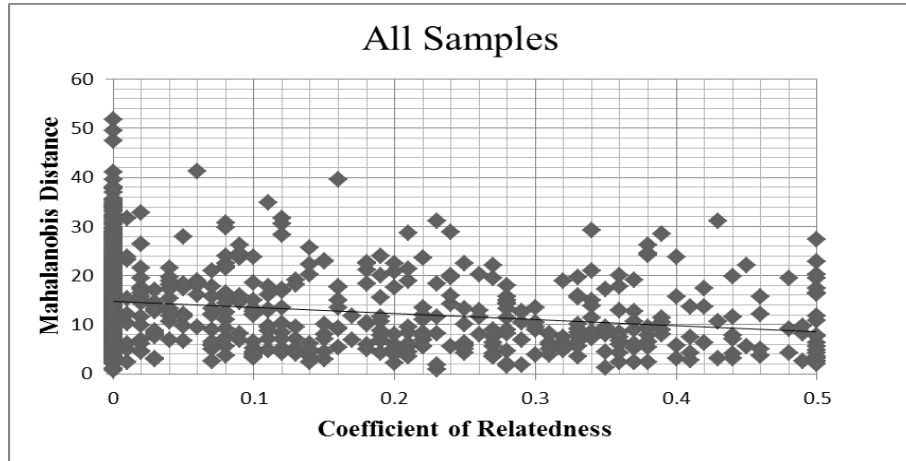


Figure 1. Correlation between genetic similarity and acoustic dissimilarity for all samples analyzed together. The scatterplot with the line of best fit show the negative correlation between genetic similarity and acoustic dissimilarity that was seen for all the samples when analyzed together ($r = -0.172$, $p = 0.001$)

Correlation between genetic relatedness and acoustic distance within each population

When I performed Mantel Tests for samples within each population, I found that the negative correlation between genetic relatedness and acoustic dissimilarity continued for the Alturas population, the Mono Lake population and the Silver lake population (Fig. 2). The correlation magnitude decreased from -0.172 for the Mono Lake population ($r = -0.135$, $p = 0.02$), but the correlation magnitude increased for the Alturas population ($r = -0.179$, $p = 0.06$) and the Silver Lake population ($r = -0.202$, $p = 0.07$). The significance of the correlation decreased when I analyzed the populations separately, and this was probably due to the decrease in sample size. When corrected for sex, the correlation for the Alturas population and for the Silver Lake population increased (Alturas: $r = -0.182$, $p = 0.04$; Silver: $r = -0.215$, $p = 0.06$), but the Mono Lake population's correlation decreased ($r = -0.131$, $p = 0.02$). Correcting for age decreased the magnitude of the correlations for the Alturas population ($r = -0.173$, $p = 0.06$), the Mono Lake population ($r = -0.134$, $p = 0.02$) and the Silver Lake population ($r = -0.178$, $p = 0.09$).

The Lundy Lake population had a positive correlation between genetic similarity and acoustic dissimilarity ($r = 0.104$, $p = 0.23$), but this result was not significant so it is not clear that

the correlation was actually reversed in this population (Fig. 2). The correlation decreased when corrected for both sex ($r= 0.100$, $p=0.23$) and age ($r= 0.102$, $p=0.22$).

