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The effects of climate and habitat change on the distribution and genetic diversity of chipmunks in the Sierra Nevada, California

Author
Rubidge, Emily

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The effects of climate and habitat change on the distribution and genetic diversity of chipmunks in the Sierra Nevada, California

by

Emily Margaret Rubidge

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University of California, Berkeley

Committee in charge

Professor Justin S. Brashares, Chair
Professor N. Maggi Kelly
Professor Craig Moritz

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ABSTRACT

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Historical changes in climate have affected the diversity and distribution of species across the globe. Recent and rapid human-induced climate change is expected to have extreme consequences for biodiversity and ecosystem integrity. Documented species' responses to recent climate change include adaptation, distributional shifts and extinctions. There is an urgent need for scientists to improve understanding of how climate change will affect not only species distribution and abundance but also genetic diversity, which is the basis for evolutionary potential and thus critical to long-term persistence. Here I investigate the effects of climate change on the diversification, distribution and genetics of chipmunks in Sierra Nevada, California.

First, I examine the role of late Pleistocene climate fluctuations on the divergence of the endemic Alpine Chipmunk (*Tamias alpinus*) from its sister taxa, western populations of the Least Chipmunk (*T. minimus*). I use one mitochondrial gene (cytochrome b) and 14 microsatellite loci to examine the evolutionary relationship between these co-distributed species. Mitochondrial sequence data revealed that that *T. alpinus* and *T. minimus* populations share mitochondrial haplotypes with no overall geneaological separation, and that diversity at this locus is better explained by geography than by species' boundaries. In contrast, the microsatellite analysis showed that although highly differentiated populations within species exist, populations of the same species are more similar to each other than they are to members of the other species. This result suggests that the two species are distinct and there is no contemporary introgression along their parapatric boundary. Coalescent analysis of the divergence history indicated a late Pleistocene splitting time (~450ka) and subsequent, though limited, gene flow between the two lineages. The divergence of *T. alpinus* during this time period provides more evidence that Pleistocene glacial cycles played an important role in diversification of species in the Sierra Nevada and North America in general.

Second, I used small mammal surveys repeated over a century to assess accuracy of species distribution models in predicting known changes in chipmunk distributions, and to identify the main environmental drivers of these shifts. Historical (1900-1940) climate, vegetation and species occurrence data were used to develop single-species and multi-species multivariate adaptive regression spline (MARS) distribution models for three species of chipmunk. Models were projected onto the current (1980-2007) environmental surface and then

tested against modern field resurveys of each species. I evaluated models both within and between time periods and found that even with the inclusion of biotic predictors, climate alone is the dominant predictor explaining the distribution of the study species within a time period. However, climate was not consistently an adequate predictor of the distributional change observed in all three species across time. For *T. alpinus*, climate alone showed the best predictive performance, strongly suggesting this species has retracted its range up in elevation due warming over the past 100 years. Modeling results showed that both climate and vegetation were factors in the collapse of *T. senex* populations in the study area and my modeling approach failed to predict the stability of the distribution over time observed in *T. speciosus*.

Third, I investigated how the climate-induced range contraction observed in *T. alpinus* over the past century has affected the species' overall diversity and population genetic structure in Yosemite National Park. I used one mitochondrial and seven microsatellite loci, amplified from both historical and modern specimens, to compare genetic structure of *T. alpinus* with that of *T. speciosus*, whose distribution remained stable over time. I found a decline in overall genetic diversity and a reduction of gene flow between local populations over time in *T. alpinus,* as expected from spatial modeling of distributions. As predicted given its relative stability through time, there were no significant genetic changes seen in *T. speciosus*.

Overall, my dissertation contributes to existing research on the diversification, climate change impacts, and conservation genetics of small mammals. Most importantly, this work exemplifies the value of natural history museums, and the use of historically collected data in contemporary biodiversity research.

This dissertation is dedicated to my parents, Nicholas and Pamela, who taught me to love and respect nature; my daughters, Maeve and Sylvie, in the hopes of preserving wild places for future generations; and my husband, Cole, for his unwavering support and encouragement.

"Every able-bodied man, woman, and child should make it his or her business, so far as possible, to seek out for himself, on foot, and preferably alone, the wild creatures, and come to some appreciation of them. That most tourists take the wrong attitude seems clear. This must be especially true of the automobile tourists. The mere fact of having 'retinized' a certain series of objects said to be extraordinary is certainly not especially conducive to edification, in the long run. Far better it is to settle down quietly in one locality, being content not to see everything at once, and earnestly strive to look out upon one's environment with the 'seeing' eye, the 'hearing' ear, and the mind that understands, or at least tries to."

Walter P. Taylor – 16 August 1915, on route to Yosemite Valley.

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I am eternally grateful to Professor Emeritus Jim Patton. Although not officially a part of my advising committee, Jim was my mentor; without him my dissertation would not have been the same. I felt that I was the envy of other graduate students to have Jim, in his own words "an old rat-trapper", guide me through my academic experience at Berkeley. Not only did Jim teach me to trap, handle, identify and sample chipmunks and much more, he showed me what it takes to be a good scientist: commitment, determination and lots of hard work (although Jim makes the work part look easy). Jim's generosity amazed me and I am lucky to have worked so closely with him.

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CHAPTER 1

General Introduction

Past climate changes have affected the abundance, genetic diversity, morphology and geographic ranges of mammal species (reviewed in Blois & Hadley 2009). In particular, late-Pleistocene climate oscillations have been implicated in the loss of mammalian community diversity (Blois et al. 2010). Range dynamics during this time period also played a significant role in the diversification of several North American taxa and the generation of richness patterns seen today (Brown 1971, Knowles 2000, Johnson & Cicero 2004). Evaluating the genetic structure of species across a landscape is a vital tool for reconstructing how past geological and climate changes have shaped the diversity across the globe. However, biologists are now under pressure to not only interpret past changes, but also to generate methods for accurately predicting species' responses to future rapid climate change. The biotic effects of recent, anthropogenic-induced climate change are already well documented across diverse taxa (Root et al. 2003), with signals of latitudinal and elevational range shifts, phenological changes, and extinctions (reviewed in Parmesan & Yoho 2003).

Mountain species are thought to be especially vulnerable to warming due to their geographic isolation, limited habitat availability, and generally restricted distribution (MacDonald & Brown 1992). Recent temperature increases and vegetation changes in the Sierra Nevada (Millar et al. 2004, Lutz et al. 2009, 2010) appear to have already affected the distributions of mammals and birds that inhabit the area (Moritz et al 2008, Tingley et al. 2009). Chipmunks (genus *Tamias*), a speciose group of small diurnal squirrels, are a group that has a complex biogeographical history likely due to past climate oscillations (e.g., Good & Sullivan 2001, Demoboski & Sullivan 2003), but that also shows signals of being affected by recent human-induced climate change (Moritz et al. 2008). In this dissertation, I examine the evolutionary history and effects of recent climate change on the distribution and population genetics of chipmunks in Sierra Nevada, California.

To accurately detect the effects of environmental change on species, biologists must rely on data collected prior to an environmental perturbation of interest. The research presented here relies heavily on both historical data (1911-1916) and contemporary data (2003-2009) collected by teams from the Museum of Vertebrate Zoology as part of a project called " The Grinnell Resurvey Project". The goal of this project is to resurvey vertebrates at sites first surveyed by Joseph Grinnell and his team between 1908-1939 across the state of California to better understand how changes in climate and vegetation over the past century have affected Californian vertebrate fauna (http://mvz.berkeley.edu/Grinnell/index.html). Results from the resurveys indicate several species appear to have changed their distribution (Moritz et al. 2008) or tracked their climatic niche through time (Tingley et al. 2009). More specifically, resurvey results indicate that chipmunk species, in particular, have shown diverse patterns of change since the original Grinnell surveys. Within Yosemite National Park (YNP), these varied changes include the apparent decline of Shadow Chipmunk (*T. senex*), an upward retraction of the lower elevational limit in Alpine Chipmunk (*T. alpinus*), and the relative stability or expansion of the Lodgepole Chipmunk (*T. speciosus*). In addition, the Grinnell Project resurvey in YNP has shown evidence of an expansion at the upper elevational limit for Yellow-pine Chipmunk (*T. amoenus*), known previously only from the east-slope of the Sierras up to 2700m in elevation (found at Mono Pass, YNP, elevation 3200m in 2008; a documented new species for YNP). In

my dissertation, I combine Grinnell Project data on chipmunk occurrences with genetic data derived from collected specimens and tissue samples, from both historical and modern eras, to examine three central questions described below.

In my first set of analyses (Chapter 2), I use both mitochondrial and nuclear genetic loci to examine the divergence history of Alpine Chipmunk (*T. alpinus*). This animal is endemic to California with a distribution restricted to the alpine areas (mainly found above 3000m) of central to southern Sierra Nevada. Although not included in the most recent phylogeny of chipmunks (Piaggio & Spicer 2001) it has been shown to be the sister taxa of the western populations of the widespread Least Chipmunk (*T. minimus*; Good et al. 2008). The objective of this chapter is to gain a better understanding of the divergence history between these two species using molecular tools. The role of late Pleistocene climate oscillations in lineage diversification are discussed in light of estimated splitting time, migration rates and effective population sizes of both species.

In my second study (Chapter 3), I use historical and modern datasets to better understand distributional changes observed in three species chipmunk (*T. alpinus*, *T. senex* and *T. speciosus*) in and around Yosemite National Park. As mentioned above, the resurvey results have shown that *T. senex* has collapsed in the park, the lower distributional limit of *T. alpinus* has retracted up in elevation, and *T. speciosus* has remained relatively stable. Here, I use historical vegetation, climate and species data to build species distribution models (SDMs) and then project these models on to the current environmental landscape to assess their accuracy with the modern presence/absence data. With this approach, I am not only able to identify the main drivers of the observed changes of these species, but also assess the predictive efficacy of SDMs in general, a popular yet relatively untested tool used in predicting species' responses to future climate change (Araujo et al. 2005a).

There are several studies investigating the effects of recent warming trends on alpine species (Inouye et al. 2001, Reale et al. 2003, Ozgul et al. 2010), but very few have examined effects at the genetic level. In my last study (Chapter 4), I use a comparative genetic approach with historical and modern data from two species of chipmunk in YNP: *T. alpinus* that has retracted its distribution, and *T. speciosus*, whose distribution has remained relatively stable over the past century. A range contraction up in elevation in a mountain species may be associated with several genetic signals. If the lower elevation populations have been extirpated, reducing the overall population size then a decrease in genetic diversity is expected. Conversely, if animals have physically moved up in elevation and there has been no reduction in overall abundance then there should be no evidence of genetic diversity loss over time. Both scenarios should produce a signal of decreased gene flow between populations as lower elevation areas become less suitable for dispersal. No significant genetic changes are expected in *T. speciosus* due to its observed stability through time. Habitat loss due to global warming poses a significant threat to genetic diversity (Ditto and Frey 2007) and this study is among the first to investigate potential for loss of genetic diversity as a result of a climate-induced range shift.

In general, the research presented in this dissertation contributes to existing research on speciation, climate change impacts, and conservation genetics. Firstly, my results add to the growing literature examining recent speciation events across taxa in the Sierras and the role of the late-Pleistocene glacial and interglacial periods on lineage divergence in this region (e.g., Schoville & Roderick 2009, Rovito 2010). Secondly, this research provides insight into the

applicability of SDMs for projecting into novel climatic environments and identifies the most likely drivers of observed distributional shifts in chipmunk species over the past century. Thirdly, this dissertation is one of few studies able to quantify the genetic effects of recent distributional shifts and the role climate change plays in shaping the genetic structure of an endemic alpine species. Finally, and perhaps most importantly, this work exemplifies the value of historical information and museum specimens, and particularly the "Grinnell data", which, having been collected in a detailed and consistent manner, made possible this novel investigation into the ecological and evolutionary effects of environmental change.

CHAPTER 2

A multilocus analysis of the evolutionary history of Alpine Chipmunk, *Tamias alpinus*, in Sierra Nevada California.

Introduction

Understanding processes that promote and maintain biodiversity is a key goal of evolutionary biology. Divergent natural selection resulting from resource heterogeneity and competitive interactions can drive population divergence and speciation (Dobzhansky 1951, Schluter 2001). Nonadaptive divergence, operating via genetic drift due to isolation and founder effects, also plays a significant role in generating patterns of species diversity. Several processes promote speciation, including vicariance events (e.g., mountain building), ecological gradients and glacial refugia. Pleistocene glacial and interglacial periods have shaped the genetic structure of taxa across the globe, as glacial refugia facilitated the formation of distinct evolutionary lineages within species (Avise *et al.* 1998, Hewitt 2004). The fragmentation of species' distributions led to local adaptations, and recurrent glacial and interglacial periods resulted in repeated secondary contact, hybridization and demographic fluctuations of species (Avise 2000).

Glaciation cycles occurring throughout the Pleistocene caused frequent shifts in species' ranges (Pielou 1991, Hewitt 1996), with important implications for models of species divergence. For example, periods of allopatry during range contractions allowed differences to accumulate between separated populations, promoting divergence (e.g., Avise & Walker 1998, Knowles 2000). Conversely, range expansions during interglacial periods may have had homogenizing effects through increased gene flow and secondary contact (e.g., Cracraft & Prum 1988, Riddle 1996, Klicka & Zink 1997). Range dynamics and the complex geological and climatic history of Sierra Nevada, California shaped the diversity of the area. The late Pleistocene in particular was a time of drastic climate fluctuations in Sierra Nevada (Gillespie & Zehfuss 2004) and this time period has been linked to intra and interspecific diversification in many taxa (e.g., Feldman & Spicer 2006, Schoville & Roderick 2009, Hull et al. 2010, Rovito 2010). The complex glacial history and associated range dynamics in Sierra Nevada provides a natural laboratory to examine species' histories, including an understanding of both the contemporary and historical processes, that promoted lineage diversification and the biodiversity patterns observed today.

California's rich mammalian fauna includes 185 species, 18 of which are endemic (Stein *et al.* 2000). Chipmunks (Genus *Tamias*) make up one of the most speciose groups of mammals in the state, having 10 of the 25 *Tamias* species worldwide. The biogeographic history, radiation and evolutionary relationships of western North American chipmunks are complex (Good & Sullivan 2001, Piaggio & Spicer 2001, Demboski & Sullivan 2003, Good *et al.* 2003, Good *et al.* 2008). The highest diversity of *Tamias* species is centered in western portion of the continent (Hoffman *et al.* 1993) and diversification within the group is thought to be linked to changes in geographic ranges through climatic cycles and subsequent shifts in habitat preference resulting from interspecific competition and niche partitioning (Hoffmann *et al*. 1981). Much of this niche partitioning has occurred over elevational gradients where one species replaces another moving up in elevation (Heller 1971, Chappell 1978, Bergstrom & Hoffmann 1991). This is especially clear in the Yosemite area where Grinnell and Storer (1924) described the elevational zonation of seven species of chipmunk that occur from 1000m on the west slope of the Sierra Nevada to over

3300m at the crest in Yosemite National Park (YNP) down to approximately 1800m on the eastern side. *T. alpinus*, an endemic of the alpine zone of the central and southern Sierra Nevada, occupies the highest elevational band (mainly above 3000m) in this transect whereas its sister taxa, western populations of *T. minimus* occupy the lowest elevation range on the eastern side (up to about 2200m). In this study, I examine the evolutionary relationship of the narrowly distributed *T. alpinus,* and its widespread sister species, the Least Chipmunk (*T. minimus*) in Sierra Nevada, California.

The evolutionary history of *T. alpinus* has never been examined. The most recent review of the *Tamias* phylogeny using mitochondrial DNA, did not include *T. alpinus* (Piaggio & Spicer 2001) although Good *et al.* (2008), using the same mitochondrial gene (cytochrome b) showed that *T. minimus* is polyphyletic and that western populations are sister to *T. alpinus*. The objective of this study is to examine the relationship between *T. alpinus* and the western group of *T. minimus*, with the goal of gaining a better understanding of the evolutionary history of *T. alpinus*. More specifically, I focus on two questions 1) did *T. alpinus* diverge from western *T. minimus* in association with late-Pleistocene glacial dynamics and 2) is there evidence for contemporary or historical introgression as reported in other species-pairs of chipmunks (Good et al. 2003, 2008). To address these questions I use a multilocus approach examining genetic variation and population structure at one mitochondrial and 14 microsatellite loci. Contrasting patterns of nucleotide variation in the mitochondrial genome with patterns of genetic variation at microsatellite markers gives us a picture of both historical and contemporary processes respectively.

Methods

Study species

The *T. minimus* species complex contains 21 recognized subspecies (Vert & Carraway 2001) and is the most widely distributed *Tamias* species (Sullivan 1985). It occurs from western central Yukon Territory southward along the eastern base of the Rocky Mountains in British Columbia (BC) eastward throughout Canada's provinces, upper Michigan and Minnesota. It is also found throughout the Great Basin with disjunct populations further south in Arizona and New Mexico (Vert & Carraway 2001). The subspecies, *T. minimus scrutator*, is the sister taxa to *T. alpinus* and occurs outside California in parts of southeastern Oregon, south-central Washington, northern and central Nevada, western Utah and southwestern Idaho (Johnson 1943). In California, this species is restricted to the northeastern and central-eastern part of the state, mainly east of Sierras, with an isolated high elevation population in Tulare County (Figure 1). For the purposes of this study, I will use *T. minimus*, to refer to the subspecies *T. m. scrutator* that inhabits the southwestern most portion of this species' range directly adjacent to that of *T. alpinus* (Figure 1). *T. alpinus* is geographically restricted to the high elevations of the central to southern Sierra Nevada (Figure 1).

T. alpinus and *T. minimus* are allopatric at the northern end of our study area; moving south of YNP there are confirmed areas of sympatry in the mountains northeast of Bishop (D. Guiliani, pers. comm.) and at the southern edge of their range(Figure 1). The only location of where both species have both been confirmed from museum specimens (collected in 1911) is Olancha Peak (elevation: 2970m), in the southern Sierras (Johnson 1943). The two species differ in morphology and habitat preferences. *T. minimus*, among other characters, is smaller in body

mass, has a longer tail, shorter ears and darker coloration than *T. alpinus* (Johnson 1943) and has a different bacular (penis bone) morphology (Clawson et al. 1994, J.L. Patton unpublished data). In California, *T. minimus* is found in arid sagebrush habitat that ranges in elevation from 1500m to above 3000m in the Sierra Nevada and mountains to the immediate east (e.g., Sweetwater, White, and Inyo ranges). *T. alpinus* is restricted to the alpine zone of Sierra Nevada at and above tree-line (2950 to 4100 m) where it occupies open granite habitat, meadow edges and talus slopes (Grinnell & Storer 1924, Johnson 1943).

Study Site and Samples

The study area is the central to southern Sierra Nevada, CA, USA, which includes the entire known range of *T. alpinus* and that of *T. minimus* to the immediate east and north (Figure 2). A total of 327 chipmunks were included in this study. The majority of samples were collected between 2003-2009, however 26 samples were taken from museum skins that were collected between 1911-1916. Chapter 4 describes live trapping and tissue sampling. Chipmunks collected in areas of potential sympatry were identified to species by small mammal expert J.L Patton (Emeritus Professor and Mammal Curator, MVZ), based on distinct morphological differences including body size, pelage color, ear and tail length. For the modern tissues, ear clips or liver tissues where used in DNA extraction. For the museum skins, I removed an approximately 3mm x 3mm square piece of skin from the lower lip. Special precautions against contamination was taken when sampling, extracting and amplifying DNA from museum skins (see Chapter 4).

DNA Extraction, microsatellite genotyping and sequencing

I used the standard Qiagen DNAeasy kit to extract DNA from samples of either liver or ear tissue following the manufacturer's protocol (Qiagen). Extractions were eluted in a total of 400µl AE buffer. An 801bp portion of the mitrochondrial gene, Cytochrome b, was amplified using universal mammal primers, MVZ05 & MVZ16 (Smith 1998). I sequenced 139 *T. alpinus* and 107 *T. minimus* samples for a total dataset of 246 sequences. The thermal cycler conditions for the mitochondrial PCRs were as follows: 94°C for 2mins, 35 cycles of 94°C for 30s, 47-50°C for 30s, 72°C for 60s and then a final extension at 72°C for 5mins. Amplicons were sequenced on an ABI 3730 Capillary Sequencer (Applied Biosystems, Inc.). Resulting sequences were edited and aligned using Sequencher 4.8 (Gene Codes Corp.).

I amplified the DNA at 14 microsatellite loci in *T. alpinus* and *T. minimus* (Loci names: EuAmMS26, EuAmMS37, EuAmMS41, EuAmMS86, EuAmMS94, AC A2, AC A101, AC A108, AC B12, AC B111, AC C2, AC C122, AC D107 and AC D115). The first five these were taken from the literature (Schulte-Hostedde *et al.* 2000), and the remaining nine were developed in *T. alpinus*. Primer sequences of all 14 loci are available in Chapter 4 (Chapter 4, Table 1). Reverse primers were fluorescently labeled with one of the following dyes: PET, NED, FAM, or HEX, forward primers were unlabeled. PCR reactions with a volume of 8.0µl contained reagents in the following concentrations: 0.5-1µl DNA template, 0.25µM each primer, 0.2mM each dNTP, 0.8µl 10X BSA, 0.8µl 10X PCR buffer (Roche), 1.5mM $MgCl_2$ and 0.4U of *Taq* DNA polymerase (Roche). The thermal cycler consisted of 94°C for 2min, followed by 30 cycles of 94°C for 40s, 51-60°C for 40s, and 72°C for 40s, and ending with a final extension at 72°C for 10mins. Locus-specific annealing temperatures are shown in Chapter 4, Table 1). PCR products were sized by capillary electrophoresis on an ABI 3730 sequencer (Applied Biosystems, Inc.), and alleles were scored manually using program GENEMAPPER Ver. 4.0 software (Applied

Biosystems, Inc.). Positive and negative controls as well as three replicate samples were run on each PCR plate for each locus. Repeat genotypes showed high repeatability.

Data Analysis

Mitochondrial DNA Analyses

Genetic variation among sequences within species was quantified as haplotype diversity (h_d), and nucleotide diversity (θ_π. θ_S).

I used two approaches to estimate genealogical relationships. First, to estimate the phylogenetic relationships of haplotypes, I used the Bayesian approach implemented in MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001). The best-fit model of nucleotide change was estimated using Akaike Information Criterion as implemented in jModeltest (Posada 2008). The model of sequence evolution ranked highest by AIC for the dataset was the Tamura-Nei model (TrN + I + Γ) but because TrN is not an option in MrBayes, and this model is a special case of the general time reversible model (GTR) I used GTR+ I + Γ. I ran four MCMC chains for 3,000,000 generations with trees sampled every 300 generations. I assessed convergence by examining the standard deviation of split frequencies, which were <0.01 after 3×10^6 generations. A burn-in period of 10^5 was discarded prior to calculating the consensus tree. Three individuals of the Panamint chipmunk (*Tamias panamintinus*) were used as an outgroup to *T. alpinus* and *T. minimus* (Good et al. 2008). Traditional phylogeny reconstruction approaches such as described above, however, make several assumptions that make them inaccurate at the population level. For example, they assume ancestral haplotypes are no longer present in the population. Therefore, our second approach was to use haplotype networks to estimate the genealogical relationship using the statistical parsimony approach (Templeton et al. 1992) as implemented in the program TCS 2.1 (Clement et al. 2000). TCS 2.1 uses statistical parsimony to connect haplotypes based on a 95% confidence interval. The number of mutational differences just before this 95% cut off is the maximum number of mutational connections between pairs of sequences. TCS 2.1 collapses sequences into haplotypes and calculates haplotype frequencies. These frequencies are used to estimate haplotype outgroup probabilities, which correlate with haploytpe age (Castelloe & Templeton 1994).

To quantify mtDNA differentiation between species and/or populations I calculated the average and the net number of nucleotide substitutions per site (*Dxy* and *Da*, Nei 1987). To visualize divergence patterns I clustered individuals by geography and used *Dxy* to produce a neighbour-joining tree in MEGA version 4 (Tamura et al. 2007). All diversity and distance calculations were estimated in program DNAsp version 5 (Librado & Rozas 2009). I used an Analysis of Molecular Variance (AMOVA) implemented in the program ARLEQUIN (Excoffier et al. 2005) to examine the population structure of haplotype diversity. F-statistic analogues (ϕ) were calculated to estimate the differentiation among groups (ϕ_{CT}) among populations within groups (ϕ_{SC}) and within populations (ϕ_{ST}). Populations were grouped according to their species designation and according to the genetic clusters determined from the STRUCTURE analysis (see *Microsatellite analysis* section). I tested the statistical significance of the AMOVA with 10100 permutations and corrected the p-values associated with the ϕ values using Bonferroni correction for multiple tests. I calculated Tajima's D (Tajima 1989) and Fu's Fs statistic (Fu 1996) and the 95% confidence interval around these statistics using the bootstrap method (with no recombination) offered in DNAsp (Librado and Rozas 2009) with 5000 replicates, to

determine if there was evidence of historical population expansion or contraction in each species.

Microsatellite analyses

I tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium (HWE) in per locus, across populations and overall with an exact test using (10000 permutations; Raymond & Rousset 1995). Bonferroni corrections for multiple tests were applied to p-values (Rice 1989).

To examine population structure without a priori definitions of "populations", I applied the Bayesian approach implemented in the software Structure 2.3.3 (Pritchard et al. 2000) to identify clusters of randomly mating individuals with minimum HW deviations and linkage disequilibrium. I ran the admixture model with correlated allele frequencies with five replicates of 10^6 Markov Chain Monte Carlo (MCMC) iterations after a burnin of 10^5 from K (number of parental populations) = 1 to K = 10. To provide the most accurate estimation of K, I used the statistic ΔK introduced by Evanno et al. (2005). I averaged coefficients of membership across the five replicates using the software CLUMMP 1.1 (Jakobsson & Rosenberg 2007) and DISTRUCT 1.1 (Rosenberg 2004) was used to plot the graphical representation of this membership. To further examine genetic structure I used the program Arelquin (Excoffier et al. 2005) to calculate pair-wise F_{ST} between the genetic clusters determined by the Bayesian analysis. To visualize the genetic distance between clusters I generated a neighbor-joining tree using the pairwise F_{ST} distances in the program MEGA version 4 (Tamura *et al.* 2007)

Divergence dynamics

The two chipmunk species are assumed to have diverged from a common ancestral population at some point in the past. To determine the time since divergence and the migration rates since the time of the split I used the coalescent-based isolation-with-migration (IM) model (Nielsen & Wakely 2001) implemented in the program IMa2 (Hey 2010). Using this program I estimated the following parameters: effective population size of *T. alpinus* (N_{eALP}), *T. minimus* (N_{eMIN}) and their common ancestor (N_{eA}), the migration rate from *T. alpinus* into *T.minimus* ($m_{ALP->MIN}$) and from *T. minimus* to *T. alpinus* ($m_{MIN->ALP}$), and finally time since divergence (t). IMa2 first uses a Bayesian Marcov Chain Monte Carlo (MCMC) approach to integrate over the space of possible genealogies and divergence times then uses the genealogies to estimate the posterior distribution of effective population sizes and migration rates to calculate joint posterior probability of all model parameters (Hey & Neilsen 2004, 2007, Hey 2010). I used 10 loci (cyt b sequences, and 9 microsatellite loci: EuAmMS26, EuAmMS41 EuAmMS86, EuAmMS94, EuAmMS37, ACA101, ACA108, ACC2, ACD115) partitioned by species in this analysis. Five microsatellite loci used in this study have complex repeat motifs and therefore may not follow a strict step-wise mutation model. These were not used in the analysis as recommended by Hey (http://groups.google.com/group/Isolation-with-Migration?pli=1). I sub-sampled the entire dataset to improve computational efficiency. Thirty individuals from the microsatellite dataset and twenty individuals from the sequence dataset were randomly chosen from each species for the analysis with assurance that each geographic area was represented. I used a two-population model where each species was considered a "population". A series of preliminary runs were used to estimate upper bounds on priors and assess mixing. Our final run consisted of 60 chains (geometric heating scheme set h_a=0.980, h_b=0.50), a burnin of 3 x 10^5 followed by 30 x 10^6 steps sampling trees from each locus every 300 steps (ESS>50). I did two replicates of the final run

starting with a different random number seed. Each run took approximately 72 days to finish (they were run simultaneously) and both returned parameter estimates that were near identical. Two hundred thousand saved genealogies (100,000 from each run) were used to calculate the joint posterior probability of the parameters in L-mode of IMa2. I estimated the locus-wide mutation rate of cytochrome b for 801bp as 4.68 $x10^{-8}$ from the literature (Zheng *et al*. 2003), and the average mutation rate for the microsatellites as1.0×10^{-4} to convert the parameter estimates into demographic units (i.e., time in years, population size in individuals and migration rates as individuals/generation).

Results

Mitochondrial sequence data

Our dataset consisted of 246 sequences with 81 variable sites, 40 singleton sites and 47 haplotypes. I found 28 haplotypes, 16 of which were unique (only found in one individual) in the *T. alpinus* dataset and 23 haplotypes, nine of which were unique in the *T. minimus* dataset (Table 1). All individual identification numbers, their locality information, cyt b sequence and haplotype are provided in Appendix 1.

The mtDNA phylogenetic tree estimated by the Bayesian analysis was weakly resolved but demonstrates a lack of clear genealogical separation between the two species (Figure 3). However, there is weak support for two clades in which both species belong (Figure 3). One clade comprised all geographic areas and both species (RED on Figure 3), whereas the other (BLUE on Figure 3) comprised only individuals of both species from the northern sampling area and two individuals from the White Mountains. Both clades included haplotypes that were shared between species. There were two divergent haplotypes comprised of *T. alpinus* individuals from the southernmost portion of their range.

There were four haplotypes that were shared by both species. The first shared haplotype (AlpMin1) was the second most frequent haplotype present in *T. alpinus* (29% of all individuals) where it was confined to individuals from the Yosemite area (T.alp-N) and two from the northern sampling area of *T. minimus* (T. min-N; Figure 2). The next shared haplotype (AlpMin2) was the most frequent haplotype found in *T. minimus* (24%) and detected in only one *T. alpinus* individual from Bullfrog Lake (T. alp-S). All *T. minimus* individuals with the AlpMin2 haplotype were from the White Mountains, the Inyo Mountains or the central part of our sampling area (Figure 2; T. min-Wht/Iny, T.min-C). Interestingly, this was the only haplotype that was shared between southern *T. alpinus* individuals and any of the *T. minimus* groups. The third shared haplotype (AlpMin3) was in low frequency (6%) and only found at one site in Yosemite National Park in *T. alpinus* (Vogelsang Lake, T. alp–N) and in one *T. minimus* sampling locality, Bohler Creek (T. min–N). The fourth and last shared haplotype (AlpMin4) was also in low frequency in both species (*T. alpinus*: 1.4%; *T. minimus*: 2.8%) from the northern part of our sampling area.

