DISPERSAL, DIVERSITY AND DIVERGENCE: EVOLUTIONARY PROCESSES IN PRAIRIE DOGS (GENUS *CYNOMYS*)

by

LOREN CASSIN SACKETT

B.A., Whitman College, 2003

A thesis submitted to the Faculty of the Graduate School of the University of Colorado in partial fulfillment of the requirement of the degree of

Doctor of Philosophy

Department of Ecology and Evolutionary Biology

2012

This thesis entitled:

Dispersal, Diversity and Divergence: Evolutionary Processes in Prairie Dogs (Genus *Cynomys*)

Written by Loren Cassin Sackett

Has been approved for the Department of Ecology and Evolutionary Biology

_____

Andrew P. Martin (Committee Chair)

_____

Sharon K. Collinge

IACUC protocol #: 1004.09

Date _____

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.

Sackett, Loren Cassin (Ph.D., Ecology and Evolutionary Biology)

Dispersal, diversity and divergence: Evolutionary processes in prairie dogs (Genus *Cynomys*)

Thesis directed by Professor Andrew P. Martin

Abstract

Evolutionary biology seeks to understand the processes driving genetic diversity within species and phylogenetic divergence among species. Patterns of diversity may be dictated by both abiotic factors and community interactions. My dissertation research focuses on the processes that influence diversity and divergence in prairie dogs across spatial and temporal scales. At the macroevolutionary scale, habitat constraints influencing metabolism, reproductive period and growth rate may act as selective agents. Gunnison's prairie dogs (*Cynomys gunnisoni*) have been proposed to exist as two subspecies that occupy distinct habitats and are morphologically distinct in portions of their range. Using complementary approaches of phylogenetic analysis, genetic clustering, and spatial analysis of environment, I examine the contributions of different ecological selection pressures to genetic diversity of Gunnison's prairie dogs throughout their range. I find that elevation and temperature determine the distribution of two unique lineages of Gunnison's prairie dogs, and that plant communities differ between habitats occupied by each subspecies. At the local scale, landscape connectivity may interact with susceptibility to pathogens to influence population structure, particularly in social species such as prairie dogs. Although some land cover types, such as intense urbanization and water bodies, prevent dispersal across them, prairie dogs can travel across multiple land cover types such as grassland, shrubland and agricultural fields. Moreover, although large highways act as dispersal barriers, small roads can actually facilitate prairie dog dispersal, particularly across water bodies. Pathogen susceptibility is likely to influence genetic structure because prairie dogs suffer approximately 99% mortality during plague outbreaks. In Boulder County, re-

colonization by black-tailed prairie dogs (*C. ludovicianus*) occurred 1 – 3 years after extirpation in well-connected colonies. Genetic diversity within the metapopulation was retained. Within some well-connected populations colonized by multiple source populations, genetic variation was maintained or increased, but diversity was lost in some isolated populations. Finally, individuals in recolonized populations had significantly higher heterozygosity than those present before plague. This dissertation will contribute to our understanding of the abiotic and biotic mechanisms of diversification in social species.

Dedication

To my mom, who has encouraged me unendingly, never let me doubt myself, reminded me to follow my passions, and pushed me to do my best in every walk of life. She has stood behind me in every endeavor and devoted herself to my success. I am deeply thankful for her decades' worth of words of encouragement.

Acknowledgments

I give my greatest thanks to Andy Martin, my incredibly supportive, passionate, and insightful advisor. Like most advisors, Andy was intellectually supportive, but his contributions far exceeded the academic realm. Andy encouraged my participation in every tangential endeavor I sought, gave me the ultimate academic freedom to pursue my interests, and stood behind me through every challenge. His honest desire for what is best for his students is humbling, and his enthusiasm for science (and for revolutionizing science education) is inspirational.

I am appreciative of the unique faculty in this department for promoting community and encouraging graduate students to become engaged scientists; especially Yan Linhart, Deane Bowers, David Stock, Andy Martin, Becca Safran, Jeff Mitton, Mike Breed and Bill Bowman. I am also fortunate to have had academic support from Alan Templeton and Ken Olsen at Washington University in St. Louis, both of whom welcomed me into their labs during the time I spent there. John Hoogland at the University of Maryland engaged in helpful discussions about prairie dog biology that contributed to the way I envision divergence in Gunnison's prairie dogs. My committee provided feedback throughout my graduate career, and always encouraged me to pursue my own interests. The graduate committee in EEB recognized the importance of training outside the University, and generously funded two trips to specialized OTS courses. These courses provided me with a network of scientists with whom I still engage in intellectual discussions, allowed me to explore interests in my specialized field, and also happened to introduced me to my life partner. For this I am ever grateful! Finally, I must extend special thanks to Rob Guralnick, who gave not only intellectual feedback on my work, but also unending emotional and moral support. He always made me believe in myself and encouraged me to see no limits to what I could achieve.

I am deeply grateful to my cohort for their support, without which I would have a very different perspective of the collaborative community of scientists; particularly Mary Kay Herzenach, Katherine McClure, Natalie Robinson, Se Jin Song, and Susan Whitehead. Other graduate students in the department played a similarly supportive role, especially Liesl Peterson Erb, Sara Hellmuth Paull, and Kallin Tea. These people are friends, colleagues, amazing scientists and wonderful people, and they made my graduate career both bearable and enjoyable. I am excited that many of them will continue to be collaborators in my future. I have also benefited from the specialized expertise of numerous other graduate students, including Brian Stucky in analyzing vocalizations of prairie dogs, Abbey Paulson in generating maps in ArcMap, and Mike Robeson in making phylogenetic inferences and mastering some computational skills. The other members of the Martin Lab were supportive and gave helpful advice for succeeding in graduate school; I thoroughly enjoyed having Ryan Jones, Jessica Metcalf and Sierra Love Stowell in my academic family.

My family has contributed greatly to everything I have ever achieved, by encouraging me endlessly and by setting the standards for success very high. They are each amazing examples of finding passion in the world around them (even in such unlikely places as West Texas road cuts), working tirelessly to achieve their goals, and pursuing their intellectual curiosity all over the planet. I especially admire my grandparents and my mom, who made sure I had every opportunity imaginable and always reminded me to find fascination in the way things work. I have enjoyed the intellectual feedback of Nic Kooyers, whose big-picture world view contributes to my ability to put my work in a larger context. I am also appreciative of the fact that he has abandoned his strict "No science at home" policy to allow me to share what I find most exciting about the world. He willingly reads drafts of my papers and proposals, hears iterations of my talks, and gives insightful feedback about all of them. His intellect is humbling, and his confidence in my abilities invokes my greatest appreciation.

I am very fortunate to have received a great deal of financial support during the course of my dissertation. The EEB department was especially generous through its funding of my research, attendance at external courses, and a writing fellowship. The Graduate School and the University of Colorado Natural History Museum also provided support on multiple occasions, as did the Boulder County Nature Association. The Colorado Division of Wildlife funded all work on Gunnison's prairie dogs. The Undergraduate Research Opportunities Program funded a suite of enthusiastic undergraduates without which my field work would not have been possible; particularly amazing were Sarah Hale, whose love of prairie dogs overcame any logistical difficulty she encountered; Max Mazzella, who in addition to everything else volunteered to collect samples from wide-awake prairie dogs whose sole goal in life was to bite through his gloves; Ben Langsten, who drove across the country to join us in the field the day after I called him; Corey Fulton, whose anthropomorphization of each prairie dog we handled (and its appropriate human-in-a-hospital scenario) made me laugh so hard I couldn't walk; and Hayden Gardner for his hilariously accurate impression of Gob from Arrested Development, which facilitated the task of enduring long days in the field. Erin Arnold Pikcilingis was the best field assistant I've ever had: she was there to support me from my first semester of TAing through 3 arduous field seasons, she is extremely organized and quietly solves problems almost before they happen. Even after the most grueling 14 weeks of living out of a truck, without running water or a bed, the only complaint I ever heard from her was that she was going to miss field work the next year.

I have enjoyed working with many undergraduate students in the classroom as well. Teaching was an unexpected source of motivation for me, and I feel lucky to have had engaged, passionate students with whom I could share the love of biology. They served as a constant reminder that one of the major reasons scientists do research is to share it with others. Interacting with students was the most rewarding part of my graduate career.

This research was supplemented by samples obtained or provided by others. The Colorado Division of Wildlife provided field crews and collected data from Gunnison's prairie dogs in Colorado. Dave Wagner at Northern Arizona University provided samples from two important populations at the western edge of the GUPD range, Kenny Bader provided a much sought after sample from Petrified Forest National Park, and Prairie Dog Pals allowed us to obtain samples from their holding facility. The Gunnison's prairie dog project never could have taken place without the support of Amy Seglund in justifying the project to the Colorado Division of Wildlife, securing funds, and providing logistical, organizational and material support for field work.

I wish to thank the Colorado Division of Wildlife, Jim Stuart and the New Mexico Department of Game and Fish, Tony Wright and the Utah Division of Wildlife Resources, and Dave Cagle and the Arizona Department of Game and Fish for permission to capture and release prairie dogs, and innumerable land management agencies for providing access to study prairie dog colonies on their properties, including: the City of Boulder Open Space and Mountain Parks Department, the City of Boulder Parks and Recreation Department, the Boulder County Parks and Open Space Department, the Jefferson County Open Space Department, Andrea Chavez and the Bureau of Land Management, the National Park Service, the Zia Department of Natural Resources, the United States Forest Service, and Bob Salter and the Santo Domingo Department of Natural Resources. I am also grateful to Paula Martin for information about previous relocation sites in New Mexico and Colorado. Finally, several private landowners graciously allowed us to trap prairie dogs on their land without asking anything in return. These include Susan Carter, Charles Redd, Keith Rush, Shane Shumway, and Paul Archuleta.

Table of Contents

List of Tables

List of Figures

Chapter 1: **Introduction**

Throughout its history, evolutionary biology has sought the goal of elucidating the processes that drive diversity within and among species (e.g., Darwin 1859; Hutchinson 1959). Since the rediscovery of Mendelian genetics (e.g., de Vries, Stamhuis et al. 1999) and its integration with Darwin's theory of natural selection (together coined the "evolutionary synthesis"), followed by the advent of molecular methods (e.g., allozyme markers, polymerase chain reaction, and DNA sequencing), scientists have become equipped to address the ways in which genetic diversity is distributed within species, and the causes and consequences of diversity. Recent advances in coalescent theory (Kingman 1982; Donnelly and Tavare 1995) and computing power have allowed us to reconstruct the evolution of various types of genes and gain a greater understanding of both shared diversity and divergence among species. These combined tools provide an opportunity to determine how genetic diversity within species contributes to genetic diversity among species, leading to the phylogenetic diversity of life on earth.

Some diversity is thought to be neutral (Kimura 1968; Ohta 1992), driven by stochastic processes such as genetic drift (Wright 1931) and allopatric speciation via the accumulation of neutral differences (Mayr 1954). Under the assumptions of neutrality, species divergence occurs over long periods of time in geographic separation, a paradigm which attempts to explain the evolution of reproductive isolation between two similar species as a byproduct of an accumulation of changes throughout the genome (Dodd 1989). Reproductive isolation is predicted to evolve more slowly under neutral processes than under strong divergent selection (Hendry et al. 2007). On the other hand, neutral genetic processes such as polyploidy may lead to speciation due to genetic incompatibility; this speciation can happen within one generation (Rieseberg and Willis 2007). Thus,

diversification of taxonomic groups can happen quickly (Palumbi 1994) even via neutral processes, or can take millions of years (Nei et al. 1983).

Other patterns of diversity are driven by adaptive processes such as predator avoidance (Nosil and Crespi 2006; Mullen et al. 2009; Rosenblum 2006), pathogen resistance (Hughes and Nei 1988; Yang et al. 2011), and responses to distinct biotic and abiotic conditions in different environments (Grant and Grant 1995; Malmquist et al. 1992; Terai et al. 2006). Adaptation to divergent conditions can explain both variation within species (Hendry et al. 2002; Seehausen et al. 2008) and divergence among species (Nosil et al. 2002; Schluter and McPhail 1992). Diversification can occur particularly quickly when genes favored in a certain environment also influence or are physically linked to those for mate choice (Hawthorne and Via 2001), but even in the absence of initial mate choice differences, we observe divergent selection pressures that influence morphological and behavioral traits or lead to reproductive isolation as a byproduct (Dodd 1989).

Divergent selection can come about through spatial variability in habitat conditions, both biotic and abiotic (Vantienderen 1991; Kooyers and Olsen 2012). A species that encounters variable environments but is still constrained by environmental conditions (i.e., not a generalist) would therefore be a useful system in which to study which types of selection favor evolutionary divergence. One such species is the Gunnison's prairie dog (*Cynomys gunnisoni*), one of five species of prairie dogs in the Sciuridae family. Prairie dogs are social, semi-fossorial rodents that require soil amenable to burrowing. Because they are social, they are conspicuous to predators and have evolved an elaborate system of alarm calls to warn family and population members of approaching predators (Slobodchikoff et al. 1991, 1998; Waring 1970). Prairie dogs live in grasslands of the western United States, southwestern Canada and northern Mexico. They occupy colonies that comprise a number of family groups called coteries, which consist of one adult male and several related females and their offspring (Hoogland 1995). Dispersal occurs primarily among coteries, but

also among colonies, and predation is thought to be high during dispersal (Garrett and Franklin 1988).

The five species are social to varying degrees, with black-tailed prairie dogs (*C. ludovicianus*) being the most social and forming large, densely aggregated colonies (Hoogland 1981, 1995), and Gunnison's prairie dogs the least social and found at densities similar to those of ancestral ground squirrels (Longhurst 1944; Pizzimenti and Hoffman 1973). Black-tailed prairie dogs may be the most social species because of their higher vulnerability to predation owing to their habit of clipping all uneaten vegetation in their colonies (Hoogland 1981). Because they are so dependent on their ability to scan for predators, they are found only in open grasslands (historically shortgrass and tallgrass prairie) at low to mid elevations with little to no slope (Merriam 1901). In contrast, Gunnison's prairie dogs are found from 1,800 to 3,000 meters (Pizzimenti and Hoffman 1973) and occupy a diverse array of habitats with complex vegetation structure, from cholla-dominated desert to high montane meadows (Pizzimenti and Hoffman 1973; Fitzgerald et al. 1994). Gunnison's prairie dog alarm calls vary depending on habitat (Perla and Slobodchikoff 2002), possibly due to the way sound travels through different vegetation structure.

Gunnison's prairie dogs' distribution in starkly different habitats ("montane" and "prairie," USFWS 2008) is well documented, but little is known about their degree of evolutionary divergence. As a result of their distinct habitats and behavioral (Renner 2003) and morphological (Hollister 1916) variation among populations, scientists have historically recognized of two distinct subspecies (Hollister 1916; Aldous 1935; Longhurst 1944; Lechleitner 1969; Pizzimenti and Hoffman 1973). However, what is unknown is the extent to which selective differences in abiotic conditions and habitat structure contribute to the early stages of genetic differentiation that may ultimately lead to evolutionary divergence. Plant community structure may be important to prairie dog divergence if it influences the way sound from mating calls travels through the habitat (Boughman 2002; Perla and

Slobodchikoff 2002). In addition, mate choice in prairie dogs is related to size, where females prefer larger males. Individuals of *C. g. zuniensis* are larger (Hollister 1916), and low-elevation sites have longer growing seasons during which males can forage to sustain body mass; thus, elevation and forage availability may play a role in divergence. Temperature may play a role in evolutionary divergence of prairie dogs because the timing of estrus is related to the date of emergence from hibernation, which varies by elevation (Longhurst 1944). Thus, dispersing male prairie dogs from lower to higher elevations could potentially mate with resident females if they arrived as females were emerging from hibernation; conversely, dispersing males from higher to lower elevations would miss the breeding season and be unable to reproduce. These ecological conditions may interact to facilitate divergence in prairie dogs, and habitat differences throughout the range of Gunnison's prairie dogs have long been proposed but never quantified. The second chapter of this dissertation examines functional differences in climatic conditions and vegetation structure between subspecies, and uses ecological information to explain genetic differences within Gunnison's prairie dogs. Because the first steps in assessing evolutionary divergence are estimating genetic differentiation and identifying ecological conditions favoring differentiation, this chapter provides a foundation for future experimental research on mechanisms of divergence between subspecies.

In addition to climate and other abiotic conditions, some of the primary forces of natural selection in animals are interactions with other organisms, including competition (Vellend 2008), predation (Dingemanse et al. 2009) and parasitism (Haldane 1949), which favor adaptations for enhanced competitive ability and predator and parasite avoidance. The relative importance of adaptation to parasites in driving evolution is unknown, although it is believed that diversity in disease-resistance loci (such as the major histocompatibility complex in mammals) is due to pathogen presence (Hughes and Nei 1988; Yang et al. 2011). Genetic diversity in response to parasitism has been shown to decrease due to population bottlenecks (Trudeau 2004), but also may

increase due to the release from competition caused by extirpations and an influx of diversity from multiple source populations (Sackett et al., in review). Parasitism is thought to be of fundamental importance to the evolution of animals (Haldane 1949; Hamilton 1982).

Social animals are predicted to be particularly susceptible to parasitism because of the high transmission potential associated with group living (Alexander 1974; Hoogland 1979). For instance, primate species with higher population densities have higher parasitism than those with smaller group size (Nunn et al. 2003). Thus, social animals provide useful systems for determining the evolutionary effects of parasitism on host genetic diversity. Black-tailed prairie dogs are one of the historically most numerous social mammals in North America; for example, one large colony in Texas was estimated in the early 1900s to contain 400 million prairie dogs (Merriam 1901). Today, as a result of eradication campaigns, land conversion to agriculture and urban areas, and the introduction of sylvatic plague, the occupied range of prairie dogs has plummeted to less than 1% of its historic size (Miller and Cully 2001). However, they still exist in often densely populated colonies with high rates of interaction among individuals (Hoogland 1995).

Prairie dogs, like most mammals, are known to harbor a number of pathogens with varying degrees of virulence, including *Yersinia pestis*, the bacterium causing sylvatic plague. *Y. pestis* is a gram-negative gamma-proteobacterium that infects almost all mammals (including humans), and its effects are particularly severe in prairie dogs. Prairie dog mortality from plague approaches 99% (Pauli et al. 2006; Cully et al. 1997; Biggins et al. 2010). The pathogen is transmitted by fleas (Siphonaptera), which prairie dogs may harbor in high numbers (e.g., over 200 on some animals; personal observation). *Y. pestis* was introduced to western North America from Asia around 1900 by the transport of *Rattus* on ships (Cully et al. 2000). Once *Y. pestis* is endemic within a region, prairie dog colonies experience metapopulation dynamics (Antolin et al. 2006) with migration (Sackett et al. 2012), periodic local extirpations due to plague (Cully and Williams 2001), and subsequent

recolonization (Roach et al. 2001). Because plague causes colony extirpation that is often, but not always, followed by recolonization, the disease is expected to have consequences on the distribution of genetic variation within and among colonies. In the third chapter of this dissertation, I investigate the influence of this recently introduced pathogen on patterns of diversity in a metapopulation of black-tailed prairie dogs.

Despite the widespread extirpations caused by *Y. pestis* and the numerical prevalence of other less virulent pathogens, little is known about how pathogens travel among populations. Predicting potential outbreaks and inferring the spread of pathogens is difficult, but can be aided by an improved understanding of dispersal of prairie dog hosts. The complex landscapes that exist today present challenges to dispersing animals that confront highways, agricultural fields and other novel barriers. Numerous anthropogenic barriers have been documented in various species (Beneteau et al. 2009; Blake et al. 2008; Coulon et al. 2006; Goverde et al. 2002; Levy et al. 2010; Pavlacky et al. 2009; Telles et al. 2007). Thus, determining how certain species respond to particular landscapes is essential to understanding patterns of pathogen spread, the degree of genetic diversity in localized populations, the relative importance of selection and genetic drift, and the evolutionary potential of the species. The objective of the fourth chapter of this dissertation is to use our knowledge of the distribution of genetic diversity and landscape features to predict how well black-tailed prairie dogs are able to disperse through certain habitat types. This information can be used to designate migration corridors in conservation, predict and prevent plague outbreaks, and manage relocation of particular populations.

The goal of this dissertation is to describe the processes that produce and maintain diversity within and among species, using prairie dogs as a study system. At the broad phylogenetic scale, I hypothesize that abiotic conditions and habitat are important determinants of the selection pressures exerted on species, with resulting morphological and behavioral adaptations that vary by habitat.

These adaptations are likely to translate into genetic divergence among species, particularly when coupled with divergent selection on mate choice (Rundle et al. 2005) or selection against immigrants (Nosil et al. 2005). At lower phylogenetic scales (such as within species), I hypothesize that parasitism influences the distribution of genetic diversity due to the interaction of landscape features with individual behavior, such as dispersal patterns among populations and territoriality. These two major questions are examined in one species expected to be limited by habitat selection pressures (Gunnison's prairie dog), and one species that is limited by repeated extirpations by a virulent pathogen (black-tailed prairie dog). In Gunnison's prairie dogs, I find that genetic structure correlates with elevation and annual temperature variation, but not precipitation. There is genetic evidence for subspecies of Gunnison's prairie dogs, but lineages exchange gene flow and are thus not yet reproductively isolated. In black-tailed prairie dogs, I find that the virulent pathogen *Yersinia pestis* alters the distribution of genetic diversity within colonies and individuals, with well-connected colonies experiencing an influx of diversity and others experiencing a precipitous decline in diversity. Of particular importance is the result that *Y. pestis* selectively eliminates individuals with the lowest degree of genetic diversity. Prairie dogs travel among colonies using primarily grassland, agricultural fields and shrubland; major roads act as barriers to dispersal. However, small roads appear to facilitate dispersal across impervious land such as waterways. Plague may travel among colonies using the same routes when trafficked by prairie dogs themselves, but more work is needed to determine alternate routes of plague movement. This research contributes to our understanding of the processes governing the distribution of genetic diversity within and among species.

Chapter 2: **Diversification of Gunnison's Prairie Dogs Across Their Range**

Abstract

Our knowledge of ecological speciation has improved markedly in recent years, but we still lack a broad understanding of the selective forces that favor evolutionary divergence. For instance, it is unknown to what degree divergent adaptation to biotic factors, such as selection for crypsis in predator avoidance, is more important than adaptation to abiotic factors, such as temperature regime. In this chapter, I examine the degree of ecological and genetic divergence between proposed subspecies of Gunnison's prairie dogs (*Cynomys gunnisoni*) that occupy distinct habitats ranging from desert to montane grasslands. I quantified ecological characteristics including climate and land cover at 48 sampling locations, and genotyped 838 prairie dogs at 15 microsatellite and two mitochondrial loci. I found high support for recognition of two subspecies of Gunnison's prairie dogs, one of which was found only in cold, high elevation sites. Despite the association between genotype and environment, I observed several colonies along the subspecies boundary that contained genotypes from both subspecies. It may be the case that Gunnison's prairie dogs are in the early stages of speciation, where local adaptation is occurring, but gene flow has not yet subsided. Further studies of adaptation, including those that perform reciprocal transplants, are needed to assess the degree of reproductive isolation between subspecies.

Introduction

Our understanding of ecological divergence has improved greatly in the last several years (Schluter 2001; Nosil et al. 2009; Hendry et al. 2007; Rundle and Nosil 2005), and has been documented in numerous systems (e.g., Mullen and Hoekstra 2008; Bush 1969; Losos et al. 1997; Johnson et al. 1998). Due to the existence of intraspecific variation in nature (Darwin 1859) and fine-scale heterogeneity of abiotic conditions (Stratton 1994; Baraloto and Couteron 2010), divergent selection likely exists ubiquitously at small scales (e.g., Funk et al. 2006; Nosil and Crespi 2006; Terai et al. 2006; Tobler et al. 2008). However, divergence often takes thousands of generations or does not occur at all. The quickest route to speciation involves a response to ecological selection that is linked to mate preference or reproductive isolation (Kirkpatrick and Ravigné 2002). Still, even in the absence of initial mate choice differences, we observe divergent selection pressures that influence morphological and behavioral traits or lead to reproductive isolation as a byproduct (Dodd 1989). The question thus emerges of which ecological conditions facilitate speciation.

The ecological speciation literature has made much progress on elucidating responses to selection at the genomic level (Nosil et al. 2009; Emelianov et al. 2004; Nosil and Feder 2012; Via and West 2008), and our theoretical understanding of the evolutionary conditions promoting divergence has vastly improved (Gavrilets 2003). For instance, speciation tends to be favored under conditions, among other things, of very strong divergent selection (Nosil et al. 2009). However, we possess little empirical evidence relating ecology to the theoretical conditions favoring divergence. It is unknown, for example, what types of selection pressures are most likely to lead to evolutionary divergence (Schluter 2001). For instance, are species interactions such as predator avoidance and pollinator shifts particularly important (Langerhans et al. 2007; Duffy et al. 2008; Rosenblum, van der Niet and Johnson 2009), or is divergent local adaptation to micro-climate more effective (Keller and Seehausen 2012)?

Spatial variability in habitat conditions—both biotic and abiotic—exists for many species and can lead to fluctuating selection (Vantienderen 1991; Kooyers and Olsen 2012). A species that encounters variable environments but is still constrained by environmental conditions (i.e., not a generalist) would therefore be a useful system in which to study which types of selection favor divergence. One such species is the Gunnison's prairie dog (*Cynomys gunnisoni*), one of five species of prairie dogs in the Sciuridae family. Prairie dogs are social, semi-fossorial rodents that require soil amenable to burrowing. Because they are social, they are conspicuous to predators and have evolved an elaborate system of alarm calls to warn family and population members of approaching predators (Hoogland 1983; Slobodchikoff et al. 1991). Alarm calls vary by species (Waring 1970) and habitat (likely due to the way sound travels through different vegetation structure; Perla and Slobodchikoff 2002).

Gunnison's prairie dogs' distribution in starkly different habitats ("montane" and "prairie," USFWS 2008) is well documented, but little is known about their degree of evolutionary divergence. As a result of their distinct habitats and behavioral (Renner 2003) and morphological (Hollister 1916) variation among populations, scientists have historically recognized of two distinct subspecies (Hollister 1916; Aldous 1935; Longhurst 1944; Lechleitner 1969; Pizzimenti and Hoffman 1973), but official recognition of subspecies was abandoned with genetic work demonstrating a lack of polymorphism in chromosome number and 4 serum proteins (Pizzimenti 1975). There are geographical differences in both alarm calls (Slobodchikoff et al. 1998) and morphology (Pizzimenti 1976a) that are linked to habitat structure; both vocalizations (alarm calls) and morphological crypsis are of paramount importance in avoidance of predators. However, it remains unknown whether these differences in behavior and morphology translate into evolutionary divergence. Previous studies have found conflicting results on genetic differentiation (Hafner 2005; Pizzimenti 1975), but none have used multiple types of markers or examined divergence in an evolutionary framework.

The goal of this study is to examine the extent to which differences in abiotic conditions and habitat structure contribute to evolutionary divergence, manifested by genetic differentiation, in Gunnison's prairie dogs.

Methods

Study locations and sample collection

Data were collected from 48 sites in New Mexico, Colorado, Arizona and Utah from 2008 – 2010 (Fig. 2.1, Table 2.1). My goal was to obtain samples from throughout the range of GUPD, with particularly high sampling effort in the geographical region where both subspecies exist. Prairie dogs were trapped by myself and collaborators in 39 of those sites, and tissue samples were collected from animals in a relocation holding facility from six additional colonies. Tissues were also obtained from one control effort, and samples from two sites were obtained from animals killed on roads. Due to geographic uniqueness (PEFO) and lack of support for their belonging to a sampled colony (RM), these samples were treated as originating from unique colonies. At each of the 39 trapped colonies, 24-68 Tomahawk traps were set and pre-baited with a corn-oat-barley mixture for at least five days with the traps held open, and each day after trapping concluded. After pre-baiting, traps were baited, set, and left for no more than 2 hours at a time. Prairie dogs were trapped for 1 – 2 weeks at each site by targeting active burrows with one to four traps (Hoogland 1995).

Figure 2.1. Sampling locations of Gunnison's prairie dogs across both portions of their range (montane = northeast section, prairie = southwest section). Subspecies range designations are based on elevation-based delineation from the U.S. Fish and Wildlife Service.

