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Origin and genetic structure of a recovering bobcat (*Lynx rufus*) population

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Abstract: Genetic analyses can provide important insights into the demographic processes that underlie recovering populations of mammals of conservation concern such as felid species. To better understand the recent and rapid recovery of bobcats (*Lynx rufus* [Schreber, 1777]) in Ohio, we analyzed samples from 4 states in the lower Great Lakes Region using 12 microsatellite DNA loci and a portion of the mtDNA control region. Our results showed that a newly-established population of bobcats in the eastern part of Ohio was genetically distinct from a multi-state population distributed across Kentucky, southern Ohio, West Virginia, and western Pennsylvania. There was no direct genetic evidence of a bottleneck or inbreeding in this population. A lack of private alleles and only slightly lower levels of allelic richness and heterozygosity compared to its neighbors suggest that the eastern Ohio population likely originated from the migration of relatively large numbers of individuals from a source population rather than re-emerging from an undetected residual population. We recommend that a management plan should define the areas occupied by the two populations in Ohio as separate management units at least for the near future.

Key Words: *Lynx rufus*; bobcat; mtDNA; microsatellites; population bottleneck; conservation genetics; management units

Introduction

Conservation biologists use analyses of genetic data to gain demographic information about endangered species or species of concern that are hard to study using conventional census techniques (Nowell and Jackson 1996; Palomares et al. 2002). For example, if populations are demographically isolated then limited migration can lead to the development of significant population differences in allele frequencies (Moritz 1994). Identifying genetically distinct populations can be used to identify putative management units (Moritz 1994). In recovering populations, genetic comparisons between new and existing populations can also identify the demographic processes that have resulted in recolonization. If a newly occupied area is sufficiently isolated, then a recent colonization by a small number of individuals will leave a genetic signature of divergence of the newly-formed population from its source through genetic drift (Ibrahim et al. 1996; Haanes et al. 2010). In contrast, sustained colonization by large numbers of immigrants will result in little or no divergence in the newly-established population.

Different patterns of within population levels of genetic variation can result when recolonization occurs via alternative processes. If a small number of individuals are the source for population reestablishment in reintroduction programs, then founder effects or genetic drift can occur (Walker et al. 2001; Clark et al. 2002). In American black bears, there is evidence that recent natural recolonizations have likely been initiated by a single, dispersing female which has resulted in low

levels of genetic variation in extant populations (Onorato et al. 2004b). Low overall genetic variation is also a common pattern in animals colonizing new habitats, and has been documented in a recently recovered population of European otters (Janssens et al. 2008). However, if the number of colonizing individuals is large and/or there is ongoing gene flow between source and newly-established populations then there will be no difference in levels of variation between the original and colonizing populations (Onorato et al. 2004b; Kendall et al. 2009).

Bobcats (*Lynx rufus* [Schreber, 1777] are currently distributed from Canada south into Mexico, and within the continental United States occur from coast to coast. There is a conspicuous absence in the upper Midwest, presumably due to the bobcat's avoidance of intensive agriculture (McDonald et al. 2008; Fig. 1a). However, the majority of states including those in the Midwest have reported an increasing trend in bobcat numbers, as well as expanding distributions (Roberts and Crimmins 2010). This trend has been attributed to increased habitat availability due to changing land-use practices (e.g., reversion of agricultural land and changes in farming practices) and more intensive harvest management at the state level (Roberts and Crimmins 2010). In Ohio, bobcats were once found throughout the state, but were extirpated by the 1850s as forests were cleared for settlement and agriculture (Trautman 1977). Since 1946, there have been 464 verified reports of bobcats in Ohio, of which 94% have occurred since 2000 (S. Prange, unpublished data). Bobcat recovery in Ohio has occurred in conjunction

with the reversion of the Ohio portion of the Western Allegheny Plateau ecoregion from farmland back to woodland (Hutchinson et al. 2003; Fig. 1b). In 2012, the bobcat was reclassified from endangered to threatened, and in 2014 it was removed from Ohio's threatened and endangered species list.

Previous genetic studies on bobcats have found evidence for and against genetic structuring among bobcat populations at different spatial scales. For example, at regional to local geographic scales, Williams (2006) and Millions and Swanson (2007) found evidence for genetic differentiation between bobcats found in the lower and upper peninsulas in Michigan. Reding (2011) found structure between populations in the Midwestern USA in habitats subdivided by intensive row-crop agriculture, and Riley et al. (2006) and Lee et al. (2012) found genetic differences between bobcat populations on either side of a major highway in southern California. In contrast, Croteau et al. (2010) found the bobcat population in southern Illinois to be genetically panmictic, as did Reid (2006) for bobcats sampled in southern Georgia and northern Florida. At a larger spatial scale, with samples from 14 states and 2 Canadian provinces, Croteau (2009) identified an isolation-by-distance effect and potential historical subdivision between western bobcats (California, Wyoming, Nevada and North Dakota) and those from the rest of the sampled range. Reding et al. (2012) analyzed 1700 samples throughout the majority of the bobcat range, and found that the data distinguished bobcats in the eastern USA from those in the western half, with no obvious physical barrier to gene flow.

One limitation of these studies is that many have focused on long-standing populations of these animals (e.g., Croteau 2009; Millions and Swanson 2007). However, over much of their USA range, bobcats are showing increases in abundance and the recolonization of areas where they were previously extirpated (Woolf and Hubert 1998; Roberts and Crimmins 2010). Natural recolonizations are rarely documented in large terrestrial mammals (Onorato et al. 2004a). Therefore, genetic studies that estimate levels of inbreeding and the likelihood of bottlenecks as bobcats recover and reclaim parts of their former range could provide valuable insights as to the demographic processes that underlie these recolonization events and elucidate the genetic characteristics of these newly re-established populations (also see Lee et al. 2012; Reding et al. 2012).

The recent and rapid recovery of bobcats in Ohio provides an opportunity to examine the demographic and genetic characteristics of a recolonization event involving a terrestrial mammal of conservation concern. To carry out such a study, we tested the predictions that 1) Ohio bobcats would consist of multiple genetic distinct populations that originated from different source populations in nearby states, 2) the genetic characteristics of Ohio bobcat populations would reflect a recent colonization event with a small number of founders with low levels of genetic variation and evidence of inbreeding, and 3) there would be genetic signatures of a bottleneck consistent with recent founder events.

Materials and methods

Sample collection

We obtained tissue samples for genetic analyses from the Ohio Department of Natural Resources ($N = 111$ for OH), the West Virginia Division of Natural Resources ($N = 25$ for WV), the Kentucky Department of Fish and Wildlife Resources ($N = 31$ for KY), and the Pennsylvania Game Commission ($N = 29$ for PA; Fig. 1b). Bobcats were obtained through road mortality and incidental captures from 2002 – 2012. Samples of skin, tongue, or other tissue were collected from specimens and stored at -20°C . Any county within these states where no samples were collected was due to a lack of available carcasses, and was not the result of purposely sampling in spatially discrete groups (Fig. 1b).

