Original Article

Using Population Genetics for Management of Bobcats in Oregon

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ABSTRACT In Oregon, USA, bobcats on either side of the Cascade Mountain Range are recognized as distinct subspecies, with *Lynx rufus fasciatus* west and *Lynx rufus pallescens* east of the Cascades. These subspecies are currently managed for harvest as separate populations primarily because of substantial differences in regional pelt values. We used genetic data to determine whether bobcats in Oregon are subdivided into genetically discernible populations that support current regional management regulations. We collected 250 tissue samples from 12 *a priori* sampling districts, and generated data from 15 microsatellite markers and approximately 1 kB of mtDNA sequence. Results of clustering analyses based on the microsatellite data indicated strong support for the presence of 2 genetic populations, generally corresponding to the 2 subspecies. Analysis of molecular variance (AMOVA) indicated significant structure between the east and west regions, which accounted for 1.7% of the total variation in microsatellites. AMOVA based on the mtDNA sequences indicated significant structure between regions, accounting for 12.8% of the mtDNA variation. With both microsatellite and mtDNA data, we observed a significant pattern of isolation-by-distance, whereby geographically proximate sampling districts were more genetically similar than were more distant districts. We identified 21 putative migrants, individuals with genotypes more likely to have originated from the opposite region, showing that the Cascade Mountain Range apparently is not an absolute barrier to gene flow. Given the potential for differential harvest effort by region based on differences in pelt values and bobcat densities, and the vulnerability of bobcats to harvest, our results support the current management framework. © 2013 The Wildlife Society.

KEY WORDS bobcat, furbearer management, harvest, *Lynx rufus*, Oregon, population genetics, subspecies, trapping.

The bobcat (*Lynx rufus*) is the most widely distributed native feline in North America, occurring from coast to coast and from southern Canada through central Mexico (Anderson and Lovallo 2003). Bobcats are defined in the Convention on International Trade in Endangered Species (CITES) Appendix II under similarity of appearance to enable effective international regulation of listed *Lynx* species. This CITES classification requires state wildlife agencies to have reliable estimates of population status and accurate harvest data to manage the species, and wildlife managers have therefore highlighted the need for a large-scale population assessment (Bluett et al. 2001). A recent survey, in part stimulated by state agency and US Fish and Wildlife Service proposals to remove bobcats from Appendix II (Association of Fish and Wildlife Agencies 2010), showed that populations have been increasing in abundance during the past 2 decades throughout the majority of jurisdictions within their geographic distribution (Roberts and Crimmins 2010).

Like many North American mammals, bobcats were described on the basis of regional physical variations (e.g., body size, cranial morphology, pelage color, and markings), and 12 subspecies are currently recognized (Hall 1981, Larivière and Walton 1997). Two of the designated subspecies occur within the state of Oregon, USA: *L. r. fasciatus* generally west of the peak of the Cascade Range, and *L. r. pallescens* generally east of the Cascades (Fig. 1; Verts and Carraway 1998). Bobcats west of the Cascades typically have a darker pelt with red coloration and relatively few spots; whereas, bobcats east of the Cascades generally have a paler pelt with gray coloration and more distinct spots. Pelts of *L. r. pallescens* averaged about US$ 252.00 in 2010, almost 4 times that of the darker *L. r. fasciatus* (Oregon Territorial Council 2010). Consequently, harvest of bobcats has been managed by region in Oregon, with counties west of the Cascades (i.e., W region) having no harvest limit and counties east of the Cascades (i.e., E region) having a harvest limit of 5 bobcats/licensed individual (Hiller 2011).

Previous population genetic studies of bobcats show mixed results regarding the potential of population structuring (Reid 2006, Riley et al. 2006, Williams 2006, Millions and Swanson 2007, Croteau et al. 2010). Until Reding...
et al. (2012) completed a nationwide genetic analysis; there had not been a study of genetic variation of bobcats in the northwestern portion of their range. Like most researchers, we anticipated population genetic structure because of the topographic variation and glacial history in this region. Such factors have been implicated in restricting gene flow in Canada lynx (L. canadensis; Rueness et al. 2003), mule deer (Odocoileus hemionus; Latch et al. 2009), and many other species (Shafer et al. 2010). Because bobcats are rarely found above 2,750 m in elevation (Young 1958), topographic features such as the Cascade Mountain Range in Oregon may serve as an east–west dispersal barrier and result in genetic differentiation between subspecies.