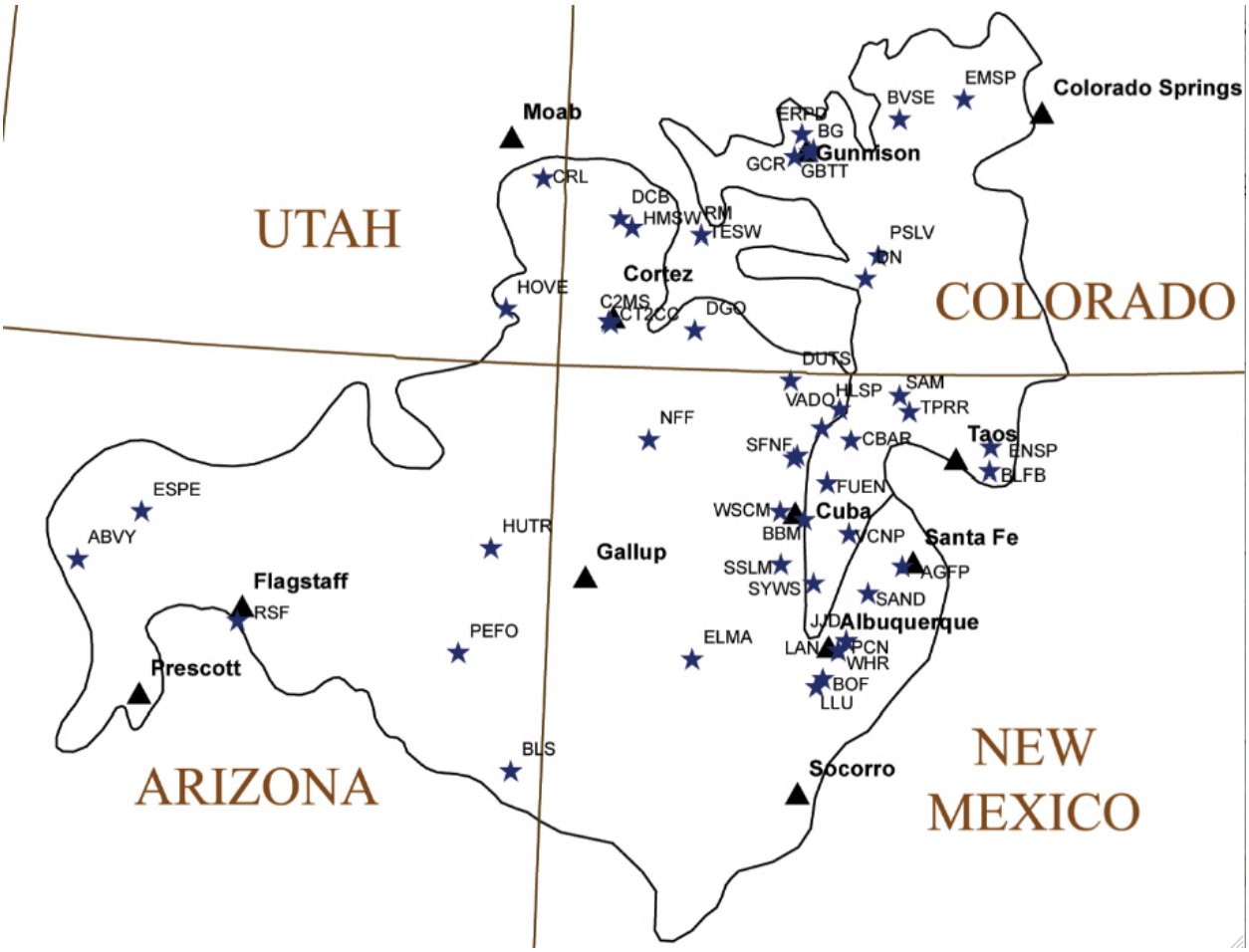


Table 2.1. Colony names, samples sizes, and location information for 48 sampling locations. Asterisks indicate admixed populations.

Colony	N	Elev(m)	Latitude	Longitude
ABVY	30	1582	35.44690222	-113.07662043
ESPE	30	1704	35.81479769	-112.56231371
RSF	3	2053	35.09731000	-111.68130000
PEFO	1	1663	34.96258000	-109.79403000
HUTR	25	1935	35.70825864	-109.55686639
BLS	7	2112	34.15812000	-109.30601000
ELMA	24	2025	34.97996276	-107.80529096
HOVE	21	1617	37.38067743	-109.52543323
CT2CC	35	1795	37.32686400	-108.62122500
C2MS	2	1822	37.31004918	-108.60458000
CRL	25	2076	38.29940341	-109.25172796
HMSW	11	2347	37.97871634	-108.44975469
DCB*	22	2012	38.03977929	-108.55449892
DGO	17	2010	35.44690222	-113.07662043
NFF	2	1918	36.50235000	-108.23574000
SSLM	25	1884	35.66269000	-107.07092000
SYWS	23	1666	35.53308000	-106.78559000
DUTS	25	2069	36.94589000	-107.01632000
WSCM	25	2172	36.03221000	-107.08239000
BBM	25	2477	35.97913000	-106.87738000
VADO	27	2121	36.61410000	-106.74035000
FUEN	25	2544	36.23647000	-106.68417000
HLSP	3	2255	36.75033000	-106.58382000
SFNF	15	2279	36.40094000	-106.97304000
CBAR	25	2326	36.53127000	-106.48307000
VCNP	24	2613	35.88247356	-106.48795164
SAND	1	1713	35.46654000	-106.31748000
AGFP	15	2011	35.65953847	-106.02468124
WHR	4	1631	35.05807000	-106.56342000
JJD	3	1810	35.13052000	-106.49680000
LAN	10	1811	35.12993000	-106.49648000
LLU	10	1480	34.80261100	-106.74791100
PCN	4	1632	35.06345000	-106.56585000
BOF	10	1485	34.86170600	-106.69146100
C. g. zuniensis **(34 populations)**	554			
TESW	16	2664	37.94497156	-107.83391243
RM	2	2664	37.94497156	-107.83391243
SAM*	1	2669	36.85083000	-106.06828000
ENSP*	25	2509	36.49235000	-105.27218000
BLFB	29	2631	36.32635000	-105.28148000
TPRR*	22	2504	36.73654000	-105.98047000
PSLV	30	2392	37.83310582	-106.26744545
DN	24	2418	37.67087596	-106.37984352
ERPD	45	2483	38.66916274	-106.96134016
GBTT	22	2367	38.54364822	-106.88965066
GCR	16	2337	38.50703655	-107.02629875
BG	8	2536	38.56174108	-106.85981616
BVSE	29	2383	38.78449772	-106.09599958
EMSP	15	2661	38.92843400	-105.52093500
C. g. gunnisoni **(14 populations)**	284			

Prairie dog trapping and processing were conducted in accordance with protocols approved by the University of Colorado's Institutional Animal Care and Use Committee and are described in detail therein (available upon request). Captured prairie dogs were anesthetized with 1 – 4% isoflurane in oxygen using a vaporizer to control the dosage (Heath et al. 1997). Processing involved collection of tissue for DNA and collection of blood for pathogen screening. Tissue for DNA analysis was collected using a 2-mm diameter ear punch (Braintree Scientific) and stored frozen in a solution of EDTA-DMSO until extraction. After processing, animals were placed back into the traps until the anesthesia wore off and they became alert, at which time they were returned to their capture locations.

Ecological analyses

Ecological characteristics of all colonies were analyzed using ArcGIS version 10 (ESRI, Redlands, CA). Land cover data were obtained from the GAP land cover database (USGS 2011) and climate data were obtained from the Worldclim database (Hijmans et al. 2005). All site locations were confirmed using Google Earth to ensure that data extracted in ArcMap corresponded to the actual sampling locations. I compared ecological characteristics between subspecies using a revised delineation based on the observed geographic distribution of genetic diversity (see results and discussion). First, I performed an ANOVA to detect differences in elevation between subspecies. I then created matrixes of pairwise elevation differences among all colonies and compared those to genetic distance matrixes using a partial Mantel test (to control for the effects of geographic distance) implemented in the Vegan package (Oksanen et al. 2010) for R (The R Foundation for Statistical Computing, http://www.r-project.org/). After classifying all sites using 19 bioclim variables, these were condensed along with elevation in a Principal Components Analysis using the ade4 package (Dray and Dufour 2007; Chessel et al. 2004) for R. I performed a randomization test

in the ade4 package to assess whether subspecies differed significantly on principal components, and confirmed this with an ANOVA on axis scores for the first two principal components. I subsequently performed ANOVAs on each temperature variable separately to determine the significance of each, comparing between subspecies. Finally, I tested for associations between subspecies and land cover type with a chi-square test. I repeated this test for three hierarchical classifications of land cover provided in the GAP database.

Phylogenetic and population genetic analyses

DNA from prairie dogs was extracted using a Qiagen DNeasy tissue kit, and 838 individuals were genotyped at 15 microsatellite loci developed in our lab (Jones et al. 2005, Sackett et al. 2009) and two mitochondrial genes, cytochrome b (primers modified from Harrison et al. 2003) and d-loop (Oshida et al. 2001). The program jModelTest (Posada 2008; Guindon and Gascuel 2003) was used to determine the mutation model of each mitochondrial gene. Microsatellite loci were examined for null alleles in the program Micro-checker (van Oosterhout et al. 2004). I tested all colonies for departures form Hardy-Weinberg equilibrium and examined linkage disequilibrium (LD) in each colony using Genepop software (Rousset 2008).

I used mtDNA to infer tree topology among lineages of Gunnison's prairie dogs using Bayesian methods implemented in MrBayes (Huelsenbeck and Ronquist 2001). I first concatenated cytochrome b and d-loop sequences, and then condensed the dataset into unique haplotypes using MacClade version 4.08 (Maddison and Maddison 2003). This resulted in an analysis of 150 unique haplotypes belonging to Gunnison's prairie dogs, in addition to 5 white-tailed prairie dog (*C. leucurus*) outgroup haplotypes from samples collected for a separate study. I allowed genes to evolve independently, with different mutation rates and models, and partitioned codons to allow a higher mutation rate at the third codon position. I examined the degree of genetic differentiation among

lineages at both mtDNA and microsatellite markers by pooling all individuals within clades and within subspecies. Differentiation was calculated for microsatellites using F_{ST} in Genepop Version 4.0 (Rousset 2008) and subsequently standardized in Genodive (Meirmans and Van Tienderen 2004) to prevent confounding diversity and differentiation (Hedrick 2005) and to control for unequal sample sizes. For mtDNA, F_{ST} was calculated in Arlequin version 3.1 (Excoffier et al. 2005). Next, I tested whether differentiation was significantly higher between some clades than others by performing an ANOVA in R.

To determine whether there was genetic support for distinct subspecies, I analyzed microsatellite genotypes using Structure software (Pritchard et al. 2000) and allowed K to vary from 1 to 9 (an upper bound much greater than the expected number of subspecies). I then assessed the relative support for each value of K using raw likelihood values and the change in likelihood with each increase in K (Evanno et al. 2005). I performed a series of analyses of molecular variance (AMOVAs) on 1) microsatellite allele identity, 2) microsatellite repeat number, and 3) mtDNA (using d-loop concatenated with cytochrome b) in Arlequin (Excoffier et al. 2005) to determine how genetic diversity was distributed within and between proposed subspecies. AMOVAs were then repeated with the revised subspecies delineation (see results and discussion). Next, in order to maximize the amount of variation between subspecies and thus determine the best-supported subspecies delineation, I repeated each AMOVA by moving one, two or three admixed colonies at a time between subspecies. The subspecies delineation that maximized the amount of variation between subspecies was selected for subsequent genetic and ecological comparisons.

Next, I assessed genetic composition of colonies within each subspecies using a principal components analysis (PCA) of genotypes across the 15 microsatellite loci, performed in the ade4 package for R. Because PCA is sensitive to missing data (e.g., it infers that individuals with missing data at the same locus are genetically similar), I removed all individuals with fewer than 50% of loci

genotyped (N=44). I then used the ade4 package to assess statistical significance of the differentiation between 1) the currently recognized subspecies and 2) the proposed revised subspecies (see results) by performing 10,000 permutations for each population grouping. Finally, I used the observed geographic distribution of microsatellite and mitochondrial diversity to revise the geographic delineation between subspecies.

Average pairwise differentiation among colonies was estimated by calculating F_{ST} as above, but by separating individuals into colonies rather than pooling them within clades/subspecies. I then used Arlequin to estimate the effective number of migrants (Nm) among colonies and between subspecies (Slatkin 1995). I determined the degree of isolation by distance using a Mantel test on the relationship between 1) linearized F_{ST} values for microsatellites, and 2) raw F_{ST} values for mtDNA on the log of geographic distance. I then compared the amount of pairwise differentiation between colonies within subspecies and between subspecies using unpaired tests for differences in means: for microsatellite F_{ST} values, which were normally distributed, I used a t-test, and for mtDNA F_{ST} values I used a Wilcoxon non-parametric test. Finally, I assessed overall differentiation between subspecies by estimating F_{ST} after pooling all individuals within subspecies.

Results

In total, I sampled 838 individuals from 48 colonies (mean = 17.5 per colony) for inclusion in the rangewide analysis (Fig. 2.1). Sites occurred at elevations ranging from 1480 meters to 2669 meters above sea level and comprised 34 colonies from *C. g. zuniensis* and 14 colonies from *C. g. gunnisoni*.

Ecological variation

Ecological comparisons were first made between the previously recognized subspecies (USFWS 2008) and subsequently between the revised subspecies delineated based on genetic data (see below). With the exception of elevation and one temperature measure, ecological differences were always greater between the revised subspecies (data reported here) than the currently recognized subspecies (data not shown). Colonies of the two subspecies were found at significantly different elevations (mean zuniensis = 1962m, mean gunnisoni = 2507m, $F=42.26$, $p << 0.001$; Fig. 2.2), and there was isolation-by-elevation even when controlling for geographic distance (microsatellites: $r=0.2125$, $p=0.001$; mtDNA: $r=0.1257$, $p=0.009$). Accordingly, colonies of *C. g. gunnisoni* were found in significantly colder sites than colonies of *C. g. zuniensis*. The principal components analysis of bioclim variables separated climatic data into axes largely corresponding to elevation, mean, and extreme precipitation and temperature measures (PC axis 1) and temperature variation measures (PC axis 2). The randomization test demonstrated that subspecies differed significantly in the climate of their habitats ($p<<0.001$), and this was confirmed using ANOVAs to examine the difference between subspecies on PC axis 1 ($p<<0.001$) and PC axis 2 ($p=0.0188$). Individual ANOVAs demonstrated that 9 of the 19 bioclim variables differed significantly between subspecies (Fig. 2.3); all nine were temperature-related measures. Subspecies were strongly divergent, for instance, in annual mean temperature (*C. g. gunnisoni* = 4.00°C, *C. g. zuniensis* = 9.37°C, $F=51.11$, $p << 0.001$) and minimum winter temperature (*C. g. gunnisoni* = -17.32°C, *C. g. zuniensis* = -9.85°C, $F=56.74$, $p << 0.001$; Fig. 2.3a).

Figure 2.2 Distribution of elevation of sites occupied by *C. g. zuniensis* (solid orange bars) and *C. g. gunnisoni* (hashed blue bars).

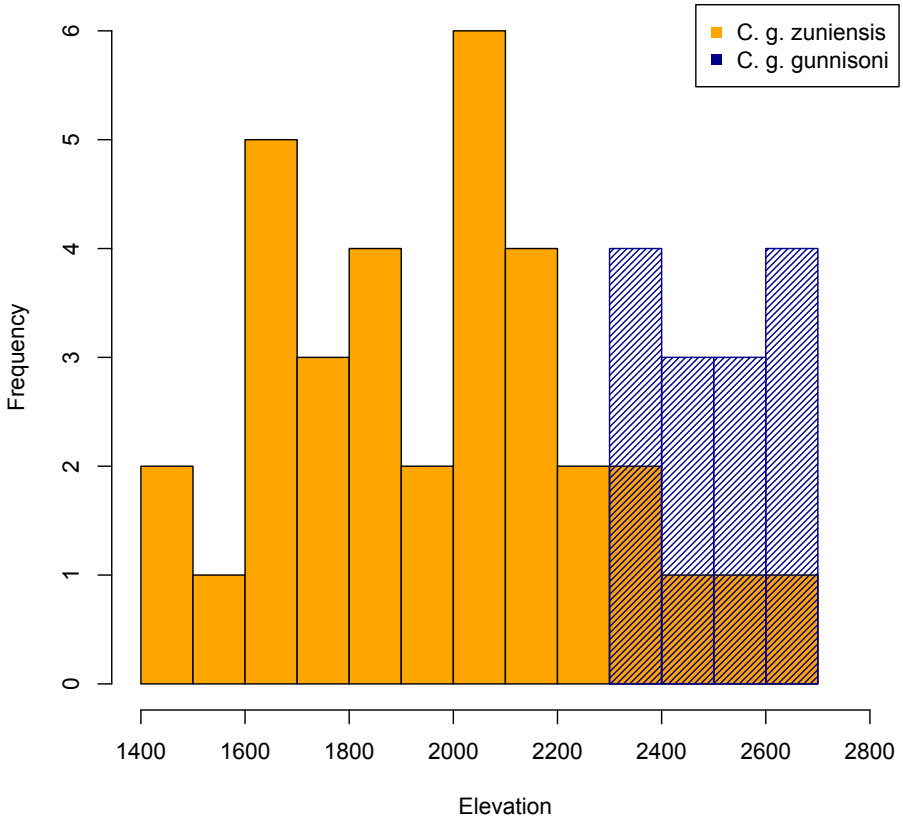
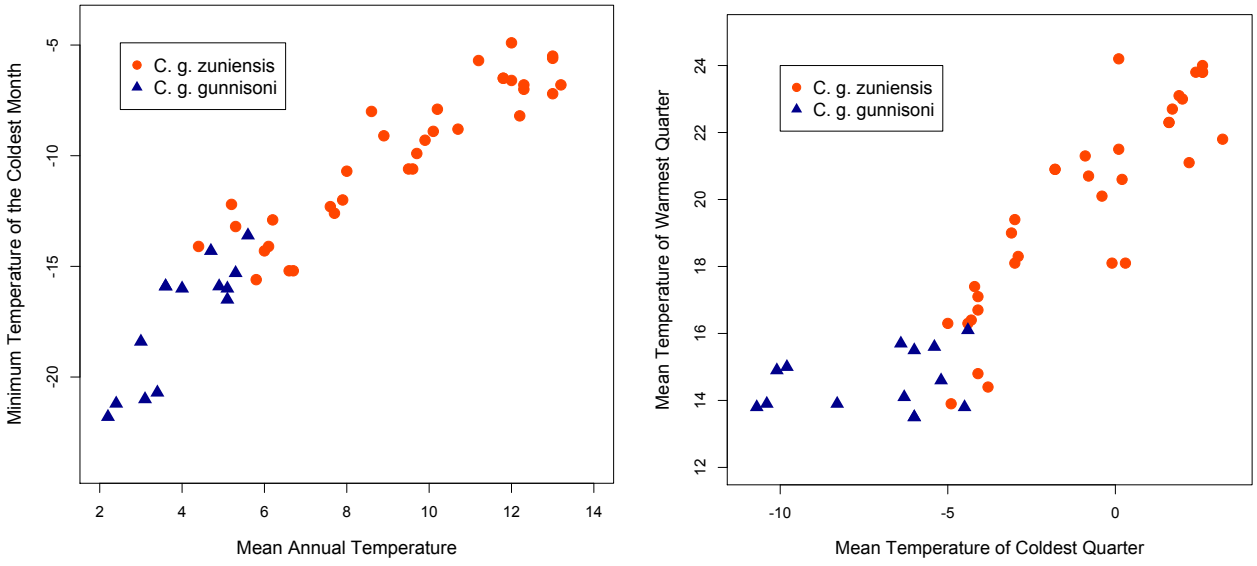


Figure 2.3 Plots of GUPD sites across 4 temperature variables from the Worldclim database: a) Mean annual temperature and mean temperature of the coldest month; b) Mean temperature of coldest and warmest quarters.



Land cover was significantly associated with subspecies identity, with the revised subspecies delineation, at three levels of hierarchical classification. At the coarsest scale, land cover in the range of GUPD was classified as either forest/woodland, grassland/shrubland, or semi-desert. Colonies of *C. g. gunnisoni* were found more often, and *C. g. zuniensis* found less often, in grassland/shrubland than expected by chance (χ^2= 7.88, p < 0.025). After dividing each land cover type into sub-classifications two times, the association between land cover and subspecies persisted. At the intermediate classification, *C. g. gunnisoni* colonies were found more often in 'Western North American grassland/shrubland' and less often in 'Western North American cool temperate woodland and scrub' than expected by chance (χ^2= 10.23, p < 0.05). Finally, at the finest classification scale, *C. g. gunnisoni* colonies were found more often than expected in 'Southern Rocky Mountain montane grassland and shrubland' and less often than expected in 'Rocky Mountain Two-needle piñon-juniper woodland' (χ^2= 16.88, p = 0.051).

Phylogenetic and population genetic analysis

Cytochrome b best fit the simple HKY + gamma mutation model including invariant sites, and d-loop best fit the GTR + gamma mutation model. All colonies except ERPD were found to be in Hardy-Weinberg equilibrium. There was no evidence of null alleles or linkage disequilibrium between markers.

The Bayesian phylogenetic analysis resolved three monophyletic clades (Fig. 2.4). One clade, hereafter the "montane" clade (denoted in dark blue), contained ten colonies of *C. g. gunnisoni* present in Colorado and four colonies of *C. g. gunnisoni* in New Mexico; the other two clades were more geographically widespread (Fig. 2.5). The analysis was unable to resolve which clade was the most recently derived (shown by the relatively low posterior probability value of the node containing two lineages, and supported by the fact that separate trees resolved different topologies with respect

to the relationships among major clades). Two individuals (the HMSW01 haplotype) could not be placed in a clade (i.e., bootstrap support for placement of these individuals was extremely low), but usually fell within the *C. g. zuniensis* clade or the intermediate clade. Removing these individuals from the analysis resulted in higher Bayesian support for nodes. Nine colonies were considered admixed, with at least 25% of individuals possessing a haplotype from a different clade than the majority of individuals. Of these, four colonies (DCB, HMSW, WSCM and TPRR) were admixed with respect to *C. g. gunnisoni* and another clade. One colony (HMSW) contained individuals from all three clades. Visualization of the distribution of the montane haplotype across the landscape shows a small zone where colonies contain haplotypes from both lineages (Fig 2.6, bright yellow) bordering its limited distribution, found primarily in Colorado.

Figure 2.4 Bayesian phylogenetic tree based on analysis in MrBayes, with cytochrome b and d-loop concatenated but allowing different mutation rates and models for each locus. Node labels are Bayesian posterior probabilities and are shown for major clades only. HMSW01 (gray haplotype) was not consistently placed in any clade; support for the geographically intermediate clade was higher when this haplotype was excluded.

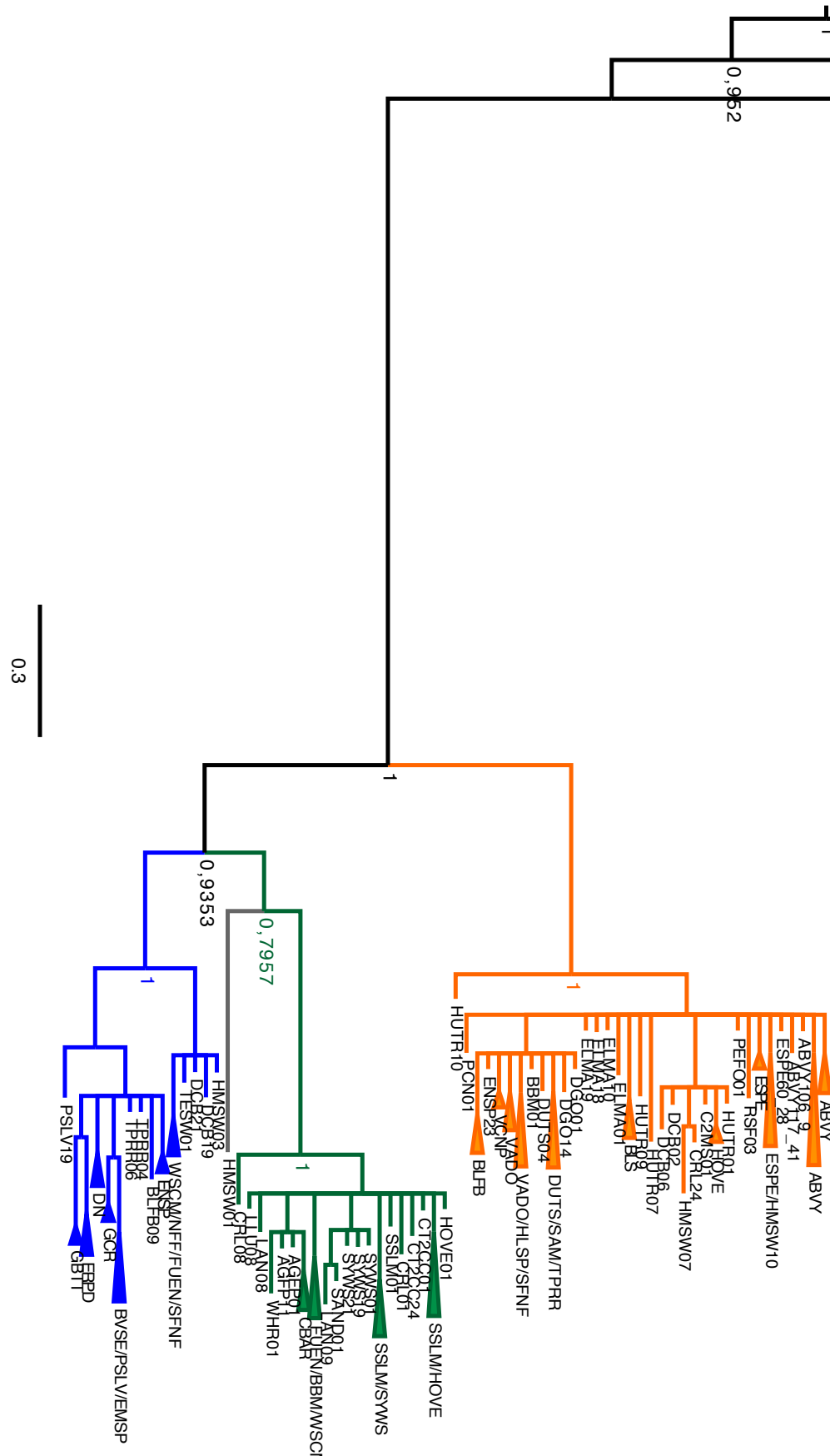

1
0,952
LCWT06
LCWT08
LCWT04
LCWT05
1
ABVY
ABVY106_9
ABVY117_41
ESPE028
ESPE/HMSW10
ESPE
RSF03
PEFO01
HUTR01
HOVE
C2MS01
CRL24
HMSW07
DCB02
DCB06
HUTR07
ELMA01
BLS
ELMA10
ELMA18
DGO01
DGO14
DUTS04
BBM01
DUTS/SAM/TPRR
VADO
VADO/HLSP/SFNF
ENSP23
BLFB
PCN01
HUTR10
1
SSLM/HOVE
HOVE01
CT2CC01
CT2CC24
CRL01
SSLM01
SSLM/SYWS
SYWS01
SYWS13
SYWS19
SAND01
SAND09
FLUEN
CBAR
FLUEN/BBM/WSCM
WHR01
AGFP01
AGFP11
LLAN08
CRL08
HMSW01
0,7957
1
WSCM/NFF/FUEN/SFNF
BLFB09
TPRR04
TPRR06
PESW01
DCB19
HMSW03
0,9353
1
BVSE/PSLV/EMSP
GCR
DN
ERPD
GBTT
PSLV19
0.3

Figure 2.5 Mitochodrial haplotype composition of 48 populations. Colors correspond to each of the three major lineages resolved in the Bayesian phylogenetic tree, with dark blue representing the montane clade.

