UNDERSTANDING FUNCTIONAL CONNECTIVITY IN SHORTGRASS AND MIXEDGRASS PRAIRIES USING THE SWIFT FOX AS A MODEL ORGANISM

By

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For Lea. May your future be as limitless as the Great Plains horizon and as wondrous as the Northern Lights.

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Graduate school is nothing if not a series of lessons, professional and personal--lessons that clarify your strengths and weaknesses, lessons in time management, in prioritization, in professionalism, in collaboration, in perseverance. Ultimately, these are lessons that test your limits and broaden your horizons; in this context, the word "education" certainly acquires a few new facets during one's graduate career. One of the most important lessons I'll be taking with me is that the pursuit of scientific knowledge is a team enterprise, and many, many people contribute even if--to keep with the pursuit theme--they are the proverbial hounds nipping at your heels ("escape," of course, equates to acquiring the Ph.D., although in Lubbock it may also imply a few bars of Mac Davis as you hit the city limits). And so, as I prepare the finishing touches on this long-sought Ph.D., I am honored and humbled as I consider the many people who have encouraged, challenged, mentored, consoled, and yes, endured me during this process.

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ABSTRACT

Habitat fragmentation and loss are the greatest sources of biodiversity loss today. The negative relationship between these phenomena and myriad ecological processes are well-documented. Chief amongst these impacts is the disruption of dispersal regimes, resulting in isolated or semi-isolated groups. Reduced dispersal in turn negatively influences gene flow between groups of individuals, resulting in reduced genetic diversity, increasing risk of inbreeding depression and, ultimately, heightened extinction risk. Thus, maintaining functional connectivity in ecosystems is high on the list of conservation priorities.

The Great Plains is a vast ecosystem characterized by habitat fragmentation natural and anthropogenic in origin. Remnant shortgrass and mixedgrass prairies, in which this study occurs, have been reduced to < 50% of their previous extensive geographic area, largely due to agricultural development. Anthropogenic impacts on connectivity are predicted to increase, resulting in loss of up to 50% of remnant native grasslands. Thus, understanding these factors' influence on grassland connectivity is critical for conservation and management in both contemporary and future time scales.

Here, I employed a landscape genetics approach to address a series of objectives, which include assessing current and historic genetic diversity and structure in swift fox populations, relating gene flow and genetic structure patterns to landscape influences, and providing insight into conservation needs for the species. In addition, I used the swift fox as a model species to elucidate connectivity patterns across two focal areas in the shortgrass and mixedgrass prairies, ultimately presenting functional connectivity maps for these regions. Finally, I developed a new method for studying connectivity networks in fragmented populations with empirically derived cost metrics, and demonstrated its utility for identifying movement corridors using least-cost path modeling; this analysis was conducted in a fragmented swift fox population wherein genetic diversity appears to be linked to inter-population movement; thus identification of movement corridors is a critical conservation need locally.

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CHAPTER I

INTRODUCTION

"…I saw also two of the small burrowing foxes of the plains, about the size of the common domestic cat, and of a reddish brown colour, except the tail, which is black."
Biddle 1814

"A small fox was killed, which appears to be the animal mentioned by Lewis and Clark, in the account of their travels, under the name of the burrowing fox…It runs with extraordinary swiftness, so much so, that when at full speed its course has been, by the hunters, compared to the flight of a bird skimming the surface of the earth. I had opportunities of seeing it run with the antelope, and appearances sanctioned the belief, that in fleetness it even exceeded that extraordinary animal…Like the corsac of Asia it burrows in the earth, in a country totally destitute of trees or bushes, and is not known to dwell in forest districts."
Say 1820; in *James 1823*

Prologue

The shortgrass and mixedgrass prairies (SMGP) historically spanned 2.3 million km^2, stretching from southern Alberta and Saskatchewan in Canada south into eastern New Mexico and western Texas in the United States, as part of the Great Plains (GP), the largest phytogeographical region in North America. These expansive prairies are home to a unique biota that exhibit behaviors and life histories evolved to maximize resilience to the ever-challenging and changeful regional climate (Speils 2010). Intense drought, widespread fires and massive herds of migratory ungulates were major drivers of disturbance and, ultimately, of grassland system persistence in the Great Plains (Knopf and Samson 1997). The sweeping prairies, endless miles of quick-burning fuels and readily available forage, nurtured these disturbances. Connectivity, therefore, was and remains a critical component for the maintenance of

ecosystem function, for the continuance of disturbance regimes and, in the end, for the persistence of the region's grasslands and associated species.

On these prairies there resides a diminutive, seemingly unremarkable canid--the swift fox (*Vulpes velox*; Say 1823). There is little about the species that immediately distinguishes it from its fellow plains natives. It is by necessity a generalist, capable of eking out a living in the land of limited diversity and harsh summer and winter climates; but then, many vertebrates of the Great Plains are generalists (Speils 2010). The species exhibits a broad geographic distribution. Swift fox were historically found throughout the SMGP and, albeit rarely, in the tallgrass prairie--yet expansive distributions are not a rarity among Great Plains species. As with many of its grassland counterparts, the species was once quite numerous, a common sight to explorers, trappers and settlers alike. For example, 10,600 swift fox pelts were collected in a single three-year period between 1835-1838 (Johnson 1969). This number was 10 times higher than red fox (*Vulpes vulpes*) pelts and 100 times higher than gray fox (*Urocyon cinereoargenteus*) pelts collected during the same period. Yet this once ubiquitous, adaptable little carnivore is now a rare species throughout most of its current range, which encompasses less than half its historic breadth. The species was considered for listing under the Endangered Species Act previously; listing was found to be warranted but precluded in 1995. In 2001, the species was removed from candidacy (Allardyce and Sovada 2003). Today, it remains a species of conservation concern and is protected at varying levels of status in all nine states encompassing its distribution in the United States. This protection varies from

regulated harvest (Kansas, New Mexico, Montana and Texas), closed harvest (Colorado, North Dakota and Oklahoma), threatened listing (South Dakota) and endangered listing (Nebraska). In Canada, it was entirely extirpated. Only via intensive reintroduction efforts was it reestablished in the Alberta and Saskatchewan provinces. Currently, Canada lists the swift fox as an endangered species.

What happened?

Some of this species' tale matches that of the fate of many other carnivores. Unregulated harvest and predator-control regimes exacted a heavy toll, catalyzing its initial and precipitous decline. However, even as the species began its downward trajectory, a new and rampant disturbance was rapidly reshaping the Great Plains landscape, replacing grassland with cropland, converting once expansive and continuous prairies into increasingly smaller and more isolated fragments. Agricultural development began in earnest in the early 1900's (Waisanen and Bliss 2002, Harman et al. 2011); soon, extensive swaths of "America's Breadbasket" were converted to fields of wheat, corn, cotton and other crops (Licht 1997). Roadways, urbanization, oil pads, coal mines and fences further subdivided grassland remnants; migratory grazing and fire ceased, allowing woody vegetation to encroach into previously open and low-statured prairie grasslands. Grassland systems in the Great Plains today represent but a fraction of their historic expanse (Samson et al. 2004), and it is the current pattern of habitat loss and fragmentation that excludes the swift fox from large portions of its historic distribution and limits its repatriation of others.

In and of itself, the negative relationship between habitat fragmentation and swift fox populations is worthy of scientific inquiry. Research on the relationships among landscape composition, inter-population dispersal and gene flow is critical if conservation and restoration of this species is to be successful in its increasingly fragmented world. Understanding the role habitat fragmentation plays in gene flow and genetic structure in and between remnant swift fox populations is a key requirement for the establishment of conservation and management protocols geared towards long-term species persistence.

However, the interactions among habitat fragmentation, gene flow and swift fox populations offers another, equally important opportunity, the chance to gain insight into the impact habitat fragmentation and loss have on functional connectivity in the once highly connected SMGP. This topic has been largely neglected by the scientific community, despite burgeoning information on the negative ramifications of habitat fragmentation on biodiversity overall. Using the swift fox, I seek to garner knowledge regarding the extent at which functional connectivity, in this case as measured by gene flow, has been reduced or altogether lost in the Great Plains. I am further focused on identifying what factors, natural or anthropogenic, influence connectivity in this region. Finally, I aim to provide managers with empirically derived surfaces which demonstrate visually the overall connectivity "map" as well as movement corridors and barriers. In the following pages, I provide an overview and justification for the central focus of my dissertation, which is an assessment of the

impact of habitat fragmentations on swift fox populations and on functional connectivity in the SMGP of the United States.

The issue at hand: habitat fragmentation

In a world of increasing anthropogenic influence, the ability to understand the relationship between human activities and wildlife conservation needs is imperative. Among the many anthropogenic impacts conservationists are tasked with studying and remediating, habitat fragmentation ranks highly (Lindenmayer and Fischer 2006, Fischer and Lindenmayer 2007). Habitat fragmentation involves the subdivision of a specific landcover type or suite of landcover types into spatially disjunct pieces and is frequently associated with habitat loss (Fahrig 2003); it can occur naturally or as a result of anthropogenic disturbance. Anthropogenic habitat fragmentation and loss are considered to be the biggest threats to biodiversity globally (Fischer and Lindenmayer 2007, Lindenmayer et al. 2008, Hanski et al. 2011).

This phenomenon has been associated with myriad deleterious effects, including creation of small populations (Fahrig 2003), disrupted migration (Collingham and Huntley 2000, Gosset et al. 2006) and dispersal (Darvill et al. 2009, Peery et al. 2010) patterns, increased presence of invasive species (Marvier et al. 2004, Hoffmeister et al. 2005, Didham et al. 2007), altered predator-prey interactions (Kareiva 1987, Ryall and Fahrig 2006), disease (Brownstein et al. 2005), social alterations (Banks et al. 2007, Walker et al. 2008), disruption of nutrient cycles (Cloern 2007), limited gene flow and increased inbreeding depression (Keller et al.

2002, Keyghobadi 2007), reduced fitness (Kolb 2008) and local or global extinction (Wilcove et al. 1998, With and King 1999).

One of the most worrisome results of fragmentation is loss of genetic diversity due to disrupted gene flow patterns and increased inbreeding (Gibbs 2001, Honnay and Jacquemyn 2007, Haag et al. 2010). Even low levels of gene flow can greatly improve individual fitness and population persistence (Vilà et al. 2003, Hogg et al. 2006, Johnson et al. 2010), thus maintaining or restoring natural gene flow regimes is a critical component of long-term species management. Although habitat fragmentation may play a natural role in shaping the genetic composition of populations over time, a rapid increase in habitat fragmentation due to anthropogenic causes, coupled with habitat loss, can negatively influence species persistence (Wilcove et al. 1998, With and King 1999).

The Great Plains: an extensive (and extensively fragmented) system

The Great Plains is the largest phytogeographical region in North America (Samson and Knopf 1994). Within it lie the shortgrass and mixedgrass prairies (SMGP), which are the focus of my research; historically, these covered approximately 162 million ha^2 (Samson and Knopf 1994). Unlike many regions in the United States, human population densities are stable or declining except in urban centers, and populations are aging overall (Parton et al. 2007), with large regions classified as "wilderness" (i.e., < two person/km^2; Gauthier and Licht 2003). This trend may present unique opportunities for proactive connectivity conservation and

restoration measures not readily available in systems with persistent and growing human populations.

Unfortunately, this opportunity has been largely neglected by the conservation community (Scott et al. 2001a, b; Samson et al. 2004; Sampson and Knopf 2006; Henwood 2010). Native grasslands are the least-protected system in the United States (3.7% of total area; Dietz and Czech 2005), a pattern reflected in grassland conservation globally (Hoekstra et al. 2005). Estimates indicate at least 70% of native prairie in the Great Plains has been lost, with only 29% of shortgrass and 52% of mixedgrass prairie remaining in an unaltered but fragmented state (Samson et al. 2004). Grasslands in the region are naturally fragmented by biotic and abiotic factors (e.g., climatic gradients, topographic relief, waterways, scrub and forest landcovers) and this fragmentation likely shapes genetic patterns in native species. However, grassland systems in the GP historically covered broad geographic regions with near endless connectivity. Today, the lack of conservation has resulted in broad-scale habitat fragmentation, largely due to agricultural development and infrastructure (Samson et al. 2004, Samson and Knopf 2006, Engle et al. 2008). Simultaneously, remnant connectivity faces emerging threats from wind energy (Pruet et al. 2009a, b) and biofuel development (Fargione et al. 2009), as well as coal, natural gas and oil extraction (Forest et al. 2004, Mehaffey et al. 2012). Globally, grassland systems are predicted to experience the greatest biodiversity losses due to cumulative impacts from multiple drivers such as global climate change and land use change (Sala et al. 2000). Sohl et al. (2012) predict up to 50% of remnant native grasslands in the GP will be lost

within the next 90 years, largely due to cultivated crop expansion, highlighting the need to immediate conservation efforts to subvert this loss.

Connectivity in the GP is a topic of conservation concern for wildlife species (Sanderson et al. 2008, Hénaux et al. 2011, Cormack Gates et al. 2012). Connectivity is important for maintaining natural disturbance regimes in grassland systems such as fire (Rodriquez Gonzalez et al. 2008) and migratory grazing (Berger et al. 2008); disruption of these regimes has led to changes in plant community composition and landscape patterns (Bragg and Steuter 1996, Weaver et al. 1996, Briggs et al. 2002) and reduced biodiversity (Fuhlendorf et al. 2008, Rooney and Leach 2010, Pillsbury et al. 2011) in the SMGP. Lack of connectivity is also linked to desertification in grassland systems (Okin et al. 2009). To date, most investigations have concerned tallgrass prairie systems (e.g., Cavitt 2000, Briggs et al. 2001, With et al. 2008, Rooney et al. 2010, Kluger et al. 2011). Furthermore, the bulk of GP connectivity research has focused on birds (e.g., Coppedge et al. 2001, Rahmig et al. 2009, Klug et al. 2010) and fishes (e.g., Taylor et al. 2008, Gido et al. 2010, Bouska and Paukert 2010, Perkin and Gido 2011; reviewed in Perkin et al. 2010), providing limited insight for nonavian, terrestrial species. In addition, few studies in the Great Plains region have directly assessed the impact of habitat fragmentation on genetic structure and diversity in native terrestrial species (but see Jungels et al. 2010, Blevins et al. 2011, Klug et al. 2011, Stronen et al. 2012). This paucity of SMGP related research limits managers' ability to develop sound conservation strategies, even as land use pressures threaten to disrupt remnant connectivity. Improving our understanding of habitat

fragmentation's influence on connectivity for grassland species is critical for developing long-term habitat conservation and management regimes, identifying dispersal corridors for protection and locating dispersal barriers for remediation, as well as anticipating further impacts from emerging development pressures.

The swift fox: a conservation concern and a conservation tool

Although once abundant throughout the shortgrass and mixedgrass prairies of North America (Johnson 1969), the swift fox experienced range-wide declines in density and overall distribution for several decades (Egoscue 1979, Scott-Brown et al. 1987, Allardyce and Sovada 2003). Currently, the species is extirpated from > 50% of its historic range (Sovada et al. 2009). Concern over the degree of decline prompted petitioning of the United States Fish and Wildlife Service (USFWS) to list the species as endangered in 1992. In 1994, the USFWS determined listing was "warranted but precluded" and the species was deemed a candidate for threatened status. In response to this finding, the Swift Fox Conservation Team (SFCT) was formed by representatives of 10 states encompassing the species' current distribution in the United States. The SFCT's primary goal was development of a list of objectives and strategies for species recovery, commonly referred to as the Conservation Assessment and Conservation Strategy (CACS; Kahn et al. 1997), a draft of which was submitted to the USFWS in 1994. Review of updated population status information combined with inaction of the CACS by the SFCT prompted the USFWS to remove the species from the candidate list in 2001. Research and conservation efforts have continued as a

result of commitments by state and federal agencies to implement the CACS (Allardyce and Sovada 2003).

Habitat loss and subsequent fragmentation have been implicated, in part, as contributing factors to the decline of the swift fox (Egoscue 1979, Scott-Brown et al. 1987, Knowles et al. 2003). The species is found primarily in the SMGP (Allardyce and Sovada 2003, Finley et al. 2005, Martin et al. 2007), although it exhibits some plasticity in habitat preferences. Although agricultural development is believed to limit recolonization by swift fox (Allardyce and Sovada 2003), several studies have reported use of agricultural lands by swift fox (Cutter 1958a, b; Kilgore 1969; Sovada et al. 2003). In western Kansas, for example, some home ranges were comprised almost exclusively of cropland (Sovada et al. 1998, 2001, 2003; Matlack et al. 2000) with no apparent detrimental effects on survival (Sovada et al. 1998, Matlack et al. 2000) or reproduction (Sovada et al. 2003). Schaughnessy (2003) detected swift fox in Oklahoma cropland, albeit lower than expected based on availability of cropland land cover; similarly, Criffield et al. (2010) documented swift fox presence in cropland during presence-absence surveys but did not assess variation in selection. However, in a separate study of habitat use and home ranges in a mixed grassland-cropland matrix, Kamler et al. (2003) and Nicholson et al. (2007) observed avoidance and, in some cases, complete exclusion of agricultural lands. Presence-absence surveys for swift fox in Colorado indicated cropland avoidance (Finely et al. 2005). Utilization (Warrick et al. 2007) and avoidance (Cypher et al. 2003) of cropland has also been reported in the closely related kit fox (*Vulpes macrotis*), indicating some

plasticity in habitat use but a general trend of agricultural avoidance. Ultimately, habitat loss and fragmentation result in remnant swift fox populations that are spatially disjunct.

Data concerning habitat fragmentation's impact on the persistence and long-term viability of swift fox populations, as well as investigations into gene flow and intra-population genetic diversity in fragmented swift fox populations, are nonexistent, despite the important implications of these factors for swift fox conservation. Previous research on swift fox has occurred primarily in continuous natural habitats (or has considered them as such) and focused largely on presence/absence surveys (Harrison et al. 2002, 2004, Schauster et al. 2002, Finley et al. 2005, Martin et al. 2007), diet (Sovada et al. 2001, Harrison 2003, Lemons et al. 2010) and mortality sources (Matlack et al. 2000, Kamler et al. 2003, Thompson and Gese 2007). Research on extra-territory movements (e.g., forays, dispersal) typically has addressed the influence of predator presence (Covell 1992, Kitchen 1999) or demographic variables (e.g., individual age, sex; Covell 1992, Kamler et al. 2004a, b), rather than habitat connectivity. Habitat loss and fragmentation may necessitate increased dispersal distances between isolated grassland fragments. Dispersal distance has been negatively correlated with swift fox survival and reproduction (Moehrenschlager and Macdonald 2003), and inhospitable habitat matrices that separate populations serve as mortality sinks for swift fox (Moehrenschlager and Somers 2004). These factors combined may lead to genetic differentiation in swift fox populations in fragmented landscapes. Although these relationships are as yet unknown in swift fox populations,

negative correlations among habitat fragmentation, dispersal and gene flow have been observed in closely related canid species (Schwartz et al. 2005, Dalén et al. 2006, Riley et al. 2006). Further research is needed to develop informed swift fox conservation strategies in fragmented habitats.

Although the swift foxes' close association with native grasslands may contribute to its imperilment, this relationship is a useful tool for studying and conserving SMGP connectivity. Historic and current distribution patterns of swift fox mirror those of the SMGP; its close association with grassland systems has resulted in a spatially disjunct distribution that reflects the fragmented condition of the short and mixed grasslands across their entire extent. This relationship is not a perfect one. As mentioned previously, in some portions of its range, it uses non-grassland habitats (Sovada et al. 1998, Matlack et al. 2000, Shaughnessy 2003, Criffield et al. 2010). This may limit the inference that can be made for more habitat-specific species. However, variation in habitat utilization patterns occurs largely along the distribution periphery. Groups at the edge of a species' distribution can be reservoirs of unique genetic variation (Petit et al. 2003), lending critical plasticity in the species' adaptation potential to future land cover changes (Hampe and Petit 2005), which are likely to stem jointly from agriculture development and climate change (Sala et al. 2000, Mehaffey et al. 2012, Sohl et al. 2012). Thus, variation in swift fox habitat preferences may improve results' inference and utility, rather than limit them.

The swift fox taxonomic designation also lends credence to its use as a model system. Carnivore species are important to conservation (Sergio et al. 2006), in part

because the guild plays integral roles in many ecosystems (Terborgh et al. 2001, Ray et al. 2005, Ripple et al. 2007, Myers et al. 2007), and can be important indicator species, especially in fragmented systems (Noss et al. 1996, Woodroffe and Ginsberg 1998) as habitat loss and fragmentation are global contributors to their decline, in part due to the large area requirements necessary to support viable populations (Crooks et al. 2011). The role of mesocarnivores, such as the swift fox, is not synonymous with that of tertiary carnivores, but their importance is nonetheless well-documented (Brashares et al. 2010). Roemer et al. (2009) provide an overview of the ecological role of mesocarnivores, which includes the potential to alter nutrient flows, change plant community composition and affect the distribution and density of prey species. Changes in mesocarnivore populations can be both indicative of perturbance in ecosystems (Roemer et al. 2002, Brashares et al. 2010) and the source of these perturbations (Crooks and Soulé 1999, Roemer et al. 2009, Brashares et al. 2010). Thus, understanding the long-term impact of habitat fragmentation on populations of swift fox may have important implications for ecosystem function in the SMGP.

Habitat fragmentation and functional connectivity: the landscape genetics approach

The field of landscape genetics (Manel et al. 2003, Holdregger and Wagner 2006) offers new and powerful means for assessing the relationships among landscape composition, habitat fragmentation and animal movements, as measured via gene flow, an important outcome of effective dispersal. Quantifying animal movement in fragmented landscapes using traditional methods is a daunting task, as the necessary

sample size to detect fragmentation effects can be financially and logistically prohibitive. Furthermore, the temporal scale required to document population-level dispersal regime disruption is beyond the scope of most research projects. Genetic data provide an opportunity to circumvent these limitations (Holdregger and Wagner 2008). Genetic indices are affected by disrupted dispersal regimes and decreased landscape connectivity in both historic and contemporary time scales (Gibbs 2001, Keyghobadi 2007). Thus, patterns of gene flow and genetic structure can be related to landscape connectivity (Storfer et al. 2007, Holdregger and Wagner 2008) and are useful for studying the impact of habitat fragmentation on dispersal (e.g., Epps et al. 2007, Coulon et al. 2010, and many others). Whereas traditional field based studies require extensive trapping and monitoring over extended time scales to capture the interaction among habitat fragmentation, connectivity and organismal movement, a genetic study can address this suite of questions with a single sampling effort, provided that samples are collected at appropriate spatial (Storfer et al. 2007, Murphy et al. 2008) and temporal (Landguth et al. 2010b) scales. Using genetic indices allows researchers to move beyond quantifying structural connectivity and begin assessing functional connectivity, which stems from the hypothesis that landscape structure hinders or facilitates dispersal (Taylor et al. 2006, Murphy and Evans 2011).

Landscape genetics is a rapidly developing, highly interdisciplinary field, incorporating theory and techniques from population biology, conservation genetics, landscape ecology and geostatistics in its exploration of genetic patterns and landscape influences (Manel et al. 2003, Holderegger and Wagner 2006, Storfer et al. 2007,

Murphy and Evans 2011). As a relatively new field, its methodologies and theory are still developing (Storfer et al. 2007, Balkenhol et al. 2009a, b, Epperson et al. 2010, Balkenhol and Landguth 2011); however, rapid development of powerful analytical approaches (Cushman et al. 2006, McRae et al. 2008, Murphy et al. 2008, Schwartz and McKelvey 2009, Legendre and Fortin 2010, Murphy et al. 2010a, b, Shirk et al. 2010, Emaresi et al. 2011, Murphy and Evans 2011, Landguth et al. 2012, Shirk et al. 2012), combined with rigorous accuracy and power assessment (Murphy et al. 2008, Cushman and Landguth 2010a, b, Landguth et al. 2010a, b, Landguth et al. 2011a, Cushman et al. 2012), have resulted in new insights and unique approaches for assessing complex ecological relationships between wildlife species and their surroundings.

Landscape genetics has provided insight into diverse topics, including looming global climate change threats (Scoble and Lowe 2010, McKelvey et al. 2011, Wasserman et al. 2011, 2012), barrier identification (Riley et al. 2006, Hapeman et al. 2011), disease spread (Biek and Real 2010, Pérez-Espona et al. 2010, Guivier et al. 2011), sex-biased dispersal (Beck et al. 2008, Chambers and Garant 2010, Pernetta et al. 2011), social interactions (Kitchen et al. 2006, Walker et al. 2007, Andrews et al. 2010), metapopulations dynamics (Spear et al. 2005, Murphy et al. 2010a, b, Cosentino et al. 2011, Cobben et al. 2012), corridor identification (Epps et al. 2007, Cushman et al. 2008, Schwartz et al. 2009, Wang et al. 2009, Braunisch et al. 2010), and diverse other biological and ecological topics, providing numerous insights into the effects of habitat fragmentation on these topics. It does have its limitations

(Storfer et al. 2010): for example, temporal and geographic scale influence results (Gauffre et al. 2009, Zellerman and Knowles 2009, Cushman and Landguth 2010a, Ortego et al. 2012), landscape effects and genetic response are not precisely synonymous (Epperson 2005, Orsini et al. 2008, Landguth et al. 2010b), approaches and metrics are still being refined (Marko and Hart 2011, Segelbacker et al. 2010, Spear et al. 2010, Cushman and Landguth 2010b), landscape composition can affect power (Jaquiéry et al. 2011, Cushman et al. 2012, Graves et al. 2012) and influential factors can vary regionally, potentially limiting inference of one study to another area (Short Bull et al. 2011). However, overall, research in this developing field signifies considerable growth in our ability to elucidate landscape/species interactions and ultimately, inform conservation efforts.

A brief summary of findings

Here, I summarize the contribution of this research to three areas: swift fox conservation, understanding SMGP connectivity and management and the field of landscape genetics. I provide an assessment of current genetic diversity and structure patterns across the species' distribution in the United States (Chapter 2). This unique analysis provides important new information for population management within and across political boundaries and has implications for species restoration and reintroduction programs. I identified unusual genetic patterns and, potentially, introgression with kit fox, highlighting important topics for further study (Chapter 2). I modeled functional connectivity across two broad subsections of the species'

distribution, testing the impact of biotic, abiotic and anthropogenic factors on this important process (Chapter 3). The results of this analysis have considerable implications for species management currently and under projected climate change scenarios. Specifically, these results highlight the need for improved grassland connectivity management in the SMGP. Furthermore, my research underscores the importance of connectivity conservation in light of predicted extensive grassland loss (Sohl et al. 2012) and global climate change (e.g., Parmesan 2006). These results demonstrate that the swift fox, despite its impressive dispersal capabilities (Ausband and Moehrenschlager 2009), experiences reduced gene flow where grasslands are fragmented, an important finding given current and predicted future land use patterns in the SMGP (Mehaffey et al. 2012, Sohl et al. 2012). Long-distance movements are unlikely to conserve genetic diversity under the current fragmentation regime, and I surmise genetic diversity will further decrease as new pressures expand in the SMGP. I next provide a focused genetic diversity analysis of a swift fox population of conservation concern due to low population density and spatially disjunct geographic distribution (Mote et al. 1998, Harrison et al. 2004, Criffield et al. 2010, Schwalm et al. in press) as well as patchy legal protection (Chapter 4). These results imply that gene flow may be higher than predicted in this fragmented swift fox population and indicate this result hinges on maintenance of extant inter-population dispersal. To assist managers in conservation planning, I developed a functional connectivity model for the region and, importantly, provided preliminary insight into movement corridor location in this highly fragmented area (Chapter 5).

My research also has clear implications for the SMGP and its associated biota. First, I show that gene flow in the SMGP has been reduced from historic levels, and conclude preliminarily that this reduction is anthropogenic in origin (Chapter 2). This conclusion is further explored in an assessment of functional connectivity in terrestrial systems across two broad geographic areas in the SMGP; I demonstrate the importance of both natural and anthropogenic forces in shaping this connectivity (Chapter 3). Given the dispersal capabilities and habitat plasticity exhibited by my model species, the functional connectivity estimate I provide is likely a liberal one. This highlights the need for further research focusing on sessile species, colonial species, etc. to elucidate conservation and restoration needs for a range of nonavian terrestrial SMGP species. Regardless, this analysis demonstrates that while remnant SMGP is relatively extensive, terrestrial functional connectivity is highly impacted at broad and fine scales. Importantly, common anthropogenic activities in the GP (e.g., agriculture, urbanization) are associated with this reduction. These factors are expected to increase at local and regional scales in the future (Mehaffey et al. 2012, Sohl et al. 2012); thus my research also serves as a warning regarding eminent, extensive reduction of remnant functional connectivity in SMGP. In addition to improving our understanding of what factors influence functional connectivity in SMGP, I provide visual representation of landscape connectivity, arming managers with a roadmap from which connectivity restoration can be targeted to maximize improvement for SMGP species. Finally, my research raises a cautionary flag for connectivity persistence as global climate change impacts climate variables that highly

influence connectivity in the region, and brings to attention the need to develop proactive conservation strategies for future landscape change.

As previously discussed, the field of landscape genetics is a growing discipline with considerable utility for conservation and management issues. I offer the following contributions to this field. First, although researchers have begun expanding the spatial extent of their studies (e.g., Quéméré et al. 2010), projects that offer inference across a species' distribution remain limited, despite their importance for understanding local and regional differences in landscape/genetic interactions (Anderson et al. 2010). By conducting my inquiry at broad geographic scales, I illustrate the utility and importance of this approach for more inclusive, species-level assessments as well as the generation of broad, more universal conclusions that may have implications for multiple species or regions (Chapters 2 and 3). I demonstrate that the processes influencing functional connectivity can lack stationarity (Chapter 3), potentially leading to erroneous conclusions when attempting to apply research results from one region to another, a concern emphasized by Short Bull et al. (2011). I demonstrate the utility of newly developed methodologies (Murphy et al. 2010, Murphy and Evans 2011) for testing landscape/genetic interactions (Chapter 3 and 5) and extend these applications, presenting a new method for assessing connectivity networks in fragmented systems (Chapter 5). Although traditional methods of least cost path connectivity networks in landscape genetics are well-tested and demonstrate considerable power (Cushman et al. 2006, Braunish et al. 2010, Shirk et al. 2010, Carranza et al. 2012), they were insufficient for overcoming noisy ecological data and

correlated cost distances present in my data set. Furthermore, the methodologies required arbitrary designation of resistance values, which has been criticized (Epps et al. 2007, Rayfield et al. 2010, Spear et al. 2010, Sawyer et al. 2011). I present an approach in which resistance values are empirically derived from genetic ancestry values, with no *a priori* cost designation, circumventing this criticism. I believe this approach has considerable utility for connectivity assessment, barrier identification and corridor conservation in fragmented populations and landscapes.

CHAPTER II

LITTLE FOX ON THE PRAIRIE: GENETIC STRUCTURE AND DIVERSITY THROUGHOUT THE DISTRIBUTION OF A GRASSLAND SPECIES IN THE UNITED STATES

INTRODUCTION

Habitat fragmentation involves the subdivision of a specific landcover type or suite of landcover types into spatially disjunct pieces, and is frequently associated with habitat loss (Fahrig 2003); it is considered to be one of the greatest threats to biodiversity (Wilcove et al. 1998, Lindenmayer et al. 2008, Hanski 2011). Fragmentation occurs naturally or as a result of anthropogenic disturbance and can negatively influence genetic diversity, gene flow and genetic structure in and among populations (Gibbs 2001, Frankham 2006, Keyghobadi 2007). Reduced gene flow and genetic diversity have significant negative implications for population persistence, including decreased fecundity, fitness, survival, and recruitment and reduced adaptive plasticity (Keller et al. 2002; Frankham 2005b). Although habitat fragmentation may play a natural role in shaping the genetic composition of populations over time, a rapid increase in habitat fragmentation due to anthropogenic causes, coupled with habitat loss, can negatively influence species persistence (Wilcove et al. 1998, With and King 1999).

