DESCRIBING THE GENETIC DIVERSITY AND POPULATION RELATIONSHIPS OF THE MONTANE SUBSPECIES OF GUNNISONS PRAIRIE DOG, *CYNOMYS GUNNISONI GUNNISONI*.

by

SEAN PATRICK STREICH

B.S., Colorado State University, 2009

This thesis is submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Master of Science

Department of Ecology and Evolutionary Biology

2018

This Thesis Entitled:
Describing the Genetic Diversity and Population Relationships of the Montane Subspecies of Gunnison's Prairie Dog, *Cynomys gunnisoni gunnisoni*

Written by Sean P. Streich

_____
Andrew Martin, PhD. Thesis Advisor

_____
Nolan Kane, PhD. Committee Member

_____
Brett Melbourne, PhD. Committee Member

Date_____

IACUC protocol # 2553

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Streich, Sean P. (M.S., Ecology and Evolutionary Biology)

Describing the Genetic Diversity and Population Relationships of the Montane Subspecies of Gunnison's Prairie Dog, *Cynomys gunnisoni gunnisoni*

Thesis directed by Professor Andrew P. Martin

.

ABSTRACT

Loss of biodiversity is a concern all over the world. While species level extinction results in the loss of whole unique organisms, reductions in range and population numbers can lead to population level extinction events resulting in the loss of unique and adaptive genetic and phenotypic diversity. Understanding the genetic relationships within and between populations across a species range will lead to a better understanding of how each population is related to another and can inform on practices to better manage and promote the survival and growth of populations. This study uses genetic data from the montane subspecies of the Gunnison's prairie dog, *Cynomys gunnisoni gunnisoni* to evaluate and compare populations across the entire range of the subspecies. Prairie dogs are colonial ground squirrels whose numbers have fallen by up to 99% of historic levels. Continued pressures on prairie dog populations, including the plague, a disease which causes extreme moralities in prairie dog populations, have kept population numbers low and resulted in reductions of gene flow between extant populations, higher inbreeding, and increased possibilities of localized extinctions. Genetic markers show that genetic relationships between populations show high levels of population structure, varying degrees of diversity and low evidence of migration events occurring between sampled colonies. These results can inform on how species management activities are addressed in the future. Additionally, the mitochondrial genome of *C.g.g* was sequenced and incorporated into the *Cynomys* phylogeny, providing further insight into the evolution of the evolution of the Genus *Cynomys.*

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Andrew Martin, for his guidance throughout my time as a graduate student. I thank my committee members, Brett Melbourne and Nolan Kane for their advice and guidance. I would like to thanks the Martin, Taylor, Safran, and Kane labs for their support and advice, and help as a student and researcher at CU, Boulder. Additional I was fortunate to have help in the lab and the field from many lab and field assistants with special thanks to Liza Hasan, Kelly McCahill, Soeil Gaylord who made my field work possible and Kyle Keepers who greatly helped me with genome assembly.

I would also like to thank the Colorado Parks and Wildlife for providing funding towards this study. I would also like to acknowledge the support from individuals within Colorado Parks and Wildlife who were influential during my Master's degree, starting with Dr. Michael Miller, who facilitated and supported my admittance to graduate school. The members of the Wildlife Health Program's section of the CPW, in particular Dan Tripp and Karen Griffin have assisted me greatly with field and laboratory work. I would also like to thank the State Biologists who worked with me in obtaining conducting field work, in particular Amy Seglund and Nate Seaward. I am also thankful for the help and support given to me by the biologists and field technicians from the Gunnison and San Luis Valley Bureau of Land Management and the Gunnison and Rio Grand National Forests.

Thank you to the EBIO Departmental Research Grant program for funding towards making my field work possible and to the IACUC, IBC, and Occupational Health organizations for their help in completing and submitting protocols.

CONTENTS

TABLES

FIGURES

Chapter 1
Data Analysis on Evaluating Genetic Diversity of the Montane Gunnison's Prairie Dog, *Cynomys gunnisoni gunnisoni*

1.1 Introduction:

1.1.1 Prairie dogs

Prairie dogs are colonial semi-fossorial members of the family *Scuiridae* inhabiting the grassland and brush ecosystems of the Great Plains and Rocky Mountain regions of western North America (Clark et al. 1971; Pizzimenti, 1975; Seglund and Schnurr 2010). Two subgenera encompass five species of prairie dogs. *Leucocrossuromys* includes the Utah (*Cynomys parvidens*), Gunnison's (*Cynomys gunnisoni*), and white-tailed (*Cynomys leucurus*) prairie dogs, while *Cynomys* includes the Mexican (*Cynomys mexicanus*) and black-tailed (*Cynomys ludovicianus*) prairie dogs (Clark et al. 1971; Pizzimenti, 1975; Seglund et al. 2006). Two species are currently listed under the Endangered Species Act, *C. parvidens* (Threatened) and *C. mexicanus* (Endangered), while *C. gunnisoni*, *C. ludovicianus* and *C. leucurus* have not warranted listing on each of their last reviews (USFWS).

Current habitat occupied by prairie dogs is estimated to only be about one to two percent of the historic levels (Hoogland et al. 1999, 2006; Slobodchikoff et al. 2009). The drastic reduction in prairie dog abundance coincided with the economic development of the western United States for agriculture, rangeland, urbanization, energy, and mining (Seglund and Schnurr 2010). Furthermore, the introduction of *Yersinia pestis*, the bacterium that causes bubonic and sylvatic plague, in the 1900s has dramatically compounded these reductions due to the 95-100% mortality rate caused in prairie dogs (Cully, 1989; Cully and Williams 2001; Rayor, 1985; Seglund and Schnurr 2010).

Prairie dogs are a keystone species; therefore their presence or absence has effects on a wide variety of other species (Bangert and Slobodchikoff 2006). Prairie dog colonies provide habitat, shelter, and food for numerous species including hawks, eagles, badgers, foxes, and coyotes (Davidson et al. 2012;

Martínez-Estévez et al. 2013; Miller et al. 1994). Efforts for prairie dog conservation simultaneously promote the conservation of other species of concern in Colorado, such as black-footed ferrets (*Mustela nigripes)*, mountain plovers (*Charadrius montanus)*, burrowing owls (*Athene cunicularia*), and ferruginous hawks (*Buteo regalis)* (Seglund and Schnurr 2010). In addition, prairie dogs change the ecology of the soil and flora communities by continuous clipping, digging, and soil aeration (Sierra-Corona et al. 2015; Kaiser et al. 2016). Herbivores such as pronghorn (*Antilocapra Americana)*, bison (*Bison bison*), and cattle have been observed to prefer grazing on prairie dog colonies over surrounding areas (Hoogland et al. 2006; Sierra-Corona et al. 2015).

1.1.2 Gunnison's Prairie Dog

The Gunnison's prairie dog is found throughout the grassland, brush, and montane ecosystems of southern Colorado, New Mexico, southeast Utah, and northern Arizona (Sackett et al. 2014; Seglund et al. 2005). The Gunnison's prairie dog includes two subspecies: the montane *C.g. gunnisoni* and lowland *C.g. zuniensis* (Sackett et al. 2014). The boundary between these two sub-species was recently evaluated during an analysis comparing pheno- and genotypic differences, concluding that the subspecies boundary should be slightly redrawn from previous estimates (Sackett et al. 2014). *C.g. gunnisoni* has a smaller range and its distribution is more fragmented by landscape features in the southern Rocky Mountains then the *C.g. zuniensis* subspecies (Sackett et al. 2014). The range of *C.g. gunnisoni* spans the north-central part of New Mexico and the Colorado regions of Gunnison, San Luis Valley, South Park, the Telluride area, and the mountainous region around the Pikes Peak region. Most of the range of *C.g gunnisoni* resides inside of the state of Colorado (Fig. 1; Sackett et al. 2014).

Gunnison prairie dogs exist on the landscape as a set of spatially discrete colonies that experience metapopulation dynamics (Roach et al. 2011). A species' metapopulation is a network of suitable habitat patches in a matrix of largely unsuitable habitat and each patch can receive immigrants from one or more colonies (Hanski, 1998). Colonies can be extirpated by plague, anthropogenic activities, or stochastic

events, and patches may be recolonized by individuals from nearby colonies (Sackett et al. 2012). Migration events allow individuals to move between colonies, and the level of connectivity between each colony in the metapopulation differs due to population size, dispersal rate, distance, and landscape (Hanski and Gilpin 1991; Roach, et al. 2011; Sackett et al. 2012). A key aspect of metapopulation dynamics is that habitat patches are connected through the ability for individuals to migrate. Localized extirpation of prairie dog colonies has accelerated over time because of plague and the subsequent loss of colonies, coupled with land use activities by humans, has caused a decline in occupancy from historic levels, effectively lowering the number of source colonies that can promote emigration and therefor reducing the movement on individuals across the landscape reducing recolonization rates. The predicted result is a decline in connectivity of colonies and the degradation of metapopulation structure (Hanski and Gilpin 1991; Den Boer, 1968). This loss of network connectivity can reduce or stop migration and gene flow between occupied patches, limiting the ability of genetic exchange across colonies or populations (Den Boer, 1968). Moreover, the loss of inter-colony connectivity can increase the probability of colony extirpation from genetic effects (e.g. decline fitness due to inbreeding depression), the effects of environmental stochasticity (e.g., localized environmental events that wipe out a colony because it is small and has a restricted range), the effects of demographic stochasticity (e.g. there are large swings in sex ratio that cause bottlenecks in population size), and localized outbreaks of disease (e.g. plague goes through and wipes all out individuals because colonies are small).

Thus, the demographic connectivity of colonies is a key part of maintaining prairie dogs on the landscape. However, the connectivity which currently exists between *C. g. gunnisoni* colonies is unknown. It is likely, though, that there is variation in colony connectivity as a consequence of regional landscape features and the population size of colonies. Additionally, as plague alters occupancy and population numbers colony connectivity is likely ever changing as colonies are extirpated and colonized.