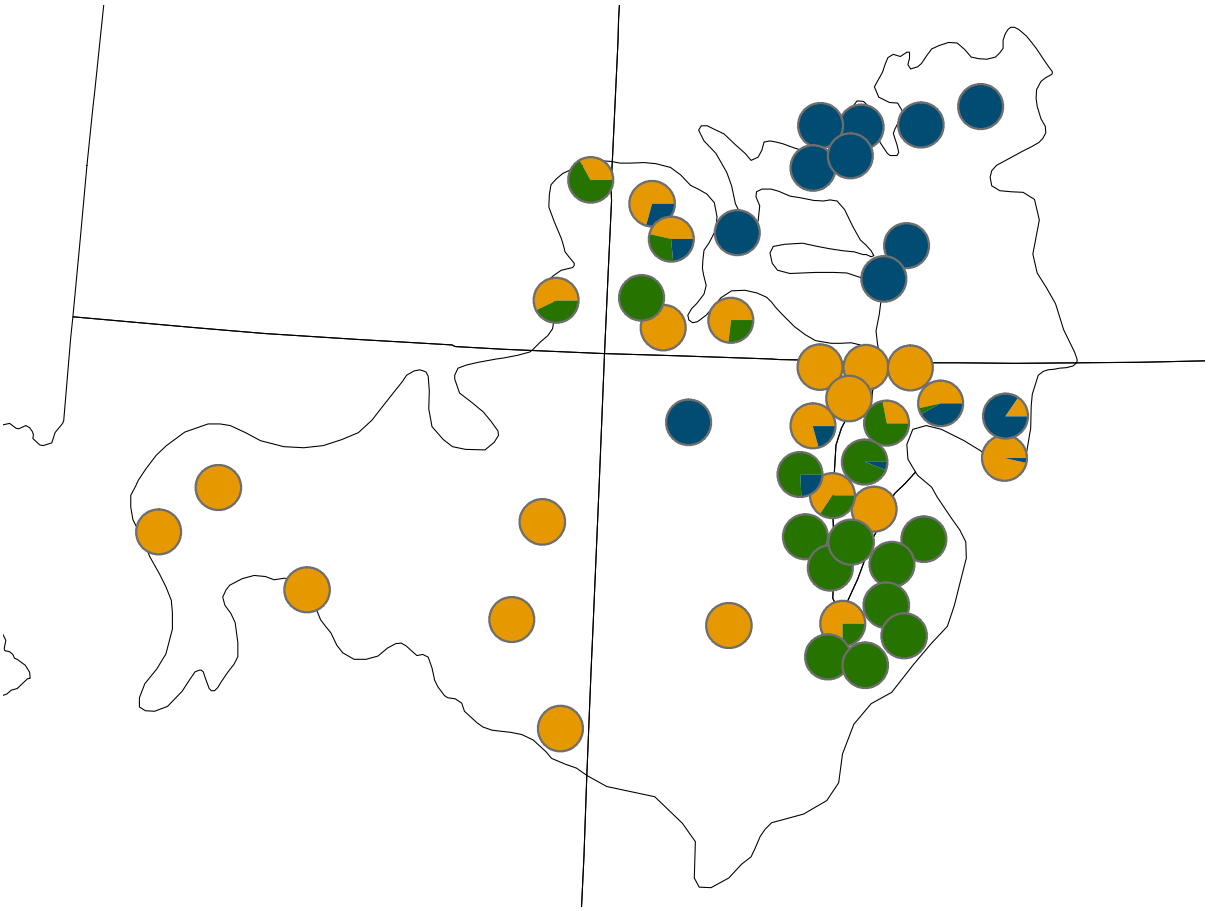
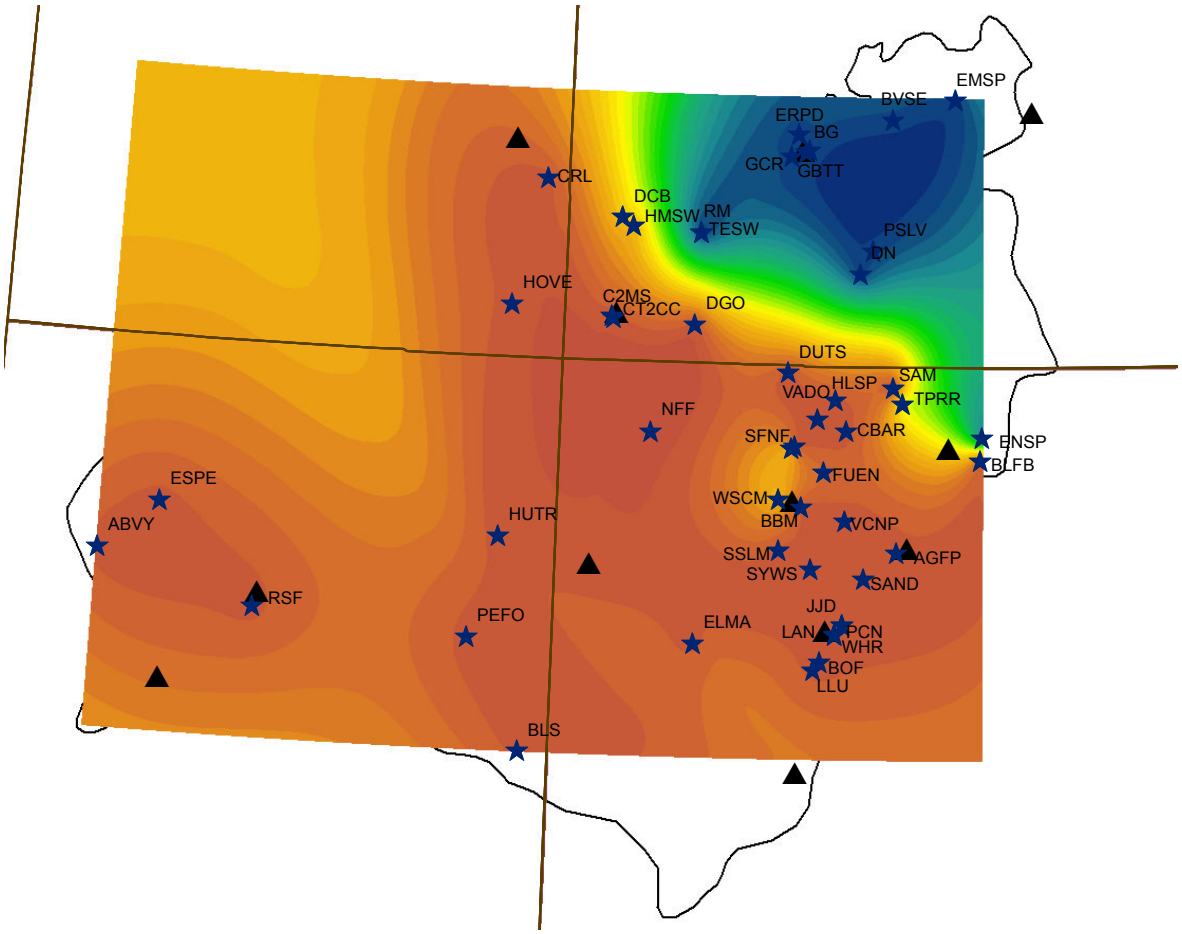


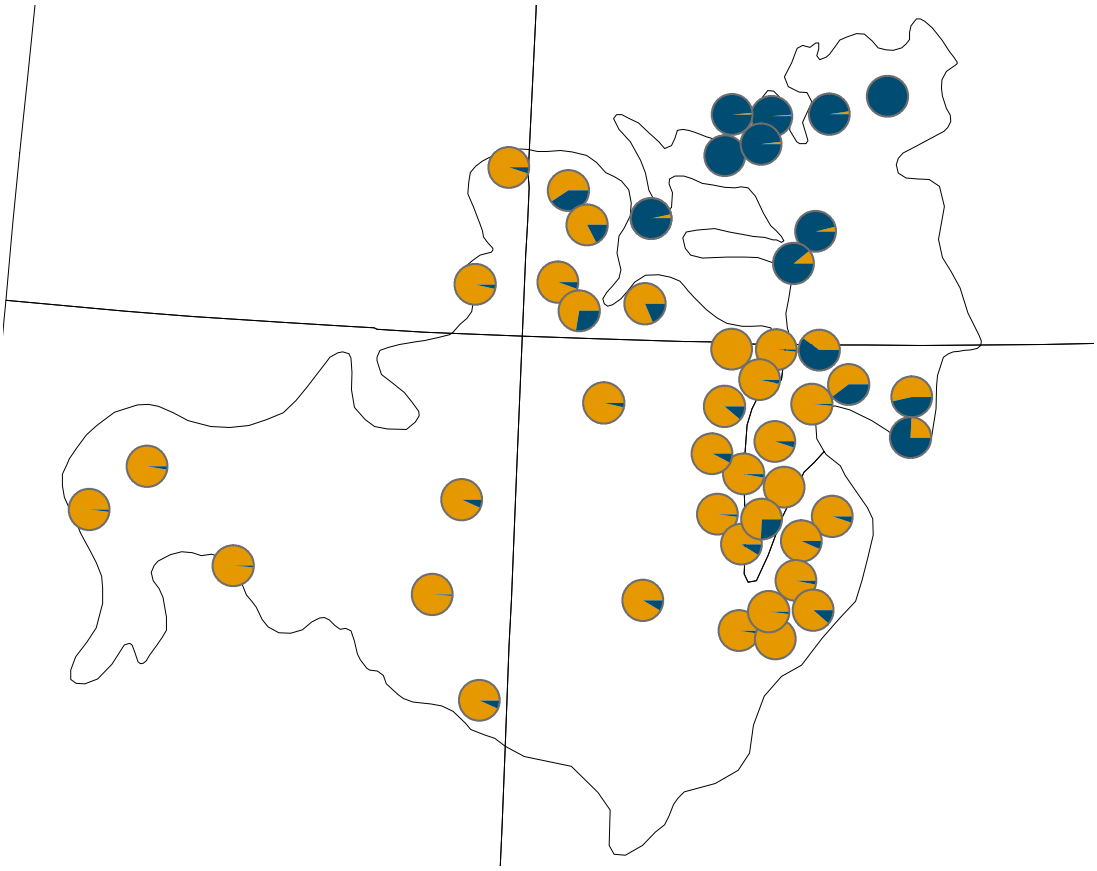
Figure 2.6 Distribution of the montane mitochondrial haplotype through the range of GUPD, where dark blue represents the highest proportion (100%) of the montane haplotype, green and bright yellow are intermediate, and orange represents 0% proportion of the montane haplotype.



Differentiation was highest between *C. g. gunnisoni* and the intermediate clade (microsatellite F_{ST}=0.1067, mtDNA F_{ST}=0.8580); F_{ST} values were significantly higher than between other clades (microsatellite F = 52.315, p << 0.001; mtDNA F = 13.056, p << 0.001). Patterns of differentiation between the other two clades varied between microsatellite and mtDNA: with microsatellites, differentiation was fairly high between *C. g. gunnisoni* and *C. g. zuniensis* (F_{ST}=0.084), and low between *C. g. zuniensis* and the intermediate clade (F_{ST}=0.026); however, with mtDNA, differentiation was higher between *C. g. zuniensis* and the intermediate clade (F_{ST}=0.7822) than between *C. g. gunnisoni* and *C. g. zuniensis* (F_{ST}=0.7523).

The Structure analysis of microsatellite genotypes provided the highest degree of support for two major genotype clusters, which largely corresponded to the previously proposed subspecies (Fig. 2.7). However, six colonies thought to belong to the *C. g. gunnisoni* subspecies (VADO, CBAR, FUEN, BBM, VCNP and SYWS) clustered instead with *C. g. zuniensis* populations. All ten Colorado colonies belonging to the montane cluster also belonged to the montane mitochondrial clade. Four NM colonies (SAM, TPRR, BLFB and ENSP) and 3 CO colonies (DCB, HMSW and C2MS) were considered admixed because they contained individuals assigned to both clusters (Fig. 6). Of these, two colonies (SAM and C2MS) were represented by only one and two individuals, respectively. Four colonies (TPRR, ENSP, DCB and HMSW) contained individuals with both the montane and prairie mitochondrial clades. All 7 of these admixed colonies were on the boundary between subspecies.

Figure 2.7 Map of structure-inferred microsatellite clusters in each population for K=2.



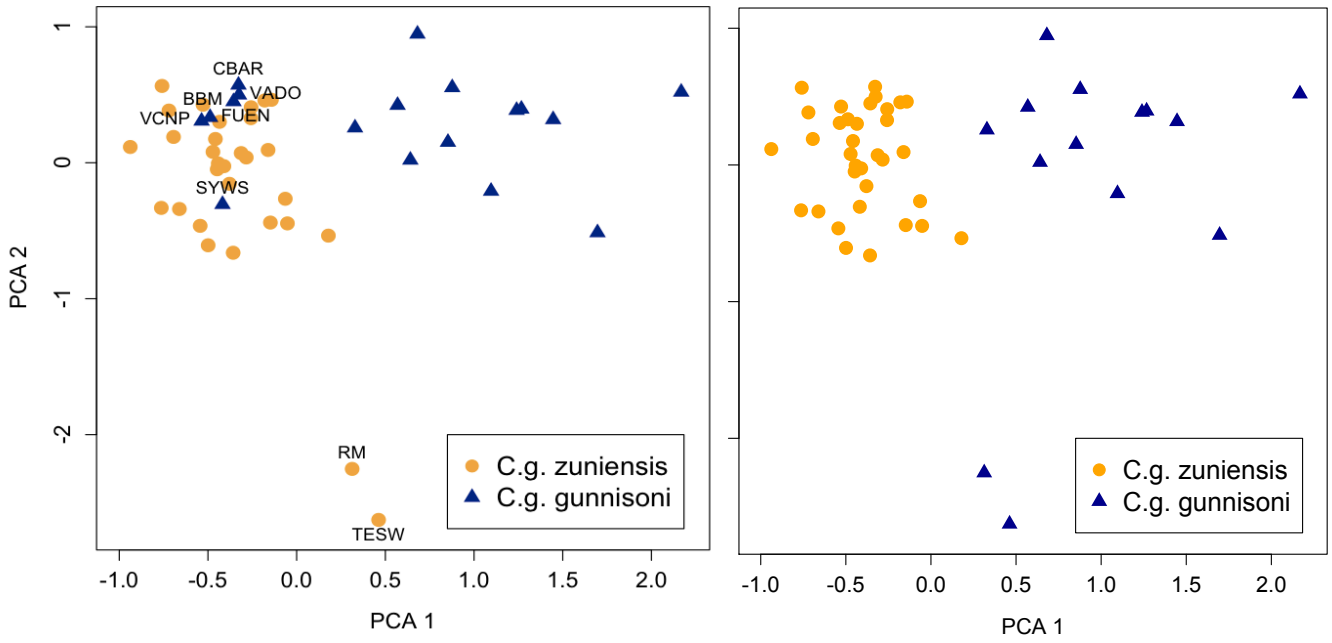
The initial AMOVA using microsatellite allele identity indicated that only 3.21% of the microsatellite variation was attributed to differences between subspecies, 24.72% among colonies, and 72.07% within colonies; similar results were obtained when using microsatellite repeat number (Table 2.3). With mitochondrial data, 11.20% of the variation was apportioned to between-subspecies differences, 65.37% among colonies, and 23.43% within colonies. However, when separating colonies into the new subspecies delineations (based initially on Structure clustering, Fig 2.7), the AMOVA attributed more than twice as much variation to among-subspecies differences (7.35% for microsatellite allele size and 31.16% for mtDNA; Table 2.2). Changing the groupings 1-3 colonies at a time did not markedly increase the amount of variation attributed to among-subspecies variation, with one exception: mtDNA supported grouping BLFB, on the boundary between subspecies (Fig 2.1), with *C. g. zuniensis*. However, I chose to leave this colony in *C. g. gunnisoni* for three reasons: 1) this change also decreased the amount of among-species variation in microsatellites, 2) the ecological characteristics of this site were highly similar to other *C. g. gunnisoni* sites, and 3) the principal components analysis (see below) suggests this colony is genotypically more similar to *C. g. gunnisoni*.

Table 2.2 Percentage of variation attributed to differences among subspecies, among colonies, and individuals. All AMOVAs were conducted in Arlequin and significance was assessed by permutation test. All were highly significant ($p<0.001$) except where noted by an asterisk ($p<0.05$).

	Microsatellite allele sizes		Microsatellite repeat number		mtDNA	
	Old	New	Old	New	Old	New
Among subspecies	3.21	7.35	4.21	9.05	11.20*	31.16
Among colonies	24.72	22.35	24.40	21.65	65.37	48.28
Among individuals	72.07	70.30	71.39	69.31	23.43	20.56

The principal components analysis of genotypes recovered three distinct groups of colonies corresponding to the two subspecies and two outlier populations located within one kilometer of each other. One of these populations (RM) consisted of two road kill animals, and the other comprised 16 individuals; both populations were characterized by unusually low genetic diversity (e.g., observed heterozygosity <0.2 and allelic richness <2, data not shown). Whether or not I included these populations in the PCA, our randomization tests demonstrated that the previously delineated subspecies were not significantly different ($p>0.1$) but that using the revised subspecies delineation, subspecies were genotypically distinct ($p=0.009$; Fig. 2.8).

Figure 2.8 Principal Components Analysis (PCA) plot of genotype space across 15 loci, showing a) the originally described subspecies, and b) the revised subspecies delineation. Populations originally belonging one subspecies that were more genetically similar to the other are labeled in part a).



Pairwise F_{ST} values among colonies averaged 0.292 for microsatellites and 0.692 for mtDNA, and was higher between than within subspecies ($p<<0.001$ for microsatellites and mtDNA). Conversely, mean effective migration between colonies was higher within (microsatellite Nm=1.572, mtDNA Nm=0.377) than between subspecies (microsatellite Nm=0.430, $p<<0.001$; mtDNA Nm=0.120, $p<<0.001$). When pooling individuals within subspecies, the estimated number of effective migrants between subspecies per generation was 2.136 for microsatellite DNA and 0.970 for mtDNA. With microsatellite markers, a Mantel test indicated only marginally significant isolation by distance ($r = 0.0929$, $p=0.087$) across the range of Gunnison's prairie dogs. However, mtDNA showed significant isolation by distance ($r = 0.2091$, $p = 0.001$). Differentiation between subspecies (with populations within each subspecies pooled) was significant (microsatellite F_{ST} = 0.085, mtDNA F_{ST} = 0.3404, $p<<0.001$). Pairwise sequence divergence between subspecies was 1.07%.

Discussion

I determined that Gunnison's prairie dogs comprise ecologically and genetically distinct lineages, which correspond to a new proposed subspecies delineation (Fig 2.9). Evidence from both mitochondrial and microsatellite DNA suggests that the GUPD in the montane region of their range in Colorado form a distinct group that also includes four admixed colonies in New Mexico. These colonies, coinciding with *C. g. gunnisoni*, occur in starkly different environments and may represent a unique evolutionary trajectory within the species.

Figure 2.9 Maps of the range of each GUPD subspecies: left, the distribution originally proposed by USFWS, and right, the distribution proposed based on our genetic and ecological data.

Ecological variation

Gunnison's prairie dogs were found in distinct habitats that coincide with their revised designation as "montane" and "prairie" subspecies: *C. g. gunnisoni* colonies existed at higher elevations and in colder sites than *C. g. zuniensis*. Moreover, while *C. g. zuniensis* occurred in a variety of habitats ranging from semi-desert to piñon-juniper woodland, *C. g. gunnisoni* colonies were strongly associated with montane grasslands. This suggests that the *C. g. gunnisoni* subspecies has become specialized to a high montane environment.

Phylogenetic and population genetic analysis

The Bayesian and spatial analyses revealed three spatially disparate mitochondrial clades, one of which was restricted in distribution and corresponded to the montane (*C. g. gunnisoni*) subspecies (Figs. 2.4-2.6). Although my phylogenetic analysis was unable to resolve which clade was the most recently derived, the estimates of differentiation consistently showed the *C. g. gunnisoni* clade to be

the most distinct. The population clustering algorithm implemented in Structure resolved two clusters that agreed with the revised subspecies designation (discussed below). Furthermore, the principal components analysis and AMOVAs supported the separation of colonies into two major groups corresponding to the revised subspecies. Collectively, the genetic results support two major findings: 1) there is strong evidence for the existence of distinct subspecies, and 2) the view of the subspecies delineation currently held (USFWS 2008) requires revision.

Most colonies belonged to a microsatellite cluster that corresponded with its mitochondrial clade (e.g., colonies in Arizona contained the *C. g. zuniensis* mitochondrial clade and belonged to the *C. z. zuniensis* microsatellite cluster). In particular, colonies at the northeastern and southwestern range edges were unambiguously part of either the *C. g. zuniensis* (Arizona) or *C. g. gunnisoni* (eastern Colorado) clades. However, with both microsatellite and mtDNA, we detected six colonies in the center of the species' range that contained haplotypes and genotypes from both subspecies. Of the six colonies that were admixed with respect to both microsatellite and mtDNA (DCB, HMSW, SAM, TPRR, BLFB and ENSP; Figs 2.5, 2.7), only one (SAM) fell in a different microsatellite cluster (i.e., montane) than its corresponding mitochondrial clade (i.e., prairie), and this colony consisted of only one sampled individual.

It is somewhat surprising that I did not detect significant isolation-by-distance in our microsatellite data, which encompasses colonies throughout the range of Gunnison's prairie dogs. However, the detection of isolation-by-distance relies on the assumption of drift-migration equilibrium, which is unlikely to be true in prairie dogs, whose populations experience frequent local extinctions due to sylvatic plague. The discrepancy between our microsatellite and mitochondrial data may lie in the smaller effective population size of the mitochondrial genome, or in the fact that prairie dogs exhibit sex-biased dispersal (Garrett and Franklin 1988). Additional support for this

reasoning arises from the significant geographic structure among regions in mtDNA (as demonstrated by the AMOVA) when compared to nuclear DNA.

Revision of subspecies delineation

The previously defined subspecies of Gunnison's prairie dogs were delineated based on historical descriptions of differences in morphology and habitat type (Hollister 1916; Pizzimenti and Hoffman 1973), as well as recently defined high-elevation versus low-elevation sites (USFWS 2008). In the present paper, I propose a revised subspecies delineation based on several lines of evidence from the observed ecological and genetic data. First, the ANOVAs examining each temperature measure separately showed larger differences between revised than previously proposed subspecies in all but one case. Second, the proportion of genetic variation attributed to between-subspecies differences (AMOVAs) was substantially higher with the revised than the previously proposed subspecies. Third, the geographic distribution of mitochondrial clades and microsatellite genotype clusters demonstrate support for a unique montane subspecies that exists in a more restricted distribution than previously thought (namely, it is limited to Colorado and far northern New Mexico). Fourth, genetic differentiation was highest between *C. g. gunnisoni* and the geographically intermediate clade, some colonies of which belonged to the previously designated montane subspecies. Finally, the principal components analysis of genotypes did not detect significant differences between the previously defined subspecies, but did resolve significant differences between the revised subspecies. Further work should confirm that morphological differences between subspecies persist with the revised designation.

Implications for evolutionary divergence of subspecies

In addition to isolation-by-distance, I observed isolation-by-habitat (Wagner and McCune 2009). The mitochondrial clade (and microsatellite cluster) corresponding to *C. g. gunnisoni* was significantly associated with higher elevations and colder temperatures, even when controlling for the effects of geographic distance. Abiotic habitat differences were paralleled by differences in plant communities between subspecies. Plant community structure may be important to prairie dog divergence if it influences the way sound from mating calls travels through the habitat (Boughman 2002; Perla and Schlobodchikoff 2002). However, it is unlikely that consuming different species of vegetation plays a role in divergence because prairie dogs are diet generalists and tend to eat whatever forage is available (Pizzimenti and Hoffman 1973). Temperature may contribute to evolutionary divergence of prairie dogs because the timing of estrus is related to the date of emergence from hibernation (Hoogland 1997; 1998; personal communication), which varies by elevation (Longhurst 1944). Thus, dispersing male prairie dogs from lower to higher elevations could potentially mate with resident females if they arrived as females were emerging from hibernation; conversely, dispersing males from higher to lower elevations would miss the breeding season and be unable to reproduce. This leads to the prediction of asymmetrical gene flow from lower to higher elevations. It is unknown whether *C. g. gunnisoni* individuals possess physiological adaptations for living at high elevations (e.g., altered hemoglobin properties) that may act, for instance, through reduced hybrid fitness, as barriers to gene flow.

The degree of sequence divergence between subspecies (1.07%) is analogous to the degree of differentiation at the same or similar loci between other currently recognized subspecies described within Sciuridae (Hoisington-Lopez et al. 2012; Wettstein et al. 1995), which supports their treatment as distinct subspecies. The significant associations of subspecies with habitat further corroborate the recognition of unique subspecies of Gunnison's prairie dogs. Future work should

focus on determining whether local adaptation exists by characterizing genetic markers under selection, using genomic methods such as identifying outlier loci that show higher differentiation among habitats than found across the genome. In addition, research should attempt to quantify the degree of adaptation to different habitats by performing reciprocal transplants of prairie dogs and estimating fitness of each subspecies in each environment. This type of study could be coupled with ongoing management plans that relocate prairie dogs, by moving them from one habitat type to another and assessing fitness in each habitat type. Our current understanding suggests that the two subspecies should be managed separately because of their unique habitats and degree of genetic differentiation.

In this system, the ecological factors driving local adaptation to divergent conditions are probably primarily abiotic. There is no evidence of distinct predator communities between subspecies or unique diets in each habitat type, despite differences in plant communities. Reproductive isolation may be likely to occur in conjunction with habitat isolation due to physical limitations of dispersal, selection against immigrants (Nosil et al. 2005), timing of estrus (Hoogland 1997; 1998) and physiological adaptations leading to reduced hybrid fitness, but seems unlikely to arise as a result of predator avoidance or food preference. These hypotheses should be tested through reciprocal transplants in Gunnison's prairie dogs, and in other mammalian species experiencing multiple divergent selection pressures.

Conclusions

The present study is the most thorough examination of genetic divergence in Gunnison's prairie dogs to date, and the first to quantitatively characterize habitat differences between subspecies. Redefining the delimitation between subspecies of Gunnison's prairie dog better encapsulates the ecological and genetic diversity of the species. Although it has been argued that

subspecies are genetically indistinguishable (Pizzimenti 1975), this view of a monotypic subspecies is based on insufficient genetic evidence (i.e., four serum proteins, three of which are also monomorphic for the same alleles in white-tailed prairie dogs (*C. leucurus*), Pizzimenti 1976b). Several lines of evidence support that the subspecies should be treated distinctly: 1) There is a high degree of genetic divergence between subspecies, 2) genetic differentiation is higher between than within subspecies, and this is not an artifact of geographic distance, 3) the subspecies are morphologically distinct in size (Hollister 1916), 4) they occupy habitats that influence the degree of time spent hibernating, and the timing of emergence from hibernation, estrus and reproduction. In order to best preserve the evolutionary processes acting within Gunnison's prairie dogs, treatment of two unique subspecies is advised.

Chapter 3: **Does Sylvatic Plague Reduce Genetic Diversity in Black-Tailed Prairie Dogs? Variable Effects Across Scales**

Abstract

Introduced diseases can cause dramatic declines in—and even the loss of—natural populations. Extirpations may be followed by low recolonization rates, leading to inbreeding and a loss of genetic variation, with consequences on population viability. Conversely, extirpations may create vacant habitat patches that individuals from multiple source populations can colonize, potentially leading to an influx of variation. I tested these alternative hypotheses by sampling 15 colonies in a prairie dog metapopulation during 7 years that encompassed an outbreak of sylvatic plague, providing the opportunity to monitor genetic diversity before, during and after the outbreak. Analysis of 9 microsatellite loci revealed that within the metapopulation, there was no change in diversity. However, within extirpated colonies, patterns varied: In half of the colonies, genetic diversity after recovery was less than the pre-plague conditions, and in the other half, diversity was greater than the pre-plague conditions. Finally, analysis of variation within individuals revealed that prairie dogs present in recolonized colonies had higher heterozygosity than those present before plague. Although most colonists were immigrants, we confirmed plague survivorship in 6 founders; these individuals had significantly higher heterozygosity than expected by chance. Collectively, these results suggest that high immigration rates can maintain genetic variation at a regional scale despite simultaneous extirpations in spatially proximate populations. Thus, virulent diseases may increase genetic diversity of host populations by creating vacant habitats that allow an influx of genetic diversity. Furthermore, even highly virulent diseases may not eliminate individuals randomly; rather, they may act as selective forces that remove the most inbred individuals.

Introduction

Genetic variation is of fundamental importance to natural populations because it enables adaptation to novel conditions (Lavergne and Molofsky 2007), confers resistance to pathogens (de Bellocq et al. 2008), and protects against inbreeding depression (Reed and Frankham 2003). The level of genetic diversity within a population reflects that combined forces of drift and selection acting on variation generated by mutation, recombination, and migration. Selection happens as a consequence of abiotic conditions and biotic interactions, including competition (Vellend 2008), predation (Dingemanse et al. 2009), parasitism (Lachish et al. 2011), and disease caused by pathogens (Hawley and Fleischer 2012).

Pathogens have long been recognized as potentially important influences on host evolutionary dynamics (Haldane 1949; Hamilton 1982). The effects pathogens have on host genetic diversity (e.g., Trudeau et al. 2004) may vary, in part, depending on pathogen virulence (namely, the incidence of host mortality due to pathogens; Lenski and May 1994) and whether there are nearby populations that can serve as source areas for recolonization (Hoban et al. 2010; Teacher et al. 2009). At low and intermediate virulence, pathogens may increase adaptive genetic diversity of their host populations due to diversifying selection at resistance loci (Schulte et al. 2010). In contrast, highly virulent pathogens can cause dramatic reductions in population size and population extirpation (Caillaud et al. 2006, Cully and Williams 2001). In populations with dramatic declines, the population bottleneck rapidly erodes genetic variation, thereby increasing inbreeding (Lachish et al. 2011) and susceptibility to other pathogens (Spielman et al. 2004; Valsecchi et al. 2004), potentially leading to a positive feedback cycle.

The effect of pathogens on genetic variation will also vary depending on demographic connectivity of populations in a complex landscape, which likely influences both rates of local extinction (hereafter, "extirpation") and recolonization. Well-connected populations may have

higher probabilities of extirpation because pathogen spread is rapid (Hess 1996; Collinge et al. 2005), resulting in reduction of variation. Alternatively, well-connected colonies may facilitate recolonization by migration from multiple populations (Fahrig and Merriam 1994), a situation that increases variation. Less connected populations may have lower probabilities of disease incidence (Real and Biek 2007), but correspondingly fewer source populations for recolonization (Hanski 1998). There are a number of possible scenarios depending on rates of colony extirpation by pathogens, rates of recolonization, and the number of possible source populations for recolonization. If, for instance, extirpation rate is high, recolonization rate is low, and there are few source populations, disease outbreaks are expected to erode genetic variation (Harrison and Hastings 1996). By contrast, slow rates of extirpation, rapid recolonization, and multiple source populations would buffer against the loss of genetic diversity. Therefore, landscape context can contribute to the effects of pathogens on genetic variation within and among populations (Biek and Real 2010).

One highly virulent pathogen infecting mammals is the bacterium *Yersinia pestis*, the agent of sylvatic plague. Plague is transmitted by fleas and was introduced to western North America from Asia around 1900 (Cully et al. 2000); its effects are particularly severe in naïve hosts such as prairie dogs (*Cynomys spp*; family Sciuridae). Prairie dogs comprise five species of social burrowing rodents inhabiting the grasslands of western North America; their populations are organized into colonies consisting of several groups of related individuals (Hoogland 1995). Prairie dog mortality from plague approaches 99% (Pauli et al. 2006; Cully et al. 1997; Biggins et al. 2010). Once *Y. pestis* is endemic within a region, prairie dog colonies experience metapopulation dynamics (Antolin et al. 2006) with plague-induced extirpations (Cully and Williams 2001) and subsequent recolonization (Roach et al. 2001). In Boulder County, Colorado, movement of prairie dogs among colonies is dependent on the intervening landscape matrix, where urbanization suppresses movement but other

land types facilitate movement (Sackett et al. 2012). Because plague causes colony extirpation that is often, but not always, followed by recolonization, the disease is expected to have consequences on the distribution of neutral genetic variation within and among colonies.