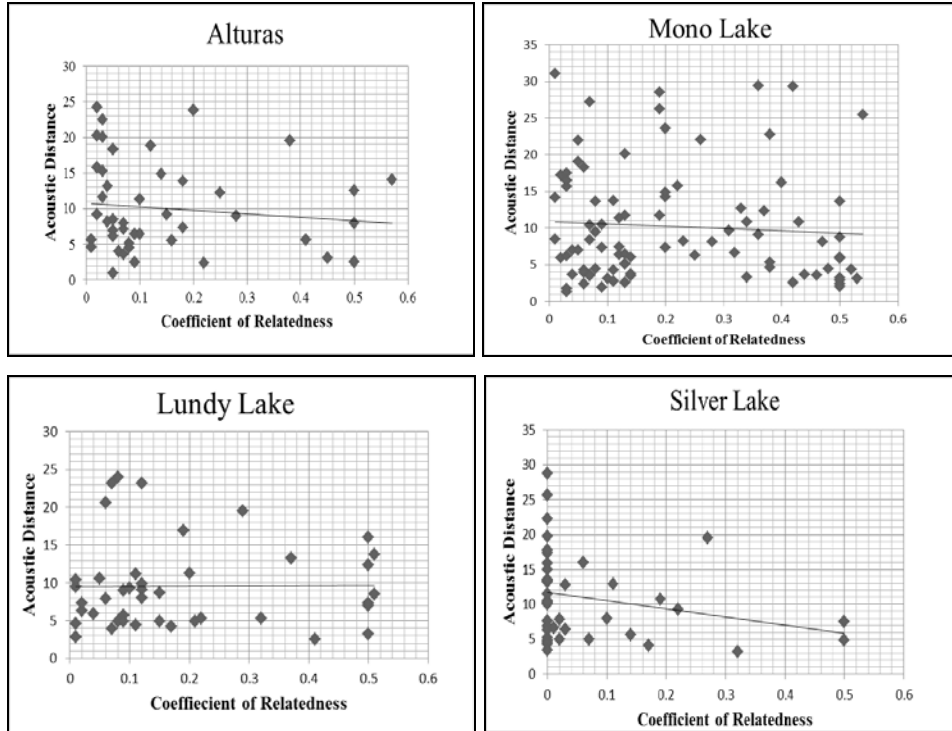


Figure 2. Correlation between genetic similarity and acoustic dissimilarity for each population. Scatterplots with lines of best fit for each population show the negative correlation between genetic similarity and acoustic dissimilarity for the Alturas population ($r = -0.179$, $p = 0.06$) the Mono Lake population ($r = -0.135$, $p = 0.02$) and the Silver Lake population ($r = -0.202$, $p = 0.07$). The Lundy Lake population had a positive correlation between genetic similarity and acoustic dissimilarity but this was not a significant correlation ($r = 0.104$, $p = 0.23$)

Correlation between genetic relatedness and acoustic distance within each population

The Lundy Lake population and the Silver Lake population had the smallest genetic distance whereas the Silver Lake population and the Mono Lake population had the largest genetic distance (complete summary in Table 1). Similarly, the Lundy Lake population and the Silver Lake population had the smallest acoustic distance. However, the Alturas population and the Mono Lake population had the largest acoustic distance (complete summary in Table 2). There was a very small positive correlation between the population genetic distances and the population acoustic distances but the correlation was not significant ($r = 0.01$, $p > 0.05$).

Table 1. Genetic distances between populations (Fst). The smallest genetic distance is between Lundy Lake and Silver Lake while the largest genetic distance is between Mono Lake and Silver Lake.

Population	Alturas	Mono Lake	Lundy Lake	Silver Lake
Alturas	—			
Mono Lake	0.1972	—		
Lundy Lake	0.1762	0.1632	—	
Silver Lake	0.2303	0.2399	0.1367	—

Table 2. Acoustic distances between populations. The smallest acoustic distance is between the Lundy Lake and Silver Lake populations. The largest acoustic distance is between the Alturas population and the Mono Lake population.

Population	Alturas	Mono Lake	Lundy Lake	Silver Lake
Alturas	—			
Mono Lake	3.534	—		
Lundy Lake	3.282	2.110	—	
Silver Lake	1.162	1.972	1.103	—

Mahalanobis distances between related and unrelated individuals

The Mahalanobis distances were significantly higher for the unrelated individuals than for the related individuals ($p < 0.001$, nonparametric Wilcoxon rank sum test) (Fig. 3). The median Mahalanobis distance for related individuals was 8.3 and the median for unrelated individuals was 13.5.