1.1.3 Objectives of this Analysis

In this analysis we provide indirect estimates of two key demographic parameters of *C. g. gunnisoni:* long-term effective population size (Ne) and inter-colony migration (Nm) (or connectivity). The degree that colonies are isolated at a regional scale will be evaluated among six Individual Population Areas (IPAs), including South Park, Southeast, Southwest, Gunnison, and the San Luis Valley (Seglund and Schnurr 2010) and New Mexico. Inferences are based on an analysis of microsatellite genotypes and mitochondrial DNA sequences (Sackett et al. 2014). Specifically, we use estimates of genetic diversity for inferring effective population size and estimates of genetic structure for inferring regional and local connectivity among populations.

1.2.1 Genetic diversity

1.2.1 Background

Estimates of genetic diversity within populations are proxies for estimating key demographic information that reflects processes over ecological and short evolutionary time frames. This is because genetic diversity, typically denoted as theta, is proportional to effective population size multiplied by the mutation rate. Effective population size is a key demographic parameter because it is defined as the number of individuals that successfully contribute offspring to future generations. Based on estimates of theta from samples of genotypes or DNA sequences, we can compare effective population size among different populations and infer cases where there may have been recent extreme bottlenecks or persistent issues that can potentially compromise viability or signal an elevated risk of extirpation.

I used microsatellite genotypes to estimate nuclear gene diversity and DNA sequences to estimate cytoplasmic (mitochondrial) gene diversity. These two markers have different modes of inheritance. Nuclear genes are biparentally inherited whereas mitochondrial genes are uniparentally inherited through

mothers. The difference in inheritance can be leveraged to infer the most likely historical scenarios for specific colonies and regions (Table 1).

Nuclear diversity	Mitochondrial diversity	Example inferences
Low	Low	Small long-term effective population size, recent colonization, extreme bottleneck
High	Low	Recent bottleneck mostly due to reduction of reproductive females, male biased immigration, lower effective population size of females relative to males
Low	High	Asymmetric hybridization (females of one x males of the focal species)
High	High	Hybridization, long term large effective population size

Table 1-1. **Population inferences** based on comparison of relative levels genetic diversity for nuclear and mitochondrial gene markers.

1.2.2 Results of Genetic Diversity

My analysis focuses on 12 widely separated sites across the range of *C. g. gunnisonni* (Figure 1; Table 2). I focused on estimating the average pairwise differences between individuals within colonies, denoted pie (Tajima 1983). Pie estimates $2Mu_{Micro}$ and Mu_{mtDNA} , where M is $2N_E$ and u is the mutation rate, for diploid (microsatellites) and haploid (mtDNA) markers, respectively.

IPA or Region	State	Site	Individuals Sampled
Southwest	CO	TESW	18
Gunnison	CO	BG	6
Gunnison	CO	ERPD	42
Gunnison	CO	GBTT	21
Gunnison	CO	GCR	11
South Park	CO	EMSP	15
Southeast	CO	BVSE	29
San Luis Valley	CO	DN	24
San Luis Valley	CO	PSLV	27
New Mexico	NM	BLFB	29
New Mexico	NM	ENSP	25
New Mexico	NM	TPRR	22
Total		12	269

Table 1-2. Sample Locations: *C. g. gunnisoni* were collected from 12 localities across six regions in Colorado and New Mexico. See figure 1 for geographic location of each sample size.

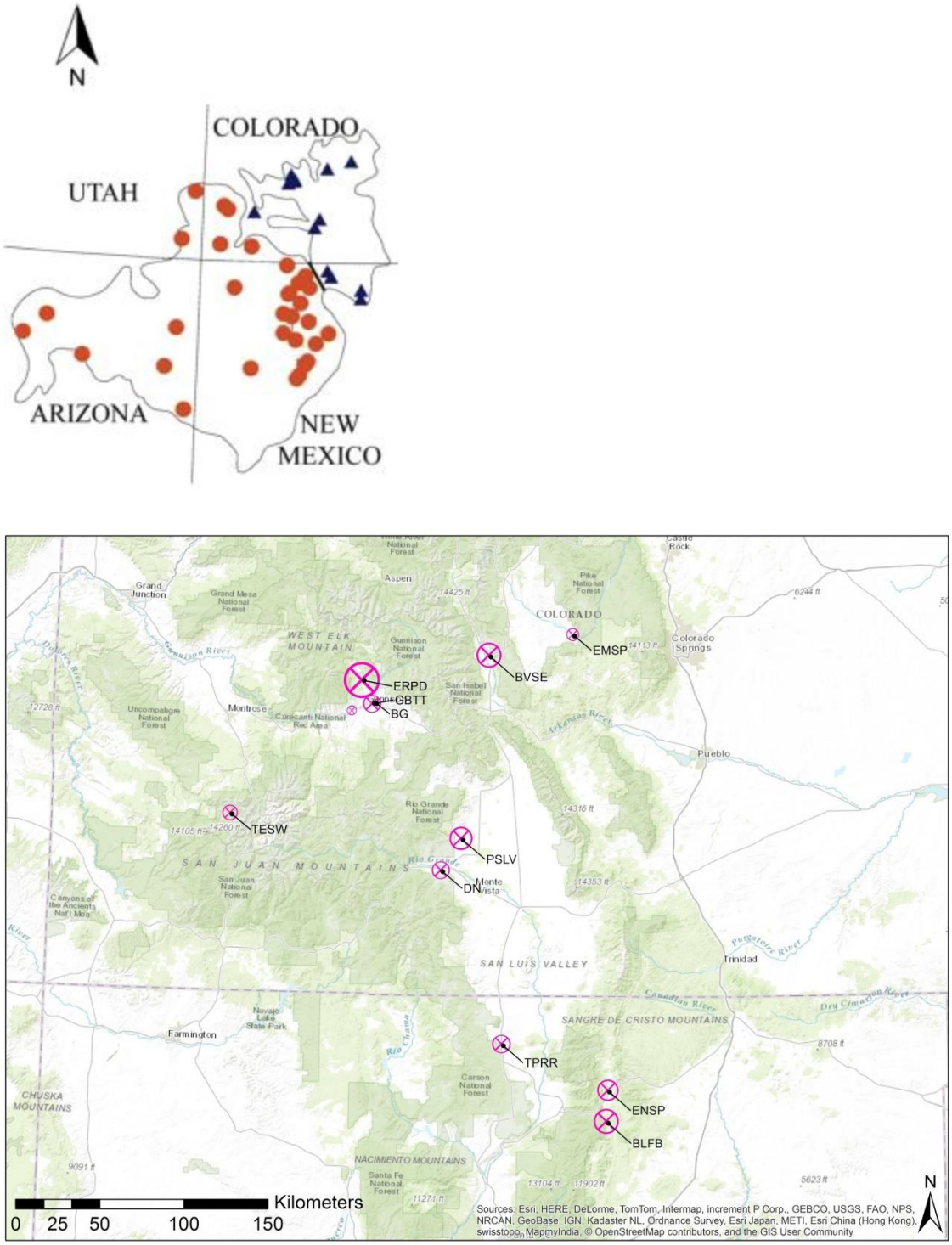


Figure 1-1. Sampeling Locations and Subspecies Distribution: Above: The ranges for the two subspecies of *C.g. gunnisoni*: *zuniensis* is depicted with orange circles and *gunnisoni* with blue triangles (Sackett et al. 2014). Below: Geographic locations of the colonies included in the study. Symbols are sized proportional to sample size (see Table 1).

I discovered three different types of colonies with respect to the relative magnitude of nuclear and mtDNA gene diversity (Figure 2). There are two sites (TESW and EMSP) where both nuclear and mtDNA variation were low, suggesting recent colonization by relatively few individuals or a recent population bottleneck. Second, two sites (TPRR and ENSP) exhibited an excess of mtDNA diversity relative to the expectations based on the nuclear genotype variation, suggesting these populations may have some individuals with hybrid ancestry (between *C. g. gunnisonni* and *C. g. zuniensis*) (see Sackett et al. 2014). Finally, nine populations surveyed appeared to have less mtDNA diversity than expected (relative to microsatellite diversity) that may be indicative of a system in which there are fewer females contributing to the ancestry of a population than males. This can happen in a system, like prairie dogs, where females tend to be philopatric and males disperse among colonies.