Laboratory methods

We extracted DNA from tissue samples using the DNeasy Blood and Tissue Kit (Qiagen) with the addition of $2\ \mu\text{l}$ Rnase A (10mg/ml). We then genotyped samples at 12 microsatellite loci: BC1AT, BCE5T, BCG8T (Faircloth et al. 2005), Lc109 (Carmichael et al. 2000), FCA23, FCA26, FCA35, FCA43, FCA90, FCA96, FCA126, AND FCA132 (Menotti-Raymond et al. 1999). These primers have previously been used to investigate population structure, genetic diversity, and sex-biases dispersal in bobcats (e.g. Millions and Swanson 2007; Croteau et al. 2010). Samples were genotyped at 3 – 5 loci in each of three multiplex PCR reactions (Table 1). We performed PCR in $10\text{-}\mu\text{l}$ reactions containing $5\ \mu\text{l}$ of Multiplex PCR Master Mix (Qiagen), $1\ \mu\text{l}$ of primer mix (containing $2\ \mu\text{M}$ of each primer), and $3.5\ \mu\text{l}$ of RNase-free H_2O . Thermal cycling followed a touchdown

program with an initial activation step at 95 °C for 15 min followed by 8 cycles of 94 °C for 30 s, annealing temperature for 90 s decreasing 0.5 °C per cycle, 72 °C for 60 s, followed by 21 cycles at the lowest annealing temperature, and a final extension at 60 °C for 30 min (Table 1). Fragments were sized with the NAUOX size standard described in DeWoody et al. (2004) and products were run on a 3100 Genetic Analyzer (ABI). Alleles were identified and binned using GENEMAPPER 3.7 software (ABI).

To generate additional genetic information from these samples, we also amplified and sequenced approximately 600bp of the left domain of the mtDNA control region of all samples using primers CR1 and CR2R (Palomares et al. 2002). Structure and variation in this region has been previously used to examine genetic variation and spatial structure in lynx (Hellborg et al. 2002; Rueness et al. 2003) and bobcats (Croteau 2009). This region contains an 80-bp repeat (Lopez et al. 1996) in the repetitive segment (RS-2) that varied in the number of repeats across samples (Jae-Heup et al. 2001; Hellborg et al. 2002). Due to the difficulty of aligning the sequences (Croteau 2009) and potential issues with heteroplasmy (Hoelzel et al. 1994), we included only 138 bp of nonrepetitive sequence upstream of RS-2 in the analysis.

To generate sequence, we amplified this region using PCR in 10- μ l reaction volumes consisting of 5.5 μ l ddH₂O, 1 μ l MgCl₂-free 10x PCR buffer, 1 μ l BSA (10 mg/ml), 0.2 μ l dNTPs (10 μ M), 0.3 μ l each of forward and reverse primer (10

μM), 0.6 μl MgCl_2 (50mM), and 0.05 μl Platinum Taq DNA polymerase (5 U/ μl). Amplifications included an initial incubation at 94°C for 3 min, followed by 30 cycles of 94°C for 20 s, 56°C for 20 s, 72°C for 60 s, and a final extension at 72°C for 5 min. We precipitated PCR products using a Polyethylene Glycol/EtOH procedure and resuspended them with ddH₂O. We sequenced in the forward and reverse direction using primers CR1 and CR2R with the Big Dye 3.1 Cycle Sequencing Kit (ABI). We cleaned sequencing products with Sephadex and ran them on a 3100 Genetic Analyzer (ABI). We edited sequences using ALIGNER (CodonCode) and aligned them in BIOEDIT (Hall 1999) using CLUSTALW (Thompson et al. 1994).

Assessing genetic variation

The samples were divided into 7 *a priori* sample locations based on spatial clustering as follows: E OH = eastern Ohio, S OH = southern Ohio, W KY = western Kentucky, E KY = eastern Kentucky, WV = West Virginia, W PA = western Pennsylvania, and E PA = eastern Pennsylvania (Fig. 1b). Departures from Hardy-Weinberg equilibrium for each microsatellite loci and sample location were assessed using the Markov chain method in GENEPOP v4.2 (Raymond and Rousset 1995) and critical *P* values were corrected for multiple tests using the Benjamini and Yekutieli (B-Y) method (Narum 2006). The frequency of potential null alleles at each locus in each sample location was estimated using the EM algorithm of Dempster et al. (1977) in GENEPOP v4.2 (Raymond and Rousset 1995). To calculate an error rate for the microsatellite and mtDNA data

in our study, we randomly chose a subset of 18 samples for microsatellite and 6 samples for mtDNA to be amplified and genotyped blindly a second time. The genotypes obtained were compared to the original runs, and the number of mismatches was counted (Bonin et al. 2004; Hoffman and Amos 2005).

For a preliminary assessment of levels of genetic variation at each of the 7 *a priori* sample locations we used FSTAT v2.9.3.2 (Goudet 2001) to determine the number of alleles, observed and expected heterozygosity, and allelic richness (corrected for sample size) for each microsatellite locus. To assess genetic variation within genetically distinct populations as defined by STRUCTURE (see below), we again used FSTAT (Goudet 2001) to calculate expected heterozygosity and allelic richness (corrected for sample size), and GENEPOP (Raymond and Rousset 1995) to calculate observed heterozygosity. CONVERT (Glaubitz 2004) was used to indicate the number of private alleles per genetically distinct population and ADZE v1.0 (Szpiech et al. 2008) was used to estimate private allelic richness using a standardized sample size of 17 individuals. In ADZE, the estimated private allelic richness is the number of private alleles expected in a population based on the rarefaction method when sample sizes differ across populations (Szpiech et al. 2008). For mtDNA data, we calculated the number of haplotypes, haplotypic (gene) diversity (h) and nucleotide diversity (π) per genetically distinct population in DnaSP (Rozas et al. 2003).

Defining genetically distinct populations

To identify possible genetically distinct populations (e.g. Moritz 1994), we assessed genetic differentiation in three ways. First, we used the Bayesian clustering method implemented in STRUCTURE v2.3.4 to infer the number of distinct genetic groups observed in our microsatellite data (Pritchard et al. 2000). Each STRUCTURE run consisted of a burn-in of 100,000 Markov Chain Monte Carlo (MCMC) iterations followed by 300,000 iterations using the admixture model with sample locations (E OH, S OH, W KY, E KY, WV, W PA, E PA) as priors and correlated allele frequencies (Falush et al. 2003) as recommended in Gilbert et al. (2012). In addition, runs of different lengths were also performed to check for consistency among runs. We performed 20 runs for each value of K ranging from 1 to 7 after initial results suggested that Ohio samples should be further subdivided into two separate populations (i.e., eastern and southern; Fig. 1b). To confirm that burn-in was adequate, we checked for convergence in time series data plots of values of summary statistics estimated by the program. To determine the most likely value of K suggesting the number of populations, we used the Evanno et al. (2005) method implemented in the program STRUCTURE HARVESTER (Earl 2009) which determines the second-order rate of change in the distribution of $L(K)$. We provide both the bar plot showing individual assignments for the given K and the ΔK graph as recommended by Gilbert et al. (2012). The estimated membership of individuals (using mean values of q) assigned to different clusters based on *a priori* sample locations was calculated as mean $\pm$ SE.