This chapter examines the effects of pathogen extirpations on genetic variation at three scales. At the scale of the metapopulation, variation could either decrease due to population extirpation, or be maintained by recolonization (Whitlock and McCauley 1990; Bohonak 1999). At the scale of the colony, genetic diversity could decline due to bottleneck and founder effects (Tsutsui et al. 2000; Leberg 1992), which would be exacerbated if immigration occurs from only one or few source colonies (Pannell and Charlesworth 1999), as may be expected in isolated colonies or those surrounded by urbanization (Magle et al 2010; Sackett et al. 2012). Alternatively, recolonization from multiple sources would replenish variation within a colony after recolonization (Slatkin 1977). At the scale of the individual, those remaining after widespread extirpations could be a random subset of the original population (for instance, if they were not exposed to the pathogen because of stochastic effects), leading to a loss of within-individual genetic diversity due to inbreeding that results from founder effects (Trudeau et al. 2004). Conversely, surviving individuals could have higher within-individual genetic diversity (heterozygosity) because in many systems, more inbred individuals suffer higher mortality from infection than less inbred individuals (Coltman et al. 1999; Valsecchi et al. 2004). Higher within-individual heterozygosity could also result from admixture following recolonization from multiple sources.

To test the hypotheses of how *Y. pestis* influences the distribution of neutral genetic diversity in black-tailed prairie dogs (*C. ludovicianus*, hereafter "prairie dog") at different scales, I sampled 15 prairie dog colonies before, during, and after a plague epizootic. Using nine microsatellite markers, I examined the effects of plague-induced colony extirpation and subsequent recolonization on neutral genetic diversity in prairie dogs at three scales: within the metapopulation, within colonies and

within individuals. At the metapopulation scale, I investigated regional temporal changes in the degree of genetic diversity and isolation by distance before extirpation and after recolonization. At the colony scale, I examined changes in genetic diversity and temporal differentiation within colonies and estimate immigration rates into extirpated colonies. At the individual scale, I calculated observed heterozygosity (as the proportion of heterozygous loci) before plague and after recolonization. I found that within the metapopulation, plague did not change genetic diversity; within colonies, genetic diversity changed depending on the number of inferred source populations; and within individuals, genetic diversity increased.

Methods

Study location and sample collection

Data were collected from 15 colonies in Boulder County from 2003 – 2009 (Fig. 3.1; Table 3.1). Of these, six colonies were extirpated by plague in 2006-2007 and subsequently re-colonized, four were extirpated and not recolonized, two could not be sampled after recolonization, and three were not affected by plague. Because prairie dogs are diurnal and highly social, extirpations were easy to document when they occurred. Sampled colonies were separated by pairwise distances varying from 1.5 – 36 kilometers, and varied in their surrounding habitat matrix (Johnson and Collinge 2004; Collinge et al. 2005). At each colony, 49-104 Tomahawk traps were placed with even distribution throughout the colony, baited, set, and left for up to 2.5 hours. Prairie dogs were trapped for 1 – 2 weeks at each site by targeting active burrows with one to four traps (Hoogland 1995).

Fig. 3.1 Locations of sampled populations and plague events in Boulder County, CO. Light orange represents colonies extirpated by plague in 2005; medium orange represents colonies extirpated by plague in 2006; dark purple represents colonies extirpated by plague after 2007 or not at all.

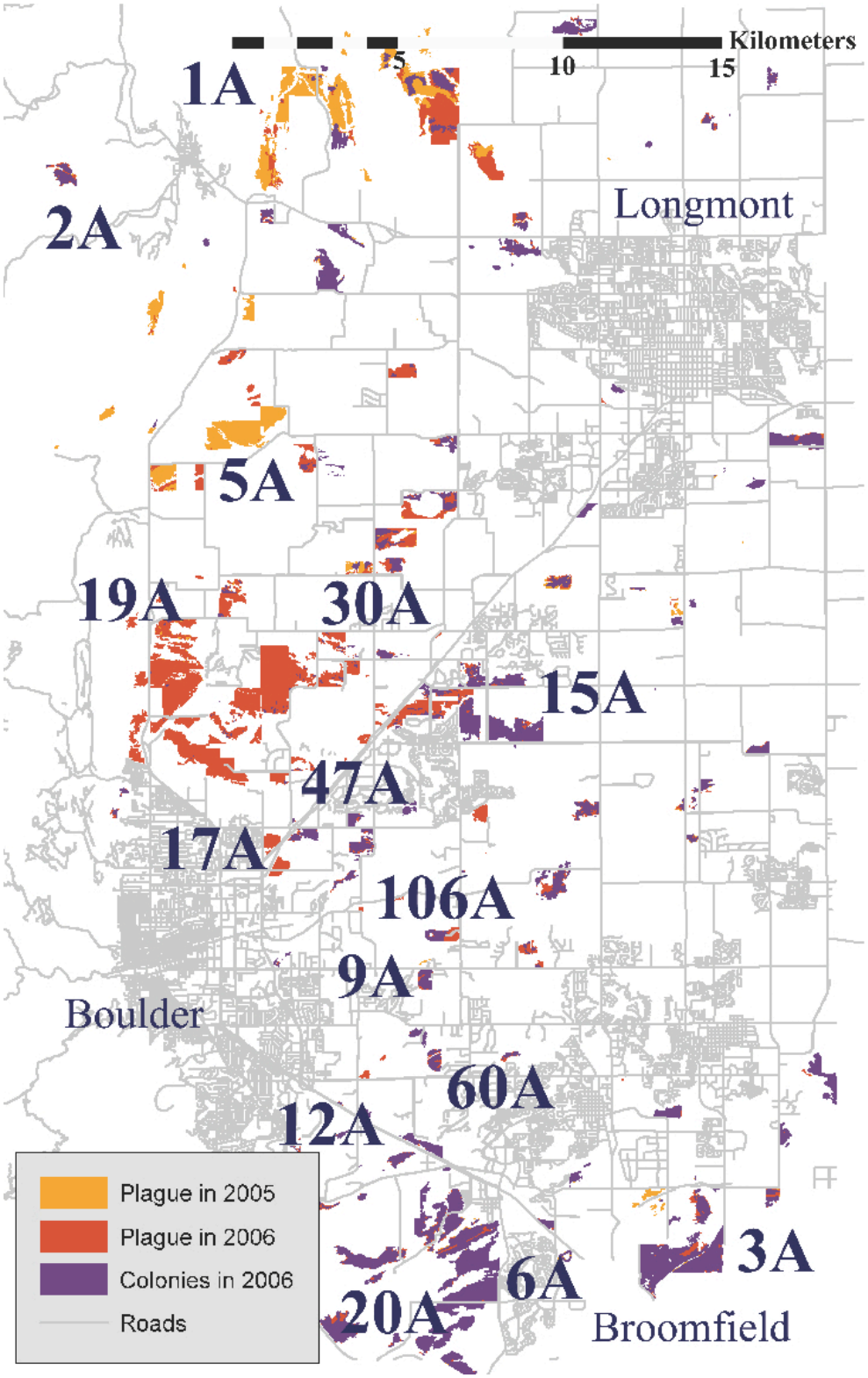


Table 3.1 Colony names and locations, samples sizes and descriptive statistics. N_{total} is the total number of prairie dogs genotyped before and after plague in each colony; N_{03} - N_{09} denote the number of unique individuals genotyped in each year. For cost reasons, only a subset of individuals captured in control colonies and pre-plague colonies was genotyped; thus, numbers reflect capture rates only in recolonized colonies (first 6). Å - Allelic richness was estimated in Fstat to control for unequal sample sizes (given in parentheses). Observed and expected heterozygosity were calculated in Arlequin; F_{IS} was calculated and significance was evaluated by permutation analysis in Fstat (significant deviations from random mating are denoted with asterisks). ♮ denotes colonies with confirmed plague survivors (N=2 each); § represents colonies that were extirpated and not recolonized during our study.

Colony	N_{total}	N_{03}	N_{04}	N_{05}	N_{06}	N_{07}	N_{08}	N_{09}	Latitude	Longitude	Å(N)	H_e	H_o	F_{is}
1A	79	16	49	14					40.244130	-105.226589	4.506 (23)	0.663	0.681	-0.027
1Apost	31					9	9	13	40.244130	-105.226589	3.935 (23)	0.588	0.615	-0.048
12A	11			11	0				39.961195	-105.246726	3.746 (10)	0.624	0.574	0.085
12Apost	55					12	31	12	39.961195	-105.246726	3.910 (10)	0.664	0.713	-0.074*
17A	39	2	10	27					40.047172	-105.243323	4.233 (26)	0.667	0.668	-0.002
17Apost♮	37					7	16	14	40.047172	-105.243323	4.422 (26)	0.706	0.699	0.019
19A	94	24	63	7	0				40.104762	-105.276544	4.409 (24)	0.684	0.655	0.043
19Apost♮	74					15	21	38	40.104762	-105.276544	4.465 (24)	0.694	0.718	-0.018
30A	59		22	19	18				40.101002	-105.22196	3.976 (10)	0.660	0.647	0.020
30Apost	11					0	0	11	40.101002	-105.22196	3.312 (10)	0.591	0.683	-0.165*
47A	61		33	28					40.070321	-105.170399	5.078 (25)	0.686	0.653	0.049*
47Apost♮	49					6	10	33	40.070321	-105.170399	4.197 (25)	0.647	0.698	-0.072*
2A	85	35	35	15					40.219082	-105.309569	4.517 (22)	0.667	0.662	0.007
2Apost	73					25	14	34	40.219082	-105.309569	4.472 (22)	0.665	0.668	-0.005
3A	99	18	42	39					39.940286	-105.094591	4.102 (9)	0.659	0.659	-0.001
3Apost	9					9			39.940286	-105.094591	4.111 (9)	0.649	0.605	0.071
9A	59	29	30						40.015889	-105.189321	4.683 (41)	0.641	0.613	0.046
9Apost	41					27		14	40.015889	-105.189321	4.667 (41)	0.591	0.583	0.014
5A§	37	19	18						40.154402	-105.25251	4.591 (26)	0.632	0.645	-0.021
6A§	49	16	33						39.912180	-105.18057	4.555 (26)	0.717	0.689	0.039
15A§	36	24	12						40.108112	-105.21019	4.737 (26)	0.685	0.721	-0.053
20A§	48	16	32						39.920672	-105.22091	4.663 (26)	0.665	0.657	0.015
60A	4		3	1					39.998259	-105.192919	3.111 (4)	0.607	0.611	-0.008
106A	47		47						40.022840	-105.179904	4.260 (26)	0.566	0.594	-0.052

Prairie dog trapping and processing were conducted in accordance with protocols approved by the University of Colorado's Institutional Animal Care and Use Committee and are described in detail therein (available upon request). Captured prairie dogs were anesthetized under close supervision with 1 – 4% isoflurane in oxygen using a precision, calibrated vaporizer to control the dosage (Heath et al. 1997). Processing involved collection of ear tissue for DNA; collection of blood for pathogen screening; and insertion of a Passive Integrated Transponder (PIT) tag for future identification. Tissue for DNA analysis was collected using a 2-mm diameter ear punch (Braintree Scientific) and stored frozen in a solution of EDTA-DMSO until DNA extraction. After processing, animals were placed back into the traps until the anesthesia wore off and they became alert, at which time they were released at their capture locations.

Blood samples were sent to the Centers of Disease Control and Prevention in Fort Collins, CO for detection of plague antibodies. Presence of these antibodies indicates exposure to *Y. pestis*, and allows for evaluation of survival of prairie dogs exposed to plague. DNA from prairie dogs was extracted using a Qiagen tissue kit, and individuals were genotyped at 9 unlinked microsatellite loci (Jones et al. 2005; Sackett et al. 2009). Loci were examined for null alleles in the program Micro-checker (van Oosterhout et al. 2004). I tested all loci in all populations for deviations from neutrality using the Fdist algorithm (Beaumont and Nichols 1996) implemented in Lositan (Antao et al. 2008) and following the protocol of Bryja et al. (2007).

Analysis of genetic variation within the regional metapopulation

Genetic variation in the metapopulation (across six recolonized and three control colonies) was examined before and after the plague epizootic. I estimated the number of alleles per locus and allelic richness using permutation analysis in the program Fstat (Goudet 1995) to control for unequal sample sizes, and then tested for changes in the number of alleles and allelic richness using

Wilcoxon tests for difference in means, implemented in R (the R Project for Statistical Computing, www.r-project.org). I then calculated spatial differentiation using theta, an unbiased estimator of F_{ST} (Weir and Cockerham 1984), in Fstat and assessed the significance of differentiation by performing Fisher exact tests in Genepop (Rousset and Raymond 1997) and performing a Bonferroni correction. Average pairwise F_{ST} values in the metapopulation (9 colonies only) were compared before plague and after recolonization with a Wilcoxon test. Estimates of F_{ST} were corroborated by allele size-based estimates designed for microsatellites (Slatkin 1995; Rousset 1996). In addition, estimates of differentiation are confounded by the degree of diversity present; therefore, I verified all inferences of differentiation by calculating standardized estimates of F_{ST} (Hedrick 2005) by dividing by the maximum possible values (obtained in the program recodeData, Meirmans 2006) and re-assessed differentiation. All inferences were the same with un-adjusted and standardized F_{ST} values, except where noted. The degree of isolation by distance was determined before plague and after recolonization by performing Mantel tests in in the Vegan package (Oksanen et al. 2010) for R using linearized F_{ST} values (Slatkin 1995) and the natural log of geographic distance (Rousset 1997). The degree of isolation by distance was then compared before plague and after recolonization by performing separate regressions in Genepop, estimating the slopes and determining whether the 95% confidence intervals (estimated by bootstrapping over loci) for the slopes overlapped.

I used GeneClass2 (Piry et al. 2004) to assess migration rates among colonies and characterize founding individuals as immigrants or residents. Because there were unsampled colonies in the landscape, my goal was not to assign individuals to actual source populations, but rather to estimate the number of immigrants in recolonized colonies and estimate the number of putative source populations. Using the recommended frequencies-based method (Paetkau et al. 1995), I inferred immigrant status by choosing individuals that were assigned to their colony of capture with a probability less than 0.05 (as in Berthier et al. 2006). Individuals were assumed to originate in one

of the 15 colonies present before plague (i.e., I did not allow them to be assigned to a post-recolonization colony) or a nearby unsampled colony with a genetic signature similar to the sampled colony. Therefore, individuals assigned to particular colonies (especially extirpated colonies) should be interpreted as originating from a population with a genetic signature that matches the source colony, rather than originating necessarily from that colony.

Analysis of genetic variation within colonies

Genetic variation at the colony scale was assessed within each colony before plague and after recolonization. I tested all colonies for significant departures from Hardy-Weinberg equilibrium both before and after the plague epizootic, controlling for multiple tests with a Bonferroni correction, using Genepop software (Rousset 2008). I estimated allelic richness within colonies before plague and after recolonization using permutation analysis in the program Fstat (Goudet 1995) to control for unequal population size, and then tested for changes in allelic richness using the Wilcoxon test for difference in means, implemented in R. I also examined allelic richness in each year following recolonization. I used a linear model in R to test for a relationship between allelic richness in recolonized colonies and the number of inferred source colonies from the GeneClass2 analysis. I then calculated average relatedness among individuals within a colony relative to all individuals in the metapopulation both before plague and after recolonization using permutation analysis in SPAGeDi (Hardy and Vekemans 2002) with the kinship coefficient of Ritland (1996), recommended for microsatellite studies (Vekemans and Hardy 2004). Next, I assessed whether the degree of linkage disequilibrium increased within each colony (indicative of a founder effect) after recolonization by comparing |D'| values estimated in MIDAS software (Gaunt et al. 2006) with a Wilcoxon test. An unpaired test was used because MIDAS uses information from all allele combinations, some of which were not present either before plague or after recolonization.

I calculated temporal differentiation within colonies by estimating F_{ST} in the program Fstat and performing 10,000 permutations (Goudet 1995; Waples 1989), which allowed me to assess whether colonists were genetically different from residents present before plague. To confirm that temporal changes in genotypes were not expected in the absence of extirpations, I estimated the degree of temporal differentiation in control colonies and compared this to the degree of differentiation in extirpated colonies with a Wilcoxon test in R. Finally, I performed a Principal Components Analysis (PCA) in the ade4 package for R (Dray and Dufour 2007; Chessel et al. 2004) on individual genotypes within each colony before plague and after recolonization. I performed 10,000 randomizations of genotypes within a colony to assess whether genotypes were significantly different over time, and whether plague survivors (see results) had different genotypes than other individuals in their colonies.

Analysis of genetic variation within individuals

Genetic variation at the individual scale was examined using observed heterozygosity of individuals (calculated as the proportion of loci that were heterozygous) before plague and after recolonization. After determining that heterozygosity values were normally distributed, I used unpaired t-tests to assess the difference in mean observed heterozygosity between 1) all extirpated colonies before plague and after recolonization, 2) all control colonies before and after 2006, and 3) inferred immigrants and residents (non-immigrants). For individual colonies, I used a randomization procedure because of small samples sizes (R script available in supporting online information) to test for significant differences in individual heterozygosity by randomly drawing observed heterozygosity values from pre-plague colonies. The number of values drawn was equal to the actual number of individuals in post-recolonization colonies. This sampling procedure was repeated 10,000 times to generate a probability distribution of observing particular average

heterozygosity values, and the percentage of times simulated post-recolonization heterozygosity was lower than the average from the pre-plague distribution was reported as a p-value. This randomization procedure was used because of low samples sizes (e.g., 11 individuals in post-recolonization colony 30A), and was conducted in R.

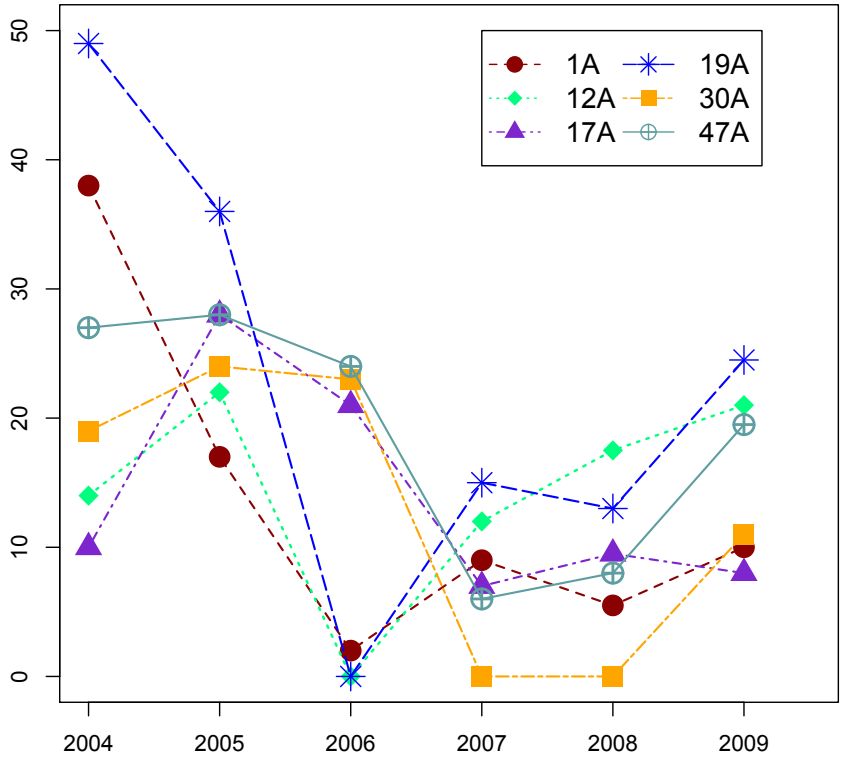
The genotypes of six individuals that survived plague (see results) were compared to those of pre-plague and post-recolonization individuals by performing a permutation test on principal components scores in the ade4 package for R. Next, to compare observed heterozygosity of survivors to a random sample of prairie dogs in the same colonies, I randomly sampled six individuals from those colonies and calculated average heterozygosity. I repeated this sampling 10,000 times and generated a distribution of observed heterozygosity values based on individuals present in the same colonies. The proportion of times survivor heterozygosity was lower than the simulated average heterozygosity was reported as a p-value.

Results

During seven field seasons, I captured 1187 prairie dogs from 15 colonies (Figs. 3.1 – 3.2, Table 3.1). I obtained samples before and after a plague epizootic from nine colonies, including six that were extirpated by plague and three control colonies that were not exposed to plague. Four colonies were extirpated and not recolonized; of these, two were not recolonized within 3 years, and two were not recolonized within 5 years (personal observation). These and two others for which I obtained no post-recolonization data were excluded from analyses involving temporal comparisons. Rates of population growth after recolonization varied from 11 to 47 individuals captured two years after plague (Fig. 3.2, Table 3.1). The majority of individuals in recolonized colonies were captured (estimated by visual counts and within-trapping session recapture rates). By re-typing a subset of individuals, I estimated the genotyping error rate to be 2.1%; errors were distributed randomly

across individuals and loci. Null alleles were not detected at any loci. Finally, departures from neutrality were not detected with the Fdist method (Beaumont and Nichols 1996).

Fig. 3.2 Capture rates in six colonies from 2004-2009; number of captures represents the number of individuals trapped in the first sampling week of each year, even if they were not genotyped. Only the first week of trapping is shown to control for unequal sampling effort.



Genetic variation within the regional metapopulation

The degree of allelic richness across all colonies within the metapopulation was not significantly different before plague (6.456) and after recolonization (6.247, Wilcoxon p=0.631), even when including the colonies present before plague that were not recolonized (6.397; p=0.500). All colonies were significantly differentiated from each other, with the exception of 60A (N=4; Table 3.2). Average pairwise F_{ST} among 9 colonies was significantly higher after recolonization (0.1136) than before plague (0.0867, Wilcoxon p=0.019). When using standardized F_{ST} (Hedrick

2005), differentiation was only marginally significantly higher after recolonization (p=0.1). The isolation-by-distance signal present before plague (Mantel p=0.029, r = 0.4418) was evident after recolonization (Mantel p = 0.011, r = 0.4856; Table 3.3), and the degree of isolation by distance was significantly higher after recolonization (slope and 95% CI of regression after: 0.040 [0.025, 0.080]) than before plague (slope and 95% CI of regression before: 0.016 [-0.002, 0.027]; Table 3.3).

Table 3.2 Pairwise Fst values estimated in Fstat for all study colonies, both a) before plague and b) after recolonization. Spatial Fst values are below the diagonal; temporal Fsts (where applicable) are along the diagonal. A - represents colonies with no post-plague data. A ⊘ denotes that differentiation is not significant; all other pairs of populations were significantly differentiated.

a) before plague

	1A	2A	3A	5A	6A	9A	12A	15A	17A	19A	20A	30A	47A	60A	106A
1A	0.113														
2A	0.082	0.006⊘													
3A	0.080	0.106	0.006⊘												
5A	0.092	0.086	0.119	-											
6A	0.087	0.098	0.060	0.097	-										
9A	0.084	0.106	0.064	0.148	0.089	0.002⊘									
12A	0.125	0.160	0.121	0.171	0.048	0.106	0.098								
15A	0.117	0.052	0.098	0.094	0.082	0.113	0.154	-							
17A	0.082	0.103	0.104	0.080	0.057	0.095	0.121	0.099	0.015						
19A	0.061	0.068	0.102	0.054	0.065	0.085	0.095	0.057	0.042	0.050					
20A	0.061	0.089	0.068	0.089	0.057	0.028	0.085	0.092	0.072	0.070	-				
30A	0.057	0.082	0.089	0.093	0.064	0.0865	0.115	0.078	0.080	0.060	0.044	0.069			
47A	0.067	0.062	0.085	0.039	0.059	0.070	0.108	0.064	0.038	0.044	0.054	0.061	0.063		
60A	0.077	0.146	0.111	0.178	0.098	0.030⊘	0.101	0.132	0.132	0.100	0.022⊘	0.079	0.103	-	
106A	0.113	0.140	0.098	0.169	0.128	0.024	0.143	0.146	0.158	0.127	0.066	0.110	0.121	0.047⊘	-

b) after recolonization

	1A	2A	3A	5A	6A	9A	12A	15A	17A	19A	20A	30A	47A	60A	106A
1A	0.113														
2A	0.090	0.006⊘													
3A	0.220	0.096	0.006⊘												
5A	0.148	0.070	0.124	-											
6A	0.174	0.075	0.064	-	-										
9A	0.237	0.136	0.067	0.132	0.090	0.002⊘									
12A	0.179	0.096	0.054	0.092	0.057	0.082	0.098								
15A	0.139	0.052	0.092	-	-	0.103	0.083	-							
17A	0.127	0.069	0.087	0.090	0.045	0.072	0.069	0.048	0.015						
19A	0.153	0.085	0.088	0.096	0.062	0.060	0.079	0.056	0.028	0.050					
20A	0.163	0.084	0.051	-	-	0.043	0.040	-	0.050	0.052	-				
30A	0.238	0.112	0.066	0.086	0.107	0.078	0.082	0.084	0.091	0.059	0.082	0.069			
47A	0.155	0.082	0.135	0.077	0.087	0.110	0.096	0.051	0.044	0.060	0.098	0.097	0.063		
60A	0.270	0.149	0.079⊘	-	-	0.026⊘	0.118	-	0.078	0.057	-	0.099	0.134	-	
106A	0.260	0.125	0.067	-	-	0.040	0.101	-	0.106	0.073	-	0.069	0.116	-	-

Table 3.3 Analysis results for the prairie dog metapopulation before plague and after recolonization.

Parameter and Time Frame	Before Plague	After Recolonization
Average number of alleles per locus	6.556	6.333
Allelic richness	6. 456 (9 colonies)	6. 247 (no change; p = 0.631)
Allelic richness	6. 397 (15 colonies)	6. 247 (no change; p = 0.500)
Average pairwise differentiation (F_{ST} implemented in Fstat)	0.0867	0.1136 (higher; p=0.019)
Isolation by distance (Mantel test on linearized F_{ST} and natural log of geographic distance)	r = 0.4418, p = 0.029	r = 0.4856, p = 0.011
Isolation by distance (regression on linearized F_{ST} and natural log of geographic distance; slope and 95% confidence intervals)	b = 0.029 [-0.002, 0.027]	b = 0.040 [0.025, 0.080]

Following recolonization of six colonies, I identified 95 out of 258 founders (36.8%) as immigrants. The remaining founders were inferred to originate from a nearby unsampled source colony with a genetic signature indistinguishable from the recolonized colony. Similarly, founders that were assigned to extirpated colonies (e.g., one founder of colony 1A originated in colony 47A, Table 3.4) were interpreted as either migrants from a nearby unsampled, not extirpated colony with a similar genetic signature, or as a migrant leaving before the colony was extirpated. For example, recolonization of colony 1A began in 2007; thus, migrants from 47A may have arrived in 2007 before colony 47A was extirpated (Fig. 3.1). Some colonies were repopulated from a large number of source colonies, while others were repopulated from a small number of sources (Table 3.4). The proportion of immigrants in each colony ranged from 20.4% (colony 47A) to 46.7% (colony 19A). My estimate of migration rate is higher than previously observed in prairie dogs in urban landscapes (Magle et al. 2010), but seems consistent with the rapid repopulation of extirpated colonies.

Table 3.4 Pairwise migration values inferred in GeneClass2. Inferred source colonies are across the top; colonies in which recolonizing individuals were sampled are on the left. Individuals in this table are those that were assigned with probability <0.05 to the colony in which they were sampled and thus are inferred immigrants, which represent 36.8% of individuals present after recolonization. The remaining 63.2% (not shown) are inferred to have colonized from the same population in which they were sampled (or a geographically close unsampled population with an indistinguishable genetic signature) and are not considered immigrants. "Other" signifies that the individual did not assign to any sampled colony with at least a probability of 0.05 and is inferred to have originated from an unsampled colony. "Ncol" is the number of inferred source colonies (including the sampled colony); M is the proportion of colonists that are inferred immigrants.

	1A	2A	3A	5A	6A	9A	12A	15A	17A	19A	20A	30A	47A	60A	106A	other	Ncol	M
1A				2				2		1		2	1			1	7	0.290
12A			1	3	5			9			8	1					7	0.491
17A		1			1		1	2			1		2	1		2	9	0.297
19A	1				5	1	1		2		3		3	9	6	1	11	0.467
30A				1				2									3	0.273
47A				2				6			1			1			5	0.204

Genetic variation within colonies

Within colonies, no systematic departures from Hardy-Weinberg equilibrium were observed either before plague or after recolonization. In colonies that experienced plague, changes in allelic richness varied widely when compared with control colonies, which showed no change in richness. There was a decline in richness in three recolonized colonies (1A ($p=0.164$), 30A ($p<0.001$), and 47A ($p << 0.001$); Table 3.1), but the other three colonies (12A, 17A, and 19A) experienced a slight, but not significant, increase in allelic richness (Fig. 3.3). Allelic richness did not change systematically throughout recolonization (Fig. 3.4). Richness in post-recolonization colonies was positively related to the number of inferred source colonies (adjusted $R^2 = 0.631$, $p = 0.037$; Table 3.5). Changes in relatedness among individuals in a colony were consistent with the changes in allelic diversity (Fig. 3.3): average relatedness increased in the three colonies that experienced a decline in allelic diversity (1A ($p << 0.001$), 30A ($p=0.026$), and 47A ($p << 0.001$); Table 3.5). On the other hand, average relatedness decreased in the three colonies that did not exhibit a significant decrease in allelic

richness (12A (p=0.001), 17A (p<<0.001), and 19A (p=0.211)). The variance in relatedness among individuals decreased significantly (p<0.02) in three colonies (12A, 19A, and 30A), but was unrelated to the number of individuals sampled or the mean change in relatedness. Linkage disequilibrium (measured by |D'|) increased significantly in the three colonies that lost allelic diversity (1A: p<<0.001; 30A, 47A: p=0.004; 3.5), which may be indicative of founder effects. LD did not change in two colonies (17A and 19A, p>0.1) and decreased in one colony (12A, p <<0.001).

Fig. 3.3 Plot of the change in allelic richness (light blue) and relatedness (dark purple) within colonies before plague and after recolonization. Error bars represent one standard error; solid lines are for relatedness and dotted lines are for richness.