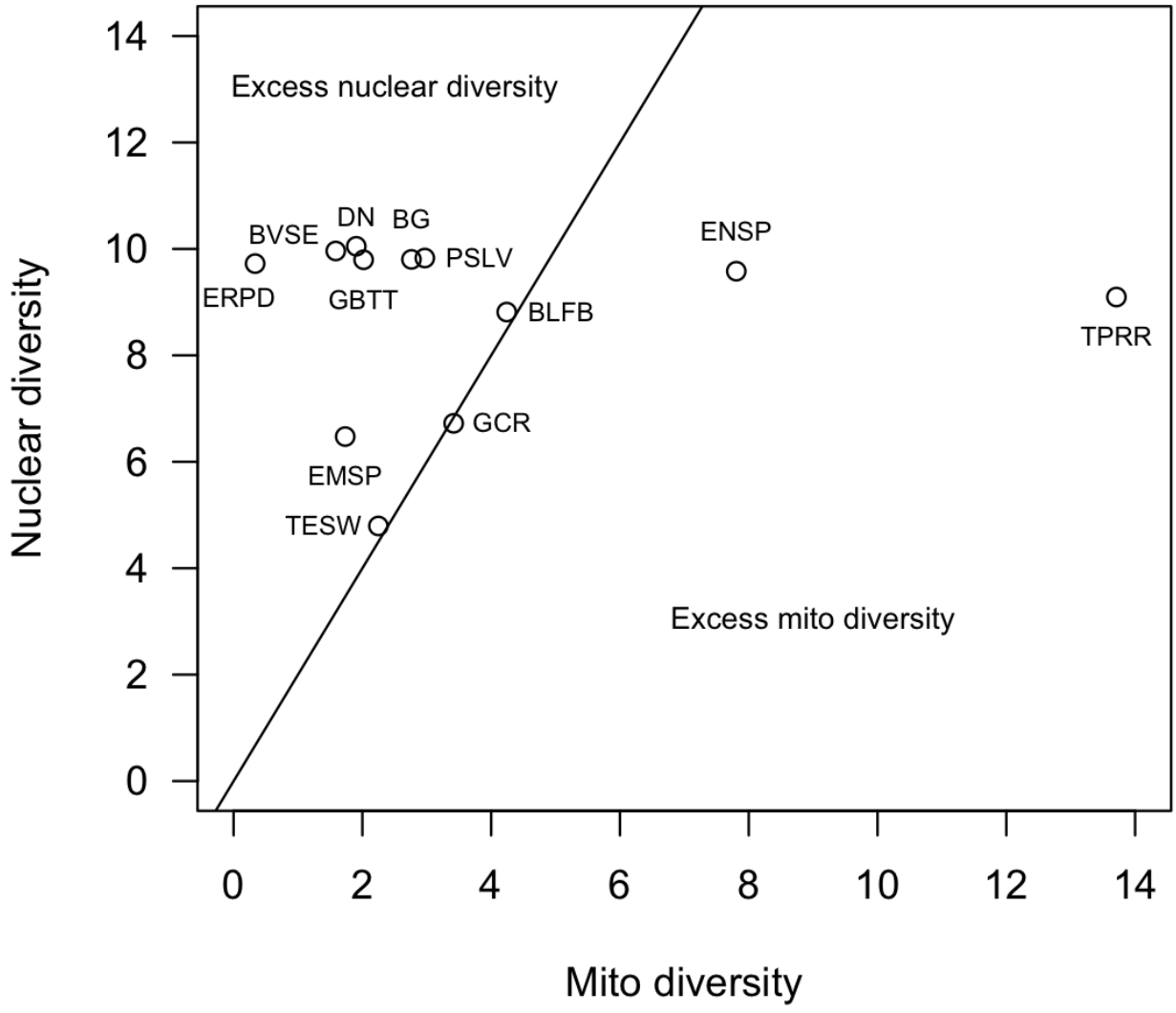


Figure 1-2. DNA Diversity Comparison: Plot of within population variation estimated based on pairwise comparisons among individuals for the nuclear genome (from microsatellite genotypes) and the mitochondrial genome (from DNA sequences) for the 12 populations surveyed. The solid line represents an expectation from theory (nuclear variation is expected to be *at least* two times the variation in the mitochondrial genome). ENSP and TPRR have evidence of admixture between *zuniensis* and *gunnisoni* (Sackett et al. 2014).

1.2.3 Genetic structure

Genetic structure is a proxy for estimating the degree of demographic isolation of populations. Low genetic structure is an indication that two or more populations have exchanged migrations relatively recently whereas high genetic structure suggests two or more populations have been isolated for multiple

generations. Two approaches are used to estimate population structure: hierarchical analysis of variance (F_{ST}) and Bayesian assignment methods (using STRUCTURE).

F_{ST} is a measure of the amount of genetic variation is partitioned among geographically separated colonies. In particular, $F_{ST} = (H_t - H_w)/H_t$, where H_t is the total genetic variation from two distinct populations and H_w is the average genetic variation within the two populations being compared. Larger values of F_{ST} indicate greater genetic differentiation among populations. The value of FST is interpreted in the context of the theoretical expectations that $F_{ST} \approx 1/(4Nm + 1)$, where Nm is the number of migrants that move between a pair of populations per generation (Hedrick 1999). Because Nm is an indication of demographic connectivity, higher values of Nm correspond with *lower* values of F_{ST}. This makes sense because as populations become more demographically connected, the genetic differentiation between two populations should be limited or absent. By contrast, when populations are isolated, Nm can approach zero, resulting in an F_{ST} that approaches 1. F_{ST} values of 1 indicate that the two populations each lack variation and do not share any alleles. Importantly, the estimated value of F_{ST} is influenced by history (how recently two populations exchanged migrants) and the amount of variation in each population. F_{ST} is valuable for making inferences in a comparative framework: in other words, F_{ST} provides the basis for inferring the isolation of populations relative to other populations.

There are at least three factors that influence the relative magnitude of F_{ST} for microsatellite and mtDNA data. The first factor reflects differences between the two markers in the amount of genetic diversity within populations. Microsatellites tend to be more variable than mitochondrial DNA because of the higher rate of mutation observed during replication. Because F_{ST} is a ratio of the average within population to the total variation for the two populations combined, namely $1 - D_W/D_T$ (D is genetic distance), greater variation translates into larger values of D_W/D_T and a depression of F_{ST} (or similar analog statistics) (Hedrick 1999). So, on average, F_{ST} values for microsatellites are smaller than for DNA sequence markers. The second fact reflects the fact that mtDNA is uniparentally-inherited only through

females; thus, would also expect higher F_{ST} because of the lower effective population size (Hudson and Turelli 2003). Finally, and perhaps most importantly, there is a sex-bias in mammalian dispersal, with males tending to disperse more than females (Greenwood 1980), although both males and females disperse in prairie dogs (Garrett and Franklin 1988). Because mtDNA is maternally-inherited whereas microsatellite genotypes are transmitted from both males and females, greater F_{ST} values for mtDNA may reflect, in part, the tendencies for females remain in the natal colony and for males to disperse (Hoogland 1999).

1.2.4 Genetic Structure Results

Estimates of F_{ST} were calculated for pairs of *C. g. gunnisoni* colonies across a large range based on the variation among microsatellite genotypes and mitochondrial DNA sequences. The results are summarized in table 3 and figure 3. There were three noteworthy results. First, mtDNA F_{ST} values are overwhelmingly higher than nuclear F_{ST}. This makes sense as effective population size is effectively half as mitochondrial DNA is maternally inherited while nuclear DNA is contributed by males and females. In addition, the higher apparent isolation of colonies for mtDNA relative to nuclear genes may reflect female philopatry and male-biased dispersal. Table 3. Matrix of pairwise F_{ST} values for the microsatellite genotypes (below the diagonal) and mitochondrial DNA (above the diagonal). Bold values are significant at $P < 0.01$.

	TESW	ENSP	BLFB	TPRR	PSLV	DN	ERPD	GBTT	GCR	BG	BVSE	EMSP
TESW		**0.60**	**0.84**	**0.48**	**0.69**	**0.81**	**0.90**	**0.79**	**0.74**	**0.73**	**0.82**	**0.78**
ENSP	**0.43**		**0.71**	**0.31**	**0.48**	**0.31**	**0.64**	**0.53**	**0.46**	**0.40**	**0.63**	**0.53**
BLFB	**0.44**	**0.16**		**0.40**	**0.82**	**0.86**	**0.91**	**0.85**	**0.82**	**0.80**	**0.88**	**0.86**
TPRR	**0.40**	**0.17**	**0.22**		**0.40**	**0.48**	**0.61**	**0.49**	**0.42**	**0.31**	**0.59**	**0.48**
PSLV	**0.37**	**0.24**	**0.27**	**0.20**		**0.72**	**0.74**	**0.50**	**0.21**	**0.28**	**0.73**	**0.65**
DN	**0.43**	**0.21**	**0.23**	**0.19**	**0.21**		**0.86**	**0.75**	**0.70**	**0.71**	**0.81**	**0.77**
ERPD	**0.40**	**0.22**	**0.23**	**0.20**	**0.18**	**0.13**		0.12	**0.59**	**0.49**	**0.91**	**0.92**
GBTT	**0.43**	**0.19**	**0.19**	**0.18**	**0.20**	**0.17**	**0.11**		**0.37**	-0.09	**0.84**	**0.81**
GCR	**0.59**	**0.31**	**0.35**	**0.32**	**0.24**	**0.22**	**0.15**	**0.22**		**0.12**	**0.76**	**0.69**
BG	**0.47**	**0.22**	**0.21**	**0.18**	**0.17**	**0.13**	0.08	0.07	**0.25**		**0.81**	**0.75**
BVSE	**0.38**	**0.21**	**0.20**	**0.16**	**0.17**	**0.16**	**0.17**	**0.16**	**0.26**	**0.16**		**0.59**
EMSP	**0.51**	**0.27**	**0.26**	**0.28**	**0.31**	**0.32**	**0.31**	**0.28**	**0.42**	**0.34**	**0.24**	

Table 1-3. Matrix of pairwise F_{ST} Values: F_{ST} values for the microsatellite genotypes (below the diagonal) and mitochondrial DNA (above the diagonal). Bold values are significant at $P < 0.01$.