To complement the STRUCTURE analysis, we identified an optimal number of genetic clusters and probabilistically assigned samples to groups with adegenet, as implemented in R 2.12 (R development core team 2013; www.r-project.org). Adegenet performs model free K-means clustering, which, in contrast to STRUCTURE, does not rely on assumptions such as HWE and LD within groups (Jombart et al. 2010). Specifically, we first used the find.clusters function in adegenet to identify the optimal clustering solution based on Bayesian Information Criterion (BIC) for possible K values 1–10. We then used the optimal clustering solution to perform Discriminant Analysis of Principal Components (DAPC), which is a multivariate analysis that minimizes within-group variance while maximizing among-group variance (Jombart et al. 2010). We plotted the identified clusters along the first two discriminant functions to visualize how variation is partitioned among the identified groups, and we also obtained posterior probabilities of group membership for each sample based on the DAPC analysis.

We analyzed levels of genetic differentiation between the same 7 *a priori* sample locations based on F_{ST} values generated using FSTAT (Goudet 2001). We assessed whether there was evidence for isolation by distance using F_{ST} values between the 7 individual sample locations generated using microsatellite data. This analysis was performed using IBD Web Service (Jensen et al. 2005). For mtDNA data, we used ARLEQUIN (Schneider et al. 2000) to calculate pairwise F_{ST} based on haplotype frequencies and used DnaSP (Rozas et al. 2003) to

calculate overall F_{ST} . In order to compare the overall F_{ST} values from each type of marker, we applied the correction described in Crochet (2000) of $F_{ST(mitochondrial)}$ = $4 F_{ST(nuclear)} / [1 + 3F_{ST(nuclear)}]$.

Estimates of contemporary migration

To identify recent immigrants within genetically distinct populations, we used assignment tests (e.g. Waser and Strobeck 1998) implemented in the program GENECLASS v2 (Piry et al. 2004). Each individual's probability of genetic assignment to the population from which it was collected was estimated using Bayesian probabilities based on the similarity of its multilocus genotype to genotypes found in each population (Rannala and Mountain 1997). We used a threshold of $\geq 90\%$ for the likelihood scores in assigning individuals to a population.

We also used a Bayesian method implemented in BAYESASS v1.3 to estimate rates of recent immigration (i.e., within the last 1–3 generations) with microsatellite genotypes in BAYESASS (Wilson and Rannala 2003). This program does not assume Hardy-Weinberg equilibrium within populations and provides an estimate of the mean posterior distribution of m for all population pairs, which is the proportion of individuals in location i that have location j as their ancestral location. This provides both the proportion of residents and the proportion of immigrants in each population. The program was run for 3×10^6 iterations, 2000 sampling frequency, and the first 1×10^6 iterations were

discarded as burn-in. We report the proportion of residents in both Ohio sampling locations to compare with the results reported with GENECLASS.

Inbreeding and bottlenecks

We estimated levels of inbreeding by calculating F_{IS} (the inbreeding coefficient) for samples from genetically distinct bobcat populations using microsatellite data in FSTAT (Goudet 2001). Because the recent recolonization of Ohio suggests the possibility that populations have undergone a bottleneck, we used two methods implemented in BOTTLENECK v1.2.02 to detect whether there was a genetic signature of such a phenomenon (Cornuet and Luikart 1996; Piry et al. 1999). First, we used Wilcoxon's test, which examines whether populations exhibit a greater level of heterozygosity than predicted in a population at drift-migration equilibrium. This test is most sensitive at detecting bottlenecks within the last 2–4 N_e generations. We performed 10,000 simulations under the stepwise mutation model (SMM) and the two-phase model (TPM). Second, we examined whether the allele frequency followed a normal L-shaped distribution since a mode-shift discriminates recently bottlenecked populations from stable populations. This test is based on the idea that nonbottlenecked populations at mutation-drift equilibrium are expected to have a large proportion of alleles at low frequency and a smaller proportion of alleles at intermediate frequencies (L-shape distribution; Luikart et al. 1998). Due to the relatively recent time frame of bobcat recolonization in Ohio, the mode-shift test should be more appropriate in detecting recent bottlenecks than analyses based on heterozygote excess.

Results

Genetic variation and population differentiation

We genotyped 194 bobcats from seven sample locations (circled in Fig. 1b) at 12 microsatellite loci. The number of alleles per locus ranged from six (FCA23 and FCA43) to 14 (FCA35 and BCG8T; Table 1). Allelic richness varied from 3.94–9.47 and observed and expected heterozygosities for each locus were moderate to high and ranged from 0.56–0.89 (Table 1). No microsatellite loci deviated from Hardy-Weinberg equilibrium and estimates of the frequency of possible null alleles were $\leq 9\%$ (Table 1). The genotyping error rate was 0% for the 16 samples amplified and genotyped a second time.

STRUCTURE, which was used to estimate the number of genetic clusters of populations among the 7 sample locations (E OH, S OH, W KY, E KY, WV, W PA, E PA; circled in Fig. 1b), showed that the optimal number of clusters was $K = 3$ (mean $\text{LnP}(K) = -7944.1$; Fig. 2a). We found that 84% (48/57 total) of individuals from the *a priori* population in E OH were assigned to one cluster with a mean estimated membership of 0.914 (SE = 0.077), 100% (18/18) of E PA bobcats were assigned to a second cluster with a mean estimated membership of 0.957 (SE = 0.044), and 89% (48/54) of the S OH bobcats were assigned to separate multi-state cluster with a mean estimated membership of 0.885 (SE = 0.107) that included samples from the remaining *a priori* sample locations (Fig. 2b).

The results from the adegenet cluster analyses broadly support the STRUCTURE results. Based on K values evaluated using BIC scores there were 4 clusters of genetically distinct samples in the data: all contain a mixture of samples from different locations but like the Structure results are dominated by samples from specific locations (Fig. S1a). E OH and S OH samples are largely found in different clusters supporting the conclusion that they are genetically distinct at least to a limited extent: 54% (31/57 total) of E OH samples but only 13% (7/54) of S OH are assigned to Cluster 1 whereas 48% (26/54) of S OH samples but only 11% (6/57) of E OH are assigned to Cluster 4 (Fig. S1b).

