A MULTI-SCALE INVESTIGATION OF MOVEMENT PATTERNS AMONG BLACK-TAILED PRAIRIE DOG COLONIES

by

RACHEL M. PIGG

B.S., Rhodes College, 2006

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

Abstract

Dispersal remains one of the most important, yet least understood, life history traits. As the vehicle of gene flow among populations, dispersal can both relieve inbreeding depression and prevent local adaptation. Regionally, dispersal can stabilize or destabilize metapopulations, given its critical roles in disease transmission among populations as well as recolonization following local extinction events. Furthermore, in light of climate change and increasing habitat loss and fragmentation, the ability to navigate through unfamiliar, unsuitable habitat between populations is essential to the long-term survival of a species across its range. In my dissertation, I present a multi-scale investigation of factors affecting gene flow and disease transmission among populations of a keystone species and an agricultural pest of the North American prairie: the black-tailed prairie dog (*Cynomys ludovicianus*). Black-tailed prairie dogs are social, ground-dwelling squirrels that live in spatially isolated populations called colonies. First, we conducted a landscape genetic analysis of black-tailed prairie dogs throughout a large portion of their current range. Our estimates of gene flow indicate that the genetic neighborhood size of both male and female prairie dogs reaches 40-60 km within short-grass prairie, whereas colonies within mixed-grass prairie are more isolated. At a broad scale, we observed isolation-by-distance among colonies and great influence of grassland productivity on genetic connectivity; however, neither distance nor landscape characteristics greatly explained observed genetic differentiation among colonies separated by < 50 km. Last, we investigated whether landscape features could predict disease transmission patterns of sylvatic plague among colonies in short-grass prairie and found evidence that pastures act as corridors for plague transmission. Our results indicate that black-tailed prairie dogs are more resilient to habitat loss and fragmentation than other obligate grassland species and likely capable of transmitting sylvatic plague over long distances. Taken together, these studies illustrate how a multi-scale approach can reveal complexities of dispersal dynamics that would otherwise remain undetected.

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Acknowledgements

Many people contributed to the completion of my dissertation, certainly more than I could possibly mention here. First, I must thank my great advisor, Dr. Jack F. Cully, Jr. It's been quite the adventure! Thanks for your patience, your encouragement, and your sense of humor. I am also tremendously grateful to my research committee: Drs. Samantha M. Wisely, Brett K. Sandercock, and Joanna Whittier. Your continued support and your thoughtful comments on my work have been invaluable. In particular, I must single out Dr. Samantha M. Wisely for her commitment to my landscape genetic studies and her investment in my training. Your collaboration made this dissertation possible!

It took a small army to gather the data presented in this dissertation. I thank my friend, labmate, roommate, and fellow prairie dog enthusiast Amanda Goldberg for sharing her time and knowledge with me. You made fieldwork infinitely more fun! Next, I'm extremely grateful to Charles Lee, who gathered tissue samples from private lands throughout Kansas. I also thank Dr. Tammi L. Johnson, whose previous work inspired me to investigate the influence of landscape features on the disease dynamics she described in her thesis. Of course, I am also indebted to many field and laboratory technicians, but I must particularly thank both Amy Berner and Paige Harper. I am a better field and molecular ecologist for having known the two of you!

Numerous government agencies and private citizens made great contributions to my dissertation that were essential to its completion. For funding as well as technical support, I thank The Nature Conservancy, the Jack H. Berryman Institute, the Great Plains Landscape Cooperative, the United States Geological Survey, and the National Science Foundation. I am also indebted to several private landowners throughout Kansas for permitting my colleagues to collect samples on their property. For ensuring our field seasons ran smoothly, I must thank the National Park Service and United States Forest Service. In particular, I am grateful to NPS and USFS employees Felix Revello, Kevin McMurry, George Elmore, Fran Pannebaker, Robert Manasek, Karl Zimmerman, Shephanie Shively, Joe Hartman, and Andrew Chappell. Of course, I also thank employees of the many campgrounds that housed our field crews throughout the western Great Plains. Your hospitality was greatly appreciated!

I owe much of my success to the faculty, staff, and graduate students at Kansas State University. My sincerest thanks go to Drs. David A. Haukos, Kimberly A. With, David A. Rintoul, Brian S. Spooner, Ashley Rhodes, Martha Smith-Caldas, W. Alice Boyle, and Eva A. Horne for investing in my professional development as a research scientist and undergraduate educator. I also thank the administrative staff for their guidance and their patience. In particular, I am indebted to Becki Bohnenblust, Joyce Brite, Tari Philips, and Diane Ukena. Of course, I also must say a few words about my fellow graduate students. There are too many of you to name here, but please know I'm so grateful to have met you! You are the greatest reward from my graduate school experience. In particular, I thank all current and former members of the Kansas Cooperative Fish and Wildlife Research Unit, the Avian Ecology Laboratory, and the former Molecular Ecology Laboratory. For their specific contributions to my dissertation, my course work, and/or my future as an educator, I thank Claudia Ganser, Dr. Andrew Gregory, Zachary Lounsberry, Gina Barton, Andrea Severson, Emily Blevins, Derek Moon, Andrew Ricketts, and my wonderful roommates Alba Liliana Baquero Gomez and Eunbi Kwon.

Last, but certainly not least, I thank my family and friends outside the University for their support throughout the many years I have invested in my education. To my amazing undergraduate advisor, Dr. Alan P. Jaslow: thanks for your friendship and your continued interest in my future. To my wonderful, life-long friends Sonia Grevenitis, Virginia Terhar, Susanna Farmer, Kristen Henry, Jaclyn Rath, and Justin Bradford: your encouragement has helped more than you know. To my siblings, Kathryn and Charles Pigg: thanks for keeping me grounded, for distracting me when I needed distraction, and for never letting me take myself too seriously. Finally, to my wonderful parents, Woodard and Pamela Pigg: I do not have words to express how blessed I am to call you mine. Thank you for never giving up on me and for believing in me when I did not believe in myself. I love you.

Dedication

In honor of my parents:
H. Woodard Pigg, Jr. and Pamela K. Pigg

AND

In memory of two friends and scholars:
Ron E. VanNimwegen and Kara N. Bayless

Chapter 1 - Introduction

Dispersal is defined as the movement of an organism from one residence to another suitable location for residency (Bowler and Benton 2005). Variation among individuals in the spatial extent and propensity of dispersal has far-reaching effects across ecological scales and disciplines (Hanski 1999, Clobert et al. 2001, Nathan et al. 2008). For individual organisms, dispersal carries fitness costs and benefits that depend on both the internal condition of the individual and the external conditions of the environment (Clobert et al. 2001, Nathan et al. 2008). At a population level, the stability and long-term survival of populations depends upon achieving an appropriate balance between immigration into and emigration out of a population. These observations laid the foundation for the metapopulation concept.

Coined by Levins (1969), metapopulations are formed when habitat loss and fragmentation create isolated patches of suitable habitat surrounded by unsuitable habitat, often called the matrix (Fahrig and Merriam 1994, Lidicker and Koenig 1996, Kindlemann and Burel 2008). The response of individual organisms to this landscape structure creates a group of somewhat-isolated, local populations, where the long-term persistence of each population, and of the metapopulation as a whole, depends on dispersal rates among populations (McCullough 1996, Hanski 1999). Constant, moderate dispersal rates decrease the extinction risk faced by each population and allow for recolonization events that rescue populations following local extinction events (Hanski 1999); however, frequent dispersal can carry disease quickly throughout the metapopulation, threatening regional survival (Hess 1996) or preventing local adaptation of populations, as immigrants flood the gene pool locally and create source-sink dynamics regionally (Pulliam 1988, Dias et al. 1996). Infrequent dispersal is also a threat. When dispersal is rare, increasingly isolated populations can become inbred, which could increase a population’s extinction risk (Gilpin and Soule 1986).

Due to such far-reaching implications, understanding factors affecting dispersal among populations (sometimes called connectivity) has been the focus of a multitude of studies on plant and animal systems (Clobert et al. 2001). Dispersal is composed of 3 distinct stages: (1) emigration, (2) interpatch movement, and (3) immigration (Clobert et.

al 2001). Much of the previous research in dispersal has sought to quantify emigration and immigration rates and to identify intrinsic and extrinsic factors associated with changes to those rates. Comparatively less work has been done to identify characteristics of the matrix that facilitate or hinder movement among populations (Wiens 2001, Hawkes 2009). My dissertation seeks to address this knowledge gap through an investigation of movement patterns among populations of an obligate grassland mammal: the black-tailed prairie dog (*Cynomys ludovicianus*).

Black-tailed prairie dog ecology

Black-tailed prairie dogs are highly social, diurnal, ground-dwelling rodents that live in populations called colonies (Hoogland 1995, Dobson et al. 1997, Devilliard et al. 2004). Black-tailed prairie dogs (hereafter, "prairie dogs") are a highly conspicuous species, given not only their diurnal habits, but also their raised burrow entrances and their tendency to clip vegetation surrounding those entrances. These behaviors have led to the characterization of prairie dogs as ecosystem engineers and a keystone species of the North American prairie ecosystem, enhancing species diversity by providing beneficial ecosystem services (Kotliar et al. 1999, 2006). Despite the ecological significance of this species, their abundance has declined greatly in the past 200 years (Hoogland 1995, 2006). Land conversion, government and private pest control, recreational shooting, and the introduction of the exotic disease sylvatic plague have decreased the area they occupy by 98% (Cully and Williams 2001, Cully et al. 2006, Luce et al. 2006). Consequently, prairie dogs exist in metapopulations composed of colonies in various degrees of isolation (Koford 1958, Hoogland 1995, Roach et al. 2001, Antolin et al. 2006).

Prairie dog colonies can contain thousands of residents and cover several square kilometers (Koford 1958, Hoogland 1995). Within these colonies, black-tailed prairie dogs organize themselves into family groups called coteries (Hoogland 1995). A coterie typically consists of one adult male, 3 or 4 adult females, and their non-breeding yearling and juvenile offspring (Garrett and Franklin 1988, Hoogland 1995). An observational study of one colony in the mixed-grass prairie of South Dakota reported that most females remain in their natal coterie their entire lives, while males typically spend no more than two years in any coterie (Hoogland 1995). Their breeding season lasts from February to April, with juveniles emerging from their natal burrows in May (Hoogland 1995). Intercolonial dispersal, i.e., dispersal between colonies and through the matrix, begins at the end of this breeding season and usually continues until June (Garrett and Franklin 1988). Alternatively, intracolonial dispersal, i.e., dispersal between coteries within the same colony, occurs throughout the year.

Over the years, visual observations, radiotracking technology, and genetic studies have provided some information concerning the intercolonial and intracolonial dispersal patterns of this small mammal. Results from observational studies in mixed-grass prairie (Garrett et al. 1982, Halpin 1987, Hoogland 1995) suggest that intracolonial dispersal is frequent and strongly male-biased. Intercolonial dispersal patterns have proven more difficult to characterize.

Observational studies of intercolonial dispersal

Hoogland (1995) observed 28 male and 21 female immigrants to his study colony over a 16-year period, suggesting that intercolonial dispersal, unlike intracolonial dispersal, was not male-biased. A study of dispersal by Garrett and Franklin (1988) countered that adult intercolonial dispersers were more often female than male, while juvenile intercolonial dispersers were more often male. Garrett and Franklin (1988) also attempted to determine survivorship of dispersers. Of 27 animals, only 15 successfully immigrated into new colonies, while the survival rate among philopatric prairie dogs remained high (90%). Predation emerged as the most common cause of mortality (73%), while conspecific aggression and hazardous landscape features accounted for the other deaths. The results of these field studies suggest that successful intercolonial dispersal is affected by both age and sex (Hoogland 1995).

Molecular approaches

Gene flow is a measure of effective dispersal, i.e. dispersal events culminating in successful reproduction at the new residence (Hanski 2001). A variety of methods to estimate gene flow from genetic data have been developed, but the most frequently reported metrics in black-tailed prairie dog studies are F-statistics (Wright 1978, Antolin et al. 2006). Of these previous studies, two used allozymes to characterize the degree of gene flow among colonies ≤ 50 km apart (Chesser 1983, Daley 1992) and found only moderate genetic differentiation between colonies, suggesting intercolonial dispersal occurs more frequently than observational studies suggest. This conclusion agrees with reports from more recent investigations that utilized microsatellite markers rather than allozymes to study gene flow among colonies (Roach et al. 2001, Magle et al. 2010, Jones and Britten 2010, Sacket et al. 2012).

Roach et al. (2001) uncovered further support for regular intercolonial dispersal by implementing assignment tests (Cornuet et al. 1999). Assignment tests determine the most likely population of origin for an individual based on allelic frequencies (Hamilton 2009). In Roach et al. (2001), > 30% of the sampled prairie dogs were either immigrants to their colony of capture or offspring of immigrants. Chi-squared tests showed no significant difference between the numbers of male and female immigrants, providing further evidence that intercolonial dispersal is not sex- or age-biased (Roach et al. 2001).

Of the studies presented here, none have attempted to examine gene flow at a broader, range-wide scale. Such a study could provide valuable information concerning the limits of prairie dog dispersal capabilities and the size and structure of metapopulations.

Corridors and barriers to movement among colonies

Researchers are only beginning to investigate the complex influence of environmental characteristics of the matrix on prairie dog dispersal. Hypotheses concerning the effects of certain vegetation types, topographic features, and anthropogenic features have been proposed by a number of authors (Koford 1958, Knowles 1986, Garrett and Franklin 1988, Hoogland 1995, Magle et al. 2010, Sackett et al. 2012). Roach et al. (2001) collected genetic samples from 13 colonies in the short-grass prairie of Pawnee National Grassland in Colorado and found that dry-creek drainages may act as corridors for dispersal; however, the adjusted-R^2 value for this model reveals that drainage distance only accounts for a small amount of the observed genetic differentiation among colonies ($R^2 = 0.43$; Roach et al. 2001). Consequently, other factors not included in the models of Roach et al. (2001) might be more predictive of prairie dog dispersal. Recent studies by Magle et al. (2010) and Sacket et al. (2012)

show evidence that intense urbanization decreases connectivity, while roadways play a small role in facilitating movement through urban and agricultural development. Surprisingly, no previous study of gene flow among colonies has explored the influence of climatic variables on observed genetic connectivity patterns in spite of their predictive power in studies of sylvatic plague dynamics in prairie dog metapopulations.

Sylvatic plague ecology in prairie dog metapopulations

One consequence of dispersal among populations is the potential spread of disease across broad spatial scales (Hess 1996). If this disease has a high mortality rate, numerous, synchronized extinctions will threaten metapopulation stability. For prairie dogs, one disease with the potential to destabilize populations is sylvatic plague (Cully and Williams 2001). Sylvatic plague is a flea-borne disease caused by the bacterium *Yersinia pestis*, which likely first arrived in North America near the turn of the 20th century. Over the past 100 years, this generalist bacteria has spread quickly across the continent through various flea vectors and mammalian hosts, periodically causing epizootic outbreaks with the potential to affect human populations (Enscore et al. 2002, Ray and Collinge 2006, Holt et al. 2009). Recent studies have created predictive models of plague occurences by relating temporal patterns of disease spread to climatic variables (Parmenter et al. 1999, Enscore et al. 2002, Collinge et al. 2005a, Ray and Collinge 2006) and colony spatial characteristics (Collinge et al. 2005b, Cully et al. 2010, Johnson et al. 2011). Investigations of the influence of landscape features, however, are less prevalent in the literature. Collinge et al. (2005b) determined that the percent cover of roads, lakes, and streams on the land surrounding prairie dog colonies had a negative effect on the spread of plague to those colonies. Johnson et al. (2011) revealed conflicting results concerning the magnitude and direction of the effect of these landscape variables on the probability of plague transmission between colonies. The studies used different metrics to quantify landscape variation within their models, which may explain their disparate results; however, neither metric choice may adequately represent the landscape within their models.

Dissertation objectives

In this dissertation, I present three studies that explore the effects of environmental features on movement among prairie dog colonies. First, in Chapter 2, we show the influence of grassland productivity on broad scale genetic connectivity patterns extending across the longitudinal breadth of current prairie dog range. This study represents the most widespread investigation of prairie dog movement to date. Next, in Chapter 3, we present a characterization of movement patterns among prairie dog colonies within short-grass prairie, which expands on past reports of the effect of sex on prairie dog dispersal behavior and provides a genetic neighborhood size for prairie dogs within short-grass prairie that can inform management practices. In Chapter 4, we create predictive models of sylvatic plague transmission among colonies in short-grass prairie that demonstrate the complex role of landscape features in the spatial spread of this exotic disease. Last, in Chapter 5, we briefly summarize our major findings and offer suggestions for future research directions.

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Chapter 2 - Grassland productivity influences broad scale connectivity patterns among black-tailed prairie dog colonies

Rachel M. Pigg, Samantha M. Wisely, Charles Lee, and Jack F. Cully, Jr.

Abstract

Climatic variables and landscape features influence connectivity among populations of a variety of taxa. Connectivity ensures long-term persistence of a species across its current range, and so, understanding what factors affect that connectivity is essential for effective species management and conservation. Here, we use a landscape genetic approach to identify variables predictive of observed connectivity patterns among populations of a North American grassland rodent, the black-tailed prairie dog (*Cynomys ludovicianus*), from the geographic center of its range to the eastern periphery. We found western populations to be highly connected, whereas eastern populations show significant genetic differentiation; however, our investigation of inbreeding within eastern populations produced equivocal results. Our landscape genetic analyses reveal that at broad spatial scales, average annual precipitation has a strong correlation with connectivity, while landscape features, such as urban and agricultural development or stream networks, do not. Our results demonstrate the resilience of prairie dogs to habitat loss and fragmentation, the great influence of grassland productivity on prairie dog movement abilities, and the importance of including climatic variables in broad scale landscape genetic analyses.

Introduction

Climate change, habitat loss, and fragmentation constitute three of the most significant threats to biodiversity worldwide (Fahrig 1997, Travis 2003, Wiegand et al. 2005, Olden et al. 2006, Dawson et al. 2011). Results from recent predictive models of climate change indicate grassland ecosystems are likely to experience the most dramatic changes in biodiversity by the year 2100 (Sala et al. 2000, Ceballos et al. 2010). In North America, grasslands are classified into three prairie types: short-grass prairie in the west, tall-grass prairie in the east, and a mixed-grass prairie type forming an ecotone between the two (Borchert 1950, Sala et al. 1988). While tallgrass prairie is threatened by increased urbanization, agricultural development, climate change, and woody encroachment, short-grass prairie also faces an uncertain future in the wake of increased drought severity and agricultural development. To quantify the extent of such threats to an ecosystem and its associated fauna, most studies measure changes in site specific response variables, including demographic rates, local extinction probabilities, and behavioral shifts, particularly in life history traits (Burke and Nol 2000, Warren et al. 2001, Davidson et al. 2002). More recently, however, focus has shifted to determining how evolutionary forces affect a between-site process: connectivity.

Connectivity, as the term implies, quantifies the extent to which geographically isolated populations are connected to one another through both natal and breeding dispersal (Taylor et al. 1993, Nathan et al. 2008). Although a multitude of strategies exist to estimate connectivity, the use of landscape genetic tools has become increasingly prevalent in recent literature (Manel et al. 2003; Manel and Holdregger 2013). Landscape genetic techniques have allowed investigators to make valuable contributions to our understanding of organisms' resiliency to habitat and climate change. Examples of such contributions abound in the literature, but recent examples include the detection of critical dispersal corridors for the endangered Ethiopian mountain nyala (Atickem et al. 2013), the identification of habitat features driving the invasion ecology of the South American capybara (Campos-Krauer and Wisely 2011), and the creation of predictive models for the future genetic structure of European alpine plants under different climate change scenarios (Jay et al. 2012). Here, we use a landscape genetic approach to investigate the effects of climatic variables and landscape features on connectivity among populations of a keystone species of the North American grassland: the black-tailed prairie dog (*Cynomys ludovicianus*).

Black-tailed prairie dogs are ground-dwelling sciurids and a keystone species of prairie ecosystems (Kotliar et al. 1999). This keystone status implies that a significant portion of grassland biodiversity is preserved by the presence and activity of this species (Mills et al. 1993); however, black-tailed prairie dogs are also considered agricultural pests, so wildlife managers cull populations via poisoning and recreational shooting throughout most of the species' current range. Consequently, black-tailed prairie dogs (hereafter, "prairie dogs") occupy less than 2% of their historic range (Hoogland 1995). Today, prairie dogs live in discrete spatial units called colonies, easily distinguishable from the surrounding landscape by their clipped vegetation and raised burrow entrances (Koford 1958). These two colony features demonstrate the reliance of prairie dogs on visual cues to detect predators. Any landscape feature that limits their vision field could lead to decreased survival or decreased dispersal propensity.

When colonies occur at high density across a landscape, they are collectively referred to as a colony complex. Effective dispersal among colonies, i.e., dispersal followed by successful social integration and reproduction at the new colony, is rarely observed (Halpin 1987, Garrett and Franklin 1988). Consequently, recent studies of intercolonial dispersal almost exclusively use population genetic methods to characterize regional connectivity patterns (Roach et al. 2001, Antolin et al. 2006, Magle et al. 2010, Sackett et al. 2012). Previous studies have characterized connectivity within a single colony complex or, more specifically, an area of ≤ 60 km diameter containing multiple colonies (Antolin et al. 2006). No study has investigated the effects of climate on connectivity or quantified connectivity at broader spatial scales, but previous studies have given us an appreciation of the variance in connectivity throughout prairie dog range. Towards the geographic center in eastern Colorado and western Kansas, prairie dog colonies are highly connected and show no sign of inbreeding depression (Foltz and Hoogland 1983, Daley 1992, Roach et al. 2001), whereas at the edges, more limited movement patterns have been reported, notably in western Colorado (Magle et al. 2010, Sackett et al. 2012), northern Montana (Jones and Brittan 2010), and New Mexico (Chesser 1983). Potential causes for decreased intercolony dispersal include increased urbanization and human population density, decreased habitat quality, and pervasive, synchronized local extinction events caused by the exotic disease sylvatic plague.

Sylvatic plague, caused by the bacterium *Yersinia pestis*, has been known to cause local extinctions throughout colony complexes on a roughly 10-year cycle (Cully and

Williams 2001). The mechanism responsible for epizootic events is not known, but Collinge et al. (2005) noted correlations with high precipitation and moderate temperatures in the years preceding the epizootic. It is also unknown whether plague poses a significant risk to the long-term survival of prairie dogs throughout their range. Interestingly, although sylvatic plague can dramatically affect colonies west of the 100th meridian, it has rarely been observed in any species east of this longitudinal boundary, including prairie dogs (Cully et al. 2000). The 100th meridian also roughly divides the short-grass and mixed-grass prairie types (Borchert 1950), suggesting that mixed grass prairie may limit movement and, consequently, disease transmission among colonies. Whether this limitation is due to increased urbanization or agricultural development in mixed grass prairie or to increased vegetation height in mixed grass prairie has not been investigated.

To our knowledge, no study has investigated connectivity or genetic isolation among colonies along the eastern edge of prairie dog range, where average annual precipitation and, consequently, grassland productivity and vegetation heights are highest (Sala et al. 1988). Only one study has compared any western and eastern colony characteristics directly. Lomolino and Smith (2001) monitored changes in colony area and geographic isolation over a 10-year period throughout Oklahoma. Their observations of decreasing area and increasing geographic isolation through time led them to question the viability of prairie dog colonies throughout their study area, but most imminently in their easternmost survey sites. They speculated that increasing human population density and land use led to their observations, but did not test these hypotheses, nor did they consider the possible role of grassland productivity in shaping the temporal and spatial patterns they observed.

Building on the work of Lomolino and Smith (2001), Avila-Flores et al. (2012) investigated drivers of change in colony size and geographic isolation along the southwestern edge of prairie dog range in New Mexico. The authors found that, contrary to the expectations of Lomolino and Smith (2001) for eastern colonies, southwestern colonies were relatively unaffected by agricultural development and urbanization; however, a persistent and pervasive drought greatly influenced colony size and isolation, likely because the drought decreased forage quality and quantity and, consequently, prairie dog survival. Given these results, it is plausible that colonies along the eastern edge of prairie dog range would show a similar sensitivity to precipitation patterns over landscape variables, but the specific mechanism driving the sensitivity in the east (such as increased visual barriers to predator detection, rather than reduced forage quality and quantity) may differ. Whether this sensitivity extends to between-site processes, such as connectivity, remains unexplored.