Phenotypic differences may or may not be indicative of intraspecific variation associated with population dynamics. Now, molecular techniques provide a means to independently test whether subspecies or other intraspecific classifications are biologically relevant entities (DeYoung and Honeycutt 2005). Not only have several scientists questioned the biological significance of taxonomic distinctions among contiguous bobcat populations that lack clear geographical breaks (Read 1981, McCord and Cardoza 1982), but wildlife management agencies are under pressure to justify regulations based on wildlife population structure (T.L. Hiller, personal communication). Our objectives were to assess whether bobcats in Oregon constitute a single, panmictic (i.e., freely interbreeding) population or are subdivided by the Cascade Mountain Range into 2 genetically discernible populations that are geographically concordant with the subspecies designations. Our study was designed to test the assumption that bobcats were effectively being managed by treating the subspecies as biological units (i.e., differing harvest regulations for E and W regions primarily as the result of regional pelt characteristics).

STUDY AREA

Oregon included diverse topographical features that ranged in elevation from sea level at the Pacific Ocean coast to >3,420 m at Mt. Hood in the Cascade Mountain Range. The western third of Oregon (W region; total of approx. 98,000 km²) was composed primarily of the northwestern forest, forage, and specialty land resource region, and 44% of this region was federally owned lands (Natural Resources Conservation Service 2006). Forested areas in this region included the Coast Range and western slopes of the Cascades, which were lowland conifer–hardwood forests.

Figure 1. Genetic assignments (based on microsatellite markers) of bobcats collected in Oregon, USA, 2009–2010. Analyses are based on (a) STRUCTURE analysis with no location prior, (b) STRUCTURE analysis with location prior, (c) BAPS aspatial clustering of individuals, and (d) BAPS spatial and aspatial clustering of groups (districts and counties). Size of each pie reflects the number of individuals genotyped per county. Individuals assigned to the west cluster with q ≥ 0.75 are colored black (non-admixed) and q < 0.75 (admixed) are colored dark gray. Individuals assigned to the east cluster with q ≥ 0.75 (non-admixed) are colored white and q < 0.75 (admixed) are colored light gray. Numbers indicate sampling district.
dominated by Douglas-fir (Pseudotsuga menziesii) and western hemlock (Tsuga heterophylla); the eastern slopes of the Cascades, which were primarily ponderosa pine (Pinus ponderosa) forests, but also Douglas-fir and white oak (Quercus garryana); and southwestern Oregon, which were mixed conifer–hardwood forests that consisted of a diverse mixture of dominants, including Douglas-fir, white fir (Abies concolor), sugar pine (P. lambertiana), ponderosa pine, and incense cedar (Calocedrus decurrens; Chappell et al. 2001).

The eastern two-thirds of Oregon (E region) had diverse topography and vegetation characteristics. The northeastern portion of Oregon consisted of a mixture of the regions of northwestern wheat and range (56,850 km²), and Rocky Mountain Range and forest (30,644 km²). Land use of the former was primarily croplands (e.g., wheat, barley, and oats) and livestock grazing, whereas the latter included the Blue Mountains, with large expanses of forest that consisted of western juniper (Juniperus occidentalis), ponderosa pine, Douglas-fir, subalpine fir (A. lasiocarpa), Engelmann spruce (Picea engelmannii), lodgepole pine (Pinus contorta), and western larch (Larix spp.), depending on slope, aspect, and elevation (Natural Resources Conservation Service 2006). Much of southeastern Oregon was in the western range and irrigated region (56,979 km²), which consisted of shrub–steppe vegetation dominated by sagebrush (Artemisia spp.) and antelope bitterbrush (Purshia tridentata; Chappell et al. 2001, Natural Resources Conservation Service 2006). Distribution of human population of 3.4 million somewhat reflected the west–east differences in land use, with the highest human densities occurring in the Willamette Valley (US Census Bureau 2002).

Climate was spatially variable within the state. Mean annual precipitation ranged from about 20 cm in Harney County (southeastern OR) to about 325 cm in Polk County (northeastern OR); mean annual daily temperature ranged from 3.1°C in Klamath County (southcentral OR) to 13.0°C in Douglas County (southwestern OR; Southern Regional Climate Center 2010).

METHODS

We collected tissue samples from 250 mandibles of bobcats harvested in Oregon during the 2009–2010 season. Bobcat hunters and trappers in Oregon were required to provide the Oregon Department of Fish and Wildlife with information on sex and date and county of harvest for each bobcat, as well as to submit mandibles for aging purposes (i.e., extraction of lower canine tooth for cementum annuli analysis) and present pelts for tagging requirements. To collect a tissue sample from each mandible, we used a sterile disposable scalpel to remove a piece of tissue, which was stored at room temperature in a 5 mL vial filled with silica desiccant. We divided the state of Oregon into 12 a priori sampling districts (districts 1–6 in the W region; 7–12 in E) based on geographic proximity and ecological similarity (Fig. 1), and in each district we obtained 10–30 samples, a reasonable sample size for detecting population genetic structure (Kalinowski 2005). Our sampling scheme resulted in 125 samples collected from each of the east and west regions, with the crest of the Cascade Mountain Range dividing regions.