Finally, as an alternative way of assessing structure, for microsatellite data, we calculated pair-wise values of F_{ST} and found that even though values were low all 21 comparisons were significantly different from zero after correction (Table 2) with an overall $F_{ST} = 0.037$ (95% CI: 0.029–0.046, $P < 0.001$). This means that all seven *a priori* sample locations show some level of genetic distinctiveness from each other. A significant isolation-by-distance effect was found among the 7 *a priori* sample locations ($R = 0.71$, $P = 0.005$; Fig. 3). For mtDNA data, we found that 9 pair-wise comparisons of F_{ST} were significant after B–Y correction (Table 2). The overall level of population differentiation in mtDNA ($F_{ST} = 0.240$) was an order of magnitude larger than that based on microsatellite data alone. After correction following Crochet (2000), the overall F_{ST} value observed for mtDNA

was 0.133, which is still roughly an order of magnitude larger than that observed for microsatellites.

Within-population genetic variation for populations based on the three clusters from STRUCTURE was relatively high with measures slightly lower in E OH as compared to the multi-state population (S OH, WV, W KY, E KY, and W PA; Table 3a). Expected heterozygosity ranged from 0.73 in E OH to 0.80 in the multi-state population with a value of 0.74 in E PA. Allelic richness averaged across loci varied from 6.0 for E PA to 7.5 for the multi-state population that includes S OH but was 6.4 for E OH (Table 3a). The number of private alleles without accounting for differences in sample size across populations was zero in E OH, 14 in the multi-state population including S OH, and 1 in E PA. When sample size was standardized at 17 individuals, the number of private alleles was still the lowest in E OH compared to the other two populations (Fig. 4).

MtDNA haplotype designations were identical for the six samples that provided informative sequence in two independent runs, resulting in an error rate of 0%. We identified 6 unique mtDNA haplotypes and the number of haplotypes ranged from 2–6 per genetically distinct population (Table 3b). The haplotypes in the E OH population are a subset of those found in the multi-state population that includes S OH. Haplotype diversity varied between 0.111 in E PA to 0.568 in the multi-state population (S OH, WV, W KY, E KY, and W PA) and nucleotide diversity was low across populations (range 0.001–0.005).

Estimates of contemporary migration

We found that GENECLASS could assign 150 of 194 individuals to one of the three populations with $\geq 90\%$ likelihood. Not surprisingly, most of these individuals were classified as residents in that they were assigned to their population of collection ($\geq 80\%$ for all three populations; Table 4). Nine bobcats (20.5%) likely moved into E OH from the multi-state population to the south (S OH, WV, W KY, E KY, and W PA), compared to eight migrants (8.8%) in the opposite direction (Table 4). While it was estimated that there was no migration between E OH and E PA likely due to the large distance between them, results suggest that 1–2 bobcats migrated between the multi-state population and E PA (Table 4). Multiple runs of BAYESASS provided similar results, with the proportion of residents in each of the three populations ranging from 0.88–0.96.

Inbreeding and bottlenecks

The coefficient F_{IS} suggested that inbreeding levels within each population were low, with 0.000 for E OH and 0.043 for the multi-state population containing S OH (Table 3a). The populations of bobcats in both E OH and E PA showed no genetic evidence of a bottleneck based on results from both Wilcoxon's test and the mode-shift test (Table 5; Fig. 5). For the multi-state population of bobcats from S OH, WV, KY, and W PA, the Wilcoxon's test under the TPM was significant for heterozygote excess, whereas the SMM and the mode-shift test suggested no recent genetic bottleneck (Table 5; Fig. 5).

Discussion

This study was focused on using genetics to make inferences about the dynamic processes associated with the range expansion and natural recolonization of a large mammal. Our major results are that 1) based on analyses of population structure and genetic differentiation we found evidence for a separate population of bobcats in eastern Ohio 2) but given that overall levels of genetic variation were moderate to high and there was no evidence for a bottleneck, the numbers of founders was likely relatively large and/or there was recent migration of individuals between source and recolonized populations. We discuss the implications of these results below.

A number of results support the scenario that the genetic distinctiveness of the eastern Ohio population developed through a founder effect affecting a limited number of recolonizing animals (e.g. Walker et al. 2001; Randi et al. 2003; Haanes et al. 2010) rather than the re-emergence of a small undetected residual population in the area that had experienced high levels of genetic drift (Szpiech et al. 2008). First, the eastern Ohio population contains no private alleles or haplotypes as are predicted to be present under the re-emergence hypothesis (Szpiech et al. 2008). The variation observed is a limited subset of that presence in potential source populations. Second, our assignment test and immigration results show direct evidence for movement of individuals from a potential source population (the multi-state cluster) consistent with a colonization scenario. Other

studies of recolonizing populations of mammals have shown similar patterns suggesting similar colonization dynamics. For example, a study on natural recolonization of river otters in Missouri reported significant genetic differentiation among some newly-founded populations, little to no loss of genetic variation, and the presence of the most common mtDNA haplotypes range-wide in recolonized populations (Mowry et al. 2015). In Scandinavian wolverines, founding effects were also inferred in significant subdivision and a loss of genetic variation, and no private alleles were found in the recolonizing populations (Walker et al. 2001). Thus, this pattern of recolonization seems to be common among populations of terrestrial mammals recolonizing areas where species were previously found until extirpation by humans.

Only a limited loss of genetic variation in the recovering bobcat population in eastern Ohio has occurred. Other studies on bobcats in the USA and Canada have found very similar levels of heterozygosity and allelic richness using the same loci (Millions and Swanson 2007; Croteau 2009; Reding et al. 2012). We also found no evidence of inbreeding in any of the populations, including the recently recolonized eastern Ohio population. Rapid inbreeding in small populations produces increased homozygosity and can reduce fitness (Lande 1988). While the overall inbreeding statistic F_{IS} was significant across the 10 groups covering the USA in Reding et al. (2012), levels were not significant in the Pennsylvania groups which were close to our study populations. Another study found no evidence of inbreeding in bobcat populations (Lee et al. 2012). While

levels of genetic variation are slightly lower in eastern Ohio, at this time there is little concern about the genetic 'health' of bobcats in the newly recolonized areas.