Few, if any, natural systems remain unaltered by anthropogenic habitat fragmentation (Noss and Csuti 1997). However, research efforts on the impacts of habitat fragmentation are not uniform across ecosystems, and conservation efforts

show similar bias (Scott et al. 2001a; Scott et al. 2001b, Henwood 2010). The shortgrass and mixedgrass prairie systems cover approximately 1.8 million km^2 of a 2.1 million km^2 region of the Great Plains in North America (Licht 1997). Native grasslands are the least-protected ecosystem in the United States (3.7% of total area; Dietz and Czech 2005). Estimates indicate at least 70% of native prairie in the Great Plains has been lost, with only 29% of shortgrass prairies and 52% of mixedgrass prairies remaining in a native but fragmented state (Sampson et al. 2004). Despite the considerable level of fragmentation in this region, few studies in the Great Plains region have directly assessed the impact of habitat fragmentation on genetic structure in native species. Not only is this problematic for contemporary conservation, but this lack of knowledge is extremely detrimental in light of predicted land-use/land-cover changes, which estimate another 30-50% of remnant grassland may be lost (Sohl et al. 2012).

The swift fox (*Vulpes velox*) provides a model system for examining the impact of fragmentation, both natural and anthropogenic in origin, on genetic diversity and structure in the shortgrass and mixedgrass prairies of the Great Plains (SMGP). The swift fox is native to North America and is found primarily in the SMGP. Its historic distribution spanned the region from its northern to southern extent in close association with the aforementioned grassland communities. Swift fox were extirpated from the majority of their historic range by the 1950's, due primarily to predator control programs and conversion of grassland to farmland, and are of conservation concern throughout most of their extant range (Allardyce *and* Sovada

2003). Currently, the species' distribution is closely associated with the presence of remnant native grasslands throughout most of its range (Finley et al. 2005, Kamler et al. 2003), although some use of cropland has been observed (Sovada et al. 2001, Criffield et al. 2010). As a result, the species exhibits a spatially disjunct distribution that generally reflects the fragmented condition of the SMGP. Although the association between habitat fragmentation and genetic patterns has not yet been assessed in swift fox populations, a correlation between these factors has been observed in closely related canid species (Schwartz et al. 2005; Dalén et al. 2006, Riley et al. 2006). Moreover, habitat loss and fragmentation may necessitate increased dispersal distances for swift fox attempting to move between grassland fragments. Dispersal distance has been negatively correlated with swift fox survival and reproduction (Moehrenschlager and Macdonald 2003), potentially leading to genetic differentiation in fragmented landscapes.

The objectives of this research were threefold. This first goal was to identify potential barriers, either natural or anthropogenic in origin, that negatively impact gene flow in SMGP species, providing managers and researchers with a starting point for studies of other SMGP species. Potential barrier location and type was hypothesized *a priori* based on factors affecting grassland continuity (Figure 2.1). The second goal was to assess genetic diversity and structure in swift fox populations, explore changes to this historic structure from historic to contemporary time scales, and ultimately provide insight into the interaction between habitat fragmentation and swift fox populations for conservation planning. Finally, by conducting this inquiry at

a broad spatial scale, this research provides an example of the complexity and diversity of information available for landscape genetics studies and highlights the importance of this approach for a holistic understanding of landscape-level influences on gene flow in wildlife populations. Studying the relationship among gene flow, genetic structure and landscape structure at broad scales may reveal patterns and processes not obvious at finer scales, as well as provide a more complete understanding of genetic patterns in widespread species inhabiting fragmented landscapes (Anderson et al. 2010).

METHODS

Study site

The study area encompassed the distribution of the swift fox in SMGP in the United States, spanning approximately 477,673 km^2 (Figure 2.1; from Sovada et al. 2009). The SMGP is characterized by flat to gently rolling expanses of native grassland with limited areas of topographic relief, interspersed with moderate to high levels of agricultural development, and moderate to low levels of transportation infrastructure, human population densities and urbanization.

Sample collection

I accumulated a data set comprised of 589 blood, hair root and tissue samples collected between 1996 and 2008, with the majority (n = 523) collected between 2004 and 2009, or roughly the time required for one swift fox generation. Samples were acquired through a variety of channels, including opportunistic collection from road-

killed individuals and furbearer harvest, live capture for translocation or research purposes, inadvertent take from predator control programs and from museum collections. The sample set spans the known distribution of the species in the United States (Figure 2.1, left) with the following exceptions. First, I was unable to collect samples from a small native population in south-western South Dakota. Second, I did not include samples from translocated populations in South Dakota. Capture locations and dates overlapped extensively between this study and translocation events. Therefore, assessing allele frequencies, genetic diversity indices and gene flow rates would be uninformative and extremely biased. However, I did include samples from along the border between Montana and Canada despite likely interbreeding between native Montana swift fox and reintroduced populations in Canada, which stem from multiple source populations in the United States. Temporal and spatial overlap between sampling for this study and previous translocation events was minimal, as translocations in this area ceased at least 13 years prior to sample collection for this study. In addition, I chose to include four samples that were collected outside the known swift fox distribution in western Wyoming and along the North Dakota/Canada border or in the far western portion of the North Dakota/South Dakota border, where the swift fox was believed to be extirpated until the unexpected collection of road-killed specimens (Figure 2.1). These samples were of particular interest to managers, who were interested in identifying source populations.

DNA extraction, PCR and genotyping

I extracted whole genomic DNA from samples using the QIAGEN DNeasy tissue and blood kit (QIAGEN Inc, Valencia, California, USA) except that I used a 12-hour incubation at step 3 and 210 µl of ethanol at step 6. I next extracted genetic data using microsatellite and mitochondrial DNA markers. For microsatellite data, I used 16 primer sets (Ostrander et al. 1993; Fredholm and Wintero 1995; Francisco et al. 1996, Cullingham et al. 2007; Table 2.1) previously used for swift fox (Kitchen et al. 2005; Cullingham et al. 2007).

Microsatellite primers were divided into three multiplexes using the QIAGEN Multiplex Kit (QIAGEN Inc, Valencia, California, USA). For tissue samples, PCR conditions (7 µl final volume) for multiplex one were 2X Master Mix, 5X Q solution, 0.05 µM each primer for locus CXX173, 0.06µM each primer for loci CXX377 and FH2054, 0.09 µM each primer for locus CXX20, 0.10 µM each primer for loci CPH3 and CXX250 and 0.19 µM each primer for locus CXX403. The PCR thermoprofile included initial denaturation for 15 min at 95ºC, 14 cycles of 30 s at 94ºC, 90 s at 55ºC (decreasing 0.3ºC per cycle to 50.8ºC) and 1 min at 72ºC, followed by 20 cycles of 30 s at 94ºC, 90 s at 51ºC and 1 min at 72ºC, then final elongation at 60ºC for 30 min.

PCR conditions for multiplex two were 2X Master Mix, 5X Q solution, 0.03 µM each primer for locus CXX263, 0.08 µM each primer for locus VVE2-111, 0.15 µM each primer for locus CXX2062, 0.25 µM each primer for locus VVE5-33 and 0.30 µM each primer for locus CXX109. PCR conditions for multiplex three were 2X Master Mix, 5X Q solution, 0.06 µM each primer for locus VVE-M19, 0.08 µM each primer for locus VVE3-131, 0.10 µM each primer for locus VVE-M25 and 0.25 µM

each primer for locus VVE2-110. The PCR thermoprofile for multiplex two and three included initial denaturation for 15 min at 95ºC, 12 cycles of 30 s at 94ºC, 90 s at 53ºC (decreasing 0.3ºC per cycle to 49.4ºC) and 1 min at 72ºC, followed by 25 cycles of 30 s at 94ºC, 90 s at 47ºC and 1 min at 72ºC then final elongation at 60ºC for 30 min. I subsequently removed VVE-M25 from analysis due to inconsistent peaks, which rendered allele identification subjective.

Quantity and quality of DNA in hair root and blood samples is often lower than in tissue samples, so generating a usable product can require modifications to the PRC profile. I did not change PCR conditions for blood or hair root samples but added 5 additional cycles at the 4th step of the thermoprofiles. PCR products were run on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Inc., Carlsbad, California, USA) and allele size, number of alleles and individual genotypes were determined using GeneMapper 3.7 (Applied Biosystems, Inc., Carlsbad, California, USA). I checked data for genotyping error using a randomly selected portion of the sample set; PCR analysis was repeated for this random sample and compared to the original sample for agreement. Because DNA quantity and quality are potentially higher in tissue samples than in hair root and blood samples, leading to differences in error risk, I randomly selected and reanalyzed 11% of tissue samples (n = 48) and 17% of hair and blood samples (n = 23).

For mitochondrial DNA analysis, I sequenced a 250-base section of the mitochondrial control region (Ward et al. 1991). Due to logistic and financial constraints, I was unable to analyze the entire sample set. Instead, I randomly selected

100 samples (17%) but subjectively included the oldest samples (e.g., those collected prior to 1999, $n = 8$) to provide the best possible measure of historic genetic structure and ensured that the entire species distribution was represented in the final sample set (Figure 2.2). The laboratory protocol was comprised of the following steps. Step 1, DNA amplification: PCR conditions (10 µl final volume) were 1.5X $MgCl_2$, 1.5X Goldtaq buffer, 0.3 µM each primer (Thr-L15997 and Control H16401), 0.1 µM AmpliTaq Gold, 10 µM DNTPs with a thermoprofile of initial denaturation for 10 min and 30 s at 95ºC, 20 cycles of 30 s starting at 51ºC and decreasing 0.2ºC per cycle, 1 min at 72ºC, 20 s at 95ºC, followed by 20 cycles of 30 s at 48ºC, then final elongation at 72ºC for 8 min. Step 2, DNA cleanup: following manufacturer protocol, I mixed 5 µl of product from step 1 with 2 µl of exosap, then ran the mixture at 37ºC for 15 min, 80ºC for 15 min and 4ºC for 10 min. Step 3, sequencing: PCR conditions (10 µl final volume) were 2X Big Dye, 2.4x sequencing buffer and 2X primer (Control H16401), with a thermoprofile of initial denaturation for 3 min at 90ºC, 25 cycles of 30 s at 95ºC, 15 s at 50ºC and 4 min at 60ºC, followed by cool down at 4ºC for 10 min. Sequencing products were run on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Inc , Carlsbad, California, USA). Sequences were aligned using the program Sequencher (Gene Codes Corporation, Ann Arbor, Michigan USA) and grouped into unique haplotypes using the program MacClade (Maddison and Maddison 2000).

A priori *genetic structure hypothesis*

Prior to testing for genetic structure, I predicted the location of genetically distinct groups, based on the presence of landscape features that I believed might be significant barriers to swift fox dispersal. I identified both natural and anthropogenic forms of dispersal barriers, including river systems, mountain ranges, regions characterized by rugged topography, urbanized areas, high-traffic roads and agricultural development. In total, I identified 8 regions that I hypothesized might currently be genetically distinct from one another (Figure 2.1, left).

Analysis of historic genetic structure

I constructed a minimum spanning network using HapStar (Teacher and Griffiths 2010) to assess mitochondrial haplotype phylogenetic relationships, then recreated the resulting network such that nodes were weighted by sample size using Gephi (https://gephi.org/). I used the geographic location of these haplotypes to assess potential historic patterns of genetic structure in a landscape context.

Analysis of current genetic diversity and structure

I conducted both spatial and aspatial analysis of the nuclear dataset. I chose STRUCTURE v. 2.3 (Pritchard et al. 2000; Hubisz et al. 2009) for aspatial analysis and TESS (Francois et al. 2006; Durand et al. 2009) for spatial analysis. I calculated the total number of groups (K) and assigned individuals to these groups using Bayesian clustering and assignment methods available in these programs.

Using the admixture model and correlated allele frequencies in the program STRUCTURE, I identified the optimal K value by first testing $K = 1 - 15$ with a burn-in period of 50,000 iterations and a total run time of 500,000 iterations for each of 10

replicates per candidate *K* value. If support for a specific value was not clear based on log-likelihood values, I selected a *K* value using the delta *K* statistic described by Evanno et al. (2005). I next assigned individuals to groups using a *q* value of ≥ 0.70, then analyzed each resulting group individually in STRUCTURE using the above analysis framework, except that *K* = 1 - 6, to check for support for further subdivision. I continued with this approach until the majority of individuals were admixed (i.e., *q* < 0.70) or individuals from multiple groups lacked cohesive geographic grouping. I used the extension XTools Pro (Data East Corporation, Novosibirsk, Russia) in ArcMap 9.3 (ESRI, Redlands, California, USA) to create Thiessen polygons around samples for comparison to TESS analysis results.

The program TESS considers the spatial configuration of samples during clustering and assignment tests. To do so, the program first constructs a neighborhood network of pair-wise connections between neighboring samples; these connections can exceed expected dispersal capabilities of the study organism. Average straight-line post-release movement distances (Fritts et al. 1997) and straight-line natural dispersal distance (Shields 1987) for swift fox vary by region and sex but are typically < 30 km on average (Allardyce and Sovada 2003; Kamler et al. 2004a). However, at least three long-distance movements have been recorded for swift fox: 411 km (Shaun Grassel, pers. com.), 298 km (Kevin Honness, pers. com.) and 191 km (Ausband and Moehrenschlager 2009). Thus, I removed all connections between samples in the neighborhood network greater than the mean for known long-distance movements (300 km).

TESS uses an interaction parameter, Ψ, to weight the relationship between geographic and genetic information on subsequent groupings. As Ψ increases, the weight placed on spatial location increases and the weight placed on genetic information decreases (Francoise et al. 2006). Choosing an appropriate interaction parameter is an important consideration when samples occur in clumps, as exhibited in portions of the sample distribution for this study, because a high Ψ value may mask genetic patterns by arbitrarily creating groups and assigning individuals based on sample aggregation. Conversely, a low Ψ value can result in an overabundance of groups, which is equally unlikely to represent the true nature of the populations being studied. Following program manual guidelines, I tested a range of Ψ values from 0.20 to 0.80 and selected Ψ = 0.60 for this study. This decision was based on associated log-likelihood values and production of sample aggregations that were biologically reasonable.

To identify the optimal *K* value using TESS, I used a burn-in period of 10,000 iterations and 50,000 total iterations for 20 replicates for each integer value of $K = 5$ - 15. The estimated number of clusters stabilized at $K = 6$. Following program guidelines, I next conducted the final analysis using 200 replicates at $K = 7$ with the same burn-in and total iterations previously defined and selected the top 20% of replicates ($n = 40$) for further use.

Both STRUCTURE and TESS produce *q* values for each individual; these indicate the percent ancestry associated with each putative population. I used the program CLUMPP (Jakobsson et al. 2007) to compile two final *q* values for each

individual; one *q* value was calculated using the values from the 10 iterations associated with optimal *K* determined by STRUCTURE, and one value was calculated from the 40 highest ranked iterations for the optimal *K* determined by TESS. I assigned individuals to a source population for each clustering method using the corresponding *q* value and considered them admixed if $q < 0.70$.

Genetic structure can occur as a result of geographic distance, where individuals are more closely related to neighboring individuals than to individuals further away in space, a process known as isolation by distance (IBD; Wright 1943). I used SpaGEDI (Hardy and Vekemans 2002) to calculate individual-based pair-wise genetic distance (Rousset's *a*; Rousset 2000) and Euclidean distance matrices. I used zt (Bonnet and Van de Peer 2002) to conducted a Mantel test (Mantel 1967) using the genetic and geographic distance matrices to assess the influence of IBD in the dataset.

I tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LE) in each group with FSTAT (Goudet 1995), using a Bonferroni correction (Rice 1989) at a nominal α value of 0.05. I calculated allelic richness using HP-Rare (Kalinowski 2005), which adjusts calculations for unequal samples sizes. I calculated observed and expected heterozygosity using Arlequin 3.5 (Excoffier and Lischer 2010). I conducted pair-wise comparisons of between-population F_{st} in Arlequin 3.5 (Excoffier and Lischer 2010) to test the significance of differentiation between populations. For these analyses, I grouped individuals according to sampling location rather than population of origin.

RESULTS

Genotyping error rates

I detected an overall genotyping error rate of 1.0%. Genotyping error rates were 0.6% for tissue samples and 2.0% for hair and blood samples. All errors in the hair and blood subsample ($n = 6$) were associated with a single sample, and 80.0% of errors in the tissue subsample ($n = 5$) were associated with a single sample.

Historic genetic diversity and structure analysis

I detected nine polymorphisms consisting of eight transitions, one transversion and one substitution. These represented six unique haplotypes in the mitochondrial DNA dataset (n = 4, 8, 4, 4, 76 and 4 for haplotypes 1, 2, 3, 4, 5, and 6, respectively). Of these, haplotype 5 was distributed throughout the study area excluding the northernmost region, whereas the other six haplotypes were geographically localized to varying degrees (Figure 2.1, right). When I constructed a minimum spanning network using these six haplotypes, I found that most haplotypes were centralized around haplotype 5, differing by one base except for haplotype 3 (Figure 2.2), which differed by 6 – 8 nucleotides.

Current genetic diversity and structure analysis

The total number of populations detected differed between STRUCTURE and TESS analysis methods; log-likelihood and delta *K* results are presented in Appendix A. Results from STRUCTURE indicate three hierarchies of genetic structure in the data (Figure 2.3), which can be interpreted as increasing from coarse scale to fine scale genetic structure with each consecutive hierarchy. At the coarsest scale ($K = 2$),

I found distinct northern and southern groups, with considerable admixture where the two intersected spatially. At the median scale ($K = 6$), each of the groups detected at $K = 2$ was subdivided into three distinct subgroups. The majority of these subgroups were located in the south-central portion of the species' range. At the finest scale of genetic structure ($K = 10$), all new groups were detected in the south-central portion of the species' range, specifically northern New Mexico, Texas, Oklahoma and far southern Colorado. I detected seven genetic groups using the spatial analysis of genetic structure in TESS (Figure 2.5). The majority of these subgroups were located in the south-central portion of the species' range. Breaks between genetic groups at moderate levels of genetic structure (Figures 2.3 and 2.4) correspond to hypothesized gene flow boundaries (Figure 2.1, left) in many but not all instances.

I found evidence of IBD in the swift fox distribution (Mantel $r = 0.18$, $P = 0.01$). Pair-wise F_{st} comparisons between populations in TESS and each hierarchy of STRUCTURE were all highly significant ($P \leq 0.001$; Table 2.2), but differentiation varied from low to moderate (F_{st} range = 0.02 – 0.25; Table 2.2). The analysis of HWE and LE showed few violations of equilibrium; no groups and no individual loci or locus-locus comparisons were consistently out of equilibrium (Table 2.3). Allelic richness and heterozygosity were low overall and comparable across genetic groupings.

DISCUSSION

Here I discuss genetic diversity and structure patterns throughout the current swift fox distribution in the United States, starting with comments on model agreement and following with research implications for three areas. First, I discuss results in the context of SMGP connectivity, a subject for which there is a paucity of information. Next, I review the implications of my findings for swift fox management and conservation. Finally, I close by summarizing the unique and diverse advantages I observed in working at a broad geographic scale. Assessing conservation needs at a local scale can overlook regional patterns (e.g., "the tyranny of the local"; Groves 2003). The results imply genetic structure results from diverse sources across the SMGP, a pattern that narrowly focused analysis would have likely failed to detect.

Model agreement

The number of groups identified differed between spatial (i.e., TESS) and aspatial (i.e., STRUCTURE) analysis. Spatial results corresponded most closely to the median level of genetic structure detected in aspatial analysis (K = 7 and 6 in TESS and STRUCTURE, respectively); I suggest that general model agreement implies the greatest support for mid-level genetic structure patterns. However, given that the boundaries defining groups at finer scales are more recently derived (see below), I further suggest fine-scale structure that lacks multi-model support may represent emergent disturbances not immediately detectable across analytical methods.

Habitat fragmentation and gene flow in the Great Plains

Prior to analysis, I identified potential gene flow barriers that represented both anthropogenic and natural sources of grassland fragmentation (Figure 2.1). Genetic

differentiation is synonymous with many of these boundaries, regardless of type, implying that gene flow in Great Plains species is impacted by a complex suite of landscape influences, and, importantly, that these factors are not universal across the SMGP. Connectivity conservation will require strategies that reflect this complex relationship. However, my assessment is limited to one vagile, relatively widespread species. I encourage further research on this topic using other grassland species, which may also be negatively affected (*sensu* Semple Delaney et al. 2010), especially those that are sessile, exhibit short generation times, possess smaller effective population sizes and exhibit otherwise different life history traits than does the swift fox. Several of the breaks I observed occurred in association with agricultural development, a prevalent landscape feature in the SMGP, potentially indicating widespread reduction in SMGP connectivity. This result is especially critical given predicted extensive future native grassland loss (Sala et al. 2000; Rashford et al. 2011, Sohl et al. 2012).

Although genetic structure in the SMGP appears to have increased in contemporary time scales, my results indicate restricted gene flow and genetic substructure also occur naturally in the SMGP. Groups experiencing restricted gene flow over long time periods often house unique genetic variation (Petit et al. 2003; Provan and Bennet 2008, Diekmann and Serrão 2012); conserving this variation is critical for species resilience and recovery as grasslands are further disrupted by land use and global climate change (Frankham 2005b, Hampe and Petit 2005, Cobben et al.

2011). These results highlight regions that may be of particular interest for further investigation and, if warranted, conservation under these scenarios.

Swift fox conservation implications

Genetic structure

I observed extensive genetic structure across the swift fox distribution in the United States. Isolation by distance contributes to this pattern. The analysis of differentiation (F_{st}) supports this result in part, as groups that were spatially proximate were less differentiated than groups that were geographically distant. However, the explanatory power of the Mantel test was moderate to low, implying additional factors that also shape genetic structure. Genetic structure as measured by nuclear DNA was lower than predicted but higher than historic levels as indicated by mitochondrial DNA, indicating reduced connectivity in contemporary time scales.

I detected hierarchical structure in nuclear DNA analysis, implicating three levels of genetic structure in the swift fox distribution. At the coarsest level of genetic structure ($K = 2$), I observed northern and southern groups, with extensive admixture at their junction. The source of this pattern is unclear; however, I offer two competing potential explanations for further research. These groups may represent a response to climatic influences (e.g., temperature, precipitation) along a latitudinal gradient. Differentiation along an environmental cline has been recorded in other North American canids (Geffen et al. 2004). Conversely, these groups may correspond to two expanding source populations, representing species recovery post-extirpation,

which are loosely subdivided by heterogeneous landscapes within the region of admixture.

At the median hierarchy of genetic structure ($K = 6$ and $K = 7$ in STRUCTURE and TESS, respectively), group boundaries often correspond to naturally occurring habitat fragmentation sources. For example, unique genetic groups occur on either side of the Arkansas River, the Conchas and Canadian Rivers and their associated drainage system, topographic relief along the Colorado-New Mexico border (Barriers D, F, and E, respectively, in Figure 2.1) and multiple rivers and mountain ranges in Wyoming and Montana. Unique mitochondrial haplotypes occur in association with these boundaries as well. The presence of historic genetic groupings has important implications for swift fox conservation, as groups may contain unique genetic diversity. Developing management regimes which maintain genetic diversity in the swift fox distribution may require targeting multiple regions characterized by unique genetic characteristics.

Fine-scale genetic structure ($K = 10$) may reflect localized or emergent gene flow influences. That these factors were not detected except at fine scales could imply that associated boundaries represent semi-permeable gene flow barriers. Conversely, given the lag time between habitat fragmentation and subsequent genetic response (Landguth et al. 2010b), fine-scale structure may instead implicate emergent disturbances. Fine-scale structure occurs where landscape heterogeneity is high and results largely from agricultural development, a recent (Parton et al. 2007) and ongoing (Licht 1997, Mehaffey et al. 2012, Sohl et al. 2012) disturbance in the SMGP.

In addition to landscape influences, fine-scale genetic structure may be an artifact of swift foxes' variable breeding regimes. Cooperative breeding has been documented in the closely related kit fox (Ralls et al. 2001), and both Kitchen et al. (2006) and Kamler et al. (2004b) reported this reproductive strategy in swift fox; Kitchen et al. (2005) also detected kinship clustering. At a fine scale, these behaviors may increase genetic structure such that regions where these behaviors are common are identified as distinct groups (Storz 1999). Indeed, the study areas of both Kitchen et al. (2005; 2006) and Kamler et al. (2004b) are represented by unique groups, but only at the finest scale of genetic structure observed. The diverse and potentially synergistic effects of these three scenarios represent an area rich with research possibilities.

Interestingly, while both STRUCTURE and TESS results did not support the presence of two distinct groups in Wyoming, mitochondrial DNA analysis revealed two unique haplotypes corresponding loosely to genetic structure predictions for that area. These results may indicate a recent increase in gene flow locally. Swift fox were extirpated or nearly so from much of their range by the 1950's but subsequently expanded back into some areas of their historic distribution (Allardyce and Sovada 2003). The location of remnant populations is not known, nor are repatriation patterns well-understood. Potentially, the lack of contemporary genetic structure in this region results from recent recolonization, or genetic mixing of a small remnant population with extensive outside immigration (Wade and McCauley 1988).

Genetic diversity

Diversity measures were relatively uniform across groups, excluding allelic richness measures for $K = 2$, which were considerably higher. Population size and geographic extent are positively related to allelic richness (Frankham 1996), so it is not surprising allelic richness was higher at $K = 2$. Genetic diversity measures were low, similar to reported values for closely related, imperiled canids (Schwartz et al. 2005, Smith et al. 2006; but see Dalén et al. 2006). Allelic richness and observed heterozygosity were consistently highest in the central portion of the species' range and decreased towards the limits of the species' distribution. These results are consistent with diversity expectations at the edge of a species' distribution based on the central-marginal hypothesis (Yeh and Layton 1979; Lammi et al. 1999; Eckert et al. 2008). However, if this decline is associated with isolation via habitat fragmentation, peripheral groups may require additional conservation efforts to maintain genetic diversity and ameliorate extinction risk. In addition, peripheral groups may exhibit similar levels of genetic diversity as central groups but house unique variation critical for adaptation to developing selection pressures (Petit et al. 2003; Hampe and Petit 2005, Diekmann and Serrão 2010).

The group located in southern New Mexico is associated with consistently high F_{st} values, even with groups that are geographically close. This result is striking considering groups that are farther from each other in space are less differentiated than this group is from even its closest neighbors. These results imply that the effects of factors influencing genetic structure patterns in southern New Mexico are especially strong, relative to other regions. This region is separated from the rest of the species'

distribution by the Conchas and Canadian Rivers and their drainage systems, which may form an impermeable dispersal barrier. However, if this is the case, it is unclear why high differentiation was not associated with other, analogous dispersal barriers (e.g., the South Platte River in Colorado or the Medicine Bow Mountains in Wyoming). Mitochondrial results may help elucidate this situation. The unique haplotype I detected (i.e., haplotype 3) locally was more similar to kit fox (*Vulpes macrotis*) haplotypes than swift fox haplotypes in GenBank (Benson et al. 2006). The two species are closely related and believed to hybridize locally (Mercure et al. 1993), so the presence of this haplotype in swift fox samples may represent interbreeding between swift and kit fox. Simultaneously, the Allee effect (Allee 1931) may amplify expression of this haplotype locally while the Canadian River limits its transfer to northern swift fox populations. Hybridization can impact allele frequencies (Roy et al. 1994), offering further explanation for the unusually high F_{st} values in this area. Conversely, its presence may represent incomplete lineage sorting, wherein some alleles are polyphyletic between kit and swift fox (Avise 2004). Whether the differentiation I observed is an artifact of habitat fragmentation, hybridization, evolutionary history or a combination of these factors warrants further investigation. Low genetic variability and high levels of differentiation, coupled with low population density, may indicate this population is worthy of increased conservation efforts. Furthermore, populations at the southern edge of a species' distribution may be disproportionately important for species adaptation under global climate change scenarios (Hampe and Petit 2003; Cobben et al. 2011; Sexton et al. 2011).

The group in Montana is likewise associated with low nuclear and mitochondrial genetic diversity. The species was nearly extirpated locally, while it was extinct and subsequently reintroduced in nearby Canada (Carbyn et al. 1994), potentially explaining reduced genetic variation. What is more difficult to explain, however, is the identity of the haplotype found in this region (i.e., haplotype 2). Outside of Montana, haplotype 2 is found in a restricted geographic area in Wyoming that excludes source populations used for reintroduction to Canada (Carbyn 1998). Why, then, is it the only haplotype I detected in Montana? I offer two possible explanations. First, perhaps its presence in Wyoming and Montana indicates historic gene flow between the two areas. Second, perhaps haplotype 2 was translocated to Canada, unusually successful in the reintroduced population, and subsequently replaced the native haplotype in Montana. This would require its presence in source populations, which I did not observe. Has it been lost in source populations since translocations ceased? Neither hypothesis explains the absence of haplotype 5 from Montana, however. In either scenario, I would expect it to be present, given its commonality range-wide. Might it have been absent or at least uncommon during translocations, or even earlier? If this is the case, then haplotype 5's panmictic distribution may represent recent recolonization events following population decline. The ability to resolve these questions is constrained by the limited number of samples for which I have mitochondrial information, and historic panmixia is the most parsimonious explanation these results offer. However, the questions I pose here beg further inquiry. Certainly the recolonization hypothesis is worthy of further

examination, as it may lead to important insights into how species repatriate fragmented habitats after population decline and extirpation.

Assessing genetic patterns at broad geographic scales

The term "scale" has many possible definitions (Dungan et al. 2002); here I refer to scale in the context of spatial extent. In landscape ecology, the importance of spatial scale is a well-recognized issue (Turner et al. 1999). When assessing the relationship between pattern and process, the power to detect process influence is directly associated with scale choice (Wu 2004). Choosing the appropriate scale is a subject of considerable concern in landscape genetics as well (Storfer et al. 2007; Balkenhol et al. 2009, Anderson et al. 2010, Cushman and Landguth 2010a). I conducted this research at a broad spatial scale, the extent of which exceeded many landscape genetics studies. The benefits of this approach included identification of localized influences that occur in a limited portion of the species' range, identification of genetic patterns that are likely to only be detected at broad scales, assessing the influence of diverse fragmentation sources (e.g., rivers, mountains, agriculture, urban development) individually and in concert, as well as the opportunity to generate general inferences that transcend species-specific implications and provide insight into ecosystem-wide impacts. I acknowledge that logistic and financial constraints present significant limitations but encourage individuals to design studies that assess genetic patterns and landscape correlates at broad scales when possible. Including samples from museum collections or harvested animals and collaboration with other

researchers is likely to increase the potential for broadening the spatial extent of a study, as it did in the research presented here.