We extracted DNA using DNeasy (Qiagen, Valencia, CA) purification kits and genotyped individual bobcats at 15 autosomal microsatellite markers developed from the domestic cat (Felis catus): FCA008, FCA031, FCA043, FCA077, FCA082, FCA090, FCA096, FCA132, FCA149, FCA391, FCA559 (Menotti-Raymond et al. 1999), and FCA740 (Menotti-Raymond et al. 2005); Canada lynx: Lc109 and Lc111 (Carmichael et al. 2000); and bobcat: BCAST (Faircloth et al. 2005). Each locus was amplified separately using the M13-tailed primer method (Boutin-Ganache et al. 2005). The total polymerize chain reaction (PCR) volume was 10 μL, composed of 1× PCR buffer with 2 mM MgSO₄ (ID Labs, London, ON, Canada), 0.2 mM dNTPs, 0.3 μM fluoresently labeled M13 primer, 0.3 μM reverse primer, 0.02 μM M13-tailed forward primer, 0.4 U IDPROOF DNA Polymerase (ID Labs), and 10–20 ng of template DNA. The PCR profile was 95°C/5 minutes (95°C/20 sec, X°C/20 sec, 72°C/30 sec) × X cycles, 72°C/20 minutes (X variable conditions defined in Table 1). To verify the sex of each bobcat, we used 2 PCR tests developed specifically for felids and based on Y-chromosome deletions in the amelogenin and zinc-finger regions (Pilgrim et al. 2005). For each, the total PCR volume was 10 μL, composed of 1× PCR buffer with 2 mM MgSO₄, 0.2 mM dNTPs, 1.0 μM reverse primer, 1.0 μM fluoresently labeled M13 primer, 0.08 μM M13-tailed forward primer, 15 μg BSA, 0.5 U IDPROOF, and 10–20 ng of template DNA. The PCR profile was 94°C C/5 minutes (94°C C/1 min, X°C C/1 min, 72°C C/30 sec) × 30 cycles, 72°C C/7 minutes (see Table 1). We combined analysis of markers into 5 gel sets, each consisting of 2–4 loci (Table 1). The samples were analyzed on an ABI 3730×1 DNA Analyzer (Applied Biosystems, Foster City, CA) at the Iowa State University DNA Facility. Alleles were scored using the software Genemapper (Applied Biosystems).

We amplified a 949-base-pair portion of the mtDNA NADH dehydrogenase subunit 5 (ND5) gene using primers we designed, ND5-DR1F (5’-TCATCCCGCTAGCACTTTTC-3’) and ND5-DR3R (5’-AAGGGATGTGGCCATGAG-3’). Total PCR volume was 10 μL, composed of 1× PCR buffer with 2 mM MgSO₄, 0.2 mM dNTPs, 0.3 μM each primer, 0.4 μU DNA Polymerase, and 10–20 ng of template DNA. The PCR profile was 95°C 8/5 minutes (94°C 8/1 min, 72°C 8/30 sec, 72°C 8/45 sec) × 30 cycles, 72°C 8/10 minutes. Polymerase chain reaction products were cleaned using the ExoSAP method (Werle et al. 1994) and submitted to the Iowa State University DNA Facility for cycle sequencing and analysis on an ABI 3730×1 DNA Analyzer. Both directions were sequenced with the same primers used for PCR. To check data quality, we randomly selected 25 samples (10%) and repeated extraction, PCR, and analysis steps for microsatellites, sex identification markers, and mtDNA (DeWoody et al. 2006).

For the total sample, regional scale (E, W), and each of the 12 sampling districts, we used Genepop 4.0 (Rousset 2008).
to perform tests of linkage disequilibrium for each pair of microsatellite loci, deviation from Hardy–Weinberg equilibrium (HWE), and estimate $F_{ST}$ (Weir and Cockerham 1984). We used Arlequin 3.11 (Excoffier et al. 2005) to calculate expected ($H_E$) and observed ($H_O$) heterozygosities, and Fstat 2.9.3 (Goudet 2001) to calculate allelic richness (AR) corrected for differences in sample size. We used Arlequin to calculate pair-wise $F_{ST}$ as a measure of differentiation among sampling districts. To test for a pattern of isolation-by-distance, we performed a Mantel test (Mantel 1967) between a genetic distance matrix $[F_{ST}/(1 − F_{ST})]$ and a geographic distance matrix (ln-transformed km) with 1,000 permutations in Arlequin. We also used Arlequin to perform an analysis of molecular variance (AMOVA) and a geographic distance matrix (ln-transformed km) with 1,000 permutations in Arlequin. We also used Arlequin 3.11 (Excoffier et al. 2005) to identify unique haplotypes and generate Arlequin input files. To measure genetic differentiation, we used Arlequin to estimate pair-wise $\Phi_{ST}$ values between sampling districts and to perform an AMOVA to test for differentiation between east and west regions. To test for a pattern of isolation-by-distance, we performed a Mantel test between a genetic distance matrix $[\Phi_{ST}/(1 − \Phi_{ST})]$ and a geographic distance matrix (ln-transformed km) using 1,000 permutations in Arlequin. To visualize relationships among haplotypes, we constructed a median joining network using the program Network 4.6 (Bandelt et al. 1999).

