Conserv Genet (2012) 13:1637–1651
DOI 10.1007/s10592-012-0416-0

RESEARCH ARTICLE

Microsatellites and mitochondrial DNA reveal regional population structure in bobcats (*Lynx rufus*) of North America

Emily K. Croteau · Edward J. Heist · Clayton K. Nielsen · Jennifer R. Hutchinson · Eric C. Hellgren

Received: 27 September 2011 / Accepted: 27 September 2012 / Published online: 9 October 2012
© Springer Science+Business Media Dordrecht 2012

Abstract Genetic analyses can facilitate large-scale conservation planning for wide-ranging species capable of long-distance dispersal. Bobcats (*Lynx rufus*) are the most broadly distributed native felid in North America, and are managed on a "state-by-state" basis. Little is known about the distribution of genetic diversity across bobcats' range. We examined genetic differentiation among bobcats from throughout their distributional range in North America using 10 microsatellite loci and mitochondrial control region sequence to elucidate patterns of genetic diversity. Both markers revealed significant regional genetic differentiation. Additionally, genetic diversity estimates, population expansion statistics, and the haplotype network elucidated from mitochondrial DNA analyses, suggest that populations in the West and East/Midwest experienced historical population expansions, with the East/Midwest likely undergoing periodic range contractions and extirpations during bobcat colonization. Microsatellite data revealed significant regional genetic differentiation between the Midwest and East, as well as the West, suggesting that recent barriers to gene flow may be affecting dispersal of bobcats. These analyses indicate that conservation of forested areas will be crucial for maintaining gene flow throughout bobcats' range as human populations increase and that multi-state consortia may be a more appropriate way to manage bobcats as this scenario will conserve both historical and current levels of genetic diversity.

Keywords *Lynx rufus* · DNA microsatellites · Mitochondrial DNA · Genetic diversity · Gene flow · Population structure · North America

Electronic supplementary material The online version of this article (doi:10.1007/s10592-012-0416-0) contains supplementary material, which is available to authorized users.

E. K. Croteau · E. C. Hellgren
Cooperative Wildlife Research Laboratory, Southern Illinois University, Carbondale, IL 62901-6504, USA

E. K. Croteau (✉)
Biology Department, Valdosta State University,
1500 North Patterson Street, Valdosta, GA 31698, USA
e-mail: emily.croteau@gmail.com

E. J. Heist
Fisheries and Illinois Aquaculture Center, Southern Illinois University Carbondale, Life Sciences II, 1125 Lincoln Drive, Carbondale, IL 62901-6511, USA

C. K. Nielsen
Cooperative Wildlife Research Laboratory and Department of Forestry, Southern Illinois University, Carbondale, IL 62901-6504, USA

J. R. Hutchinson
Duke Human Vaccine Institute, Duke University Medical Center, 106 Research Drive, 4120 MSRB II, MC 103020, Durham, NC 27710, USA

E. C. Hellgren
Center for Ecology, Southern Illinois University, Carbondale, IL 62901, USA

Introduction

Patterns in genetic diversity and differentiation are the result of a combination of historical and contemporary effects on evolutionary forces including genetic drift, gene flow and natural selection (Allendorf 1983; Avise 2000; Hedrick 2000; Hartl and Clark 2007). Historical events, including range contraction or expansion in conjunction with large-scale landscape changes (e.g. glacial periods) initially shape patterns of genetic diversity (Hewitt 2000);

however, contemporary gene flow and anthropogenic habitat changes continue to alter the genetic landscape of species' ranges and population structure (Lande 1988). Understanding the factors that influence genetic structure and how they are interrelated is crucial to species conservation and management (Lande 1988; Amos and Harwood 1998).

Species with expansive ranges, long-distance dispersal capabilities, and continuous distributions can benefit from analyses of genetic diversity to inform large-scale conservation planning. For example, in most cases species are managed with respect to government-imposed (e.g. state or province) boundaries. However, mobile species do not recognize such artificial boundaries and thus management at these levels may not be appropriate. As such, genetic analyses can clarify the most suitable level for management. Studies of wide-ranging and vagile species are numerous and have focused on a host of vertebrates such as: marine mammals (Mendez et al. 2008), terrestrial mammals (von Holdt et al. 2010), fishes (Ward et al. 1997; Castro et al. 2007), birds (Hull et al. 2008), and reptiles (Shillinger et al. 2008). The goal of such work was to effectively manage genetic diversity given the biology of the species and to consider the conservation implications of such results.

Examinations of genetic diversity and population structure in fields has been useful to large-scale management efforts because some felids are economically important, many have threatened conservation status, and because they function in many ecosystems as apex carnivores (Ray et al. 2005; Roemer et al. 2009). Schwartz et al. (2002) examined large-scale spatial synchrony in population dynamics of Canada lynx (*Lynx canadensis*). Estimates of gene flow indicated that large numbers of lynx disperse long distances from the core of their range, creating a wave of immigrants that drive cycle-like synchrony in Western lynx populations. To maintain population connectivity, Schwartz et al. (2002) concluded that international efforts would be necessary to appropriately manage Canada lynx populations. Conversely, molecular analyses of Eurasian lynxes (*L. lynx*) from Finland and the Baltic states indicated that populations from the two regions were genetically distinct and might warrant separate management (Hellborg et al. 2002). In addition, restricted gene flow in jaguars (*Panthera onca*) due to major geographical barriers like the Amazon River and Darien Straits produced measurable genetic differentiation (Eizirik et al. 2001). Eizirik et al. (2001) recommended that historical barriers to dispersal should be used to define operational conservation units in future management of this species.

Bobcats (*L. rufus* (Schreber, 1777)) are solitary, highly mobile, polygamous carnivores (Larivière and Walton 1997; Anderson and Lovallo 2003) and are widely distributed in North America, ranging throughout the contiguous U.S. and portions of Canada and Mexico (Lopez-Gonzalez et al. 1998; Anderson and Lovallo 2003). Twelve subspecies are recognized within their distribution (Hall 1981). The classification of subspecies is based mostly on differences in pelage coloration (Young 1958); however, Samson (1979) confirmed these taxa using multivariate analyses of cranial characters. Bobcats are considered habitat generalists, but typically require woody cover for a variety of activities, including dispersal (Anderson and Lovallo 2003).

Bobcats are considered furbearers or game mammals in North America and as such, their management is based primarily on regulations prescribed by state or provincial wildlife conservation agencies. During the last few decades, bobcats have increased in many U.S. states (Woolf and Hubert 1998), and continue to do so (Roberts and Crimmins 2010). The U.S. allows harvest of bobcats in 39 states while others prohibit it due to their relative rarity, especially in the Midwest (spp *L. r. rufus*) where land-use practices (i.e., intensive row-crop agriculture) have rendered the landscape less suitable for bobcats. In Canada, bobcats are at their northern range limit, but are hunted or trapped in seven of the eight provinces in which they are native. Bobcats are considered common in the southern regions of provinces in Western Canada (e.g. British Columbia, Alberta) and in the Atlantic provinces (e.g. New Brunswick and Nova Scotia). Hunting and trapping has been halted in Quebec since 1991 due to large declines in harvest (Lavoie et al. 2010) but populations have since been found to be stable or increasing (Roberts and Crimmins 2010). In Mexico, five states permit hunting; there is no evidence of declines in central and southern Mexico (Hansen 2007).

By virtue of its high vagility, broad distribution, international economic importance, and role in many North American ecosystems as an apex carnivore, the bobcat serves as an excellent model for exploring questions related to partitioning of genetic diversity, degree of genetic differentiation and how these relate to current management practices. Specifically, management based on patterns in genetic diversity and differentiation (rather than the current management strategy) may be more appropriate since bobcats most likely do not adhere to artificial boundaries such as state and provincial lines. The ability of bobcats to disperse long distances (Knick and Bailey 1986; Kamler et al. 2000; Nielsen and Woolf 2003; Johnson et al. 2010) suggests that gene flow is high and genetic differentiation low. However, some studies indicate that gene flow has been restricted at smaller scales due to natural and anthropogenic barriers (Riley et al. 2006; Millions and Swanson 2007). By understanding range-wide patterns in genetic diversity, recommendations for bobcat management can be made that maintain both historical and present connections in order to

preserve the maximal amount of genetic diversity necessary for the species to continue to thrive and adapt.

Genetic structure in bobcats has been assessed at small scales (Croteau et al. 2010; Janecka et al. 2006, 2007; Millions and Swanson 2006, 2007; Riley et al. 2006); however, range-wide population genetic structure and its relevance to current management policy have not been assessed. In this study, we used microsatellite and mitochondrial DNA to examine genetic structure of bobcats in North America. The objectives were to identify patterns of genetic diversity and genetic differentiation among populations to gain an understanding of how past demographic events and current population isolation have influenced these aspects of population structure. The implications of such research may aid in future management of this wide-ranging species.