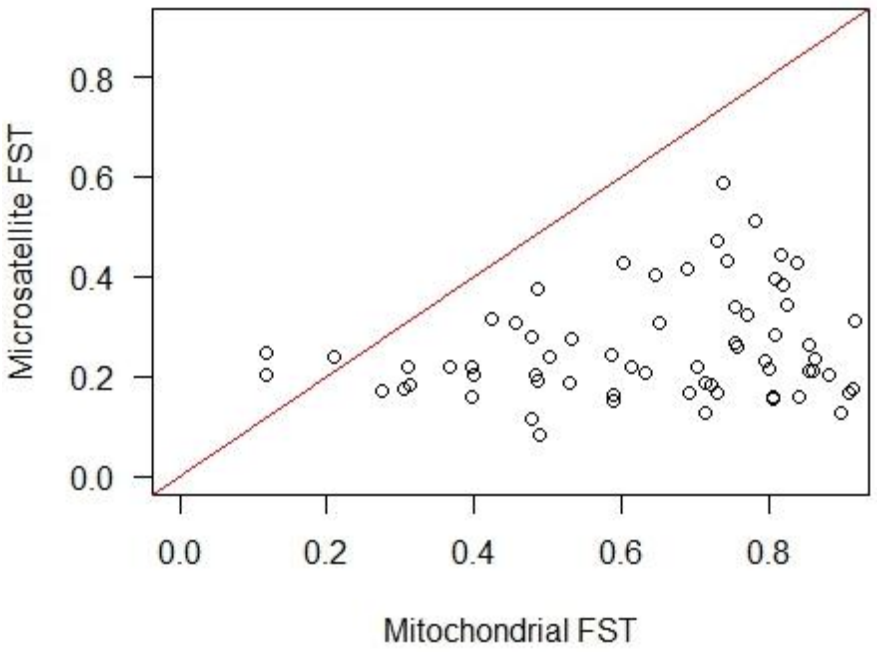


Figure 1-3. Microsatellite and Mitochondrial F_{ST} Comparisons: Plot of pairwise F_{ST} values for all pairs of populations showing that mtDNA values are larger, on average, than estimates from nuclear genotypes.

The second relevant result is that two of the colonies (TESW and EMSP) are genetically divergent, and clearly demographically isolated, from all other colonies for both sets of markers. TESW is the only colony without any private alleles, emphasizing both the low amount of genetic diversity and that the divergence observed in the colony is a result of divergent allele frequencies and not from alleles unique to the population (Figure 4).

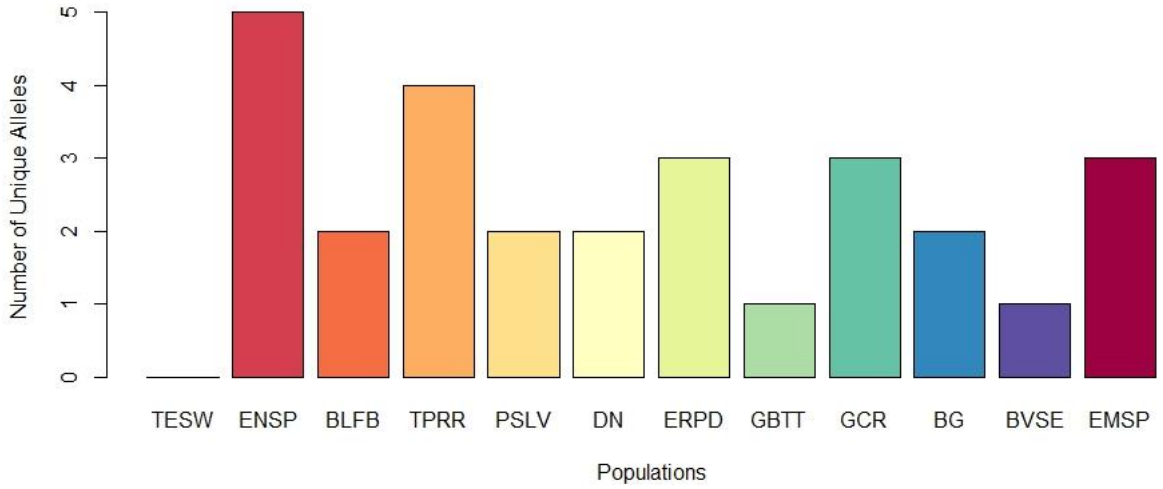


Figure 1-4. Unique Alleles: The number of unique microsatellite alleles observed at each population.

The third relevant result is that all of the sampled colonies are genetically isolated in the sense that it is unlikely individuals have moved between any pair of populations recently (in time frames measured in a least 10s of generations) (Table 4 and 5). Table 4 shows the low pairwise migration estimates between Individual Population Areas used in this study from mitochondrial and nuclear markers. The only populations sampled with a lack of statistically-detectable genetic structure (estimated using F_{ST}) were three of the colonies sampled near Gunnison (GBTT, GCR, and BG).

	TESW	NM	SLV	GUNNI	BVSE	EMSP
TESW		0.668	0.320	0.132	0.110	0.140
NM	0.496		0.900	0.478	0.500	0.674
SLV	0.523	1.843		0.493	0.329	0.477
GUNNI	0.426	1.597	2.713		0.114	0.978
BVSE	0.400	1.612	2.146	1.468		0.353
EMSP	0.240	1.050	0.774	0.693	0.780	

Table 1-4: Nm Values Between Population Areas: TESW (southwest), NM (New Mexico), SLV (San Luis Valley), BVSE (southeast), and EMSP (South Park). Upper triangle is mitochondria values and lower triangle is microsatellite values. Nm values are representative of the number of migrants between populations per generation. This data is visualized in figure 4.

	TESW	ENSP	BLFB	TPRR	PSLV	DN	ERPD	GBTT	GCR	BG	BVSE	EMSP
TESW		0.337	0.314	0.370	0.417	0.328	0.380	0.332	0.175	0.279	0.400	0.240
ENSP	0.331		1.331	1.252	0.803	0.924	0.886	1.092	0.562	0.886	0.967	0.660
BLFB	0.097	0.202		0.895	0.688	0.825	0.822	1.061	0.474	0.923	0.991	0.696
TPRR	0.532	1.139	0.754		0.986	1.093	0.973	1.176	0.541	1.116	1.272	0.644
PSLV	0.224	0.546	0.113	0.763		0.946	1.109	0.988	0.788	1.200	1.247	0.564
DN	0.118	1.116	0.079	0.537	0.194		1.745	1.237	0.891	1.695	1.350	0.523
ERPD	0.057	0.275	0.050	0.314	0.173	0.081		1.963	1.417	2.774	1.191	0.550
GBTT	0.130	0.444	0.085	0.531	0.495	0.164	3.781		0.894	3.316	1.326	0.639
GCR	0.177	0.598	0.106	0.681	1.887	0.213	0.349	0.862		0.768	0.721	0.349
BG	0.184	0.764	0.124	1.105	1.312	0.201	0.524	-6.234	3.764		1.316	0.484
BVSE	0.110	0.291	0.067	0.349	0.185	0.121	0.048	0.095	0.160	0.120		0.780
EMSP	0.140	0.441	0.085	0.549	0.267	0.148	0.046	0.119	0.227	0.164	0.353	

Table 1-5. Pairwise estimate of Nm between sites: Estimating gene flow. Upper triangle is mitochondria values and lower triangle is microsatellite values.

Variation in F_{ST} among colonies reflects the effects of three processes. Genetic drift and selection within populations causes populations to become different. Movement of individuals between populations causes populations to become more similar. The effect of genetic drift is most evident from isolation by distance plots in which variation in F_{ST} is compared with the geographic distance between pairs of populations. There is clear evidence for an effect of geographic distance on F_{ST} estimates of population differentiation (Figure 9). An effect of natural selection can be estimated by performing a similar analysis in which the influence of geographic distance is held constant and the variation in F_{ST} related to a factor that may influence organismal performance. There were substantial differences in the elevation among sampled colonies and there is clear evidence of the effects of elevation on selection in rodents (Novillo and Ojeda

2014; Ferro and Barquez 2009; Patterson et al. 1989). The resulting test is referred to as isolation by environment. In this case, after controlling for the effects of geographic distance, there was a demonstrable effect of elevation of the degree of nuclear genome differentiation between pairs of populations (Mantel r = 0.567, p = 0.001; Figure 5). These results suggest that a significant fraction of the genetic variation among colonies may reflect the effects of selection due to elevational differences across the landscape.

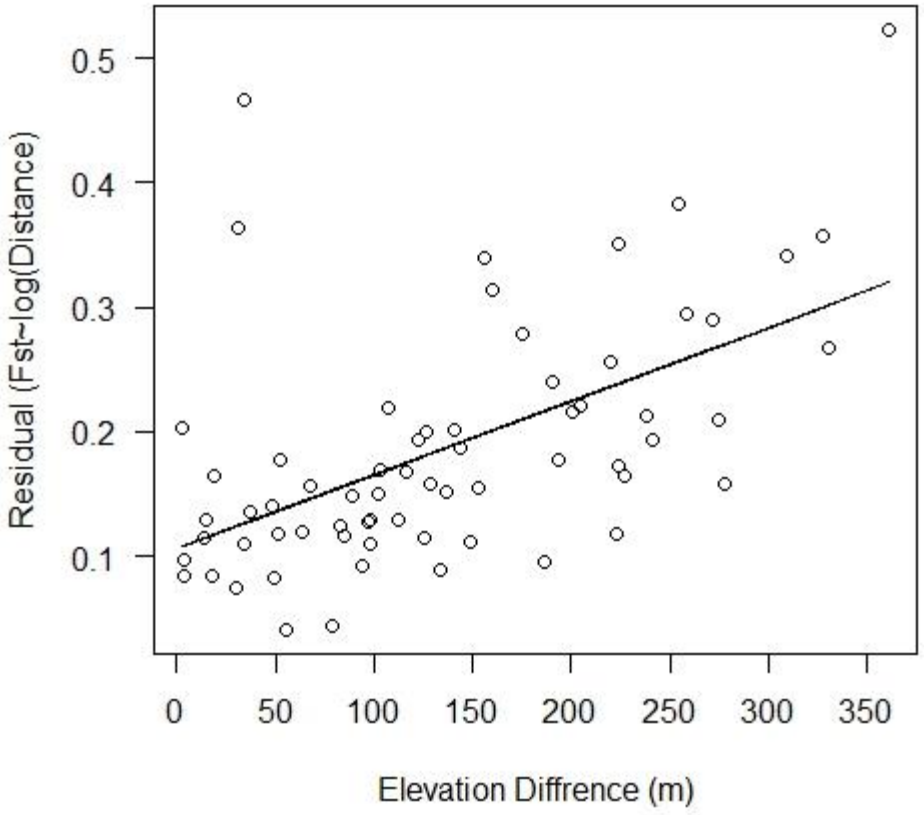


Figure 1-5. Mantel Tests:
Microsatellite F_{ST} values:

Upper: Isolation by distance: Microsatellite F_{ST} plotted against the log of pairwise Euclidian distance (mantel: r=0.2401, p=0.045, method=spearman)

Lower: Isolation by elevation: After controlling for the effects of geographic distance there is a demonstrable effect of elevation of the degree of differentiation between pairs of populations (Mantel r = 0.567, p = 0.001m, method=spearman).