To quantify the number of genetically unique populations present in Oregon, we applied both aspatial and spatial multi-locus Bayesian clustering techniques to the microsatellite data. These approaches identify the most likely number of populations present in a data set and sort individuals into clusters so as to minimize departures from Hardy–Weinberg and linkage equilibrium. We used the aspatial method implemented in Program Structure 2.3.3 (Pritchard et al. 2000), performing 10 independent runs of 300,000 iterations following a burn-in period of 100,000 iterations for each value in the range $K = 1–14$. We used the admixture model and allowed allele frequencies to be correlated among populations (Falush et al. 2003). We determined the most likely number of populations in our data set as the $K$ with the highest maximum log-likelihood value $[L(K)]$ and $\Delta K$ statistic (Evanno et al. 2005). To assign individuals to genetic clusters, we performed a final run (500,000 burn-in and 1,000,000 iterations) at the inferred $K$ and used the estimated values of the proportion of an individual’s genome attributed to each of the identified genetic clusters ($q$). We assigned individuals to the cluster with the highest $q$-value, but considered individuals with $q < 0.75$ to be potentially admixed (ancestry from multiple clusters). We repeated the entire Structure procedure, but included sampling district information in the model to assist clustering by modifying the prior to prefer clustering solutions that correlate with the sampling locations (Hubisz et al. 2009). This approach is still considered aspatial because no information on the actual geographic locations of the sampling units was incorporated.

We also performed aspatial and spatial clustering using the program Baps 5.4 (Corander et al. 2008). We performed aspatial mixture clustering of individuals, aspatial mixture clustering of groups of individuals based on the 12 sampling districts, spatial mixture clustering of individuals with

### Table 1. Properties of the 15 microsatellite and 2 sex identification loci used in study of bobcats in Oregon, USA, 2009–2010.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Repeat motif$^a$</th>
<th>Set</th>
<th>Annealing temp. (°C)</th>
<th>No. of cycles</th>
<th>Dye$^b$</th>
<th>Allele size range (base-pair)</th>
<th>No. of alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCA090</td>
<td>Di</td>
<td>1</td>
<td>50</td>
<td>30</td>
<td>6-FAM</td>
<td>246</td>
<td>109–129</td>
</tr>
<tr>
<td>FCA149</td>
<td>Di</td>
<td>1</td>
<td>50</td>
<td>35</td>
<td>HEX</td>
<td>242</td>
<td>136–156</td>
</tr>
<tr>
<td>Lc109</td>
<td>Di</td>
<td>1</td>
<td>48</td>
<td>35</td>
<td>6-FAM</td>
<td>229</td>
<td>180–200</td>
</tr>
<tr>
<td>FCA391</td>
<td>Tetra</td>
<td>1</td>
<td>50</td>
<td>35</td>
<td>HEX</td>
<td>238</td>
<td>208–240</td>
</tr>
<tr>
<td>FCA008</td>
<td>Di</td>
<td>2</td>
<td>50</td>
<td>30</td>
<td>6-FAM</td>
<td>248</td>
<td>144–160</td>
</tr>
<tr>
<td>FCA096</td>
<td>Di</td>
<td>2</td>
<td>50</td>
<td>35</td>
<td>HEX</td>
<td>245</td>
<td>187–211</td>
</tr>
<tr>
<td>BCEST</td>
<td>Tetra</td>
<td>2</td>
<td>50</td>
<td>35</td>
<td>6-FAM</td>
<td>241</td>
<td>257–281</td>
</tr>
<tr>
<td>FCA740</td>
<td>Tetra</td>
<td>2</td>
<td>50</td>
<td>32</td>
<td>HEX</td>
<td>242</td>
<td>334–362</td>
</tr>
<tr>
<td>FCA043</td>
<td>Di</td>
<td>3</td>
<td>50</td>
<td>30</td>
<td>6-FAM</td>
<td>239</td>
<td>132–142</td>
</tr>
<tr>
<td>Lc111</td>
<td>Di</td>
<td>3</td>
<td>48</td>
<td>35</td>
<td>HEX</td>
<td>229</td>
<td>157–217</td>
</tr>
<tr>
<td>FCA031</td>
<td>Tetra</td>
<td>3</td>
<td>50</td>
<td>35</td>
<td>6-FAM</td>
<td>229</td>
<td>238–258</td>
</tr>
<tr>
<td>FCA559</td>
<td>Tetra</td>
<td>4</td>
<td>51</td>
<td>35</td>
<td>HEX</td>
<td>245</td>
<td>117–137</td>
</tr>
<tr>
<td>FCA077</td>
<td>Di</td>
<td>4</td>
<td>48</td>
<td>35</td>
<td>6-FAM</td>
<td>247</td>
<td>148–172</td>
</tr>
<tr>
<td>FCA132</td>
<td>Di</td>
<td>4</td>
<td>47</td>
<td>32</td>
<td>HEX</td>
<td>247</td>
<td>182–198</td>
</tr>
<tr>
<td>FCA082</td>
<td>Di</td>
<td>4</td>
<td>50</td>
<td>30</td>
<td>6-FAM</td>
<td>244</td>
<td>248–266</td>
</tr>
<tr>
<td>Amelogenin</td>
<td>Di</td>
<td>1</td>
<td>50</td>
<td>30</td>
<td>HEX</td>
<td>235</td>
<td>213–233</td>
</tr>
<tr>
<td>Zinc-finger</td>
<td>Tetra</td>
<td>1</td>
<td>51</td>
<td>30</td>
<td>6-FAM</td>
<td>248</td>
<td>180–183</td>
</tr>
</tbody>
</table>