Why is it that only a limited loss of genetic variation has occurred during the recolonization of bobcats in Ohio, and no evidence of a bottleneck? One possibility is likely that these recolonization events involved relatively large numbers of founding individuals. We also found genetic evidence of substantial recent migration of bobcats in both directions connecting the eastern Ohio population to the large population to the south (Table 4). High recent gene flow has also been documented in other large carnivores like the wolverine (Walker et al. 2001). Our evidence for a significant isolation by distance relationship suggests that bobcat populations do not generally consist of discrete genetically isolated populations but that migration between populations is constrained by distance. At large scales, isolation by distance can explain genetic differentiation in wolf (Geffen et al. 2004), puma (McRae et al. 2005), and river otters (Blundell et al. 2002). We view the genetic signature of differentiation in bobcats as likely a transitory non-equilibrium feature that will erode over time as populations come into migration-drift equilibrium. The statistically significant but low levels of genetic differentiation may already be evidence for this. In work on other recolonizing species, Missouri river otters have retained genetic diversity levels similar to those of the source populations, but genetic structure also has not reached an equilibrium between migration and genetic drift 30 years after the start of reintroduction efforts (Mowry et al. 2015). Another study on the genetic

structure of recovering European otters also found significant genetic differentiation and moderately high heterozygosity, and showed that some populations were partially admixed with no recent bottlenecks observed (Randi et al. 2003). Still, evidence for present-day genetic differentiation among bobcat populations implies some level of demographic independence and so based on Moritz's (1994) management unit criteria, we recommend that for the near future the two Ohio populations be managed as separate management units, which will require the coordination of agencies in Ohio, Kentucky, West Virginia, and Pennsylvania. Other recent work by Croteau et al. (2012) proposed that multi-state consortia might be a more appropriate way to manage bobcats as this scenario will conserve both historical and current levels of genetic diversity.

Another reason for the minimal loss of genetic variation during the recolonization of bobcats in Ohio is that populations grew rapidly avoiding the effects of genetic drift due to small population size. All of the Western Alleghany Plateau ecoregion in Ohio (approximately 30,750 km² in southeastern Ohio) appears to be suitable bobcat habitat with no apparent barriers to movement (see Fig. 1b). Based on camera surveys during 2008, bobcat occupancy (MacKenzie et al. 2002) of this area was only about 35% (95% CI = 13 - 64%; S. Prange, unpublished data). In the absence of dispersal barriers, a higher population growth rate of the eastern Ohio population and the spatial clustering of bobcats could be due to higher food availability. This area differs from areas to the south in that it contains some of the most heavily mined counties; at the center of the eastern Ohio population is

Noble County with approximately 45% of its land consisting of reclaimed surface mines (ODNR 2014). The relationship between reclaimed mineland and bobcat population growth is unknown; however, foxes likely use reclaimed surface mines in West Virginia because of the presence of seasonally important food items such as small mammals (Yearsley and Samuel 1980).

Rapid growth of a small number of recolonizing animals containing a subset of the genetic make-up of the source population may account for why the eastern and southern Ohio populations are genetically differentiated even though they appear spatially contiguous. It is possible that the eastern population has potentially greater habitat quality and food availability, which has allowed the original founders to quickly build up the population, whereas southern Ohio may be more dependent on continuous dispersal from neighboring states (i.e. sink habitat). Eventually, we predict that all suitable habitat will be used and the species should become panmictic within the southeastern portion of the state. For large carnivores, such as American black bears (Pelletier et al. 2011) and lynx (Schwartz et al. 2002), microsatellite markers have shown panmictic population structure where topographic barriers to dispersal are absent. As a result of these processes, distinct population structure should be assessed on a regular basis until bobcats expand further into unoccupied habitat, the eastern population is no longer spatially distinguishable, and the need to account for two management units is no longer required for conservation actions.

Range expansions into both eastern and southern Ohio likely came from the south and east. These states contain healthy bobcat populations and a higher proportion of patchy forested landscapes that are likely correlated with bobcat presence (Lovallo and Anderson 1996; Woolf et al. 2002). Additionally, primary bobcat habitat in Ohio exists in the forested southeastern hill country, whereas the western portion of Ohio consists largely of agricultural lands that are typically avoided by bobcats (Woolf et al. 2002; Tucker et al. 2008). Consequently, bobcat sightings in western Ohio are practically nonexistent. Bobcats are considered rare in Indiana, which borders Ohio to the west, with most sightings occurring in the southern portion of Indiana near its border with Kentucky. Similarly, although bobcats are common in northern Michigan and the Upper Peninsula, they are uncommon in the southern half of the state where bobcat trapping is prohibited.

The recent recolonization of bobcats in both eastern and southern Ohio requires animals to cross the Ohio River (see Fig. 1b). Some studies have found that landscape elements may limit dispersal (Riley et al. 2006; Lee et al. 2012). However, range-wide studies by both Reding et al. (2012) and Croteau (2009) indicated that the Mississippi River was not a major barrier to gene flow for bobcats. Bobcats are considered good swimmers (Young 1958; Van Wormer 1964; Rue 1981; Merritt 1987), and it is possible that summer reductions in river flow or ice cover during winter provide opportunities for dispersal (Croteau 2009). In contrast, populations of bobcats were found to be genetically isolated by the Straits of Mackinac between the upper and lower peninsulas in Michigan (Millions

and Swanson 2007). The extent to which bridges and other structures are used to cross major rivers is unknown, but may represent another method of movement.

We also found that the northeast Pennsylvania population in our study was genetically isolated from all of the other sampling localities, including the population in nearby southwest Pennsylvania. Since much of Pennsylvania is forested, it is possible that interstate highways such as I-80 act as barriers to gene flow. However, we found a significant effect of isolation by distance, implying that this is related to overall geographic distance and not anthropogenic barriers to dispersal. Southeastern Ohio was unglaciated during the last ice age and has extensive patches of deciduous forest habitat, but also major highways. In fact, both interstate highways I-70 and I-77 run through the eastern population and I-77 largely bisects it north to south, suggesting that they are not major barriers to movement. Millions and Swanson (2007) investigated the impact of natural and artificial barriers to dispersal on population structure of bobcats and found no evidence that a greater density of roads in the lower peninsula of Michigan resulted in population structure. In contrast, Lee et al.'s (2012) recent work suggests that urban development, including freeways, was a physical barrier that has reduced bobcat movement and gene flow between some isolated groups of individuals but not others.

Based on the known number of vehicle-related mortalities of bobcats in Ohio, we project that there are a minimum of ~450 bobcats in Ohio (S. Prange, unpublished data). Our genetic analyses suggest that these are historically divided into two relatively independent management units that are growing and regularly exchange individuals. We recommend that population structure should be assessed on a regular basis in this landscape until bobcats expand further into unoccupied habitat, the eastern population is no longer spatially distinguishable, and the need to define two units for management purposes no longer exists.

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References

- Blundell, G.M., Ben-David, M., Groves, P., Bowyers, R.T., and Geffen, E. 2002. Characteristics of sex-biased dispersal and gene flow in coastal river otters: I implications for natural recolonization of extirpated populations. *Mol. Ecol.* **11**: 289–303.
- Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C. et al. 2004. How to track and assess genotyping errors in population genetics studies. *Mol. Ecol.* **13**: 3261–3273.
- Carmichael, L.E., Clark, W., and Strobeck, C. 2000. Development and characterization of microsatellite loci from lynx (*Lynx canadensis*), and their use in other felids. *Mol. Ecol.* **9**: 2197–2198.
- Clark, J.D., Huber, D., and Servheen, C. 2002. Bear reintroductions: lessons and challenges. *Ursus*, **13**: 335–345.
- Cornuet, J.M., and Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–2014.
- Crochet, P.A. 2000. Genetic structure of avian populations—allozymes revisited. *Mol. Ecol.* **9**: 1463–1469.