CHAPTER III

MULTIPLE FACTORS INFLUENCE CONNECTIVITY IN SHORT AND MIXED GRASS PRAIRIES OF THE GREAT PLAINS: A SWIFT FOX CASE STUDY

INTRODUCTION

In a world of increasing anthropogenic influence, the biggest threats to biodiversity are habitat alteration (Fahrig 2003, Fischer and Lindenmayer 2007, Lindenmayer et al. 2008, Hanski 2011). This phenomenon is associated with a myriad of deleterious effects, including population subdivision and isolation (Fahrig 2003), disrupted migration (Gosset et al. 2006) and dispersal (Darvill et al. 2009) patterns, increased access for invasive species (Didham et al. 2007), altered predator-prey interactions (Ryall and Fahrig 2006), disease (Brownstein et al. 2005), social alterations (Walker et al. 2008), disruption of nutrient cycles (Cloern 2007), limited gene flow and increased inbreeding depression (Keyghobadi 2007), reduced fitness (Kolb 2008) and local or global extinction (With and King 1999, Hanksi 2011). Currently, habitat fragmentation and loss are the dominant topics of conservation-oriented research (Fazey et al. 2005). Ecologists have long struggled to quantify animal movement in fragmented landscapes, as the necessary sample size to detect fragmentation effects can be financially and logistically prohibitive. Furthermore, the temporal scale required to document population-level dispersal regime disruption is beyond the scope of most research projects. Genetic data provide an opportunity to circumvent these limitations (Holdregger and Wagner 2008). Genetic indices are affected by disrupted dispersal regimes and decreased landscape connectivity in both

historic and contemporary time scales (Gibbs 2001, Frankham 2006, Keyghobadi 2007). Whereas traditional field-based studies require extensive trapping and monitoring over extended time scales to capture the interactions among habitat fragmentation, connectivity and organism movement, a genetic study can address this suite of effects with a single sampling effort, provided that samples are collected at appropriate spatial and temporal scales (Storfer et al. 2007, Murphy et al. 2008, Anderson et al. 2010, Landguth et al. 2010b).

The Great Plains (GP) is the largest phytogeographic region in North America. Grassland systems are the dominant natural land-cover type; historically, these prairies exhibited continuity at broad geographic scales, save for limited interruption from river drainages and geologic features. Currently, < 50% of native grassland persists (Samson et al. 2004) and what remains is fragmented by natural and anthropogenic factors. Unlike many regions in the United States, human population densities in the GP are stable to declining, excluding some urban centers, and populations are aging overall (Parton et al. 2007). Large regions are classified as "wilderness" (i.e., < two persons/km^2; Gauthier and Licht 2003), resulting in unique opportunities for connectivity conservation and restoration. Although the threat of urbanization has abated for most of the GP region, remnant grassland systems represent a fraction of their historic spatial extent (Samson et al. 2004), while increasing agricultural (Sohl et al. 2012) and energy development (Forest et al. 2004, Pruet et al. 2009, Mehaffey et al. 2012) risks disrupting extant connectivity. Therefore, understanding habitat fragmentation's influence on connectivity for grassland species is critical for

developing long-term habitat conservation and management regimes, identifying dispersal corridors for protection and locating dispersal barriers for remediation and anticipating further impacts from emerging development pressures.

Here, I provide a novel assessment of functional connectivity for swift fox populations, as measured by gene flow. Further, I identify variables that enhance or impede this connectivity. I use the swift fox (*Vulpes velox*) as a model system for studying functional connectivity in grassland systems of the GP. The species is native to the shortgrass and mixedgrass prairies (SMGP) of the region. Swift fox have been extirpated from > 50% of their historic range (Sovada et al. 2009), and their current distribution is generally associated with remnant grassland habitats, reflecting the fragmented condition of these systems. The swift fox is sensitive to many of the natural and anthropogenic factors that fragment GP grassland systems (e.g., Kamler et al. 2003, Finley et al. 2005, Nicholson et al. 2007; but see Sovada et al. 1998, Matlack et al. 2000); thus I suggest that gene flow in swift fox populations reflects regional grassland connectivity. This is in part supported by previous research which indicates genetic structure in swift fox populations corresponds to grassland fragmentation (Chapter 2). I conducted my inquiry across two broad geographic areas located in the short and mixed grass prairie (SMGP) of the GP. To my knowledge, functional connectivity in these regions has not been studied at a similar spatial extent previously. Thus, this research provides critical insight into this important process in these understudied systems.

While the GP encompasses three grassland communities, the tallgrass, mixedgrass and shortgrass prairies, the inference herein is limited to the shortgrass and mixedgrass prairies. I feel this focus is warranted. The GP has been a subject of limited conservation focus overall (Scott et al. 2001), but existing conservation and research is biased towards tallgrass systems. Although tallgrass prairie is the rarest and most fragmented of the three GP grassland communities, the SMGP extent has also experienced severe declines, with roughly 52% of shortgrass and 29% of mixedgrass prairies remaining (Samson et al. 2004); this alone is worthy of conservation concern. Importantly, the very fact that more SMGP remains in a native state provides opportunities for proactive conservation measures (e.g., Jordan 1993) not feasible in more heavily impacted systems. Unfortunately, the paucity of research on SMGP leaves the current status of these communities unclear and limits the ability of managers to develop sound conservation strategies. Thus, my research provides unique and necessary information concerning these understudied grassland systems.

METHODS

Landscape metrics and focal study areas

I identified a suite of land cover (e.g., grass, forest, scrub, hydrography), land use (e.g., total agricultural development, wheat, corn), geomorphometric (e.g., compound topographic index, elevation relief ration, heat load index, and relative slope position), anthropogenic (e.g., non-agricultural disturbance, road infrastructure) and climatic (e.g., frost-free period, mean annual precipitation, growing season precipitation, degree days > 5°C) variables that potentially influence genetic

connectivity in the SMGP (Table 3.1); data sources, references and ecological justification for individual variables are found in Table 3.1. I transformed categorical data to continuous data, which generally have more power to detect landscape impacts on genet flow (Cushman and Landguth 2010b), by first reclassifying raster data sets to binary format, where 1 = variable of interest and 0 = all other data; this was completed using the raster calculator function in ArcMap 9.3 (ESRI, Redlands, California, USA) and resulted in a unique raster for each variable. I next used a script in ArcInfo (ESRI, Redlands, CA, USA) to calculate the proportion of each target variable individually in a 1.0-km moving window across the raster dataset. I calculated topographic morphology metrics from digital elevation models (DEM) at 30 m resolution in ArcInfo. Elevation relief ration, a measure of topographic complexity, was calculated at 1.0 km, 10 km and 30 km scales, representing home range size and upper and lower extremities of swift fox dispersal distances (Kamler et al. 2003, Nicholson et al. 2007). I assessed climatic influence using variables generated by the Rehfeldt (2006) climate model. Finally, I included distance in models by calculating a first-order polynomial interaction between x and y coordinates across DEMs in ArcMap.

These factors' relative influence on gene flow may lack stationarity across the SMGP, thus including multiple study areas is desirable to elucidate landscape and gene flow interaction (Segelbacher et al. 2010, Short Bull et al. 2011). I identified two focal areas between which I hypothesized the relative influence of individual landscape metrics on gene flow may vary and, further, where sample density and distribution were suitable for functional connectivity analysis (*sensu* Murphy et al.

2008, Landguth et al. 2011b). The first study area ("CO") encompasses 132,480 km^2 in south-eastern Colorado, western Kansas, the Oklahoma panhandle, the far-northern portion of the Texas panhandle and north-eastern New Mexico (Figure 3.1). The region is characterized by extensive grassland fragmentation resulting from anthropogenic disturbance, largely due to agricultural development, as well as natural sources of fragmentation (e.g., scrub, forest, topography). The second study area ("WY") encompasses 105,860 km^2 in south-eastern Wyoming, including portions of south-western South Dakota, north-eastern Nebraska and north-western Colorado (Figure 3.1). Grassland systems in this area are primarily fragmented by naturally occurring factors (e.g., forest, scrub, topography) with limited anthropogenic influence relative to the CO study area.

Genetic structure analysis

For each study area, I assessed genetic structure in swift fox samples (N = 367 for CO and N = 124 for WY) with genetic data for 15 microsatellite loci using program STRUCTURE (Pritchard et al. 2000, Hubisz et al. 2009) to identify the optimal number of genetic groups (*K*) in each study area (Evanno et al. 2005) and produced ancestry values for each individual per group (Jakobsson et al. 2007). Detailed laboratory protocols and genetic structure analysis methods are presented in Appendix B.

To calculate my response variable, I interpolated the STRUCTURE *q* values using an Inverse Distance Weighted interpolator in ArcGIS 9.3 (ESRI, Redlands, California, USA) to generate continuous genetic surfaces for each genetic group in

each study area (Murphy et al. 2008). Using this approach, I was able to generate a data distribution that was distance-conditioned across the range of genetic variability and generated samples that captured the lower tail of said distribution (see next for sampling method).

Functional connectivity analysis

Using the Hawth's Analysis Tools (Beyer 2004) point intersect option in ArcMap, I collected data for genetic ancestry (response) and landscape (predictor) variables at 2000 randomly generated points in each study area. I then used Random Forests (RF; Breiman 2001) implemented in R (Liaw and Weiner 2002, R Development Core Team 2007) to build functional connectivity models in each study area. Random Forests is ideal for this analysis because it is nonparametric, robust to noisy data, does not over-fit models and makes accurate predictions when analyzing complex, nonlinear ecological relationships (Cutler et al. 2007, Evans et al. 2011). Furthermore, the program produces estimates of variation explained (RF pseudo-R^2) and error (mean squared error, MSE) and ranks variables by importance, improving interpretability. For my analysis, I took the following steps. First, I tested for variable multicollinearity using QR-decomposition (Murphy et al. 2010). Upon evidence of collinearity, I chose variables based on known influence on swift fox movement and habitat use (e.g., Kamler et al. 2003, Finley et al. 2005, Nicholson et al. 2007). Next, I iterated 1000 bootstrapped regression trees for each genetic group identified by STRUCTURE analysis, withholding 34% of the data in each iteration for error estimation (i.e., out of bag sample, or OOB). When K was greater than two, I also

developed categories using fuzzy set theory (Zadeh 1965, Kampichler et al. 2000) and then produced 1000 classification trees using these categorical data. Finally, I applied a model selection procedure using the Model Improvement Ratio (MIR) described by Murphy et al. (2010). RF outputs a model ranking all variables in order of importance; the MIR trims this "master" model to a final model by promoting parsimony, minimized MSE and maximized percentage of variation explained (RF pseudo-R^2)

Understanding which factors influence functional connectivity is an important first step in effective conservation planning. However, the complexity of these relationships can be difficult to conceptualize and translate into practical application. In application, mapped predictions of landscape-level connectivity are often the ultimate need. Thus, as a final step, I produced maps of predicted functional connectivity (Murphy and Evans 2011) in each study area using RF model outputs in the following steps. For $K = 2$, I first predicted individual group models to a raster data layer with 30 m resolution using the packages raster and rgdal in R. This approach predicted ancestry based on model variable measures for each pixel in the raster. Next, I used a 1.0-km moving window in ArcInfo to calculate minimum and maximum values across the raster, assuming a large difference in ancestry values equated to high landscape resistance. Finally, I estimated connectivity by subtracting minimum ancestry from maximum ancestry. For $K > 2$, steps one and two remained the same. However, in the third step, I created connectivity maps by calculating the coefficient of variation across raster layers.

RESULTS

Genetic structure analysis

I detected support for varying levels of genetic structure in each study area (Appendix B). In the CO area, results indicated support for two and four genetic groups, whereas in the WY area I found evidence of two and five genetic groups. Varying levels of genetic structure in each study area may correspond to localized effects on gene flow. Therefore, I analyzed functional connectivity at both levels of genetic structure per study area.

Functional connectivity analysis

Multicollinearity tests indicated correlations between the variables growing season precipitation, degree-days > 5°C and percentage agricultural disturbance. I removed each of these variables individually but multicollinearity between the remaining two variables persisted, necessitating selection of a single variable. I retained percent agricultural disturbance based on previous research that demonstrated a negative relationship between agricultural development and swift fox movement and habitat selection (Kamler et al. 2003, Finley et al. 2005, Nicholson et al. 2007).

Using Random Forests, I built connectivity models for CO at $K = 2$ and $K = 4$ and WY at $K = 2$ and $K = 5$. Model variables, pseudo-R^2 and error estimates are summarized in Table 3.2. I observed high predictive power (pseudo-R^2 range 64.23% to 93.89%) and low error (MSE range 0.001 to 0.007) for RF regression analysis. The OOB error estimates for categorical RF analysis were marginally high (Appendix C), indicating poor model fit, so I excluded these models from further analysis. In a given

study area, model variables were generally consistent, but rank varied (Figures 3.2, 3.3 and 3.4). Between study areas, top-ranking variables were generally similar, but low-ranking variables differed (Figures 3.2, 3.3 and 3.4). Overall, distance and climate (i.e., frost-free period and mean annual precipitation) were highly ranked, regardless of study area, whereas anthropogenic variables were more frequently ranked in CO area models and natural landcover variables were more frequently ranked in WY area models. Finally, I generated functional connectivity maps for each level of genetic structure observed in the two study areas (Figures 3.5 and 3.6). These maps indicate different gene flow regimes and functional connectivity patterns in each study area and at each level of genetic differentiation. Where $K = 2$, connectivity is high throughout each study area, excluding regions of intense localized disruption. However, when $K > 2$, connectivity was severely reduced at broad geographic scales in both study areas.

DISCUSSION

Connectivity is a topic of conservation concern that has received considerable scientific investigation (Sanderson et al. 2008, Hénaux et al. 2011, Cormack Gates et al. 2012). However, the bulk of GP connectivity research has focused on birds (e.g., Coppedge et al. 2001, Rahmig et al. 2009, Klug et al. 2010) and fishes (e.g., Taylor et al. 2008, Gido et al. 2010, Bouska and Paukert 2010, Perkin and Gido 2011), providing limited insight for terrestrial species. My research demonstrates that while remnant SMGP is relatively extensive, terrestrial functional connectivity has been reduced and requires increased conservation and management.

Study area comparison

I found that while some underlying restrictions in functional connectivity were shared between study areas, functional connectivity was more constrained by anthropogenic disturbance in the CO study area than in the WY study area. At the coarsest level of genetic structure ($K = 2$), I found natural landcover types (e.g., scrub, grass and forest) influenced gene flow in the WY area, while anthropogenic influences (e.g., agricultural and nonagricultural development) were featured in CO models. At the finest level of genetic structure ($K = 4$ and $K = 5$ for CO and WY, respectively), I observed an increase in anthropogenic influence in WY but only in some models, indicating localized impacts. Anthropogenic influences rank in all models in the CO study area, indicating universal influence in the region.

These results have several implications. First, functional connectivity is influenced by different factors in different SMGP regions; managers should carefully consider these differences when implementing conservation measures in the SMGP. Second, fine-scale genetic structure is influenced by different factors than coarse scale genetic structure between regions; holistic management will need to account for these differences at both local and regional scales. Finally, I found that even when it is a limited landscape feature, anthropogenic disturbance negatively impacts grassland connectivity; thus managers should not assume that limited anthropogenic disturbance automatically translates to higher functional connectivity in prairie systems.

In the CO area, functional connectivity is impacted by both agricultural and nonagricultural forms of anthropogenic disturbance in each genetic group across both

levels of genetic structure. When $K = 2$, roads are clearly defined as a resistant factor along the western edge of the study area (Figure 3.5). Roads are a high source of mortality for swift fox (Sovada et al. 1998) and many other Great Plains species (Smith-Patten and Patten 2008); their impact on gene flow in canid populations has been demonstrated previously (Riley et al. 2006). However, roads can act as dispersal corridors for some species (Balkenhol and Waits 2009). Where road networks are limited, secondary roads appear to facilitate gene flow in swift fox populations outside of this area (D. Schwalm, unpublished data). The results presented here indicate that, past an unknown threshold, road networks can limit connectivity in swift fox populations. The visible road network I detected partially corresponds with concentrated urbanization along the Rocky Mountain Front Range, an area expected to experience extensive human population growth (Parton et al. 2007); thus, it is likely connectivity will be further reduced in this area.

Despite ranking in the $K = 2$ model for CO, the influence of agricultural development is not clearly visible on the corresponding connectivity surface. In fact, the area of highest agricultural development largely corresponds to the region of highest connectivity. This implies gene flow is not impeded by cropland, corresponding with Sovada et al. (1998) and Matlack et al. (2000), who found swift fox were not detrimentally affected by agricultural development. However, agriculture's influence on connectivity is more apparent in fine-scale genetic structure ($K = 4$), with the highest resistance to gene flow reflecting agricultural development in the center of the study area (Figure 3.6). This indicates greater agricultural impacts on

fine-scale genetic structure and local gene flow. I offer two alternative explanations for these observations. First, it is possible agriculture is more conducive to gene flow than extensive road networks at broad geographic scales, but at fine scales, localized agricultural effects are dominant. Alternatively, it is possible genetic patterns do not fully reflect the agricultural development's impact on gene flow. Agricultural development represents a recent and ongoing disturbance in the SMGP. Although genetic impacts exhibit a slight lag behind landscape disturbance effects, they can be detected relatively quickly (Murphy et al. 2008, Landguth et al. 2010b), so the low impact of agriculture at broad scales may reflect its emergent status as a disturbance factor in the Great Plains.

Although model selection and rank differed for many variables, the climatic variables frost-free period and mean annual precipitation ranked highly across all models in both study areas. This influence is an important finding in light of burgeoning evidence of the negative effects from global climate change on genetic diversity and connectivity (Parmesan 2006, McKelvey et al. 2011, Cobben et al. 2012, Wasserman et al. 2012). Frost-free period and mean annual precipitation drive vegetation community composition and structure in the SMGP; in addition, they influence crop type, planting time and duration in agricultural systems, especially where dryland practices predominate. Thus, these variables represent key processes shaping functional connectivity at multiple scales. Global climate change will impact these factors, likely resulting in drastic alteration of current gene flow regimes as vegetation communities and agricultural practices shift in type and location (Parmesan

2006, Cobben et al. 2012). These impacts are not well-understood for the SMGP, however. My results highlight an urgent need for further research investigating future global climate change influence on functional connectivity in the shortgrass and mixedgrass prairies.

Model limitations

Although the swift fox is a useful model organism for studying SMGP connectivity, it is certainly not an all-encompassing one (*sensu* Wiens et al. 2008); thus, this assessment would be improved through study of other grassland species with different life histories, including colonial species (e.g., prairie dog, *Cynomys ludovicianus*), migratory species (e.g., pronghorn, *Antilocapra americana*), and rare species or those with limited ranges (e.g., black-footed ferret, *Mustela nigripes*, lesser prairie chicken, *Tympanuchus pallidicinctus*). One obvious choice would be species with lower mobility relative to swift fox. My analysis shows even the swift fox, which typically disperses 10-35 km (Kamler et al. 2003, Nicholson et al. 2007), but is capable of dispersing > 190 km (Ausband and Moehrenschlager 2009), can be negatively influenced by relatively narrow bands of anthropogenic disturbance. For example, anthropogenic and agricultural development along the Arkansas River in CO (Figure 3.6, "A") form a concentrated area of resistance despite being < 30 km at its widest point. That this area represents a formidable barrier for the mobile swift fox may imply even greater reduction in connectivity for species less vagile or more habitat-specific; it unclear, based on these results, what dispersal capabilities are necessary to override extant connectivity limitations in the SMGP. Similar research

conducted with different model species may further elucidate the interaction between movement capabilities and habitat specificity on functional connectivity in the SMGP, reveal additional barriers and identify other factors influencing connectivity in the SMGP.

In a natural system where landscape impacts gene flow minimally, one of the primary drivers of genetic differentiation is geographic distance, a process known as isolation by distance (Wright 1943). Distance was highly ranked and exhibited considerable explanatory importance in connectivity models, implying some of the limited connectivity I observed may represent natural genetic patterns. Conversely, it could be an artifact of sample aggregation and density. For example, distance ranks higher in WY models than in CO models. The WY study area had half as many samples as the CO study area and these samples were more spatially clumped. Distance may be less influential if sample density and distribution were improved in the WY study area. However, the current sample distribution reflects lower species density and distribution in the WY study area relative to the CO study area; thus, it is also possible that distance's importance in WY models accurately reflects reduced connectivity resulting from spatially disjunct habitats.

CONCLUSIONS

Brown et al. (2003) and Hull Sieg et al. (1999) argued that appropriate land-use planning was a critical conservation priority for the Great Plains. These results indicate that prioritizing connectivity management in land-use planning is an

important consideration for the SMGP. While remnant SMGP extent gives the illusion of continuity, functional connectivity is quite limited at fine scales. Furthermore, given the delay between landscape-level alteration and genetic response (Landguth et al. 2010b), these models may underestimate habitat fragmentation's effect on functional connectivity in the SMGP. At minimum, these results raise a warning in the face of emerging land-use pressures in the SMGP. Concentrated efforts to develop and implement conservation strategies that maintain remnant SMGP in its native state are critical for connectivity conservation given ongoing and rapid development for agricultural use (Sohl et al. 2012) coal, natural gas and oil extraction (Forest et al. 2004), wind energy development (Pruet et al. 2009) and biofuel production (Mehaffey et al. 2012). Further, given the relationship between altered grazing and fire regimes and subsequent woody vegetation encroachment in grassland systems (Briggs et al. 2002), the strong resistance observed where grasslands transition to a grass/scrub matrix in WY is also worthy of management concern and further investigation.

Given the extensive fragmentation observed that stems from recent land-use changes, extinction debt (Tilman et al. 1994, Cousins et al. 2009, Kuussaari et al. 2009) is a potential concern. This phenomenon results from the lag between habitat alteration and subsequent species extirpation; it reflects the possibility that species may be present in contemporary times but doomed to extinction in the long term as a result of the loss of resources necessary for population viability. As such, conservation planning that maintains extant grassland connectivity but fails to increase

it may not effectively preserve native species long-term (e.g., Kraus et al. 2010). Thus, I suggest managers develop grassland restoration protocols that not only increase the proportion of native grassland but also intervening matrix quality (Prugh et al. 2008). As a starting point, managers may consider using grassland restoration to improve connectivity among Forest Service National Grasslands in New Mexico, Texas and Colorado, as these areas represent the most extensive regions of protected grasslands and could, potentially, serve as stepping stones and source populations in a connectivity network. Given the variation I observed in which variables influence connectivity between study areas, managers will need to tailor management to match local and regional influences. The considerable influence of distance in both study areas indicates steps which increase species distribution and reduce gaps between occupied areas are likely to benefit swift fox genetic connectivity. While managers can do little to influence frost free period and mean annual precipitation, the most influential factors in connectivity models, much can be done to influence landcover structure. For example, my results indicate functional grassland connectivity in the CO study area can be improved by 1) reducing the proportion of agricultural development and increasing the proportion of grassland, perhaps via Conservation Reserve Program or similar private landowner agreements and 2) mitigating the impacts of roadways, possibly through the use of wildlife overpasses or underpasses, although their efficacy for swift fox should be tested and site selection should be informed by movement corridor identification prior to large-scale implementation. Grassland restoration and/or swift fox translocation may be effective tools in this

endeavor, although appropriate sites must be carefully selected. The observed variation adds further weight to the argument for conducting connectivity analysis in multiple study areas to more fully elucidate regional variation and develop appropriate conservation strategies (Short Bull et al. 2011).

These results indicate connectivity planning in light of land conversion and climate change should be a focus for SMGP management and conservation. Thomas et al. (2004) estimate 15.7% of grassland species are "committed to extinction" globally. This loss may be even higher in extensively fragmented systems (Pyke 2004) such as the SMGP. Lack of spatial cohesion can lead to species' inability to shift distributions when pressured by climate change (Opdam and Wascher 2004) and can drastically alter spatial extent and gene flow patterns for species where connectivity is closely tied to climate gradients (Wasserman et al. 2012). Pyke (2004) cautions that losses will be greatest where current conservation is limited, and grasslands are the least-protected biome in the US (Scott et al. 2001, Dietz and Czech 2005). I second the argument that "climatically under-represented areas should be included in systematic conservation planning priorities" (Pyke 2004:181) and call for greater research and development of conservation strategies to mediate landscape and climate change impacts in the SMGP. Predicting the impact of land conversion and climate scenarios on connectivity is a necessary next step for conservation in the SMGP.

CHAPTER IV

GENE FLOW AND GENETIC DIVERSITY IN A FRAGMENTED SWIFT FOX POPULATION: IMPLICATIONS FOR MANAGEMENT

INTRODUCTION

The swift fox (*Vulpes velox*) is native to the shortgrass and mixedgrass prairies of the Great Plains and is a species of conservation concern throughout most of its present distribution. Widespread predator controls coupled with broad-scale grassland conversion for agricultural purposes has led to precipitous population declines over the past ~100 years (Allardyce and Sovada 2003). Current evidence indicated that swift fox has been extirpated from > 50% of its historic range (Sovada et al. 2009). The species has shown signs of recovery in parts of its range but remains rare in the southern portion of its distribution, with remnant populations occurring in spatially disjunct grassland fragments (Harrison et al. 2004, Criffield et al. 2010, Schwalm et al. in press). In this area, remnant grassland represents < 50% of its historic extent, with the majority of grassland loss stemming from agricultural development (Samson and Knopf 1994, Samson et al. 2004).

Regardless of species, fragmented populations are of conservation concern because they may experience restricted immigration, resulting in reduced gene flow, diminished genetic diversity, inbreeding depression and genetic drift (Gibbs 2001, Keller and Waller 2002, Keyghobadi 2007). Ultimately, these factors increase extinction risk (With and King 1999, Brook et al. 2002, O'Grady et al. 2006). In swift fox, previous research has indicated the presence of a regional genetic group located in

parts of Colorado, New Mexico, Oklahoma and Texas; this group is comprised of three genetic subgroups (Chapter 1). Gene flow between groups is unknown at local scales but may be impacted by habitat fragmentation resulting from agricultural development. Although the swift fox is a capable disperser (Ausband and Moehrenschlager 2009), both dispersal distance (Moehrenschlager and Macdonald 2003) and movement through a hostile habitat matrix (Moehrenschlager and Somers 2004) have been shown to increase mortality, potentially reducing gene flow in fragmented habitats. Swift fox are known to avoid cropland locally (Kamler et al. 2003, Nicholson et al. 2007), and agricultural development has been shown to impede gene flow elsewhere in the species range' (Chapter 2); thus, agricultural development may disrupt gene flow locally, but this is unknown. Given extensive habitat loss, population fragmentation and low population densities, an assessment of regional and local gene flow and genetic diversity is therefore necessary to inform long-term swift fox conservation planning.

My objectives were to quantify extant genetic diversity and structure, compare these metrics to range-wide values, and evaluate movement between subgroups, ultimately using the results to offer insight into regional swift fox management. I hypothesize genetic differentiation will be highest and genetic diversity will be lowest where groups occur in fragmented grasslands. Furthermore, I predict the western portion of the population will act as an immigrant source, given its larger group size and greater population density locally.

Study Area

The study area encompasses north-eastern New Mexico, south-eastern Colorado, the Oklahoma panhandle and the far northern portion of the Texas panhandle, covering approximately 70,350 km^2 (Figure 4.1). This region historically was comprised of shortgrass and mixedgrass prairie communities but has experienced extensive grassland loss and fragmentation primarily due to agricultural development over the past ~100 years. Although the rate of loss has slowed since the 1940's (Parton et al. 2007), agricultural development continues to be the major factor determining habitat availability and grassland connectivity across multiple spatial scales. Grassland conversion is more extensive in the eastern portion of the study area (Figure 4.1); overall, less than 50% of shortgrass and mixedgrass prairie remains in a native state throughout the region (Samson and Knopf 1994, Samson et al. 2004).

Methods

I used genetic structure analysis previously completed for this region as a starting point (Chapter 1). These analyses indicated the study area contained one genetic group comprised of three subgroups. I assigned 142 swift fox samples, collected between 1997-2008, to source populations (e.g., eastern, central and western groups; Figure 4.1) based on the highest ancestry (*q)* value produced by genetic structure analysis using program STRUCTURE (Pritchard et al. 2000, Hubisz et al. 2009; see Appendix D for genetic analysis details). These groupings were used to calculate by-group genetic indices and identify putative immigrants (see below). The

boundaries of these groups were clear except along the New Mexico/Oklahoma/Texas intersection (hereafter the NM/OK/TX intersection; Figure 4.1), where samples from western and central groups were equally represented and no clear group delineation was apparent. Combining samples from different ancestry groups or admixed regions in genetic diversity analysis can produce erroneous results due to violation of Hardy-Weinberg expectations (Hartl and Clark 2007). I therefore excluded samples from this location (n = 31) from further analysis.

I calculated the correlation between genetic (Rousset's a, Rousset 2000) and geographic distance to test the influence of isolation by distance (IBD; Wright 1943) using the ecodist package (Goslee and Urban 2007) in R version 2.14.0 (R Core Development Team 2007). I assessed differentiation between groups using pair-wise F_{st} calculations (Arlequin 3.5, Excoffier and Litcher 2010). For each group, I measured observed and expected heterozygosity (Excoffier and Litcher 2010), F_{is} (FSTAT 2.9, Goudet 2002) and allelic richness and private allelic richness, correcting for uneven sample sizes with rarefaction (HP-Rare, Kalinowski 2005). For these analyses, individuals were included in the group where they were geographically located. I considered an individual an immigrant if it was located in a group other than that of its genetic origin. I then totaled the number of immigrants from each potential source group and each potential recipient group to assess immigration and emigration rates between subgroups. As an additional estimate of inter-population movement, I calculated the number of migrants (N_em) following Avis (1994:207), where:

$$\mathrm{N}em = \frac{1 - Fst}{4Fst}$$

RESULTS

After removing samples from the NM/OK/TX intersection (see methods), samples sizes were 48, 38 and 25 individuals for the eastern, central and western groups, respectively. Differentiation among groups was low but significant ($P < 0.001$; Table 4.1) and increased from east to west across the study area. I observed a moderate correlation between genetic and geographic distance (Mantel $r = 0.23$, CI = 0.20-0.27, $P = 0.001$), implicating isolation by distance as a factor in observed genetic structure. I found no differences in genetic diversity indices (e.g., expected and observed heterozygosity, mean allelic and private allelic richness) among groups (Table 4.2); these values were also similar to range-wide measures. I found little evidence of inbreeding based on F_{is} measures; although generally higher than the range-wide average (Table 4.2), these values were low overall. I identified 25 immigrants using STRUCTURE (Table 4.3); the central group produced the most immigrants ($n = 15$) and the eastern group received the most immigrants ($n = 14$). Immigration rates between groups was lower when estimated using F_{st} (Table 4.4)

DISCUSSION

Although I anticipated reduced genetic diversity, increased genetic differentiation and less evidence of inter-population movement amongst swift fox populations where grassland fragmentation was prevalent, I found the opposite. Based on these results, differentiation is lowest between groups in fragmented habitats, e.g., eastern and central groups. Furthermore, these results indicate the regional swift fox

population is genetically robust. Genetic diversity indices were similar amongst subgroups and mirrored range-wide values, offering no indication of reduced gene flow or inbreeding in the regional population. These indices are comparable to those reported for a different swift fox population (Kitchen et al. 2005) and higher than reported values for the closely related San Joaquin kit fox (*Vulpes macrotis mutica*; Schwartz et al. 2005), potentially implying that genetic diversity in the study area is reasonably robust. I acknowledge the limitations of F_{is} for assessing contemporary landscape impacts on inbreeding (Keyghobadi et al. 2005, Segelbacher et al. 2008). Unfortunately, traditional inbreeding calculations require pedigree knowledge (*sensu* Alho et al. 2009), which I lack, meaning that the estimates I present are indirect estimates and the best possible measures of inbreeding given current knowledge. Using expected heterozygosity to assess inbreeding can underestimate this phenomenon in contemporary timescales; this metric may instead reflecting historic patterns (Balloux et al. 2004, Slate et al. 2004). Therefore, the estimation of inbreeding I present is likely conservative and may reflect historic genetic patterns; I caution against an overly optimistic view of swift fox genetic diversity in the study area.