We also used Bayesian assignment methods implemented in STRUCTURE (Pritchard et al. 2000). In this method, individuals are assigned to groups and a likelihood of the data given the assignment of individuals to groups calculated. A heuristic assessment for choosing the best number of groups (Figure 6) revealed that 3 and 12 groups best explained the data (Figure 7). In the K = 3 analysis, colonies from the Gunnison, San Luis Valley and Southeast IPAs grouped together (group 1), Telluride was by itself (group 2), and the New Mexico populations clustered together (group 3). Interestingly, the South Park colony was split between groups 2 and 3. This is best interpreted as uncertainty in the assignment South Park individuals to one of these two groups. In the K = 12 analysis, there were 11 distinct groups and one group that introduced uncertainty in the assignment of some individuals (the yellow color in the figure). The two colonies that grouped together in this analysis were the two that were the most geographically close near Gunnison. The inference of 11 groups (with uncertainty in assignment of some of the Gunnison individuals) is concordant with evidence of statistical significant structure based on F_{ST}.

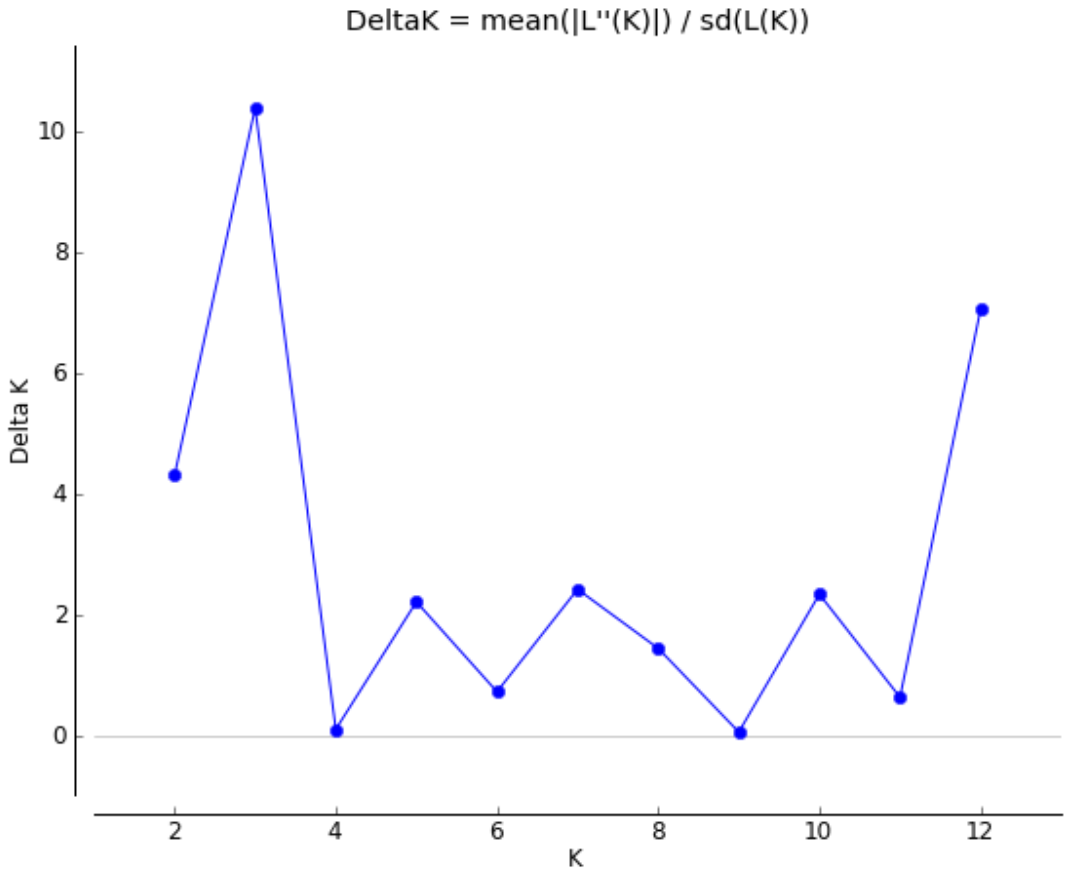
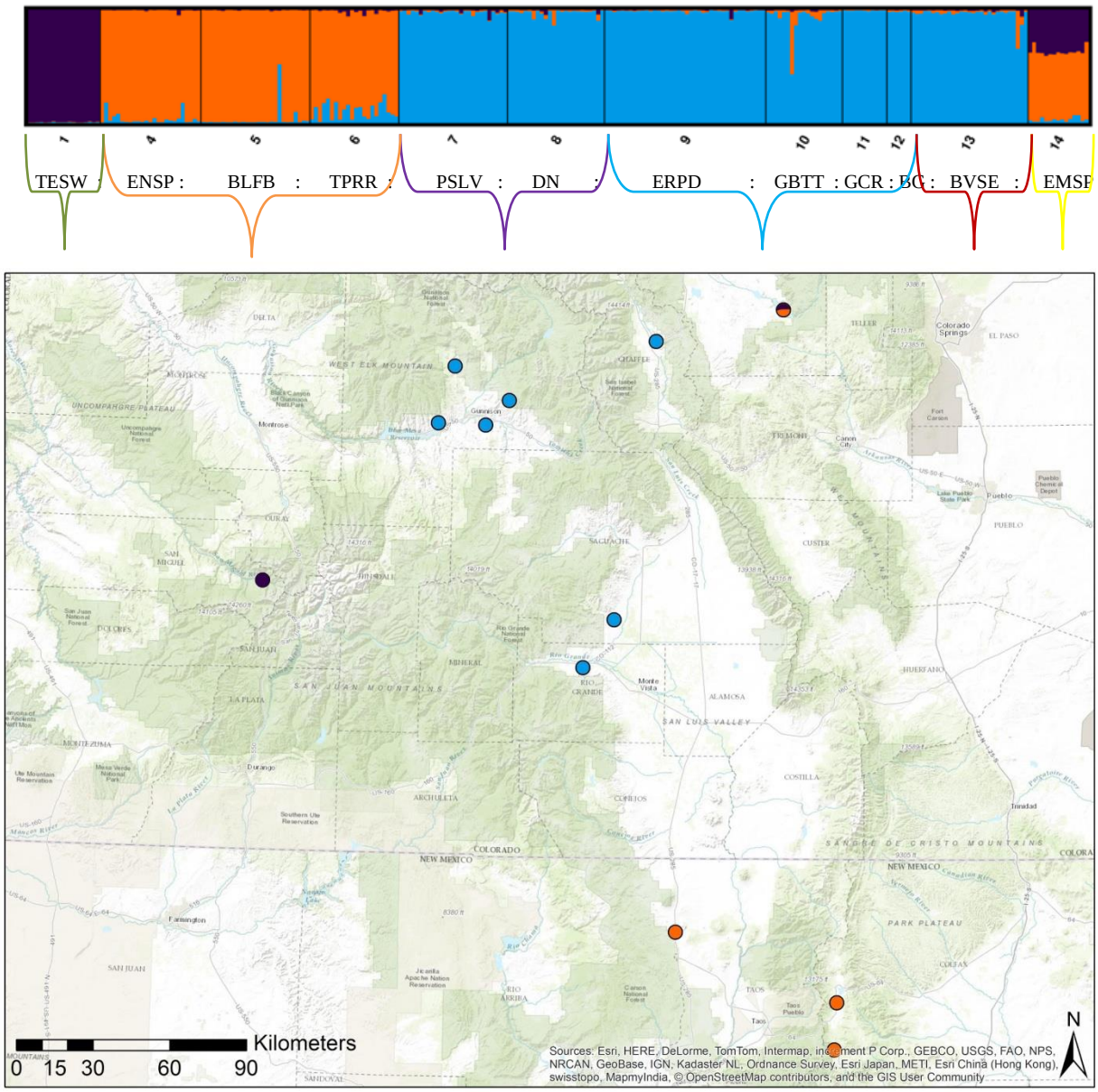


Figure 1-6. Likelihood of number of Structured Populations: Plot of the difference in likelihood scores for different values of K (number of groups) based on STRUCTURE analysis. Peaks in the graph correspond with values of K that are better supported by the data than other values of K (Pritchard et al. 2000).

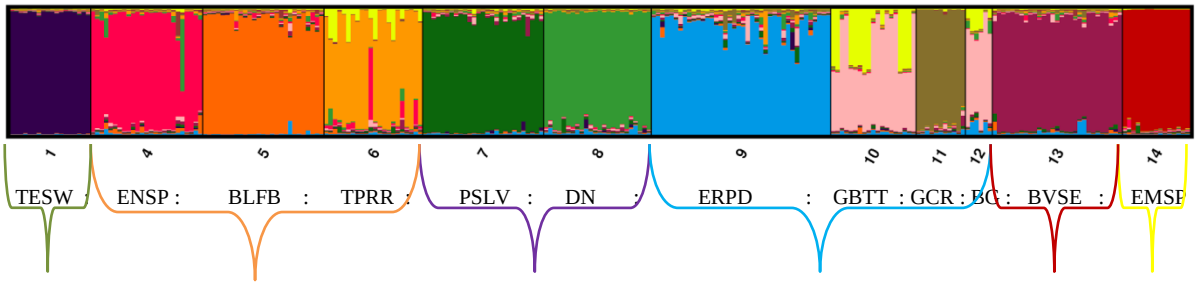
K=3

Mean(LnProb) = -9739.740, Mean(similarity score) = 0.867



K = 12

Mean(LnProb) = -7364.229, Mean(similarity score) = 0.856.