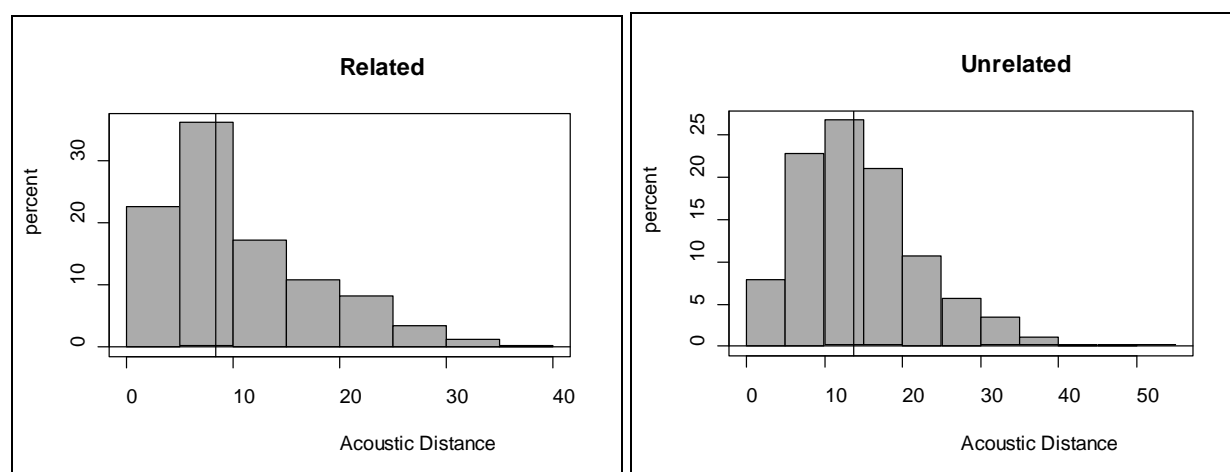


Figure 3. Histograms of Mahalanobis distances for related and unrelated pairs. The Mahalanobis distances for unrelated individuals ($R < 0.12$) are greater on average than the Mahalanobis distances of related individuals ($R \geq 0.12$, $p < 0.001$). The vertical line on each histogram indicates the median Mahalanobis distance for the Related (median=8.35) and Unrelated pairs (median=13.8)

Acoustic structure between populations

A majority of the calls from the Alturas, Mono Lake and Silver Lake populations were correctly classified to population. These values were significantly higher than was expected due to chance ($p < 0.001$, chi-squared goodness of fit test). The Mono Lake population had the highest percent of calls correctly classified, followed by the Alturas population and then the Lundy Lake population (Table 4). Calls from the Silver Lake population were only correctly classified 3% of the time, and this was not significant compared to chance ($p > 0.1$, Chi-squared goodness of fit test).

Table 3. Cross-Validation Table for Discriminant Function Analysis between Populations. Calls from Alturas, Mono Lake, and Lundy Lake populations were correctly assigned to their population significantly more than expected due to chance (Chi-squared goodness of fit test, $p < 0.001$). However, Silver Lake calls were only insignificantly assigned correctly 3% of the time ($p > 0.05$).

	Alturas	Mono	Lundy	Silver	p-value
Alturas	69%	18%	12%	0.7%	$P < 0.001$ ***
Mono	6%	84%	10%	0.2%	$P < 0.001$ ***
Lundy	12%	33%	55%	0.6%	$P < 0.001$ ***
Silver	35%	36%	26%	3%	$P > 0.1$

DISCUSSION

Through this study, I have shown that Belding's ground squirrels have more similar alarm calls to their relatives than to unrelated individuals. This relationship between genetic relatedness and alarm call similarity has implications for understanding the variation of alarm call structures within and between populations, and may allow for the analysis of alarm calls to diagnose the genetic health of a population by understanding its acoustic structure. In the future, it will be important to thoroughly record and analyze the acoustic structures of the major Belding's ground squirrel populations to understand how alarm call structures are changing and being impacted, particularly by human factors.

Kin Recognition through Acoustic Structure

The evidence that closely related individuals sound more similar to each other than unrelated individuals indicates that alarm calls encode information about relatedness. Several bird species, including bell miners, long-tailed tits and macaroni penguins, use calls to recognize related individuals, (McDonald and Wright 2011, Sharp and Hatchwell 2006, Searby and Jouventin 2004). Baboons have also shown to be capable of kin recognition through calls and it is possible that other mammalian species are capable of using calls for a mechanism of kin recognition (Cheney and Seyfarth 1999). Through phenotypic recognition, animals are able to distinguish who their relatives are by recognizing their own phenotypic traits in others. Belding's ground squirrels could identify their relatives by recognizing the similar sounding individuals as kin.