These results imply that the genetic diversity and differentiation patterns I observed may be driven by high levels of migration between eastern and central groups. Nearly 30% (14 of 48) of samples in the eastern group were from other groups, with the vast majority (13 of 14) originating from the central group. In total, 76% (19 of 25) of observed immigrants were exchanged between the central and

eastern groups. Given high grassland fragmentation in this part of the study area, this finding is initially counterintuitive. However, it is important to note that swift fox are capable of exhibiting some tolerance of agricultural development at local scales. Although Kamler et al. (2003) reported cropland avoidance by swift fox in Texas, Criffield et al. (2010) found swift fox present in cropland in Oklahoma. This flexibility may facilitate movement between groups despite agricultural encroachment, implying gene flow may persist in fragmented swift fox populations where they utilize cropland. However, I caution that this interaction has not been quantified and requires further investigation, ideally with field studies (e.g., telemetry). Results presented in Chapter 5 indicate that agricultural development reduces gene flow in this region.

There seems to be limited migration into and out of the western group. Perhaps not coincidentally this group is also associated with the highest measures of genetic differentiation, suggesting a greater degree of isolation relative to the other subgroups. This was surprising, given the greater geographic extent and larger population size in this area. However, while the swift fox population is larger in the west, the species is still rare. Potentially, the western group functions below carrying capacity currently. If this is the case, dispersal from west to east may remain at low levels unless swift fox numbers increase in the west; this requires further investigation, however. In addition, this result may well be exacerbated by the exclusion of samples along the NM/OK/TX intersection. Although necessary to avoid subjective group assignment, this decision resulted in exclusion of 31 samples from the western population that might otherwise have been designated as immigrants. At minimum, removal of these samples resulted

in reduced representation of the western group outside of its geographic bounds and resulted in a more conservative estimate of gene flow from this group to the eastern and central groups. One might predict swift fox in suboptimal (i.e., fragmented, nonnative) habitats would readily disperse into high quality (e.g., connected, native) habitats, so the limited number of immigrants found in the western population is unexpected. I suspect the sample distribution may have contributed to this result. Although swift fox are present throughout grasslands in the New Mexico portion of the study area, I lack samples for eastern New Mexico. Immigrants from the central and eastern groups may be present but unrepresented due to this gap in the sample distribution. Dispersal distances for swift fox in the study area range between 10 and 25 km (Nicholson et al. 2007), well short of the distance between samples in eastern/central and western subgroups. Thus, the frequency of immigrants in the western group may under-represent movement from eastern and central subgroups.

Overall, these results demonstrate that movement between groups in fragmented habitats may be higher than expected and, in addition, that this movement can be a critical component in maintaining genetic diversity in small populations. Moreover, I find that the presence of large, neighboring populations does not automatically correlate with greater immigration or gene flow to associated small populations. These results highlight the importance of maintaining dispersal between fragmented populations, given potential isolation from other, larger populations.

MANAGEMENT IMPLICATIONS

Movement between subgroups in the regional population appears to be a critical process for maintaining genetic diversity in fragmented habitats. Thus, swift fox management should emphasize connectivity conservation at a multi-state level. This will require identifying and conserving extant corridors, which may be few in number and narrow in width in this impacted region. Sohl et al. (2012) predict as much as 50% of remnant grassland will be lost within the region in the next ~ 90 years; preemptive grassland conservation is therefore critical for connectivity conservation. Although patches of grassland are conserved as part of the Forest Service National Grassland system (e.g., the Rita Blanca and Kiowa National Grasslands), the vast majority of shortgrass and mixedgrass prairie in the study area is privately owned and vulnerable to further development. Thus, managing for connectivity will require coordination between private landowners and state and federal agencies for success.

In the study area, swift fox management varies by state. In all four states the swift fox is listed as a furbearer; however, in Colorado and Oklahoma the season is closed, whereas in New Mexico and Texas, swift fox can be harvested during regulated seasons. These results imply that genetic diversity in the eastern group, which exists primarily in Texas, is maintained by high immigration from the central group; furthermore, population numbers in the Texas population appear to benefit from immigration, with nearly 30% of resident foxes originating from other sources. At minimum, I encourage managers to afford the swift fox in Texas greater protection. Reliance on support from other populations is a tenuous option, given anticipated

future connectivity reductions in the face of grassland conversion. Given the aforementioned limitations of the sample distribution in New Mexico, I am hesitant to extend recommendations for this region, but urge caution in management decisions, given the limited indication of gene flow my results imply.

CHAPTER V

CONNECTIVITY NETWORKS IN A FRAGMENTED GRASSLAND SYSTEM: BUILDING LEAST-COST PATHS WITH NOVEL, EMPIRICALLY DERIVED RESISTANCE COSTS TO IDENTIFY MOVEMENT CORRIDORS FOR A RARE MESOCARNIVORE

INTRODUCTION

Maintaining connectivity in wildlife populations is an increasingly challenging task for conservationists (Crooks and Sanjayan 2006). Yet it is the difficulty of this undertaking that underscores its necessity. Habitat fragmentation and loss are steadily reducing habitat availability and degrading remnant quality, subdividing wildlife populations into isolated fragments, interrupting population-level processes and ushering in an era of unprecedented biodiversity loss (Lindenmayer and Fisher 2006). As the anthropogenic footprint continues to expand, identifying extant movement corridors and connectivity networks ranks highly in land-planning priorities. Even so, the process for elucidating linkages in fragmented habitats remains unresolved. Numerous procedures have been defined (Cushman et al. 2008, McRae et al. 2008, Pinto and Keitt 2009, Braunisch et al. 2010, Dyer et al. 2010, Shirk et al. 2010, Carranza et al. 2012), but the most frequently employed method involves calculating effective geographical distance (Michels et al. 2001) by building least-cost paths (LCP) between remnant habitat patches, populations or individual locations (Chetkiewicz et al. 2006, Rouget et al. 2006, Huber et al. 2010). Briefly, this methodology seeks to predict animal movement corridors based on landscape resistance or facilitation. Researchers assign values to pixels in rasterized landscape

data; these values are based on perceived resistance or "cost" associated with movement through the represented variable (e.g., land-cover or land-use type, topographic metric, temperature/moisture gradients, etc.) in each pixel. Costs rank from high to low; these values represent the cost scheme, which is unique to each variable of interest based on known or expected ecological or biological effect on movement. After classifying individual raster datasets with their associated cost schemes, rasters are summed (e.g., with a raster calculator in ArcGIS); the output layer represents the hypothesized relationship between organism movement and landscape structure. Potential movement paths are then evaluated between source and destination points (e.g., habitat patches, individual locations) and the most likely path is selected based on the lowest cumulative cost, summed across all pixels along the path, or the "least-cost" path.

The accuracy of this methodology has been challenged (Rothley 2005) and bears further refinement (Spear et al. 2010, Sawyer et al. 2011). One constraint of these approaches is the use of expert opinion to parameterize cost surfaces (Spear et al. 2010, Sawyer et al. 2011). Least-cost path models are sensitive to cost scheme choices (Andelman et al. 2002, Adriaensen et al. 2003, Rayfield et al. 2010), and variation in cost schemes can lead to discrepancies in final path designation and location (Epps et al. 2007), ultimately limiting interpretability and applicability. Researchers are not cavalier in their decision to use expert opinion; this approach is born largely of necessity, given the difficulty of assembling robust animal movement and habitat utilization data to generate resistance costs. In response to criticisms

levied at cost subjectivity, many well-vetted means of modeling connectivity and corridor identification have been developed in which models are built and tested using empirical (e.g., genetic) data, improving inference and accuracy. These innovative and powerful analytical methodologies partially address the shortcomings of opinion-derived resistance costs through iterative, multi-model regimes that test varying cost schemes, selecting the most supported scheme for corridor assessment (Cushman et al. 2006, Epps et al. 2007, Cushman et al. 2008, Schwartz et al. 2009, Wang et al. 2009, Shirk et al. 2010, McKelvey et al. 2011, Short Bull et al. 2011, Wasserman et al. 2011).

Even so, the specter of subjectivity lingers and interest in developing empirically derived costs persists (Braunisch et al. 2010, Wang and Summers 2010, Garroway et al. 2011, Sawyer et al. 2011). In addition, the aforementioned approaches are characterized by extensive and computationally cumbersome analysis, with hundreds (Cushman et al. 2006, Short Bull et al. 2011) to tens of thousands (Wang et al. 2009) of models assessed. To address these challenges, I developed a novel LCP modeling approach using resistance surfaces parameterized with cost values derived purely from empirical genetic data using a straightforward analytical framework. The methodology for generating these surfaces was first described in Murphy et al. (2010) and Murphy and Evans (2011) but has not previously been utilized in a LCP modeling framework. I demonstrate the approach in a fragmented swift fox (*Vulpes velox)* population spanning 70,350 km^2 in Texas, Oklahoma, Colorado and New Mexico, wherein correlated cost-distances defeated LCP

methodologies more traditionally utilized by landscape geneticists (e.g. Cushman et al. 2006). I close by offering comments on implementation, as well as implications for swift fox management.

METHODS

Study area

I conducted my inquiry in a region previously identified as a unique genetic group with population sub-structure comprised of three sub-groups (Chapter 2). The region encompasses 70,350 km^2 in north-eastern New Mexico, south-eastern Colorado, the Oklahoma panhandle and the far northern portion of the Texas panhandle (Figure 5.1). Swift fox occur in shortgrass and mixedgrass prairies (SMGP); in this study area, these systems are relatively continuous on the west and highly fragmented in the east due to agricultural development, which swift fox generally avoid locally (Kamler et al. 2003, Finley et al. 2005, Nicholson et al. 2007; but see Criffield et al. 2010). Genetic diversity in eastern populations appears to be maintained by immigration between sub-groups (Chapter 4); given predicted agricultural intensification and grassland loss in the area (Sohl et al. 2012), identification of movement corridors is a critical component of long-term species conservation.

Genetic and landscape variable calculation

I assessed genetic structure in 142 swift fox samples (Figure 5.1) using data for 15 microsatellite loci in STRUCTURE (Pritchard et al. 2000, Hubisz et al. 2009),

identified the optimal number of genetic groups (*K*) (Evanno et al. 2005) and produced ancestry values (*q*) for each individual per group (Jakobsson et al. 2007). Detailed laboratory protocols and genetic structure analysis methods are presented in Appendix D. I used the Inverse Distance Weighted interpolator in ArcGIS 9.3 (ESRI, Redlands, California, USA) to generate a continuous genetic surface of *q* values for each genetic group (Murphy et al. 2008). These surfaces represent the response variables in further analysis.

I identified a suite of land cover (e.g., grass, forest, scrub, hydrography), land use (e.g., agricultural development, wheat, corn), topographic (e.g., compound topographic index, elevation relief ration, heat load index, and relative slope position), anthropogenic (e.g., non-agricultural disturbance, road infrastructure) and climatic (e.g., frost-free period, mean annual precipitation, growing season precipitation, degree days > 5°C) variables that potentially influence genetic connectivity in the SMGP (Table 3.1); data sources, references and ecological justification for individual variables are found in Table 3.1. I transformed categorical data to continuous data, which generally have more power to detect landscape impacts on gene flow (Cushman and Landguth 2010) by first reclassifying raster data sets to binary format, where 1 = variable of interest and 0 = all other data; this was completed using the raster calculator function in ArcMap 9.3 (ESRI, Redlands, California, USA) and resulted in a unique raster for each variable. I next used a script in ArcInfo (ESRI, Redlands, California, USA) to calculate the proportion of each target variable individually in a 1.0-km moving window across the raster dataset. I calculated topographic

morphology metrics from digital elevation models (DEM) at 30 m resolution in ArcInfo. Elevation relief ration, a measure of topographic complexity, was calculated at 1.0-km, 10-km and 30-km scales, representing home range size and upper and lower extremities of swift fox dispersal distances (Kamler et al. 2003, Nicholson et al. 2007). I assessed climatic influence using variables generated by the Rehfeldt (2006) climate model. Finally, I included distance in models by calculating a first-order polynomial interaction between x and y coordinates across DEMs in ArcMap. Variable calculation and ecological justification are further detailed in Table 3.1. These variables represent the predictor variables in further analysis.

Developing resistance surfaces

Using the Hawth's Analysis Tools (Beyer 2004) point intersect option in ArcMap, I collected data for genetic ancestry (response) and landscape (predictor) variables at 2000 randomly generated points in the study area. I then used Random Forests (RF; Breiman 2001) in R (Liaw and Weiner 2002, R Core Team 2007) to assess the influence of climate, topography, hydrography, land cover, land use and anthropogenic development on functional connectivity, as measured by interpolated *q* values. Random Forests is ideal for this analysis because it is nonparametric, does not over fit models, and makes accurate predictions when analyzing complex ecological relationships across broad spatial scales (Prasad et al. 2006, Cutler et al. 2007). Additionally, the program produces estimates of variation explained (RF pseudo-R^2) and error (mean squared error, MSE), and ranks variables by importance, improving interpretability. For the Random Forests analysis, I took the following steps. First, I

tested for variable multicollinearity using QR-decomposition (Murphy et al. 2010); upon evidence of collinearity, I retained variables known to influence to swift fox movement (e.g., Kamler et al. 2003, Finley et al. 2005, Nicholson et al. 2007) and removed variables that were either unsupported by the literature or redundant with retained variables. Next, I iterated 1000 bootstrapped regression trees for each genetic group, withholding 34% of the data in each iteration for error estimation (i.e., out of bag sample, or OOB). I selected a final model for each genetic group using the Model Improvement Ratio (MIR) described by Murphy et al. (2010). RF outputs a model ranking all variables in order of importance; the MIR trims this "master" model to a final model by promoting parsimony, minimized MSE and maximized percentage of variation explained (via the RF pseudo-R^2). I then generated three surfaces (one per group) by predicting ancestry models to individual raster datasets with 30 m resolution using the packages raster, rgdal and dismo in R. In the final step, I calculated resistance costs for each surface by quantifying mean and summed ancestry values across all three surfaces in ArcInfo, then re-scaled values from 0-1. Mean and summed connectivity were calculated separately, resulting in two layers representing different connectivity scenarios. Assuming an inverse relationship between mean or summed connectivity and landscape resistance, low connectivity values represent high resistance in the resulting surfaces. To adjust for computational limitations, I upscaled the surfaces from 30 m to 240 m pixel size using the bilinear resample function in ArcGIS.

Least-cost path modeling and corridor identification

I developed connectivity networks for each resistance surface using the program UNICOR (Landguth et al. 2012). UNICOR implements a version of Dijkstra's algorithm (Dijkstra 1959) adjusted to improve computational efficiency (Landguth et al. 2012). In short, the program calculates cost distances along all potential links in a graph network of all pair-wise connections between data points and selects the path with lowest cost, i.e. the least-cost path, as the most likely movement path. For the analysis, I sub-sampled the original 142 samples by enforcing a 5.0-km distance between samples, thereby reducing overrepresentation of densely sampled locations in the final corridor estimates. I retained 62 individual samples, between which I calculated LCPs for all pair-wise combinations, generating a connectivity network across the study area. Standard least-cost paths are one pixel in width; however, this relatively deterministic output is unlikely to represent individual movement variation (McRae et al. 2008, Pinto and Keitt 2009, Sawyer et al. 2011); therefore, I smoothed the paths using a Gaussian kernel density function, producing final paths 2.2-km wide. This width roughly reflects the width of two swift fox territories. I next summed the connectivity networks produced with the two resistance surfaces, postulating that multi-model selection infers greater support for a given connection.

RESULTS

I observed multicollinearity between growing season precipitation, degree days > 5°C and percent agricultural disturbance; I retained percent agricultural disturbance

based on previous research that demonstrated a negative relationship between agricultural development and swift fox movement and habitat selection (Kamler et al. 2003, Finley et al. 2005, Nicholson et al. 2007) and removed growing season precipitation and degree days > 5°C. The three Random Forests models explaining genetic ancestry showed high explanatory power (pseudo-R^2 range = 83.6%-93.6%) and low mean squared error (range = 0.0002-0.0006). Ranked variables differed between models (Figure 5.2); all models ranked distance and climate variables highly but differed in type and rank of selected land cover/land use variables. Predicted minimum and summed connectivity surfaces are shown in Figure 5.3 and 5.4, respectively; both original (30 m) and upscaled (240 m) resolution surfaces are shown for comparison. The LCP connectivity networks differ between these two estimates (Figure 5.5). These two models agree most frequently where high-use paths are designated (Figure 5.6).

DISCUSSION

Here, I present a novel approach to corridor building and connectivity network assessment using resistance surfaces with cost values derived from genetic data. I demonstrate the utility of using Random Forests analysis to produce empirically derived resistance costs for modeling movement (as measured by gene flow). To my knowledge, this is the first application of this approach, although a limited number of other empirically derived resistance costs have been developed (Braunish et al. 2010, Wang and Summers 2010, Garroway et al. 2011). This approach improves on existing

least-cost path methods in that it eliminates the need for subjective cost assignment and increases computational efficiency without sacrificing power, accuracy or scale of inquiry.

Scope and limitations

A suite of research has demonstrated the applicability and power of causal-modeling or information-theoretic approaches to connectivity modeling; these iteratively test a range of potential costs derived from expert opinion to build final, empirically derived surfaces (Cushman et al. 2006, Cushman et al. 2008, Schwartz et al. 2009, Wang et al. 2009, McKelvey et al. 2011, Wasserman et al. 2011, 2012). The contribution of these methodologies to researchers' capacity for exploring complex landscape/genetic interactions is undeniable. However, LCP development is inherently sensitive to the cost regime used (Andelman et al. 2002, Rayfield et al. 2010); therefore, building cost schemes that originate from empirically tested landscape/genetic relationships circumvents this subjectivity and represents an improvement on extant methodologies.

Another drawback to these methodologies is the required exploration of 100's to 10,000's of competing models. This process can be cumbersome and time-consuming. This method I present here has improved efficiency over traditional methods. Although the time required to assemble appropriate genetic and landscape data is equivocal between approaches, the Random Forests model and resulting resistance surfaces used here were generated in < 12 hours; subsequent least-cost paths were generated in < 8 hours per analysis. Previous analysis using traditional causal-

modeling approaches took > 4 weeks, by comparison (D. Schwalm, unpublished data). Model power and precision were not sacrificed, as evidenced by the high pseudo-R^2 values and low mean squared error observed in this analysis. Nor was geographical scale limited; in this example, I completed analysis over 70,350 km^2. Additionally, this efficiency allows rapid analysis across multiple study areas or scales. Short Bull et al. (2011) demonstrated the importance of assessing localized influences on gene flow, given nonstationarity in variable effects. Furthermore, Huber et al. (2010) demonstrated the importance of building LCPs at regional and local scales within the same area to account for varying needs across scales. Although I conducted this inquiry at a single scale for this project, these methods could readily be applied in multiple locations or spatial scales, addressing the admonitions of Short Bull et al. (2011) and Huber et al. (2010), respectively.

However, I offer several cautionary comments. First, like all modeling frameworks, RF functions on a "garbage in, garbage out" basis; practitioners must choose variables that have biological or ecological justification, which are measured at ecologically relevant temporal and spatial scales, and which are not co-confounding (e.g., correlated). Importantly, grain size in resistance surfaces is a critical consideration, especially if linear features (e.g., roads) are a hypothesized barrier, as these can develop "cracks" when upscaled to a larger cell size (Rothley 2005; see also Figures 5.4 and Figure 5.5). In this analysis, gaps were present in road features, and in some instances LCPs appeared to cross roads arbitrarily where these breaks occurred. In traditional LCP analysis, roads are often buffered to account for this issue; although

I did not test this method, it could potentially be implemented in the raw data layers prior to RF analysis. However, I am unsure if this is appropriate, as buffered roads may change the weights assigned in RF analysis. Alternatively, a road layer with assigned resistance values, perhaps using the mean road-related resistance values from RF models, could be added to the resistance surface. However, this would introduce the issue of subjectively derived costs, which I was trying to avoid; furthermore, this approach may not represent organism responses accurately. Specifically, in this study area, I found that roads were not assigned consistent resistance values; cost varied not only with road type but also by location along the road length. A road overlay would mask these differences. This analysis was conducted at 240 m resolution due to computational limitations. To reduce the gap occurrence, I encourage researchers to either work at finer scales (e.g., 30-60 m) or develop methods of filling "cracks". In the absence of these approaches, researchers should not imply strong inference where corridors arbitrarily correspond with gaps in linear features.

Finally, as presented this approach does not allow for hypothesis-testing. However, layers representing specific barriers or other fragmentation-related theories could be included in future applications. Random Forests performs best with a large number of variables (Hastie et al. 2009), so testing hypotheses with a limited number of variable sets may not be appropriate, although this has yet to be tested formally. Although I did not include specific barriers in this analysis, RF analysis accurately identified suspected gene flow barriers in previously completed work (Chapter 3). Research that *a priori* identifies putative gene flow barriers and assesses their support

in an RF framework could easily be conceptualized, although it is beyond the scope of this inquiry.

The minimum and mean resistance scenarios produced disparate results in corridor location in some instances, highlighting the sensitively of LCP modeling to resistance cost schemes (Spear et al. 2010, Rayfield et al. 2010, Sawyer et al. 2011). Given this sensitivity, I recommend LCP modeling be conducted with a series of surfaces that vary costs based on ecological expectations and assess model agreement to yield more concrete corridor outputs.

Swift fox connectivity

As the LCP method I have presented is still being developed, the implications I present for swift fox are preliminary; these will be revisited after the method is refined, so I encourage refraining from making management decisions strictly from the results presented here.

Despite difference between models, I identified numerous locations wherein both models produced congruent paths; generally, these were portions of the high-traffic paths identified independently by each model, but not exclusively so. In addition, in many cases where high-traffic paths were not congruent, they occurred alongside each other, implying movement in the combined area is important for swift fox dispersal.

Overall, both models produced more paths in the fragmented habitats on the eastern side of the study area. Although greater in number, these paths garnered weak support in general. Most represented single-use paths between individual pairs of

points, as opposed to movement corridors between multiple sets of points. When modeling LCPs, I did not impose cost thresholds (*sensu* Shaw et al. 2011); as a result, paths were automatically generated between all points regardless of total resistance. Many eastern paths travel through highly inhospitable areas, an implausible scenario given high swift fox mortality associated with dispersal distance and unsuitable matrix composition (Moehrenschlager and Macdonald 2003, Moehrenschlager and Somers 2004). I caution against concluding these paths represent important connections between swift fox groups. Instead, I argue that they are likely an artifact of analytical design; I intend to build thresholds into future analyses to test this hypothesis. The current results detect few corridors in this area that were high-traffic, implying connectivity between locations is limited locally, restricted to a few corridors and otherwise exposes swift fox to high risk by requiring movement through unsuitable habitats. Conserving corridors identified by both models should be considered a priority, given limited connectivity overall; likewise, grassland restoration should be undertaken to improve connectivity between isolated groups in the eastern portion of the study area.

In the western half of the study area, paths are fewer in number and generally enjoy greater multi-model support or represent high selection in an individual connectivity model. It is in this area that I observe paths crossing roads where cracks in the resistance surface occur; therefore, I caution against inference of path location where this occurs. However, the high movement and multi-model support observed indicates that swift fox gene flow relies on connectivity within these regions.

Revisiting this analysis with fine-scaled resistance surfaces should refine corridor location appropriately, improving utility for conserving conservation in swift fox populations.

Table 2.1. Size range and observed polymorphism per microsatellite locus used for analyzing 589 swift fox samples.

Multiplex	Locus	Size range (bp)	# of Alleles
1	FH2054[1]	167-191	7
	CPH3[2]	150-160	6
	CXX20[3]	120-144	9
	CXX173[3]	123-129	4
	CXX250[3]	131-153	10
	CXX377[3]	173-193	8
	CXX403[3]	269-281	5
2	CXX109[3]	165-170	6
	CXX263[3]	94-122	5
	CXX2062[3]	135-156	6
	VVE2-111[4]	125-141	5
	VVE5-33[4]	190-226	9
3	VVE-M19[4]	227-287	27
	VVE2-110[4]	231-358	40
	VVE3-131[4]	157-189	6

(Francisco et al. 1996) [1]
(Fredholm et al. 1995) [2]
(Ostrander et al. 1993) [3]
(Cullingham et al. 2007) [4]

Table 2.2. Pairwise F_{st} between *a priori* hypothesized groups plus putative groups detected using the programs STRUCTURE and TESS. The number of individuals per group is shown in parenthesis. $P < 0.001$ for all comparisons.

Analysis Method					Group			
Hypothesized K = 8	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Group 1 (42)	-	0.06	0.06	0.05	0.06	0.07	0.09	0.25
Group 2 (43)	-	-	0.02	0.03	0.05	0.06	0.09	0.23
Group 3 (85)	-	-	-	0.04	0.03	0.02	0.06	0.17
Group 4 (119)	-	-	-	-	0.03	0.02	0.06	0.17
Group 5 (98)	-	-	-	-	-	0.03	0.03	0.19
Group 6 (55)	-	-	-	-	-	-	0.04	0.16
Group 7 (132)	-	-	-	-	-	-	-	0.16
Group 8 (15)	-	-	-	-	-	-	-	-
STRUCTURE K = 2			Group 1				Group 2	
Group 1 (314)			-				0.04	
Group 2 (275)			-				-	
STRUCTURE K = 6	Group 1		Group 2	Group 3	Group 4		Group 5	Group 6
Group 1 (45)	-		0.05	0.04	0.25		0.09	0.05
Group 2 (131)	-		-	0.03	0.22		0.09	0.05
Group 3 (113)	-		-	-	0.18		0.06	0.02
Group 4 (14)	-		-	-	-		0.16	0.17
Group 5 (153)	-		-	-	-		-	0.03
Group 6 (133)	-		-	-	-		-	-
STRUCTURE K = 10	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Group 1 (45)	-	0.05	0.05	0.25	0.09	0.07	0.13	0.09	0.13	0.06
Group 2 (131)	-	-	0.03	0.22	0.10	0.05	0.12	0.08	0.10	0.05
Group 3 (113)	-	-	-	0.17	0.07	0.02	0.09	0.05	0.07	0.03
Group 4 (14)	-	-	-	-	0.17	0.15	0.19	0.16	0.25	0.19

Table 2.2, Continued

Group 5 (56)	-	-	-	-	-	0.05	0.05	0.02	0.10	0.04
Group 6 (42)	-	-	-	-	-	-	0.04	0.03	0.07	0.03
Group 7 (41)	-	-	-	-	-	-	-	0.02	0.12	0.05
Group 8 (46)	-	-	-	-	-	-	-	-	0.09	0.03
Group 9 (16)	-	-	-	-	-	-	-	-	-	0.09
Group 10 (86)	-	-	-	-	-	-	-	-	-	-

TESS K = 7	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Group 1 (135)	-	0.05	0.02	0.04	0.04	0.17	0.07
Group 2 (45)	-	-	0.06	0.05	0.09	0.25	0.13
Group 3 (99)	-	-	-	0.04	0.04	0.15	0.08
Group 4 (131)	-	-	-	-	0.09	0.22	0.13
Group 5 (169)	-	-	-	-	-	0.16	0.04
Group 6 (14)	-	-	-	-	-	-	0.20
Group 7 (56)	-	-	-	-	-	-	-

Table 2.3. Number of samples (*N*), mean observed (H_o) and expected (H_e) heterozygosity, allelic richness (A), and results of Hardy Weinberg Equilibrium (HWE) and linkage disequilibrium (LE) tests per population defined by STRUCTURE and TESS. The number of loci (HWE) or locus to locus comparisons (LE) significantly ($P < 0.05$) deviant from expectations are shown in parenthesis. Observed and expected heterozygosity were calculated using the program Arlequin v. 3.5; HWE, and LE were calculated using FSTAT using a Bonferroni correction at a nominal p value = 0.05, and A was calculated using the program HP-Rare, which uses rarefaction to adjust for differing samples sizes.

Population	*N*	H_o	H_e	A	HW	LE
STRUCTURE (K = 2)						
Group 1	314	0.53	0.59	8.59	(7)	
Group 2	275	0.63	0.65	9.10	(7)	
STRUCTURE (K = 6)						
Group 1	45	0.53	0.59	3.77	(1)	(1)
Group 2	131	0.63	0.65	4.26		(1)
Group 3	113	0.66	0.68	4.64		
Group 4	14	0.50	0.53	3.95		
Group 5	153	0.66	0.70	4.50	(2)	
Group 6	133	0.66	0.69	4.60		
STRUCTURE (K = 10)						
Group 1	45	0.53	0.59	3.77	(1)	(1)
Group 2	131	0.63	0.65	4.26		(1)
Group 3	113	0.66	0.68	4.64		
Group 4	14	0.50	0.53	3.95		
Group 5	56	0.66	0.68	4.34		
Group 6	42	0.71	0.73	4.90		
Group 7	41	0.66	0.67	4.16		
Group 8	46	0.66	0.72	4.59	(2)	
Group 9	16	0.63	0.60	3.50		
Group 10	85	0.66	0.67	4.41		
TESS (K = 7)						

Table 2.3, continued

Group 1	135	0.65	0.67	4.53	
Group 2	45	0.53	0.59	3.70	(1)
Group 3	99	0.68	0.71	4.73	
Group 4	131	0.63	0.65	4.34	
Group 5	109	0.67	0.69	4.42	(1)
Group 6	14	0.50	0.53	3.95	
Group 7	56	0.61	0.66	4.13	

Table 3.1. Metrics used in functional connectivity assessment. Percent variables were calculated using a circular 1km moving window. Elevation relief ratio was calculated at 1 km, 10 km and 30 km scales, reflecting swift fox home range size and low and high extremities of average dispersal. Calculations were completed using an Arc Macro scrip in ArcInfo.

Variable	Abbreviation	Source	Variable explanation	Ecological justification
Distance	xy	DEM[1]	1st order polynomial between x and y	Isolation by distance (Wright 1942)
Land Cover				
Percent grass	grass	NLCD[2]	Percent of class 71	Swift fox prefer grassland habitat (Finley et al. 2005, Kamler et al. 2003)
Percent forest	forest	NLCD[2]	Percent of classes 41, 42, 43	Swift fox prefer grassland habitat (Finley et al. 2005, Kamler et al. 2003)
Percent scrub	scrub	NLCD[2]	Percent of class 52	Higher mortality in scrub habitats (Thompson and Gese 2007)
Hydrography	hydro	GDD[3]	Percent rivers and streams	Streams and rivers may represent a dispersal barrier
Land use				
Percent wheat	wheat	NASS[4]	Percent of classes 24, 225, 236	Dominant crop type in study area
Percent corn	corn	NASS[4]	Percent of class 1	Dominant crop type in study area
Percent agricultural disturbance	pdistag	TNC[5]	Percent agricultural development	Swift fox avoid cropland in much of their range (Finley et al 2005, Kamler et al. 2003)
Topography				
Compound topographic index	cti	DEM[1]	Water accumulation capacity (McCune and Keon 2002)	Wet areas may represent a dispersal barrier for terrestrial species
Elevation relief ratio	err	DEM[1]	Elevational complexity (Evans 1972)	Swift fox prefer limited topographic relief (Harrison and Whitaker-Hoagland 2003)
Heat load index	hli	DEM[1]	Measure of solar intensity (Moore et al. 1993)	Influences vegetation structure
Relative slope position	rsp	DEM[1]	Pposition between ridge (1) and valley (0) (Murphy et al. 2010)	Ridge tops provide visual relief and are frequently used by carnivores
Anthropogenic development				
Percent non-ag disturbance	pdistan	TNC[5]	Percent non-agricultural development (e.g., roads, mines, natural gas extraction, etc)	Anthropogenic disturbance may impact swift fox
Percent all roads	rds	TIGER[6]	Percent of low and high traffic roads	Roads are a source of mortality (Matlack et al. 2000, Kamler et al. 2003)

Table 3.1., continued

Percent busy roads	bsyrds	TIGER[6]	Percent of high traffic roads	Roads are a source of mortality (Matlack et al. 2000, Kamler et al. 2003); busy roads may be a higher risk
Climate				
Frost free period	ffp	CM[7]	# days between last and first freeze	Influences vegetation structure, crop type and duration
Mean annual precipitation	map	CM[7]	Mean annual precipitation	Increasing values lead to taller vegetation
Growing season precipitation	gsp	CM[7]	Mean growing season precipitation	Increasing values lead to taller vegetation
Degree days > 5°C	dd5	CM[7]	Total degrees for all days >5°C	Influences vegetation structure, crop type and duration

[1]Digital Elevation Model, USGS National Map; [2]National Land Cover Database; [3]GIS Data Depot; [4]2006 Cropland Data Layer, National Agricultural Statistics Service; [5]The Nature Conservancy; [6]2006 Tiger road data; U. S. Census Bureau; [7]Climate Model, Rehfeldt et al. 2006.