Methods

Sample area and DNA extraction

Ear, tongue, or liver tissues were collected from 587 wild bobcats during 1995–2006 (Fig. 1). Tissue samples were collected during field research and harvest in various states/provinces, by several universities, state agencies and individuals, and were stored at room temperature in a 20 % DMSO solution saturated with NaCl until DNA extraction. Liver segments obtained by necropsy were frozen at −70 °C. Total genomic DNA was extracted from these tissues using the DNeasy tissue kit (Qiagen Inc., Valencia, CA).

Samples were obtained from 16 U.S. states and two Canadian provinces (Table 1): Arkansas (AR), California (CA), Florida (FL), Illinois (IL), Indiana (IN), Kentucky (KY), Louisiana (LA), Maine (ME), Michigan [Upper and Lower Peninsulas: UP and LP, respectively], Minnesota (MN), Missouri (MO), New Brunswick (NB), North Dakota (ND), Nova Scotia (NS), Nevada (NV), Texas (TX), Wisconsin (WI), and Wyoming (WY). Samples from Michigan were separated into UP and LP because a large water barrier isolates these regions, and because previous studies demonstrated significant genetic differences between these locations (Millions and Swanson 2006, 2007). As a result of this division, our study included 19 populations representing eight of the twelve putative subspecies (Hall 1981). In most cases, county-level location information was provided for the tissue sample, however, given the method of tissue collection, in some cases, only state-level location information was available (e.g. MN, LA). Because bobcats are managed by state or provincial regulations, we initially defined sample populations based upon these state/provincial boundaries and populations were named using the abbreviation for that state/province (except in the case of Michigan wherein populations were named LP and UP).

Fig. 1 Map of locations for bobcats sampled from 1995 to 2006 throughout North America. Areas shaded in *grey* indicate county or state where tissue samples were taken

Table 1 Number of individuals surveyed (*n*), observed heterozygosity (H_o), expected heterozygosity (H_e), total allelic richness and regional cluster as indicated by STRUCTURE (Pritchard et al. 2000) for 17 populations of bobcats in North America surveyed at 10 microsatellite loci

Population	*N*	H_o	H_e	Total allelic richness	Regional cluster
CA	1	–	–	–	–
NV	48	0.75	0.76	4.85	West
WY	43	0.72	0.76	5.03	West
ND	30	0.70	0.77	5.04	West
TX	22	0.73	0.76	4.66	West
MN	20	0.76	0.75	4.64	East
LA	22	0.67	0.76	4.89	East
MO	50	0.76	0.76	4.86	Midwest
WI	12	0.79	0.74	4.57	East/Midwest
IL	146	0.74	0.75	4.78	Midwest
IN	31	0.75	0.74	4.65	Midwest
UP	30	0.72	0.74	4.59	Midwest
LP	24	0.58	0.64	3.65	East
KY	35	0.75	0.76	4.75	Midwest
FL	10	0.72	0.73	4.61	East
ME	13	0.53	0.61	3.73	East
NB	9	0.66	0.68	4.04	East
NS	37	0.66	0.63	3.79	East
AR	4	–	–	–	
Overall	587	0.71	0.71	4.5	

Marker development and analysis

We used both microsatellite markers and mitochondrial DNA to address our research questions. Ten microsatellite loci developed in other felid species were amplified using polymerase chain reaction (PCR). Six microsatellite primer pairs were originally developed for the domestic cat (*Felis catus*; FCA023, FCA043, FCA045, FCA077, FCA090,

FCA096; Menotti-Raymond and O'Brien 1995; Menotti-Raymond et al. 1999), three were designed for the Canada lynx (LC109, LC110, LC111; Carmichael et al. 2000) and one was designed for the bobcat (BCE5T; Faircloth et al. 2005). See Croteau et al. (2010) for PCR reaction conditions and thermal cycling profiles for all microsatellite primer sets.

PCR products were diluted in loading buffer (1:1) containing deionized formamide, blue dextran EDTA, and ROX-labeled size standard (The Gel Company MRK-400 size standard; The Gel Company, San Francisco, CA), and resolved on a 36-cm-long 5 % denaturing Long Ranger gel (Cambrex Bio Science, East Rutherford, NJ) and run at 2,500 scans/h for 2.5 h using an ABI 377 automated genotyper. Gel images were analyzed using Genescan version 3.1.2 software and alleles were defined using Genotyper version 2.5 genotyping software (PE Applied Biosystems, Applera Corporation, Valencia, CA). Genotypes were typically re-run at least three times to ensure there were no genotyping errors. MICROCHECKER version 2.3.3 (van Oosterhout et al. 2004) was used to check for the occurrence of null alleles.

The control region of the mitochondrial DNA genome was amplified by PCR using two primers developed for this study (CatPro 5′-CAAGGAAGAAGCAACAGC3′ and Cat 12S 5′ TRRAGGGCATTTTCACCG3′) anchoring at positions 16,249 and 1,018, respectively, of the domestic cat (*Felis catus*) mtDNA genome (GenBank Accession Number U20753; Lopez et al. 1996), and amplifying approximately 1,742 base pairs (bp). A subset of samples was utilized for DNA sequencing, rather than the entire sample set of 587 due to technical and time limitations. Specifically, a small sample of individuals was selected from each population (e.g. state or province) to ensure that a sufficient sample size was obtained and the geographic area of the sample area was accurately represented. However, in some cases sample size was lower than intended due to poor sequence resolution. PCR reactions were performed in 50 µl volumes using Thermoprime ABGene 2X concentrated PCR mastermix (Thermo Fisher Scientific, Rockford, IL), 0.5 µmol of each primer and 30–50 ng of DNA per reaction. Conditions for amplification consisted of a denaturing step of 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, with a final extension of 72 °C for 5 min. PCR products were purified using QIAquick PCR purification kits (Qiagen Inc., Valencia, CA). Three primers were designed to sequence portions of the first hypervariable segment (HVS-1) and the conserved central portion of the bobcat control region: forward primer LruFwd3 5′-CACTATCAGCACCCAAAGC-3′ mapping to position 16,269 of the domestic cat mitochondrial genome (position 3 in the fragment); reverse primer, LruRev2 5′-GACCCCGCATAGAGAATAAG-3′ mapping to position 160 in domestic cat (position 882 of the fragment); and internal primer LruRevSeq 5′-AGGATTGCTGGTTTCT CG-3′ mapping to position 16,862 in domestic cat (position 574 of the fragment). These primers produced 813 base pairs (bp) of sequence. Cycle sequencing reactions were performed in an Eppendorf Mastercycler using a 96 °C denaturation step for 4 min, followed by 99 cycles of 96 °C for 30 s, 50 °C for 15 s, and 60 °C for 4 min. Fragments were purified using Sephadex G-50 columns, electrophoresed on a 36 cm long, 5 % denaturing Long Ranger gel (Cambrex Bio Science, east Rutherford, NJ), and run at 1,200 scans/h for 7 h using an ABI 377 automated sequencer (Applied Biosystems). Gel images were analyzed using Genescan version 3.1.2 software (PE Applied Biosystems, Applera Corporation, Valencia, CA); sequences were examined and aligned using SEQUENCHER version 4.7 (Gene Codes Corporation).

The portion of the control region that was amplified for this study contains a region of tandem repeats (Hoelzel et al. 1994) that vary in size, number of variable sites, and number of repeats (Lopez et al. 1996; Freeman et al. 2001). Due to the uncertainty of aligning the repeats among individuals, only 464 bp of non-repetitive sequence flanking the repeat region was used for analysis. In cases where alignment was difficult due to poor amplification and/or sequence resolution, individuals were re-sequenced for that fragment and re-aligned. This resulted in the re-sequencing of approximately 50 % of individuals for any given fragment to ensure correct alignment.

Analysis of genetic diversity

Patterns of contemporary genetic variation within each population was assessed for microsatellites by calculating observed and expected heterozygosity in GENETIX 4.05 (Belkhir et al. 2001) and polymorphic characteristics of the microsatellites, such as the number of alleles, allelic richness adjusted for sample size, and allele frequencies, were calculated using FSTAT 2.9.3.2 (Goudet et al. 2002). Linkage disequilibrium (LD) between pairs of loci across all populations was assessed using GENEPOP 4.0 (Rousset 2008). Significance values for deviations from Hardy–Weinberg equilibrium (HWE) were calculated using an exact test ($P < 0.05$; Guo and Thompson 1992; Raymond and Rousset 1995) as implemented in GENEPOP 4.0 (Rousset 2008). This method can identify significant deviations in HWE resulting in an excess or deficiency of homozygotes as indicated by positive or negative F_{IS} scores. Tests for both LD and HWE were corrected for multiple comparisons using a sequential Bonferroni adjustment (Holm 1979; Rice 1989).

