

Impact of Natural and Artificial Barriers to Dispersal on the Population Structure of Bobcats

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ABSTRACT We investigated population structure and genetic diversity for bobcats (*Lynx rufus*) in Michigan, USA, which are distributed throughout the upper peninsula (UP) and the northern half of the lower peninsula (LP) of Michigan. Specifically, we assessed the influence of natural and artificial barriers to dispersal on the genetic population structure of the bobcat across Michigan, as well as in each peninsula. We used 5 microsatellite markers and the statistical package STRUCTURE to identify populations and assign individuals to their population of origin. STRUCTURE identified one population in each peninsula, indicating that the UP and LP are genetically isolated by the Straits of Mackinac which divide the UP and LP. Despite a greater density of roads in the LP, we found no evidence that they have led to intra-peninsular population structure. Our results suggest that, from a genetic standpoint, management agencies do not need to be concerned about the fragmenting effects of roads when producing management plans for bobcats. (JOURNAL OF WILDLIFE MANAGEMENT 71(1):96–102; 2007)

DOI: 10.2193.2005-563

KEY WORDS bobcat, dispersal barriers, landscape genetics, *Lynx rufus*, Michigan, microsatellites, roads.

Improvements in molecular genetic and statistical tools led to the emergence of landscape genetics, a combination of population genetics and landscape ecology (Manel et al. 2003). Landscape genetics shows how landscape features influence population structure by correlating genetic discontinuities with landscape or environmental features. Manel et al. (2003) defined these features as the biotic, climatic, edaphic, and other conditions that encompass an organism's immediate habitat. Determining the effects of landscape features on genetic variation is integral to understanding how landscapes influence gene flow, dispersal, and population structure.

Several recent studies increased our understanding of how landscape features influence the genetic population structure of many taxa, including invertebrates (Keyghobadi et al. 1999, Keller et al. 2004), fish (Castric et al. 2001, Wofford et al. 2005), amphibians (Vos et al. 2001, Funk et al. 2005), birds (Piertney et al. 1998, Caizergues et al. 2003), and mammals (Anderson et al. 2004, Trizio et al. 2005). These studies have resulted from improved methods for analyzing molecular data (Pritchard et al. 2000, Corander et al. 2003, Mank and Avise 2004) that do not require researchers to rely on a priori assumptions about population structure (Pearse and Crandall 2004, Evanno et al. 2005). These new methods let the data define populations rather than using sampling location as a surrogate when defining genetic population structure (Pearse and Crandall 2004). By correlating genetically defined population structure with landscape features, the genetic structure highlights true barriers to dispersal.

The distribution of bobcats (*Lynx rufus*) in Michigan, USA (Fig. 1), on 2 peninsulas separated by the Straits of Mackinac, provides an opportunity to apply landscape

genetic methods to investigate the effect of natural and artificial landscape features on dispersal, population structure, and genetic variation. Given the unlikelihood that bobcats are crossing the straits often enough to genetically homogenize populations, the straits should form a natural barrier to bobcat dispersal, potentially leading to the formation of 2 genetically distinct populations.

The distribution of bobcats in Michigan and North America (Fig. 1) also provides an opportunity to observe the potential differences in genetic variation that may exist between the upper peninsula (UP) and the northern half of the lower peninsula (LP). Bobcats in the UP are connected to a presumably continuous population across North America (Fig. 1). However, if the Straits of Mackinac act as a barrier to movement, the LP may be isolated from this continuous North American population. If the LP is genetically distinct from the UP, it should have lower levels of genetic diversity in relation to the UP given its isolation from the continuous North American distribution.

The geography of Michigan and the distribution of the bobcat also allowed us to determine the effects of potential barriers to dispersal on intra-peninsular population structure. It is less clear which natural features may constitute barriers to movement and subsequent creation of genetically distinct populations within Michigan's peninsulas. However, there are many artificial barriers to dispersal that have the potential to influence wildlife movements (e.g., urban areas, agricultural areas, roads). Of these potential barriers, roads are a ubiquitous barrier type and have become items of concern in recent years because of their proliferation (Forman 1998, Forman et al. 2003). Roads act as barriers by disrupting movement patterns through road avoidance, inaccessibility, or by direct mortality caused by vehicle collisions (Forman 1998, Forman and Alexander 1998, Jaeger et al. 2005). If the barrier effect is severe enough, it