Summary statistics are for the entire data set ($n = 250$).

$^a$ Di, dinucleotide; Tetra, tetranucleotide; set, loci simultaneously electrophoresed.

$^b$ $N_s$, number of samples successfully analyzed.
geographic coordinates for each animal consisting of the centroid of the county of harvest, and spatial mixture clustering of groups of individuals with geographic coordinates for each group consisting of the centroid of the district. For approaches involving clustering of groups, we also used county instead of district. In all 4 analyses, we performed the mixture clustering with the maximum number of populations set to a range of \( K = 1–14 \). Results from each run were stored and merged by the program, which selected the optimal \( K \)-value based on the partition with the maximum likelihood \( [L(K)] \) and highest probability \( (P) \). The assignments from the mixture analysis were then used to perform admixture analysis, resulting in the estimation of ancestry \( (q) \) values for each individual. Again, we assigned each individual to the group with the highest \( q \)-value, but considered individuals with \( q < 0.75 \) to be admixed.

**RESULTS**

All 25 of the samples repeated for error-checking matched 100% with the initial microsatellite genotype, genetic sex identification, and sequence data, which indicated overall high genetic data quality. The 2 independent PCR sex tests provided concordant results and indicated that our total data set included 147 males, 101 females, and 2 unknowns (i.e., did not amplify). One sample failed to amplify any microsatellite loci, but the remaining samples were genotyped at an average of 14.5 loci (range 7–15). Total allele counts ranged from 6 to 11 alleles (Table 1). We found no evidence for linkage disequilibrium or deviation from HWE among the 15 loci in any sampling district, so all loci were used in the analyses.

Sampling districts had similar levels of genetic diversity, as measured by \( H_E \) and AR (Table 2). Based on the microsatellite data, pair-wise \( F_{ST} \) estimates ranged from effectively 0 to 0.083 (Table 3), and 27 of 66 comparisons were statistically significant after sequential Bonferroni adjustment. Sampling districts 1 and 2 (in northwestern OR) appeared most differentiated from other districts, accounting for 17 of the significant comparisons and generating the highest \( F_{ST} \)-values. We observed a significant pattern of isolation-by-distance \( (r = 0.422, P = 0.002; \text{Fig. 2a}) \). Results of AMOVA indicated weak but significant structure between east and west regions \( (F_{CT} = 0.017, P < 0.01) \), which accounted for 1.7% of the total genetic variation. The vast majority of genetic variation (97.0%) resided within populations, and an additional 1.3% occurred among populations within regions. First-generation assignment tests identified 21 individuals as \( F_0 \) migrants (Fig. 3). Nine migrants were detected in the east region and 12 in the west region; 8 of the migrants were genetically identified as females and 13 as males. Most (76%) of the migrants were located in counties bordering the Cascade Mountain Range.