The statistical parsimony haplotype network for *T. alpinus* and *T. minimus* had a 95% parsimony limit of 12 steps (Figure 4). There are four groups of haplotypes that are separated by at least 5 base pair changes and show some geographic structure (A-D, Figure 4) and three out of four of these contain individuals of both species (Group A, C, & D). The fourth group is made up of *T. alpinus* haplotypes from the southern portion of their range. This group corresponds to the

ALP-S labeled tips on the phylogenetic tree in Figure 3. There are two differentiated northern groups, one of which is also supported by the Bayesian phylogeny. Group D in the statistical parsimony network is represented in the Blue clade in the tree. The southernmost *T. minimus* samples are represented in Group C of the network are 10 mutational steps from shared haplotype AlpMin2 found in the northern part of our sampling area. Two *T. alpinus* haplotypes were more than 12 steps away from the others in the network and each other (two divergent haplotypes shown in Figure 3 – green and black branches) so are not shown in Figure 4.

Diversity measures for mtDNA by species and geographic location are summarized in Table 1. There were no significant signals of population expansion or decline (or deviations from neutrality) in either species or geographic populations of species according to Tajima's D or Fu's Fs statistics in any of the groups tested (Table 1). There is some suggestion of geographic differences in historical demography in that northern populations of both species have positive values for Tajima's D and Fs, whereas southern populations of each have negative values. However, none of these are significantly different from zero.

The AMOVA attributed 28.63% ($\phi_{CT} = 0.28$, $p = 0.34$) of the genetic variation across haplotypes to be between species, 37.6% ($\phi_{SC}=0.52$, $p<0.001$) to be among populations within species and 33.8% ($\phi_{ST}=0.66$, $p<0.0001$) of the variation to differences within species. The AMOVA run on the 6 genetic clusters resulting from the STRUCTURE analysis of nuclear loci (see next section) attributed 62% ($\phi_{CT} = 0.62$, $p = 0.14$) to variation among groups, 5.1% ($\phi_{SC}=0.13$, $p<0.001$) among populations within groups and 32.9% ($\phi_{ST}=0.67$, $p<0.001$) to variation within populations. These AMOVA analysis reveals that the mtDNA variation is not best explained by differences among species or geographic groups, but rather differences within species and populations.

The average and the net number of nucleotide substitutions per site were lower between species (Dxy = 0.018, Da=0.003) than between the northern and southern *T. alpinus* populations (Dxy = 0.021, Da = 0.005). The northern *T. alpinus* population was most similar to the northern *T. minimus* population (Dxy = 0.015, Da = 0.003, Table 2). The southern *T. minimus* samples are the most genetically distinct group sampled, being most different from the northern *T. minimus* population (Dxy = 0.024, Da = 0.017). The neighbor-joining tree shows that genetic similarity across populations is better explained by geography than by species boundaries (Figure 5).

Microsatellite Data

Deviations from H-W equilibrium were observed across all loci within species however, this is not unexpected given known genetic substructure within species. Within geographic groups, there were deviations from HWE in the *T. alpinus* N group but again, there is known substructure in this group (Chapter 4) so deviations from HWE are expected. A high frequency null allele was detected at the D107 locus in the *T. alpinus* (Chapter 4). I ran the STRUCTURE analysis with and without this locus and the results did not change, therefore, I chose to run the analysis with all 14 loci. No significant linkage disequilibrium was detected after Bonferonni correction. Genetic diversity was highest in the T. min-N group (A=7.6; H_e=0.84), followed by T. min –Wht/Iny (A=6.8; $H_e = 0.84$; Table 3). The northern *T. alpinus* samples had the lowest genetic diversity of all sampled groups (A=4.7, He = 0.63).

Population structure

The estimated number of parental populations for the dataset using the Evanno method was K=2, however, K=6 had a higher mean likelihood value (Figure 6a & b) and reflected geographic groups of each species (Figure 7b). Higher K values produced clusters within the T. alp-N group (results not shown) but did not greatly increase the likelihood scores (Figure 6a). The results of the cluster analyses at K=2 separated individuals into two groups by species, with admixture in the T. alp-S sample (Figure 7a). The individual membership bar graph for K=6 (Figure 7b) shows 6 clusters that correspond clearly with geographic groups. Based on cluster membership percentages, there appears to be some gene flow between T. min-N, T.min-C and T.min-Wht/Iny, while T.min-S is well differentiated. The T.alp-S sample now appears as a distinct cluster, with one exception. There is one *T. alpinus* individual from Bullfrog Lake (MVZ224480) in the T.alp-S geographic group that was assigned to T.Min-N based on its genotype at 14 loci.This individual is one of the four *T. alpinus* individuals which had the divergent haplotype shown in green on the tree (location shown with green triangle on the map of Figure 3).

The pairwise F_{ST} values between clusters showed significant differentiation across all clusters and ranged from 0.018 between T.min-N and T.min-C to 0.227 between T.alp-N and T.min-S (Table 2). In contrast to the results for mtDNA, the neighbor-joining tree based on F_{ST} shows that groups of the same species are genetically more similar to each other than to the other species (Figure 9) although the southern populations of both species (T. alp-S and T.min-S) are both differentiated from their more northern conspecifics.

Coalescent analysis of divergence history

Using IMa2, I estimated the following parameters: effective population size of *T. alpinus* (Ne_{ALP}) , *T. minimus* (Ne_{MIN}) and the common ancestor (Ne_A) the migration rate from *T. alpinus* into *T.minimus* ($m_{ALP->MIN}$) and from *T. minimus* to *T. alpinus* ($m_{MIN->ALP}$) and time since divergence (t) (Table 4). The split between *T. alpinus* and *T. minimus* lineages was estimated by IMa2 to have occurred in the mid-Pleistocene, at approximately 450 ka. There is a sharp peak in the posterior density plot at this value however, the plot plateaus at a low, but non- zero value for higher values of t, including when a higher upper bound on the divergence time prior is used (results not shown). The mean effective population size (N_e) of *T. alpinus* was estimated to be much smaller than *T. minimus* with non-overlapping confidence intervals (T. alp mean N_e = 430,625. 95% HPD 230,019- 648,519; T. min mean N_e = 1,448,317, 95% HPD 833,365-2,095,096). The size of the daughter populations is small compared to the ancestral population (N_{eA}= 6,680,761). Migration estimates between the two species showed that migration into *T. minimus* from *T. alpinus* was higher ($2N_m$=0.5441) than from *T.minimus* into *T. alpinus* ($2N$m=0.002) however the confidence intervals for these parameters broadly overlap (Table 2; Figure 8).

Discussion

I examined the evolutionary relationship of *T. alpinus* and *T. minimus* using cytochrome b and microsatellites to help elucidate the divergence history of *T. alpinus* in Sierra Nevada. Microsatellite analysis was used to get a more contemporary view of this relationship and to examine the population genetic structure within species. I found that *T. alpinus* and *T. minimus* populations share mitochondrial haplotypes with no overall geneaological separation, and that diversity at this locus is better explained by geography than it is by species' boundaries. This

result indicates either recent speciation of *T. alpinus* from *T. minimus* with retention of ancestral polymorphism, or historical or contemporary hybridization. In contrast to the sequence data, the nuclear analysis revealed that the two species are genetically distinct. Although there are highly differentiated populations within species, populations of the same species are more similar to each other than they are to members of the other species. This result indicates that hybridization is not currently widespread along the parapatric boundary between *T. minimus* and *T. alpinus.* Coalescent analysis of divergence history indicated late Pleistocene divergence and subsequent, albeit limited, gene flow between the two lineages. Overall, the analyses suggest divergence and historical, but not contemporary, introgression during the climatic cycles of the late Pleistocene.

Differentiating the genetic signature of incomplete lineage sorting and historical hybridization is difficult however, distinguishing between the two is important in addressing non-concordance among characters in closely related species (Avise & Ball 1990). The spatial pattern of genetic variation across species can help to provide an objective assessment of which process is more likely to have occurred because each should produce a specific spatial pattern (Goodman *et al*. 1999, Good *et al.* 2003). The results of our mitochondrial sequences show no strong spatial structure within species, as both the Bayesian tree and haplotype network show, the two species are completely intermingled across the landscape. Recent hybridization should show a clustered pattern, where introgressed alleles are more common at or near the contact zone of the two species; in contrast, ancestral polymorphism should be diffuse and uniform across space (Good *et al*. 2003). However, the spatial patterns described above assume a contact zone between species, and at present, I was unable to obtain samples of both species from the same location (even in areas in which they were known to occur together in the past). The one site where they were collected together in 1911 (Olancha Peak) showed distinct and divergent haplotypes between species. The *T. minimus* individual's haplotype was a unique haplotype and is clustered with the southern *minimus* group in the haplotype network (Group C, Figure 4) whereas the *T. alpinus* from that location and 3 other *T. alpinus* from the area collected at the same time, were clustered in the southern *T. alpinus* group in the network.

There were several potential problems with the parameter estimates using IMa2, in particular, the divergence time estimate. The first is that our microsatellite data exhibit genetic structure within lineages (recall that in this analysis, each species was considered a "population"), which may lead to an overestimation of divergence time (Wakeley 2000). Second, the right tail of the posterior density plot reached low but non-zero values potentially making the 95% HPD high and low estimates unreliable. Furthermore, the mutation rate I used for cytochrome b in this analysis was taken from the literature from data on deer mice (*Peromyscus sp*., Zheng *et al.* 2003) as there is no estimate available for *Tamias*. Other *Tamias* studies have used a general mutation rate estimated for all mammals (Good *et al.* 2008) that is two orders of magnitude faster than the mutation rate I used, and another source suggests a mutation rate of three orders of magnitude faster for mammals (Ho *et al.* 2005). The divergence time estimate is calculated using the mutation rate, and an underestimation of the mutation rate will lead to overestimation of divergence time. Given these caveats, the estimate of divergence time between *T. alpinus* and *T. minimus* from IMa2 is about 450ka with some support of it being as recent as 110ka (the lower 95% HPD of t). If I proceed assuming that this is somewhat accurate, this timeframe occurs in the mid to late Pleistocene, a time of extreme climate fluctuations in the Sierra Nevada including several major glaciations with prolonged glacial rather than interglacial periods (Gillespie & Zehfuss 2004).

The Pleistocene shaped the genetic structure and distributions of many species (reviewed in Hewitt 2004) and its role in the speciation in several North American taxa is clear (e.g., Knowles 2000, Knowles 2001, Johnson & Cicero 2004). It is plausible that a founding population of *T. alpinus* became isolated in a glacial refugium from an adjacent *T. minimus* population. The IMa2 analysis supports this notion with a parameter estimate for the effective population size of *T. alpinus*, although fairly large (N_{eALP} = 430,625), significantly smaller than the estimate of the effective population size of *T. minimus* (N_{eMIN} = 1,448,317). Furthermore, the estimates of migration between species were low (95% HPD high <1 in both directions) suggesting minimal gene flow between species through time. Incomplete lineage sorting is especially probable where effective populations sizes are large relative to the time since divergence (Maddison & Knowles 2006), which is likely for this dataset. The evidence presented here suggests recent speciation of *T. alpinus* and adds to the growing number of other studies that have shown the importance of glacial refugia and recently derived species in the Sierra Nevada, including salamanders (Rovito 2010) and insects (Schoville & Roderick 2009).

Hybridization may play an important role in the evolution of species (Arnold 1992, Seehausen 2004, Riesberg 1997) and although the genetic pattern observed in this study is likely due to recent speciation, the potential for hybridization should not be overlooked. It was previously accepted that the morphological differences in bacular morphology in western chipmunks mechanically prevented hybridization between species and was considered a strong pre-mating barrier to gene flow (Adams & Sutton 1968, Patterson & Thaeler 1982). However, several recent studies have documented both historical and ongoing hybridization in two non-sister species of *Tamias* and suggest that it may be more common in the genus than previously thought (Good et al. 2003, Good et al. 2008, Hird et al. 2009). Our analyses suggest little current introgression across the parapatric boundaries and low post-divergence migration; however, the potential for gene flow between these species where they co-occur in the southern portion of their range does exist. One apparent hybrid individual that was morphologically determined to be a *T. alpinus*, and contained a divergent *T. alpinus* haplotype appeared to be more similar to *T. minimus* than *T. alpinus* based on 14 microsatellite loci (Figure7b). The assignment of this individual to the T.min-N population is puzzling because the two localities are far apart geographically (at least 80km). Due to the lack of confirmed sympatry along the crest of the southern Sierras except at the single, peripheral locality of Olancha Peak, it has been challenging to capture both species at the same locality where a there is direct potential for hybrid matings.

Study limitations and future work

This analysis provides useful insights into the divergence history of a range-restricted alpine endemic species and its sister taxa, however, there were several limitations to this study. As mentioned, population structure violates an assumption of the isolation with migration model. The two *T. alpinus* populations included are so differentiated that at K=2, the STRUCTURE analyses did not place the southern individuals solely in the "*alpinus*" cluster but grouped them as a mixed population of both the *alpinus* and *minimus* clusters (Figure 7). Because of this differentiation within *T. alpinus* as well as evidence of population structure within *T. minimus*, in further analyses, perhaps excluding the southern *T. alpinus T. minimus* individuals in the IM model will generate better estimates and thus more insight into the divergence between these two populations. A final potential problem with IM analyses is that our loci give contrasting genealogical patterns (mitochondrial data is more similar between species than the microsatellite

data) and running these two sets separately may improve convergence and estimates of splitting time. Good *et al.* (2008) report convergence issues while using this model in IMa and suggest contrasting histories across loci as a potential cause.

Another limitation is sampling. There is the large sampling gap of *T. alpinus* between the northern and southern tip of their distribution and our sample size at the northern tip of their range is much greater than in the south (total T.alp-N n=149 vs T. alp-S n=26). This distributional gap results from a lack of sampling effort in the high country habitat of *T. alpinus* where access is by foot in federally designated wilderness. Sampling for this species was targeting in the Yosemite area for other goals of this dissertation, which is why there is a much greater sample size from this region. In the future, a targeted effort to sample *T. alpinus* in the central and southern portion of their range should be undertaken.

A comparison of our results with sequencing nuclear introns and a morphological analysis would greatly enhance our understanding of the relationship between these two species (e.g., Good et al. 2008). Nuclear introns are expected to retain ancestral polymorphisms longer than mtDNA because of a larger effective population size and slower mutation rate. If speciation is recent, and the pattern observed is a retention of ancestral polymorphisms in the mitochondrial DNA, then I would expect to also observe this in nuclear sequence data. However, as observed in another species-pair of chipmunks (Good et al. 2008), if the nuclear genes indicate well-supported divergence between species, then the intermingled pattern observed pattern at cyt b across species is likely due historical hybridization. Lastly, a concurrent analysis of morphological differences between these two populations shows marked differences at several characters and in the future, the genetic analysis presented here will be coupled with the morphological analyses to provide a more complete picture of the evolutionary relationship between these two species.

Conclusions

This study was the first to examine the evolutionary history of the Alpine Chipmunk endemic to the Sierra Nevada. I provided evidence of recent speciation between this species and its closest relative. Our results showed an interesting and complex pattern of shared and intermingled haplotypes across species and highly differentiated populations within species and it has barely scratched the surface to unraveling the processes that may have caused these patterns. Increased sampling and the addition of more genetic data will further improve our understanding of this speciation event. *T. alpinus* appears to be under threat due to recent climate change (Chapter 4) and a clear understanding of this species history, will aid in understanding and predicting its persistence and evolutionary potential to adapt under future environmental change.

Table 1. Sample size, number of haplotypes detected, haplotype diversity (h_d) nucleotide diversity (θ_π, θ_S) and tests of population expansion/contraction (Tajima's D, Fu' Fs statistics) in *T. minimus* and *T. alpinus*, and geographic groups of each species (Figure 2) at the mitochondrial gene, Cyt b.

Species	**N**	**No. of haplotypes**	**h_d**	**θ_π**	**θ_S**	**Tajima's D**	**Fu's Fs**
T. alpinus	139	28	0.797	0.014	0.021	-1.581	0.094
T. minimus	107	23	0.891	0.016	0.012	0.932	1.624
Geographic groups							
T. alp-N	113	12	0.695	0.012	0.008	0.9191	7.317
T. alp-S	26	17	0.951	0.026	0.038	-1.492	-0.489
T. min-N	40	11	0.863	0.012	0.010	1.015	3.186
T. min-C	11	4	0.764	0.002	0.002	0.433	0.164
T. min- Wht/Iny	26	6	0.517	0.008	0.008	0.0976	4.225
T. min-S	30	6	0.655	0.001	0.002	-1.309	-1.697

Table 2. Pairwise comparisons of *Tamias* populations. Pairwise Dxy values from the mtDNA data are given above the diagonal and pairwise Fst-values from the microsatellite data below the diagonal. Statistically significant Fst values shown in bold font (P<0.005, 10100 permutations)

	T. alp-N	T. alp-S	T. min-N	T. min-C	T. min-Wht/Iny	T. min-S
T. alp-N	-	0.020	0.014	0.017	0.016	0.023
T. alp-S	**0.116**	-	0.022	0.020	0.021	0.024
T. min-N	**0.167**	**0.092**	-	0.017	0.015	0.024
T. min-C	**0.166**	**0.103**	**0.018**	-	0.005	0.019
T. min-Wht/Iny	**0.167**	**0.114**	**0.028**	**0.042**	-	0.020
T. min-S	**0.227**	**0.168**	**0.118**	**0.124**	**0.147**	-

Table 3. Sample size (N), average allelic richness (*A*; corrected for differences in sample size), observed and expected heterozygosity (H_o, H_e & standard deviation (sd)) in *T. alpinus* and *T. minimus* at 14 microsatellite loci.

Species	Locality	N	*A*	H_o (sd)	H_e (sd)
T. alpinus	North (YNP)	149	4.7	0.57 (0.19)	0.63 (0.21)
	South	17	6.0	0.71 (0.19)	0.75 (0.17)
T. minimus	North	57	7.6	0.73 (0.14)	0.84 (0.07)
	White/Inyo Mtns	33	6.8	0.71 (0.18)	0.82 (0.09
	Central	42	6.4	0.62 (0.12)	0.78 (0.10)
	South	29	5.1	0.58 (0.23)	0.68 (I0.18)

Table 4. Joint posterior probability parameter estimates from IMa2 runs for *T. alpinus* and *T. minimus*.

	N_e	N_{eA}	t	$2N_{mALP->MIN}$	$2N_{mMIN->ALP}$
T. alpinus	430625	6680761	446538	0.5441	0.002
HPD95 low-high	230019-648513	778846-18058846*	115 -*	0.1986-0.9981*	0-0.9463*
T. minimus	1448317				
HPD95 low-high	833365-2095096				

*HPD may not be useful because posterior density does not reach low levels near upper (N_{eA}, t) or lower (2*N*m) limit of prior. In the case of N_{eA} & t, even with higher values set for the prior, the posterior density plot plateaus at low but nonzero values (see Figure 8).

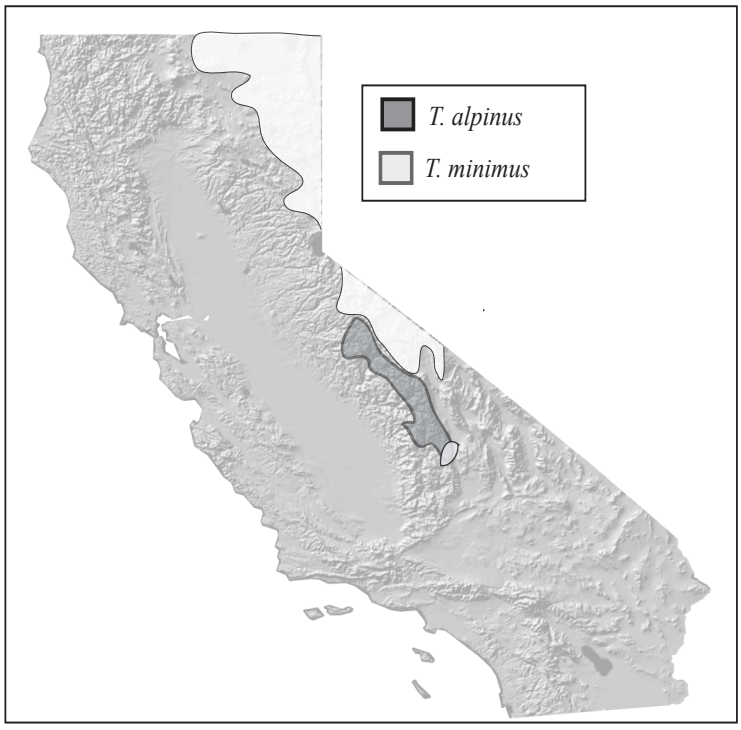


Figure 1. Distribution of *T. alpinus* (dark gray) and *T. minimus* (light gray) in California

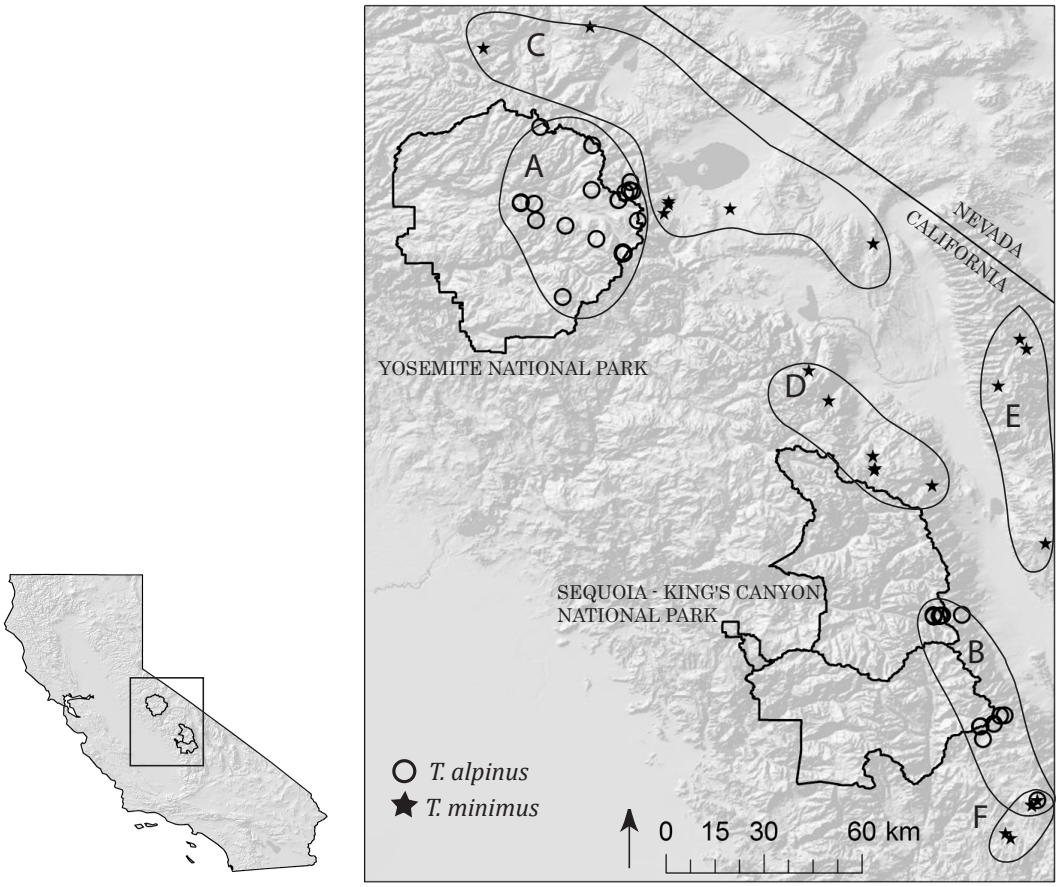


Figure 2. Map of sampling localities in the Sierra Nevada, California. Open circles indicate sampling sites of *T. alpinus*, black stars show *T. minimus* sampling sites. Polygons labeled with letters show geographic groupings used in population structure analyses: A)T.alp-N; B)T.alp-S; C)T.min-N; D)T.min-C; E)T.min-Wht/Iny; F)T.min-S.

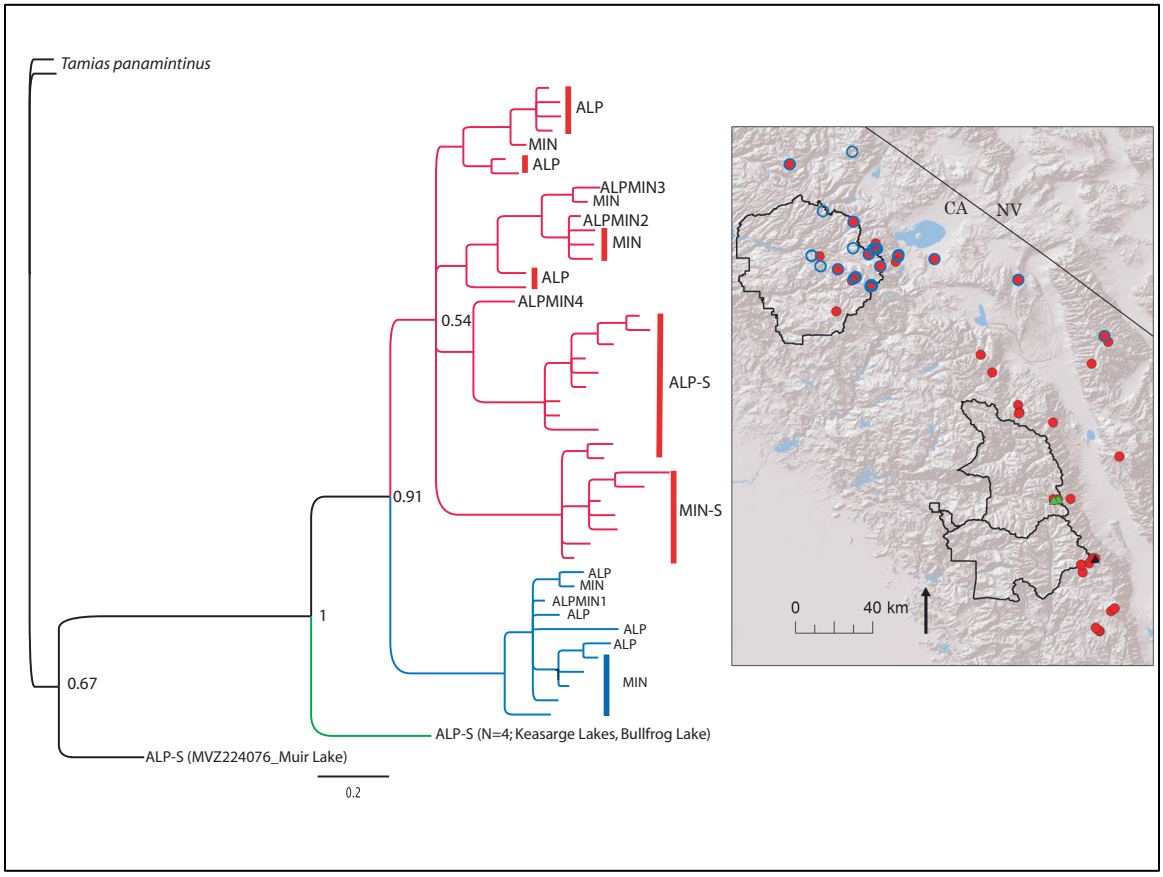


Figure 3. Bayesian estimate (GTR + I + Γ) of phylogeny of cyt b for 47 haplotypes of *T. alpinus*, *T. minimus* and *T. panamintinus* (outgroup). Posterior probabilities of major mtDNA clades are shown. The species that haplotypes belong, including shared haplotypes (AlpMin1, 2, 3, & 4), are labeled at tips. Map shows geographic location of each clade. The red solid circles represent individuals with haplotypes within the "red" clade. Blue open circles represent individuals with haplotypes within the "blue" clade and green triangles show the individuals with the divergent Alp-S haplotype (n=4) and the black triangle show the unique haplotype that is divergent from the other haplotypes. Scale represents expected changes per site.