Table 3.2. Models of variables influencing gene flow at two scales of genetic structure in CO and WY study areas. Random Forests pseudo-R^2, mean squared error (MSE) and out-of-bag error (OOB) are also shown; variables are ranked from highest to lowest importance.

Regression Analysis	Selected variables (in ranked order)	psuedo-R^2	MSE
CO study area			
K = 2	ffp, map, xy, pdistag, pdistan, scrub	93.89	0.005
K = 4			
Group 1	ffp, map, xy, scrub, pdistag, pdistan	91.09	0.004
Group 2	ffp, xy, map, pdistag, pdistan, wheat	85.73	0.006
Group 3	ffp, map, xy, pdistag, pdistan, scrub	95.12	0.005
Group 4	map, ffp, xy, pdistag, scrub, pdistan	87.07	0.006
WY study area			
K = 2	xy, ffp, map, scrub, grass, forest	82.14	0.001
K = 5			
Group 1	xy, ffp, map, scrub, grass, pdistan	81.08	0.007
Group 2	xy, ffp, map, grass, scrub, pdistan	75.80	0.003
Group 3	map, xy, ffp, scrub, grass, forest	72.85	0.002
Group 4	xy, map, ffp, pdistan, scrub, grass	64.23	0.004
Group 5	map, xy, ffp, scrub, grass, forest	74.04	0.001
Categorical Analysis		OOB	
CO study area	ffp, map, xy, pdistag, pdistan, scrub	19.30	
WY study area	xy, map, ffp, scrub	24.85	

Table 4.1. Pair-wise differentiation between genetic sub-groups (F_{st}); samples sizes per group are shown in parentheses. $P < 0.001$ in all cases.

	Eastern (48)	Central (38)	Western (25)
Eastern (48)	-	-	-
Central (38)	0.02	-	-
Western (25)	0.08	0.04	-

Table 4.2. Mean expected and observed heterozygosity, allelic richness, private allelic richness, and F_{is} in each genetic subgroup and range-wide; sample sizes are shown in parenthesis. Range-wide measures are taken from Chapter 2 for reference.

	Eastern (48)	Central (38)	Western (25)	Range-Wide (589)
H_e (sd)	0.67(0.11)	0.65(0.14)	0.61(0.21)	0.64(0.15)
H_o (sd)	0.67(0.12)	0.72(0.11)	0.65(0.17)	0.70(0.15)
Allelic Richness	5.57	6.01	5.39	
Private Allelic Richness	0.38	0.60	0.38	
F_{is}	0.00	0.10	0.07	0.01

Table 4.3. Number of putative immigrants (n = 25) per source-recipient group pairs, as measured using the program STRUCTURE.

	Recipient Group (# individual samples)		
Source Group	Eastern (48)	Central (38)	Western (25)
Eastern (48)	-	6	1
Central (38)	13	-	2
Western (25)	1	2	-

Table 4.4. Number of immigrants ($N_e m$) exchanged between groups, as measured using F_{st}.

	Group (# individual samples)		
Group (# samps)	Eastern (48)	Central (38)	Western (25)
Eastern (48)	-	-	-
Central (38)	8.76	-	-
Western (25)	3.08	5.44	-

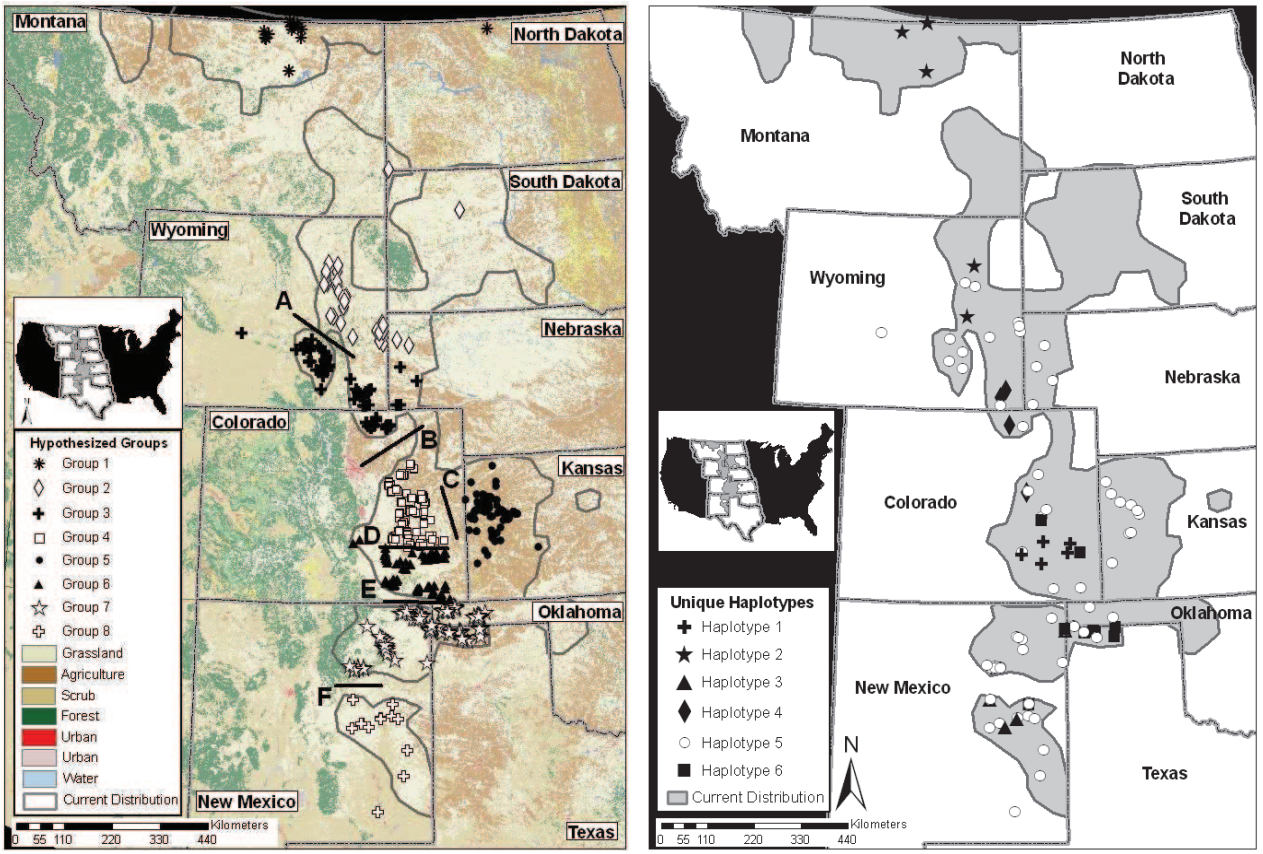

Figure 2.1. Geographic location of hypothesized genetically distinct swift fox groups and general gene flow boundaries (left) and unique swift fox haplotypes (right). Barrier A corresponds with the Medicine Bow Mountains; Barrier B corresponds with the South Platte River, scrub and agricultural development; Barrier C corresponds with agricultural development; Barrier D

corresponds with the Arkansas River and agricultural development; Barrier E corresponds with a region of rugged topography; Barrier F corresponds with the Conchas and Canadian Rivers. Groups 1 and 2 are separated by a broad region encompassing several mountain ranges and river systems.

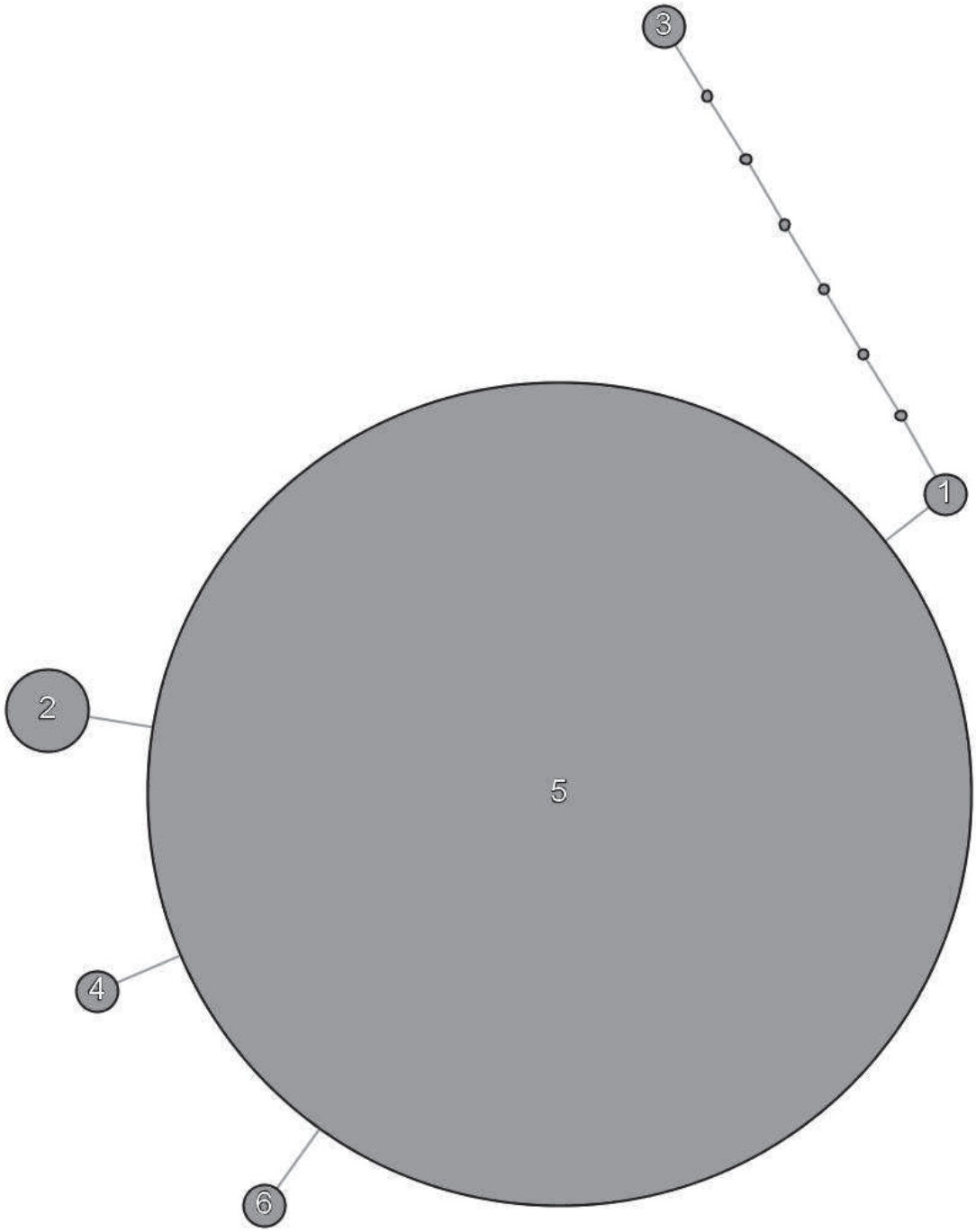

Figure 2.2. Minimum spanning network demonstrating the relationship between 6 unique swift fox haplotypes detected in a 250 base mitochondrial control region sequence. Haplotypes are weighted by sample size.

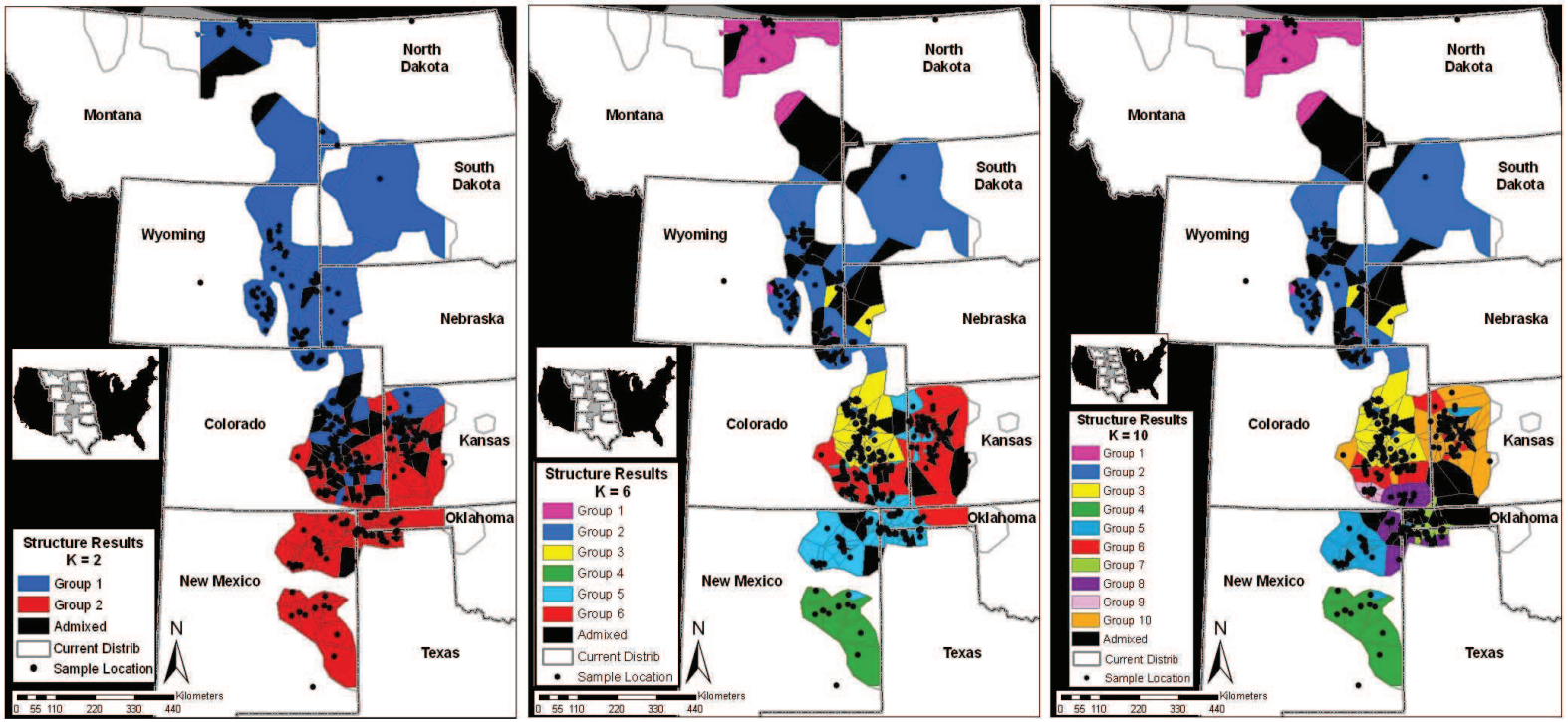

Figure 2.3. Genetic structure detected in the swift fox distribution using a hierarchical analysis approach in the program STRUCTURE. We detected three levels of genetic structure in our data set; these proceed from coarse to fine scale structuring from left to right in this figure. We found two unique groups in the first hierarchy of genetic structure, six groups in the second hierarchy, and ten groups in the third and final hierarchy.

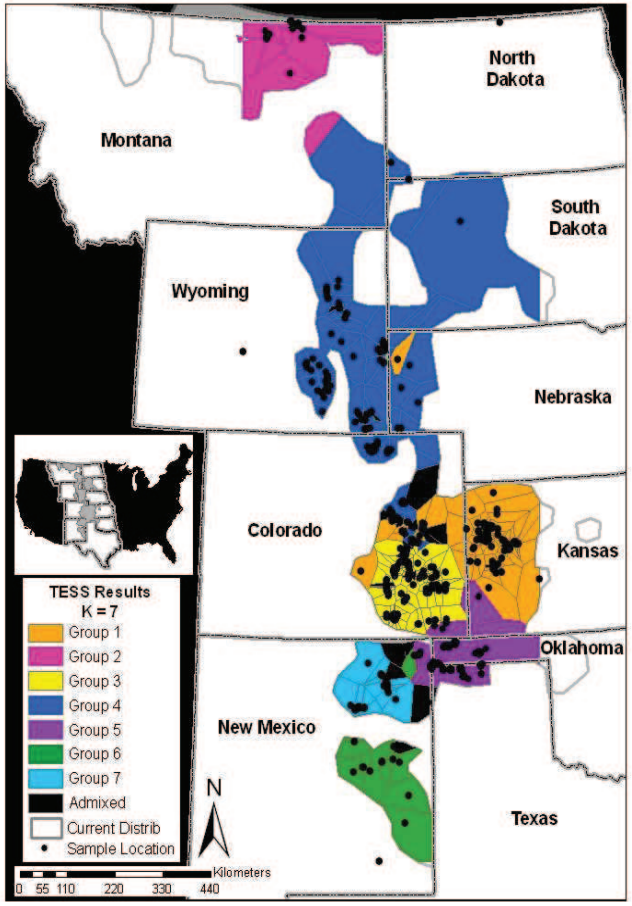

Figure 2.4. Genetic structure detected in the swift fox distribution using the program TESS. We detected seven distinct groups using this analysis.

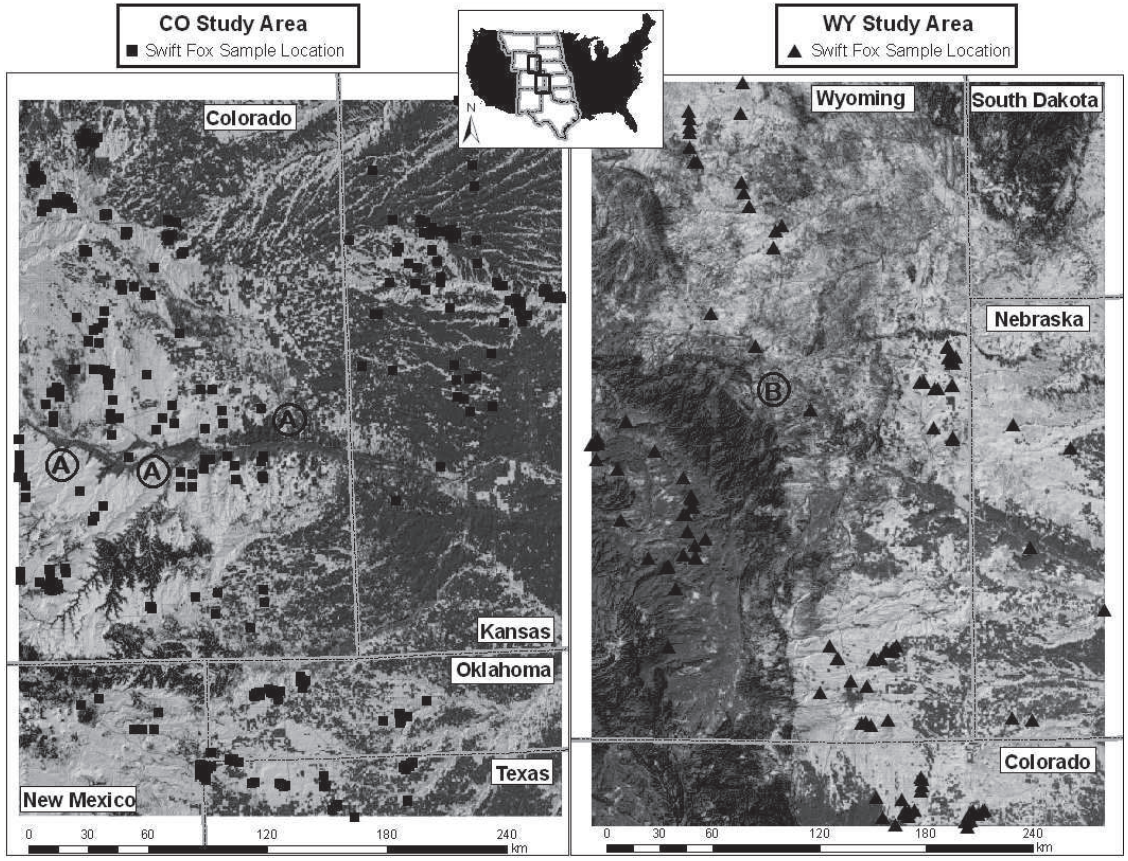

Figure 3.1. CO (left) and WY (right) study areas. A percent grassland layer is draped over a hill shade to provide information on topographic relief and native grassland composition in each study area; grassland is represented in white, non-grassland in black or gray. As reference points, the Arkansas River (A) and the Medicine Bow Mountains (B) are labeled.

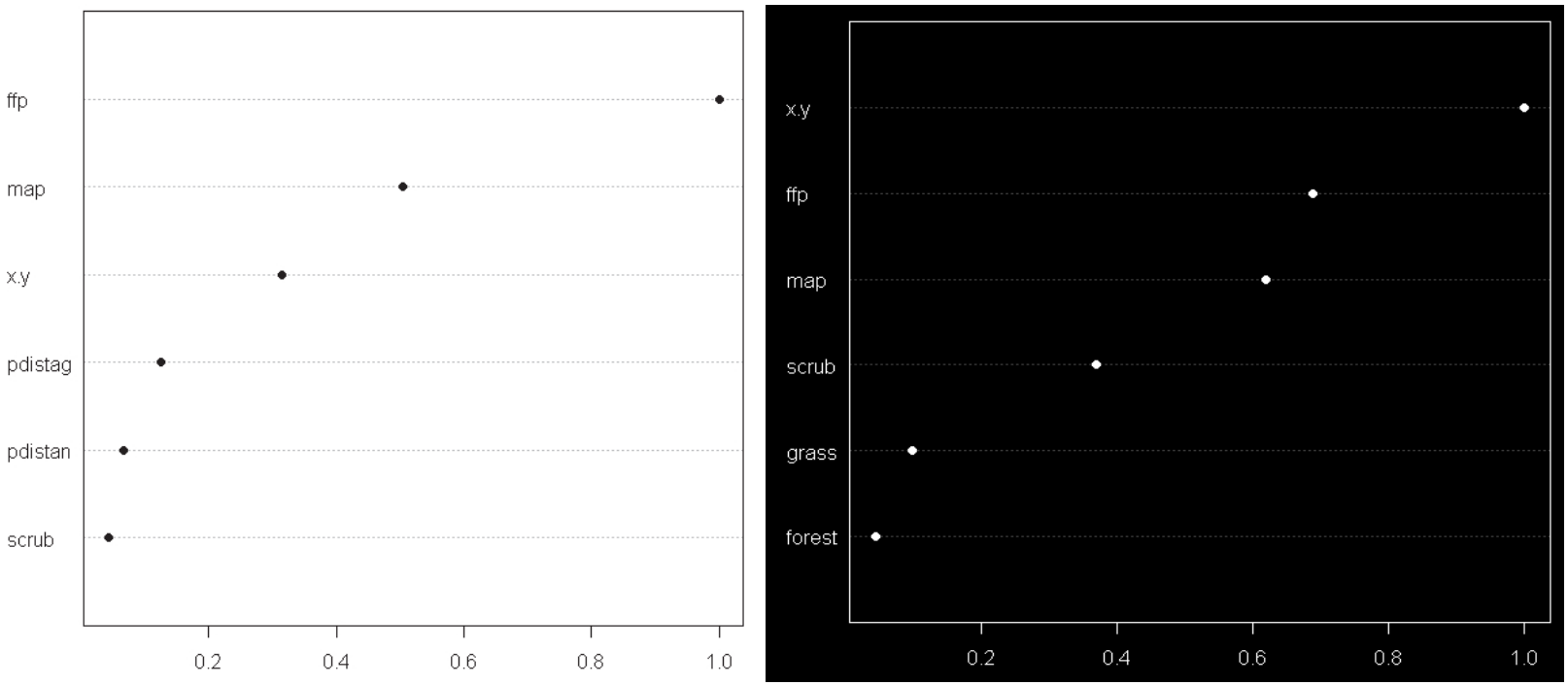

Figure 3.2. Variable importance, based on model improvement ratios, for coarse scale (K = 2) genetic structure in CO (left) and WY (right) study areas. Variable abbreviations are provided in Table 1.

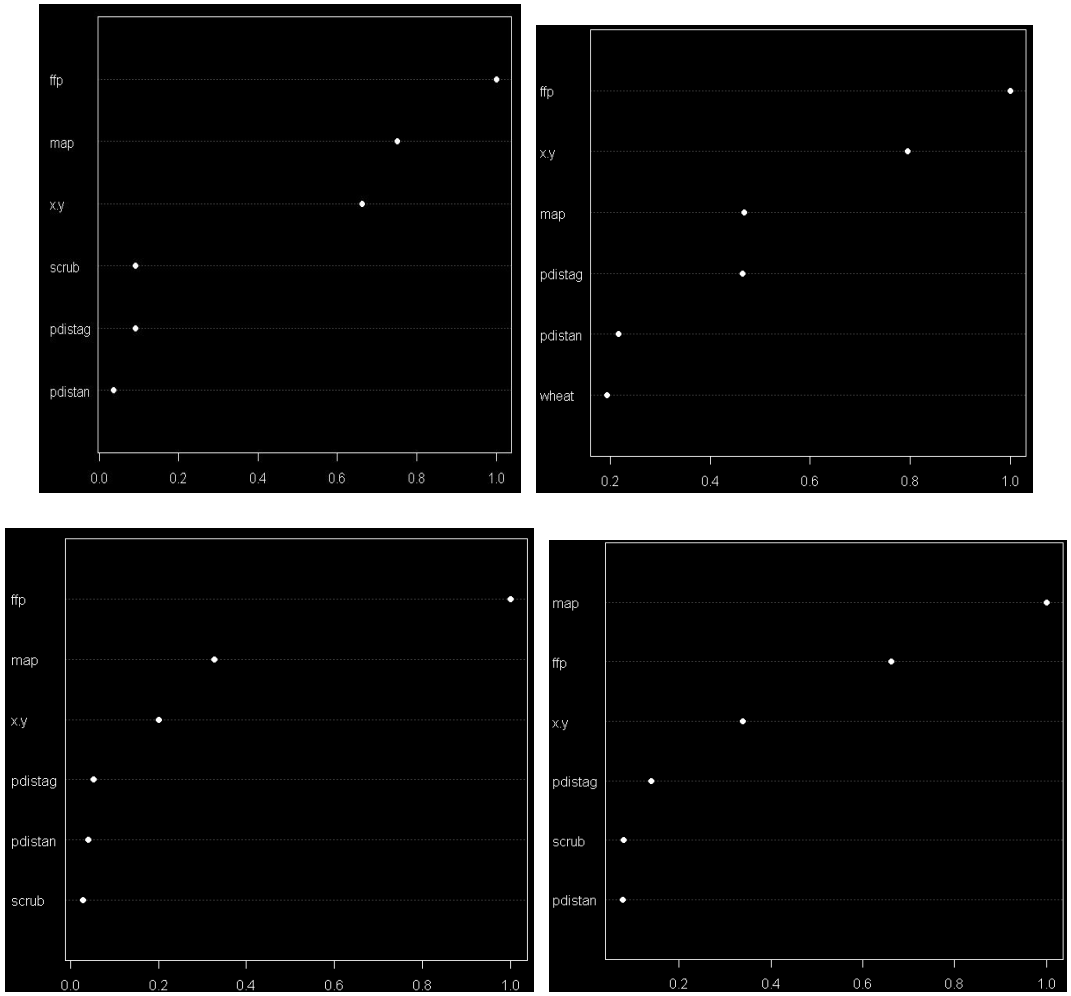

Figure 3.3. Variable importance, based on model improvement ratios, for models of each genetic group (K = 4) in the CO study area for fine scale genetic structure. Graphs are arranged in numerical order from left to right, starting with group 1. Variable abbreviations are provided in Table 3.1.

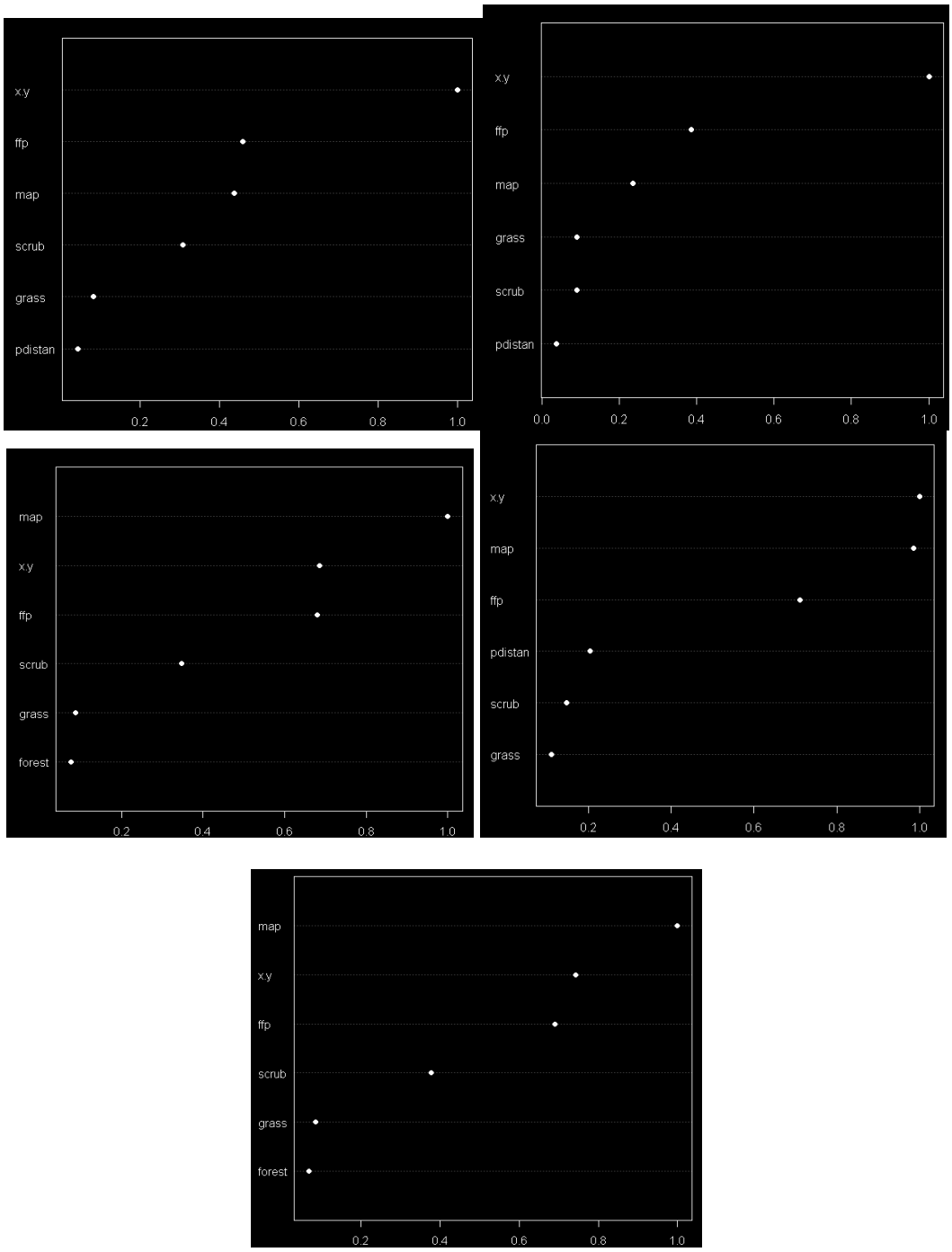

Figure 3.4. Variable importance, based on model improvement ratios, for models of each genetic group (K = 5) in the WY study area for fine scale genetic structure.