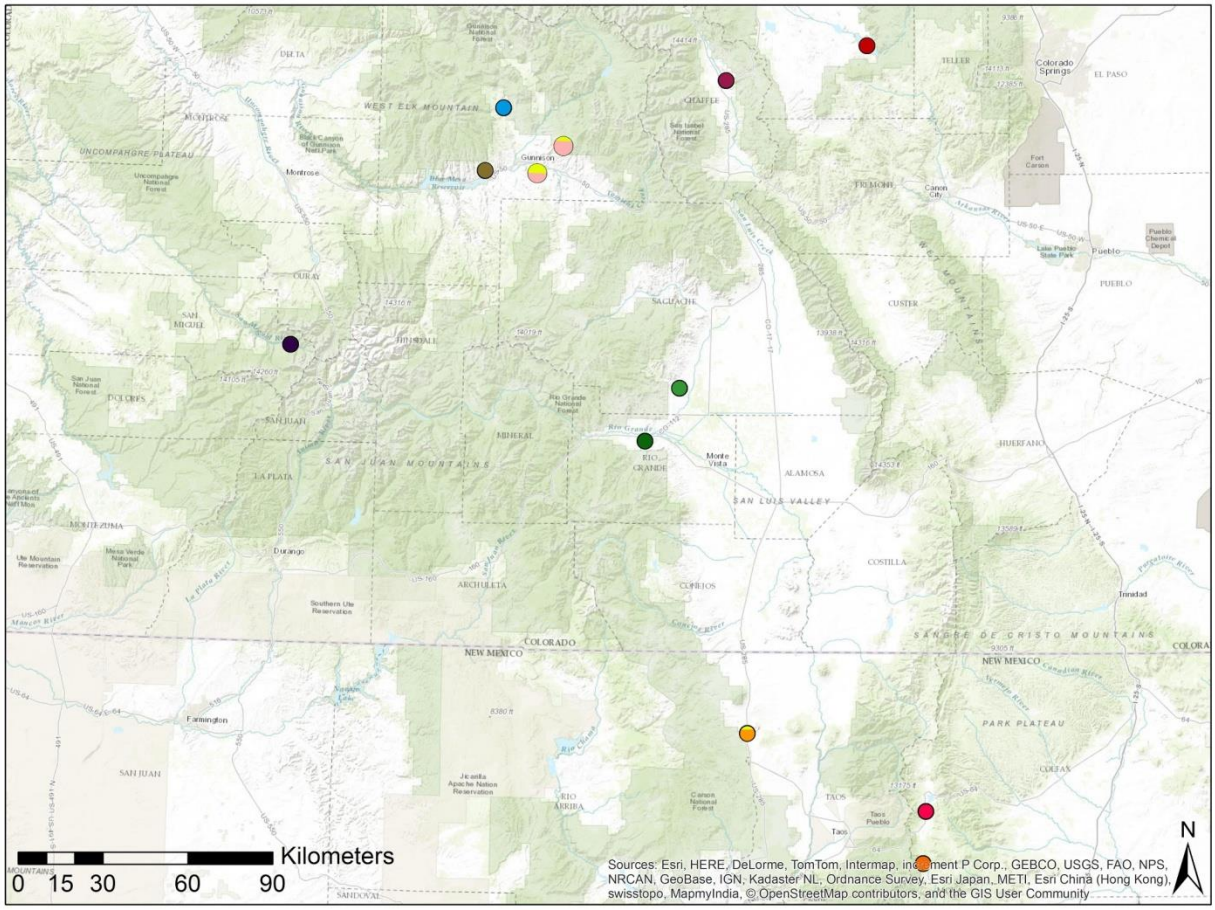


Figure 1-7. STRUCTURE Plots for K = 3 and K = 12: Each vertical line is an individual. Different colors correspond with different genotype groups. The height of a bar indicates probability of assignment to a particular group (Pritchard et al. 2000; Kopelman et al.). Brackets under each STRUCTURE plot indicate population areas. Green=southwest, orange=New Mexico, purple = SLV, blue= Gunnison, red= southeast, yellow = South Park. Maps following each STRUCTURE plot show each population colored to match corresponding grouping from STRUCTURE. The four Gunnison colonies are shown further apart from each other than in actuality for easier visualization between populations.

1.3 Conclusions

1.3.1 Key inferences

1) All populations, with the exception of two geographically close Gunnison colonies, are genetically distinct
2) Estimates of inter-colony gene flow are low, implying the sampled colonies are demographically isolated from each other
3) Two colonies (TESW) and (EMSP) have extraordinary low genetic diversity indicative of small effective population size, recent colonization by few founders, or a recent population bottleneck
4) There is a clear signal of isolation by elevation suggesting prairie dogs may be adapted to different elevations

1.3.2 Management implications

1) May be necessary to move individuals between colonies to maintain or increase genetic diversity in the face of colony isolation and the loss of variation due to drift.
2) Movement of individuals should involve colonies at similar elevation.
3) Special management practices, such as reintroductions, in the South Park and Southwest regions may need to be implemented if genetic diversity is to increase.
4) Plague management may allow managed colonies to become stable occupied patches, which should increase migration to surrounding patches, and possibly reduce bottle neck events.

5) Maintaining long term patch occupancy and the ability to strategically introduce individuals to key unoccupied patches may lead to colony networks becoming re-established and increased migration rate and gene flow within population areas.

1.3.3 Recommendations for additional research

1) It would be useful to sample additional colonies within population areas that are under-sampled, including South Park, Telluride, Southeast, Southwest, and the San Luis Valley. This would enable estimates of demographic connectivity within regions.
2) Move prairie dogs into colonies with low genetic diversity and study the survival of immigrant individuals and their genes; ideally, individuals are moved from different elevations to assess whether elevation influences survival.
3) Examine the effects of survival and resulting genetic mixing by moving prairie dogs between colonies in the same population area to moving prairie dogs between population areas.
4) Investigate the dynamics of hybridization between *C. g. gunnisoni* and *C. g. zuniensis*.
5) Explore the evolution of adaptation to elevation. Identify the underlying genes and traits which allow for living at high elevation.

Chapter II:
The Complete Mitochondrial Genome of The Gunnison's Prairie Dog (*Cynomys gunnisoni*) and Phylogenetic Relationship within the Genus *Cynomys*

2.1 Abstract:

The mitochondrial genome of the Gunnison's prairie dog, *Cynomys gunnisoni*, is described and incorporated into the *Cynomys* phylogeny. Using seven novel mitochondrial genomes and existing genomes from the related *Cynomys leucurus* and *Cynomys ludovicianus* species along with a sister genus, *Ictidomys tridecemlineatus,* we further examined the genus phylogeny and determined intra-species relationships.

2.2 Introduction:

Prairie dogs are colonial semi-fossorial members of the family *Sciuridae*. Because of the transformative effects of their herbivory and tunneling, they are considered ecosystem engineers of grassland and brush ecosystems of the Great Plains and Rocky Mountain regions of western North America (Clark et al. 1971; Pizzimenti 1975; Seglund and Schnurr 2010). The two subgenera include five species of extant prairie dogs. Subgenus *Leucocrossuromys,* the white-tailed prairie dogs, include the Gunnison's (*Cynomys gunnisoni*), Utah (*Cynomys parvidens*), and white-tailed (*Cynomys leucurus*) prairie dogs. (Pizzimenti and Hoffman 1973). The subgenus *Cynomys,* the black-tailed prairie dogs, includes the Mexican (*Cynomys mexicanus*) and black-tailed (*Cynomys ludovicianus*) prairie dogs (Clark et al. 1971; Pizzimenti 1975; Seglund et al. 2006).

Previous studies using mtDNA sequences from the cytochrome b gene led to an inference of the origination of the genus about 3 million years ago (Harrison et al. 1993). In an effort to better characterize the divergence among lineages within the genus, we assembled complete mitochondrial genome sequences from seven individuals from three of the distinct species. These sequences include five novel genomes of *Cynomys gunnisoni*, all of which come from the montane subspecies (*C.g.gunnisoni)*, and a

single individual each from *C. leucurus* and *C. ludovicianus.* Additionally, existing mitochondrial genomes of *Cynomys* and the putative sister genus (*Ictidomys tridecemlineatus*) were obtained to use as a basis for estimating origination time and for providing a resource for more fine-scale population genetic and historical biogeographic inferences across the taxon's distribution.

2.3 Methods:

Whole genomic DNA was extracted from frozen spleen samples using Qiagen DNEasy Blood and Tissue Kits. DNA libraries were prepared by the BioFrontiers Institute at the University of Colorado, Boulder using Nextera Library prep kits. DNA was sequenced on an Illumina Next Seq500 using paired-end 2 x 150 bp reads. Sequence data was assembled using SPAdes v. 3.11 (Nurk et al. 2013). One annotation for *Cynomys gunnisoni* was submitted to GenBank (accession MG450794) and oriented to start with the cytochrome c oxidase I gene (*cox1*) (Fig 1).