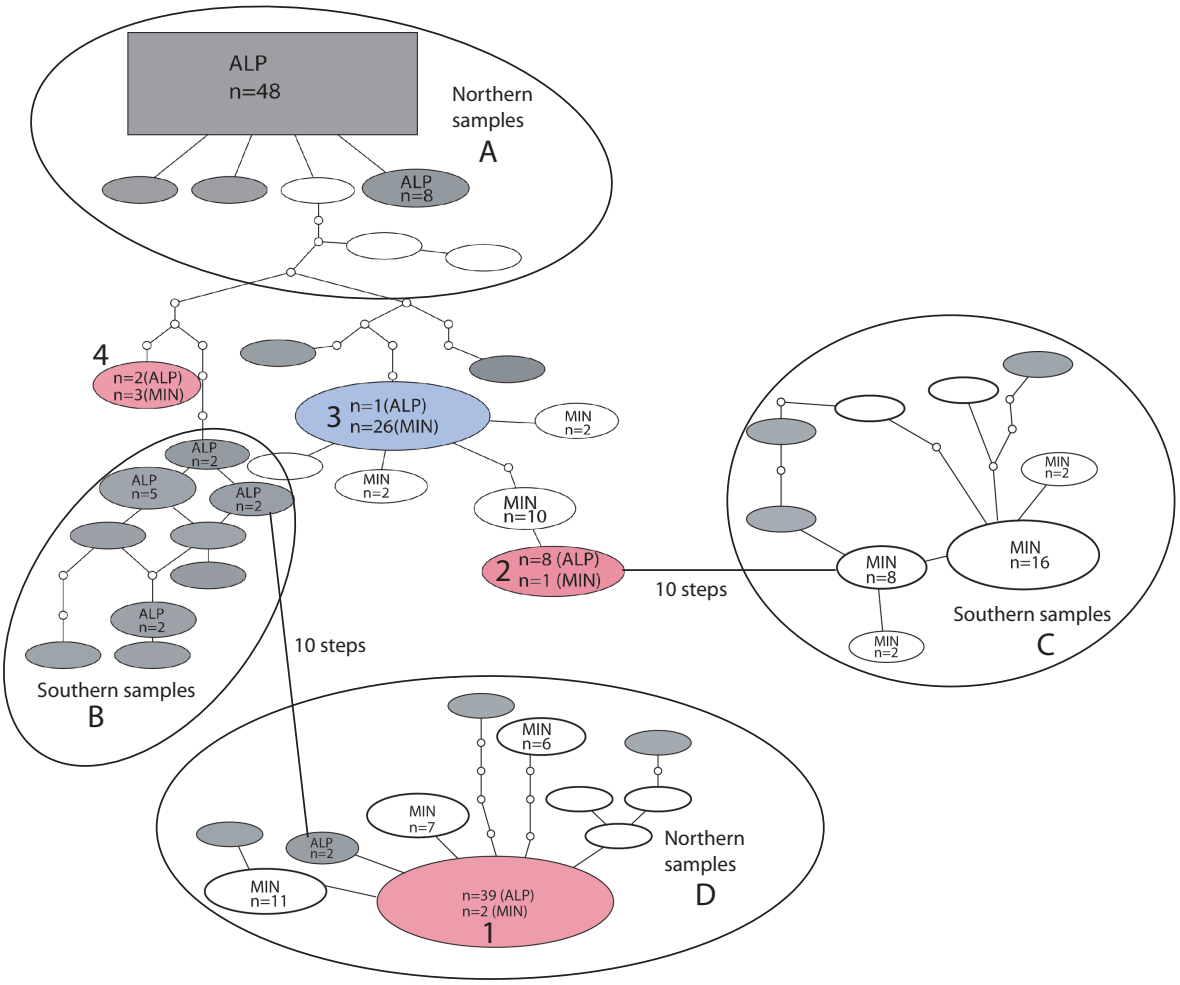


Figure 4. 95% Statistical parsimony haplotype network for cyt b. Haplotypes are indicated as ovals and scaled by frequency (also noted within oval unless haplotype is unique). Gray represents *T. alpinus* haplotypes, white represents *T. minimus* haplotypes and colored shapes represent shared haplotypes 1) AlpMin1, 2) AlpMin2, 3) AlpMin3, and 4) AlpMin4. Rectangle indicates ancestral haplotypes estimated by the program TCS 2.1 based on haplotype frequency. Large ovals show groups of haplotypes that are present in individuals from the same geographic region (Labeled A through D). If haplotypes are not encompassed within an oval, then no geographic pattern exists (i.e, found throughout sampling area).

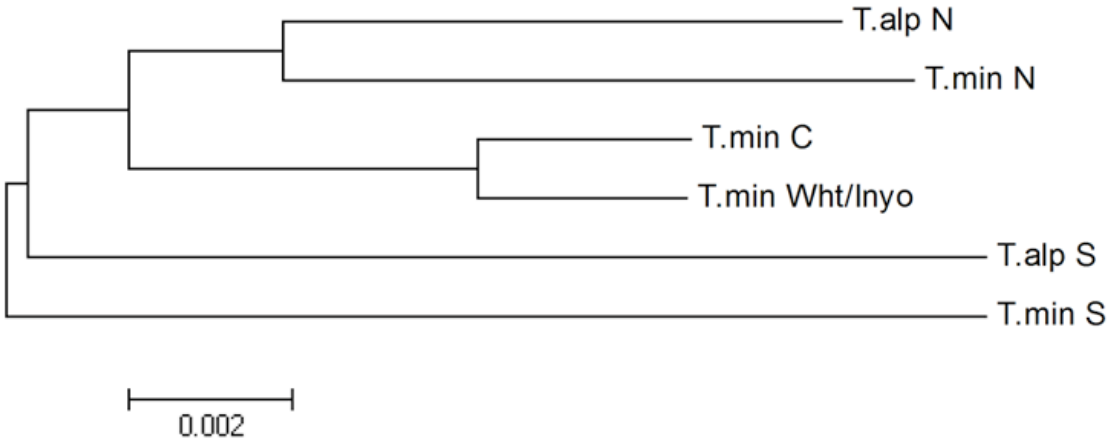
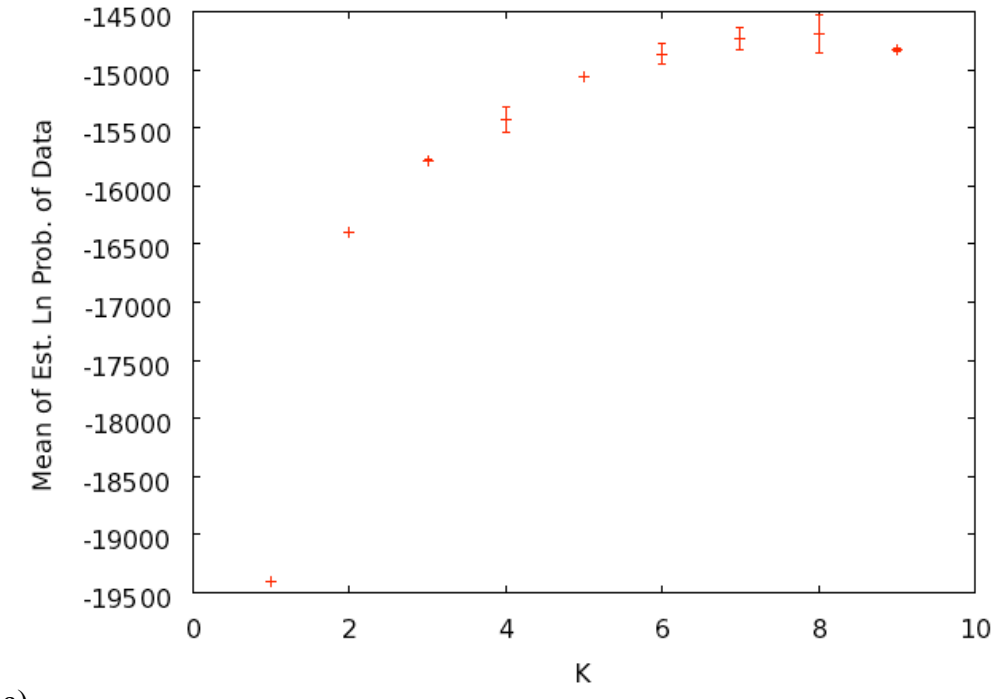
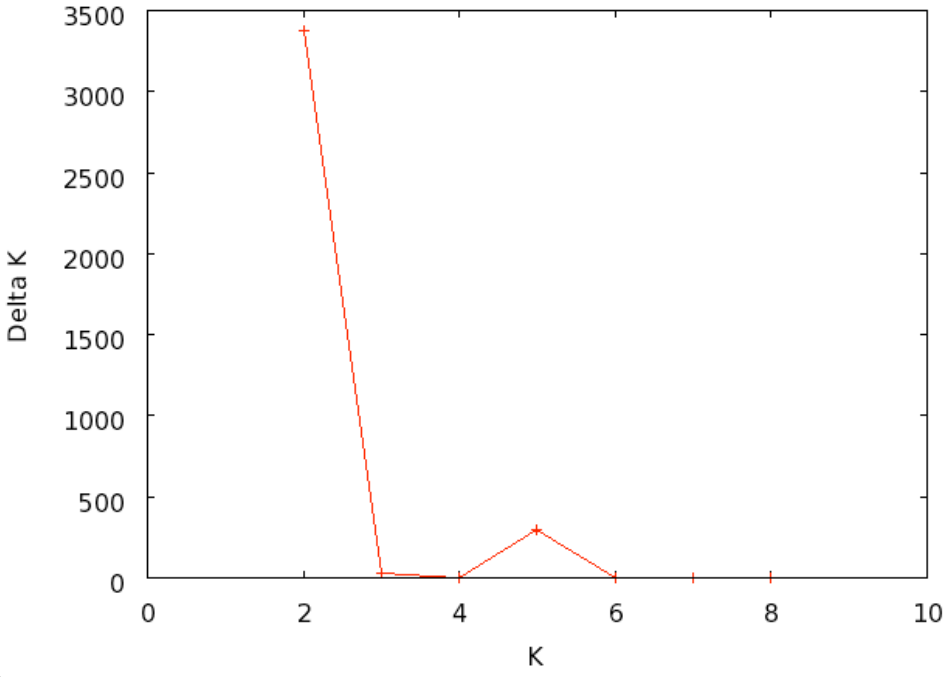


Figure 5. NJ tree of the relationships among geographic groups of *T. alpinus* and *T. minimus* based on the average number of nucleotide substitutions per site (Dxy, Nei 1987) at cyt b.



a)



b)

Figure 6. Estimation of the true number of clusters using ΔK (Evanno et al 2005); a)Mean likelihood values (±SD) over 5 runs for each K, asymptotes at K=6; b) ΔK values, where uppermost level is the true number of clusters, which in this case is K=2.

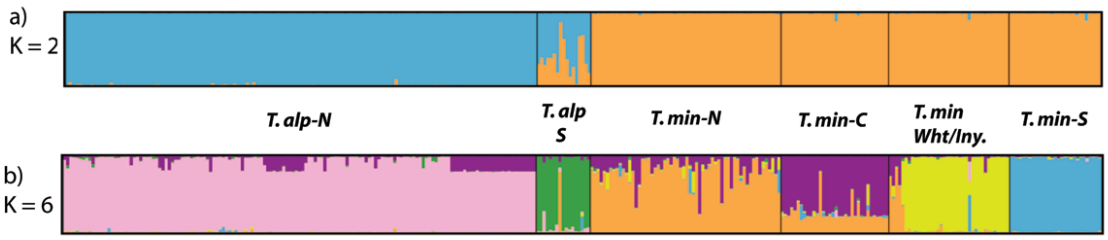


Figure 7. Bayesian analysis of nuclear genetic structure of *Tamias* populations based on 14 microsatellite loci. Each individual is represented by a vertical line, which is partitioned into colored segments that indicate individual's membership in (a) 2 or (b) 6 parental populations.

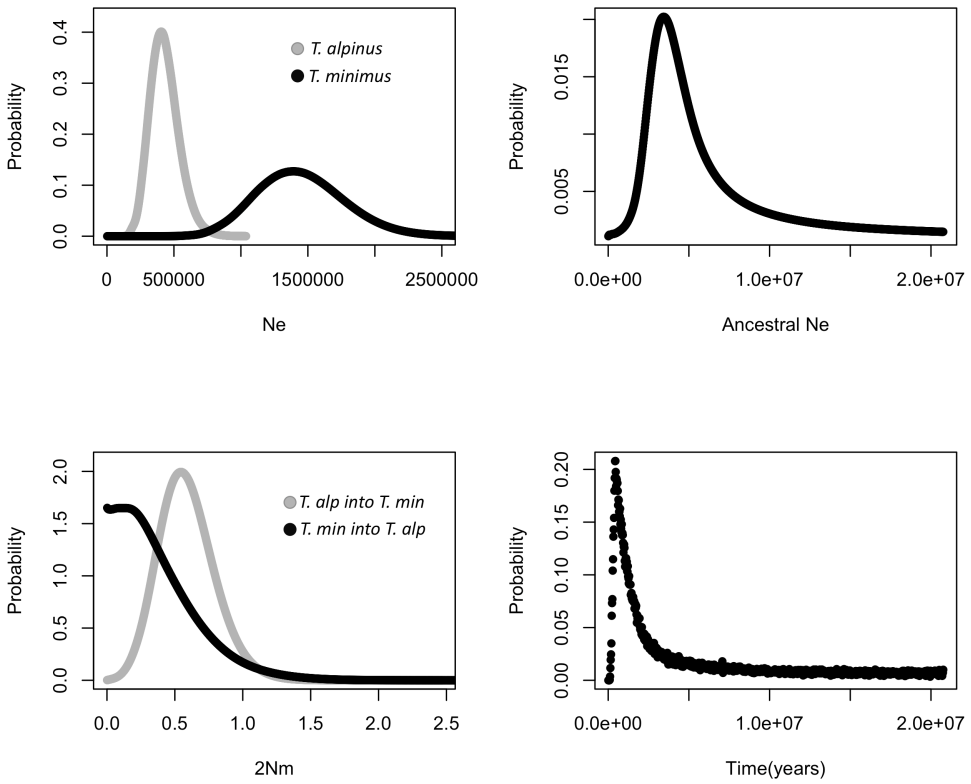


Figure 8. Posterior density plots of parameters from Isolation with Migration (IMa2) analysis. Top left: effective population size of *T. alpinus* and *T. minimus*; top right: ancestral effective population size; bottom left: effective number of migration events per generation between *T. alpinus* and *T. minimus*; bottom right: divergence time between *T. alpinus* and *T. minimus*.

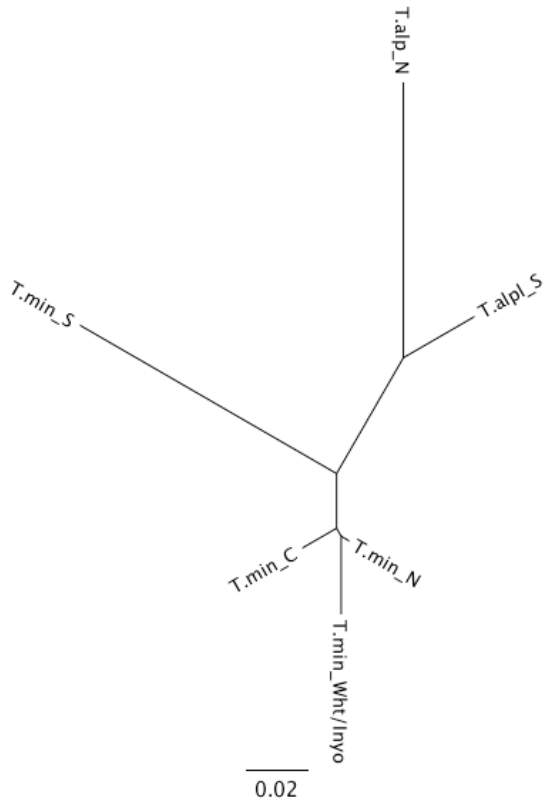


Figure 9. Unrooted NJ tree of the relationship among populations using F_{ST} (Weir and Cockerham 1984) based on 14 microsatellite loci.

CHAPTER 3

The role of climate, habitat, and species co-occurrence as drivers of change in small mammal distributions over the past century.

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Introduction

A pressing challenge for biodiversity conservation in the 21st century lies in forecasting species' responses to the direct and indirect effects of climate change (Barnard & Thuiller, 2008). The complexity of these effects and the evidence for the idiosyncratic nature of species' responses to past climate change makes this arguably the most difficult problem confronting biologists today (Brown *et al.,* 1997; Jackson & Overpeck, 2000; Walther *et al.,* 2002). Novel climates are anticipated in the future (Williams & Jackson, 2007), which further exacerbate our ability to accurately predict how species will respond. Forecasting in the face of this uncertainty requires that we develop a deeper understanding of the ecological and environmental factors that drive changes in distribution at multiple spatiotemporal scales.

Correlative models are widely used to predict the effects of climate change on species' distributions (Thomas *et al.,* 2004; Thuiller *et al.,* 2005; Lawler *et al.,* 2006). These models are based on the observed relationship between a species and its environment (Guisan & Zimmermann, 2000) and when mapped using geographic information systems (GIS) are referred to as predictive distribution maps (Guisan & Thuiller, 2005). A strong criticism of this approach in predicting species responses to climate change is that they are difficult to validate across time (Araujo *et al.,* 2005a; Hijmans & Graham, 2006) so their usefulness as predictive tools remains relatively untested (but see Araujo *et al.*, 2005b; Kharouba *et al*., 2009). Another criticism of correlative models is that they tend to rely solely on abiotic variables while excluding biotic factors such as species interactions, vegetation and dispersal (Davis *et al.,* 1998; Pearson & Dawson, 2003; Hampe, 2004; Barnard & Thuiller, 2008; but see Preston *et al.,* 2008). In this study, we are in the unique position to address some of these criticisms. Using historical surveys and contemporary resurveys of chipmunks in Yosemite National Park, California, we examine the environmental drivers of changes in distribution over the past century.

The three study species examined in the study, *Tamias alpinus*, *T. senex*, and *T. speciosus* occupy different elevational zones in Yosemite. Recently, Moritz *et al.* (2008) showed by comparing early 20th century surveys with modern resurveys that these chipmunk species have

responded differently to environmental change over the past century. The Alpine Chipmunk (*T. alpinus*), which occupies the highest elevational zone, retracted its lower elevational range upwards overtime. Meanwhile, the Lodgepole Chipmunk (*T. speciosus*), which occupies the mid-elevational zone, did not significantly change its distribution. Finally, the Shadow Chipmunk (*T. senex*), which occupies the low to mid-elevational zone, experienced massive range collapse and is now extremely rare in the study area. Moritz *et al.* (2008) suggest warmer temperatures as the main driver of the observed shifts for these species and the broader community of small mammals, but did not explicitly test alternative hypotheses or whether climate was acting indirectly on distributions through changes in vegetation or species interactions.

Interspecific competition is likely to be a factor where chipmunk species co-occur (Heller, 1971; Chappell, 1978). However, the study species do differ in their microhabitat preferences (Chappell, 1978; Waters & Zabel, 1998; Waters *et al.,* 2001). Laboratory physiological studies of these species suggest they have comparable climatic tolerances and have similar thermoneutral zones (Heller & Gates, 1971; Heller & Poulson, 1972), whereas field based physiological studies suggest that higher altitude chipmunks are slower to recover from heat stress (Chappell *et al*., 1978). Given the previous physiological and behavioral work on *Tamias* species (Heller, 1971; Heller & Gates, 1971; Heller & Poulson, 1972; Chappell, 1978; Chappell, *et al.* 1978), we hypothesize that climate, vegetation and species co-occurrence all should be important predictors of the changes in chipmunk distributions observed in Yosemite National Park. However, because of conflicting reports and the dynamic nature of species' geographic boundaries, the relative importance of each of these variables is not easily deciphered.

We use historical (1900-1940) and modern (1980-2007) climate, vegetation, and species presence-absence locality data to 'forecast' changes in chipmunk distributions. Specifically, the objective of this study is to identify drivers of observed distributional changes of three species of chipmunks in Yosemite National Park. Our approach is to include environmental variables both separately (i.e., climate-only and vegetation-only) and together to better understand their relative importance. We examine the roles of climate and vegetation in both single-species models (without congener co-occurrence) and multi-species models (with congener co-occurrence) to determine if accounting for the distribution of potential competitors improves model performance (Fig. 1).

Data and Methods

Study area and species

This study took place in and around Yosemite National Park, California (Fig. 2) and relied on data collected as part of the "Grinnell Resurvey Project through the Museum of Vertebrate Zoology (MVZ) at the University of California, Berkeley (http://mvz.berkeley.edu/Grinnell/index.html). Our study used data from the resurvey of the "Yosemite transect" published by Moritz *et al.* (2008). Detailed descriptions of the original Grinnell mammal surveys (hereafter historical surveys) from 1914-1915 and the modern resurvey of the small mammals in the Yosemite Transect from 2003-2006 are given in Moritz *et al.* (Supplementary Material, 2008). For the modern dataset, we expand on the Moritz *et al.* (2008) data with results from targeted trapping of chipmunks between May and August 2007.

Due to the limited availability of comparable vegetation data in both eras, we restricted our study to 39 sites in the historical dataset and 109 sites in the modern dataset. There are more modern than historical sites because the modern surveys were greater in their sampling extent at each general site (i.e., more traplines in the general vicinity of the historical locality) and included new survey sites (i.e., sites not sampled in the past; Fig. 2). The three focal species have overlapping but distinct distributions in California and share several life history traits (Table 1).

Species Data

Original Grinnell survey results were georeferenced from detailed field notes and maps (http://bscit.berkeley.edu/mvz/volumes.html?). Traplines within 2 km and 100 m in elevation were aggregated within each era to minimize spatial autocorrelation and account for the uncertainty in the location of historical traplines (hereafter "aggregated traplines" are referred to as "sites"). Using detailed field notes on trap captures in the historical and modern surveys, we were able to calculate the probability of detection of each species at each site following the methods of Mackenzie *et al.* (2002; Mackenzie, 2006). We did the calculations including the 2007 data following Moritz *et al.* (Supplementary Material, 2008) with one exception, instead of estimating the probability of false absence (PFA) across elevational bands, we calculated PFA for each specific site. We considered a site at which a particular species was not detected to represent a "true absence" for that species if the PFA was less than 10%. We used the presence and "true absence" data when validating the models within and between eras.

Climate Data

We used a climate dataset generated with the Anusplin interpolation algorithm on weather station data at 1 km^2 spatial resolution (Parra & Monahan, 2008). A comparison of these interpolated layers of historical and present climate indicated that our study area has become drier and on average, the minimum monthly temperature has increased by about 1°C. However, warming is not consistent across sites; low elevation sites have warmed from 0.8-2.9°C and certain high elevation sites have remained stable or become slightly cooler. This variation is not unexpected given the topological complexity of the area. The Anusplin interpolation is consistent with the available climate station data from each time period (National Climate Data Center, 2003; see Appendix 2 for more details). Nineteen bioclimatic variables were derived for each time period. We removed variables that were highly correlated (cut-off Pearson's $r < 0.85$, Elith *et al*., 2006) and selected a final set of variables that were biologically relevant to the study species. From previous research, we know that winter temperatures and timing of spring snowmelt are important factors for the survival and reproduction of alpine plants (e.g., Dunne *et al.,* 2003), non-hibernating boreal mammals (e.g., pika: Smith & Ivins, 1983, Morrison & Hik, 2007; snowshoe hares: Odonoghue & Krebs, 1992) and hibernating sciurid mammals (marmots: Inouye *et al*., 2000, red squirrels: Réale *et al.,* 2003). Therefore, we selected climatic variables based on the life history of the study species and the resources upon which they depend at emergence. Four biologically relevant variables were considered in our models: TS – temperature seasonality (standard deviation of mean monthly temperature); ATR - annual temperature range (maximum temperature of warmest month minus minimum temperature of coldest month); PWet - precipitation of wettest month; MinT - minimum temperature of coldest month.

Vegetation Data

The vegetation dataset used for this study was derived from two vegetation maps of the area representing both eras (historical: Wieslander, 1935 and modern: NatureServe, 2003). The Wieslander Vegetation Type Map (VTM) collection consists of plot data, plot maps and vegetation maps which show hand drawn polygons of forest type and their associated species across California. The VTM collection has been digitized and is available online (Kelly *et al*., 2005) and a recent analysis of spatial uncertainties in this dataset suggest that the use of these data in environmental niche modeling or multivariate analyses, such as this study, alleviate spatial error concerns (Kelly *et al.,* 2008).

Cameron *et al.* (unpub. data) reclassified both the historical and modern vegetation maps into a matching classification scheme (i.e., developed a vegetation "crosswalk") using the California Wildlife Habitat Relationship database (CWHR, California Interagency Task Force 2008). Twelve vegetation categories were recorded in the Yosemite area in both time periods. From these twelve, we chose four to six vegetation types for each species using the habitat associations recorded in the CWHR database and representing habitats that each species is known from field observation to inhabit. Multi-species vegetation models included four vegetation types that are overlapping between at least two of the three species (Fig. 1).

Model Development and Evaluation

We developed correlative distribution models using both single- and multi-species multivariate adaptive regression splines (MARS, Friedman, 1991). We constructed single- and multi-species models with different combinations of predictor variables according to the following framework: climate-only, vegetation-only and climate+vegetation. All models were run in the statistical package R 2.9.0 (R Core Development Team 2009) using the *mda* library and custom code written by Elith and Leathwick (2007). We developed 18 historical models and 12 modern models. We have a reduced set of modern models because the prevalence of *T. senex* dropped from 0.18 in the historical survey to < 0.01 in the modern resurvey with comparable detectability (Moritz *et al.,* 2008, this study). Hence, modern models were run for only *T. alpinus* and *T. speciosus*.

We evaluated the accuracy of the models both within- and between-eras. For the within-era scenario we projected the model onto the environmental landscape from the era in which it was built. In the between-era evaluation we projected models built in one era onto the environmental landscape of the modern or past era and then used the species data from the era into which it was projected to evaluate the accuracy of the model. Sites where focal species exhibited low detectability or a PFA > 0.1 were removed from the historical and modern test data sets.

The "best" models were defined using two approaches. First, we calculated Akaike Information Criterion (AIC, Burnham & Anderson, 2002) to select the top model from three candidate models (i.e., climate-only, vegetation-only, and climate+vegetation). Models were ranked based on the lowest AIC score for a given species and modeling technique (single- *vs* multi-species). Second, we assessed model prediction accuracy by examining the area under the receiver-operating characteristic curve (AUC), and the true skill statistic (TSS). AUC varies from 0-1: a score of 1 is perfect discrimination and a score of 0.5 is no different from random. TSS is defined as sensitivity (correctly classified presences) + specificity (correctly classified absences) – 1 (Allouche *et al*., 2006). We set the threshold for calculating TSS to the prevalence (# of

presences/# of sites) in the training dataset (Liu *et al.*, 2005). The values of the thresholds to calculate TSS for each species are shown in Table 1.

We determined the top performing model by examining its predictive power as measured by AUC and TSS. An AUC > 0.8 and TSS > 0.50 suggest strong predictive power (Swets, 1988, Allouche *et al*., 2006). The model for each species with the highest AUC and the highest TSS (i.e., highest cumulative accuracy score) was considered the most accurate at predicting the species' distribution either within or between eras. AUC or TSS values that differed by 0.05 or less between competing models were considered to have similar predictive performance and in these cases the model with the lowest AIC score was used to determine the top model. All models with an AUC < 0.70 and TSS < 0.40 were considered poor. For specific details of the model settings, please refer to the Appendices (Appendix 3 & 4).

Results

Based on AIC scores alone, the historical single- and multi-species climate-only models were ranked as the best models for all three species (Table 2a & b). The same was true for the modern models with the exception of *T. speciosus* for which the climate+vegetation model was ranked highest based on AIC for both single and multi-species models. For all species, across eras and modeling approaches, the vegetation-only models were ranked lowest by AIC. However, models with high AIC rankings were not always the most accurate as measured by AUC and TSS (Table 2a-d, Fig. 3). The historical multi-species models did not greatly improve the between era accuracy of models, but the inclusion of co-occurrence did improve the ability of modern models to predict distributions in the past.

Within-era Model Accuracy

Results for the single-species models show that historical models with the top AIC score (climate-only models) have high accuracy (Table 2a). The historical climate+vegetation model was ranked second best by AIC for all species and also did a good job of recovering the input data. The lowest AIC-ranked model (vegetation-only) exhibited high accuracy for *T. alpinus* and *T. speciosus*, but not for *T. senex*.

The modern climate-only model for *T. alpinus* had high accuracy (Table 2c) whereas the highest AIC-ranked climate+vegetation modern model for *T. speciosus* had low accuracy (Table 2c). In fact, all three modern within-era models for *T. speciosus* performed poorly indicating a weak model fit between the input data and the predictor variables. The addition of species co-occurrence in the multi-species models did not significantly improve model performance within eras (Table 2b & 2c).

Between-era Model Accuracy

i) Historical to Modern

The historical single-species climate-only model accurately predicted the elevational shift observed in *T. alpinus* (Figure 3b). All three historical *T. alpinus* models had high discriminatory power when predicting this species' distribution to the present, but only the climate-only model had high accuracy with both AUC and TSS. Predictive performance was not improved by adding co-occurrence into the historical *T. alpinus* models (Table 2b), but overall, the multi-species

climate-only model had higher accuracy than either of the other two single-species models (climate+vegetation and vegetation-only).

In contrast to the high accuracy of the *T. alpinus* models, all three historical *T. speciosus* models performed poorly in predicting the stability of this species' distribution to the present (Table 2b; Figure 3e-h) and in all cases an upwards shift in distribution was predicted. For *T. speciosus*, the multi-species models had slightly greater discriminatory power (AUC) but the distribution of this species was still grossly under-predicted, as reflected in the low TSS scores. All six historical models for *T. speciosus* predicted a modern shift upwards in elevation that was not empirically observed.

The third AIC-ranked vegetation-only model more accurately predicted the range collapse of *T. senex* than the top AIC-ranked climate-only model (Table 2a, Fig. 3j). In fact, both the single-species and the multi-species vegetation-only models are most accurate at predicting the observed range collapse of *T. senex*. The single-species climate+vegetation and the multi-species climate-only models do have high discriminatory power (AUC=1.0 and 0.89 respectively), but these models do not perform well when examining the threshold-dependent TSS. However, the low TSS score is likely an artifact of the testing data, which includes only one presence point. The incorrect classification of this single point results in a sensitivity of zero. Nonetheless, the predictive maps of these models both indicate a northward contraction of *T. senex*, suggesting that both climate and vegetation are related to the range collapse of this species.

ii) Modern to Historic

The single-species modern climate-only model for *T. alpinus* did not accurately predict the species' historical distribution (Table 2c). Although it had a high discriminatory power (AUC), the model under-predicted the true historical range of *T. alpinus* based on TSS. The single-species model that showed the highest accuracy in predicting the distribution back in time was the vegetation-only model, a model that did poorly at predicting the distribution within era (Table 2c). Overall, the best performing modern *T. alpinus* model at predicting the species' distribution in the past was the multi-species modern climate-only model (Table 2c, Fig. 3c).

The best modern single-species model based on AIC for *T. speciosus* was the climate+vegetation model, but this model showed lower overall accuracy at predicting the historical range of *T. speciosus* in the past than both the vegetation-only and the climate-only models (Table 2c). The most accurate modern model was the multi-species climate-only model. The climate+vegetation multi-species model, which was ranked as best in terms of AIC, did an inadequate job at predicting the historical range. In general, the best modern *T. speciosus* models did a better job at predicting historical distributions than the historical models did at predicting into the present (Table 2c, Fig. 3e-h).

Predictor Variables

The variables included in the best models for predicting species' distributions varied by species, modeling approach (single- *vs* multi-species) and era (Table 2a-d). The most common climate variables selected for the historical single-species models were "minimum temperature of coldest month" (MinT) and "precipitation of wettest month" (PWet). The climate variables

selected for the historical multi-species models were "temperature seasonality" (TS), MinT, and "annual temperature range" (ATR) and this did not change when vegetation was included in the model (Table 2b).

The most common climate variables selected for the modern single-species models were ATR and MinT. The set of predictor variables for each species in the historical era was not the same set of variables that were selected by the models for the modern era. For example, the *T. alpinus* historical single-species climate-only model included TS and MinT but the modern model of the same type included TS, MinT and ATR. Overall, TS appears to be a more important predictor of species' distributions in the present than in the past and MinT maintained its importance as a predictor in both eras.