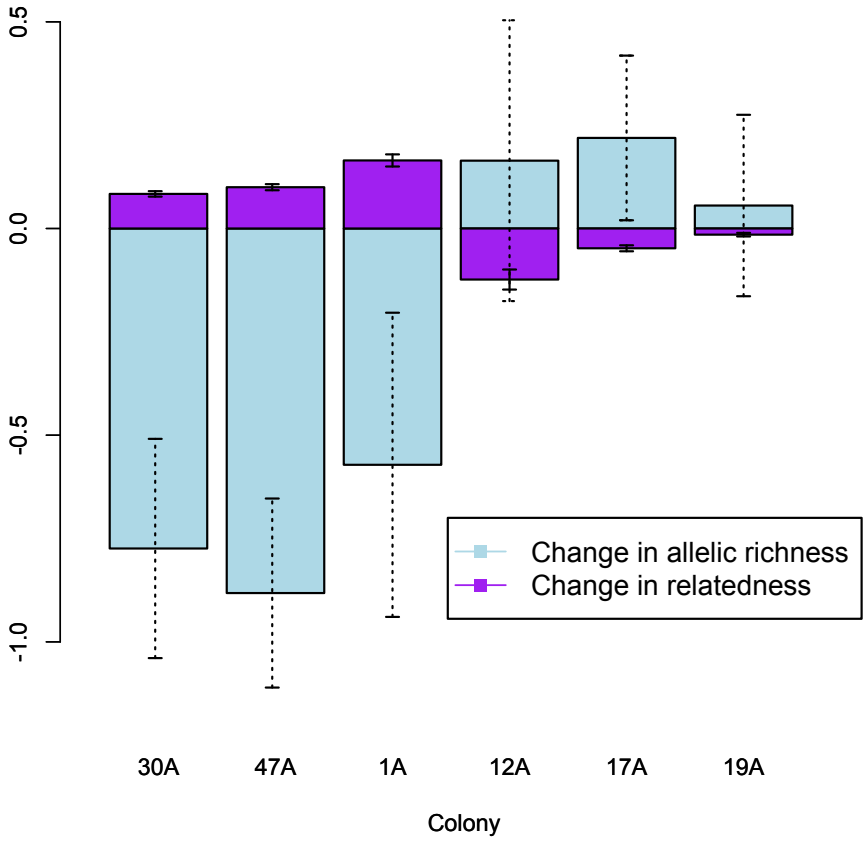


Fig 3.4 Allelic richness across years in five colonies following recolonization (Colony 30A is not shown because there are data from only one year of recolonization).

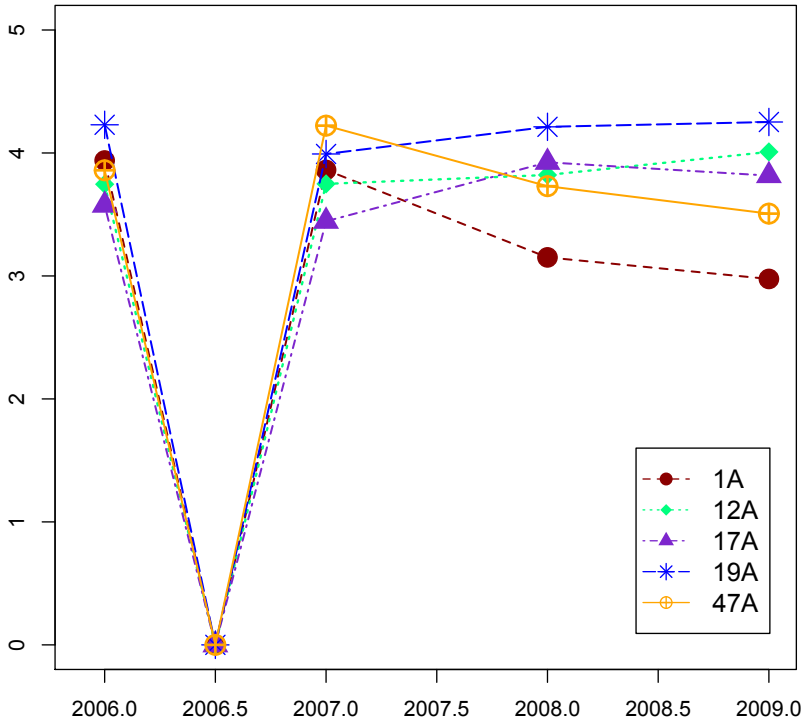


Table 3.5 Analysis results for prairie dog colonies before plague and after recolonization. ♮ denotes colonies with plague survivors; asterisks represent a significant increase in relatedness/LD, and § represent a significant decrease in relatedness/LD.

Parameter and Time Frame		Before Plague	After Recolonization
Regression on post-recolonization allelic richness and the number of inferred source colonies		N/A	p=0.037, adjusted R^2=0.631
Average kinship among individuals (coefficient from Ritland 1996)	**1A**	0.0678	0.2324*
	12A	0.2187	0.0953§
	17A ♮	0.1076	0.0597§
	19A ♮	0.0620	0.0471
	30A	0.0494	0.1331*
	47A ♮	0.0409	0.1408*
Average magnitude of linkage disequilibrium, |D'|	**1A**	0.5358	0.6218*
	12A	0.7362	0.6308§
	17A ♮	0.5532	0.5571
	19A ♮	0.5361	0.5187
	30A	0.5743	0.6577*
	47A ♮	0.5884	0.6452*

Temporal differentiation before and after 2006 was an order of magnitude higher in colonies affected by plague than control colonies (F_{ST}=0.0689 plague, 0.005 control; p=0.009; Fig. 3.5). The average spatial F_{ST} was 0.0867 (Table 3.2). Principal components analysis of genotypes showed that in some cases, post-recolonization individuals were significantly different from pre-plague individuals, suggesting that founders originated from other colonies. For instance, founders of colony 19A were more genetically similar to residents of colony 30A than to individuals present in colony 19A before extirpation (Fig. 3.6). The mean genotype within four colonies (1A, 12A, 19A, and 47A) changed over time ($p < 0.05$; Fig. 3.7a – f), with the change more pronounced in some colonies (e.g., 1A and 19A, $p << 0.001$; Fig. 3.7a, 3.7f) than others. One colony with plague survivors, 17A (Fig. 3.7e), experienced no shift in genotype space (p=0.265); 17A and 30A were probably recolonized from a source population that was genetically similar to the original colonies.

Fig. 3.5 Temporal differentiation (F_{ST}) in colonies before plague and after recolonization for extirpated colonies (light blue bars), or before and after 2006 for control colonies (dark green bars). Error bars represent one standard error. Two asterisks denote highly significant differentiation (p<0.0001); one asterisk denotes significant differentiation (p<0.01).

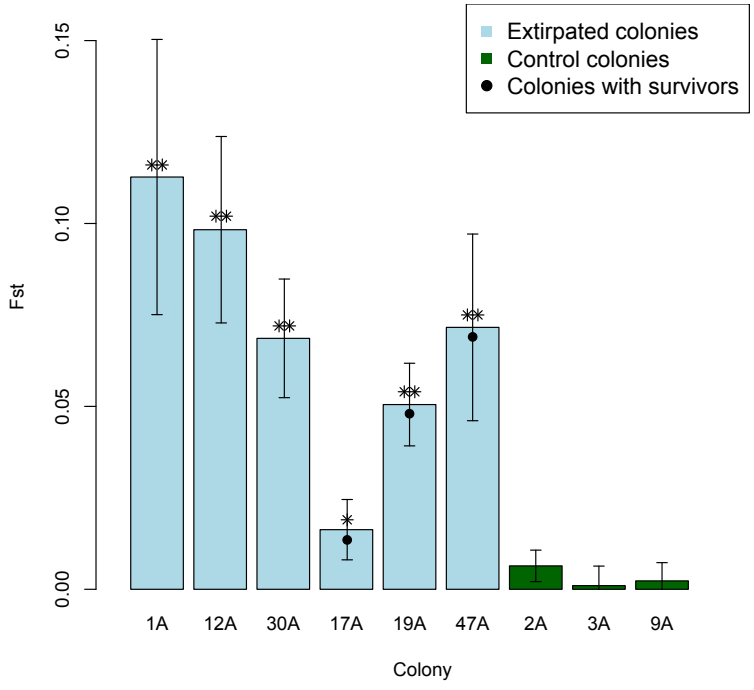


Fig 3.6 Principal Components Analysis of prairie dog genotypes in two neighboring colonies (light green, 30A, and blue, 19A). Genotypes present before plague are denoted with circles (19A) and triangles (30A); those after recolonization are denoted by plus signs (19A only). Genotypes of colonists in 19A are more similar to those in 30A than in 19A before plague.

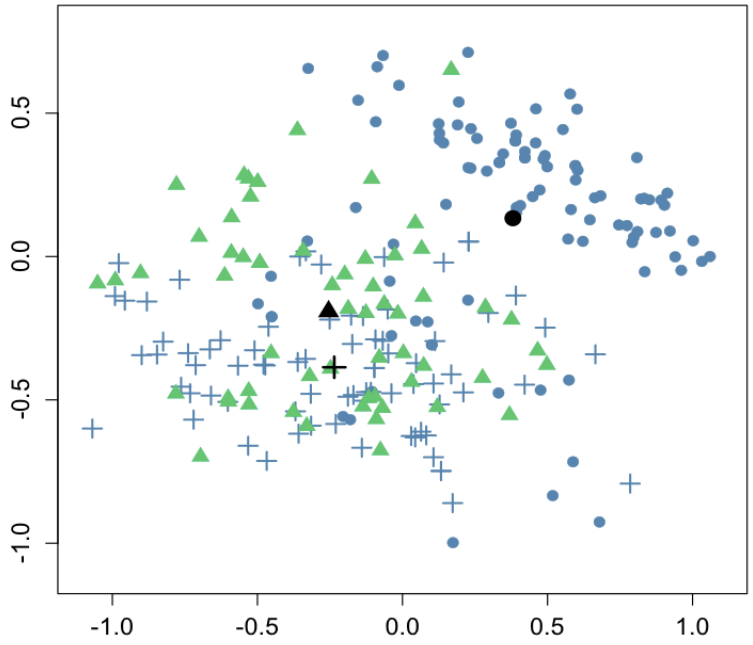
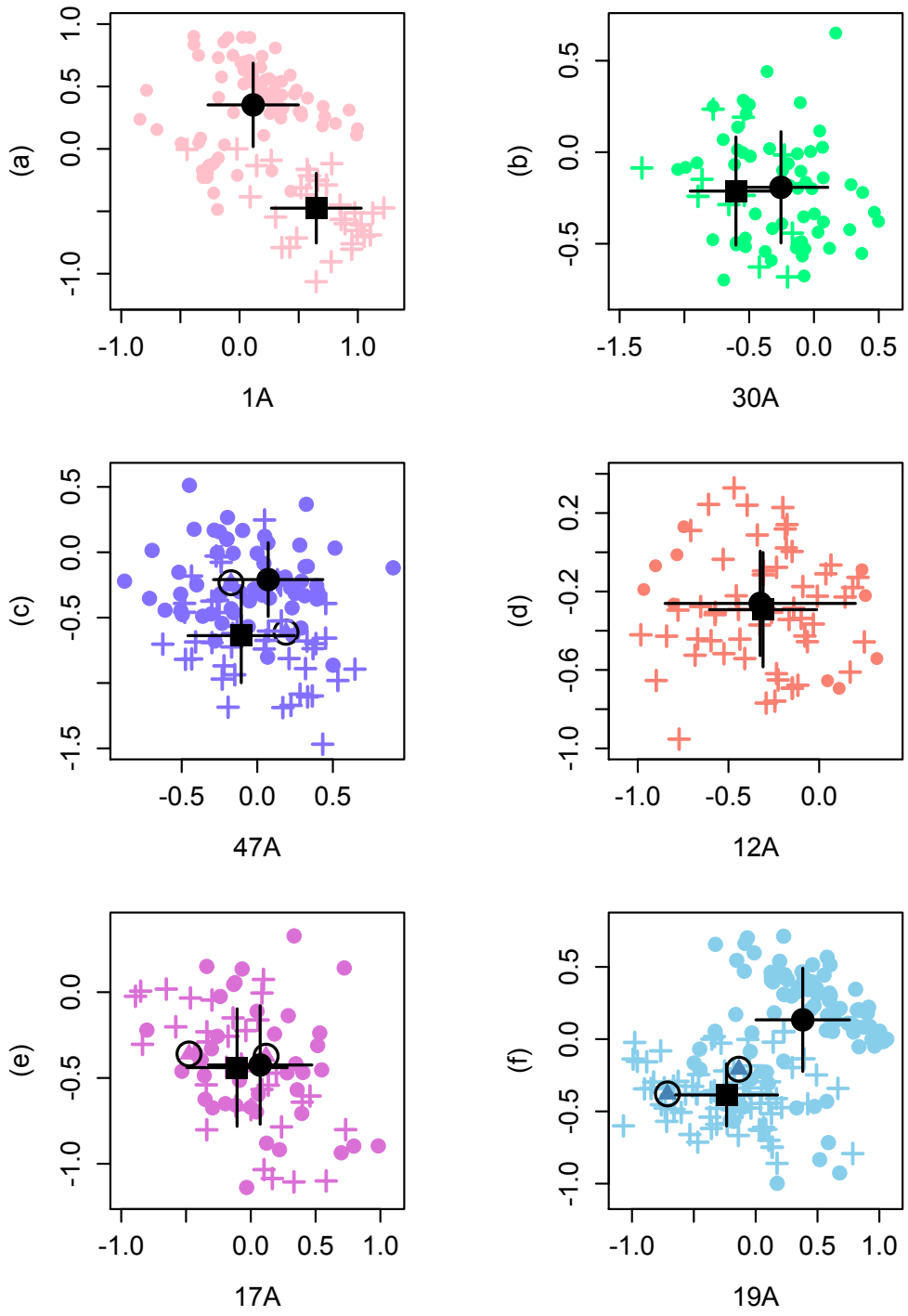


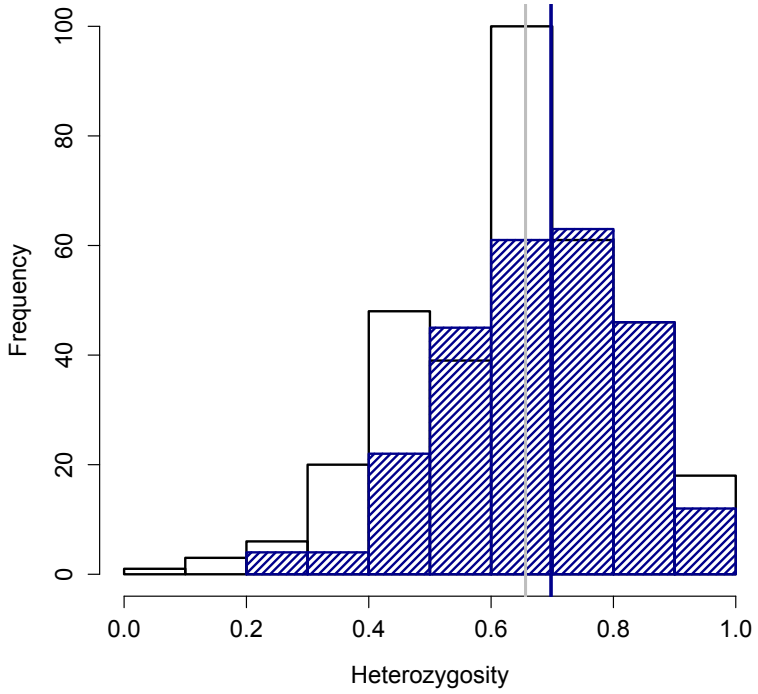
Fig 3.7 Principal Components (PC) Analysis of prairie dog genotypes in individual colonies: a) 1A, b) 30A, c) 47A, d) 12A, e) 17A, and f) 19A. Solid circles represent individuals present before plague; plus signs represent individuals that colonized populations after plague; circled triangles in three colonies (17A, 19A and 47A; d, e, and f) represent individuals that survived plague. Mean population PC scores for before plague (circles) and after recolonization (squares) are denoted in bold black symbols with lines representing one standard deviation.



Genetic variation within individuals

Observed heterozygosity in recolonized colonies ranged from 0.632 (1A; 20 individuals captured in 3 years) to 0.718 (19A; 74 individuals captured in 3 years). Across all recolonized colonies, there was a significant increase in heterozygosity after recolonization (mean H_o in pre-plague colonies = 0.656; mean H_o in recolonized colonies = 0.698; t=2.99, p=0.003). During the same time period, heterozygosity did not change in colonies that did not experience plague (t=0.083, p=0.934). Inferred migrants from GeneClass2 did not have higher heterozygosity than residents (t=0.608, p = 0.237). Recolonized colonies with plague survivors (17A, 19A and 47A; see below) and 12A exhibited significantly higher heterozygosity than before plague ($p<<0.001$), whereas the other two colonies were not different before plague and after recolonization ($p>0.1$). Interestingly, the increase in mean heterozygosity among recolonized colonies resulted partly from the loss of almost all individuals in the lowest heterozygosity classes (e.g., $H_o<0.4$; Fig. 3.8). Post-recolonization heterozygosity was approximately equal to that of a theoretical population created by culling approximately 10% of the most inbred individuals present before plague.

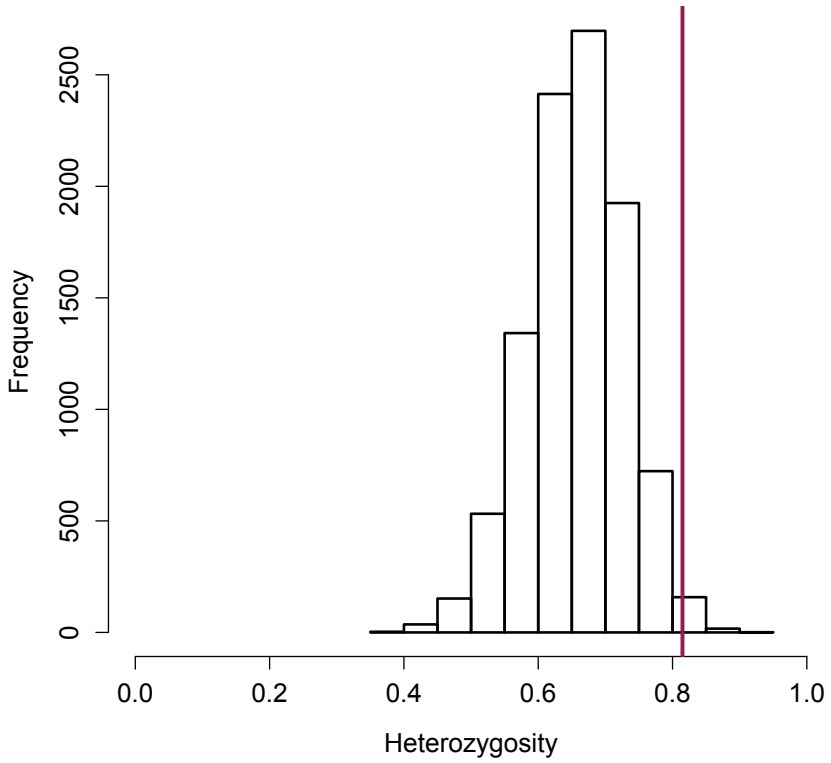
Fig 3.8 Distribution of observed heterozygosity of individuals in colonies experiencing plague. Open bars represent individuals present before plague (mean at the light gray bar); hashed bars represent individuals present after recolonization (mean at the dark blue bar). After recolonization, the distribution shifted towards higher heterozygosity, and individuals in the lowest heterozygosity classes were lost.



During 2007 and 2008, I confirmed that six individuals survived exposure to plague (assessed by plague antibodies in one year and recapture the next); two individuals survived in three colonies (17A, 19A, and 47A). Five of these animals also tested positive for antibodies in the second year of capture, indicating that plague antibodies can persist for at least a year, or that there was repeated exposure to plague. The PCA indicated that genotypes of survivors were not significantly different from other individuals in their colonies (Fig. 3.7c, e and f), and in one colony (19A, Fig. 3.7f) survivors were more genetically similar to colonists (no difference; $p=0.61$) than to pre-plague residents (marginally significant difference, $p=0.10$). In the other two colonies (17A and 47A, Fig. 3.7c and 3.7e), survivors were not genotypically distinct from residents before or after plague ($p > 0.1$). Two of the survivors, both sampled in colony 17A, were inferred immigrants. One was assigned to colony 60A, a historically plague negative colony, and the other was assigned to colony

20A, which was extirpated by plague in 2008 (Fig. 3.1; data from Boulder Open Space and Mountain Parks). The two survivors from 19A and one survivor from 47A were not excluded from their sampled colony (i.e., not inferred immigrants) but were assigned with a higher probability to colony 20A. Heterozygosity of the six plague survivors was significantly higher than expected if survival was random with respect to heterozygosity (mean survivor H_o=0.815, p=0.006; Fig. 3.9).

Fig 3.9 Simulated distribution of observed heterozygosity of individuals in colonies with plague survivors. The distribution was generated by randomly sampling six (equal to the actual number of survivors) individuals in these colonies and calculating average observed heterozygosity. This process was repeated 10,000 times. Mean heterozygosity of survivors is denoted by the solid bar.



Discussion

The effects of plague extirpation and subsequent recolonization on genetic diversity varied depending on the scale at which diversity was assessed. Within the metapopulation, there was no change in diversity. Within colonies, the magnitude and direction of change varied, with some

colonies experiencing a large decline in allelic richness and others exhibiting a slight (non-significant) increase in allelic richness. Within individuals, the processes of extirpation and recolonization preferentially eliminated individuals with the lowest heterozygosity. Furthermore, the six survivors had significantly higher heterozygosity than expected by chance. These results show that although the effects of extirpations on genetic diversity of *colonies* varied, plague and recolonization favored *individuals* with the highest genetic diversity, with the implication that pathogen-induced extirpations provide a mechanism for the maintenance of genetic variation, provided that there are disease-free populations that can serve as sources for recolonization.

Genetic variation within the metapopulation

In this system, plague extirpations did not depress allelic richness of prairie dogs at the regional scale, a pattern that held even with the inclusion of the four colonies that were never recolonized. There was, however, an increase in average pairwise differentiation and isolation by distance, likely due to reductions in population size (Wright 1931; van Treuren et al. 1991). It is probable that the maintenance of genetic diversity within this metapopulation was due to its urban landscape matrix, which may have slowed spread of the *Y. pestis* (Collinge et al. 2005) such that source populations remained at all times, allowing recolonization of extirpated populations (data from Boulder Open Space and Mountain Parks; see Fig. 1). For instance, colony 19A could have been a source colony in 2005 but not 2006, whereas colony 2A could have been a source in 2006. In contrast, a system that experienced simultaneous extirpation of all populations would likely exhibit a loss of genetic diversity (Larson et al. 2002).

Genetic variation within colonies

Three lines of evidence suggest that three colonies (1A, 30A, and 47A) experienced founder effects: there was 1) a decline in allelic richness, 2) an increase in average relatedness among individuals, and 3) an increase in LD. Founder effects were not evident in the other three recolonized colonies (12A, 17A, and 19A), which experienced a slight increase in allelic richness, a decrease in average relatedness, and a decrease or no change in LD (Table S4, Supporting information). Prairie dogs in the three re-colonized sites without founder effects (and in 47A) were clustered in spatially discrete portions of their colonies (personal observation), suggesting that they may represent distinct family groups and separate immigration events from different source populations. Coupled with the slight increase in allelic diversity and the change in genetic composition before plague and after recolonization, spatial clustering supports the idea that immigrants arrive from multiple source colonies.

The reasons for the disparity in patterns of change in diversity are probably largely dependent on the landscape context in which colonies occur (Magle et al. 2010) and the associated relative rates of extirpation and recolonization. I observed that colonies with the largest putative number of sources also contained the most genetic diversity (Tables 3.1, 3.4), whereas colonies with low migration rates from few source populations experienced a loss of diversity. Increased numbers of source populations for recolonization may thus mitigate founder effects (Kolbe et al. 2004). Thus, for the maintenance of genetic variation, the importance of connectivity for recolonization appeared to outweigh the increased risk of extinction (Hess 1996), perhaps because so few colonies escaped exposure to plague (data from Boulder Open Space and Mountain Parks). Quantifying the landscape context in relationship to colony isolation, gene flow and genetic diversity will be a fruitful avenue for further research.

Temporal genetic differentiation within colonies before plague and after recolonization may occur for three reasons. First, migration routes could vary over time, causing genetic inputs into a colony to also change over time (Mackey et al. 2011). This explanation may be particularly likely in systems where extirpations are spatially proximate, disrupting the typical dispersal corridors used by individuals. For instance, most genetic exchange likely occurs between closely situated populations (e.g., colonies 17A and 47A, Fig. 1), but if plague eliminates both populations simultaneously, then immigrants into each colony will necessarily originate from farther away (e.g., colony 106A). Second, temporal differentiation within colonies before and after plague could arise from stochastic demographic fluctuations. During colony formation, one or two genotype groups could become prevalent due to colonization order or other non-adaptive effects. Finally, only certain genotypes may be able to colonize (as in migratory culling, Bartel et al. 2011) after plague. Successful colonists may need to be excellent dispersers, resistant to plague, or both.

Genetic variation within individuals

At the individual scale, heterozygosity within individuals increased, partially reflecting the loss of individuals with the lowest heterozygosity. This suggests a cost of low heterozygosity to either the ability to successfully colonize a new population or survive plague. Migratory culling (Bartel et al. 2011) may be partially responsible for this loss of low-heterozygosity individuals; however, I found no difference in heterozygosity between inferred immigrants and residents, suggesting instead that the role of heterozygosity is linked to survival from plague. Indeed, genome-wide heterozygosity is often associated with resistance to disease (Hawley et al. 2005; Calleri et al. 2006; Pearman and Garner 2005). The overall increase in heterozygosity within post-recolonization individuals likely reflects both the selective loss of inbred individuals and the direct result of admixture of multiple genotype groups founding new populations (Table 3.4). This increase in

heterozygosity suggests that successful immigration increases after extirpations: Prairie dog colonies have high population densities and socially-structured groups that are defended territorially (Hoogland 1981); thus, immigrants are likely to experience intense competition with residents. After plague extirpations, this competition is eliminated, and immigrants may be more likely to survive. Thus, extirpation could act to effectively increase the degree of successful immigration because territories are no longer defended. This hypothesis should be further tested using behavioral observations of newly founded populations in species with territorial behavior that are subject to periodic population extirpations.

The six individuals that survived plague were not genetically differentiated from other individuals in their colonies for the microsatellite loci examined (Fig. 5); however, survivors exhibited unusually high heterozygosity (Fig. 7). Microsatellites are neutral markers; however, they provide a proxy for genome-wide heterozygosity (Da Silva et al. 2006), which correlates inversely with inbreeding and sometimes reflects diversity at non-neutral loci (Hansson and Westerberg 2002; Slate et al. 2004) such as genes that provide protection against disease (Yang et al. 2011; Penn et al. 2002; Turner et al. 2008). Heterozygosity-fitness correlations at neutral loci may be due either to linkage disequilibrium between one or several neutral markers and functional loci under selection (Hansson and Westerberg 2002) or due to negative effects on fitness owing to genome-wide homozygosity (Charlesworth and Charlesworth 1987; Hansson and Westerberg 2002). I observed a particularly strong effect of two loci (D1 and D115, Jones et al. 2005), at which all survivors were heterozygous, which may indicate LD with functionally important loci. However, the pattern persisted when removing these loci from analyses; further, these markers showed no signs of selection with the Fdist method, suggesting these genomic regions are not the only drivers of the observed effect. Although it is possible that both mechanisms are contributing to differential survivorship of highly heterozygous prairie dogs, the significant relationship between multilocus

heterozygosity and survivorship lends support to the idea that survival is conferred by the collective heterozygosity of many loci across the genome.

Implications of this study for understanding pathogen influences on diversity

The genetic consequences of plague across scales lend support to the hypothesis that extirpation creates a vacant habitat into which colonization can occur from multiple sources, leading to an increase in genetic diversity in sufficiently connected populations. The immediate decline in allelic richness in three colonies may be counteracted by immigration in subsequent years (indeed, colony 30A had just begun recolonization at the conclusion of our study and only 11 individuals were captured; see Fig. 2). The increase in diversity may occur over longer time frames and allow evolutionary forces such as relaxed selection to act through, for example, the enhanced survival of advantageous mutations (Carson 1968; Templeton 1980). If founder populations have higher additive genetic variance, they could have a more pronounced response to selection in subsequent generations. Furthermore, *Y. pestis* preferentially eliminated the most inbred individuals. High genome-wide heterozygosity may offer protection against pathogens via overdominance at many loci involved in resistance to pathogens (e.g., MHC, Hughes and Nei 1988; signaling pathways in immune response, Yang et al. 2011). The genetic consequences of extirpations have implications for the way we understand host-pathogen coevolution (Nuismer and Doebeli 2004), the evolution of virulence (Lenski and May 1994), and evolution of host resistance (Hughes and Boomsma 2007).

More research is needed to determine the circumstances under which pathogens facilitate an influx of diversity. First, the spatial arrangement of populations in a landscape can influence extinction probability and recolonization dynamics, as discussed above. Subdivided populations may be more likely to retain genetic diversity in the face of extinctions than less divided populations (Ray 2001). Second, pathogen virulence is a likely predictor of how genetic diversity is maintained

(Tobler and Schmidt 2010). A pathogen of moderate virulence would not eliminate populations (e.g., Breitschwerdt and Kordick 2000) and create a vacant habitat; thus, we would not predict an influx of diversity. Highly virulent pathogens that extirpate populations—provided some populations remain as sources for recolonization—may be the most likely to allow an influx of diversity into a vacant habitat, particularly when coupled with high levels of host movement (Cross et al. 2005). Finally, social structure and life history characteristics of the host also likely play an important role in how extirpations change host genetic diversity. Prairie dogs territorially defend their colonies (Hoogland 1981); thus, immigrants are likely to be more successful at surviving after a colony has been extirpated. In less aggregated species, however, individuals may have to travel farther to find unrelated mates, and inbreeding may thus increase after extirpations (Lachish et al. 2011). Overall, my findings suggest that pathogen-induced extirpations provide a mechanism for the maintenance of genetic variation. Further study will help resolve the conditions under which virulent pathogens allow for increased genetic diversity within their hosts (Caprio and Tabashnik 1992).