Croteau, E.K. 2009. Population genetics and phylogeography of bobcats (*Lynx rufus*) using microsatellites and mitochondrial DNA. Ph.D. dissertation, Department of Zoology, Southern Illinois University, Carbondale, IL.

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

Croteau, E.K., Heist, E.J., Nielson, C.K., Hutchinson, J.R., and Hellgren, E.C. 2012. Microsatellites and mitochondrial DNA reveal regional population structure in bobcats (*Lynx rufus*) of North America. Conserv. Genet. **13**: 1637–1651.

Dempster, A.P., Laird, N.M., and Rubin, D.B. 1977. Maximum likelihood from incomplete data via the EM algorithm. J. Roy. Stat. Soc. B. Meth. **39**: 1–38.

DeWoody, J.A., Schupp, J., Kenefic, L., Busch, J., Murfitt, L. et al. 2004. Universal method for producing ROX-labeled size standards suitable for automated genotyping. BioTechniques, **37**: 348–352.

Earl, D. 2009. Structure Harvester v0.3. Available from http://users.soe.ucsc.edu/~dearl/software/struct_harvest/ [accessed 20 June 2012].

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

Faircloth, B.C., Reed, A., and Valentine, T. 2005. Tetranucleotide, trinucleotide, and dinucleotide loci from the bobcat (*Lynx rufus*). *Mol. Ecol. Notes*, **5**: 387–389.

Falush, D., Stephens, M., and Pritchard, J.K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**: 1567–1587.

Fry, J., Xian, G., and Jin, S. 2011. Completion of the 2006 National Land Cover Database for the Conterminous United States. *Photogramm. Eng. Remote Sens.* **77**: 858–864.

Geffen, E., Anderson, M.J., and Wayne, R.K. 2004. Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Mol. Ecol.* **13**: 2481–2490.

Gilbert, K.J., Andrew, R.L., Bock, D.G., Franklin, M.T., Kane, N.C. et al. 2012. Recommendations for utilizing and reporting population genetic analyses: the reproducibility of genetic clustering using the program STRUCTURE. *Mol. Ecol.* **21**: 4925–4930.

- Glaubitz, J.C. 2004. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Mol. Ecol.* **4**: 309–310.
- Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from <http://www2.unil.ch/popgen/softwares/fstat.htm> [accessed 10 May 2010].
- Haanes, H., Røed, K.H., Flagstad, Ø., and Rosef, O. 2010. Genetic structure in an expanding cervid population after population reduction. *Conserv. Genet.* **11**: 11–20.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* **41**: 95–98.
- Hellborg, L., Walker, C.W., Rueness, E.K., Stacy, J.E., Kojola, I. et al. 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.
- Hoelzel, A.R., Lopez, J.V., Dover, G.A., and O'Brien, S.J. 1994. Rapid evolution of a heteroplasmic repetitive sequence in the mitochondrial DNA control region of

carnivores. *J. Mol. Evol.* **39**: 191–199.

Hoffman, J.I., and Amos, W. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Mol. Ecol.* **14**: 599–612.

Hutchinson, T.F., Rubino, D., McCarthy, B.C., and Sutherland, E.K. 2003. History of forests and land-use. *In* Characteristics of mixed-oak forest ecosystems in southern Ohio prior to the reintroduction of fire. *Edited by* E.K. Sutherland and T.F. Hutchinson. Northeastern Research Station, Gen. Tech. Rep. NE-299, Forest Service, United States Department of Agriculture, Newtown Square, PA. pp. 17-28.

Ibrahim, K.M., Nichols, R.A., and Hewitt, G.M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *J. Hered.* **77**: 282–291.

IUCN (International Union for Conservation of Nature). 2008. *Lynx rufus*. The IUCN Red List of Threatened Species, version 2013.1 Available from <http://maps.iucnredlist.org/map.html?id=12521> [accessed 8 Sept 2013].

Jae-Heup, K., Eizirik, E., O'Brien, S.J., and Johnson, W.E. 2001. Structure and patterns of sequence variation in the mitochondrial DNA control region of the great cats. *Mitochondrion*, **14**: 279–292.

Janssens, X., Fontaine, M.C., Michaux, J.R., Libois, R. D. Kermabon, J., Defourny, P., and Baret, P.V. 2008. Genetic pattern of the recent recovery of European otters in southern France. *Ecography*, **30**: 176–186.

Jensen, J.L., Bohonak, A.J., and Kelley, S.T. 2005. Isolation by distance, web service. *BMC Genet.* **6**: 13.

Jombart, T., Devillard S., and Balloux, F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet.* **11**: 94.

Kendall, K.C., Stetz, J.B., Boulanger, J., Macleod, A.C., Paetkau, D., and White, G. C. 2009. Demography and genetic structure of a recovering grizzly bear population. *J. Mammal.* **73**: 3–17.

Konovalov, D.A., Manning, C., and Henshaw, M.T. 2004. KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Mol. Ecol. Notes*, **4**: 779-782.

Lande, R. 1988. Genetics and demography in biological conservation. *Science*, **241**: 1455-1460.

Lee, J.S., Ruell, E.W., Boydston, E.E., Lyren, L.M., Alonso, R.S. et al. 2012. Gene flow and pathogen transmission among bobcats (*Lynx rufus*) in a fragmented urban landscape. *Mol. Ecol.* **21**: 1617–1631.

Lopez, J.V., Cevario, S., and O'Brien, S.J. 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.

Lovallo, M.J., and Anderson, E.M. 1996. Bobcat (*Lynx rufus*) home range size and habitat use in northwest Wisconsin. *Am. Midl. Nat.* **135**: 241–252.

Luikart, G., Allendorf, F.W., Cornuet, J.M., and Sherwin, W.B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Hered.* **89**: 238-247.

MacKenzie, D.I., Nichols, J.D., Lachman, G.B., Droege, S., Royle, J.A. et al. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, **83**: 2248-2255.

McDonald, P.T., Nielsen, C.K., Oyana, T.J., and Sun, W. 2008. Modelling habitat overlap among sympatric mesocarnivores in southern Illinois, USA. *Ecol. Model.* **215**: 276-286.

McRae, B.H., Beier, P., DeWald, L.E., Huynh, L.Y., and 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.

Menotti-Raymond, M., David, V.A., Lyons, L.A., Schaffer, A.A., Tomlin, J.F. 1999. A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics*, **57**: 9–23.

Merritt, J.F. 1987. Guide to mammals of Pennsylvania. University of Pittsburgh Press, Pittsburgh, PA.