Model results suggested that a minimum temperature of approximately -10ºC during the coldest month of the year is required for *T. alpinus* to occupy an area (Fig. 4a). Sites that did not exhibit this threshold had low probability of presence. This threshold temperature was present in both the historical and modern model results. The modeled probability of presence for *T. speciosus* shows a unimodal pattern in both eras, suggesting that there are lower and upper thresholds for critical temperatures; however, these limits were not constant over time (Fig. 4b). Currently, *T. speciosus* occupies both warmer and colder environments than it did in the past.

The vegetation variables included in models were different for each species as described in the methods. There were two cases where vegetation-only models outperformed or were comparable to climate models between eras. One was in predicting the range collapse observed in *T. senex*. The historical single and multi-species vegetation-only models for *T. senex* selected red fir (RFR), Juniper (JUN) and Montane Chapparal-Mixed Chaparral (MCP_MHC), and RFR and JUN respectively. The other case is the single-species modern vegetation-only model for *T. alpinus.* When projected back in time, this model performed nearly as well as the multi-species modern climate-only model. The vegetation variables in this model were barren (BAR) and Subalpine Conifer (SCN).

Discussion

This study evaluated the role of climate, habitat and occurrence of congeners in predicting known changes in chipmunk distributions over the past century. Overall, we found that even with the inclusion of biotic predictors, climate alone is the dominant predictor explaining the distribution of the study species within a time period, and this was particularly true for the historical era. However, climate was not consistently an adequate predictor of changes in all three species' distributions across time. The top model accurately predicted the observed elevational shift upslope for *T. alpinus*, but also predicted a similar upslope shift in *T. speciosus* that was not observed. Climate alone did an adequate job of explaining the distribution of *T. senex* in the historical era but it did not predict its collapse as accurately as models including vegetation.

Direct versus indirect effects of climate on chipmunk distributions

Animals and plants that live on mountaintops are thought to be especially vulnerable to climate change for two reasons: they are more extinction prone due to limited dispersal options (McDonald & Brown, 1992), and often have relatively narrow tolerances to temperature (e.g.

pika, McArthur and Wang, 1973). *T. alpinus* is an example of an alpine animal that has retracted its distribution upwards in elevation over the past century. Our results strongly support the hypothesis that a warmer modern climate is the major driver of this elevational shift rather than factors such as vegetation and competition with other chipmunk species. In particular, our results show that a minimum winter temperature, approximately -10º C during the coldest month of the year, is an important limiting factor for *T. alpinus* within the study area. The elevation at which this minimum temperature occurs appears to have moved upslope over the past century and this species has tracked it through time. Recently, as part of the Grinnellian resurvey of Californian birds, Tingley *et al*. (2009) found that several species of birds have also tracked their "climatic niche" over the past century.

It is important to note, however, that although it appears that MinT is a limiting factor in *T. alpinus'* distribution, our approach cannot determine causation. Our climate data estimate air temperatures, which are known to be important cues for hibernating sciurid mammals, particularly for springtime arousal (Inouye *et al.,* 2000), but other biologically important factors related to climate were not directly measured in this study and also vary with elevation, such as snowpack. Snowpack provides a critical insulating layer for small mammals and is an important factor for overwinter survival (Vaughan *et al*., 2000). Our results suggest that *T. alpinus* inhabits some colder areas now than it did in the past (Fig. 4). One explanation of this is that the higher the elevation the lower the MinT, but the temperature inside the hibernacula during the winter is likely warmer at higher elevations with deep snowpack, than lower elevations with less snowpack. This example demonstrates the multidimensional nature of climate-species interactions but also stresses the potential limitations of using interpolated bioclimatic variables as biologically relevant proxies.

Interestingly, the modern multi-species climate-only model is more accurate at predicting the historical distribution of *T. alpinus* than the equivalent single-species model. The multi-species model is based solely on MinT, further supporting evidence that minimum temperature is an important factor delimiting the elevational zonation of these species, and *T. alpinus* in particular. This result also suggests that perhaps simpler models with few biologically relevant predictor variables are more accurate at predicting across time than more complex models (the single-species modern climate-only model selected three: TS, MinT, and PWet). It is also possible that single-species within era models are subject to model-overfitting and therefore suffer reduced performance when projected between eras.

A key weakness of species distribution models is their high prediction error rate when projecting into novel environments and/or non-analog climates (Fitzpatrick & Hargrove, 2009). This occurs because the correlations between the environmental variables and species data in the training model may not exist in those combinations in the new environmental space (Thuiller *et al.,* 2004). An alternative hypothesis for the large prediction error when predicting a distribution across time is that the predictor variables selected by the model in one era are not tracked by the species across time and space (Broennimann *et al*., 2008). In other words, what limits a species distribution in one era may be different than what limits its distribution in another (e.g., Monahan & Hijmans, 2008). This appears to be the case for *T. speciosus*. The single-species historical climate-only model did an adequate job of recovering the species distribution within the historical era, but the climate-only model did not perform well when forecasting the current distribution or simply recovering the modern distribution from the modern input data. However,

both the single and multi-species modern climate-only models accurately predicted the historical distribution of *T. speciosus*. These results suggest that the historical distribution of *T. speciosus* in the study area was delineated by climate, primarily by minimum winter temperature and secondarily by seasonality. However, this correlative relationship between temperature and the species' distributional limit no longer exists. *T. speciosus* appears to now occupy both warmer and colder habitats than it did in the past. Perhaps this suggests that it is no longer limited by temperature; however, a more likely explanation is that it was not and is not in a stable equilibrium state with respect to these environmental variables, a simplifying assumption of most distribution models.

It is possible that the historical distribution of *T. speciosus* was in fact limited indirectly by interspecific competition and not by climate as the models suggest. It meets two congeners at both distributional boundaries: *T. senex* at the lower elevational boundary and *T. alpinus* at the upper boundary. Our results provide strong evidence that *T. alpinus* is limited by climate and moderate evidence that both climate and vegetation have played a role in the range collapse of *T. senex*. With the retraction of both of these species, *T. speciosus* may have been released from competition and responded by filling the space left following the contraction of the other two species, hence moving into both cooler and warmer areas. In addition, there was greater opportunity for interspecific interactions in the past, because the species' distributions had greater geographic overlap.

Using the historical surveys, we calculated that *T. senex* and *T. speciosus* were found together at 27% of sites out of all sites where at least one of the species was found. Currently, both species were only detected at one site (2%), the single site where we detected *T. senex*. There was a similar trend with *T. alpinus*. In the past, *T. alpinus* and *T. speciosus* were caught at the same site 35% of the time whereas today, we only caught both species at 18% of sites out of all sites where at least one was captured. Interestingly, in the past there was one site (elevation 2455m) where all three species were detected whereas currently, only *T. speciosus* was detected at that site. Although *T. speciosus'* distribution has generally remained stable overtime, Moritz *et al.* (2008) did report an elevational expansion up by 65m and down by 128m, which provides some evidence consistent with competitive release of this species at its elevational boundaries. *T. speciosus* was "likely the most common chipmunk" in Yosemite National Park during Grinnell surveys (Grinnell and Storer, 1924) and today, trap captures corrected for effort suggest that it has increased in relative abundance since the original surveys across the park (Rubidge, unpublished data). Based on this evidence and our results, it is possible that *T. speciosus'* distribution in the study area is not limited by climate but by interspecific interactions with the other two chipmunk species. However, the environmental change in the study area appears to have had an indirect and positive effect on *T. speciosus* by removing its competitors at its distributional limits.

Our study area captures the southern-most tip of the *T. senex* range. Local populations at range edges are expected to experience higher extinction rates and possess lower genetic diversity than those at the center because they tend to occur in less favorable habitats (Lawton, 1993). The local populations at the rear edge are at particular extinction risk under climate change scenarios because they already represent the warmest conditions a species inhabits (e.g. Parmesan, 1996), and perhaps do not possess the genetic variation required for adaptation to a change in conditions (Kirkpatrick & Barton, 1997; Case & Taper, 2000). Grinnell and Storer

(1924) describe *T. senex* as a "common resident" in Yosemite National Park; today, *T. senex* has virtually disappeared from the area. The collapse of *T. senex's* distribution in Yosemite is likely another example of a poleward range shift in response to warming that has been observed in other taxa (reviewed in Parmesan & Yohe, 2003). Resurvey efforts in other parts of California including a site just 190 km north of our study area report capture rates of *T. senex* similar to the historical surveys in the same area (Chris Conroy, pers. comm.). *T. senex* is the largest of the three study chipmunks and previous studies suggest that it selects dense closed-canopy old growth Jeffrey pine and Red Fir forests (Sharples, 1983; Coppeto *et al.,* 2006) and riparian habitat (Waters and Zabel, 1998). A recent study reported that between 1930 and 1990, large diameter tree density in Yosemite declined by 24% (Lutz *et al.,* 2009). Jeffrey Pine (*Pinus jeffreyi*), in particular, suffered disproportionately greater losses of large-diameter trees in the lower-elevation portions of their range. Lutz *et al.* (2009) attribute the death of old growth trees in Yosemite to increased water stress. The direct impact of climate change on vegetation has indirect effects on the species that depend upon these habitats. According to our model results, the documented change in the vegetation structure of the forests of Yosemite, as well as warming, have likely played a role in the northward retraction of the *T. senex* range. However, because this species has become so rare in the study area our power to validate the model is low.

Conclusions

Species are responding to climate change by shifting their distributions both by latitude and elevation (Parmesan, 2006). Correlative distribution models can be useful for predicting where species will occur under future climates, but the correlations do not necessarily hold through time. This study was able to assess the predictive accuracy of correlative species distribution models over a century of climate change, but we were also able to include habitat and species co-occurrence predictors to determine their relative importance. Our results demonstrate that correlative distribution models are useful in understanding species' potential responses to environmental change, but also show how changes in species-environment correlations through time can limit the predictive performance of models. With recent developments in biodiversity informatics and the increasing availability of spatiotemporally-explicit data (Graham *et al*., 2004), studies like this that are able to validate models in a projection environment, and include both abiotic and biotic predictor variables, will allow us to develop a more complete mechanistic understanding of which species respond directly or indirectly to climate change.

Table 1. Elevational zone, observed distributional change (as recorded by Moritz *et al.* 2008), prevalence and habitat description of study species in and around Yosemite National Park.

Species	***Elevational zone in study area***	***Elevation shift reported in Moritz et al. (2008)***	***Prevalence in Historical Era (# detected/# sites)***	***Prevalence in Modern Era (# detected/# sites)***	***Habitat in study area****
T. alpinus	above 3000m	Retracted 628m up	10/39 (0.26)	18/109 (0.17)	Mainly above treeline in open granite slab areas, talus slopes and at meadow edges.
T. speciosus	2000-3000m	Expanded 128m down and 65m up	12/39 (0.31)	48/109 (0.44)	Open lodgepole forest stands; present at treeline but rarely above.
T. senex	1800-2300m	Retracted 1007m up and 334m down	7/39 (0.18)	1/109 (0.01)	Dense canopy old-growth forests; Jeffrey Pine & Red Fir; riparian vegetation

References: Grinnell & Storer, 1924; Johnson, 1943; Waters & Zabel, 1998; Waters, 2001

Table 2. AIC model selection and performance statistics (AUC and TSS); a) Single-species historical models projected onto historical (within era) and modern (between era) environmental data; b) Multi-species historical models projected onto historical (within era) and modern (between era) environmental data; c) Single-species modern models when projected onto modern (within era) and historical (between era) environmental data; d) Multi-species modern models projected onto modern (within era) and historical (between era) environmental data.

a) Single-species historical models projected onto historical (within era) and modern (between era) environmental data

Model	AIC	Δ AIC	Predictors	AUC (within era)	TSS (within era)	AUC (between era)	TSS (between era)
T. alpinus							
Climate-only	-22.9	0.0	TS, MinT	1.0	1.0	0.92	0.72
Climate + Vegetation	-21.3	1.6	PWet, SCN, JUN, BAR, LPN	1.0	1.0	0.81	0.47
Vegetation-only	-17.1	5.8	SCN, JUN, BAR, LPN	0.97	0.93	0.81	0.40
T. specisosus							
Climate-only	-11.2		MinT, ATR, PWet	1.0	1.0	0.60	0.13
Climate + Vegetation	-9.5	1.6	LPN, MinT, ATR, PWet	1.0	0.92	0.62	0.08
Vegetation-only	-2.9	8.3	LPN, RFR, MCP_MCH	0.9	0.83	0.61	0.19
T. senex							
Climate-only	-15.3		TS, MinT, PWet	0.98	0.77	0.68	0.68
Climate + Vegetation	-8.0	7.3	TS, MinT, PWet, RFR, JUN	0.93	0.70	1	0.52
Vegetation-only	-2.7	12.7	JUN, RFR, MCP_MCH	0.69	0.34	1	0.69

b) Multi-species historical models projected onto historical (within era) and modern (between era) environmental data

Model	AIC	Δ AIC	Predictor Variables	AUC (within era)	TSS (within era)	AUC (between M era)	TSS (between era)
T. alpinus							
Climate-only	-25.2	0.0	TS, MinT, ATR	1.0	1.0	0.83	0.65
Climate + Vegetation	-25.2*	0.0	TS, MinT, ATR	1.0	1.0	0.83	0.65
Vegetation-only	-9.7	15.5	RFR, JUN	0.92	0.84	0.75	0.35
T. speciosus							
Climate-only	-7.8	0.0	TS, MinT, ATR	1.0	1.0	0.68	0.14
Climate + Vegetation	-7.8*	0.0	TS, MinT, ATR	1.0	1.0	0.68	0.14
Vegetation-only	-5.1	2.7	RFR, JUN	0.9	0.83	0.65	0.22
T. senex							
Climate-only	-6.0		TS, MinT, ATR	0.89	0.67	0.89	0.0
Climate + Vegetation	-6.0*		TS, MinT, ATR	0.89	0.67	0.89	0.0
Vegetation-only	0.6	5.4	RFR, JUN	0.68	0.26	1	0.71

c) Single-species modern models when projected onto modern (within era) and historical (between era) environmental data

Model	AIC	Δ AIC	Predictor Variables	AUC (within era)	TSS (within era)	AUC (between era)	TSS (between era)
T. alpinus							
Climate-only	-50.6	0.0	TS, MinT, ATR	0.93	0.72	0.88	0.0
Climate + Vegetation	-50.6*	0.0	TS, MinT, ATR	0.93	0.72	0.88	0.0
Vegetation-only	-15.6	35	BAR, SCN	0.73	0.43	0.77	0.5
T. specisosus							
Climate + Vegetation	15.2	0.0	TS, PWet, LPN	0.79	0.42	0.62	0.06
Climate-only	19.4	4.2	TS, MinT	0.76	0.34	0.92	0.43
Vegetation-only	27.4	12.2	PPN, MHW_MHC	0.62	0.25	0.79	0.57

d) Multi-species modern models projected onto modern (within era) and historical (between era) environmental data

Model	AIC	Δ AIC	Predictor Variables	AUC (within era)	TSS (within era)	AUC (between era)	TSS (between era)
T. alpinus							
Climate-only	-52.5	0.0	MinT	0.92	0.72	1	0.55
Climate + Vegetation	-43.5	9.0	TS, MinT, ATR, PWet, Jun	0.93	0.73	0.61	0.0
Vegetation-only	-17.0	35.5	RFR, JUN	0.67	0.35	0.53	0.05
T. speciosus							
Climate + Vegetation	15.8	0.0	TS, MinT, ATR, PWet, Jun	0.8	0.48	0.57	0.13
Climate-only	18.6	3.2	MinT	0.75	0.33	1	0.86
Vegetation-only	41.0	25.2	RFR, JUN	0.59	0.18	0.62	0.25

* Even with the inclusion of vegetation variables, model result is the identical to climate-only model

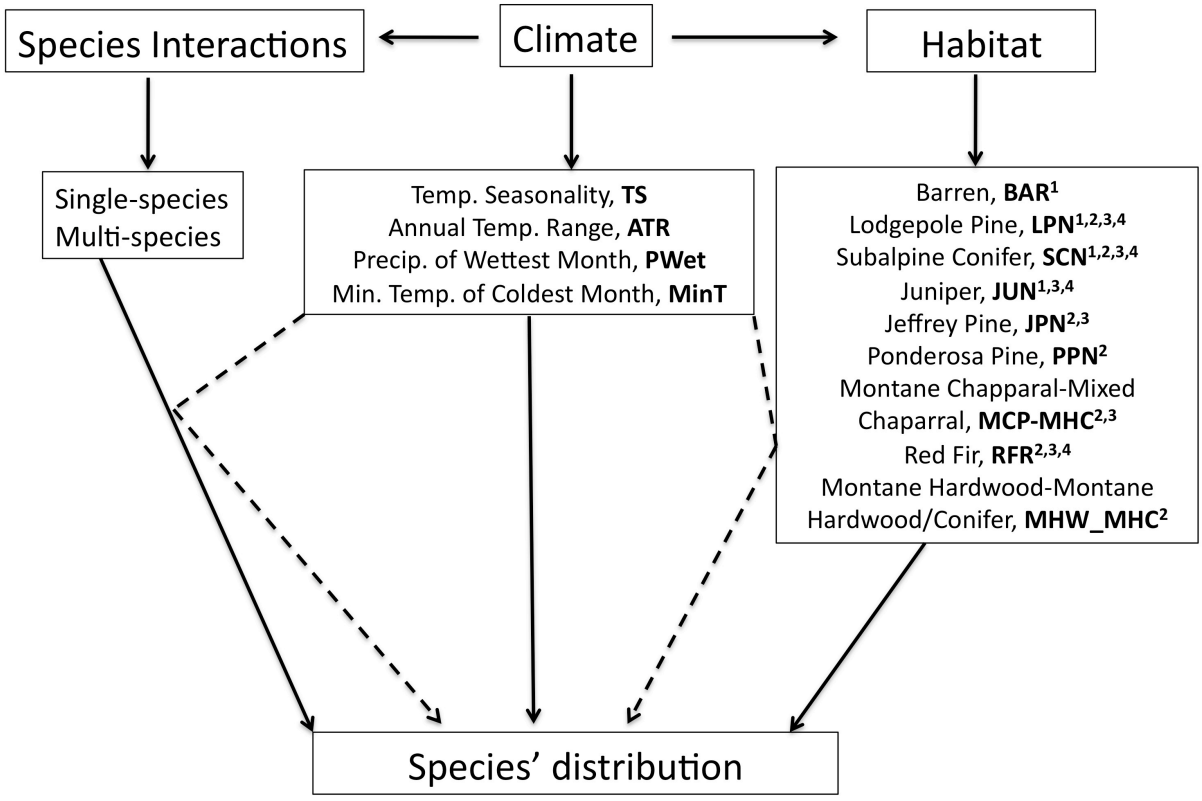


Fig. 1. Conceptual model illustrating modeling framework and the potential direct (black arrows) and indirect (dashed arrows) roles climate can play in species' distributions. Species interactions (in this study, specifically, interspecific competition) and habitat can play a direct role in limiting species' distribution or a climate-mediated (indirect role). Middle boxes indicate environmental variables and their abbreviations. For the vegetation models the included vegetation types are coded by species and modeling approach: 1 - *T. alpinus* single-species models; 2 - *T. speciosus* single-species models; 3 - *T. senex* single species models. 4 - Multi-species models.

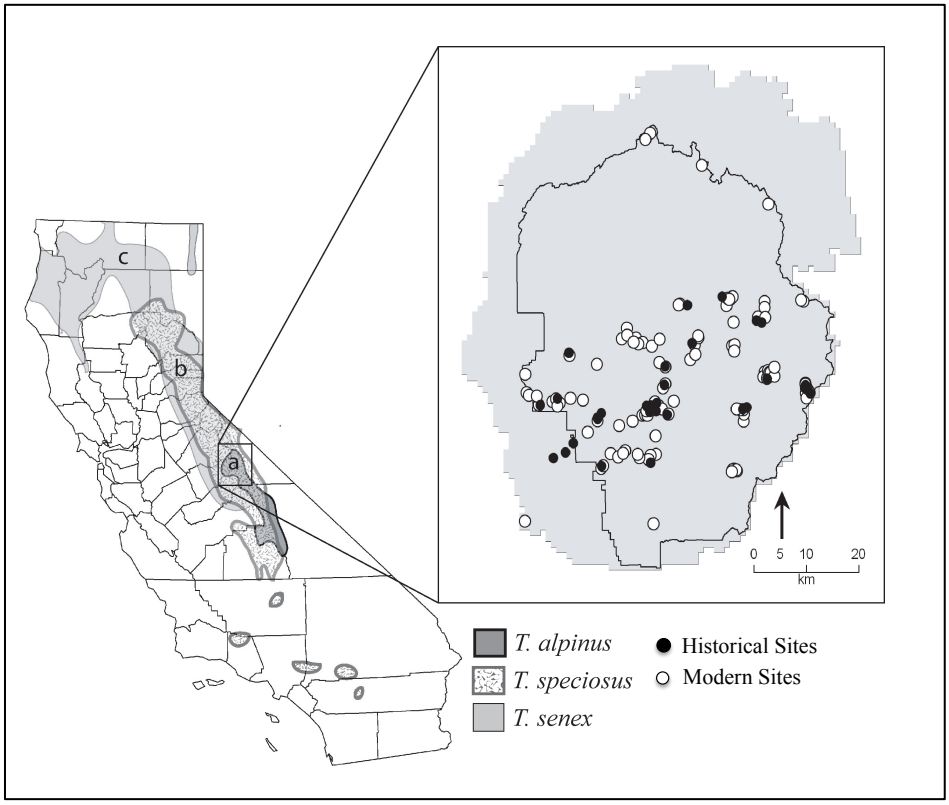


Fig. 2. State of California showing the distribution of a) *T. alpinus*, b) *T. speciosus* and c) *T. senex*. Black rectangle shows blow-up of study area. The gray shaded area is the study area and the black line shows Yosemite National Park boundary. Black dots indicate historical mammal sites (1914-1916, n=39) and white dots show modern mammal sites (2003-2007, n=109)

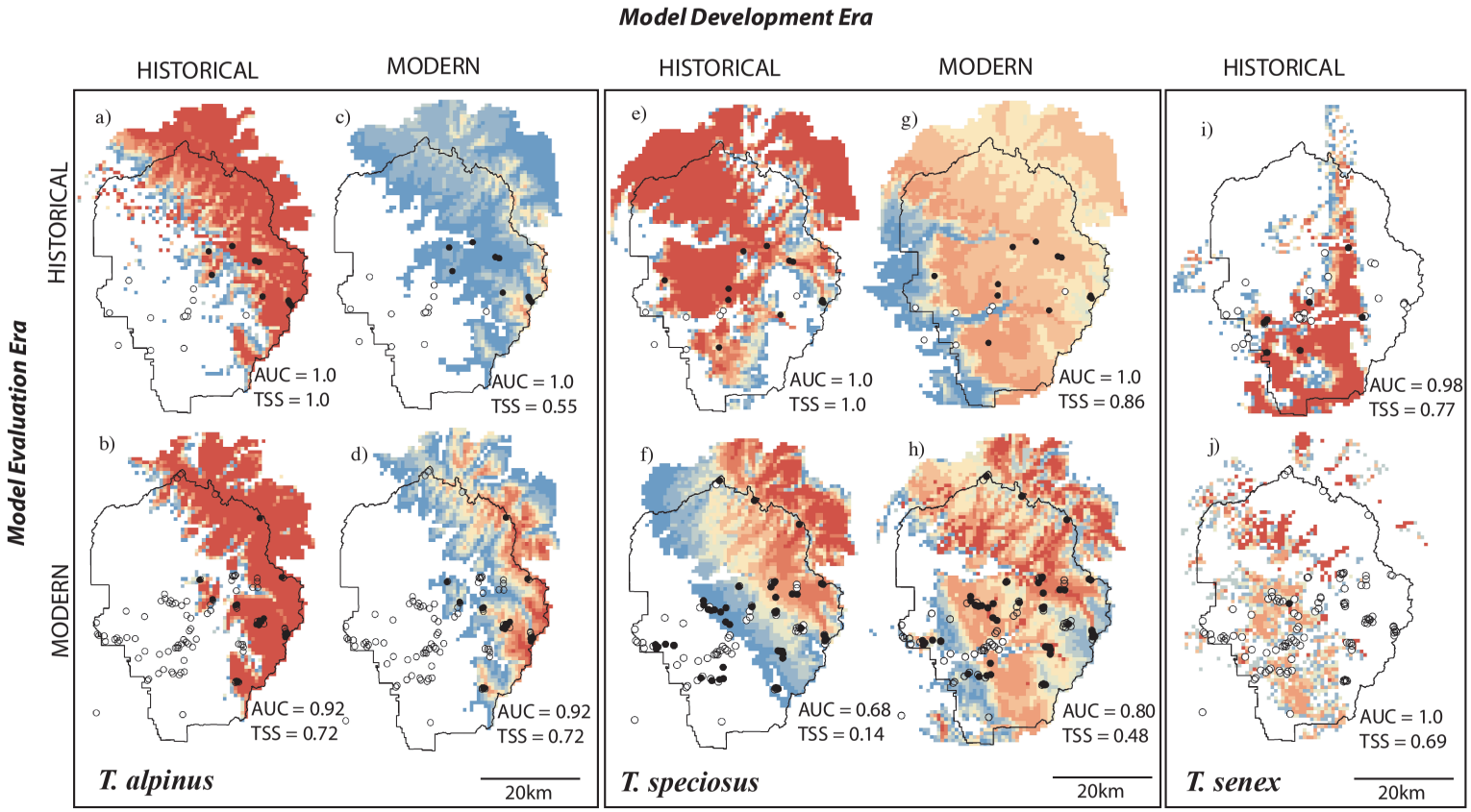


Figure. 3. Predictive distribution maps of most accurate models (based on AUC and TSS) for *T. alpinus* (a-d), *T. speciosus* (e-h) and *T. senex* (i-j). The "model development era" along the horizontal axis of the figure refers to the timeframe in which the model was built; the "model evaluation era" along the vertical axis refers to the timeframe in which the model was tested. All models evaluated in the historical era were tested with the historical P/A points. All models evaluated in the modern era were tested with modern P/A points. Black dots represent presences, open circles represent absences. Mapped top models are as follows: *T. alpinus* a) historical single-species climate-only on historical environment; b) historical single-species climate-only on modern environment; c) modern multi-species climate-only on historical environment; d) modern multi-species climate-only on modern environment; *T. speciosus*e) historical single-species climate-only on historical environment; f) historical multi-species climate-only on modern environment; g) modern multi-species climate-only on historical environment h) modern multi-species climate+vegetation on modern environment; *T. senex* i) historical single-species climate-only on historical environment; ii) historical single-species vegetation-only on modern environment.

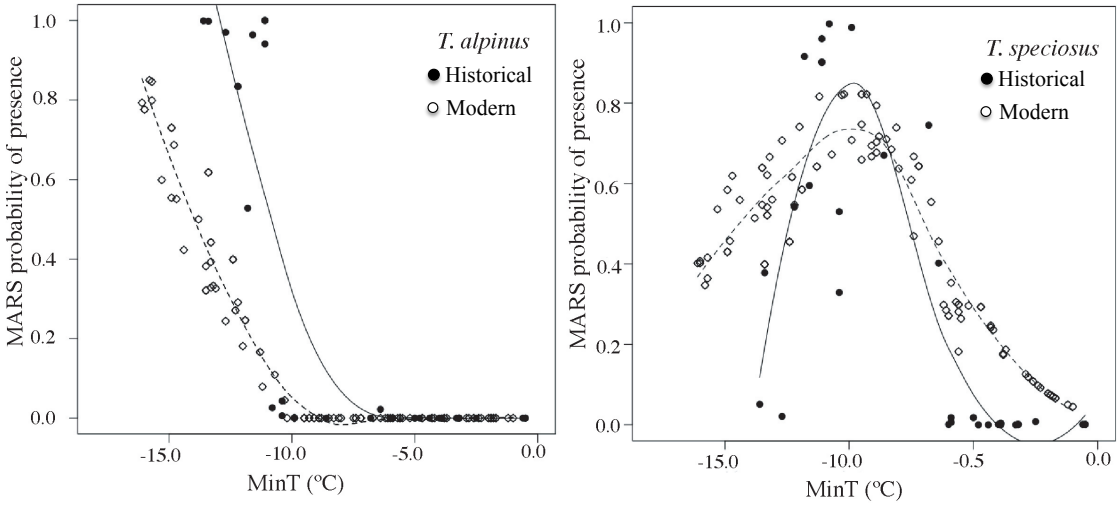


Fig. 4. The MARS predicted probability of presence of *T. alpinus* (left), and *T. speciosus* (right), across minimum monthly temperatures (MinT) in the historical (black circles), and modern (open circles) models. MARS Probabilities generated from historical and modern single-species climate-only models.

CHAPTER 4

Global warming reduces genetic diversity and gene flow in an endemic alpine small mammal species.

Introduction

Biologists have ample evidence indicating that climate change is one of the greatest threats facing biodiversity today (e.g., Root et al. 2003) but there continues to be much uncertainty in how species will respond, our ability to predict these responses, and how to mitigate and manage these effects in a conservation context. An understanding of responses to past climate changes should improve predictions to future effects. Several studies have examined the effect of climate change over geological time on mammal morphology (e.g., Smith & Betancourt 1998, Blois et al. 2008), genetics (e.g., Hadly et al. 2004), and species assembly of mammals (Blois et al. 2010). Others have examined the effects of recent climate change, over the past several decades or less, on morphology (e.g., Smith et al. 1998, Ozgul et al. 2010), phenology (Reale et al. 2003) and species' distributions (e.g., Moritz et al. 2008). Few studies, however, have been able to examine the population genetic effects of recent climate change. Here, I apply genetic analysis to modern and historical specimens skins to examine the population genetic effects of a climate-driven elevational range shift upslope over the last 100 years observed in the Alpine Chipmunk, *T. alpinus*, in Yosemite National Park (YNP), CA.