Patterns of historical genetic diversity for sample areas were assessed for the mitochondrial control region using the numbers of variable sites and haplotypes, and

transitions and transversions between haplotypes in DnaSP version 4 (Rozas et al. 2003). Haplotype diversity (*h*) and nucleotide diversity (π) within sample areas were calculated using ARLEQUIN version 3.11 (Excoffier et al. 2005). Both haplotype and nucleotide diversity are useful for inferring population expansion, demographic bottlenecks, Hardy–Weinberg equilibrium, and admixture of samples (Rogers and Harpending 1992; Avise 2000). Matrices of pairwise differences between haplotypes were computed for all analyses of molecular variation.

Analysis of historical demography

NETWORK version 4.5 (Bandelt et al. 1999) was employed to generate a median joining network. We used a network because phylogenetic trees depict sequence haplotypes on terminal branches, with internal branches representing extinct ancestral haplotypes. In intraspecific samples ancestral haplotypes are often still present, and networks are therefore a more accurate depiction of haplotype relationships (Smouse 1998).

Several neutrality statistics were used to test for historical population growth, contraction, or stability. The expansion coefficient (S/d) was calculated following Peck and Congdon (2004). S/d can indicate a population expansion or equilibrium, where large values of S/d suggest recent population expansion and small values suggest stable long-term size (Peck and Congdon 2004). To detect past population expansion, ARLEQUIN version 3.11 (Excoffier et al. 2005) was employed to calculate the Fu's F_S statistic (Fu 1997) and Tajima's D statistic (Tajima 1989). We used 100,000 simulations of Hudson's (1990) coalescent simulation algorithm to test the significance of F_S and D under the null hypothesis of selective neutrality and population equilibrium. A significantly large negative value for F_S suggests an excess of recent mutations and is taken as evidence against neutrality of mutations and population equilibrium (Fu 1997). Population expansions can cause a significant negative departure of Tajima's D from zero (Tajima 1989). Fu's F_S is thought to be more sensitive to population expansion than Tajima's D (Fu and Li 1993), but both statistics were implemented to confirm selective neutrality. Fu and Li's D* and F* statistics were calculated in DnaSP version 4 (Rozas et al. 2003), significant values are associated with selection (Fu and Li 1993). To distinguish between the effects of background selection and population expansion, a comparison of F_S to D* and F* statistics was performed. A significant F_S with non-significant D* and F* implies expansion whereas the reverse indicates selection (Fu 1997).

A mismatch distribution and raggedness statistic (rg) were calculated in ARLEQUIN version 3.11 (Excoffier et al. 2005). Mismatch distributions describe the frequency of pairwise nucleotide differences among individuals. A unimodal distribution approximating a Poisson curve indicates a population that has undergone recent expansion (Rogers and Harpending 1992; Harpending et al. 1993). A multimodal distribution is expected in a population at mutation-drift equilibrium (Slatkin and Hudson 1991). Furthermore, population growth will generate waves in the distribution that are seldom seen in equilibrium populations (Rogers and Harpending 1992). The value of rg also allows differentiation of populations that are expanding from those at equilibrium. Large values of rg indicate populations that are stationary with multimodal mismatch distributions (Harpending et al. 1993). In ARLEQUIN version 3.11, the validity of the expansion model is tested by means of the sudden expansion model using the sum of squared deviations (SSD) between the observed and the expected mismatch as a test statistic. A *P* value is obtained by calculating the number of simulated SSD that are $\geq$ the observed SSD, with a *P* value $\leq$0.05 taken as evidence of departure from the expansion model. The significance test for rg is similar to that for the mismatch distribution.

Analysis of population structure

Current population structure was examined using STRUCTURE (version 2.3.1; Pritchard et al. 2000, 2009) to infer the number of genetic clusters (*K*) in our sample and to assign individuals to these clusters. STRUCTURE employs a Bayesian clustering algorithm to determine the posterior probability of the data for a given number of genetic groups and finds the optimal *K* assuming Hardy–Weinberg and linkage equilibrium.

We estimated *K* in STRUCTURE by performing ten independent runs for each *K* ($K = 1$–18), using 100,000 burn-in steps to ensure likelihood convergence, and 750,000 Markov Chain Monte Carlo (MCMC) repetitions. We did not include prior population delineation information and assumed correlated allele frequencies and population admixture. The posterior probability was then calculated for each value of *K* using the log-likelihood of the data, and we chose the optimal *K* using an ad hoc statistic ΔK, based on the second order rate of change in the log probability of the data between successive *K* values (Evanno et al. 2005). We assessed the proportion of membership/assignment of each individual to each genetic cluster using CLUMPP (Jakobbson and Rosenberg 2007), for the ΔK that best explained the data.

Current population differentiation (F_{ST}) was measured by Weir and Cockerham's (1984) unbiased estimator θ, and determined for pairs of populations and STRUCTURE identified clusters (based on microsatellite data) using GENEPOP 4.0 (Rousset 2008). Analysis of Molecular Variation (AMOVA) of mitochondrial control region sequences was implemented to determine the extent of historical

population structure in bobcats using ARLEQUIN version 3.11 (Excoffier et al. 2005) to calculate pairwise ϕ-statistics (Excoffier et al. 1992) between populations and to quantify the percentage of variation within and among those populations. The topology of the network and STRUCTURE identified clusters were used in combination to group populations together for AMOVA analyses. The groupings that maximize the among-population variance component (ϕ_{ST}) and are statistically significant indicate the most parsimonious geographical subdivisions (Eizirik et al. 2001; Liu et al. 2007). The significance of ϕ_{ST} was tested using 10,000 permutations (ϕ_{ST}: permuting haplotypes among populations). Pairwise tests of F_{ST} and ϕ_{ST} were corrected for multiple comparisons using sequential Bonferroni (Holm 1979; Rice 1989). Mantel tests (Mantel 1967) were used to test for isolation by distance (IBD) between STRUCTURE-identified clusters using ARLEQUIN version 3.11 (Excoffier et al. 2005). Geographic distances in kilometers (km) were converted to $F_{ST}/(1 - F_{ST})$ in GENEPOP 4.0 using 100,000 permutations. Geographic distances were measured between sample locations using ArcView GIS 3.3 (Environmental Systems Research Institute Inc. 2002). When the county from which a bobcat was sampled was unknown, the centroid of the state/province was used for that locale; where the county location was known, the centroid of the counties/townships sampled was used to calculate an overall centroid representing all of the samples collected from that area. These provided a reference point for measuring geographic distance. Given that IBD is used here for inter-population analyses, both methods should result in an accurate and reliable point for calculations of geographic distance between sample areas.

Principal Coordinate Analysis (PCA) as implemented in GENALEX 6 (Peakall and Smouse 2006) was used to further identify major patterns of genetic differentiation in the microsatellites. Pairwise F_{ST} between populations was selected to form the basis of the PCA and was calculated using those methods described in GENALEX 6 (Peakall and Smouse 2006).

Results

Patterns of genetic diversity

Multilocus genotypes for 581 individuals (of 587 total individuals) were obtained representing 17 sample areas (Table 1). Approximately 4.4 % of genotypes were unobtainable within that sample. All microsatellite loci were polymorphic, with an average of 16 alleles per locus, ranging from 11 to 28 alleles. Observed heterozygosity ranged from 0.53 to 0.79 (Table 1). Allelic richness values averaged 4.5, with LP and ME exhibiting the lowest richness (also corresponding to the lowest levels of observed heterozygosity) and Western populations exhibiting the highest values (Table 1). Of 170 pairwise tests for deviations from Hardy–Weinberg equilibrium, two were significant after Bonferroni correction (adjusted $\alpha = 0.00029$), both occurring in the IL population and both indicating a slight homozygote excess ($F_{IS} = 0.018$ for LC109; $F_{IS} = 0.086$ for BCE5T). After Bonferroni correction, two significant linkages between pairs of loci across all populations were found of the 45 pairwise comparisons performed ($P < 0.001$). Examining LD on a location-by-location basis detected one significant score in IL, and three in ND ($\alpha = 0.00029$). MICROCHECKER did not detect any null alleles.

Tests for LD and HWE were performed within each cluster. Tests for LD yielded a few significant values (e.g. in the West between FCA023/LC110, and in the East between FCA043/FCA096 and FCA077/LC110; $\alpha = 0.00111$). However no pattern in the significant LD scores for specific loci was revealed. Some significant values in all clusters were found when testing for HWE. For example in the East two loci were out of HWE (all homozygous excess), in the Midwest two loci were out of HWE (one homozygous excess, one deficiency), and one locus was out of HWE in the West (homozygous excess). No pattern of significant HWE scores was evident. Significance for HWE was tested at $\alpha = 0.00166$ as per the Bonferroni correction.