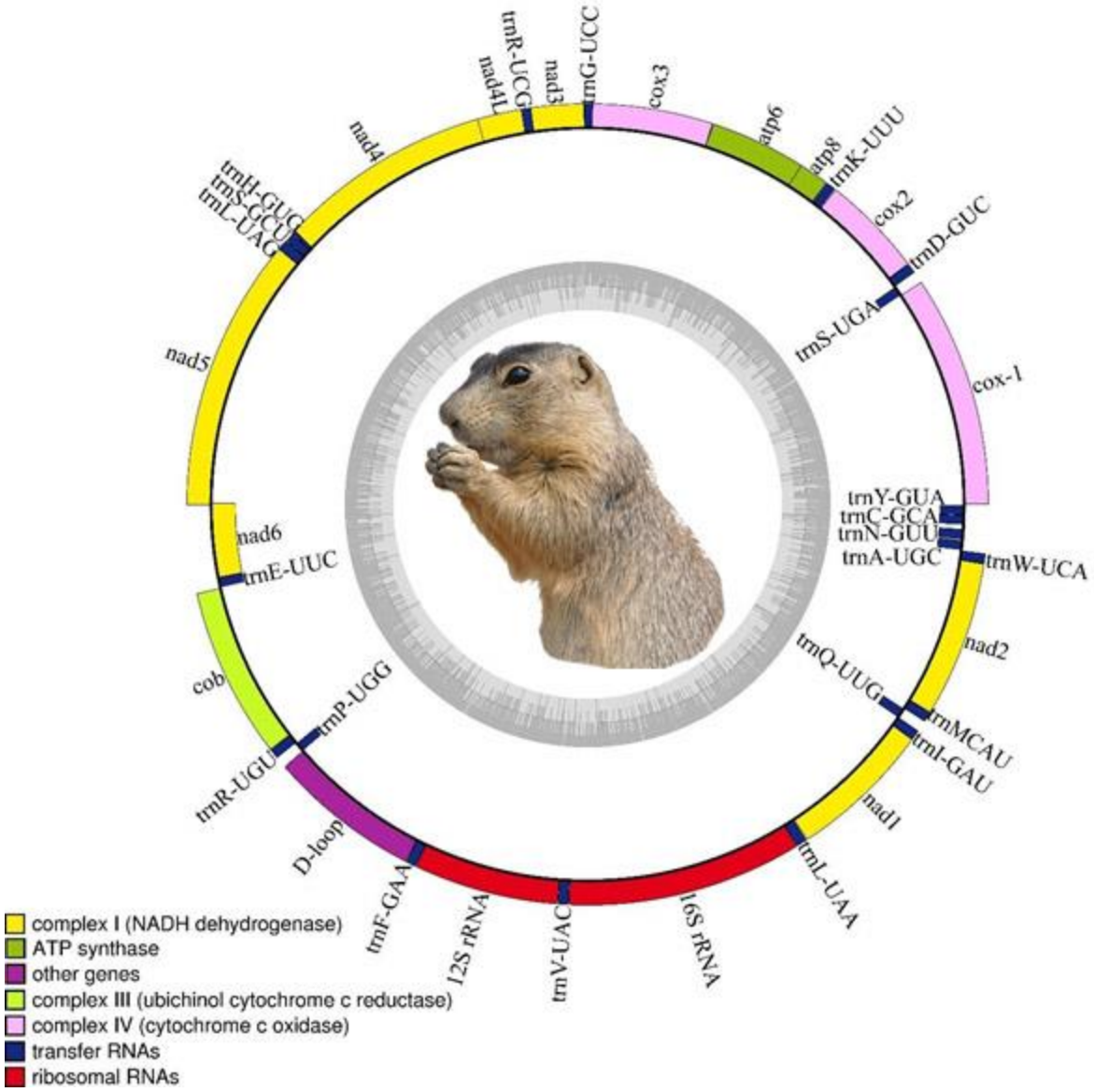


Fig. 2-1: The Complete Circularized Mitochondrial Genome of the Montane Gunnison's Prairie Dog, *Cynomys gunnisoni gunnisoni*, 16,461 bp. The inner circle is a representation of average GC content, with the 50% mark being the inner gray line (OGDRAW, Lohse et al 2007, Lohse et al. 2013).

The seven assembled mitochondrial prairie dog genomes were aligned using ClustalW with default parameters (Larkin et al 2007). Additional genomes were obtained NCBI's GenBank database which include a single *C. ludovicianus* (NCBI accession KP326310), *C. leucurus* (KP326309), and a thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*; KP698974). Alignments were curated in

MEGA7 (Kumar et al, 2016) and a bootstrap consensus tree was created using maximum likelihood with *I. tridecemlineatus* as an out-group. The Maximum Parsimony method was used to develop an initial tree for the heuristic search and the tree was drawn to scale with branch lengths proportional to the number of substitutions per site (figure 2).

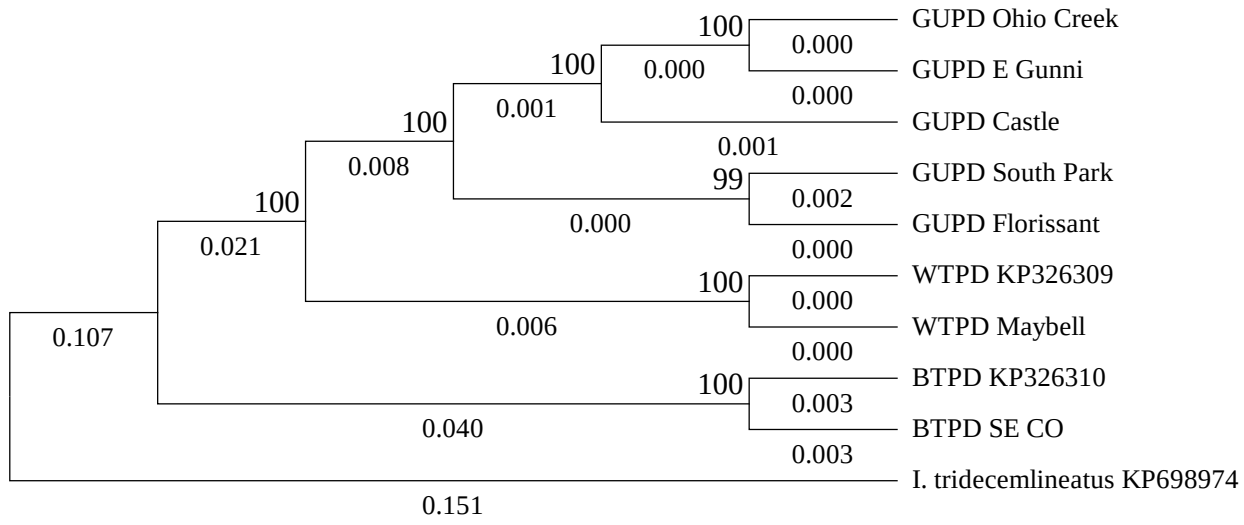


Fig 2-2. Molecular Phylogenetic Analysis by Maximum Likelihood Method.
The phylogenetic tree above shows the evolutionary history of the available mitochondrial genomes from the *Cynomys* genus. This tree was inferred using the Maximum Likelihood method based on the General Time Reversible model. A bootstrap consensus tree was created using 200 replicates and the percentage of replicate trees which show the same relationship in the bootstrap tests are shown above each branch. This analysis was conducted in MEGA7 (Kumar et al. 2016, Tamura and Nei, 1993, Tamura et al. 2012).

2.4 Results

The new *C. gunnisoni* sequences are very similar to the previously published mitochondrial genome sequences of two congeners from Li et al. 2016. The five *C. gunnisoni* mitochondrial genomes varied in length from 16404 to 16462 base pairs, a 58bp difference. There are small length differences between the two individuals of *C. leucurus* (16443 to 16454, 11 bp difference) and *C. ludovicianus* (16457 to 16466, 9 bp difference). Genetic distances, nucleotide composition, and phylogenetic trees were obtained using MEGA v.7.0.26. Percent divergence (p-distance*100) ranged from 0.02 - 0.29 within Gunnison's prairie dogs and correlation by Mantel test between genetic and geographic distance was high, though only marginally significant (r=0.63, P=0.075). P-distance between Li et al. 2016's genomes and ones newly

sequenced were comparable. The two *C. leucurus* are 0.05% divergent and the two *C. ludovicianus* are 0.54% divergent. The average percent divergence seen between *C. gunnisoni* and *C. leucurus* is 1.29%. The divergence between *C. gunnisoni* and *C. ludovicianus* and between *C. leucurus* and *C. ludovicianus* is 4.04% and 4.01% respectively. Nucleotide abundance is highly conserved. All individuals from the *Leucocrossuromys* subgenus have nucleotide frequencies of T (30.9%) C (24.2%) A (32.1%) and G (12.8%) while the two individual from the *Cynomys* subgenus show frequencies of T (30.7%) C (24.3%) A (31.9%) and G (13.0%). G/C content is similar across all prairie dog species, 37% for *C. gunnisoni* and *C. leucurus* and 37.3% for *C. ludovicianus.* Clades within *C. gunnisoni* coincide with river drainages (Fig 2), with one clade including three samples (East Gunni, Ohio Creek and Castle) sorting with the Gunnison watershed, while the other clade contains two samples, (South Park, Florissant), originated from the South Platte watershed.

2.5 Discussion

Next generation whole genome shotgun technology was utilized to explore the genomes of three species of prairie dogs and have assembled the complete mitochondrial sequences of each. The novel genome of *Cynomys gunnisoni gunnisoni* has been contributed to GenBank with the addition of a genome to the genomic resources available for black-tailed (*Cynomys ludovicianus)* and white-tailed (*Cynomys leucurus)* prairie dogs. These additional genomic resources will improve the understanding of genetic relationships within and among these prairie dog species. Genetic distance among species supports current phylogenetic relationships, within species variation is lower than among species, and that geographic distance is likely associated with genetic distance. The number of complete mitochondrial genome sequences of *Cynomys* has been increased from two to nine using whole genome next generation sequencing technology, adding one new species and replication within 3 species. These data support previous phylogenetic hypotheses and indicate that there is important variation within species across their respective geographic ranges.

