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Patterns and processes of spatial genetic structure in a mobile and continuously distributed species, the bobcat (*Lynx rufus*)

by

Dawn Marie Reding

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Ecology and Evolutionary Biology

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ABSTRACT

Population structure, the term used to describe the reproductive and demographic cohesiveness of con-specific individuals, is a fundamental concept in ecology and evolution. Despite the importance, patterns and processes of population structure are poorly understood, particularly for highly mobile species with broad distributions. For these organisms, the ability to disperse across large distances and occupy diverse habitats should promote gene flow and limit intraspecific genetic differentiation. However, significant genetic structure is often detected even in the absence of obvious movement barriers, indicating that the factors influencing population subdivision are not always clear. In this dissertation, I examined the patterns and processes of spatial genetic structure over three spatial scales in a mobile and abundant carnivore, the bobcat (*Lynx rufus*). At the local scale, I integrated telemetry, landscape, and genetic data to test whether habitat fragmentation influences movement behavior of bobcats, and whether these movement constraints translate into fine-scale genetic structuring of bobcats within an agricultural landscape. Despite observing an influence of habitat heterogeneity on bobcat movement behavior, whereby bobcats preferentially moved through forests surrounded by perennial habitat, I did not detect a signature of a landscape effect in the fine-scale genetic structure. However, much of Iowa’s landscape was predicted to pose a high level of resistance to bobcat movement, likely impeding connectivity with bobcat populations in neighboring states. At the regional scale, I characterized spatial genetic structure across 15 Midwestern states to delineate populations and identify landscape characteristics influencing recent expansions of bobcats into areas from which they had been extirpated. I identified 6 genetic populations separated by both physical (large expanses of row cropping and a major waterway) and cryptic (zones of sharp changes in habitat type)
boundaries. As predicted by the fine-scale analysis, results indicated that bobcats do not readily disperse through this agriculturally-modified landscape, and the newly-established populations in Iowa and northern Missouri are closely linked with bobcats to the southwest, but have had little genetic input from populations to the north and east. At the continental scale, I analyzed genetic data from across the entire United States to determine whether landscape features or other factors generate deeper, broad-scale genetic divergences that warrant recognition as distinct subspecies. The primary signature involved a longitudinal cline with a transition zone occurring along the Great Plains in the central U.S., distinguishing bobcats in the eastern part of the country from those in the western half. Results implicated historical processes as the primary cause of the observed continental-scale genetic patterns, and demographic evidence supported a scenario of post-glacial expansion from two disjunct Pleistocene refugia, which likely were isolated by the aridification of the Great Plains grasslands during interglacial periods. Although genetic patterns were loosely congruent with most subspecific designations, the data supported only two historically independent units: eastern and western bobcats. Collectively, the data indicate that despite the bobcat’s mobility and broad niche, population genetic structure is evident and characterized by complex combinations of clines, clusters, and isolation-by-distance arising from habitat heterogeneity, restricted dispersal, and historical processes.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Population structure, the term used to describe the reproductive and demographic cohesiveness of con-specific individuals, is a fundamental concept in ecology and evolution. Species generally are not homogenous units of randomly interacting individuals experiencing the same abiotic and biotic conditions, but rather develop an internal structure whereby some individuals are more connected than others (Nunney 2001). Such structure is created by both endogenous and exogenous forces (Templeton 2006). For example, an organism’s innate movement capabilities, habitat specificity, or behavioral traits (e.g., social structure, territoriality, or natal philopatry) can greatly limit interactions across its range. In addition, habitat fragmentation, environmental variability, and historical events such as glacial cycles or volcanic eruptions can disrupt connectivity. The structure that develops from such intrinsic and extrinsic factors can affect a wide range of ecologically and evolutionarily important processes, including population regulation, spread of disease, extinction and recolonization events, local adaptation, and even speciation.

Despite the importance to ecology, evolution, and conservation, patterns and processes of population structure are poorly understood for most species. Delimiting populations and identifying the factors responsible for intraspecific structuring are particularly challenging for species that are abundant, continuously distributed, highly mobile, and/or habitat generalists. For these organisms, including many medium and large-bodied mammals, it is often unclear what, if anything, limits movement and gene flow. However, significant genetic structure has increasingly been detected in mobile mammals (Sacks et al. 2004; McRae et al. 2005; Pilot et al. 2006), indicating that the factors
influencing population subdivision, such as distance and habitat heterogeneity, are not always clear.

Perhaps the most influential factor in generating genetic differentiation among wild populations is that of geographical distance. Isolation-by-distance refers to the commonly observed pattern whereby genetic similarity among populations or individuals decreases as the geographic distance between them increases. This pattern results from spatially limited dispersal; individuals living nearby to one another are more likely to interbreed than geographically distant ones (Wright 1943). For highly mobile species capable of dispersing large distances, however, extensive gene flow is expected to prevent the accumulation of such spatial genetic differentiation (Wayne & Koepfli 1996). Indeed, an absence of genetic structure across large areas has been reported in gray wolves (*Canis lupus*; Roy *et al.* 1994; Vilà *et al.* 1999), Canada lynx (*Lynx canadensis*; Schwartz *et al.* 2002), coyotes (*Canis latrans*; Lehman & Wayne 1991; Roy *et al.* 1994), and Arctic fox (*Vulpes lagopus*; Dalén *et al.* 2005).

Even for large, mobile mammals, however, dispersal is finite and may actually be quite limited relative to the extent of their broad geographic ranges. While maximum dispersal distances are often highlighted to support the expectation of genetic panmixia, most individuals generally move much shorter distances. Furthermore, it is usually not known whether these exceptionally long dispersal events result in gene flow (Cegelski *et al.* 2006). Thus, the potential for genetic panmixia due to high mobility has likely been overestimated for many species. In support of this idea, recent studies have uncovered isolation-by-distance patterns in species where such patterns had previously been missed: wolves (Geffen *et al.*
2004; Pilot et al. 2006); Canada lynx (Rueness et al. 2003); coyotes (Sacks et al. 2004); and Arctic fox (Geffen et al. 2007).

Although the isolation-by-distance model has been useful in explaining patterns of genetic differentiation in natural populations, one can often achieve greater insight by taking into consideration characteristics of the landscape in which these organisms exist (Manel et al. 2003; Scribner et al. 2005; Storfer et al. 2007). In this arena, research has primarily focused on the impacts of physical barriers to dispersal. Major topographic obstacles, such as water bodies, mountain ranges, and roadways, are frequently cited as factors limiting gene flow in terrestrial populations (Epps et al. 2005; Antolin et al. 2006). Such landscape effects on genetic structure are primarily expected in species that are habitat-specialists and demonstrate low mobility, since gaps between suitable patches would be difficult to cross. In contrast, landscape should have little effect on population subdivision of highly mobile habitat-generalists. Recent research, however, suggests this simplistic view may underestimate the importance of habitat barriers in limiting gene flow even in large mammals (Ernest et al. 2003; McRae et al. 2005; Riley et al. 2006).

In addition to geographical distance and topographic barriers, ecological factors may play a major role in promoting and maintaining genetic subdivision within continuously-distributed species. Recent studies suggest significant phenotypic and genetic differentiation can develop among widespread, mobile canids, ungulates, and felids found in different habitat types or climatic zones (Carmichael et al. 2001; Rueness et al. 2003; Geffen et al. 2004; Sacks et al. 2004; Stenseth et al. 2004; Pilot et al. 2006; Pease et al. 2009). Proposed mechanisms range from natal-habitat-biased dispersal, perhaps stemming from development of hunting/foraging strategies specific to local habitat and food types, to spatial differences in
seasonality and timing of mating and reproduction. Furthermore, paleoclimatic changes such as glaciation events can leave a lasting mark on current population genetic structure, though such an influence should be greater in species with boreal distributions (Runck & Cook 2005), or species that specialize on certain habitat types (i.e., forests or deserts) that may have been isolated during the Pleistocene (Wooding & Ward 1997; Rowe et al. 2006).

Overall, the potential for structure in the absence of obvious natural breaks makes a priori population characterization difficult for widely-dispersing habitat generalists. In these species, spatial genetic data may be most useful for elucidating population connectivity. The rapidly evolving field of landscape genetics, which combines methods and concepts from population genetics, landscape ecology, and spatial statistics, has emerged as a new research area to facilitate the study of gene flow in relation to spatial and environmental characteristics. Since Manel et al.’s (2003) seminal paper on the topic, an increasing number of studies on a range of taxa have been published (see Storfer et al. 2007 for review). However, the field is still in its infancy and much is yet to be explored. For most species, the factors mediating gene flow are likely complex, and may vary depending on the spatial scale under consideration (e.g., Trapnell & Hamrick 2004). Therefore, much insight could be gained by examining patterns at varying spatial scales, from a local population on up to the entire species distribution.

In this dissertation, I apply a landscape genetics approach to investigate the patterns and processes of population structure in a mobile habitat-generalist, the bobcat (*Lynx rufus*). Bobcats are one of the most common and broadly-distributed species in North America, ranging from southern Canada to central Mexico and from California to Maine (Anderson & Lovallo 2003). These medium-sized felids are consummate habitat-generalists, thriving in a
wide range of environments including deserts, coniferous and deciduous forests, subtropical wetlands, and prairie-woodland mixes. Though a strict carnivore, its diet is diverse and includes lagomorphs, rodents, deer, birds, reptiles, fish, and insects (Larivièreme & Walton 1997). Behaviorally, bobcats are solitary, territorial, and have a polygynous mating system (Anderson & Lovallo 2003). They are capable of dispersing long distances (>150 km) (Knick & Bailey 1986; Johnson et al. 2010; Gosselink et al. 2011), though dispersal distances vary greatly among individuals and study areas (Anderson & Lovallo 2003). Based on government surveys, Roberts & Crimmins (2010) estimate the total U.S. bobcat population at approximately 2-3 million individuals, and 38 states allow harvest of this valuable furbearer (Unites States Government 2010). The species was also apparently abundant in the past, as it is commonly found in Pleistocene fossil deposits (Graham & Lundelius 2010).

Collectively, these characteristics – habitat and prey generalist, solitary, long-distance disperser, currently and historically abundant and widespread – predict little genetic differentiation in this species. However, given the discovery of cryptic population subdivision in other mobile animals, it is unlikely bobcats represent a single panmictic unit, particularly over large spatial extents. A few population genetic studies have been published for this species. Analyses of genetic structure within the state of Michigan have consistently identified two bobcat populations, one on each peninsula, which are isolated by the Straits of Mackinac, a major waterway (Millions & Swanson 2006, 2007; Williams 2006). Riley et al. (2006) found that despite observed dispersal, bobcat populations on either side of the 10-lane Ventura Freeway in southern California were genetically differentiated from one another and from bobcats in northern California. However, Croteau et al. (2010) found the bobcat
population in southern Illinois to be genetically panmictic, as did Reid (2006) for bobcats sampled in southern Georgia and northern Florida. At a larger spatial scale, with samples from 14 states and 2 Canadian provinces, Croteau (2009) identified an isolation-by-distance effect and potentially evidence for distinct genetic groups, though the patchy sampling makes distinguishing between clines vs. clusters difficult. Together, the findings of these local and regional studies support the potential for population structuring in this species, but general patterns and processes are still unknown.

As a top predator and furbearer, the bobcat is a species of significant ecological and economic importance. Like many predators, bobcats are susceptible to habitat alteration (Crooks 2002). As a result of large-scale landscape changes coupled with unregulated harvest (Woolf & Hubert 1998), the species was largely eliminated from the agricultural Midwest and the heavily populated mid-Atlantic states by the mid-1900s (Deems & Pursley 1978). Although the bobcat now appears to be increasing in abundance and reclaiming parts of its former range (Roberts & Crimmins 2010), it is still protected in 9 states (United States Government 2010). For example, in the state of Iowa, bobcats were completely extirpated from this agriculturally-modified landscape and placed on the state endangered species list in 1977. Bobcats have since expanded into portions of the state, prompting its removal from the endangered and threatened list, but it is still absent from a large portion of the area and is recognized as a Species of Greatest Conservation Need (Zohrer 2006). In addition, the Mexican bobcat (L. r. escuinipae) was placed on the federal endangered species list in 1976. However, in 2005 the U.S. Fish and Wildlife Service (USFWS) announced their finding that a petition to delist the subspecies was warranted, citing that the available information indicates it may not constitute a separate subspecies (USFWS 2005). The entire species is
listed in Appendix II of the Convention on International Trade in Endangered Species (CITES) due to its similarity of appearance to other listed felids, requiring close monitoring of hunting and trading (Unites States Government 2010). Given these facts, knowledge of population structure would be highly beneficial for properly managing and conserving this species, for example by helping to resolve subspecific designations, identifying landscape features that facilitate and limit movements, identifying impediments to recolonization following local extirpation, predicting how disease could potentially spread across the landscape, evaluating the impact of landscape changes on population dynamics and persistence, and identifying the appropriate spatial scale at which management efforts should be directed.

**Research Objectives**

In this dissertation, I examined the patterns and processes of spatial genetic structure in bobcats across three spatial scales: local, regional, and continental. Specifically, my objectives were to:

1. Combine radio-telemetry, genetic, and geographic data to test whether habitat heterogeneity influences local movements of bobcats within a highly fragmented landscape, and whether these movement constraints translate into fine-scale genetic structuring of the population.

2. Delineate populations in the Midwest United States region and identify landscape characteristics influencing genetic structure and contemporary recolonization of areas from which bobcats had been extirpated.
3. Test whether ecological variation or other factors shape broad-scale patterns of genetic variation across the range of this species.

**Dissertation Organization**

This dissertation is composed of five chapters, including a general introduction (Chapter 1), three manuscripts written for submission to peer-reviewed scientific journals (Chapters 2-4), and a general conclusion (Chapter 5).

Chapter 2 focuses on the bobcat population within Iowa. Since European settlement, the landscape of Iowa has undergone significant alterations; once comprised of a diverse array of land cover types including prairies, forests, and wetlands, Iowa is now dominated by annual row crops (Wagner & Gobster 2007). These changes have contributed to the formation of a highly fragmented landscape from the perspective of a bobcat. Research suggests that, even within a population, habitat fragmentation can have a substantial effect on landscape connectivity and the dispersal of animals, reducing gene flow and generating fine-scale patterns of genetic differentiation (Coulon *et al.* 2004). In this chapter, I combine telemetry, genetic, and landscape data to test whether habitat fragmentation influences movement behavior and generates fine-scale genetic structuring of bobcats within an agricultural landscape.

Chapter 3 focuses on bobcats within the Midwest Cornbelt. Here, the species had been largely eliminated from most areas (Deems & Pursley 1978) as a result of habitat loss and unregulated harvest (Rolley 1987; Woolf & Hubert 1998). While the bobcat is rebounding in the fringes of the region (Linde 2010), it remains largely absent from the core of the area. It is unclear whether this distribution gap, or other potential dispersal barriers,
may restrict gene flow across the region and contribute to population genetic structure. Using spatial genetic data, I delineate populations in the region and identify potential landscape characteristics influencing recent expansions of bobcats into areas from which they had been extirpated.

Chapter 4 focuses on bobcats across the entire United States, the majority of the bobcat’s range. At this continental scale, ecological and environmental variation may play a more prominent role in shaping broad-scale patterns of genetic variation. Bobcats can be found in a diverse range of habitat types, and phenotypic variation loosely parallels environmental variation (Read 1981; Wiggington & Dobson 1999). Many animals preferentially disperse to habitat resembling their natal habitat (Davis & Stamps 2004), which may generate habitat-specific genetic subdivision in bobcats, as has been observed in some other carnivore species (Geffen et al. 2004; Sacks et al. 2008). For this chapter, my goal was to quantitatively assess the spatial genetic patterns in this species to test whether environmental heterogeneity or other factors generate deeper, broad-scale genetic divergences that warrant recognition as distinct subspecies.

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CHAPTER 2. THE INFLUENCE OF LANDSCAPE RESISTANCE: DISCORDANCE BETWEEN MOVEMENT BEHAVIOR AND FINE-SCALE PATTERNS OF GENE FLOW IN BOBCATS (Lynx rufus)

A paper to be submitted to Landscape Ecology

Dawn M. Reding

Abstract

Environmental heterogeneity can constrain the movement of individuals and consequently genes across a landscape, influencing demographic and genetic processes. Whereas many studies have used telemetry data to gain insight into how animals respond to and utilize complex landscapes, rarely has this information been applied to generating predictions about patterns of dispersal and gene flow. In this study, my aim was to link information on landscape structure, movement behavior of individuals, and patterns of genetic variation to gain a mechanistic understanding of how spatial heterogeneity may influence movement and gene flow of bobcats in the fragmented landscape of Iowa, USA. I used resource selection functions developed from empirical movement paths of 23 animals to parameterize landscape resistance surfaces, and applied least-cost path analysis to generate measures of effective geographic distance between collection locations of 625 bobcats. Using Mantel and partial Mantel tests, I evaluated whether landscape connectivity, as predicted by the movement models, influences gene flow in bobcats and leads to a departure from a pure isolation-by-distance pattern. I found that bobcats showed a strong preference for forest over any other land cover type, and that incorporating information on habitat composition both along the path and at a broader spatial scale around the path provided the
best model of bobcat movement. Measures of effective geographic distance based on the landscape resistance models were significantly correlated with genetic distance, but not once the effects of Euclidean distance were accounted for. Thus, despite the impact of habitat heterogeneity on bobcat movement behavior, I was unable to detect a signature of a landscape effect in fine-scale genetic structure. A number of factors, including the recent age of Iowa’s bobcat population and the scale of the analysis, may have contributed to this discordance between movement behavior and gene flow. However, much of Iowa’s landscape is predicted to pose a high level of resistance to bobcat movement, which may impede connectivity with bobcat populations in neighboring states.

**Introduction**

Dispersal, be it the movement of individuals or genes, is one of the key forces shaping the ecology and evolution of natural populations and can greatly influence, for example, the spread of disease, local adaptation, and extinction/recolonization events. Early theoretical models dealing with dispersal in population ecology (*e.g.*, correlated random walks – Karieva & Shigesada 1983; diffusion models – Turchin 1998) and population genetics (*e.g.*, isolation-by-distance – Wright 1943; stepping stone model – Kimura & Weiss 1964) made a simplifying assumption that the underlying environment is spatially homogenous. The landscapes wild populations actually inhabit, however, depart markedly from the idealized world, and are better viewed as complex mosaics of patches varying in type, size, shape, and arrangement (*Turner et al.* 2001), or perhaps even more realistically as continuous multidimensional gradients (*McGarigal & Cushman* 2005). This environmental heterogeneity, whether natural or anthropogenic, can impose physical and behavioral
constraints on the movement of individuals/genes across a landscape, influencing demographic and genetic processes. Landscape connectivity, the term used to describe the interaction between landscape structure (i.e., composition and configuration) and the movement response of organisms (Taylor et al. 1993; Tischendorf & Fahrig 2000), has been a focus in population ecology for more than two decades (Fahrig & Nuttle 2005). Only recently, however, has landscape connectivity been explicitly and formally incorporated into the population genetics discipline (Manel et al. 2003).

Landscape genetics, the emerging research area that integrates concepts and tools from landscape ecology, spatial statistics, and population genetics, provides a framework for quantitatively assessing the relationship between gene flow and landscape structure (Manel et al. 2003; Balkenhol et al. 2009). One of the most common approaches for evaluating the importance of organism-environment interaction in regards to gene flow has been the correlation of genetic distances between individuals/populations with that of Euclidean (assumes a spatially homogenous landscape) or effective (takes into account the influence of a heterogeneous landscape) geographic distances (Spear et al. 2010; Storfer et al. 2010). The approach is based on isolation-by-distance (IBD) theory (Wright 1943), which predicts that genetic similarity among individuals decreases as the geographic distance between them increases. This pattern results from spatially limited dispersal; individuals living nearby to one another are more likely to interbreed than geographically distant ones. In heterogeneous landscapes, however, straight-line geographical distances may not adequately reflect the true pattern of movement, leading to a break-down in the predicted correlation between genetic and geographic distance. Recent studies have demonstrated that measures of geographic distance which reflect landscape connectivity often explain a greater proportion of the

With the proliferation of GIS data and tools, effective geographic distances are increasingly being quantified via resistance grids (a.k.a. cost or friction grids), spatially explicit representations of the landscape where each pixel is associated with a cost reflecting the degree to which the area facilitates or impedes movement of the study organism (Sork & Waits 2010; Spear et al. 2010). These resistance grids may represent a singular aspect of the landscape, such as land cover classification (Stevens et al. 2006), slope (Epps et al. 2007), or snow cover (Schwartz et al. 2009), or may combine several landscape elements to form a multivariate representation of friction to movement or gene flow (Cushman et al. 2006; Shirk et al. 2010). Given the resistance surfaces as models of landscape connectivity, researchers can then generate effective measures of geographical distance by proceeding to least-cost path analysis (Adriaensen et al. 2003), which calculates the pathway between two locations resulting in the least accumulated cost, or to circuit-theory analysis (McRae 2006), which calculates a metric analogous to the amount of electrical current that could flow between two locations. Resistance surfaces are also applied in corridor design (Beier et al. 2009), invasive species management (Gonzales & Gergel 2007), disease modeling (Ellis et al. 2010) and other non-genetics purposes. Regardless of the ultimate application, one of the most challenging but vital steps in the process is the assignment of resistance values to different landscape components (Spear et al. 2010).
For most studies to date, the assignment of resistance values has been largely subjective, often relying on expert opinion (Spear et al. 2010; Beier et al. 2008). The way in which species perceive and interact with their environment, however, may not correspond to our assumptions, and the use of empirical movement data offers a more robust approach than subjective methods. Indeed, Pullinger & Johnson (2010) found that expert-based friction models performed poorly relative to empirically-based models in predicting long-distance animal movements. Few studies, however, have used actual movement data to parameterize friction grids (but see Richard & Armstrong 2010; Pullinger & Johnson 2010; Chetkiewicz & Boyce 2009; Cushman & Lewis 2010; Stevens et al. 2006). The dependency on expert opinion is a major weakness of landscape-resistance modeling efforts to date.