Graphs are arranged in numerical order from left to right, starting with group 1.

Variable abbreviations are provided in Table 3.1.

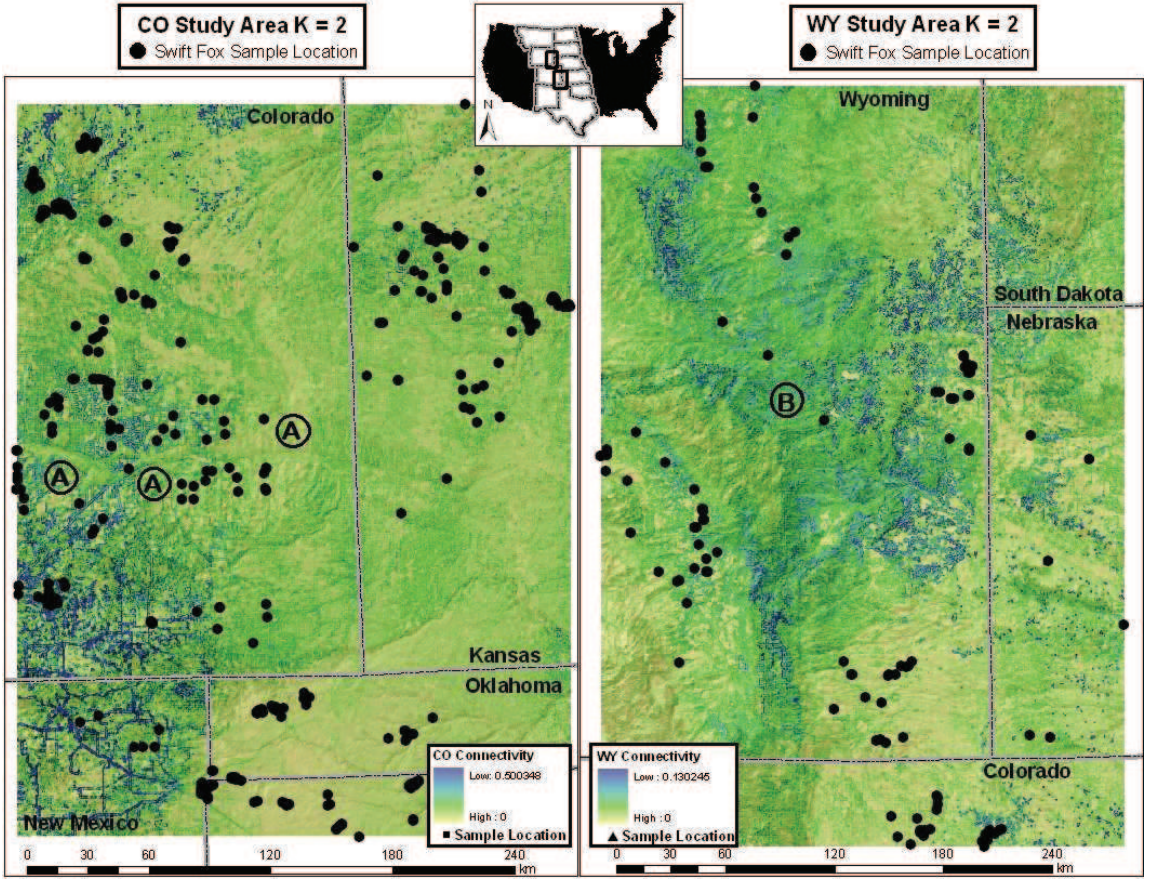

Figure 3.5. Functional connectivity in CO and WY study areas for coarse level genetic structure (K = 2). Light shading indicates low connectivity, whereas dark shading indicates high connectivity. As reference points, the Arkansas River (A) and the Medicine Bow Mountains (B) are labeled.

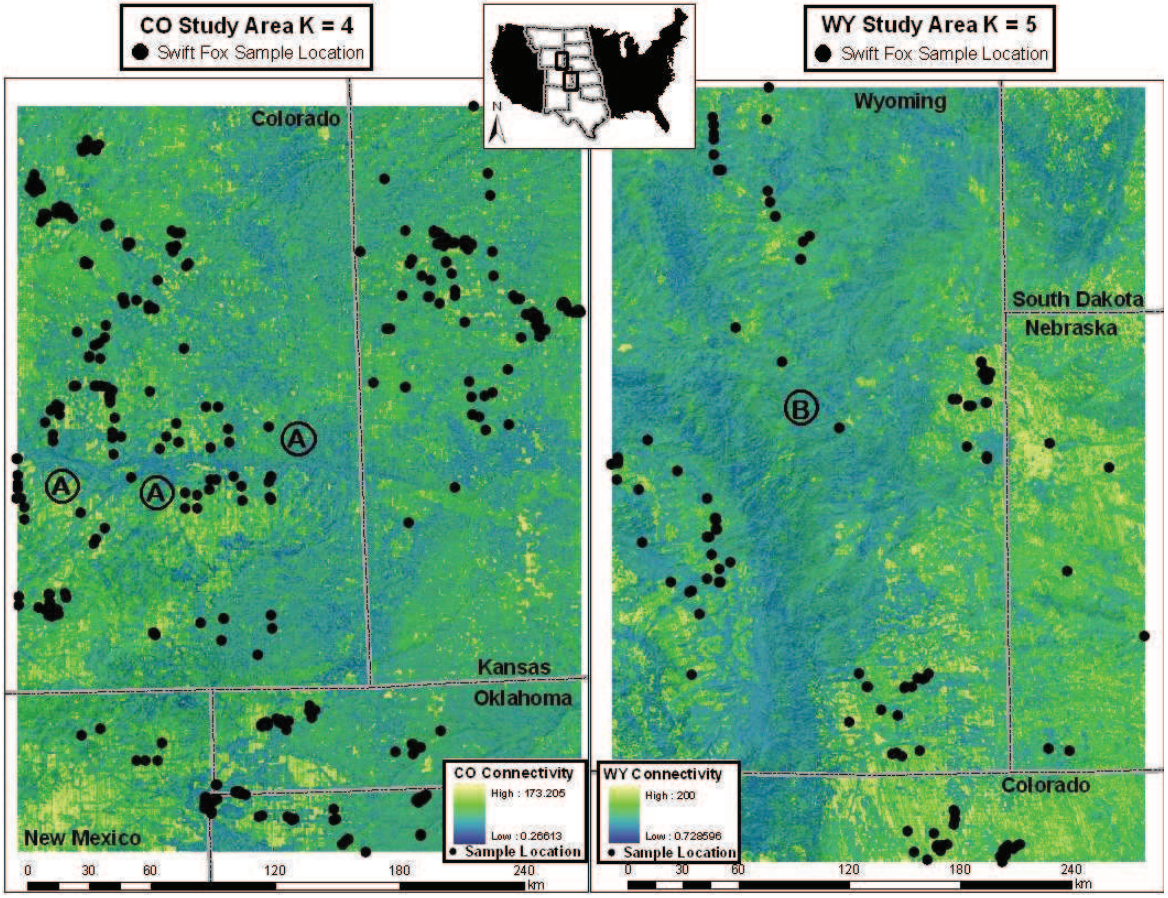

Figure 3.6. Functional connectivity in CO and WY study areas for fine scale genetic structure (K = 4 and 5 groups, respectively). Light shading indicates low connectivity, whereas dark shading indicates high connectivity. As reference points, the Arkansas River (A) and the Medicine Bow Mountains (B) are labeled.

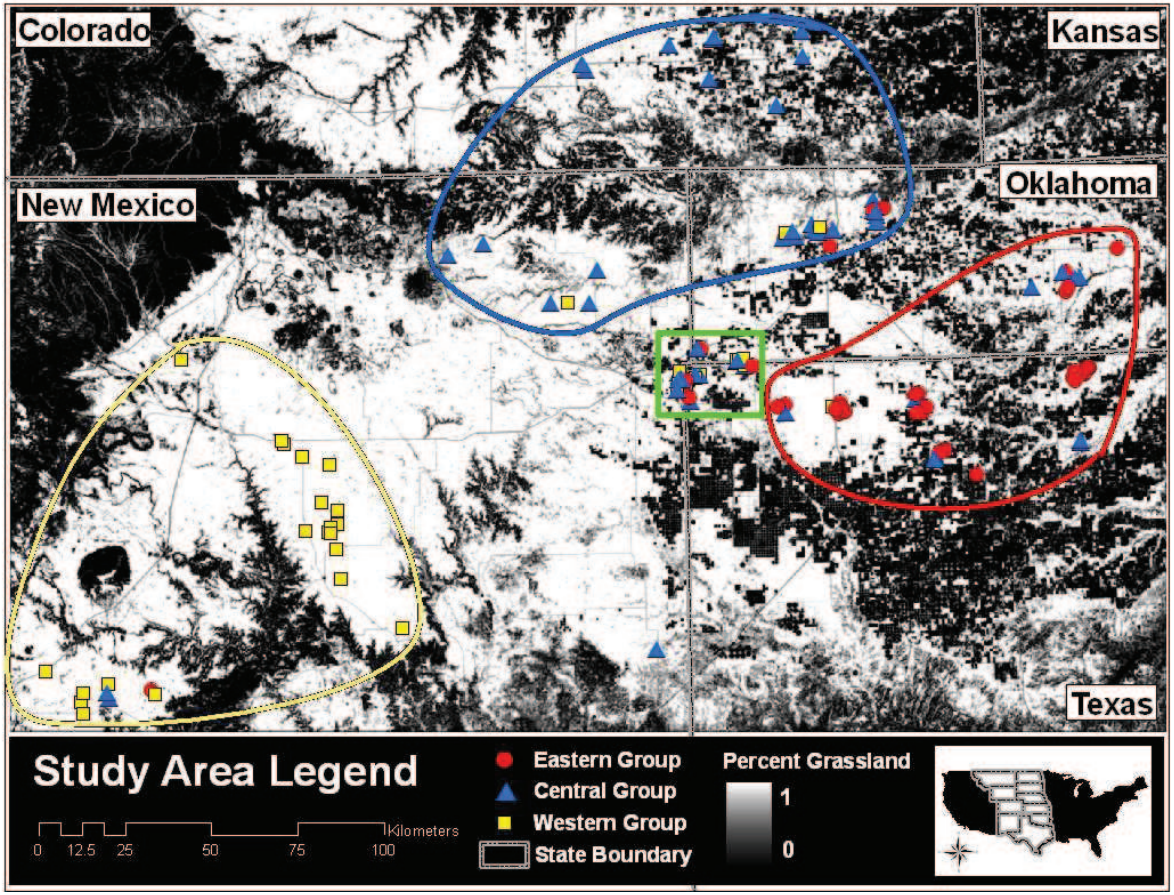

Figure 4.1. Study area encompassing the swift fox genetic group in southern Colorado, Oklahoma, Texas and northeastern New Mexico. The percentage grassland (calculated from National Land Cover Data) is shown to illustrate landscape heterogeneity and fragmentation. Genetic subgroups (e.g., eastern, central and western) are shown with different symbols; geographic groupings are

delineated with color-coordinated polygons. Samples excluded from analysis are indicated by the green box at the intersection of New Mexico, Oklahoma and Texas state boundaries.

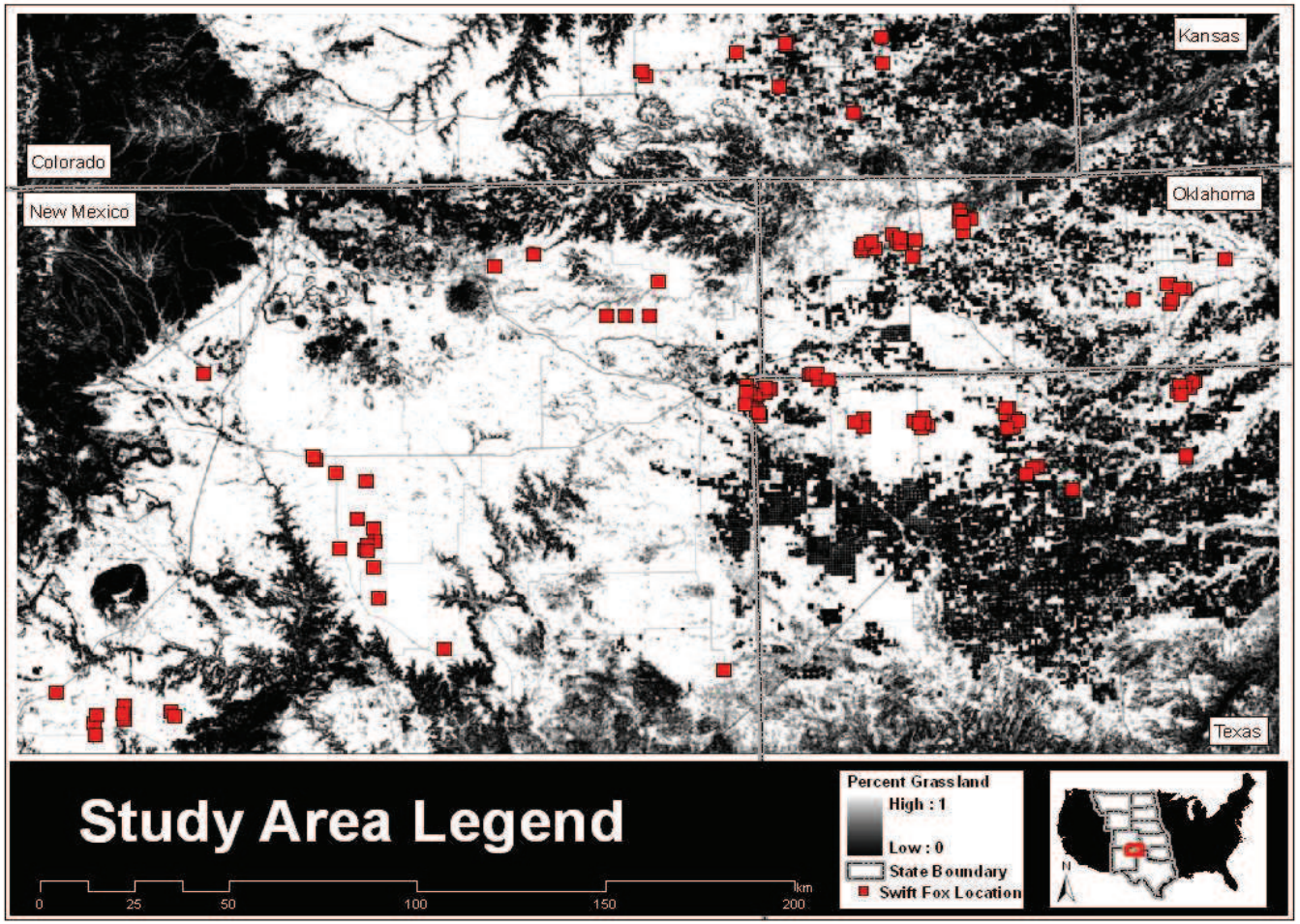

Figure 5.1. Study area, with individual swift fox locations shown as red squares. Landscape heterogeneity is illustrated using a percentage of grassland layer, calculated from National Land Cover Data (USGS) with a 1.0-km moving window.

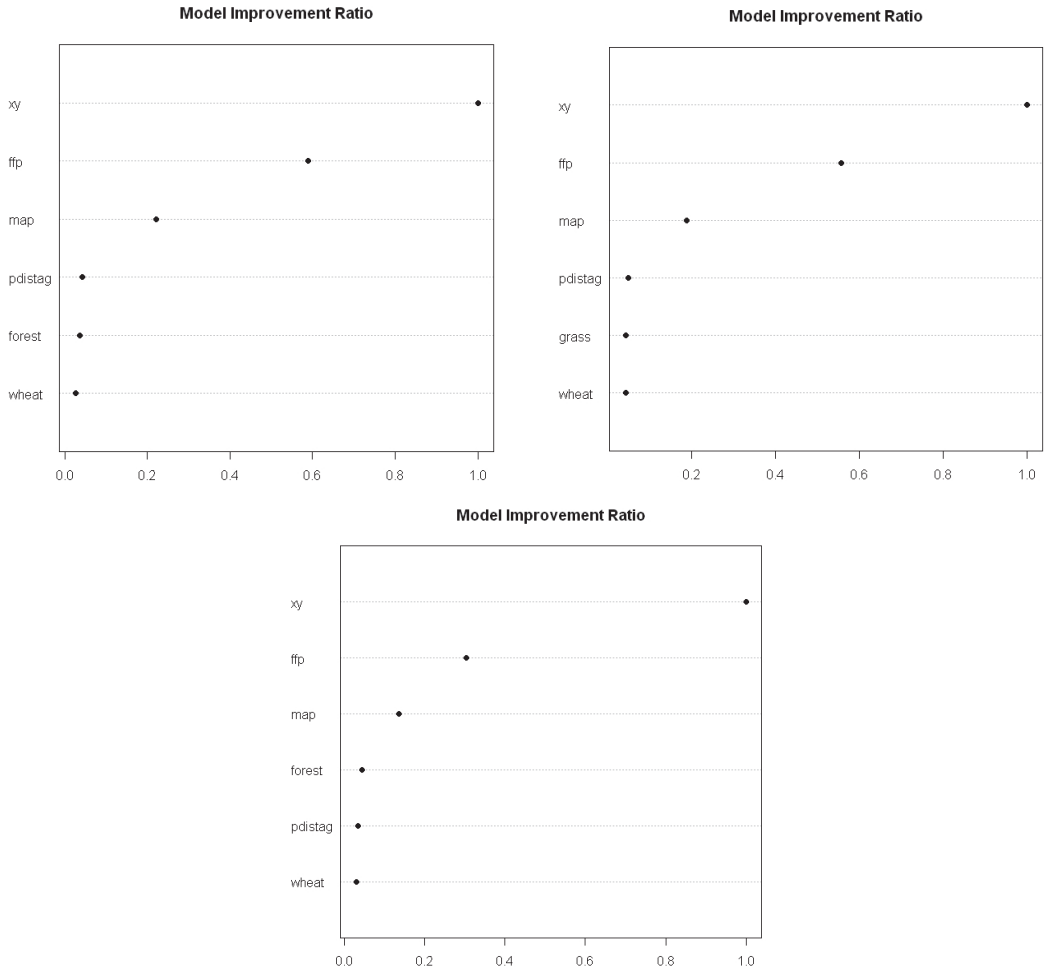

Figure 5.2. Model improvement ratios for Random Forests analysis; graphs are shown sequentially from top, left to bottom for each of three unique genetic groups in the study area.

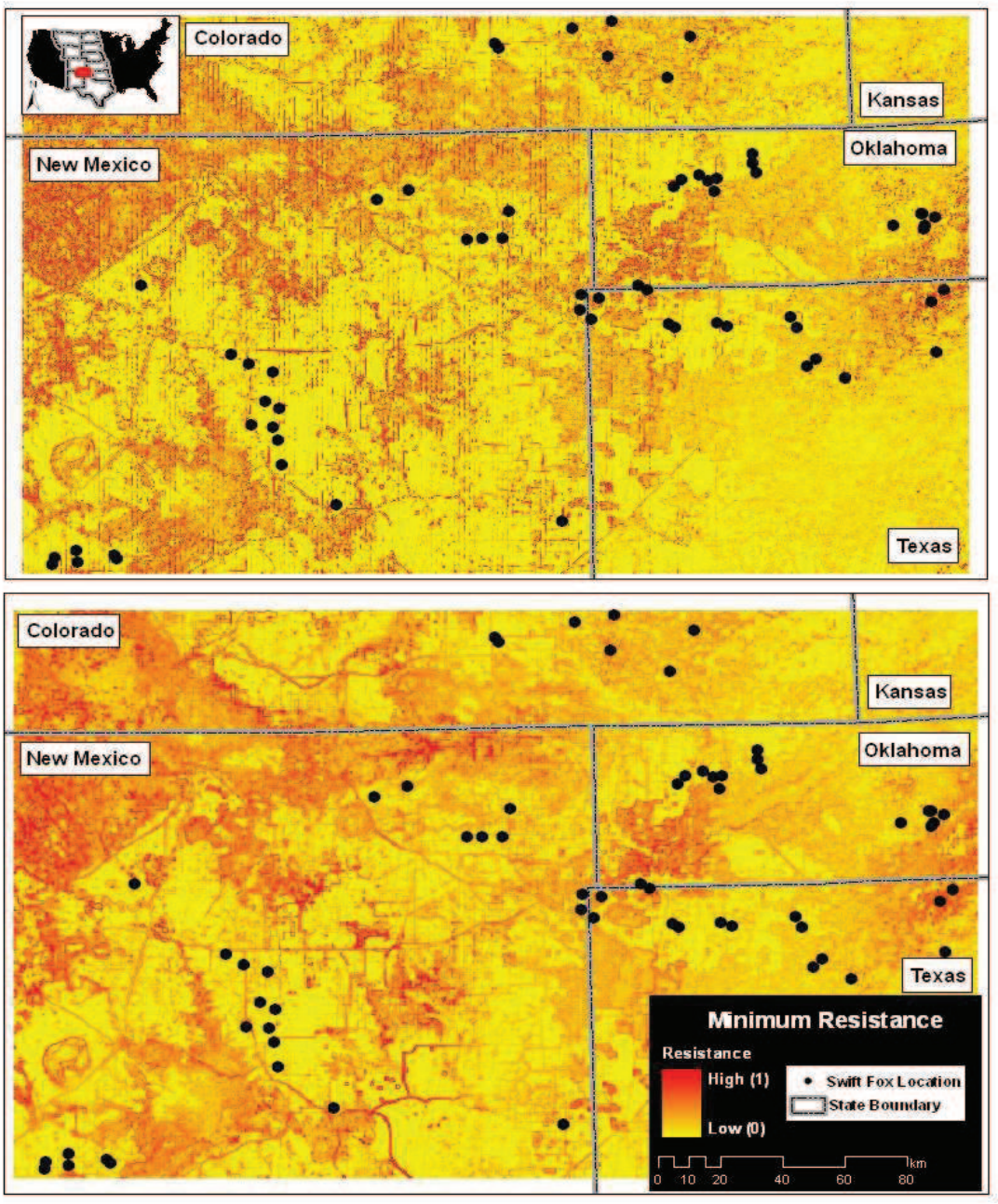

Figure 5.3. Minimum resistance surface as predicted using Random Forests modeling results. For reference, the original surface (30 m resolution) is shown on bottom and the upscaled surface (240 m resolution) is shown on top. The upscaled surface was used in least-cost path modeling due to computational limitations.

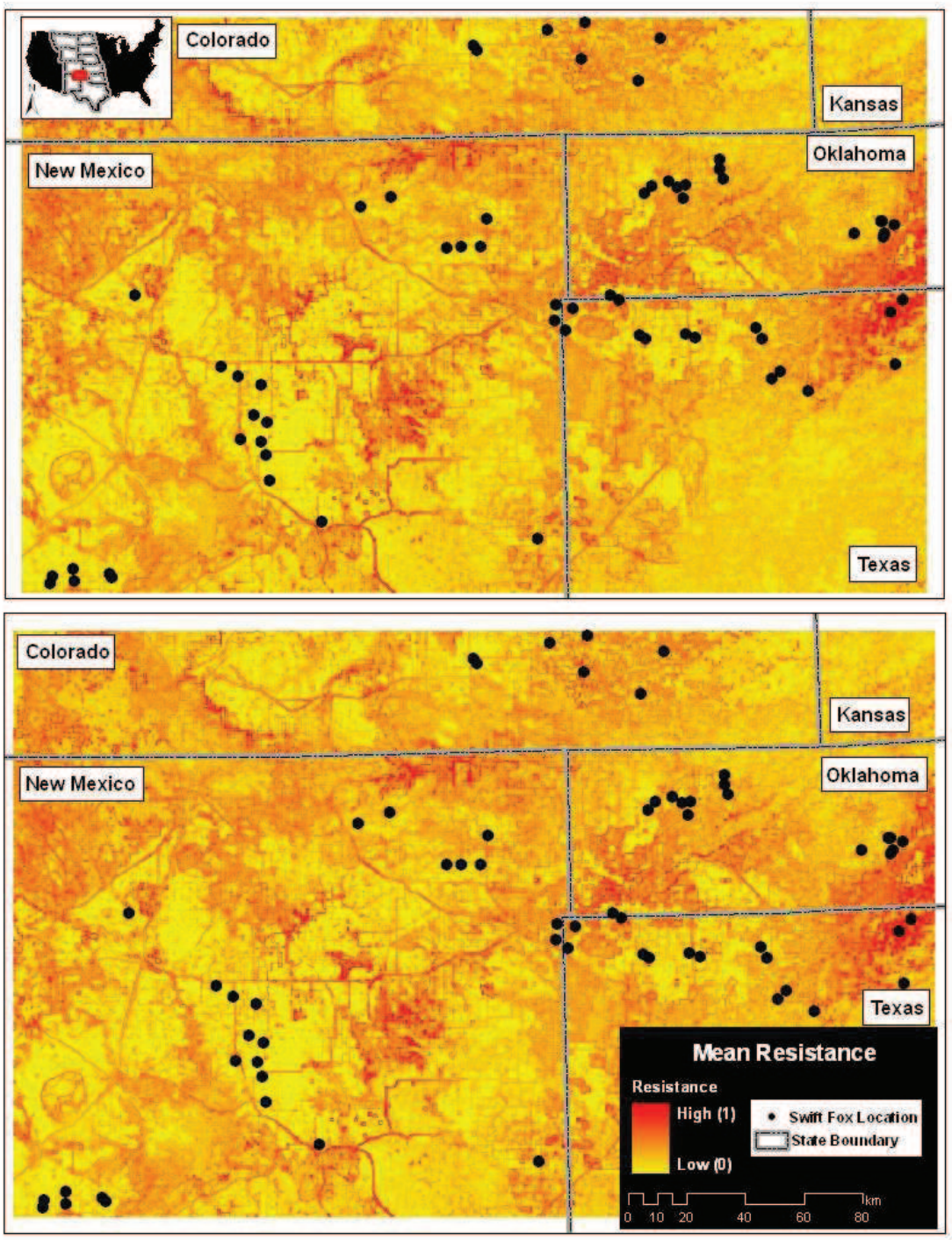

Figure 5.4. Mean resistance surface as predicted using Random Forests modeling results. For reference, the original surface (30 m resolution) is shown on bottom and

the upscaled surface (240 m resolution) is shown on top. The upscaled surface was used in least-cost path modeling due to computational limitations.

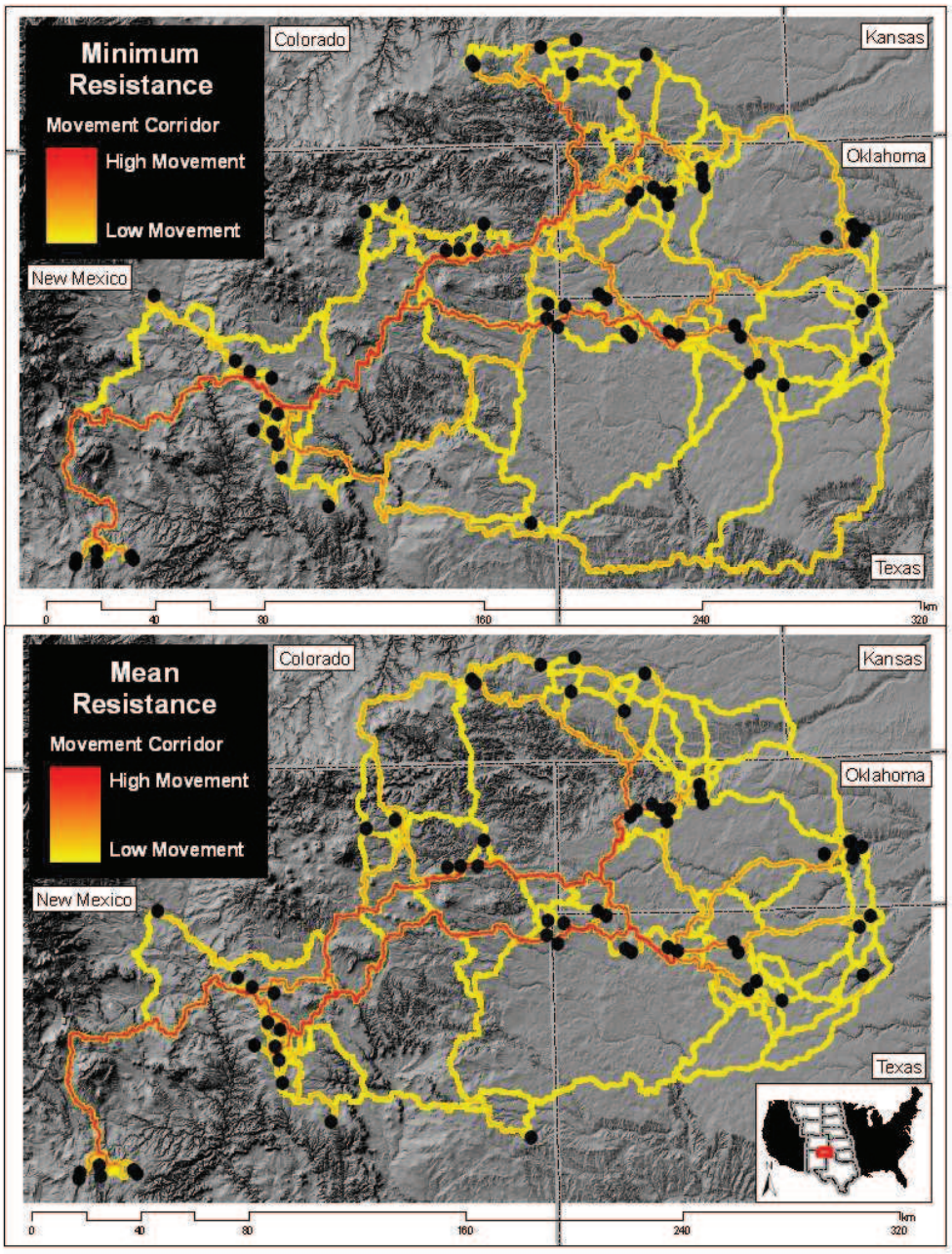

Figure 5.5. Least-cost paths calculated using minimum (top) and mean (bottom) resistance surfaces. Paths are smoothed with a Gaussian kernel density estimator, and are 2.2-km in width. Yellow color indicates low support (e.g., selected by few pair-wise point connections), whereas red indicates high support (e.g., selected by many

pair-wise point connections). Paths are displayed over a topographic hill shade for viewing ease, but the reader should recall topography was not selected in Random Forests models.