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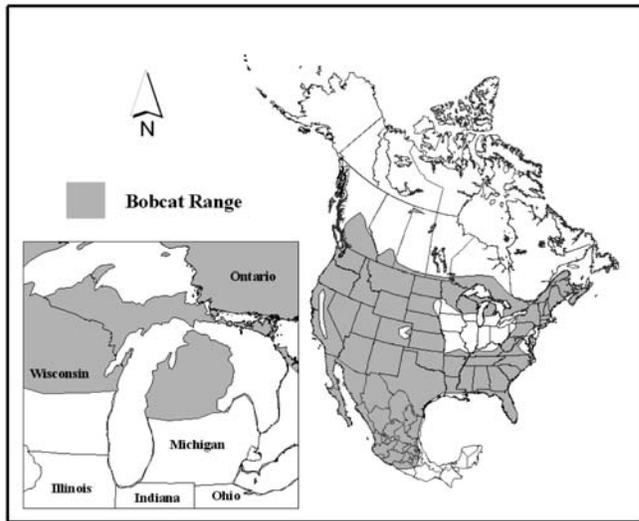


Figure 1. Approximate distribution of bobcats in North America and Michigan, USA, based on Anderson and Lovallo (2002).

may lead to the fragmentation of habitat and subdivision of populations (Jaeger et al. 2005), resulting in genetic differentiation (Gerlach and Musolf 2000, Keller and Largiadèr 2003).

Roads influence wildlife movement patterns in a variety of species (deMaynadier and Hunter 2000, Lode 2000, Vos et al. 2001, Keller and Largiadèr 2003), including mammals (Oxley et al. 1974, Bruinderink and Hazebroek 1996, Dyer et al. 2002, McDonald and St. Clair 2004), carnivores (Beier 1995, Gibeau and Heuer 1996, Blanco et al. 2005, Dickson et al. 2005), and specifically bobcats (Lovallo and Anderson 1996, Riley 1999, Tigas et al. 2002, Cain et al. 2003, Riley et al. 2003). Although bobcats will cross various types of roads, high mortality and avoidance of particular types of roads may lead to the genetic differentiation of bobcat populations. Although other artificial barriers to movement, such as agricultural or urban areas, could potentially influence bobcats, studies have not shown these barriers to influence bobcat movements in the same manner as roads. This could be because of the bobcats' ability to live in a wide range of suitable habitats (reviewed in Rolley 1987, Anderson and Lovallo 2002) and to persist in close proximity to, or within, urban or residential areas (Harrison 1998, Haas 2000, Tigas et al. 2002).

Our study addressed 3 questions: 1) Do the Straits of Mackinac act as a natural barrier to dispersal between the UP and LP?; 2) If population structure exists between the UP and LP, are there differences in levels of genetic variation among populations?; and 3) Have artificial barriers to dispersal influenced intra-peninsular population structure?

STUDY AREA

Our study area included the entire upper peninsula of Michigan and the northern half of the lower peninsula where bobcats exist in large numbers, an area covering

approximately 104,000 km² (Fig. 1). The peninsulas are separated by the Straits of Mackinac (Fig. 2), which connect Lake Michigan and Lake Huron and are 6 km apart at the narrowest point. Although observed daily bobcat movement and dispersal distances (reviewed in Anderson and Lovallo 2002) could accommodate movement across the straits during winter when ice cover was possible, there is evidence that this is a rare event. In a 2-year study of winter river crossings by large mammals on the St. Mary's River (0.2–7 km wide), which connects Lake Superior to Lake Huron, researchers made no observations of any bobcat crossings (Amacher 1983). Further, even though the Straits of Mackinac and the St. Mary's River freeze over regularly in the winter, ice-breaking activity maintained an approximately 35-m channel through the ice in both locations (S. P. Kasten, United States Coast Guard Group Sault Ste. Marie, personal communication).

METHODS

Sample Collection and DNA Extraction

We obtained tissue samples from the Michigan Department of Natural Resources (MI-DNR) from bobcats harvested during the 2001–2002 hunting and trapping season (Fig. 3). Because of harvest regulations during the 2001–2002 hunting and trapping season, there is a chance that bobcats were poached from the LP and reported as being harvested in the UP (Millions and Swanson 2006). We removed individuals identified as poached by the assignment test within STRUCTURE from subsequent analyses. We extracted DNA from tissue of 343 individuals; hunters reported harvesting 206 in the UP and 137 in the LP. We performed extractions with Qiagen DNeasy kits (Qiagen, Valencia, CA) following published protocols (Qiagen 2001), and we measured DNA quantity and purity with an Eppendorf Biophotometer (Brinkman Instruments Inc., Westbury, NY). We diluted samples to a working stock of