Millions, D.G., and Swanson, B.J. 2007. Impact of natural and artificial barriers to dispersal on the population structure of bobcats. *J. Wildl. Manage.* **71**: 96–102.

Mowry, R.A., Schneider, T.M., Latch, E.K., Gompper, M.E., Beringer, J., and Eggert, L.S. 2015. Genetics and the successful reintroduction of the Missouri river otter. *Anim. Conserv.* **18**: 196–206.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. *Trends Ecol. Evol.* **9**: 373–375.

Narum, S.R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conserv. Genet.* **7**: 783–787.

Nowell, K., and Jackson, P. 1996. The wild cats: a status survey and conservation action plan. IUCN/SSC Cat Specialist Group, Gland, Switzerland and Cambridge, UK.

Ohio Division of Wildlife. 2014. GIS Web Mapping Services. Available from <http://geospatial.ohiodnr.gov/map-services> [accessed 21 June 2014].

Onorato, D.P., Hellgren, E.C., Van Den Bussche, R.A., and Doan-Crider, D.L. 2004a. Phylogeographic patterns within a metapopulation of black bears (*Ursus americanus*) in the American Southwest. *J. Mammal.* **85**: 140–147.

Onorato, D.P., Hellgren, E.C., Van Den Bussche, R.A., and Skiles Jr., J.R. 2004b. Paternity and relatedness of American black bears recolonizing a desert montane island. *Can. J. Zool.* **82**: 1201–1210.

- Palomares, F., Godoy, J.A., Piriz, A., O'Brien, S.J., and Johnson, W.E. 2002. Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. *Mol. Ecol.* **11**: 2171–2182.
- Pelletier, A., Obbard, M.E., White, B.N., Doyle, C., and Kyle, C.J. 2011. Small-scale genetic structure of American black bears illustrates potential postglacial recolonization routes. *J. Mammal.* **92**: 629–644.
- Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L. et al. 2004. GENECLASS2: a software for genetic assignment and first-generation migration detection. *J. Hered.* **95**: 536–539.
- Piry, S., Luikart, G., and Cornuet, J.M. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered.* **90**: 502–503.
- Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Influence of population structure using multilocus genotype data. *Genetics*, **155**: 945-959.
- Randi, E., Davoli, F., Pierpaoli, M., Pertoldi, C., Madsen, A.K., and Loeschcke, V. 2003. Genetic structure in otter (*Lutra lutra*) populations in Europe: implications for conservation. *Anim. Conserv.* **6**: 9 –100.

Rannala, B., and Mountain, J.L. 1997. Detecting immigration by using multilocus genotypes. *Proc. Natl. Acad. Sci. USA* **94**: 9197-9201.

Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* **86**: 248–249.

Reding, D.M. 2011. Patterns and processes of spatial genetic structure in a mobile and continuously distributed species, the bobcat (*Lynx rufus*). Ph.D. dissertation, Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA.

Reding, D.M., Bronikowski, A.M., Johnson, W.E., and Clark, W.R. 2012. Pleistocene and ecological effects on continental-scale genetic differentiation in the bobcat (*Lynx rufus*). *Mol. Ecol.* **21**: 3078–3093.

Reid, A.E. 2006. Spatial genetic structure of four bobcat populations in the southeastern U.S. M.Sc. thesis, University of Georgia, Athens, GA.

Riley, S.P.D., Pollinger, J.P., Sauvajot, R.M., York, E.C., Fuller, T.K. et al. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. *Mol. Ecol.* **15**: 1733–1741.

Roberts, N.M., and Crimmins, S.M. 2010. Bobcat population status and

management in North America: evidence of large-scale population increase. *J. Fish Wildl. Manage.* **1**: 169–174.

Rozas, J., Sánchez-DelBarrio, J.C., Messegyer, X., and Rozas, R. 2003. DnsSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**: 2496–2497.

Rue, L.L. 1981. Furbearing animals of North America. Crown Publishers, Inc., New York, NY.

Rueness, E.K., Stenseth, N.C., O'Donoghue, M., Boutin, S., Ellegren, H. et al. 2003. Ecological and genetic spatial structuring in the Canadian lynx. *Nature*, **425**: 69–72.

Schneider, S., Roessli, D., and Excoffier, L. 2000. Arlequin: a software for population genetics data analysis, version 2.0. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Schwartz, M.K., Mills, L.S., McKeveley, K.S., Ruggiero, L.F., and Allendorf, F.W. 2002. DNA reveals high dispersal synchronizing the population dynamics of Canada lynx. *Nature*, **415**: 520–522.

Szpiech, Z.A., Jakobsson, M., and Rosenberg, N.A. 2008. ADZE: a rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics*, **24**: 2498–2504.

Thompson, J.D., Higgins, D.J., and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680.

Trautman, M.B. 1977. The Ohio country from 1750 to 1977 – a naturalist's view. Ohio Biological Survey Note No. 10, Ohio State University, Columbus, OH.

Tucker, S.A., Clark, W.R., and Gosselink, T.E. 2008. Space use and habitat selection by bobcats in the fragmented landscape of south-central Iowa. *J. Wildl. Manage.* **72**: 1114–1124.

Van Wormer, J. 1964. The world of the bobcat. Liippincott, Philadelphia, PA.

Walker, C.W., Vila, C., Landa, A., Linden, M., and Ellegren, H. 2001. Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations. *Mol. Ecol.* **10**: 53–63.

Waser, P., and Strobeck, C. 1998. Genetic signatures of interpopulation dispersal. *Trends Ecol. Evol.* **13**: 43–44.

Williams, B.W. 2006. Applications of Genetic Methods in Furbearer Management and Ecology: Case Studies of Fishers, American Martens and Bobcats. M.Sc. thesis, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI.

Wilson, G.A., and Rannala, B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163**: 1177–1191.

Woolf, A., and Hubert, G.F. 1998. Status and Management of Bobcats in the United States over Three Decades: 1970s-1990s. *Wildl. Soc. Bull.* **26**: 287-293.

Woolf, A., Nielsen, C.K., Weber, T., and Gibbs-Kieninger, T.J. 2002. Statewide modeling of bobcat, *Lynx rufus*, habitat in Illinois, USA. *Biol. Conserv.* **104**: 191–198.

Yearley, E.F., and Samuel, D.E. 1980. Use of reclaimed surface mines by foxes in West Virginia. *J. Wildl. Manage.* **44**: 729–734.

Young, S.P. 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, D.C.

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Table 1. Characteristics of 12 microsatellite loci in bobcats (*Lynx rufus*) from seven sampling locations in the southern Great Lakes region, USA.