Of the 587 individuals, 185 were sequenced for a portion of the mitochondrial control region, and represented 19 sample areas (Table 2). Thirty-eight unique haplotypes (GenBank Accession Numbers FJ204789–FJ204826) were identified, with 35 of the 464 sites polymorphic (7.3 %) and 26 of which were parsimony informative. Of the 35 pairwise substitutions, we found 31 transitions, three transversions, and one insertion-deletion. Among all samples, h was 0.817 ($\pm$0.025) and π was 0.0077. The mean number of nucleotide differences between haplotypes was 3.79 and ranged from 2.44 to 7.56 (Table 2).

Historical demography

The haplotype network illustrated a clear distinction between haplotypes found in most Western bobcats and those from the rest of the range. The branching pattern in Eastern/Midwestern haplotypes approximated a star-like shape, with a common haplotype having several rare haplotypes that differed from it by a single substitution. Western bobcats were separated by several mutational differences from Eastern/Midwestern bobcats with a common haplotype (Fig. 2). Overall, the network approached a "dumbbell-like" shape, which has been suggested to occur in a species that has experienced two population expansions (Avise 2000). Haplotype 1 was most common in the central portion of the range ($n = 73$ individuals) and included 79.5 % of individuals from the Midwestern populations (IL,

Table 2 Population locations, sample sizes (*n*), number of haplotypes (H#), haplotype diversity (*h*), percent nucleotide diversity (π), and mean population pairwise differences (mean pop pair diffs) for mtDNA control region variation within 19 areas in 185 bobcats sampled from 1995 to 2006 throughout North America

Population	*n*	H#	*h*	%	Mean pop pair differences
CA	1	1	1	N/A	N/A
NV	13	6	0.859	1.069	6.215
WY	8	6	0.929	1.486	7.557
ND	12	7	0.894	1.365	6.142
TX	3	3	1	0.576	3.067
MN	12	2	0.409	0.648	3.623
LA	11	7	0.891	0.534	3.221
AR	4	4	1	0.792	3.499
MO	8	2	0.25	0.054	2.439
WI	7	2	0.476	0.411	2.974
IL	15	6	0.829	0.514	3.348
IN	17	7	0.75	0.454	3.045
UP	12	2	0.167	0.144	2.591
LP	14	1	0	0	2.443
KY	10	7	0.933	0.643	3.428
FL	9	5	0.861	0.336	3.204
ME	12	2	0.53	0.344	2.865
NB	5	4	0.9	0.648	3.623
NS	12	3	0.53	0.35	3.246
Overall	185	38	0.817	0.773	3.587

Population acronyms are listed in the text

IN, KY, LP, MN, MO, UP and WI), 1.4 % of individuals from the Western populations (ND), 13.6 % of individuals from the Eastern populations (ME and NS), and the remaining 5.5 % from other populations (AR, LA and FL). Interestingly, 58 % of the individuals possessing haplotype 2 (the second most numerous haplotype) were from Eastern populations (ME, NB, and NS) and haplotype 26 was the most common Western haplotype appearing in eight individuals from three Western populations. Also, some of the one-step branches derived from haplotype 1 led to haplotypes that occurred in widely dispersed populations. For example, haplotype 3 was found in FL, IL, LA, MO, MN, and TX.

The network suggested two separate population expansions occurred (Avise 2000), one comprising Western haplotypes ("Left Side") and another comprising all other haplotypes (mostly Midwestern/Eastern but some western samples; "Right Side"). For clarification the "Right Side" includes those Western samples associated with the right side of the network. The mismatch distributions for those haplotypes on the "Left" and "Right" sides of the network fit the sudden expansion model ($P = 0.500$ and $P = 0.557$ respectively). Also, the rg index was low for each mismatch distribution (rg = 0.045, $P = 0.448$ for "Left Side"; rg = 0.032, $P = 0.783$ for "Right Side"). Tajima's D was negative for both areas but not significantly different from 0 (D = - 0.52, $P = 0.342$ for "Left Side"; D = - 1.39, $P = 0.063$ for "Right Side"), whereas Fu's F_S was significantly negative in both cases ($F_S = -7.50$, $P < 0.000001$ for "Left Side"; $F_S = -27.1$, $P < 0.000001$ for Right Side") indicating a departure from population stability and further supporting the hypothesis of recent expansion. Furthermore, Fu and Li's F* and D* were not significant for either side (F* = 0.125, $P > 0.10$, D* = 0.253, $P > 0.10$ for "Left Side"; F* = −2.29; $0.10 > P > 0.05$, D* = −2.18, $0.10 > P > 0.05$, for "Right Side"), suggesting that selection was not responsible for the observed pattern of demography. The expansion coefficient was moderate for the "Left Side" (S/d = 4.51) and large for the "Right Side" (S/d = 11.11).

Population structure

Determining *K* from STRUCTURE using the ad hoc statistic ΔK clearly indicated that *K* is equal to 3 (Fig. 3). At $K = 3$, mean α varied very little between runs (mean α = 0.141–0.147) and there was clear asymmetric assignment of individuals to each cluster (Pritchard et al. 2009). Assignments made by CLUMPP to the three clusters designated by STRUCTURE included one cluster containing 95, 92, 90, and 71 % from WY, NV, ND, and TX respectively, another cluster containing 92, 91, 77, 71, 50, and 36 % of individuals from MO, KY, IN, IL, UP and WI respectively and a third cluster containing 100, 100, 92, 96, 89, 65, 59, and 36 % of individuals from NS, FL, ME, LP, NB, MN, LA and WI respectively (Fig. 4 and Online Resource 1). Percentages are based on a majority-rule assignment. These three clusters will be referred to as West which includes sample areas WY, NV, ND, and TX, Midwest which includes sample areas MO, KY, IN, IL, UP, and WI and East which includes sample areas NS, FL, ME, LP, NB, MN, and LA based on the origin of the majority of individuals assigning to each. Given the equal assignment of WI individuals to two clusters, upon looking further at the data, WI was placed in the Midwest cluster because individuals more strongly assigned to this cluster than to the East cluster. In a few cases, a large percentage of individuals within a given population assigned to two or more regional clusters. For example, 41 % of individuals from LA also assigned to Midwest, 40 % of individuals from UP also assigned to East, 35 % of individuals from MN also assigned to Midwest, and 27 % of individuals from WI also assigned to the West (Online Resource 1). For all populations with a large number of individuals assigning to other clusters, there was no spatial segregation of those individuals. However, this could not be determined for MN or LA due to the lack of specific location information for those populations.

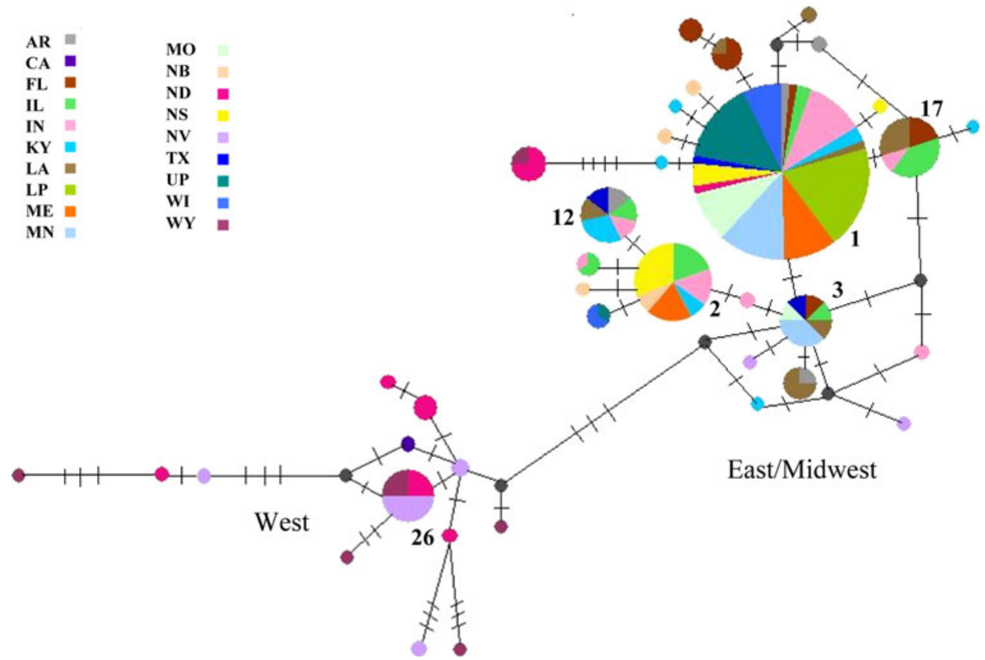

Fig. 2 Median joining network depicting relationships among 38 mitochondrial control region haplotypes among 19 areas in 185 bobcats sampled from 1995 to 2006 throughout North America. Haplotypes are represented by *circles* and areas of circles are proportional to haplotype frequency. *Cross marks* on branches define the number of substitutions between connected haplotypes, and *numbers* indicate haplotype

