2011

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

Dawn Marie Reding
Iowa State University

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

Program of Study Committee:
William R. Clark, Co-Major Professor
Anne M. Bronikowski, Co-Major Professor
Julie A. Blanchong
Fredric J. Janzen
John D. Nason

Iowa State University

Ames, Iowa

2011

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I would like to thank my co-advisors, Drs. William R. Clark and Anne M. Bronikowski, for the opportunity to work in their labs and for their continued support and guidance throughout this entire process. Both afforded me a tremendous amount of flexibility and independence in developing this project, while at the same time helped keep me on course. I benefited tremendously from our collective working relationship. I marvel at Anne's fearlessness and appreciated our open conversations about the ups and downs of this profession. I found Bill's passion for his job and commitment to mentoring inspiring, and I will always value his advice and friendship. They never failed to make me feel supported, encouraged, and challenged. Perhaps above all else, though, I appreciate that they demonstrated confidence in my abilities and treated me as a colleague.

One of the benefits of having two major professors is the opportunity to be a member of two active labs. In Anne's lab, I interacted with a tremendous group of women: Tonia Schwartz, Amanda Sparkman, Gaby Palacios, Liz Addis, and Megan Manes. In Bill's lab, my outstanding co-workers included Stephanie Tucker, Dale Tessin, Stephanie Linde, and Mike Ervin. Graduate school would not have been nearly as enjoyable without our collaborations, conversations, and friendships. I would also like to express gratitude to my committee members (Drs. Julie Blanchong, Fredric Janzen, and John Nason) and collaborators (Drs. Warren Johnson, Sam Cushman, and Michael Schwartz) for their insight and helpful comments. In addition, Kristy Bellinger, Hana Yoon, and Carolyn Carter were great research mentees and probably taught me as much as I taught them. I am grateful for all of my interactions with the students, faculty, and staff in the Ecology and Evolutionary Biology (EEB) program and the Ecology, Evolution, and Organismal Biology (EEOB)

department, as well as the overall education and support I received at Iowa State University. I found the GIS Facility (Robin McNeely, Josh Obrecht, and Jill Sherwood) and AES Statistical Consulting Group (Man-Yu Yum, Anna Peterson, Dan Able, Jia Liu, Dennis Lock, and Karl Pazdernik) particularly helpful.

The project would not have been possible without the cooperation of many furharvesters, furbuyers, state and federal agencies, and research biologists in collecting or donating samples and information. The importance of their contributions cannot be overstated, as they helped me build an impressive data set. For samples and information, I thank: Ron Andrews, Dave Hoffman, and Iowa Dept. of Natural Resources wildlife and law enforcement staff; John Erb, Carolin Humpal, Gary Nelson, Minnesota Dept. of Natural Resources; Jeff Berringer, Dave Hamilton, Nick Laposha, Tom Strother, Chris Morrow, Matt Wolken, Ed Higdon, Bill Lenhart, and the many conservation agents with the Missouri Dept. of Conservation; John Olson, Brian Dhuey, William Ishmael, Sara Kehrli, Rebecca Roth, Jasmine Batten, Steve Hoffman, James Tomasko, Michael Worland, Wisconsin Dept. of Natural Resources; Matt Peek, Rob Riggin, Daryl Fisher, Kansas Dept. of Wildlife and Parks; Blake Sasse, Arkansas Game and Fish Commission; Bob Bluett, Illinois Dept. of Natural Resources; Scott Johnson, Daniel Belwood, Indiana Dept. of Natural Resources; Andy Lindbloom, South Dakota Game, Fish and Parks; Sam Wilson, Bob Meduna, Ben Rutten, Tom Krolikowski, Tom Welstead, Jon Reeves, Dustin Darveau, Lance Hastings, Bob Wood, Dirk Greene, Nebraska Game and Parks Commission; the staff of Ak-Sar-Ben Aquarium and Indian Cave State Park; Laura Patton, Kentucky Dept. of Fish and Wildlife Resources; Gray Anderson, Tennessee Wildlife Resources Agency; Dick Hoar, Erik Bartholomew, Oklahoma Dept. of Wildlife Conservation; Dwayne Etter, Michigan Dept. of

Natural Resources; Stephanie Tucker, Dorothy Fecske, North Dakota Game and Fish; David Hastings; Virgie Fisher; Paul Kapfer, University of Minnesota; Emily Croteau, Clay Nielsen, Southern Illinois University; Kim Scribner, Michigan State University; Frank Ammer, Thomas Serfass, Adam Brandt, Frostburg State University; Gene Rhodes, Jennifer Fike, Purdue University; Brian McMillan, North America Fur Auctions; Petska Furs; Warren Johnson, Laboratory of Genomic Diversity; Michael Schwartz, Kristy Pilgrim, U.S. Forest Service; Erin Boydsten, USGS; Gretchen Fowles, New Jersey Division of Fish and Wildlife; Kent Ware, U.S. Fish and Wildlife Service; Don Whittaker, Richard Green, Tim Hiller, Oregon Dept. of Fish and Wildlife; Justin Touchstone, North Florida Nuisance Control; Christina Kocer, Connecticut Dept. of Environmental Protection; Jeffery McCrady, Richard Rogers, West Virginia Dept. of Natural Resources; Tab Pitre, Pitre's Fur Co.; Patrick Tate, New Hampshire Fish and Game; Victor Nettles; Bryant White, Association of Fish and Wildlife Agencies; Michelle Hiltz, Alberta Innovates – Technology Futures; Roberta Newbury, University of British Columbia Okanagan; Colleen Offenbuttel, North Carolina Wildlife Resources Commission; Aimee Rockhill, North Carolina State University; Jon Rachael, John Nelson, Jennifer Struthers, Idaho Fish and Game; Sean Carrell, Washington State Fish and Wildlife; Fred Robinette, Clinton Peters, Kathleen Smith, Andrew Jernigan, Cory Burch, Anni Mitchell, Rick Spratt, Joe Davis, Kurt Hodges, David Johnson, Florida Fish and Wildlife Commission; Shannon Chandler, Todd Menke, Timothy McDermott; Leck Fuston, Miller Walker, Mike Bodenchuk, USDA-APHIS; Pete Figura, Jesus Garcia, California Dept. of Fish and Game; Susan VandeWoude, Kevin Crooks, Justin Lee, Scott Carver, Colorado State University; Charles Kilpatrick, University of Vermont; Kevin Bunnell, Heather Bernales, Justin Dolling, Utah Division of Wildlife Resources; James

Gallaspy, R.P. Trapping; Edmond Mouton Jr., Louisiana Dept. of Wildlife and Fisheries; Ricky Flynt, Mississippi Dept. of Wildlife, Fisheries, and Parks; Brian Giddings, Montana Fish, Wildlife, and Parks; Kevin Lansford, Nevada Dept. of Wildlife; Ron Day, Arizona Game and Fish; Nathan Roberts, Cornell University; Matthew Lovallo, Pennsylvania Game Commission; Frederic Winslow, New Mexico Dept. of Game and Fish; Michelle Gallagher, Chad Bishop, John Broderick, Colorado Division of Wildlife; Steve Tessmann, Wyoming Game and Fish; John Young, Texas Parks and Wildlife Dept.; Mike Sievering, Alabama Dept, of Conservation and Natural Resources; Jay Butfiloski, South Carolina Dept. of Natural Resources; Michael Fies, Virginia Dept. of Game and Inland Fisheries; Laura Hajduk, Massachusetts Division of Fisheries and Wildlife; Kim Royar, Vermont Fish and Wildlife Dept.; Walter Jakubas, John DePue, Maine Dept. of Inland Fisheries and Wildlife. Any omission is accidental and I apologize for the oversight.

Funding and logistical support for this project was largely provided by the Iowa DNR and State Wildlife Grant T-1-R-14. Though many DNR employees assisted in some way with this study, I am especially grateful to Todd Gosselink, Jim Coffey, and Ron Andrews for the large role they played in getting this research started and keeping field operations running smoothly – I always enjoyed my visits to Chariton and the carcass parties. A steady crew of capable technicians also helped move this research forward: Derek Broman, Jennifer Timmer, Jaclyn Comeau, Jim Kaufmann, Alicia Andes, Chad Tucker, Sara Hansen, Scott Williams, Eric Schroder, and Dan Fredrickson. Additional funding was provided by the National Science Foundation, Iowa Science Foundation, and ISU's EEOB department, EEB program, Office of Biotechnology, and CIAG.

Last but certainly not least, I would like to thank my family (both the Redings and Wollenziens) for their unrelenting patience, support, love, and good food. They kept me grounded and reminded me what is really important in life. My parents, Steve and Cathy, always emphasized the value of education and inspired me to work hard to achieve my goals. My parents-in-law, David and Norene, went above and beyond to help out when I was writing. I am lucky to have such great people in my life. Especially my husband, Ben, who was my motivator, distraction, guinea pig, punching bag, assistant, friend, or basically whatever I needed him to be at the moment. This dissertation is every bit yours as it is mine.

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 (Larivière & 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 (Unites 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.

References

Anderson EM, Lovallo MJ (2003) Bobcat and lynx. In: *Wild Mammals of North America: Biology, Management, and Conservation* (eds. Feldhamer GA, Thompson BC, Chapman JA), pp. 758-786. Johns Hopkins University Press, Baltimore, MD.

Antolin M, Savage L, Eisen R (2006) Landscape features influence genetic structure of black-tailed prairie dogs (*Cynomys ludovicianus*). *Landscape Ecology*, **21**, 867-875.

Carmichael LE, Nagy JA, Larter NC, Strobeck C (2001) Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. *Molecular Ecology*, **10**, 2787-2798.

Cegelski CC, Waits LP, Anderson NJ, Flagstad O *et al*. (2006) Genetic diversity and population structure of wolverine (*Gulo gulo*) populations at the southern edge of their current distribution in North America with implications for genetic viability. *Conservation Genetics*, **7**, 197-211.

Coulon A, Cosson JF, Angibault JM, Cargnelutti B, Galan M, Morellet N, Petit E, Aulangnier S, Hewison AJM (2004) Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. *Molecular Ecology*, **13**, 2841-2850.

Crooks KR (2002) Relative sensitivities of mammalian carnivores to habitat fragmentation. *Conservation Biology*, **16**, 488-502.

Croteau EK (2009) *Population genetics and phylogeography of bobcats (Lynx rufus) using microsatellites and mitochondrial DNA*. PhD dissertation, Southern Illinois University at Carbondale.

Croteau EK, Heist EJ, Nielsen CK (2010) Fine-scale population structure and sex-biased dispersal in bobcats (*Lynx rufus*) from southern Illinois. *Canadian Journal of Zoology*, **88**, 536-545.

Dalén L, Fuglei E, Hersteinsson P, *et al.* (2005) Population history and genetic structure of a circumpolar species: the arctic fox. *Biological Journal of the Linnean Society*, **84**, 79-89.

Davis JM, Stamps JA (2004) The effect of natal experience on habitat preferences. *Trends in Ecology and Evolution*, **19**, 411-416.

Deems Jr EF, Pursley D (1978) *North American furbearers, their management, research, and harvest status in 1976*. The International Association of Fish and Wildlife Agencies, Washington, DC.

Epps CW, Palsboll PJ, Wehausen JD, Roderick GK *et al*. (2005) Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecology Letters*, **8**, 1029-1038.

Ernest HE, Boyce WM, Bleich VC, May B *et al*. (2003) Genetic structure of mountain lion (*Puma concolor*) populations in California. *Conservation Genetics*, **4**, 353-366.

Geffen E, Anderson MJ, Wayne RK (2004) Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular Ecology*, **13**, 2481-2490.

Geffen E, Waidyaratne S, Dalen L, Angerbjorn A, *et al*. (2007) Sea ice occurrence predicts genetic isolation in the Arctic fox. *Molecular Ecology*, **16**, 4241-4255.

Gosselink T, Roberts S, Clark W, Reding D, Linde S (2011) Distribution and population dynamics of bobcats in Iowa. Final Report. State Wildlife Grant T-1-R-14. Iowa Department of Natural Resources, Des Moines, IA.

Graham RW, Lundelius Jr EL (2010) FAUNMAP II: new data for North America with a temporal extension for the Blancan, Irvingtonian and early Rancholabrean. FAUNMAP II Database, version 1.0. http://www.ucmp.berkeley.edu/faunmap.

Johnson SA, Walker HD, Hudson CM (2010) Dispersal characteristics of juvenile bobcats in south-central Indiana. *Journal of Wildlife Management*, **74**, 379-385.

Knick ST, Bailey TN (1986) Long distance movements by two bobcats from southeastern Idaho. *The American Midland Naturalist*, **116**, 222-223.

Larivière S, Walton LR (1997) *Lynx rufus*. *Mammalian Species*, **563,** 1-8.

Lehman N, Wayne RK (1991) Analysis of coyote mitochondrial DNA genotype frequencies: estimation of effective number of alleles. *Genetics*, **128**, 405-416.

Linde SA (2010) *Predicting favorable habitat for bobcats (Lynx rufus) in Iowa*. MS thesis, Iowa State University.

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

McRae BH, Beier P, Dewald LE, Huynh LY, Keim P (2005) Habitat barriers limit gene flow and illuminate historical events in a wide-ranging carnivore, the American puma. *Molecular Ecology*, **14**, 1965-1977.

Millions DG, Swanson BJ (2006) An application of Manel's model: Detecting bobcat poaching in Michigan. *Wildlife Society Bulletin,* **34**, 150-155.

Millions DG, Swanson BJ (2007) Impact of natural and artificial barriers to dispersal on the population structure of bobcats. *Journal of Wildlife Management,* **71**, 96-102.

Nunney L (2001) Population structure. In: *Evolutionary ecology: concepts and case studies* (eds. Fox CW, Roff DA, Fairbairn DJ), pp. 70-83. Oxford University Press, New York, NY.

Pease KM, Freedman AH, Pollinger JP, McCormack JE, Buermann W, Rodzen J, Banks J, Meredith E, Bleich VC, Schaefer RJ, Jones K, Wayne RK (2009) Landscape genetics

of California mule deer (*Odocoileus hemionus*): the roles of ecological and historical factors in generating differentiation. *Molecular Ecology*, **18**, 1848-1862.

Pilot M, Jedrzejewski W, Branicki W, Sidorovich VE, Jedrzejewska B, Stachura K, Funk SM (2006) Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology*, **15**, 4533-4553.

Read JA (1981) *Geographic variation in the bobcat (Felis rufus) in the south-central United States*. MS thesis, Texas A&M University.

Reid AE (2006) *Spatial genetic structure of four bobcat populations in the southeastern U.S.* MS thesis, University of Georgia.

Riley SPD, Pollinger JP, Sauvajot RM, York EC *et al*. (2006) A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology*, **15**, 1733-1741.

Roberts NM, Crimmins SM (2010) Bobcat population status and management in North America: evidence of large-scale population increase. *Journal of Fish and Wildlife Management*, **1**, 169-174.

Rolley RE (1987) Bobcat. In: *Wild furbearer management and conservation in North America* (eds. Novak M, Baker JA, Obbard ME, Malloch B), pp. 671-681. Ontario Ministry of Natural Resources, Toronto, Ontario, Canada.

Rowe KC, Heske EJ, Paige KN (2006) Comparative phylogeography of eastern chipmunks and white-footed mice in relation to the individualistic nature of species. *Molecular Ecology*, **15**, 4003-4020.

Roy MS, Geffen E, Smith D, Ostrander E, Wayne RK (1994) Patterns of differentiation and hybridization in North American wolf-like canids revealed by analysis of microsatellite loci. *Molecular Biology and Evolution*, **11**, 553-570.

Rueness EK, Stenseth NC, O'Donoghue M, Boutin S, *et al*. (2003) Ecological and genetic spatial structuring in the Canadian lynx. *Nature*, **425**, 69-72.

Runck AM, Cook JA (2005) Postglacial expansion of the southern red-backed vole (*Clethrionomys gapperi*) in North America. *Molecular Ecology*, **14**, 1445-1456.

Sacks BN, Brown SK, Ernest HB (2004) Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species history. *Molecular Ecology*, **13**, 1265-1275.

Schwartz MK, Mills LS, McKelvey KS, Ruggiero LF, Allendorf FW (2002) DNA reveals high dispersal synchronizing the population dynamics of Canada lynx. *Nature*, **415**, 520-522.

Scribner KT, Blanchong JA, Bruggeman DJ, Epperson BK, *et al*. (2005) Geographical genetics: conceptual foundations and empirical applications of spatial genetic data in wildlife management. *Journal of Wildlife Management*, **69**, 1434-1453.

Stenseth NC, Ehrich D, Rueness EK, *et al.* (2004) The effect of climatic forcing on population synchrony and genetic structuring of the Canadian lynx. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 6056-6061.

Storfer A, Murphy MA, Evans JS, Goldberg CS, *et al*. (2007) Putting the 'landscape' in landscape genetics. *Heredity*, **98**, 128-142.

Templeton AR (2006) *Population genetics and microevolutionary theory*. John Wiley & Sons, Inc., Hoboken, NJ.

Trapnell DW, Hamrick JL (2004) Partitioning nuclear and chloroplast variation at multiple spatial scale in the neotropical epiphytic orchid, *Laulia rubescens*. *Molecular Ecology*, **13**, 2655-2566.

United States Fish and Wildlife Service (2005) Endangered and threatened wildlife and plants: 12-month petition finding and proposed rule to delist the Mexican bobcat (*Lynx rufus escuinapae*). *Federal Register*, **70**, 28895-28900.

Unites States Government (2010) Proposal to de-list the bobcat from Appendix II. *Proceedings of the 15th Meeting of the Conference of the Parties*. Secretariat of CITES: Doha, Qatar.

Vilà C, Amorim IR, Leonard JA, *et al.* (1999) Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. *Molecular Ecology*, **8**, 2089-2103.

Wagner MM, Gobster PH (2007) Interpreting landscape change: measured biophysical change and surround social context. *Landscape and Urban Planning*, **81**, 67-80.

Wayne RK, Koepfli KP (1996) Demographic and historical effects on genetic variation of carnivores. In: *Carnivore behavior, ecology, and evolution* (ed. Gittleman JL), pp. 453-484. Cornell University Press, Ithaca, NY.

Wigginton JD, Dobson FS (1999) Environmental influences on geographic variation in body size of western bobcats. *Canadian Journal of Zoology*, **77**, 802-813.

Williams BW (2006) *Applications of genetic methods in furbearer management and ecology: case studies of fishers, American martens and bobcats*. MS thesis, Michigan State University.

Wooding S, Ward R (1997) Phylogeography and Pleistocene evolution in the North American black bear. *Molecular Biology and Evolution*, **14**, 1096-1105.

Woolf A, Hubert Jr GF (1998) Status and management of bobcats in the United States over three decades: 1970s-1990s. *Wildlife Society Bulletin*, **26**, 287-293.

Wright S (1943) Isolation by distance. *Genetics*, **28**, 114-138.

Zohrer JJ (2006) The Iowa wildlife action plan: securing a future for fish and wildlife. Iowa Department of Natural Resources, Des Moines, IA.

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

genetic variability than simple Euclidean distance (Arter 1990; Keyghobadi *et al.* 1999; Michels *et al.* 2001; Coulon *et al.* 2004; Spear *et al.* 2005; Vignieri 2005; Broquet *et al.* 2006; Cushman *et al.* 2006; Stevens *et al.* 2006; Pérez-Espona *et al.* 2008; Schwartz *et al.* 2009).

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 (≥ 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.*, "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 (β) 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 (Belsey *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):

$$w(x) = \exp(\beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_p X_p) / 1 + \exp(\beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_p X_p)$$

Here, β_1 to β_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 * (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 * (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 ID*PROOF* 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) × 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.

Acknowledgments

Funding was provided by State Wildlife Grant T-1-R-14, Iowa Department of Natural Resources, Iowa State University, and through a grant from the Iowa Science Foundation administered by the Iowa Academy of Science. I thank my collaborators, S. Cushman and W. Clark, for their intellectual contributions to this research. I also acknowledge S. Tucker, T. Gosselink, J. Coffey, D. Broman, J. Timmer, J. Kaufmann, and J. Comeau for field assistance; E. Schroder, D. Fredrickson, H. Yoon, and K. Bellinger for laboratory assistance; J. Sherwood, R. McNeely, and J. Olbrecht for GIS assistance; and K. Pazdernik for statistical assistance.

References

Adriaensen F, Chardon JP, De Blust G, *et al.* (2003) The application of 'least-cost' modelling as a functional landscape model. *Landscape and Urban Planning*, **64**, 233-247.

Anderson CD, Epperson BK, Fortin MJ, *et al.* (2010). Considering spatial and temporal scale in landscape-genetic studies of gene flow. *Molecular Ecology*, **19**, 3565-3575.

Arter HE (1990) Spatial relationship and gene flow paths between populations of the alpine snail *Arianta arbustorum* (Pulmonata: Helicidae). *Evolution*, **44**, 966-980.

Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography*, **32**, 818-830.

Beier P, Majka DR, Newell SL (2009) Uncertainty analysis of least-cost modeling for designing wildlife linkages. *Ecological Applications*, **19**, 2067-2077.

Beier P, Majka DR, Spencer WD (2008) Forks in the road: choices in procedures for designing wildland linkages. *Conservation Biology*, **22**, 836-851.

Belsey DA, Kuh E, Welsch RE (1980) *Regression diagnostics*. John Wiley & Sons, New York, NY.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques*, **31**, 24-28.

Boyce MS, Mao JS, Merrill EH, *et al.* (2003) Scale and heterogeneity in habitat selection by elk in Yellowstone National Park. *Ecoscience*, **10**, 421-431.

Broquet T, Ray N, Petit E, *et al.* (2006) Genetic isolation by distance and landscape connectivity in the American marten (*Martes americana*). *Landscape Ecology*, **21**, 877-889.

Bruggeman JE, Garrott RA, White PJ, Watson FGR, Wallen R (2007) Covariates affecting spatial variability in bison travel behavior in Yellowstone National Park. *Ecological Applications*, **17**, 1411-1423.

Burnham KP, Anderson DR (1998) *Model selection and inference: a practical information-theoretic approach*. Springer, New York, NY.

Carmichael LE, Clark W, Strobeck C (2000) Development and characterization of microsatellite loci from lynx (*Lynx canadensis*), and their use in other felids. *Molecular Ecology,* **9**, 2197-2198.

Chetkiewicz CLB, Boyce MS (2009) Use of resource selection functions to identify conservation corridors. *Journal of Applied Ecology*, **46**, 1036-1047.

Compton BW, Rhymer JM, McCollough M (2002) Habitat selection by wood turtles (*Clemmys insculpta*): an application of paired logistic regression. *Ecology,* **83**, 833-843.

Corander J, Siren J, Arjas E (2008) Bayesian spatial modeling of genetic population structure. *Computational Statistics,* **23**, 111-129.

Coulon A, Cosson JF, Angibault JM, *et al.* (2004) Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. *Molecular Ecology*, **13**, 2841-2850.

Coulon A, Morellet N, Goulard M, *et al.* (2008) Inferring the effects of landscape structure on roe deer (*Capreolus capreolus*) movements using a step selection function. *Landscape Ecology*, **23**, 603-614.

Cushman SA, Chase MJ, Griffin C (2010) Mapping landscape resistance to identify corridors and barriers for elephant movement in southern Africa. In: *Spatial complexity, informatics and wildlife conservation* (eds. Cushman SA, Huettman F), pp. 349-368. Springer, Tokyo.

Cushman SA, Landguth EL (2010) Spurious correlations and inference in landscape genetics. *Molecular Ecology*, **19**, 3592-3602.

Cushman SA, Lewis JS (2010) Movement behavior explains genetic differentiation in American black bears. *Landscape Ecology*, **25**, 1613-1625.

Cushman SA, McKelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. *American Naturalist*, **168**, 486-499.

Deems J, E. F., Pursley D (1978) *North American furbearers: their managment, research, and harvest status in 1976.* International Association of Fish and Wildlife Agencies, Washington, DC.

Devore J, Peck R (1986) *Statistics: the exploration and analysis of data*. West Publishing Company, St. Paul, MN.

Ellis AM, Vaclavik T, Meentemeyer RK (2010) When is connectivity important? A case study of the spatial pattern of sudden oak death. *Oikos*, **119**, 485-493.

Epps CW, Wehausen JD, Bleich VC, Torres SG, Brashares JS (2007) Optimizing dispersal and corridor models using landscape genetics. *Journal of Applied Ecology*, **44**, 714-724.

Etherington TR (2011) Python based GIS tools for landscape genetics: visualizing genetic relatedness and measuring landscape connectivity. *Methods in Ecology and Evolution*, **2**, 52-55.

Fahrig L, Nuttle WK (2005) Population ecology in spatially heterogeneous environments. In: *Ecosystem Function in Heterogeneous Landscapes* (eds. Lovette GM, Jones CG, Turner MG, Weathers KC, pp. 95-118. Springer, New York, NY.

Faircloth BC, Reid A, Valentine T, *et al.* (2005) Tetranucleotide, trinucleotide, and dinucleotide loci from the bobcat (*Lynx rufus*). *Molecular Ecology Notes,* **5**, 387-389.

Fortin D, Beyer HL, Boyce MS, *et al.* (2005) Wolves influence elk movements: behavior shapes a trophic cascade in Yellowstone National Park. *Ecology,* **86**, 1320-1330.

Gillies CS, Hebblewhite M, Nielsen SE, *et al.* (2006) Application of random effects to the study of resource selection by animals. *Journal of Animal Ecology*, **75**, 887-898.