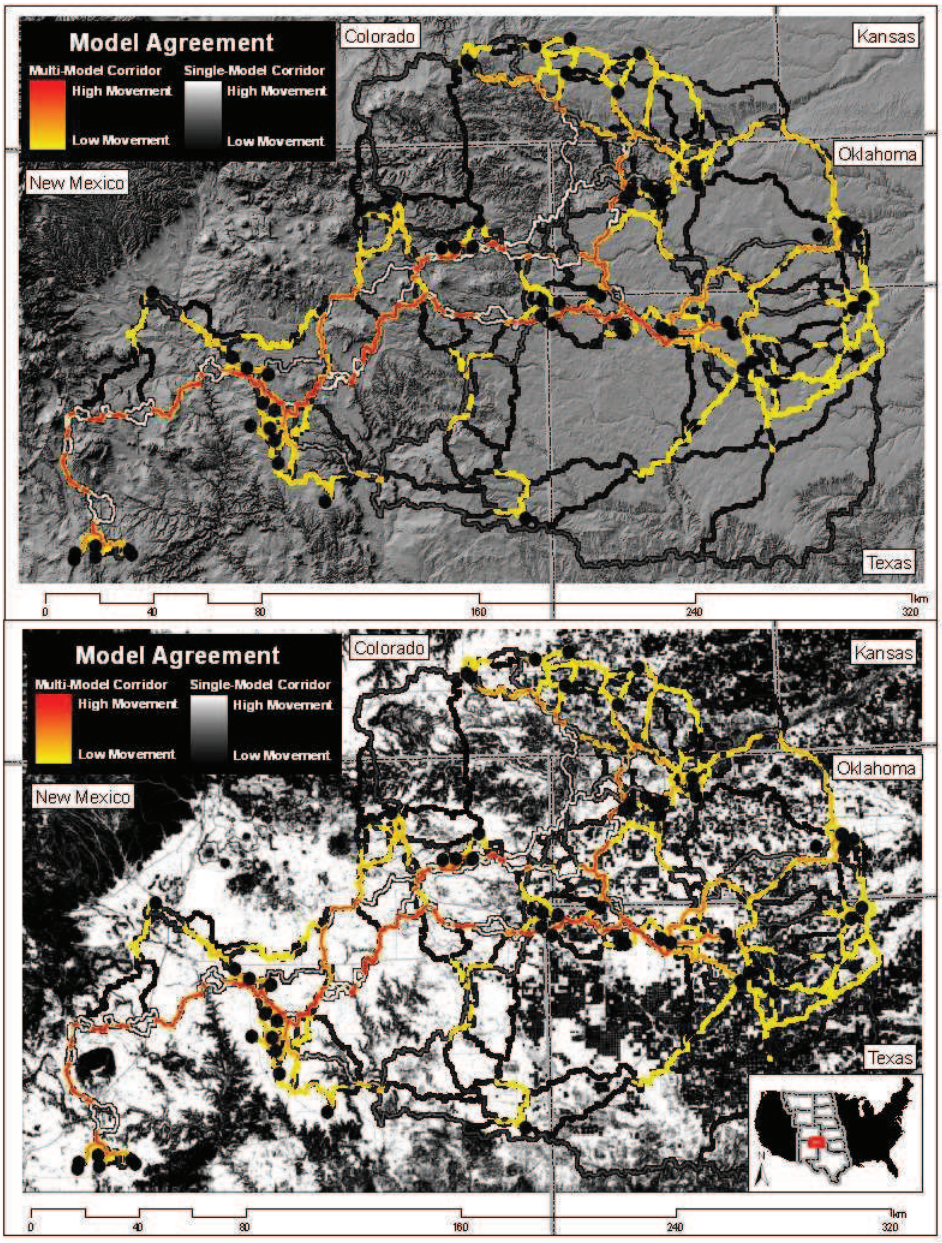

Figure 5.6. Model agreement between minimum and mean resistance least-cost paths. Paths in color represent agreement between models, with predicted movement increasing from yellow to red. Individual model paths are shown in gray scale, with predicted movement increasing from black to white. Corridors are displayed over a

topographic hill shade (top) to improve viewing. However, topography was not selected in Random Forests models, so paths are also displayed over percent grassland (bottom) to illustrate path location in association with landscape heterogeneity.

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APPENDIX A

Results of group identification (K) using the program STRUCTURE

I detected 3 hierarchies of genetic structure using the program STRUCTURE. These results are presented by hierarchy, below.

Hierarchy one:
In the first round of analysis, log-likelihood values did not clearly identify an optimal *K* value (Figure A.1); however, I detected support for two genetic groups using the delta *K* statistic (Figure A.2). Thus, in the first hierarchy of genetic structure, I concluded that there are two genetic groups.

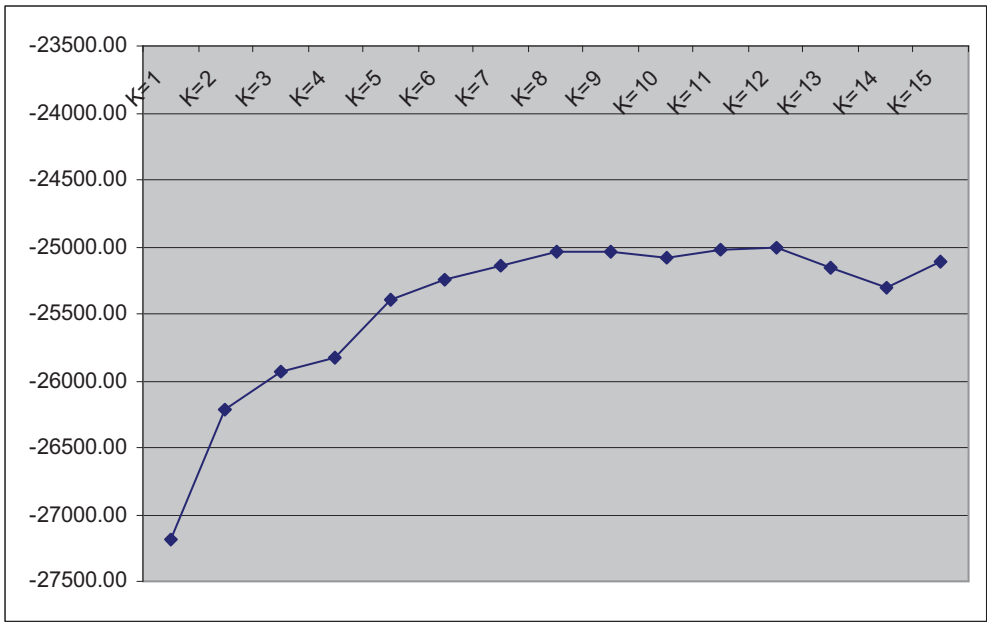

Figure A.1. Log-likelihood values for the first hierarchy of STRUCTURE analysis; no clear support for any specific *K* value was observed.

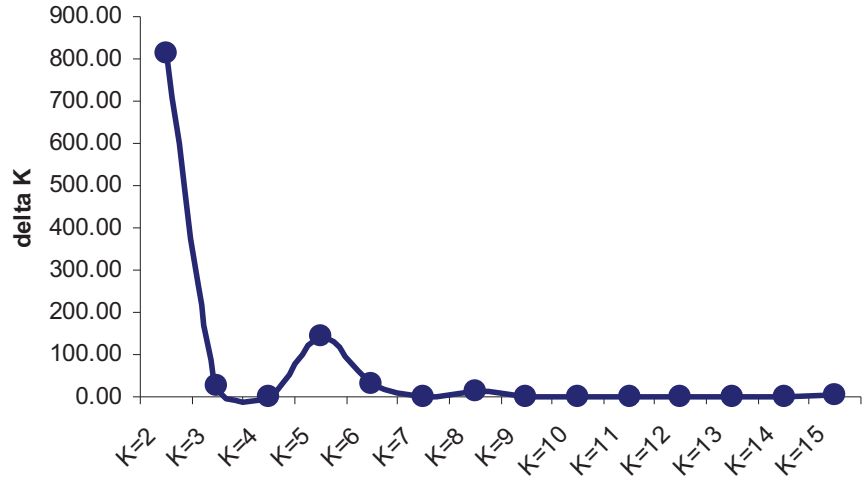

Figure A.2. Delta *K* values for genetic structure analysis, showing strong support for *K* = 2 and lower support for *K* = 5.

Hierarchy two:
I next analyzed genetic structure in each of the two groups present in the first genetic structure hierarchy. I found each of these groups contained three unique genetic groups. Log-likelihood and delta *K* graphs for each of these analyses are shown below.

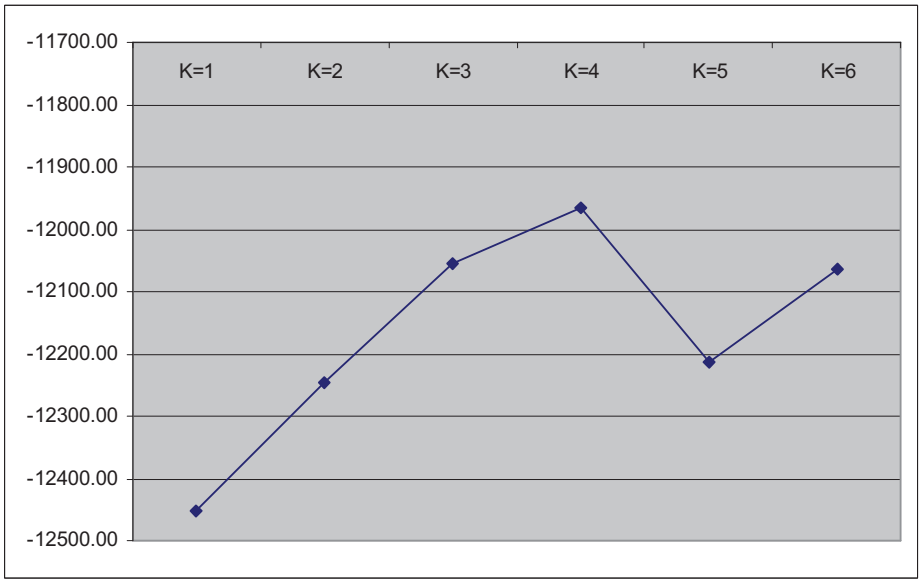

Figure A.3. Log-likelihood values for genetic structure analysis of group 1, hierarchy 1; this result implies greatest support for *K* = 4.

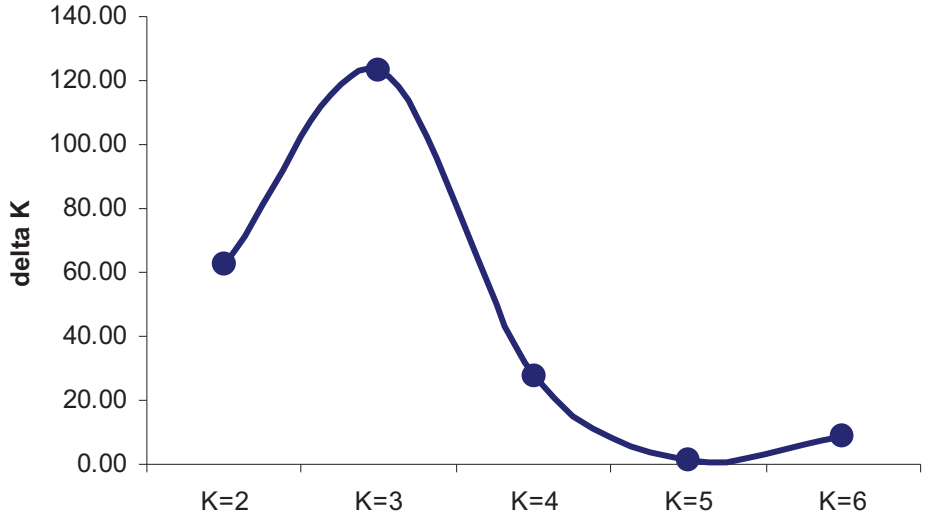

Figure A.4. Delta *K* values for genetic structure analysis for the genetic structure analysis of group 1, hierarchy 1; this result implies greatest support for $K = 3$. I chose this, the more conservative estimate of genetic structure, for further analysis.

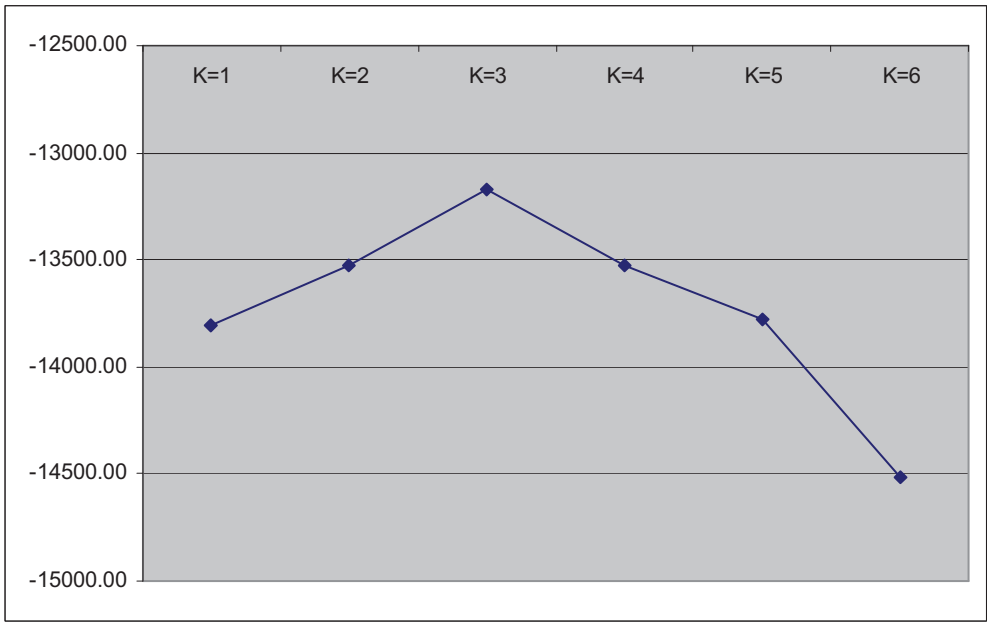

Figure A.5. Log-likelihood values for genetic structure analysis for genetic structure analysis of group 2, hierarchy 1; this result implies greatest support for $K = 3$.

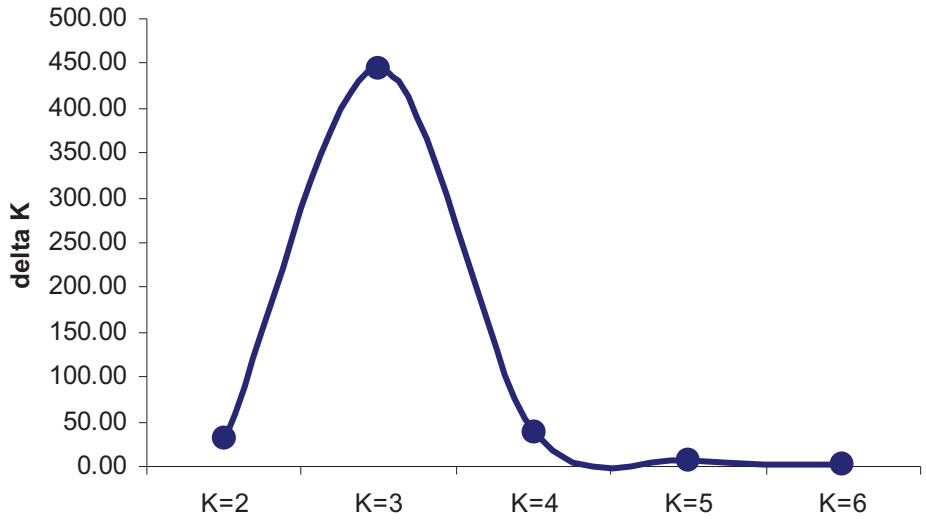

Figure A.6. Delta *K* values for genetic structure analysis for genetic structure analysis of group 2, hierarchy 1; this result implies greatest support for $K = 3$.

Hierarchy 3:
I next assessed genetic structure in each of the six genetic groups identified in the second hierarchy. Of these, analysis indicated some groups were further subdivided and some represented a single group. Upon further investigation, some of the subdivisions detected lacked biological or ecological justification; in these instances, I concluded that a single genetic groups was detected. In total I detected 10 genetic groups in the third hierarchy. The associated log-likelihood and delta *K* figures are presented below.

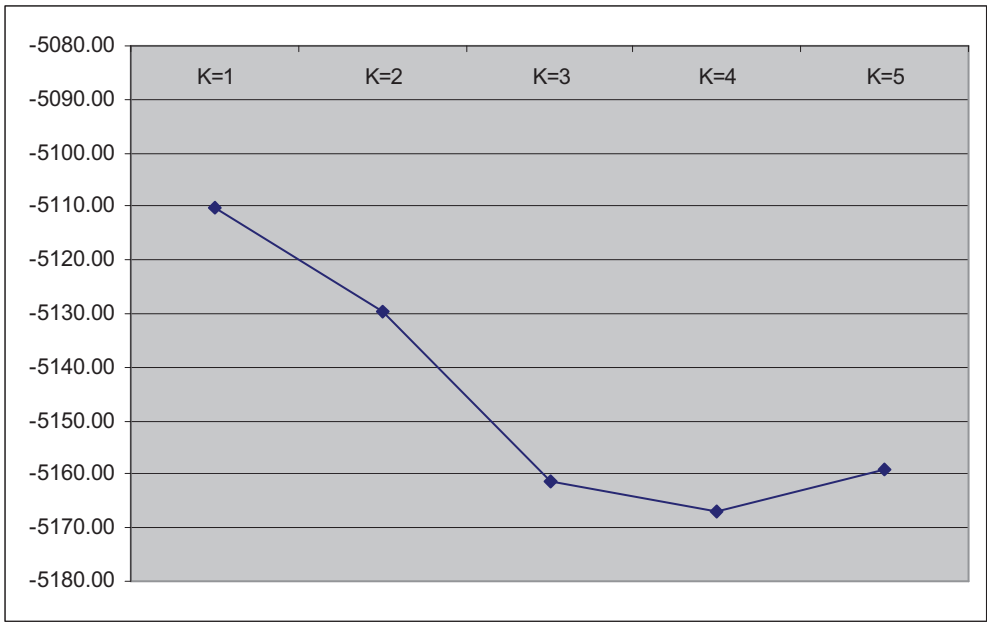

Figure A.7. Log-likelihood values for genetic structure analysis for genetic structure analysis of group 1, hierarchy 2; this result implies greatest support for $K = 1$.

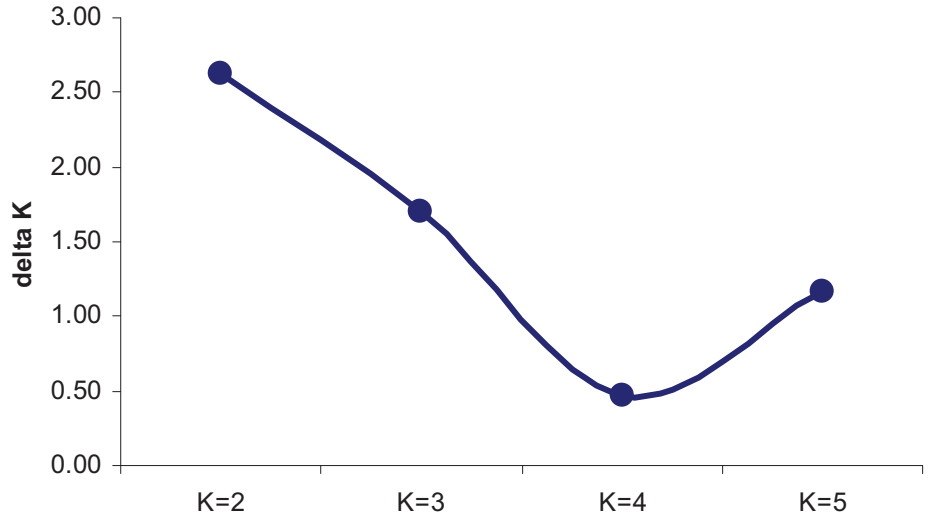

Figure A.8. Delta K values for genetic structure analysis for genetic structure analysis of group 1, hierarchy 2; this result implies greatest support for $K = 1$. Based on this result and log-likelihood values (Figure A.7), I concluded that further subdivision of this group was not supported.

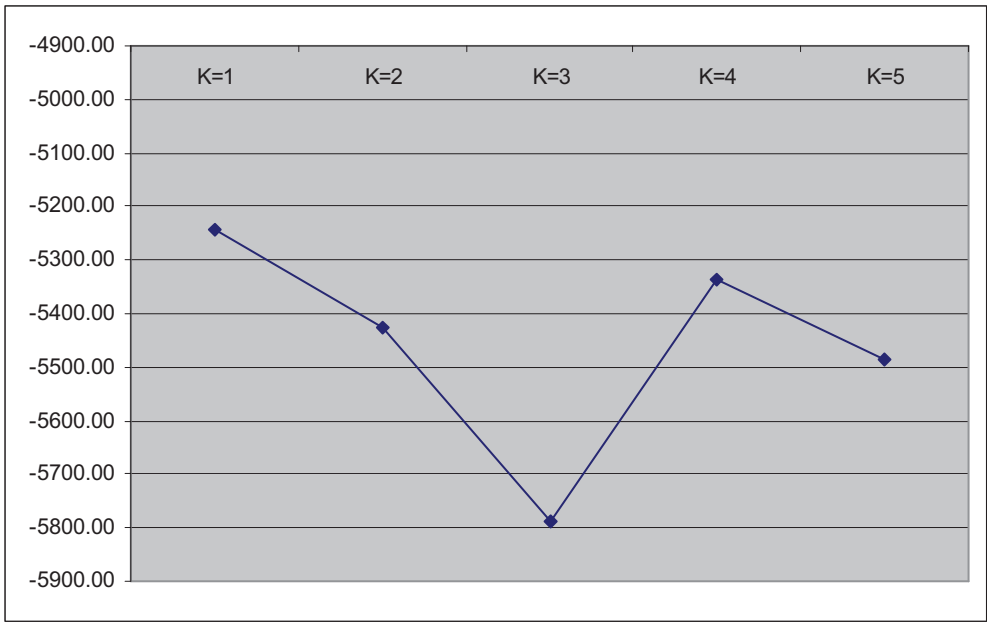

Figure A.9. Log-likelihood values for genetic structure analysis for genetic structure analysis of group 2, hierarchy 2; this result implies greatest support for $K = 1$.

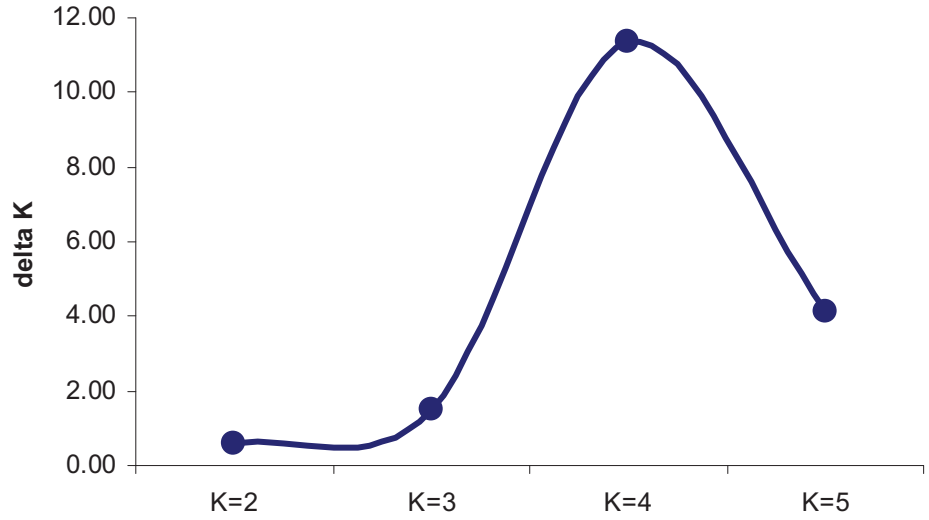

Figure A.10. Delta K values for genetic structure analysis for genetic structure analysis of group 2, hierarchy 2; this result implies greatest support for $K = 4$. Samples were highly admixed at $q = 0.70$ and lacked geographic cohesion, thus I concluded that there was not support for further subdivision of this group.

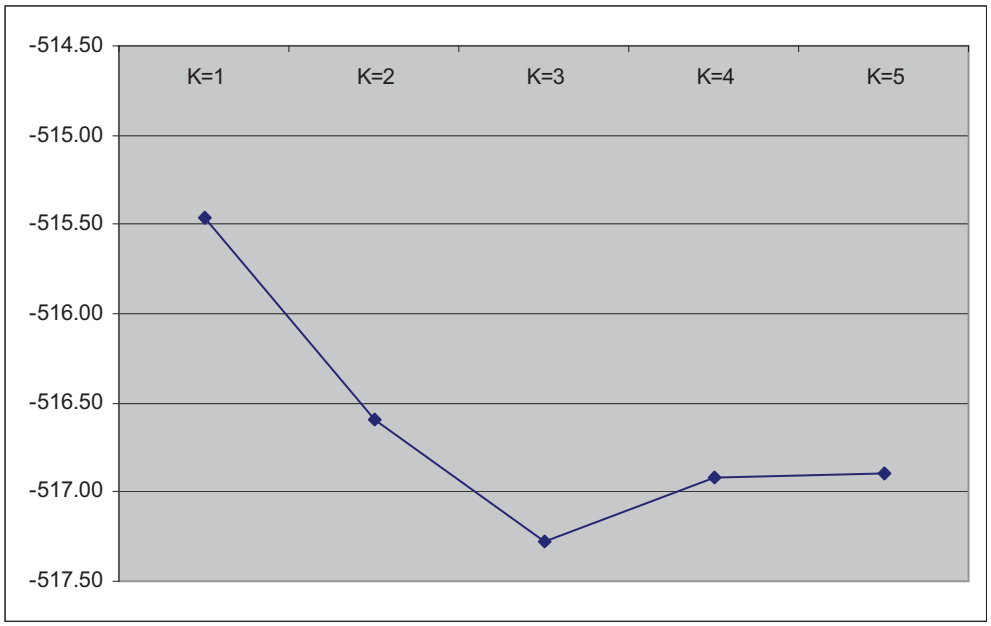

Figure A.11. Log-likelihood values for genetic structure analysis for genetic structure analysis of group 3, hierarchy 2; this result implies greatest support for $K = 1$.

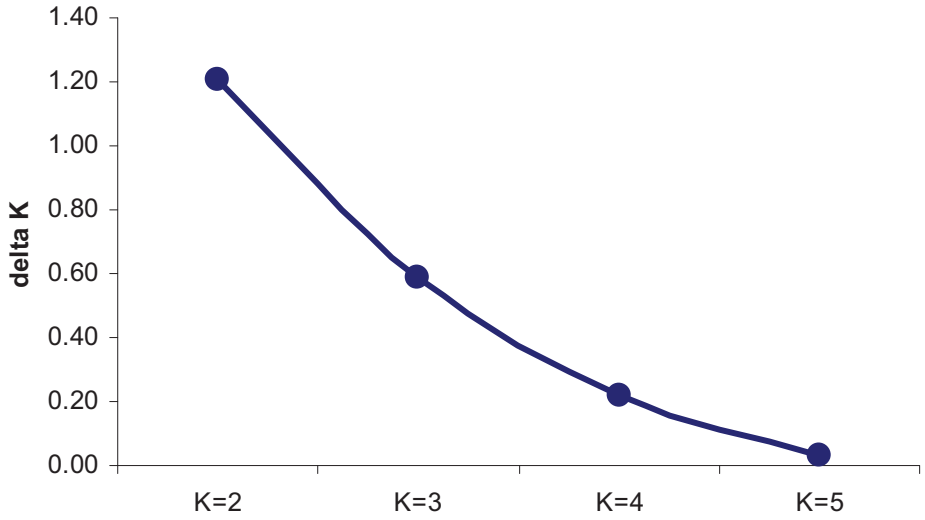

Figure A.12. Delta K values for genetic structure analysis for genetic structure analysis of group 3, hierarchy 2; this result implies greatest support for $K = 1$. Based on this result and log-likelihood values (Figure A.11), I concluded that further subdivision of this group was not supported.

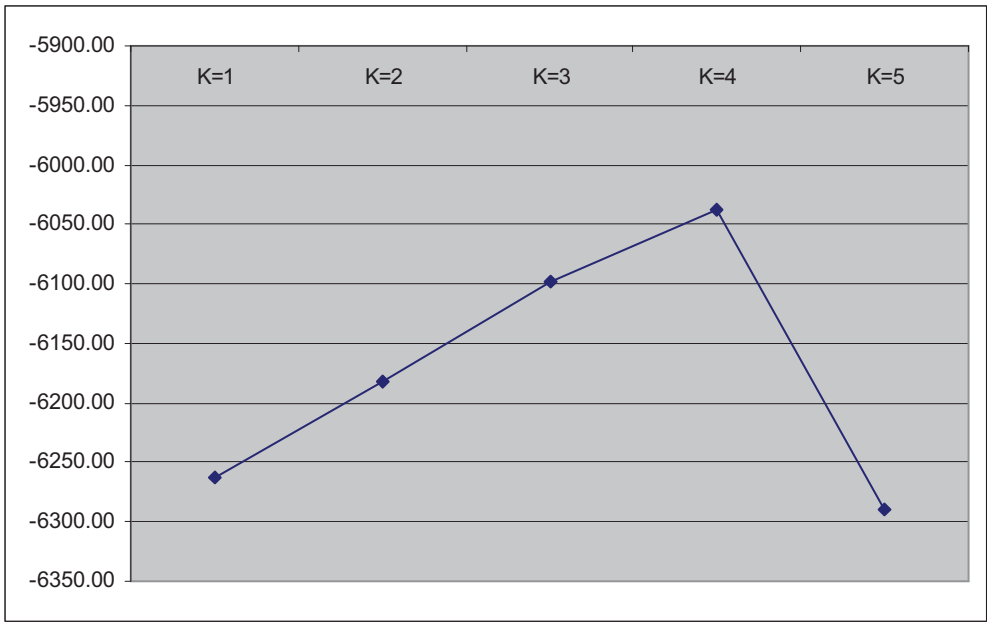

Figure A.13. Log-likelihood values for genetic structure analysis for genetic structure analysis of group 4, hierarchy 2; this result implies greatest support for $K = 4$. However, samples were highly admixed at $q = 0.70$ and lacked geographic cohesion.

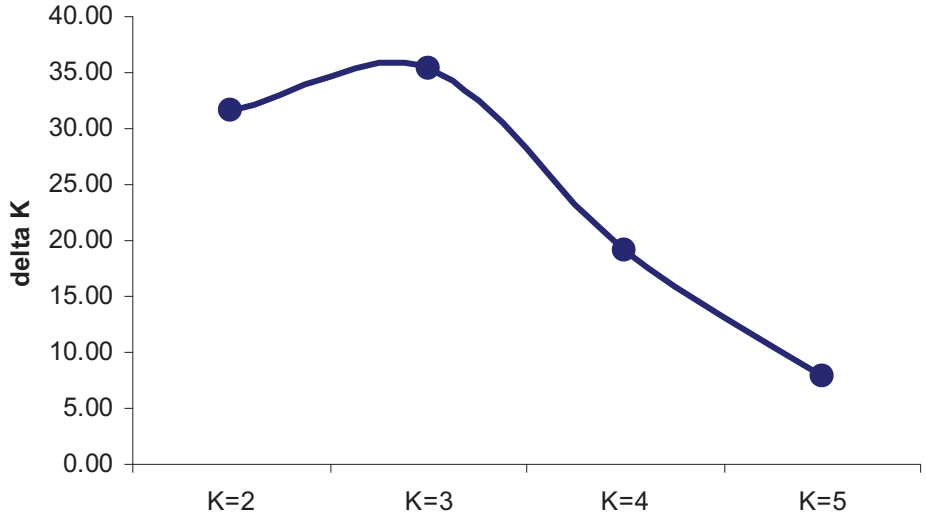

Figure A.14. Delta K values for genetic structure analysis for genetic structure analysis of group 4, hierarchy 2; this result implies greatest support for $K = 3$. Samples were highly admixed at $q = 0.70$ and lacked geographic cohesion; based on this and the results in Figure A.13, I concluded there was not support for further subdivision of this group.