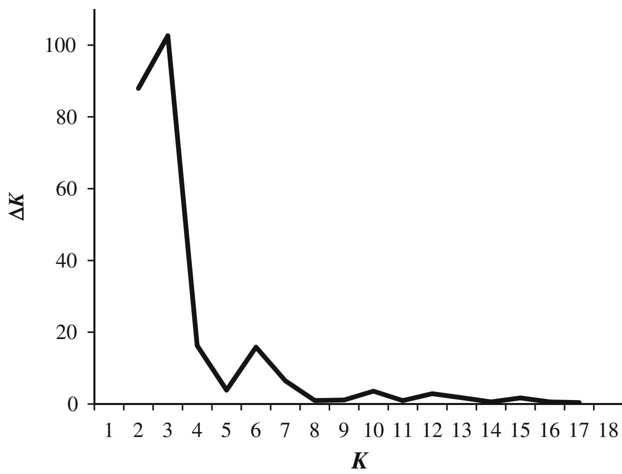

Fig. 3 Number of populations (K) estimated using STRUCTURE (Pritchard et al. 2000) for bobcats sampled from 1995 to 2006 throughout North America. Determination of the number of populations (K) based on the rate of change (ΔK) in the log probability of the data (as determined by STRUCTURE) between successive K values. The rate of change in log-likelihood values (ΔK) was estimated using the method of Evanno et al. (2005) where the maximum ΔK indicates the most likely number of populations

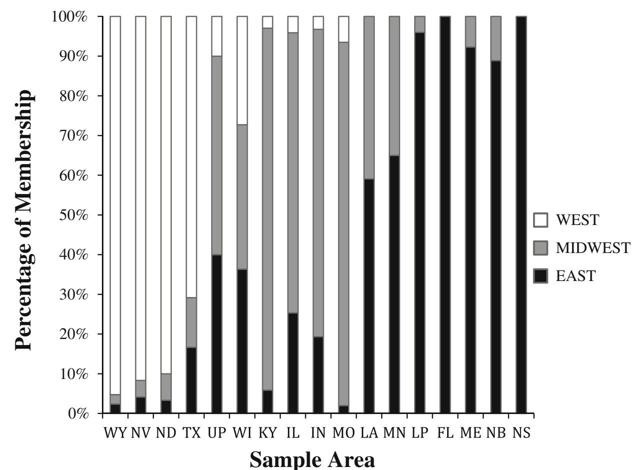

Fig. 4 Percentage of individuals assigned to each of the three clusters using majority-rule consensus and microsatellite data, as identified by STRUCTURE and determined by the method of Evanno et al. (2005) for bobcats from each population sampled from 1995 to 2006 throughout North America

Figure 3 illustrates moderately high ΔK, at $K = 4, 6, 10$ and 12, indicating potential hierarchical structuring (Pritchard et al. 2009). Given this possibility, subsequent STRUCTURE runs using the identical parameters as the previous runs were performed within each of the three clusters to elucidate whether these clusters could be further subdivided. We used the ad hoc statistic ΔK to reveal the number of clusters. These analyses subdivided the West cluster into two clusters; however, all populations assigned to each of the two clusters with almost equal frequency. The Midwest was subdivided into five clusters, with KY and MO assigned to one cluster (II), and WI and UP assigned to one cluster (IV). IL and IN had weak assignment probabilities and assigned to the same four clusters (I, II, IV, V). The East was subdivided into two clusters with FL, LA, LP, and MN assigning to one cluster and ME, NB and NS assigning to the other cluster.

F_{ST} between the West and Midwest was 0.0542, West and East 0.0862, and Midwest and East 0.0373. F_{ST} between the three clusters was moderate and significant

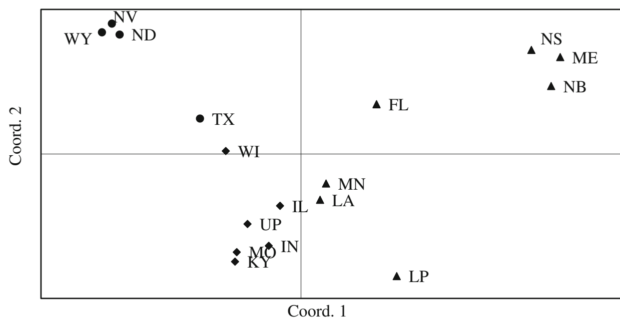

Fig. 5 PCA plot indicating genetic distances between sample areas of bobcats sampled from 1995 to 2006 throughout North America. PCA is based on pair-wise F_{ST} values using microsatellite data. *Circles* indicate those sample areas that clustered to West, *triangles* those that clustered to East, and *diamonds* those that clustered to the Midwest. Overall, the first and second axes explained 38 and 20 % of genetic variation respectively

($P < 0.000001$). Mantel tests did not support significant isolation by distance within the East cluster ($P = 0.417$), Midwest cluster ($P = 0.68$), or West cluster ($P = 0.074$). For comparison within clusters, population pairwise F_{ST} between the 17 populations ranged from 0 to 0.159. Using the Bonferroni correction (adjusted $\alpha = 0.00037$) six comparisons were not significant (ME and NB; WI and MN, WI and FL, WI and TX, WI and UP, and MN and LA; Online Resource 2). Population pair-wise F_{ST} values were used for the PCA plot and illustrated a clear separation between STRUCTURE identified clusters. WY, NV, and ND from the West cluster grouped closely together with TX plotted further away but in the same general area. Sample areas from the Midwest cluster grouped tightly together with the exception of WI. Sample areas in the East cluster had a much broader distribution than the other clusters, with those areas further East grouping together (Fig. 5). Overall, the first and second axes explained 38 and 20 % of genetic variation respectively.

Pairwise comparisons of ϕ_{ST} among sampled areas ranged from −0.414 to 0.964, with 25.1 % of those significant after Bonferroni correction (adjusted $\alpha = 0.00029$; Online Resource 3). The highest significant ϕ_{ST} values occurred between Western populations (CA, WY, NV, and ND) and all other populations. Hierarchical analysis of genetic variation using AMOVA revealed significant genetic structure among all populations (i.e. no grouping of populations) with ϕ_{ST} of 0.347 ($P < 0.00001$; Table 3). When groups of populations were used in AMOVA analyses, the grouping based on the topology of the network maximized ϕ_{ST} [i.e. Western populations (NV, WY, CA, ND) versus the remaining populations; $\phi_{ST} = 0.546$; Table 3]. AMOVA using three groups of populations (West versus Midwest versus East as revealed by STRUCTURE) resulted in a ϕ_{ST} of 0.329 ($P < 0.00001$). AMOVA using four, five and six groups of populations did not produce higher ϕ_{ST} values (Table 3). A Mantel test detected significant correlation between genetic and geographic distance when comparing all sample sites using ϕ_{ST} ($P = 0.0001$, $r = 0.510$).

Discussion

Both microsatellites and mitochondrial DNA confirmed little genetic structure over a broad range in bobcats. However, significant regional genetic differentiation was revealed between sample areas in the West and East/Midwest using mitochondrial DNA, and the West, Midwest and East using microsatellites. The regional population structure in bobcats was most likely established by historical processes that include separation via regional barriers and repeated expansions and contractions in population size resulting in population bottlenecks and genetic drift. The phylogeographic structure determined by this study suggests that bobcats as a species are mobile with no long-standing barriers to gene flow (Avise 2000) within regions. However, over time contemporary processes may have acted upon the historical genetic structure to initiate differentiation between the Midwest and East.