Gonzales EK, Gergel SE (2007) Testing assumptions of cost surface analysis-a tool for invasive species management. *Landscape Ecology,* **22**, 1155-1168.

Gosselink T, Roberts S, Clark W, Reding D, Linde S (2011) Distribution and population dynamics of bobcats in Iowa. Final Report. State Wildlife Grant T-1-R-14. Iowa Department of Natural Resources, Des Moines, IA.

Hardy OJ, Vekemans X (1999) Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. *Heredity*, **83**, 145-154.

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

Johnson SA, Walker HD, Hudson CM (2010) Dispersal characteristics of juvenile bobcats in south-central Indiana. *Journal of Wildlife Management,* **74**, 379-385.

Karieva PM, Shigesada N (1983) Analyzing insect movement as a correlated random walk. *Oecologia*, **56**, 234-238.

Keyghobadi N, Roland J, Strobeck C (1999) Influence of landscape on the population genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae). *Molecular Ecology*, **8**, 1481-1495.

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561-576.

Knick ST, Bailey TN (1986) Long distance movements by two bobcats from southeastern Idaho. *The American Midland Naturalist*, **116**, 222-223.

Levey DJ, Bolker BM, Tewksbury JJ, Sargent S, Haddad NM (2005) Effects of landscape corridors on seed dispersal by birds. *Science*, **309**, 146-148.

Linde SA (2010) *Predicting favorable habitat for bobcats (Lynx rufus) in Iowa*. MS thesis, Iowa State University.

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

Manly BFJ, McDonald LL, Thomas D (2002) *Resource selection by animals: statistical design and analysis for field studies.* Kluwer, Boston, MA.

Mantel N (1967) Detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209-220.

McGarigal K, Cushman S (2005) The gradient concept of landscape structure. In: *Issues and Perspectives in Landscape Ecology* (eds. Wiens J, Moss M), pp. 112-119 Cambridge University Press, Cambridge.

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

Menotti-Raymond M, David VA, Lyons LA, *et al.* (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics,* **57**, 9-23.

Menotti-Raymond MA, David VA, Wachter LL, Butler JM, O'Brien SJ (2005) An STR forensic typing system for genetic individualization of domestic cat (*Felis catus*) samples. *Journal of Forensic Sciences,* **50**, 1061-1070.

Michels E, Cottenie K, Neys L, *et al.* (2001) Geographical and genetic distances among zooplankton populations in a set of interconnected ponds: a plea for using GIS modelling of the effective geographical distance. *Molecular Ecology*, **10**, 1929-1938.

Morales JM, Ellner SP (2002) Scaling up animal movements in heterogeneous landscapes: the importance of behavior. *Ecology*, **83**, 2240-2247.

Oksanen J, Blanchet FG, Kindt R, *et al.* (2010) vegan: Community Ecology Package. R package version 1.17-5. http://CRAN.R-project.org/package=vegan

Pérez-Espona S, Pérez-Barbería FJ, McLeod JE, *et al.* (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Molecular Ecology*, **17**, 981-996.

Pullinger MG, Johnson CJ (2010) Maintaining or restoring connectivity of modified landscapes: evaluating the least-cost path model with multiple sources of ecological information. *Landscape Ecology*, **25**, 1547-1560.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution,* **43**, 223-225.

Richard Y, Armstrong DP (2010) Cost distance modelling of landscape connectivity and gap-crossing ability using radio-tracking data. *Journal of Applied Ecology,* **47**, 603-610.

Rousset F (2000) Genetic differentiation between individuals. *Journal of Evolutionary Biology*, **13**, 58-62.

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

Schick RS, Loarie SR, Colchero F, *et al.* (2008) Understanding movement data and movement processes: current and emerging directions. *Ecology Letters*, **11**, 1338-1350.

Schwartz MK, Copeland JP, Anderson NJ, *et al.* (2009) Wolverine gene flow across a narrow climatic niche. *Ecology*, **90**, 3222-3232.

Shirk AJ, Wallin DO, Cushman SA, Rice CG, Warheit KI (2010) Inferring landscape effects on gene flow: a new model selection framework. *Molecular Ecology*, **19**, 3603-3619.

Sork VL, Waits L (2010) Contributions of landscape genetics - approaches, insights, and future potential. *Molecular Ecology*, **19**, 3489-3495.

Spear SF, Balkenhol N, Fortin MJ, McRae BH, Scribner K (2010) Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis. *Molecular Ecology*, **19**, 3576-3591.

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

Stevens VM, Verkenne C, Vandewoestijne S, Wesselingh RA, Baguette M (2006) Gene flow and functional connectivity in the natterjack toad. *Molecular Ecology*, **15**, 2333-2344.

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

Taylor PD, Fahrig L, Henein K, Merriam G (1993) Connectivity is a vital element of landscape structure. *Oikos*, **68**, 571-573.

Tischendorf L, Fahrig L (2000) How should we measure landscape connectivity? *Landscape Ecology*, **15**, 633-641.

Tucker SA, Clark WR, Gosselink TE (2008) Space use and habitat selection by bobcats in the fragmented landscape of south-central Iowa. *Journal of Wildlife Management*, **72**, 1114-1124.

Turchin P (1998) *Quantitative analysis of movement: measuring and modeling population redistribution in animals and plants*. Sinauer Associates, Sunderland, MA.

Turner MG, Gardner RH, O'Neill RV (2001) *Landscape ecology in theory and practice*. Springer, New York, NY.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535-538.

Vignieri SN (2005) Streams over mountains: influence of riparian connectivity on gene flow in the Pacific jumping mouse (*Zapus trinotatus*). *Molecular Ecology*, **14**, 1925-1937.

Wagner MM, Gobster PH (2007) Interpreting landscape change: measured biophysical change and surround social context. *Landscape and Urban Planning*, **81**, 67-80.

Wang YH, Yang KC, Bridgman CL, Lin LK (2008) Habitat suitability modelling to correlate gene flow with landscape connectivity. *Landscape Ecology*, **23**, 989-1000.

Wasserman TN, Cushman SA, Schwartz MK, Wallin DO (2010) Spatial scaling and multi-model inference in landscape genetics: *Martes americana* in northern Idaho. *Landscape Ecology*, **25**, 1601-1612.

Weir BS (1996) *Genetic Data Analysis II*. Sinauer.

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

Wright S (1943) Isolation by distance. *Genetics*, **28**, 114-138.

Xian G, Homer C, Fry J (2009) Updating the 2001 National Land Cover Database land cover classification to 2006 by using Landsat imagery change detection methods. *Remote Sensing of Environment*, **113**, 1133-1147.

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.

Locus	Species	Repeat motif	Chr.	N	Size range (bp)	No. alleles	H_E	H_O	F_{IS}
BCE5T[1]	Bobcat	Tetra	unknown	600	257-318	7	0.704	0.693	0.0159
Lc109[2]	Canada lynx	Di	unknown	620	182-202	10	0.759	0.784	-0.0328
Lc110[2]	Canada lynx	Di	unknown	622	92-104	8	0.714	0.735	-0.0290
Lc111[2]	Canada lynx	Di	unknown	618	157-217	7	0.667	0.647	0.0299
FCA008[3]	Domestic cat	Di	A1	621	132-160	9	0.773	0.763	0.0120
FCA023[3]	Domestic cat	Di	B1	615	151-163	6	0.792	0.779	0.0164
FCA026[3]	Domestic cat	Di	D3	619	143-159	8	0.645	0.638	0.0106
FCA031[3]	Domestic cat	Di	E3	620	238-258	11	0.721	0.702	0.0269
FCA043[3]	Domestic cat	Di	C2	622	130-140	6	0.754	0.712	0.0560
FCA045[3]	Domestic cat	Di	D4	609	166-178	7	0.561	0.557	0.0071
FCA077[3]	Domestic cat	Di	C2	620	152-168	9	0.717	0.708	0.0119
FCA082[3]	Domestic cat	Di	E1	603	246-266	11	0.844	0.828	0.0200
FCA090[3]	Domestic cat	Di	A1	619	117-129	7	0.762	0.746	0.0206
FCA096[3]	Domestic cat	Di	E2	622	191-219	11	0.836	0.826	0.0117
FCA126[3]	Domestic cat	Di	B1	603	132-154	8	0.770	0.760	0.0131
FCA132[3]	Domestic cat	Di	D3	612	182-196	8	0.850	0.822	0.0331
FCA149[3]	Domestic cat	Di	B1	622	138-158	10	0.805	0.822	-0.0209
FCA391[3]	Domestic cat	Tetra	B3	609	210-236	8	0.667	0.655	0.0179
FCA559[3]	Domestic cat	Tetra	B1	618	105-141	8	0.793	0.777	0.0200
FCA740[4]	Domestic cat	Tetra	C1	618	334-358	6	0.727	0.739	-0.0170
FCA741[3]	Domestic cat	Tri	D1	616	170-188	7	0.546	0.563	-0.0318
FCA742[4]	Domestic cat	Tetra	D4	622	115-135	6	0.640	0.613	0.0424

Locus name, species developed from, and repeat motif (tetra: tetranucleotide; di: dinucleotide; tri: trinucleotide) were taken from the cited reference: [1]Faircloth *et al.* 2005; [2]Carmichael *et al.* 2000; [3]Menotti-Raymond *et al.* 1999; [4]Menotti-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 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.

		Observed Paths			Random Paths				
Variable	**AB**	$\bar{x}$	**SD**	**Range**	$\bar{x}$	**SD**	**Range**	**β**	***P***
Water (100 m buffer)	W100	0.0052	0.0204	0-0.2042	0.0243	0.1098	0-1	-8.443	0.041
Development (100 m buffer)	D100	0.0046	0.0111	0-0.0633	0.0202	0.0684	0-0.9688	-25.179	<0.001
Forest (100 m buffer)	F100	0.3967	0.2414	0-1	0.1936	0.209	0-1	3.918	<0.001
Grassland (100 m buffer)	G100	0.3727	0.2338	0-0.9269	0.4357	0.2603	0-1	-1.122	0.002
Cropland (100 m buffer)	C100	0.1978	0.2289	0-1	0.2869	0.288	0-1	-1.617	<0.001
Road (100 m buffer)	R100	0.0229	0.0316	0-0.2330	0.0393	0.0588	0-0.9378	-10.429	<0.001
Water (750 m annulus)	W750	0.0076	0.0193	0-0.1281	0.0242	0.0948	0-0.9936	-9.495	0.034
Development (750 m annulus)	D750	0.0132	0.0237	0-0.1895	0.0207	0.053	0-0.7283	-6.492	0.076
Forest (750 m annulus)	F750	0.2325	0.1323	0.0036-0.7749	0.1961	0.1498	0-0.9788	1.999	<0.001
Grassland (750 m annulus)	G750	0.4647	0.1568	0.0316-0.8964	0.4371	0.1763	0-0.9297	1.352	0.022
Cropland (750 m annulus)	C750	0.2465	0.1801	0-0.8107	0.2823	0.2159	0-0.9805	-1.324	0.011
Road (750 m annulus)	R750	0.0354	0.0224	0-0.1876	0.0396	0.0349	0-0.3684	-5.177	0.119

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.

	Path				Annulus				Combined			
Variable	**β**	**SE**	**Wald**	***P***	**β**	**SE**	**Wald**	***P***	**β**	**SE**	**Wald**	***P***
Water (100 m)	-8.862	4.207	4.44	0.035	-	-	-	-	-2.191	4.834	0.21	0.650
Development (100 m)	-13.521	7.063	3.66	0.056	-	-	-	-	-17.464	7.541	5.36	0.021
Forest (100 m)	3.582	0.430	69.43	<0.001	-	-	-	-	6.290	0.607	107.42	<0.001
Cropland (100 m)	-0.036	0.466	0.01	0.939	-	-	-	-	0.302	0.647	0.22	0.641
Road (100 m)	-3.559	2.956	1.45	0.229	-	-	-	-	-2.481	3.056	0.66	0.417
Water (750 m)	-	-	-	-	-10.142	4.378	5.37	0.021	-10.814	5.207	4.31	0.038
Development (750 m)	-	-	-	-	-5.602	4.132	1.84	0.175	4.219	4.705	0.80	0.370
Forest (750 m)	-	-	-	-	0.956	0.769	1.55	0.214	-6.726	1.112	36.60	<0.001
Cropland (750 m)	-	-	-	-	-1.331	0.661	4.06	0.044	-1.393	0.917	2.30	0.129
Road (750 m)	-	-	-	-	0.317	4.231	0.01	0.940	3.282	4.628	0.50	0.478

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 (AIC), and AIC differences (ΔAIC). Variables are abbreviated as in Table 2.

Model	**Variables**	***K***	**AIC**	**ΔAIC**
Path	W100 + D100 + F100 + C100 + R100	5	957.965	44.23
Annulus	W750 + D750 + F750 + C750 + R750	5	1071.993	158.26
Combined	W100 + D100 + F100 + C100 + R100 + W750 + D750 + F750 + C750 + R750	10	913.735	0.00

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 $P < 0.05$.

Mantel or partial Mantel test	***r***	***P***
IBD	0.05766	**0.003**
Path	0.04968	**0.009**
Annulus	0.05808	**0.002**
Combined	0.05587	**0.005**
Suitability	0.06082	**0.002**
Path.IBD	-0.00371	0.578
Annulus.IBD	0.00744	0.349
Combined.IBD	0.00315	0.450
Suitability.IBD	0.02411	0.145

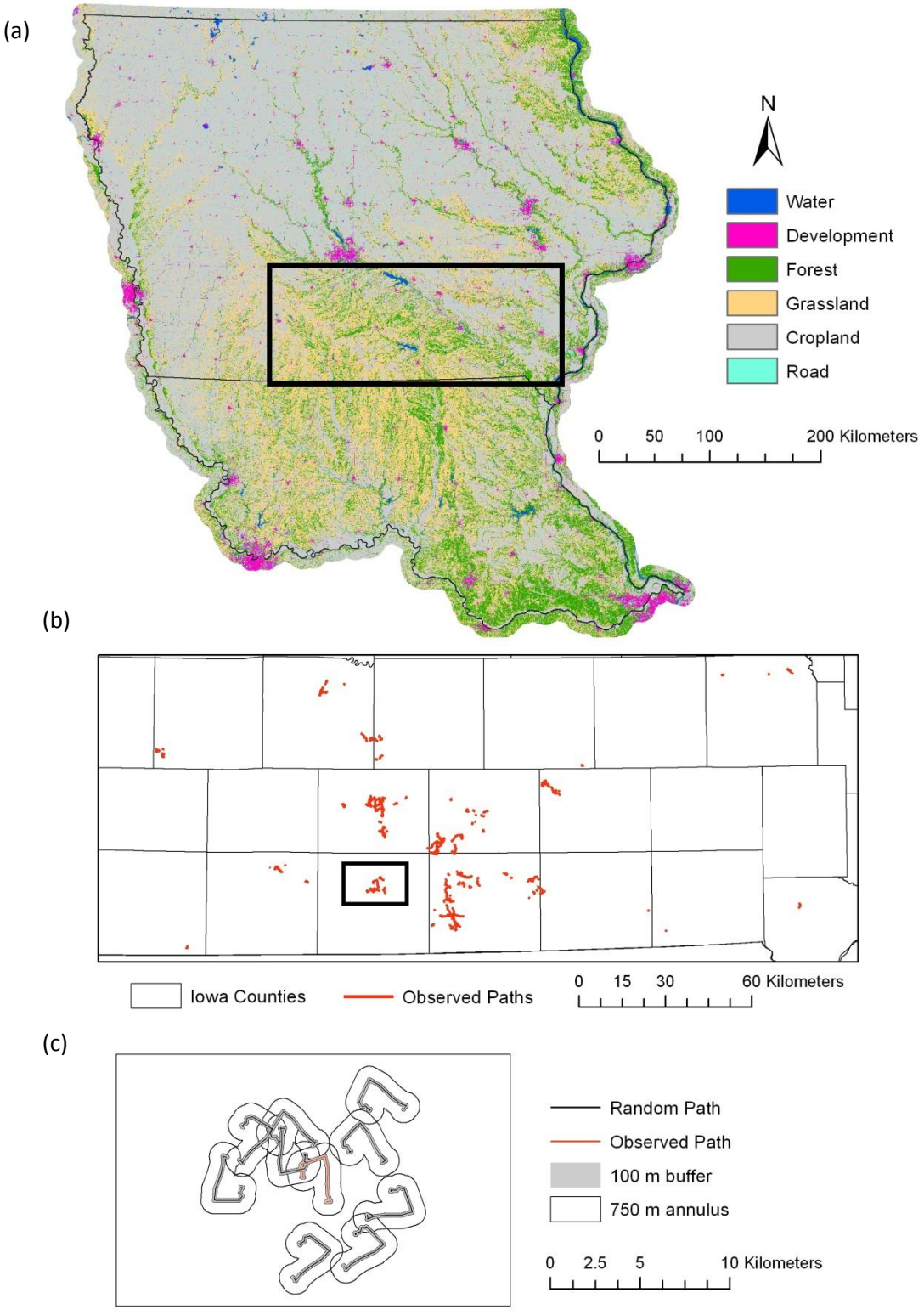
(a)
N
Water
Development
Forest
Grassland
Cropland
Road
0 50 100 200 Kilometers
(b)
Iowa Counties
Observed Paths
0 15 30 60 Kilometers
(c)
Random Path
Observed Path
100 m buffer
750 m annulus
0 2.5 5 10 Kilometers

Fig. 1

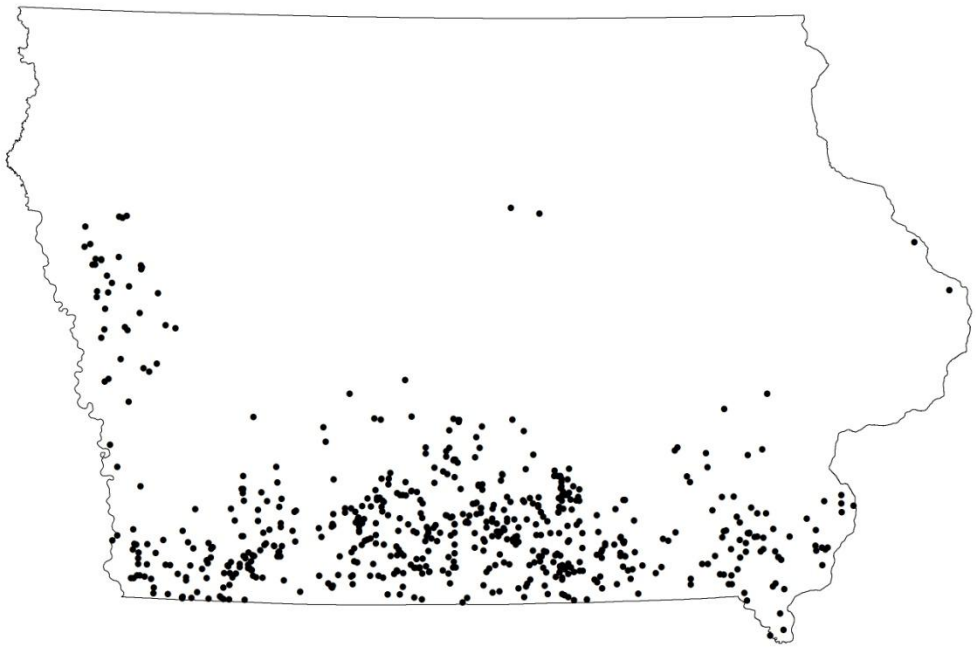

Fig. 2

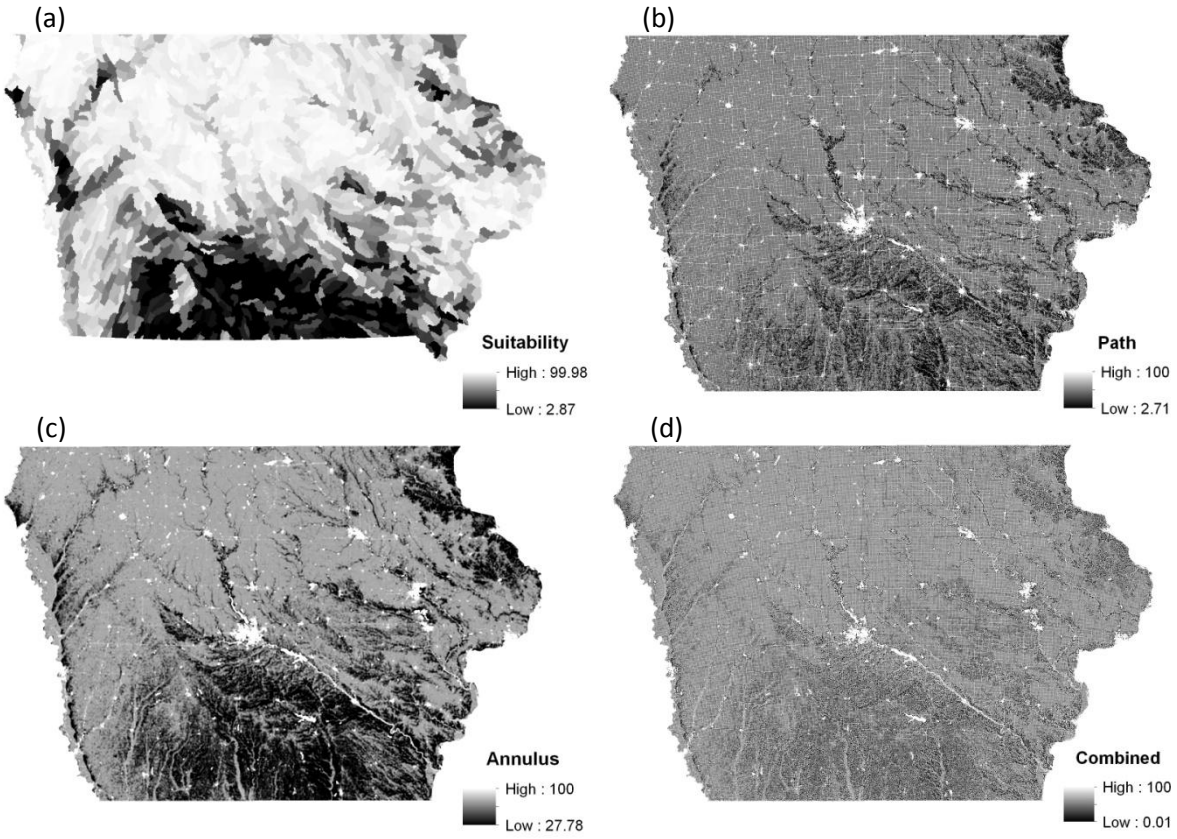
(a)
Suitability
High : 99.98
Low : 2.87
(b)
Path
High : 100
Low : 2.71
(c)
Annulus
High : 100
Low : 27.78
(d)
Combined
High : 100
Low : 0.01

Fig. 3

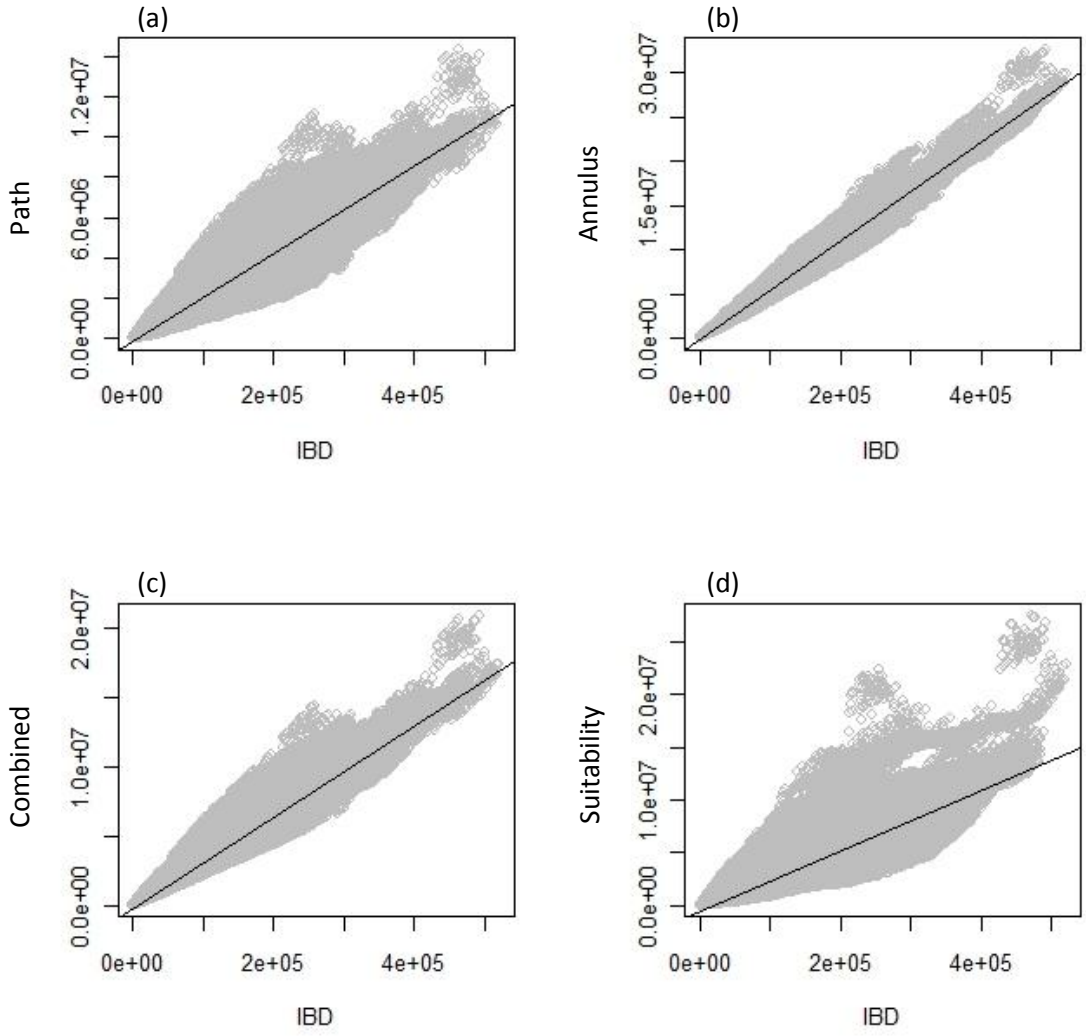
(a)
Path
0.0e+00
6.0e+06
1.2e+07
0e+00
2e+05
4e+05
IBD
(b)
Annulus
0.0e+00
1.5e+07
3.0e+07
0e+00
2e+05
4e+05
IBD
(c)
Combined
0.0e+00
1.0e+07
2.0e+07
0e+00
2e+05
4e+05
IBD
(d)
Suitability
0.0e+00
1.0e+07
2.0e+07
0e+00
2e+05
4e+05
IBD