Chapter 4: **Connectivity of Prairie Dog Colonies in an Altered Landscape: Inferences from Analysis of Microsatellite DNA Variation**

Abstract

Connectivity of populations influences the degree to which species maintain genetic diversity and persist despite local extinctions. Natural landscape features are known to influence connectivity, but global anthropogenic landscape change underscores the importance of quantifying how human-modified landscapes disrupt connectivity of natural populations. Grasslands of western North America have experienced extensive habitat alteration, fragmenting populations of species such as black-tailed prairie dogs (*Cynomys ludovicianus*). Population sizes and the geographic range of prairie dogs have been declining for over a century due to habitat loss, disease, and eradication efforts. In many places, prairie dogs have persisted in the face of emerging urban landscapes that carve habitat into smaller and smaller fragments separated by uninhabitable areas. In extreme cases, prairie dog colonies are completely bounded by urbanization. Connectivity is particularly important for prairie dogs because colonies suffer high probabilities of extirpation by plague, and dispersal permits recolonization. Here I explore connectivity of prairie dog populations using analyses of 11 microsatellite loci for 9 prairie dog colonies spanning the fragmented landscape of Boulder County, Colorado. Isolation-by-resistance modeling suggests that wetlands and high intensity urbanization limit movement of prairie dogs. However, prairie dogs appear to move moderately well through low intensity development (including roads) and freely through cropland and grassland. Additionally, there is a marked decline in gene flow between colonies with increasing geographic distance, indicating isolation by distance even in an altered landscape. These results suggest that prairie dog colonies retain some connectivity despite fragmentation by urbanization and agricultural development.

Introduction

Wildlife populations are distributed discontinuously across the landscape, leading to varying degrees of spatial and genetic connectivity among populations. Through the burgeoning field of landscape genetics, we have gained a greater understanding of the natural barriers that structure populations and mediate gene flow across a landscape (e.g. Manel et al. 2003; Perez-Espona et al. 2008; Spear et al. 2005). Human alteration of the landscape further divides populations and may interrupt or redirect existing corridors among them (Collinge 2009). Features with demonstrated effects on the connectivity of populations include highways (Coulon et al. 2006), deforestation (Pavlacky et al. 2009), urbanization (Telles et al. 2007), agriculture (Levy et al. 2010) and dams (Beneteau et al. 2009). Furthermore, landscape changes can alter migration corridors (Antonio et al. 2007), create asymmetrical gene flow (Barrowclough et al. 2004), or decrease the magnitude of gene flow without altering its direction (Goverde et al. 2002). With an increasing percentage of global land being converted to agriculture (34%; Ramankutty et. al 2008) and urban sprawl (increasing twice as fast as human population growth; DeCoster 2000), many species encounter complex human-modified landscapes. Depending on dispersal ability of the organism, genetic connectivity of different species will be influenced to varying degrees by each form of habitat alteration.

Population connectivity in black-tailed prairie dogs (*Cynomys ludovicianus*, hereafter "prairie dogs"), a social mammal important in prairie ecosystems, was historically maintained by their occurrence in large, continuous swaths of grassland. In natural landscapes, dispersal among colonies probably happens every generation, can occur over relatively large distances, and likely employs corridors such as dry creek beds or ravines (Garrett and Franklin 1988; Roach et al. 2001). In the last 200 years, the occupied range of prairie dogs has declined by over 99% (Miller and Cully 2001) from the combined actions of land conversions, eradication campaigns, and extirpation by sylvatic plague. Consequently, prairie dog populations (equivalent to colonies for the purpose of this paper) in many

places now occupy discrete patches of grassland surrounded by a matrix of uninhabitable land including urban sprawl, agricultural fields and hay fields (Johnson and Collinge 2004).

Landscapes in which colonies are mostly bounded by urban or agricultural land may inhibit inter-colony movement of animals, and such conditions can accelerate inbreeding and prevent recolonization if the colony is extirpated; however, these landscapes may also limit the spread of diseases that can move through well-connected systems (Hess 1996). Conversely, landscapes that support well-connected colonies most closely resemble the native conditions for prairie dogs and presumably limit localized inbreeding; nonetheless, well-connected colonies may be prone to colonization by pathogens (Hess 1996; Jesse et al. 2008; Lopez et al. 2005; Trudeau et al. 2004) such as the plague pathogen *Yersinia pestis*. Pathogens may experience different influences on connectivity than a host if the pathogen has multiple modes of dispersal (Jones and Britten 2010), but pathogens and parasites that rely on their hosts for dispersal (Brinkerhoff et al. 2011), such as flea transmission of *Y. pestis* (Stapp et al. 2009), will be influenced by similar constraints on connectivity as their hosts.

Recent plague events in Boulder County, Colorado (in 2005-2009, confirmed by the Centers for Disease Control and Prevention) were more geographically restricted than in the past, spreading through the county over the course of several years (Boulder Open Space and Mountain Parks, unpublished data), possibly due to the disruption of migration corridors caused by urbanization. In natural habitats and in urbanized landscapes, extirpated colonies are re-colonized within a few years (Roach et al. 2001; Antolin et al. 2006). However, if urbanization and other types of land alteration restrict movement of prairie dogs, re-colonization from a smaller number of source colonies may result in founder effects (Templeton 2006) and inbreeding. If movement is limited, the increase in colony isolation due to plague extirpation may be amplified in an altered landscape such as Boulder County, where humans and prairie dogs share a mosaic of different habitat types. Urbanization that isolates colonies (Magle et al. 2010) may disrupt connectivity; in contrast, habitat conversion to

agriculture may facilitate dispersal due to increased vegetation cover or by providing refuge from predators. Here I develop a model for the connectivity of black-tailed prairie dog colonies in a complex landscape by integrating the habitat matrix with estimates of genetic similarity among colonies across Boulder County. I show clear effects of various landscape characteristics and demonstrate that there may be a complex network of corridors that facilitates connectivity among prairie dog colonies, with different forms of landscape alteration contributing to connectivity in distinct ways. Finally, I discuss how interrupted connectivity in complex landscapes may contribute to metapopulation dynamics in the context of pathogen-mediated extinctions.

Methods

Genotyping and estimating differentiation among colonies

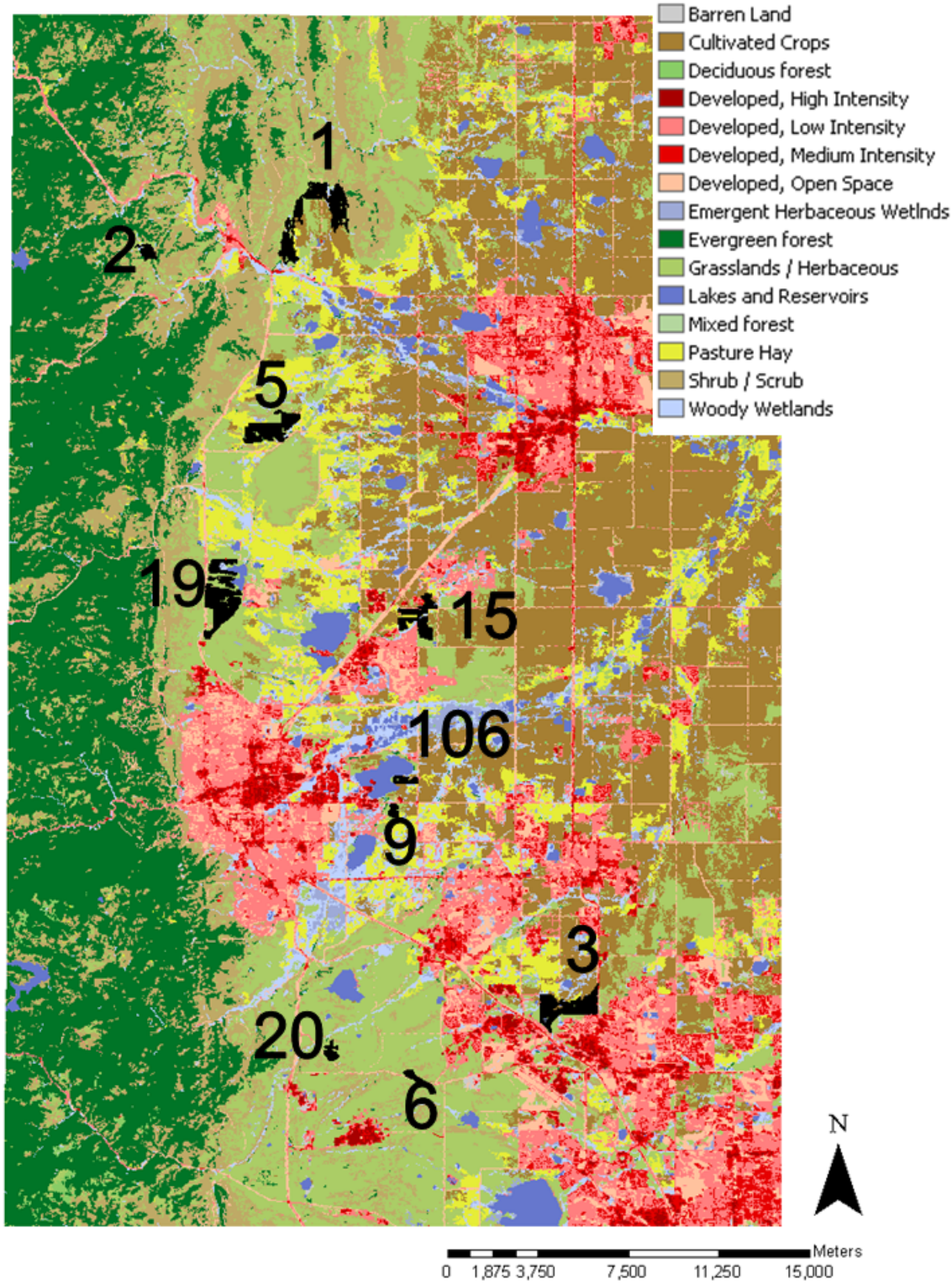
The genetic analysis focused on ten colonies located throughout Boulder County (Table 4.1) with relatively large sample sizes. Of the 1200 ha of land occupied by prairie dog colonies in Boulder County, study colonies were chosen to represent populations bounded by varying degrees of urbanization (Johnson and Collinge 2004) and surrounded by a range of habitat matrix types (Figure 4.1). Sampled colonies were separated by pairwise distances varying from 1.5-36 kilometers. No attempt was made to sample at regular spatial intervals; instead, colonies were selected based upon their surrounding habitat matrix. Eight of the ten colonies were affected by plague in 1994, as determined by local plague records kept by land and wildlife managers (Collinge et al. 2005). Prairie dog trapping and processing were conducted in accordance with protocols approved by the University of Colorado's Institutional Animal Care and Use Committee and are described in detail therein (available upon request). For each of the ten colonies sampled, 49 Tomahawk traps were set on a 150 x 150 meter grid in the approximate center of the colony and pre-baited with a corn-oat-barley mixture for five days with the traps held open. After pre-baiting, traps were baited, set, and

left for three hours at a time for the course of a week. Captured prairie dogs were temporarily immobilized using isoflurane anesthesia while blood, fleas, and ear tissue were collected. One half milliliter of blood was collected from the femoral vein, and tissue was obtained by clipping ½ cm from the outer edge of the ear. Both adult and juvenile prairie dogs were processed, but for animal safety reasons, blood was not obtained from juveniles under 300g. When each animal had recovered from anesthesia, it was released at the trap location where it was captured.

Table 4.1. List of colony names and their locations, area and population densities. Population density is based on visual counts averaged from 2003 and 2004. Colony 20 was excluded from IBR analysis due to its relocation history.

Colony number	Latitude	Longitude	Area (hectares)	Density (prairie dogs/hectare)
1	40.24454	-105.227273	190.46	64
2	40.21930	-105.310418	22.59	36
3	39.93955	-105.096410	153.43	31
5	40.15440	-105.252508	132.53	11
6	39.91218	-105.180573	19.09	14
9	40.01136	-105.191460	16.18	36
15	40.10811	-105.210192	42.50	42
19	40.10466	-105.276360	201.21	27
20	39.92067	-105.220905	24.10	17
106	40.02252	-105.180065	22.26	19

Fig. 4.1 Map of Boulder County showing land cover types (data from the National Land Cover Database 2001) and location of prairie dog colonies used to establish nodes for Circuitscape (in black) and sites for which we determined multilocus microsatellite genotypes (indicated with numbers).



Tissue samples collected during 2003 and 2004 were stored until extraction at 4°C in a solution of EDTA and DMSO. DNA extraction was performed using a Qiagen Tissue kit according to protocol, and extracted DNA was stored at -80°C until genetic analysis. DNA from 557 Boulder County prairie dogs was amplified at 11 microsatellite loci by polymerase chain reaction (PCR) (Jones et al. 2005). PCR products were analyzed on a LICOR 4200 sequencer and genotypes determined using GeneImagIR software. I estimated error rates by repeating amplifications and genotyping for approximately 10% of the data. Because a population of prairie dogs consists of overlapping generations and I did not expect genotype frequencies to change in one year, all samples for each colony sampled in 2003 and 2004 were pooled. Animals recaptured in multiple years were typed only once. Tests of Hardy-Weinberg expectations were carried out using Arlequin (Schneider et al. 2000). Colony heterozygosity was calculated using Microsatellite Analyzer (Dieringer and Schlotterer 2003).

I estimated migration between pairs of colonies using assignment methods implemented in GeneClass (Piry et al. 2004) under two scenarios: one in which resident and migrant classification were based on assignment test values (assuming an individual was born in the colony it was assigned to with highest log likelihood), and one in which I tested for migration in the previous one or two generations using 10,000 MCMC replications and a threshold value of 0.01; that is, if individuals assigned to the sampled colony with probability less than 0.01, they were inferred migrants (Paetkau et al. 1995; Paetkau et al. 2004).

Models of prairie dog movement potential

Landscape features may influence gene flow among populations by affecting dispersal rates; therefore, models more robust to spatial heterogeneity than simple isolation-by-distance (IBD) measures are required (McRae 2006). I used an isolation-by-resistance (IBR) approach (McRae

2006; McRae and Beier 2007; McRae et al. 2008) for predicting demographic connectivity among prairie dog colonies in the complex landscape of Boulder County. IBR predicts a positive relationship between genetic differentiation and the resistance distance, a distance metric that exploits relationships between the distances among populations and the ecological resistance estimated during simulated random walks (McRae 2006). IBR is conceptually similar to the least cost path (LCP)-based distance approach, but allows for the possibility of multiple pathways of connectivity, and pathways of varying width (McRae and Beier 2007), and therefore offers a more biologically realistic scenario of individual movement among populations. IBR is robust to violations of certain assumptions, including that of migration-drift equilibrium (McRae and Beier 2007).

IBR is based in electric circuit theory, analyzing a landscape as if it were a circuit board and treating organisms (and therefore gene flow) as electrical current. IBR calculates the resistance distance by simultaneously considering all possible pathways connecting population pairs (McRae and Beier 2007), creating a theoretical "circuit board". Populations (in this case, colonies) are represented as sources or grounds, while the landscape matrix is composed of a raster grid of values that correlate to relative conductance or resistance values associated with landscape features. Conductance or resistance values are fitted to the observed genetic relationships among populations (e.g. pairwise F_{ST} values) through simulation, with fits constrained by known species-habitat associations (McRae 2006). For example, higher conductance values are assigned to landscape cells that are known to contain preferred habitat for dwelling or dispersal (e.g. grassland), and lower conductance values are assigned to cells known to contain habitat that is not preferred or that inhibits dispersal (e.g. water). Using the program Circuitscape, IBR can be analyzed by estimating the resistance encountered along all possible paths (circuits) among colonies (current sources or grounds). The result is that better, more numerous and/or wider pathways between nodes reduce the resistance distance separating them. IBR analysis provides a flexible and efficient tool to

understand effects of landscape features on genetic structure, and to predict genetic and evolutionary consequences of landscape change.

In this study, I modeled gene flow among 10 prairie dog colonies, out of 369 known colonies located in Boulder County. Colonies were represented by their polygon centroids as single raster cells (30 x 30 meters) in ASCII format. Using the National Land Cover Data (NLCD) 2001 layer obtained from USGS, which extended beyond the area of sampled prairie dog colonies, we assigned estimated conductance values to the 15 classes of land cover found within the study area (Figure 4.1; Homer et al. 2004). After condensing functionally similar land types into eight single classes (e.g., forest and shrubland), I used a hierarchical approach in which I evaluated 128 initial models representing all possible combinations of eight land cover types in two conductance categories, with each landscape variable (Table 4.2) initially assigned a conductance value—or ease of prairie dog movement—of either 100 (low resistance, e.g. movement within prairie dog colonies) or zero ("infinite resistance"). For all models, the land use types in which prairie dog colonies were sampled (grasslands and barren land) were included in the high conductance category (failure to include these habitat types in the high conductance, C=100 category resulted in infinite resistance between prairie dog colonies) and lakes were included in the low conductance, C = 0 category (since we know prairie dogs do not inhabit or move across lakes).

Table 4.2. List and descriptions of National Land Cover Database (NLCD) land classes used in Circuitscape resistance model (adapted from Homer et al. 2004).

NLCD LAND CLASSES		
Code	**Title (assigned class)**	**Description**
LR	Lakes and Reservoirs (1)	open water bodies
LID	Developed, Low Intensity (2)	impervious surfaces such as roads and suburban environments
MID	Developed, Medium Intensity (3)	most commonly include single-family housing units and surrounding areas
HID	Developed, High Intensity (4)	highly developed areas where people reside or work in high numbers (e.g. apartment complexes, row houses and commercial/industrial where impervious surfaces account for 80 to 100 percent of the total cover)
HW	Emergent Herbaceous Wetlands (5)	areas where perennial herbaceous vegetation accounts for greater than 80 percent of vegetative cover *and* the soil or substrate is periodically saturated with or covered with water
WW	Woody Wetlands (5)	areas where forest or shrubland vegetation accounts for greater than 20 percent of vegetative cover *and* the soil or substrate is periodically saturated with or covered with water
DOS	Developed, Open Space (6)	areas that included large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes
DF	Deciduous Forest (7)	areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover
EF	Evergreen Forest (7)	areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover
MF	Mixed Forest (7)	areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover, deciduous or evergreen species accounting for less than 75 percent of total tree cover
SS	Shrub/Scrub (7)	areas dominated by shrubs
BL	Barren Land (8)	areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other accumulations of earthen material
CC	Cultivated Cropland (8)	areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards, and land that is actively tilled
GH	Grasslands/Herbaceous (8)	areas dominated by grasses
PH	Pasture Hay (8)	areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle

After determining the ten best models in the initial step, I iteratively refined the models by adding additional intermediate conductance categories (varying degrees of prairie dog movement) to which I assigned land cover types that did not clearly fall into either conductance category in the ten best models (e.g., developed open space). The approach of increasing complexity in subsequent models (as in Lee-Yaw et al. 2009) allowed me to isolate the effects of one land class at a time by assessing the change in model fit after changing its conductance. A total of 203 models were evaluated; among the tested models were those that assigned low conductance values to heavy urbanization, as inferred by Magle et al. (2010), and higher conductance values to low-intensity development, with small roads potentially acting in a similar facilitative fashion to dry creek beds (Roach et al. 2001). Prairie dog colonies were mapped with Geographic Information System (GIS) tools such that colonies surrounded by heterogeneous land cover were treated as nodes surrounded by discrete areas with varying resistance. Circuitscape was run in pair-wise mode (i.e. testing each colony's connection to every other colony), using a connection scheme where gene flow was allowed between the neighboring 8 cells (through the creation of 8 undirected edges). The edge conductance between any two grid cells (nodes) was based on the average of the conductance (on a scale of 0 to 100) assigned to each cell; thus, both nodes and edges were set as conductive. Resistance distance matrices output for each model were compared to pair-wise normalized F_{ST} values for the 9 colonies using partial Mantel tests (controlling for the effects of geographic distance) implemented in the Vegan package for R (The R Foundation for Statistical Computing, http://www.r-project.org/).

Finally, using historic records of prairie dog relocation, I assessed whether relocation influenced genetic structure of prairie dog colonies in our system. Relocation has the potential to interfere with inferences based on genetic differentiation, and because it is a common management strategy in many systems, it is important to assess its effect on these inferences. One relocation event in 1996 totaling 100 individuals involved one of the study sites (20) as a destination; six additional

events from 1995-2001 moved 1008 animals to colonies within two kilometers of this site (data from City of Boulder Open Space and Mountain Parks). Therefore, I re-ran all of our models with this colony excluded to determine whether relocation influenced connectivity estimates. Several smaller scale relocation events placed prairie dogs (average = 38 per event) in colonies within two kilometers of two other study sites (1 and 3), so I also re-ran our models with these colonies excluded.

Results

Genetic differentiation

Genetic effects of relocation could not be detected in colonies 1 or 3. However, I did detect a signal of relocation in colony 20, the destination of a large relocation effort from 1995-2001. Although one-month survival rates in mid-autumn averaged only 21.2%, re-running the models with this colony excluded improved model fit markedly. Furthermore, excluding this colony from a Mantel test of isolation by distance reversed the pattern (no isolation by distance with colony 20 included, r=0.1004, p=0.279) to one of significant isolation by distance (r=0.3654, p=0.033). Therefore, I removed this colony from analyses of connectivity, and all reported values exclude this colony.

I obtained samples from 557 prairie dogs in 10 colonies (mean 56, range 35-87). After omitting colony 20 from analyses of connectivity because of its history of relocation, 510 individuals in 9 colonies remained. For the eleven loci surveyed, the number of alleles per locus ranged from 6 to 14, with a mean of 9.3. All pair-wise F_{ST} comparisons among the 10 colonies were significant. Values ranged from 0.0588 to 0.194; average pair-wise F_{ST} was 0.109 (Table 2.3). Average heterozygosity within colonies ranged from 0.595 to 0.767 (Table 3); across all colonies average heterozygosity was 0.663. Removing colony 20 had no noticeable effect on average F_{ST}, global allele frequencies or average heterozygosity. Observed heterozygosities were similar to Hardy-Weinberg

expectations, with the exception that locus C116 showed a deficiency of heterozygotes in 3 colonies (6, 9, and 20). This locus was the most variable, suggesting that the departure from HW expectations may reflect the presence of null alleles. Because null alleles do not appreciably affect estimates of migration among colonies (Hauser et al. 2006), we included this locus in our analyses. There was no evidence of null alleles at other loci. Based on re-typing of 10% of our data, the estimated genotyping error rate was 2.8%; errors were approximately randomly distributed across loci and individuals.

Table 4.3. Linearized F_{ST} values (above diagonal) and heterozygosity (along diagonal). Average number of individuals scored for the 11 loci is provided in parentheses below colony number.

	1	2	3	5	6	9	15	19	20	106
	(61)	(67)	(59)	(37)	(48)	(57)	(35)	(87)	(47)	(46)
1	0.714	0.103	0.064	0.093	0.07	0.136	0.133	0.083	0.061	0.125
2		0.610	0.122	0.109	0.123	0.194	0.119	0.075	0.116	0.183
3			0.767	0.111	0.049	0.095	0.119	0.106	0.064	0.097
5				0.652	0.107	0.131	0.108	0.059	0.091	0.133
6					0.682	0.116	0.107	0.089	0.062	0.112
9						0.595	0.142	0.134	0.063	0.054
15							0.660	0.097	0.1	0.129
19								0.670	0.096	0.141
20									0.659	0.077
106										0.614

Evaluation of connectivity models assumes that animals move across the landscape; therefore, I tested this assumption by estimating the fraction of sampled individuals from a colony inferred to be migrants using Bayesian assignment methods implemented in GeneClass (Piry et al. 2004). Using a relaxed criterion for assigning individuals to particular colonies (namely that individuals were assumed to be born in the colony to which it was assigned with the highest log

likelihood), a total of 70 out of 557 individuals surveyed were inferred migrants. When I used a more stringent criterion for estimating migration between colonies (assuming a threshold value for assignment as resident of 0.01), 22 individuals were inferred migrants. Because I did not sample all colonies across Boulder County, I could not confidently assign whether the inferred migrants moved between sampled colonies; nonetheless, these results support the assumption that prairie dogs move across the landscape of Boulder County.

Explaining connectivity among colonies: evaluating models

My general approach was to use IBR models for estimating connectivity and to choose among the many possible models using partial Mantel correlations between the estimated connectivity and the degree of genetic differentiation among colonies. For each test of a particular model, I controlled for the effect of log-transformed geographic distance because there was a significant effect of geographic distance on the degree of genetic differentiation between colonies (Mantel test, $r = 0.3654$, $p = 0.033$). Of the models evaluated for the two conductance categories (0 and 100), there were some that provided good explanations of the estimated genetic differentiation among colonies; in particular, there were 11 significant ($p<0.01$) models with high correlation coefficients ($r > 0.6$). Typically, the step-wise addition of habitat types to the resistant (C=0) category initially improved the explanation of the estimated genetic distance between populations followed by a steep decline in model fit (Figure 4.2); when all habitat types except type 8 (grassland, barren land, pasture/hay and cropland) were resistant to movement, the model explained the estimated genetic differentiation poorly ($r = -0.2201$, $p = 0.93$). The simplest model that was among the eleven best models included only two habitat types that acted as barriers to conductance: lakes (type = 1) and wetlands (type = 5) (Table 4.4).

Fig. 4.2 Model fit (r value) based on the number of habitat classes in the infinite resistance category. Each subsequent model represents the previous model plus one additional land class; the best model for each number of land classes is represented. Because we observe genetic connectivity among colonies in Boulder County, models allowing movement through few land cover types provide poor explanations of the degree of genetic differentiation.

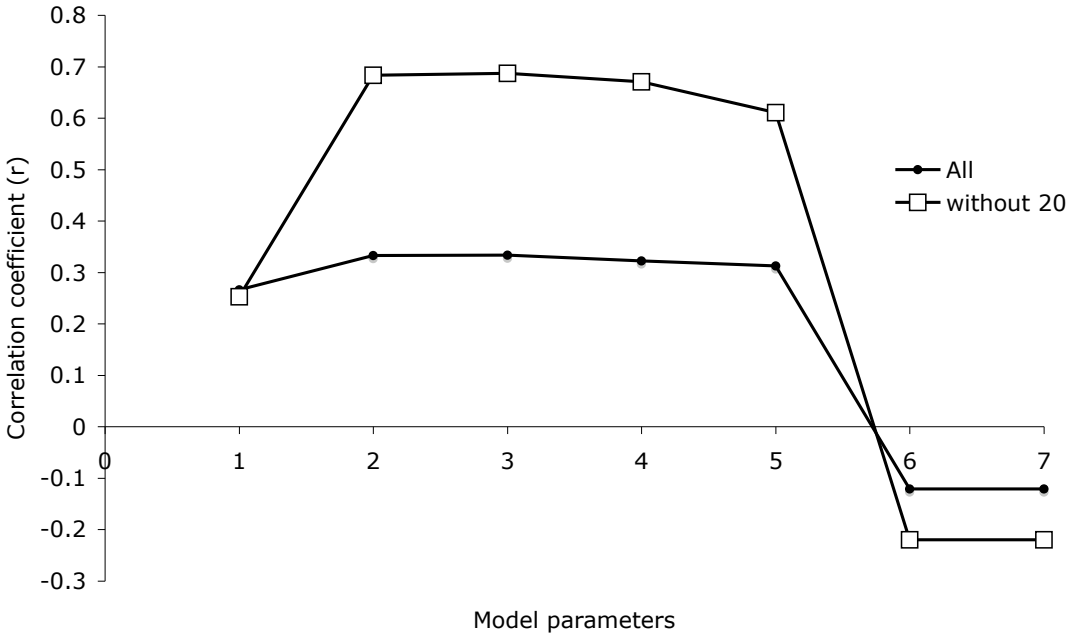


Table 4.4. Model scores for stepwise addition of variables to a particular conductance class. Representative models are shown from two sets of models evaluated with respect to the number of conductance categories (4 conductance categories not shown). Landscape features: 1 = lakes and reservoirs; 2 = low intensity development; 3 = medium density development; 4 = high intensity development, 5 = wetlands; 6 = developed open space; 7 = forest (all types included), shrubs; 8 = grassland, pasture/hay, cropland, barren land. Model 5, the simplest model with good fit, is indicated in bold for comparison.