In this study, I chose to parameterize landscape resistance models using the resource selection function (RSF), a well-developed and utilized concept in the ecological literature that models resource selection by comparing landscape variables at sites used by an animal to those that were unused or potentially available (Manly et al. 2002). RSFs are commonly used to evaluate habitat suitability or home range selection, focusing on point locations as the unit of analysis. Recently, however, the RSF approach has been extended to analyze animal movements either at the level of steps (step selection function: SSF; Fortin et al. 2005; Coulon et al. 2008) or entire paths (path selection function: PSF; Cushman et al. 2010). For the large part, the wealth of data derived from the RSF and its derivatives has yet to be incorporated into landscape genetics.

In this study, my aim was to link information on landscape structure, movement behavior of individuals, and patterns of genetic variation to gain a mechanistic understanding of how spatial heterogeneity may influence movement and ultimately gene flow of bobcats.
(Lynx rufus) in the fragmented landscape of Iowa, USA. Specifically, my objectives were to: 1) analyze empirical movement paths collected via radio telemetry using the path selection function approach to test whether landscape structure at different spatial extents influences movements of bobcats in Iowa; 2) use the path selection models to parameterize landscape resistance surfaces; 3) use least-cost path analysis to generate measures of effective geographic distance between collection locations of individual bobcats based on the landscape resistance models, as well as a habitat suitability model and an isolation-by-distance model; 4) use an individual-based genetics approach (i.e., where individual genotypes rather than populations are the unit of analysis) to test whether landscape connectivity influences gene flow in bobcats and leads to a departure from a pure isolation-by-distance pattern.

**Methods**

**Study system**

The landscape of Iowa has undergone significant alterations since European settlement. Once comprised of a diverse array of land cover types including prairies, forests, and wetlands, the state is now dominated by annual row crop agriculture (Wagner & Gobster 2007) (Fig. 1a). This large scale landscape change, coupled with unregulated harvest, resulted in the extirpation of bobcats from Iowa for much of the 20th century (Deems & Pursely 1978). Sightings of bobcats increased by the early 1990s, and during the past two decades the cats have naturally recolonized a portion of the state. By modeling habitat suitability with relative abundance and presence-absence data, Linde (2010) found that forest-grassland associations figured prominently in predicting favorable bobcat habitat and
that intensely cultivated areas, primarily in the northern two-thirds of the state, are essentially unsuitable for bobcats. Investigating the fragmented landscape from the perspective of a bobcat, Tucker et al. (2008) found that home range selection in Iowa bobcats was closely tied to patches of forest associated with other perennial habitat like grassland, whereas row crops were generally avoided. Together, these findings predict that individuals may be less prone to disperse across expanses of agricultural lands or other unpreferred/unsuitable habitat, which would lead to a departure from a pure isolation-by-distance pattern of genetic variation.

Movement data

To improve our understanding of bobcat movement behavior, I followed 23 bobcats (11 females, 12 males) fitted with very high frequency (VHF) radio collars between February 2008 and March 2009. These animals were located in south-central Iowa (Fig. 1b), where bobcats are most prevalent (Linde 2010). See Tucker et al. (2008) for details of animal-handling and telemetry procedures. All animal handling followed approved Iowa State University Institutional Animal Care and Use Committee protocol #5-03-5447-W under Iowa DNR Scientific Permit SC 126.

I monitored individual movements for a total of 144 sessions (range = 1-10 sessions per individual), where each session represents a ~6-hour sampling period with locations obtained approximately every 20 minutes. Locations consisted of visuals (i.e., animal was spotted by an observer and the actual location was recorded with GPS after the animal left; \( n = 57 \)), biangulations (2 bearings; \( n = 8 \)), and triangulated locations (\( \geq 3 \) bearings; \( n = 2632 \)).
From the triangulated locations, I calculated the distribution of the error ellipse polygons and removed locations with errors greater than 3.0 times the interquartile range above the 75\% quantile \( (i.e., \text{“extreme outliers”}; \) Devore & Peck 1986). I also removed biangulated locations, since there is no way to evaluate location error. I calculated the time lapse between successive locations in this reduced data set. In 4 instances, the time lapse exceeded 60 minutes and thus each of these 4 paths were divided into 2, resulting in a total of 148 paths.

Case-control design

I used a case-control design common in RSF analysis, but instead of individual locations (Compton et al. 2002; Boyce et al. 2003) or steps (Fortin et al. 2005; Coulon et al. 2008) as the unit of analysis, I focused on entire paths (Bruggeman et al. 2007; Cushman & Lewis 2010). The data set consisted of 148 cases, each consisting of an observed path paired with 39 random paths (Fig. 1c). I generated the random paths using ArcGIS (ESRI) and Geospatial Modelling Environment (GME; Spatial Ecology, LLC) to shift the X and Y coordinates of each of the actual paths by a random value between -5000 to 5000 m and to simultaneously rotate it by a random value between 0 and 360°. The 5 km radius was based on the radius of a circle with an area equivalent to the mean annual male home range in this area (Gosselink et al. 2011). The choice of 39 random paths was based on a compromise between adequately sampling the available landscape area while limiting overlap among the paths.
Landscape parameters

I downloaded the 2006 National Land Cover Data (Xian et al. 2009) at 30 m resolution for the region within a 10 km buffer around the entire state of Iowa and northern Missouri (i.e., north of the Missouri River). I collapsed the original 15 land cover classes into 6 habitat classes that I hypothesized would be functionally relevant to bobcats: 1) Water (open water); 2) Development (developed areas of low, medium, and high-intensity; barren); 3) Forest (deciduous, evergreen, and mixed forest; woody wetlands); 4) Grassland (shrub/scrub; grassland/herbaceous, pasture/hay, emergent herbaceous wetlands); 5) Cropland (cultivated crops); 6) Open space development (primarily road ditches). I applied a 100 m buffer around each path, reflecting the approximate radius of a circle with an area equivalent to the mean error ellipse associated with the telemetry data (see Results). I used GME to calculate the proportion of each land cover type within each of the buffers. To test whether movement paths of bobcats are also influenced by the landscape context of the habitat, I created an annular ring (hereafter “annulus”) extending 650 m around the existing 100 m buffer and again calculated proportions of land cover types. The 750 m total buffer radius is equivalent to 3× the median step length.

Conditional logistic regression

To compare the landscape composition of observed and random paths, I applied fixed-effects conditional logistic regression using PROC LOGISTIC in SAS 9.2 (SAS Institute, Inc.). This study consisted of a 1:N matched case-control design, with one observed path matched to 39 available paths. Conditional logistic regression takes this
clustering in the data into account, and results of the model are conditional upon each stratum. Thus, no intercept is estimated.

For each of the 12 individual land cover variables, I calculated univariate descriptive statistics (mean, standard deviation, range). I also conducted univariate conditional logistic regression analyses and estimated coefficients ($\beta$) to contrast the observed and random paths. I then tested three different multivariate models: the “Path” model was constructed with each of the land cover proportions within the 100 m buffers as predictor variables; the “Annulus” model was constructed with land cover proportions within the 750 m annulus as predictor variables; the “Combined” model was constructed by combining both the 100 m buffer and 750 m annulus variables. In all multivariate models, Grassland was used as the reference class, and I considered only the main effects (i.e., no interactions). I screened each model for multicollinearity between predictor variables by calculating variance inflation factors (VIFs), with the intent to disregard models containing predictors with VIFs >10 (Belsley et al. 1980). I ranked the models by AIC values (Burnham & Anderson 1998) and obtained parameter estimates for each. Since the sampling design was unbalanced, with a varying number of paths per individual, and since response to landscape could vary among individuals (Gillies et al. 2006), I also performed a jackknife procedure by repeating the analysis 23 times, removing a different individual bobcat each time, to verify that no particular individual biased the coefficients.

Using the model coefficients, I estimated a Path Selection Function (PSF):

$$w(x) = \frac{\exp(\beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_p X_p)}{1 + \exp(\beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_p X_p)}$$
Here, $\beta_1$ to $\beta_p$ are the coefficients estimated by conditional logistic regression and associated with the variables $X_1$ and $X_p$, respectively. The PSF score, $w(x)$, ranges from 0 to 1 and reflects the probability of a path being selected by an animal.

**Resistance Surfaces**

I reclassified the land cover layer such that each 30 m × 30 m cell of a given habitat class had a value of 1 and all other classes a 0. Thus, I created 6 new layers, one for each of the land cover types. For each of these layers, I then used the ArcGIS Spatial Analyst Neighborhood tool to use a moving window approach to calculate the proportion of the given habitat present within a 100 m radius of each focal cell and to create new raster layers reflecting these proportions. I repeated these calculations but using for the window an annulus with an inner radius of 100 m and outer radius of 750 m.

I used the path selection function scores, $w(x)$, from the Path, Annulus, and Combined models to generate three landscape resistance surfaces using the formula: cell cost $= 100 \times (1 - w(x))$. Thus, cell values could potentially range from 0 to 100, and cells with the highest predicted probability of use resulted in the lowest costs of movement. I used ArcGIS Spatial Analyst Raster Calculator to apply the formula and create each resistance layer. Given the large spatial extent of the study area, I resampled the rasters to 90 m resolution using bilinear interpolation to reduce the number of cells from ~380 million to ~40 million. The resampling was necessary to reduce computation times in the subsequent least-cost path analysis.

In addition to the three landscape resistance models, I created a resistance surface based on a habitat suitability model. I used the 5-variable model developed at the HUC_12
watershed level by Linde (2010). In this model, each watershed was given a value between 0 and 1 indicating the probability of bobcat presence \( (Pr) \). In this model, cell cost = \( 100 \times (1 – Pr) \), such that watersheds with high predicted probability of bobcat presence resulted in the lowest costs of movement. Again, the final raster had a 90 m cell size.

Finally, I created a 90 m resolution isolation-by-distance “IBD” model where each cell was given a cost of 1. This model assumes a homogenous landscape across the entire region, and was viewed as the null model.

**Genetics**

I took tissue samples from 625 geo-referenced individuals (Fig. 2) that were either live-captured animals \( (n = 159) \) or carcasses from road-killed, legally harvested, or incidentally trapped animals \( (n = 466) \) collected from within the state of Iowa in 2001-2008. I extracted DNA using DNeasy (Qiagen) or IDPure (IDLabs) purification kits following manufacturer protocols. I used the M13-tailed primer method (Boutin-Ganache et al. 2001) to individually amplify 22 microsatellite markers (Table 1) that were developed from the bobcat, Canada lynx \( (Lynx canadensis) \), or domestic cat \( (Felis catus) \). Total PCR volume was 10 µl, including 1× PCR buffer with 2 mM MgSO\(_4\) (IDLabs), 0.2 mM dNTPs, 0.3 µM fluorescently labeled M13 primer, 0.3 µM reverse primer, 0.02 µM M13-tailed forward primer, 0.4 U IDPROOF DNA Polymerase (IDLabs), and 10-20 ng of template DNA. The PCR profile was 95 °C/5 min, \( (95 °C/20 s, 47-50 °C/20 s, 72 °C/30 s) \times 30-35 \) cycles, 72 °C/20 min. I combined PCR products from each sample into gel sets (each consisting of three to five loci), analyzed them on an ABI 377 sequencer at Iowa State University’s DNA Facility, and scored alleles using the software Genotyper 3.7 (ABI).
I used program Microchecker (van Oosterhout et al. 2004) to evaluate whether any loci showed evidence of scoring errors such as null alleles, large allele dropout, and stuttering (1000 iterations, Bonferroni-adjusted confidence interval). I used program GenePop 4.0 (Rousset 2008) to estimate number of alleles, observed and expected heterozygosities, and Weir and Cockerham’s (1984) \( F_{IS} \) for each locus. In addition, I tested for deviation from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium (LE) for each locus (10,000 dememorization, 500 batches, 5000 iterations), applying a sequential Bonferroni adjustment (nominal \( \alpha = 0.05 \)) to account for multiple tests (Rice 1989). To determine whether significant population subdivision exists across Iowa, I: 1) used GenePop to perform a global test (i.e., across loci) of heterozygote deficiency and 2) used BAPS 5.2 (Corander et al. 2008) software to perform spatial clustering of individuals, with five runs each for the maximum number of populations \( K \) set to \( K = 2 - 4 \). I calculated Rousset’s \( a_r \) (Rousset 2000) as a measure of individual-level genetic distance using program Spagedi (Hardy & Vekemans 2002).

*Least-cost path analysis*

I used the landscape connectivity cost-distance tool in the Landscape Genetics ArcToolbox (Etherington 2011) to calculate matrices of LCP cost-distances between each of the 625 samples for each of the 5 models (Path, Annulus, Combined, Suitability, IBD). These cost-distance values reflect the minimum possible combination of the distance that would be traveled and the cost of the landscape that would be traversed between two points. To reduce processing time, I first clipped the rasters to a more limited spatial extent, but for which preliminary analyses indicated would still contain the least-cost paths.
Mantel tests

To evaluate the hypothesized resistance models, I used Mantel tests (Mantel 1967) in the R software package Vegan (Oksanen et al. 2010) to calculate the correlation between genetic and geographic distance. I used a permutation procedure (999 replicates) to evaluate the statistical significance of the relationships. I considered the most supported model to be the one with the highest significant correlation. To determine whether the landscape resistance models retained a significant relationship with genetic distance after partialling out the effects of Euclidean distance, I performed partial Mantel tests (999 replicates) in Vegan.

Results

Following the removal of 26 extreme outliers (error ellipse area > 215,646 m$^2$) and 8 biangulated locations, the telemetry data set consisted of $n = 2663$ points. The mean position error of the remaining triangulated locations was 112 m. The 148 routes consisted of an average of 18 points (range 4 – 20) and a total of 2515 steps (i.e., vectors connecting two successive locations). Among steps in which the animal moved (i.e., step length > 0 m) and time lapse did not exceed 20 minutes, mean step length was 246.6 m ($n = 1520$; range 1 – 2632 m).

Univariate tests revealed that observed movement paths had a higher proportion of forest, but a lower proportion of all other land cover types, in the immediate surroundings (100 m buffer) compared to the random paths (Table 2). In the broader spatial context (750 m annulus), observed movement paths were located in areas containing a higher proportion
of both forest and grassland, but lower proportion of cropland and water, than expected by chance (Table 2).

None of the 3 a priori path selection models (Path, Annulus, Combined) contained predictor variables with VIFs exceeding 10, and thus all were considered as candidate models in the conditional logistic regression analyses. Based on the coefficients from the Path model (Table 3), bobcats strongly selected for forested habitat in their immediate surroundings, but avoided water (note this is now relative to grassland). A similar pattern of habitat preference emerged at the broader landscape scale, with the Annulus model indicating significant avoidance of water and cropland relative to grassland (Table 3). The Combined model, however, had the lowest AIC score and thus was identified as the most parsimonious of the candidate models (Table 4). According to this full multivariate model, probability of path use was positively related to forest cover at the path level, but negatively related at the annulus level when all of the other variables are taken into consideration. These two forest variables were the most significant habitat variables (according to Wald statistics) in the model (Table 3). Results of the jackknife procedure indicated the unbalanced sampling design likely did not bias my interpretations, as all parameter values were similar across runs (data not shown). Visual representations of the resistance surfaces based on the habitat suitability map and the three path selection models (Path, Annulus, Combined) are presented in Fig. 3a-d.

The Microchecker analysis revealed no significant problems with null alleles, large allele dropout, or stuttering in any of the microsatellite loci. Furthermore, none of the markers were out of HWE after applying the Bonferonni correction (Table 1). However, 8 pairs of loci exhibited significant linkage disequilibrium. Given that some of the locus-pairs
suspected of linkage are known to be located on different chromosomes in the domestic cat (Table 1), and that many factors other than physical linkage (e.g., population growth or admixture) can also lead to nonrandom association of alleles (Weir 1996), I chose to retain data from all loci in this analysis. I did detect an overall heterozygote deficiency among the samples ($P = 0.002$), which may be indicative of internal population structure (i.e., Wahlund effect) such as isolation-by-distance or discrete clusters. BAPS results, however, indicated the most likely number of populations present in the data set was 1 (posterior $p = 1.0$).

Cost distance values derived from the 4 landscape resistance models (Path, Annulus, Combined, and Suitability) were highly correlated with those obtained from the IBD null model (Fig. 4), with the Annulus distances being the most similar to Euclidean distances ($r = 0.985, P = 0.001$) and the Suitability model the least ($r = 0.810, P = 0.001$). All 5 models showed significant correlations with genetic distances (Table 5), with the Suitability model explaining the largest proportion of genetic variability. However, none of the landscape models were significantly correlated with genetic distance once the effects of Euclidean distance were accounted for (Table 5).

**Discussion**

My analysis of movement paths indicated that bobcats do respond to habitat heterogeneity when moving through the landscape, and the response depends not only on the habitat composition in the immediate surroundings but the broader landscape context as well. Along movement paths, bobcats showed a strong preference for forest over any other land cover type. This preference is still evident but diminished over a greater spatial extent (750 m), indicating that in this landscape bobcats prefer forest interspersed with other landcover
types such as road ditches and grasslands. Relative to availability, cropland was avoided at both scales. This finding is concordant with the results of Tucker et al. (2008) and Linde (2010), which also supported a preference for forest associated with perennial habitats.

Even within a population, habitat fragmentation can have a substantial effect on landscape connectivity and the dispersal of animals, reducing gene flow and generating fine-scale patterns of genetic differentiation (Coulon et al. 2004; Wasserman et al. 2010). However, despite the impact of habitat heterogeneity on bobcat movement behavior, I was unable to detect a signature of a landscape effect in fine-scale genetic structure. Although each of the measures of effective distances from the four landscape models (Path, Annulus, Combined, Suitability) were significantly correlated with genetic distances, these effective distances did not account for genetic variation any better than the pure isolation-by-distance model that assumes a homogeneous environment. Furthermore, the relationships between effective geographic distances and genetic distances were no longer significant after accounting for the effect of Euclidean distance. Interestingly, although presence-absence data for habitat suitability models do not incorporate any information on movement behavior, here it predicted genetic variation equally as well as the movement-based models.

Given the high correlations between the measures of effective geographic distance to Euclidean distance, my landscape models may be too similar to the IBD null model to be able to separate out their effects from pure distance effects. Detecting a landscape effect may be especially challenging given that correlations based on individual data are likely to be inherently weak because of the high level of variability among individuals. The observed correlation values are not out of line with other studies with individual-based data: Wang et al. 2008 (r = 0.123-0.161); Wasserman et al. 2010 (r = 0.168-0.206); Coulon et al. 2004 (r <
0.031); Shirk et al. 2010 ($r = 0.181-0.723$); Schwartz et al. 2009 ($r = 0.074-0.265$). Thus, if a strong landscape effect on fine-scale genetic structure did exist, I expect I should have been able to detect it with these data.

Several factors may have contributed to the discordance between movement behavior and genetic structure. First, although the landscape of Iowa is highly altered, composition and configuration metrics are largely consistent across the south-central region in which the majority of the samples were collected (Linde 2010). Thus, least-cost paths between samples are not very divergent from straight-line paths (Fig. 5). In addition, bobcats are capable of dispersing long (>100 km) distances (Knick & Bailey 1986; Johnson et al. 2010; Gosselink et al. 2011). Thus, habitat heterogeneity may exert little influence on dispersal and gene flow at this spatial scale, but perhaps more of a landscape effect would be observed if I examined a broader spatial area for which habitat fragmentation levels are more variable.

Another point to consider is that the bobcat population in Iowa is relatively new to the landscape, and it may simply take more time for the IBD pattern and landscape effects to develop (Anderson et al. 2010). Based on simulations of isolation-by-distance, Hardy & Vekemans (1999) showed that although the time needed to reach equilibrium (i.e., correlogram stabilizes) can be quite long (> 250 generations), particularly for large populations, detectable genetic structure emerges quite quickly (~ 8 generations). Similarly, Cushman & Landguth (2010) found through simulations that distance and landscape effects can be detected almost immediately in spatial genetic patterns (< 10 generations), but do not reach equilibrium for several hundred generations. Furthermore, when stipulating an isolation-by-landscape resistance process, Cushman & Landguth (2010) discovered that simple Mantel tests based on Euclidean distance produced strong, spurious correlations of
similar magnitude to those produced by the correct effective distances, and that it took more than 40 generations for partial Mantel tests to be able to identify the correct process with power greater than 0.90. Assuming a generation time of ~2.3 years (Gosselink et al. 2011), I estimate bobcats have been on Iowa’s landscape for only approximately 7 - 20 generations. Thus, a temporal lag in genetic response may explain the inability to disentangle a landscape effect from a pure distance effect.

It is important to point out that the movement data were taken during normal home range use, and I made the assumption that these movements are representative of the type of dispersal movements leading to gene flow. Although the movement paths I observed represent the best available data for bobcats, it is unclear how well such local behaviors link up with dispersal movements. For example, Levey et al. (2005) found that local movements of birds could be scaled up to predict long-distance dispersal of seeds, whereas Morales & Ellner (2002) found that models successful in describing small-scale, within-patch movements of beetles failed to predict movements at larger spatial scales. Movement models may need to incorporate behavioral complexity, such as accounting for “state switching” between foraging vs. migrating behavior (Schick et al. 2008). More directional movement paths, or ones taken over longer spatial and temporal periods, may provide a stronger fit to gene flow. However, observations of dispersal events of bobcats from south-central Iowa generally support my conclusions based on local movement paths, as dispersal was found to be biased in an east-west direction and only rarely did animals attempt to disperse north into areas of high landscape resistance (Gosselink et al. 2011).

By comparing habitat composition of observed paths to available paths, I found that bobcats preferentially move through forested areas associated with perennial habitats.
Models of landscape resistance based on these habitat selection results indicate a large portion of Iowa’s landscape, namely the northern 2/3 of the state, is predicted to generate a high level of resistance to movement for bobcats. Although I could not discern a landscape effect in fine-scale genetic structure within Iowa, these large blocks of high resistance may impede connectivity with bobcat populations in neighboring states and lead to genetic subdivision at the regional scale.

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**References**


**Figure Legends**

**Fig. 1.** Spatially nested maps showing: (a) land cover classes in the study area; (b) locations of actual bobcat movement paths; and (c) an actual path with 9 of its associated random paths, each surrounded by a 100 m buffer and 750 m annulus. Note the spatial extent of the maps in (b) and (c) are shown as rectangles in each preceding map.

**Fig. 2.** Locations of \(n = 625\) geo-referenced bobcat DNA samples collected in Iowa from 2001-2008.

**Fig. 3** Resistance surfaces for bobcat movement based on the (a) Habitat Suitability model; (b) Path model; (c) Annulus model; and (d) Combined (Path + Annulus) model.