Results from a resurvey of mammals in YNP showed evidence that several species of small mammal have changed their range over the past century likely due to the indirect and direct effects of an observed ~1-3.7ºC increase in minimum temperatures in the area (Moritz et al. 2008). Further research suggests that the Alpine Chipmunk (*Tamias alpinus*) has retracted its elevational range upwards as a direct result of this warming (Rubidge et al. in press). Here, I examine how the range change in *T. alpinus* has affected its overall diversity and population genetic structure in Yosemite National Park. I use a comparative approach examining historical and modern datasets in two species of chipmunk: one whose range has changed (*T. alpinus*), and a related species (Lodgepole chipmunk, *T. speciosus*) with a similar ecology but whose range has remained stable over time. Comparison with the relatively stable, largely co-distributed species enables me to attribute changes unique to *T. alpinus* to effects of climate-driven range retraction

The two study species are mid to high elevational species. *T. alpinus* lives in the alpine zone mainly above treeline (2880-4100m), whereas the Lodgepole Chipmunk occupies the mid to high elevation area from about 1800-3000 m in the study area (Grinnell & Storer 1924). These species are sympatric at or just above treeline but have different habitat preferences. *T. speciosus* occupies open lodgepole and whitebark pine forest stands whereas *T. alpinus* prefers open habitat, granite slab, and talus slopes (Grinnell & Storer 1924, Johnson, 1943). In 1915, *T. alpinus* was found at sites as low as 2377m whereas today, the lowest elevation it has been detected is 2888m, which suggests a 511m upwards shift in this animals' distribution in the study area. By contrast, *T. speciosus*, has not changed its distribution significantly over the past century (Moritz *et al.* 2008). Rubidge *et al.* (in press) showed that climate change, independent of species interactions, has been the main driver in the upslope contraction observed in *T. alpinus*,

and found little evidence that *T. speciosus'* distribution is limited by climate or habitat in the study area.

A range contraction up in elevation in a mountain species may be associated with several genetic signals. If the lower elevation populations of this species have become extinct, then I expect a decline in genetic diversity from the past to the present. However, if these populations have physically moved up to higher ground without a decline in overall population size (or local extinctions), then I do not expect to see a change in genetic diversity. Further, to the extent that fragmentation of the historical distribution has reduced gene flow among montane habitat islands, I would predict an increase in genetic divergence between populations. Using direct genetic comparison between a large series of specimens collected in the early 20th century and specimens and tissues obtained from recent resurveys of the same area, I can test these predictions directly. My specific predictions for this study are as follows. The range contraction observed in *T. alpinus* populations in the study area has 1) resulted in a decrease in overall genetic diversity (measured by haplotype diversity, allelic richness, and heterozygosity) between the past and the present; 2) Populations that have become spatially fragmented on a local scale will exhibit reduced gene flow as compared to more homogenous historical populations resulting in an increase in isolation between population and population structure; *T. speciosus'* range has remained relatively stable through time and I therefore predict no change in genetic diversity or population structure between the past and the present.

Methods

Study Site and Sampling

This study took place in and around Yosemite National Park (YNP), CA, USA (Figure 1). Yosemite National Park was established in 1890 and is 3027 km^2 in size. It covers a large elevational gradient from about 657m to 3997m. Vegetation in the park is comprised of six major vegetation types that cover about 84% of the park: subalpine coniferous forest, upper montane forest, lower montane coniferous forest, broadleaved upland forest and woodland, scrub and chaparral, and grassland and meadow community. The remainder of the park has less than 20% vegetation cover (Lutz et al. 2010).

This study uses tissues collected by the "Grinnell Resurvey Project" where field teams from the Museum of Vertebrate Zoology at the University of California, Berkeley, have been revisiting vertebrate survey sites originally sampled between 1910-1939 across California by research teams led by Dr. Joseph Grinnell. Museum skins used in this study are from the original specimens collected by the Grinnell team from 1915-1916 and housed in the mammal collection in the Museum of Vertebrate Zoology, UC Berkeley. I removed an approximately 3mm x 3mm square piece of skin from the lower lip of 88 *Tamias alpinus* and 59 *T. speciosus* museum specimens collected from several areas across YNP between 1915 and 1916 (Figure 1). For the modern dataset, resurvey teams live-trapped animals using Sherman traps at the original "Grinnellian" sites between 2003-2008. I used mainly non-lethal sampling (ear clips) but also collected whole specimens for the museum collection. Where specimens were collected, I used liver tissue in our DNA extractions. In situations where I did not detect the species at the historical site after repeated annual visits, near-by but higher elevation areas were sampled for comparison. This only occurred in the case of *T. alpinus*; resurvey teams were unable to detect it at six localities at or near historical areas (Figure 1a.).

DNA Extraction

All extractions and PCR set-up on historical samples were conducted in a separate laboratory devoted to ancient DNA research. I followed the museum skin DNA extraction protocol described in Mullen and Hoekstra (2009). After sampling a small piece of lip tissue, I placed the sample in 95% ethanol and refreshed the ethanol roughly every 3 hours over a 24-hour period to wash the sample of salts and PCR inhibitors. Following these washes, each skin sample was carefully removed of hair and shaved into smaller pieces with a scalpel and placed into a 1.5 ml locking Eppendorf tube. Between each sample, the forceps and scalpel were washed in 10% bleach, rinsed in 95% ethanol and flamed to avoid cross contamination. I extracted DNA using a Qiagen DNeasy Tissue Extraction kit with the following modifications. First I diluted the AE Buffer to 1:10 in RNAse-free H_2O and warmed it to 70°C prior to elution. Second, I applied two elutions of 50 µl of warm 1:10 AE Buffer to the spin columns and allowed this elution step to incubate at room temperature for 5 min prior to the final spin. I conducted a negative extraction (sterilized forceps in extraction buffer) alongside all historical skin extractions. The negative extraction was run along with a negative PCR control in reactions to test for contamination between samples. I used the standard Qiagen DNAeasy kit to extract DNA from the modern tissue samples. Final extractions from modern tissues were eluted in a total of 400µl AE buffer. The extractions and PCR of each dataset (historical and modern) for both species were separated physically, as mentioned above, but also temporally to avoid cross-contamination between historical and modern DNA.

Microsatellite Analysis

Twenty microsatellites were screened on modern DNA samples of both *T. alpinus* and *T. speciosus* first and then subsequently tested on museum samples. Eleven of these were taken from the literature (Schulte-Hostedde et al. 2000), and nine were developed specifically for this study, by Genetic Identification Services from *T. alpinus* DNA (Table 1). After multiple tests and PCR optimization trials, only 5 (EuAmMS26, EuAmMS37, EuAmMS41, EuAmMS86, EuAmMS94, Table 1) of the 11 published primers gave high quality amplification in both *T. speciosus* and *T. alpinus*. All five of these also gave reliable signal in the museum skins. Nine of the markers developed specifically for *T. alpinus* gave strong signal on the modern data but only three of the nine resulted in reliable amplification in the museum skins of both species (AC A101, AC D107 and AC D115).

PCR reactions for both modern and historic samples were carried out in a volume of 8.0µl. For modern samples, the PCR reagents were as follows: 0.5-1µl DNA template, 0.25µM each primer, 0.2mM each dNTP, 0.8µl 10X BSA, 0.8µl 10X PCR buffer (Roche), 1.5mM $MgCl_2$ and 0.4U of *Taq* DNA polymerase (Roche). PCRs for the historic samples included: 1-3µl DNA template, 0.25µM each primer, 0.2mM each dNTP, 0.8µl 10X BSA, 0.8µl 10X JumpStart PCR buffer (Sigma), 3mM $MgCl_2$ and 0.5U of JumpStart *Taq* DNA polymerase (Sigma). For both historic and modern reactions, the reverse primers were fluorescently labeled with one of the following dyes: PET, NED, FAM, or HEX, forward primers were unlabeled. The thermal cycler conditions for the modern and historical PCRs differed only in the initial denaturation step and the number of cycles. The conditions for the modern samples consisted of 94°C for 2min, followed by 30 cycles of 94°C for 40s, 51-60°C for 40s, and 72°C for 40s, and ending with a final extension at 72°C for 10mins. For the historical samples, the initial denaturation was 95°C for 10min and the middle steps were run for 44 cycles as opposed to 30. Locus-specific

annealing temperatures are shown in Table 1. PCR products were sized by capillary electrophoresis on an ABI 3730 sequencer (Applied Biosystems, Inc.), and alleles were scored manually using program GENEMAPPER Ver. 4.0 software (Applied Biosystems, Inc.). Each skin specimen was genotyped at least 3 times and in some cases, 4 times if runs were not consistent. If samples did not amplify at a minimum three of the seven loci, they were discarded from further analyses as were considered unreliable. Replicates of modern data showed no inconsistencies.

Mitochondrial DNA Analysis

I amplified a portion of the mitrochondrial gene, Cytochrome b, to examine changes in mitochondrial genetic diversity overtime. For the modern samples, I used universal mammal primers, MVZ05 & MVZ16 (Smith 1998) to amplify an 801bp region of this gene. Because the historical DNA was degraded, I was only able to amplify a fragment less than 400bp; therefore, I developed three pairs of genus specific primers to amplify shorter fragments that could be pieced together to complete a 780bp sequence (Table 1) for the historical DNA samples. To ensure the species-specific primers were not causing any irregularities in the sequences, I also used these primers on five modern DNA samples and compared the results with the sequences using the MVZ universal primer pair. I sequenced 15 historical and 97 modern samples of *T. alpinus* and 13 historical and 44 modern *T. speciosus* samples. The thermal cycler conditions for the mitochondrial PCRs were as follows: 94°C for 2mins, 35 cycles of 94°C for 30s, 47-50°C for 30s, 72°C for 60s and then a final extension at 72°C for 5mins. Historical samples were sequenced in both the forward and reverse direction and PCR'd and sequenced at least twice to ensure repeatability of resulting sequence. Amplicons were sequenced on an ABI 3730 Capillary Sequencer (Applied Biosystems, Inc.). Resulting sequences were edited and aligned using Sequencher 4.8 (Gene Codes Corp.).

Data Analysis

Microsatellite analyses

I tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium (HWE) in each population using the heterozygosity deficit test implemented in Genepop 4.0. I applied Bonferroni corrections separately for each species and time period. Because of significant deviations from HWE in at certain loci in historical populations (See Results) I used the program FreeNa (Chapuis & Estoup 2007) to estimate the frequency of null alleles and to generate a dataset corrected for null alleles that was used to examine their influence on estimates of genetic differentiation.

The program FSTAT was used to estimate genetic differentiation among populations (F_{ST} analogue θ_{ST}; Weir and Cockerham 1984) and tested for significance by bootstrapping across loci to generate 95% confidence intervals for overall θ_{ST}. In addition, to examine population structure without a priori definitions of "populations" and to avoid issues of uneven sampling between populations on pairwise θ_{ST} measures, I applied the Bayesian approach implemented in the software Structure 2.3.3 (Pritchard et al. 2000) to identify clusters of randomly mating individuals with minimum HW deviations and linkage disequilibrium. I did this on all four datasets separately, historical *T. alpinus (*HAlp*)*, modern *T. alpinus (*MAlp*)*, historical *T. speciosus* (HSpec), and modern *T. speciosus* (MSpec), to examine changes in population

structure for each species over time. I ran the admixture model with correlated allele frequencies with five replicates of 10^6 Markov Chain Monte Carlo (MCMC) iterations after a burnin of 10^5 from K (number of parental populations) = 1 to K = 8. The upper bound for K represents the number of sampling localities corresponding to the hypothesis that each sampling locality is a distinct population. To provide the most accurate estimation of K, I used the statistic ΔK introduced by Evanno *et al*. (2005). I averaged coefficients of membership across the five replicates using the software CLUMMP 1.1 (Jakobsson & Rosenberg 2007) and DISTRUCT 1.1 (Rosenberg 2004) was used to plot the graphical representation of this membership.

To examine changes in genetic diversity over time I pooled samples park-wide into a historical and modern dataset for each species. I estimated Nei's measure of gene diversity (H_S, Nei & Kumar 2000) using the program FSTAT, as this statistic is unbiased by sample size and does not appear to be seriously affected by null alleles (Chapuis et al. 2008). I also examined allelic richness at each locus and the presence of private alleles using the hierarchical rarefaction approach implemented in HP-RARE to correct for differences in sample size between eras (Kalinowski 2005). To statistically compare mean diversity measures between the historical and modern datasets, I used either a Welch Two-sample T-test when data fit assumptions of normality or a Wilcoxon Rank Sum test when they did not. All comparative diversity statistical analyses were run in R package (R Core Development Team 2010).

I secondarily examined changes in population structure and diversity at the per site level assuming that each locality was a distinct population. Although there are statistical biases introduced when examining pairwise F_{ST} measures with uneven sample sizes between populations, I conducted these analyses to compare with the Bayesian cluster analysis and to examine changes in isolation by distance over time. I used the program FreeNA to calculate pairwise F_{ST} measures for each "population" (sample sizes in Table 2 & 3) and tested for significance by bootstrapping across loci to generate 95% confidence intervals. In addition, when significant structuring was found, I used the program Isolation by Distance Web Service (IBDWS; Jensen et al. 2005) to conduct a Mantel test to test for patterns of spatially limited gene flow in both species using log(genetic distance) and log(geographic distance) with 5000 randomizations. Pairwise F_{ST} and Euclidian distances were used.

Mitochondrial DNA Analyses

Temporal changes in mtDNA diversity were investigated by comparing estimates of mitochondrial haplotype diversity (h_d), and nucleotide diversity (π) calculated in the program DNAsp 5.0 (Librado & Rozas 2009). To quantify mtDNA differentiation between populations I calculated ϕ_{ST} using Arlequin 3.11 (Excoffier et al. 2005) where haplotypes were grouped into populations based on sampling locality. In addition, haplotype networks for each species were estimated using the statistical parsimony method (Templeton et al.1992) with a 95% connection limit implemented in TCS 1.21 computer software (Clement et al. 2000).

Results

Seven of the eight loci used in the analyses gave consistent and repeatable amplification results. One locus, D107, however, showed a high frequency null allele in all four datasets (HTalp, MTalp, HTspec, MTspec) and was removed from further analyses. Although genotypes

were repeated at least three and in some cases four times with consistent results, both historical datasets appeared to suffer from allelic dropout as some populations showed a significant heterozygote deficiency at certain loci (see Appendix 5 for test results). However, the loci that deviated from HWE were not consistent across populations, or overall, so this deviation was not likely due to the presence of a true null allele. In addition, there was no heterozygote deficiency, and thus no indication of the presence of null alleles in either of the modern datasets. Allelic dropout and null alleles should have the same affect on the dataset (i.e., heterozygote deficiency). Most historical populations of both species showed the null allele frequencies of <0.07, although some populations showed frequencies at certain loci as a high 0.28 in the HTalp and 0.25 in the HTasp datasets. The FreeNA-corrected dataset, however, yielded similar levels of pair-wise genetic differentiation, or overall θ_{ST} suggesting the data are robust to genotyping errors. Nevertheless, to err on the conservative side, I have used the FreeNA corrected values for both historical datasets. No linkage disequilibrium was detected between any loci in any of the datasets.

Changes in diversity

I examined park-wide changes in genetic diversity between the two eras pooling all samples from the historical era and all samples from the modern era for each species (Table 4). For the microsatellite DNA comparison between historical and modern *T. alpinus* populations, I observed a downward, though non-significant, trend in gene diversity (HAlp H_S = 0.75, SE = 0.023; MAlp H_S = 0.71, SE = 0.023, W = 35, p = 0.21) and a significant decline in average allelelic richness (t = 1.919, df = 11.5, p=0.040) and the frequency of private alleles (W=39, p = 0.002) over time. Six of the 7 loci lost at least one allele from the past to the present (Appendix 5 Figure 1). In contrast to *T. alpinus*, I did not observe any significant changes in overall gene diversity, allelic richness or frequency of private alleles in the historical/modern comparison of *T. speciosus* populations (Table 4).

For *T. alpinus*, neither haplotype diversity nor nucleotide diversity changed between the historical and modern datasets (Table 4), however, there were three haplotypes present in the historical samples that were not detected in the modern samples, despite a much larger sample size in the latter (N_{modern} = 97 versus $N_{historical}$ = 15). Only one haplotype (HM1) out of the 12 detected in all *T. alpinus* samples was shared between the historical and modern datasets (Figure 4). The remaining 9 haplotypes were only present in the modern dataset, although uneven sampling between eras may be responsible for the lack of detecting low frequency haplotypes in the historical dataset. There were two high frequency haplotypes in the *T. alpinus* samples that were 2.7% different from one another (Figure 4). Thirty-eight percent of individuals sequenced were the TAlp_HM1 haplotype, which was shared between the historical and modern datasets, and 33% were the TAlp_M1 haplotype that was not detected in the historical dataset. Although 8/15 historical samples sequenced were only 1bp different (haplotype H1) from the high frequency M1 haplotype (Figure 4). Six out of the 12 haplotypes were identified as unique (only occurring in one individual; HAlp: 2 unique haplotypes, MAlp: 4 unique haplotypes) and the remaining 3 haplotypes ranged in frequency from about 2-9%. Nine haplotypes were identified in the *T. speciosus* samples. The two haplotypes detected in the historical dataset were both identified in the modern dataset and the remaining 7 were only found in the modern dataset. Seventy-two percent of all *T. speciosus* samples sequenced were identified as haplotype

TSpec_HM1 (Figure 4). The remaining haplotypes were either low frequency (4%-6%) or unique haplotypes. A trend towards an increase in both haplotype diversity and nucleotide diversity over time was observed in *T. speciosus,* though this could be an artifact of larger sample in the modern (N_{modern} = 44) versus historical samples ($N_{historical}$ = 13).

Changes in population structure

The estimate from microsatellie loci of the global θ_{ST} for the historical *T. alpinus* dataset was low and the 95% confidence interval overlapped with zero (θ_{ST} = 0.026, 95% CI: 0.000 – 0.044). In contrast, the modern *T. alpinus* dataset showed a significant increase in among population diversity over time (W = 5, p = 0.01), with a global θ_{ST} = 0.086 (95% CI: 0.060 - 0.112). The historical *T. speciosus* population was not significantly structured (global θ_{ST} = 0.0185, 95% CI: -0.007 – 0.049). The modern dataset did show increased structure (θ_{ST} = 0.029, 95% CI: 0.013 – 0.045) however, this increase between the two time periods was non-significant (W = 19, p = 0.535). The results of the cluster analyses also suggested an increase in population structure over time in *T. alpinus* but no change in *T. speciosus* (Figure 2a-d). The estimated number of parental populations for HAlp was 2 (Figure 3a). Although K=3 had a slightly larger ΔK value than K=2, increasing the number of parental populations from 2 to 3 did not clarify any further structuring when examining the individual membership bar graph (Figure 2a). In contrast, MAlp showed much stronger structure than HAlp with a K=4 (Figure 3a, Figure 2b). A comparison of the cluster analysis in *T. speciosus* between the past and the present shows that population structure in this species in the study area is low, and has remained low through time (K=2 for both HSpec and MSpec; Figure 2b, Figure 3b).

Measures of pair-wise ϕ_{ST} between populations indicated no significant structure in haplotype diversity in *T. alpinus* historically (average pairwise ϕ_{ST} <0.0), however one population in the modern dataset (Vogelsang Lake) was significantly differentiated from three other populations (Upper Lyell Canyon ϕ_{ST} = 0.48, p<0.0001; East-YNP ϕ_{ST} = 0.49, p<0.001; and Mono Pass ϕ_{ST} = 0.55 p<0.0001). However, this differentiation is likely due to the presence of one haplotype (M5, Figure 4) found in 8/9 individuals from Vogelsang, that was not detected anywhere else in the park. The haplotype network analysis of *T. alpinus* indicated two differentiated groups with three low frequency haplotypes situated in between, however, these two groups do not appear to have strong geographic structure when plotted on the landscape (Figure 4).

The geographic distribution of the 4 clusters identified from the microsatellite analysis in MAlp suggest reduced gene flow overtime between the localities around Tuolumne meadows (D, E, F, Figure 2b), the eastern park border (Locality B, Mono Pass), the high alpine areas near Lyell Glacier and Vogelsang Lake (G, H Figure 2b) and the Clark Mountain Range (C, Figure 2b). The greatest genetic differentiation was observed between Young Lakes (E) and the Clark Mountain Range (C. Ottoway Lakes) with a pairwise F_{ST} of 0.151. Mono Pass (B) and the Clark Mountain Range (C) had the second greatest pairwise F_{ST} of 0.127. Comparing the pair-wise F_{ST} between populations within the historical *T. alpinus* populations and modern *T. alpinus* populations showed that genetic differentiation between populations has increased in magnitude and significance overtime (Table 5a&b, Figure 6a). Although there is evidence of increased differentiation between populations in the modern *T. speciosus* dataset (Table 6a&b) as

compared to the historical dataset, overall the average pairwise F_{ST} values did not change between the two time points (Figure 6b). Only the MAlp dataset showed significant isolation-by-distance ($Z = -40.37$, $r = 0.42$, $p=0.03$) and although overall θ_{ST} was significant for MSpec, there was no significant isolation-by-distance ($Z = - 75.45$, $r = 0.018$, $p = 0.43$).

Discussion

Genetic diversity is an essential component of biological diversity as it provides the basis for genetic adaptations and thereby defines the evolutionary potential of a population (Frankham and Kingsolver 2004). Species under threat from climate change will likely be under strong selection to adapt quickly if they are unable to move to more suitable habitat and genetic diversity is the basis for this adaptation. Theoretically, a contracting and fragmenting population will exhibit a loss of neutral genetic diversity and decreased gene flow over time. This study may be the first to use a comparative approach to document genetic effects of population decline and fragmentation due a climate-induced shift in a terrestrial species' distribution (see Fontaine et al. 2010, for marine example). Our results strongly suggest that an observed distributional change in Alpine Chipmunks has resulted in a decline in overall genetic diversity and has reduced gene flow between local populations in Yosemite National Park, California over the past century. Importantly, these effects are not seen in *T. speciosus*, a species sampled over the same region and time-span, but with a relatively stable geographic range.

Modern genetic data are often used in conservation studies to investigate the effects of habitat fragmentation on genetic structure and inform re-introduction programs (reviewed in DeSalle and Amato 2004). Our results demonstrate the significance of adding a historical component for detecting genetic changes on a recent timescales and prior to the environmental disturbance. As with another recent study that used a comparative approach examining the genetic effects of recent declines in bumblebees (Lozier et al. 2009), it also highlights the value of comparing genetic changes in a species that appears to have changed its distribution or population size over time with a related species that has remained relatively stable under the same environmental change. Both historical datasets should be subject to similar problems associated with the use of ancient DNA such as DNA degradation, amplification success and sequence errors (reviewed in Paabo et al. 2004), yet, the different patterns observed in each species is clear. The *T. alpinus* population in YNP has lost alleles that were present in the past and certain localities have become more isolated from each other. In contrast, *T. speciosus*, whose distribution in the study area has remained stable, does not show any significant changes in genetic diversity or population structure as expected.

Genetic diversity

Average allelic richness declined significantly from the past to the present in *T. alpinus* populations in the study area, with 6/7 loci losing at least one allele. Gene diversity (H_S), although lower in the modern dataset, did not significantly change overtime. This result is not necessarily surprising given that alleles are lost more quickly than heterozygosity in declining populations and that there must be a dramatic population decline to detect changes in gene diversity over recent time. Lozier et al. (2009), using computer simulations, showed that a substantial population size change is needed to dramatically impact H_s; a 99% reduction in effective population size resulted in only a 0.19 reduction in H_S over 58 generations. Between our two sampling time periods, there have been approximately 88-93 generations and a loss of

0.04 in H_s in *T. alpinus* populations. Although, I observed a decrease in diversity over time at microsatellite markers, there was virtually no change in mtDNA diversity between the two time points. However, there has been substantial turnover of specific haplotypes with three haplotypes found in the historical dataset not detected in the much larger modern dataset and a high frequency modern haplotype was not detected in the historical samples. Comparing the haplotype richness between eras in this circumstance is difficult because there was a huge discrepancy in sample sizes ($N_{Historical} = 15$ $N_{Modern} = 97$). The absence of the three historical haplotypes in the extensively sampled modern era suggests diversity loss. However, the network analysis revealed that the geographic structure of haplotype diversity (or lack there of) has not changed over time. All three haplotypes that were not detected in the modern dataset were closely related to haplotypes still present in the study area.

In contrast to the decrease in nuclear genetic variation observed in *T. alpinus* populations, microsatellite diversity measures in *T. speciosus* populations in the study area have remained relatively stable between the time periods, as expected. Interestingly, both haplotype and nucleotide diversity has increased in the modern *T. speciosus* dataset but it is difficult to determine if this is a sampling size effect ($N_{Historical} = 13$ $N_{Modern} = 44$) or recent immigration of new *T. speciosus* haplotypes into the study area. The two haplotypes detected in the historical dataset were found in the modern dataset providing no evidence of loss of haplotype richness overtime as observed in the *T. alpinus* populations.

Population structure

The spatial genetic structure of natural populations has important consequences on ecological and evolutionary processes over both contemporary and long-term timescales. The literature suggests that most mammal species show some genetic subdivision, however the scale of subdivisions varies greatly and is affected by complex interactions between dispersal ability, social structure and the environment (e.g., Lidicker & Patton 1987; Waser & Elliot 1991, Goosens et al. 2001, Burton et al. 2002). Our results provide evidence of an increase in genetic structure resulting from a climate-driven distributional change in an alpine mammal. In the past, there was no significant genetic structure in the historical datasets of the either study chipmunk species. This lack of genetic structure is not surprising given the relatively small spatial scale of the study (average distance between sampling sites for *T. alpinus* is 21.5 km and 26.6 km for *T. speciosus*). The geographic scale of genetic structure reported in other chipmunks varies by species. For the Eastern chipmunk, *Tamias striatus*, Chambers and Garant (2010) report an average pairwise F_{ST} of 0.127 across seven sites in an area 35, 000 km^2 (over ten times the size of Yosemite) with an average distance between sites of 211km. In contrast, microgeographic structure has been detected in Yellow-Pine Chipmunk (*T. amoenus*) in Southwest Alberta across sites less than 15km away from each other (Schulte-Hostedde et al. 2001). Schulte-Hostedde et al. (2001) suggest that the strong effect of genetic drift in the small populations of these animals outweigh substantial immigration rates (pairwise F_{ST} between three study sites ranged from 0.036-0.083).

Both modern datasets, showed some degree of structure however, only the modern *T. alpinus* dataset showed a significant overall increase in global θ_{ST} from 0.026 in the past to 0.086 today. Overall θ_{ST} increased from 0.019 – 0.029 overtime in the *T. speciosus* dataset, but this was a non-significant increase. There is the issue of power when detecting genetic subdivision and larger datasets have more power to detect population genetic structure if it exists. It is possible

that the increase in structure observed in modern datasets is a result of larger sample sizes in the modern era, however, one would expect a similar increase in both datasets across time because the discrepancy in sample sizes is similar. Furthermore, the cluster analysis, which is less biased by sample size, showed a stronger population structure in *T. alpinus* overtime whereas the *T. speciosus* populations remained similarly structured through time. Therefore, the disappearance of *T. alpinus* at lower elevations, particularly the area in and around Tuolumne Meadows, has disrupted the gene flow that was once widespread across the landscape.

Measures of relative abundance from historical and contemporary trap data corrected for effort suggest that *T. alpinus* was more abundant than *T. speciosus* in Tuolumne Meadow in Grinnell's time (Rubidge, unpublished data), whereas today, *T. speciosus* is the only *Tamias* species present after extensive and multi-year surveying. Tuolumne Meadow sits at approximately 2700m and is a large meadow surrounded by large granite domes, talus slopes and boulder fields. In the past, when this area supported a large *T. alpinus* population it likely provided more connectivity up the through the Tuolumne River headwaters near the eastern portion of the Park (Mono Pass), and up Lyell Canyon and Vogelsang Lake. In addition, if lower elevation areas were more hospitable to this species in the past, then there was also likely more opportunity for genes from the Tuolumne area to reach populations in the Clark Mountain Range through Upper Lyell and Vogelsang Lake following a stepping-stone model of gene flow. The presence of isolation by distance in the MAlp dataset support that this as a feasible interpretation of the observed increased structure in today's populations. The reduction in overall abundance in the study area associated with the lower elevation populations disappearing appears to have effectively increased the geographic distance between sampling localities.

Study limitations

Joseph Grinnell had remarkable foresight and recognized the value of his research as a baseline for future studies[1]. Although he and his colleagues left an extremely valuable dataset of detailed field notes, photographs and specimens, they could not have foreseen the use of their specimens in a study of this kind. I am limited not only by the historical sample size per locality but also by sampling extent. In addition, the quality of the DNA extracted from 90+ year-old specimens is much lower than from fresh tissues preserved in ethanol. Although there is no way to overcome the sample size constraint, I did attempt to tackle the DNA quality issue through repeat genotyping and removing individuals and/or loci that did not give consistent results. I found signals of allelic dropout in both of our historical datasets, but I still detected alleles in the past that were not present today. Therefore, the historical estimates of genetic diversity are potentially lower than the true historical value resulting in a reduced estimate of genetic diversity loss over time. The lack of significant change in genetic diversity and population structure observed in *T. speciosus* further supports our conclusion that allelic dropout is not a determining factor in observed patterns of our results.

[1] "… the greatest purpose of our museum… will not be realized until the lapse of many years, possibly a century…and this is that the student of the future will have access to the original record of faunal conditions in California and the west, wherever we now work" Joseph Grinnell, 1910.

Conservation Implications and Conclusions

Climate change is responsible for several documented elevational and latitudinal distributional changes observed across taxa (Parmesan & Yoho 2003, Parmesan 2006), and these distributional changes may result in increasing isolated and fragmented populations. Isolated populations are more vulnerable to extinction for several reasons including both intrinsic effects such as genetic drift and extrinsic factors such as disease or habitat destruction (Sih et al. 2000, Macallum & Dobson 2002, Gaggiotti & Hanksi 2004). Ditto and Frey (2007) found a positive relationship with habitat island size and genetic variation and a negative relationship with island isolation and genetic variation for two species of chipmunk (the least chipmunk; *T. minimus*, *T. alpinus'* sister species) and the Colorado chipmunk (*T. quadrivatturus*). Testing various habitat-loss scenarios under climate change, they found that models predicted decreases in genetic variation (polymorphism, heterozygosity and allelic diversity) due to global warming and warned that erosion of genetic diversity for such populations should be a major conservation concern (Ditto and Frey 2007). Our study represents perhaps the first to document such erosion of genetic diversity due to climate change in a terrestrial mammal. This elevational range shift and apparent decrease in relative abundance of *T. alpinus* is not limited to the study area, but has also been observed in the Southern Sierra Nevada where the Grinnell Resurvey project is ongoing (Patton, Rowe, et al. unpublished data). As the climate warms, this endemic mountain species is likely to retract its range further up in elevation, becoming more and more isolated over time and its long-term persistence is threatened. I feel that the genetic integrity of species needs to be considered in conservation and climate adaptation management plans to ensure the preservation of genetic diversity, connectivity and ultimately the long term evolutionary potential of species' under threat of range shifts due to climate change.