Population history and structure

It is evident from the "dumbbell" shaped network that bobcats have experienced two population expansions (Avise 2000). Smaller π estimates in Midwestern and Eastern North America compared to Western areas indicate that expansion took place in the West first with subsequent expansion in the Midwest and East (Zink et al. 2000). It is plausible that during the Pleistocene, Western populations of bobcats existed south of glacial ice and subsequently expanded into central and Eastern areas of the U.S. following glacier retreat. Hewitt (1996) suggested that organisms that are mobile and capable of long-distance dispersal are best able to track climate changes. Given the mobility of bobcats, it is probable that during periods of suitable climate conditions they gradually increased their distribution by dispersing into new areas. The glacial and interglacial cycles that occurred during the Pleistocene would have resulted in continual expansions and contractions of populations in the Midwestern and Eastern US. As a result, multiple extirpations and founder events would have occurred in northern-most Midwestern and Eastern areas because they were the last areas to be exposed due to glacial retreat. The continual cycle of colonization and extinction would ultimately lead to differentiation from the West. Additionally, it appears that dispersal back to the West after colonization of the Midwest/East was limited, suggesting that barriers to dispersal may have formed over

Table 3 Number of groups defined, source of variation, percent variation, values of ϕ_{ST}, and p values (*P*) determined by Analysis of Molecular Variation (AMOVA), for the mtDNA control region among 19 areas in 185 bobcats sampled from 1995 to 2006 throughout North America. Population acronyms are listed in the text

Populations	Source of variation	Percent of variation	ϕ_{ST}	*P*
Defined by state and provincial boundaries	Among populations	34.69	0.347	<0.000001
	Within populations	65.31		
2 Population	Among populations	54.64	0.546	<0.000001
Population 1: AR, IL, IN, KY, MO, FL, LA, LP, ME	Within populations	45.36		
MN, NB, NS, TX, UP, WI				
Population 2: NV, WY, CA, ND				
3 Populations	Among populations	32.91	0.329	<0.000001
Population 1: IL, IN, KY, MO, UP, WI	Within populations	67.09		
Population 2: AR, FL, LA, LP, MN, ME, NB, NS				
Population 3: NV, WY, CA, ND, TX				
4 Populations	Among populations	33.98	0.340	<0.000001
Population 1: IL, IN, KY, MO	Within populations	66.02		
Population 2: AR, FL, LP, ME, NS, NB				
Population 3: NV, WY, CA, ND, TX				
Population 4: LA, MN, UP, WI				
5 Populations	Among populations	35.91	0.359	<0.000001
Population 1: IL, IN, KY, MO	Within populations	64.09		
Population 2: AR, FL, LP				
Population 3: NV, WY, CA, ND, TX				
Population 4: LA, MN, UP, WI				
Population 5: ME, NB, NS				
6 Populations	Among populations	35.53	0.353	<0.000001
Population 1: IL, IN	Within populations	64.47		
Population 2: KY, MO				
Population 3: WI, UP				
Population 4: AR, FL, LA, LP, MN				
Population 5: NV, WY, CA, ND, TX				
Population 6: ME, NB, NS				

time. This is supported by the moderate microsatellite F_{ST} values between the West and Midwest (0.0542) and West and East (0.0862), and the few to no individuals assigning to the West from either the East or Midwest (outside of WI; Fig. 4; Online Resource 2). It is likely that the Great Plains or large rivers (e.g. Mississippi) reinforced regional differentiation by limiting dispersal back into the West. Additionally, positive isolation by distance was evident over the entire study area signifying that gene flow is increasingly less likely at great distances. The additive effect of geographic barriers and large distances between populations could have further enhanced the patterns of population structure found here.

The analyses of microsatellite data corroborate the control region population structure analyses to a degree, identifying the West as discrete but also distinguishing between Midwest and East. This result indicates that current processes are likely maintaining the historically present geographic structure (i.e. structure between the West and Midwest/East); however, the microsatellite data also indicate subdivision within the Midwest/East. The increased structure observed with microsatellite data is in contrast to what one would expect given the philopatric nature of female bobcats (Croteau et al. 2010; Janecka et al. 2007) and maternal inheritance of mtDNA. This result might reflect increased power in the microsatellites in detecting population structure or incomplete lineage sorting in mtDNA. Alternatively, it is likely that habitat fragmentation in the North American interior, and recent population recovery affect genetic differentiation between and within the East, Midwest and West. First, woody cover is a crucial habitat feature for bobcats (Anderson 1987; Rolley 1987; Nielsen and Woolf 2002; Woolf et al. 2002; Anderson and Lovallo 2003), and the patchy distribution of woody cover coupled with widespread agricultural development in the Midwest may sufficiently impede dispersal

and gene flow between regions and specific sample areas. Multi-scale changes in habitat composition, landscape characteristics, and metropolitan areas have been recognized as impediments to dispersal in other mobile carnivores (e.g. cougars: Ernest et al. 2003; McRae et al. 2005 and wolverines, *Gulo gulo*: Kyle and Strobeck 2001). Secondly, noticeable decreases of bobcats range-wide, and most significantly in the Midwest, resulting from increased agricultural land use, recreational hunting and trapping, and predator control in the twentieth century (Brown and Yeager 1943; Deems and Pursley 1978; Erickson et al. 1981; Hamilton and Fox 1987; Woolf and Hubert 1998) and subsequent increases in density, might have impacted allele frequencies. Following bobcat decreases, they were protected under federal legislation (i.e. the Convention on International Trade in Endangered Species (CITES) and the U.S. Endangered Species Act (ESA 1973)) and bobcat numbers rebounded (in the Midwest especially; Woolf and Hubert 1998), and continue to do so (Roberts and Crimmins 2010). Expansions of small, isolated bobcat populations may have shifted allele frequencies and contributed to genetic differentiation between and within the Midwest and East. Nevertheless, the Midwest and East are not entirely distinct from one another. Assignment of individuals to other clusters, and weak assignment probabilities imply that there is some gene flow between the Midwest and East. Bobcat life history indicates that they readily disperse and undertake long-distance dispersal (Knick and Bailey 1986; Kamler et al. 2000; Nielsen and Woolf 2003; Johnson et al. 2010), suggesting that gene flow between the Midwest and East is plausible.

Patterns of genetic diversity

Microsatellite diversity was moderate to high among bobcat sample areas, and although the diversity measures here are not directly comparable to other studies due to differences in microsatellites used, number of loci and species, our estimates are similar or greater than other bobcat studies and mobile North American medium-sized cats (e.g. Schwartz et al. 2002; Riley et al. 2006; Millions and Swanson 2007; Campbell and Strobeck 2006). On the other hand, average mtDNA diversity was low in comparison to other medium-sized cats (423 base pair fragment control region analyzed in ocelots (*Leopardus pardalis*) and margays (*L. wiedii*; Eizirik et al. 1998)).

Significant HWE scores indicating homozygote excesses in the microsatellite data were evident in a few cases within clusters. However, given that there was no consistency to the pattern of loci out of HWE, we do not think the assumption of random mating was violated. Loci out of HWE could also be a result of sex-biased dispersal behavior in bobcats (Croteau et al. 2010; Janecka et al. 2007), further fine scale sub-structuring within clusters, a clumped sampling scheme, not sampling all individuals that would be contained within the cluster and specifying a cluster to a sample area even though the assignment values were weak.

The relatively high levels of genetic variation historically present and maintained throughout the Western portion of bobcat range suggest that bobcats have persisted at large effective population sizes in this region and gene flow is ongoing. Extensive gene flow within this area will counteract the negative effects of genetic drift and potentially introduce new alleles (Hartl and Clark 2007). Low values of nucleotide and haplotype diversity in the Midwest and East suggest that populations have historically experienced small effective population sizes (Avise 2000). Additionally, small values of π can be attributed to stochastic events (e.g. drastic changes in the environment and/or founder and bottleneck effects) that occur during expansion, which can lead to a reduction in genetic variation within the newly occupied range (Zink et al. 2000). The current low levels of microsatellite variation in the East are most likely a result of population isolation and genetic drift. Bobcats have thriving populations in the East and Midwest, as evidenced by hunting and trapping seasons and growing populations (Woolf and Hubert 1998; Roberts and Crimmins 2010); however, the ability of bobcats to easily disperse between the East and other portions of bobcats' range may be hindered due to barriers such as urban development and agricultural land use in the Midwest. Since Eastern populations are at the range boundary, it is likely that anthropogenic land use, and possibly geographic distance, play a role in the degree of connectivity of Eastern populations to other populations at the range core.

Conservation implications

The potential of a species to evolve, adapt and respond to environmental change is primarily dependent upon genetic diversity (Frankham et al. 2002). The World Conservation Union (IUCN) recognized that the preservation of genetic diversity is one of three priorities essential to conserving biodiversity as a whole (McNeely et al. 1990). Furthermore, genetic diversity has shown to be essential to ecosystem recovery and function (Reusch et al. 2005; Frankham 2005). Given this, identifying both historical and contemporary patterns of genetic diversity is a vital component of managing species and populations for conservation.