**Fig. 4.** Relationship between Euclidean distances (IBD model) and each of the four measures of effective geographic distances: (a) Path model; (b) Annulus model; (c) Combined (Path + Annulus) model; (d) Habitat Suitability model.
Fig. 5. Least-cost paths between $n = 625$ bobcat samples based on the Combined model of landscape resistance.
Table 1. Summary of microsatellite loci characteristics.

<table>
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<tr>
<th>Locus</th>
<th>Species</th>
<th>Repeat motif</th>
<th>Chr.</th>
<th>N</th>
<th>Size range (bp)</th>
<th>No. alleles</th>
<th>$H_E$</th>
<th>$H_O$</th>
<th>$F_{IS}$</th>
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<td>Domestic cat</td>
<td>Di</td>
<td>D4</td>
<td>609</td>
<td>166-178</td>
<td>7</td>
<td>0.561</td>
<td>0.557</td>
<td>0.0071</td>
</tr>
<tr>
<td>FCA077</td>
<td>Domestic cat</td>
<td>Di</td>
<td>C2</td>
<td>620</td>
<td>152-168</td>
<td>9</td>
<td>0.717</td>
<td>0.708</td>
<td>0.0119</td>
</tr>
<tr>
<td>FCA082</td>
<td>Domestic cat</td>
<td>Di</td>
<td>E1</td>
<td>603</td>
<td>246-266</td>
<td>11</td>
<td>0.844</td>
<td>0.828</td>
<td>0.0200</td>
</tr>
<tr>
<td>FCA090</td>
<td>Domestic cat</td>
<td>Di</td>
<td>A1</td>
<td>619</td>
<td>117-129</td>
<td>7</td>
<td>0.762</td>
<td>0.746</td>
<td>0.0206</td>
</tr>
<tr>
<td>FCA096</td>
<td>Domestic cat</td>
<td>Di</td>
<td>E2</td>
<td>622</td>
<td>191-219</td>
<td>11</td>
<td>0.836</td>
<td>0.826</td>
<td>0.0117</td>
</tr>
<tr>
<td>FCA126</td>
<td>Domestic cat</td>
<td>Di</td>
<td>B1</td>
<td>603</td>
<td>132-154</td>
<td>8</td>
<td>0.770</td>
<td>0.760</td>
<td>0.0131</td>
</tr>
<tr>
<td>FCA132</td>
<td>Domestic cat</td>
<td>Di</td>
<td>D3</td>
<td>612</td>
<td>182-196</td>
<td>8</td>
<td>0.850</td>
<td>0.822</td>
<td>0.0331</td>
</tr>
<tr>
<td>FCA149</td>
<td>Domestic cat</td>
<td>Di</td>
<td>B1</td>
<td>622</td>
<td>138-158</td>
<td>10</td>
<td>0.805</td>
<td>0.822</td>
<td>-0.0209</td>
</tr>
<tr>
<td>FCA391</td>
<td>Domestic cat</td>
<td>Tetra</td>
<td>B3</td>
<td>609</td>
<td>210-236</td>
<td>8</td>
<td>0.667</td>
<td>0.655</td>
<td>0.0179</td>
</tr>
<tr>
<td>FCA559</td>
<td>Domestic cat</td>
<td>Tetra</td>
<td>B1</td>
<td>618</td>
<td>105-141</td>
<td>8</td>
<td>0.793</td>
<td>0.777</td>
<td>0.0200</td>
</tr>
<tr>
<td>FCA740</td>
<td>Domestic cat</td>
<td>Tetra</td>
<td>C1</td>
<td>618</td>
<td>334-358</td>
<td>6</td>
<td>0.727</td>
<td>0.739</td>
<td>-0.0170</td>
</tr>
<tr>
<td>FCA741</td>
<td>Domestic cat</td>
<td>Tri</td>
<td>D1</td>
<td>616</td>
<td>170-188</td>
<td>7</td>
<td>0.546</td>
<td>0.563</td>
<td>-0.0318</td>
</tr>
<tr>
<td>FCA742</td>
<td>Domestic cat</td>
<td>Tetra</td>
<td>D4</td>
<td>622</td>
<td>115-135</td>
<td>6</td>
<td>0.640</td>
<td>0.613</td>
<td>0.0424</td>
</tr>
</tbody>
</table>

Locus name, species developed from, and repeat motif (tetra: tetranucleotide; di: dinucleotide; tri: trinucleotide) were taken from the cited reference: ¹Faircloth et al. 2005; ²Carmichael et al. 2000; ³Menotti-Raymond et al. 1999; ⁴Menotti-Raymond et al. 2005. Chromosomal location (Chr.) was determined from the NCBI Map Viewer for the domestic cat genome, available at http://www.ncbi.nlm.nih.gov/mapview. The number of genotypes (N), size range of alleles, number of alleles, expected ($H_E$) and observed heterozygosity ($H_O$), and $F_{IS}$ were based on analysis of 625 Iowa bobcat samples.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Observed Paths</th>
<th>Random Paths</th>
<th>AB</th>
<th>×</th>
<th>SD</th>
<th>Range</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (100 m buffer)</td>
<td></td>
<td></td>
<td>W100</td>
<td>0.0052</td>
<td>0.0204</td>
<td>0-0.2042</td>
<td>0.0243</td>
<td>0.1098</td>
</tr>
<tr>
<td>Development (100 m buffer)</td>
<td></td>
<td></td>
<td>D100</td>
<td>0.0046</td>
<td>0.0111</td>
<td>0-0.0633</td>
<td>0.0202</td>
<td>0.0684</td>
</tr>
<tr>
<td>Forest (100 m buffer)</td>
<td></td>
<td></td>
<td>F100</td>
<td>0.3967</td>
<td>0.2414</td>
<td>0-1</td>
<td>0.1936</td>
<td>0.209</td>
</tr>
<tr>
<td>Grassland (100 m buffer)</td>
<td></td>
<td></td>
<td>G100</td>
<td>0.3727</td>
<td>0.2338</td>
<td>0-0.9269</td>
<td>0.4357</td>
<td>0.2603</td>
</tr>
<tr>
<td>Cropland (100 m buffer)</td>
<td></td>
<td></td>
<td>C100</td>
<td>0.1978</td>
<td>0.2289</td>
<td>0-1</td>
<td>0.2869</td>
<td>0.288</td>
</tr>
<tr>
<td>Road (100 m buffer)</td>
<td></td>
<td></td>
<td>R100</td>
<td>0.0229</td>
<td>0.0316</td>
<td>0-0.2330</td>
<td>0.0393</td>
<td>0.0588</td>
</tr>
<tr>
<td>Water (750 m annulus)</td>
<td></td>
<td></td>
<td>W750</td>
<td>0.0076</td>
<td>0.0193</td>
<td>0-0.1281</td>
<td>0.0242</td>
<td>0.0948</td>
</tr>
<tr>
<td>Development (750 m annulus)</td>
<td></td>
<td></td>
<td>D750</td>
<td>0.0132</td>
<td>0.0237</td>
<td>0-0.1895</td>
<td>0.0207</td>
<td>0.053</td>
</tr>
<tr>
<td>Forest (750 m annulus)</td>
<td></td>
<td></td>
<td>F750</td>
<td>0.2325</td>
<td>0.1323</td>
<td>0.0036-0.7749</td>
<td>0.1961</td>
<td>0.1498</td>
</tr>
<tr>
<td>Grassland (750 m annulus)</td>
<td></td>
<td></td>
<td>G750</td>
<td>0.4647</td>
<td>0.1568</td>
<td>0.0316-0.8964</td>
<td>0.4371</td>
<td>0.1763</td>
</tr>
<tr>
<td>Cropland (750 m annulus)</td>
<td></td>
<td></td>
<td>C750</td>
<td>0.2465</td>
<td>0.1801</td>
<td>0-0.8107</td>
<td>0.2823</td>
<td>0.2159</td>
</tr>
<tr>
<td>Road (750 m annulus)</td>
<td></td>
<td></td>
<td>R750</td>
<td>0.0354</td>
<td>0.0224</td>
<td>0-0.1876</td>
<td>0.0396</td>
<td>0.0349</td>
</tr>
</tbody>
</table>

Table 2. Univariate descriptive statistics including mean, standard deviation, and range of habitat proportions found within the 100 m buffer and 750 m annulus of observed and random movement paths. The estimated coefficients (β) and associated probabilities (P) testing the null hypothesis (β = 0), which were obtained from univariate conditional logistic regression, are provided to illustrate the differences between observed and random paths for each of the habitat types. AB: abbreviation of variable name.
Table 3. Estimated coefficients, standard errors, Wald statistics, and associated probabilities of land cover variables included in the three candidate logistic models describing path selection by bobcats in south-central Iowa.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Path</th>
<th></th>
<th></th>
<th></th>
<th>Annulus</th>
<th></th>
<th></th>
<th></th>
<th>Combined</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>Wald</td>
<td>P</td>
<td>β</td>
<td>SE</td>
<td>Wald</td>
<td>P</td>
<td>β</td>
<td>SE</td>
<td>Wald</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (100 m)</td>
<td>-8.862</td>
<td>4.207</td>
<td>4.44</td>
<td>0.035</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-2.191</td>
<td>4.834</td>
<td>0.21</td>
<td>0.650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Development (100 m)</td>
<td>-13.521</td>
<td>7.063</td>
<td>3.66</td>
<td>0.056</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-17.464</td>
<td>7.541</td>
<td>5.36</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest (100 m)</td>
<td>3.582</td>
<td>0.430</td>
<td>69.43</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.290</td>
<td>0.607</td>
<td>107.42</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cropland (100 m)</td>
<td>-0.036</td>
<td>0.466</td>
<td>0.01</td>
<td>0.939</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.302</td>
<td>0.647</td>
<td>0.22</td>
<td>0.641</td>
<td></td>
<td></td>
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<tr>
<td>Road (100 m)</td>
<td>-3.559</td>
<td>2.956</td>
<td>1.45</td>
<td>0.229</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-2.481</td>
<td>3.056</td>
<td>0.66</td>
<td>0.417</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (750 m)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-10.142</td>
<td>4.378</td>
<td>5.37</td>
<td>0.021</td>
<td>-10.814</td>
<td>5.207</td>
<td>4.31</td>
<td>0.038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Development (750 m)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-5.602</td>
<td>4.132</td>
<td>1.84</td>
<td>0.175</td>
<td>4.219</td>
<td>4.705</td>
<td>0.80</td>
<td>0.370</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest (750 m)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.956</td>
<td>0.769</td>
<td>1.55</td>
<td>0.214</td>
<td>-6.726</td>
<td>1.112</td>
<td>36.60</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cropland (750 m)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-1.331</td>
<td>0.661</td>
<td>4.06</td>
<td>0.044</td>
<td>-1.393</td>
<td>0.917</td>
<td>2.30</td>
<td>0.129</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Road (750 m)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.317</td>
<td>4.231</td>
<td>0.01</td>
<td>0.940</td>
<td>3.282</td>
<td>4.628</td>
<td>0.50</td>
<td>0.478</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Performance of three candidate models of path selection by bobcats in south-central Iowa. Statistics include the number of parameters \((K)\), Akaike’s Information Criterion \((\text{AIC})\), and AIC differences \((\Delta\text{AIC})\). Variables are abbreviated as in Table 2.

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables</th>
<th>(K)</th>
<th>AIC</th>
<th>(\Delta\text{AIC})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Path</td>
<td>(W_{100} + D_{100} + F_{100} + C_{100} + R_{100})</td>
<td>5</td>
<td>957.965</td>
<td>44.23</td>
</tr>
<tr>
<td>Annulus</td>
<td>(W_{750} + D_{750} + F_{750} + C_{750} + R_{750})</td>
<td>5</td>
<td>1071.993</td>
<td>158.26</td>
</tr>
<tr>
<td>Combined</td>
<td>(W_{100} + D_{100} + F_{100} + C_{100} + R_{100} + W_{750} + D_{750} + F_{750} + C_{750} + R_{750})</td>
<td>10</td>
<td>913.735</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 5. Mantel and partial Mantel correlations ($r$) between spatial and genetic pairwise distances among 625 individual bobcats in Iowa. Spatial distances are based on a null model of distance only (IBD), three different models of landscape resistance developed from analysis of movement paths (Path, Annulus, Combined), and a model of landscape resistance based on habitat suitability (Suitability). A period separates the main spatial matrix on the left from the covariate matrix on the right that is partialed out in the partial Mantel test. For each of the four landscape resistance models, partial Mantel tests were conducted to partial out the effects of Euclidean distance (e.g., Path.IBD). Significant values ($P$) are based on 999 permutations. Bold values indicate $P < 0.05$.

<table>
<thead>
<tr>
<th>Mantel or partial Mantel test</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD</td>
<td>0.05766</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>Path</td>
<td>0.04968</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Annulus</td>
<td>0.05808</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Combined</td>
<td>0.05587</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>Suitability</td>
<td>0.06082</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Path.IBD</td>
<td>-0.00371</td>
<td>0.578</td>
</tr>
<tr>
<td>Annulus.IBD</td>
<td>0.00744</td>
<td>0.349</td>
</tr>
<tr>
<td>Combined.IBD</td>
<td>0.00315</td>
<td>0.450</td>
</tr>
<tr>
<td>Suitability.IBD</td>
<td>0.02411</td>
<td>0.145</td>
</tr>
</tbody>
</table>
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
CHAPTER 3. SPATIAL GENETIC STRUCTURE AND NATURAL RECOLONIZATION OF A MOBILE CARNIVORE, THE BOBCAT (*Lynx rufus*), WITHIN AN AGRICULTURAL LANDSCAPE

A paper submitted to *Molecular Ecology*

Dawn M. Reding

Abstract

Many terrestrial mammals are widely dispersing organisms with large, continuous ranges, challenging our ability to define populations and estimate reproductive and demographic cohesiveness. Yet understanding population connectivity is important for many species with distributions influenced by human activity. The bobcat, once extirpated from much of the American Midwest, has recently recolonized parts of its former range, but remains absent from the intensively-cultivated core of the region. Through a large-scale effort, I examined the genetic relationships of bobcats across the Midwest to: 1) assess population differentiation; 2) determine whether the intensive agricultural zone or other landscape features operate as dispersal barriers; and 3) identify the primary sources of recolonization. Using a panel of 19 microsatellite markers and 949 bp mtDNA, I analyzed 1447 bobcat samples collected from 15 states. I identified 6 genetic populations separated by both physical and cryptic boundaries. Physical barriers included large expanses of row cropping and the Straits of Mackinac waterway. Two cryptic barriers occur along zones of sharp changes in habitat type, suggesting that ecological variation may also figure prominently in shaping regional population structure. Newly-established populations in Iowa and northern Missouri are closely linked with bobcats to the southwest (eastern Kansas and
southern Nebraska), but have had little genetic input from populations to the north and east. These results indicate that even a highly mobile species does not readily disperse through this agriculturally-modified landscape, thus affecting the population connectivity and recolonization process of bobcats and likely other species.

**Introduction**

Populations represent groups of individuals that could potentially interact reproductively and demographically with each other (Waples & Gaggiotti 2006). Thus, delimiting populations and identifying the factors responsible for intraspecific structuring are of central importance to the fields of ecology, evolution, and conservation biology. For species that exist in discrete patches with limited dispersal, defining populations can be relatively straightforward, and such a model forms the basis of classical population genetic and ecological theory, e.g., *Wright’s Island Model* (Wright 1931), *natural populations* (Andrewartha & Birch 1954), and *metapopulations* (Levins 1969). But for species that are continuously distributed, highly vagile, and habitat generalists, delineating populations can be a difficult task as it is often unclear what, if anything, limits movement and gene flow.

Carnivores particularly challenge our understanding of the classical population concept. They typically disperse across large distances and occupy diverse habitats, which potentially promotes gene flow and limits intraspecific genetic differentiation (Lehman & Wayne 1991; Schwartz *et al.* 2002). However, significant genetic structure has increasingly been described in mobile carnivores (McRae *et al.* 2005; Pilot *et al.* 2006; Sacks *et al.* 2004), indicating that the factors influencing population subdivisions, such as distance and habitat heterogeneity, are not always clear. The potential for structure in the absence of obvious
natural breaks makes *a priori* population characterization difficult and points to the importance of individual-based spatial genetic data for elucidating population connectivity in these species (Manel *et al.* 2003).

Many carnivores have experienced significant declines in abundance and distribution resulting from human activities (Gittleman *et al.* 2001). In recent years, however, several species have naturally expanded into portions of their former ranges. In North America, bobcats (*Lynx rufus*) (Tucker *et al.* 2008), gray wolves (*Canis lupus*) (Fuller *et al.* 1992), brown bears (*Ursus arctos*) (Pyare *et al.* 2004), and cougars (*Puma concolor*) (Riley & Malecki 2001) have experienced recent range expansions, partly due to improved protection and habitat management. Since the factors affecting gene flow are poorly understood, investigating spatial genetic structure could greatly enhance our understanding of these contemporary recolonizations and future changes in distributions (Rueness *et al.* 2003a; Williams & Scribner 2010).

In this study, I examine the regional spatial genetic structure of a mobile, solitary, broadly-distributed carnivore, the bobcat. Bobcats are consummate habitat-generalists and were historically found throughout most of North America, including the prairies and woodlands of the American Midwest (Hall 1981; Young 1958). But overexploitation due to the fur trade and predator control, combined with large scale conversion of suitable habitat to row crop agriculture (Rolley 1987; Woolf & Hubert 1998), resulted in the extirpation of bobcats from a large portion of the Midwest by the mid-1900s (Fig. 1) and prompted several states to limit bobcat harvest (Deems & Pursley 1978). Today, the bobcat is more abundant and widely-distributed in the Midwest and is reclaiming parts of its former range. Most notably, bobcats are now relatively common in northern Missouri (Schwartz & Schwartz
2001) and southern and western Iowa (Linde 2010), places where 30 years ago they were effectively absent. The source populations for these range expansions are not known, and it is not clear whether or how bobcat may continue to spread throughout remaining unoccupied areas of the Midwest.

Because bobcats are capable of dispersing hundreds of kilometers (Johnson et al. 2010; Gosselink et al. 2011), it is possible that the Midwest represents one panmictic population with recolonizing bobcats coming from established sources in every direction throughout the region. However, the species remains largely absent from the most intensely cultivated areas of corn (*Zea mays*) – soybean (*Glycine max*) agriculture in the heart of the region known as the Corn Belt (Linde 2010). Thus, expanses of agricultural lands or other potential landscape barriers may limit dispersal across the Midwest, leading to the formation of several distinct populations. If such structure exists, only one or a few of these populations may be the primary source for recolonization of nearby areas.

In this study, I used an individual-based genetics approach to characterize regional population structure of bobcats in the Midwest. My goals were to identify 1) how many genetically different bobcat populations exist in the Midwest; 2) whether intensive agriculture or other landscape features influence connectivity among populations; and 3) the primary source(s) for bobcats recolonizing Iowa and northern Missouri, as well as rare occurrences in the heart of the Corn Belt.
Methods

Study area and sample collection

The study area consisted of a 15-state region in the Midwestern United States (Fig. 1). Bobcats likely were extirpated from the core of the study area by the mid-1900s, but began rebounding into the fringes of the Corn Belt in the 1990s (Woolf & Hubert 1998). Bobcats remain fully protected from harvest across the center of the Corn Belt (Fig. 1), generally corresponding to areas in which bobcats are thought to be rare or absent, except that bobcats are relatively common in southern Illinois (Woolf et al. 2002) and Indiana (Johnson et al. 2010).

I obtained tissue samples \((n = 1447)\) from live-captured, road-killed, legally harvested, and incidentally trapped bobcats collected between 1999-2009 (Fig. 1). Location information for sampled individuals ranged from precise geographical coordinates to a small proportion referenced only to a specific state or county. I used MapSource 4.07 (Garmin) to translate place-names to spatial coordinates. If a polygon (e.g., county, township, section) was provided as the location, I used ArcGIS 9.3 (ESRI) to calculate the mean center of the polygon, which I used as the spatial coordinates. If multiple individuals were collected at a given location, I used ArcGIS and the HawthsTools extension (Beyer 2004) to either: 1) construct a 500-m buffer around the place-name point and located the samples at unique, random locations within the buffer; or 2) locate the samples at unique, random locations within the polygon. I performed the relocations to visualize sample points on maps and to use spatial models requiring unique coordinates for each sample point.

In examining recolonization, I focused on bobcats in northern Missouri (i.e., north of the Missouri River) \((n = 97)\) and Iowa \((n = 628)\), as well as 23 bobcats (including 4 from
Iowa) collected from the core of the Corn Belt (Fig. S1), as bobcats were previously extirpated from these areas.

Microsatellite genotyping

I extracted DNA using either the DNeasy (Qiagen) or IDPURE (IDLabs) purification kits. I genotyped individuals at 19 autosomal microsatellite markers that were variable in bobcat and developed from domestic cat (*Felis catus*): FCA008, FCA023, FCA026, FCA031, FCA043, FCA045, FCA077, FCA082, FCA090, FCA096, FCA132, FCA149, FCA391, FCA559 (Menotti-Raymond et al. 1999), and FCA740 (Menotti-Raymond et al. 2005); Canada lynx (*Lynx canadensis*): Lc109, Lc110, Lc111 (Carmichael et al. 2000); and bobcat: BCE5T (Faircloth et al. 2005). Each locus was amplified separately using the M13-tailed primer method (Boutin-Ganache et al. 2001). Total PCR volume was 10 µl, including 1× PCR buffer with 2 mM MgSO$_4$ (IDLabs), 0.2 mM dNTPs, 0.3 µM fluorescently labeled M13 primer, 0.3 µM reverse primer, 0.02 µM M13-tailed forward primer, 0.4 U IDPROOF DNA Polymerase (IDLabs), and 10-20 ng of template DNA. The PCR profile was 95 °C/5 min, (95 °C/20 s, X °C/20 s, 72 °C/30 s) × X cycles, 72 °C/20 min (Table 1). I combined PCR products from each sample into four gel sets, each consisting of three to five loci (Table 1), and analyzed them on an ABI 3100 sequencer (ABI) at Iowa State University’s DNA Facility. Alleles were scored using the software Genotyper 3.7 (ABI).