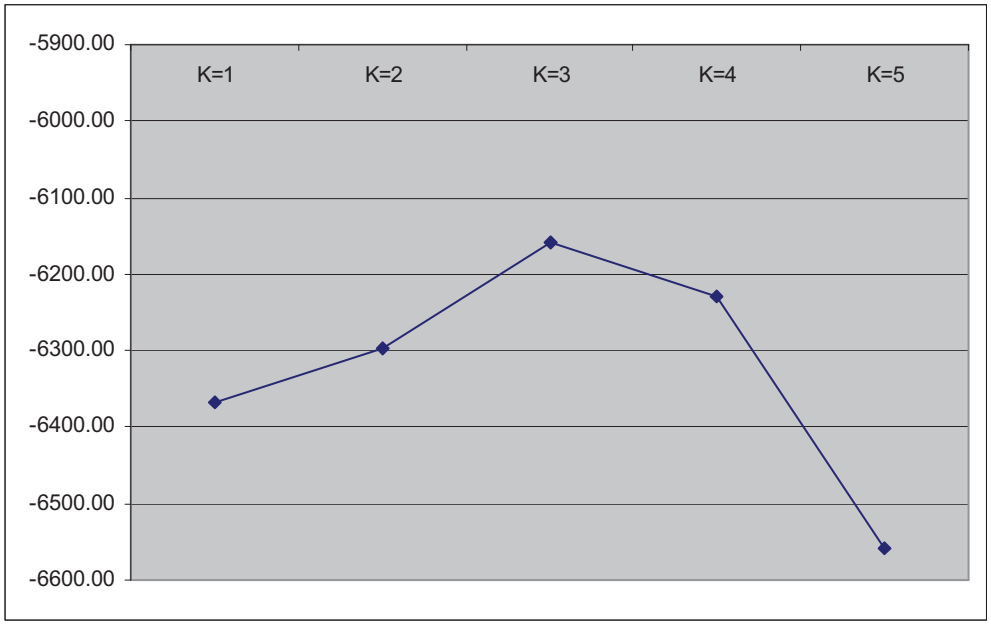

Figure A.15. Log-likelihood values for genetic structure analysis for genetic structure analysis of group 5, hierarchy 2; this result implies greatest support for $K = 3$.

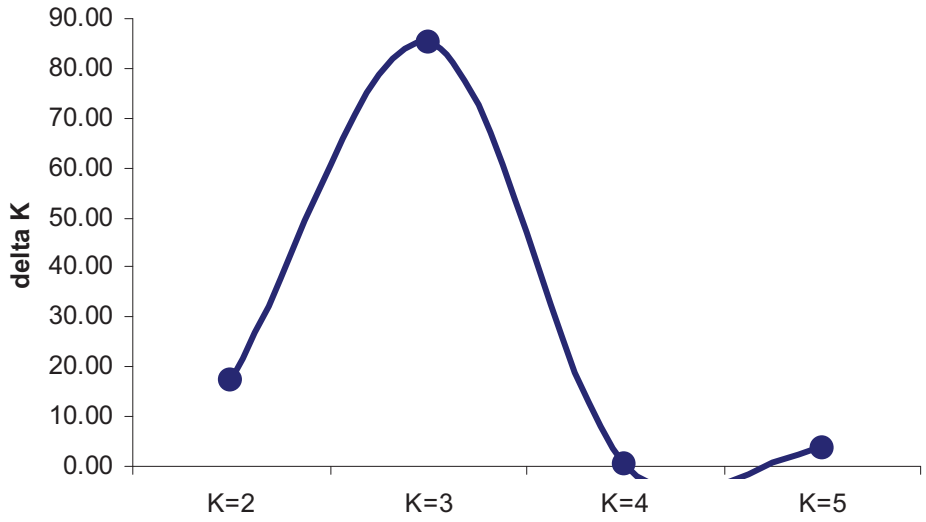

Figure A.16. Delta K values for genetic structure analysis for genetic structure analysis of group 5, hierarchy 2; this result implies greatest support for $K = 3$. This group was subsequently split into 3 groups.

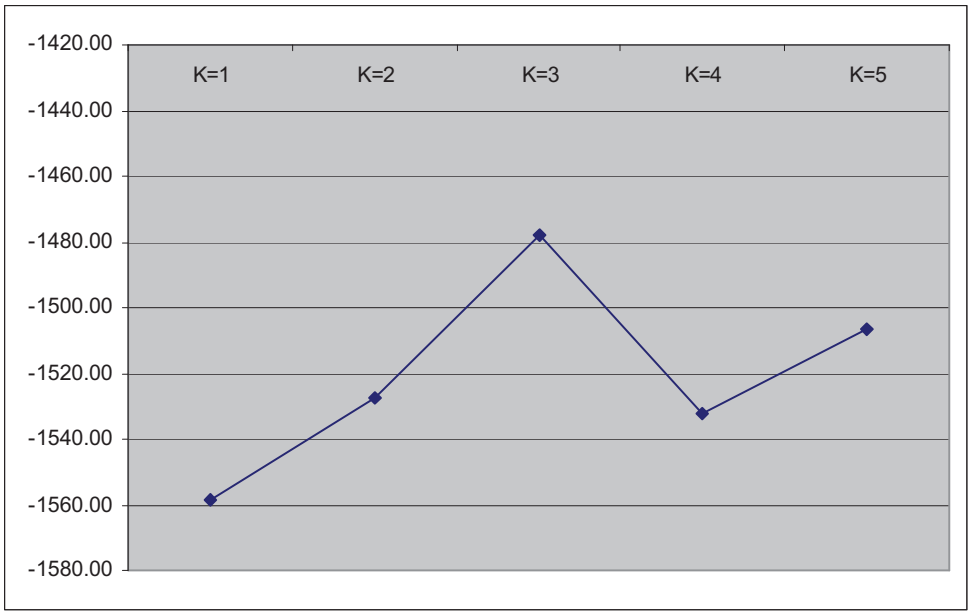

Figure A.17. Log-likelihood values for genetic structure analysis for genetic structure analysis of group 6, hierarchy 2; this result implies greatest support for $K = 3$.

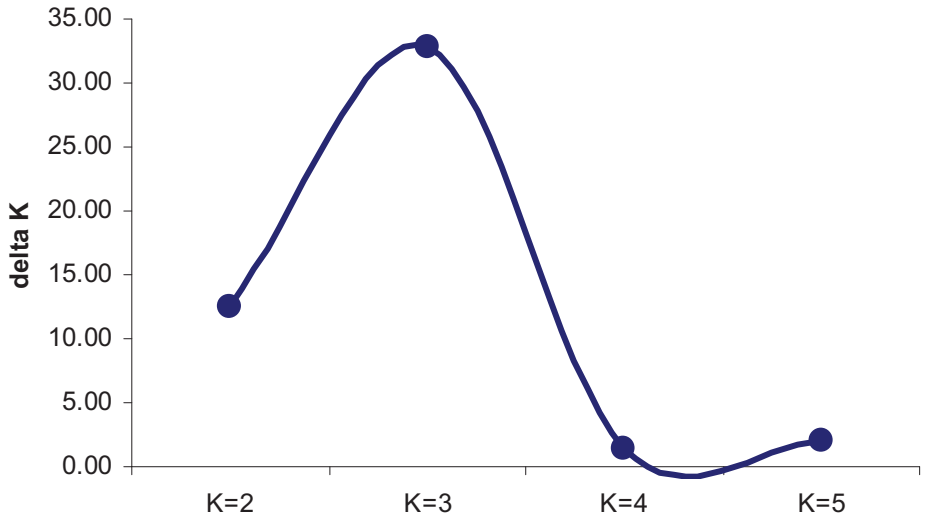

Figure A.18. Delta K values for genetic structure analysis for genetic structure analysis of group 6, hierarchy 2; this result implies greatest support for $K = 3$. This group was subsequently split into 3 groups.

APPENDIX B

DNA extraction, PCR and genotyping

I extracted whole genomic DNA from samples using the QIAGEN DNeasy tissue and blood kit (QIAGEN) with the following modifications to the manufacturer's protocol: 12 hour incubation at step 3 and 210 µl of ethanol at step 6. I next generated genetic data from 589 blood, hair and tissue samples using microsatellite and mitochondrial markers. For microsatellite data, we used 16 primer sets designed from the canid genome (Ostrander et al. 1993; Fredholm & Wintero 1995; Francisco et al.1996, Cullingham et al. 2007; Table 1) and previously used for swift fox (Kitchen *et al.* 2005; Cullingham *et al.* 2007). Microsatellite primers were divided into three multiplexes using the QIAGEN Multiplex Kit (QIAGEN). For tissue samples, PCR conditions (7µl final volume) for multiplex 1 were 1 µl Master Mix, 0.5 µl Q solution, 0.11µM each primer for locus CXX173, 0.11µM each primer for locus CXX377, 0.15µM each primer for locus FH2054, 0.23µM each primer for locus CXX20, 0.15µM each primer for loci CPH3 and CXX250, and 0.30µM each primer for locus CXX403. The PCR thermoprofile included initial denaturation for 15 m at 95ºC, 14 cycles of 30 s at 94ºC, 90 s at 55ºC (decreasing 0.3ºC per cycle to 50.8ºC) and 1 m at 72ºC, followed by 20 cycles of 30 s at 94ºC, 90 s at 51ºC and 1 m at 72ºC, then final elongation at 60ºC for 30 m. PCR conditions for multiplex 2 were 1 µl Master Mix, 0.5 µl Q solution, 0.07µM each primer for locus CXX263, 0.23µM each primer for locus VVE2-111, 0.36µM each primer for locus CXX2062, 0.71µM each primer for locus VVE5-33, and 0.29µM each primer for locus CXX109. PCR conditions for multiplex 3 were 1 µl Master Mix, 0.5 µl Q solution, 0.07µM each primer for locus VVE-M19, 0.10µM each primer for locus VVE3-131, 0.21µM each primer for locus VVE-M25, and 0.29µM each primer for locus VVE2-110. The PCR thermoprofile for multiplex 2 and 3 included initial denaturation for 15 m at 95ºC, 12 cycles of 30 s at 94ºC, 90 s at 53ºC (decreasing 0.3ºC per cycle to 49.4ºC) and 1 m at 72ºC, followed by 25 cycles of 30 s at 94ºC, 90 s at 47ºC and 1 m at 72ºC then final elongation at 60ºC for 30 m. I excluded marker VVE-M25 from further analysis due to inconsistent and irregular allele peak patterns, which rendered allele categorization highly subjective.

Quantity and quality of DNA in hair and blood samples is often lower than in tissue samples, thus generating a usable product can require modifications to the PCR profile. I did not change PCR conditions for blood or hair samples, but added 5 additional cycles at the 4th step of the thermoprofiles. PCR products were run on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Inc.) and allele size, number of alleles and individual genotypes were determined using GeneMapper 3.7 (Applied Biosystems, Inc.). I checked data for genotyping error using a randomly selected portion of our sample set. Because DNA quantity and quality is potentially higher in tissue samples than in hair and blood samples, leading to differences in error risk, we reanalyzed 11% of tissue samples (n = 48) and 17% of hair and blood samples (n = 23).

Genetic structure analysis

Using the admixture model and correlated allele frequencies in the program STRUCTURE v. 2.3 (Pritchard et al. 2000; Hubisz et al. 2009), I identified the optimal *K* value by first testing *K* = 1 - 8 with a burn-in period of 100,000 iterations and a total run time of 600,000 iterations for each of 15 replicates per candidate K value. I selected a *K* value using the delta *K* statistic described by Evanno et al. (2005). Log-likelihood and delta *K* graphs are shown below.

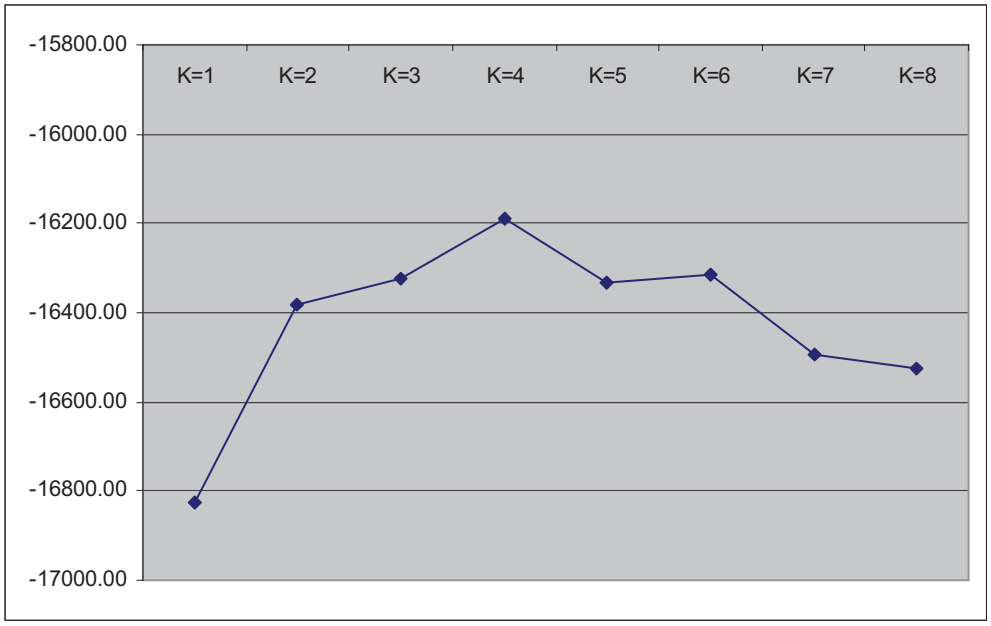

Figure A.1. Log-likelihood (ln) values for each potential genetic group (*K*) in the CO study area. These values imply the greatest support for 4 unique genetic groups.

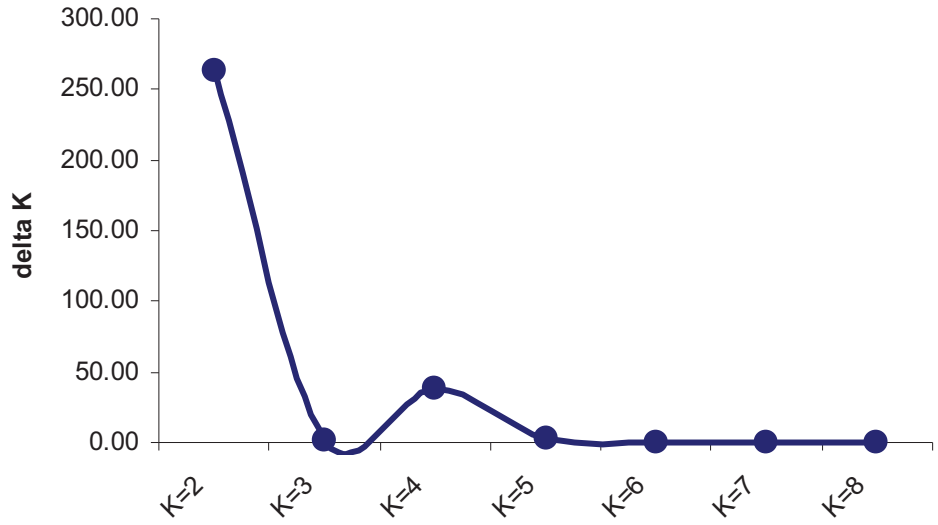

Figure A.2. Results of genetic group identification in the CO study area using the delta *K* statistic (Evanno et al. 2005). I observed support for unique genetic groupings at *K* = 2 and *K* = 4.

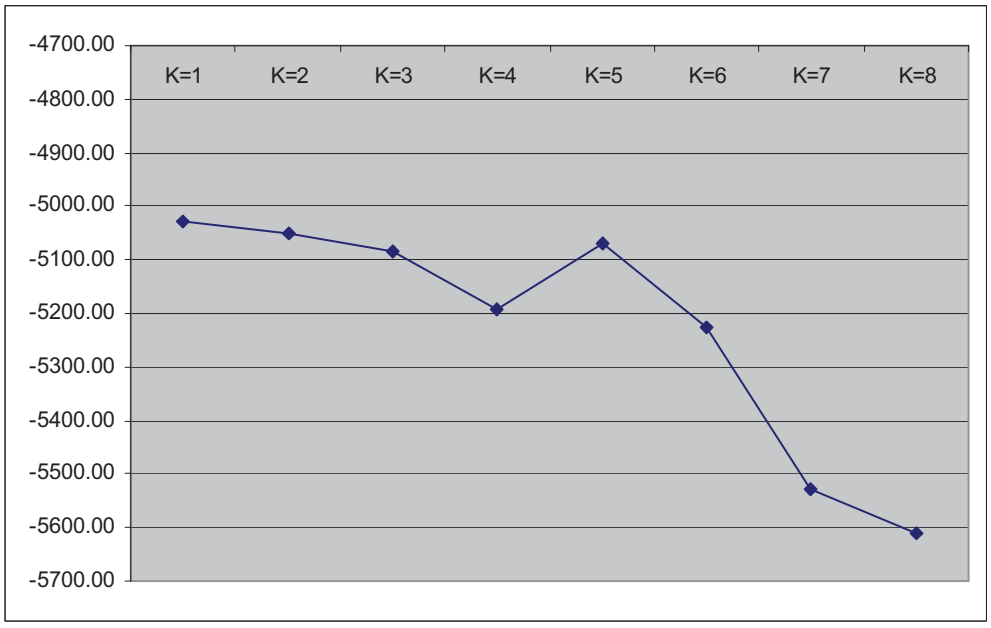

Figure A.3. Log-likelihood (ln) values for each potential genetic group (*K*) in the WY study area. These results are somewhat unclear, offering mixed support for both 1 and 5 unique genetic groups.

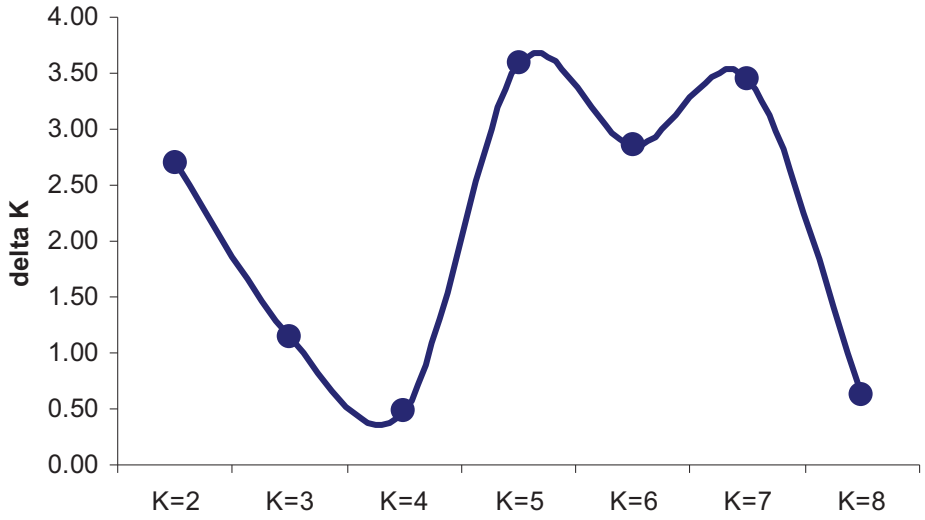

Figure A.4. Results of genetic group identification in the WY study area using the delta *K* statistic (Evanno et al. 2005). I observed support for unique genetic groupings at $K = 2$, $K = 5$ and $K = 7$. However, groupings at $K = 7$ lacked biological or ecological support when viewed geographically, and may have been driven by sampling closely related individuals, thus I did not conduct connectivity analysis at $K = 7$.

APPENDIX C

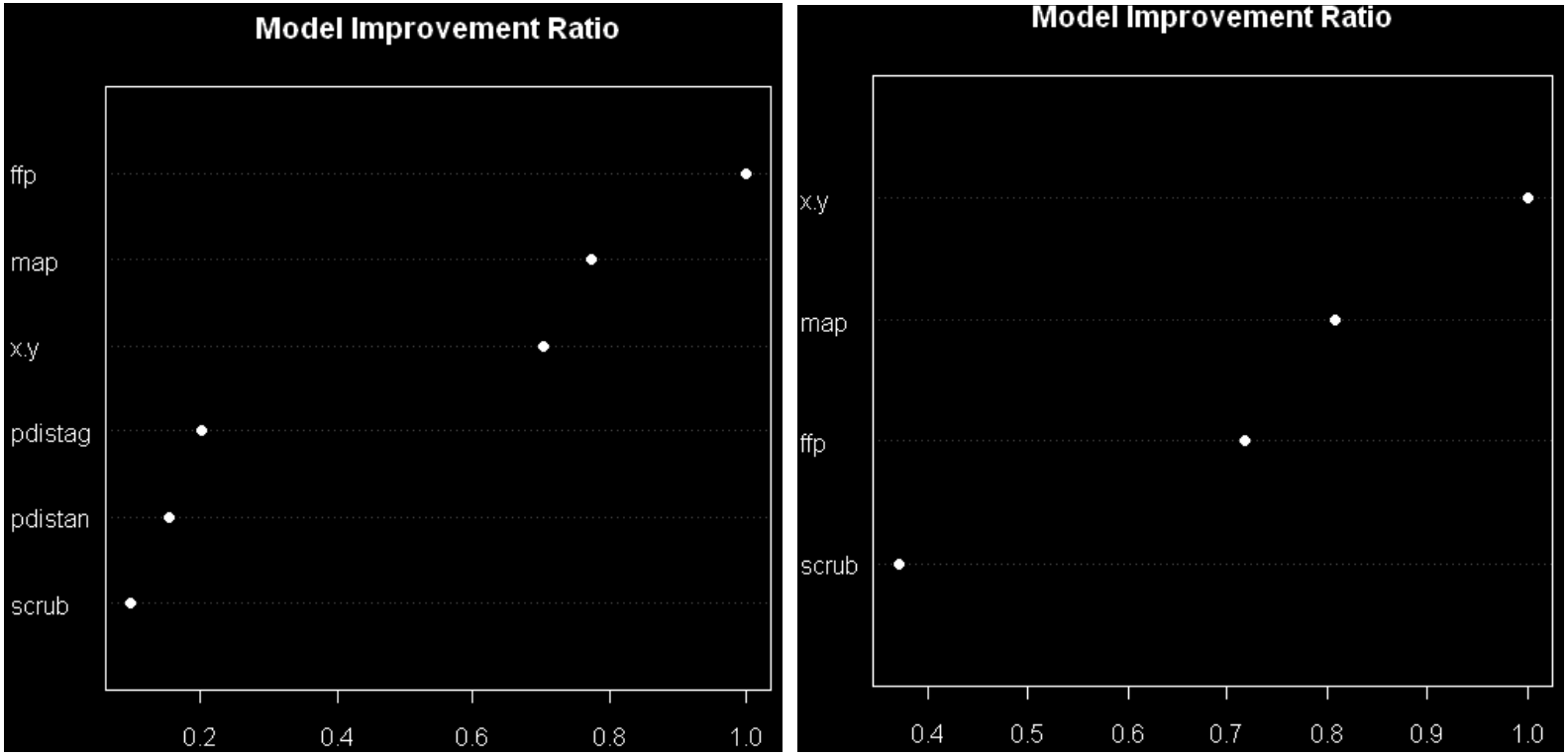

Figure B.1. Model improvement ratio graphs for categorical analysis of CO (left) and WY (right) study areas with 4 and 5 genetic groups, respectively.

APPENDIX D

DNA extraction, PCR and genotyping procedures

I extracted whole genomic DNA from samples using the QIAGEN DNeasy tissue and blood kit (QIAGEN) with the following modifications to the manufacturer's protocol: 12 hour incubation at step 3 and 210 µl of ethanol at step 6. I next generated genetic data from 589 blood, hair and tissue samples using microsatellite and mitochondrial markers. For microsatellite data, we used 16 primer sets designed from the canid genome (Ostrander et al. 1993; Fredholm & Wintero 1995; Francisco et al.1996, Cullingham et al. 2007; Table 1) and previously used for swift fox (Kitchen *et al.* 2005; Cullingham *et al.* 2007). Microsatellite primers were divided into three multiplexes using the QIAGEN Multiplex Kit (QIAGEN). For tissue samples, PCR conditions (7µl final volume) for multiplex 1 were 1 µl Master Mix, 0.5 µl Q solution, 0.11µM each primer for locus CXX173, 0.11µM each primer for locus CXX377, 0.15µM each primer for locus FH2054, 0.23µM each primer for locus CXX20, 0.15µM each primer for loci CPH3 and CXX250, and 0.30µM each primer for locus CXX403. The PCR thermoprofile included initial denaturation for 15 m at 95ºC, 14 cycles of 30 s at 94ºC, 90 s at 55ºC (decreasing 0.3ºC per cycle to 50.8ºC) and 1 m at 72ºC, followed by 20 cycles of 30 s at 94ºC, 90 s at 51ºC and 1 m at 72ºC, then final elongation at 60ºC for 30 m. PCR conditions for multiplex 2 were 1 µl Master Mix, 0.5 µl Q solution, 0.07µM each primer for locus CXX263, 0.23µM each primer for locus VVE2-111, 0.36µM each primer for locus CXX2062, 0.71µM each primer for locus VVE5-33, and 0.29µM each primer for locus CXX109. PCR conditions for multiplex 3 were 1 µl Master Mix, 0.5 µl Q solution, 0.07µM each primer for locus VVE-M19, 0.10µM each primer for locus VVE3-131, 0.21µM each primer for locus VVE-M25, and 0.29µM each primer for locus VVE2-110. The PCR thermoprofile for multiplex 2 and 3 included initial denaturation for 15 m at 95ºC, 12 cycles of 30 s at 94ºC, 90 s at 53ºC (decreasing 0.3ºC per cycle to 49.4ºC) and 1 m at 72ºC, followed by 25 cycles of 30 s at 94ºC, 90 s at 47ºC and 1 m at 72ºC then final elongation at 60ºC for 30 m. I excluded marker VVE-M25 from further analysis due to inconsistent and irregular allele peak patterns, which rendered allele categorization highly subjective.

Quantity and quality of DNA in hair and blood samples is often lower than in tissue samples, thus generating a usable product can require modifications to the PCR profile. I did not change PCR conditions for blood or hair samples, but added 5 additional cycles at the 4th step of the thermoprofiles. PCR products were run on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Inc.) and allele size, number of alleles and individual genotypes were determined using GeneMapper 3.7 (Applied Biosystems, Inc.). I checked data for genotyping error using a randomly selected portion of our sample set. Because DNA quantity and quality is potentially higher in tissue samples than in hair and blood samples, leading to differences in error risk, we reanalyzed 11% of tissue samples (n = 48) and 17% of hair and blood samples (n = 23).

Genetic structure analysis

Using the admixture model and correlated allele frequencies in the program STRUCTURE v. 2.3 (Pritchard et al. 2000; Hubisz et al. 2009), I identified the optimal *K* value by first testing *K* = 1 - 8 with a burn-in period of 100,000 iterations and a total run time of 600,000 iterations for each of 15 replicates per candidate K value. I selected a *K* value using the delta *K* statistic described by Evanno et al. (2005). Log-likelihood and delta *K* graphs are shown below.

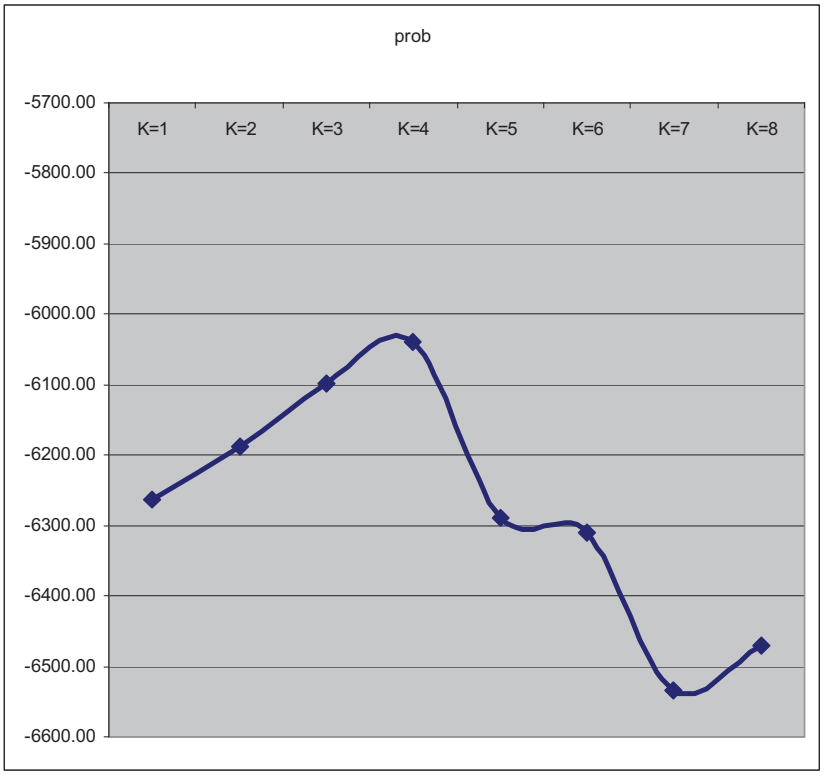

Figure C.1: Log-likelihood graph for genetic groupings (K) 1-8. Here, support was highest for four genetic groups; however, the presence of group 4 appeared to be driven by a small number (n = 2) of individual samples.

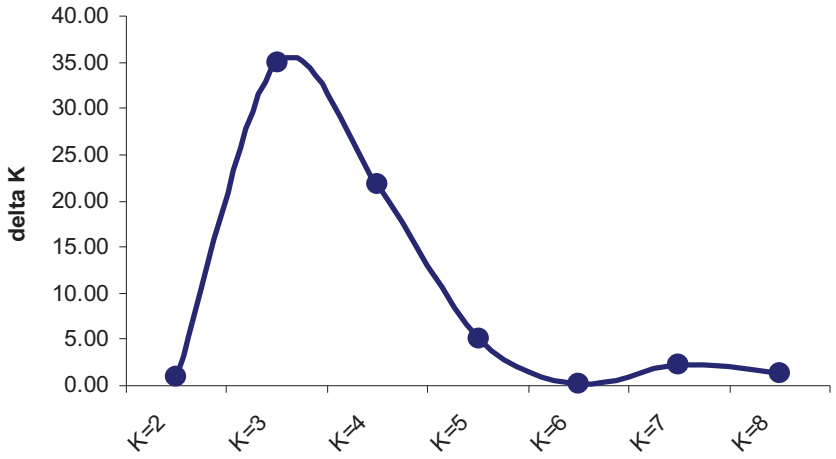

Figure C.2: Results of genetic group identification delta *K* statistic (Evanno et al. 2005). I observed support for three unique genetic groupings.