Table 1. Primer name, sequences, repeat motif, PCR product range, annealing temperature (T_a, ºC), and number of alleles for *T. alpinus* (T.alp) and *T. speciosus* (T.spec) pooled across eras.

Primer	Sequence or Reference	Repeat Motif	T_a	Range	No. Alleles *T. alp*	No. Alleles *T. spec*
Microsatellite Primers						
Eu26	Schulte-Hostedde et al 2000	$(CA)_{20}$	55	140-200	13	12
Eu37	Schulte-Hostedde et al 2000	$(GA)_{17}$	55	120-160	12	11
Eu41	Schulte-Hostedde et al 2000	$(GT)_{16}$	55	120-160	10	9
Eu86	Schulte-Hostedde et al 2000	$(AC)_{21}$	55	140-180	9	9
Eu94	Schulte-Hostedde et al 2000	$(GT)_{14}$	51	90-140	7	9
AC A2	F 5'-ACA TCC ACT TTA CTC CCC A AC TC-3'	$(TTTC)_9(TAC)_8$	60	240-340	*	*
	R 5' AGA GGC TAT GTC AGG AGG ATC'3					
AC B111	F 5'- AAT ACC TAT CCA CCC ATC ATC -3'	$(GTCT)_8(CT)_6(CA)_{16}$	57	230-245	*	*
	R 5' TTC CCT CAA GCA GTC ATA AG -3'					
AC B12	F 5'-AAA GGA ACC CTC TAC ACC AC -3'	$(TG)_{11}(TCTG)_7$	58	250-300	*	*
	R 5'-ACC GAA ACC ACT CAG AAC AG -3'					
AC A101	F 5' CTA GCC TCA GCC ACT CAG TG -3'	$(TAG)_{10}(AAT)_6$	58	120-190	8	7
	R 5'- GGT TCT ACC TTT GAG CCA CAC -3'					
AC A108	F 5'- CTG CTG CCT CTT CTT CTT C-3'	$(TAC)_{18}$	60	250-280	*	*
	R 5' CCC ATA ATC CCA GTG ACT C- 3'					
AC C2	F 5' CTG GGA ACA TAG TCT TTA GGC -3'	$(ATCC)_8$	60	110-160	*	*
	R 5' GTG AAT GGG CAG ATG ATT AG -3'					
AC D115	F 5' CTT TGC CAC ATC TGA CTT G -3'	$(TCTA)_9$	58	160-200	8	7
	R 5' ACT GGG TAG ACT GAG AAA TAC G -3'					
AC D107	F 5' TAA TTC AAC TCT GGG AAC ACT G -3'	$(TATC)_{10}$	57	170-200	*	*
	R 5' GAA AGG AGC CTA AGC TGT TG -3'					
AC C122	F 5' CCC ATC CAT CTA TCT GTT CAT C-3'	$(TA)_3TT(TCCA)_9$	58	100-190	*	*
Tamias specific cytochrome b primers		**Product size (bp)**				
TAMH4F	ATG ACA AAC ATC CGC AAA AC					
TAMH3R	TCC GTT AGC GTG TAT GTA TCG	239	50			
TAMH5F	CCG ATA CAT ACA CGC CAA CG					
TAMH2R	TCC TCA TGG GAG AAC GTA GC	171	50			
TAMH6F	GCT ACG TTC TCC CAT GAG GA					
TAMH6R	TCC TTG GAG ACC CAG ACA AC	390	50			

* primer sets that were screened but not used in final analyses due to poor amplification success in the historical samples or the presence of null alleles

Table 2. *Tamias alpinus* sampling localities, the elevational range the species was detected, and sample sizes for microsatellite analysis (Msat *N*) and the mitochondrial analysis (cytb *N*) for each locality in both the historical (H) and modern (M) time period.

Historical Locality	**Elevational range detected (m)**	**Msat N (cytb N)**	**Paired Modern Locality**	**Elevational range detected (m)**	**Msat N (cytb N)**
Mt. Hoffman/Ten Lakes	2438-3246	16 (3)	Mt. Hoffman/ Ten Lakes	2935 - 2959	11 (6)
Tuolumne Meadows	2377 - 3002	19 (4)	Young Lakes	3054	18 (9)
			Upper Cathedral Lakes	2984	12 (6)
East YNP	2895 - 3048	8 (3)	East YNP	2952 - 3165	8 (8)
			Mono Pass	3131-3252	23 (17)
Upper Lyell Canyon	2743 - 3352	16 (2)	Upper Lyell Canyon	3121 - 3277	33 (31)
Vogelsang Lake	2804 - 3200	9 (2)	Vogelsang Lake	3024 - 3170	14 (10)
Mt. Clark	3048	6 (1)	Clark Range/ Ottoway Lakes	2970	27 (7)
Mitochondrial Analysis only					
Not sampled in past			Upper Return Creek	3013	2
Not sampled in past			Kerrick Meadow	2888	1

Table 3. *Tamias speciosus* sampling localities, the range of elevations that the species was detected at that site, and sample sizes for microsatellite analysis (Msat *N*) and the mitochondrial analysis (cytb *N*) for each locality in both the historical (H) and modern (M) time period.

Historical Locality	**Elevational range detected (m)**	**Msat *N* (cytb *N*)**	**Paired Modern Locality**	**Elevational range detected (m)**	**Msat N (cytb N)**
Crane Flat	1874 - 1920	2 (2)	Crane Flat	1886 -2106	23 (3)
Porcupine Flat	2459	12 (2)	Porcupine Flat	2572 - 2590	5 (4)
Glen Aulin	2377	4 (1)	Glen Aulin	2377 - 2491	10 (10)
Tuolumne Meadows	2621-3048	17 (2)	Tuolumne Meadows	2600 - 2823	18 (6)
Mono Meadow	2225	5 (2)	Mono Meadow	2350 - 2159	14 (2)
Vogelsang	2834-2956	3 (1)	Vogelsang	3160	4 (4)
Merced Lake	2225	4 (1)	Merced Lake	2199 - 2266	21 (4)
Upper Lyell Canyon	2743-3291	12 (2)	Upper Lyell Canyon	2988 - 3121	20 (8)
Mitochondrial Analysis only					
Not sampled			Upper Return Creek	3013	2
Not sampled			Kerrick Meadow	2888	1

Table 4. Observed heterozygosity (H_o), gene diversity (H_S), average allelic richness (A), average no. of private alleles per loci (P_A), haplotype diversity (H_d), nucleotide diversity (π), Tajima's D and Fu's F_S, for historical and modern *T. alpinus* and *T. speciosus* datasets. A and P_A have been corrected for differences in samples size between the eras. Standard error (SE) is shown where applicable.

Species	**Era**	**H_s (SE)**	***A***	***P_A***	**H_d (SE)**	**π (SE)**
T. alpinus	H	0.75 (0.023)	9.23	3	0.638 (0.024)	0.0112 (0.003)
	M	0.71 (0.023)	7.27	0.89	0.666 (0.0028)	0.0118 (0.003)
T. speciosus	H	0.76 (0.032)	7.56	0.83	0.182 (0.043)	0.0002 (0.000)
	M	0.75 (0.041)	8.12	1.39	0.52 (0.015)	0.001 (0.004)

*Significant values P<0.01

Table 5a. Pair-wise F_{ST} (corrected for null alleles) for the historical *T. alpinus* dataset. Values in bold indicate values significantly greater than zero ($p < 0.05$) after bootstrapping.

Locality	MtH/Ten Lakes	Tuolumne Meadows	Ellery Lake	Upper Lyell	Vogelsang
Tuolumne Meadows	0.009285				
Ellery Lake	-0.000900	0.013958			
Upper Lyell	0.012010	**0.019462**	0.061164		
Vogelsang	-0.001325	**0.046783**	0.010877	0.061619	
Mt. Clark	0.034775	0.038961	0.036932	0.070186	0.058056

Table 5b. Pair-wise F_{ST} for the modern *T. alpinus* dataset. Values in bold indicate values significantly greater than zero ($p < 0.05$) after bootstrapping.

Locality	MtH/Ten Lakes	Young Lakes	Cathedral Lakes	Ellery Lakes	Mono Pass	Upper Lyell	Vogelsang
Young Lakes	0.048140						
Cathedral Lakes	0.038905	**0.097905**					
Ellery Lakes	**0.087404**	**0.070553**	**0.114917**				
Mono Pass	**0.114072**	**0.073456**	**0.118256**	**0.061982**			
Upper Lyell	**0.068075**	**0.064080**	**0.099405**	**0.039359**	**0.072141**		
Vogelsang	**0.064746**	**0.080454**	**0.087316**	**0.054547**	**0.109947**	0.016560	
Ottoway Lakes	**0.119395**	**0.151391**	0.095501	**0.055986**	**0.126989**	**0.083345**	**0.090617**

Table 6a. Pair-wise F_{ST} (corrected for null alleles) for historical *T. speciosus* dataset. Values in bold indicate values significantly greater than zero ($p < 0.05$) after bootstrapping.

Locality	Crane Flat	Upper Lyell Canyon	Vogelsang	Mono Meadow	Tuolumne Meadow	Glen Aulin	Merced Lake
Upper Lyell Canyon	0.0165						
Vogelsang	0.073	-0.001					
Mono Meadow	-0.003	0.046	0.116				
Tuolumne Meadow	0.005	0.009	0.051	0.000			
Glen Aulin	0.071	0.069	**0.071**	0.116	0.082		
Merced Lake	0.044	0.024	0.100	0.027	0.038	0.148	
Porcupine Flat	-0.012	0.007	0.041	-0.005	-0.015	0.075	0.022

Table 6b. Pairwise F_{ST} for *T. speciosus* modern dataset. Values in bold indicate values significantly greater than zero ($p < 0.05$) after bootstrapping.

Locality	Crane Flat	Upper Lyell Canyon	Vogelsang	Mono Meadow	Tuolumne Meadow	Glen Aulin	Merced Lake
Upper Lyell Canyon	0.026						
Vogelsang	0.034	0.022					
Mono Meadow	0.016	0.013	0.024				
Tuolumne Meadow	**0.064**	0.046	0.071	**0.071**			
Glen Aulin	0.013	0.019	0.034	-0.005	**0.034**		
Merced Lake	0.007	0.000	0.025	0.026	0.065	0.092	
Porcupine Flat	-0.002	0.009	0.045	-0.019	**0.085**	-0.026	0.020

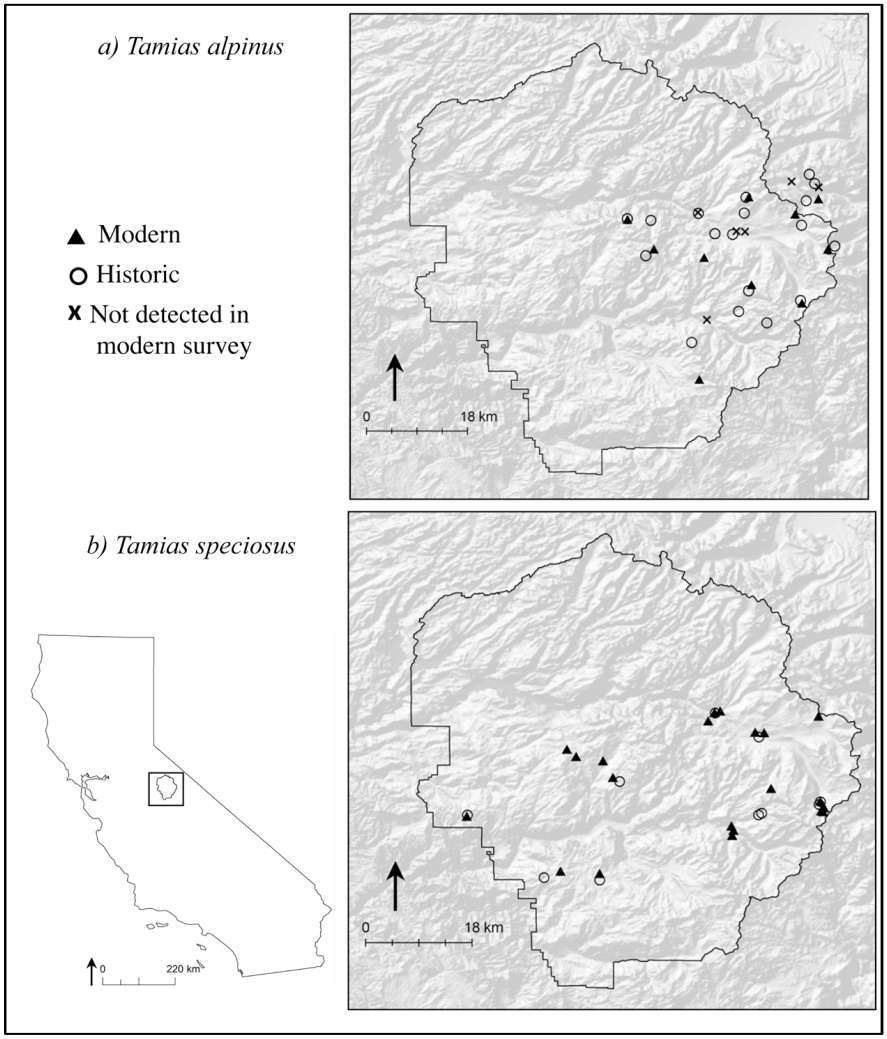


Figure 1. Study area and sampling localities. Historical sampling localities (1915-1916) are shown in open circles and modern in black triangles (2003-2008) for a) *Tamias alpinus* and b) *Tamias speciosus*. Black crosses show sites that were sampled repeatedly in the present era but species was not detected (probability of false absence at these sites is <10% see Moritz et al. 2008 for more details). Inset shows state of California with the box around study area.

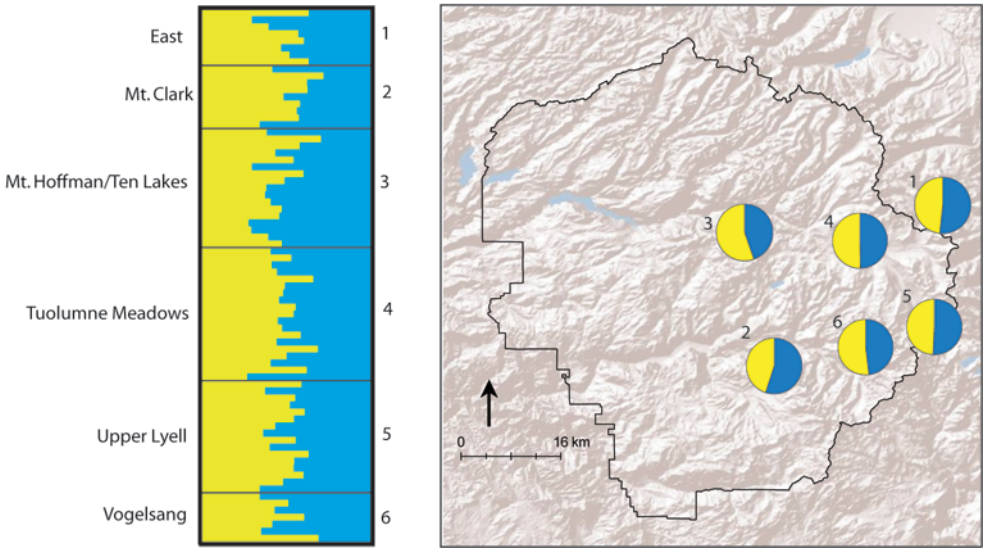


Figure 2a. Bayesian analysis of the nuclear genetic structure of historical *T. alpinus* populations based on seven microsatellite markers. Each individual is represented by a thin horizontal line in bar graph on the left, which is partitioned into colored segments that indicate the individuals' membership in one of two clusters. Locality names to the left of the bar graph and numbers on the right correspond to numbered pie charts on the map. Pie charts represent the sum of all individuals' membership in each cluster at each locality.

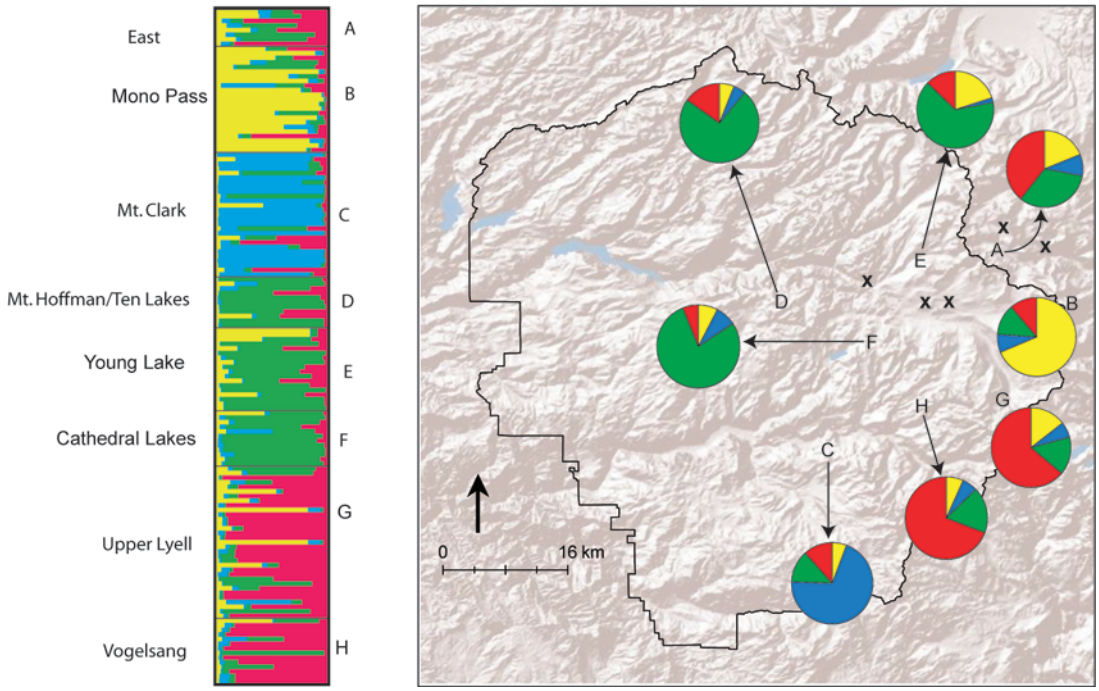


Figure 2b. Bayesian analysis of the nuclear genetic structure of modern *T. alpinus* populations based on seven microsatellite markers. Each individual is represented by a thin horizontal line in bar graph on the left, which is partitioned into colored segments that indicate the individuals' membership in one of four clusters. Locality names to the left of the bar graph and numbers on the right correspond to numbered pie charts on the map. Pie charts represent the sum of all individuals' membership in each cluster at each locality. Pie charts were enlarged in order to visualize segments clearly and arrows indicate which pie chart is associated with each site.

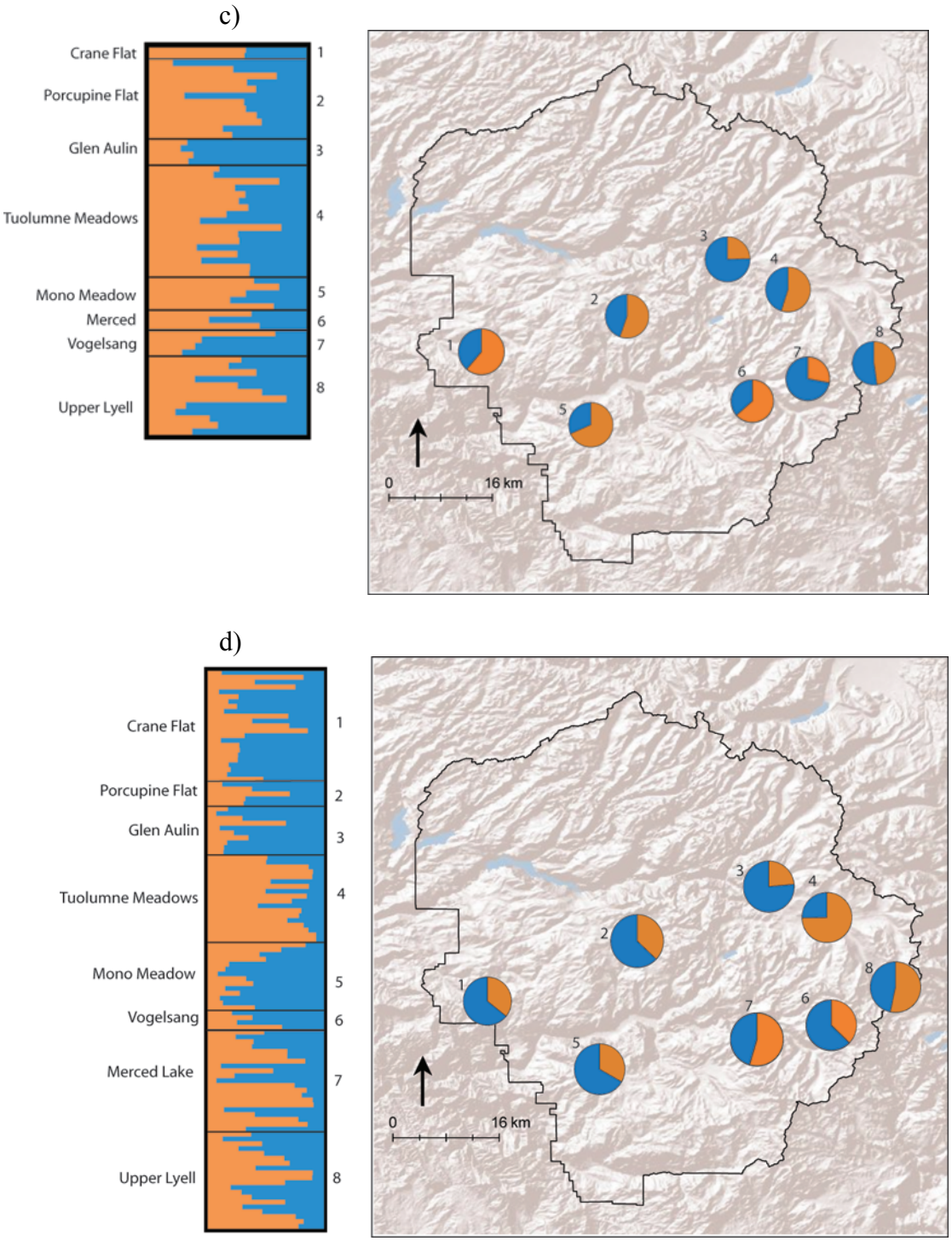
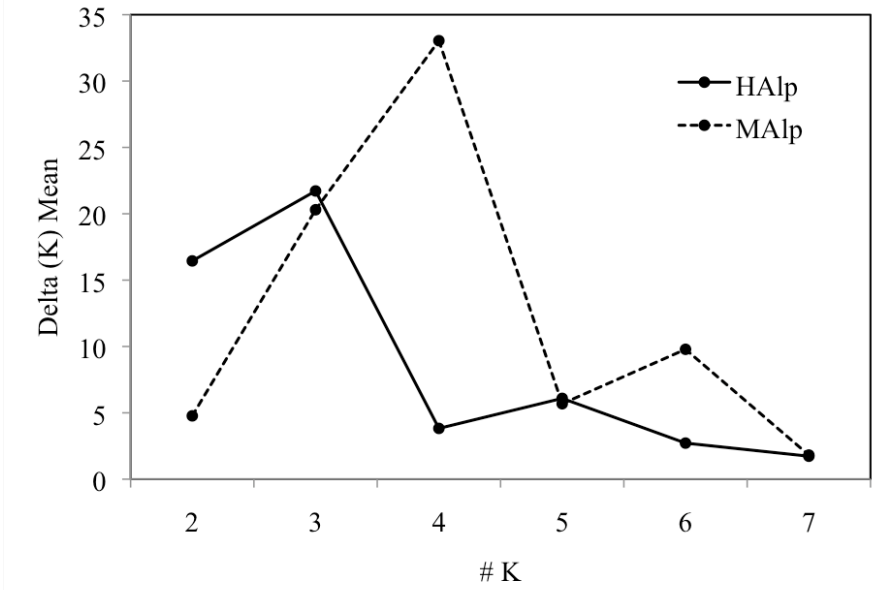


Figure 2c & d. Bayesian analysis of the nuclear genetic structure of historical (c) and modern (d) *T. speciosus* populations based on seven microsatellite markers. Each individual is represented by a thin horizontal line in bar graph on the left, which is partitioned into colored segments that indicate the individuals' membership in one of two clusters. Locality names to the left of the bar graph and numbers on the right correspond to numbered pie charts on the map. Pie charts represent the sum of all individuals' membership in each cluster at each locality.

a)



b)

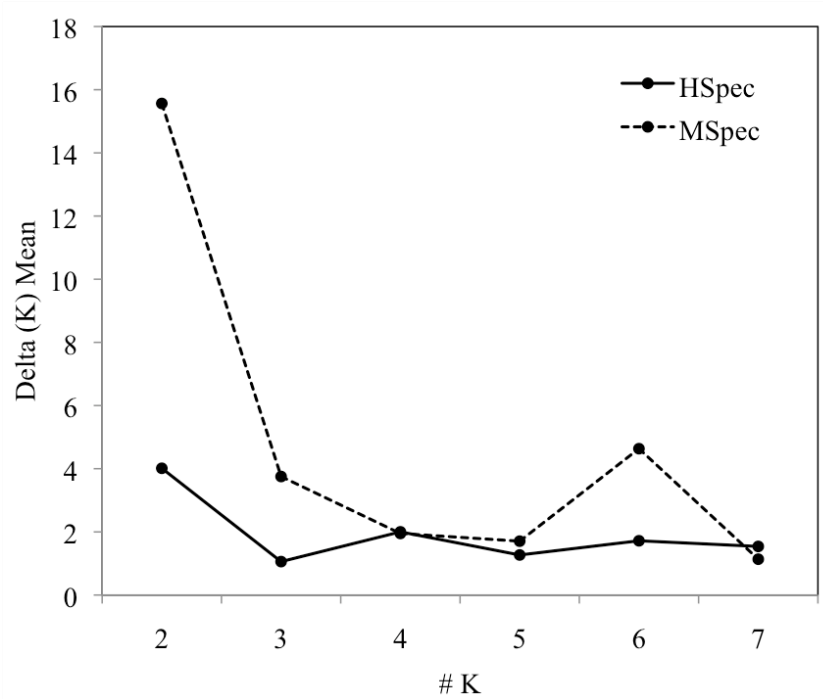
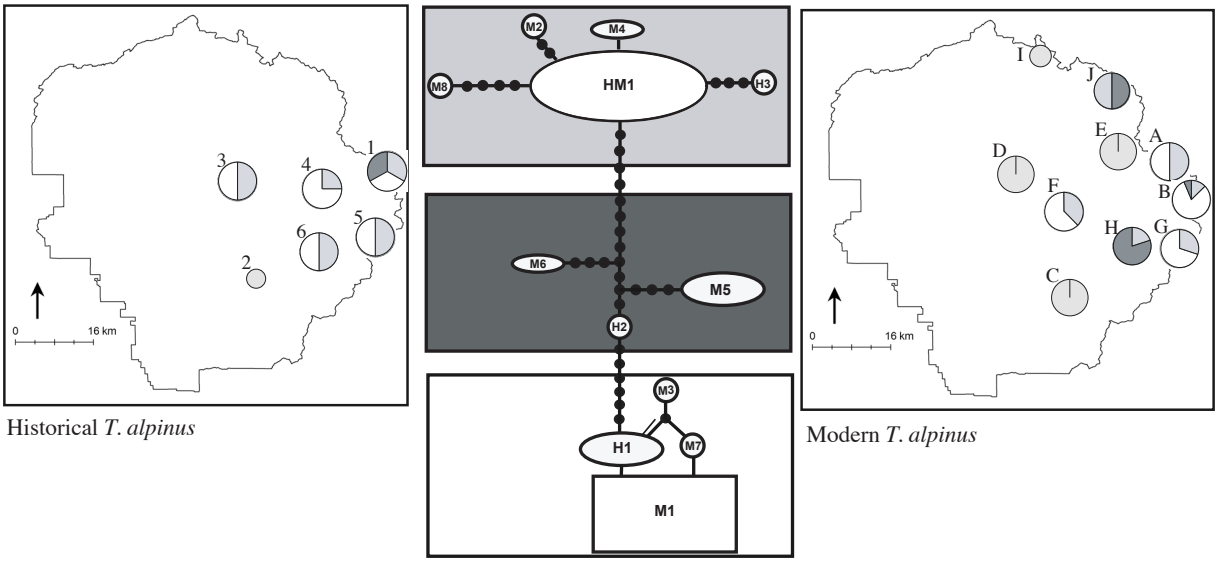


Figure 3. Estimating population structure with the Δ*K* method (Evanno et al 2005) where the highest Δ*K* value represents the true number of clusters. a) historical *T. alpinus* dataset (HAlp – black line) and the modern *T. alpinus* dataset (MAlp – dashed line). The K for HAlp is 2 and 4 for MAlp. b) historical *T. speciosus* dataset (HSpec – black line) and the modern *T. speciosus*

dataset (MSpec – dashed line). The estimated K for both *T. speciosus* datasets equals 2.



Historical *T. alpinus*

Modern *T. alpinus*

Figure 4. Haplotype network of cytochrome b gene in historical and modern *T. alpinus* samples from the past (left) and the present (right) localities in the study area. Pie charts represent the proportion of haplotype group shown with the corresponding color in the network. "HM" haplotypes are shared between historical and modern datasets; "H" haplotypes were found only in the historical dataset and "M" haplotypes only in the modern dataset. Circles represent unique haploytypes whereas the size of the ovals and rectangle indicate the frequency of respective haplotype. Sample size and locality elevation shown in Table 2; 1. East-YNP, 2. Mt. Clark, 3. Mt. Hoffman/Ten Lakes, 4. Tuolumne Meadows, 5. Upper Lyell Canyon, 6. Vogelsang Lake; A. East-YNP, B. Mono Pass, C. Clark Range (Ottoway Lakes), D. Mt. Hoffman/Ten Lakes, E. Young Lake, F. Upper Cathedral Lake, G. Upper Lyell Canyon, H. Vogelsang Lake, I. Kerrick Meadow, J. Upper Return Creek.