Acoustic Variation between Populations

The correlation that I found between genetic relatedness and acoustic structure supports the idea different populations of Belding's ground squirrels have different acoustic structures. Limited genetic flow or even complete genetic isolation could cause alarm calls to diverge and different dialects to form between the populations. This is echoed by data from the Grinnell Resurvey Project, which indicate that the population at the Mono Lake site is genetically distinctive and appears to be becoming genetically isolated from the surrounding populations (Morelli et al. in prep). The population at the Mono Lake site was also acoustically distinct, with 84% of the calls being correctly assigned to the population. The acoustic distinction mirrors the genetic distinction and this suggests that the genetic structure and the acoustic structure are related not only at the individual level but also at the population level. In addition to the implications these results have for understanding vocal variation at the population level, this genetic fingerprint in the acoustic structure indicates that acoustic structure could be used to diagnose the genetic health of a population.

Factors affecting acoustic structure of a population

Genetic variation is only one of the factors that may be shaping the acoustic structure of each population. Variation in other factors may also be influencing the acoustic structure of these populations. Researchers have suggested that the alarm call structure may be impacted by the complexity of the habitat, such that call characteristics that allow the caller to be heard may develop (Slododchikoff and Coast 1980). The Mono Lake site is a county park, both the Lundy Lake site and the Silver Lake site are located on campgrounds, and the Modoc County site is an alfalfa field. At the Lundy Lake and Silver Lake camp grounds there were trees and shrubs as well as manmade structures such as fire pits and picnic tables. This additional habitat complexity could shape the acoustic structure of these populations.

Human presence has also been found to impact call structure by forcing populations to adapt their calls to be heard through human noise pollution and altered habitats (Barber et al. 2010). There was a steady flow of cars driving at the campgrounds and the park, so it is possible that human impacts were putting selective pressure on the alarm calls and that the variation in selective pressure between the populations influenced the inter-population acoustic variation.

Relationship between Acoustic Structure and Relatedness in Other Ground Squirrel Species

The relationship between genetic relatedness and acoustic similarity may also exist in other ground squirrel species that have similar social structures to Belding's ground squirrels and who would also benefit from identifying their relative's call. Other alarm-calling ground squirrel species, such as the Richardson's ground squirrel, also have populations where many closely related females live in close proximity to each other (Michener and Michener 1977, Armitage 1984). In the case of Richardson's ground squirrels, they have also been shown to have individually distinguishable alarm calls (Hare and Atkins 2001). The cause of this acoustic variation may also be related to the genetic variation; therefore, the genetic fingerprint of the population should be reflected in the acoustic structure.

Limitations and Future Directions

One limitation I encountered was that although I had enough samples of the “chirp” call type to analyze and compare the calls to the genetic relatedness, I did not have enough of the “trill” call type to draw conclusions about the acoustic similarity of this call. The trills may be linked to genetic relatedness through completely different call parameters than the chirps or they may not have a relationship to genetic relatedness at all. It would be interesting in future studies to investigate whether “trill” calls are more similar between genetically related individuals.

Another limitation on my project was that the sizes of the populations varied greatly, so although I was able to collect similar sample sizes at each site, the percentage of the total population that I sampled varied. The Mono Lake County population was contained on a small irrigated lawn, and within two days of trapping we were recapturing many of the same individuals. This leads me to believe that we thoroughly sampled this site. However, at the Alturas site there were more than a thousand Belding's ground squirrels interconnected throughout the ranch that our site was situated on. I did trap in several locations on the ranch, but I cannot make thorough conclusions about the overall genetic or acoustic structure at this site because I did not have a robust sample size in this location, and I am unsure about the percentage of the populations that I sampled at the Lundy Lake and Silver Lake sites. In future studies it will be important to conduct more extensive sampling to understand acoustic structure of all major populations of Belding's in California. This data could then be analyzed to understand if there is a connection between genetic flow barriers and acoustic isolation.

Broader Implications

The link between genetic relatedness and acoustic structure revealed in this study could be used better understand genetic flow between populations. Populations with short Mahalanobis distances can be identified as isolated, and this information can be used to predict whether a population is inbred. It is particularly important to understand the genetic health of Belding's ground squirrel because they have disappeared from 42% of the California sites that they were found at just 100 years ago. The populations that remain are becoming more isolated, and the species is in danger of losing genetic variation (Morelli et al. in prep) and this may result in the loss of phenotypic variation including acoustic variation.

Recording and analyzing alarm calls is much easier, faster and less expensive than genetic sampling and analysis. In addition to the methodological benefits, acoustic studies may be a less invasive way to understand genetic structure as one does not need to handle the animal or take a tissue sample from them. However, in order to record a sufficient amount of alarm calls, I had to keep the squirrels in traps for up to an hour. Being in a trap could cause the animal to experience stress and ultimately weaken the animal, so it is important to consider the impact that an acoustic study could have on an animal if it was necessary to the study to keep the animal in a trap for a long period of time. Ultimately, acoustic studies could provide an efficient means to get a preliminary understanding of genetic structure.