For each locus, a number of individuals ( $\bar{x} = 72$, range 14 – 168) were re-amplified, -electrophoresed, and -scored, allowing for an ad hoc measure of genotype scoring error. I also used the program Microchecker (Van Oosterhout et al. 2004) to test for the presence of null alleles, stuttering, and large allele dropout.
To infer the number of bobcat populations (K) in the Midwest and assign individuals to the populations, I used both aspatial and spatial Bayesian clustering techniques and investigated congruence between the two approaches (Corander et al. 2008; Latch et al. 2006). The aspatial method, implemented in the program Structure 2.3.1 (Pritchard et al. 2000), uses only genetic data and sets a uniform prior such that all clustering solutions, even spatially unstructured ones, are a priori equally likely. I performed Structure analysis in a hierarchical approach (Evanno et al. 2005; Pritchard et al. 2009). Structure tends to detect the uppermost hierarchical level of structure present in a data set and attempts to explain the most variation by preferentially separating out the most distinctive or the largest clusters. In the data set, the greatest amount of variation was explained by separating out the heavily sampled Iowa (and surrounding areas) population from the others (K = 2) (see Results). In order to detect potential substructure, I assigned individuals to one of these two groups based on the highest q-value – an estimate of the proportion of an individual’s genome attributed to each of the identified genetic clusters – and subsequently analyzed each group separately.

In all Structure analyses, I performed 10 independent runs at each value of K = 1-9. Each run consisted of 100,000 replicates following a burn-in of 50,000. I used the admixture model and allowed the allele frequencies to be correlated among populations (Falush et al. 2003). To align and average each individual’s cluster membership coefficients (q) across the 10 replicate runs for each K, I used the program Clumpp 1.1.1 (Jakobsson & Rosenberg 2007), selecting the Greedy algorithm and 20 random input orders. To help choose the most likely number of populations in the dataset, I examined two metrics. First, for each value of K, I averaged the maximum log-likelihood, L(K), across runs. Second, I calculated the ΔK
statistic, which is based on the rate of change between successive $K$ values (Evanno et al. 2005). To assess whether the inferred genetic clusters made biological sense (Pritchard et al. 2009), I plotted the Structure-assigned individuals on a map of the study area to visualize the geographical congruence of the clusters. For the $K$ chosen as the best scenario, I assigned each individual to the group with the highest $q$-value. Individuals were displayed using ArcGIS software and color coded with respect to their cluster assignment. To take potential admixture (mixed ancestry) into account, I flagged bobcats for which $q < 0.75$, an arbitrary cutoff representing the amount of ancestry equivalent to 1 grandparent from outside the assigned group (Robinson et al. 2007).

The spatial method, implemented in the program BAPS 5.2 (Corander et al. 2008), incorporates coordinate data to assign a non-uniform prior such that neighboring individuals are favored to assign to the same genetic cluster. I performed spatial mixture clustering of individuals for 10 runs with the maximum number of populations ($K_{\text{max}}$) set to 20. Results from each run were stored and merged by the program, and the optimal $K$ value was selected as the partition with the maximum likelihood ($L(K)$) and highest posterior probability ($p$). The assignments from the mixture analysis were then used to perform admixture analysis with the recommended parameter values, including 100 iterations for individuals, 20 iterations for reference individuals, and 200 reference individuals from each population. Similar to the Structure analyses, I assigned individuals to the group with the highest $q$-value, flagging potentially admixed individuals ($q < 0.75$).

To examine whether the different sampling densities across the region (specifically Iowa and Indiana were intensely sampled) could potentially skew the clustering results, I repeated the Structure and BAPS procedures after making sampling density more uniform.
First, I overlaid a 20 km square grid on a map of the samples and used HawthsTools to randomly select a single sample from each sampled grid cell. This resulted in a selection of 755 samples. The grid size was chosen because it balanced sampling density while maintaining large sample sizes. A coarser grid size of 50 km yielded highly concordant results (not shown). Then, I used the PFROMPOPFLAGONLY option in Structure to prevent non-selected samples from contributing to allele frequency estimates during the clustering analysis, while still estimating ancestry for all individuals. In BAPS, I performed analyses using just the subset of 755 individuals.

Population Genetic Analyses

I used Genepop 4.0 (Rousset 2008) to test for linkage disequilibrium and deviations from Hardy-Weinberg (HW) equilibrium for each of the 19 microsatellite loci in the total sample and each inferred population. To quantify the direction and degree of deviation from HW equilibrium, I also used Genepop to estimate $F_{IS}$ (Weir & Cockerham 1984). For measures of genetic diversity, I calculated expected ($H_E$) and observed ($H_O$) heterozygosities for each locus as well as the average across loci using Arlequin 3.11 (Excoffier et al. 2005), and allelic richness (AR), corrected for difference in sample size, using Fstat version 2.9.3 (Goudet 2001). I also used Arlequin to calculate pairwise $F_{ST}$ as a measure of differentiation between the inferred populations. In all assessments of significance, I used the sequential Bonferroni method to account for multiple comparisons (Rice 1989).

To evaluate the potential effect of sampling closely related individuals on estimates of population structure, I used GenAlEx 6.2 (Peakall & Smouse 2006) to calculate Queller and Goodnight (1989) pairwise relatedness values among individuals within each genetic cluster.
For clusters in which the proportion of closely related ($r \geq 0.45$) pairs exceeded 1%, I removed one individual from each pair with $r \geq 0.45$ and re-calculated pairwise $F_{ST}$ estimates (Rosel et al. 2009).

**Clines vs. Clusters**

Clustering approaches assume discrete structure exists. If instead allele frequencies vary continuously over space, conforming to an isolation-by-distance (IBD) pattern, cluster assignments could be misleading (Frantz et al. 2009; Pritchard et al. 2009; Schwartz & McKelvey 2009). Therefore, before interpreting population genetic structure and inferring barriers to gene flow, I took steps to account for the potentially confounding effect of geographical distance by: 1) testing for IBD; and 2) including spatial coordinates as a covariable in a multivariate analysis.

To test for an IBD pattern, I used GenAlEx to conduct individual-based Mantel tests (Mantel 1967) between pairwise Euclidean distance (km, ln-transformed) and linear pairwise genetic distance (Smouse & Peakall 1999), with significance assessed through 999 permutations. Since an overall pattern of IBD could result simply from higher genetic similarity of individuals within populations (which are spatially proximate) than among populations (Murphy et al. 2008), I performed the IBD analysis separately for each population identified from the cluster analysis, in addition to the total sample.

To examine whether the genetic clusters explain genetic variation over and above that expected due simply to geographical distance alone, I performed a partitioning of the genetic distance matrix using distance-based redundancy analysis (dbRDA) (Legendre & Anderson 1999; McArdle & Anderson 2001), a form of multivariate multiple regression shown to have
very low type-I error rates (Balkenhol et al. 2009). The relationship between the genetic distance matrix and each of two sets of predictor variables (geographic coordinates and cluster assignment) was first analyzed separately using dbRDA marginal tests. I then performed conditional dbRDA for cluster assignment, having first fit geographic coordinates as a covariable. Statistical significance was tested using 999 permutations of the response matrix or the multivariate residual matrix. I used program DISTLM v.5 (Anderson 2004) for all dbRDA analyses.

**Mitochondrial DNA Analysis**

To provide insight from another genetic marker system, I selected 210 individuals for mtDNA sequencing to evenly span the study area and represent each of the six populations inferred from clustering (see Results). I amplified a 949 bp portion of the NADH dehydrogenase subunit 5 (ND5) gene using primers I designed, ND5-DR1F (5'-TCATCCCGTAGCCTTTTCT-3') and ND5-DR3R (5'-AAGGGATGTGGCAATGAGAG-3'). Total PCR volume was 10 µl, including 1× PCR buffer with 2 mM MgSO$_4$, 0.2 mM dNTPs, 0.3 µM each primer, 0.4 U DNA Polymerase, and 10-20 ng of template DNA. The PCR profile was 95 °C/5 min, (94 °C/30 s, 57 °C/45 s, 72 °C/45 s) × 30 cycles, 72 °C/10 min. PCR products were cleaned using the ExoSAP method (Werle et al. 1994) and submitted to the Iowa State University DNA Facility for cycle sequencing and analysis on an ABI 3730xl DNA Analyzer. Both directions were sequenced with the same primers used for PCR. I used Sequencher 4.6 (Gene Codes Corporation) to edit, assemble, and align sequences, and DnaSP 5.10 (Librado & Rozas 2009) to identify unique haplotypes and generate Arlequin input files. To measure genetic differentiation, I used Arlequin and the
Tamura and Nei (1993) model to estimate pairwise $\Phi_{ST}$ values between genetic clusters defined by microsatellite analysis. I also constructed a median joining network using the program Network 4.5 (Bandelt et al. 1999).

**Results**

*Genotyping*

The 1447 samples were genotyped at an average of 18.6 (1.2, SD) loci, with all individuals genotyped for a minimum of 10 of the 19 markers. Allele counts ranged from 7 to 15 (Table 1). The Microchecker results indicated no consistent problems with large allele dropout, null alleles, or stuttering for any loci. Of the 1368 replicated genotypes, I found only two mismatches, one (of 114; 0.88%) for Lc111 and one (of 108; 0.93%) for FCA090. The overall low incidence (0.1%) of mismatches supports the quality of the genotypic data and suggests errors are unlikely to have significantly influenced my analyses.

*Population Inference Using Bayesian Clustering Analyses*

Results of initial Structure analysis with all samples indicated strongest support for $K = 2$ (Iowa and surrounding areas vs. all other samples), based on $\Delta K$ and the assignment plots, though the L($K$) values increased asymptotically with higher $K$ (Fig. 2a). Subsequent analysis of just the cluster consisting of Iowa and surrounding areas did not provide evidence of additional substructure (Fig. 2b). There was little improvement in likelihood with increasing $K$, and the cluster assignments lacked geographic congruence. Although $\Delta K$ was highest for $K = 2$, there is no way to evaluate $K = 1$ with this method (Evanno et al. 2005). The second cluster, however, did show evidence of substructure (Fig. 2c). The likelihood
values increased until $K = 5$, and then reached a plateau. The $\Delta K$ statistic also yielded the highest peak at $K = 5$, and the groups were geographically concordant. Thus, $K = 6$ (the first cluster and the 5 subgroups of the second cluster) was the best scenario using the hierarchical approach (Fig. S2).

Results of the BAPS analysis were highly concordant with the hierarchical Structure analysis. Using all samples, $K = 6$ was the optimal solution ($L(K) = -88229.6$; posterior $p = 1.0$), with the clusters (Fig. 1) matching those identified in the hierarchical Structure analysis (Fig. S2). The six clusters were classified as: 1) Central: Iowa, northwestern Missouri, eastern Kansas, and southern Nebraska; 2) Western: North and South Dakota, northern Nebraska, and parts of western Kansas and Oklahoma; 3) Northern: Minnesota, Wisconsin, and the Upper Peninsula (UP) of Michigan; 4) Michigan: the Lower Peninsula (LP) of Michigan and a few from the UP; 5) Southeastern: southeastern Missouri, Arkansas, Illinois, Indiana, Kentucky, and Tennessee; and 6) Indiana: a small cluster located in the Crane Naval Surface Warfare Center, the study area of telemetry work conducted by Johnson et al. (2010).

Reducing the data set to even sampling density yielded similar results in both Structure and BAPS analyses. Using the UPDATEREFROMPOPFLAG option in Structure, $K = 5$ was the best scenario for this data set since it yielded the highest $L(K)$ value (Fig. 2d) and captured the majority of the spatial genetic variation. Although the $\Delta K$ statistic indicated strongest support for $K = 2$ (Fig. 2d), visual observation of individual assignments clearly indicates additional substructure exists beyond $K = 2$, the majority of which was captured at $K = 5$. The five clusters were identical to those identified in the hierarchical analysis, with the omission of the Indiana cluster, which instead fell in with the Southeastern cluster (Fig.
S3). Using just the subset of 755 individuals, \( K = 5 \) was also the optimal solution in BAPS analysis \( (L(K) = -47327.8; p = 1.0) \), again with the Indiana cluster being absorbed into the Southeastern cluster (Fig. S4).

In all four scenarios (Structure \( K6 \), BAPS \( K6 \), Structure \( K5 \), and BAPS \( K5 \)), the genetic clusters were geographically well-defined, but the boundaries typically showed some degree of overlap. Potentially admixed individuals \( (q < 0.75) \) were found throughout the region, particularly at the interfaces of different clusters, and account for between 3 to 37% of the individuals assigned to each cluster (Table 2). BAPS analysis showed fewer admixed individuals than Structure. The 23 individuals sampled within the core of the Corn Belt showed signatures from several genetic populations (Northern = 5, Southeastern = 7, Central = 4, admixed = 7, based on BAPS \( K6 \)), indicating multiple potential sources (Fig. 1, Fig. S1). Within Iowa (Central = 595, Southeastern = 9, admixed = 24) and northern Missouri (Central = 82, Southeastern = 10, admixed = 5), however, nearly all individuals (93.4%) were assigned to the Central population (Fig. 1).

**Population Genetic Analyses**

In the total sample, all loci except Lc110 showed significant deviations from HW equilibrium due to heterozygote deficiencies (data not shown), suggesting underlying population structure (Wahlund effect). All four clustering methods reduced departures from HW equilibrium, and only for the Structure \( (K5) \) assignments did two loci (FCA391 and FCA043, both in the Southeastern population) still show significant deviation following sequential Bonferroni corrections. Substructure also led to linkage disequilibrium in the total sample, with 61 of 171 locus pairs exhibiting a significant pattern. Separating the samples
into populations minimized the number of locus pairs in linkage disequilibrium (31 pairs for Structure \((K6)\), 4 pairs for Structure \((K5)\), 3 pairs for BAPS \((K6)\), and 1 pair for the BAPS \((K5)\)). Since no locus pairs showed evidence of consistent linkage across populations, I assume loci are independent. Both the Michigan and Indiana populations had significantly lower genetic diversity \((H_E\) and AR averaged over 19 loci) than each of the other populations (Table 2) (Tukey-Kramer HSD, \(P < 0.05\)), except \(H_E\) did not differ significantly between Michigan and Central \((P = 0.06)\).

All populations were significantly differentiated from one another based on pairwise \(F_{ST}\) estimates, and highly concordant results were achieved whether Structure vs. BAPS or five vs. six populations were considered (Table 3). Populations were moderately differentiated \((F_{ST} \approx 0.03 - 0.05)\), and the Indiana and Michigan clusters were most differentiated from the other populations \((F_{ST} \approx 0.08 - 0.15)\). Populations had few closely-related individuals, as 0 to 0.16% of pairs had \(r \geq 0.45\), except in the Indiana population where approximately 3% of pairs exceeded this level. After removing 18 individuals from the Indiana population, pairwise \(F_{ST}\) values declined slightly, but were still highly significant (see Table 3). This finding along with the data on admixture, HW and linkage equilibrium, and pairwise \(F_{ST}\), supported the classification of Indiana as a legitimate sixth population and indicated the BAPS assignments for \(K = 6\) best represented the genetic structure of bobcats in the Midwest.

**Clines vs. Clusters**

Based on the Mantel tests, there was a weak but significant univariate correlation between geographic and genetic distance in the total sample \((r = 0.288, P < 0.001)\) as well as
within each of the six Baps clusters except the Northern (Fig. S5). The dbRDA results also indicated a weak but significant relationship between genetic and spatial variation across the study area (pseudo-F = 12.60, $P = 0.001$), with coordinates explaining 1.7% of the total genetic variation. Cluster assignment also showed a significant but higher relationship with genetic variation (pseudo-F = 18.36, $P = 0.001$), accounting for 6.0% of the total variation. This relationship was significant even when spatial variation was taken into account by fitting coordinates as covariables in the analysis (pseudo-F = 14.12, $P = 0.001$), providing evidence that the clustering solutions are real and not artifacts of forcing a purely IBD pattern into discrete populations.

*Mitochondrial DNA Analysis*

I detected 21 unique haplotypes among the 210 samples (Table 2). The haplotype network (Fig. 3a) indicated two major clades, separated by six substitutions. Haplotypes from Clade 1 were found throughout the region (Fig. 3b). All four of the haplotypes from Clade 2 were found only in samples from the western portion of the study area (except for one bobcat in southeastern MN; Fig. 3b). The six BAPS-defined populations showed strong levels of mtDNA genetic differentiation (Table 4), and no haplotype was found in all regions. In general, the geographic distribution of haplotypes closely mirrored the patterns observed with the microsatellite data (Fig. 3b and Fig. S6), except no differentiation was detected between the Northern and Michigan clusters. Both were dominated (Northern: 72.5%; Michigan: 78.9%) by H1, which was uncommon in other locations. The Indiana population was similarly dominated (93.3%) by a single haplotype (H12), which elsewhere was only detected in two Wisconsin bobcats. The other three populations were more diverse, but still
showed notable patterns, with H3 common (32.6%) and restricted primarily to the Southeastern population, H5 to the Central population (47.3%), and the four Clade 2 haplotypes to the Western population (45.7%). H2 was common and found throughout the three southern populations, but was absent north of the Corn Belt. Within the core of the Corn Belt, two individuals located in northeastern IN possessed haplotype H21 (Fig. S1), unique to these two individuals, and thus may be more connected to unsampled populations farther to the east.

**Discussion**

*How many genetic populations?*

Despite the bobcat’s broad range and high mobility, both mtDNA and microsatellite data reject a null hypothesis of a single, panmictic population of bobcats in the Midwest. Instead, six genetic clusters were identified, and the patterns were remarkably concordant among the different markers and methods employed. Only the Indiana cluster was not supported across all analyses. However, this population is primarily restricted to a small area in Indiana, and analyses using uniform sampling density greatly (from 50 to 5) reduced the number of individuals from this area, likely resulting in too few samples to adequately characterize allele frequencies. With the complete data set, it is possible that the clustering algorithms may simply be picking up on family structure in Indiana (Anderson & Dunham 2008), but I found no evidence to support this conclusion, as removing closely related individuals did not affect the results. Instead, the Indiana population may represent an isolated and/or remnant population. The majority of individuals assigned to this cluster came from a four-county region in south-central Indiana, one of the largest contiguous forested
landscapes in the state (Johnson et al. 2010). In addition, the majority of this forest region has been protected from hunting because it is located on a military base. Thus, a small population may have persisted here during times of persecution and land cover change. The discovery of a dominant haplotype not found in neighboring states also points to a potentially unique history for this population. Fine-scale structuring may also exist in other areas within the study region, but were not detected in this analysis.

Both microsatellite and mtDNA data revealed similar genetic patterns. A notable exception, however, is that although mtDNA pairwise $F_{ST}$ values were generally higher than microsatellite pairwise $F_{ST}$ values, the Northern and Michigan populations were differentiated based on microsatellite but not mtDNA data. The discrepancy between mtDNA and nuclear markers is unlikely to have arisen due to sex-biased dispersal. Females are the philopatric sex in bobcats (Janecka et al. 2007); thus the pattern in the Northern and Michigan populations is opposite of what would be expected. Both Northern and Michigan populations showed low mtDNA diversity and were dominated by haplotype H1. One possible explanation is that the two populations have a shared evolutionary history following the glacial retreat during the Pleistocene. Expansions from glacial refugia may also explain the two distinct mtDNA clades, where the western edge of the study area represents the convergence of two bobcat lineages. In a study of bobcat mtDNA control region sequences, Croteau (2009) also discovered two clades: Western and Eastern/Midwestern. Since historical as well as contemporary forces are likely influencing current patterns, a full-scale study of the phylogeography and demographic history of this species across its entire range would help to further elucidate the causes of the genetic patterns and discrepancies between markers.
What landscape features influence population structure?

It appears both physical and cryptic barriers distinguish the six bobcat populations (Fig. 1). The physical barriers include the Straits of Mackinac, which limits movement of bobcats between the UP and LP of Michigan, and the Corn Belt distribution gap, which separates the northern bobcats from the rest of the Midwest and may also help to isolate the Indiana cluster. In addition, two cryptic barriers delineate a continuous distribution of bobcats south of the Corn Belt into three separate populations (Western, Central, and Southeastern).

Physical Barriers. The Straits of Mackinac, a waterway formed during the Pleistocene that connects Lakes Michigan and Huron, was previously identified as a barrier to gene flow in this species (Millions & Swanson 2006, 2007; Williams 2006) based on microsatellite analysis of Michigan samples. The strait is > 6 km in width with a 35 m shipping channel maintained through the ice in the winter. Though this is a formidable barrier, dispersal is conceivable since bobcats have been found on islands in northern Lake Michigan (Baker 1983) and observed crossing major rivers (Johnson et al. 2010). I observed five individuals genetically assigned to the LP Michigan population but located in the UP, but no Northern individuals located in the LP. All Michigan samples in this study came from harvested animals used by Williams (2006), with hunters and trappers reporting take locations. Since the bag limit is higher in the UP than the LP, Williams (2006) and Millions and Swanson (2006, 2007) have suggested the directional bias in genetic misassignment may be due to poaching and/or misreporting of harvest locations. However, the observed genetic pattern could also be due to biased dispersal between the peninsulas.
In contrast, the upper Mississippi and Missouri Rivers did not have the same effect. Major rivers are certainly perceived as barriers by some individuals, as Gosselink et al. (2011) observed dispersing radio-collared animals abruptly change course upon encountering one, but they also documented individuals crossing both the Mississippi and Missouri Rivers, and Johnson et al. (2010) documented bobcats crossing the Ohio and Wabash Rivers. Whether bobcats travel across highway or railroad bridges, swim across during times of low flow, or walk across ice in winter, evidently there are enough crossing and mating opportunities to keep populations on either side genetically connected.

The other physical barrier I observed was the intensely cultivated agricultural zone. In Iowa, bobcats prefer patches of forest, and to a lesser extent grasslands, while row crops are avoided (Linde 2010; Tucker et al. 2008). Nielsen and Woolf (2002) found similar patterns in southern Illinois. These habitat selection data suggest individuals may be less prone to disperse across agricultural fields. Indeed, Gosselink et al. (2011) found that bobcats in southern Iowa frequently dispersed in an east-west direction or to the south, but rarely dispersed north into landscapes dominated by row crop agriculture. Similarly, Johnson et al. (2010) found that dispersal movements of radio-collared bobcats in south-central Indiana were directionally biased to the south and east, thereby avoiding intensive row cropping. Supporting the idea that the Corn Belt functions as a dispersal barrier, I found that populations on either side were significantly differentiated, and I did not observe a single bobcat in the Northern or Michigan populations with a compelling (support from both microsatellite and mtDNA) genetic signature of a southern population, and vice-versa. Although two individuals in southern Arkansas were assigned (q ≥ 0.75) to the Northern population based on microsatellite data, both possessed haplotype H3 – absent from the
Northern population and common to the Southeastern population in which they were located – suggesting they likely were not migrants, but could potentially be the offspring of earlier migrants or unsanctioned translocations.