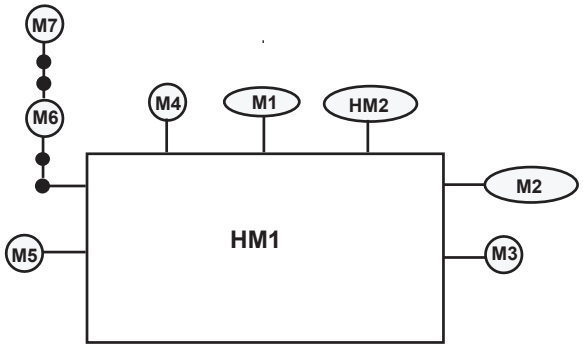
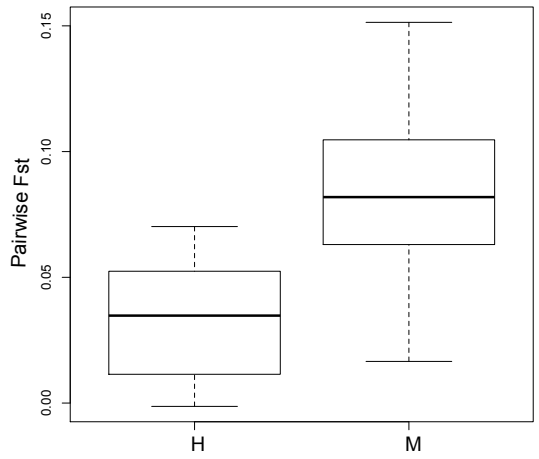


Figure 5. Haplotype network of cytochrome b gene in historical and modern samples of *T. speciosus.* "HM" haplotypes are shared between historical and modern datasets; "H" haplotypes were found only in the historical dataset and "M" haplotypes only in the modern dataset. Circles represent unique haploytypes whereas the size of the ovals and rectangle indicate the frequency of haplotype. Map not shown as very little genetic or geographic structure observed.

a)



b)

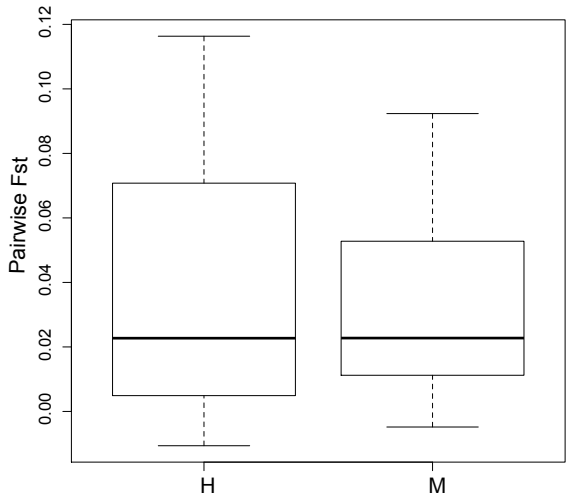


Figure 6. Boxplot comparing average pairwise F_{ST} values between a) *T. alpinus* populations across study area and b) *T. speciosus* populations across study area in the past (H – historical) and the present (M- Modern).

CHAPTER 5

General Conclusions

Human-induced climate change is expected to have extreme consequences for biodiversity and ecosystem integrity (Thomas et al. 2004, Parmesan 2006). There is an urgent need for scientists to improve understanding of how climate change will affect not only species distribution and abundance but also genetic diversity, which is the basis for evolutionary potential and thus critical to long-term persistence (Reed & Frankham 2003). Assessing species changes relative to historical data is a powerful way to improve such understanding, yet such baseline data are rarely available. Fortunately, Joseph Grinnell, founding director of the Museum of Vertebrate Zoology at the University of California, Berkeley, had the foresight to create a valuable baseline for the vertebrate fauna of California (Grinnell 1910). Grinnell and colleagues left a rich legacy of detailed field notes, photographs and museum specimens for hundreds of species distributed across the state in the early 20th century, doing so specifically to facilitate future assessments of the effects of environmental change. In this dissertation, I capitalized on this legacy, using matched historical and contemporary data on chipmunk species s in California's Sierra Nevada to examine distributional and genetic changes and their drivers over the past century. In addition, I examined the evolutionary history of Alpine Chipmunk (*Tamias alpinus*) to gain a more complete understanding of underlying processes that led to the origin of this range-restricted alpine species which is endemic to Sierra Nevada, CA.

Summary of key findings

Diversification within western chipmunks (genus *Tamias*) has been linked to changes in geographic ranges through climatic cycles and to the species' strong association with distinct ecological communities partitioned by habitat preferences and interspecific competition (Johnson 1943, Heller 1971, Hoffman et al. 1981, Chappell 1978). In the first study (Chapter 2) I contributed to the literature investigating the evolution of western chipmunks through my examination of the divergence history between *T. alpinus* and western populations of *T. minimus* in Sierra Nevada, California. I employed mitochondrial (cytochrome b) and nuclear (microsatellites) genetic analyses to address this question. The mitochondrial analysis provided a historical view of lineage divergence whereas the microsatellite analysis provided a more contemporary view and enabled me to examine the population genetic structure within species. I found that *T. alpinus* and *T. minimus* populations share mitochondrial haplotypes with no overall geneaological separation, and that diversity at this locus is better explained by geography than by species' boundaries. Two main processes could have produced this pattern: 1) recent speciation of *T. alpinus* from *T. minimus* with retention of geographically structured ancestral polymorphism, or 2) historical and/or contemporary hybridization between species. In contrast to the mitochondrial results, the microsatellite analysis showed that although highly differentiated populations within species exist, populations of the same species are more similar to each other than they are to members of the other species. This result revealed that the two species are distinct and there is no contemporary introgression along their parapatric boundary. Coalescent analysis of divergence history indicated late Pleistocene divergence and subsequent, though limited, gene flow between the two lineages. From these results I concluded that these two species diverged and hybridized during the climatic cycles of the late Pleistocene, and at present there is no ongoing hybridization or introgression.

Species distribution models are commonly used to predict species responses to climate change (e.g., Thomas et al. 2004, Thuiller et al. 2005, Lawler et al. 2006). However, their usefulness in conservation planning and policy is controversial because they are difficult to validate across time and space (Pearson et al. 2003, Araujo et al. 2005a). In my second study (Chapter 3) I used the small mammal surveys repeated over a century in Yosemite National Park (YNP) to assess accuracy of model predictions. Historical (1900-1940) climate, vegetation and species occurrence data were used to develop single-species and multi-species multivariate adaptive regression spline (MARS) distribution models for three species of chipmunk (*T. alpinus*, *T. senex* and *T. speciosus*). Models were projected onto the current (1980-2007) environmental surface and then tested against modern field resurveys of each species. I found that climate alone was the dominant predictor explaining the distribution of the study species within a time period. However, climate was not consistently an adequate predictor of the distributional change observed in all three species' across time. The models showed good predictive power for two out of three study species. For *T. alpinus*, I found that climate alone appeared to drive its observed elevational upslope range contraction. Results for *T. senex* suggested that both warming and vegetation change were likely responsible for this species' distributional collapse in YNP. By contrast, observed temporal stability in the distribution of the third species, *T. speciosus*, was not predicted by the models. These results demonstrated that correlative distribution models are useful in understanding species' potential responses to environmental change, but also showed how changes in species-environment correlations through time can limit predictive performance.

Predicting changes in distribution is a mainstay of investigating climate change impacts (e.g., Thomas et al. 2004), yet surprisingly little empirical work has endeavored to quantify how such changes impact genetic diversity within affected populations (and thus their capacity for evolutionary adaptation). In the final chapter (Chapter 4), I investigated how the climate-induced range contraction observed in *T. alpinus* over the past century has affected the species' overall diversity and population genetic structure in Yosemite National Park. Specifically, I used one mitochondrial and seven microsatellite loci, amplified from both historical and modern specimens, to compare genetic structure of *T. alpinus* with that of *T. speciosus*, whose distribution remained stable over time. Comparison with the relatively stable, largely co-distributed species allowed me to attribute changes seen in *T. alpinus* to effects of climate-driven distributional contraction. The genetic correlations of the contraction met my predictions as follows: a comparison of past and present populations of *T. alpinus* showed a decline in overall genetic diversity and a reduction of gene flow between local populations over time. As expected given its relative stability through time, there were no significant genetic changes seen in *T. speciosus*. This study is perhaps the first to document the erosion of genetic diversity due to climate change in a terrestrial mammal species.

Directions for future work

There are many potential avenues of future work to follow this research. Although I have provided a preliminary examination of the evolutionary history of *T. alpinus*, understanding of the complex relationship between *T. alpinus* and its sister taxa (*T. minimus*) would be enhanced by the addition of more genetic markers (e.g., nuclear gene sequences), targeted sampling of *T. alpinus* in between its northern and southern range boundaries, and analysis of morphological differences between species.

In regards to the species distribution modeling chapter, there are several follow up studies that would greatly improve our understanding of recently observed range dynamics in chipmunks, including *T. alpinus*. One obvious avenue is behavior: a better understanding of the role of species' interactions, specifically interspecific competition, in setting the distributional boundary between *T. alpinus* and *T. speciosus* is needed. Although my modeling approach included multi-species models, it did not explicitly incorporate underlying mechanisms like competition into the models. Another avenue for future work is to investigate the habitat requirements of these species, including diet and microclimate analyses. A comparison of diet from the past to the present using isotope analyses will allow us to better understand any diet shifts associated with observed range changes over time. Lastly, expanding the spatial scale of distribution modeling to include the entire range of the study species (including recently resurveyed sites in the southern Sierras), as well as comparing different modeling methods (e.g, Boosted Regression Trees, Maxent etc, reviewed in Elith et al. 2006), may enhance model accuracy, particularly for species in which my modeling approach failed.

Research examining the effects of climate-induced range shifts on genetic diversity and population genetic structure could be expanded to include the entire range of *T. alpinus* once the Grinnell resurvey is complete. In addition, similar studies on other species have apparently undergone range changes within the study area would be useful for testing the generality of my results. For example, both Belding's Ground Squirrel (*Urocitellus beldingi*) and Golden-Mantled Ground Squirrel (*Callospermophilus lateralis*) appear to have contracted their lower elevational limit in the Yosemite area (Moritz et al. 2008). The large number of museum specimens collected in the original Grinnell survey at each locality, and the resurvey of these sites including tissue collection, will allow comparative genetic studies such as the one presented here. The comparison of genetic effects of distributional changes across the small mammal community should strengthen inference on the genetic effects of climate-induced habitat fragmentation.

Overall, my dissertation contributes to existing research on the diversification, climate change impacts, and conservation genetics of small mammals. Most importantly, this work exemplifies the value of natural history museums, and the use of historically collected data in contemporary biodiversity research.

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Appendix for Chapter 2: A multilocus analysis of the evolutionary history of Alpine Chipmunk, *Tamias alpinus*, in Sierra Nevada California

Appendix 1

Table A1. ID/Catalogue numbers of all samples sequenced at cytochrome b. Summarized information includes location information, haplotype number, network group, clade and whether or not sample was included in microsatellite analysis. Samples from museum skins were not run with the microsatellites because only a subset of microsatellite (7) amplify the historical samples. (Table on following page

ID/Catologue No.	Species	Location	Lattitude	Longnitude	Msat	Haplotype	Network	Clade
14931	*T. alpinus*	W slope Olancha Peak, Sierra Nevada	36.26623	-118.11771	No	Hap_16	B	RED
14932	*T. alpinus*	W slope Olancha Peak, Sierra Nevada	36.26623	-118.11771	No	Hap_17	B	RED
14933	*T. alpinus*	W slope Olancha Peak, Sierra Nevada	36.26623	-118.11771	No	Hap_18	B	RED
224075	*T. alpinus*	Muir Lake, Cottonwood Lakes Basin	36.49873	-118.20772	Yes	Hap_28	C	RED
224076	*T. alpinus*	Muir Lake, Cottonwood Lakes Basin	36.49873	-118.20772	Yes	Hap_29	N/A	BLACK
224077	*T. alpinus*	Muir Lake, Cottonwood Lakes Basin	36.49873	-118.20772	Yes	Hap_21	B	RED
224078	*T. alpinus*	Muir Lake, Cottonwood Lakes Basin	36.49873	-118.20772	Yes	Hap_21	B	RED
14973	*T. alpinus*	Cottonwood Lakes, Sierra Nevada Mts.	36.49835	-118.21991	No	Hap_17	B	RED
14903	*T. alpinus*	Cirque Peak, Sierra Nevada	36.47629	-118.23710	No	Hap_13	B	RED
14904	*T. alpinus*	Cirque Peak, Sierra Nevada	36.47629	-118.23710	No	Hap_13	B	RED
14908	*T. alpinus*	Whitney Meadow, Sierra Nevada	36.43413	-118.26710	No	Hap_14	B	RED
14909	*T. alpinus*	Whitney Meadow, Sierra Nevada	36.43413	-118.26710	No	Hap_15	C	RED
206401	*T. alpinus*	Siberian Outpost, Sequoia National Park	36.46957	-118.27374	Yes	Hap_22	B	RED
206403	*T. alpinus*	Siberian Outpost, Sequoia National Park	36.4685	-118.27449	Yes	Hap_24	B	RED
206402	*T. alpinus*	Siberian Outpost, Sequoia National Park	36.46895	-118.27458	Yes	Hap_23	C	RED
206400	*T. alpinus*	Siberian Outpost, Sequoia National Park	36.46829	-118.2748	Yes	Hap_21	B	RED
17576	*T. alpinus*	Kearsarge Pass, Onion Valley, Sierra Nevada	36.77450	-118.32419	No	Hap_19	B	RED
206398	*T. alpinus*	Kearsarge Lakes, Kings Canyon National Park	36.77289	-118.37895	Yes	Hap_20	N/A	GREEN
206397	*T. alpinus*	Kearsarge Lakes, Kings Canyon National Park	36.7724	-118.3834	Yes	Hap_21	B	RED
206396	*T. alpinus*	Kearsarge Lakes, Kings Canyon National Park	36.7713	-118.3857	Yes	Hap_20	N/A	GREEN
206399	*T. alpinus*	Kearsarge Lakes, Kings Canyon National Park	36.77128	-118.38596	Yes	Hap_18	B	RED
224480	*T. alpinus*	Bullfrog Lake, Kings Canyon National Park	36.769664	-118.401769	Yes	Hap_20	N/A	GREEN
224481	*T. alpinus*	Bullfrog Lake, Kings Canyon National Park	36.769664	-118.401769	Yes	Hap_21	B	RED
224482	*T. alpinus*	Bullfrog Lake, Kings Canyon National Park	36.769664	-118.401769	Yes	Hap_25	N/A	GREEN
224483	*T. alpinus*	Bullfrog Lake, Kings Canyon National Park	36.773289	-118.402803	Yes	Hap_26	-	RED
224484	*T. alpinus*	Bullfrog Lake, Kings Canyon National Park	36.773289	-118.402803	Yes	Hap_27*	-	RED
222200	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
222203	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_7*	B	RED
JLP24301	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
JLP24302	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
JLP24310	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T174	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T175	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T180	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T184	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T191	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_3*	D	BLUE
T195	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T196a	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T196b	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T197a	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
T197b	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED

ID/Catologue No.	Species	Location	Lattitude	Longnitude	Msat	Haplotype	Network	Clade
T198	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_3*	D	BLUE
T38	*T. alpinus*	Mono Pass	37.85461	-119.21362	Yes	Hap_1	A	RED
EMR91	*T. alpinus*	Head of Ellery Lake	37.93635	-119.22753	Yes	Hap_1	A	RED
EMR90	*T. alpinus*	Head of Ellery Lake	37.93557	-119.22878	Yes	Hap_3*	D	BLUE
23335	*T. alpinus*	Warren Fork of Lee Vining Creek	37.96006	-119.23465	No	Hap_10	A	RED
23334	*T. alpinus*	near Ellery Lake, Tioga Rd.	37.93806	-119.23593	No	Hap_3*	D	BLUE
23338	*T. alpinus*	near Tioga Rd.	37.93248	-119.24846	No	Hap_11	D	BLUE
217179	*T. alpinus*	Upper Lyell Canyon, YNP	37.76458	-119.25213	Yes	Hap_1	A	RED
217180	*T. alpinus*	Upper Lyell Canyon, YNP	37.76458	-119.25213	Yes	Hap_1	A	RED
217181	*T. alpinus*	Upper Lyell Canyon, YNP	37.76458	-119.25213	Yes	Hap_1	A	RED
217182	*T. alpinus*	Upper Lyell Canyon, YNP	37.76458	-119.25213	Yes	Hap_2	D	BLUE
217183	*T. alpinus*	Upper Lyell Canyon, YNP	37.76458	-119.25213	Yes	Hap_1	A	RED
217184	*T. alpinus*	Upper Lyell Canyon, YNP	37.76458	-119.25213	Yes	Hap_1	A	RED
217185	*T. alpinus*	Upper Lyell Canyon, YNP	37.76458	-119.25213	Yes	Hap_1	A	RED
EMR37	*T. alpinus*	Upper Lyell Canyon , YNP	37.76458	-119.25213	Yes	Hap_3*	D	BLUE
EMR32	*T. alpinus*	Upper Lyell Canyon, YNP	37.76595	-119.25273	Yes	Hap_2	D	BLUE
EMR38	*T. alpinus*	Upper Lyell Canyon, YNP	37.76612	-119.25287	Yes	Hap_4	D	RED
EMR33	*T. alpinus*	Upper Lyell Canyon, YNP	37.76405	-119.25298	Yes	Hap_1	A	RED
216270	*T. alpinus*	Upper Lyell Canyon, YNP	37.76807	-119.25506	Yes	Hap_3*	D	BLUE
217178	*T. alpinus*	Upper Lyell Canyon, YNP	37.76807	-119.25506	Yes	Hap_1	A	RED
217186	*T. alpinus*	Upper Lyell Canyon, YNP	37.76807	-119.25506	Yes	Hap_1	A	RED
EMR36	*T. alpinus*	Upper Lyell Canyon, YNP	37.76417	-119.25540	Yes	Hap_3*	D	BLUE
EMR35	*T. alpinus*	Upper Lyell Canyon, YNP	37.76415	-119.25542	Yes	Hap_1	A	RED
EMR31	*T. alpinus*	Upper Lyell Canyon, YNP	37.76397	-119.25582	Yes	Hap_1	A	RED
EMR34	*T. alpinus*	Upper Lyell Canyon, YNP	37.76390	-119.25598	Yes	Hap_1	A	RED
EMR29	*T. alpinus*	Upper Lyell Canyon, YNP	37.76353	-119.25625	Yes	Hap_1	A	RED
EMR30	*T. alpinus*	Upper Lyell Canyon, YNP	37.76353	-119.25625	Yes	Hap_1	A	RED
EMR50	*T. alpinus*	Upper Lyell Canyon, YNP	37.76330	-119.25773	Yes	Hap_3*	D	BLUE
EMR39	*T. alpinus*	Upper Lyell Canyon, YNP	37.76228	-119.25847	Yes	Hap_3*	D	BLUE
217188	*T. alpinus*	Upper Lyell Canyon, YNP	37.75675	-119.25855	Yes	Hap_8	A	RED
EMR48	*T. alpinus*	Upper Lyell Canyon, YNP	37.76150	-119.25870	Yes	Hap_1	A	RED
EMR51	*T. alpinus*	Upper Lyell Canyon, YNP	37.76155	-119.25885	Yes	Hap_1	A	RED
EMR41	*T. alpinus*	Upper Lyell Canyon, YNP	37.76072	-119.25923	Yes	Hap_3*	D	BLUE
EMR45	*T. alpinus*	Upper Lyell Canyon , YNP	37.76072	-119.25923	Yes	Hap_3*	D	BLUE
EMR46	*T. alpinus*	Upper Lyell Canyon , YNP	37.76072	-119.25923	Yes	Hap_1	A	RED
217189	*T. alpinus*	Upper Lyell Canyon, YNP	37.76358	-119.25996	Yes	Hap_1	A	RED
EMR72	*T. alpinus*	Gaylor Lakes, YNP	37.91122	-119.26625	Yes	Hap_3*	D	BLUE
EMR85	*T. alpinus*	Gaylor Lakes, YNP	37.91062	-119.26761	Yes	Hap_3*	D	BLUE
EMR91b	*T. alpinus*	Gaylor Lakes, YNP	37.91062	-119.26761	Yes	Hap_3*	D	BLUE
EMR82	*T. alpinus*	Gaylor Lakes, YNP	37.91008	-119.26835	Yes	Hap_1	A	RED
EMR76	*T. alpinus*	Gaylor Lakes, YNP	37.90986	-119.26875	Yes	Hap_1	A	RED

ID/Catologue No.	Species	Location	Lattitude	Longnitude	Msat	Haplotype	Network	Clade
EMR81	*T. alpinus*	Gaylor Lakes, YNP	37.90912	-119.26948	Yes	Hap_1	A	RED
207203	*T. alpinus*	Evelyn Lake, YNP	37.80485	-119.32759	Yes	Hap_6*	-	RED
207204	*T. alpinus*	Evelyn Lake, YNP	37.80485	-119.32759	Yes	Hap_6*	-	RED
207205	*T. alpinus*	Evelyn Lake, YNP	37.80485	-119.32759	Yes	Hap_6*	-	RED
207206	*T. alpinus*	Evelyn Lake, YNP	37.80485	-119.32759	Yes	Hap_3*	D	BLUE
207207	*T. alpinus*	Evelyn Lake, YNP	37.80485	-119.32759	Yes	Hap_6*	-	RED
207199	*T. alpinus*	E end Fletcher Lake, YNP	37.79778	-119.33617	Yes	Hap_3*	D	BLUE
216271	*T. alpinus*	Upper Return Creek, Virginia Canyon, YNP	38.06129	-119.33899	Yes	Hap_3*	D	BLUE
216272	*T. alpinus*	Upper Return Creek, Virginia Canyon, YNP	38.06129	-119.33899	Yes	Hap_7*	B	RED
222201	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_3*	D	BLUE
222202	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_3*	D	BLUE
222206	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_3*	D	BLUE
JLP24337	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_3*	D	BLUE
JLP24338	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_3*	D	BLUE
JLP24345	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_3*	D	BLUE
JLP24365	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_3*	D	BLUE
JLP24373	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_9	D	BLUE
JLP24374	*T. alpinus*	Middle Young Lake, YNP	37.93876	-119.34047	Yes	Hap_3*	D	BLUE
207209	*T. alpinus*	Vogelsang Lake, YNP	37.78769	-119.3469	Yes	Hap_6*	-	RED
207210	*T. alpinus*	Fletcher Lake, YNP	37.78769	-119.3469	Yes	Hap_6*	-	RED
207211	*T. alpinus*	Outlet Creek, Vogelsang Lake, YNP	37.78769	-119.3469	Yes	Hap_6*	-	RED
219990	*T. alpinus*	Upper Cathedral Lake, YNP	37.84117	-119.41206	Yes	Hap_3*	D	BLUE
219991	*T. alpinus*	Upper Cathedral Lake, YNP	37.84117	-119.41206	Yes	Hap_1	A	RED
219992	*T. alpinus*	Upper Cathedral Lake, YNP	37.84117	-119.41206	Yes	Hap_1	A	RED
219993	*T. alpinus*	Upper Cathedral Lake, YNP	37.84117	-119.41206	Yes	Hap_1	A	RED
219994	*T. alpinus*	Upper Cathedral Lake, YNP	37.84117	-119.41206	Yes	Hap_3*	D	BLUE
219995	*T. alpinus*	Upper Cathedral Lake, YNP	37.84117	-119.41206	Yes	Hap_1	A	RED
219998	*T. alpinus*	Lower Ottaway Lake, YNP	37.64537	-119.41978	Yes	Hap_1	A	RED
220000	*T. alpinus*	Lower Ottaway Lake, YNP	37.64537	-119.41978	Yes	Hap_1	A	RED
220001	*T. alpinus*	Lower Ottaway Lake, YNP	37.64537	-119.41978	Yes	Hap_1	A	RED
220003	*T. alpinus*	Lower Ottaway Lake, YNP	37.64537	-119.41978	Yes	Hap_1	A	RED
220004	*T. alpinus*	Lower Ottaway Lake, YNP	37.64537	-119.41978	Yes	Hap_1	A	RED
220005	*T. alpinus*	Lower Ottaway Lake, YNP	37.64537	-119.41978	Yes	Hap_1	A	RED
220006	*T. alpinus*	Lower Ottaway Lake, YNP	37.64537	-119.41978	Yes	Hap_1	A	RED
216690	*T. alpinus*	Kerrick Meadow, YNP	38.11129	-119.4818	Yes	Hap_3*	D	BLUE
EMR69	*T. alpinus*	Mt. Hoffman; Northwest of May Lake, YNP	37.85449	-119.49239	Yes	Hap_3*	D	BLUE
EMR66	*T. alpinus*	Mt. Hoffman; Northwest of May Lake, YNP	37.85478	-119.49276	Yes	Hap_3*	D	BLUE
EMR71	*T. alpinus*	Mt. Hoffman; Northwest of May Lake, YNP	37.85548	-119.49289	Yes	Hap_3*	D	BLUE
23344	*T. alpinus*	Ten Lakes Trail above Ten Lakes, YNP	37.90135	-119.49807	No	Hap_10	A	RED
23345	*T. alpinus*	ridge near Ten Lakes, YNP	37.90135	-119.49807	No	Hap_12	-	RED
EMR62	*T. alpinus*	Ten Lakes Pass, YNP	37.90510	-119.53470	Yes	Hap_3*	D	BLUE

ID/Catologue No.	Species	Location	Lattitude	Longnitude	Msat	Haplotype	Network	Clade
EMR52	*T. alpinus*	Ten Lakes Pass, YNP	37.90290	-119.53505	Yes	Hap_3*	D	BLUE
EMR53	*T. alpinus*	Ten Lakes Pass, YNP	37.90290	-119.53505	Yes	Hap_5	D	BLUE
216306	*T. minimus*	Badger Flat	36.97099	-118.09535	Yes	Hap_27*	-	RED
216307	*T. minimus*	Badger Flat	36.97099	-118.09535	Yes	Hap_27*	-	RED
216308	*T. minimus*	Badger Flat	36.97099	-118.09535	Yes	Hap_27*	-	RED
216309	*T. minimus*	Badger Flat	36.97099	-118.09535	Yes	Hap_27*	-	RED
14976	*T. minimus*	Little Brush Meadow, Olancha Peak, Sierra Nevada	36.26623	-118.11771	Yes	Hap_41	C	RED
224487	*T. minimus*	Little Brush Meadow, Olancha Peak, Inyo National Forest	36.25424	-118.132301	Yes	Hap_30	C	RED
224485	*T. minimus*	Little Brush Meadow, Olancha Peak, Inyo National Forest	36.25278	-118.133606	Yes	Hap_30	C	RED
224486	*T. minimus*	Little Brush Meadow, Olancha Peak, Inyo National Forest	36.25278	-118.133606	Yes	Hap_31	C	RED
217106	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_27*	-	RED
217107	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_27*	-	RED
217111	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_27*	-	RED
217112	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_27*	-	RED
217113	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_27*	-	RED
217114	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_27*	-	RED
217116	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_27*	-	RED
217117	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_27*	-	RED
217118	*T. minimus*	N. Fork Crooked Creek	37.50348	-118.14757	Yes	Hap_44	-	RED
219907	*T. minimus*	south fork Cottonwood Creek, White Mts.	37.53101	-118.16508	Yes	Hap_27*	-	RED
219908	*T. minimus*	south fork Cottonwood Creek, White Mts.	37.53101	-118.16508	Yes	Hap_44	-	RED
219909	*T. minimus*	south fork Cottonwood Creek, White Mts.	37.53101	-118.16508	Yes	Hap_27*	-	RED
219910	*T. minimus*	south fork Cottonwood Creek, White Mts.	37.53101	-118.16508	Yes	Hap_27*	-	RED
219911	*T. minimus*	south fork Cottonwood Creek, White Mts.	37.53101	-118.16508	Yes	Hap_45	D	BLUE
222659	*T. minimus*	Broder/Monache Meadow	36.159921	-118.187395	Yes	Hap_36	C	RED
222660	*T. minimus*	Broder/Monache Meadow	36.159921	-118.187395	Yes	Hap_36	C	RED
222661	*T. minimus*	Broder/Monache Meadow	36.162908	-118.1914	Yes	Hap_37	C	RED
222662	*T. minimus*	Broder/Monache Meadow	36.162908	-118.1914	Yes	Hap_36	C	RED
222663	*T. minimus*	Broder/Monache Meadow	36.162908	-118.1914	Yes	Hap_37	C	RED
222664	*T. minimus*	Broder/Monache Meadow	36.162908	-118.1914	Yes	Hap_37	C	RED
222665	*T. minimus*	Broder/Monache Meadow	36.162908	-118.1914	Yes	Hap_37	C	RED
222666	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
222667	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
222668	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_38	C	RED
222669	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
222670	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
222671	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
222672	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_36	C	RED
222673	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
JAC399	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
JAC401	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_36	C	RED