It is also important to understand the factors that contribute to the acoustic structure of these vocally dependent animals so that we can understand how changing those factors will impact the species. Human impacts on the natural habitats of Belding's ground squirrels, such as habitat fragmentation, could alter the genetic flow between populations and this in turn could alter the acoustic structure of many populations.

ACKNOWLEDGEMENTS

Dr. Toni Lyn Morelli and Dr. Stacie Hooper contributed countless hours of support and direction for this project. Without their expertise, enthusiasm and patience this project would not have been possible. Dr. Craig Moritz and Dr. Brenda McCowan provided funding, equipment, and essential guidance throughout this project, and I will be forever grateful for their support. Thanks also to Ilaria Mastroserio, who dedicated an entire week to assist with this field work of this project. Marisa Lim, Lindsey Eastman and Lydia Smith provided help and advice for the genetic analysis of this project. Patina Mendez, Kurt Spreyer, Melissa Eitzel and Seth Shonkoff have also been extremely helpful by constructing a wonderfully organized thesis class, full of engaging lectures and workshops. Patina Mendez and Melissa Eitzel have been especially crucial to my thesis by editing my work, giving essential advice along the way and providing moral support during stressful times. Jacob Finkle, Uriel Garcia, Carolina Méndez and Ming Stephens were amazing work group members and Ming Stephens in particular carefully edited and provided comments for many drafts of my thesis. Thanks as well to Jaclyn Iaquina, Marissa Montjoy, Austin Roughton and Kimberly Lam for their wonderful comradery for the past year

and half. Also, my family and friends have been supportive not only through this thesis but throughout my entire life. Amy Kastely, J. Kastely, Joe Kastely, Molly Porter-Kastely, KT McCorkmick, Iris Ayala, Gerardo Yeverino, Zoe Burr, Ashanti Shih, Anika Tuba and Rusty Nye have been the greatest support system I could have ever hoped for. Finally, thanks to all of the Belding's Ground Squirrels who have shown me again and again that the animal world is more complicated and amazing than I ever dreamed was possible. I promise I will stop catching you now, and leave you in peace.

Scientific collecting permit through California Natural Resources Agency, Department of Fish and Game, Permit ID Number 10774

REFERENCES

- Armitage, K. B. 1981. Sociality as a life-history tactic of ground squirrels. *Oecologia* 48: 36-49
- Barber, J.R., K.R. Crooks, and K.M. Fristrup. 2010. The costs of chronic noise exposure for terrestrial organisms. *Trends in Ecological Evolution* 25: 180-189
- Boersma, P. 2001. Praat, a system for doing phonetics by computer. *Glott International*, 5: 341-345
- Chapman, C. A., and D. M. Weary. 1990. Variability in spider monkeys' vocalizations may provide basis for individual recognition. *American Journal of Primatology* 22: 279-284
- Charlton, B. D., Z. Zhihe, and R. J. Snyder. 2009. Vocal cues to identity and relatedness in giant pandas (*Ailuropoda melanoleuca*). *Journal of the Acoustic Society of America* 125: 2721-2732
- Cheney, D. L., and R. M. Seyfarth. 1999. Recognition of other individuals' social relationships by female baboons. *Animal Behaviour* 58: 67-75
- Cooper, W. E. 2011. Escape strategy and vocalization during escape by American bullfrogs (*Lithobates catesbeianus*). *Amphibia-Reptilia* 32: 213-221
- Da Silva, A., G. Luikart, D. Allainé, P. Gautier, P. Taberlet, and F. Pompanon. 2003. Isolation and characterization of microsatellites in European alpine marmots (*Marmota marmota*). *Molecular Ecology Notes* 3: 189-190
- Goossens, B., L. Graziani, L. P. Waits, E. Farand, S. Magnolon, J. Coulon, M. Bel, P. Taberlet, and D. Allainé. 1998. Extra-pair paternity in the monogamous alpine marmot revealed by nuclear DNA microsatellite analysis. *Behavioral Ecology and Sociobiology* 43: 281-288