Here, we address the conservation concerns presented by climate change, habitat loss, and fragmentation to the long-term persistence of prairie dogs throughout their range via a broad-scale landscape genetic study, spanning six states and > 700 km. First, we describe connectivity patterns among prairie dog colonies east of the 100th meridian and compare those patterns to colonies in the west. We expected to observe significantly higher genetic differentiation and genetic isolation among eastern colonies in mixed-grass prairie than among western colonies in short-grass prairie. Second, we sought to identify environmental characteristics predictive of connectivity among colonies throughout our study area. We hypothesized that while the influence of landscape features and average annual precipitation on connectivity would increase in the east, given increased vegetation heights and urban and agricultural development in mixed-grass prairie. Last, based on our results, we speculate on the long-term survival and distribution of prairie dogs, given climate change projections for North America and the confounding factor of sylvatic plague.

Materials and Methods

Study area

We selected 14 sites between 36°N and 42°N latitude across the longitudinal breadth of black-tailed prairie dog distribution (Figure 2.1). As expected, mixed-grass prairie dominated the eastern portion of this study area, but transitioned into short-grass prairie at roughly the 100th meridian (Borchert 1950). West of this meridian, short-grass prairie predominated, although semiarid sand-plains and shrubland became increasingly prevalent towards the southwest.

To quantify and compare genetic variation across our study area, we selected 14 locations in which to concentrate our sampling efforts. Seven of these locations occur west of the 100th meridian, while the remaining 7 sites were to the east. Given low colony densities (< 2 colonies per 10 km^2) in the eastern portion of our study area, we only sampled 1 colony per location east of the 100th meridian (Table 2.1). As 10 km corresponds to the greatest observed dispersal distance of a prairie dog (Knowles 1985), a single colony is likely representative of the genetic variation at each of the seven eastern locations. Most western locations were represented by more than one colony, depending on the density of colonies observed at these locations. The number of colonies sampled at each location varied from 1 to 13. All colonies included in the analyses described below were represented by 9-35 individuals (Table 2.1).

Sample collection

We collected tissue samples from 1127 prairie dogs in 52 colonies via two methods (Table 2.1). First, between 2009 and 2012, we opportunistically obtained tail and/or muscle tissue from culled prairie dogs during wildlife damage control efforts on private lands in Kansas. Second, we collected ear tissue samples from live-trapped prairie dogs on National Grassland and National Park lands between 2010 and 2012. We established trap lines at 1 to 4 sites within each colony, dependent on colony size and prairie dog density, and placed approximately 80 to 100 collapsible, single-door Tomahawk traps near active burrows on each colony. We used surgical scissors and/or ear punches (Med Vet International) to harvest ~2 mm diameter ear clippings from captured prairie dogs. All tissue samples were preserved in 95% ethanol for later DNA extraction. Kansas State University's Institutional Animal Care and Use Committee approved all capture, handling, and sampling procedures (Protocol Number 2889), which followed guidelines established by the American Society of Mammalogists (Sikes et al. 2011).

Molecular methods

We used Qiagen DNeasy Blood and Tissue Kits (Valencia, CA) to extract DNA from our samples according to the manufacturer's protocol. We diluted all extractions to a concentration of 0.25 ng/µL prior to primer optimization for all microsatellite and mitochondrial loci.

For our multilocus microsatellite analyses, we optimized primers for 19 polymorphic loci from previously published literature (Table 2.2; Stevens et al. 1997, May et al. 1997, Jones et al. 2005, Sackett et al. 2009) and used an M-13 universal primer (Schuelke 2000) labeled with an Operon Biotechnologies fluorescent dye (HEX, PET, NED, or FAM; Huntsville, AL) to distinguish individual loci from one another when in mixture. We genotyped individuals via the ABI 3730 DNA Analyzer at the DNA Sequencing and Genotyping Facility at Kansas State University, using the 500-LIZ size standard (Applied Biosystems, Foster City, CA) and program GeneMarker (v1.95; Holland and Parson 2011) to determine the specific fragment length of all observed alleles. To evaluate allelic dropout rates at each locus, we randomly selected 10% of homozygous and 10% of heterozygous samples to rerun. We used program Arlequin (v3.5.1.4; Excoffier et al. 2005) to test all loci for deviations from Hardy-Weinberg equilibrium (HWE; 1,000,000 Markov Chain Monte Carlo simulations) and linkage disequilibrium (Fisher's exact test; 5000 permutations). Finally, we used Micro-Checker to test for the presence of null alleles at all loci (v2.2.3; van Oosterhout 2004). We excluded loci that deviated significantly from HWE from analyses that assume HWE.

For our mitochondrial DNA analyses, we amplified and sequenced a 697 bp region of the cytochrome b gene using previously published primers (Irwin et al. 1991, Harrison et al. 2003, Herron et al. 2004). From our total sample set of 1127 individuals, we systematically selected a subset of 46 individuals to represent diversity at this locus throughout our study area. Bidirectional sequencing was completed using ABI 3730 DNA Analyzers in the Molecular Ecology Lab at the University of Florida as well as the University of Kentucky's AGTC Sequencing Center. We then compiled consensus sequences using program CLC Main Workbench (v6; CLC bio) and aligned all samples using the ClustalW approach in program MEGA (v5.1; Tamura et al. 2007).

Analyses of genetic differentiation and population structure

We used several independent methods to assess genetic diversity within our study area using our microsatellite data. First, we calculated allelic richness and observed vs. expected heterozygosity for each colony using program GenAlEx (v6.5; Peakall and Smouse 2006). To determine whether significantly higher genetic differentiation existed among colonies east of the 100th meridian than among western colonies, we compared the two groups via a one-sided significance test of the OS_x statistic (10,000 permutations) calculated by FSTAT (v.2.9.3; Goudet 2001).

Beyond testing our east-west hypothesis, we also investigated whether significant population structure exists throughout our study area. First, we explored our data by creating a visual representation of genetic clusters in a 2-diminsional principal coordinate analysis (PCoA) implemented in program GenAlEx. We then compared our PCoA results to a Bayesian clustering analysis conducted in program STRUCTURE (v2.3.4; Pritchard et al. 2000, Evanno et al. 2005). In the STRUCTURE analysis, we used an admixture model in which K varied from 1 to 13, corresponding to the number of locations in which at least one colony was successfully sampled and genotyped across our study area (200000 Markov Chain Monte Carlo simulations; burnin period = 50,000). We compiled and visualized our STRUCTURE output using a sequence of programs, including STRUCTURE Harvester (web v0.6.93; Earl and vanHoldt 2011), CLUMPP (v1.1.2; Jakobsson and Rosenberg 2007), and DISTRUCT (v1.1; Rosenberg 2004). Based on the uncertain results from program STRUCTURE (see Results), we also ran an analysis of molecular variance (AMOVA) in program Arlequin to better judge the significance of our observations.

We investigated the impact of historical processes on the results of our microsatellite analyses by calculating diversity indices from our mitochondrial data. We used program DnaSP (v5; Librado and Rozas 2009) to calculate haplotype number and diversity. We also ran two tests for selective neutrality: Tajima's *D* (Tajima 1989) and R_2 (Ramos-Onsins and Rozas 2002). Ramos-Onsins and Rozas (2002) found that R_2 performed better than the more commonly reported F_S (Fu and Li 1993) when evaluating neutrality for small sample sizes, such as ours. Last, we visualized the relationship among our haplotypes by creating a minimum-spanning haplotype network using program Network (v4.6.1.1; Bandelt et al. 1999).

Analyses of genetic isolation

We calculated and compared inbreeding indices at both the colony and individual levels. At the individual level, we calculated homozygosity by loci (HL; Aparicio et al.

2006) for all individuals using IR macroN3 (Amos et al. 2001). We then used a one-sided Kruskal-Wallis comparison test in program R (v2.12.2; R Core Team) to determine whether significantly higher levels of this index occurred in individuals found east of the 100th meridian as opposed to west. At the population level, we tested whether we observed significantly higher F_{IS} indices among our eastern colonies using the aforementioned OS_x statistic in FSTAT.

To further characterize colony isolation, we conducted an assignment test to identify immigrants in each of our study colonies. We used program GeneClass2 (v2; Piry et al. 2004) to determine the likelihood that an individual was captured in its natal colony. Specifically, the program used Markov Chain Monte Carlo (MCMC) simulations to identify individuals with a likelihood of originating from their colony of capture less than some set threshold (Paetkau et al. 2004). We ran 10,000 MCMC simulations and identified immigrants at two probability thresholds: 0.05 and, the more conservative, 0.01. If immigrants were found, we did not try to identify their colony of origin, as many more colonies exist in our study area than the 52 colonies we sampled. Instead, we used a one-sided Pearson's chi-square statistic in R to evaluate whether the proportion of immigrants observed in western colonies differed significantly from eastern colonies.

Landscape genetic analyses

To determine whether patterns of genetic differentiation among colonies conform to expectations of isolation-by-distance (IBD), we regressed genetic distance against geographic distance separating each colony pair. We approximated pairwise genetic distances by calculating Slatkin's linearized F_{ST} in Arlequin (Slatkin 1995). We then calculated pairwise geographic distances using the Point Distance tool in ArcGIS (v10.1; ESRI). To test the IBD hypothesis, we ran a Mantel test (9999 permutations; Manly 1991) on these two distance matrices in GenAlEx.

We then conducted a landscape genetic analysis to identify climatic or landscape variables that influence gene flow among our colonies at the broadest spatial scale. Specifically, we created and tested isolation-by-resistance models (IBR; McRae 2006) using programs ArcGIS, Circuitscape (v3.5.8; McRae et al. 2008), and GenAlEx. First, we surveyed the literature for landscape and climatic variables that may act as drivers or inhibitors of gene flow among prairie dog colonies. The variables we selected, their hypothesized impact on gene flow, and the dataset(s) used to visualize these variables in ArcGIS are given in Table 2.3. While we considered investigating effects of temperature within our study area, we ultimately excluded this climatic variable because our sampling design did not adequately represent the north-south temperature gradient within our study area, given the large gap between our most northerly location and all other locations (Figure 2.1).

We created IBR landscape input data by transforming our source datasets into rasterized resistance surfaces. For discrete landscape features, e.g., roads, we created categorical IBR models by assigning high resistance values to hypothesized barriers (R = 100 ohms) within the raster and low resistance values to hypothesized corridors (R = 1 ohms). All other cells within the raster were given an intermediate, constant value (R = 50 ohms), which approximates the IBD model within cells where the feature is absent. Additionally, for each model containing a single feature, we created and tested its reciprocal model. For example, if we hypothesized a certain feature functioned as a barrier to gene flow, we created two IBR models: one in which the feature was coded as a barrier and a second in which the feature was coded as a corridor. This procedure allowed us to test both our hypothesis and an alternate hypothesis within the same modeling framework. If our hypothesis was correct, we expected the original model to perform well and the reciprocal model to perform poorly.

Previous investigations of prairie dog dispersal informed our hypotheses regarding the effect of each landscape feature on observed gene flow among our colonies (Table 2.3). Dry stream networks are prevalent features in short-grass prairie and may serve as corridors, while active streams are prevalent features in wetter mixed-grass prairie and may act as barriers to movement (Garrett and Franklin 1988, Roach et al. 2001). We also hypothesized that high intensity urban development would act as a barrier (Magle et al. 2010), while low intensity development, such as rural or suburban roadways, may be movement corridors (Sackett et al. 2012). Increased agricultural practices would lead to increased human activity and rodenticide use, so we hypothesized that agricultural lands would also act as a barrier (Tilman et al. 2001).

For our climatic variable, we investigated the influence of average annual precipitation, given its strong, positive association with grassland productivity (Sala et al. 1988) as well as our expectation that increased vegetation height may hinder prairie dog movement in the eastern portion of our study area. We created a continuous resistance surface (R = 1-100 ohms) using precipitation data from the PRISM Climate Group. To more directly compare the results of this continuous climate IBR model to our landscape IBR models, we also created continuous IBR models of our landscape variables, in addition to the previously described categorical IBR models. In these continuous landscape IBR models, resistance values correspond to the density of the feature within a raster cell, rather than simply the presence or absence of that feature (Table 2.3). In all our IBR models, raster cell size was 1.5-km by 1.5-km, which equals or exceeds the size of every colony we sampled.

We used program Circuitscape to calculate pairwise resistance distances separating our colonies within each IBR model. These resistance distances are calculated using circuit theory, which views each colony within the IBR model as a source of electrical current, while the IBR model itself (with the landscape or climatic variables coded within) provides the electrical circuit that allows current to move among populations. Connectivity between population pairs is predicted by (1) the resistance values assigned to cells separating those populations and (2) the width and redundancy of pathways connecting those populations.

To test the fit of our IBR models, we used Mantel tests (10,000 permutations) of resistance distance against genetic distance implemented in GenAlEx. We evaluated our landscape and climate variables in the following manner. First, for all IBR models containing a single landscape or climate variable, we determined the fit of both the model containing our hypothesized effect and the reciprocal model (Table 2.3). Variables that fit our observed genetic data well (p-value of Mantel's $R < 0.01$) were kept in our candidate variable set only if the reciprocal model did not fit the data (p-value > 0.01). Variables that passed this initial quality control test were further tested via partial Mantel tests against our IBD model in program R. Landscape or climatic variables that passed both quality control measures were used to create multivariate IBR models. We adopted this conservative approach to eliminate nuisance variables from our candidate set for two reasons: (1) the use and misuse of Mantel tests in landscape genetics is currently under debate among scholars (Raufaste and Roussett 2001, Cushman and Landguth 2010b, Legendre and Fortin 2010) and (2) our clustered sampling design and its resulting, highly pruned network made the use of other techniques, such as gravity modeling, equally questionable (Garroway et al. 2008, Murphy et al. 2010, Jacoby et al. 2012).

To identify the most influential variables within our candidate set, we ranked all IBR models against our IBD model using an AIC model ranking procedure. We calculated AIC using the equation:

$$\mathrm{AIC} = 2K + n * \ln(\mathrm{RSS}/n),$$

where K is the number of variables in the model, n is the number of colonies, and RSS is the residual sum of squares from the Mantel test (Burnham and Anderson 2002). The only model in which $K = 1$ was the IBD model, as all IBR models include distance. We also applied a correction for small sample size ($\mathrm{AIC_C}$), as suggested by Burnham & Anderson (2002). The model with the lowest AICc value is best-fit; however, alternative models with $\Delta\mathrm{AIC_C} \leq 2$ from the best model are equally well-supported (Burnham & Anderson 2002). All other models are less likely, and models with $\Delta\mathrm{AIC_C} > 10$ should not be considered (Burnham and Anderson 2002).

Results

Of the tissue samples we collected from 1127 prairie dogs in 52 colonies, we successfully genotyped 1096 individuals from 49 colonies at 19 microsatellite loci (Table 2.2). Based on results of our quality control procedures, our 19 loci showed no evidence of significant allelic dropout. Our genotyping error rate was ≤ 2.4% at all loci, and we found no consistent patterns of significant linkage disequilibrium across our populations. We did observe heterozygote deficiencies at two of our loci: GS14 and C116; however, excluding these loci from calculations of genetic isolation and differentiation did not appreciably change our results.

Genetic differentiation

From our 19 microsatellite loci, allelic richness within our colonies ranged between 2.5 to 6.4 alleles, while our calculations of observed and expected heterozygosity revealed an excess of heterozygotes in most colonies across our study area (Table 2.3). In accordance with our hypothesis of less connectivity among colonies within mixed-grass prairie, F_{ST} was significantly greater among colonies east of the 100th meridian than west (eastern $F_{ST} = 0.24$; western $F_{ST} = 0.07$; $P < 0.0001$). Our PCoA (Figure 2.2) also showed that eastern colonies were on independent evolutionary trajectories from each other and from the western colonies, demonstrating that the influence of genetic drift on eastern colonies exceeded gene flow. Among the western colonies, our PCoA and STRUCTURE analysis also provided evidence of significant differentiation between Logan County colonies and the three National Grasslands in the south (Figure 2.3), but results from our AMOVA indicated that this separation accounts for only 6% of the observed genetic variation among our colonies (Table 2.5). Alternatively, 90% of the variation was explained by individual-level heterozygosity, suggesting a high degree of connectivity throughout our region and only moderate differentiation among our widely dispersed sampling locations ($F_{CT} = 0.06$; Table 2.5). In addition, further examination of our STRUCTURE results demonstrated a steadily increasing mean likelihood with increasing K, rather than the presence of a horizontal asymptote, as would be expected from true population structure (Figure 2.3A). This pattern suggested that isolation-by-distance is a significant force acting on genetic variation among colonies within our study area.

From our mtDNA analyses, we found 15 polymorphic sites and 14 haplotypes of the cytochrome b gene among our 46 sequenced individuals. Half of these individuals (50%) compose our largest single haplogroup. Average haplotype diversity (H_d) was 0.74 ± 0.07 and nucleotide diversity (π) was 0.002 ± 0.0003, providing evidence that prairie dog within our study area may have experienced a fairly recent and rapid period of expansion from a single refugium. Our tests of neutrality supported this conclusion (Tajima's $D = -1.97$, $P < 0.05$; $R_2 = 0.042$, $P < 0.0001$), as did the starburst topology of our haplotype network (Figure 2.4). Therefore, within the range of latitudes we sampled, current prairie dog populations likely evolved from a single genetic refugium following the last glacial maximum.

Genetic isolation

We found evidence of a trend across our study area of increasing individual-level homozygosity (HL) with increasing average annual precipitation (Figure 2.5). When we compared eastern and western individuals, we found significantly higher homozygosity-by-loci in the east (observed difference = 255.34, Kruskal-Wallis statistic = 64.18; $P < 0.01$). At the population level, however, we found no difference between eastern and western colonies (Eastern $F_{IS} = -0.04$, Western $F_{IS} = -0.05$; $P = 0.39$). From assignment tests in GeneClass2, we found 37 migrants west of the 100th meridian and 8 migrants east of the meridian at our most conservative threshold ($p = 0.01$; $\chi^2 = 0.29$, df = 1, $P = 0.30$). At a threshold of 0.05, however, we found significantly more migrants west of the 100th meridian (west migrants = 111, east migrants= 10; $\chi^2 = 3.16$, df = 1, $P = 0.04$).

Isolation-by-distance

We found significant pairwise F_{ST} values among most colony pairs within our study area (Figure 2.6). The five insignificant F_{ST} values ($P > 0.05$) occurred between colonies from the same sampling location (i.e., one of our 14 locations shown in Figure 2.1).

We detected a significant signal of IBD across our study area (Mantel's R = 0.61, $P < 0.0001$; Figure 2.7A). When we decomposed this pairwise regression, however, the different slopes we observed provide evidence of an interaction effect between distance and the positions of the colonies relative to the 100th meridian (Figure 2.7B). The steeper slope among eastern colonies than among western colonies indicates the presence of a variable not included in the IBD model that either enhances the isolating effect of distance in the east and/or relieves the effect in the west. Additional support for this conclusion is the intermediate slope observed for colony pairs in which one colony is in the west while the other is in the east. These observations, along with our previous results of greater genetic differentiation and isolation in the east, gave us grounds to investigate the effect(s) of landscape and/or climatic features on connectivity across our study area, in attempts to pinpoint the most likely mechanism(s) driving decreased movement in the east.

Landscape genetics

From our single feature IBR models, none of the landscape models with continuous coding performed well enough to include in our model ranking procedure or in our multivariate models (Table 2.6A). In fact, only three single feature IBR models satisfied our model selection criteria: medium-high intensity development as a discrete barrier, agricultural development as a discrete barrier, and average annual precipitation as directly proportional to resistance. We combined these features to create multivariate models (Table 2.6A), and the results of our AIC_C model ranking procedure are shown in Table 2.6B. This procedure identified average annual precipitation as the most significant driver of connectivity patterns among colonies throughout our study area, even outperforming our IBD model.

Discussion

Our study is the first to use both nuclear and mitochondrial genetic variation to characterize movement patterns among prairie dog colonies along an environmental gradient. Our results demonstrate the resilience of prairie dogs to habitat loss and fragmentation; however, greater average annual precipitation predicted reduced connectivity among colonies east of the 100th meridian, suggesting that increased grassland productivity and vegetation heights may limit dispersal among colonies in mixed-grass prairie. We conclude that prairie dogs are a highly mobile species, but caution that precipitation extremes challenge prairie dogs' ability to maintain connectivity throughout their geographic range. Changing precipitation patterns could ultimately lead to a range shift or contraction for this species, as climate change scenarios predict that southwestern droughts and eastern precipitation increases will continue (Sala et al. 2000, Ceballos et al. 2010). To more accurately assess the likelihood of long-term persistence for prairie dogs throughout their current range, future studies should continue to investigate the influence of climatic variables on metapopulation dynamics across spatial scales.

Frequent movement among colonies west of the 100^{th} meridian

Similar to previous studies of gene flow among prairie dog colonies, our results suggest that emigration and immigration events occur frequently among colonies, particularly in the western portion of our study area. The western portion is composed almost entirely of shortgrass prairie, although mixed-grass prairie becomes more prevalent in the northwest. Global and pairwise F_{ST} values among our western colonies were similar to or slightly lower than F_{ST} values reported by most previous studies that quantified prairie dog genetic diversity using microsatellite loci (Antolin et al. 2006, Sacket et al. 2012). Two notable exceptions are Magle et al. (2010) and Jones and Britten (2010), who both reported remarkably high genetic differentiation among colonies in shortgrass prairie.

Although one should always proceed with caution when making comparisons among population genetic studies, ecological differences may explanations the disparate results. Colonies from Magle et al. (2010) were within the city limits of Denver, Colorado, a landscape with a much higher human population density than any of our regional locations. Denver is also located at a higher elevation than our study sites, which exposes prairie dogs to higher annual average precipitation and lower average minimum temperatures than our shortgrass prairie locations. Our landscape genetic analysis suggests that high precipitation predicts low intercolony connectivity, while previous studies of facultative torpor in prairie dogs implicate sufficiently low temperatures with extended periods of decreased above-ground activity (Harlow and Menkins 1986, Lehmer et al. 2006). While our sampling design precluded our inclusion of temperature in our IBR models, we suspect that temperature gradients also drive both fine and broad scale movement patterns in our study area.

Physiological responses of prairie dogs to decreased temperature may also explain the limited intercolony movement observed by Jones and Britten (2010). The authors sampled colonies from rural Montana, where prairie dogs experience longer, colder winters than prairie dogs within our study area (Lehmer et al. 2006). Aside from increased incidence and duration of torpor brought on by such weather, long winters also lead to shortened growing seasons, possibly leaving individuals with less time to prepare for or to make long-distance movements throughout the spring and summer. The warmer, shorter winters within southern prairie dog range allow for longer growing seasons and lower incidences of facultative torpor; however, it should be noted that drought conditions can also cause torpor in prairie dogs (Lehmer et al. 2006). Though speculative, these ideas provide interesting avenues for future investigations of factors influencing prairie dog movement. Taken together, the results from Magle et al. (2010), Jones and Britten (2010), and our study show that prairie dogs are sensitive to climatic variation and urban development. Disentangling the relative strengths of those pressures will require further study.

Genetic drift and inbreeding east of the 100th meridian

In the eastern portion of our study area, we found significant evidence of genetic drift among our colonies, indicating that gene flow occurs less frequently east of the 100th meridian than west. Global and pairwise F_{ST} values among our eastern colonies were similar to values reported in Denver and Montana, which imply that each of these areas lie within less connected, peripheral habitat for prairie dogs. However, while the individual-level statistic, HL, was significantly higher in the east, the population-level statistic, F_{IS}, was not. Previous studies have also investigated inbreeding among prairie dogs (Chesser 1983, Foltz and Hoogland 1983, Dobson et al. 1997, Trudeau et al. 2004; Winterrowd et al. 2005); however, the location of these studies, the molecular markers used, and the inbreeding indices reported vary widely, making overall conclusions about prairie dogs' ability to avoid inbreeding depression and the factors affecting that ability difficult to draw.