Fig. 4

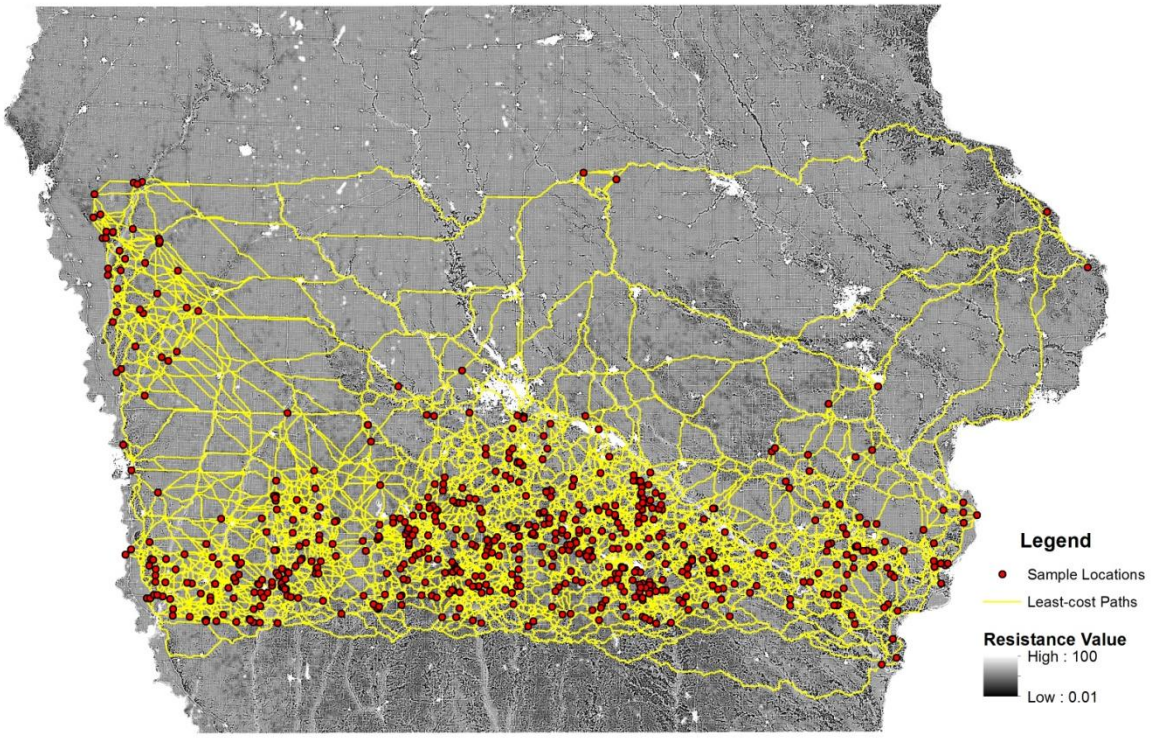
Legend
Sample Locations
Least-cost Paths
Resistance Value
High : 100
Low : 0.01

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 ID*PURE* (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 ID*PROOF* 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.

Population inference using Bayesian clustering analyses

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 $K = 1\text{-}9$. 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_{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'-TCATCCCCGTAGCACTTTTC-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 Φ_{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 ΔK 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 ΔK 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 ΔK 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 UPDATEFROMPOPFLAG 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 ΔK 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 (*K*6), 4 pairs for Structure (*K*5), 3 pairs for BAPS (*K*6), and 1 pair for the BAPS (*K*5)). 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 ($q \geq 0.75$) 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 ($q \geq 0.75$) 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.

Acknowledgments

This work was supported by State Wildlife Grant T-1-R-14, the Iowa DNR, Iowa State University, and a grant from the Iowa Science Foundation administered by the Iowa Academy of Science. The project would not have been possible without the cooperation and support of many furharvesters, furbuyers, state agencies, and biologists – too numerous to list individually here though their efforts are every bit deserving – in collecting samples and information. For providing comments on earlier drafts, I thank W. Johnson, A. Bronikowski, W. Clark, T. Gosselink, J. Blanchong, F. Janzen, M. Palacios, T. Schwartz, and M. Manes. I also thank R. Andrews, J. Coffey, S. Tucker, Iowa DNR wildlife and law enforcement staff, D. Broman, J. Timmer, J. Comeau, J. Kaufmann, M. Purvis, C. Duncan, N. McCann, B. Orning-Tschampl, J. Bolis, H. Stricker, C. Tucker, M. Jones, S. Hansen, J. Kanine, S. Williams, K. Bellinger, H. Yoon, D. Fredrickson, E. Schroder, and B. Wollenzien for field and lab assistance. Part of this work was carried out by using the resources of the

Computational Biology Service Unit from Cornell University which is partially funded by Microsoft Corporation.

References

Anderson EC, Dunham KK (2008) The influence of family groups on inferences made with the program Structure. *Molecular Ecology Resources*, **8**, 1219-1229.

Anderson EM, Lovallo MJ (2003) Bobcat and lynx. In: *Wild Mammals of North America: Biology, Management, and Conservation* (eds. Feldhamer GA, Thompson BC, Chapman JA), pp. 758-786. Johns Hopkins University Press, Baltimore, Maryland.

Anderson MJ (2004) DISTLM v.5: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model. Department of Statistics, University of Auckland, New Zealand.

Andrewartha HG, Birch LC (1954) *The distribution and abundance of animals.* The University of Chicago Press, Chicago, IL.

Baker RH (1983) *Michigan Mammals.* Michigan State University Press, East Lansing, MI.

Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography*, **32**, 818-830.

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37-48.

Beyer HL (2004) Hawth's Analysis Tools for ArcGIS. Available at http://www.spatialecology.com/htools.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques*, **31**, 24-28.

Carmichael LE, Clark W, Strobeck C (2000) Development and characterization of microsatellite loci from lynx (*Lynx canadensis*), and their use in other felids. *Molecular Ecology*, **9**, 2197-2198.

Carmichael LE, Nagy JA, Larter NC, Strobeck C (2001) Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. *Molecular Ecology*, **10**, 2787-2798.

Commission for Environmental Cooperation (1997) *Ecological regions of North America: toward a common perspective.* Commission for Environmental Cooperation, Montreal, Canada.

Corander J, Siren J, Arjas E (2008) Bayesian spatial modeling of genetic population structure. *Computational Statistics*, **23**, 111-129.

Croteau EK (2009) *Population genetics and phylogeography of bobcats (Lynx rufus) using microsatellites and mitochondrial DNA*. PhD thesis, Southern Illinois University at Carbondale.

Davis JM, Stamps JA (2004) The effect of natal experience on habitat preferences. *Trends in Ecology & Evolution*, **19**, 411-416.

Deems J, E. F., Pursley D (1978) *North American furbearers: their managment, research, and harvest status in 1976.* International Association of Fish and Wildlife Agencies, Washington, DC.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

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

Faircloth BC, Reid A, Valentine T, *et al.* (2005) Tetranucleotide, trinucleotide, and dinucleotide loci from the bobcat (*Lynx rufus*). *Molecular Ecology Notes*, **5**, 387-389.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, **164**, 1567-1587.

Frantz AC, Cellina S, Krier A, Schley L, Burke T (2009) Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? *Journal of Applied Ecology*, **46**, 493-505.

Fuller TK, Berg WE, Radde GL, Lenarz MS, Joselyn GB (1992) A history and current estimate of wolf distribution and numbers in Minnesota. *Wildlife Society Bulletin*, **20**, 42-55.

Geffen E, Anderson MJ, Wayne RK (2004) Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular Ecology*, **13**, 2481-2490.

Gittleman JL, Funk SM, Macdonald DW, Wayne RK (2001) *Carnivore Conservation.* Cambridge University Press, Cambridge, UK.

Gosselink T, Roberts S, Clark W, Reding D, Linde S (2011) Distribution and population dynamics of bobcats in Iowa. Final Report. State Wildlife Grant T-1-R-14. Iowa Department of Natural Resources, Des Moines, IA.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hall ER (1981) *The mammals of North America.*, 2nd edn. John Wiley and Sons, New York, NY.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics,* **23**, 1801-1806.

Janecka JE, Blankenship TL, Hirth DH, *et al.* (2007) Evidence for male-biased dispersal in bobcats *Lynx rufus* using relatedness analysis. *Wildlife Biology,* **13**, 38-47.

Johnson SA, Walker HD, Hudson CM (2010) Dispersal characteristics of juvenile bobcats in south-central Indiana. *Journal of Wildlife Management,* **74**, 379-385.

Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295-302.

Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs,* **69**, 1-24.

Lehman N, Wayne RK (1991) Analysis of coyote mitochondrial-DNA genotype frequencies - estimation of the effective number of alleles. *Genetics,* **128**, 405-416.

Levins R (1969) Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America*, **15**, 237-240.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics,* **25**, 1451-1452.

Linde SA (2010) *Predicting favorable habitat for bobcats (Lynx rufus) in Iowa*. MS thesis, Iowa State University.

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

Mantel N (1967) Detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209-220.

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology,* **82**, 290-297.

McRae BH, Beier P, Dewald LE, Huynh LY, Keim P (2005) Habitat barriers limit gene flow and illuminate historical events in a wide-ranging carnivore, the American puma. *Molecular Ecology,* **14**, 1965-1977.

Menotti-Raymond M, David VA, Lyons LA, *et al.* (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics,* **57**, 9-23.

Menotti-Raymond MA, David VA, Wachter LL, Butler JM, O'Brien SJ (2005) An STR forensic typing system for genetic individualization of domestic cat (*Felis catus*) samples. *Journal of Forensic Sciences,* **50**, 1061-1070.

Millions DG, Swanson BJ (2006) An application of Manel's model: Detecting bobcat poaching in Michigan. *Wildlife Society Bulletin,* **34**, 150-155.

Millions DG, Swanson BJ (2007) Impact of natural and artificial barriers to dispersal on the population structure of bobcats. *Journal of Wildlife Management,* **71**, 96-102.

Murphy MA, Evans JS, Cushman SA, Storfer A (2008) Representing genetic variation as continuous surfaces: an approach for identifying spatial dependency in landscape genetic studies. *Ecography*, **31**, 685-697.

Nielsen CK, Woolf A (2002) Habitat-relative abundance relationship for bobcats in southern Illinois. *Wildlife Society Bulletin*, **30**, 222-230.

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

Pease KM, Freedman AH, Pollinger JP, *et al.* (2009) Landscape genetics of California mule deer (*Odocoileus hemionus*): the roles of ecological and historical factors in generating differentiation. *Molecular Ecology*, **18**, 1848-1862.

Pilgrim KL, McKelvey KS, Riddle AE, Schwartz MK (2005) Felid sex identification based on noninvasive genetic samples. *Molecular Ecology Notes* **5**, 60-61.

Pilot M, Jedrzejewski W, Branicki W, *et al.* (2006) Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology*, **15**, 4533-4553.

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

Pritchard JK, Wen X, Falush D (2009) Documentation for STRUCTURE software: Version 2.3., Chicago, IL.

Pyare S, Cain S, Moody D, Schwartz C, Berger J (2004) Carnivore re-colonisation: reality, possibility and a non-equilibrium century for grizzly bears in the southern Yellowstone ecosystem. *Animal Conservation*, **7**, 71-77.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic-markers. *Evolution*, **43**, 258-275.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution,* **43**, 223-225.

Riley SJ, Malecki RA (2001) A landscape analysis of cougar distribution and abundance in Montana, USA. *Environmental Management,* **28**, 317-323.

Riley SPD, Pollinger JP, Sauvajot RM, *et al.* (2006) A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology,* **15**, 1733-1741.

Robinson SJ, Waits LP, Martin ID (2007) Evaluating population structure of black bears on the Kenai Peninsula using mitochondrial and nuclear DNA analyses. *Journal of Mammalogy,* **88**, 1288-1299.

Rolley RE (1987) Bobcat. In: *Wild furbearer management and conservation in North America* (eds. Novak M, Baker JA, Obbard ME, Malloch B), pp. 671-681. Ontario Ministry of Natural Resources, Toronto, Ontario.

Rosel PE, Hansen L, Hohn AA (2009) Restricted dispersal in a continuously distributed marine species: common bottlenose dolphins *Tursiops truncatus* in coastal waters of the western North Atlantic. *Molecular Ecology,* **18**, 5030-5045.

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

Rueness EK, Jorde PE, Hellborg L, *et al.* (2003a) Cryptic population structure in a large, mobile mammalian predator: the Scandinavian lynx. *Molecular Ecology,* **12**, 2623-2633.

Rueness EK, Stenseth NC, O'Donoghue M, *et al.* (2003b) Ecological and genetic spatial structuring in the Canadian lynx. *Nature,* **425**, 69-72.

Sacks BN, Brown SK, Ernest HB (2004) Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species history. *Molecular Ecology,* **13**, 1265-1275.

Schwartz CW, Schwartz ER (2001) *The wild mammals of Missouri, 2nd revised edition.* University of Missouri Press, Columbia, MO.

Schwartz MK, Cushman SA, McKelvey KS, Hayden J, Engkjer C (2006) Detecting genotyping errors and describing American black bear movement in northern Idaho. *Ursus,* **17**, 138-148.

Schwartz MK, McKelvey KS (2009) Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. *Conservation Genetics,* **10**, 441-452.

Schwartz MK, Mills LS, McKelvey KS, Ruggiero LF, Allendorf FW (2002) DNA reveals high dispersal synchronizing the population dynamics of Canada lynx. *Nature,* **415**, 520-522.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity,* **82**, 561-573.

Stenseth NC, Ehrich D, Rueness EK, *et al.* (2004) The effect of climatic forcing on population synchrony and genetic structuring of the Canadian lynx. *Proceedings of the National Academy of Sciences of the United States of America,* **101**, 6056-6061.

Tammeleht E, Remm J, Korsten M, *et al.* (2010) Genetic structure in large, continuous mammal populations: the example of brown bears in northwestern Eurasia. *Molecular Ecology,* **19**, 5359-5370.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial-DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512-526.

Tucker SA, Clark WR, Gosselink TE (2008) Space use and habitat selection by bobcats in the fragmented landscape of south-central Iowa. *Journal of Wildlife Management,* **72**, 1114-1124.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535-538.

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology,* **15**, 1419-1439.

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

Werle E, Schneider C, Renner M, Volker M, Fiehn W (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research,* **22**, 4354-4355.

Williams BW (2006) *Applications of genetic methods in furbearer management and ecology: case studies of fishers, American martens and bobcats*. MS thesis, Michigan State University.

Williams BW, Scribner KT (2010) Effects of multiple founder populations on spatial genetic structure of reintroduced American martens. *Molecular Ecology*, **19**, 227-240.

Woolf A, Hubert GF (1998) Status and management of bobcats in the United States over three decades: 1970s-1990s. *Wildlife Society Bulletin*, **26**, 287-293.

Woolf A, Nielsen CK, Weber T, Gibbs-Kieninger TJ (2002) Statewide modeling of bobcat, *Lynx rufus*, habitat in Illinois, USA. *Biological Conservation*, **104**, 191-198.

Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97-159.

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 ΔK 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 (*K*6) 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.

Marker	Repeat motif	Set	Annealing temp. (°C)	No. cycles	Dye	N	Allele size range (bp)	No. alleles
Lc110	Di	1	48	30	HEX	1422	92-104	10
FCA 043	Di	1	50	30	6-FAM	1438	130-142	7
FCA 023	Di	1	50	30	HEX	1418	151-163	7
Lc109	Di	1	48	35	6-FAM	1433	180-204	13
FCA 391	Tetra	1	50	35	HEX	1409	206-236	10
FCA 008	Di	2	50	30	6-FAM	1439	132-172	11
FCA 045	Di	2	50	35	HEX	1411	150-178	8
BCE5T	Tetra	2	50	35	6-FAM	1401	257-318	8
FCA 740	Tetra	2	50	35	HEX	1429	330-362	9
FCA 090	Di	3	50	30	6-FAM	1423	117-129	7
FCA 149	Di	3	50	35	HEX	1423	132-158	14
Lc111	Di	3	48	35	6-FAM	1403	151-217	14
FCA 096	Di	3	50	35	HEX	1422	191-219	14
FCA 031	Di	3	50	35	6-FAM	1426	232-258	12
FCA 559	Tetra	4	53	35	6-FAM	1416	105-141	10
FCA 026	Di	4	47	32	HEX	1429	141-169	15
FCA 077	Di	4	48	35	6-FAM	1434	148-168	10
FCA 132	Di	4	47	32	HEX	1378	182-198	9
FCA 082	Di	4	47	32	6-FAM	1330	246-268	12

Table 2. Summary of microsatellite and mtDNA genetic variation for six bobcat populations inferred from cluster analysis.

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

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 ($r \geq 0.45$) removed from the population. All comparisons were statistically significant ($P < 0.0001$) based on 10,000 permutations.

K	*Population*	*Central*	*Western*	*Northern*	*Michigan*	*Southeastern*	*Indiana*
6	***Central***	-	0.051	0.047	0.090	0.035	0.119/0.101
	Western	0.051	-	0.052	0.111	0.040	0.137/0.116
	Northern	0.047	0.047	-	0.089	0.024	0.121/0.102
	Michigan	0.091	0.108	0.089	-	0.076	0.153/0.129
	Southeastern	0.035	0.037	0.025	0.075	-	0.082/0.062
	Indiana	0.118/0.100	0.130/0.109	0.119/0.099	0.151/0.127	0.080/0.059	-
5	***Central***	-	0.050	0.047	0.089	0.033	
	Western	0.048	-	0.050	0.108	0.039	
	Northern	0.047	0.045	-	0.092	0.025	
	Michigan	0.085	0.101	0.085	-	0.074	
	Southeastern	0.043	0.044	0.032	0.072	-	

Table 4. Population pairwise Φ_{ST} values based on the mtDNA data. All comparisons were statistically significant ($P < 0.0001$) except Northern – Michigan ($P > 0.05$).

Population	*Central*	*Western*	*Northern*	*Michigan*	*Southeastern*
Western	0.329	-			
Northern	0.423	0.483	-		
Michigan	0.399	0.391	0.009	-	
Southeastern	0.090	0.350	0.225	0.217	-
Indiana	0.546	0.462	0.586	0.602	0.426

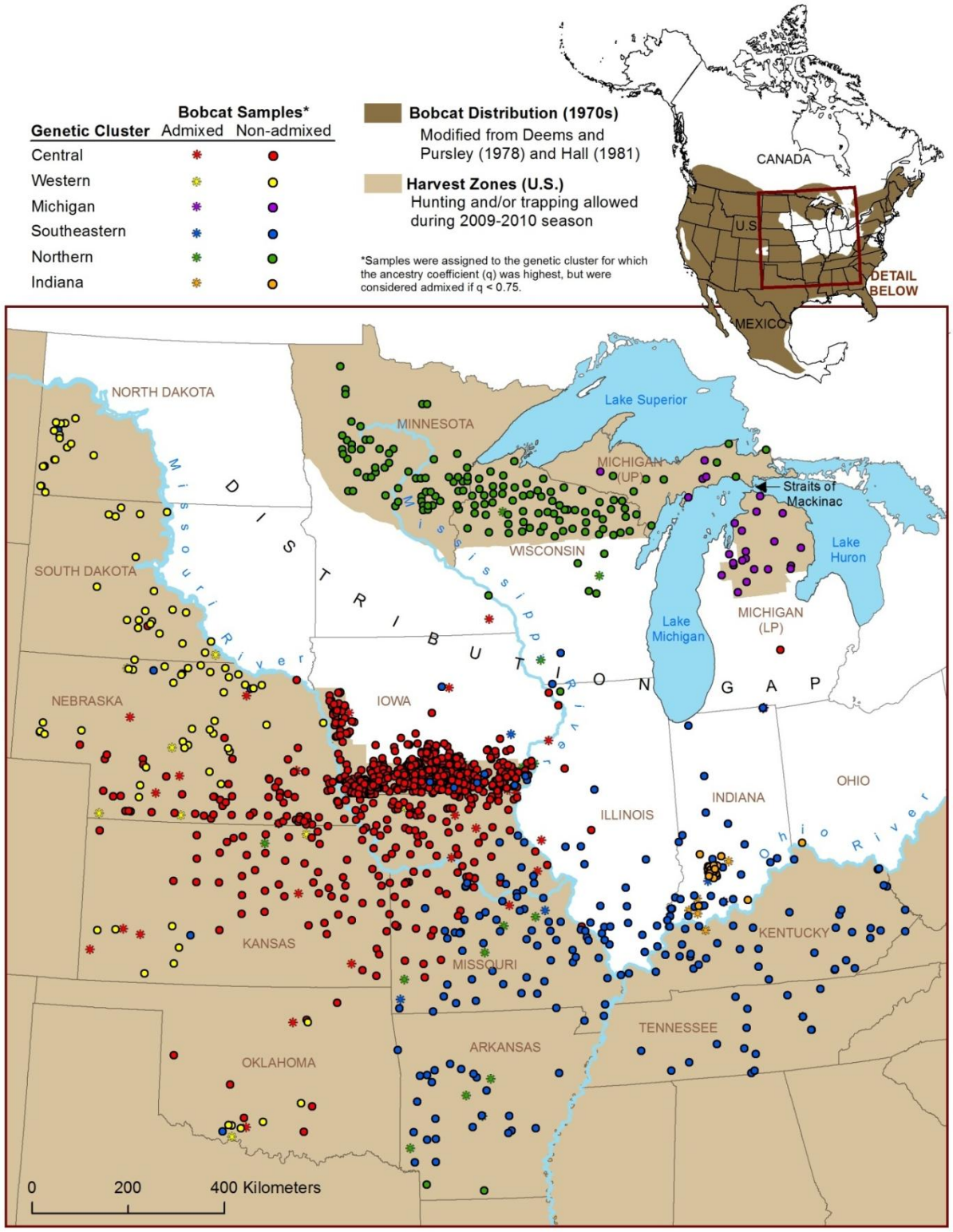
Bobcat Samples*
Genetic Cluster Admixed Non-admixed
Central
Western
Michigan
Southeastern
Northern
Indiana
Bobcat Distribution (1970s)
Modified from Deems and Pursley (1978) and Hall (1981)
Harvest Zones (U.S.)
Hunting and/or trapping allowed during 2009-2010 season
*Samples were assigned to the genetic cluster for which the ancestry coefficient (q) was highest, but were considered admixed if q < 0.75.
CANADA
U.S.
MEXICO
DETAIL BELOW
NORTH DAKOTA
MINNESOTA
Lake Superior
MICHIGAN (UP)
Straits of Mackinac
WISCONSIN
Lake Huron
SOUTH DAKOTA
Missouri River
DISTRIBUTION GAP
Mississippi River
Lake Michigan
MICHIGAN (LP)
NEBRASKA
IOWA
ILLINOIS
INDIANA
OHIO
Ohio River
KANSAS
MISSOURI
KENTUCKY
TENNESSEE
ARKANSAS
OKLAHOMA
0 200 400 Kilometers