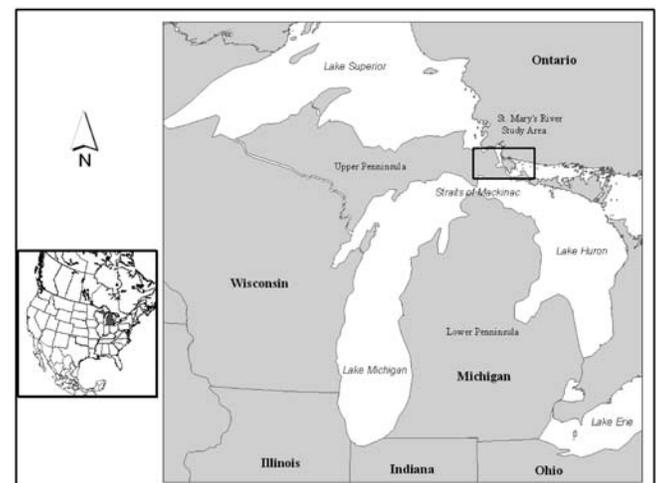


Figure 2. Location of Straits of Mackinac and St. Mary's River, Michigan, USA.

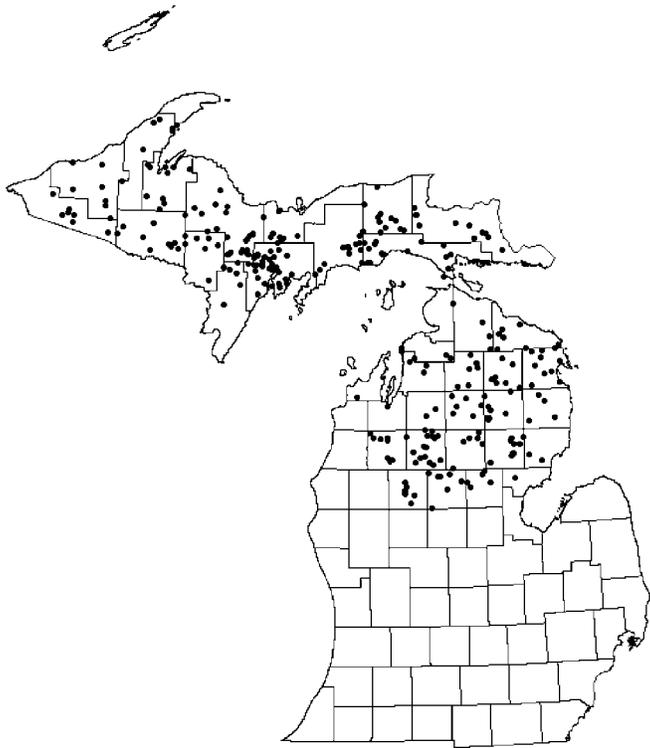


Figure 3. Harvest locations of 206 upper peninsula and 137 lower peninsula bobcats used in this study from the 2001 to 2002 harvest season, Michigan, USA.

15 ng/ μ L, and we stored any remaining tissue and extracted DNA at -20° C.

Microsatellite Amplification

We typed all bobcats at 5 microsatellite loci using 2 Canada lynx (*Lynx canadensis*) primers (Lc109 and Lc111; Carmichael et al. 2000) and 3 domestic cat (*Felis catus*) primers (Fca8, Fca43, and Fca90; Menotti-Raymond and O'Brien 1995). We fluorescently labeled all reverse primers with FAM, TET, or HEX. We performed polymerase chain reaction in a 20- μ L cocktail containing 75 ng genomic DNA, 250 μ M deoxyribonucleotides, 0.16 μ M of each primer, 1 \times HotMaster *Taq* buffer, and 1.5 units of HotMaster *Taq* polymerase (Brinkman Instruments Inc.). We conducted amplification on an Eppendorf MasterGradient Thermocycler (Brinkman Instruments Inc.), which consisted of initial denaturation step for 2 minutes at 94° C followed by 3 cycles of 20 seconds at 94° C, 20 seconds at 52° C, and 5 seconds at 72° C. We followed this step with 33 cycles of 15 seconds at 94° C, 20 seconds at 52° C, and 5 seconds at 72° C, followed by a terminal extension step of 1 minute at 72° C. Following amplification, we analyzed samples on an ABI 310 Genetic Analyzer using GeneScan Analysis 3.1.2 software (Applied Biosystems, Foster City, CA). We determined genotypes using Genotyper 2.0 software (Applied Biosystems).