- Goslee, S. C., and D. L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* **22**: 1-19.
- Hamilton, W. D. 1964. The genetical evolution of social behaviour. *Journal of Theoretical Biology* **7**: 1-16
- Hanslik, S., and L. Kruckenhauser. 2000. Microsatellite loci for two European sciurid species (*Marmota marmot*, *Spermophilus citellus*)
- Hare, J. F., and B.A. Atkins. 2001. The squirrel that cried wolf: reliability detection by juvenile Richardson's ground squirrels (*Spermophilus richardsonii*). *Behavioral Ecology and Sociobiology* **51**: 108-112
- Holekamp, K. E. 1984. Natal dispersal in Belding's ground squirrels (*Spermophilus beldingi*). *Behavioral Ecology and Sociobiology* **16**: 21-30
- Hollen, L. I., and A. N. Radford. 2009. The development of alarm call behavior in mammals and birds. *Animal Behaviour* **78**:791-800
- Hooper, S. L. 2011. Impacts and Applications: Developing a bioacoustic tool for mammals and measuring the effects of highway noise on a mammalian communication system, using ground squirrels as a model. Dissertation. University of California at Davis, Davis, California, U.S.A.
- Kalinowski, S.T., A.P. Wagner, and M.L. Taper. 2006. ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes* **6**: 576-579
- Kyle, C. J., T. J. Karels, B. Clark, C. Strobeck, D. S. Hik, and C. S. Davis. 2004. Isolation and characterization of microsatellite markers in hoary marmots (*Marmota caligata*). *Molecular Ecology* **4**: 749-751
- Long, A. M., N. P. Moore, and T. J. Hayden. 1998. Vocalizations in red deer (*Cervus elaphus*), sika deer (*Cervus nippon*), and red x sika hybrid. *Journal of Zoology* **244**: 123-134
- May, B., T. A. Gavin, P. W. Sherman, and T. M. Korves. 1997. Characterization of microsatellite loci in the Northern Idaho ground squirrel *Spermophilus brunneus brunneus*. *Molecular Ecology* **6**: 399-400
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209-220
- Mateo, J. M. 2010. Alarm calls elicit predator-specific physiological responses. *Biology Letters* **6**: 623-625

- Mateo, J. M., and W. G. Holmes. 1999. How rearing history affects alarm-call responses of Belding's ground squirrels (*Spermophilus beldingi*, Sciuridae). *Ethology* **105**: 207-222
- Matrosova, V. A., I. A. Volodin, E. V. Volodina, and N. A. Vasilieva. 2010. Stability of acoustic individuality in the alarm calls of wild yellow ground squirrels *Spermophilus fulvus* & contrasting calls from trapped & free-ranging callers. *Naturwissenschaften* **97**: 707-715
- McCowan, B., and S. L. Hooper. 2002. Individual acoustic variation in Belding's ground squirrel alarm chirps in the High Sierra Nevada. *Journal of Acoustical Society of America* **111**: 1157-1160
- McDonald, P.G., and J. Wright. 2011. Bell miner provisioning calls are more similar among relatives and are used by helpers at the nest to bias their effort towards kin. *Proceeding of the Royal Society Biological Sciences* **278**: 3403-3411
- Michener, G.R., and D.R. Michener. 1977. Population structure and dispersal in Richardson's ground squirrels. *Ecology* **58**:359-368
- Mitani, J. C., T. Hasegawa, J. Gros-Louis, P. Marler, and R. Byrne. 1992. Dialects in chimpanzees? *American Journal of Primatology* **27**: 233-243
- Owens, N. M., and J. D. Goss-Custard. 1976. The adaptive significance of alarm calls given by shorebirds on their winter feeding grounds. *Evolution* **30**: 397-398
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>
- Raymond, M., and F. Rousset . 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Heredity*, **86**:248-249
- Rousset, F. 2008. Genepop'007: a complete reimplementaion of the Genepop software for Windows and Linux. *Mol. Ecol. Resources* **8**: 103-106
- Schibler, F. and M. B. Manser. 2007. The irrelevance of individual discrimination in meerkat alarm calls. *Animal Behaviour* **74**: 1259-1268
- Schneiderova, I., and R. Policht. 2010. Alarm calls of the European ground squirrel *Spermophilus citellus* & the Taurus ground squirrel *S. taurensis* encode information about caller identity. *The International Journal of Animal Sound & Its Recording* **20**: 29-43
- Searby, A., and P. Jouventin. 2004. Acoustic recognition in macaroni penguins: an original signature system. *Animal Behaviour* **67**: 615-625
- Sharp, S. P., and B.J. Hatchwell. 2006. Development of family specific contact calls in the long-tailed Tit *Aegithalos caudatus*. *Ibis* **148**: 649-656