Fig. 1

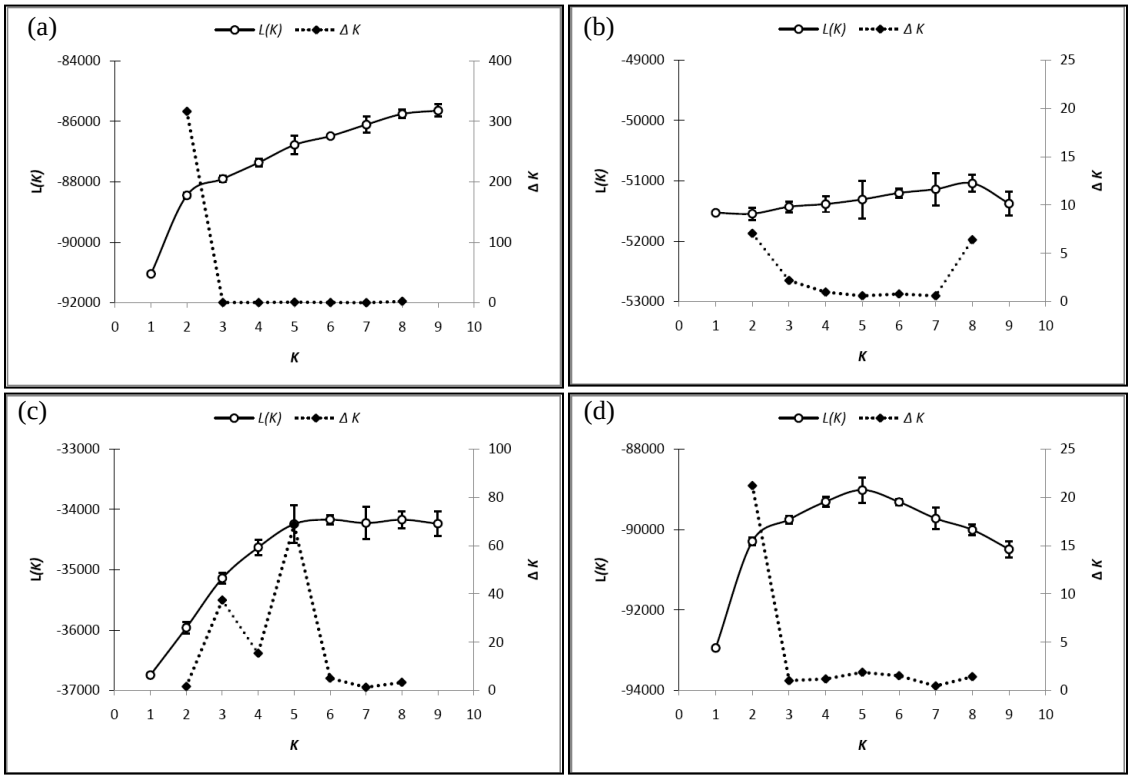
(a)
L(K)
Δ K
-84000
-86000
-88000
-90000
-92000
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10
K
(b)
L(K)
Δ K
-49000
-50000
-51000
-52000
-53000
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
K
(c)
L(K)
Δ K
-33000
-34000
-35000
-36000
-37000
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
K
(d)
L(K)
Δ K
-88000
-90000
-92000
-94000
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
K

Fig. 2

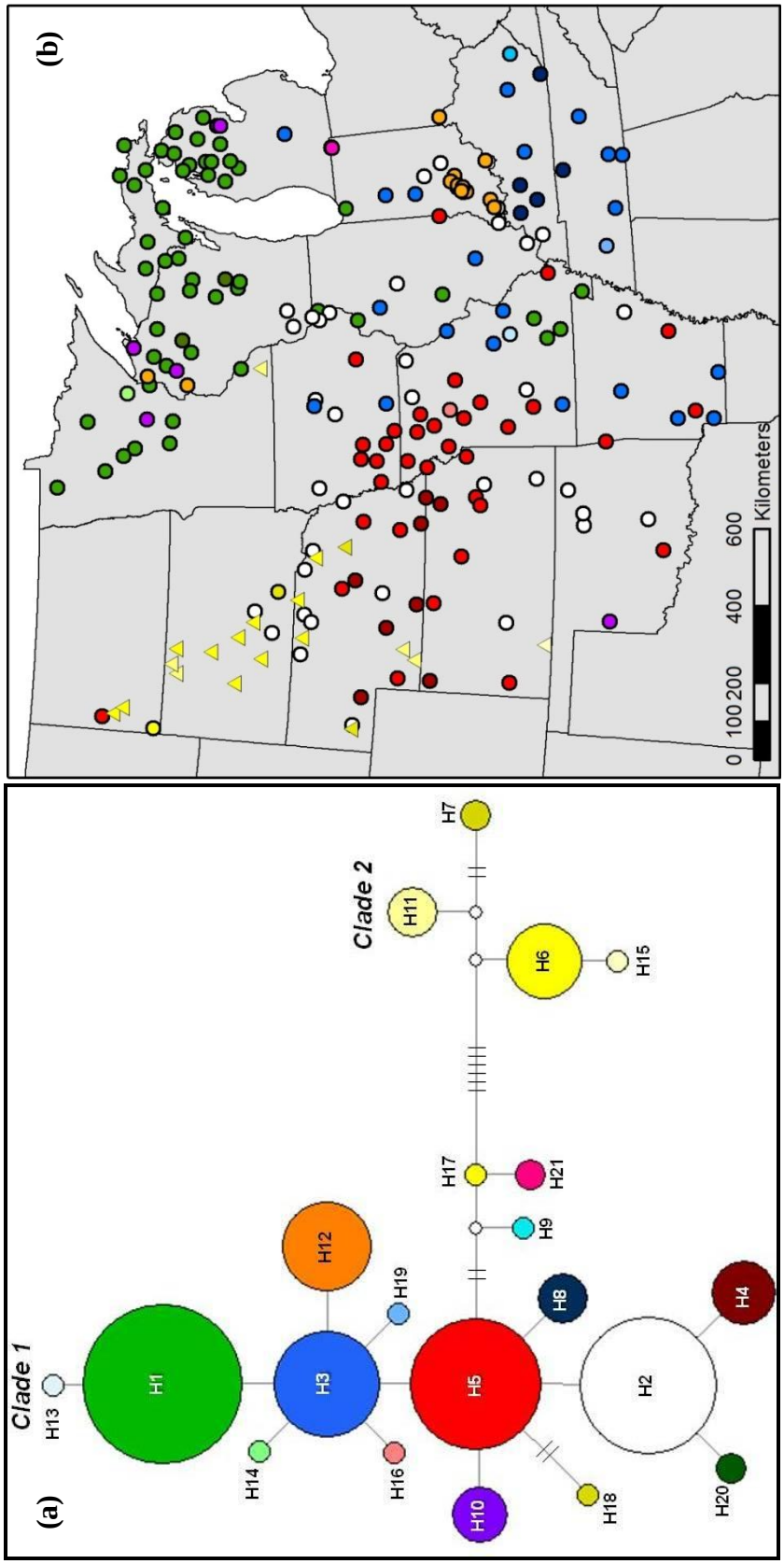
(a)
Clade 1
Clade 2
H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
(b)
0 100 200 400 600 Kilometers

Fig. 3

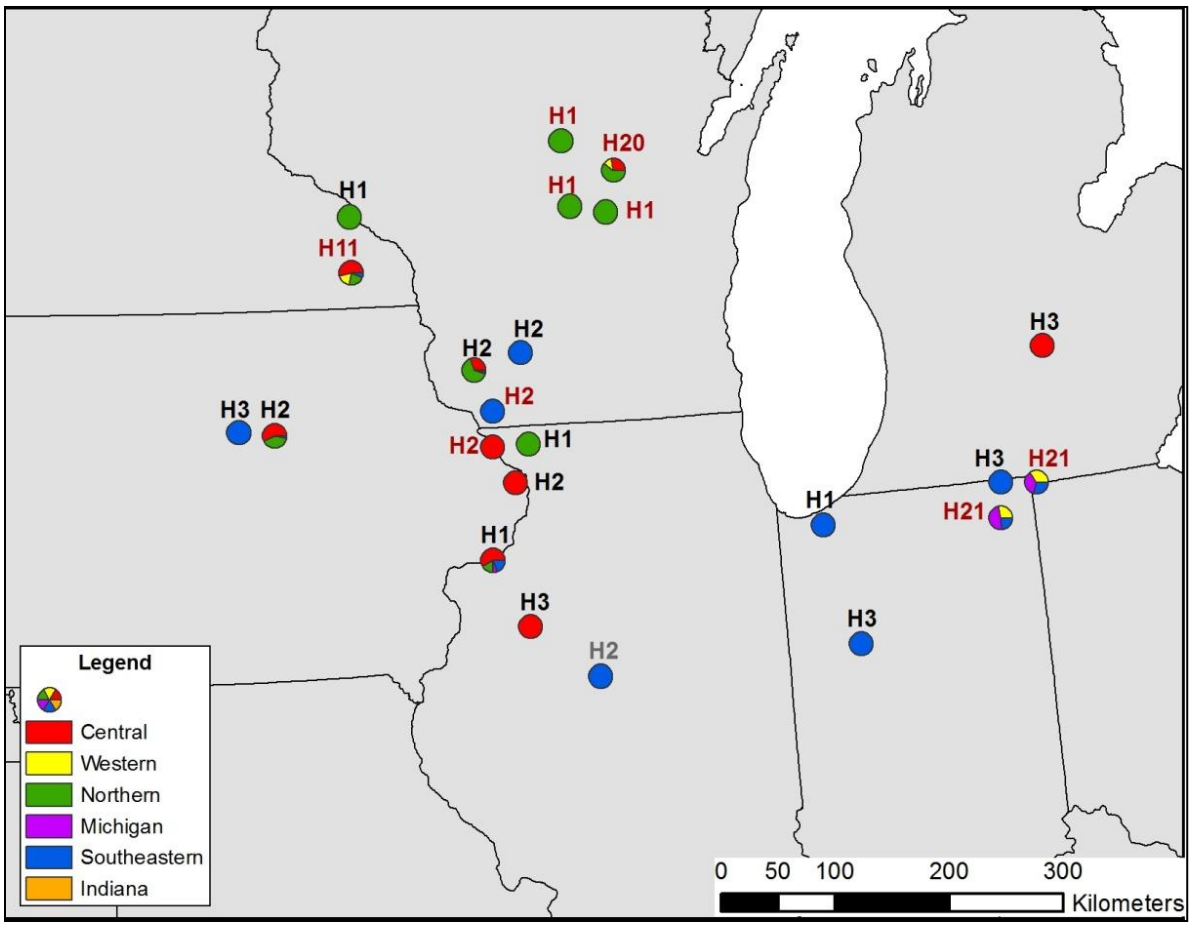
H1
H20
H1
H1
H1
H11
H2
H2
H2
H3 H2
H2
H1
H2
H1
H3
H2
H3
H3
H21
H21
H1
H3
Legend
Central
Western
Northern
Michigan
Southeastern
Indiana
0 50 100 200 300
Kilometers

Fig. S1

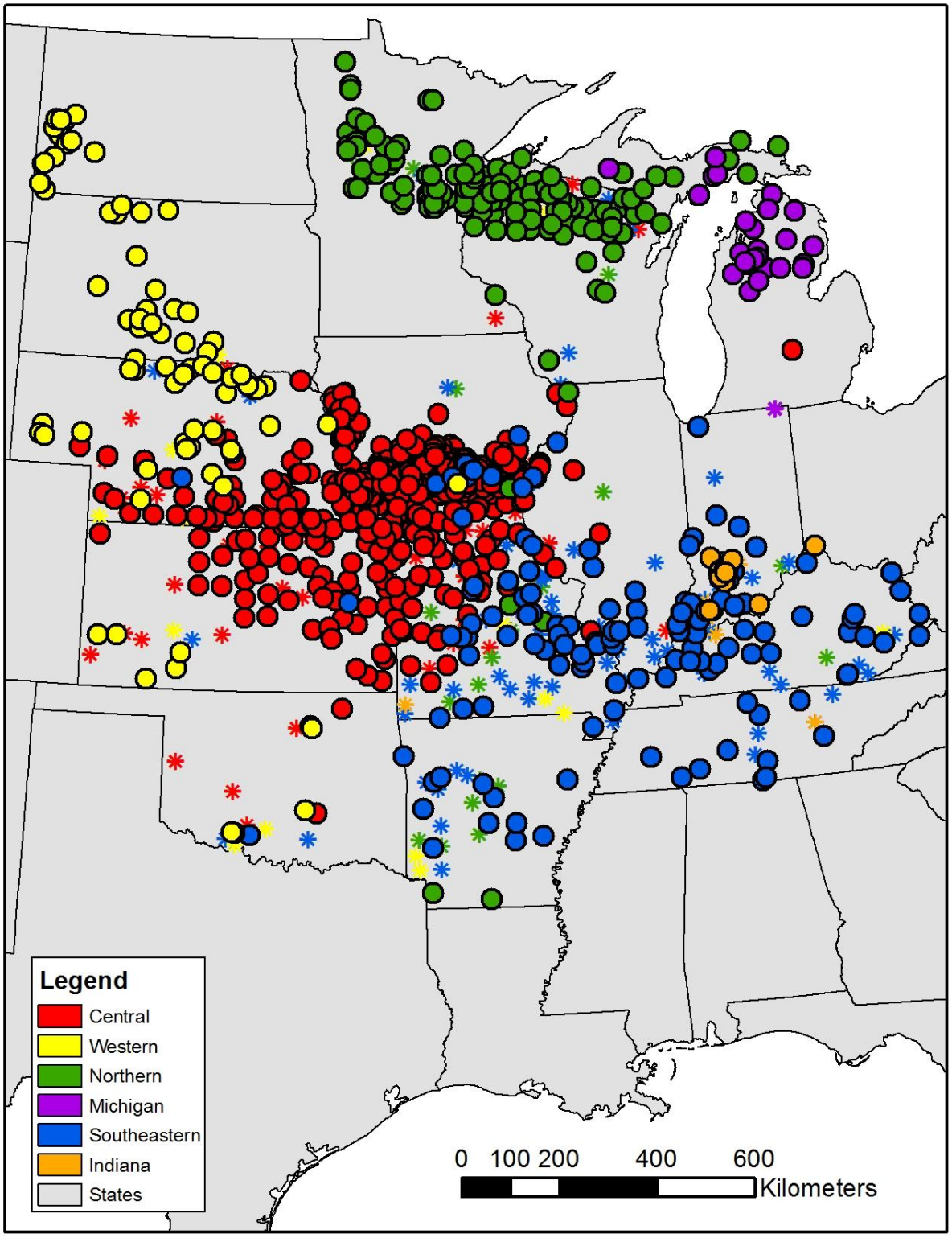
Legend
Central
Western
Northern
Michigan
Southeastern
Indiana
States
0 100 200 400 600
Kilometers

Fig. S2

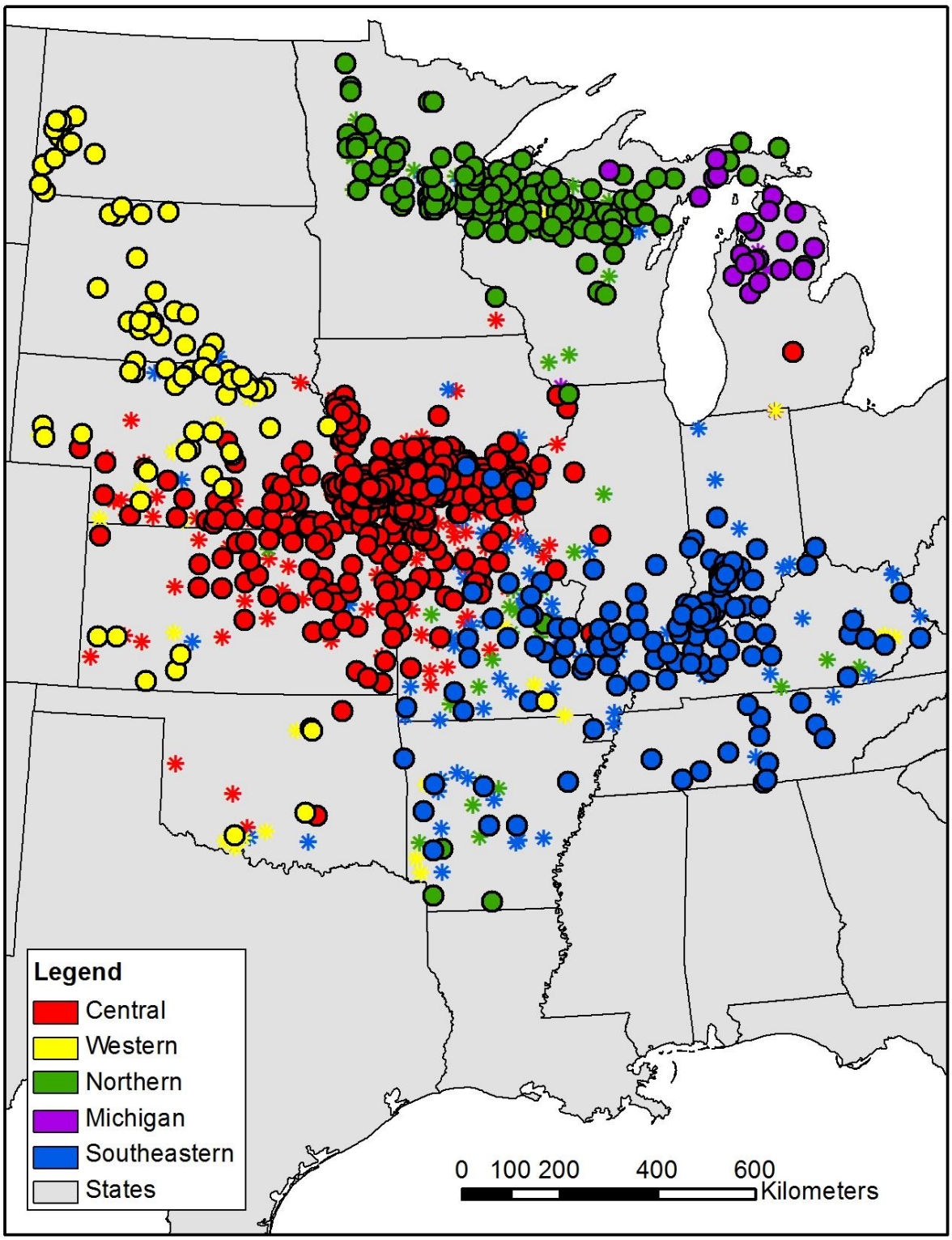
Legend
Central
Western
Northern
Michigan
Southeastern
States
0 100 200 400 600
Kilometers

Fig. S3

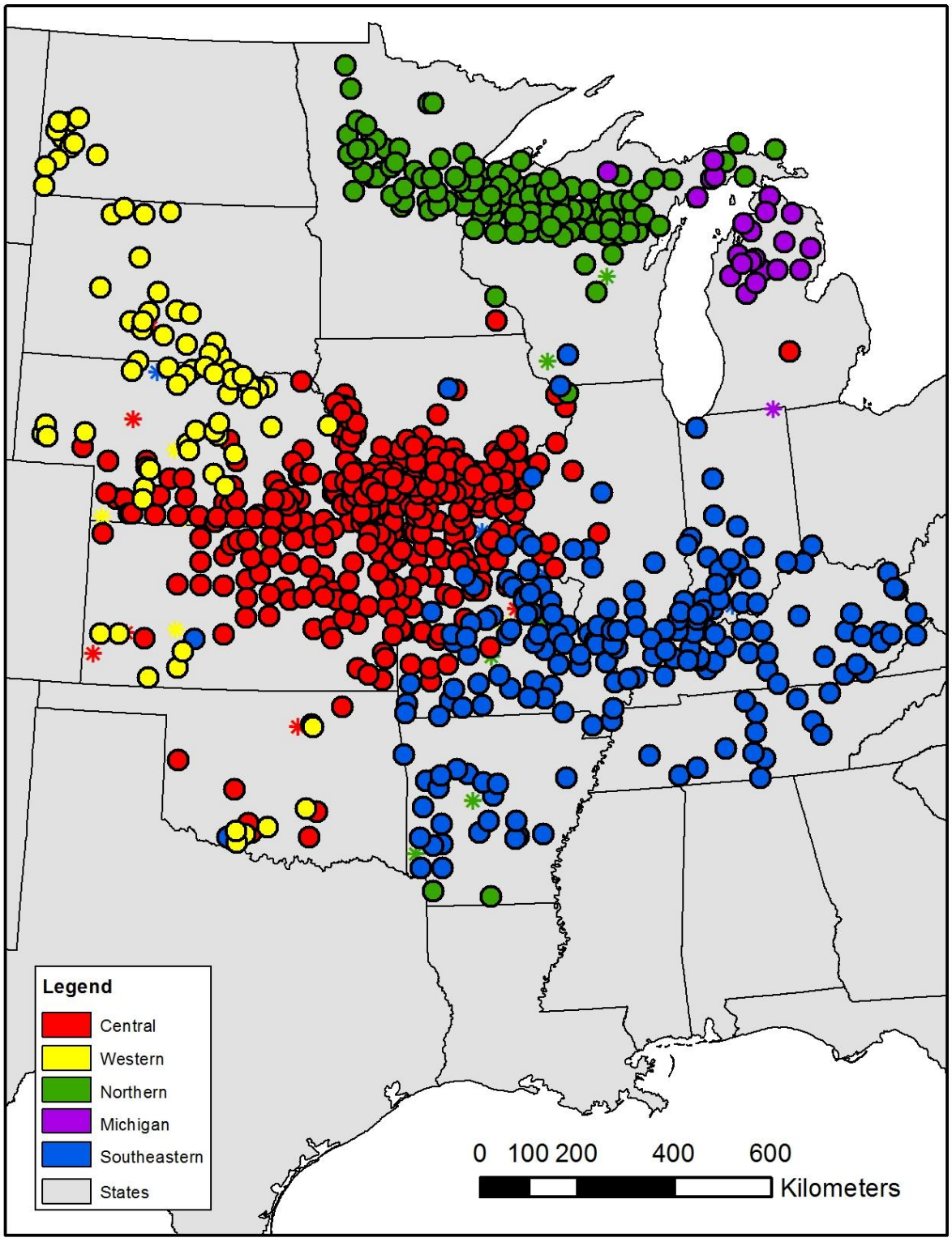
Legend
Central
Western
Northern
Michigan
Southeastern
States
0 100 200 400 600
Kilometers

Fig. S4

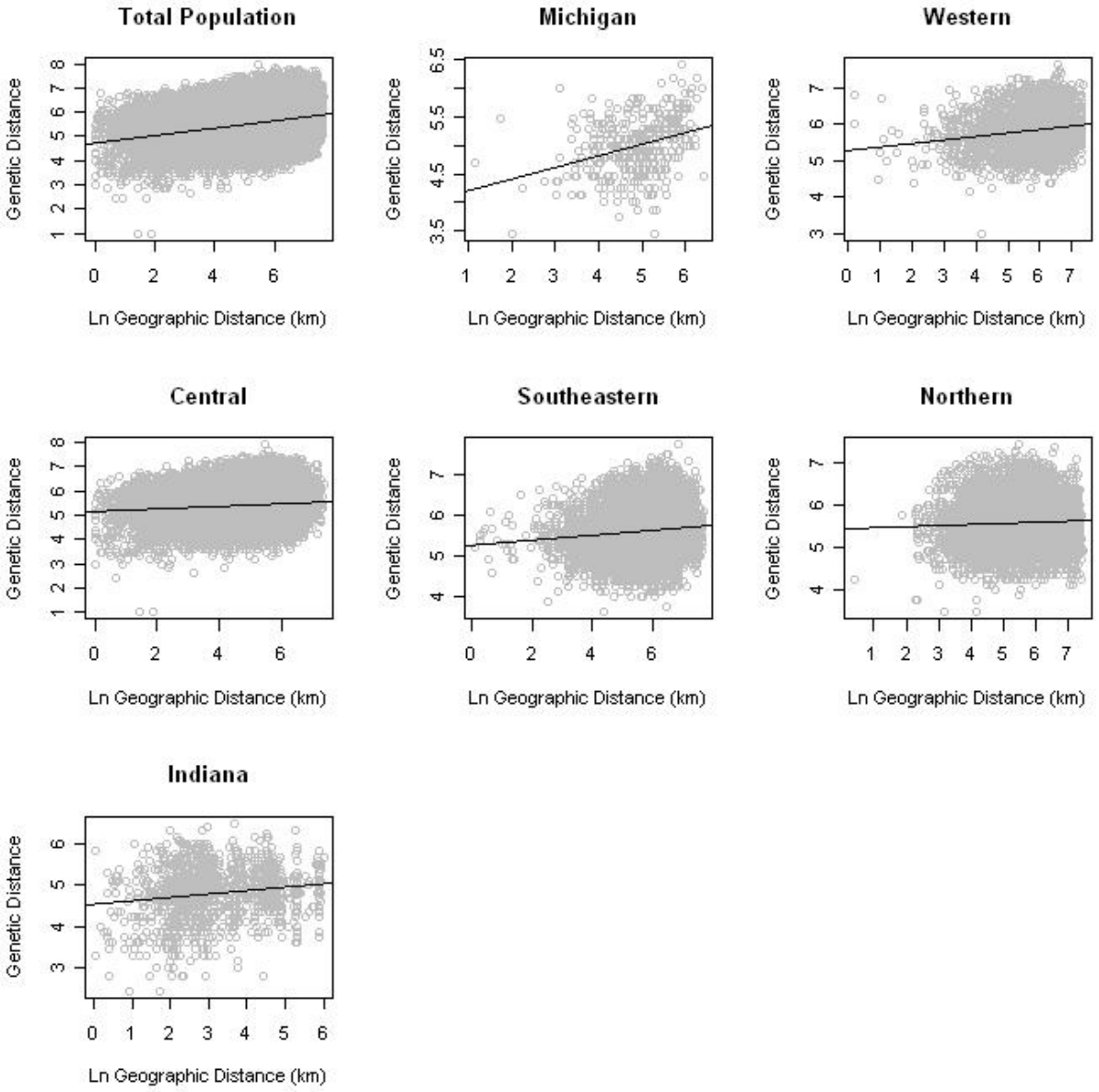
Total Population
Genetic Distance
Ln Geographic Distance (km)
Michigan
Genetic Distance
Ln Geographic Distance (km)
Western
Genetic Distance
Ln Geographic Distance (km)
Central
Genetic Distance
Ln Geographic Distance (km)
Southeastern
Genetic Distance
Ln Geographic Distance (km)
Northern
Genetic Distance
Ln Geographic Distance (km)
Indiana
Genetic Distance
Ln Geographic Distance (km)