Population Subdivision

We analyzed population structure using the Bayesian clustering program STRUCTURE 2.1 (Pritchard et al.

2000) because of its ability to identify cryptic population structure (Rueness et al. 2003, Beaumont and Rannala 2004, Sacks et al. 2004, Swanson et al. 2005, Wright et al. 2005). We tested 4 models using all combinations of admixture or no admixture with correlated or independent allele frequencies. Each model included all 343 genotypes and tested for $K = 1-5$, where K is the number of potential populations. We followed a burn-in of 100,000 iterations of the Markov chain by a run of 100,000 iterations and repeated this 10 times at each K . These parameters produced consistent estimates of the posterior probability of having K populations in replicate runs.

Once we identified population clusters, we assigned individual bobcats to a population using the assignment test within STRUCTURE. We used a threshold value of 0.9 to assign individuals to a population and to identify potential dispersers or poached individuals (Manel et al. 2003, Millions and Swanson 2006).

We then performed individual STRUCTURE analyses for each peninsula to test for the presence of intra-peninsular population structure. We used the same 4 models, assumptions, and run conditions as the initial statewide STRUCTURE analyses.

We analyzed population clusters identified by STRUCTURE using GENEPOP 3.4 (Raymond and Rousset 1995) to test for deviations from Hardy-Weinberg equilibrium, for genotypic linkage (Bonferroni corrections applied for multiple tests), and for genic differentiation. We calculated F_{ST} values using Arlequin version 2.000 (Schneider et al. 2000).

We determined differences in genetic variation by comparing observed heterozygosity and the number of alleles per locus between all identified population clusters. We compared the number of alleles per locus in each population cluster after adjusting for sample size by resampling 1,000 times from the largest observed population cluster (Rueness et al. 2003, Beaumont and Rannala 2004, Sacks et al. 2004, Swanson et al. 2005, Wright et al. 2005) using the program Resampling Stats (Simon 1997).

Quantification of Roads

We calculated the prevalence of roads in each peninsula in ArcView GIS 3.2a. We included all counties that allowed bobcat hunting and trapping in the analyses and estimated road coverage and density using Census 2000 TIGER/Line Data (<www.esri.com/data/download>). We included only roads that were part of Michigan's trunkline (i.e., state, United States national highways, and interstate highways) in the analyses (Feature Class Codes: A1X-A2X), based on the assumption that these roads were more likely to influence bobcats through avoidance or mortality (Lovallo and Anderson 1996, Riley 1999, Tigas et al. 2002, Cain et al. 2003). We defined the influence of roads on the landscape by total road density (km/km^2) and the creation of patches by these road types, which included patch density ($\text{no.}/100 \text{ km}^2$) and patch size (km^2 ; Saunders et al. 2002). We defined patches as any polygon created by the intersection of trunkline roads and compared median patch size (km^2)

Table 1. Inference of the number of populations of bobcats in Michigan, USA, using the admixture and correlated allele frequency model in STRUCTURE for bobcats harvested during the 2001–2002 hunting and trapping season.

<i>K</i>	Log $P(X K)$	Log $P(K X)$
1	−4,811.1	approx. 0
2	−4,569.9	1.00
3	−4,603.4	approx. 0
4	−4,574.4	approx. 0
5	−4,594.3	approx. 0

using a Mann–Whitney test ($\alpha = 0.05$), due to the non-normal distribution of the data.

RESULTS

The results of the STRUCTURE analysis identified 2 populations using each of the 4 models (i.e., the log probability of the data was greatest at $K = 2$ clusters; Table 1). Individual STRUCTURE analyses for the UP and LP identified only one population in each peninsula (Table 2). For the purposes of defining populations and conducting the assignment test, we used the model that assumed admixture and correlated allele frequencies; therefore, we show only these results (Tables 1, 2).

The assignment test within STRUCTURE assigned 15 individual bobcats from the UP (i.e., reported as being harvested there) to the LP based on a threshold assignment value of 0.9. As the threshold for assignment increased, the number of bobcats assigned from the UP to the LP decreased to 12 at a threshold of 0.95, and 10 animals at a threshold of 0.999. We did not assign any animals in the opposite direction, i.e., we did not genetically assign animals to the UP that were reportedly captured in the LP. It is likely that the 15 individuals identified by STRUCTURE as dispersers from the LP to the UP were poached (Millions and Swanson 2006); therefore, we removed them from subsequent analyses.