Gene/region	Start Position	Stop Position	Length (bp)	Strand
cox1	1	1542	1542	+
tRNA-Ser	1613	1545	67	-
tRNA-Asp	1617	1685	69	+
cox2	1686	2369	684	+
tRNA-Lys	2441	2644	67	+
atp8	2441	2644	204	+
atp6	2602	3282	681	+
cox3	3282	4085	804	+
tRNA-Gly	4066	4135	70	+
nad3	4136	4492	357	+
tRNA-Arg	4483	4549	67	+
nad4L	4551	4847	297	+
nad4	4841	6265	1425	+
tRNA-His	6219	6287	69	+
tRNA-Ser	6288	6347	60	+
tRNA-Leu	6348	6417	70	+
nad5	6418	8235	1818	+
nad6	8744	8219	524	-
tRNA-Glu	8812	8744	67	-
cob	8817	9956	1140	+
tRNA-Thr	9957	10025	69	+
tRNA-Pro	10098	10031	66	-
D-loop	10099	11113	1015	+
tRNA-Phe	11114	11184	71	+
12S rRNA	11185	12150	966	+
tRNA-Val	12151	12220	70	+
16S rRNA	12221	13786	1565	+
tRNA-Leu	13787	13860	73	+
nad1	13864	14820	956	+
tRNA-Ile	14820	14888	68	+
tRNA-Gln	14957	14886	71	-
tRNA-Met	14961	15029	68	+
nad2	15030	16073	1043	+
tRNA-Trp	16072	16139	67	+
tRNA-Ala	16211	16143	68	-
tRNA-Asn	16289	16217	72	-
tRNA-Cys	16387	16321	66	-
tRNA-Tyr	16453	16388	65	-

Table 2-1: *C.g.g* Mitochondrial Genome Description: Gene or region names, start and stop location, length and strand of the *Cynomys gunnisoni* mitochondrial genome.

References Cited

Bangert RK, Slobodchikoff CN. 2006. Conservation of prairie dog ecosystem engineering may support arthropod beta and gamma diversity. Journal of Arid Environments 6:100–115

Biodiversity Response to Climate Change in the Middle Pleistocene: The Porcupine Cave Fauna from Colorado, edited by Anthony D. Barnosky, University of California Press, 2004. ProQuest Ebook Central, https://ebookcentral.proquest.com/lib/ucb/detail.action?docID=224672.

Clark TW, Hoffman RS, Nadler CF. 1971. Cynomys leucurus. American Society. Mammal. Mammalian Species No. 7.

Cully JF. 1989. Plague in prairie dog ecosystems: importance for black-footed ferret management in T. W. Clark, D. Hinckley, and T. Rich, editors. The prairie dog ecosystem: managing for biological diversity. Bureau of Land Management, Billings, Montana.

Cully JF, Williams SE. 2001. Interspecific comparisons of sylvatic plague in prairie dogs. Journal of Mammalogy 82:894-905

Davidson AD, Detling JK, Brown JH. 2012. Ecological roles and conservation challenges of social, burrowing, herbivorous mammals in the world's grasslands. Frontiers in Ecology and the Environment 10(9) 477-486

Den Boer PJ. Spreading of Risk and Stabilization of Animal Numbers. 1968. Acta Biotheoretica 18(1):165-94

Ferro LI, Barquez RM. Species richness of nonvolant small mammals along elevational gradients in northwestern Argentina. 2009. Biotropica. 41 (6): 759-767.

Garrett M G and W L Franklin. 1988. Behavioral ecology of dispersal in the black-tailed prairie dog. J. Mammal. 69: 236-250. https://doi.org/10.2307/1381375

Greenwood PJ. Mating systems, philopatry and dispersal in birds and mammals. 1980. Animal Behavior. 28:1140-1162

Goodwin H. Thomas, Pliocene-Plistocene Biogeographic History of Prairie Dogs, Genus Cynomys (Sciuridae). Journal of Mammalogy, Vol. 76 No1, 1995, 100-122.

Hanski I, Gilpin M. 1991. Metapopulation dynamics: brief history and conceptual domain. Biological Journal of the Linnean Society of London 42:3-16

Hanski I. 1998. Metapopulation dynamics, Nature 58:41-49.

Hedges SB, Dudley J, & Kumar S. 2006. TimeTree: A public knowledge-base of divergence times among organisms. Bioinformatics 22: 2971-2972

Hedges SB, Marin J, Suleski M, Paymer M, and Kumar S. 2015 Tree of Life Reveals Clock-Like Speciation and Diversification. Mol Biol Evol 32: 835-845.

Hedrick P. 1999. Highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313-318.

Hoogland J L. 1999. Philopatry, dispersal and social organization of Gunnison's prairie dogs.J. Mammal. 80: 243-251

Hoogland JL, Cannon KE, DeBarbieri LM, Manno TG. 2006. Selective predation on Utah prairie dogs. The American Naturalist 168(4): 546–552.

Hudson R R and M Turelli. 2003. Stochasticity overrules the "three-times rule": Genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. Evolution 57: 182-190.

Kaiser SA, Taylor SA, Chen N, Sillett TS, Bondra ER, Webster MS. 2016. A comparative assessment of SNP and microsatellite markers for assigning parentage in a socially monogamous bird. Molecular Ecology Resources.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA Mayrose I. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15(5): 1179-1191

Kumar S, Stecher G, Suleski M, Hedges SB (2017) TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. Mol Biol Evol 34 (7): 1812-1819

Kumar S, Stecher G, and Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. 2016. Molecular biology and evolution. 33.7: 1870-1874.

Larkin MA. Blackshields G Brown NP, Chenna R. McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, and Higgins DG. 2007. Bioinformatics 23(21): 2947-2948. doi:10.1093/bioinformatics/btm404

Lohse M, Drechsel O, and Bock R. 2007. OrganellarGenomeDRAW (OGDRAW) - a tool for the easy generation of high-quality custom graphical maps of plastid and mitochondrial genomes. *Curr. Genet.* 52:267-274.

Martínez-Estévez L, Balvanera P, Pacheco J, Ceballos G. 2013. Prairie Dog Decline Reduces the Supply of Ecosystem Services and Leads to Desertification of Semiarid Grasslands. PLoS One 8(10)

Miller RF, Svejcar TJ, West NE. 1994. Implications of livestock grazing in the Intermountain sagebrush region: plant composition. Pages 101-146. in M. Vavra, W. A. Laycock, and R. D. Pieper, editors. Ecological implications of livestock herbivory in the west. Society for Range Management, Denver, Colorado.

Novillo A, Ojeda RA. Elevation patterns in rodent diversity in the dry Andes: disentangling the role of environmental factors. 2014. Journal of Mammalogy. 95 (1): 99-107

Nurk S. et al. 2013 Assembling Genomes and Mini-metagenomes from Highly Chimeric Reads. Deng M., Jiang R., Sun F., Zhang X. Research in Computational Molecular Biology. RECOMB 2013. Lecture Notes in Computer Science, Vol 7821. Springer, Berlin, Heidelberg

Patterson BD, Meserve PL, Lang BK. Distribution and abundance of small mammals along an elevational transect in temperate rainforests of chile. 1989. Journal of Mammalogy. 70 (1): 67-78.

Pizzimenti JJ. 1975. Evolution of the prairie dog genus Cynomys. Lawrence: Museum of Natural History, University of Kansas

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rayor LS. 1985. Effects of Habitat Quality on Growth, Age of First Reproduction, and Dispersal in Gunnison Prairie Dogs (Cynomys-Gunnisoni). Canadian Journal of Zoology 63(12)

Roach JL, Stapp P, Van Horne B, Antolin MF. 2011. Genetic Structure of a Metapopulation of Black-tailed Prairie Dogs. American Society of Mammalogists. Journal of Mammalogy 82(4): 946-959

Sackett LC, Etchberger LK, Mazzella MN, Lim DD, Martin AP. 2010. Characterization of 18 microsatellite loci for three species of prairie dogs. Lored Molecular Ecology Resources 10(1):232-236.

Sackett LC, Cross TB, Jones RT, Johnson WC, Ballare K, Ray C, Martin AP. 2012. Connectivity of prairie dog colonies in an altered landscape: Inferences from analysis of microsatellite DNA variation. Conservation Genetics 13(2)

Sackett LC, Seglund AE, Guralnick RP, Mazzella MM, Wagner DM, Busch JD, Martin AP. 2014. Evidence for two subspecies of Gunnison's prairie dogs (*Cynomys gunnisoni*), and the general importance of the subspecies concept. Biological Conservation 174:1–11

Seglund AE, Ernst AE, Grenier M, Luce B, Puchniak A, Schnurr P. 2006. White-tailed prairie dog conservation assessment. Western Association of Fish and Wildlife Agencies. Laramie, Wyoming. Unpublished Report. 137 pp

Seglund AE. Schnurr PM. 2010. Colorado Gunnison's and White-tailed Prairie Dog Strategy. Colorado Parks and Wildlife. Denver, CO.

Sierra-Corona R, Davidson A, Fredrickson EL, Luna-Soria H, Suzan-Azpiri H, Ponce-Guevara E, Ceballos G. 2015. Black-Tailed Prairie Dogs, Cattle, and the Conservation of North America's Arid Grasslands. PLoS One 10(3)

Slobodchikoff CN, Perla BS, Verdolin JL. 2009. Prairie Dogs: Communication and Community in an Animal Society. Cambridge, MA: Harvard University Press.

Tamura K and Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochndrail DNA in Humans and Chimpanzees. Molecular Biology and Evolution 10: 512-526.

USFW 12 month finding on GUPD. VOL. 78, No. 220. Nov. 14, 2013. Docket No. FWS–R6–ES–2013–0115; 4500030113