Cryptic Barriers. Within contiguous bobcat populations south of the Corn Belt, I observed two genetic boundaries at locations with no obvious gene flow barriers. The first genetic boundary, however, cuts diagonally across Missouri and coincides nearly perfectly with a shift between two major ecoregions: the Great Plains (grasslands with little forest) and the Eastern Temperate Forest (relatively dense and diverse forest cover) (Fig. S7). Natal-habitat biased dispersal, whereby individuals preferentially disperse into habitat similar to that in which they were born, may be a possible mechanism responsible for this pattern (Davis & Stamps 2004). Thus, ecological distinctions such as differences in prey type and cover habitat may be important in separating populations along ecotones. Although bobcats do not form social groups, which might facilitate behavioral divergences among individuals from different ecotypes, offspring stay with their mothers for an extended period of time (9 months to 2 years), learning to live and hunt within their mother’s territory (Anderson & Lovallo 2003) and perhaps developing preferences for prey and habitat.

A similar phenomenon may be working to separate Western bobcats from the Central bobcats, coinciding with a shift between the cooler northern and warmer southern Great Plains ecoregions in central Nebraska (Fig. S7). This pattern is less clear, however, as the Western population seems to extend below the ecotone into western Kansas and Oklahoma. Additional samples will be needed from these states and areas to the south and west in order to clarify patterns at the edge of our study area. Furthermore, although the observed genetic patterns suggest sharp changes in habitat type may play a major role in promoting and
maintaining genetic subdivision among seemingly continuous populations, this hypothesis should be further explored, for example, by focusing radiotelemetry studies along ecotones to test whether dispersal movements are biased towards natal-habitat type.

*Recolonization of bobcats*

Bobcats in Iowa and northwestern Missouri show close affinity to bobcats from Kansas and southern Nebraska. Nearly all (677 of 724) individuals in these recolonized areas were assigned (q ≥ 0.75) to the Central population, 19 individuals located in eastern Iowa and Missouri were assigned to the Southeastern population, and the remaining individuals had admixed proportions. Thus, bobcats in Iowa and northern Missouri have primarily reestablished themselves from the west/southwest, where the habitat is more similar (Fig. S7). This southwest connection is consistent with reports that bobcat first reappeared in western Missouri, then extended into northwestern Missouri, and later into the northeastern portion of the state (Schwartz & Schwartz 2001). There appears to be little genetic connectivity between Iowa and neighboring states to the north, including Wisconsin, Minnesota, and the Dakotas, indicating the core of the Corn Belt functions as a major barrier to dispersal and influences the recolonization process of bobcats into the region. It is also interesting that the established bobcat population in the Ozarks of southern Missouri has not contributed much to expansion into northern Missouri, perhaps due to the differences in habitat types between the two areas.

Given the genetic assignments of the 23 individuals within the Corn Belt, it appears bobcats may be moving into the unoccupied area from both fronts, as individuals were assigned to populations north and south of the Corn Belt. Although it appears bobcats are
capable of dispersing to these areas, a better understanding of the habitat suitability throughout the Corn Belt will be necessary to predict expansion, since the habitat they encounter may not be suitable to support self-sustaining populations (Linde 2010).

Furthermore, development of models predicting bobcat movement paths from potential source populations (e.g., least-cost paths) would also be beneficial to highlight dispersal corridors important for the recolonization process. Now that I have characterized the regional genetic structure, this information can be used to identify the population of origin of future bobcats caught in the Corn Belt, giving us greater insight into how bobcats are recolonizing this area and aiding in the conservation of this species.

Broader Implications

In this study, I examined the genetic structure of a widespread mammal over a spatial scale rarely examined using individual-based methods (but see Tammeleht et al. 2010). Though data-intensive, such an approach can contribute to our understanding of factors influencing gene flow in these organisms. My results indicate that factors such as spatial distance, ecological variation, and topographic barriers can lead to genetic structure even among contiguous populations of a highly mobile species.

These findings and others suggest the potential for genetic panmixia due to high mobility has likely been overestimated for many carnivores (Geffen et al. 2004; Rueness et al. 2003b; Sacks et al. 2004). Although researchers often highlight maximum dispersal distances to support the expectation of genetic panmixia, most individuals generally move much shorter distances and not all individuals disperse. For example, Johnson et al. (2010)
found that only one out of five radiocollared juvenile female bobcats left its natal range, and although all 11 juvenile males dispersed, distances varied considerably.

In addition, recent studies also suggest significant phenotypic and genetic differentiation can develop among widespread, mobile canids, ungulates, and felids found in different habitat types or climatic zones (Carmichael et al. 2001; Geffen et al. 2004; Pease et al. 2009; Pilot et al. 2006; Rueness et al. 2003b; Sacks et al. 2004; Stenseth et al. 2004). Proposed mechanisms range from natal-habitat-biased dispersal, perhaps stemming from development of hunting/foraging strategies specific to local habitat and food types, to spatial differences in seasonality and timing of mating and reproduction. Given the accumulating evidence, ecological and environmental variation may play a more prominent role in shaping fine – and even broad-scale – patterns of genetic variation than previously realized.

Landscape effects are primarily expected in species that are habitat-specialists and demonstrate low mobility, since gaps between suitable patches would be difficult, if not impossible, to cross. But landscape features do not necessarily need to operate as impenetrable barriers to exert significant influence on population structure. Recent studies have found that water bodies (Millions & Swanson 2007), mountain ranges (Rueness et al. 2003b), and roadways (Riley et al. 2006) can restrict gene flow even in highly mobile carnivores, and I found that an intense agricultural zone functions as a dispersal barrier in bobcats. Farming landscapes have been shown to restrict dispersal in some species at finer-scales (Schwartz et al. 2006), but the American Midwest is unique in the sheer magnitude of the scale involved. I show that even a highly vagile species does not readily disperse through this intensively row-cropped region, so the Corn Belt likely functions as a major barrier for many species.
Together with other recent findings, this study supports the notion that space and habitat heterogeneity play an important role in structuring even mobile, widely dispersing organisms. This has clear implications for the population connectivity and recolonization process of not only bobcats, but other predators attempting to regain former habitat in the Midwest and elsewhere. By improving our understanding of the mechanisms controlling gene flow in these ecologically and economically important species, we will be better equipped to facilitate the recolonization process and evaluate the impact of landscape changes on ecological dynamics, evolutionary processes, and species persistence.

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Young SP (1958) *The bobcat of North America*. Wildlife Management Institute, Washington, DC.

**Figure Legends**

**Fig. 1.** Map of study area showing locations of bobcat samples (*n* = 1447), color coded according to assignment in 1 of 6 genetic populations identified by BAPS analysis. Asterisks indicate admixed individuals with <75% of their ancestry estimated to be from a single genetic population. Map also shows current harvest zones, with protected areas generally reflecting regions of bobcat absence or rarity. Upper map shows the approximate distribution of bobcats in the 1970s, indicating the extirpation of the species from Iowa and portions of several other states.

**Fig. 2.** Plots of Structure log-likelihood values and the $\Delta K$ measure at each *K* for: (a) all samples; (b) samples assigned to cluster one (Iowa and surrounding areas) in the hierarchical analysis; (c) samples assigned to cluster two in the hierarchical analysis; (d) all samples using only the 755 selected samples to update allele frequencies. Note the axes have different scales.
**Fig. 3.** (a) Median-joining network illustrating relationships among mtDNA haplotypes found in 210 bobcat samples. Each circle represents a unique haplotype, with size proportional to frequency. Small white circles represent missing or ancestral haplotypes not present in the data set. Lines connecting haplotypes are one mutation long unless otherwise denoted by the number of hash marks. Numbers are the haplotype names. (b) Map showing the spatial distribution of haplotypes, color coded as in (a). The circles represent Clade 1 haplotypes, while triangles designate Clade 2 haplotypes.

**Fig. S1.** Map of 23 individuals located in core of the Corn Belt region. Individuals are shown as a pie chart, indicating proportion of ancestry from each of 6 genetic clusters as determined by BAPS analysis. The mtDNA haplotype ID is provided next to the sample. The IDs labeled in black = males, burgundy = females, and gray = unknown, according to results of a PCR test developed specifically for felids based on Y-chromosome deletions in the amelogenin region (Pilgrim *et al.* 2005).

**Fig. S2.** Map of study area showing locations of bobcat samples (*n* = 1447), color coded according to assignment in 1 of 6 genetic populations identified by hierarchical Structure analysis. Asterisks indicate admixed individuals with <75% of their ancestry estimated to be from a single genetic population.

**Fig. S3.** Map of study area showing locations of bobcat samples (*n* = 1447), color coded according to assignment in 1 of 5 genetic populations identified by Structure analysis using only a subset of 755 samples to update allele frequencies (PFROMPOPFLAGONLY option).
Asterisks indicate admixed individuals with <75% of their ancestry estimated to be from a single genetic population.

**Fig. S4.** Map of study area showing locations of bobcat samples \((n = 755)\), color coded according to assignment in 1 of 6 genetic populations identified by BAPS analysis. Asterisks indicate admixed individuals with <75% of their ancestry estimated to be from a single genetic population.

**Fig. S5.** Plots of \(ln\)-transformed geographic distance vs. linear genetic distance for the total population and for each of 6 subpopulations inferred from BAPS analyses.

**Fig. S6.** (a) Median-joining network illustrating relationships among mtDNA haplotypes found in 210 bobcat samples. Unique haplotypes are displayed as pie charts, with different colors reflecting the proportion of samples from each genetic cluster based on BAPS \((K6)\) analysis. Size of the pie is proportional to haplotype prevalence. Small white circles represent missing or ancestral haplotypes not present in the data set. Lines connecting haplotypes are one mutation long unless otherwise denoted by the number of hash marks. Numbers are the haplotype identifications. (b) Map showing the locations of the 210 samples that were sequenced, color coded according to assignment in 1 of 6 genetic populations identified by BAPS analysis.

**Fig. S7.** Map of study area showing Level II ecological regions of North America (Commission for Environmental Cooperation 1997) and bobcat samples color coded
according to BAPS assignments. The red lines indicate transition zones that seem to coincide with cryptic genetic boundaries in a continuous bobcat population.
Table 1. Properties of the 19 microsatellite loci used in this study. Summary statistics are for the entire data set ($n = 1447$). Di: dinucleotide; Tetra: tetranucleotide; Set: loci simultaneously electrophoresed; N: number of samples successfully analyzed.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Repeat motif</th>
<th>Set</th>
<th>Annealing temp. (°C)</th>
<th>No. cycles</th>
<th>Dye</th>
<th>N</th>
<th>Allele size range (bp)</th>
<th>No. alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lc110</td>
<td>Di</td>
<td>1</td>
<td>48</td>
<td>30</td>
<td>HEX</td>
<td>1422</td>
<td>92-104</td>
<td>10</td>
</tr>
<tr>
<td>FCA 043</td>
<td>Di</td>
<td>1</td>
<td>50</td>
<td>30</td>
<td>6-FAM</td>
<td>1438</td>
<td>130-142</td>
<td>7</td>
</tr>
<tr>
<td>FCA 023</td>
<td>Di</td>
<td>1</td>
<td>50</td>
<td>30</td>
<td>HEX</td>
<td>1418</td>
<td>151-163</td>
<td>7</td>
</tr>
<tr>
<td>Lc109</td>
<td>Di</td>
<td>1</td>
<td>48</td>
<td>35</td>
<td>6-FAM</td>
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<td>180-204</td>
<td>13</td>
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<tr>
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<td>35</td>
<td>HEX</td>
<td>1409</td>
<td>206-236</td>
<td>10</td>
</tr>
<tr>
<td>FCA 008</td>
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<td>50</td>
<td>30</td>
<td>6-FAM</td>
<td>1439</td>
<td>132-172</td>
<td>11</td>
</tr>
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<td>50</td>
<td>35</td>
<td>HEX</td>
<td>1411</td>
<td>150-178</td>
<td>8</td>
</tr>
<tr>
<td>BCE5T</td>
<td>Tetra</td>
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<td>50</td>
<td>35</td>
<td>6-FAM</td>
<td>1401</td>
<td>257-318</td>
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<td>HEX</td>
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<td>330-362</td>
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<td>3</td>
<td>50</td>
<td>30</td>
<td>6-FAM</td>
<td>1423</td>
<td>117-129</td>
<td>7</td>
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<td>Di</td>
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<td>50</td>
<td>35</td>
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<tr>
<td>Lc111</td>
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<td>48</td>
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<td>1422</td>
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<td>14</td>
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<td>50</td>
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<td>6-FAM</td>
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<td>6-FAM</td>
<td>1416</td>
<td>105-141</td>
<td>10</td>
</tr>
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<td>Di</td>
<td>4</td>
<td>47</td>
<td>32</td>
<td>HEX</td>
<td>1429</td>
<td>141-169</td>
<td>15</td>
</tr>
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<td>148-168</td>
<td>10</td>
</tr>
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<td>32</td>
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<td>1378</td>
<td>182-198</td>
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<td>47</td>
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<td>6-FAM</td>
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<td>246-268</td>
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</table>
Table 2. Summary of microsatellite and mtDNA genetic variation for six bobcat populations inferred from cluster analysis.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>AM</th>
<th>H_O</th>
<th>H_E</th>
<th>F(IS)</th>
<th>AR</th>
<th>N</th>
<th>AM</th>
<th>H_O</th>
<th>H_E</th>
<th>F(IS)</th>
<th>AR</th>
<th>N</th>
<th>#H</th>
<th>h (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1447</td>
<td>221</td>
<td>0.73</td>
<td>0.77</td>
<td>0.05</td>
<td>10.5</td>
<td>1447</td>
<td>78</td>
<td>0.73</td>
<td>0.77</td>
<td>0.05</td>
<td>10.5</td>
<td>210</td>
<td>21</td>
<td>0.8629 (0.0112)</td>
</tr>
<tr>
<td>Central</td>
<td>873</td>
<td>71</td>
<td>0.73</td>
<td>0.74</td>
<td>0.02</td>
<td>6.6</td>
<td>880</td>
<td>37</td>
<td>0.73</td>
<td>0.74</td>
<td>0.01</td>
<td>6.6</td>
<td>55</td>
<td>8</td>
<td>0.7131 (0.0475)</td>
</tr>
<tr>
<td>Western</td>
<td>99</td>
<td>18</td>
<td>0.74</td>
<td>0.79</td>
<td>0.07</td>
<td>7.4</td>
<td>99</td>
<td>10</td>
<td>0.73</td>
<td>0.79</td>
<td>0.08</td>
<td>7.4</td>
<td>35</td>
<td>8</td>
<td>0.7765 (0.0464)</td>
</tr>
<tr>
<td>Northern</td>
<td>169</td>
<td>32</td>
<td>0.72</td>
<td>0.75</td>
<td>0.04</td>
<td>6.3</td>
<td>163</td>
<td>17</td>
<td>0.72</td>
<td>0.75</td>
<td>0.04</td>
<td>6.3</td>
<td>40</td>
<td>7</td>
<td>0.4718 (0.0955)</td>
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<tr>
<td>Michigan</td>
<td>27</td>
<td>2</td>
<td>0.65</td>
<td>0.66</td>
<td>0.00</td>
<td>5.0</td>
<td>27</td>
<td>2</td>
<td>0.65</td>
<td>0.66</td>
<td>0.00</td>
<td>5.0</td>
<td>19</td>
<td>3</td>
<td>0.3743 (0.1296)</td>
</tr>
<tr>
<td>Southeastern</td>
<td>227</td>
<td>84</td>
<td>0.74</td>
<td>0.76</td>
<td>0.03</td>
<td>7.1</td>
<td>227</td>
<td>6</td>
<td>0.74</td>
<td>0.76</td>
<td>0.04</td>
<td>7.1</td>
<td>46</td>
<td>9</td>
<td>0.8116 (0.0327)</td>
</tr>
<tr>
<td>Indiana</td>
<td>52</td>
<td>14</td>
<td>0.64</td>
<td>0.62</td>
<td>-0.03</td>
<td>4.8</td>
<td>51</td>
<td>6</td>
<td>0.63</td>
<td>0.62</td>
<td>-0.03</td>
<td>4.7</td>
<td>15</td>
<td>2</td>
<td>0.1333 (0.1123)</td>
</tr>
</tbody>
</table>

N: number of individuals; AM: number of admixed individuals (q < 0.75); H_O: observed heterozygosity; H_E: gene diversity; F(IS) values calculated after Weir & Cockerham (1984); AR: allelic richness (adjusted to the smallest sample size); #H: number of haplotypes; h: haplotype diversity; SD: standard deviation.
Table 3. Population pairwise F_{ST} values for the microsatellite Structure analysis (below the diagonal) and BAPS analysis (above the diagonal) for each of the six (top) and five (bottom) population scenarios. For the Indiana comparisons, values to the right of the slash reflect comparisons made with potential relatives (r ≥ 0.45) removed from the population. All comparisons were statistically significant (P < 0.0001) based on 10,000 permutations.

<table>
<thead>
<tr>
<th>K</th>
<th>Population</th>
<th>Central</th>
<th>Western</th>
<th>Northern</th>
<th>Michigan</th>
<th>Southeastern</th>
<th>Indiana</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Central</td>
<td>-</td>
<td>0.051</td>
<td>0.047</td>
<td>0.090</td>
<td>0.035</td>
<td>0.119/0.101</td>
</tr>
<tr>
<td></td>
<td>Western</td>
<td>0.051</td>
<td>-</td>
<td>0.052</td>
<td>0.111</td>
<td>0.040</td>
<td>0.137/0.116</td>
</tr>
<tr>
<td></td>
<td>Northern</td>
<td>0.047</td>
<td>0.047</td>
<td>-</td>
<td>0.089</td>
<td>0.024</td>
<td>0.121/0.102</td>
</tr>
<tr>
<td></td>
<td>Michigan</td>
<td>0.091</td>
<td>0.108</td>
<td>0.089</td>
<td>-</td>
<td>0.076</td>
<td>0.153/0.129</td>
</tr>
<tr>
<td></td>
<td>Southeastern</td>
<td>0.035</td>
<td>0.037</td>
<td>0.025</td>
<td>0.075</td>
<td>-</td>
<td>0.082/0.062</td>
</tr>
<tr>
<td></td>
<td>Indiana</td>
<td>0.118/0.100</td>
<td>0.130/0.109</td>
<td>0.119/0.099</td>
<td>0.151/0.127</td>
<td>0.080/0.059</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Central</td>
<td>-</td>
<td>0.050</td>
<td>0.047</td>
<td>0.089</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Western</td>
<td>0.048</td>
<td>-</td>
<td>0.050</td>
<td>0.108</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Northern</td>
<td>0.047</td>
<td>0.045</td>
<td>-</td>
<td>0.092</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Michigan</td>
<td>0.085</td>
<td>0.101</td>
<td>0.085</td>
<td>-</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Southeastern</td>
<td>0.043</td>
<td>0.044</td>
<td>0.032</td>
<td>0.072</td>
<td>-</td>
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Table 4. Population pairwise Φ_{ST} values based on the mtDNA data. All comparisons were statistically significant \((P < 0.0001)\) except Northern – Michigan \((P > 0.05)\).

<table>
<thead>
<tr>
<th>Population</th>
<th>Central</th>
<th>Western</th>
<th>Northern</th>
<th>Michigan</th>
<th>Southeastern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western</td>
<td>0.329</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Northern</td>
<td>0.423</td>
<td>0.483</td>
<td>-</td>
<td>0.009</td>
<td>-</td>
</tr>
<tr>
<td>Michigan</td>
<td>0.399</td>
<td>0.391</td>
<td>0.009</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Southeastern</td>
<td>0.090</td>
<td>0.350</td>
<td>0.225</td>
<td>0.217</td>
<td>-</td>
</tr>
<tr>
<td>Indiana</td>
<td>0.546</td>
<td>0.462</td>
<td>0.586</td>
<td>0.602</td>
<td>0.426</td>
</tr>
</tbody>
</table>
Fig. 1
Fig. 2
Fig. S1
Fig. S4
Fig. S5
Fig. S7
CHAPTER 4. CONTINENTAL-SCALE PATTERNS OF GENETIC VARIATION IN
THE CONTINUOUSLY DISTRIBUTED BOBCAT (*Lynx rufus*)

A paper to be submitted to *Molecular Ecology*

Dawn M. Reding

**Abstract**

From both an evolutionary and ecological standpoint, an intriguing issue is how population structure and subspecific divergence can arise in widespread, mobile species, particularly when geographic isolation is not an obvious factor. The bobcat is one of the most common and broadly-distributed mammals in North America. As a habitat-generalist capable of dispersing long distances and exhibiting only subtle morphological variation across its range, minimal genetic differentiation is predicted in this species. However, the patterns and mechanisms of genetic population structure, and the relevance of morphologically-based subspecies designations, remain largely unexplored for many abundant and widespread organisms such as the bobcat. In this study, I sampled over 1700 geo-referenced bobcats collected from throughout the majority of its range, including 9 of the 12 recognized subspecies, and assessed genetic variation at 15 microsatellite loci and approximately 1 KB of mtDNA sequence. Complex genetic patterns emerge, but the primary signature involves a longitudinal cline with a transition, or suture zone, occurring along the Great Plains in the central U.S. and distinguishing bobcats in the eastern part of the country from those in the western half. The divergence is evident in both marker types, and supported by multiple statistical approaches including spatial principal components analysis, Bayesian clustering analysis, distance-based redundancy analysis, and haplotype network
construction. Demographic evidence, including significantly negative $F_s$ values and unimodal mismatch distributions, support a scenario of post-glacial expansion from two disjunct Pleistocene refugia, which likely were separated by the aridification of the Great Plains grasslands during interglacial periods. Although genetic patterns were loosely congruent with most subspecific designations, results support only two historically independent units: eastern and western bobcats. Collectively, the data indicate that despite the bobcat’s mobility and broad niche, population genetic structure is evident and characterized by complex combinations of clines, clusters, and isolation-by-distance. Results implicate historical processes as the primary cause of the observed continental-scale genetic patterns (i.e., post-glacial expansion from Pleistocene refugia), though environmental and ecological forces may have played a role in promoting and maintaining the structure.