The results of the GENEPOP 3.4 analyses of the STRUCTURE-defined populations showed that none of the loci deviated from Hardy–Weinberg equilibrium in either population (following Bonferroni correction). No loci showed evidence of linkage disequilibrium (following Bonferroni correction).

We detected significant genic differentiation at all loci

Table 2. Inference of the number of populations of bobcats in the upper (UP) and lower (LP) peninsulas of Michigan, USA, using the admixture and correlated allele frequency model in STRUCTURE for bobcats harvested during the 2001–2002 hunting and trapping season.

<i>K</i>	UP		LP	
	Log $P(X K)$	Log $P(K X)$	Log $P(X K)$	Log $P(K X)$
1	−2,751.2	0.9442	−1,621.6	1.0000
2	−2,756.7	0.0039	−1,664.3	0.0000
3	−2,754.1	0.0520	−1,658.5	0.0000
4	−2,775.1	0.0000	−1,651.3	0.0000
5	−2,812.6	0.0000	−1,674.4	0.0000

between the UP and LP populations ($P < 0.001$). Observed heterozygosity was significantly higher in the UP than in the LP (Table 3; $\chi^2 = 115.4$; $P < 0.001$). Loci Lc109, Lc111, and Fca43 each had significantly more alleles in the UP than in the LP (Table 3; $P < 0.001$), after adjusting for sample size (resampling 1,000 times from the larger UP population). We observed a significant F_{ST} value of 0.06 (range: 0.04–0.13; $P < 0.0001$) between the UP and LP populations.

There was a significant difference in the prevalence of roads in the UP and LP. Road density in the UP (0.07 km/km²) was lower than in the LP (0.12 km/km²). This was similar to patch density, where the UP (0.09 patches/100 km²) had lower patch density than the LP (0.18 patches/100 km²). We found a significant difference in the median patch size between the UP (935.5 km²) and the LP (467.2 km²; $W = 1,850.0$; $P = 0.002$).

DISCUSSION

Despite the difference in the prevalence of roads between the peninsulas, there was no evidence of intra-peninsular population structure. This may be due to the relatively short time period over which the LP has experienced influence from the effects of roads. Most interstates in Michigan were constructed and expanded between the 1950s and the mid-1960s (Lewis 1997, Forman et al. 2003), or approximately 12–14 bobcat generations ago (Hoppe 1980). Therefore, an insufficient amount of time may have passed for the influence of roads to impact bobcat population structure in the state.

However, our analyses of bobcat populations in Michigan was consistent with the hypotheses that the Straits of Mackinac were a barrier to gene flow and that the UP and LP are 2 genetically distinct populations. The separation of the UP and LP into 2 genetically distinct populations has led to differences in genetic diversity between the peninsulas, with the LP exhibiting lower diversity in relation to the UP. The reduced genetic diversity found in the LP likely results from the relative isolation the LP experiences compared to the UP. The bobcats of the UP exist as a continuous population with the rest of the range through their western connection with Wisconsin, USA. However, the LP bobcats are isolated from the UP bobcats by the Straights of Mackinac. Even though we found genetic evidence that some individuals appear to belong to the other peninsula than the one in which hunters captured them, this is more likely due to poaching than to dispersal (Millions and Swanson 2006). If dispersal were occurring between the 2 peninsulas, we would expect to see at least some animals that were captured in the LP assigned to the UP. Instead, all of the animals genetically assigned to a population other than their capture population were assigned from the UP to the LP. This pattern is more consistent with a poaching scenario than dispersal (Millions and Swanson 2006), supporting the idea of inter-peninsula isolation.

The LP bobcats also experienced isolation from the south given the extirpation of bobcats in northern Illinois, Indiana,

Table 3. Genetic diversity indices for 5 bobcat microsatellite loci in the upper (UP) and lower (LP) peninsulas of Michigan, USA (2001–2002).

Population	<i>n</i> ^b	Individual loci ^a													
		Lc109		Lc111		Fca8		Fca43		Fca90		\bar{x}			
		A	H _O	A	H _O	A	H _O	A	H _O	A	H _O	A	SE	H _O	SE
UP	206	11	0.77	3	0.44	6	0.74	6	0.69	6	0.75	6.4	1.3	0.68	0.06
LP	137	8	0.73	2	0.47	6	0.69	3	0.46	6	0.70	5.0	1.1	0.61	0.06

^a *n* = number of individuals analyzed.