All 250 samples yielded the full 949-base-pair mtDNA sequence, and we detected 18 unique haplotypes (Table 2). The haplotype network (Fig. 4a) indicated 2 major clades, separated by 7 substitutions. Representative haplotypes from both clades were consistently found in both regions of the state; however, each specific haplotype was generally restricted to either the east or west region (Fig. 4b). Pair-wise \( \Phi_{ST} \) estimates ranged from effectively 0 to 0.436 (Table 3), and 10 of 66 comparisons were statistically significant (Table 3). Again, we observed a significant pattern of isolation-by-distance \( (r = 0.433, P = 0.005; \text{Fig. 2b}) \). The highest \( \Phi_{ST} \)-values and all but one of the significant comparisons occurred between districts in the east versus west regions. Indeed, results of AMOVA indicated significant structure

### Table 2. Summary of microsatellite and mtDNA genetic variation for different groupings of bobcats in Oregon, USA, 2009–2010.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Microsatellites</th>
<th>mtDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( N ) ( H_O ) (SD)( b )</td>
<td>( H_E ) (SD)( b )</td>
</tr>
<tr>
<td>Total</td>
<td>249 0.717 (0.073) 0.750 (0.077) 0.042 (0.042) 9.04 (1.76)</td>
<td>250 18 0.848 (0.010)</td>
</tr>
<tr>
<td>East</td>
<td>125 0.736 (0.081) 0.754 (0.076) 0.023 (0.060) 8.44 (1.78)</td>
<td>125 15 0.802 (0.023)</td>
</tr>
<tr>
<td>West</td>
<td>124 0.699 (0.071) 0.730 (0.085) 0.038 (0.065) 7.95 (1.61)</td>
<td>125 10 0.742 (0.030)</td>
</tr>
<tr>
<td>District 1 (W)</td>
<td>25 0.708 (0.109) 0.685 (0.109) 0.044 (0.130) 4.07 (0.99)</td>
<td>25 4 0.767 (0.033)</td>
</tr>
<tr>
<td>District 2 (W)</td>
<td>20 0.687 (0.102) 0.696 (0.095) 0.002 (0.158) 4.35 (1.02)</td>
<td>20 2 0.505 (0.056)</td>
</tr>
<tr>
<td>District 3 (W)</td>
<td>19 0.717 (0.108) 0.732 (0.074) 0.018 (0.135) 4.75 (0.99)</td>
<td>20 4 0.605 (0.101)</td>
</tr>
<tr>
<td>District 4 (W)</td>
<td>20 0.650 (0.124) 0.692 (0.113) 0.057 (0.135) 4.55 (1.07)</td>
<td>20 6 0.726 (0.075)</td>
</tr>
<tr>
<td>District 5 (W)</td>
<td>20 0.679 (0.121) 0.728 (0.092) 0.069 (0.117) 4.71 (0.99)</td>
<td>20 5 0.653 (0.076)</td>
</tr>
<tr>
<td>District 6 (W)</td>
<td>20 0.747 (0.090) 0.761 (0.088) 0.013 (0.107) 5.06 (1.02)</td>
<td>20 7 0.868 (0.039)</td>
</tr>
<tr>
<td>District 7 (E)</td>
<td>20 0.720 (0.125) 0.729 (0.097) 0.010 (0.129) 4.89 (1.19)</td>
<td>20 5 0.795 (0.045)</td>
</tr>
<tr>
<td>District 8 (E)</td>
<td>10 0.743 (0.165) 0.782 (0.089) 0.057 (0.172) 5.22 (1.09)</td>
<td>10 7 0.911 (0.077)</td>
</tr>
<tr>
<td>District 9 (E)</td>
<td>20 0.719 (0.131) 0.738 (0.107) 0.029 (0.100) 5.13 (1.10)</td>
<td>20 5 0.653 (0.076)</td>
</tr>
<tr>
<td>District 10 (E)</td>
<td>20 0.692 (0.145) 0.734 (0.096) 0.058 (0.148) 4.98 (1.09)</td>
<td>20 6 0.747 (0.074)</td>
</tr>
<tr>
<td>District 11 (E)</td>
<td>20 0.779 (0.092) 0.756 (0.077) 0.032 (0.085) 5.19 (0.94)</td>
<td>20 9 0.759 (0.071)</td>
</tr>
<tr>
<td>District 12 (E)</td>
<td>25 0.744 (0.081) 0.755 (0.059) 0.012 (0.120) 5.05 (0.81)</td>
<td>25 9 0.837 (0.048)</td>
</tr>
</tbody>
</table>

West (districts 1–6) and east (districts 7–12) regions were separated by the crest of the Cascade Mountain Range. SD, standard deviation.

\( a \) \( N \), no. of individuals.
\( b \) \( H_O \), observed heterozygosity.
\( c \) \( H_E \), gene diversity.
\( d \) \( F_{IS} \)-values calculated after Weir and Cockerham (1984).
\( e \) AR, allelic richness (adjusted to the smallest sample size). Values for AR (SD) were based on a minimum sample size of 229 individuals for total, 109 for east and west, and 7 for each district.
\( f \) \( n \), number of haplotypes.
\( g \) \( h \), haplotype diversity.
Table 3. Population pair-wise $F_{CT}$ values based on 15 microsatellites below diagonal, and population pair-wise $\Phi_{ST}$ values based on 949-base-pair mtDNA above diagonal, of bobcats from sampling districts in Oregon, USA, 2009–2010.