Fig. S5

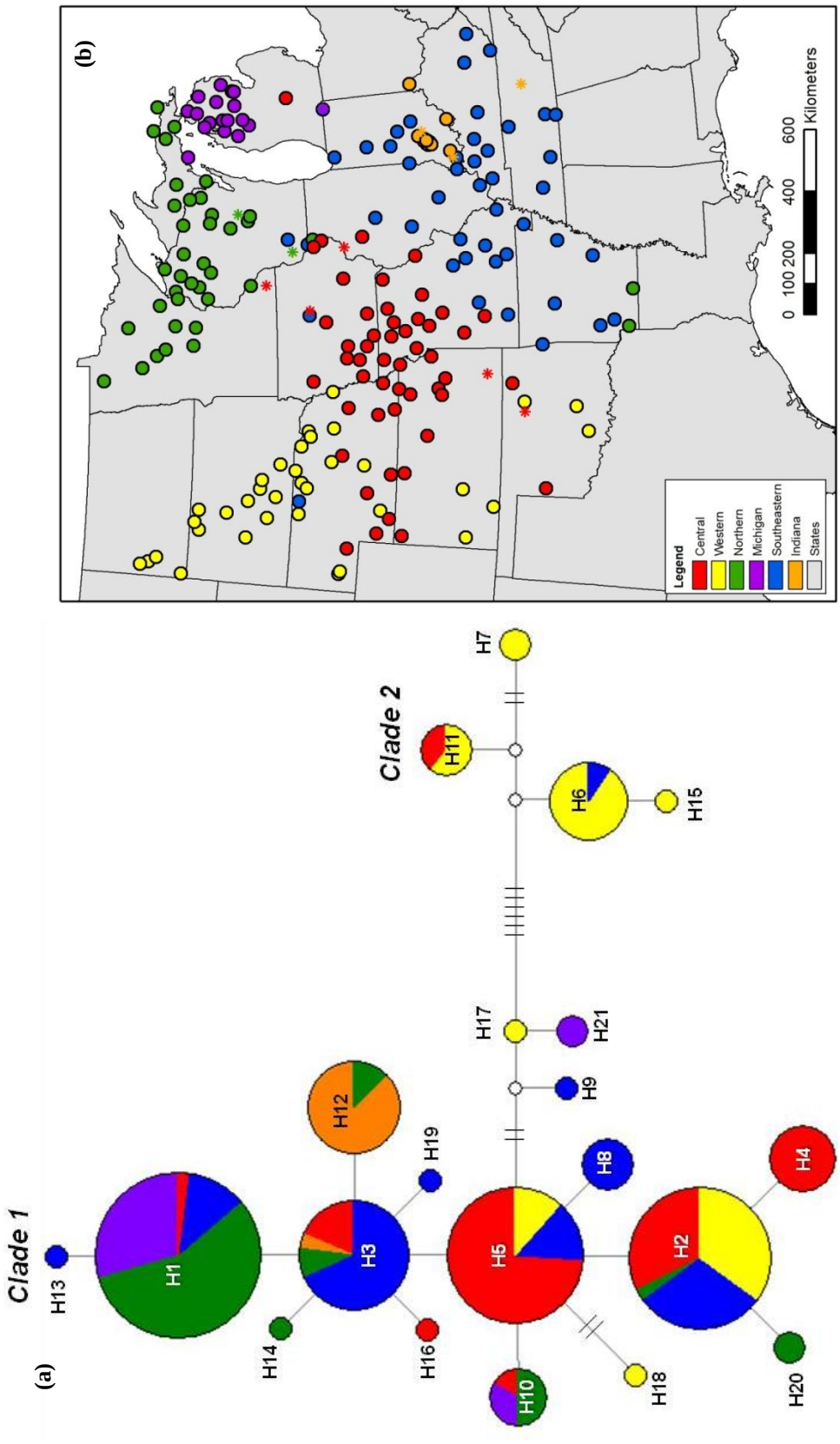
(a)
Clade 1
Clade 2
H13
H1
H14
H3
H12
H19
H16
H5
H10
H8
H9
H17
H21
H18
H2
H4
H20
H11
H7
H6
H15
(b)
Legend
Central
Western
Northern
Michigan
Southeastern
Indiana
States
0 100 200 400 600
Kilometers

Fig. S6

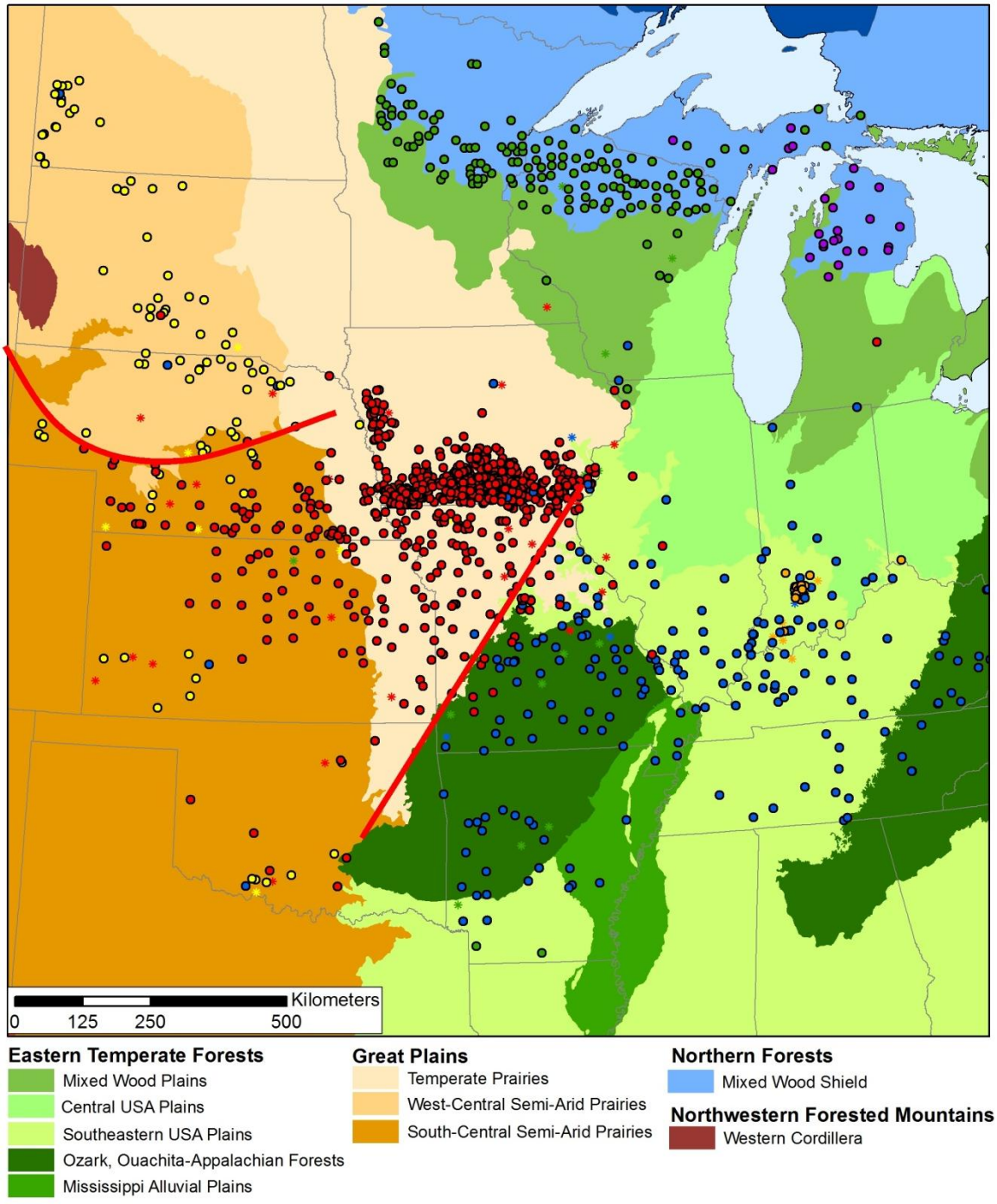
Kilometers
0 125 250 500
Eastern Temperate Forests
Mixed Wood Plains
Central USA Plains
Southeastern USA Plains
Ozark, Ouachita-Appalachian Forests
Mississippi Alluvial Plains
Great Plains
Temperate Prairies
West-Central Semi-Arid Prairies
South-Central Semi-Arid Prairies
Northern Forests
Mixed Wood Shield
Northwestern Forested Mountains
Western Cordillera

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 (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 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 (Unites 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 $MgSO^4$ (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 ID*PROOF* 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 amplifed 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'-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.

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 ≤ 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_{max} (the maximum number of populations) for the range K_{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, LnP(D), for each K, averaged across the 10 runs; 2) calculate the Δ*K* statistic, which is based on the rate of change in LnP(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 ΔK 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 (π) 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 θ_0 and θ_1 describe the population sizes before and after the expansion, while τ 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 τ is estimated from the mismatch distribution, μ 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 1.22×10^{-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 Φ_{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 heterozyogotes 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 (*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; *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 LnP(D) rose sharply for the first few K and then reached a plateau at approximately K = 12, whereas ΔK values peaked at K = 2 (Fig. 6). Evanno *et al.* (2005) showed that when hierarchical structure exists in a data set, the ΔK method detects the uppermost level of population structure. In the bobcat data, the pattern of ΔK 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 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 $\leq$ 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 θ_0 to θ_1, as 95% CIs around θ_0 always included 0 whereas CIs around θ_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 θ_0 and θ_1 differed significantly. Based on estimates of τ, 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 Φ_{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

This work was supported by State Wildlife Grant T-1-R-14, the Iowa DNR, Iowa State University, a National Science Foundation Doctoral Dissertation Improvement Grant, and a grant from the Iowa Science Foundation administered by the Iowa Academy of Science. The project would not have been possible without the cooperation and support of many furharvesters, furbuyers, state and federal agencies, and biologists in collecting and donating samples and information. I thank B. Wollenzien, D. Broman, J. Kaufmann, A. Andes, C. Carter, K. Bellinger, H. Yoon, D. Fredrickson, and E. Schroder for field and laboratory assistance.

References

Adams SM, Lindmeier JB, Duvernell DD (2006) Microsatellite analysis of the phylogeography, Pleistocene history and secondary contact hypotheses for the killifish, *Fundulus heteroclitus*. *Molecular Ecology*, **15**, 1109-1123.

Als TD, Hansen MM, Maes GE, *et al.* (2011) All roads lead to home: panmixia of European eel in the Sargasso Sea. *Molecular Ecology*, **20**, 1333–1346.

Anderson EC, Dunham KK (2008) The influence of family groups on inferences made with the program Structure. *Molecular Ecology Resources*, **8**, 1219-1229.

Anderson EM, Lovallo MJ (2003) Bobcat and lynx. In: *Wild Mammals of North America: Biology, Management, and Conservation* (eds. Feldhamer GA, Thompson BC, Chapman JA), pp. 758-786. Johns Hopkins University Press, Baltimore, MD.

Anderson MJ (2003) DISTLM *forward*: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model using forward selection. Department of Statistics, University of Auckland, New Zealand.

Anderson MJ (2004) DISTLM v.5: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model. Department of Statistics, University of Auckland, New Zealand.

Anderson MJ, Ford RB, Feary DA, Honeywill C (2004) Quantitative measures of sedimentation in an estuarine system and its relationship with intertidal soft-sediment infauna. *Marine Ecology Progress Series*, **272**, 33-48.

Anderson MJ, Legendre P (1999) An empirical comparison of permutation methods for tests of partial regression coefficients in a linear model. *Journal of Statistical Computation and Simulation*, **62**, 271-303.

Arbogast, BS (1999) Mitochondrial DNA phylogeography of the new world flying squirrels (Glaucomys): implications for Pleistocene biogeography. *Journal of Mammalogy*, **80**, 142-155.

Aubry KB, Statham MJ, Sacks BN, Perrine JD, Wisely SM (2009) Phylogeography of the North American red fox: vicariance in Pleistocene forest refugia. *Molecular Ecology*, **18**, 2668-2686.

Avise JC, Walker D, Johns GC (1998) Speciation durations and Pleistocene effects on vertebrate phylogeography. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 1707-1712.

Bailey (1983) Delineation of ecosystem regions. *Environmental Management*, **7**, 365-373.

Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography*, **32**, 818-830.

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37-48.

Bartlein PJ, Anderson KH, Anderson PM, *et al.* (1998) Paleoclimate simulations for North America over the past 21,000 years: features of the simulated climate and comparisons with paleoenvironmental data. *Quaternary Science Reviews*, **17**, 549-585.

Beyer HL (2004) Hawth's Analysis Tools for ArcGIS. Available at http://www.spatialecology.com/htools.

Boulet M, Couturier S, Cote SD, *et al.* (2007) Integrative use of spatial, genetic, and demographic analyses for investigating genetic connectivity between migratory, montane, and sedentary caribou herds. *Molecular Ecology***, 16**, 4223-4240.

Boursot P, Auffray JC, Brittondavidian J, Bonhomme F (1993) The evolution of house mice. *Annual Review of Ecology and Systematics*, **24**, 119-152.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques*, **31**, 24-28.

Brown DM, Brenneman RA, Koepfli KP, *et al.* (2007) Extensive population genetic structure in the giraffe. *BMC Biology*, **5**, 57.

Carling MD, Zuckerberg B (2011) Spatio-temporal changes in the genetic structure of the Passerina bunting hybrid zone. *Molecular Ecology*, **20**, 1166-1175.

Carmichael LE, Clark W, Strobeck C (2000) Development and characterization of microsatellite loci from lynx (*Lynx canadensis*), and their use in other felids. *Molecular Ecology*, **9**, 2197-2198.

Carmichael LE, Krizan J, Nagy JA, *et al.* (2007) Historical and ecological determinants of genetic structure in arctic canids. *Molecular Ecology*, **16**, 3466-3483.

Carmichael LE, Nagy JA, Larter NC, Strobeck C (2001) Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. *Molecular Ecology*, **10**, 2787-2798.

Commission for Environmental Cooperation (1997) *Ecological regions of North America: toward a common perspective.* Commission for Environmental Cooperation, Montreal, Canada.

Corander J, Siren J, Arjas E (2008) Bayesian spatial modeling of genetic population structure. *Computational Statistics*, **23**, 111-129.

Croteau EK (2009) *Population genetics and phylogeography of bobcats (Lynx rufus) using microsatellites and mitochondrial DNA*. PhD dissertation, Southern Illinois University at Carbondale.

Croteau EK, Heist EJ, Nielsen CK (2010) Fine-scale population structure and sex-biased dispersal in bobcats (*Lynx rufus*) from southern Illinois. *Canadian Journal of Zoology*, **88**, 536-545.

Cullingham CI, Kyle CJ, Pond BA, White BN (2008) Genetic structure of raccoons in Eastern North America based on mtDNA: implications for subspecies designation and rabies disease dynamics. *Canadian Journal of Zoology*, **86**, 947-958.

Culver M, Johnson WE, Pecon-Slattery J, O'Brien SJ (2000) Genomic ancestry of the American puma (*Puma concolor*). *Journal of Heredity*, **91**, 186-197.

Deems EF Jr, Pursley D (1978) *North American furbearers: their managment, research, and harvest status in 1976.* International Association of Fish and Wildlife Agencies, Washington, DC.

Earl DA (2011) Structure harvester v0.6.1. Available at http://taylor0.biology.ucla.edu/structureHarvester/ (accessed May 2011).

Edwards S, Claude J, Van Vuuren BJ, *et al.* (2011) Evolutionary history of the Karoo bush rat, *Myotomys unisulcatus* (Rodentia: Muridae): disconcordance between morphology and genetics. *Biological Journal of the Linnean Society*, **102**, 510-526.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

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

Faircloth BC, Reid A, Valentine T, *et al.* (2005) Tetranucleotide, trinucleotide, and dinucleotide loci from the bobcat (*Lynx rufus*). *Molecular Ecology Notes*, **5**, 387-389.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, **164**, 1567-1587.

Feldhamer GA, Thompson BC, Chapman JA (2003) *Wild Mammals of North America: Biology, Management, and Conservation.* Johns Hopkins University Press, Baltimore, MD.

Fisher DO, Owens IPF (2004) The comparative method in conservation biology. *Trends in Ecology & Evolution*, **19**, 391-398.

Fontaine MC, Baird SJE, Piry S, *et al.* (2007) Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. *BMC Biology*, **5**, 30.

Fontanillas P, Depraz A, Giorgi MS, Perrin N (2005) Nonshivering thermogenesis capacity associated to mitochondrial DNA haplotypes and gender in the greater white-toothed shrew, *Crocidura russula*. *Molecular Ecology*, **14**, 661-670.

Francois O, Durand E (2010) Spatially explicit Bayesian clustering models in population genetics. *Molecular Ecology Resources*, **10**, 773-784.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915-925.

Geffen E, Anderson MJ, Wayne RK (2004) Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular Ecology*, **13**, 2481-2490.

Gosselink T, Roberts S, Clark W, Reding D, Linde S (2011) Distribution and population dynamics of bobcats in Iowa. Final Report. State Wildlife Grant T-1-R-14. Iowa Department of Natural Resources, Des Moines, IA.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Graham RW, Lundelius Jr EL (2010) FAUNMAP II: new data for North America with a temporal extension for the Blancan, Irvingtonian and early Rancholabrean. FAUNMAP II Database, version 1.0. http://www.ucmp.berkeley.edu/faunmap.

Grahame JW, Wilding CS, Butlin RK (2006) Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. *Evolution*, **60**, 268-278.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140-1162.

Guillot G, Leblois R, Coulon A, Frantz A (2009) Statistical methods in spatial genetics. *Molecular Ecology*, **18**, 4734-4756.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**, 696-704.

Hall ER (1981) *The mammals of North America.* 2nd edn. John Wiley and Sons, New York, NY.

Hall ER, Kelson KR (1959) The mammals of North America. Ronald Press, New York, NY.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247-276.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965-1978.

Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution*, **22**, 1561-1568.

Homyack JA, Vashon JH, Libby C, *et al.* (2008) Canada lynx-bobcat (*Lynx canadensis* x *L. rufus*) hybrids at the southern periphery of lynx range in Maine, Minnesota and New Brunswick. *American Midland Naturalist*, **159**, 504-508.

Hooge PN, Eichenlaub B (1997) Animal movement extension to ArcView. Version 1.1. Alaska Science Center, Biological Science Office, U.S. Geological Survey, Anchorage, AK.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754-755.

Hugall A, Moritz C, Moussalli A, Stanisic J (2002) Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). *Proceedings of the National Academy of Sciences USA*, **99**, 6112-6117.

Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282-291.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801-1806.

Janečka JE, Blankenship TL, Hirth DH, *et al.* (2007) Evidence for male-biased dispersal in bobcats *Lynx rufus* using relatedness analysis. *Wildlife Biology*, **13**, 38-47.

Johnson SA, Walker HD, Hudson CM (2010) Dispersal characteristics of juvenile bobcats in south-central Indiana. *Journal of Wildlife Management*, **74**, 379-385.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403-1405.

Jombart T, Devillard S, Dufour AB, Pontier D (2008) Revealing cryptic spatial patterns in genetic variability by a new multivariate method. *Heredity*, **101**, 92-103.

Jombart T, Pontier D, Dufour AB (2009) Genetic markers in the playground of multivariate analysis. *Heredity*, **102**, 330-341.

Kamler JF, Gipson PS, Snyder TR (2000) Dispersal characteristics of young bobcats from northeastern Kansas. *The Southwestern Naturalist*, **45**, 543-546.

Knick ST, Bailey TN (1986) Long distance movements by two bobcats from southeastern Idaho. *The American Midland Naturalist*, **116**, 222-223.

Kloor K (1999) Lynx and biologists try to recover after disastrous start. *Science*, **285**, 320-321.

Kozak KH, Graham CH, Wiens JJ (2008) Integrating GIS-based environmental data into evolutionary biology. *Trends in Ecology & Evolution*, **23**, 141-148.

Larivière S, Walton LR (1997) *Lynx rufus*. *Mammalian Species*, **563,** 1-8.

Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295-302.

Latch EK, Heffelfinger JR, Fike JA, Rhodes OE (2009) Species-wide phylogeography of North American mule deer (*Odocoileus hemionus*): cryptic glacial refugia and postglacial recolonization. *Molecular Ecology*, **18**, 1730-1745.

Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, **69**, 1-24.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics,* **25**, 1451-1452.

Lister AM (2004) The impact of Quaternary Ice Ages on mammalian evolution. *Philosophical Transactions of the Royal Society B-Biological Sciences,* **359**, 221-241.

Lopez JV, Culver M, Stephens JC, Johnson WE, O'Brien SJ (1997) Rates of nuclear and cytoplasmic mitochondrial DNA sequence divergence in mammals. *Molecular Biology and Evolution*, **14**, 277-286.

Lovallo MJ (2001) Status and management of bobcat in Pennsylvania. Pages 74-79 in Woolf A, Nielsen CK, Bluett RD, editors. *Proceedings of a Symposium on Current Bobcat Research and Implications for Management*. The Wildlife Society 2000 Conference, Nashville, TN.

Mantel N (1967) Detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209-220.

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology,* **82**, 290-297.

McCord CM, Cardoza JE (1982) Bobcat and lynx. In: *Wild mammals of North America* (eds. Chapman JA, Feldhammer GA), pp. 728-766. The Johns Hopkins University Press, Baltimore, MD.

McRae BH, Beier P, Dewald LE, Huynh LY, Keim P (2005) Habitat barriers limit gene flow and illuminate historical events in a wide-ranging carnivore, the American puma. *Molecular Ecology,* **14**, 1965-1977.

Menotti-Raymond M, David VA, Lyons LA, *et al.* (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics,* **57**, 9-23.

Menotti-Raymond MA, David VA, Wachter LL, Butler JM, O'Brien SJ (2005) An STR forensic typing system for genetic individualization of domestic cat (*Felis catus*) samples. *Journal of Forensic Sciences*, **50**, 1061-1070.

Miller Jr GS, Kellogg R (1955) List of North American Recent mammals. *Bulletin of the United States National Museum*, **205**, 1-954.

Möller LM, Wiszniewski J, Allen SJ, Beheregaray LB (2007) Habitat type promotes rapid and extremely localised genetic differentiation in dolphins. *Marine and Freshwater Research*, **58**, 640-648.

Moritz C (1994) Defining evolutionarily significant units for conservation. *Trends in Ecology & Evolution*, **9**, 373-375.

Musiani M, Leonard JA, Cluff HD, *et al.* (2007) Differentiation of tundra/taiga and boreal coniferous forest wolves: genetics, coat colour and association with migratory caribou. *Molecular Ecology*, **16**, 4149-4170.

Nichols RA, Hewitt GM (1994) The genetic consequences of long-distance dispersal during colonization. *Heredity*, **72**, 312-317.

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

Pease KM, Freedman AH, Pollinger JP, *et al.* (2009) Landscape genetics of California mule deer (*Odocoileus hemionus*): the roles of ecological and historical factors in generating differentiation. *Molecular Ecology*, **18**, 1848-1862.

Peterson RL, Downing SC (1952) Notes on the bobcat (*Lynx rufus*) of eastern North America with the description of a new race. *Contributions of the Royal Ontario Museum*, **33**, 1-23.

Pilot M, Jedrzejewski W, Branicki W, *et al.* (2006) Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology,* **15**, 4533-4553.

Platt A, Horton M, Huang YS, *et al.* (2010) The scale of population structure in *Arabidopsis thaliana*. *PLoS Genetics,* **6**, e1000843.

Placyk JS, Burghardt GM, Small RL, *et al*. (2007) Post-glacial recolonization of the Great Lakes region by the common gartersnake (*Thamnophis sirtalis*) inferred from mtDNA sequences. *Molecular Phylogenetics and Evolution*, **43**, 452-467.

Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253-1256.

Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution*, **16**, 37-45.

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

Read JA (1981) *Geographic variation in the bobcat (Felis rufus) in the south-central United States*. MS thesis, Texas A&M University.

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.

Reding DR, Clark WR, Bronikowski AM (2011) Population genetics of bobcats (*Lynx rufus*) in Oregon. Final Report. Iowa State University, Ames, Iowa.

Remington CL (1968) Suture-zones of hybrid interaction between recently joined biotas. In: *Evolutionary Biology* (eds. Dobzhansky T, Hecht MK, Steere WC), pp. 321-428. Plenum, New York, NY.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.

Roberts NM, Crimmins SM (2010) Bobcat population status and management in North America: evidence of large-scale population increase. *Journal of Fish and Wildlife Management*, **1**, 169-174.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552-569.

Rolley RE (1987) Bobcat. In: *Wild furbearer management and conservation in North America* (eds. Novak M, Baker JA, Obbard ME, Malloch B), pp. 671-681. Ontario Ministry of Natural Resources, Toronto, Ontario, Canada.

Rosenberg N A, Mahajan S, Ramachandran S, *et al.* (2005) Clines, clusters, and the effect of study design on the inference of human population structure. *PLoS Genetics*, **1**, e70.

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

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

Rowe KC, Heske EJ, Brown PW, Paige KN (2004) Surviving the ice: northern refugia and postglacial colonization. *Proceedings of the National Academy of Sciences*, **101**, 10355-10359.

Rowe KC, Heske EJ, Paige KN (2006) Comparative phylogeography of eastern chipmunks and white-footed mice in relation to the individualistic nature of species. *Molecular Ecology*, **15**, 4003-4020.