- Sherman, P. W. 1977. Nepotism and the evolution of alarm calls. *Science* **197**: 1246-1253
- Slobodchikoff, C. N., and R. Coast. 1980. Dialects in the alarm calls of prairie dogs. *Behavioral Ecology and Sociobiology* **7**: 49-53
- Stevens, S., J. Coffin, and C. Strobeck. 1997. Microsatellite loci in Columbian ground squirrels *Spermophilus columbianus*. *Molecular Ecology* **6**: 493-495
- Tunner, H. G., and W. Hoedl. 1978. Warning or alarm call and distress call in *Rana-ridibunda-ridibunda salientia ranidae*. *Zoologischer Anzeiger* **200**: 386-390
- Volodina E. V., V. A. Matrosova, and I. A. Volodin. 2010. An unusual effect of maturation on the alarm call fundamental frequency in two species of ground squirrels. *The International Journal of Animal Sound & Its Recording* **20**: 87-98
- Wright, T. F. 1996. Regional dialects in the contact call of a parrot. *Proceedings: Biological Sciences* **263**: 867-872

APPENDIX A. Microsatellite data**Table A1. Summary of primer data for microsatellite analysis .** N_A = Number of alleles, T_A = Annealing temperature (C°), N_C = Number of cycles

Locus	Reference	Sequence	N_A	Range	T_A	N_C
IGS1	May et al. 1997		9	85-101	53	34
IGS6	May et al. 1997	(CA)28	11	101-125	68/58	20/18
IGS9d	May et al. 1997	(AAC)12	3	126-132	48	34
IGSBP1	May et al. 1997	(GCA)10	5	90-104	53	34
MA018	da Silva et al. 2003	(CA)13	12	293-315	68/58	20/18
GS14	Stevens et al. 1997	(TG)30	4	225-231	64/54	20/18
GS17	Stevens et al. 1997	(TG)16	5	149-171	52	34
GS22	Stevens et al. 1997	(TG)18	10	168-186	64/54	20/18
GS25	Stevens et al. 1997	(TG)17	17	129-163	60/50	20/18
ST10	Hanslik and Kruckenhouse 2000	(CA)12	10	118-140	56	34
2g2	Kyle et al. 2004	(GT)17	13	121-153	64/54	20/18
SS-Bib18	Goossens et al. 1998		8	131-149	60	34

APPENDIX B. Acoustic Parameters

Table B1. Description of acoustic parameters. Fifteen independent call parameters used perform discriminant function analysis. Table reproduced with permission (Hooper 2011)

Acoustic Parameter	Description
Duration	Temporal length of call, measured in seconds
Minimum Frequency	Lowest frequency attained by fundamental frequency, measured in Hz
Maximum Frequency	Highest frequency attained by fundamental frequency, measured in Hz
Mean Frequency	Average frequency of the fundamental frequency across the call, measured in Hz
Standard Deviation of Frequency	Standard deviation of the fundamental frequency, measured in Hz
Time of Max. Fund. Frequency	Relative position of max. fundamental frequency measured in milliseconds from the start of the call
Maximum Frequency Location	Location of max. fundamental frequency, given as a proportion of the duration
Time of Min. Fund. Frequency	Relative position of min. fundamental frequency measured in milliseconds from the start of the call
Minimum Frequency Location	Location of min. fundamental frequency, given as a proportion of the duration
Jitter Factor	Weighted measure of the amount of frequency modulation by calculating the sum of the absolute value of the difference between two sequential frequencies
Shimmer	Weighted measure of the amount of amplitude modulation by calculating the average absolute difference between consecutive amplitude differences
Max. Harmonic-to-noise Ratio (HNR)	Max. ration of the energy of harmonic components to noise energy in the call
Min. HNR	Max. ration of the energy of harmonic components to noise energy in the call
Mean HNR	Average of the HNR across the call
Standard Deviation of HNR	Standard deviation of the HNR