For our study, two possible scenarios could explain our inability to detect significant inbreeding at the population-level for geographically isolated eastern colonies. First, migration events among our eastern colonies may occur frequently enough to overcome inbreeding. Dispersal theory predicts that only one migrant per generation is necessary to prevent significant loss of genetic diversity at the population-level (Mills and Allendorf 1996). The evolutionary response to habitat loss and fragmentation is expected to be nonlinear, as populations initially respond by producing migrants capable of increasingly longer dispersal distances until they reach some physiological threshold, at which point dispersal propensity decreases dramatically (Hampe and Petit 2005, Colbert et al. 2012). Daley (1992) also suggested that, while geographically isolated colonies likely receive few migrants, those migrants may more easily integrate into existing social groups than migrants to large, densely populated colonies; however, further study is required to support that claim.

A second possible explanation could be the existence of a lengthy time lag between the geographic isolation of a colony and subsequent genetic isolation. Polygynous mating systems, such as the prairie dog system, are known to maintain outbred populations in other species for quite some time following geographic isolation (Storz 1999, Storz et al. 2001). As temporal effects could have major implications on management recommendations and predictions, future studies of prairie dog genetic diversity should consider including a temporal dimension to address this hypothesis.

Historical patterns of expansion from a single genetic refugium

Results from our investigation of cytochrome B diversity in the mitochondrial genome show that the prairie dog populations within our study area have expanded from a single genetic refugium following the last glacial maxima. To our knowledge, little work has been done to characterize the biogeographic history of prairie dogs. Goodwin (1995) utilized the relatively complete fossil record available for prairie dogs to create maps of range expansions and contractions for the species through time. From his work, we know that the oldest known prairie dog fossils were found within the western portion of our study area and, further, that prairie dogs have consistently resided in our study area since their first appearance in the fossil record. Goodwin also reported that, while black-tailed prairie dog range expanded and contracted along its southern edge during the Pleistocene, there is no fossil record of black-tailed prairie dogs residing above approximately the north 42^{nd} parallel prior to the last glacial maximum. This absence from the fossil record suggests that the expansion of black-tailed prairie dogs into the northern United States and Canada is a relatively recent event. Further study could provide additional insights into the timing of northern expansion, as well as expectations for prairie dog persistence in the east in light of projected precipitation changes in the region (Hampe and Petit 2005).

Gene flow predicted by precipitation patterns across our study area

Of all the variables we tested in our landscape genetic analyses, including isolation-by-distance alone, our proxy for grassland productivity, average annual precipitation, was best predictor of observed gene flow among our colonies. This result, when combined with evidence from previous studies of increasing geographic isolation and colony area in response to changing precipitation patterns (Lomolino and Smith 2001, Avila-Flores et al. 2012), convincingly demonstrate the strong influence of precipitation patterns on prairie dog movement frequency. In fact, results of our modeling procedure suggest that the influence of precipitation at broad scales supersedes any of the landscape variables previously implicated as drivers of prairie dog movement (Roach et al. 2001, Magle et al. 2010, Sackett et al. 2012); however, further study is necessary before we can draw conclusions about the relative influence of these variables on gene flow across scales.

It is also important to note that the strength of the influence of any variable can change depending on the scale of the investigation (Cushman and Landguth 2010a). While precipitation and grassland productivity drive patterns at broad scales, finer scale investigations of movement may show little influence of climatic variables and great influence of the landscape. We suggest that future regional-scale landscape genetic studies include both climatic and landscape variables within their candidate model set. In addition, we propose that these studies utilize a comparative or multi-scale study design to provide managers with both scale-specific and region-specific expectations for the influence of these variables on connectivity.

Conversation implications

While results from this study demonstrate the resilience of prairie dogs to various evolutionary pressures, we stress the importance of maintaining stepping stone colonies to preserve connectivity across prairie dog range, from its core in the shortgrass prairie to its periphery within mixed-grass prairie. Populations along the periphery face unique evolutionary challenges, and while some degree of genetic isolation allows for local adaptation to those challenges, complete isolation could lead to inbreeding depression and, ultimately, local extinction (Colbert et al. 2012). We propose an adaptive management strategy, in which managers monitor geographically isolated colonies for signs of inbreeding depression and consider intervention should it develop (McRae et al. 2012). Intervention measures could include translocation of prairie dogs from nearby active colonies or burning regimes to curb woody encroachment and vegetative overgrowth brought on by increased precipitation. Additionally, incentives for private landowners to sustain prairie dog colonies on their land could be considered, particularly in the portions of prairie dog range where little public land exists.

Aside from connectivity concerns, the long-term persistence of this species faces another challenge from sylvatic plague. Although we have equated genetic connectivity with prairie dog movement throughout this study, it is important to note that measures of gene flow only quantify effective dispersal patterns (Spear et al. 2010). In contrast, disease movement among populations does not require successful reproduction by migrants, only successful arrival. Consequently, our measures of genetic connectivity and factors that affect it may or may not limit the flow of disease across the landscape. Further, multiple mammalian species could spread plague or its vector, fleas, among colonies, so our concentration on visualizing prairie dog movement patterns may be too restrictive to make extrapolations about this particular disease system (Cully and Williams 2001, Collinge et al. 2005). That being said, genetic connectivity does give us a minimum measure of intercolony movement on which to base predictions of disease spread. Our results suggest that limited movement among colonies within mixed-grass prairie may partly explain why plague dynamics do not spread east of the 100th meridian (Lomolino et al. 2004). We emphasize the importance of further investigations of movement of plague through the landscape, as understanding its limitations will allow researchers and managers to make more informed projections of prairie dogs' long-term persistence across North America's grasslands.

Acknowledgements

We thank the Jack H. Berryman Institute, The Nature Conservancy, the United States Fish and Wildlife Service's Great Plains Landscape Conservation Cooperative, and the United States Geological Survey for their financial support of this project. For land access privileges, we thank the National Park Service, the National Forest Service, and various private landowners throughout central and eastern Kansas. In particular, we extend our gratitude to the staff of Comanche National Grassland, Cimarron National Grassland, Kiowa-Rita Blanca National Grassland, Fort Larned National Historic Site, and Bent's Old Fort National Historic Site for allowing our field crew to live on-site. We also thank the Kansas Cooperative Fish and Wildlife Research Unit and the DNA Sequencing and Genotyping Facility at Kansas State University, the Molecular Ecology Laboratory at the University of Florida, the AGTC Sequencing Center at the University of Kentucky, and USDA APHIS for their technical support. This project would not have been possible without the assistance of our colleagues Amanda Goldberg, Claudia Ganser, Paige Harper, John Hargrove, and numerous field and laboratory technicians.

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Table 2.1: Geographic locations and names of colonies sampled from each of our 14 sampling locations, 2010-2012. Numbers at left correspond to the locations in Figure 2.1. Shaded colonies were inadequately sampled and not included in analyses.

	Locations	Coordinates	Colony Name	Number Sampled	Tissue Type	Year
1	Scotts Bluff National Monument	41.852196 N 103.71806 W	Scotts Bluff	20	Ear	2010
2	Sand Creek Massacre National Historic Site	38.549910 N 102.49291 W	Sand Creek	2	Ear	2010
3	Bent's Old Fort National Historic Site	38.790389 N 103.41845 W	Bent's Fort	20	Ear	2010
4	Comanche National Grassland	37.210663 N 102.94990 W	Oklarado	20	Ear	2010
		37.239602 N 102.93876 W	South Fork	21	Ear	2010
		37.249332 N 102.91531 W	SN Ranch	26	Ear	2010
		37.291788 N 102.70682 W	Liberty	31	Ear	2010
		37.312052 N 102.66911 W	Three Awn	21	Ear	2010
		37.277875 N 102.66063 W	Lonestar	22	Ear	2010
		37.065583 N 102.57350 W	Hallmark 2	27	Ear	2010
		37.062280 N 102.54864 W	Hallmark 1	20	Ear	2010
		37.033736 N 102.54752 W	Ute Canyon	22	Ear	2010
5	Kiowa-Rita Blanca National Grasslands	36.520688 N 103.05877 W	KW 46	25	Ear	2012
		36.524449 N 103.05284 W	KW 43	22	Ear	2012

		36.507508 N 103.02630 W	KW 44	25	Ear	2012
		36.533302 N 102.72571 W	RB 128	23	Ear	2012
		36.523192 N 102.71315 W	RB 126	24	Ear	2012
		36.558274 N 102.67620 W	RB 132	15	Ear	2012
		36.387952 N 102.73027 W	RB 23	1	Ear	2012
		36.388185 N 102.66858 W	RB 33	25	Ear	2012
		36.334863 N 102.68334 W	RB 37	27	Ear	2012
6	Cimarron National Grassland	36.995051 N 102.03081 W	State Line	20	Ear	2011
		37.203615 N 102.03082 W	Pasture 47	35	Ear	2010
		37.212956 N 101.99535 N	Pasture 7	22	Ear	2010
		37.257415 N 101.96971 W	North Fork	22	Ear	2010
		37.168721 N 101.80209 W	Pasture 81	20	Ear	2011
		31.175974 N 101.78694 W	RD N15	23	Ear	2011
		37.193706 N 101.70507 W	North Lowe	20	Ear	2011
		37.094075 N 101.62231 W	RD 735	18	Ear	2011
		37.096852 N 101.60804 W	RD 734	20	Ear	2011
		37.094245 N 101.56822 W	RD 733	18	Ear	2011

7	Logan County, Kansas	38.981222 N 101.44653 W	BD Highway	9	Tail	2009
		38.971611 N 101.40899 W	BD East Road	15	Tail	2009
		38.977111 N 101.42671 W	BD North Highway	15	Tail	2009
		38.900361 N 101.31017 W	HD Boys Northwest	13	Tail	2009
		38.896472 N 101.29903 W	HD Boys Northeast	23	Tail	2009
		38.887528 N 101.30369 W	South Boys	29	Tail	2009
		38.812750 N 101.15267 W	HD Northeast	32	Tail	2009
		38.780694 N 101.14578 W	BT Northeast	25	Tail	2009
		38.790389 N 101.17983 W	HD Schoolhouse	29	Tail	2009
		38.790000 N 101.20639 W	HD Lone Butte	30	Tail	2009
		38.855833 N 100.98481 W	TNC West	24	Tail	2009
		38.850833 N 100.97100 W	TNC South Trap	30	Tail	2009
		38.884750 N 100.96450 W	TNC North Long	16	Tail	2009
		38.833944 N 100.93086 W	TNC East	32	Tail	2009
8	WaKeeny, Kansas	38.924620 N 99.893460 W	WaKeeny	30	Muscle	2012
9	Minneapolis, Kansas	39.234500 N 97.526370 W	Minneapolis	30	Muscle	2011
10	Fort Larned National	38.121910 N	Fort Larned	20	Ear	2010

	Historic Site	99.239185 W				
11	Macksville, Kansas	37.929950 N 98.936920 W	Macksville	30	Muscle	2011
12	Norwich, Kansas	37.511300 N 97.850750 W	Norwich	18	Muscle	2011
13	Anthony, Kansas	37.141061 N 98.091731 W	Anthony	9	Muscle	2011
14	Hardtner, Kansas	37.015000 N 98.706470 W	Hardtner	16	Muscle	2011

Table 2.2: Allelic richness, heterozygosity, and inbreeding coefficients reported for 19 microsatellite loci in 1096 prairie dogs. Shaded loci violated expectations of HW equilibrium.

Locus	Number of Alleles	Size Range	H_E	H_O	F_S	Source
A2	14	220-248	0.723 (0.011)	0.744 (0.016)	-0.029 (0.018)	Jones et al. 2005
A8	13	265-291	0.657 (0.016)	0.704 (0.020)	-0.076 (0.023)	
A104	10	189-207	0.735 (0.013)	0.759 (0.019)	-0.033 (0.018)	
A111	10	181-199	0.635 (0.016)	0.657 (0.020)	-0.033 (0.020)	
A115	9	189-205	0.702 (0.013)	0.719 (0.016)	-0.027 (0.015)	
A119	10	111-133	0.692 (0.012)	0.837 (0.022)	-0.208 (0.022)	
C116	14	190-242	0.744 (0.016)	0.630 (0.021)	0.149 (0.026)	
D1	7	192-216	0.670 (0.012)	0.747 (0.024)	-0.111 (0.025)	
D2	8	300-328	0.681 (0.022)	0.705 (0.028)	-0.037 (0.024)	
D6	6	186-206	0.558 (0.013)	0.626 (0.023)	-0.118 (0.028)	
D12	7	204-228	0.684 (0.010)	0.784 (0.018)	-0.140 (0.019)	
D115	9	193-225	0.682 (0.012)	0.778 (0.020)	-0.136 (0.021)	
GS14	17	241-275	0.756 (0.009)	0.650 (0.024)	0.141 (0.030)	Stevens et al. 1997
IGS-1	9	103-119	0.673	0.717	-0.058	May et al. 1997

			(0.021)	(0.030)	(0.024)	
A105	6	204-216	0.606 (0.015)	0.612 (0.021)	-0.002 (0.022)	Sackett et al. 2009
A109	9	324-346	0.547 (0.025)	0.572 (0.028)	-0.046 (0.018)	
C101	15	300-356	0.770 (0.018)	0.788 (0.022)	-0.025 (0.018)	
D109	17	401-489	0.691 (0.020)	0.706 (0.026)	-0.013 (0.023)	
TAGA27	9	220-252	0.663 (0.021)	0.691 (0.027)	-0.037 (0.019)	

H_E =average estimated heterozygosity across populations; H_O = average observed heterozygosity across populations; $F_S = (H_E-H_O)/H_E$

Table 2.3: Landscape and climatic variables used in isolation-by-resistance models. We include our hypothesized effect of each variable on gene flow among colonies, the source(s) of that hypothesis, the alternate hypothesis, the GIS layers used to create the model(s), and the resistance values assigned to the variable in question. If the univariate model supported both our hypothesis and the alternate hypothesis for a variable, we discarded at a statistical nuisance and did not include it in multivariate models. We show discarded variables below in grey. (H = hypothesis; *AH = alternate hypothesis*)

Variable	Hypothesis	Hypothesis Source(s)	GIS Layer Source
Landscape variables:			
Categorical coding → presence/absence:	***barrier = 100 Ω; corridor = 1 Ω; other = 50 Ω****		
Continuous coding → density rescaled:	***barrier = 50-100 Ω; corridor = 1-50 Ω****		
Stream Networks	H1: ephemeral and intermittent streams = corridors	Garrett and Franklin 1988; Roach et al. 2001	National Hydrography Dataset
	H2: permanent streams = barriers		
	AH1: ephemeral and intermittent streams = barriers		
	AH2: permanent streams = corridors		
Urban Development	H1: low intensity development = corridor	Magle et al. 2010; Sackett et al. 2012	National Landcover Dataset 2006
	H2: medium-high intensity development = barrier		
	AH1: low intensity development = barrier		
	AH2: medium-high intensity development = corridor		
Agricultural Development	H: agricultural lands = barriers	Tilman et al. 2001	
	AH: agricultural lands = corridors		
Climate variable:			
Continuous coding →	***rescaled to 1 – 100 Ω*, depending on direction of hypothesized effect***		

Average Annual Precipitation	H: precipitation amount is directly proportional to resistance *AH: precipitation amount is inversely proportional to resistance*	Avila-Flores et al. 2012	PRISM Climate Group 1981-2010 Annual Normals

* an ohm (Ω) is a unit of resistance

Table 2.4: Indices of genetic diversity and isolation for each colony from which we genotyped >8 individuals at the 19 microsatellite loci shown in Table 2.2. The standard error of each estimate is given in parentheses. Numbers at the far left corresponds to sampling locations shown in Figure 2.1.

	Colony	N	N_A	H_O	H_E	F_S
1	Scott's Bluff	20.00	4.526	0.566	0.591	0.048
		(0.00)	(0.23)	(0.05)	(0.03)	(0.05)
3	Bent's Fort	20.00	4.895	0.726	0.668	-0.087
		(0.00)	(0.32)	(0.03)	(0.02)	(0.04)
4	Comanche					
	Oklarado	19.95	5.421	0.675	0.674	0.003
		(0.05)	(0.26)	(0.04)	(0.03)	(0.04)
	South Fork	21.00	5.000	0.639	0.600	-0.070
		(0.00)	(0.35)	(0.05)	(0.04)	(0.04)
	SN Ranch	25.95	5.474	0.706	0.689	-0.031
		(0.05)	(0.30)	(0.03)	(0.02)	(0.03)
	Liberty	28.84	5.947	0.691	0.683	-0.007
		(0.16)	(0.35)	(0.04)	(0.02)	(0.04)
	Three Awn	21.00	5.947	0.689	0.710	0.029
		(0.00)	(0.40)	(0.04)	(0.02)	(0.04)
	Lonestar	21.95	5.368	0.669	0.666	-0.007
		(0.05)	(0.37)	(0.03)	(0.02)	(0.04)
	Hallmark 2	27.00	5.474	0.682	0.660	-0.028
		(0.00)	(0.35)	(0.04)	(0.03)	(0.04)
	Hallmark 1	19.95	5.421	0.734	0.680	-0.072
		(0.05)	(0.40)	(0.04)	(0.03)	(0.04)
	Ute Canyon	22.00	5.895	0.667	0.679	0.030
		(0.00)	(0.31)	(0.04)	(0.02)	(0.04)
5	Kiowa-Rita Blanca					
	KW 46	24.95	6.368	0.749	0.737	-0.019
		(0.05)	(0.43)	(0.04)	(0.04)	(0.04)
	KW 43	22.00	6.053	0.739	0.712	-0.037
		(0.00)	(0.40)	(0.05)	(0.03)	(0.05)

	KW 44	24.95	5.632	0.789	0.703	-0.124
		(0.05)	(0.44)	(0.03)	(0.02)	(0.04)
	RB 128	23.00	5.947	0.785	0.731	-0.080
		(0.00)	(0.39)	(0.04)	(0.02)	(0.04)
	RB 126	24.00	6.368	0.787	0.719	-0.097
		(0.00)	(0.37)	(0.03)	(0.02)	(0.03)
	RB 132	14.84	5.684	0.783	0.716	-0.106
		(0.09)	(0.45)	(0.04)	(0.27)	(0.05)
	RB 33	27.00	6.263	0.762	0.726	-0.050
		(0.00)	(0.04)	(0.04)	(0.04)	(0.05)
	RB 37	25.00	6.053	0.735	0.707	-0.036
		(0.00)	(0.37)	(0.05)	(0.02)	(0.06)
6	Cimarron					
	State Line	20.00	5.842	0.721	0.702	-0.027
		(0.00)	(0.35)	(0.03)	(0.03)	(0.03)
	Pasture 47	34.95	5.789	0.715	0.698	-0.029
		(0.05)	(0.03)	(0.03)	(0.02)	(0.04)
	Pasture 7	20.00	5.158	0.737	0.692	-0.062
		(0.00)	(0.33)	(0.03)	(0.02)	(0.03)
	North Fork	22.00	6.000	0.742	0.721	-0.033
		(0.00)	(0.34)	(0.03)	(0.02)	(0.03)
	Pasture 81	20.00	5.789	0.697	0.685	-0.023
		(0.00)	(0.35)	(0.03)	(0.02)	(0.03)
	RD N15	24.95	5.947	0.736	0.710	-0.037
		(0.05)	(0.30)	(0.03)	(0.02)	(0.04)
	North Lowe	20.00	6.000	0.737	0.710	-0.050
		(0.00)	(0.34)	(0.02)	(0.03)	(0.03)
	RD 735	18.00	5.737	0.737	0.692	-0.066
		(0.00)	(0.30)	(0.03)	(0.02)	(0.03)
	RD 734	19.95	5.789	0.757	0.724	-0.049
		(0.05)	(0.24)	(0.03)	(0.02)	(0.04)
	RD 733	17.95	5.158	0.713	0.678	-0.059
		(0.05)	(0.25)	(0.03)	(0.32)	(0.05)

7	Logan County					
	BD Highway	8.947	5.105	0.752	0.699	-0.080
		(0.05)	(0.03)	(0.04)	(0.02)	(0.05)
	BD East Road	15.00	3.522	0.698	0.701	-0.004
		(0.00)	(0.19)	(0.03)	(0.02)	(0.04)
	BD North	15.00	5.737	0.747	0.705	-0.064
		(0.00)	(0.34)	(0.03)	(0.02)	(0.04)
	HD Boys Northwest	13.00	5.421	0.713	0.697	-0.023
		(0.00)	(0.33)	(0.04)	(0.04)	(0.04)
	HD Boys Northeast	22.95	5.789	0.739	0.712	-0.037
		(0.05)	(0.33)	(0.03)	(0.02)	(0.03)
	South Boys	28.95	6.053	0.760	0.719	-0.065
		(0.05)	(0.45)	(0.03)	(0.02)	(0.05)
	HD Northeast	32.00	6.263	0.745	0.721	-0.037
		(0.00)	(0.40)	(0.02)	(0.01)	(0.03)
	BT Northeast	25.00	5.842	0.771	0.728	-0.062
		(0.00)	(0.34)	(0.03)	(0.01)	(0.04)
	HD Schoolhouse	28.95	6.105	0.720	0.706	-0.024
		(0.05)	(0.37)	(0.03)	(0.02)	(0.04)
	HD Lone Butte	29.95	5.579	0.743	0.714	-0.040
		(0.05)	(0.28)	(0.02)	(0.01)	(0.02)
	TNC West	23.95	5.895	0.767	0.716	-0.082
		(0.05)	(0.30)	(0.03)	(0.02)	(0.04)
	TNC North Long	16.00	5.632	0.760	0.690	-0.103
		(0.00)	(0.36)	(0.03)	(0.02)	(0.03)
	TNC East	30.74	6.368	0.749	0.714	-0.054
		(0.17)	(0.43)	(0.02)	(0.02)	(0.03)
8	WaKeeny	30.00	3.895	0.661	0.582	-0.145
		(0.00)	(0.26)	(0.04)	(0.03)	(0.04)
9	Minneapolis	30.00	5.211	0.609	0.630	0.027
		(0.00)	(0.36)	(0.03)	(0.03)	(0.03)
10	Fort Larned	20.00	5.316	0.637	0.678	0.069
		(0.00)	(0.38)	(0.04)	(0.03)	(0.04)

11	Macksville	29.84	5.421	0.728	0.669	-0.094
		(0.16)	(0.40)	(0.03)	(0.02)	(0.04)
12	Norwich	18.00	2.474	0.406	0.409	0.001
		(0.00)	(0.21)	(0.05)	(0.05)	(0.04)
13	Anthony	9.000	3.368	0.544	0.496	-0.068
		(0.00)	(0.19)	(0.07)	(0.05)	(0.06)
14	Hardtner	16.00	3.211	0.503	0.445	-0.116
		(0.00)	(0.37)	(0.07)	(0.06)	(0.06)

Indices are: N = number of genotyped individuals (averaged over all loci); N_A = average allelic richness; H_E =average expected heterozygosity; H_O = average observed heterozygosity; $F_S = (H_E-H_O)/H_E$

Table 2.5: Results from analysis of molecular variance (AMOVA; Arlequin). Based on the results of our PCoA (Figure 2.2) and structure analysis (Figure 2.3), we merged locations 4-6 (Figure 2.1) into one group. Otherwise, we coded each sampling location as a unique group in the AMOVA.

Source of variation	Sum of Squares	Variance Components	Percentage Variation
Among groups	755.522	0.441	5.89
Among populations within groups	1020.564	0.426	5.68
Among individuals within populations	6793.515	-0.127	-1.70
Within individuals	7390.000	6.753	90.12
Total	15959.600	7.747	

Average F-statistics over all loci: F_{IS} = -0.01917; F_{SC} = 0.06036; F_{CT} = 0.05891; F_{IT} = 0.09876

Table 2.6: Results from isolation-by-resistance (IBR) model selection and evaluation procedures. Results from models containing a single landscape or climate feature are shown in (A). Single-feature IBR models that met our criteria for inclusion in multivariable landscape and climate model development and AIC$_C$ model evaluation are shown in bold. Results from AIC$_C$ model ranking of all acceptable IBR models and our isolation-by-distance model are shown in (B).