Understanding what drives patterns of genetic diversity and population structure is especially important in populations of wide-ranging species because lack of gene flow between populations within the range can lead to isolation and small population size which will eventually result in inbreeding, loss of genetic variability and higher extinction

risk (Frankam et al. 2002). From our results, we can infer that bobcats are maintaining gene flow, and thus genetic diversity. This result is no surprise given their dispersal capabilities (Knick and Bailey 1986; Kamler et al. 2000; Nielsen and Woolf 2003; Johnson et al. 2010). Gene flow is most likely influencing the growth and stability of populations as documented by Roberts and Crimmins (2010).

Despite high abundance and improved status of the bobcat throughout much of its range (Woolf and Hubert 1998; Roberts and Crimmins 2010), impending changes in the composition and structure of bobcat habitat are not likely to be conducive to gene flow. These habitat changes are already at work as evidenced by the microsatellite analyses. Furthermore, closer inspection of F_{ST} results show that bobcats exhibit moderate genetic differentiation (Hartl and Clark 2007) between many samples areas (47 % of pairwise F_{ST} estimates ≥ 0.05; Online Resource 2), demonstrating that several populations may be experiencing some level of isolation. However, the clumped sampling scheme may artificially inflate F_{ST} results (Schwartz and McKelvey 2009). Regardless, these results coupled with STRUCTURE analyses from within clusters and some loci out of HWE support a finer level of substructuring. It has been suggested that the most significant barriers to gene flow in wildlife populations are anthropogenic in nature, in the form of roads, agriculture, and human settlement (Kyle et al. 2000; Kyle and Strobeck 2001). As human populations increase and expand into exurban areas, and if former bobcat habitat is converted to row-crop agriculture for biofuel production (Bies 2006; U.S. Department of Agriculture 2007), fragmentation and loss of habitat will increase, resulting in reduced gene flow and increased subdivision of bobcat populations. Therefore, it will become necessary to preserve contiguous tracts of habitat to allow for bobcat dispersal to maintain historical connections that facilitate gene flow and maintain present levels of genetic diversity.

Of more immediate conservation concern is how to properly manage bobcat populations given our findings. Although smaller-scale studies can better the understanding of local patterns of population structure, large-scale studies are important for assessing the range-wide distribution of genetic diversity of a species, and can provide vital information for conservation planning. Bobcats historically have been managed by state and provincial conservation agencies on a state-by-state basis. These boundaries mean little to bobcats given the size of their home ranges and dispersal capabilities. Thus, referring to regional differences among genetic variation may be more appropriate for determining the most suitable strategy for bobcat conservation. We suggest that wildlife biologists and agency administrators consider implementing regional (i.e., multi-state) management plans for bobcats that maintain historical connections and mitigate limitations to gene flow within regions to prevent loss of genetic diversity. Furthermore, future management of bobcats would benefit from a more rigorous landscape genetics approach that pinpoints specific anthropogenic or natural barriers to gene flow and the effects that landscape composition, configuration and matrix quality have on spatial genetic variation. This approach would allow biologists to identify areas that are isolated, habitat/landscape variables that impede gene flow and the potential for corridor development to ensure effective dispersal.

Acknowledgments We thank C. Albright, C. Carlton, G. Duncan, R. Earle, J. Emmerich, J. Erb, J. Ermer, H. Ernest, B. Ettinger, W. Jakubus, L. Jahnke, J. Janecka, S. Johnson, B. Lanka, D. Lutz, G. Matthews, J. Mitchell, J. Nemick, M. O'Brien, J. Olson, B. Parker, A. Roth, B. Rudd, J. Stevens, T. Stevens and Stevens Fur Company, S. Smith, D. Wagner, B. Weeks, and R. Woostenhulme, for providing tissue samples analyzed in this study, and most notably B. Bluett and the Illinois Department of Natural Resources (IDNR) who coordinated this effort. Special thanks to A. Woolf for initiating this work. Thanks to C. Krajewski and K. Ibrahim for providing comments on previous drafts of this manuscript. Additional thanks to F. Anderson, A. Cordoba, A. Schrey, and J. Sipiorski for assistance with molecular work and statistical analyses. Also, thank you to several anonymous reviews of previous versions of this manuscript. Funding was provided by the IDNR Special Funds Program, State Furbearer Fund to A.W and the Cooperative Wildlife Research Laboratory (CWRL). Additional support was provided to E.C. by CWRL, the Department of Zoology and Southern Illinois University—Carbondale Graduate School.

References

Allendorf FW (1983) Isolation, gene flow, and genetic differentiation among populations. In: Schonewald-Cox CM, Chambers SM, MacBryde B, Thomas WL (eds) Genetics and conservation: a reference for managing wild animal and plant populations. Benjamin Cummings, Menlo Park

Amos W, Harwood J (1998) Factors affecting levels of genetic diversity in natural populations. Philos Trans R Soc Lond B 353:177–186

Anderson EM (1987) A critical review and annotated bibliography of literature on the bobcat. Colorado Division of Wildlife, Special Report Number 62

Anderson EM, Lovallo MJ (2003) Bobcat and lynx. In: Feldhamer GA, Thompson BC, Chapman JA (eds) Wild mammals of North America: biology, conservation, and management, 3rd edn. Johns Hopkins University Press, Baltimore, pp 758–786

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) Genetix 4.02. Logiciel sous Windows TM pour la génetique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000 Université de Montpelier II, Montpelier

Bies L (2006) The biofuels explosion: is green energy good for wildlife? Wildl Soc Bull 34:1203–1205

Brown LG, Yeager LE (1943) Survey of the Illinois fur resource. Ill Nat Hist Surv Bull 22:434–504

Campbell V, Strobeck C (2006) Fine-scale genetic structure and dispersal in Canada lynx (*Lynx canadenxis*) within Alberta, Canada. Can J Zool 84:1112–1119

Carmichael LE, Clark W, Strobek C (2000) Development and characterization of microsatellite loci from lynx (*Lynx canadensis*), and their use in other felids. Mol Ecol 9:2197–2198

Castro ALF, Stewart BS, Wilson SG, Hueter RE, Meekan MG, Motta PJ, Bowen BW, Karl SA (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). Mol Ecol 16:5183–5192

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. Evolution 21:550–570

Croteau EK, Heist EJ, Nielsen CK (2010) Fine-scale population structure and sex-biased dispersal in bobcats (*Lynx rufus*) from southern Illinois. Can J Zool 88:536–545

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

Eizirik E, Bonatto SL, Johnson WE, Crawshaw PG, Vie JC, Brousset DM, O'Brien SJ, Salzano FM (1998) Phylogeographic patterns and evolution of the mitochondrial DNA control region in two neotropical cats (Mammalia, Felidae). J Mol Evol 47:613–624

Eizirik E, Kim JH, Menotti-Raymond M, Crawshaw PG Jr, O'Brien SJ, Johnson WE (2001) Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). Mol Ecol 10:65–79

Endangered Species Act (1973) 16 USC Sec. 1531

Environmental Systems Research Institute, Inc. (1992–2002) Arcview Version 3.3 Redlands, California

Erickson DW, Hamilton DA, Sampson FW (1981) The status of the bobcat in Missouri. Trans Mo Acad Sci 15:49–59

Ernest HB, Boyce WM, Bleich VC, May B, Stiver SJ, Torres SJ (2003) Genetic structure of mountain lion (*Puma concolor*) populations in California. Conserv Genet 4:353–366

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes. Application to human mitochondrial DNA. Genetics 131:479–491

Excoffier L, Laval LG, Schneider S (2005) Arlequin version 3.0: an integrated software package for population genetic data analysis. Evol Bioinform 1:47–50

Faircloth BC, Reid A, Valentine T, Hyung ES, Terhune TM, Glenn TC, Palmer WE, Nairn CJ, Carroll JP (2005) Tetranucleotide, trinucleotide, and dinucleotide loci from the bobcat (*Lynx rufus*). Mol Ecol Notes 5:387–389

Frankham R (2005) Conservation biology: ecosystem recovery enhanced by genotypic diversity. Heredity 95:183

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge

Freeman AR, MacHugh DE, McKeown S, Walzer C, McConnell DJ, Bradley DG (2001) Sequence variation in the mitochondrial DNA control region of wild African cheetahs (*Acinonyx jubatus*). Heredity 86:355–362

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

Fu Y-X, Li WH (1993) Statistical tests of neutrality of mutations. Genetics 133:693–709

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. Mol Ecol 11: 1103–1114

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361–372

Hall ER (1981) The mammals of North America. Wiley, New York

Hamilton DA, Fox LB (1987) Wild furbearer management in the midwestern United States. In: Novak M, Baker JA, Obbard ME, Malloch B (eds) Wild furbearer conservation and management in North America. Ontario Ministry of Natural Resources, Toronto, pp 1100–1115

Hansen K (2007) Bobcat: master of survival. Oxford University Press, Inc., New York