ID/Catologue No.	Species	Location	Lattitude	Longnitude	Msat	Haplotype	Network	Clade
JAC403	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
JAC404	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
JAC405	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_38	C	RED
JAC412	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
JAC413	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_36	C	RED
JAC415	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
JAC417	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_36	C	RED
JAC418	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_36	C	RED
KCR487	*T. minimus*	Broder/Monache Meadow	36.175368	-118.205312	Yes	Hap_37	C	RED
217271	*T. minimus*	Silver Canyon	37.40205	-118.22488	Yes	Hap_27*	-	RED
221244	*T. minimus*	below Big Pine Creek Campground, Sierra Nevada, Inyo C	37.12927	-118.40589	Yes	Hap_47	-	RED
221274	*T. minimus*	Wier Lake, Sierra Nevada, Inyo Co.	37.17597	-118.56301	Yes	Hap_27*	-	RED
221275	*T. minimus*	Wier Lake, Sierra Nevada, Inyo Co.	37.17597	-118.56301	Yes	Hap_27*	-	RED
221260	*T. minimus*	South Lake, Sierra Nevada, Inyo Co.	37.17082	-118.56561	Yes	Hap_27*	-	RED
221263	*T. minimus*	South Lake, Sierra Nevada, Inyo Co.	37.17082	-118.56561	Yes	Hap_46	-	RED
221266	*T. minimus*	South Lake, Sierra Nevada, Inyo Co.	37.17082	-118.56561	Yes	Hap_47	-	RED
221270	*T. minimus*	South Lake, Sierra Nevada, Inyo Co.	37.17082	-118.56561	Yes	Hap_47	-	RED
221276	*T. minimus*	south end Adobe Valley, Mono Co.	37.792	-118.56752	Yes	Hap_48	-	RED
221277	*T. minimus*	south end Adobe Valley, Mono Co.	37.792	-118.56752	Yes	Hap_33	D	BLUE
221278	*T. minimus*	south end Adobe Valley, Mono Co.	37.792	-118.56752	Yes	Hap_34	D	BLUE
221247	*T. minimus*	South Fork Bishop Creek, Sierra Nevada, Inyo Co.	37.20996	-118.56908	Yes	Hap_27*	-	RED
221249	*T. minimus*	South Fork Bishop Creek, Sierra Nevada, Inyo Co.	37.20996	-118.56908	Yes	Hap_27*	-	RED
221251	*T. minimus*	South Fork Bishop Creek, Sierra Nevada, Inyo Co.	37.20996	-118.56908	Yes	Hap_27*	-	RED
221252	*T. minimus*	South Fork Bishop Creek, Sierra Nevada, Inyo Co.	37.20996	-118.56908	Yes	Hap_27*	-	RED
224150	*T. minimus*	Pine Creek, Sierra Nevada	37.36174	-118.69017	Yes	Hap_27*	-	RED
224151	*T. minimus*	Rock Creek, Sierra Nevada	37.44426	-118.74413	Yes	Hap_27*	-	RED
224152	*T. minimus*	Rock Creek, Sierra Nevada	37.44426	-118.74413	Yes	Hap_27*	-	RED
224153	*T. minimus*	Rock Creek, Sierra Nevada	37.44426	-118.74413	Yes	Hap_27*	-	RED
208328	*T. minimus*	Mono Mills	37.88811	-118.96021	Yes	Hap_40	-	RED
208329	*T. minimus*	Mono Mills	37.88811	-118.96021	Yes	Hap_40	-	RED
208330	*T. minimus*	Mono Mills	37.88811	-118.96021	Yes	Hap_33	D	BLUE
208331	*T. minimus*	Mono Mills	37.88811	-118.96021	Yes	Hap_40	-	RED
208332	*T. minimus*	Mono Mills	37.88811	-118.96021	Yes	Hap_7*	B	RED
202384	*T. minimus*	William's Butte	37.90753	-119.12848	Yes	Hap_33	D	BLUE
219225	*T. minimus*	William's Butte	37.90753	-119.12848	Yes	Hap_7*	B	RED
219226	*T. minimus*	William's Butte	37.90753	-119.12848	Yes	Hap_42	D	BLUE
219229	*T. minimus*	William's Butte	37.90753	-119.12848	Yes	Hap_40	-	RED
219230	*T. minimus*	William's Butte	37.90753	-119.12848	Yes	Hap_40	-	RED
219231	*T. minimus*	William's Butte	37.90753	-119.12848	Yes	Hap_40	-	RED
219232	*T. minimus*	William's Butte	37.90753	-119.12848	Yes	Hap_43	D	BLUE
219233	*T. minimus*	William's Butte	37.90753	-119.12848	Yes	Hap_40	-	RED

ID/Catologue No.	Species	Location	Lattitude	Longnitude	Msat	Haplotype	Network	Clade
208551	*T. minimus*	Bohler Creek	37.90028	-119.12977	Yes	Hap_6*	-	RED
208554	*T. minimus*	Bohler Creek	37.90028	-119.12977	Yes	Hap_39	B	RED
216299	*T. minimus*	Bohler Creek	37.90028	-119.12977	Yes	Hap_33	D	BLUE
216302	*T. minimus*	Bohler Creek	37.90028	-119.12977	Yes	Hap_33	D	BLUE
216303	*T. minimus*	Bohler Creek	37.90028	-119.12977	Yes	Hap_33	D	BLUE
216304	*T. minimus*	Bohler Creek	37.90028	-119.12977	Yes	Hap_40	-	RED
221279	*T. minimus*	Sawmill Canyon, Sierra Nevada, Mono Co.	37.87559	-119.14156	Yes	Hap_7*	B	RED
221280	*T. minimus*	Sawmill Canyon, Sierra Nevada, Mono Co.	37.87559	-119.14156	Yes	Hap_40	-	RED
224154	*T. minimus*	Swager Canyon, Sweetwater Mts.	38.38664	-119.34457	Yes	Hap_3*	D	BLUE
224140	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_32	D	BLUE
224141	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_3*	D	BLUE
224142	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_33	D	BLUE
224143	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_32	D	BLUE
224144	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_34	D	BLUE
224145	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_32	D	BLUE
224146	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_32	D	BLUE
224147	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_32	D	BLUE
224148	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_35	A	RED
224149	*T. minimus*	Sonora Pass	38.32799	-119.63721	Yes	Hap_32	D	BLUE

* haplotypes shared by both species
- no labeled network group; see Figure 4 in main text
N/A: haplotype more than 12 steps from network

Appendices for Chapter 3 (Appedix 2, 3, & 4): The role of climate, vegetation, and species co-occurrence as drivers of change in small mammal distributions over the past century.

Appendix 2: Comparison of available past and present interpolated climate layers with weather station climate data.

We conducted a simple analysis examining how well the interpolated climate surfaces used in our analyses recovered the weather station data collected from the appropriate timeframe. The climate surfaces we used in our analyses were developed using the Anusplin algorithm at 1 km^2 resolution from raw weather station data (Parra and Monahan, 2008). Recently, and after completion of the modeling for this study, a new finer resolution (~800 m^2) PRISM (parameter-elevation regression on independent slopes model) climate database became available through the PRISM Climate Research group (http://www.prism.oregonstate.edu/). Previously, PRISM was only available at 4x4 km resolution. For this comparison, we used the 800 m^2 PRISM data. These data are also derived from weather station data but using a different interpolation method. We calculated average MinT (minimum temperature of coldest month) from available weather stations for each available time periods (1900-1940 = historical; 1980-2007 = modern). We chose MinT for the comparison because it appeared to be an important predictor in many of our distribution models. We calculated the average MinT value for the pixel in which the weather station lies for the time period in which the data were collected to determine how well each of the interpolated climate surfaces could recover the weather station data (Table 1.). There are nine weather stations near the Yosemite study area. For six of the nine sites the Anusplin interpolated climate data correctly estimated the true weather station measured temperature within 0.5°C. At two sites, Anusplin overestimated the raw temperature data (warmer) and at one site, Anusplin underestimated the raw temperature data (colder). PRISM on the other hand overestimated the value of MinT at eight of the nine sites and underestimated it at one of the sites. None of the temperature estimates from PRISM were within 0.5°C of the recorded weather station data for that time period. Parra and Monahan (2008) found that when examining the ability of each climate dataset to recover data across the state of California, the Anusplin data had less bias but more variance whereas PRISM had more bias but less variance.

The Anusplin climate surface that we used in this analysis indicated warming across all low elevation sites. At sites at 2600m, there was no change in minimum temperature, whereas over 2600m we observed cooling (Fig. S1). PRISM indicated consistent warming across all sites regardless of elevation (Fig. S2). We completed these analyses in order to further examine these differences and feel confident with our use of Anusplin, given its performance at recovering weather station data for MinT.

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Table A2. Comparison of two climate interpolations at recovering weather station data for weather stations in and around Yosemite National Park, CA, USA

Weather Station Name	Era of measured data H: 1900-1940 M: 1980-2005	Average measured temperature at weather station across era MinT (°C)	Average pixel value for Anusplin MinT at weather station from respective era (°C)	Average pixel value for Prism MinT at weather station from respective era (°C)	Climate Interpolation layer with more accurate recovery of measured weather station data (°C)
Cherry Valley Dam	M	-2.4	-2.0	-1.1	Anusplin same Prism warmer
Dudleys	H	-4.6	-1.7	-0.8	Both warmer
Ellery Lake	H	-13.2	-13.0	-10.6	Anusplin same Prism warmer
Gem Lake	H	-11.0	-10.9	-7.9	Anusplin same Prism warmer
Lake Eleanor	H	-4.0	-4.2	-2.1	Anusplin same Prism warmer
Lee Vining	M	-6.8	-8.6	-7.6	Both colder
Mono Lake	M	-8.4	-8.0	-7.8	Anusplin same Prism warmer
New Melones	M	1.2	1.3	2.4	Anusplin same Prism warmer
South Entrance YNP	M	-4.0	-2.4	-3.1	Both warmer

Interpolated data
Same: within 0.5°C
Warmer: >0.5°C; Colder: <0.5°C

Fig. A1. Anusplin climate trends overtime. Circles represent historical sites. Red circles show sites that have warmed, black circles have remained the same and blue circles have cooled over the century. Triangles show closest weather stations to Yosemite National Park.

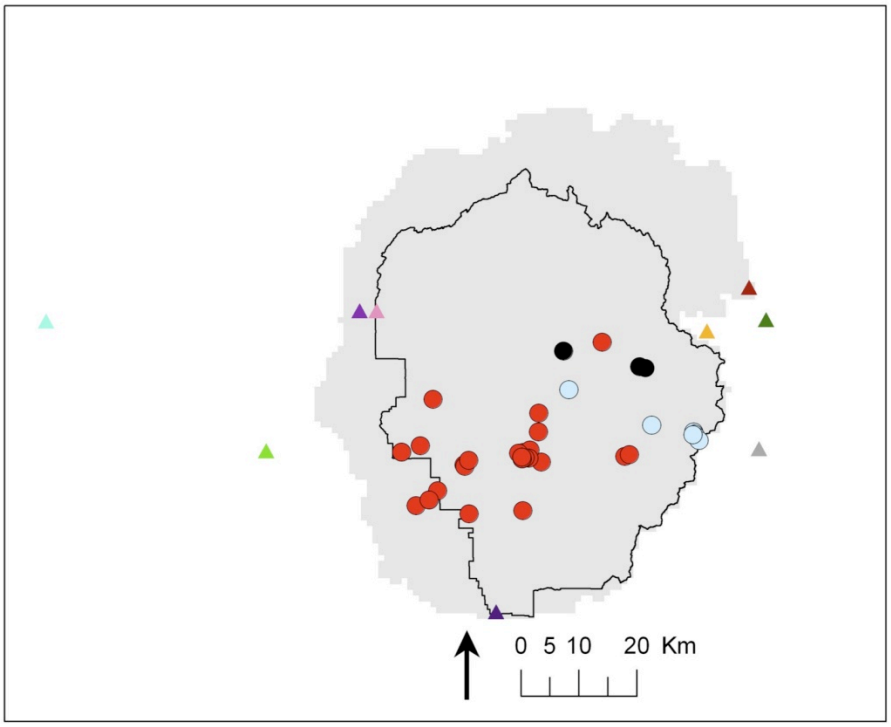
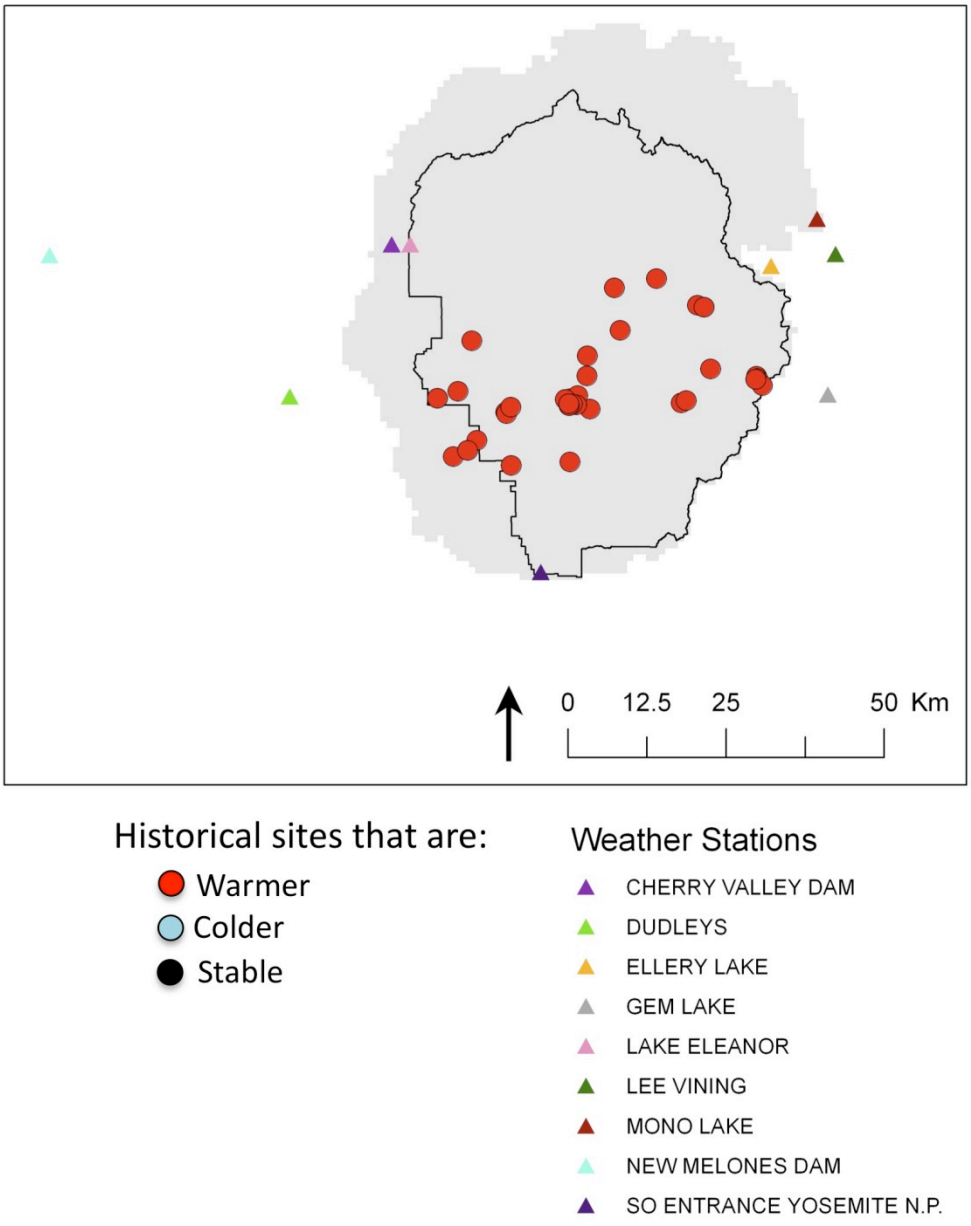


Fig. A2. PRISM climate trends overtime**.** Circles represent historical sites. Warming was consistent across all sites (red circles). Triangles show closest weather stations to Yosemite National Park.



Appendix 3: Detailed description of MARS model development and model evaluation

Model Development

We developed correlative distribution models using both single- and multi-species multivariate adaptive regression splines (MARS, Friedman, 1991; Leathwick *et al.,* 2005; Elith *et al.* 2006; Elith & Leathwick, 2007). Currently, there are several methods available for modeling species' distributions, ranging from relatively simple bioclimatic envelope models, (e.g., BIOCLIM, DOMAIN) to more complex flexible non-linear approaches (General Additive models GAM, MARS) or maximum-entropy models (MAXENT, Phillips *et al.,* 2006) to other machine-learning techniques (boosted regression trees BRT, Genetic Algorithm for Rule-set Prediction GARP). The MARS approach is to fit "piecewise linear basis functions" or linear segments to the data (Elith & Leathwick, 2007). We chose MARS from the large number of alternative methods because i) it utilizes both presence and absence data, ii) is capable of fitting non-linear relationship between a species' presence and its environment, iii) has been shown to perform well in most training environments when compared to similar modeling techniques (Elith *et al*., 2006) and is computationally efficient and its outputs are easily integrated into GIS. Importantly, MARS also allows us to examine how the inclusion of species co-occurrence affects model performance. All models were run in the statistical package R 2.9.0 GUI (R Core Development Team 2009) using the *mda* library and custom code written by Elith and Leathwick (2007). This code was written specifically to adapt MARS to presence-absence data. It does this by first fitting a least-squares MARS model, and then extracting the basis functions and refitting the model as a generalized liner model (GLM) with a binomial error distribution (Leathwick *et al*., 2005). We set MARS parameters for all models as follows: family = "quasibinomial"; penalty = 1 (more conservative approach for small dataset), and degree = 1 (no interactions). Model outputs were transferred and projected into ArcView 3.2 (ESRI) using the avenue code provided by Elith and Leathwick (2007). Outputs were visualized and converted into grids for further analyses in ArcMap 9.2 (ESRI).

We developed 18 historic models (3 competing models x 3 species x 2 modeling approaches) and 12 modern models (3 competing models x 2 species x 2 modeling approaches). We have a reduced set of modern models because the resurvey results detected *T. senex* at only one site. Its prevalence had gone from 0.18 in the historic survey to less than 0.01 in the modern resurvey with comparable sampling effort and detectability (Moritz *et al.* 2008, this study). It is not feasible to build a species distribution model using only one presence point; hence, modern models were run for *T. alpinus* and *T. speciosus* only. The modern presence/absence data for *T. senex*, however, allows us the opportunity to assess which model best predicts the observed range collapse.

Model Evaluation

We evaluated the accuracy of the models in two ways: "within-era" and "between-era". Therefore model performance was evaluated on 36 scenarios for the historic models, and 24 for the modern models. For the within-era scenario we projected the model onto the environmental landscape on which it was built to determine if the species' distribution could be accurately predicted from the data. We used the species data with which the models were built for this assessment, with the exception of removing sites where PFA > 0.1. We chose not to do the alternative, which is to split our data into training and testing sets for within-era evaluation because our sample sizes are already restricted. In addition, it is the between-era prediction, not

the within era prediction, that addresses our question of whether or not species distribution models are effective at predicting distributional changes across time. In the between-era evaluation we projected the model built in one era onto the environmental landscape of the other era and then used the species data from the era into which it was projected to test the accuracy of the model projection. For example, a historical model was built with historical species, climate and vegetation data then projected onto the modern climate and vegetation GIS layers. The resulting predictive distribution map was then tested with the modern resurvey species point data.

Model accuracy was assessed by examining the area under the receiver-operating characteristic curve (AUC), and the "true skill statistic" (TSS). AUC is a commonly used measure of model accuracy (Fielding & Bell, 1997, Elith *et al.,* 2006). Lobo *et al.* (2008) recently highlighted its drawbacks; the most relevant to this is study is that because it is a discriminatory index, it represents the likelihood that a presence will have higher predicted probability than an absence, regardless of model fit (see Lobo *et al.* 2008 for more detail). In other words, you can have a model that over or under predicts a species' distribution with good discriminatory power. The advantage of using AUC is that it is threshold independent and easily compared across different models and species. However, because it can be misleading for the reason described above we also calculated the true skill statistic (TSS).

TSS is an alternative to the widely used Cohen's Kappa statistic, which is criticized for its dependence on the prevalence of the testing data. This dependence makes Kappa inappropriate for comparisons of model accuracy between species or regions (Allouche *et al.,* 2006). TSS is defined as sensitivity (i.e., correctly classified presences) + specificity (i.e., correctly classified absences) – 1. We set the threshold for calculating TSS to the prevalence (# of presences/#sites) in the training dataset. We chose this threshold approach not only because it is robust (Liu *et al.,* 2005) but also because it makes sense in terms of our comparison of ranges across time. The prevalence of all three species appear to have changed over time and incorporating this difference into the model allowed us to assess this range shift in terms of its assumed baseline (prevalence in the training model).

Model accuracy of between-era models was assessed using the independent data on species' detections for the era into which the model was projected. The test points only included presences and absences that had a PFA of less than 10%. AUC and TSS were generated using the PresenceAbsence package (Freeman & Moisen, 2008) in R 2.9.0 GUI statistical program. The threshold to produce a binary output of predicted presence-absence was set to the prevalence in the training dataset. The species specific thresholds were as follows for the historic (H) and modern (C) models: *T. alpinus* (H = 0.26, C = 0.17), *T. senex* (H = 0.18, C = 0.01), *T. speciosus* (H = 0.31, C = 0.45).

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Appendix 4: Examination of sampling and sample size effects on modeling results

There are two potential limitations of our methods: 1) we did not use range-wide species data to build our models and therefore may not be including the entire environmental space each species can inhabit and 2) the sample size difference between the two eras is large and may have an affect on the modeling results. Our study was restricted by the available historical information. And although there is historical climate data available for the entire distribution of all three species, the objective of this study was to include both vegetation and co-occurrence to evaluate changes to the elevational zonation of these chipmunks. Unfortunately historical vegetation data is not available for the entire range of any of the three species, and the species only co-occur in our study area. We were, therefore, interested in the question at a local scale and restricted to the area with both historical and modern vegetation data.

The second potential issue, sample size, is also mostly due to the historical dataset. Increasing the modern dataset will not help and in fact it would further increase the discrepancy between the eras. Studies have shown that sample size can have a large effect on species distribution models but it is more important to have an unbiased sample rather than a large one (Hernandez *et al.,* 2006, Wisz *et al.,* 2008). We have 39 sites in the historical dataset versus 109 in the modern dataset. To evaluate the effect of sample size on model results, we conducted an analysis where we re-sampled 39 sites from the modern dataset, keeping the prevalence the same, one hundred times. We reran the models on each subset sampled and determined that sample size occasionally did have an affect on which predictor variables were chosen but not on the direction of the correlation. In general, the majority of models on the reduced sample size selected the same predictor variables and generated a comparable probability surface when projected onto the environmental landscape. Therefore, we are confident that the historical models built using fewer sites than the modern dataset does not affect the overall results and conclusions of the study.

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Appendix for Chapter 4: Global warming reduces genetic diversity and gene flow in an endemic alpine small mammal species

Appendix 5

Table A3: Allele size and frequency for each era for seven microsatellite loci for 1) *T. alpinus* and 2) *T. speciosus*

1) *T. alpinus*

	EuAmMS26 Allele Size & Frequency											
Era	165	167	169	171	173	175	177	179	181	183	185	190
Grinnell	0.06	0.06	0.49	0.07	0.03	0.02	0.01	0.01	0.18	0.03	0.03	0.01
Modern	0.02	0.07	0.49	0.11	0.01	0.00	0.02	0.00	0.20	0.08	0.00	0.00

	EuAmMS41 Allele Size & Frequency									
Era	133	141	145	147	149	151	153	155	157	159
Grinnell	0.01	0.01	0.06	0.06	0.20	0.22	0.25	0.17	0.02	0.02
Modern	0.00	0.00	0.03	0.02	0.35	0.35	0.13	0.12	0.00	0.01

	EuAmMS86 Allele Size & Frequency														
Era	136	146	149	151	153	155	157	159	162	164	166	168	172	174	176
Grinnell	0.02	0.01	0.13	0.02	0.33	0.26	0.06	0.04	0.05	0.06	0.02	0.01	0.02	0.00	0.00
Modern	0.00	0.00	0.06	0.03	0.30	0.20	0.04	0.03	0.14	0.16	0.01	0.01	0.00	0.00	0.01

	EuAmMS94 Allele Size & Frequency									
Era	99	101	103	105	107	109	111	113	115	117
Grinnell	0.01	0.03	0.02	0.00	0.39	0.22	0.23	0.09	0.01	0.01
Modern	0.00	0.00	0.00	0.07	0.00	0.57	0.11	0.23	0.01	0.00

	EuAmMS37 Allele Size & Frequecy								
Era	114	130	132	134	136	138	140	142	143
Grinnell	0.20	0.01	0.01	0.01	0.03	0.19	0.54	0.01	0.00
Modern	0.20	0.00	0.00	0.00	0.08	0.22	0.46	0.03	0.02

	AC A101 Size & Frequency								
Era	125	132	135	139	162	165	168	171	174
Grinnell	0.01	0.07	0.45	0.01	0.09	0.17	0.10	0.09	0.02
Modern	0.00	0.02	0.34	0.00	0.05	0.35	0.07	0.16	0.01

	AC D115 Allele Size & Frequency										
Era	160	164	168	176	180	184	188	192	196	200	204
Grinnell	0.00	0.02	0.00	0.10	0.27	0.34	0.24	0.02	0.00	0.00	0.02
Modern	0.01	0.03	0.00	0.07	0.28	0.41	0.16	0.01	0.01	0.01	0.00

2) *T. speciosus*

EuAmMS26 Allele Size & Frequency												
Era	153	155	157	159	161	163	165	167	169	171	173	175
Grinnell	0.03	0.00	0.02	0.08	0.04	0.37	0.28	0.03	0.09	0.04	0.03	0.00
Modern	0.00	0.02	0.02	0.06	0.09	0.27	0.27	0.08	0.07	0.09	0.01	0.02

EuAmMS 37 Allele Size & Frequency											
Era	132	134	136	138	140	143	145	146	147	149	152
Grinnell	0.06	0.14	0.14	0.14	0.19	0.13	0.16	0.00	0.05	0.00	0.00
Modern	0.16	0.12	0.11	0.16	0.17	0.13	0.11	0.01	0.02	0.01	0.00

	EuAmMS41 Allele Size & Frequency									
Era	131	135	137	139	141	143	145	147	149	159
Grinnell	0.02	0.06	0.00	0.25	0.25	0.19	0.17	0.06	0.00	0.00
Modern	0.00	0.21	0.02	0.23	0.12	0.25	0.08	0.06	0.01	0.02

	EuAmMS86 Allele Size & Frequency								
Era	141	143	145	147	157	159	161	163	165
Grinnell	0.02	0.00	0.00	0.00	0.01	0.58	0.13	0.24	0.02
Modern	0.04	0.01	0.02	0.01	0.04	0.59	0.17	0.11	0.03

	EuAmMS94 Allele Size & Frequency						
Era	101	103	105	107	109	111	113
Grinnell	0.03	0.02	0.08	0.24	0.29	0.22	0.13
Modern	0.01	0.02	0.09	0.14	0.49	0.19	0.07

AC A101 Allele Size & Frequency							
Era	140	143	146	150	153	156	159
Grinnell	0.01	0.30	0.30	0.11	0.16	0.10	0.02
Modern	0.00	0.26	0.22	0.16	0.14	0.20	0.02

AC D115 Allele Size & Frequency							
Era	157	161	165	169	173	177	181
Grinnell	0.01	0.40	0.09	0.26	0.21	0.02	0.01
Modern	0.02	0.58	0.23	0.08	0.04	0.04	0.00

Chapter 4: Appendix 5 (cont.)

Table A4. Sample size (N) and inbreeding coefficients (F_{IS}; Weir & Cockerham 1984), a measure of heterozygote deficit, for each population (defined by locality), for each species and time period (A-D) at seven microsatellite loci. F_{IS} values range from -1 to 1 with positive values indicating a heterozygote deficit, negative values a heterozygote excess and 0 equal to expected value under random mating. Bold numbers indicate F_{IS} values significantly different from 0 at an alpha level of 0.05 corrected for multiple tests (0.05/13 = 0.003). Tests were calculated using Genepop 4.0.10 (10000 dememorization, 100 batches, 10000 iterations/batch).

A. *T. alpinus* Grinnell Era

Locality	N	EuAmMS26	EuAmMS41	EuAmMS86	EuAmMS94	EuAmMS37	ACA101	ACD115
Mt. Hoffman/Ten Lake	16	**0.588**	**0.665**	0.382	0.393	**0.563**	**0.460**	**0.829**
Tuolumne Meadows	19	0.404	0.017	0.352	0.176	**0.576**	0.106	**0.606**
East YNP	8	0.593	0.657	0.515	0.139	**0.692**	0.100	0.556
Upper Lyell Canyon	16	0.327	**0.422**	**0.587**	0.006	0.375	0.396	**0.359**
Vogelsang Lake	9	0.379	0.286	0.200	-0.111	0.400	0.000	0.200
Mt. Clark	6	0.118	0.333	0.636	-0.013	0.216	0.500	0.100

B. *T. alpinus* Modern Era

Locality	N	EuAmMS26	EuAmMS41	EuAmMS86	EuAmMS94	EuAmMS37	ACA101	ACD115
Mt. Hoffman/Ten Lake	11	0.105	0.111	-0.200	-0.045	-0.094	-0.118	0.040
Young Lakes	18	-0.116	0.019	0.177	0.155	-0.005	0.172	0.137
Upper Cathedral Lakes	12	-0.222	-0.111	0.069	-0.152	-0.006	-0.183	0.052
East YNP	8	-0.493	0.164	-0.235	-0.167	0.000	0.087	-0.241
Mono Pass	23	-0.088	0.081	-0.030	0.102	0.039	0.405	0.270
Upper Lyell Canyon	33	-0.138	0.189	-0.072	-0.174	-0.175	0.163	-0.125
Vogelsang Lake	14	-0.052	-0.135	0.170	0.294	0.235	0.004	-0.024
ark Range/Ottoway La	27	-0.179	-0.113	-0.010	0.383	0.025	-0.076	-0.229

C. *T. speciosus* Grinnell Era

Locality	N	EuAmMS26	EuAmMS41	EuAmMS86	EuAmMS94	EuAmMS37	ACA101	ACD115
Crane Flat	2	0.500	0.000	-	-0.333	0.000	0.500	-1.000
Porcupine Flat	12	0.285	0.140	**0.583**	-0.048	-0.076	-0.089	0.149
Glen Aulin	4	-	0.700	-0.091	-0.600	0.053	-0.412	0.200
Tuolumne Meadows	17	**0.418**	**0.323**	0.054	-0.114	-0.131	0.037	0.028
Mono Meadow	5	0.360	0.059	0.200	0.059	-0.111	-0.280	0.368
Vogelsang Lake	3	-	-0.143	1.000	-0.500	-0.091	0.500	-1.000
Merced Lake	4	0.111	0.200	-	-0.200	0.100	0.429	-0.143
Upper Lyell Canyon	12	0.355	0.191	0.265	0.009	0.150	-0.134	0.281

D. *T. speciosus* Modern Era

Locality	N	EuAmMS26	EuAmMS41	EuAmMS86	EuAmMS94	EuAmMS37	ACA101	ACD115
Crane Flat	23	0.2388	0.1781	0.2511	-0.0458	-0.0464	0.0455	0.0725
Porcupine Flat	5	0.0857	0.0303	-0.2903	0.3514	0	0.0303	0.0769
Glen Aulin	10	0.04	0.1871	0.3651	0.2394	-0.0318	0	-0.1532
Tuolumne Meadows	18	-0.043	0.0868	-0.2364	0.2295	-0.1134	-0.2944	0.0286
Mono Meadows	14	0.2378	0.0744	0.1236	0.0114	0.5	0.0338	0.2806
Vogelsang Lake	4	0.2	-0.2632	-0.0909	0.5	-0.2	-0.5	0.1429
Merced Lake	21	0.2565	0.0935	0.0952	-0.07	-0.0336	0.0078	0.0023
Upper Lyell Canyon	20	0.2232	-0.0125	0.0331	-0.0071	**0.3695**	-0.0487	0.0598