A.

Model		Mantel's R, *p≤*	Partial Mantel, *p≤*	Model Abbrev.
Single landscape or climate variable:				
Ephemeral & Intermittent Streams:				
Categorical:	Corridors	0.197, *0.063*		
	Barriers	0.439, *0.0001*	0.034, *0.399*	
Continuous:	Corridors	0.554, *0.0001*		
	Barriers	0.543, *0.0001*		
Permanent Streams:				
Categorical:	Barrier	0.565, *0.0001*	R = 0.206, *0.076*	
	Corridor	-0.174, *0.114*		
Continuous:	Barrier	0.600, *0.0001*		
	Corridor	0.486, *0.0001*		
Agricultural Development:				
Categorical:	**Barrier**	**0.585, *0.0001***	**R = 0.227, *0.028***	**AgriB**
	Corridor	-0.041, *0.465*		
Continuous:	Barrier	0.581, *0.0001*	R = 0.209, *0.072*	

Corridor	0.227, *0.029*		
Low Intensity Urban Development:			
Categorical: Corridors	0.722, *0.0001*		
Barriers	0.423, *0.0001*		
Continuous: Corridors	0.552, *0.0001*		
Barriers	0.557, *0.0001*		
Medium-High Intensity Urban Development:			
Categorical: Barrier	**0.658, *0.0001***	**R = 0.530, *p* = *0.0001***	**HDevB**
Corridor	-0.138, *0.130*		
Continuous: Barrier	0.562, *0.0001*		
Corridor	0.546, *0.0001*		
Average Annual Precipitation:			
Directly Proportional to Resistance	**0.805, *0.0001***	**R = 0.702, *p* = *0.0001***	**PrecipD**
Indirectly Proportional to Resistance	0.226, *0.022*		
Multivariate landscape and climate models:			
Agricultural Development: Barrier + Medium-High Intensity Urban Development: Barrier	0.589, *0.0001*	R = 0.261, *p* = *0.005*	AgriB + HDevB
Agricultural Development: Barrier + Precipitation: Direct	0.552, *0.0001*	R = 0.267, *p* = *0.009*	AgriB + PrecipD
Medium-High Intensity Urban Development: Barrier + Precipitation: Direct	0.722, *0.0001*	R = 0.559, *p* = *0.0001*	HDevB + PrecipD
Agricultural Development: Barrier +	0.600, *0.0001*	R = 0.279, *p* = *0.003*	Full Model

Medium-High Intensity Urban Development: Barrier +
Precipitation: Direct

B.

Model Abbrev.	K	AIC_C	ΔAIC_C	Relative Likelihood	Akaike ω	Conclusion
PrecipD	2	-54.55	0	1	0.970	substantial support
HDevB + PrecipD	3	-45.83	-8.71	0.013	0.012	considerably less support
Distance Only	1	-44.84	-9.71	0.008	0.008	considerably less support
HDevB	2	-44.61	-9.94	0.007	0.007	considerably less support
AgriB	2	-41.51	-13.04	0.001	0.001	essentially no support
AgriB + HDevB	3	-39.35	-15.20	0.0005	0.0005	essentially no support
AgriB + PrecipD	3	-38.06	-16.49	0.0003	0.0002	essentially no support
Full Model	4	-37.40	-17.15	0.0002	0.0002	essentially no support

Figure 2.1: Map of study area, showing our 14 sampling locations (red points) relative to the 100th meridian (white line) and average annual precipitation (blue gradient). We sampled one colony per location, except at locations 4-7, where multiple colonies were sampled. Map insert shows the position of our study area (red rectangle) within the known range of black-tailed prairie dogs (cream area).

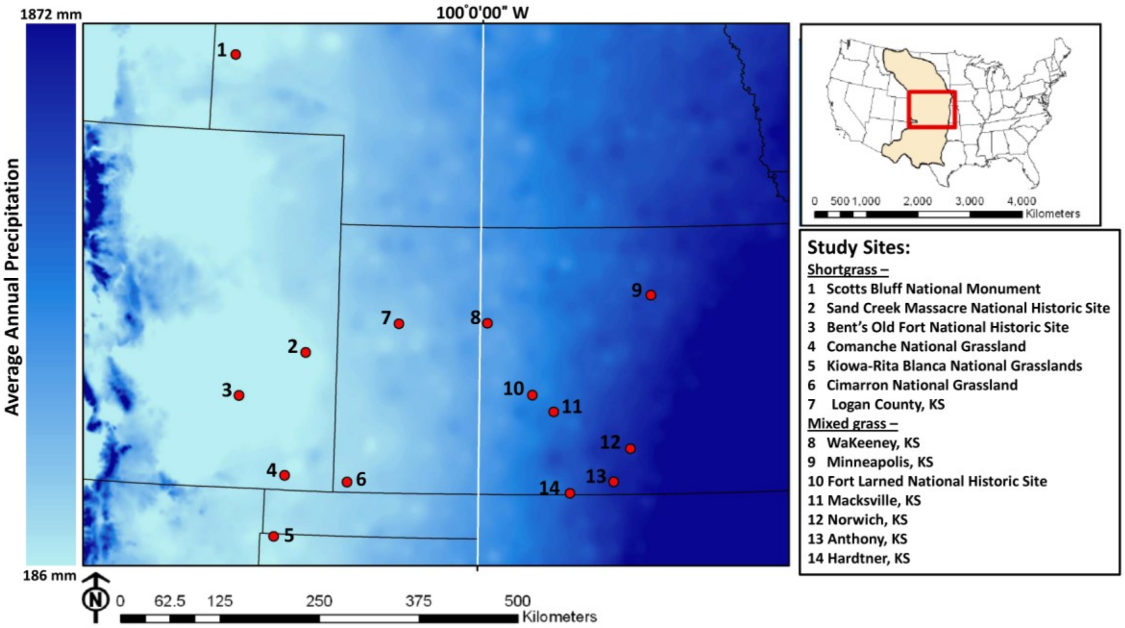


Figure 2.2: Principal coordinate analysis (PCoA) using allelic richness observed within each colony. We depict colonies found west of the 100th meridian in green and eastern colonies in red. Numbers correspond to one of 9 sampling locations shown on Figure 2.1 in which we sampled only one colony. We encircled locations in which we sampled multiple colonies (locations 4-7).

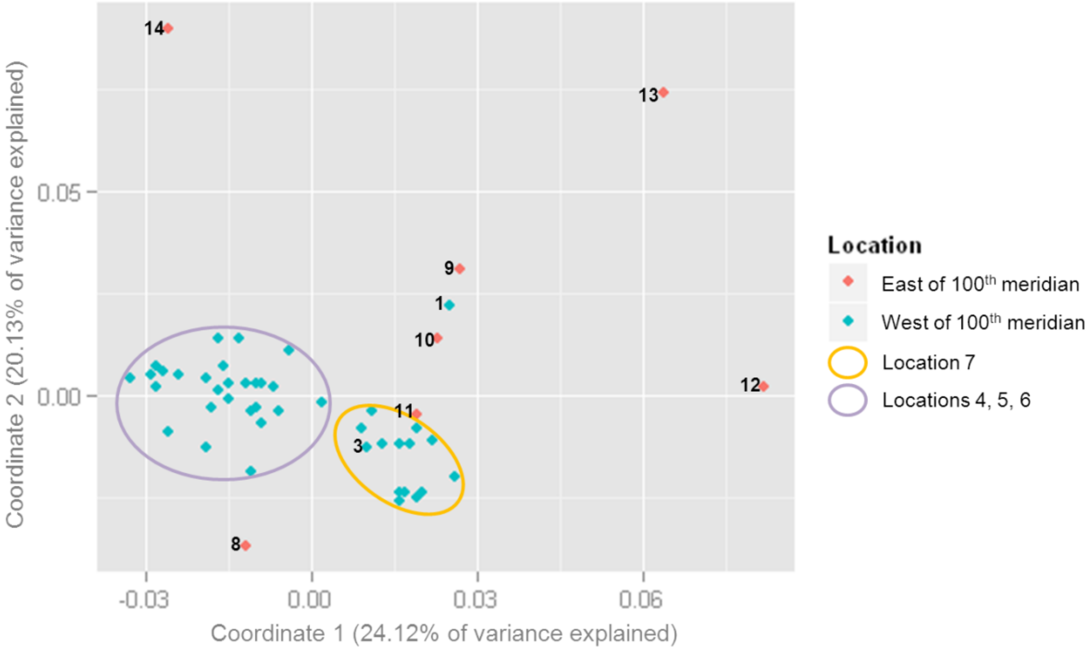


Figure 2.3: Results from a Bayesian clustering analysis in program STRUCTURE, showing a rapidly ascending mean estimate of the likelihood probability (A), Delta K (B), and the STRUCTURE barplot for K = 2 (C) for comparison to Figure 2.2. Numbers on the barplot (C) correspond to our 14 sampling locations, shown in Figure 2.1.

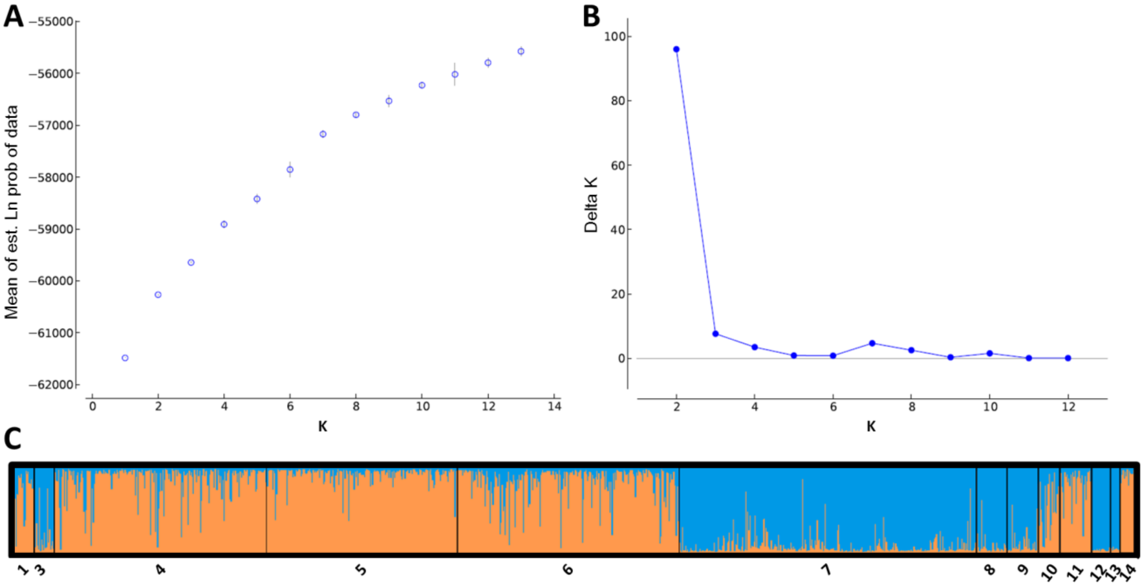


Figure 2.4: Haplotype network from Program Network, suggesting all sequenced individuals descended from a single genetic refugium. Node size corresponds to the number of individuals possessing the haplotype, while colors within the pie chart depict the location of individuals possessing that haplotype relative to the 100th meridian. Internode length corresponds to the number of base-pair mutations separating each haplotype.

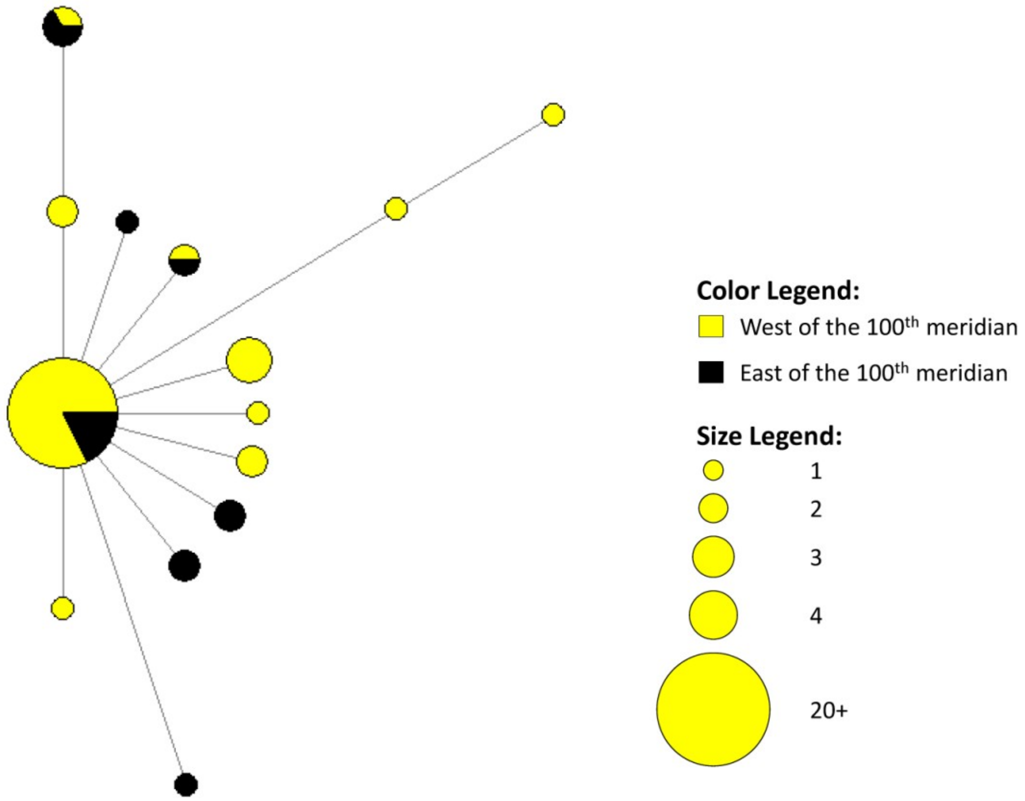


Figure 2.5: Homozygosity by loci (HL) within each sampling location relative to average annual precipitation from 1981 to 2010 (mm; PRISM Climate Group). Numbers assigned to locations correspond to those shown in Figure 2.1.

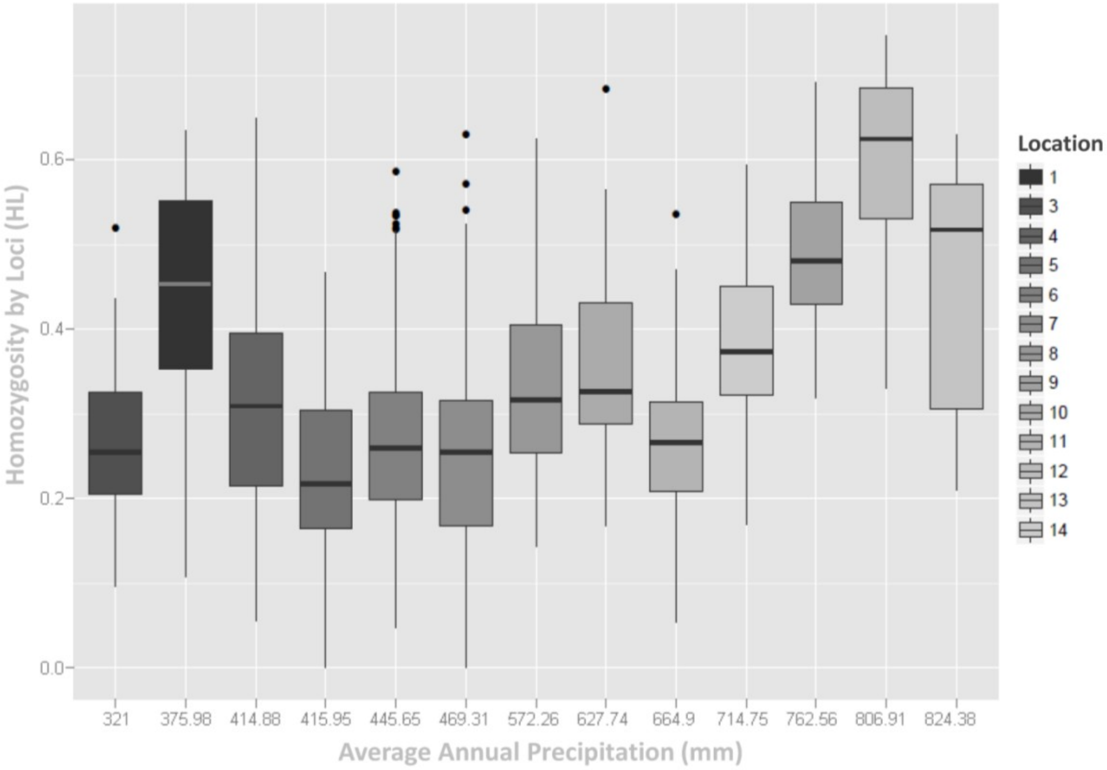


Figure 2.6: Heatmap of F_{ST} values separating all colony pairs in our study area (Linearized F_{ST}; Slatkin 1995). Cells containing a dash (-) distinguish insignificant values ($P > 0.05$). Locations in which we sampled more than one colony are labeled by name along the axes, while locations in which we sampled only one colony are labeled by number (see Figure 2.1).

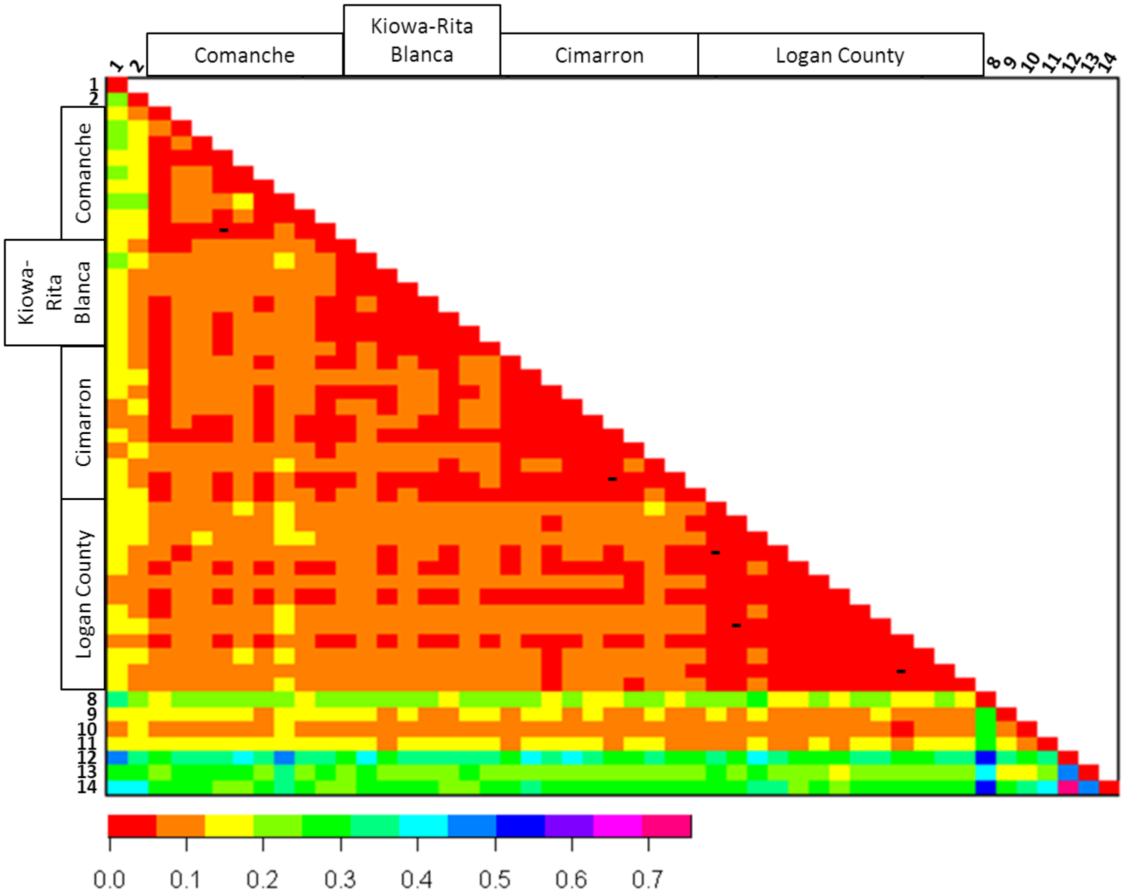


Figure 2.7: IBD model (A) and a decomposed pairwise regression of that model (B), demonstrating a possible interaction effect between geographic distance and the location of colony pairs relative to the 100th meridian.

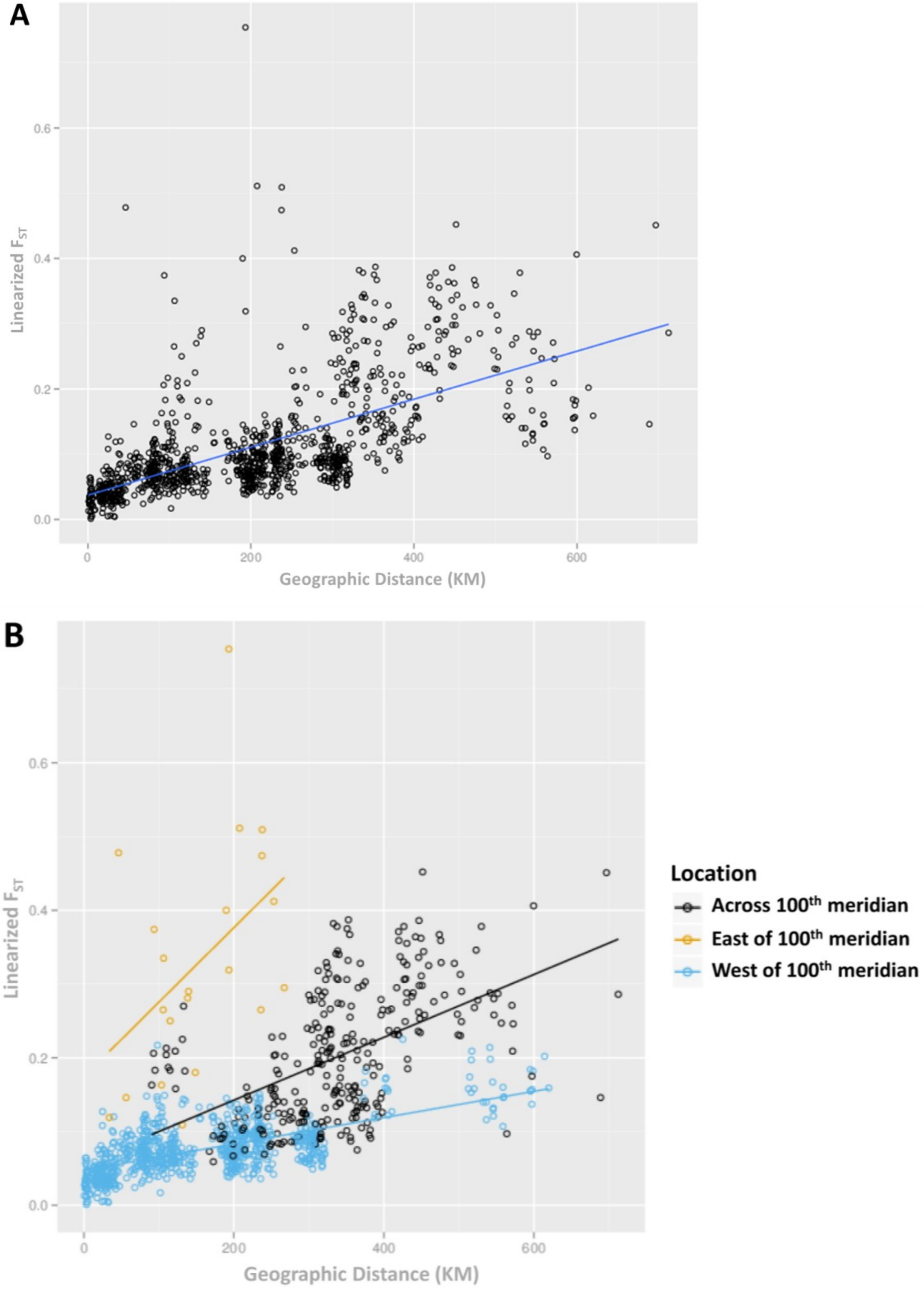
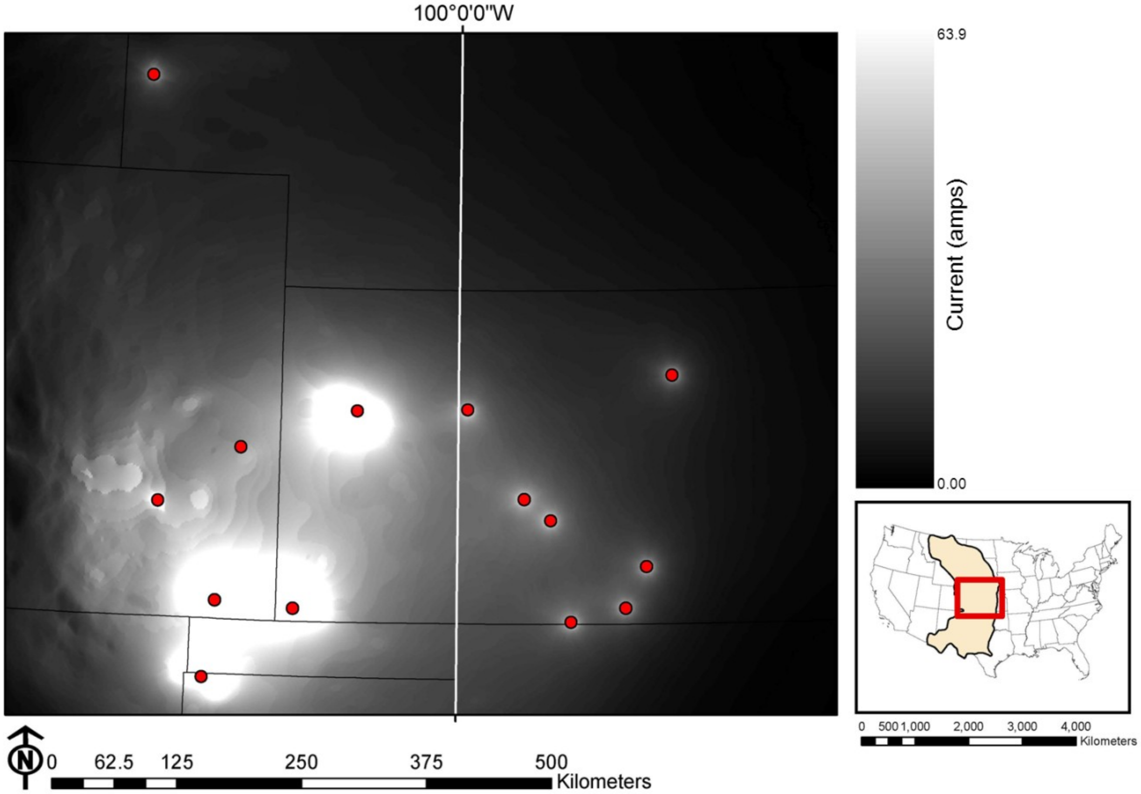


Figure 2.8: Current map of our best IBR model, according to results from our AIC$_c$ model ranking procedure (Table 2.6B). This model depicts effects of both precipitation and distance on connectivity among our 14 locations (red points). Lighter areas correspond to areas of high connectivity, while dark areas correspond to areas of low connectivity. For location identity, see Figure 2.1.