Harpending HC, Sherry ST, Rogers AR, Stoneking M (1993) The genetic structure of human populations. Curr Anthropol 34:483–496

Hartl DL, Clark AG (2007) Principles of population genetics, 4th edn. Sinauer Associates, Sunderland

Hedrick PW (2000) Genetics of populations, 2nd edn. Jones and Bartlett, Sudbury

Hellborg L, Walker CW, Rueness EK, Stacy JE, Kojola I, Valdmann H, Vila C, Zimmermann B, Jakobsen KS, Ellegren H (2002) Differentiation and levels of genetic variation in northern European lynx (*Lynx lynx*) populations revealed by microsatellites and mitochondrial DNA analysis. Conserv Genet 3:97–111

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linn Soc 58:247–276

Hewitt G (2000) The genetic legacy of the quaternary ice ages. Nature 405:907–913

Hoelzel AR, Lopez JV, Dover GA, O'Brien SJ (1994) Rapid evolution of a heteroplasmic repetitive sequence in the mitochondrial DNA control region of carnivores. J Mol Evol 39: 191–199

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Stat 6:65–70

Hudson RR (1990) Gene genealogies and the coalescent process. In: Futuyama D, Antonovics JD (eds) Oxford surveys in evolutionary biology. Oxford University Press, New York, pp 1–44

Hull JM, Anderson R, Bradbury M, Estep JA, Ernest HB (2008) Population structure and genetic diversity in Swainson's hawks (*Buteo swainsoni*): implications for conservation. Conserv Genet 9:305–316

Jakobbson 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, Tewes ME, Kilpatrick CW, Grassman LI Jr (2006) Kinship and social structure of bobcats (*Lynx rufus*) inferred from microsatellite and radio-telemetry data. J Zool 269:494–501

Janecka JE, Blankenship TL, Hirth DH, Kilpatrick CW, Tewes ME, Grassman LI Jr (2007) Evidence for male-biased dispersal in bobcats *Lynx rufus* using relatedness analysis. Wildl Biol 13:38–47

Johnson SA, Walker HD, Hudson CM (2010) Dispersal characteristics of juvenile bobcats in South-Central Indiana. J Wildl Manage 74:379–385

Kamler JF, Gipson PS, Snyder TR (2000) Dispersal characteristics of young bobcats from Northeastern Kansas. Southwest Nat 45: 543–546

Knick ST, Bailey TN (1986) Long-distance movements by two bobcats from southeastern Idaho. Am Midl Nat 116:222–223

Kyle CJ, Strobeck C (2001) Genetic structure of North American wolverine (*Gulo gulo*) populations. Mol Ecol 10:337–347

Kyle CJ, Davis CS, Strobeck C (2000) Microsatellite analysis of North American pine marten (*Martes Americana*) populations from the Yukon and Northwest Territories. Can J Zool 78:1150–1157

Lande R (1988) Genetics and demography in biological conservation. Science 241:1455–1460

Larivière S, Walton LR (1997) Lynx rufus. Mamm Species 24:1–8

Lavoie M, Collin P-Y, Lemieux F, Jolicoeur H, Canac-Marquiz P, Larivière S (2010) Understanding fluctuations in bobcat harvest at the northern limit of their range. J Wildl Manage 73:870–875

Liu Z, Ren B, Wei F, Long Y, Hao Y, Ming L (2007) Phylogeography and population structure of the Yunnan snub-nosed monkey (*Rhinopithecus bieti*) inferred from mitochondrial control region DNA sequence analysis. Mol Ecol 16:3334–3349

Lopez JV, Cevario S, O'Brien SJ (1996) Complete nucleotide sequences of the domestic cat (*Felis catus*) mitochondrial genome and a transposed mtDNA tandem repeat (Numt) in the nuclear genome. Genomics 33:229–246

Lopez-Gonzalez CA, Gonzalez-Romero A, Laundre JW (1998) Range extension of the bobcat (*Lynx rufus*) in Jalisco, Mexico. Southwest Nat 43:103–110

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

McNeely JA, Miller KR, Reid WV, Mittermeier RA, Werner TB (1990) Conserving the world's biological diversity. IUCN, World Resources Institute, Conservation International, WWF-US and the World Bank, Washington

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. Mol Ecol 14:1965–1977

Mendez M, Rosenbaum HC, Bordino P (2008) Conservation genetics of the franciscana dolphin in Northern Argentina: population structure, by-catch impacts, and management implications. Conserv Genet 9:419–435

Menotti-Raymond MA, O'Brien SJ (1995) Evolutionary conservation of ten microsatellite loci in four species of Felidae. J Hered 86:319–322

Menotti-Raymond MA, David AV, Lyons LA, Shaffer AA, Tomlin JF, Hutton MK, O'Brien SJ (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). Genomics 56:9–23

Millions DG, Swanson BJ (2006) An application of Manel's model: detecting bobcat poaching in Michigan. Wildl Soc Bull 34:150–155

Millions DG, Swanson BJ (2007) Impact of natural and artificial barriers to dispersal on the population structure of bobcats. J Wildl Manag 71:96–102

Nielsen CK, Woolf A (2002) Habitat-relative abundance relationship of bobcats in southern Illinois. Wildl Soc Bull 30:222–230

Nielsen CK, Woolf A (2003) Dispersal of juvenile male bobcats (*Lynx rufus*) in southern Illinois. Trans Ill Acad Sci 96:313–318

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Resour 6:288–295

Peck DR, Congdon BC (2004) Reconciling historical processes and population structure in the sooty tern *Sterna fuscata*. J Avian Biology 35:327–335

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. Available from: http://pritch.bsd.uchicago.edu/structure.html

Ray JC, Hunter L, Zigouris J (2005) Setting conservation and research priorities for larger african carnivores. WCS Working Paper No 24. Wildlife Conservation Society, NY

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Reusch TBH, Ehlers A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc NatL Acad Sci USA 102:2826–2831

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

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

Roberts NM, Crimmins SM (2010) Bobcat population status and management in North America: evidence of large-scale population increase. J Fish Wildl Manage 1:169–174

Roemer GW, Gompper ME, van Valkenburgh B (2009) The ecological role of the mammalian mesocarnivore. Bioscience 59:165–173

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569

Rolley RE (1987) Bobcat. In: Novak M, Baker JA, Obbard ME, Malloch B (eds) Wild furbearer conservation and management in North America. Ontario Ministry of Natural Resources, Toronto, pp 672–681

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Notes 8:103–106

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Samson FB (1979) Multivariate analysis of cranial characters among bobcats with a preliminary discussion of the number of subspecies. Bobcat Research Conference. Natl Wildl Fed Sci Tech Ser 6:80–86

Schwartz MK, McKelvey KS (2009) Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. Conserv Genet 10:441–452

Schwartz MK, Scott Mills L, McKelvey KS, Rugglero LF, Allendorf FW (2002) DNA reveals high dispersal synchronizing the population dynamics of Canada lynx. Nature 415:520–522

Shillinger GL, Palacios DM, Bailey H, Bograd SJ, Swithenbank AM, Gaspar P, Wallace BP, Spotila JR, Paladino FV, Piedra R, Eckert SA, Block BA (2008) Persistent Leatherback Turtle Migrations Present Opportunities for Conservation. PLoS Biol 7:e171

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 123:603–613

Smouse P (1998) To tree or not to tree. Mol Ecol 7:399–412

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymnorphisms. Genetics 123:585–595

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: molecular evolutionary genetic analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

United States Department of Agriculture (2007) USDA announces results of intentions to re-enroll and extend CRP contracts. United States Department of Agriculture: news. http://www.usda.gov. Accessed 25 February 2008

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Von Holdt BM, Stahler DR, Banges EE, Smith DW, Jimenez MD, Mack CM, Niemeyer CC, Pollinger JP, Wayne RK (2010) A novel assessment of population structure and gene flow in grey wolf populations of the Northern Rocky Mountains of the United States. Mol Ecol 19:4412–4427

Ward RD, Elliott NG, Innes BH, Smolenski AJ, Grewe PM (1997) Global population structure of yellowfin tuna, *Thunnus albacares*, inferred from allozyme and mitochondrial DNA variation. Fish Bull 95:566–575

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

Woolf A, Hubert GF Jr (1998) Status and management of bobcats in the United States over three decades: 1970 s–1990 s. Wildl Soc Bull 26:287–293

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

Young SP (1958) The bobcat of North America: its history, life habits, economic status and control, with list of currently recognized subspecies. The Wildlife Management Institute, Washington, DC

Zink RM, Barrowclough GF, Atwood JL, Blackwell-Rago RC (2000) Genetics, taxonomy, and conservation of the threatened California gnatcatcher. Conserv Biol 14:1394–1405