**Introduction**

In both terrestrial and aquatic environments, one can find numerous species that are highly mobile and distributed continuously across their ranges. Without geographic or habitat barriers to dispersal, unimpeded gene flow should limit the development of population genetic structure and result in either (1) a genetically panmictic population, when dispersal is unbounded relative to the species’ range and mating occurs at random (e.g., Als et al. 2011), or more commonly (2) a simple pattern of isolation-by-distance, when dispersal is local and mating occurs more frequently among neighbors (e.g., Platt et al. 2010). In North America, mammals such as striped skunk (*Mephitis mephitis*), raccoon (*Procyon lotor*), long-tailed weasel (*Mustela frenata*), gray fox (*Urocyon cinereoargenteus*), bobcat (*Lynx rufus*), etc. are mobile, habitat-generalists that occur across much of the continent with
essentially continuous ranges (Feldhammer et al. 2003). Many of these taxa, however, are subdivided into subspecies based on regional differences in physical appearance (Hall 1981). Such taxonomic distinctions imply that discrete subpopulations exist, but in many cases, we do not know whether subspecific designations actually reflect intraspecific genetic structure (Brown et al. 2007) or rather a plastic response to varying environmental conditions (Edwards et al. 2011).

Whereas many population genetic studies have concentrated on species of conservation concern (Frankham et al. 2002), which often are specialists with restricted or fragmented distributions (Fisher & Owens 2004), the patterns and mechanisms of genetic structure in abundant and widespread organisms have received less attention. This is a sizeable knowledge gap, not only in regards to these ecologically and often economically important species, but to our general understanding of whether and how differentiation can emerge without the overriding element of geographic isolation (i.e., development of cryptic population structure). Several recent studies provide evidence that environmental and behavioral mechanisms (e.g., natal-habitat-biased dispersal, prey preferences, development of specific hunting strategies, temporal mismatch in breeding due to seasonality) may drive ecologically-based niche divergence among groups of individuals and lead to discrete genetic subdivision within continuously-distributed species (Carmichael et al. 2001, 2007; Geffen et al. 2004; Sacks et al. 2004, 2005, 2008; Pilot et al. 2006; Boulet et al. 2007; Möller et al. 2007; Musiani et al. 2007; Pease et al. 2009). These observations are somewhat limited, either by a regional scope or focus on social organisms (social groups likely facilitate behavioral divergences), and further work is needed to identify whether the phenomenon of ecological divergence applies more broadly to other species in different areas. In addition, at
continental scales, historical forces such as glaciation events can leave a lasting mark on current population genetic structure, though such an influence should be greater in species with boreal distributions (Runck & Cook 2005), or species that specialize on certain habitat types (i.e., forests or deserts) that may have been isolated during the Pleistocene (Wooding & Ward 1997; Rowe et al. 2006).

Bobcats are one of the most common and broadly-distributed species in North America, ranging from southern Canada to central Mexico and from California to Maine (Fig. 1) (Anderson & Lovallo 2003). These medium-sized felids are consummate habitat-generalists, thriving in a wide range of environments including deserts, coniferous and deciduous forests, subtropical wetlands, and prairie-woodland mixes. Though strict carnivores, their diet is diverse. In general, lagomorphs and small rodents are the primary prey items across its range, but bobcats are also known to consume deer, other carnivores, domestic animals, birds, reptiles, fish, and insects (Larivière & Walton 1997). Behaviorally, bobcats are solitary, territorial, and have a polygynous mating system (McCord & Cardoza 1982). They are capable of dispersing long distances (>150 km) (Knick & Bailey 1986; Johnson et al. 2010; Gosselink et al. 2011), though dispersal distances vary greatly among individuals and study areas (Anderson & Lovallo 2003). Like many mammals (Greenwood 1980), dispersal is generally male-biased with females exhibiting a stronger pattern of philopatric behavior (Janečka et al. 2007; Croteau et al. 2010; Johnson et al. 2010), but both sexes are capable of long-distance dispersal (Kamler et al. 2000). Despite anthropogenic changes across North America, bobcats have retained the vast majority of their original range and now appear to be increasing and expanding into once-extirpated areas such as portions of the agricultural Midwest and heavily populated mid-Atlantic states (Deems & Pursley 1978;
Woolf & Hubert 1998; Lovallo 2001; Roberts & Crimmins 2010). Based on government surveys, Roberts & Crimmins (2010) estimate the total U.S. bobcat population at approximately 2-3 million individuals, and 38 states allow harvest of this valuable furbearer (United States Government 2010). The species was also apparently abundant in the past, as it is commonly found in Pleistocene fossil deposits (Graham & Lundelius 2010).

Collectively, these characteristics – habitat and prey generalist, solitary, long-distance disperser, currently and historically abundant and widespread – predict little genetic differentiation across the range of this species. Morphological examinations of specimens, however, have detected regional differences in body size, cranial morphology, and pelage color and markings (Peterson & Downing 1952; Hall & Kelson 1959; Samson 1979; Read 1981; Sikes & Kennedy 1992; Wigginton & Dobson 1999). To account for such regional variation, Hall (1981) delineated 12 subspecies (Fig. 1). Perhaps coincidentally, subspecific boundaries often correspond with transitions between major ecological regions (i.e., ecotones) or steep clines in climatic variables, suggesting environmental and ecological factors may work to differentiate this species. No broad-scale, comprehensive genetic study has been conducted to confirm whether patterns of genetic differentiation support the subspecific designations (but see Croteau 2009 and Reding 2011).

In this study, I present a population genetic analysis of the bobcat across the entire United States, its primary range. My goal was to quantitatively assess the spatial genetic patterns in this species to test whether it exhibits a simple pattern of isolation-by-distance, or whether more complex patterns emerge from ecological or historical processes. To achieve this goal, I sampled over 1700 geo-referenced individuals collected from throughout the majority of the bobcat range, including 9 of the 12 recognized subspecies, and assessed
genetic variation at 15 microsatellite loci and ~ 1 KB of mtDNA sequence using both individual and population-based analyses. I quantify how well subspecies designations explain the genetic variation compared to other potential groupings. I also test for environmental correlates, including elevation and several temperature and precipitation measurements, to patterns of genetic structure. In addition, I perform several demographic analyses to investigate potential influences of historical processes, such as Pleistocene glaciation events, on current genetic patterns. Through this continental-scale survey, I provide insight into the potential mechanisms involved in establishing and maintaining population divergence in this common North American carnivore.

Methods

Sample collection

The sample set consists of tissue and DNA (n = 1704) from live and dead bobcats collected between 1994-2011 (Fig. 1). These samples include data from a stratified random subset of those used in regional examinations in Oregon (n = 108) (Reding et al. 2011) and the Midwest (n = 755) (Reding 2011), as well as additional samples obtained and analyzed specifically for this study (n = 841). In total, the sample represents 45 states and includes 5 samples from Mexico. Given the scope of the study area, sampling was accomplished in large part through contributions from state and federal agencies, several researchers, and private hunters/trappers. In addition, I collected samples from bobcat pelts at North America Fur Auctions (NAFA) and other fur dealers, making use of the fact that bobcats are CITES regulated (United States Government 2010) and require documentation for sale and transport. In many cases, I was able to use state-specific tag numbers on the pelts to track information
collected by state agencies, including date of harvest and location information such as township, county, or game management unit. Although sampling locations for some of these pelts are not extremely precise (county, management unit, or region of state; \( n = 772 \)), and there is a chance of occasional reporting errors in the locations (either intentionally or accidentally), I contend overall errors are minimal given the large sample size and spatial extent of the study area. Furthermore, many of the samples are associated with precise geographical coordinates or other fine-scale location data (\( n = 517 \)).

In processing location data, I used MapSource 4.07 (Garmin) to translate place-names to spatial coordinates. If a polygon (e.g., county, township, section) was provided as the location, I used ArcGIS 9.3 (ESRI) to calculate the mean center of the polygon, which I used as the spatial coordinates. If multiple individuals were collected at a given location, I used ArcGIS and the HawthsTools extension (Beyer 2004) to either: 1) construct a 500-m buffer around the place-name point and located the samples at unique, random locations within the buffer; or 2) locate the samples at unique, random locations within the polygon. I performed the relocations to visualize sample points on maps and to use spatial models requiring unique coordinates for each sample point. The Mexico samples did not have location data and thus were assigned spatial coordinates for visualization purposes only and omitted from spatial analyses. All coordinates were projected in the USA Contiguous Albers Equal Area Conic USGS coordinate system.

Laboratory analysis

Procedures for data acquisition for the Oregon and Midwest samples follow Reding et al. (2011) and Reding 2011, respectively. For new tissue samples, I extracted DNA using
DNeasy (Qiagen) or IDPure (IDLabs) purification kits and genotyped individuals at 15 autosomal microsatellite markers developed from domestic cat (*Felis catus*): FCA008, FCA031, FCA043, FCA077, FCA082, FCA090, FCA096, FCA132, FCA149, FCA391, FCA559 (Menotti-Raymond *et al.* 1999), and FCA740 (Menotti-Raymond *et al.* 2005); Canada lynx (*Lynx canadensis*): Lc109 and Lc111 (Carmichael *et al.* 2000); and bobcat: BCE5T (Faircloth *et al.* 2005). Each locus was amplified separately using the M13-tailed primer method (Boutin-Ganache *et al.* 2001). The total PCR volume was 10 µl, including 1× PCR buffer with 2 mM MgSO₄ (IDLabs), 0.2 mM dNTPs, 0.3 µM fluorescently labeled M13 primer, 0.3 µM reverse primer, 0.02 µM M13-tailed forward primer, 0.4 U IDPROOF DNA Polymerase (IDLabs), and 10-20 ng of template DNA. The PCR profile was 95 °C/5 min, (95 °C/20 s, X °C/20 s, 72 °C/30 s) × X cycles, 72 °C/20 min (Table 1). I combined analysis of markers into 4 gel sets, each consisting of 3 to 5 loci (Table 1). The samples were analyzed on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, California) at Iowa State University’s DNA Facility. Alleles were scored using the software Genemapper 4.0 (Applied Biosystems).

I amplified a 949 bp portion of the mtDNA NADH dehydrogenase subunit 5 (ND5) gene using primers I designed, ND5-DR1F (5’-TCATCCCCGTAGCACTTTTC-3’) and ND5-DR3R (5’-AAGGGATGTGCAATGAGAG-3’). Total PCR volume was 10 µl, including 1× PCR buffer with 2 mM MgSO₄, 0.2 mM dNTPs, 0.3 µM each primer, 0.4 U DNA Polymerase, and 10-20 ng of template DNA. The PCR profile was 95 °C/5 min, (94 °C/30 s, 57 °C/45 s, 72 °C/45 s) × 30 cycles, 72 °C/10 min. PCR products were cleaned using the ExoSAP method (Werle *et al.* 1994) and submitted to the Iowa State University
DNA Facility for cycle sequencing and analysis on an ABI 3730xl DNA Analyzer. Both directions were sequenced with the same primers used for PCR.

**Microsatellite data analysis: standard estimates for total sample**

For all data analysis procedures using the microsatellite data, I omitted samples with fewer than 8 loci genotyped, as well as 2 New York individuals suspected to be bobcat-lynx hybrids based on mtDNA sequence (see Results). With the remaining samples \(n = 1680\), I calculated for each locus the total number of alleles, observed \(H_O\) and expected \(H_E\) heterozygosity, and Weir & Cockerham’s (1984) \(F_{IS}\), and tested for deviations from Hardy-Weinberg (HW) and linkage equilibrium using Genepop 4.0 (Rousset 2008). To account for multiple tests, I applied the sequential Bonferroni correction (Rice 1989) to significance values (nominal \(\alpha = 0.05\)).

To test for an overall positive relationship between geographic and genetic distance across the study area, I used Genalex 6.41 (Peakall & Smouse 2006) to calculate pairwise genetic distance (Smouse & Peakall 1999) and pairwise geographic distance (km, \(\ln\)-transformed) between all sample pairs and applied a Mantel test (Mantel 1967) with 999 permutations.

**Spatial principal components analysis**

Multivariate analyses, such as principal components analysis, offer several advantages for genetic analysis in that they do not rely on assumptions of HW and linkage equilibrium, and they can reveal genetic clines as well as discrete populations (Jombart et al. 2009). I performed spatial principal components analysis (sPCA) on the microsatellite data
to examine genetic structure. This reduced space ordination method, developed by Jombart et al. (2008), takes into account not only the genetic variation between individuals, but also their spatial autocorrelation, by finding synthetic variables that optimize the product of allele frequency variance and Moran’s $I$, an index of spatial autocorrelation. Highly positive eigenvalues, therefore, reflect axes with both a large variance and global (i.e., positive spatial autocorrelation) structure. I used the adegenet package (Jombart 2008) of the software R (R Development Core Team) to perform sPCA as well as a permutation procedure to test for a significant global pattern in the data. I used a distance-based connection network such that individuals separated by $\leq$ 250 km (which resulted in all samples having at least one connection) were considered neighbors, though experimentation with other distances and connection networks yielded concordant results (data not shown).

Bayesian clustering analysis

Geographic patterns of genetic structure can often entail complex combinations of clines, clusters, and patterns of isolation-by-distance (Francois & Durand 2010), and multiple analysis methods can provide complimentary information regarding such patterns (Balkenhol et al. 2009; Safner et al. 2011). Thus, as another means of exploring patterns of genetic structure, I employed both spatial and aspatial Bayesian clustering methods using the programs BAPS 5 (Corander et al. 2008) and Structure 2.3.3 (Pritchard et al. 2000), respectively. Both operate by minimizing the linkage and Hardy-Weinberg disequilibria that would result if individuals from separate populations were incorrectly grouped into a common population (Latch et al. 2006). However, the prior for the clustering is uniform (all solutions equally likely) in Structure, whereas the prior includes spatial dependence in BAPS.
Thus, in Structure, “populations” do not have to be geographically congruent, while in BAPS, since coordinates of individuals are included in the model, partitions that are geographically congruent are favored. The spatial approach can strengthen the ability to detect weak structure, but if the molecular data are overwhelming, the spatial and aspatial methods should give similar results (Corander et al. 2008).

In BAPS, I first performed a mixture analysis using the spatial clustering of individuals model. The analysis consisted of 5 iterations of each value of $K_{\text{max}}$ (the maximum number of populations) for the range $K_{\text{max}} = 1-20$. This step determines the optimum number of genetic clusters in the sample based on the partition with the maximum likelihood ($L(K)$) and highest posterior probability ($p$), and then assigns each individual to a cluster. In the second step, I performed an admixture analysis conditional on the assignments from the previous step. I used 500 simulations from the observed allele frequencies to estimate admixture coefficients ($q$), which provide the proportion of an individual’s genotype attributed to each of the identified populations.

To provide support for the clusters inferred from the spatial method, I performed Structure analysis with 10 independent runs at each value of the fixed parameter $K$ (the number of populations), from $K = 1-20$. Each run consisted of 300,000 replicates of MCMC following a burn-in of 100,000 replicates. I used the admixture model and allowed the allele frequencies to be correlated among populations (Falush et al. 2003). I then used Structure Harvester (Earl 2011) to collate data from the multiple runs and to: 1) calculate the estimated posterior probability of the data, $\ln P(D)$, for each $K$, averaged across the 10 runs; 2) calculate the $\Delta K$ statistic, which is based on the rate of change in $\ln P(D)$ between successive $K$ values (Evanno et al. 2005); and 3) generate input files for Clumpp 1.1.2 (Jakobsson &
Rosenberg 2007). I used program Clumpp to align and average each individual’s \(q\)-values across the 10 replicate runs for each \(K\), employing the Greedy algorithm and 100 random input orders. I considered the \(\Delta K\) statistic, values of LnP(D), and biological interpretation to infer the number of genetic clusters. All Structure runs were performed on the freely available Bioportal (www.bioportal.uio.no).

**Population-level analysis based on clustering results**

I used the BAPS assignments to define putative population “boundaries” and allow for traditional population genetic analysis. For each of the inferred populations, I used all individuals for which \(q \geq 0.75\) for the given population and constructed a 90% probability distribution using a fixed kernel estimator with least squares cross validation (Worton 1989) in the Animal Movement extension (Hooge & Eichenlaub 1997) for ArcView (ESRI). I then classified all individuals (whether admixed or not) within the distribution polygon as belonging to the given population. I excluded individuals in areas of overlapping distribution polygons, with some exceptions (see Results). I then used K-means clustering in Geospatial Modeling Environment (Spatial Ecology, LLC) to break large populations into sets of “subpopulations” based on X and Y coordinates. This approach simply groups geographically proximate samples; it does not use the genetic data. I used ArcGIS 10 (ESRI) to calculate the geographic mean center of samples assigned to each subpopulation, which I used as the spatial coordinates for the given subpopulation.

For each population and subpopulation, I calculated standard genetic estimates following the same procedures outlined for the total sample. In addition, I used Fstat 2.9.3 (Goudet 2001) to calculate allelic richness (AR), corrected for differences in sample size. I
used Arlequin 3.5 (Excoffier et al. 2005) to calculate pairwise $F_{ST}$ as a measure of differentiation between the inferred populations, with significance assessed through 20,000 permutations. I also used Arlequin to conduct a Mantel test (999 permutations) to test for a positive relationship between genetic ($F_{ST}/(1-F_{ST})$) and ln-transformed geographic distance between subpopulations (Rousset 1997).

**Analyses of Molecular Variance (AMOVAs)**

To evaluate how well subspecies designations account for microsatellite genetic variation observed in bobcats, I conducted a two-level (individuals, subspecies) AMOVA in Arlequin. I compared these results to those obtained by grouping individuals by ecoregion (level 1 ecological regions of North America; Commission for Environmental Cooperation 1997), as well as by grouping individuals according to BAPS assignments from the mixture analysis.

**Environmental Correlates**

To examine whether environmental factors may be important in differentiating bobcats, I conducted a series of distance-based redundancy analyses (dbRDA), a form of multivariate multiple regression (Legendre & Anderson 1999; McArdle & Anderson 2001), to test for a relationship between genetic differentiation among individuals (dependent variable) and several geographic and climate variables (predictor variables) (Geffen et al. 2004; Pilot et al. 2006; Carmichael et al. 2007). The variable sets include: 1) X and Y coordinates (i.e., longitude and latitude); 2) elevation; 3) temperature (annual mean temperature, mean diurnal range, temperature seasonality (standard deviation *100),
maximum temperature of warmest month, minimum temperature of coldest month); and 4) precipitation (annual precipitation, precipitation seasonality (standard deviation *100), precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter). The temperature and precipitation variables represent 11 of the 19 BIOCLIM variables from WorldClim (version 1.4; Hijmans et al. 2005), interpolated to 1-km spatial resolution. The elevation data were also at 1-km resolution from WorldClim.

The relationship between the genetic distance matrix and each individual predictor variable was first analyzed separately using dbRDA marginal tests with 999 unrestricted permutations of the genetic distance matrix. I also tested the relationship to each of the four sets of variables. Next, partial dbRDA was performed for the elevation, temperature, and precipitation predictor variables (both individually and as sets), having first fit X and Y coordinates as covariables, to examine the extent to which each predictor variable explains genetic differentiation above and beyond that explained by geographic distance alone. Statistical significance was tested using 999 permutations of the multivariate residual matrix under the reduced model (Anderson & Legendre 1999). The marginal and partial dbRDA analyses were conducted in DISTLM v.5 (Anderson 2004). Finally, I performed a stepwise forward selection procedure to identify the subset of environmental variables or sets of variables that provide the best model explaining genetic differences among individuals (Anderson et al. 2004; Pilot et al. 2006; Carmichael et al. 2007). I used program DISTLM forward 1.3 (Anderson 2003), with significance assessed with 999 permutations of the multivariate residual matrix under the reduced model. Several of the environmental variables are themselves correlated, but this sequential approach allowed me to control for these
correlations since the proportion of variation explained by each variable is conditional on the variables already included in the model.

mtDNA analysis

I used Sequencher 4.6 (Gene Codes Corporation) to edit, assemble, and align sequences, and DnaSP 5.10 (Librado & Rozas 2009) to identify unique haplotypes and generate input files for several programs. I examined relationships among the haplotypes using Bayesian phylogenetic methods, with a homologous Canada lynx sequence (Genbank accession AY598481) for the outgroup. I used jModeltest (Guindon & Gascuel 2003; Posada 2008) to find the best-fitting model of evolution (HKY + G) using the Bayesian information criterion. I then used MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) to create a Bayesian tree using two independent runs of four MCMC chains running simultaneously for three million generations with trees sampled every 100 generations (total of 30,001 trees per run). I determined convergence using the average standard deviation of split frequencies, graphical output of the log probability values, and the potential scale reduction factor (PSRF). I discarded the first 20,001 trees in each run. The Bayesian consensus tree and posterior probabilities (BPP) were computed from the remaining 20,000 sampled trees from the combined runs. Since intraspecific genealogies are often not well represented by bifurcating trees (Posada & Crandall 2001), I also constructed a median joining network using program Network 4.5 (Bandelt et al. 1999). The haplotypes were nested into hierarchical clades to visualize higher-order patterns of association (Templeton & Sing 1993). I omitted two bobcats from New York that shared a lynx-like haplotype and may represent hybrids (see
Results). I used Arlequin to calculate basic estimates such as haplotype \(h\) and nucleotide \(\pi\) diversities.