Runck AM, Cook JA (2005) Postglacial expansion of the southern red-backed vole (*Clethrionomys gapperi*) in North America. *Molecular Ecology*, **14**, 1445-1456.

Sacks BN, Bannasch DL, Chomel BB, Ernest HB (2008) Coyotes demonstrate how habitat specialization by individuals of a generalist species can diversify populations in a heterogeneous ecoregion. *Molecular Biology and Evolution*, **25**, 1384-1394.

Sacks BN, Brown SK, Ernest HB (2004) Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species history. *Molecular Ecology*, **13**, 1265-1275.

Sacks BN, Mitchell BR, Williams CL, Ernest HB (2005) Coyote movements and social structure along a cryptic population genetic subdivision. *Molecular Ecology*, **14**, 1241-1249.

Safner T, Miller MP, McRae BH, Fortin MJ, Manel S (2011) Comparison of Bayesian clustering and edge detection methods for inferring boundaries in landscape genetics. *International Journal of Molecular Sciences*, **12**, 865-889.

Samson FB (1979) Multivariate analysis of cranial characteristics among bobcats, with a preliminary discussion of the number of subspecies. In: *Proceedings of the 1979 bobcat research conference: current research on biology and management of Lynx rufus. Scientific and Technical Series 6* (eds. Blum L, Escherich PC), pp. 80-86. National Wildlife Federation, Washington, DC.

Schneider S, Excoffier L (1999) Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics*, **152**, 1079-1089.

Schwartz MK, Pilgrim KL, McKelvey KS, *et al.* (2004) Hybridization between Canada lynx and bobcats: genetic results and management implications. *Conservation Genetics*, **5**, 349-355.

Seton ET (1929) *Lives of game animals*. Doubleday, Doran & Co., Inc., Garden City, NY.

Shafer ABA, Cullingham CI, Cote SD, Coltman DW (2010) Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology*, **19**, 4589-4621.

Sikes RS, Kennedy ML (1992) Morphologic variation of the bobcat (*Felis rufus*) in the eastern United States and its association with selected environmental variables. *American Midland Naturalist*, **128**, 313-324.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mtDNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555-562.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561-573.

Stone KD, Flynn RW, Cook JA (2002) Post-glacial colonization of northwestern North America by the forest-associated American marten (*Martes americana*, Mammalia: Carnivora: Mustelidae). *Molecular Ecology*, **11**, 2049-2063.

Swenson NG (2006) GIS-based niche models reveal unifying climatic mechanisms that maintain the location of avian hybrid zones in a North American suture zone. *Journal of Evolutionary Biology*, **19**, 717-725.

Swenson NG, Howard DG (2005) Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *American Naturalist*, **166**, 581-591.

Swenson NG, Howard DJ (2004) Do suture zones exist? *Evolution*, **58**, 2391-2397.

Templeton AR (2006) *Population genetics and microevolutionary theory*. John Wiley & Sons, Inc., Hoboken, NJ.

Templeton AR, Sing CF (1993) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics*, **134**, 659-669.

Unites States Government (2010) Proposal to de-list the bobcat from Appendix II. Proceedings of the 15th Meeting of the Conference of the Parties. Secretariat of CITES: Doha, Qatar.

Waltari E, Hijmans RJ, Peterson AT, *et al.* (2007) Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *PLoS One,* **2**, e563.

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

Werle E, Schneider C, Renner M, Volker M, Fiehn W (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research,* **22**, 4354-4355.

Wigginton JD, Dobson FS (1999) Environmental influences on geographic variation in body size of western bobcats. *Canadian Journal of Zoology*, **77,** 802-813.

Wooding S, Ward R (1997) Phylogeography and Pleistocene evolution in the North American black bear. *Molecular Biology and Evolution*, **14**, 1096-1105.

Woolf A, Hubert GF (1998) Status and management of bobcats in the United States over three decades: 1970s-1990s. *Wildlife Society Bulletin*, **26**, 287-293.

Worton BJ (1989) Kernel methods for estimating the utilization distribution in home-range studies. *Ecology*, **70**,164-168.

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 ΔK 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 Φ_{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.

Marker	Chr	Set	Annealing temp. (°C)	No. cycles	Dye	N	Allele size range (bp)	No. alleles	H_O	H_E	F_{IS}
FCA 090	A1	1	50	30	6-FAM	1662	109-131	9	0.739	0.787	0.061
FCA 149	B1	1	50	35	HEX	1657	134-158	13	0.804	0.885	0.091
Lc109	-	1	48	35	6-FAM	1657	180-204	13	0.725	0.828	0.124
FCA 391	B3	1	50	35	HEX	1640	206-240	11	0.689	0.746	0.076
FCA 008	A1	2	50	30	6-FAM	1672	132-172	14	0.738	0.801	0.078
FCA 096	E2	2	50	35	HEX	1625	187-219	16	0.787	0.865	0.090
BCE5T	-	2	50	35	6-FAM	1651	257-326	12	0.740	0.798	0.073
FCA 740	C1	2	50	32	HEX	1657	330-362	9	0.747	0.798	0.064
FCA 043	C2	3	50	30	6-FAM	1664	126-142	8	0.681	0.762	0.106
Lc111	-	3	48	35	HEX	1636	151-217	15	0.647	0.727	0.110
FCA 031	E3	3	50	35	6-FAM	1653	232-260	14	0.759	0.818	0.072
FCA 559	B1	4	51	35	HEX	1658	105-141	10	0.681	0.721	0.056
FCA 077	C2	4	48	35	6-FAM	1670	148-172	12	0.719	0.780	0.078
FCA 132	D3	4	47	32	HEX	1661	182-198	9	0.802	0.858	0.066
FCA 082	E1	4	50	30	6-FAM	1614	246-268	12	0.788	0.857	0.080

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_O: 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).

Group	N	H_O (SD)	H_E (SD)	F_{IS}	AR
Total	1680	0.736 (0.047)	0.802 (0.050)	0.082*	11.78
Coastal Oregon (navy)	52	0.689 (0.091)	0.718 (0.080)	0.037	5.83
navy1	27	0.689 (0.124)	0.712 (0.086)	0.033	5.11
navy2	25	0.688 (0.109)	0.712 (0.102)	0.035	5.48
Western (yellow)	518	0.749 (0.061)	0.781 (0.063)	0.041	7.00
yellow1	89	0.743 (0.097)	0.767 (0.078)	0.032	6.30
yellow2	62	0.786 (0.077)	0.785 (0.069)	-0.002	6.52
yellow3	58	0.754 (0.082)	0.760 (0.076)	0.008	6.06
yellow4	60	0.733 (0.078)	0.757 (0.073)	0.033	6.04
yellow5	76	0.745 (0.063)	0.761 (0.059)	0.022	6.09
yellow6	68	0.741 (0.082)	0.785 (0.052)	0.056*	6.21
yellow7	38	0.772 (0.080)	0.791 (0.057)	0.024	6.63
yellow8	38	0.742 (0.106)	0.783 (0.068)	0.053	6.47
yellow9	29	0.720 (0.078)	0.750 (0.055)	0.041	5.66
Central (red)	291	0.742 (0.074)	0.762 (0.056)	0.028	6.26
red1	57	0.748 (0.083)	0.770 (0.058)	0.030	5.91
red2	54	0.717 (0.083)	0.746 (0.071)	0.039	5.53
red3	91	0.747 (0.096)	0.768 (0.056)	0.027	5.74
red4	89	0.747 (0.097)	0.750 (0.061)	0.003	5.54
Northern (green)	112	0.718 (0.092)	0.748 (0.076)	0.042	5.83
green1	38	0.720 (0.100)	0.749 (0.082)	0.039	5.47
green2	30	0.715 (0.158)	0.740 (0.072)	0.035	5.20
green3	44	0.720 (0.097)	0.741 (0.082)	0.028	5.30
Michigan (purple)	17	0.675 (0.102)	0.655 (0.099)	-0.032	4.25
Southeastern (blue)	332	0.735 (0.077)	0.775 (0.074)	0.052	6.91
blue1	34	0.732 (0.130)	0.774 (0.071)	0.055*	5.85
blue2	30	0.721 (0.114)	0.760 (0.079)	0.052	6.22
blue3	49	0.781 (0.085)	0.774 (0.063)	-0.009	6.18
blue4	50	0.688 (0.079)	0.728 (0.091)	0.055	5.76
blue5	35	0.753 (0.157)	0.749 (0.100)	-0.006	5.83
blue6	56	0.740 (0.118)	0.766 (0.093)	0.034	6.34
blue7	45	0.732 (0.058)	0.773 (0.062)	0.054	6.19
blue8	33	0.729 (0.118)	0.754 (0.089)	0.033	5.95
Florida (pink)	24	0.724 (0.084)	0.728 (0.106)	0.006	5.48
Pennsylvania (brown)	45	0.728 (0.112)	0.744 (0.077)	0.025	5.84
brown1	24	0.733 (0.109)	0.739 (0.097)	0.010	5.32
brown2	21	0.723 (0.154)	0.729 (0.086)	0.008	5.39
Upper New England (teal)	68	0.666 (0.094)	0.688 (0.086)	0.031	5.22
teal1	21	0.645 (0.124)	0.659 (0.091)	0.012	4.64
teal2	47	0.672 (0.110)	0.690 (0.094)	0.024	4.92
Oklahoma mixture	65	0.785 (0.051)	0.792 (0.063)	0.006	7.24
OKmixture1	29	0.801 (0.068)	0.796 (0.063)	-0.007	6.64
OKmixture2	36	0.771 (0.071)	0.787 (0.067)	0.021	6.46

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 % var the percentage of variation explained by a given predictor or predictor set. For the sequential tests, % var represents the cumulative effect of variables. The top-down sequence of variables corresponds to the sequence indicated by forward selection.

	Marginal tests			**Conditional tests**			**Sequential tests**		
Variable	***F***	***P***	**% var**	***F***	***P***	**% var**	***F***	***P***	**% var**
(a) Results from single predictors									
X coordinate	142.96	0.001	7.87	-	-	-	142.96	0.001	7.87
Precip of Warmest Quarter	116.16	0.001	6.49	21.91	0.001	1.18	22.15	0.001	9.08
Precip of Driest Quarter	79.00	0.001	4.51	17.43	0.001	0.94	16.26	0.001	9.95
Y coordinate	16.25	0.001	0.96	-	-	-	11.00	0.001	10.54
Min Temp of Coldest Month	12.26	0.001	0.73	13.66	0.002	0.74	9.37	0.001	11.04
Elevation	82.29	0.001	4.69	12.76	0.001	0.69	19.45	0.001	12.07
Annual Precip	53.35	0.001	3.09	10.54	0.002	0.57	6.95	0.001	12.43
Precip of Coldest Quarter	12.30	0.001	0.73	13.28	0.001	0.72	11.59	0.001	13.04
Precip of Wettest Quarter	22.26	0.001	1.31	10.58	0.002	0.58	7.19	0.001	13.41
Max Temp of Warmest Month	12.55	0.001	0.74	9.31	0.003	0.51	5.23	0.001	13.68
Precip Seasonality	32.07	0.001	1.88	12.25	0.002	0.67	3.38	0.001	13.86
Annual Mean Temp	15.63	0.001	0.93	4.70	0.031	0.26	3.42	0.001	14.03
Mean Diurnal Range	55.89	0.001	3.23	10.43	0.001	0.57	3.80	0.001	14.23
Temp Seasonality	12.60	0.001	0.75	14.04	0.001	0.76	2.38	0.001	14.35
(b) Results from predictor sets									
Precip	34.73	0.001	11.11	14.05	0.001	4.40	34.73	0.001	11.11
Coordinates	77.85	0.001	8.52	-	-	-	17.40	0.001	12.92
Temp	34.11	0.001	9.27	12.06	0.001	3.19	4.01	0.001	13.96
Elevation	82.29	0.001	4.69	12.76	0.001	0.69	7.61	0.001	14.35

Table 4. Summary of diversity and demographic statistics for an eastern and western clade of bobcat mtDNA haplotypes.

Estimates	Eastern (subclade A)	Western (subclades C/D)
Sample size	594	424
Haplotypes	35	28
h	0.822 ± 0.009	0.783 ± 0.017
π	0.0016 ± 0.0010	0.0025 ± 0.0015
Mean no. pairwise differences	1.485 ± 0.899	2.416 ± 1.314
Fu's F_S (*P*-value)	-27.23 (< 0.001)	-10.42 (0.015)
SSD (*P*-value)	0.0046 (0.015)	0.0185 (0.331)
τ (95% CI)	1.605 (1.424-1.980)	3.793 (0.598-7.168)
θ_0 (95% CI)	0 (0-0.069)	0 (0-1.067)
θ_1 (95% CI)	∞ (19.469-∞)	4.314 (2.168-∞)
Estimated expansion time	26,738 – 37,178 ybp	11,228 – 134,590 ybp

h: haplotype diversity; π: nucleotide diversity; SSD: sum of squared deviations between observed and expected mismatch distributions; τ: parameter reflecting time to expansion; θ_0: parameter reflecting population size prior to expansion; θ_1: parameter reflecting population size after expansion; ybp: years before present.

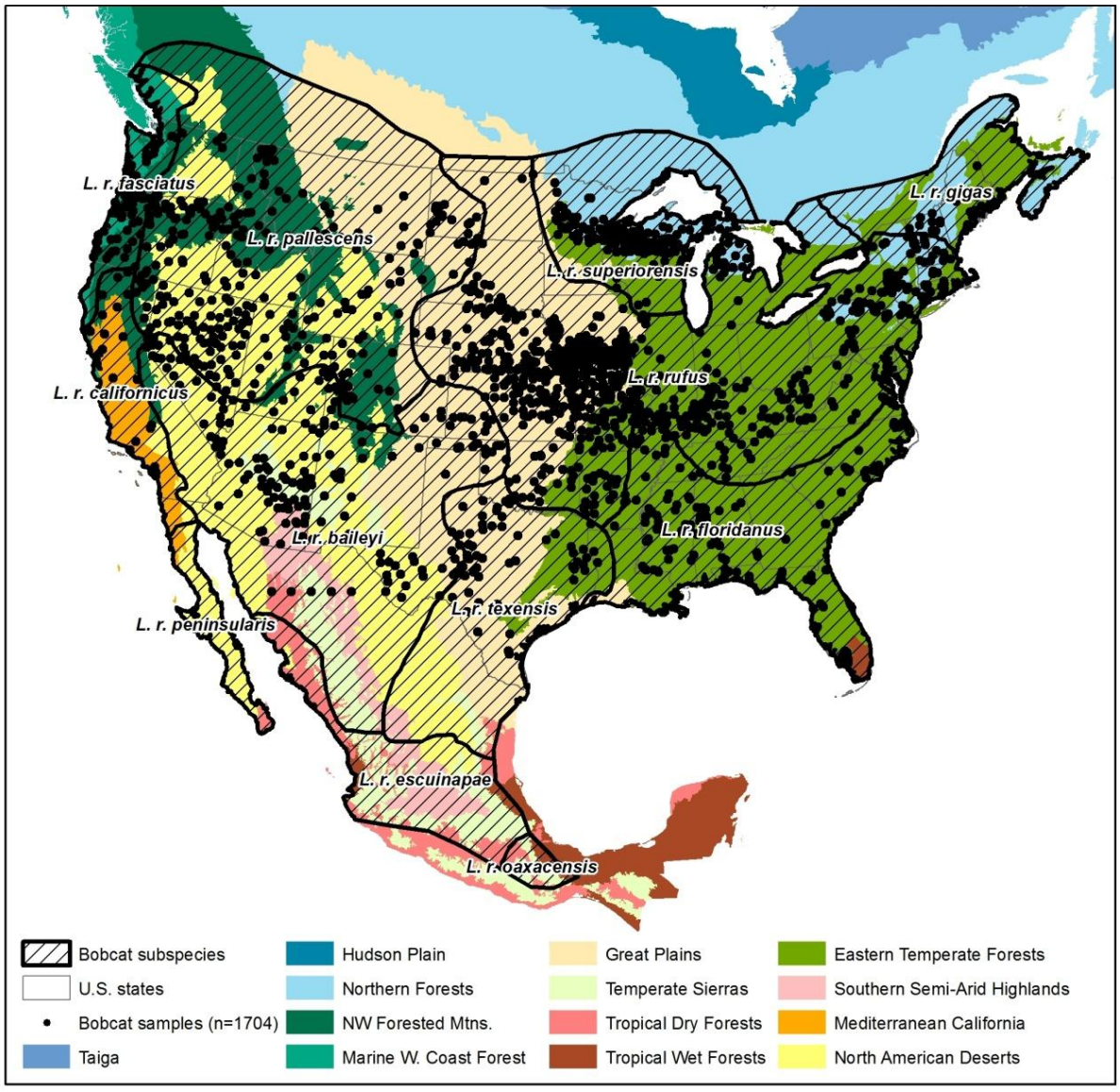
L. r. fasciatus
L. r. pallescens
L. r. californicus
L. r. peninsularis
L. r. baileyi
L. r. texensis
L. r. escuinapae
L. r. oaxacensis
L. r. superiorensis
L. r. rufus
L. r. floridanus
L. r. gigas
Bobcat subspecies
U.S. states
Bobcat samples (n=1704)
Taiga
Hudson Plain
Northern Forests
NW Forested Mtns.
Marine W. Coast Forest
Great Plains
Temperate Sierras
Tropical Dry Forests
Tropical Wet Forests
Eastern Temperate Forests
Southern Semi-Arid Highlands
Mediterranean California
North American Deserts

Fig. 1

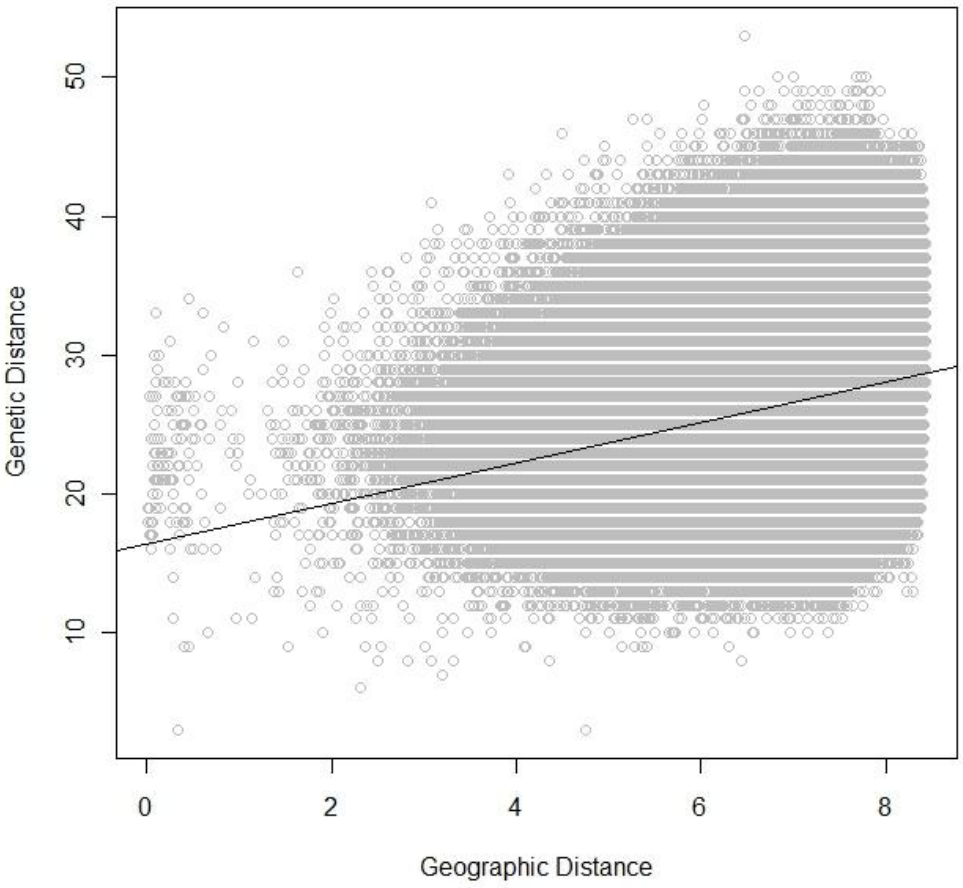

Fig. 2

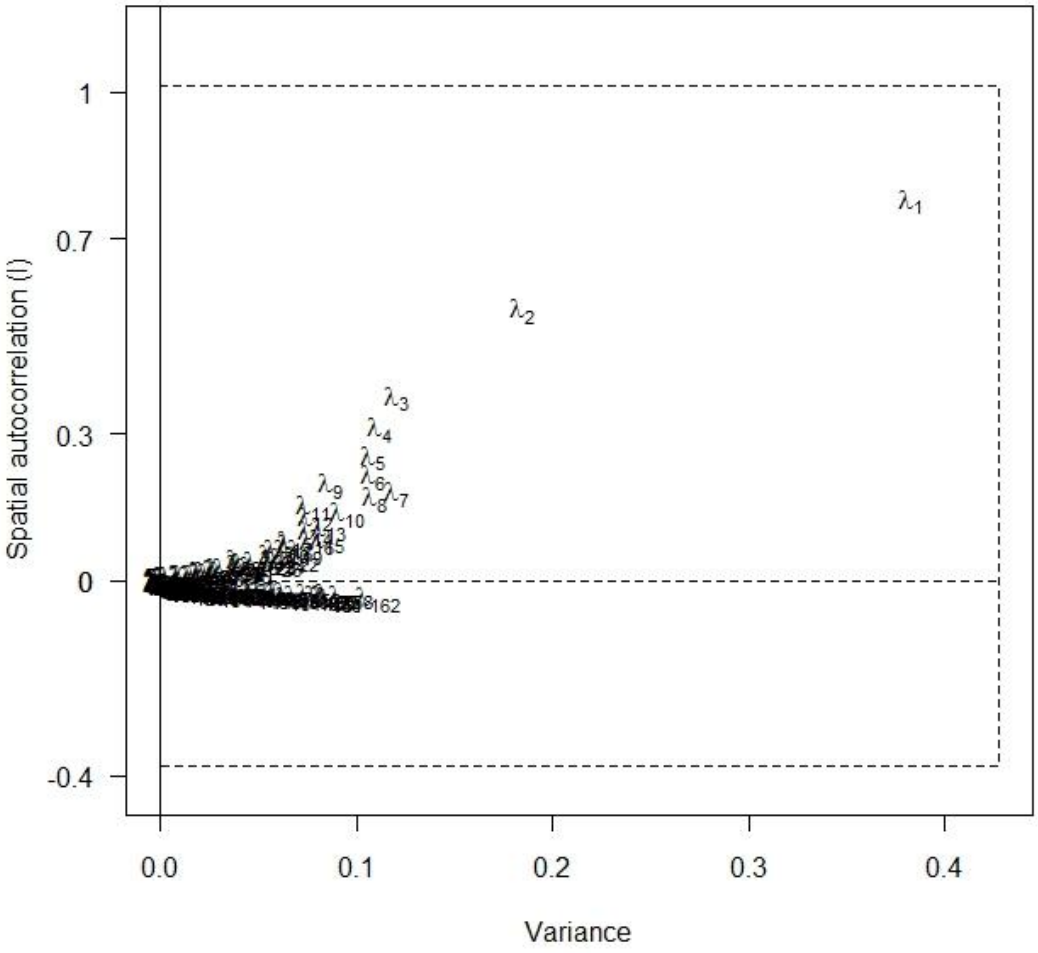
Spatial autocorrelation (I)
1
0.7
0.3
0
-0.4
0.0
0.1
0.2
0.3
0.4
Variance
λ1
λ2
λ3
λ4
λ5
λ6
λ7
λ8
λ9
λ10
λ11
162