Chapter 3 - Little evidence of sex-bias or landscape influence on intercolonial dispersal within black-tailed prairie dog colony complexes

Rachel M. Pigg, Samantha M. Wisely, and Jack F. Cully, Jr.

Abstract

Metapopulation stability and structure is maintained by effective dispersal among populations. Here, we used a genetic approach to quantify effective dispersal among populations of black-tailed prairie dogs (*Cynomys ludovicianus*) throughout four distinct management units in the western Great Plains. We report the spatial extent of gene flow within these areas, as well as the influence of sex and landscape features on gene flow among populations. Our results reveal extensive gene flow among prairie dog colonies separated by great distances (40 - 50 km) and limited influence of isolation-by-distance or landscape features on effective dispersal. We also observed scale-dependent male-biased dispersal, with female relatives clustered locally, but dispersing with equivalent propensity as males at broader spatial scales. As local scale observations were not predictive of dispersal dynamics across scales, this study illustrates the utility of a multi-scale approach when describing the dispersal behavior of a species.

Introduction

A metapopulation is a group of spatially clustered populations that regularly exchange migrants (Hanski 1999). Migrants maintain gene flow among populations, which prevents inbreeding and recolonizes areas after a local extinction event. However, gene flow can also negatively affect a metapopulation by causing rapid spread of disease and preventing local adaptation. Consequently, metapopulation dynamics are complex and difficult to monitor and predict, yet essential to the regional survival of species existing in metapopulations.

In the western Great Plains, black-tailed prairie dogs (*Cynomys ludovicianus*; hereafter, "prairie dogs") are a keystone species of the prairie ecosystem that currently exist in metapopulations (Kotliar et al. 1999). Locally, prairie dogs are found in spatially isolated populations called colonies. Colonies regularly exchange migrants, creating metapopulations called colony complexes; however, multiple factors threaten the long-term persistence of colonies and, by extension, complexes. First, recreational shooting, poisoning, and other culling methods occur frequently on private and public lands throughout prairie dog range (Hoogland 2006). Such culling is meant to mitigate disturbance to cropland caused by prairie dogs and/or prevent possible competition between prairie dogs and cattle; however, such practices are likely unnecessary (Augustine and Springer 2013) and potentially harmful to the regional persistence of prairie dogs and other species that require their presence to thrive (Kotliar et al. 1999). A second concern for prairie dog metapopulation stability is habitat alteration due to increasing agricultural and urban development within prairie dog range (Hoogland 2006). Habitat loss and fragmentation may disrupt dispersal among colonies and/or eliminate key stepping stones within the population network, making migrant exchange over large geographic distances increasingly infrequent. Last, and perhaps of greatest concern, prairie dogs are highly susceptible to the exotic disease sylvatic plague (Cully and Williams 2001). Plague was introduced to North American from Asia around 1900 and has caused local extinctions of colonies for at least 70 years. On an ~10-year cycle, plague erupts across a colony complex, causing synchronized extinction events throughout the metapopulation. Neither the cause of these epizootic events, the identity of the reservoir host(s), or the agent responsible for spreading plague among colonies is currently known, although some environmental correlates have been identified (Gage and Kosoy 2005, Cully et al. 2006).

Colonies that survive these threats may still be at risk. Local extinctions throughout the region intensify the geographic isolation of surviving colonies. If geographic isolation leads to genetic isolation, inbreeding depression could result, which carries its own extinction risk (Lacy 1997, Saccheri et al. 1998). Some researchers have proposed sex-biased dispersal as a means to avoid inbreeding depression (Pusey 1987). Male or female-biased dispersal is observed in numerous taxa (Greenwood 1980, Lawson Handley and Perrin 2007). Among mammals, male-biased dispersal is common, particularly in polygynous mating systems where male reproduction is limited by mate access, while female reproduction is limited by resource access (Dobson 1982).

Prairie dogs are a polygynous mammal. Related females tend to cluster together locally in family groups called coteries, while males disperse among coteries within a colony (Hoogland 1995). Short distance, intracolonial dispersal is certainly male-biased; however, it is unknown whether intercolony dispersal exhibits the same sexual bias. Genetic analyses and telemetry data have detected adult female prairie dogs moving among colonies (Garrett and Franklin 1989, Roach et al. 2001); however, both the sample sizes and the geographic extent of these studies are limited, making overall conclusions about sex-biased dispersal at this scale difficult to discern.

Prairie dog metapopulation dynamics are well-documented throughout their current range (Antolin et al. 2006, Jones and Britten 2010, Magle et al. 2010, Sackett et al. 2012). Most recent studies use gene flow to indirectly estimate effective dispersal among colonies (Antolin et al. 2006). Here, the term "effective dispersal" describes permanent movement from one colony to another that results in successful reproduction at the new colony. Taken together, these studies suggest that prairie dogs move frequently among colonies; however, each study used different molecular markers and different metrics to quantify gene flow, making comparisons among studies difficult (Balloux and Lugon-Moulin 2002, Nybom 2004, Kalinowski 2005).

Only one previous study has quantified the spatial limit of gene flow within their study area (Jones and Britten 2010). Quantifying the spatial extent of relatedness among local populations, as well as identifying any barriers to gene flow, would allow prairie dog metapopulation dynamics to inform the spatial bounds of management units across prairie dog range. These units could then coordinate their monitoring, control, and/or conservation efforts to more effectively manage colonies in their area.

In this study, our aims were to describe general patterns of effective dispersal within black-tailed prairie dog colony complexes and to identify both intrinsic and extrinsic factors influencing those patterns. We replicated our study design in 4 colony complexes located near the geographic center of black-tailed prairie dog range (Figure 3.1). We then identified the spatial scale at which distinct prairie dog genetic neighborhoods emerge. Second, we used a multi-scale approach to determine whether the polygynous mating system of prairie dogs results in sex-biased dispersal patterns within and/or among colonies. Finally, we employed a landscape genetic approach to empirically test hypotheses concerning the effect of the landscape composition and configuration on colony genetic diversity and intercolonial connectivity.

Materials and Methods

Study areas

Our study areas consisted of four prairie dog colony complexes located near the geographic center of current prairie dog range (Figure 3.1). Three of our study areas are national grasslands, specifically the Carrizo Unit of Comanche National Grassland in Colorado, Kiowa-Rita Blanca National Grassland in New Mexico, Oklahoma, and Texas, and Cimarron National Grassland in SW Kansas. Our final study area in Logan County, Kansas contains both private land and land managed by The Nature Conservancy (Figure 3.1C).

Colonies are numerous throughout each of these study areas, making sampling all colonies impossible. Consequently, we developed a study design to address our specific objectives. Within each study area, we chose 3-4 circular sites, each 10-km in diameter. This distance corresponds to the longest observed dispersal distance travelled by an individual prairie dog (Antolin et al. 2006). Each circular site contained at least two active colonies, and site boundaries did not overlap. Further, we positioned our sites to create pairs of sites separated by 10-km and by 30-km within each study area (Figure 3.1). In study areas of sufficient size, we also created pairs of sites separated by 20-km and by 40-km. This systematic design ensured adequate sampling within each distance class for our autocorrelation analyses (described below).

Sample collection

We collected tissue samples from prairie dogs trapped in 2-4 active prairie dog colonies within each circular site (Figure 3.1). We acquired tissue samples by one of two methods. On colonies within national grasslands, we trapped prairie dogs using 80-100 single-door collapsible Tomahawk traps placed near active burrow entrances. While we evenly distributed these traps throughout small colonies, we chose to cluster traps at multiple trapping locations within larger colonies to ensure that our dataset adequately represented the genetic diversity of the entire colony, despite our limited trap number. For each trapped animal, we recorded trap location, sex, weight, and forearm length, and we collected an approximately 2-mm diameter ear clipping using surgical scissors or an ear punch (Med Vet International). Ear clippings were stored in 95% ethanol for later DNA extraction. All animal trapping and handling procedures followed guidelines established by the American Society of Mammalogists (Sikes et al. 2011) and were approved by Kansas State University's Institutional Animal Care and Use Committee (Protocol Number 2889).

We acquired tissue samples from prairie dogs within Logan County, Kansas in a different manner. With the cooperation of The Nature Conservancy (TNC) and the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS), we received frozen tail clippings and muscle tissue from culled prairie dogs throughout the county. Each tissue sample arrived labeled with a colony name. While a location for each colony was provided, each prairie dog's physical condition, sex, and specific location within a colony were unknown.

Molecular methods

Our DNA extraction procedures and multilocus microsatellite genotyping procedures are reported in Chapter 2. Also in Chapter 2, we describe our quality control methods and results for 19 previously characterized microsatellite loci (Sackett et al. 2009, Jones et al. 2005, Stevens et al. 1997, May et al. 1997).

Identifying the spatial extent of genetic neighborhoods

To determine the average genetic neighborhood size for prairie dogs within our study areas, we implemented a genetic autocorrelation analysis in program GenAlEx (v6.5; Peakall and Smouse 2006). Genetic neighborhood size is an approximation of the spatial extent of multi-generational movement patterns among prairie dog colonies.

The autocorrelation statistic (r) calculated by GenAlEx uses multilocus data to calculate a correlation coefficient ranging from -1 to 1, similar to Moran's *I*, and its calculation requires both pairwise genetic and geographic distance matrices. A negative value indicates genetic dissimilarity, while positive values indicate genetic similarity. In this study, we quantified pairwise genetic distance between colonies as Slatkin's linearized F_{ST}, calculated in Arlequin (v3.5.1.4; Excoffier et al. 2005). We then calculated the geographic distances among colonies using the Point Distance tool in ArcGIS (v10.1; ESRI). We used the geographic center of each colony to approximate the colony's location. Tests for significance have been previously described by Peakall et al. (2003). We utilized the random permutation procedure, with the number of permutations set to 10,000.

Comparison of male and female dispersal abilities

We again employed the genetic autocorrelation analyses and randomization procedures available in GenAlEx to compare the genetic neighborhood sizes of male and female prairie dogs. Further, we implemented a multi-scale approach to investigate whether sex-biased dispersal was scale-dependent by varying the bin size used in our autocorrelation analyses. First, we used a bin size of 0.1 km, followed by 1 km, and finally 10 km. If sex-biased dispersal occurs at any or all of these scales, one of two outcomes is possible: (1) the philopatric sex will display positive autocorrelation within the shortest distance class, while the dispersing class will not or (2) both sexes will display positive autocorrelation, but the philopatric sex will have a smaller genetic neighborhood than the dispersing sex. If no or negligible sexual bias is present, both sexes will display a similar genetic neighborhood size and structure. For all autocorrelation analyses, we used the Geographic Distance tool in GenAlEx to generate a matrix of geographic distances among individuals. Genetic distances among individuals were also quantified within GenAlEx, using a method designed for codominant loci described by Peakall (1995) and again by Smouse and Peakall (1999). To test for significance of observed autocorrelation, we again utilized a random permutation procedure, with 10,000 permutations.

Although the autocorrelation coefficient (r) calculated by GenAlEx is correlated with genetic relatedness, r cannot substitute for a relatedness measure (Banks et al. 2005, Temple et al. 2006). Consequently, we also calculated Queller & Goodnight's estimator of relatedness (1989) with GenAlEx. This statistics varies from -1 to 1. Values greater than zero indicate that pairs of individuals are closely related, while values less than 0 indicate that pairs are unrelated (Queller and Goodnight 1989). We again implemented a multi-scale approach, similar to our autocorrelation analyses. Using a one-tailed t-test with unequal variances, we compared relatedness among pairs of males and among pairs of females within 0.1 km, 0.1-1 km, and 1-10 km.

Landscape genetic analyses

As the efficacy of many analytical procedures commonly used in landscape genetic studies is currently under debated (Balkenhol et al. 2009, Bolliger et al. 2014), we used a hypothesis-driven, conservative approach to test various predictive models of the influence of landscape features and distance on effective dispersal among prairie dog colonies. We describe this approach in detail in Chapter 2, but highlight significant changes from Chapter 2's methodology below.

Based on results from analyses described above, this criterion limited our investigations to colonies separated by ≤ 60 km. This distance encompasses the spatial extent in which we observed significant genetic autocorrelation (~ 40-50 km) as well as a 10-20 km barrier region about that extent. Gene flow among colonies was quantified again by Slatkin's linearized F_{ST}. We used isolation-by-resistance (IBR) modeling to consider alternate hypotheses concerning the effect(s) of landscape features on prairie dog intercolonial movement patterns. IBR models were created as described in Chapter 2. All our models were categorically coded based on the presence or absence of the landscape feature of interest. We determined the location and distribution of landscape features within our study areas from both the 2006 National Landcover Dataset and the National Hydrograph Dataset. All models contained a ≥ 30 km buffer region surrounding our colonies to control for the influence of map boundary on pairwise resistance calculations (Koen et al. 2010). Cell size for all models was 150 m x 150 m, to ensure that we preserved as much landscape heterogeneity as possible, given the computational limitations of the Circuitscape program (v. 3.5.8; McRae et al. 2008). We assigned hypothesized barriers a resistance value of 100 ohms, whereas we assigned hypothesized corridors a resistance value of 1 ohm. All other cells were given an intermediate resistance value of 50 ohms.

We investigated a variety of hypotheses in our landscape genetic analyses based on expert opinion or the results of previous work. First, we considered the influence of stream networks on connectivity. Based on results from previous work (Roach et al. 2001, Sackett et al. 2012), we predicted permanent streams would act as a barrier to movement, while temporary streams may assist movement among colonies by providing dry, low-lying corridors through the landscape with some degree of vegetative cover from predators. Second, we tested the influence of road networks on prairie dog movement, as they may have either a negative or positive effect on dispersal frequencies, depending on human population density in the study area (Sackett et al. 2012). Next, we turned our focus to land-use effects, testing the influence of open grasslands, pasturelands, and cropland on prairie dog dispersal. We hypothesized that grassland and pastureland would assist dispersal, while cropland may hinder movement among colonies. Last, we believed shrubland within our study areas may act as a movement corridor for prairie dogs, as higher, perennial vegetation would provide natural cover from predators for multiple generations of prairie dogs (Koford 1958). For all these hypotheses, we also created reciprocal models of each hypothesized effect (Chapter 2). Our hypotheses were supported if the reciprocal model was insignificant.

IBR models and our null hypothesis of isolation-by-distance (IBD) were compared by calculated Pearson's product-moment correlation coefficient (r) in program R (v. 2.15.2; Manly 1991). We implemented a permutation test in program R to determine the significance of observed correlations ($p < 0.05$, 10,000 permutations). This methodology is comparable to a Mantel test, without the need for matrices (Manly 1991). For IBR models with significant r-values, we also calculated partial Pearson's correlation coefficients to control for the relationship between genetic and geographic distances among colony pairs in our IBR models.

Results

We established 13 circular sites, from which we captured 903 prairie dogs from 40 colonies over a 4-year period (Table 3.1). Out of 903 sampled individuals, sex was recorded for 607. Descriptive statistics for our 19 microsatellite loci and 40 sampled populations are reported in Chapter 2.

Spatial extent of genetic autocorrelation

Our autocorrelogram shows significant positive autocorrelation among colony pairs separated by ≤ 40 km; however, insignificant autocorrelation observed at distance class 50 km may be a function of the small sample size within that class rather than an observed pattern (n = 19). Consequently, our results indicate that effective dispersal among prairie dog colonies leads to genetic similarity among colonies separated by as much as 50 km.

Sex-biased dispersal within and among colonies

Spatial autocorrelation analyses show remarkably similar patterns between male and female genetic similarity across scales (Figure 3.3). At the finest scale (Figure 3.3A), our graphs show evidence that related females are more clustered than males; however, sample size differences drive this disparity (for distance class 1 km: $n_{females} = 32$, $n_{males} = 4$). Sample size differences may also contribute to dissimilarities at other scales we investigated, but this explanation is less convincing for our broadest scale autocorrelograms, where sample size for both males and females exceed 40 at all distance classes, creating small confidence intervals and envelopes for both sexes (Figure 3.3C). Consequently, our results provide some evidence that female prairie dogs are more frequently successful in intercolonial, long-distance dispersal than males.

Results from our multi-scale relatedness comparison support these results (Figure 3.4). We found that females were significantly more related to nearest neighbors within a colony (0-0.1 km; *t*-statistic = 2.63, p = 0.004) and to nearest neighbor colonies (1-10 km; *t*-statistic = 4.03, p < 0.0001) than their male counterparts. However, when relatedness among males and females from the same colony were compared (0.1-1 km), male mean relatedness was greater than female mean relatedness, although the difference was insignificant (*t*-statistic = -1.44, p = 0.075).

Landscape genetic analyses

Results from our landscape genetic analyses are shown in Table 3.3. Our null isolation-by-distance (IBD) model showed a weak relationship between genetic and geographic distances among colony pairs separated by $\leq$ 60 km (Figure 3.5; r = 0.14, p = 0.03). Among our IBR models, only the model depicting shrubland as a movement corridor achieved a significant correlation with gene flow after controlling for IBD, but the portion of variance explained by shrubland corridors was quite small (partial r = 0.14, p = 0.04).

Discussion

To our knowledge, our study is the first to provide general, rather than solely site-specific, information concerning patterns of effective dispersal among colonies across spatial scales. We also demonstrate that sex-biased dispersal in this species is a scale-dependent phenomenon and provide suggestions for future investigations into intrinsic and extrinsic factors influencing the dispersal dynamics of this species.

Widespread gene flow within colony complexes

We observed positive genetic autocorrelation among colonies separated by at least 40 km and possibly as much as 50 km. These distances are far greater than the genetic neighborhood size reported by Jones and Britten (<35 km; 2010), but the authors included only 6 colonies from northern Montana, which lies at the northern edge of prairie dog range (Figure 3.1). Greater differentiation among colonies is expected along the periphery, given the influence of multiple founder events and population bottlenecks as the range expands northward (Hampe and Petit 2005). Therefore, both the location and small sample size of Jones and Britten (2010) may account for the disparity in our results.

Absence of male-biased dispersal across scales

Female relatives were spatially clustered within a colony, but we did not detect male-biased dispersal across scales. Our results demonstrate that sex-biased dispersal in prairie dogs is a scale-dependent phenomenon, with male-biases likely at fine spatial scales and female-biases likely at broad scales. Consequently, we add prairie dogs to a growing list of small mammals that exhibit differential sex-bias across scales (Fontanillas et al. 2004, Gauffre et al. 2009, Yannic et al. 2012) and dispersal dynamics much more complicated than classic dispersal theory predicts (Greenwood 1980, Lawson Handley and Perrin 2007).

In their review of mammalian dispersal patterns, Lawson Handley and Perrin (2007) suggest that dispersal dynamics for highly social species, like the prairie dog, are driven by complex interactions among inbreeding avoidance, kin cooperation, and competition. In polygynous systems, male-biased dispersal will only occur if mate competition among male kin exceeds resource competition among female kin, but caution that the relative strengths of mate competition and resource competition likely differ across scales. Specifically, resource competition would become an increasingly significant driver and would apply equivalent pressure to both sexes at broad spatial scales, leading to the loss of sex-bias in long-distance dispersal (Fontanillas et al. 2004). Effects of mate competition should only emerge at local scales.

For prairie dogs, past studies implicate inbreeding avoidance and kin competition as drivers of short distance dispersal dynamics within colonies (Halpin 1987, Hoogland 1995, Hoogland 2013), as these evolutionary forces only require short distance, intracolonial movements to escape or alleviate. Intracolonial dispersal occurs year round, but most frequently during the breeding season (Halpin 1987, Hoogland 1995). Alternatively, intercolonial or long-distance dispersal occurs following juvenile emergence, when population density across the colony reaches its maximum for the year (Halpin 1987, Garrett and Franklin 1988, Hoogland 1995). If all coteries successfully produce offspring, resource competition would maximize throughout the colony for both sexes following juvenile emergence and would apply equivalent pressure to males and females, requiring a long distance, intercolonial move to escape or alleviate. By this reasoning, we propose that the mechanism driving long distance movement is resource competition, resulting from variance among populations in availability or quality of food, space, or shelter following juvenile emergence (McPeek and Holt 1992, Newby 2005, Yannic et al. 2012).

Frequent female effective dispersal among neighboring colonies

We found significantly higher measures of relatedness among females from different colonies separated by < 10 km than among their male counterparts. We expect that similar numbers of male and female immigrants arrive at neighboring colonies each generation; however, the ease of immigration experienced by each sex may differ (Lawson Handley and Perrin 2007). Female immigrants may integrate into existing coteries with greater ease than their male counterparts or, alternatively, female immigrants may more easily attract a mate to establish a new coterie. Greater immigration success among females has been observed for other social mammals, including hamadryas baboons and spider monkeys (Sigg et al. 1982, McFarland Symington 1987), but female vs. male immigration success has not been intensely investigated for prairie dogs. Hoogland (1995) reported greater reproductive success among immigrant males than females; however, this observation comes from a single colony in mixed-grass prairie, which may not represent typical immigration success throughout prairie dog range (Chapter 2).