To test for evidence of demographic expansion resulting from post-glacial recolonization, I used Arlequin to calculate Fu’s \(F_S\) (Fu 1997) for different phylogenetic subunits indicated by the haplotype network and phylogenetic tree. Recent demographic expansions lead to significantly negative values for Fu’s \(F_S\). I further evaluated evidence for sudden population growth in each identified clade by constructing mismatch distributions of pairwise nucleotide differences between haplotypes. Mismatch distributions are usually ragged in populations at demographic equilibrium, but unimodal in populations having experienced a recent expansion (Slatkin & Hudson 1991; Rogers & Harpending 1992). I estimated the parameters of a demographic expansion: \(\tau = 2\mu t\), \(\theta_0 = 2\mu N_0\), and \(\theta_1 = 2\mu N_1\) (Schneider & Excoffier 1999). The parameters \(\theta_0\) and \(\theta_1\) describe the population sizes before and after the expansion, while \(\tau\) reflects the time to expansion. I employed a parametric bootstrap method (1000 permutations) to obtain confidence intervals for the parameters and to test the validity of the sudden expansion model, using the sum of squared deviations (SSD) between the observed and expected mismatch distributions as the test statistic (Schneider & Excoffier 1999). I estimated the time since expansion from the equation \(t = \tau/2\mu\), where \(\tau\) is estimated from the mismatch distribution, \(\mu\) is the mutation rate for the entire sequenced region, and \(t\) is measured in units of generations. I assumed a generation time of 2.3 years for bobcats (Gosselink et al. 2011) and a mutation rate of \(1.22 \times 10^{-8}\) substitutions/site/year for the feline ND5 region (Lopez et al. 1997).

To measure genetic differentiation based on mtDNA, I used Arlequin to estimate pairwise \(\Phi_{ST}\) values between the subpopulations defined in the microsatellite analysis. I also
conducted AMOVAs to evaluate how well subspecies designations and ecoregions account for the pattern of mtDNA genetic variation observed in bobcats.

**Results**

*Standard estimates for total sample*

I expected the global data set to exhibit signs of the Wahlund effect (*i.e.*, reduction of heterozygosity caused by population structure) if population structure existed within the study area. Indeed, all 15 microsatellite markers deviated from HW equilibrium, each showing a significant deficit of heterozygotes relative to random-mating expectations (Table 1). In addition, 50 of the 105 locus pairs deviated from linkage equilibrium after sequential Bonferroni adjustments and all possible pairs had p-values < 0.05, despite the fact these loci are unlikely to be physically linked (Table 1). Thus, this non-random association of alleles may also be attributed to population structure. Results of the Mantel test (Fig. 2) indicated a significant relationship between genetic and geographic distance in the total sample ($r = 0.243; P = 0.001$).

*Spatial principal components analysis*

In sPCA, Jombart *et al.* (2008) recommends using an abrupt decrease of the eigenvalues to indicate the boundary between strong and weak structures. For this dataset, the first eigenvalue was considerably larger than the others, and the second eigenvalue also stood out, but I evaluated the first 5 positive principal components (Fig. 3). The scores from the first principal component revealed a longitudinal (east-west) cline, differentiating bobcats in the eastern U.S. from those in the western half of the country, but with genotypes in the
center of the range having less extreme scores than those nearer the coasts (Figs. 4a & 4b). This pattern exhibited a strong signal of positive spatial autocorrelation ($I = 0.778$) and represented a considerable proportion of the entire genetic variability ($\text{var} = 0.383$) (Fig. 3). The scores of the second principal component differentiated bobcats in the central U.S. from others (Figs. 4c & 4d) ($I = 0.555; \text{var} = 0.185$). Subsequent scores were more challenging to interpret (results not shown). The permutation test confirmed the existence of at least one global pattern ($P = 0.001$).

**Bayesian clustering analysis**

In the BAPS analysis, $K = 10$ was the optimal solution ($L(K) = -87041.8; p = 0.927$) (Figs. 5a & 5b). Similar to the sPCA, bobcats in eastern and western U.S. were differentiated, with a third population detected between them. BAPS recognized several additional populations in the northern U.S., and clusters of individuals in southern Florida and southern California were also identified. The 10 inferred populations include: coastal Oregon (navy), Western (yellow), southern California (orange), Central (red), Northern (green), Lower Peninsula of Michigan (purple), Southeastern (blue), southern Florida (pink), Pennsylvania (brown), and upper New England (teal) (Figs. 5a & 5b).

In the Structure analysis, values of $\text{LnP}(D)$ rose sharply for the first few $K$ and then reached a plateau at approximately $K = 12$, whereas $\Delta K$ values peaked at $K = 2$ (Fig. 6). Evanno *et al.* (2005) showed that when hierarchical structure exists in a data set, the $\Delta K$ method detects the uppermost level of population structure. In the bobcat data, the pattern of $\Delta K$ suggests the presence of a hierarchical structure, with the two clusters at the first level corresponding to the east-west cline observed through sPCA (Fig. 7). The $\text{LnP}(D)$ values
suggest additional substructure exists beyond \( K = 2 \). Though less distinct, the patterns of individual q-value proportions for \( K > 2 \) largely match the populations detected by BAPS (Figs. 8a-h).

*Population-level analysis based on BAPS results*

The southern California (orange) population only included 13 individuals (\( q \geq 0.75 \)) and its 90% probability distribution was completely subsumed by the broad Western (yellow) population, thus I grouped it with the Western population and considered 9 main populations (Fig. 9). The coastal Oregon (navy) and Western (yellow) polygons also overlapped extensively, though not completely. I omitted from the Western population all individuals that fell within the coastal Oregon 90% distribution, and I conservatively included in the coastal Oregon population only individuals that fell within its 85% distribution so as to limit overlap. Since many individuals were excluded from Oklahoma and surrounding areas, I also classified an “OKmixture” population for the purpose of calculating population-level summary statistics. Guided by K-means clustering, the populations were subdivided into 32 spatially-defined subpopulations (34 including 2 OKmixture subpopulations), each consisting of 17-91 individuals (Table 2; Fig. 9).

In contrast to the total sample, the populations and subpopulations more closely met the expectations of random mating. No loci in any of the populations or subpopulations exhibited significant departure from HW equilibrium, and global tests (i.e., across loci) indicated only two subpopulations (yellow6 and blue1) with a significant heterozygote deficiency. Only one locus pair (FCA008-FCA043 in blue3) exhibited significant linkage disequilibrium. Estimates of genetic diversity were similar across subpopulations, with
expected heterozyosity ($H_E$) and allelic richness (AR) ranging from 0.655-0.796 and 4.25-6.64 respectively (Table 2). Estimates of pairwise $F_{ST}$ ranged from 0 (red2/red3) to 0.194 (navy1/teal1) (Fig. 10). The Mantel test indicated a strong, positive relationship between $F_{ST}/(1-F_{ST})$ and geographic distance ($r = 0.701; P < 0.001$), but the strength of the relationship was greater for comparisons made between subpopulations from different BAPS populations (Fig. 11).

**Analyses of Molecular Variance (AMOVAs)**

The AMOVAs provide similar support for all three grouping strategies (BAPS assignment, subspecies, ecoregion), though the BAPS assignments explained a higher proportion of microsatellite genetic variation than did subspecies or ecoregion. Most of the variation exists within groups, while among subspecies accounts for 4.34% of the variation, ecoregions 4.20%, and BAPS assignments 6.12% ($P < 0.001$).

**Environmental Correlates**

Each of the geographic and climate variables I tested with dbRDA showed a significant relationship to genetic differentiation, explaining 0.73-7.87% of the total genetic variation (Table 3). Although longitude was the best single predictor (7.87%), latitude only accounted for 0.96% of the variation in the response matrix. Each of the predictor variables were still significantly associated with genetic differentiation after accounting for the influence of geographic distance (*i.e.*, by including X and Y coordinates as covariables), but in general they explained little additional variation. Summer precipitation (Precipitation of Warmest Quarter) was the best predictor based on the conditional tests, accounting for an
additional 1.18% of the variation on top of that already explained by spatial coordinates. In the sequential tests, the X coordinate was the first variable fit, followed by two precipitation variables and the Y coordinate (Table 3). In total, the 14 variables explained 14.35% of the total genetic variation. The importance of precipitation was also evident in the analyses based on predictor sets. Precipitation explained the largest percentage of variation (11.11%) and was the first predictor set fit in the sequential model (Table 3).

**mtDNA**

Among 1130 individuals sequenced, I identified 74 unique haplotypes, including one (haplotype H69, found in two individuals) that differed from the other haplotypes by at least 75 substitutions, but from the Canada lynx sequence by only a single substitution. Among the 73 bobcat haplotypes, there were 79 substitutions (75 transitions and 4 transversions) observed at 78 polymorphic sites across the 949 bp region. Overall haplotype diversity = 0.917, nucleotide diversity = 0.007, and mean number of pairwise differences = 6.67.

Both the median-joining network (Fig. 12) and the Bayesian tree (Fig. 13) supported two main clades (Eastern: subclade A; Western: subclades C and D) that diverged from more ancestral haplotypes (subclade B). Haplotypes from subclades C and D on the network are separated by more than 7 substitutions from any others. These C and D haplotypes also group together on the Bayesian tree with high support (BPP = 0.96), and geographically are only found in the western U.S. (Fig. 14). Although subclades C and D each form monophyletic groups, they have BPP ≤ 0.8 as well as overlapping geographic ranges in the west. Haplotypes from subclade A also form a well supported group (BPP = 1.0), and they are found primarily in the eastern U.S., though some are also scattered across the west.
Haplotypes from subclade B are basal on the tree and do not form a monophyletic clade. These ancestral haplotypes are less common and primarily restricted to either the northwest or southeast parts of the country.

For individuals falling into the Eastern clade (subclade A; n = 594), $F_S$ was significantly negative (Table 4) and the mismatch distribution showed a distinct unimodal peak (Fig. 15a), indicative of recent demographic expansion. The test statistic, however, rejected the expansion model, though this is likely attributed to the large number of samples resulting in high power to detect even slight deviations from the model. Furthermore, the analysis estimated a significant change in population size from $\theta_0$ to $\theta_1$, as 95% CIs around $\theta_0$ always included 0 whereas CIs around $\theta_1$ did not. For individuals in the Western clade (subclades C and D; n = 424), $F_S$ was also significantly negative (Table 4), and the mismatch distribution was generally unimodal and did not differ significantly from the expected distribution under the stepwise expansion model (Fig. 15b). Again, estimates of $\theta_0$ and $\theta_1$ differed significantly. Based on estimates of $\tau$, the time of expansion ranged from approximately 27,000 - 37,000 years BP for the Eastern clade, and 11,000 - 135,000 for the Western clade (Table 4).

Pairwise $\Phi_{ST}$ values between the subpopulations ranged from 0 – 0.871 (Fig. 16). An AMOVA indicated that 53.17% of the variation occurred among populations, 5.47% among subpopulations within populations, and 41.36% within subpopulations ($P < 0.001$). Grouping individuals into the 9 subspecies accounts for 48.56% of the mtDNA genetic variation I observed, based on results of an AMOVA ($P < 0.001$). Similarly, the 10 ecoregions explain 49.15% of the variation ($P < 0.001$).
Discussion

The overriding pattern of genetic structure across the bobcat’s range is one of a longitudinal cline, with a transition zone occurring along the Great Plains in the central U.S. separating eastern and western bobcats. This pattern is evident in both microsatellite and mtDNA data, and is supported by different statistical approaches including spatial PCA and Bayesian clustering analysis. Although an overall pattern of isolation-by-distance is also evident in the data, several lines of evidence indicate the observed cline cannot be solely attributed to a process of finite dispersal leading to mating among neighbors. First, although longitude was the single most important predictor variable in the dbRDAs, latitude contributed very little to the models. If isolation-by-distance is the driving mechanism, both spatial variables should contribute fairly equally, given that the sampling region covers a broad area in terms of both longitudinal and latitudinal distances. Second, comparisons made between subpopulations belonging to different genetic clusters showed higher levels of genetic differentiation ($F_{ST}$) than comparisons made between subpopulations assigned to the same genetic cluster. Given that these comparisons were made over similar geographical distances, such a distinction supports the hypothesis that other factors besides distance alone are involved (McRae et al. 2005; Rosenberg et al. 2005; Fontaine et al. 2007; Guillot et al. 2009). Finally, the mtDNA haplotype network and phylogenetic tree support two monophyletic and geographically structured lineages, primarily corresponding to eastern and western bobcats.

Genetic clines commonly arise due to genetic admixture at secondary contact zones, places where formerly isolated populations meet up following range expansion and produce hybrids, and post-glacial recolonization from Pleistocene refugia is a common cause of
secondary contact (Boursot et al. 1993; Adams et al. 2006). Climate oscillations during the Pleistocene have played a pivotal role in differentiating North American vertebrates, as species contracted and expanded their ranges in response to cycles of glacial advance and retreat (Hewitt 1996; Avise et al. 1998; Lister 2004). In their treatise on North American mammal distributions, Hall & Kelson (1959) highlight the most common zoogeographic pattern within the Temperate region (the majority of the bobcat’s range) is a distinction between eastern and western sister species. They postulated that this pattern stems from the aridification of the Great Plains region during Pleistocene interglacial periods, which separated species, particularly forest specialists, into disjunct eastern and western refugia. Indeed, through paleoclimate simulations from the last glacial maximum (LGM; ~21 Ka) to the present, Bartlein et al. (1998) detected a sharp decrease in precipitation in the continental interior as the most recent ice sheets receded. The Great Plains grasslands is a common “suture zone”, not only for mammals, but for North American biota in general (Remington 1968; Swenson & Howard 2004, 2005; Swenson 2006). Here, ranges of formerly isolated sister taxa now meet with various levels of interbreeding (e.g., lazuli and indigo buntings; Passerina amoena and P. cyanea; Carling & Zuckerberg 2011), while for others the arid grasslands still represent a sizable distribution gap separating eastern and western counterparts (e.g., eastern and western chipmunks; Tamias (Tamias) striatus and Tamias (Neotamias) spp.; Hall & Kelson 1959).

Phylogeographic analysis of North American mammals has provided evidence for Pleistocene refugia for a number of forest-associated species, including black bear (Ursus americanus; Wooding & Ward 1997), eastern chipmunk (Rowe et al. 2004; Rowe et al. 2006), white-footed mouse (Peromyscus leucopus; Rowe et al. 2006), flying squirrels
(Glaucomys spp.; Arbogast 1999), and American marten (Martes americana; Stone et al. 2002). These data indicate that despite the bobcat’s broad niche, it was isolated into separate eastern and western refugia during the Pleistocene as well. The mtDNA haplotype network and phylogenetic tree support distinct western and eastern clades, and the haplotypes ancestral to the more derived clades are restricted to the western and southeastern U.S. Following range expansions, ancestral haplotypes are generally less widespread than derived haplotypes and are likely to be centered on the origins of expansion (Templeton 2006). The location of the ancestral B-subclade haplotypes, therefore, may pinpoint the approximate location of Pleistocene refugia, which includes broad areas of both the southeastern and western U.S. Further supporting a Great Plains barrier during the Pleistocene, bobcat fossils from the range 35-10 Ka have been found in several eastern and western states, as well as eastern Wyoming and southern Texas, but have not been found in the Great Plains (Graham & Lundelius 2010).

Results of the demographic analyses, including significantly negative $F_S$ values and unimodal mismatch distributions further support a scenario of post-glacial expansion. My estimates of time since expansion are based on several major assumptions, including mutation rate and generation time, and they should be interpreted cautiously. Indeed, Ho et al. (2005) found that using observed sequence divergences between species can underestimate actual mutation rates. Since the mutation rate is based on substitutions among felid species, I may be overestimating the time since expansion. Though approximate, my estimates still put the expansion in the range of the Late Pleistocene, and likely the LGM.

Although the isolation of bobcats into separate refugia is initially surprising, Aubry et al. (2009) also found that the phylogeographic signature of the North American red fox, a
habitat generalist with conspecifics throughout the Holarctic, reflects long-term isolation in two disjunct forest refugia south of the ice sheets. However, prior to European settlement, red foxes were not as widespread in North America as their current distribution and likely had more of a boreo-montane distribution, reflecting their strong association with forests and parklands. Although bobcats are not a forest-specialist per se, they do require structure in their habitat, such as trees or rocky outcroppings, and so widespread grasslands devoid of such structure may have indeed posed a barrier for this species. It is difficult to say how common bobcats were in the Great Plains grasslands prior to European settlement and fire suppression.

An intriguing question is whether the bobcat suture zone in the Great Plains is merely the midpoint of glacial refugia, or if ongoing factors are involved in its formation and maintenance. In addition to secondary contact, genetic clines can result from adaption along an environmental gradient (Fontanillas et al. 2005; Grahame et al. 2006). Today, the Great Plains region represents a major shift in climatic types, with the transition between Bailey’s (1983) Dry and Humid Temperate Domains. Swenson (2006) hypothesized that similar past and present selective pressures are acting to maintain this suture zone and, using ecological niche modeling for four avian hybrids and their parental species, concluded that temperature is the most important environmental variable in promoting and maintaining niche divergence in this system. In contrast, the dbRDA results indicated precipitation was more important than temperature in explaining bobcat genetic differentiation. Large sample sizes contributed to my ability to detect statistically significant relationships between genetic and environmental variables, and additional work is needed to interpret the biological significance of the dbRDA findings. Specifically, ecological niche models projected onto
historical climate models would help to identify potential refugial locations and gain further insight into the role of environmental variation in differentiating this species (Hugall et al. 2002; Walteri et al. 2007; Kozak et al. 2008; Pease et al. 2009). In addition, I examined only neutral genetic variation, and inferences about selective pressures should examine adaptive genes.

In addition to the east-west cline, I observed a number of putative genetic clusters in the northern portions of the bobcat’s range. These clusters may be the result of physical dispersal barriers, founder effects, ecological differences, or a combination of these factors. The Great Lakes region and New England both were covered by ice during the LGM, which persisted until ~10-15 Ka. Once appropriate habitat became available, post-glacial recolonization likely happened relatively quickly, as long-distance dispersers at the leading edge expanded into the region (i.e., the pioneer model; Nichols & Hewitt 1994; Hewitt 1996). Such a founding event leaves a signature of reduced genetic diversity (Ibrahim et al. 1996), which has been observed for a number of species in this region (Placyk et al. 2007). Indeed, subpopulations in these regions (i.e., upper New England, Northern, and Michigan) had trends for lower levels of genetic diversity, both in mtDNA and microsatellite data. In addition to founder effects, divergence of these subpopulations could be reinforced by habitat loss and extirpation in the Corn Belt (Reding 2011) and mid-Atlantic region. Furthermore, these areas are dominated by coniferous forests, and ecological distinctions such as differences in cover habitat or prey type may be important in separating these populations from bobcats in deciduous forests to the south. The central U.S. cluster is a complicated situation. The cohesiveness of this group may be a historical pattern, as it matches up with the Prairie Peninsula, a distinct region of tallgrass prairie. Thus, ecological factors may again
be important in generating genetic boundaries between grassland habitats vs. forest. The pattern, however, may also be influenced by recent recolonization (Reding 2011).

Another distinct region was coastal Oregon and Washington. Based on mtDNA and microsatellite patterns, bobcats along the Pacific Northwest coast seem to have a unique evolutionary history from their more continental relatives. This area’s uniqueness is particularly evident in the mtDNA data, as I observed virtually no subclade C haplotypes, which are otherwise common across the west, and one haplotype (H34 from subclade D) that was exclusive to this region. The Pacific Northwest has an interesting and complex history that has been the focus of many phylogeographic studies (see Shafer et al. 2010 for review). A common pattern that has emerged in many taxa is an east-west split along the Cascade-Sierra range. Here, persistent glacial ice and high elevation areas may have worked to isolate populations well after the LGM, and the mountains may continue to restrict gene flow (Latch et al. 2009). The pattern may also be attributed to post-Pleistocene recolonization of the Pacific Northwest coastal areas from the postulated glacial refugium on the Haida Gwaii archipelago (Shafer et al. 2010).

I also observed distinct genetic clusters in southern Florida and southern California. These areas may indeed be unique, and the occurrence of some private haplotypes supports such a hypothesis. However, sampling was aggregated in these areas, with 20-30 individuals collected from a more spatially limited area. Thus, clustering algorithms may be picking up on family structure (Anderson & Dunham 2008), or reflect population structure at a finer scale. Additional sampling in these regions, to cover a broader spatial extent, would help in assessing the relative uniqueness of bobcats in southern California and Florida.
As a minor note, I documented two cases of potential bobcat-lynx hybrids, likely beyond the F1 generation since the lynx signal was detected with the mtDNA but not microsatellites. Hybridization between these sister taxa has been documented previously in Minnesota, Maine, and New Brunswick (Schwartz et al. 2004; Homyack et al. 2008). In the 1980s, New York released 83 Canada lynx from the Yukon Territory in a failed reintroduction attempt (Kloor 1999). These samples may represent descendants of the translocated animals.

Finally, these genetic results are relevant to the delineation of subspecies in bobcats. Recent studies of mammals in all or parts of their ranges show that genetic patterns often do not match taxonomic subspecies (Culver et al. 2000; Cullingham et al. 2008). Patterns of genetic variation, however, should be concordant with subspecies breaks if morphologically based subspecies are to be considered valid. In bobcats, the criteria used to define subspecies generally consist of such subtle morphological differences that, without knowledge of geographic location, it is often not feasible to accurately assign individuals to their respective taxa (Peterson & Downing 1952; Rolley 1987). Several authors, therefore, have questioned the biological significance of taxonomic distinctions among contiguous bobcat populations that lack clear geographical breaks or reliable, distinguishing characters (Read 1981; McCord & Cardoza 1982). Although this study does indicate a high level of connectivity in large portions of the bobcat’s range, I did observe patterns loosely congruent with subspecific designations. Most dramatic is the split between eastern and western subspecies (i.e., L. r. texensis/rufus vs. L. r. pallescens/baileyi). The genetic data also support the distinctiveness of L. r. pallescens in coastal Oregon and Washington, as well as the northern populations in the Great Lakes (L. r. superiorensis) and New England (L. r. gigas). However, the
interpretation of these data depends on one’s definition of subspecies. Under the conservative criterion of reciprocal monophyly on a DNA sequence-based tree (Moritz 1994), my results support two historically independent units representing eastern and western bobcats.

This broad-scale survey of spatial genetic variation clearly indicates that despite possessing many characteristics predicted to limit genetic differentiation, bobcat population genetic structure exhibits complex patterns. The analyses suggest historical processes may be the primary cause of the observed patterns (i.e., post-glacial expansion from Pleistocene refugia), though environmental and ecological forces may have played a role in promoting and maintaining the structure. More in-depth demographic analyses, including additional sequence from multiple gene regions (both nuclear and mtDNA), would be helpful to gain a clearer understanding of the demographic history of this species, including timing of expansions and specific locations of refugia. In addition, unique haplotypes discovered in the few Mexico samples included in this study suggest that further genetic variation resides in bobcats from this country and warrants additional sampling. Even so, with large sample sizes collected over a broad area, I now have among the most complete pictures of intraspecific spatial genetic patterns for any North American species, providing a firm foundation for future work testing specific hypotheses related to phylogeography, barriers to dispersal, divergent selection across environmental gradients, and potential impacts of climate change.
Acknowledgments

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References


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Reding DM (2011) Patterns and processes of spatial genetic structure in a mobile and continuously distributed species, the bobcat (*Lynx rufus*). PhD dissertation. Iowa State University.