Conductance categories = 2

Model	C = 0	C = 100	r	p
N0		1,2,3,4,5,6,7,8	-0.277	0.972
N1	1	2,3,4,5,6,7,8	0.2523	0.089
N5	**1,5**	**2,3,4,6,7,8**	**0.6835**	**0.001**
N20	1,5,4	2,3,6,7,8	0.6870	0.001
N55	1,5,4,6	2,3,7,8	0.6701	0.001
N85	1,5,4,6,3	2,7,8	0.6103	0.002
N100	1,5,4,6,3,2	7,8	-0.2201	0.926
N121	1,5,4,6,3,2,7	8	-0.2201	0.930

Conductance categories = 3

Model	C = 0	C = 25	C = 100	r	p
N5	**1,5**		**2,3,4,6,7,8**	**0.6835**	**0.001**
N142	1,5	2	3,4,6,7,8	0.6916	0.001
N147	1,5	2,4	3,6,7,8	0.6910	0.003
N154	1,5	2,4,6	3,7,8	0.6885	0.001
N171	1,5	2,4,6,3	7,8	0.6810	0.001
N5	**1,5**		**2,3,4,6,7,8**	**0.6835**	**0.001**
N145	1,5	6	2,3,4,7,8	0.6905	0.002
N150	1,5	6,3	2,4,7,8	0.6915	0.003
N155	1,5	6,3,4	2,7,8	0.6865	0.002
N171	1,5	6,3,4,2	7,8	0.6810	0.001

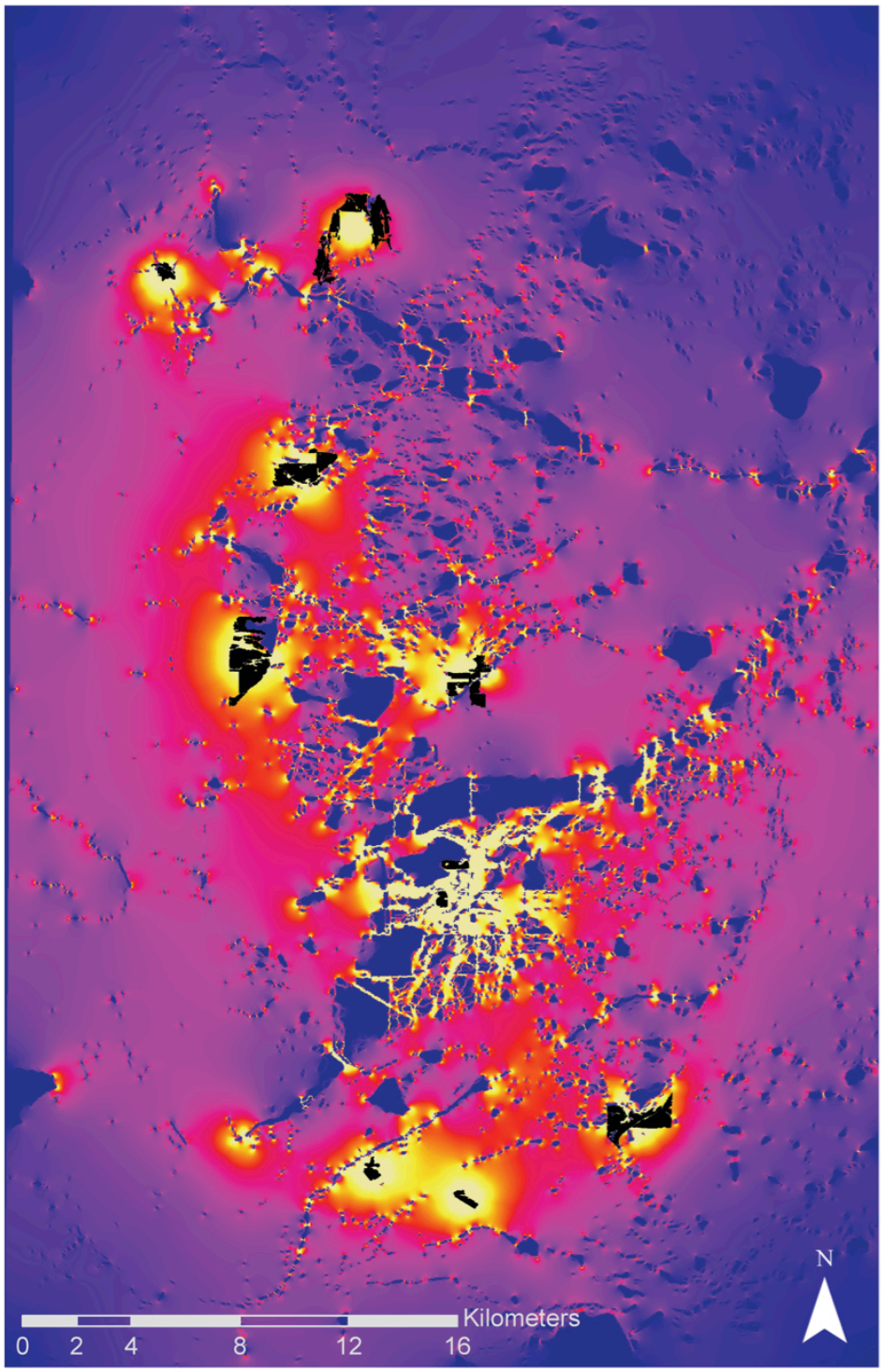
In all but one of the models with more than two conductance categories (N=24 models), the correlation between genetic differentiation and resistance increased, although the magnitude of increase relative to the two conductance class models was slight (Table 4.4). Nonetheless, when the habitat categories with roads (namely 2 and 6) were moved from high to low conductance, there was

an improvement in model performance, suggesting roads may reduce (but not eliminate) connectivity. Similarly, when medium and high intensity development (namely suburbanization and urbanization) were included in the lower conductance category (C = 25), there was a modest increase in the correlation coefficient, suggesting that buildings (and associated landscape features) inhibit prairie dog movement, although the magnitude of effect is much smaller than the estimated effects of lakes and wetlands. Placing both road types in the infinite resistance category resulted in the worst models ($r < 0$, $p > 0.9$). Including an additional conductance category (C = 0, 25, 60 and 100) did not notably improve model performance.

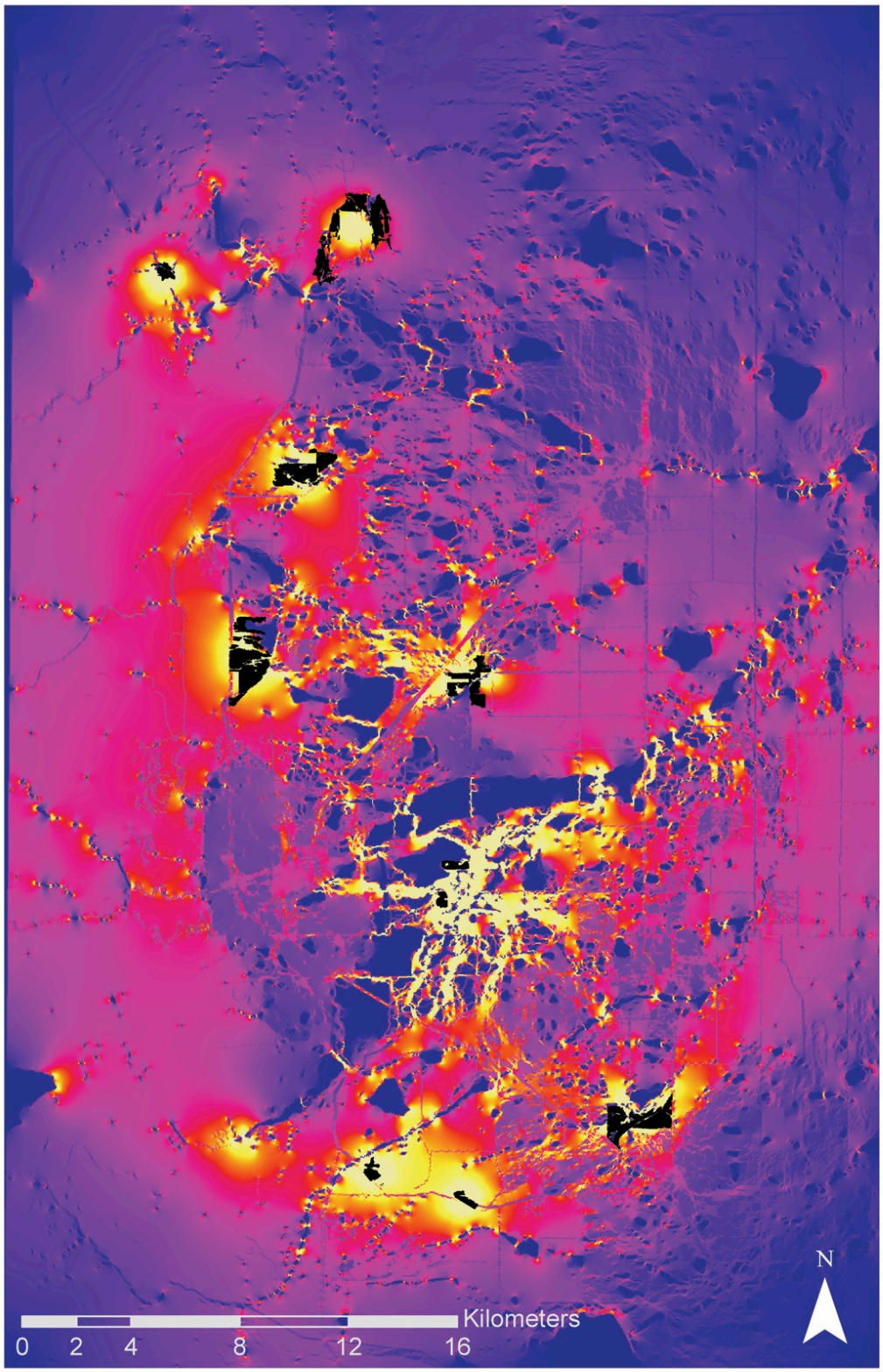
Of the many models that yielded roughly equivalent explanations of genetic differentiation, I selected two for visualization. The model with only lakes and wetlands in the C = 0 category (model N5) is perhaps the most defensible model because it involves postulating the fewest habitat features as barriers to prairie dog movement (Figure 4.3a). I also chose to visualize the model with roads (LID = 2 and OS = 6, model N148) in the low conductance (C = 25) category (Figure 4.3b). These models demonstrate that much of the land near where colonies occur is characterized by high connectivity, but colonies are separated from other populations by land cover allowing less movement. For instance, in model N148 (Figure 4.3b), the City of Boulder (directly west of the "blue zone"), which consists of numerous small roads, appears as a moderate barrier to movement. Roads in the northeastern portion of the county appear as light blue lines inhibiting dispersal across them. A model with suburbanization and urbanization (MID = 3 and HID = 4, model N158) in the C = 25 category was not noticeably different than the simple model (model 5). The degree of similarity between models N5 and N158 showed that modification of the landscape by urbanization had little influence on inferred connectivity.

Fig. 4.3 Heat maps of inferred connectivity of prairie dogs across Boulder County based on models from Circuitscape: a) the simplest model with good fit (model 5; r=0.6835, p=0.001); b) a model with roads at low conductance (model 148; r=0.6894, p=0.001); black represents prairie dog colonies, brighter colors indicate greater connectivity, blue indicates no connectivity.

a)



b)



Overall, the set of best models of prairie dog connectivity (or conductivity) for Boulder County exhibited several features. First, there were several "islands" where colonies were relatively isolated from all other colonies; especially the northernmost colonies (e.g., model N148, Figure 4.3b). Second, the county was bisected into a northern and southern section by a large swath of area in the center of the county with very low probabilities of movement (i.e. the "blue zone"; Figure 4.3). The blue zone included lakes and a perennial stream (Boulder Creek), and surrounding wetlands that trend west to east. Third, roads in the eastern part of the county appeared as barriers or areas of low connectivity, but in other places, roads (and the strip of grassland along the edges of roads) appeared to be corridors of connectivity, especially across wetlands.

Discussion

My estimated maps of connectivity among prairie dog colonies within Boulder County provide evidence for a network of corridors that connect colonies separated by wetlands and urbanization. To test the effects of roads on connectivity, I moved the land types with roads (developed open space and low intensity development) from a high to low conductance category; the resulting models provided better estimates of the degree of genetic differentiation, suggesting that roads may inhibit movement. However, moving roads to a zero conductance category resulted in very poor models. One explanation is that roads may have contradictory effects depending on context. In some cases, roads may facilitate movement because open space along the margins of roads may provide an easy means of movement. Remarkably, prairie dog colonies often exist in the median of highways, completely bounded by busy roads, an observation underscoring that prairie dogs do manage successful crossings of major roads. In other contexts, roads may inhibit movement, especially relatively large roads that dissect the County. Furthermore, perpendicular road

crossings and parallel road corridors running in the direction of prairie dog movement may exhibit potentially contrasting influences on connectivity.

The overall inhibitory effect of roads on prairie dog movement has implications for the spread of plague among populations, as well as subsequent recolonization of extirpated colonies. The existence of roads likely suppresses prairie dog mediated movement of *Y. pestis* among colonies (Collinge et al. 2005). This hypothesis is consistent with the restricted geographic distribution and slower spatial spread that characterized the most recent plague outbreak in Boulder County (Boulder Open Space and Mountain Parks, unpublished data). However, once populations are extirpated, the existence of roads could slow the recolonization process, limit the number of source populations, or prevent colonies from being recolonized altogether. Colonization from few sources is predicted to lead to founder effects and inbreeding (Templeton 1980). If extirpated populations are unable to be recolonized, the species may go locally extinct. The concomitant suppression of extinction and recolonization suggests that intermediate degrees of connectivity may lead to persistence of metapopulations where a virulent pathogen extirpates populations (via moderate barriers slowing the spread of the pathogen while still allowing sufficient recolonization).

These results provide a foundation for further refinement of various models of connectivity, which can be assessed with data from a large number of populations. For instance, the effects of roads could be estimated directly by sampling on either side of multiple types of roads (e.g., divided vs. undivided highways, dirt roads, and roads running parallel vs. perpendicular to potential dispersal corridors). Landscape modifications may change the direction or overall length of corridors, leading to gene flow patterns that are altered in magnitude (Riley et al. 2006; Templeton et al. 2007) or direction (Moore et al. 2008; Spear and Storfer 2010). In fact, estimates of the number of effective migrants in our system are an order of magnitude lower than those inferred using similar methods in the natural landscape of the nearby Pawnee National Grasslands (Roach et al. 2001). Therefore, it is

important to note that in altered landscapes, the magnitude of dispersal can be dramatically lower than in natural landscapes. Understanding the magnitude and direction of prairie dog movement will allow us to better predict when populations may be prone to invasion by *Y. pestis*, and to control plague outbreaks in areas of concern. For instance, knowledge of the most widely used habitat types for dispersal could allow for quarantine of infected populations, culling to prevent cross-species transmission or human exposure, or flea dusting of colonies located on dispersal corridors from infected populations. Furthermore, knowledge of dispersal corridors in a complex landscape could lead to predictions of the speed, spatial extent and pattern of local extinctions within a metapopulation that experiences extirpations in a non-random, spatial context (e.g., when extinctions are caused by a transmissible disease).

One challenge to evaluating models in the immense parameter space inherent in complex landscapes is that there are a large number of potentially suitable models. The more complex the landscape, the more data are required to distinguish among potential hypotheses. In systems with a high degree of gene flow, data from many populations are likely needed to discern the effects of different combinations of land cover conductance (for example, low conductance for OS and high conductance for LID versus the opposite). I have restricted my search through the parameter space to a limited number of land cover conductance combinations with potentially large effects, in keeping with the modest dataset on population genetics. In some cases, estimates of genetic differentiation among nine colonies were not sufficient to choose among very different models of connectivity. For instance, correlation coefficients of models with high-intensity urbanization allowing high conductance (e.g., Model 142, $r = 0.6916$) or providing high resistance (Model 169, $r = 0.6906$) were very similar, suggesting the effect of HID development on connectivity is very small. While this may be true, it is more likely that the lack of effect of HID reflects the location of

sampled colonies, and sampling a set of colonies around HID may provide a stronger signal. Nonetheless, urbanization, by itself, does not appear to strongly inhibit prairie dog movement.

An additional consideration in estimates of population connectivity is whether humans have managed those populations. Breeding programs and relocation are common management practices for many wildlife populations, and both have the potential to change genetic structure in a landscape. Our results, which inferred that one colony was affected by relocation practices, demonstrate the importance of explicitly addressing the effects of relocation on inferences drawn from genetic structure of populations. Many studies ignore the potential effects of management actions on population structure, but it is important to address human sources of genetic structure as they become increasingly common.

Prairie dogs in Boulder County inhabit an increasingly modified grassland environment that is carved into patches of grassland separated by urbanization, agriculture, and other land uses. As in most landscapes, some populations are more isolated than others by landscape features (e.g., roads and rivers) and changes in land cover (e.g. from grassland to agriculture or forest). Isolated colonies may also contribute to preserving regional genetic diversity (Templeton 2006) and be more insulated from the spread of pathogens such as *Y. pestis* (Collinge et al. 2005; Hess 1996). However, colonies that evolve in greater isolation may lose genetic variation over time, be more subject to genetic drift that overwhelms selection, accumulate deleterious mutations, or diverge from other populations (Templeton 2006). Thus, if roads isolate prairie dog colonies, they may be more protected from plague; however, they may see a concomitant loss of genetic diversity because of reduced recolonization. It is important to consider the mechanisms that contribute to isolation in certain populations, and how isolated populations may contribute to evolution of the species (e.g., Templeton et al. 1990; Wright 1931) and disease transmission among populations.

Conclusions

The conceptual approach of landscape resistance modeling is a useful means of inferring how various land cover types affect an organism's ability to move from one population to another, with implications for trafficking of diseases such as sylvatic plague. Prairie dog connectivity in a complex landscape matrix is sustained by pasture, cropland and small roads, but impeded by large highways and heavy urbanization. Dispersal corridors among populations may promote the spread of pathogens, but they are critical to maintain genetic diversity within populations and to allow for re-colonization of extirpated demes. An intermediate degree of connectivity (both in terms of distance to nearest population and number of connected colonies) may be ideal in systems where a virulent pathogen periodically extirpates populations, such that disease transmission among populations is slowed, but recolonization is not hampered. The impacts of human alteration of natural landscapes change connectivity in complex ways (Collinge 2009; Storfer et al. 2010), thereby influencing which populations are connected to each other. Thus, understanding how various landscape matrices influence connectivity of different species will inform strategies for preserving and managing these corridors. Maintaining some degree of connectivity in complex, human-altered landscapes is crucial to the persistence of species across the globe, as an increasing percentage of land is converted to human uses such as urban centers and agriculture. Connectivity among populations allows recolonization to rescue extirpated populations, maintains genetic diversity within populations, and ultimately facilitates the long-term persistence of species.

Conclusion

Ecological and genetic evidence support the idea that Gunnison's prairie dogs should be separated into two distinct subspecies. However, redefining the subspecies delineation is necessary to aptly characterize the ecological and genetic diversity within the subspecies. The argument for a monotypic subspecies (Pizzimenti 1975) appears to be flawed, based on several lines of evidence: 1) There is a high degree of genetic differentiation between subspecies, 2) reciprocal monophyly exists within clades, 3) genetic differentiation is higher between than within subspecies, and this is not an artifact of geographic distance, 4) the subspecies are morphologically distinct in body size (Hollister 1916), 5) they occupy distinct habitats that influence the timing of emergence from hibernation, estrus and reproduction. Further work on mate choice and fitness in various environments would be insightful.

I find that the pathogen *Yersinia pestis* influences the spatial distribution of genetic diversity within a metapopulation of black-tailed prairie dogs and selectively eliminates individuals with the lowest degree of genetic diversity. My results suggest that although extirpations cause *populations* to lose or retain diversity depending on landscape context, plague and recolonization preferentially eliminate *individuals* with the lowest genetic diversity. High genome-wide heterozygosity may offer protection against pathogens via overdominance at many loci involved in resistance to pathogens (e.g., MHC, Hughes and Nei 1988; signaling pathways central to immune response, Yang et al. 2011). Therefore, although the long-term response to the disease is unknown, sylvatic plague promotes genetic diversity within individual prairie dogs in our system. These findings support the notion that genetic diversity is beneficial (Mattila and Seeley 2007; Johnson et al. 2006); more interesting, however, is the inference that virulent pathogens should drive an evolutionary increase in the genetic diversity of their hosts. The genetic consequences of extirpations have implications for the way we understand host-pathogen coevolution (Nuismer and Doebeli 2004), the evolution of

virulence (Lenski and May 1994), and evolution of host resistance (Hughes and Boomsma 2007). The persistence of only the individuals with the highest genetic diversity may provide a path for the evolution of resistance, which requires the maintenance of genetic variation.

The fact that *Y. pestis* influences genetic structure of its host has global implications—in terms of both conservation and evolutionary theory—for host species' adaptation to novel parasites, particularly in highly social mammals. Introduced diseases exist on every continent (Best and Kerr 2000; Gage and Kosoy 2005; Gardner et al. 1997; Levin et al. 2009; Russell et al. 2008; Senapin et al. 2007; van der Putten et al. 2005), and host species' persistence depends in part on how they respond to this novel selection pressure. When virulent pathogens selectively eliminate individuals with the lowest genetic diversity, they prevent the success of these individuals due to stochastic processes, thereby promoting the retention of diversity within individuals and populations. In this way, pathogens may actually increase the mean fitness of populations and increase their evolutionary potential.

Pathogens act in conjunction with other selective pressures to maintain diversity. For instance, populations are connected to each other via dispersal corridors that are required for genetic variation to move among populations. Connectivity among populations is influenced by the surrounding habitat matrix, which may be more complex in contemporary landscapes consisting of a medley of agricultural and urban use. In black-tailed prairie dogs inhabiting a complex landscape matrix, connectivity matrix is sustained by pasture, cropland and small roads, but impeded by large highways and heavy urbanization. Dispersal corridors among populations may promote the spread of pathogens, but they are critical to maintain genetic diversity within populations and to allow for re-colonization of extirpated demes. An intermediate degree of connectivity (both in terms of distance to nearest population and number of connected colonies) may be ideal in systems where a

virulent pathogen periodically extirpates populations, such that disease transmission among populations is slowed, but recolonization is not hampered.

In the case of Gunnison's prairie dogs, abiotic habitat characteristics (namely, temperature) are associated with genetic divergence between the two subspecies. Although it is not yet clear whether climate itself promotes divergence or divergence is merely a result of allopatry, constraints on duration of female reproductive receptivity and size-biased female mate choice may play a role in divergence. Therefore, at the phylogenetic scale, abiotic selection pressures may be particularly important in facilitating divergence within and among species. At the regional scale, pathogens can interact with landscape and host characteristics to influence the distribution of host genetic diversity within species. It will be instructive to determine whether and under what circumstances pathogens can drive divergence among species due to differential responses to infection and mate preference, for instance, for individuals in better body condition or with behavioral defenses to pathogen. Finally, as we continue to learn more about the specific types of selection acting on species divergence (e.g., pollinator shifts, predation avoidance, and climate), we will begin to develop theory about which types of selection facilitate divergence that will contribute to our understanding of the diversity of life on earth.

Bibliography

Acevedo-Whitehouse K, TR Spraker, E Lyons, SR Melin, F Gulland, RL Delong, W Amos 2006. Contrasting effects of heterozygosity on survival and hookworm resistance in California sea lion pups. *Molecular Ecology* 15: 1973-1982.

Alexander RD 1974. The evolution of social behavior. *Annual Review of Ecology and Systematics* 5: 325-383.

Altizer S, A Dobson, P Hosseini, P Hudson, M Pascual, P Rohani 2006. Seasonality and the dynamics of infectious diseases. *Ecology Letters* 9: 467-484.

Antao T, A Lopes, RJ Lopes, A Beja-Pereira, G Luikart 2008. LOSITAN: A workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics* 9: 323-327.

Antolin MF, Savage LT, Eisen RJ (2006) Landscape features influence genetic structure of black-tailed prairie dogs (Cynomys ludovicianus). *Landscape Ecology* 21: 867-875.

Antonio RR, Agostinho AA, Pelicice FM, Bailly D, Okado EK, Dias JHP (2007) Blockage of migration routes by dam construction: Can migratory fish find alternative routes? *Neotropical Ichthyology* 5: 177-184.

Barrowclough GF, Groth JG, Mertz LA, Gutiérrez RJ (2004) Phylogeographic structure, gene flow and species status in blue grouse (*Dendragapus obscurus*). *Molecular Ecology* 13: 1911-1922.

Bartel RA, KS Oberhauser, JC de Roode, SM Altizer 2011. Monarch butterfly migration and parasite transmission in eastern North America. *Ecology* 92: 342-351.

Beaumont MA, RA Nichols 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London B* 263: 1619-1626.

Beneteau CL, Mandrak NE, Heath DD (2009) The effects of river barriers and range expansion of the population genetic structure and stability in Greenside Darter (Etheostoma blennioides) populations. *Conservation Genetics* 10: 477-487

Berthier K, N Charbonnel, M Galan, Y Chaval, J-F Cosson 2006. Migration and recovery of the genetic diversity during the increasing density phase in cyclic vole populations. *Molecular Ecology* 15: 2665-2676.

Best SM, PJ Kerr 2000. Coevolution of host and virus: The pathogenesis of virulent and attenuated strains of myxoma virus in resistant and susceptible European rabbits. *Virology* 267: 36-48.

Biek R, LA Real 2010. The landscape genetics of infectious disease emergence and spread. *Molecular Ecology* 19: 3515-3531.

Biggins DE, JL Godbey, KL Gage, LG Carter, JA Montenieri 2010. Vector control improves survival of three species of prairie dogs (*Cynomys*) in areas considered enzootic for plague. *Vector-borne and Zoonotic Diseases* 10: 17-26.

Biggins DE, MY Kosoy 2001. Influences of introduced plague on North American mammals: implications from ecology of plague in Asia. *Journal of Mammalogy* 82: 906-916.

Blake S, SL Deem, S Strindberg, F Maisels, L Momont, IB Isia, I Douglas-Hamilton, WB Karesh, MD Kock 2008. Roadless wilderness area determines forest elephant movements in the Congo Basin. *PLoS One* 3:e3546.

Bohonak AJ 1999. Dispersal, gene flow, and population structure. *Quarterly Review of Biology* 74: 21-45.

Breitschwerdt EB, DK Kordick 2000. Bartonella infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clinical Microbiology Reviews* 13: 428-438.

Brinkerhoff RJ, H Kabeya, K Inoue, Y Bai, S Maruyama 2010. Detection of multiple Bartonella species in digestive and reproductive tissues of fleas collected from sympatric mammals. *The ISME Journal* 4: 955-958.

Brinkerhoff RJ, Martin AP, Jones RT, Collinge SK (2011) Population genetic structure of the prairie dog flea and plague vector, *Oropsylla hirsuta*. *Parasitology* 138: 71-79.

Bruckman SV, J Krauss, I Steffan-Dewenter 2010. Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes. *Journal of Applied Ecology* 47: 799-809.

Bryja J, N Charbonnel, K Berthier, M Galan, JF Cosson 2007. Density-related changes in selection pattern for major histocompatibility complex genes in fluctuating populations of voles. *Molecular Ecology* 16: 5084-5097.

Caillaud D, F Levréro R Cristescu, S Gatti, M Dewas, M Douadi, A Gautier-Hion, M Raymond, N Ménard 2006. Gorilla susceptibility to Ebola virus: The cost of sociality. *Current Biology* 16: R489-R491.

Calleri DV, EM Reid, RB Rosengaus, EL Vargo, JFA Traniello 2006. Inbreeding and disease resistance in a social insect: effects of heterozygosity on immunocompetence in the termite *Zootermopsis angusticollis*. *Proceedings of the Royal Society B* 273: 2633-2640.

Caprio MA, BE Tabashnik 1992. Gene flow accelerates local adaptation among finite populations – simulating the evolution of insecticide resistance. *Journal of Economic Entomology* 85: 611-620.

Carlsson J 2008. Effects of microsatellite null alleles on assignment testing. *Journal of Heredity* 99: 616-623.

Carpenter JA, DJ Obbard, X Maside, FM Jiggins 2007. The recent spread of a vertically transmitted virus through populations of *Drosophila melanogaster*. *Molecular Ecology* 16: 3947-3954.

Carson HL 1968. The population flush and its genetic consequences. In: Lewontin RC, editor. *Population Biology and Evolution*. Syracuse, NY: Syracuse University Press. p 123-137.

Ceballos G, A Davidson, R List, J Pacheco, P Manzano-Fischer, G Santos-Barrera, J Cruzado 2010. Rapid decline of a grassland system and its ecological and conservation implications. *PLoS One* 5: e8562.

Chakraborty R, M Nei 1977. Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. *Evolution* 31: 347-356.

Chapin FS, GR Shaver, AE Giblin, KJ Nadelhoffer, JA Laundre 1995. Responses of Arctic tundra to experimental and observed changes in climate. *Ecology* 76: 694-711.

Chapuis, MP and A Estoup 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24: 621-631.

Charlesworth D, B Charlesworth 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237-268.

Chessel D, AB Dufour, J Thioulouse 2004. The ade4 package I. One-table methods. *R News* 4: 5-10.

Collinge SK 1996. Ecological consequences of habitat fragmentation: implications for landscape architecture and planning. *Landscape and Urban Planning* 36: 59-77.

Collinge SK (2009) Ecology of fragmented landscapes. Johns Hopkins University Press, Baltimore.

Collinge, SK, WC Johnson, C Ray, R Matchett, J Grensten, JF Cully Jr, KL Gage, MY Kosoy, JE Loye, and AP Martin 2005. Landscape structure and plague occurrence in black-tailed prairie dogs on grasslands of the western USA. *Landscape Ecology* **20**: 941-955.

Coltman DW, WD Bowen, JM Wright 1998. Birth weight and neonatal survival of harbor seal pups are positively correlated with genetic variation measured by microsatellites. *Proceedings of the Royal Society of London B* 265: 803-809.

Coltman DW, JG Pilkington, JA Smith, JM Pemberton 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53: 1259-1267.

Coulon A, Guillot G, Cosson JF, Angibault JMA, Aulagnier S, Cargnelutti B, Galan M, Hewison AJM (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Molecular Ecology* 15: 1669-1679.

Cox-Foster DL, S Conlan, EC Holmes, G Palacios, JD Evans, NA Moran, PL Quan, T Briese, M Hornig, DM Geiser et al. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318: 283-287.

Cross PC, JO Lloyd-Smith, PLF Johnson, WM Getz 2005. Duelling timescales of host movement and disease recovery determine invasion of disease in structured populations. *Ecology Letters* 8: 587-595.