Collectively, results from our multi-scale analyses of sex-biased dispersal demonstrate that local dispersal dynamics do not always predict movement patterns at broader spatial scales. We strongly urge future research employ a multi-scale approach to more accurately characterize the dispersal dynamics of their species, particularly when that species is highly social (Lawson Handley and Perrin 2007).

Little observed influence of distance or landscape features on gene flow

Similar to previous studies using microsatellite markers to quantify gene flow among prairie dog colonies, we observed a significant, but small effect of isolation by distance within colony complexes (Roach et al. 2001, Antolin et al. 2006, Sackett et al. 2012). Previous studies have found influence of landscape features on intercolonial connectivity but we did not discern an appreciable amount of influence in our study areas (Roach et al. 2001, Sackett et al. 2012). Our landscape genetic analyses provided some evidence that shrubland acts as a corridor to gene flow, possibly by providing prairie dogs with cover from predators, but the correlation coefficient of the model was low, suggesting that the relationship between shrubland corridors and connectivity is not strong.

Unexplained variation in gene flow within our study areas may result from unexplored at-site characteristics of the colonies we sampled. For instance, forage quality or population density at-site may contribute to dispersal dynamics within a complex, but their influence has not been fully investigated (but see Newby 2005). Colony size may also predict dispersal, as it has been implicated as a predictor of disease incidence during plague epizootic events (Cully et al. 2010, Johnson et al. 2011). If prairie dogs contribute greatly to plague spread, than colony size would predict dispersal dynamics as well. Given recent evidence of adaptive resistence to plague among prairie dogs in Colorado and Texas (Rocke et al. 2010), combined with and Hoogland's (2013) observation that prairie dogs disperse with greater propensity when their kin have died or moved away, prairie dogs may indeed contribute greatly to plague spread within our study areas. Our conjecture finds further support from our observations of gene flow among colonies across scales (Chapter 2). Consequently, we suggest that at-site characteristics shown to be predictive of plague dynamics should inform hypotheses of prairie dog dispersal in future studies.

Management implications

Metapopulation management is inherently difficult, as it involves monitoring species at local and regional scales to ensure that recommendations are appropriate. Our results demonstrate that, at the center of prairie dog range in short-grass prairie, colonies are highly connected. In addition, as both sexes disperse widely, local extinction events are unlikely to threaten the long-term survival of colony complexes within our study areas; however, we caution that prairie dog colonies at the periphery of the range may not display the same dispersal dynamics as we observed. Indeed, gene flow estimates reported by Magle et al. (2010) and Jones and Britten (2010) reveal lower connectivity among prairie dog colonies within the western and northern edge of their range. The same is true for the eastern edge, as our own report of genetic differentiation among colonies demonstrates (Chapter 2). Consequently, we suggest thoughtful, coordinated regulation of control efforts throughout prairie dog range, but particularly along the periphery, and further stress the importance of monitoring colony size and density in areas known to experience plague epizootic events.

Acknowledgements

We thank the Jack H. Berryman Institute, The Nature Conservancy, and the United States Fish and Wildlife Service Great Plains Landscape Conservation Cooperative for their financial support of this project. For land access privileges, we thank the National Forest Service and various private landowners in Logan County and other sites in Kansas. We are also grateful for technical support provided by USDA APHIS, the Molecular Ecology Laboratory at the University of Florida, the DNA Sequencing and Genotyping Facility at Kansas State University, and the USGS Kansas Cooperative Fish and Wildlife Research Unit at Kansas State University. This project would not have been possible without assistance and support from colleagues Amanda Goldberg, Claudia Ganser, Paige Harper, and numerous field and laboratory technicians.

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Table 3.1: Geographic location of each colony sampled with our four study sites. We provide the names of each colony and the number of male and female individuals sampled.

Colony Complex	Site #	Coordinates	Colony Name	Number of Males	Number of Females	Year
Comanche National Grassland	1	37.210663 N 102.94990 W	Oklarado	9	10	2010
		37.239602 N 102.93876 W	South Fork	10	11	2010
		37.249332 N 102.91531 W	SN Ranch	13	13	2010
	2	37.291788 N 102.70682 W	Liberty	11	18	2010
		37.312052 N 102.66911 W	Three Awn	10	11	2010
		37.277875 N 102.66063 W	Lonestar	7	15	2010
	3	37.065583 N 102.57350 W	Hallmark 2	11	16	2010
		37.062280 N 102.54864 W	Hallmark 1	9	11	2010
		37.033736 N 102.54752 W	Ute Canyon	9	13	2010
Kiowa-Rita Blanca National Grasslands	4	36.520688 N 103.05877 W	KW 46	11	14	2012
		36.524449 N 103.05284 W	KW 43	10	12	2012
		36.507508 N 103.02630 W	KW 44	10	15	2012
	5	36.533302 N 102.72571 W	RB 128	6	17	2012
		36.523192 N 102.71315 W	RB 126	8	15	2012
		36.558274 N 102.67620 W	RB 132	4	11	2012
	6	36.388185 N 102.66858 W	RB 33	10	15	2012
		36.334863 N 102.68334 W	RB 37	9	18	2012
Cimarron National Grassland		36.995051 N 102.03081 W	State Line	7	13	2011
	7	37.203615 N 102.03082 W	Pasture 47	22	13	2010
		37.212956 N 101.99535 N	Pasture 7	9	10	2010
		37.257415 N 101.96971 W	North Fork	14	8	2010
	8	37.168721 N	Pasture 81	9	11	2011

		101.80209 W				
		31.175974 N 101.78694 W	RD N15	11	12	2011
		37.193706 N 101.70507 W	North Lowe	13	7	2011
	9	37.094075 N 101.62231 W	RD 735	8	10	2011
		37.096852 N 101.60804 W	RD 734	6	13	2011
		37.094245 N 101.56822 W	RD 733	4	14	2011
				Number of Both Sexes		
Logan County, Kansas	10	38.981222 N 101.44653 W	BD Highway		9	2009
		38.971611 N 101.40899 W	BD East Road		15	2009
		38.977111 N 101.42671 W	BD North Highway		15	2009
	11	38.900361 N 101.31017 W	HD Boys Northwest		13	2009
		38.896472 N 101.29903 W	HD Boys Northeast		23	2009
		38.887528 N 101.30369 W	South Boys		29	2009
	12	38.812750 N 101.15267 W	HD Northeast		32	2009
		38.780694 N 101.14578 W	BT Northeast		25	2009
		38.790389 N 101.17983 W	HD Schoolhouse		29	2009
		38.790000 N 101.20639 W	HD Lone Butte		30	2009
	13	38.855833 N 100.98481 W	TNC West		24	2009
		38.884750 N 100.96450 W	TNC North Long		16	2009
		38.833944 N 100.93086 W	TNC East		32	2009

Table 3.2: Simplification of landcover classes provided by the 2006 National Landcover Dataset (NLCD2006) prior to our landscape genetic analyses. From 20 original classes, we deleted classes of no interest in this study and combined ten remaining classes to create five distinct cover types for our analyses.

Cover Type	NLCD2006 class included
Developed Land	21 – Developed Land, Open Space 22 – Developed, Low Intensity 23 – Developed, Medium Intensity 24 – Developed, High Intensity
Shrubland	51 – Dwarf Shrub 52 – Shrub/Scrub
Grassland	71 – Grassland/Herbaceous 72 – Sedge/Herbaceous
Pastureland	81 – Pasture/Hay
Cropland	82 – Cultivated Crops

Table 3.3: Isolation-by-resistance (IBR) model selection and evaluation procedures. Of all tested landscape variables, only one IBR model achieved significant correlation and partial correlation with observed gene flow in our study areas. This model coded shrubland as a dispersal corridor among prairie dog colonies.

Model	**Pearson's r, *p≤***	**Partial Pearson's r, *p≤***
Ephemeral & Intermittent Streams:		
H: Corridor	0.10, *0.08*	
AH: Barrier	-0.10, *0.93*	
Permanent Streams:		
H: Barrier	0.08, *0.14*	
AH: Corridor	0.11, *0.06*	
Agricultural Development:		
H: Barrier	0.14, *0.02*	0.06, *0.41*
AH: Corridor	-0.10, *0.92*	
Grassland		
H: Corridor	0.11, *0.42*	
AH: Barrier	0.07, *0.17*	
Pastureland		
H: Corridor	0.11, *0.05*	
AH: Barrier	0.11, *0.06*	
Urban Development:		
H: Barrier	0.05, *0.24*	
AH: Corridor	0.03, *0.34*	
Shrubland		
H: Corridor	**0.16, *0.01***	**r_p = 0.14, *0.04***
AH: Barrier	-0.0009, *0.55*	

Figure 3.1: Map of our study sites within three national grasslands and one Kansas county against the 2006 National Landcover Database: (A) the Carrizo Unit of Comanche National Grassland, (B) Kiowa-Rita Blanca National Grasslands, (C) Logan County, Kansas, and (D) Cimarron National Grassland. At left is a regional view of our study areas, with our 10-km circles depicted in white. Also in white are grassland or county boundaries, while state boundaries are black. The map insert places our study areas in the contiguous United States and current prairie dog range (cream). At right are four high resolution maps of each grassland or county, in which we depict our 10-km circles in black outline and our specific collection sites (colonies) within each circle in white. At Cimarron National Grassland, one collection site existed outside our 10-km circles in the southwestern corner of the grassland.

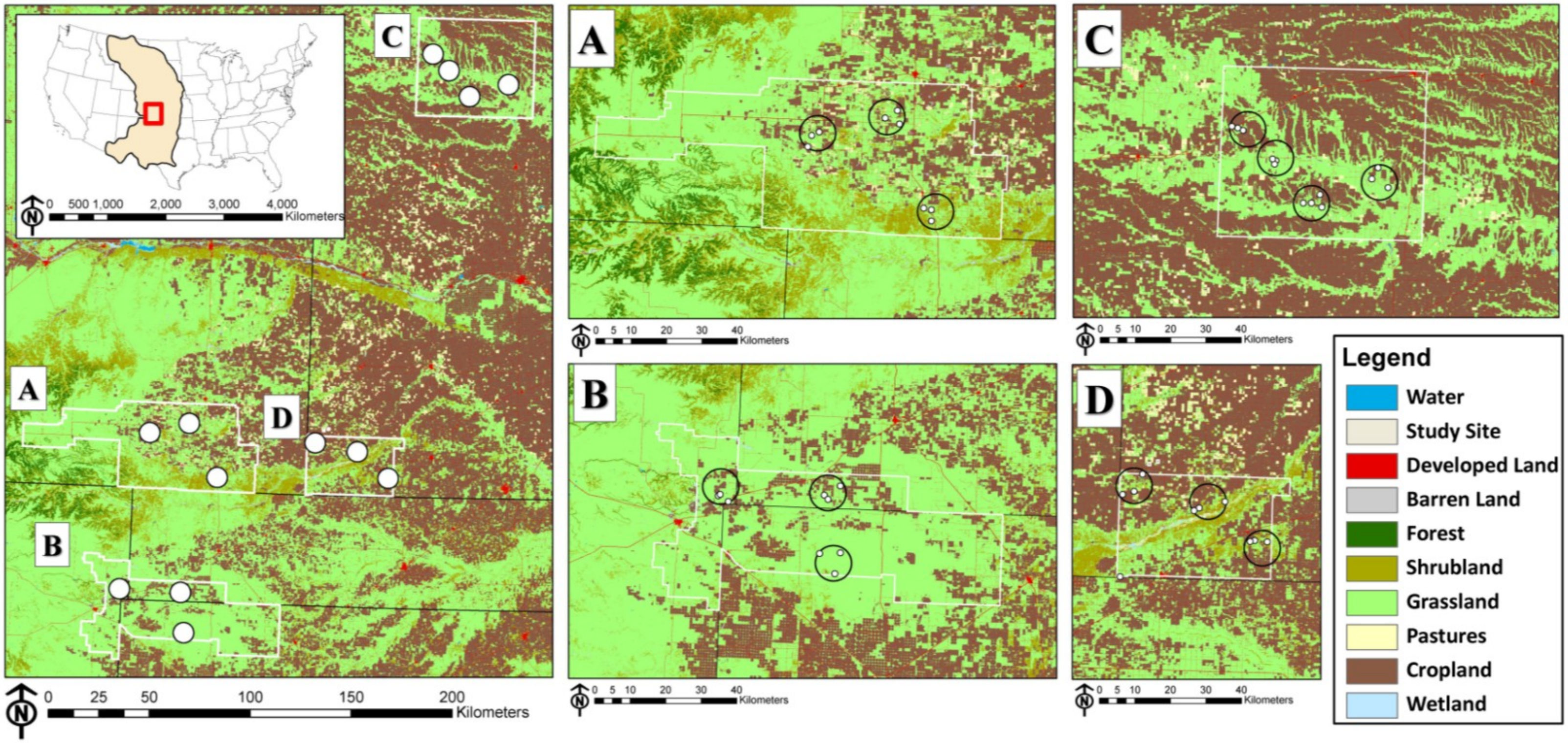


Figure 3.2: Spatial genetic autocorrelogram depicting observed genetic autocorrelation (r ± standard error) among colonies within our study areas by a solid line. Dashed lines mark upper (U) and lower (L) bounds of the 95% confidence envelope. If observed autocorrelation within a particular distance class lies outside this confidence envelope, then r represents significant positive or negative autocorrelation among colony pairs within that distance class. Observations within the envelope (or with confidence intervals overlaying the envelope) are insignificant.

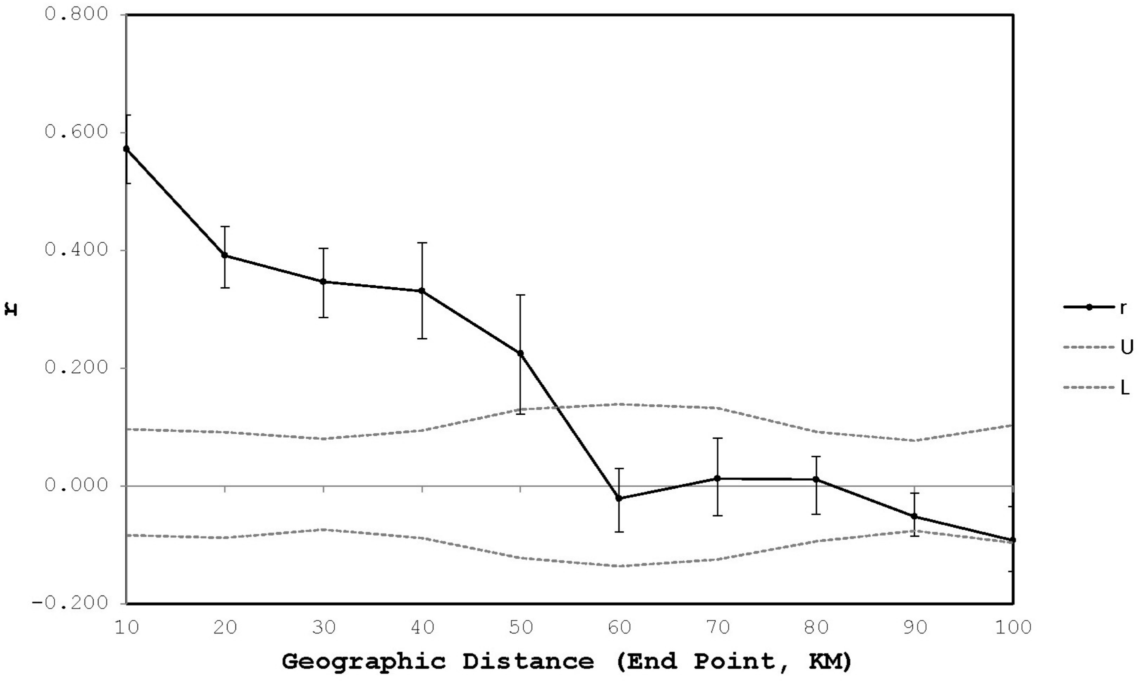
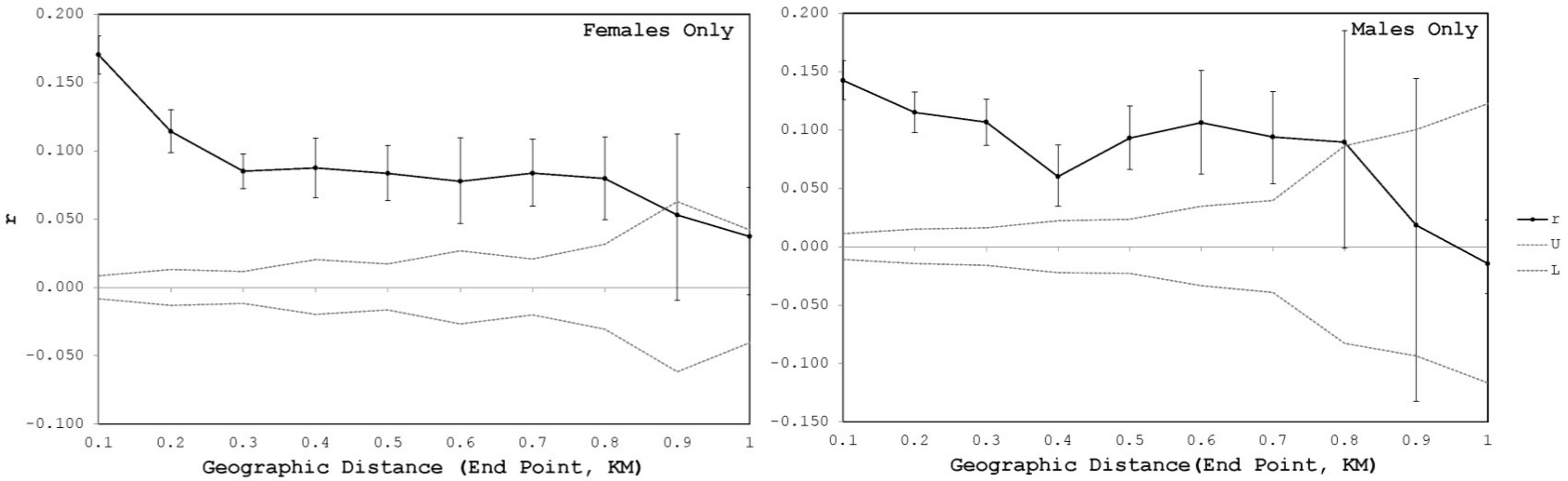
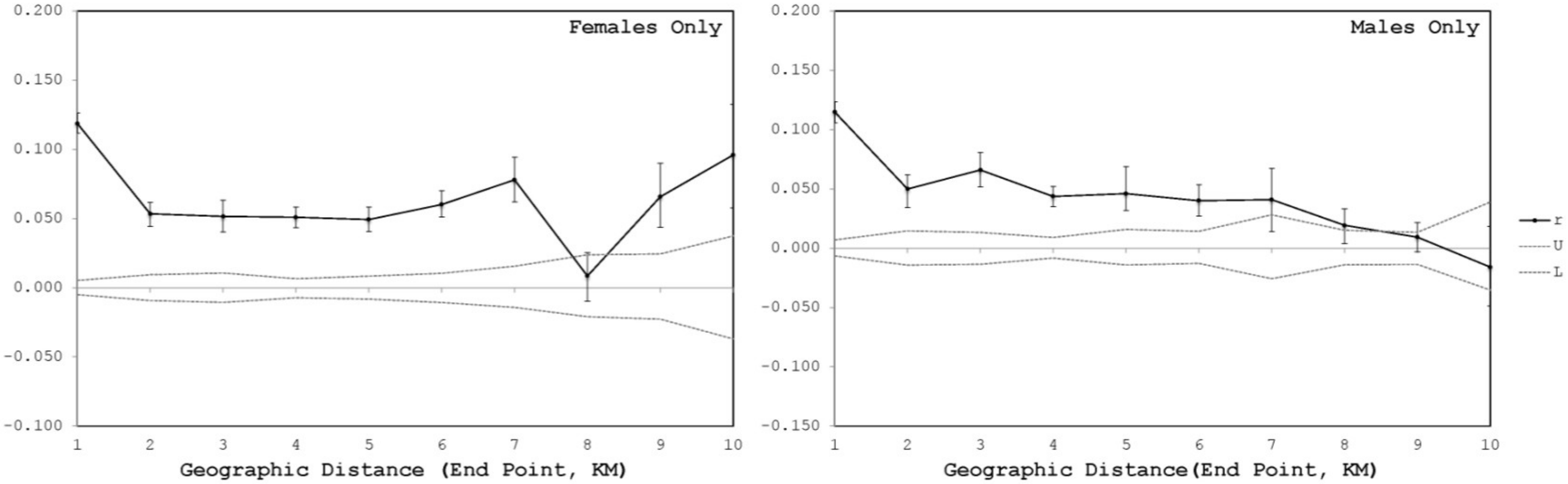


Figure 3.3: Spatial genetic autocorrelograms for females (left) and males (right) at three spatial scales: (A) distances ≤ 1 km, bin size 0.1 km, (B) distances ≤ 10 km, bin size 1 km, and (C) distances ≤ 100 km, bin size 10 km. Solid lines depict observed spatial autocorrelation (r ± standard error) among pairs of individuals, while dashed lines mark upper (U) and lower (L) bounds of the 95% confidence envelope.

A.



B.


Females Only
Males Only
r
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0.150
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1
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Geographic Distance (End Point, KM)
Geographic Distance(End Point, KM)
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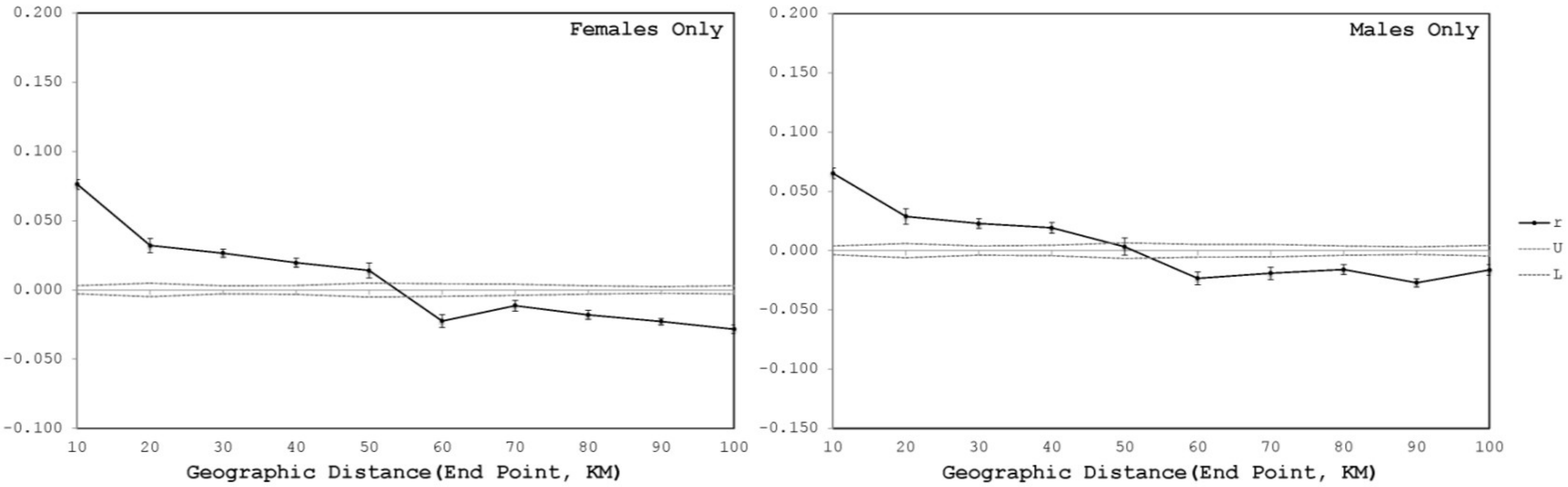
C.


Females Only
Males Only
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50
60
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80
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100
Geographic Distance(End Point, KM)
Geographic Distance(End Point, KM)
r
U
L

Figure 3.4: Coefficient of genetic relatedness (r) among females and males at three spatial scales. Locally (< 0.1 km) and among nearby colonies (1-10 km), pairs of female individuals are significantly more related than male pairs. No significant difference was observed at intermediate distances (0.1-1 km).