<table>
<thead>
<tr>
<th>District</th>
<th>1 (W)</th>
<th>2 (W)</th>
<th>3 (W)</th>
<th>4 (W)</th>
<th>5 (W)</th>
<th>6 (W)</th>
<th>7 (E)</th>
<th>8 (E)</th>
<th>9 (E)</th>
<th>10 (E)</th>
<th>11 (E)</th>
<th>12 (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (W)</td>
<td>0.032</td>
<td>0.069</td>
<td>−0.017</td>
<td>0.134</td>
<td>0.031</td>
<td>0.008</td>
<td>−0.035</td>
<td>0.155</td>
<td>0.116</td>
<td>0.119</td>
<td>0.126</td>
<td></td>
</tr>
<tr>
<td>2 (W)</td>
<td>0.017</td>
<td>0.029</td>
<td>0.004</td>
<td>0.163</td>
<td>0.048</td>
<td>0.099</td>
<td>−0.020</td>
<td>0.256</td>
<td>0.204</td>
<td>0.241</td>
<td>0.229</td>
<td></td>
</tr>
<tr>
<td>3 (W)</td>
<td>0.024</td>
<td>0.008</td>
<td>−0.036</td>
<td>0.077</td>
<td>0.043</td>
<td>0.020</td>
<td>−0.042</td>
<td>0.204</td>
<td>0.156</td>
<td>0.159</td>
<td>0.168</td>
<td></td>
</tr>
<tr>
<td>4 (W)</td>
<td>0.036</td>
<td>0.044</td>
<td>0.007</td>
<td>0.012</td>
<td>0.277</td>
<td>0.152</td>
<td>0.181</td>
<td>0.436</td>
<td>0.384</td>
<td>0.317</td>
<td>0.368</td>
<td></td>
</tr>
<tr>
<td>5 (W)</td>
<td>0.028</td>
<td>0.027</td>
<td>0.008</td>
<td>0.004</td>
<td>0.097</td>
<td>0.053</td>
<td>−0.059</td>
<td>0.073</td>
<td>0.031</td>
<td>0.104</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>6 (W)</td>
<td>0.049</td>
<td>0.054</td>
<td>0.006</td>
<td>0.008</td>
<td>0.012</td>
<td>0.004</td>
<td>−0.026</td>
<td>0.108</td>
<td>0.065</td>
<td>0.018</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>7 (E)</td>
<td>0.026</td>
<td>0.034</td>
<td>0.011</td>
<td>0.002</td>
<td>0.016</td>
<td>−0.011</td>
<td>0.007</td>
<td>0.076</td>
<td>0.027</td>
<td>0.064</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td>8 (E)</td>
<td>0.083</td>
<td>0.075</td>
<td>0.029</td>
<td>0.032</td>
<td>0.037</td>
<td>0.028</td>
<td>0.017</td>
<td>0.032</td>
<td>−0.024</td>
<td>0.055</td>
<td>−0.020</td>
<td></td>
</tr>
<tr>
<td>9 (E)</td>
<td>0.061</td>
<td>0.057</td>
<td>0.011</td>
<td>0.014</td>
<td>0.017</td>
<td>0.009</td>
<td>0.002</td>
<td>0.014</td>
<td>0.003</td>
<td>0.111</td>
<td>−0.036</td>
<td></td>
</tr>
<tr>
<td>10 (E)</td>
<td>0.050</td>
<td>0.045</td>
<td>0.013</td>
<td>0.014</td>
<td>0.022</td>
<td>0.016</td>
<td>0.006</td>
<td>0.013</td>
<td>0.008</td>
<td>0.001</td>
<td>−0.007</td>
<td></td>
</tr>
<tr>
<td>11 (E)</td>
<td>0.055</td>
<td>0.057</td>
<td>0.019</td>
<td>0.022</td>
<td>0.023</td>
<td>0.006</td>
<td>0.013</td>
<td>0.020</td>
<td>0.004</td>
<td>0.006</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

West (districts 1–6) and east (districts 7–12) regions were separated by the crest of the Cascade Mountain Range. Comparisons with asterisks were statistically significant after sequential Bonferroni correction.

between the 2 regions ($F_{CT} = 0.128$, $P < 0.01$), accounting for 12.0% of the total genetic variation. Again, most of the genetic variation (82.7%) resided within populations, and an additional 4.5% existed among populations within regions.