Fig. 3

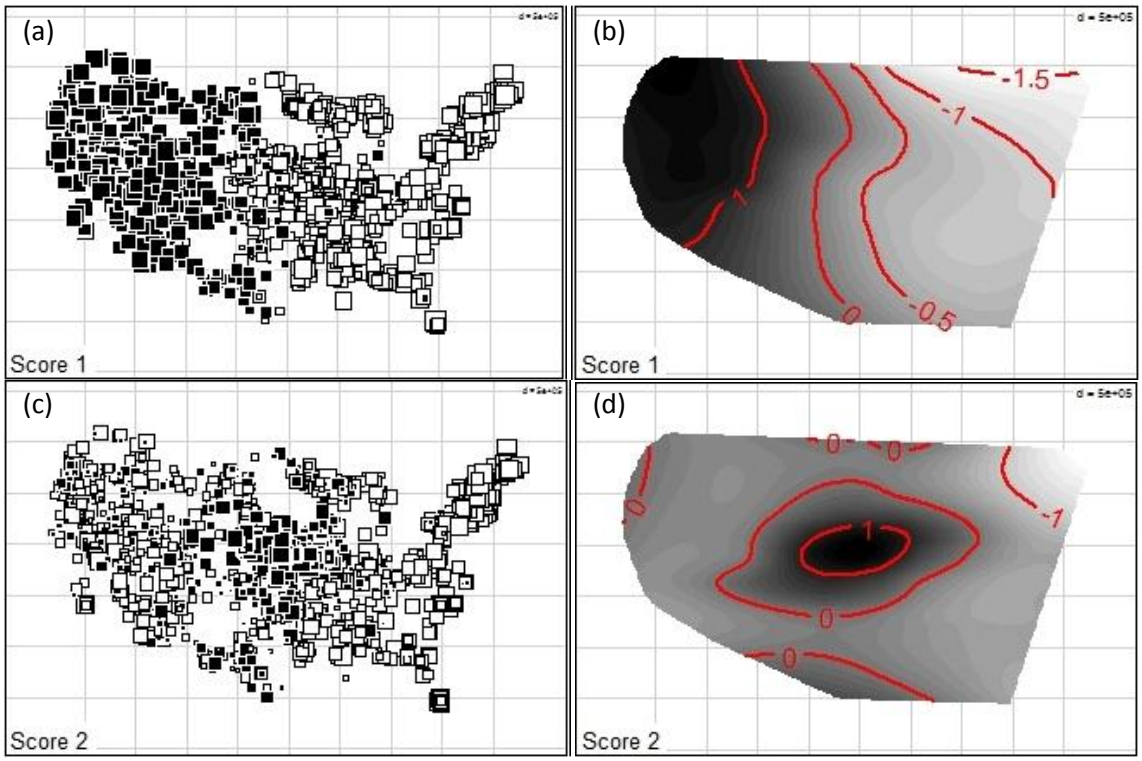

Fig. 4

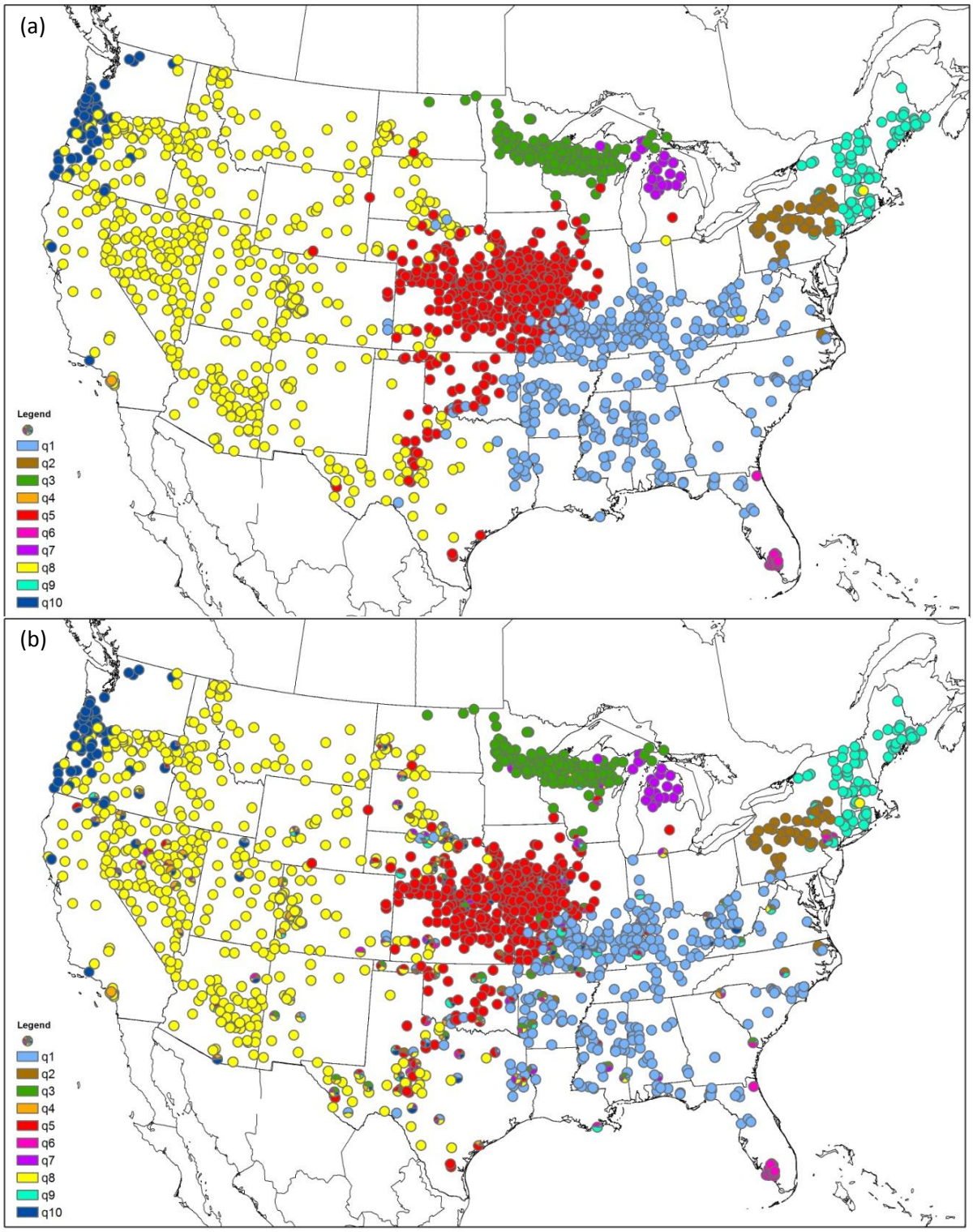
(a)
Legend
q1
q2
q3
q4
q5
q6
q7
q8
q9
q10
(b)
Legend
q1
q2
q3
q4
q5
q6
q7
q8
q9
q10

Fig. 5

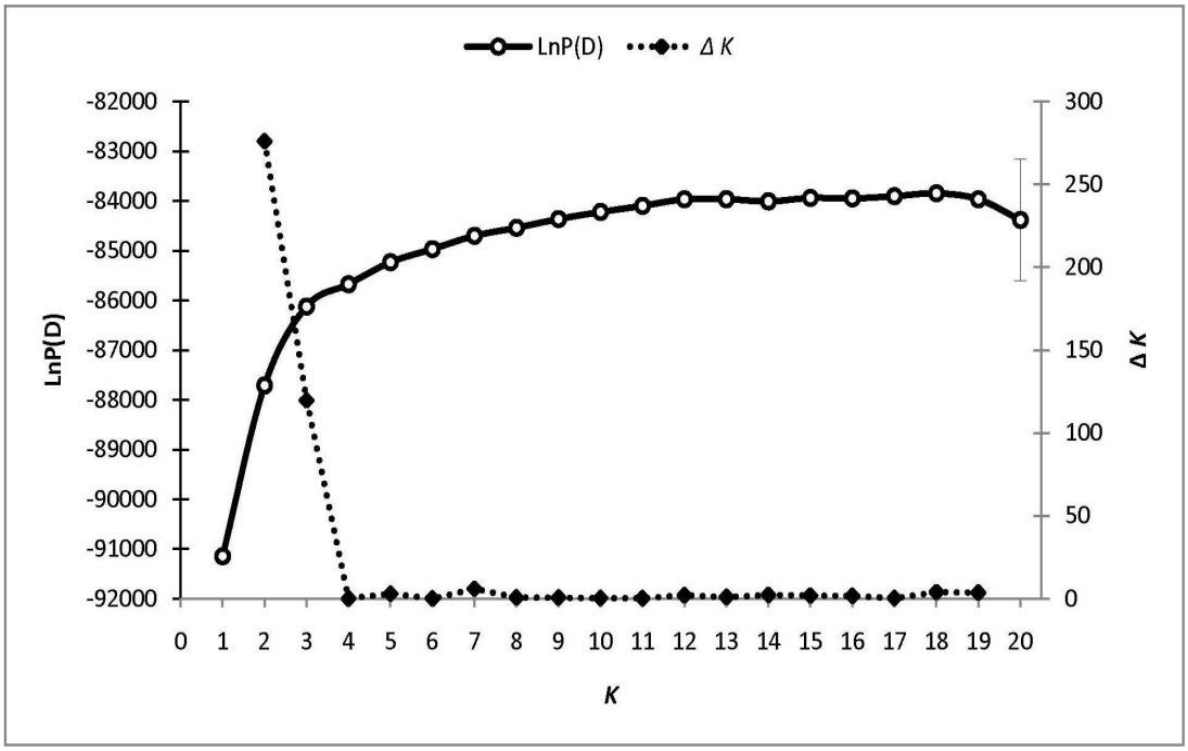
LnP(D)
Δ K
-82000
-83000
-84000
-85000
-86000
-87000
-88000
-89000
-90000
-91000
-92000
LnP(D)
300
250
200
150
100
50
0
Δ K
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
K

Fig. 6

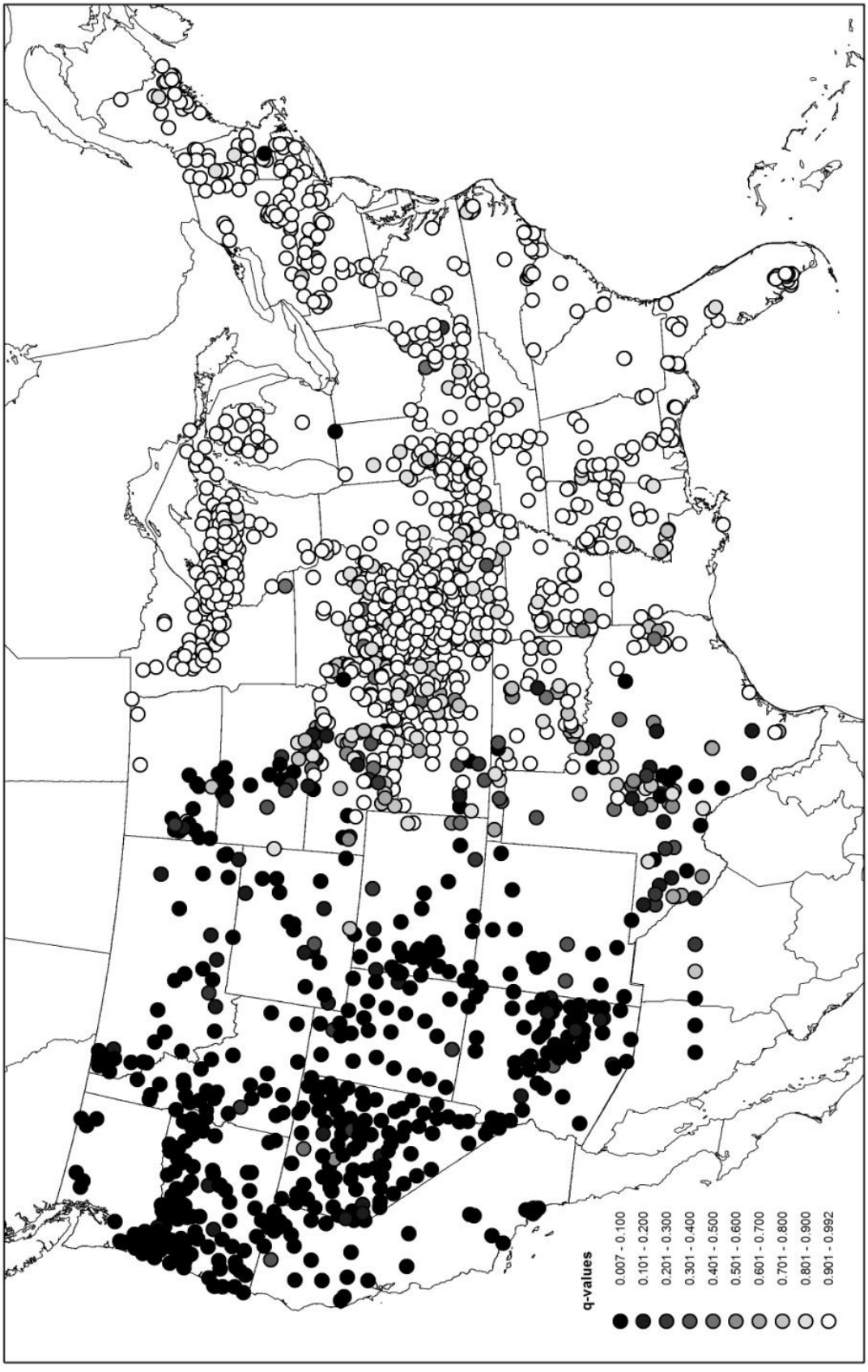
q-values
0.007 - 0.100
0.101 - 0.200
0.201 - 0.300
0.301 - 0.400
0.401 - 0.500
0.501 - 0.600
0.601 - 0.700
0.701 - 0.800
0.801 - 0.900
0.901 - 0.992

Fig. 7

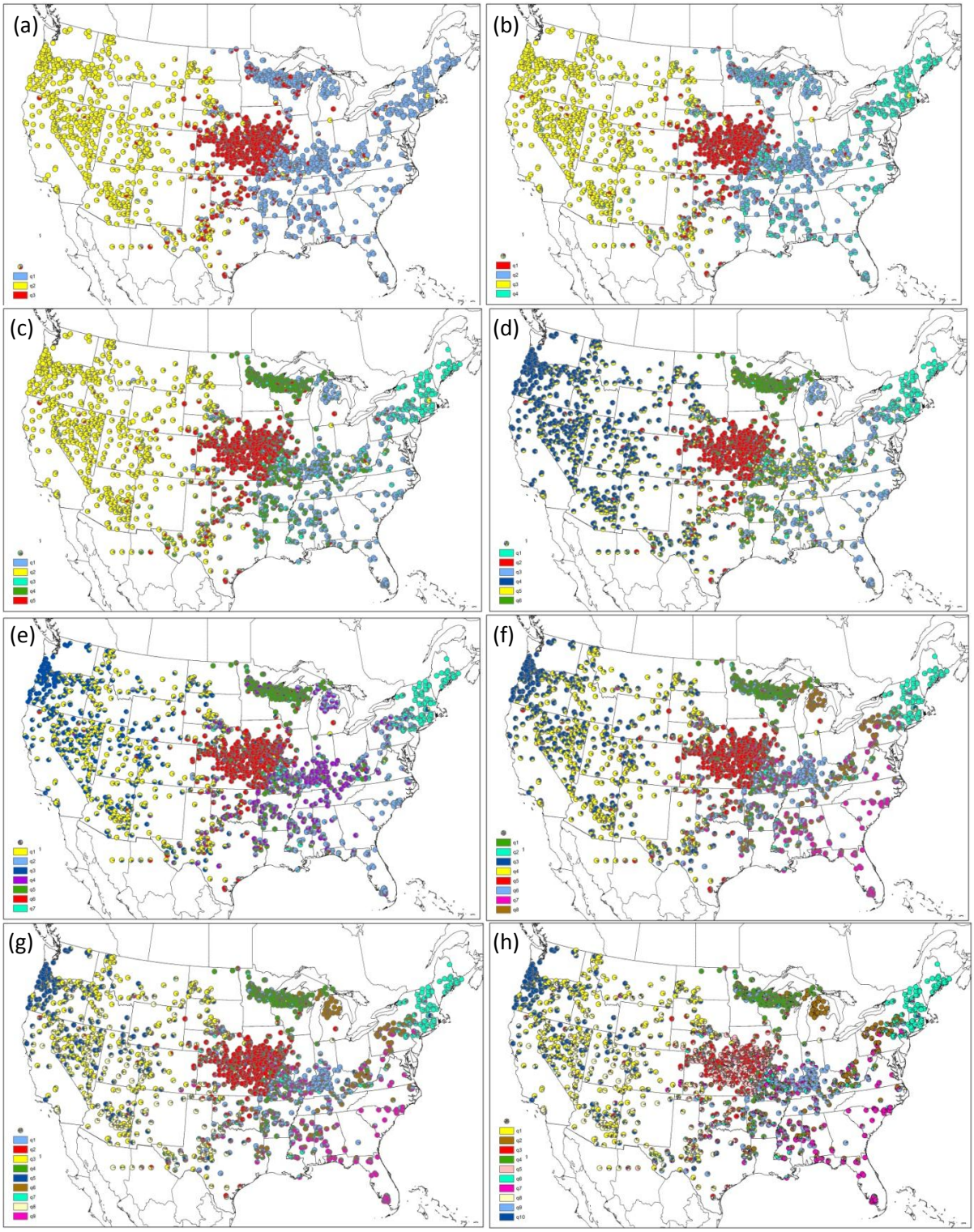

Fig. 8

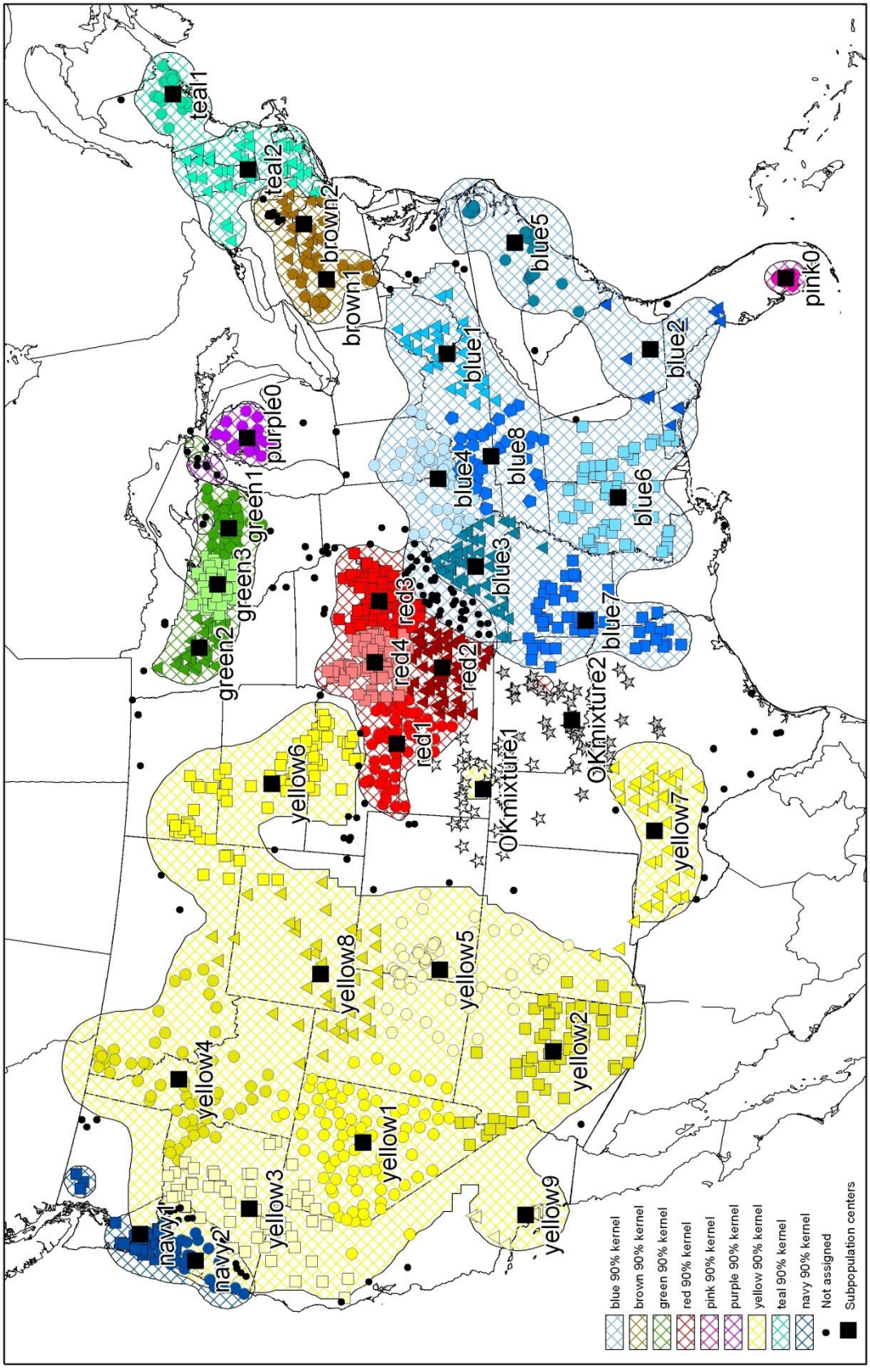
teal1
teal2
brown2
brown1
blue5
pink0
blue1
blue2
purple0
blue4
blue8
blue6
green1
green3
green2
blue3
red3
red4
blue7
red2
red1
OKmixture2
OKmixture1
yellow6
yellow7
yellow8
yellow5
yellow4
yellow2
yellow1
yellow3
navy1
navy2
yellow9
blue 90% kernel
brown 90% kernel
green 90% kernel
red 90% kernel
pink 90% kernel
purple 90% kernel
yellow 90% kernel
teal 90% kernel
navy 90% kernel
Not assigned
Subpopulation centers

Fig. 9

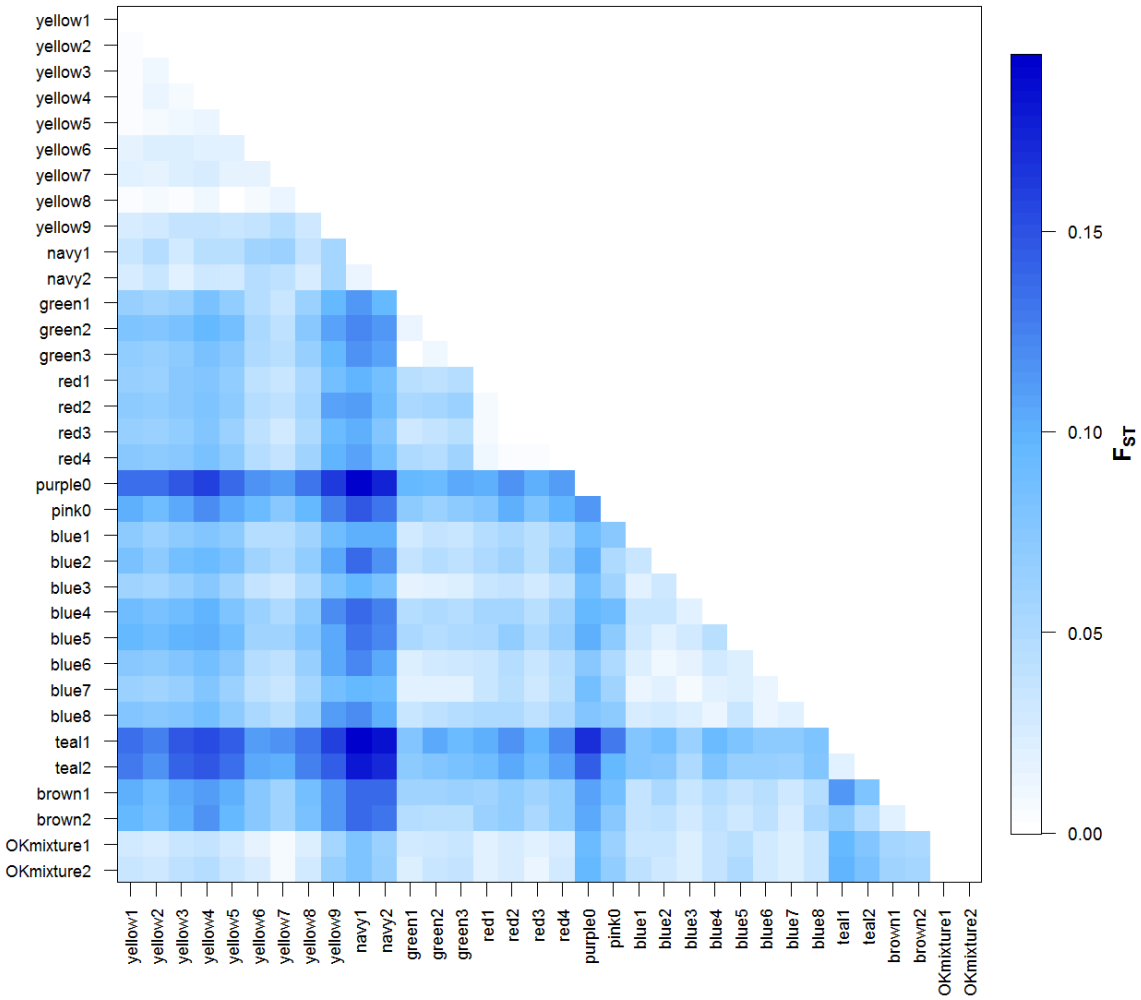
yellow1
yellow2
yellow3
yellow4
yellow5
yellow6
yellow7
yellow8
yellow9
navy1
navy2
green1
green2
green3
red1
red2
red3
red4
purple0
pink0
blue1
blue2
blue3
blue4
blue5
blue6
blue7
blue8
teal1
teal2
brown1
brown2
OKmixture1
OKmixture2
0.15
0.10
0.05
0.00
FST