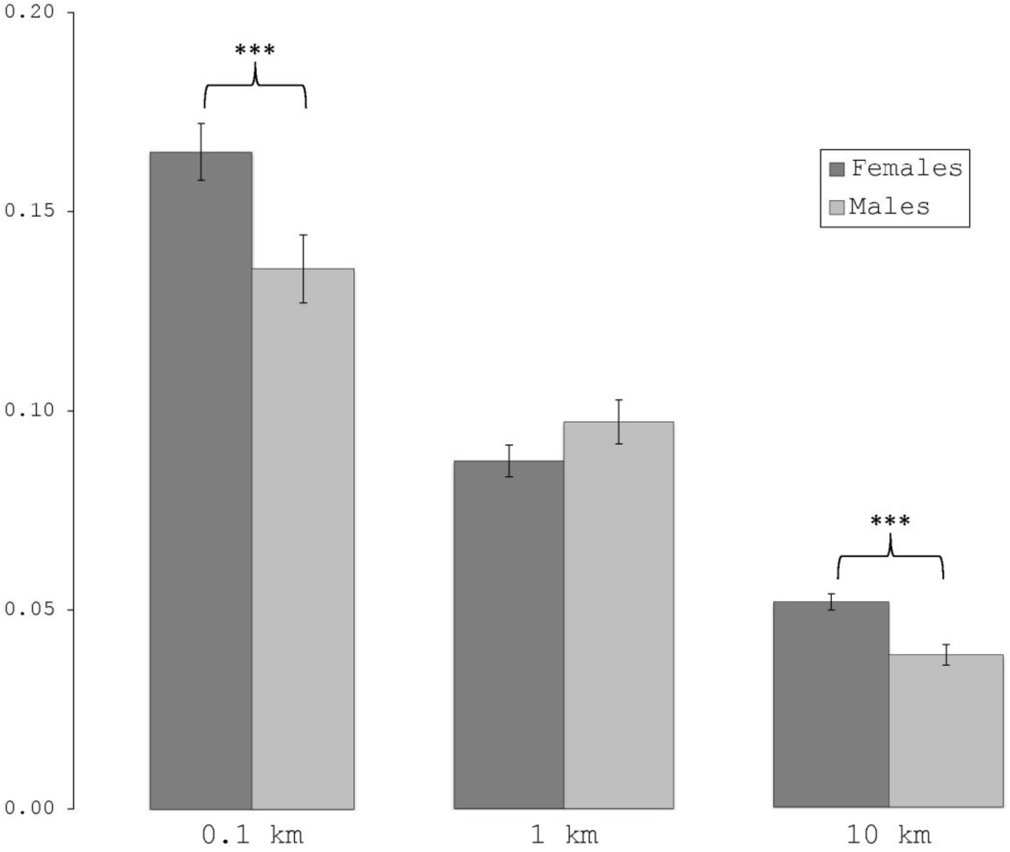
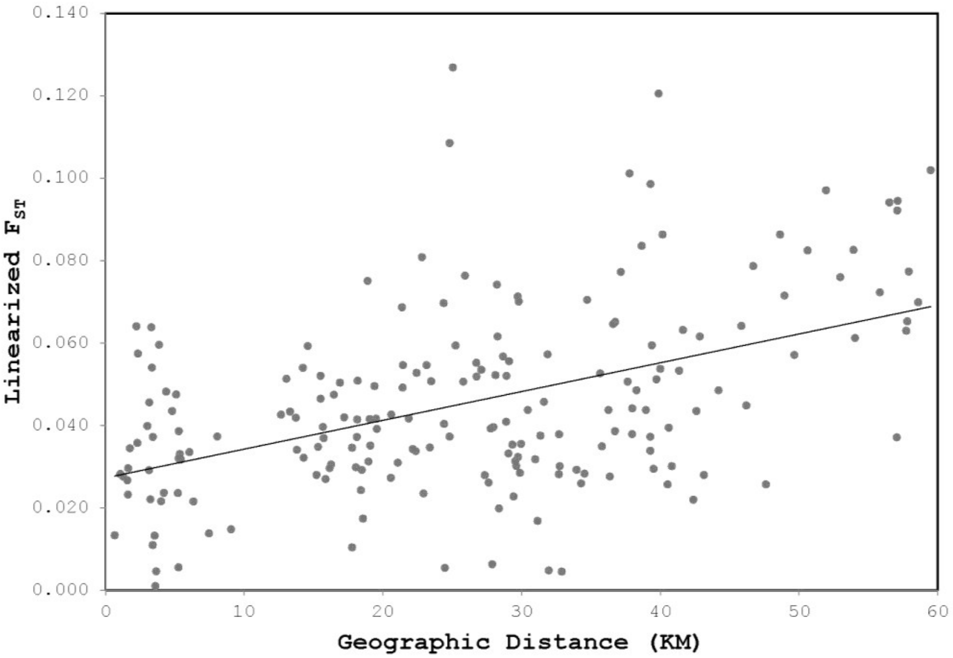


Figure 3.5: Results from our linear model of isolation-by-distance among colony pairs separated by < 60 km (Pearson's r = 0.14, p ≤ 0.03).



Chapter 4 - Landscape features predict sylvatic plague transmission among black-tailed prairie dog colonies

Rachel M. Pigg and Jack F. Cully, Jr.

Abstract

Predicting disease risk to wildlife populations is inherently difficult, particularly for vector-borne diseases in which multiple species participate in transmission at regional scales. Here, we use circuit theory and random forest modeling to identify landscape variables predictive of sylvatic plague transmission among black-tailed prairie dog colonies in Cimarron and Comanche National Grasslands. Our results indicate that pasturelands provide corridors for plague transmission, while other features, such as roads and stream networks, may serve as barriers or corridors in different landscape contexts. We discuss the utility of circuit theory beyond isolation-by-resistance modeling and encourage managers and future researchers to consider circuit theory when developing models to predict disease transmission trajectories.

Introduction

Sylvatic plague, a vector-borne disease caused by the bacterium *Yersinia pestis*, is an exotic disease in North America (Cully and Williams 2001). Many native mammals are susceptible, but mortality rates are particularly high among black-tailed prairie dogs (*Cynomys ludovicianus*). Black-tailed prairie dogs (hereafter, "prairie dogs") are social, ground-dwelling squirrels that live in colonies throughout the western Great Plains (Hoogland 1995). Unlike other rodent hosts of plague, prairie dogs are highly conspicuous due to their diurnal habits, raised burrow entrances, and tendency to clip vegetation within and surrounding the colony. Such conspicuous activities make prairie dogs ideal subjects for plague transmission studies (Johnson et al. 2011), as colony losses are apparent and can be easily monitored by wildlife managers and citizen scientists throughout the western Great Plains.

The daily activities of prairie dogs also contribute to soil nutrient cycling and modify vegetation height and composition within the colony, leading to the classification of the prairie dog as a keystone species in prairie ecosystems (Kotliar et al. 1999). Keystone status implies that any force affecting prairie dog survival could create a cascade of changes to prairie community composition. Further, at a regional scale, prairie dog colonies function as a metapopulation. A metapopulation is a group of isolated, local populations whose long-term, regional persistence depends on rates of local extinction and recolonization (Hanski 1999). Frequent or synchronized local extinctions could destabilize a metapopulation, leading to a regional decline in this keystone species (Collinge et al. 2008).

Sylvatic plague has the potential to destabilize prairie dog metapopulations (Hess 1996, Cully and Williams 2001). Locally, the disease has an individual mortality rate of >95% and spreads quickly via infected fleas or direct contact with infected or dead mammals. Regionally, mechanisms that assist the spread of plague are less clear, but no less efficient (Johnson et al. 2011). Past studies suggest that water bodies and roads affect intercolony transmission rates (Lomolino et al. 2004, Stapp et al. 2004, Collinge et al. 2005, Snäll et al. 2008, Cully et al. 2010, Johnson et al. 2011). The most consistently supported factors are colony spatial characteristics, such as colony size and proximity to infected colonies (Johnson et al. 2011). While colony characteristics may indeed be the most predictive variable for plague transmission, previous studies have used simple metrics, such as percent cover and proximity, to characterize landscape patterns surrounding prairie dog colonies. Simple metrics may not adequately describe landscape composition and configuration, making the detection of their influence on transmission less likely (Cushman et al. 2008, Uuemaa et al. 2009). Furthermore, numerous studies describe the strong influence of landscape features on the transmission of other vector-borne diseases, such as Lyme disease (Brownstein et al. 2005, Tran and Waller 2013) and malaria (Eisen and Wright 2001, Bonneaud et al. 2009).

In light of these concerns, the goal of this study was to identify landscape features predictive of plague transmission by utilizing a landscape connectivity metric calculated in the program Circuitscape (McRae 2006, McRae and Beier 2007, McRae et al. 2008). Circuitscape employs circuit theory to produce species-specific landscape connectivity models which perform well as predictors of gene flow among populations (McRae and Beier 2007). To our knowledge, no study has used Circuitscape to model other types of ecological flow, such as disease transmission.

Circuitscape views populations as sources and sinks of electrical current. For our purposes here, current corresponds to predicted rates of disease transmission through the landscape, while the landscape itself provides the circuit through which current flows. Within the circuit, each landscape feature resists current flow to varying, hypothesized degrees. Those features with little resistance act as transmission corridors, while features having great resistance act as barriers and slow transmission. Current calculations also require information concerning the width and redundancy of features within the circuit (McRae 2006), and, consequently, the connectivity metrics produced by Circuitscape integrate both landscape composition and configuration into their predicted transmission rates.

We employed program Circuitscape to determine whether landcover types within the western Great Plains could explain observed patterns of plague transmission among prairie dog colonies in a dataset previously reported by Johnson et al. (2011). From expert opinion and the results of past studies, we hypothesized that dry-creek drainage systems, grassland, and pastures would serve as corridors to plague transmission (Koford 1958, Roach et al. 2001), while water bodies and roadways would serve as barriers (Collinge et al. 2005, Snäll et al. 2008). We also investigated the effect of agricultural development and shrubland on prairie dog dispersal, hypothesizing that agricultural land may hinder plague transmission, while shrubland may assist transmission by providing natural cover for agents carrying infected fleas (Koford 1958, Johnson et al. 2011).

Materials and Methods

Study areas

Prairie dog activity was monitored at colonies on public lands throughout Cimarron National Grassland, Kansas and the Carrizo Unit of Comanche National

Grassland, Colorado (Figure 4.1; Johnson et al. 2011). The presence of fresh scat, clipped vegetation, audible prairie dog calls, or signs of recent digging at burrow entrances identified active colonies. For colonies > 2 ha in size, we attributed a decrease of >90% in a colony's active area over a 1-to-2 year period to plague: the only known cause of such abrupt extinction of large prairie dog colonies in the absence of poisioning (Cully and Williams 2001). For colonies < 2 ha, we attributed an extinction to plague only if the nearest neighboring colony of > 2 ha had been lost to plague in the same year or in the previous year.

Landscape characterization

To determine the spatial distribution of various landscape features within our study areas, we downloaded the 2006 National Landcover Database (NLCD) and the National Hydrography Database (NHD), made available by the United States Geological Survey. Given the exploratory nature of our analysis, we chose to investigate the influence of all prevalent features within our study area; however, we simplified our analysis by reclassifying default feature classifications as shown in Table 4.1. All data manipulations were performed in ArcMap (v.10.1; ESRI). Briefly, from the NLCD, we (1) removed forested lands, barren land, and perennial ice or snow, given their low prevalence in our study area and (2) combined all categories of developed lands, herbaceous lands, shrublands, and water and wetlands into single categories (Table 4.1). Following this reclassification, we created a raster file for each feature identified in Table 4.1 and coarsened all raster files to a 150-m x 150-m cell size. We chose this spatial grain to preserve as much spatial heterogeneity as possible, while staying within the computational limitations of Circuitscape. We also transformed polyline shapefiles from the NHD into raster files with a 150-m x 150-m cell size. We chose to create unique raster files for permanent streams and ephemeral/intermittent streams, as the presence of water within stream networks may change the direction of their effect on plague transmission among colonies. The spatial extent of all raster files included a ≥20-km border surrounding our outermost colonies.

To prepare our raster files for input into Circuitscape, we coded the presence of a landscape feature according to its hypothesized resistance to transmission among our colonies. We assigned 1 Ω to cells containing hypothesized corridors and 100 Ω to hypothesized barriers, while all other cells in our raster files received an intermediate value of 50 Ω. For all features included in our analyses (Table 4.1), we created two input files for Circuitscape: one in we coded the feature as a corridor and a second in which the feature was a barrier.

Connectivity estimation

We used a landscape connectivity metric calculated by the program Circuitscape to predict the likelihood of plague-induced colony extinctions in our study area (v. 4.0; McRae 2006). Circuitscape views the landscape as an electrical circuit, in which populations serve as sources or sinks for electrical current, while landscape features serve to inhibit or assist the flow of that current by applying a high or low resistance to the circuit(s) connecting the populations (McRae 2006, McRae and Beier 2007, McRae et al. 2008). In our analyses, colonies served as populations, while the electrical circuit provided pathways for disease transmission among those colonies. As aforementioned, Circuitscape is most commonly used to create isolation-by-resistance models in landscape genetic studies; however, the resistance distance metric used in these models may be an inappropriate metric to estimate disease transmission, primarily because resistance distance does not incorporate direction. Disease transmission proceeds from a source to a sink, while gene flow in landscape genetics studies is often presumed to be bidirectional. Consequently, we chose to use current (I) as our predictive metric for disease transmission, as it depends on both the location of the battery in your circuit (i.e., infected, “source” colonies) and the resistance of the circuit to current flow (i.e., hypothesized influence of landscape features on transmission).

We simplified our Circuitscape calculations by representing colonies as points, rather than regions. Point files represented the centroid of each colony, calculated in ArcMap. As aforementioned, colonies extirpated by plague in the initial year of the epizootic (2005; Figure 4.1) served as current sources. All other mapped colonies were coded as electrical sinks (i.e., grounds) because we expected the disease to flow from infected to uninfected colonies just as current flows from a source to an electrical ground. We transformed the point shapefiles of colony centroids into ArcInfo ASCII grid files for input into Circuitscape. Our categorical response variable was colony presence or absence in 2006, where an absence was assumed indicative of plague transmission. We limited our investigation to this 1-year interval because, after 2006, new and numerous sources appear in Cimarron and Comanche. As a result, our sample size of remaining uninfected colonies is drastically reduced in both grasslands, making patterns more difficult to resolve. Further, after 2006, the likelihood that other, unmonitored sources (such as colonies on private land or other mammalian individuals and populations) are participating in regional disease dynamics increases exponentially, further reducing our ability to detect landscape influence on disease transmission simply by monitoring one species.

To emulate disease transmission from 2005-2006 in Circuitscape, we first transformed our landscape raster files into the ArcInfo ASCII grid format required for Circuitscape input files. For each landscape feature and its hypothesized transmission effect, Circuitscape produced a current map in pairwise mode, using the four-neighbor cell-connection scheme. We transformed this current map from a grid file into a raster file in ArcMap, then quantified the total current flowing within 750-m radius of each sink colony centroid using the Geospatial Modelling Environment (v. 0.7.2; Beyer et al. 2010). We chose a 750-m radius, as it was the shortest length required to create a circle that enclosed the largest colony in our study areas. Total current flowing within the 750-m radius (1500-m diameter) circles served as a surrogate for the probability of plague transmission from source colonies during the first year of the epizootic (Figure 4.2). This value was the metric we used to evaluate each variable’s predictive performance, and we refer to it as total local current.

Variable importance and selection

We used a nonparametric, recursive partitioning method called random forests to evaluate the importance of each variable in our predictive models (Breiman 2001, Strobl et al. 2009a). We chose random forest modeling because this method makes few assumptions of the data, is relatively insensitive to multicollinearity, and performs well with a large set of candidate predictors regardless of sample size (Cutler et al. 2007). Random forests first create numerous classification (or regression) trees using both a random subsample of the data (termed “in-bag”) and a random selection of predictor variables, and then apply a modeling averaging procedure to predict the response (colony presence/absence in 2006) of the “out-of-bag” samples.

We used the conditional random forest implementation “cforest” provided by the party package (Strobl et al. 2007) in program R (v. 2.15.2; R Development Core Team). We used default settings of the cforest function, except (1) we set mtry to 4, based on the number of predictor variables (Strobl et al. 2009a) and (2) ntree to 10000, to ensure our results were stable and robust. We verified this stability by rerunning our random forests, changing our random seed and varying mtry (from 3 to 5) in each iteration. Finally, we used the conditional variable importance measure “varimp” provided within the party package (Strobl et al. 2009b) to rank the importance of each variable in our candidate set. This measure is determined by randomly permuting values of the predictor and reassessing its relationship to the response variable (Strobl et al. 2009b). If permutations result in a great loss of model accuracy, then the variable importance measure is a high, positive value. Candidate variables with a variable importance measure greater than the absolute value of the lowest ranking variable are considered significant predictors of the response.

Although our primary aim was to identify landscape features affecting plague transmission among colonies, we chose to include one at-site variable due to its performance in a previous investigation of these data (Johnson et al. 2011). In that study, the size of the uninfected colony was related to its probability of infection. Therefore, we included colony size (ha) in our candidate variable set.

Model evaluation

We evaluated the performance of our random forest models by calculating the following statistics. First, we determined the out-of-bag specificity and sensitivity of each model using a contingency table, in which disease transmission leading to colony loss is considered a positive result. Next, we calculated both the c-statistic and Somer’s D provided by the Hmisc package in R (Harrell 2013). The c-statistic quantifies the probability that the accuracy of the model’s out-of-bag predictions is better than random chance (Hosmer and Lemeshow 2000) and typically varies from 0.5 (random chance, given a binomial response variable) to 1 (perfect accuracy). Models with $c \geq 0.7$ are acceptable, while models with $c \geq 8$ are strongly supported. Somewhat similarly, Somer's D quantifies the degree of association between out-of-bag predictions and our observations (Demaris 1992). Somer's D is interpreted as the proportion decrease in error achieved by using the model to predict responses, as opposed to using random assignment.

Results

In total, 52 colonies were monitored in Cimarron and 116 colonies in Comanche. Of these, five colonies from each grassland met our criteria for a likely plague epizootic in 2005, leaving 47 uninfected colonies in Cimarron and 111 in Comanche. By the end of 2006, 17 more colonies in Cimarron experienced a local extinction event, while 63 disappeared in Comanche.

Variable importance and selection

Rankings of variable importance differed between the two national grasslands (Figure 4.2). Rankings shown were stable and robust to changes in random seed, tree number, and mtry (data not shown). In Cimarron, most variables were significant predictors; however, only six outperformed distance. The six variables included waterways, pasture land, and both permanent and intermittent streams as transmission corridors, while roads and agricultural land acted as barriers to transmission. Our at-site variable, colony size in 2005, was not predictive of transmission in 2006.

In Comanche, eight variables were significant predictors; however, distance was not significant, indicating that the distance separating uninfected colonies from infected colonies in Comanche was not predictive of transmission from 2005-2006. Predictive variables included pastureland, roads, intermittent streams, and agricultural land acting as transmission corridors, while grassland acted as a barrier to transmission. One landscape feature, shrubland, performed well as a predictor regardless of whether we coded it as a barrier or corridor, indicating the likelihood of an interaction between shrubland and another, unrepresented aspect of the landscape that changes the direction of shrubland's effect on transmission rate. Colony size was ranked above any landscape variable, indicating that large colonies in Comanche had a high probability of plague-induced extinction in 2006.

Model evaluation

The sensitivity and specificity achieved by each random forest was high (Table 4.2). Out-of-bag accuracy for both models was also high, correctly classifying ~87.2% of colonies in Cimarron and ~80.9% in Comanche. Our model for Cimarron was strongly supported by our diagnostic statistics ($c = 0.875$, $D_{xy} = 0.749$), but our model for Comanche, though proficient, did not perform as well ($c = 0.775$, $D_{xy} = 0.551$).

Discussion

Our study is the first to use circuit theory to investigate the influence of landscape features on disease transmission within a metapopulation. Our current maps incorporated landscape barriers and corridors and proved quite useful in predicting plague progression through two colony complexes in Cimarron and Comanche National Grasslands. We encourage future investigators to consider circuit theory when modeling disease dynamics at landscape scales, due to both the ease of use of the program Circuitscape compared to other network modeling programs and the success of our effort here. We caution, however, that the ability of current maps to resolve patterns is limited if transmission in your disease system is complex, including multiple species and modes of transmission. In these complex systems, we suggest future studies restrict their analysis to the first year of an observed epizootic, as we have here, to reduce complexity as much as possible.

Inherent difficulty of modeling sylvatic plague transmission

As with other vector-borne wildlife diseases (Ostfeld et al. 2005, Johnson and Thieltges 2010), the maintenance and spread of sylvatic plague through a system involves multiple species of mammalian hosts and flea vectors (Gage and Kosoy 2005). As different species react differently to their environments and to infection, the spatial dynamics of plague transmission likely change through time depending on the species diversity and evenness of infectious agents. By limiting our investigation to the initial year of plague epizootic events, we avoided the complexity inherent to advanced outbreaks and were able to create accurate and precise models of disease transmission among prairie dog colonies during the first year of the event. Our attempts to create predictive models of plague transmission in later years of the infection failed consistently (data not shown). In future studies, if the aim is to identify landscape features predictive of plague transmission throughout the epidemic, a finer time scale than the yearly step used in our study may help resolve landscape patterns, as would the inclusion of colonies on private land to limit the number of unmonitored source (and sink) populations in the transmission network.

Landscape features affect transmission in complex ways

In our models for both Cimarron and Comanche National Grasslands, roads, agricultural land, and waterways (or streams) were among the highest ranked variables; however, the direction of their effects differs between the grasslands. Likely, an interaction exists between these landscape features and another variable that was not represented in our models. For roadways, surface material and/or traffic volume may influence animal movement decisions and, consequently, transmission patterns among colonies (Forman and Alexander 1998), while streambank height, slope, and vegetative cover may change the direction of the effects of waterways and drainage systems on animal movement (Tomblin and Adler 1998). Similarly, the intensity of agricultural activities fluctuates both in space and time, likely causing the effect of agricultural land to change with context. Consequently, it is unsurprising that these variables differed between the grasslands in their effect on disease transmission.

One interesting similarity among our models is the selection and high rank of pastureland as a corridor for disease transmission in both grasslands. Pastureland in Cimarron and Comanche is typically occupied by cattle. Prairie dogs are often found on pastureland throughout their range because cattle grazing shortens vegetation height, thereby encouraging the establishment of prairie dog colonies within the pasture (Koford 1958). A similar association existed historically between prairie dogs and bison, with prairie dogs frequently found in areas first grazed by bison or vice versa (Koford 1958, Fahnestock and Detling 2002). Prairie dogs benefit from the cattle's maintenance of low vegetation height throughout the pasture because it allows for improved visual detection of predators without any energy cost to the prairie dogs; however, the benefit or harm of prairie dogs to cattle has been a persistent management concern. Consequently, much research effort has been spent investigating the effects of prairie dog presence on cattle weight gain and overall health (Derner et al. 2006, Miller et al. 2007). While the debate is certainly ongoing, a recent study suggests that any loss experience by cattle is likely driven by climatic variation, primarily, with the presence of prairie dogs only enhancing the effect (Augustine and Springer 2013). Given this association between cattle and prairie dogs, it is likely that pasturelands serve as corridors for plague transmission because pastureland serves as travel routes, stopover sites, and/or new, permanent residences for dispersing prairie dogs (and their fleas; but see Jones and Britten 2012).

Our results, in context

Aspects of our results disagree with observations from past studies of plague transmission among prairie dog colonies. Choice of metric, as well as differences in our candidate variable sets, may drive this disparity, but the complexities we have described above may also contribute. For example, Collinge et al. (2005) found that percent cover of roads and waterways was negatively correlated with the spread of plague, while Snäll et al. (2008) found that climatic variables were the best predictors of plague outbreaks at regional scales. We did not test climatic variables here and only our results for Comanche agree with Collinge et al. (2005).