Locus	<i>N</i>	<i>T_a</i> (°C)	size range (bp)	<i>N_a</i>	Allelic richness	<i>H_o</i>	<i>H_s</i>	<i>P</i> -HWE	null allele freq
BCE5T ^a	192	TD59 to 55	235-287	11	6.54	0.77	0.76	0.37	0.04
FCA35 ^a	186	TD59 to 55	124-160	14	8.84	0.79	0.86	0.02	0.09
FCA90 ^a	193	TD59 to 55	101-113	8	6.24	0.76	0.80	0.38	0.08
FCA96 ^a	183	TD59 to 55	175-201	11	7.06	0.80	0.80	0.69	0.04
FCA132 ^a	194	TD59 to 55	165-179	8	6.27	0.78	0.80	0.44	0.04
BCG8T ^b	188	TD55 to 50	258-284	14	9.47	0.89	0.88	0.28	0.02
FCA23 ^b	190	TD55 to 50	132-144	6	4.93	0.77	0.75	0.33	0.09
FCA26 ^b	188	TD55 to 50	124-142	10	5.07	0.68	0.71	0.74	0.03
Lc109 ^b	189	TD55 to 50	164-186	10	6.98	0.80	0.82	0.40	0.04
BC1AT ^c	188	TD55 to 50	284-312	8	5.21	0.73	0.74	0.53	0.01
FCA43 ^c	190	TD55 to 50	114-124	6	3.94	0.56	0.57	0.94	0.01
FCA126 ^c	190	TD55 to 50	114-140	11	7.02	0.87	0.81	0.98	0.00

Note: Sample size (*N*), annealing temperature for amplification (*T_a*), size range (bp) of alleles, number of alleles (*N_a*), allelic richness, observed (*H_o*) and expected (*H_s*) heterozygosities, *P* value for exact test of Hardy-Weinberg equilibrium (*P*-HWE) and estimated null allele frequency are given above. Loci multiplexed together are denoted by superscript letters ^{a-c}.

Table 2. Pairwise F_{ST} values for seven sample locations of bobcats (*Lynx rufus*) based on microsatellite loci are given above the diagonal and mtDNA sequences are given below the diagonal.

	<i>N</i> for microsats	<i>N</i> for mtDNA	E OH	S OH	W KY	E KY	WV	W PA	E PA
E OH	57	49	---	0.024*	0.063*	0.034*	0.015*	0.049*	0.088*
S OH	54	51	0.217*	---	0.033*	0.012*	0.008*	0.018*	0.070*
W KY	14	15	0.163	0.000	---	0.037*	0.043*	0.025*	0.077*
E KY	15	14	0.034	0.264*	0.198	---	0.018*	0.032*	0.089*
WV	25	23	0.122	0.000	0.000	0.169*	---	0.025*	0.081*
W PA	11	9	0.157	0.499*	0.510*	0.158	0.439*	---	0.036*
E PA	18	18	0.124	0.480*	0.503*	0.147	0.426*	0.000	---

* denotes significance after B-Y adjustment for multiple tests

Table 3. Genetic variation of populations of bobcats (*Lynx rufus*) based on (a) microsatellites and (b) mtDNA.

(a)

Population	<i>N</i>	mean allelic richness	<i>H</i> _o (SD)	<i>H</i> _s (SD)	<i>F</i> _{IS}
E OH	57	6.4	0.73 (0.09)	0.73 (0.11)	0.000
S OH, WV, W KY, E KY, W PA	119	7.5	0.77 (0.10)	0.80 (0.08)	0.043
E PA	18	6.0	0.76 (0.12)	0.74 (0.09)	-0.042

(b)

Population	<i>N</i>	# haplotypes	(%) of mtDNA control region variants						Haplotypic (gene) diversity
			Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	
E OH	49	2	69.39%	30.61%	---	---	---	---	0.434
S OH, WV, W KY, E KY, W PA	112	6	41.96%	50.89%	3.57%	1.79%	0.89%	0.89%	0.568
E PA	18	2	94.44%	5.56%	---	---	---	---	0.111

Table 4. Estimates of contemporary migration of bobcats (*Lynx rufus*) based on assignment test results with a $\geq 90\%$ likelihood value from GENECLASS.

Population of collection	N	Population of genetic assignment			Total
		E OH	S OH, WV, W KY, E KY, W PA	E PA	
E OH	44	35 (79.5%)	9 (20.5%)	0 (0%)	100%
S OH, WV, W KY, E KY, W PA	91	8 (8.8%)	81 (89.0%)	2 (2.2%)	100%
E PA	15	0 (0%)	1 (6.7%)	14 (93.3%)	100%

Note: Values shown are the number of bobcats with percentages in parentheses and residents are shown in bold.

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Table 5. Summary of results from BOTTLENECK for 3 populations of bobcats (*Lynx rufus*) using both the two-phase model (TPM) and the stepwise mutation model (SMM) for the Wilcoxon's test along with results from the mode-shift test.

Population ID	Wilcoxon's test TPM		Wilcoxon's test SMM		Mode-shift test
	two-tailed	one-tailed for H excess	two-tailed	one-tailed for H excess	
E OH	0.42	0.21	0.11	0.95	L-shape
S OH, WV, W KY, E KY, W PA	0.003*	0.002*	0.42	0.81	L-shape
E PA	0.13	0.06	0.27	0.88	L-shape

* denotes significance after B-Y adjustment for multiple tests

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FIGURE CAPTIONS

Fig. 1. (a) Map showing the study area within North America with hash marks and the range of bobcats (*Lynx rufus*) modified from spatial data in IUCN (2008) shaded in gray. (b) Map showing where bobcat samples were obtained: the samples for Ohio are mapped by actual GIS coordinates with triangles and samples from the surrounding states are mapped by county with a circle denoting the number of samples. The Ohio River, the Western Alleghany Plateau Ecoregion, and other major land use features are marked. Dashed circles represent the seven *a priori* sample locations used in subsequent STRUCTURE analyses.

Fig. 2. STRUCTURE results for bobcats (*Lynx rufus*) showing (a) the ΔK graph from STRUCTURE HARVESTER and (b) the bar plot with individual assignments from STRUCTURE for $K = 3$ ($\ln P(D) = -7944.1$). Abbreviations for sample locations are as follows: E OH (eastern Ohio) S OH (southern Ohio); W KY (western Kentucky); E KY (eastern Kentucky); WV (West Virginia); W PA (western Pennsylvania); E PA (eastern Pennsylvania).

Fig. 3. Genetic isolation by distance of the seven *a priori* sample locations of bobcats (*Lynx rufus*) as inferred using multilocus estimates of F_{ST} values and the logarithm of the geographical distance in km ($R^2 = 0.50$, $P = 0.005$).

Fig. 4. The mean number of private alleles per locus for bobcats (*Lynx rufus*) as a function of standardized sample size for three populations of bobcats calculated in ADZE.

Fig. 5. Distribution of allele frequencies from BOTTLENECK for three populations of bobcats (*Lynx rufus*) showing an L-shape distribution.

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Figure 2a.
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Figure 2b is uploaded now as a jpg which is an acceptable file format (not ppt)
254x190mm (72 x 72 DPI)

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