Fig. 10

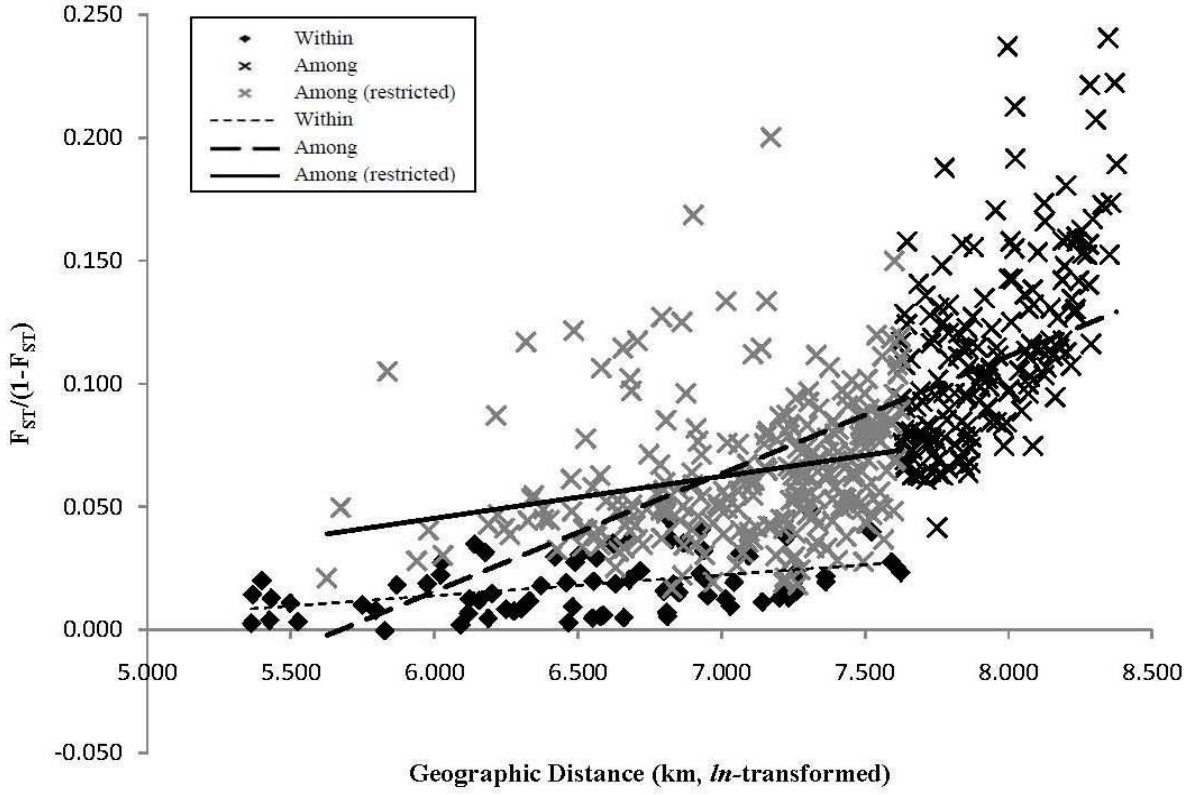
0.250
0.200
0.150
0.100
0.050
0.000
-0.050
5.000
5.500
6.000
6.500
7.000
7.500
8.000
8.500
Within
Among
Among (restricted)
Within
Among
Among (restricted)
$F_{ST}/(1-F_{ST})$
Geographic Distance (km, *ln*-transformed)

Fig. 11

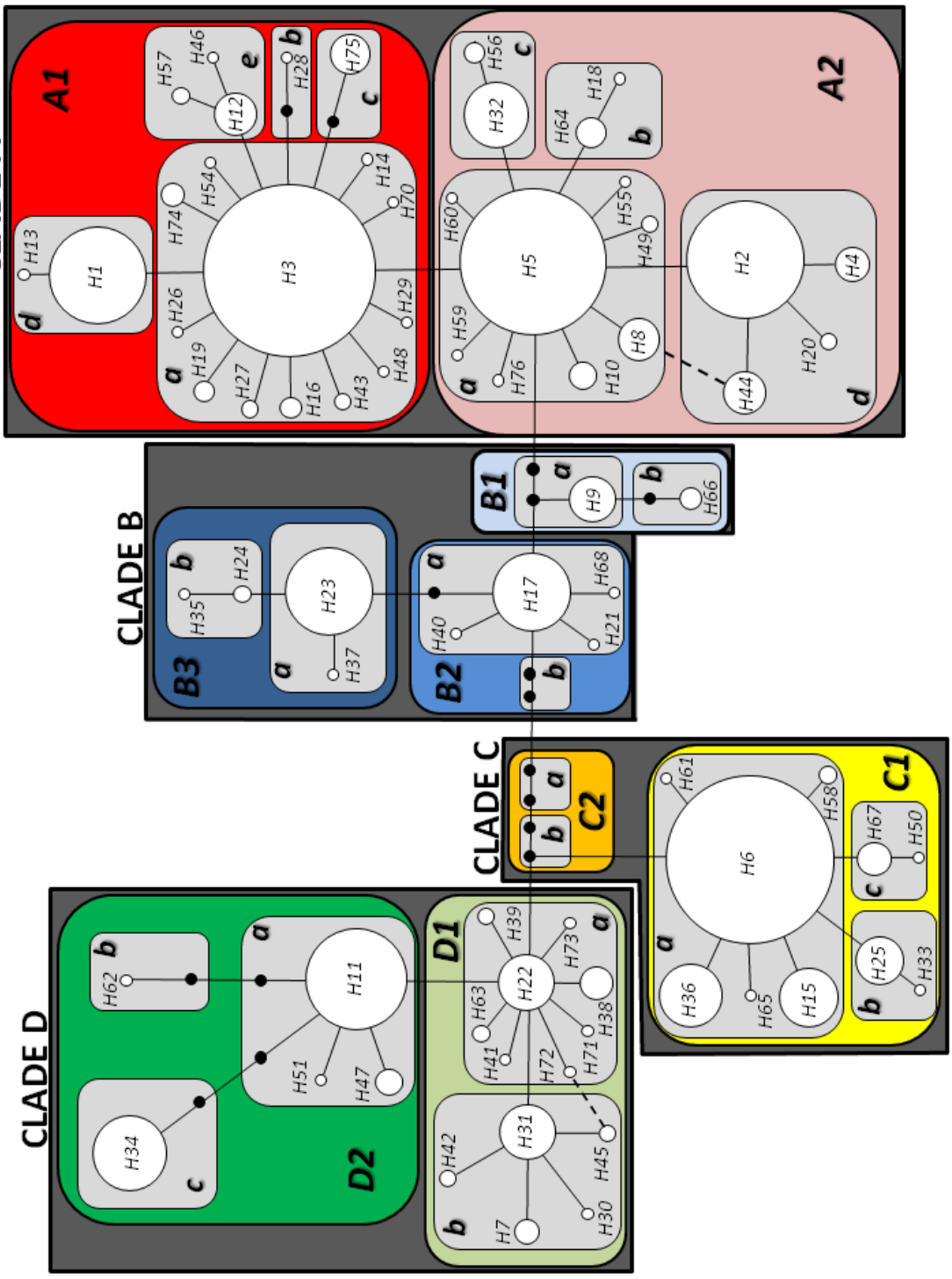
CLADE A
A1
A2
CLADE B
B1
B2
B3
CLADE C
C1
C2
CLADE D
D1
D2
H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
H23
H24
H25
H26
H27
H28
H29
H30
H31
H32
H33
H34
H35
H36
H37
H38
H39
H40
H41
H42
H43
H44
H45
H46
H47
H48
H49
H50
H51
H54
H55
H56
H57
H58
H59
H60
H61
H62
H63
H64
H65
H66
H67
H68
H70
H71
H72
H73
H74
H75
H76

Fig. 12

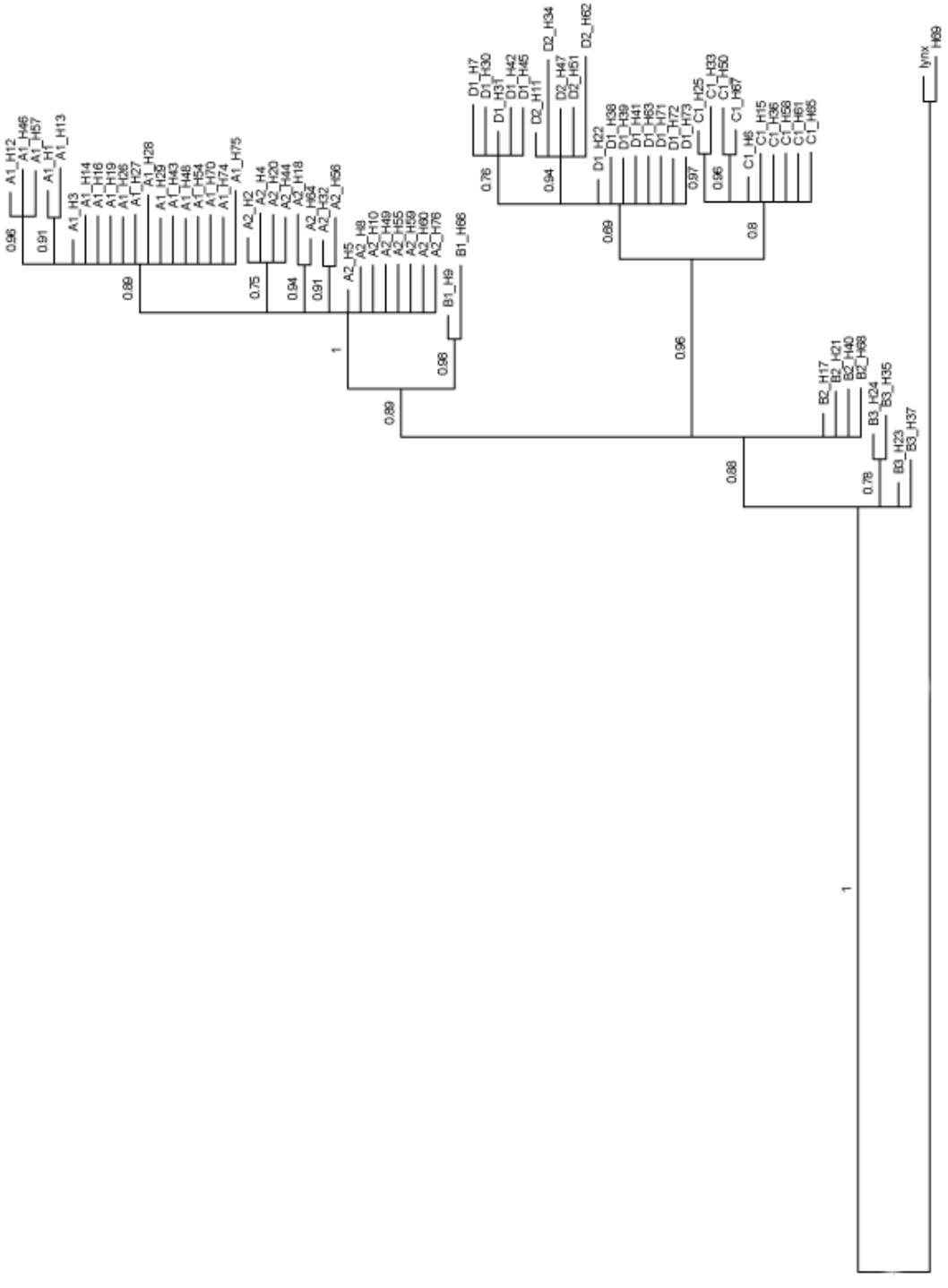
A1_H12
A1_H46
A1_H57
A1_H1
A1_H13
A1_H3
A1_H14
A1_H16
A1_H19
A1_H26
A1_H27
A1_H28
A1_H29
A1_H43
A1_H48
A1_H54
A1_H70
A1_H74
A1_H75
A2_H2
A2_H4
A2_H20
A2_H44
A2_H18
A2_H24
A2_H32
A2_H58
A2_H5
A2_H8
A2_H10
A2_H49
A2_H55
A2_H59
A2_H60
A2_H76
B1_H66
B1_H9
D1_H7
D1_H30
D1_H31
D1_H42
D1_H45
D2_H11
D2_H34
D2_H47
D2_H51
D2_H62
D1_H22
D1_H38
D1_H39
D1_H41
D1_H63
D1_H71
D1_H72
D1_H73
C1_H25
C1_H33
C1_H50
C1_H67
C1_H6
C1_H15
C1_H36
C1_H58
C1_H61
C1_H65
B2_H17
B2_H21
B2_H40
B2_H68
B3_H24
B3_H35
B3_H23
B3_H37
lynx
H89
0.96
0.91
0.89
0.75
0.94
0.91
1
0.98
0.89
0.76
0.94
0.89
0.97
0.96
0.8
0.96
0.88
0.78
1

Fig. 13

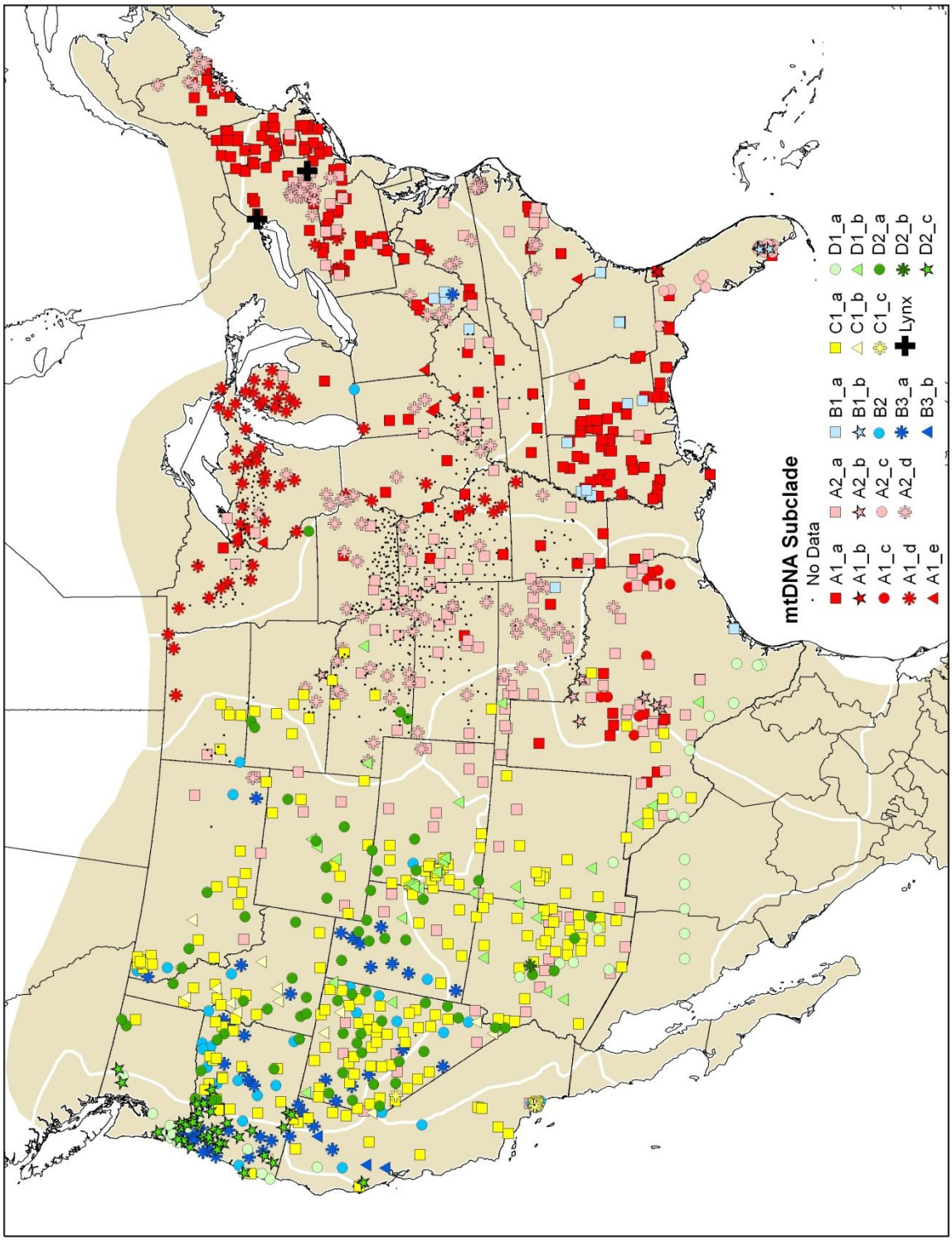
mtDNA Subclade
No Data
A1_a
A1_b
A1_c
A1_d
A1_e
A2_a
A2_b
A2_c
A2_d
B1_a
B1_b
B2
B3_a
B3_b
C1_a
C1_b
C1_c
Lynx
D1_a
D1_b
D2_a
D2_b
D2_c

Fig. 14

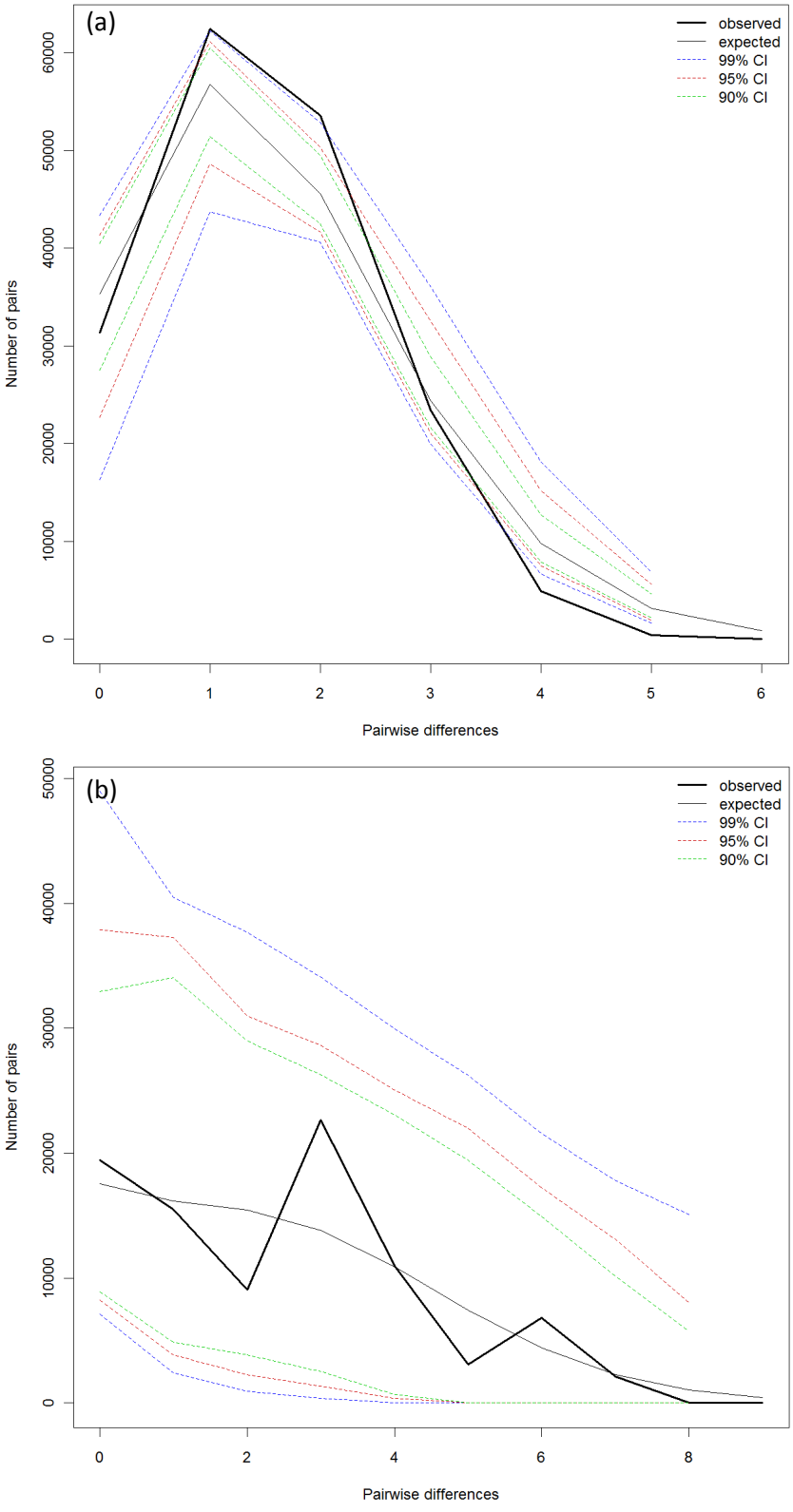
(a)
observed
expected
99% CI
95% CI
90% CI
Number of pairs
0
10000
20000
30000
40000
50000
60000
0
1
2
3
4
5
6
Pairwise differences
(b)
observed
expected
99% CI
95% CI
90% CI
Number of pairs
0
10000
20000
30000
40000
50000
0
2
4
6
8
Pairwise differences

Fig. 15

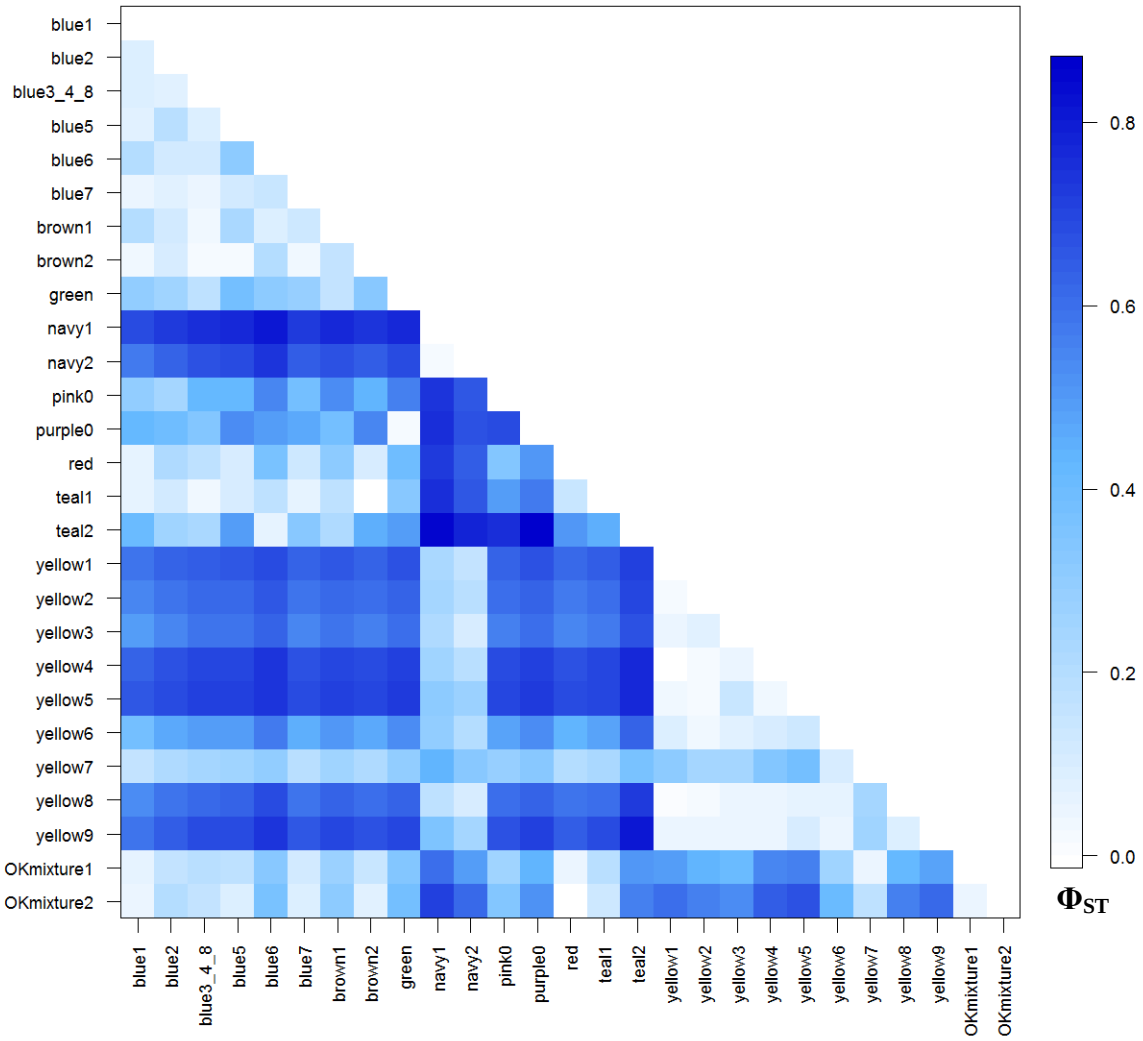
blue1
blue2
blue3_4_8
blue5
blue6
blue7
brown1
brown2
green
navy1
navy2
pink0
purple0
red
teal1
teal2
yellow1
yellow2
yellow3
yellow4
yellow5
yellow6
yellow7
yellow8
yellow9
OKmixture1
OKmixture2
0.8
0.6
0.4
0.2
0.0
ΦST

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

Aubry KB, Statham MJ, Sacks BN, Perrine JD, Wisely SM (2009) Phylogeography of the North American red fox: vicariance in Pleistocene forest refugia. *Molecular Ecology*, **18**, 2668-2686.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.

Leonard JA, Vila C, Wayne RK (2005) Legacy lost: genetic variability and population size of extirpated US grey wolves (*Canis lupus*). *Molecular Ecology*, **14**, 9-17.

Linde SA (2010) *Predicting favorable habitat for bobcats (Lynx rufus) in Iowa*. MS thesis, Iowa State University.

Roberts NM, Crimmins SM (2010) Bobcat population status and management in North America: evidence of large-scale population increase. *Journal of Fish and Wildlife Management*, **1**, 169-174.

Runck AM, Cook JA (2005) Postglacial expansion of the southern red-backed vole (*Clethrionomys gapperi*) in North America. *Molecular Ecology*, **14**, 1445-1456.

Stone KD, Flynn RW, Cook JA (2002) Post-glacial colonization of northwestern North America by the forest-associated American marten (*Martes americana*, Mammalia: Carnivora: Mustelidae). *Molecular Ecology*, **11**, 2049-2063.

Taylor PD, Fahrig L, Henein K, Merriam G (1993) Connectivity is a vital element of landscape structure. *Oikos*, **68**, 571-573.

Turner MG, Gardner RH, O'Neill RV (2001) *Landscape ecology in theory and practice*. Springer, New York, NY.

Tzedakis PC, Lawson IT, Frogley MR, Hewitt GM, Preece RC (2002) Buffered tree population changes in a Quaternary refugium: evolutionary implications. *Science*, **297**, 2044-2047.