Johnson et al. (2011) used a multi-state modeling approach to explain the same plague transmission patterns investigated by this study. In their models, the metric "distance to nearest drainage" was calculated for each colony and incorporated into various models to determine whether the organisms responsible for spreading the disease were travelling along waterways. This metric did not contribute significantly to any of their top models. The disparity between the results of Johnson et al. (2011) and our study is likely due to our study's complex connectivity metric (total local current), which incorporates not only the distance to nearest drainage, but also the length travelled along the drainage itself and the width and redundancy of paths connecting colonies (McRae

2006, McRae and Beier 2007, McRae et al. 2008). Our results do agree with some findings of Johnson et al. (2011). Primarily, we detected the same strong influence of colony size on transmission probability in Comanche; however, we did not find an association of small colony size and plague transmission probability in Cimarron. Our failure to detect this association via our random forest model may be due to a sampling difference between our two studies: while Johnson et al. (2011) included several years of observed colony die-offs in her models, we focused on the first year alone.

Management implications

Our study demonstrates that, if a plague epizootic event is detected early within a metapopulation, circuit theory can create predictive models of plague transmission among colonies by incorporating the composition and configuration of landscape features into its estimation of connectivity. We strongly advise annual monitoring of colony activity throughout areas known to experience plague epizootic events. If colony die-offs are detected and plague activity is suspected, we suggest that current maps be created using pasturelands as corridors. This variable not only performed well predicting transmission patterns in both Comanche and Cimarron, but also its performance is well-aligned with observations of prairie dog ecology and behavior throughout their range. Colony size also performed well in our own model for Comanche and in past studies; its inclusion is also advisable. While an effective plague vaccine for prairie dogs is not yet available (Abbott et al. 2012), managers may consider conservatively applying deltametrin, pyraperm, or other insecticide (Seery et al. 2003, Hoogland et al. 2004, Biggins et al. 2010) to halt disease spread to colonies located in high current areas of the predictive model.

Although the sensitivity of both our predictive models was comparable (Table 4.2), our model for Comanche had lower accuracy and specificity than our model for Cimarron. For managers actively monitoring a plague epizootic, limiting the incidence of false negatives (sensitivity) in their predictive models may take priority, as managers cannot stall or stop an epizootic event if large numbers of infected colonies are not detected. The incidence of false positives (specificity), however, would become problematic, too, if funding to control the epidemic is limited. If funds are limited, we suggest that managers target their control efforts to the largest of the colonies predicted to become infected in the coming year, since, as aforementioned, colony size has been implicated as a predictor of plague by various modeling approaches and in many locations.

In conclusion, while a multitude of factors contribute to plague transmission between prairie dog colonies in the western Great Plains, our results demonstrate that some of those factors are landscape features. If the magnitude and direction of their effects can be resolved, this knowledge will greatly assist wildlife managers in their efforts to manage prairie dog metapopulations. We strongly encourage future studies to consider the influence of landscape on plague transmission within their study areas.

Acknowledgements

We are greatly indebted to Tammi Johnson and the United States Forest Service at Cimarron and Comanche National Grasslands, who monitored colonies throughout the plague epizootic events mentioned in this study and granted us access to the data. For funding and field assistance, we thank the United States Geological Survey and the National Science Foundation (DEB-0224328). We also thank Joanna Whittier, Andrew Gregory, Keith Gido, Claudia Ganser, and attendees of the 5th Conference of the International Biogeography Society for their helpful comments on previous versions of this project.

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Table 4.1: Simplification of landcover classes provided by the 2006 National Landcover Dataset (NLCD) and the National Hydrography Dataset (NHD) prior to our Circuitscape analyses. From 20 original NLCD classes, we deleted underrepresented classes and combined remaining, similar classes to create 6 distinct cover types for our analyses.

Cover Type	NLCD Landcover Class
Developed Land	21 – Developed Land, Open Space 22 – Developed, Low Intensity 23 – Developed, Medium Intensity 24 – Developed, High Intensity
Shrubland	51 – Dwarf Shrub 52 – Shrub/Scrub
Grassland	71 – Grassland/Herbaceous 72 – Sedge/Herbaceous
Pasture Land	81 – Pasture/Hay
Cropland	82 – Cultivated Crops
Waterways	11 – Open Water 90 – Woody Wetlands 95 – Emergent Herbaceous Wetlands
Stream Type	**NHD Flowline Fcode**
Permanent Streams	46006
Intermittent Streams	46003, 46007

Table 4.2: Out-of-bag cross classification of true versus predicted colony extinctions in Cimarron (A) and Comanche (B). Darkly shaded cells highlight correctly classified colonies, while lightly shaded cells show incorrectly classified colonies. Also included are model sensitivity, specificity, accuracy, precision, and negative predictive value.

A.

		Observed Condition			
		Colony Lost	Colony Survives		
Predicted Condition	Colony Lost	15	4	0.789	Precision
	Colony Survives	2	26	0.929	Negative Predictive Value
		0.882	0.867	**0.872**	
		Sensitivity	Specificity	**Accuracy**	

B.

		Observed Condition			
		Colony Lost	Colony Survives		
Predicted Condition	Colony Lost	59	18	0.766	Precision
	Colony Survives	4	30	0.882	Negative Predictive Value
		0.937	0.625	**0.809**	
		Sensitivity	Specificity	**Accuracy**	

Figure 4.1: Map showing the locations of all monitored colonies within Comanche National Grassland (A) and Cimarron National Grassland (B) in current prairie dog range (map insert). Grassland boundaries are in grey. Red shaded colonies experienced a colony extinction event in 2005. Numbered elements in the legend correspond to landcover classes in the 2006 National Landcover Dataset (Table 4.1).

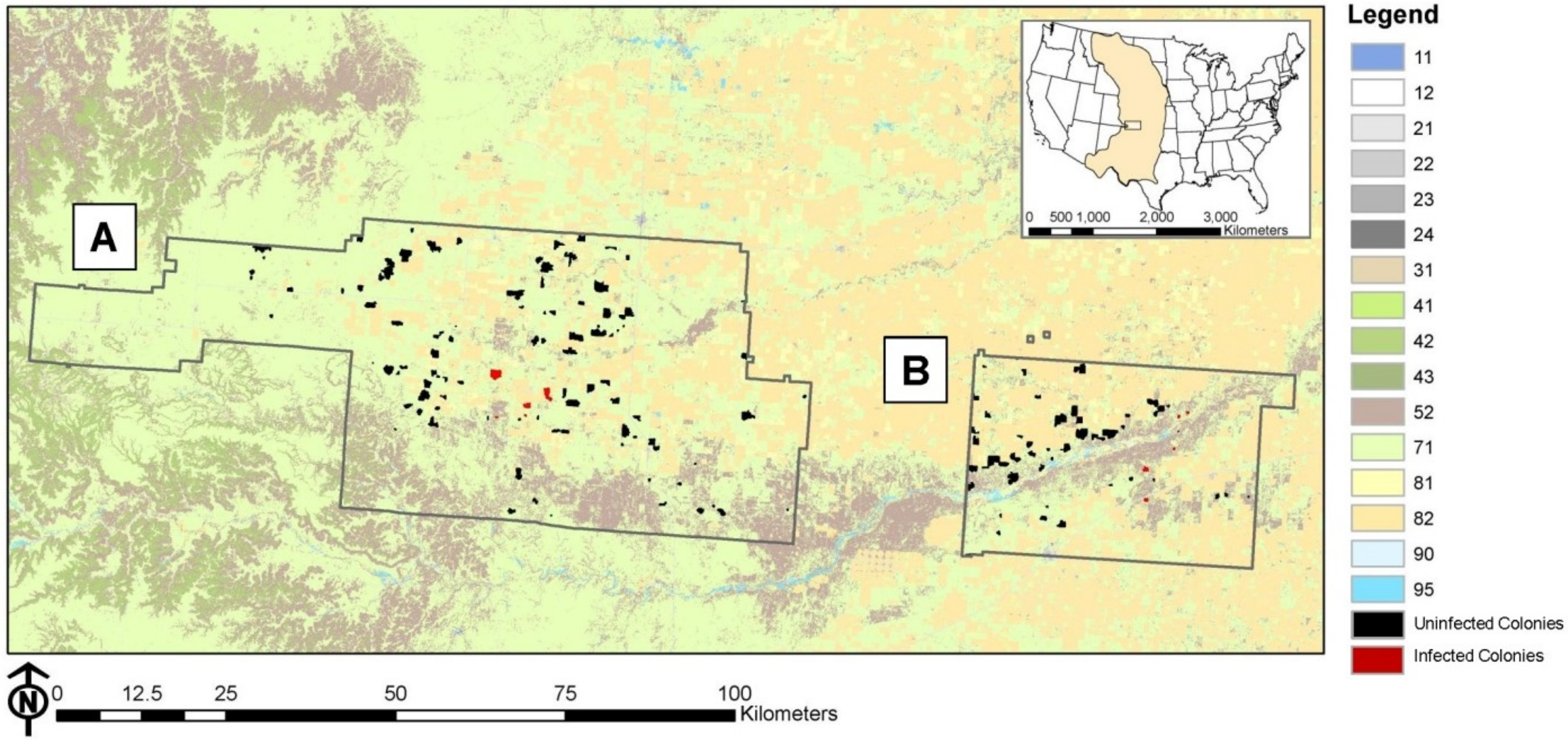
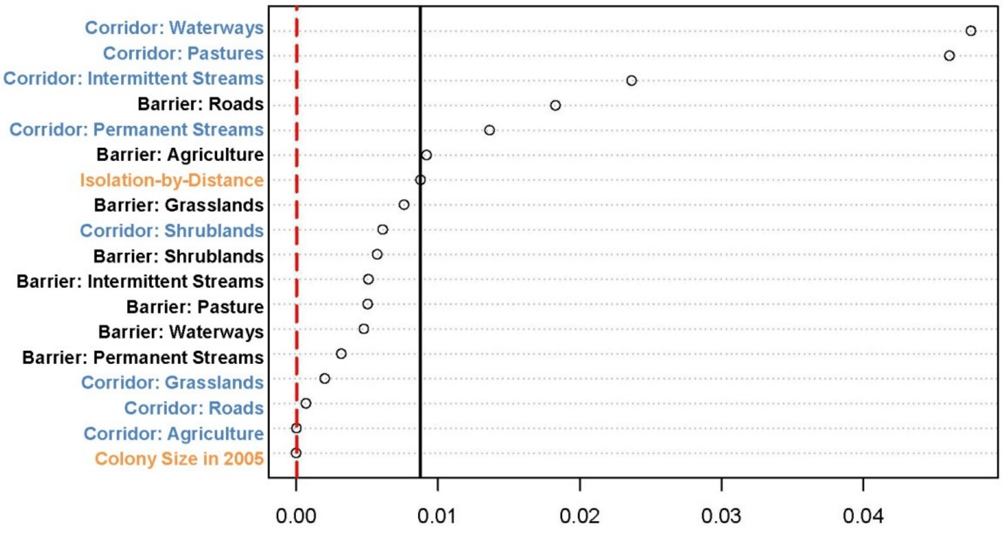


Figure 4.2: Dot plots ranking our predictor variables for Cimarron National Grassland (A) and Comanche National Grassland (B), based on conditional variable importance factors from random forest modelling (Strobl et al. 2009). Variables highlighted in blue are transmission corridors, while variables in black are transmission barriers. Colony size in 2005 and distance from current source are shown in cream. Significant predictor variables lie to the right of the dashed red line, while variables outperforming isolation-by-distance expectations lie to the right of the solid black line.

A.



B.

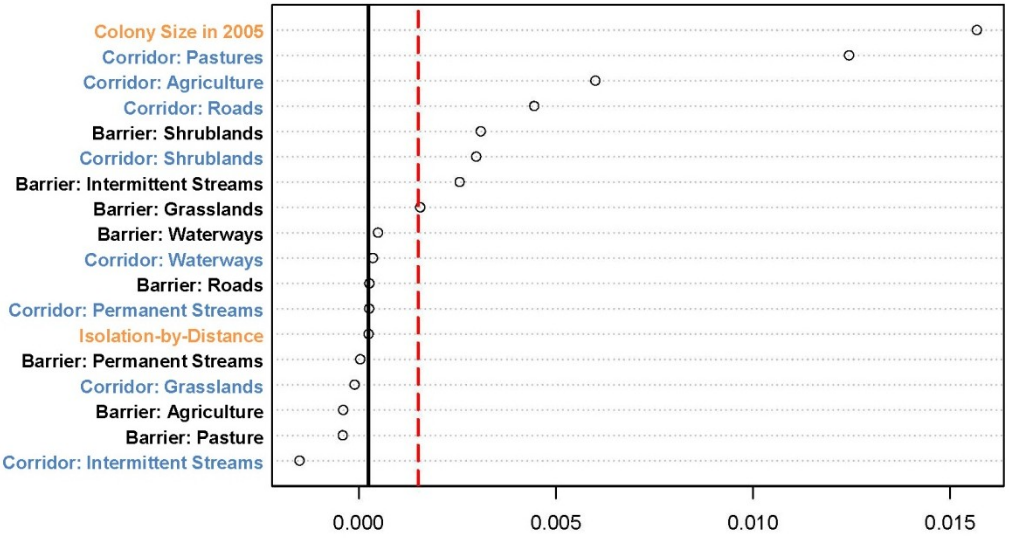
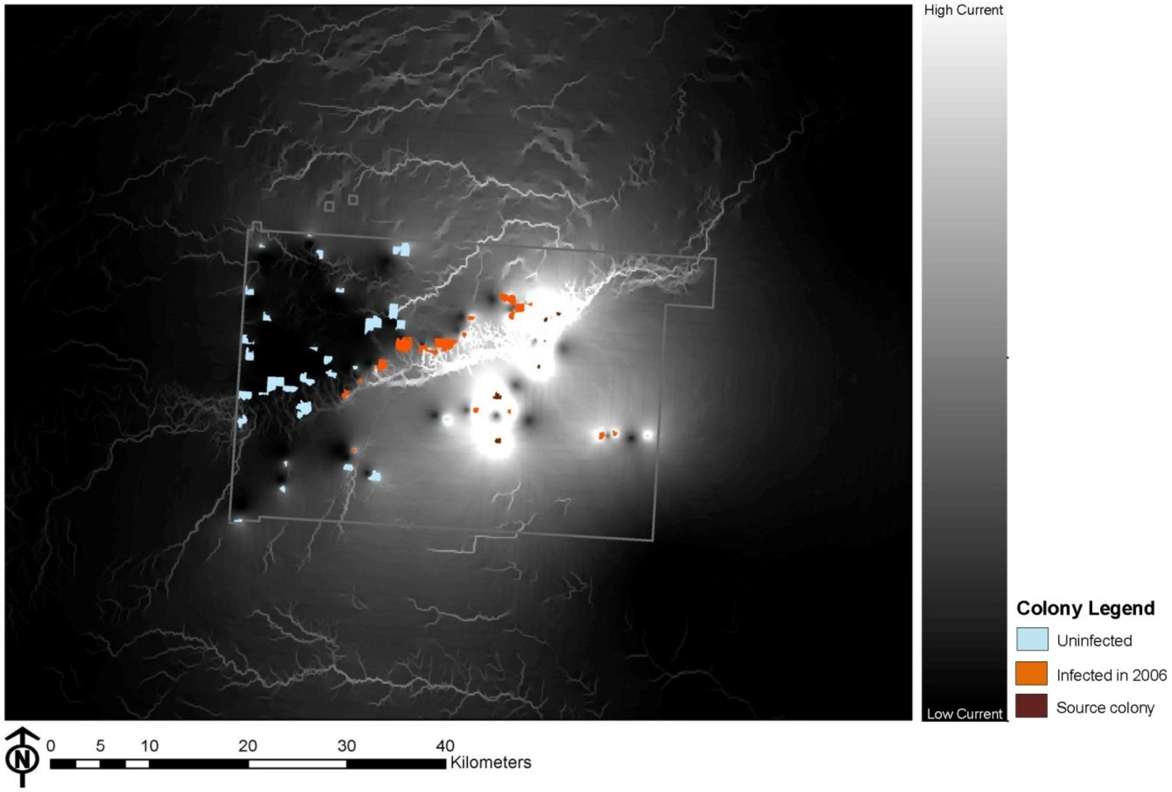
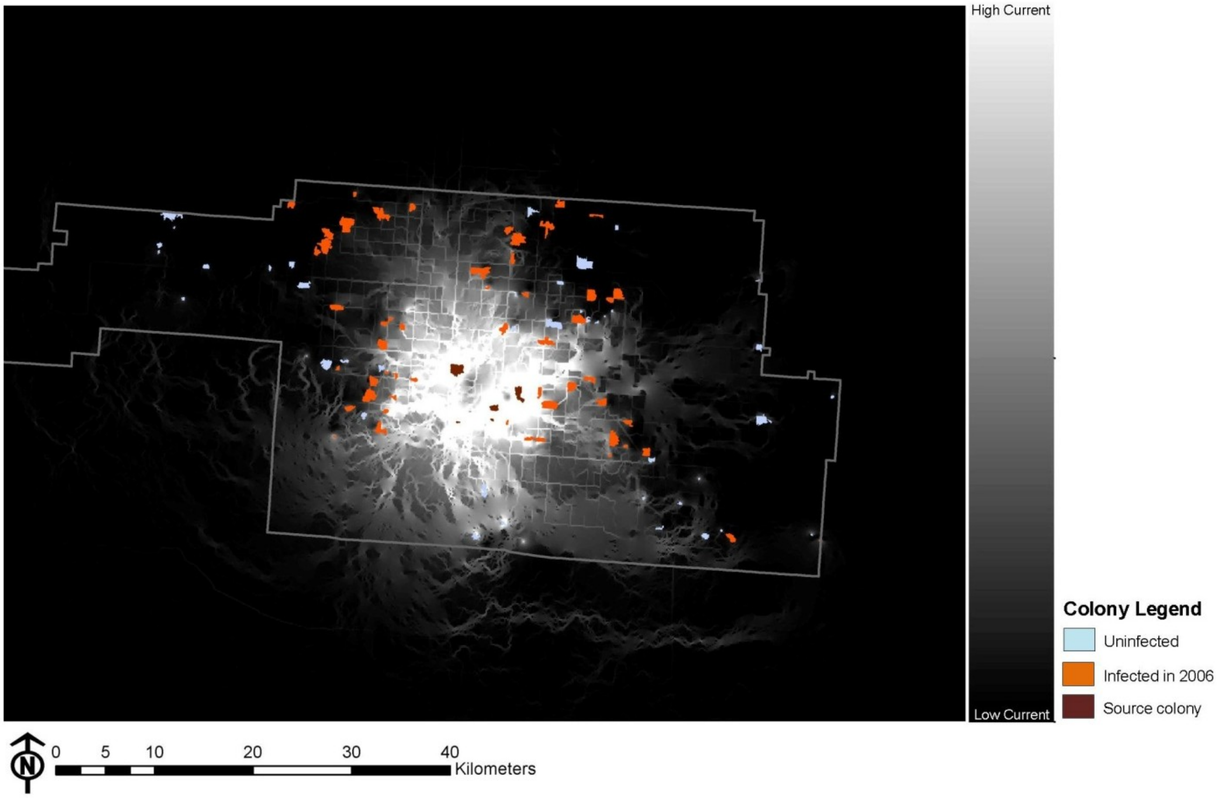


Figure 4.3: Cumulative current maps for Cimarron National Grassland (A) and Comanche National Grassland (B). Included landscape predictors were both significant and outperformed expectations of isolation-by-distance in our random forests (Figure 4.2). Disease transmission from source colonies (maroon) is most likely within lighter areas (high current) and least likely in dark areas (low current). Observed colonies losses in 2006 are shown in orange; surviving colonies are shown in light blue.

A.



B.


High Current
Low Current
Colony Legend
Uninfected
Infected in 2006
Source colony
N
0
5
10
20
30
40
Kilometers

Chapter 5 - Conclusion

In this dissertation, I have presented three studies that investigate dispersal behavior of the black-tailed prairie dog via a multi-scale investigation of gene flow and disease transmission among prairie dog colonies. These studies provide further insight into the complexities of prairie dog dispersal, which have not been previously reported. Our main findings include: (1) prairie dogs are highly resilient to habitat fragmentation and loss, (2) grassland productivity greatly influences connectivity across prairie dog range, (3) prairie dog intercolonial dispersal in short-grass prairie occurs with sufficient frequency to play a major role in sylvatic plague transmission, (4) sex-biased dispersal in this polygynous mammal is a scale-dependent phenomenon, and (5) pasturelands may facilitate plague transmission during the early stages of an epizootic event.

In Chapter 2, we showed that genetic connectivity among colonies differs depending on grassland productivity. Colonies located in short-grass prairie exchanged migrants much more frequently than colonies within mixed-grass prairie, suggesting that the dispersal dynamics of this species cannot be fully characterized by studying a single colony in either of these grassland types. Rather, prairie dog dispersal behavior varies throughout its range. Our results demonstrate the strong correlation between precipitation and connectivity at broad scales, and we expect other climatic variables, such as temperature, may also prove to be highly predictive of variations in prairie dog dispersal behavior throughout its range. While the strong correlation between precipitation and connectivity shown here revealed the influence of grassland productivity on prairie dog dispersal dynamics, an investigation of the relationship between temperature and connectivity may uncover an influence of growing season length as well.

In Chapter 3, our investigation revealed patterns that can inform multiple hypotheses concerning prairie dog dispersal behavior in future studies. Perhaps our most interesting observation in this study was the scale-dependent nature of sex-biased dispersal in prairie dogs. Although the specific mechanism(s) promoting female intercolonial dispersal remains unknown, we encourage future studies to (1) investigate the influence of forage quality and/or prairie dog density on emigration rates and to (2) compare the relative immigration success of males and females to aid in understanding of our observations. In Chapter 3, we also highlight that the genetic connectivity we've observed in our study areas supports a growing list of reports from recent literature that prairie dogs could carry plague among colonies during epizootic events in short-grass prairie. Consequently, environmental factors or colony characteristics with demonstrated influence on disease dynamics within prairie dog metapopulations can inform hypotheses concerning factors affecting prairie dog dispersal dynamics; however, environmental factors or colony characteristics implicated as predictors of genetic connectivity may underestimate disease flow, as disease transmission only requires successful arrival of a migrant and not the migrant's successful reproduction (Chapter 2).

In Chapter 4, we demonstrate the utility of program Circuitscape in studies of disease transmission. Viewing our results in the context of previous work, we suggest that wildlife managers incorporate both pastureland and colony size in their Circuitscape models to best predict the likelihood of a colony contracting plague during an epizootic event, but we caution that the effectiveness of this approach is limited to the first year of the event. Given our successful production of highly predictive models of plague transmission, we encourage future researchers to explore the utility of Circuitscape in other areas, such as invasion ecology.

Using a multi-scale approach, we have been highly successful in illuminating hidden complexities in movement patterns among black-tailed prairie dog colonies. Observations of their resiliency to habitat disturbance lead us to two broad conclusions. First, habitat specialists are not necessarily sensitive to habitat change. Though dependent on grassland habitat to thrive, prairie dogs have adapted to challenges from human disturbances and exotic pathogens. Climate change, however, may lead to a range shift for this species. Whether more sensitive grassland species that rely on prairie dog colonies for shelter, breeding habitat, or food can successfully track a prairie dog range shift is uncertain. Consequently, we stress that the resiliency of prairie dogs to habitat disturbance does not ensure the survival of the complex community supported within current prairie dog range. Prairie dogs represent an upper limit for the adaptability of North American prairie species to environmental changes; the lower limits of more sensitive species, such as the lesser prairie chicken or tiger salamander, must also be considered to preserve species diversity within North American grasslands.

Second, we stress that dispersal is a complex trait, which demands complex study designs to fully characterize its dynamics. The knowledge gained from such studies assists wildlife managers and the general public through enhanced accuracy of predictive models of animal movement and disease dynamics, more effective wildlife and disease management, and ultimately, reduction in wildlife damage and in human-wildlife conflicts. We end by encouraging future researchers to adopt similar approaches in their work.