^b A = number of observed alleles, H_O = observed proportion of heterozygotes.

and Ohio, USA. Recently, these states have managed their bobcat populations with the intention of increasing the numbers and distribution of bobcats. Although this scenario should increase gene flow into the LP, it is likely that the LP has existed as an isolated population for at least 100 years.

This recent isolation has led to a reduction in allelic diversity at 3 of the 5 loci in the LP relative to the UP, although heterozygosities were similar at all loci (Table 3). The loss of alleles without a significant difference in the observed heterozygosity at a locus suggests a recent reduction in population size (Luikart and Cornuet 1988, Cornuet and Luikart 1996, Luikart et al. 1998, Garza and Williams 2001). A recent reduction in population size is consistent with the hypothesis that the LP bobcats have only been isolated from the larger bobcat population to the south for the last 50–100 years (Young 1958, Hall 1981) because the rapid population growth in southern Michigan and the northern regions of Illinois, Indiana, and Ohio has isolated the LP populations from the continuous range (but see Woolf et al. 2000).

Because of its isolation, researchers can consider the LP an island population that experiences rare dispersal events from the UP and the south. Alternatively, researchers can consider the UP a mainland population because of its connections to bobcat populations in Wisconsin and, by extension, populations across North America. The LP displayed the tendency of island populations to have lower genetic variation than mainland populations (Frankham 1997), and when compared to the UP, the LP showed a 10% reduction in observed heterozygosity and a 22% reduction in average number of alleles per locus (Table 3). These values are especially high when compared to the results of Frankham (1997), who examined populations with long-term isolation compared to the LP bobcat population, but still only found an average reduction of 29% in variation. This may be cause for concern from management and conservation standpoints because isolated populations that experience a reduction in genetic diversity are at an increased risk of extinction (Frankham 1997). Although this is not a suggestion that the LP population is under any immediate threat, these results should serve as an indication that this population needs attention in terms of examining or revising current management strategies, as well as collecting demographic data beyond those derived from harvest records. Despite the current isolation of the LP

bobcats, managers could ameliorate the genetic concerns in the near future if the bobcat populations in the states adjacent to Michigan's southern border continue to increase and expand their range. As the bobcat population in Illinois, Indiana, and Ohio continues to grow, eventually producing healthy populations of bobcats in the northern portions of these states, the restoration of some level of gene flow will occur to the LP and should genetically stabilize the LP bobcat populations.

MANAGEMENT IMPLICATIONS

Our analysis of bobcat populations across Michigan shows that despite the increased levels of habitat fragmentation in the LP, there is no evidence that fragmentation has altered population structure within the state yet. The isolation of the LP from the UP by the Straits of Mackinac, and of the southern populations by inhospitable habitat and the Great Lakes has led to a decline in genetic variation. This should serve as a warning sign to wildlife managers that the LP is similar in many ways to an island population with low levels of connectivity to mainland populations. The potential threat from habitat fragmentation coupled with the isolation of the LP merits attention from wildlife managers, because despite the extensive dispersal abilities of bobcats, their populations still show reduced levels of genetic variation in the LP. This also suggests that the 2 other mesocarnivores found as an isolated population in the LP, black bears (*Ursus americanus*) and American marten (*Martes americana*), may be experiencing the same situation. Perhaps most importantly, our results may provide some insight into how wolves (*Canis lupus*), which have recently recolonized the LP, will respond to habitat fragmentation. The mesocarnivores isolated in the LP should be evaluated independently with unique management plans constructed to take into account the isolation found in the LP as compared to the UP. Further research addressing the genetic relatedness between the LP bobcat populations of Michigan and populations to the south and east would be of interest from a biological and management aspect given their observed dispersal patterns (Woolf et al. 2000, Nielsen and Woolf 2001).

ACKNOWLEDGMENTS

The Michigan Department of Natural resources and T. Cooley provided tissue samples. The Office of Research and Sponsored Programs at Central Michigan University, the Central Michigan University College of Graduate Studies,

the Central Michigan University Biology Department, and Sigma-Xi provided funding. We thank T. Gehring and 2 anonymous reviewers for their helpful comments and direction on the manuscript. We give special thanks to B. Potter and J. Pytko for their assistance in preparing and editing the figures. We would also like to thank everyone in the Applied Technologies in Conservation Genetics laboratory at Central Michigan University.

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Associate Editor: DeWoody.