Cully JF Jr, AM Barnes, TJ Quan, G Maupin 1997. Dynamics of plague in a Gunnison's prairie dog colony complex from New Mexico. *Journal of Wildlife Diseases* 33: 706-719.

Cully JF Jr, LG Carter, KL Gage 2000. New records of sylvatic plague in Kansas. *Journal of Wildlife Diseases* 36: 389-392.

Cully, J.F. Jr., and E.S. Williams 2001. Interspecific comparisons of sylvatic plague in prairie dogs. *Journal of Mammalogy* **82** (4): 894-904.

Da Silva A, JM Gaillard, NG Yoccoz, AJM Hewison, M Galan, T Coulson, D Allaine, L Vial, D Delorme, G Van Laere, F Klein, G Luikart 2009. Heterozygosity-fitness correlations revealed by neutral and candidate gene markers in roe deer from a long-term study. *Evolution* 63: 403-417.

Da Silva A, G Luikart, NG Yoccoz, A Cohas, D Allaine 2006. Genetic diversity-fitness correlation revealed by microsatellite analyses in European alpine marmots (*Marmota marmota*). *Conservation Genetics* 7: 371-382.

Daszak P, AA Cunningham, AD Hyatt 2000. Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* 287: 443-449.

Davies KF, CR Margules, KF Lawrence 2000. Which traits of species predict population declines in experimental forest fragments? *Ecology* 81: 1450-1461.

de Bellocq JG, N Charbonnel, S Morand 2008. Coevolutionary relationship between helminth diversity and MHC class II polymorphism in rodents. *Journal of Evolutionary Biology* 21: 1144-1150.

Decoster, LA (2000) How forests are being nibbled to death by DUCs, and what to do about it: summary of the Forest Fragmentation 2000 Conference. Annapolis, MD.

Dieringer D, Schlotterer C (2003) Microsatellite Analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* 3: 167-169.

Dingemanse NJ, F Van der Plas, J Wright, D Reale, M Schrama, DA Roff, E Van der Zee, I Barber 2009. Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proceedings of the Royal Society B* 276: 1285-1293.

Donnelly P, S Tavare 1995. Coalescents and genealogical structure under neutrality. *Annual Review of Genetics* 29: 401-421.

Dray S, AB Dufour 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* 22: 1-20.

Du ZY, FF Chen, OS Liao, HR Zhang, YF Cheni, JS Chen 2007. 2b ORFs encoded by subgroup IB strains of cucumber mosaic virus induce differential virulence on *Nicotiana* species. *Journal of General Virology* 88: 2596-2604.

Dunn PO, and DW Winkler 1999. Climate change has affected the breeding date of tree swallows throughout North America. *Proceedings of the Royal Society of London B* 266: 2487-2490.

Ellstrand NC, DR Elam 1993. Population genetic consequences of small population size-implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217-242.

Epps CW, PJ Palsboll, JD Wehausen, GK Roderick, RR Ramey, DR McCullough 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecology Letters* 8: 1029-1038.

Evans N, A Baierl, MA Semenov, P Gladders, BDL Fitt 2008. Range and severity of a plant disease increased by global warming. *Journal of the Royal Society Interface* 5: 525-531.

Excoffier, L., G. Laval, and S. Schneider 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47-50.

Fahrig L, G Merriam 1994. Conservation of fragmented populations. *Conservation Biology* 8: 50-59.

Gage KL and MY Kosoy 2005. Natural history of plague: perspectives from more than a century of research. *Annual Review of Entomology* 50: 505-528.

Gardner H, K Kerry, M Riddle, S Brouwer, L Gleeson 1997. Poultry virus infection in Antarctic penguins. *Nature* 387: 245.

Garrett MG, Franklin WL (1988) Behavioral ecology of dispersal in the black-tailed prairie dog. *Journal of Mammalogy* 69: 236-250.

Gaunt TR, S Rodriguez, C Zapata, INM Day 2006. MIDAS: software for analysis and visualisation of interallelic disequilibrium between multiallelic markers. *BMC Bioinformatics* 7: 227.

Goudet, J. 1995. Fstat version 1.2: a computer program to calculate Fstatistics. *Journal of Heredity*. 86(6): 485-486.

Goverde M, Schweizer K, Baur B, Erhardt A (2002) Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biological Conservation* 104: 293-299.

Grant PR, BR Grant 1995. Predicting microevolutionary responses to directional selection on heritable variation. *Evolution* 49: 241-251.

Haldane JBS 1949. Disease and evolution. Supplement to *La Ricerca Scientifica* 19: 68-76.

Hamilton WD 1982. Pathogens as causes of genetic diversity in their host populations. In: Anderson RM and RM May, editors. *Population Biology of Infectious Diseases*. Berlin: Springer-Verlag, p 269-296.

Hanksi I 1998. Metapopulation dyanimcs. *Nature* 396: 41-49.

Hansson B, L Westerberg 2002. On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology* 11: 2467-2474.

Hardy OJ, X Vekemans 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2: 618-620.

Harrison S, A Hastings 1996. Genetic and evolutionary consequences of metapopulation structure. *Trends in Ecology and Evolution* 11: 180-183.

Hauser L, Seamons TR, Dauer M, Naish KA, Quinn TP (2006) An empirical verification of population assignment methods by marking and parentage data: hatchery and wild steelhead (Oncorhynchus mykiss) in Forks Creek, Washington, USA. *Molecular Ecology* 15: 3157-3173.

Hawley DM, RJ Fleischer 2012. Contrasting epidemic histories reveal pathogen-mediated balancing selection on class II MHC diversity in a wild songbird. *PLoS One* 7: e30222.

Hawley DM, KV Sydenstricker, GV Kollias, AA Dhondt 2005. Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches. *Biology Letters* 1: 326-329.

Hawthorne DJ, S Via 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* 412: 904-907.

Heath RB, Delong R, Jameson V, Bradley D, Spraker T 1997: Isoflurane anesthesia in free ranging sea lion pups. *Journal of Wildlife Diseases* 33: 206-210.

Hedrick PW 2005. A standardized genetic differentiation measure. *Evolution* 59: 1633-1638.

Hendry AP, P Nosil, LH Rieseberg 2007. The speed of ecological speciation. *Functional Ecology* 21: 455-464.

Hendry AP, EB Taylor, JD McPhail 2002. Adaptive divergence and the balance between selection and gene flow: Lake and stream stickleback in the misty system. *Evolution* 56: 1199-1216.

Hess, G. 1996. Disease in metapopulation models: Implications for conservation. *Ecology* 77: 1617-1632.

Hoban SM, DS Borkowski, SL Brosi, TS McCleary, LM Thompson, JS McLachlan, MA Pereira, SE Schlarbaum, J Romero-Severson 2010. Range-wide distribution of genetic diversity in the North American tree *Juglans cinerea*: a product of range shifts, not ecological marginality or recent population decline. *Molecular Ecology* 19: 4876-4891.

Homer C, Huang CQ, Yang LM, Wylie B, Coan M (2004) Development of a 2001 National Land-Cover Database for the United States. *Photogrammetric Engineering and Remote Sensing* 70: 829-840.

Hoogland, J.L. 1995. *The black-tailed prairie dog: social life of a burrowing mammal.* The University of Chicago Press, Chicago.

Hoogland JL 1981. The evolution of coloniality in white-tailed and black-tailed prairie dogs (Sciuridae: *Cynomys leucurus* and *C. ludovicianus*). *Ecology* 62: 252-272.

Hoogland JL 1979. Aggression, ectoparasitism, and other possible costs of prairie dog (Sciuridae, *Cynomys* spp.) coloniality. *Behavior* 69: 1-35.

Huelsenbeck JP, F Ronquist 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754-755.

Hughes WOH, JJ Boomsma 2007. Genetic diversity and disease resistance in leaf-cutting ant societies. *Evolution* 58: 1251-1260.

Hughes, A. L. and Nei, M. 1988 Patterns of nucleotide substi- tution at major histocompatibility complex class I loci reveals overdominant selection. *Nature* 335, 167–170.

Jacquemyn H, K Vandepitte, I Roldan-Ruiz, O Honnay 2009. Rapid loss of genetic variation in a founding population of *Primula elatior* (Primulaceae) after colonization. *Annals of Botany* 103: 777-783.

Jesse M, Ezanno P, Davis S, Heesterbeek JAP 2008. A fully coupled, mechanistic model for infectious disease dynamics in a metapopulation: Movement and epidemic duration. *Journal of Theoretical Biology* 254: 331-338.

Johnson WC, SK Collinge 2004. Landscape effects on black-tailed prairie dog colonies. *Biological Conservation* **115** (3): 487-497.

Jones PH, Britten HB (2010) The absence of concordant population genetic structure in the black-tailed prairie dog and the flea, *Oropsylla hirsuta*, with implications for the spread of *Yersinia pestis*. *Molecular Ecology* 19: 2038-2049.

Jones RT, AP Martin, AJ Mitchell, SK Collinge, C Ray 2005. Characterization of 14 polymorphic microsatellite markers for the black-tailed prairie dog (*Cynomys ludovicianus*). *Molecular Ecology Notes* **5**(1): 71-73.

Jules ES, MJ Kauffman, WD Ritts, AL Carroll 2002. Spread of an invasive pathogen over a variable landscape: a nonnative root rot on Port Orford Cedar. *Ecology* 83: 3167-3181.

Keller L, D Waller 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17: 230-241.

Kerr P, G McFadden 2002. Immune responses to myxoma virus. *Viral Immunology* 15: 229-246.

Kimura M 1968. Evolutionary rate at the molecular level. *Nature* 217: 624-626.

Kingman JFC 1982. The Coalescent. *Stochastic Processes and their Applications* 13: 235-248.

Kolbe JJ, RE Glor, LR Schettino, AC Lara, A Larson, JB Losos 2004. Genetic variation increases during biological invasion by a Cuban lizard. *Nature* 431: 177-181.

Kutz SJ, EP Hoberg, L Polley, EJ Jenkins 2005. Global warming is changing the dynamics of Arctic host-parasite systems. *Proceedings of the Royal Society of London B* 272: 2571-2576.

Lachish S, KJ Miller, A Storfer, AW Goldizen, ME Jones 2011. Evidence that disease-induced population decline changes genetic structure and alters dispersal patterns in the Tasmanian devil. *Heredity* 106: 172-182.

La Marca E, KR Lips, S Lotters, R Puschendorf, R Ibanez, JV Rueda-Almonacid, R Schulte, C Marty, F Castro, J Manzanilla-Puppo et al. 2005. Catastrophic population declines and extinctions in Neotropical Harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37: 190-201.

Lammi A, P Siikamaki, K Mustajarvi 1999. Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. *Conservation Biology* 13: 1069-1078.

Larson S, R Jameson, M Etnier, M Fleming, P Bentzen 2002. Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. *Molecular Ecology* 11: 1899-1903.

Lavergne S, J Molofsky 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the USA* 104: 3883-3888.

Leberg PL 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution* 46: 477-494.

Lee-Yaw JA, Davidson A, McRae BH, Greens DM (2009) Do landscape processes predict phylogeographic patterns in the wood frog? *Molecular Ecology* 18: 1863-1874.

Lenski RE, RM May 1994. The evolution of virulence in parasites and pathogens – reconciliation between 2 competing hypotheses. *Journal of Theoretical Biology* 169: 253-265.

Leroy EM, P Rouquet, P Formenty, S Souquiere, A Kilbourne, JM Froment, M Bermejo, S Smit, W Karesh, R Swanepoel et al. 2004. Multiple Ebola Virus transmission events and rapid decline of Central African wildlife. *Science* 303: 387-390.

Levin II, DC Outlaw, FH Vargas, PG Parker 2009. *Plasmodium* blood parasite found in endangered Galapagos penguins (*Spheniscus mendiculus*). *Biological Conservation* 142: 3191-3195.

Levy E, Kennington WJ, Tomkins JL, Lebas NR (2010) Land clearing reduces gene flow in the granite outcrop-dwelling lizard, Ctenophorus ornatus. *Molecular Ecology* 19: 4192-4203.

Linz B, F Balloux, Y Moodley, A Manica, H Liu, P Roumagnac, D Falush, C Stamer, F Prugnolle, SW van der Merwe et al. 2007. An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* 445: 915-918.

Loiselle BA, VL Sork, J Nason, C Graham 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82: 1420-1425.

Lopez JE, Gallinot LP, Wade MJ (2005) Spread of parasites in metapopulations: an experimental study of the effects of host migration rate and local host population size. *Parasitology* 130: 323-332.

Lynch M, J Conery, R Burger 1995. Mutation accumulation and the extinction of small populations. *American Naturalist* 146: 489-518.

Mackey BH, JJ Roering, MP Lamb 2011. Landslide-dammed paleolake perturbs marine sedimentation and drives genetic change in anadromous fish. *Proceedings of the National Academy of Sciences of tte USA* 108: 18905-18909.

Magle, SB, EW Ruell, MF Antolin, and KR Crooks 2010. Population genetic structure of black-tailed prairie dogs, a highly interactive species, in fragmented urban habitat. *Journal of Mammalogy* 91: 326-335.

Malmquist HJ, SS Snorrason, S Skulason, B Jonsson, OT Sandlund, PM Jonasson 1992. Diet differentiation in polymorphic arctic charr in Thingvallavatn, Iceland. *Journal of Animal Ecology* 61: 21-35.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution* 18: 189-197.

Mayr E 1954. Change of genetic environment and evolution. Pages 157-180 in *Evolution as a process*, edited by J. Huxley, AC Hardy, EB Ford. Allen and Unwin, London.

McKinney ST, CE Fiedler, DF Tomback 2009. Invasive pathogen threatens bird-pine mutualism: implications for sustaining a high-elevation ecosystem. *Ecological Applications* 19: 597-607.

McRae BH (2006) Isolation by resistance. *Evolution* 60: 1551-1561.

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences of the USA* 104: 19885-19890.

McRae BH, Dickson BG, Keitt TH, Shah VB (2008) Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology* 89: 2712-2724.

Meirmans, P.G., and P.H. Van Tienderen 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4: 792-794.

Meirmans PG 2006. Using the AMOVA framework to estimate a standardised genetic differentiation measure. *Evolution* 60: 2399-2402.

Merriam CH 1901. The prairie dog of the Great Plains (*Cynomys ludovicianus*). *Yearbook of the United States Department of Agriculture* 257-270.

Miller SD, Cully JF Jr (2001) Conservation of black-tailed prairie dogs (*Cynomys ludovicianus*). *Journal of Mammalogy* 82: 889-893.

Moore JA, Miller HC, Daugherty CH, Nelson NJ (2008) Fine-scale genetic structure of a long-lived reptile reflects recent habitat modification. *Molecular Ecology* 17: 4630-4641.

Mullen LM, SN Vignieri, JA Gore, HE Hoekstra 2009. Adaptive basis of geographic variation: genetic, phenotypic and environmental differences among beach mouse populations. *Proceedings of the Royal Society B* 276: 3809-3813.

Muths E, PS Corn, AP Pessier, DE Green 2003. Evidence for disease-related amphibian decline in Colorado. *Biological Conservation* 110: 357-365.

Nei M, T Maruyama, C-I Wu 1983. Models of evolution of reproductive isolation. *Genetics* 103: 557-579.

Nosil P, BJ Crespi 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *Proceedings of the National Academy of Sciences of the USA* 103: 9090-9095.

Nosil P, BJ Crespi, CP Sandoval 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature* 417: 440-443.

Nosil P, TH Vines, DJ Funk 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* 59: 705-719.

Nuismer SL, M Doebeli 2004. Genetic correlations and the coevolutionary dynamics of three-species systems. *Evolution* 58: 1165-1177.

Nunn CL, S Altizer, KE Jones, W Sechrest 2003. Comparative tests of parasite species richness in primates. *The American Naturalist* 162: 597-614.

Ohta T 1992. The nearly neutral theory of molecular evolution. *Annual Review of Ecology and Systematics* 23: 263-286.

Ohta T, M Kimura 1970. Development of associative overdominance through linkage disequilibrium in finite populations. *Genetical Research* 16: 165-177.

Oksanen J, FG Blanchet, R Kindt, P Legendre, RB O'Hara, GL Simpson, P Solymos, MHH Stevens, H Wagner 2010. vegan: community ecology package. R package version 1.17-3. http://CRAN.R-project.org/package=vegan.

Paetkau D, W Calvert, I Stirling, C Strobeck 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4: 347-354.

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* 13: 55-65.

Palumbi SR 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics* 25: 547-572.

Pannell JR, B Charlesworth 1999. Neutral genetic diversity in a metapopulation with recurrent local extinction and recolonization. *Evolution* 53: 664-676.

Parmesan, C, and G Yohe 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37-42.

Pauli JN, SW Buskirk, ES Williams, WH Edwards 2006. A plague epizootic in the black-tailed prairie dog (*Cynomys ludovicianus*). *Journal of Wildlife Diseases* 42: 74-80.

Paull SH and PTJ Johnson 2011. High temperature enhances host pathology in a snail-trematode system: possible consequences of climate change for the emergence of disease. Freshwater *Biology* 56: 767-778.

Pavlacky DC, Goldizen AW, Prentis PJ, Nicholls JA, Lowe AJ (2009) A landscape genetics approach for quantifying the relative influence of historic and contemporary habitat heterogeneity on the genetic connectivity of a rainforest bird. *Molecular Ecology* 18: 2945-2960.

Peakall R, and PE Smouse 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288-295.

Pearman PB, TWJ Garner 2005. Susceptibility of Italian agile frog populations to an emerging strain of Ranavirus parallels population genetic diversity. *Ecology Letters* 8: 401-408.

Peiris JSM, MD de Jong, Y Guan 2007. Avian influenza virus (H5N1): a threat to human health. *Clinical Microbiology Reviews* 20: 243-267.

Penn, DJ, K Damjanovich, WK Potts 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proceedings of the National Academy of Sciences of the USA* 99: 11260-11264.

Perez G, GC Hunter, B Slippers, C Perez, BD Wingfield, MJ Wingfield 2009. Teratosphaeria (Mycosphaerella) nubilosa, the causal agent of Mycosphaerella leaf disease (MLD), recently introduced into Uruguay. *European Journal of Plant Pathology* 125: 109-118.

Perez-Espona S, Perez-Barberia FJ, McLeod JE, Jiggins CD, Gordon IJ, Pemberton JM (2008) Landscape features affect gene flow of Scottish Highland red deer (Cervus elaphus). *Molecular Ecology* 17: 981-996.

Piry S, A Alapetite, JM Cornuet, D Paetkau, L Baudouin, A Estoup 2004. GeneClass2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* 95: 536-539.

Purvis A, JL Gittleman, G Cowlishaw, GM Mace 2000. Predicting extinction risk in declining species. *Proceedings of the Royal Society* B 267: 1947-1952.

Ramankutty N, Evan AT, Monfreda C, Foley JA (2008) Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. *Global Biogeochem Cycles*. doi: 10.1029/2007 GB002952.

Randolph SE 2001. The shifting landscape of tick-borne zoonoses: tick-borne encephalitis and Lyme borreliosis in Europe. *Philosophical Transactions of the Royal Society of London B* 356: 1045-1056.

Ray C 2001. Maintaining genetic diversity despite local extinctions: effects of population scale. *Biological Conservation* 100: 3-14.

Real LA, R Biek 2007. Spatial dynamics and genetics of infectious diseases on heterogeneous landscapes. *Journal of the Royal Society Interface* 4: 935-948.

Reed DH, R Frankham 2003. Correlation between fitness and genetic diversity. *Conservation Biology* 17: 230-237.

Rieseberg LH, JH Willis 2007. Plant speciation. *Science* 317: 910-914.

Riley SPD, Pollinger JP, Sauvajot RM, York EC, Bromley C, Fuller TK, Wayne RK (2006) A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology* 15: 1733-1741.

Ritland K 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. *Genetical Research* 67: 175-185.

Rizzo DM and M Garbelotto 2003. Sudden oak death: endangering California and Oregon forest ecosystems. *Frontiers in Ecology and the Environment* 1: 197-204.

Roach, JL, P Stapp, B Van Horne, and MF Antolin 2001. Genetic structure of a metapopulation of black-tailed prairie dogs. *Journal of Mamalogy* **82** (4): 946-959.

Rosenblum EB 2006. Convergent evolution and divergent selection: Lizards at the White Sands ecotone. *American Naturalist* 167: 1-15.

Rousset F 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics* 142: 1357–1362.

Rousset F 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219-1228.

Rousset F 2008. Genepop'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103-106.

Rousset F, M Raymond 1997. Statistical analyses of population genetic data: old tools, new concepts. *Trends in Ecology and Evolution* 12: 313-317.

Rundle HD, SF Chenoweth, P Doughty, MW Blows 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biology* 3: e368.

Russell CA, TC Jones, IG Barr, NJ Cox, RJ Garten, V Gregory, ID Gust, AW Hampson, AJ Hay, AC Hurt et al. 2008. The global circulation of seasonal Influenza A (H3N2) viruses. *Science* 320: 340-346.

Sackett, LC, TB Cross, RT Jones, W Johnson, K Ballare, C Ray, SK Collinge, AP Martin 2012. Connectivity of prairie dog colonies in an altered landscape: inferences from analysis of microsatellite DNA variation. *Conservation Genetics* 13: 407-418.

Sackett LC, L. K. Etchberger, M. N. Mazzella, D. D. Lim, A. P. Martin 2009. Characterization of 18 microsatellite loci for three species of prairie dogs. *Molecular Ecology Resources* 10: 232-236.

Schluter D, JD McPhail 1992. Ecological character displacement and speciation in sticklebacks. *American Naturalist* 140: 85-108.

Schneider S, Roessli D, Excoffier L (2000) Arlequin Version 2.00: A software for population genetics data analysis. U Geneva, Geneva.

Schulte RD, C Makus, B Hasert, NK Michiels, H Schulenburg 2010. Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. *Proceedings of the National Academy of Sciences of the USA* 107: 7359-7364.

Seehausen O, Y Terai, IS Magalhaes, KL Carleton, HDJ Mrosso, R Miyagi, I van der Sluijs, MV Schneider, ME Maan, H Tachida et al. 2008. Speciation through sensory drive in cichlid fish. *Nature* 455: 620-626.

Senapin S, K Phewsaiya, M Briggs, TW Flegel 2007. Outbreaks of infectious myonecrosis virus (IMNV) in Indonesia confirmed by genome sequencing and use of an alternative RT-PCR detection method. *Aquaculture* 266: 32-38.

Slate J, P David, KG Dodds, BA Veenvliet, BC Glass, TE Broad, JC McEwan 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* 93: 255-265.

Slatkin M 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology* 12: 253-262.

Slatkin M 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 457-462.

Smith KF, DF Sax, KD Lafferty 2006. Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology* 20: 1349-1357.

Spear SF, Peterson CR, Matocq MD, Storfer A (2005) Landscape genetics of the blotched tiger salamander (Ambystoma tigrinum melanostictum). *Molecular Ecology* 14: 2553-2564.

Spear SF, Storfer A (2010) Anthropogenic and natural disturbance lead to differing patterns of gene flow in the Rocky Mountain tailed frog, Ascaphus montanus. *Biological Conservation* 143: 778-786.

Spielman D, BW Brook, DA Briscoe, R Frankham 2004. Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genetics* 5: 439-448.

Stahl EA, JG Bishop 2000. Plant-pathogen arms races at the molecular level. *Current Opinion in Plant Biology* 3: 299-304.

Stamhuis IH, OG Meijer, EJ Zevenhuizen 1999. Hugo de Vries on heredity, 1889-1903. Statistics, Mendelian laws, pangenes, mutations. *Isis* 90: 238-267.

Stapp P, Salkeld DJ, Franklin HA, Kraft JP, Tripp DW, Antolin MF, Gage KL (2009) Evidence for the involvement of an alternate rodent host in the dynamics of introduced plague in prairie dogs. *Journal of Animal Ecology* 78: 807-817.

Stoate C, ND Boatman, RJ Borralho, CR Carvalho, GR de Snoo, P Eden 2001. Ecological impacts of arable intensification in Europe. *Journal of Environmental Management* 63: 337-365.

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: where are we now? *Molecular Ecology* 19: 3496-3514.

Sved JA 1968. The stability of linked systems of loci with a small population size. *Genetics* 59: 543-563.

Szpiech ZA, M Jakobsson, NA Rosenberg 2008. ADZE: A rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics* 24: 2498-2504.

Teacher AGF, TWJ Garner, RA Nichols 2009. Population genetic patterns suggest a behavioural change in wild common frogs (*Rana temporaria*) following disease outbreaks (Ranavirus). *Molecular Ecology* 18: 3163-3172.

Telles MPC, Diniz JAF, Bastos RP, Soares TN, Guimaraes LD, Lima LP (2007) Landscape genetics of Physalaemus cuvieri in Brazilian Cerrado: Correspondence between population structure and patterns of human occupation and habitat loss. *Biological Conservation* 139: 37-46.

Templeton AR 2006. Population genetics and microevolutionary theory. John Wiley and Sons, Inc, Hoboken.

Templeton AR 1980a. Modes of speciation and inferences based on genetic distances. *Evolution* 34: 719-729.

Templeton AR 1980b. The theory of speciation via the founder principle. *Genetics* 94: 1011-1038.

Templeton AR, Neuwald JL, Brazeal H, Robertson RJ (2007) Restoring demographic processes in translocated populations: The case of collared lizards in the Missouri Ozarks using prescribed forest fires. *Israel Journal of Ecology and Evolution* 53: 179-196.

Templeton AR, Shaw K, Routman E, Davis SK (1990) The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden* 77: 13-27.

Terai Y, O Seehausen, T Sasaki, K Takahashi, S Mizoiri, T Sugawara, T Sato, M Watanabe, N Konijnendijk, HDJ Mrosso et al. 2006. Divergent selection on opsins drives incipient speciation in Lake Victoria cichlids. *PLoS Biology* 4: 2244-2251.

Thrall PH, JJ Burdon 1997. Host-pathogen dynamics in a metapopulation context: the ecological and evolutionary consequences of being spatial. *Journal of Ecology* 85: 743-753.

Thuiller W, O Broennimann, G Hughes, JRM Alkemade, GF Midgley, F Corsi 2006. Vulnerability of African mammals to anthropogenic climate change under conservative land transformation assumptions. *Global Change Biology* 12: 424-440.

Tobler U, BR Schmidt 2010. Within- and among-population variation in Chytridiomycosis-induced mortality in the toad *Alytes obstetricans*. *PLoS One* 5: e10927.

Tollenaere C, L Rahalison, M Ranjalahy, S Rahelinirina, JM Duplantier, C Brouat 2008. CCR5 polymorphism and plague resistance in natural populations of the black rat in Madagascar. *Infection, Genetics and Evolution* 8: 891-897.

Trudeau, K.M, H.B. Britten, and M. Restani 2004. Sylvatic plague reduces genetic variability in black-tailed prairie dogs. *Journal of Wildlife Diseases* 40: 205-211.

Tsutsui ND, AV Suarez, DA Holway, TJ Case 2000. Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences of the USA* 97: 5948-5953.

Turner JK, MM McAllister, JL Xu, RI Tapping 2008. The resistance of BALB/cJ mice to *Yersinia pestis* maps to the major histocompatibility complex of chromosome 17. *Infection and Immunity* 76: 4092-2099.

Valsecchi E, W Amos, JA Raga, M Podesta, W Sherwin 2004. The effects of inbreeding on mortality during a morbillivirus outbreak in the Mediterranean striped dolphin (*Stenella coeruleoalba*). *Animal Conservation* 7: 139-146.

van der Putten WH, GW Yeates, H Duyts, CS Reis, G Karssen 2005. Invasive plants and their escape from root herbivory: a worldwide comparison of the root-feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges. *Biological invasions* 7: 733-746.

van Oosterhout C, WF Hutchinson, DPM Wills, P Shipley 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535-538.

Van Treuren R, R Bijlsma, W van Delden, NJ Ouborg 1991. The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity* 66: 181-189.

Vekemans X, OJ Hardy 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* 13: 921-935.

Vellend M 2008. Effects of diversity on diversity: consequences of competition and facilitation. *Oikos* 117: 1075-1085.

Vitousek PM, CM DAntonio, LL Loope, M Rejmanek, R Westbrooks 1997. Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology* 21: 1-16.

Vitousek PM, HA Mooney, J Lubchenco, JM Melillo 1997. Human domination of Earth's ecosystems. *Science* 277: 494-499.

Vredenburg VT, RA Knapp, TS Tunstall, CJ Briggs 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences of the USA* 107: 9689-9694.

Waples RS 1989. Temporal variation in allele frequencies: testing the right hypothesis. *Evolution* 43: 1236-1251.

Weir BS, CC Cockerham 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.

Weishampel JF, DA Bagley, LM Ehrhart 2004. Earlier nesting by loggerhead sea turtles following sea surface warming. *Global Change Biology* 10: 1424-1427.

Weldon C, LH du Preez, AD Hyatt, R Muller, R Speare 2004. Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* 10: 2100-2105.

Whitlock MC, McCauley DE 1990. Some population genetic consequences of colony formation and extinction – genetic correlations within founding groups. *Evolution* 44: 1717-1724.

Wikelski M, J Foufopoulos, H Vargas, H Snell 2004. Galapagos birds and diseases: invasive pathogens as threats for island species. *Ecology and Society* 9: 5.

Wright S 1931. Evolution in Mendelian populations. *Genetics* 16: 97-159.

Yang H, Y Ke, J Wang, Y Tan, SK Myeni, D Li, Q Shi, Y Yan, H Chen, Z Guo, et al. 2011. Insight into bacterial virulence mechanisms against host immune response via the *Yersinia pestis*-human protein-protein interaction network. *Infection and Immunity* 79: 4413-4424.