Figure Legends

**Fig. 1.** Historic ranges of 12 bobcat (*Lynx rufus*) subspecies (Hall 1981), delineation of level 1 ecological regions of North America (Commission for Environmental Cooperation 1997), and locations of bobcat samples (*n* = 1704) used in this study.

**Fig. 2.** Relationship between Euclidean geographic distance (km, *ln*-transformed) and genetic distance based on 15 microsatellite markers, calculated for all pairwise comparisons between *n* = 1675 individual bobcat (*Lynx rufus*) samples.

**Fig. 3.** Plot showing each spatial PCA eigenvalue, decomposed into a variance and spatial autocorrelation component. The vertical dashed line indicates the maximum attainable variance, defined as the one from ordinary PCA. The two horizontal dashed lines indicate the range of variation of Moran’s I components.

**Fig. 4.** Plots of spatial PCA scores for the first (*a, b*) and second (*c, d*) global scores. In (*a, c*) each square represents the score of a genotype (white indicates negative and black positive values, and larger squares reflect greater absolute values) and is positioned by its spatial coordinates. Plots (*b, d*) show the same data but as a local interpolation of scores in gray scale and with contour lines.
**Fig. 5.** Results of BAPS analysis, with (a) displaying assignments based on mixture analysis and (b) displaying results of admixture analysis, with each individual depicted as a pie chart reflecting its ancestry coefficients ($q$) for each of the 10 inferred populations.

**Fig. 6.** Plots of Structure log-likelihood values, LnP(D), and the $\Delta K$ measure for each value of $K = 1$-20.

**Fig. 7.** Map showing each bobcat sample’s $q$-value for $K = 2$, based on Structure analysis.

**Fig. 8.** Results of Structure analysis, with maps showing proportions of ancestry for each individual for $K = 3$-10 (a-h) as miniature pie charts.

**Fig 9.** Map showing the 90% probability distributions for BAPS populations (polygons), and classification of individuals into subpopulations.

**Fig. 10.** Matrix of pairwise $F_{ST}$ values for subpopulations based on microsatellite data, depicted as a heat map.

**Fig. 11.** Plot of relationship between Euclidean geographic distance (km, $\ln$-transformed) and genetic distance based on microsatellite data ($F_{ST}/(1-F_{ST})$), calculated for all pairwise comparisons between 32 subpopulations. Diamonds represent comparisons made between subpopulations from the same BAPS population, and Xs represent comparisons made between subpopulations from different BAPS populations. The gray Xs denote comparisons
made between subpopulations with geographic distances restricted to the same range as in the within-population comparisons.

**Fig. 12.** Median-joining network illustrating relationships among 73 mtDNA haplotypes found in 1128 bobcat samples. Each white circle represents a unique haplotype, with size proportional to frequency. Haplotype names (e.g., H2) are provided in or near each circle. Small black circles represent missing haplotypes not present in the data set. Lines connecting haplotypes are one mutation long. Haplotypes are nested into hierarchical clades, with the highest level grouped by dark gray boxes (clades A to D), the second level grouped in colored boxes and labeled in upper case letter-number combinations (subclades A1 to D2), and the first level grouped by light gray boxes and labeled in lower case letters (subclades A1a to D2c).

**Fig. 13.** Bayesian tree showing genealogical relationships among mtDNA haplotypes. Labels at nodes indicate posterior probability values. Labels at tips reflect name of first level haplotype subclade, followed by haplotype name. A Canada lynx sequence was used as the outgroup.

**Fig. 14.** Distribution of haplotype subclades. Names of subclades are as in Fig. 12. White lines reflect bobcat subspecies (Hall 1981).
Fig. 15. Mismatch distributions for the Eastern clade (a), which consists of all individuals with mtDNA haplotypes from subclade A, and the Western clade (b), which consists of all individuals with mtDNA haplotypes from subclades C and D.

Fig. 16. Matrix of pairwise $\Phi_{ST}$ values for subpopulations based on mtDNA data, depicted as a heat map.
Table 1. Properties of the 15 microsatellite loci used in this study. Summary statistics are based on all samples for which at least 8 loci were successfully genotyped ($n = 1680$). Chr: chromosome location in domestic cat; Set: loci simultaneously electrophoresed; N: number of genotypes obtained; $H_O$: observed heterozygosity; $H_E$: expected heterozygosity; $F_{IS}$: Weir and Cockerham’s (1984) inbreeding coefficient.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chr</th>
<th>Set</th>
<th>Annealing temp. (°C)</th>
<th>No. cycles</th>
<th>Dye</th>
<th>N</th>
<th>Allele size range (bp)</th>
<th>No. alleles</th>
<th>$H_O$</th>
<th>$H_E$</th>
<th>$F_{IS}$</th>
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<td>A1</td>
<td>1</td>
<td>50</td>
<td>30</td>
<td>6-FAM</td>
<td>1662</td>
<td>109-131</td>
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<td>0.787</td>
<td>0.061</td>
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<td>HEX</td>
<td>1657</td>
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<td>0.885</td>
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<td>35</td>
<td>6-FAM</td>
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Table 2. Summary of microsatellite genetic variation, averaged across 15 loci, for the total bobcat sample, 10 populations, and 34 subpopulations inferred from BAPS and K-means clustering analysis. \( H_0 \): observed heterozygosity; \( H_e \): expected heterozygosity; SD: standard deviation; \( F_{IS} \): Weir and Cockerham’s (1984) inbreeding coefficient, with values significantly different from zero marked with an asterisk; AR: allelic richness, adjusted to a minimum sample size of 17 (populations) or 12 (subpopulations).

<table>
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<tr>
<th>Group</th>
<th>N</th>
<th>( H_0 ) (SD)</th>
<th>( H_e ) (SD)</th>
<th>( F_{IS} )</th>
<th>AR</th>
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<td>0.802 (0.050)</td>
<td>0.082*</td>
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<td>navy1</td>
<td>52</td>
<td>0.689 (0.091)</td>
<td>0.718 (0.080)</td>
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<td>navy2</td>
<td>27</td>
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<td>0.712 (0.086)</td>
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<td>0.741 (0.082)</td>
<td>0.028</td>
<td>5.30</td>
</tr>
<tr>
<td>Michigan (purple)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>blue1</td>
<td>34</td>
<td>0.732 (0.130)</td>
<td>0.774 (0.071)</td>
<td>0.055*</td>
<td>5.85</td>
</tr>
<tr>
<td>blue2</td>
<td>30</td>
<td>0.721 (0.114)</td>
<td>0.760 (0.079)</td>
<td>0.052</td>
<td>6.22</td>
</tr>
<tr>
<td>blue3</td>
<td>49</td>
<td>0.781 (0.085)</td>
<td>0.774 (0.063)</td>
<td>-0.009</td>
<td>6.18</td>
</tr>
<tr>
<td>blue4</td>
<td>50</td>
<td>0.688 (0.079)</td>
<td>0.728 (0.091)</td>
<td>0.055</td>
<td>5.76</td>
</tr>
<tr>
<td>blue5</td>
<td>35</td>
<td>0.753 (0.157)</td>
<td>0.749 (0.100)</td>
<td>-0.006</td>
<td>5.83</td>
</tr>
<tr>
<td>blue6</td>
<td>56</td>
<td>0.740 (0.118)</td>
<td>0.766 (0.093)</td>
<td>0.034</td>
<td>6.34</td>
</tr>
<tr>
<td>blue7</td>
<td>45</td>
<td>0.732 (0.058)</td>
<td>0.773 (0.062)</td>
<td>0.054</td>
<td>6.19</td>
</tr>
<tr>
<td>blue8</td>
<td>33</td>
<td>0.729 (0.118)</td>
<td>0.754 (0.089)</td>
<td>0.033</td>
<td>5.95</td>
</tr>
<tr>
<td>Florida (pink)</td>
<td>24</td>
<td>0.724 (0.084)</td>
<td>0.728 (0.106)</td>
<td>0.006</td>
<td>5.48</td>
</tr>
<tr>
<td>Pennsylvania (brown)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brown1</td>
<td>45</td>
<td>0.728 (0.112)</td>
<td>0.744 (0.077)</td>
<td>0.025</td>
<td>5.84</td>
</tr>
<tr>
<td>brown2</td>
<td>24</td>
<td>0.733 (0.109)</td>
<td>0.739 (0.097)</td>
<td>0.010</td>
<td>5.32</td>
</tr>
<tr>
<td>Upper New England (teal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>teal1</td>
<td>68</td>
<td>0.666 (0.094)</td>
<td>0.688 (0.086)</td>
<td>0.031</td>
<td>5.22</td>
</tr>
<tr>
<td>teal2</td>
<td>68</td>
<td>0.645 (0.124)</td>
<td>0.659 (0.091)</td>
<td>0.012</td>
<td>4.64</td>
</tr>
<tr>
<td>Oklahoma mixture</td>
<td>47</td>
<td>0.672 (0.110)</td>
<td>0.690 (0.094)</td>
<td>0.024</td>
<td>4.92</td>
</tr>
<tr>
<td>OKmixture1</td>
<td>29</td>
<td>0.801 (0.068)</td>
<td>0.796 (0.063)</td>
<td>-0.007</td>
<td>6.64</td>
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<tr>
<td>OKmixture2</td>
<td>36</td>
<td>0.771 (0.071)</td>
<td>0.787 (0.067)</td>
<td>0.021</td>
<td>6.46</td>
</tr>
</tbody>
</table>
Table 3. Tests for relationships between genetic differentiation among individual bobcats and several environmental factors using the dbRDA multivariate $F$-statistic. I analyzed 14 predictor variables individually (marginal), with spatial coordinates as covariables (conditional), and with a forward selection procedure to obtain a combined model (sequential). Analysis was repeated, treating variables as four predictor sets. $P$ indicates probability values and $\%\text{ var}$ the percentage of variation explained by a given predictor or predictor set. For the sequential tests, $\%\text{ var}$ represents the cumulative effect of variables. The top-down sequence of variables corresponds to the sequence indicated by forward selection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Marginal tests</th>
<th></th>
<th>Conditional tests</th>
<th></th>
<th>Sequential tests</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$P$</td>
<td>$%\text{ var}$</td>
<td>$F$</td>
<td>$P$</td>
<td>$%\text{ var}$</td>
</tr>
<tr>
<td>(a) Results from single predictors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X coordinate</td>
<td>142.96</td>
<td>0.001</td>
<td>7.87</td>
<td>-</td>
<td>-</td>
<td>142.96</td>
</tr>
<tr>
<td>Precip of Warmest Quarter</td>
<td>116.16</td>
<td>0.001</td>
<td>6.49</td>
<td>21.91</td>
<td>0.001</td>
<td>1.18</td>
</tr>
<tr>
<td>Precip of Driest Quarter</td>
<td>79.00</td>
<td>0.001</td>
<td>4.51</td>
<td>17.43</td>
<td>0.001</td>
<td>0.94</td>
</tr>
<tr>
<td>Y coordinate</td>
<td>16.25</td>
<td>0.001</td>
<td>0.96</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Min Temp of Coldest Month</td>
<td>12.26</td>
<td>0.001</td>
<td>0.73</td>
<td>13.66</td>
<td>0.001</td>
<td>0.74</td>
</tr>
<tr>
<td>Elevation</td>
<td>82.29</td>
<td>0.001</td>
<td>4.69</td>
<td>12.76</td>
<td>0.001</td>
<td>0.69</td>
</tr>
<tr>
<td>Annual Precip</td>
<td>53.35</td>
<td>0.001</td>
<td>3.09</td>
<td>10.54</td>
<td>0.002</td>
<td>0.57</td>
</tr>
<tr>
<td>Precip of Coldest Quarter</td>
<td>12.30</td>
<td>0.001</td>
<td>0.73</td>
<td>13.28</td>
<td>0.001</td>
<td>0.72</td>
</tr>
<tr>
<td>Precip of Wettest Quarter</td>
<td>22.26</td>
<td>0.001</td>
<td>1.31</td>
<td>10.58</td>
<td>0.002</td>
<td>0.58</td>
</tr>
<tr>
<td>Max Temp of Warmest Month</td>
<td>12.55</td>
<td>0.001</td>
<td>0.74</td>
<td>9.31</td>
<td>0.003</td>
<td>0.51</td>
</tr>
<tr>
<td>Precip Seasonality</td>
<td>32.07</td>
<td>0.001</td>
<td>1.88</td>
<td>12.25</td>
<td>0.002</td>
<td>0.67</td>
</tr>
<tr>
<td>Annual Mean Temp</td>
<td>15.63</td>
<td>0.001</td>
<td>0.93</td>
<td>4.70</td>
<td>0.031</td>
<td>0.26</td>
</tr>
<tr>
<td>Mean Diurnal Range</td>
<td>55.89</td>
<td>0.001</td>
<td>3.23</td>
<td>10.43</td>
<td>0.001</td>
<td>0.57</td>
</tr>
<tr>
<td>Temp Seasonality</td>
<td>12.60</td>
<td>0.001</td>
<td>0.75</td>
<td>14.04</td>
<td>0.001</td>
<td>0.76</td>
</tr>
<tr>
<td>(b) Results from predictor sets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precip</td>
<td>34.73</td>
<td>0.001</td>
<td>11.11</td>
<td>14.05</td>
<td>0.001</td>
<td>4.40</td>
</tr>
<tr>
<td>Coordinates</td>
<td>77.85</td>
<td>0.001</td>
<td>8.52</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Temp</td>
<td>34.11</td>
<td>0.001</td>
<td>9.27</td>
<td>12.06</td>
<td>0.001</td>
<td>3.19</td>
</tr>
<tr>
<td>Elevation</td>
<td>82.29</td>
<td>0.001</td>
<td>4.69</td>
<td>12.76</td>
<td>0.001</td>
<td>0.69</td>
</tr>
</tbody>
</table>
**Table 4.** Summary of diversity and demographic statistics for an eastern and western clade of bobcat mtDNA haplotypes.

<table>
<thead>
<tr>
<th>Estimates</th>
<th>Eastern (subclade A)</th>
<th>Western (subclades C/D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>594</td>
<td>424</td>
</tr>
<tr>
<td>Haplotypes</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>$h$</td>
<td>0.822 ± 0.009</td>
<td>0.783 ± 0.017</td>
</tr>
<tr>
<td>$\pi$</td>
<td>0.0016 ± 0.0010</td>
<td>0.0025 ± 0.0015</td>
</tr>
<tr>
<td>Mean no. pairwise differences</td>
<td>1.485 ± 0.899</td>
<td>2.416 ± 1.314</td>
</tr>
<tr>
<td>Fu’s $F_S$ ($P$-value)</td>
<td>-27.23 (&lt; 0.001)</td>
<td>-10.42 (0.015)</td>
</tr>
<tr>
<td>SSD ($P$-value)</td>
<td>0.0046 (0.015)</td>
<td>0.0185 (0.331)</td>
</tr>
<tr>
<td>$\tau$ (95% CI)</td>
<td>1.605 (1.424-1.980)</td>
<td>3.793 (0.598-7.168)</td>
</tr>
<tr>
<td>$\theta_0$ (95% CI)</td>
<td>0 (0-0.069)</td>
<td>0 (0-1.067)</td>
</tr>
<tr>
<td>$\theta_1$ (95% CI)</td>
<td>$\infty$ (19.469-$\infty$)</td>
<td>4.314 (2.168-$\infty$)</td>
</tr>
<tr>
<td>Estimated expansion time</td>
<td>26,738 – 37,178 ybp</td>
<td>11,228 – 134,590 ybp</td>
</tr>
</tbody>
</table>

$h$: haplotype diversity; $\pi$: nucleotide diversity; SSD: sum of squared deviations between observed and expected mismatch distributions; $\tau$: parameter reflecting time to expansion; $\theta_0$: parameter reflecting population size prior to expansion; $\theta_1$: parameter reflecting population size after expansion; ybp: years before present.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 6
Fig. 7
Fig. 11
Fig. 15
Fig. 16
CHAPTER 5. GENERAL CONCLUSIONS

Although the “population” is a fundamental concept in ecology and evolution, and often the unit of concern in conservation and management efforts, the patterns and processes of population structure are poorly understood for mobile species with broad distributions. The goal of this dissertation was to investigate the genetic structure of the bobcat, an abundant and mobile habitat-generalist, across three spatial scales in order to provide insight into the potential mechanisms involved in establishing and maintaining population divergence in this continuously-distributed species. Through this analysis, I found that factors such as spatial distance, habitat heterogeneity, and paleoclimatic changes can lead to genetic structure even among contiguous populations of this highly mobile species.

Environmental heterogeneity is a natural component of the landscapes that wild organisms inhabit (Turner et al. 2001). Species interact differently with their environments, and the structure of a landscape may or may not hinder the movement of individuals, depending in part on the behavioral response elicited (Taylor et al. 1993). By comparing habitat composition of observed paths to available paths, I found in Chapter 2 that bobcats in Iowa preferentially move through forested areas associated with perennial habitats. Models of landscape resistance based on these habitat selection results indicate a large portion of Iowa’s landscape, namely the northern part of the state, is predicted to generate a moderate to high level of resistance to movement for bobcats. Despite considerable effort, only a few individuals have been found in northern Iowa, including in the pocket of forested habitat in the northeast. This indicates that although bobcats are capable of dispersing to these areas, it is not a common occurrence. Even if individuals do disperse to northern Iowa, Linde’s
(2010) habitat suitability modeling suggests these regions are unlikely to sustain abundant bobcat populations. Unlike southern Iowa, where forest patches are intermingled with grassland, the forests of northeastern Iowa tend to have hard edges with the surrounding row crop fields. Thus the landscape context of forest is an important influence on local movements, dispersal, and population distribution and abundance.

Although I could not discern a landscape effect in fine-scale genetic structure within Iowa, these large blocks of high landscape resistance appear to impede connectivity of bobcats at the regional scale. In Chapter 3, I found that despite their intrinsic mobility, bobcats in the Midwest do not readily disperse across this agriculturally modified landscape. Intensive row-cropping restricts gene flow and influences the recolonization process, with most individuals in the recently expanded populations stemming from one source. The newly-established populations in Iowa and northern Missouri are closely linked with bobcats to the southwest (Kansas, southern Nebraska), but have had little genetic input from populations to the north and east (Minnesota, Wisconsin, the Dakotas, Illinois), where landscape connectivity is limited. Although landscape effects are primarily expected in species that are habitat-specialists and demonstrate low mobility, the regional genetic patterns of bobcats reveal that even a highly vagile species does not readily disperse through this intensively row-cropped region. Thus, the Corn Belt likely functions as a major barrier for many species.

The general lack of understanding of genetic processes in vagile species is especially apparent at larger spatial scales, as few studies have carefully examined spatial genetic structure beyond the local or regional scale, or sampled individuals uniformly across the landscape to reflect the continuous nature of their distributions. In Chapter 4, I collected and
analyzed a large data set of geo-referenced bobcat samples from throughout the majority of the species’ range. I found that the primary genetic signature involved a longitudinal cline with a transition zone occurring along the Great Plains in the central U.S., distinguishing bobcats in the eastern part of the country from those in the western half. Results implicated historical processes as the primary cause of the observed continental-scale genetic patterns, and demographic evidence supported a scenario of post-glacial expansion from two disjunct Pleistocene refugia. These refugia were likely isolated by the aridification of the Great Plains grasslands during interglacial periods. Such a pattern of disjunct refugia has been detected for a number of forest-dependent species (Stone et al. 2002; Runck & Cook 2005), and even for the cosmopolitan red fox (Aubry et al. 2009). The avoidance of open habitat (i.e., agricultural fields) that I observed at the local scale seems to support the idea that the arid Great Plains grasslands, which were largely devoid of trees, rocky outcroppings, or other habitat structure, posed a significant barrier to dispersal for bobcats as well.

The patterns of bobcat population genetic structure clearly demonstrate the connectivity of this species at broad spatial scales. Bobcat populations do not correspond to political boundaries, as both the regional and national analyses found that most genetic units occur across several states. These findings suggest management strategies for this species should occur beyond the state-level and involve more regional cooperation. Since local populations are highly interconnected, management decisions in one state (e.g., high harvest rates) may strongly influence bobcat populations in surrounding states. This may be especially relevant for states in which bobcats are still absent from portions of the landscape, including Iowa, as expansion may depend largely on the population dynamics in neighboring states.
With a population size estimated in the millions (Roberts & Crimmins 2010), the bobcat as an entire species is not at any immediate risk of extinction. However, by providing the raw material necessary for the ability to evolve in response to environmental change, preserving genetic diversity is important for the long-term viability of a species (Frankham et al. 2002). The delineation of subspecies is based on the importance of recognizing and protecting the genetic diversity found among groups that are on different evolutionary trajectories. For bobcats, the eastern and western clades have had distinct evolutionary histories, and may be adapted to the particular environments they inhabit. Thus, from an evolutionary standpoint, it is important in the future to maintain healthy populations from both regions. In addition, the Mexican bobcat (L. r. escuinapae) may be of particular conservation importance because of the unique ecosystems it has evolved in and the area’s likely role as a refugial population during Pleistocene glaciations. Refugial populations, which have maintained steady populations for long periods of time, generally contain the highest levels of genetic variation and thus should be of highest conservation concern (Tzedakis et al. 2002; Leonard et al. 2005). I was only able to include 5 samples from Mexico in my study, but several unique haplotypes were discovered. A comprehensive analysis of the genetic variation of Mexico’s bobcats, with comparisons made to my findings in the United States, should be a high research priority.

Collectively, the findings of this dissertation indicate that despite the bobcat’s mobility and broad niche, population genetic structure is evident and characterized by complex combinations of clines, clusters, and isolation-by-distance arising from habitat heterogeneity, restricted dispersal, and historical processes. Comparative genetic analysis with other broadly-distributed North American mammals would improve our understanding of the general mechanisms controlling gene flow in these species, helping to evaluate the
impact of future landscape changes on ecological dynamics, evolutionary processes, and species persistence.

**References**