Results of both structure analyses (i.e., without and with prior location information) indicated strongest support for $K = 2$, based on the $L(K)$ and $\Delta K$-values (Fig. 5). The 2 clusters generally corresponded to east and west regions, but there was a high level of admixture between the 2 clusters (Fig. 1). In the analysis without prior location information, 46 individuals (18.5%) had $q$-values <0.75, and including location data resulted in 96 (38.6%) admixed individuals. Individuals from west districts 1 and 2 assigned to the west cluster with little evidence of admixture, especially in the model including prior sampling data. Similarly, individuals from the eastern-most districts (i.e., 9, 10, 11, and 12) assigned to the east cluster with little evidence of admixture.

However, individuals from centrally located counties or districts, near either side of the Cascades, showed considerable admixture or assignment to both clusters.

Results of the BAPS analyses were generally concordant with the Structure analyses (Fig. 1). Only the method using spatial clustering of individuals indicated a single population. The other 3 methods (aspatial clustering of individuals; aspatial and spatial clustering of groups) indicated $K = 2$ as the optimal solution. In the approach using aspatial clustering of individuals, though no individuals were considered admixed, individuals in the central counties were assigned to the 2 clusters in fairly even proportions (Fig. 1c). The methods using aspatial and spatial groups provided identical results, both separating districts 1 and 2 from the remainder of the state, excepting a handful of individuals (Fig. 1d). Identical results were also achieved when using county as the spatial groups.

**DISCUSSION**

Our results suggested that bobcats in the east and west regions of Oregon are genetically differentiated, rather than forming a single, panmictic population. This conclusion is supported by the Bayesian clustering methods with the microsatellite data and the AMOVA results for both marker types. Based on the AMOVA results, genetic differentiation was greatest based on mtDNA data than microsatellite data, which is a common finding among mammals in which females are the philopatric sex (Avise 2004). Alternatively, the weaker structure observed with microsatellites could be due to the 4-fold larger $N_e$, and thus longer time for fixation of alleles in nuclear DNA. Our genetic data (both microsatellites and mtDNA) also fit an isolation-by-distance model, in which geographically closer individuals were more genetically similar to each other than to individuals further away.

Given the taxonomic distinction of 2 subspecies in the state, the degree of genetic differentiation was surprisingly low. We did not observe reciprocal monophyly (i.e., fixed differences) in mtDNA between the 2 subspecies, because individuals on either side of the Cascade Mountain Range
often shared the same haplotype, and pair-wise $F_{ST}$-values based on microsatellite data were relatively low. Reciprocal monophyly of mtDNA reflects long-term evolutionary independence and has often been used as a litmus test for valid subspecies (Zink 2004), ideally supported with evidence for significant divergence of allele frequencies at nuclear loci (Moritz 1994). Though potentially useful, the approach has been criticized for being too stringent and prone to sampling error, and for overemphasizing neutral rather than adaptive genetic diversity (Crandall et al. 2000). In bobcats, range-wide genetic structure appears to be hierarchical, with 2 historically independent lineages cryptically divided along the Great Plains, each of which exhibit additional substructure (Reding et al. 2012). One could argue that bobcats in Oregon may therefore be better viewed as distinct populations nested within a western subspecies. We urge caution, however, in interpreting our results relative to subspecies designation. Our goal was not to validate the taxonomic status of the subspecies, but rather to determine whether they accurately reflect the geographic pattern of population genetic structure such that they could be used for management purposes. Any description or revision of subspecies taxonomy should ideally consider multiple criteria, including not only neutral genetic variation, but also morphological, behavioral, ecological, physiological, and/or adaptive genetic data (Haig et al. 2006).

Phylogeographic studies of plants and animals in the Pacific Northwest often report a genetic pattern similar to the one we observed with bobcats—that is a division between areas along the west coast and the remainder of the continent (Shafer et al. 2010). These breaks can often be attributed to the orogeny of the Cascade–Sierra Chain 5–2 million years ago (Brunsfeld et al. 2001), or alternatively to the hypothesis that during the Pleistocene, the coastal Pacific Northwest may have served as an isolated refugium for many species (Weir and Schluter 2004), cut off from conspecifics by glacial ice in the high-elevation mountains (Dyke et al. 2003). For many species in the Pacific Northwest, gene flow has continued to be very limited across the mountains, but our results indicate gene flow is somewhat less restricted among bobcats. We identified 21 individuals with genotypes more likely to have originated from the opposite side of the Cascades. Both males and females were evenly represented among these 21 putative migrants, which suggest that both sexes are capable of crossing the mountain range. The greatest number of migrants and the most admixture of genotypes were observed in sampling districts 6 and 8. This area of Oregon is characterized by more basin and range topography and mixtures of

Figure 3. Locations of 21 first-generation migrants identified by genetics analysis of bobcats in Oregon, USA, 2009–2010. Stars indicate counties where migrants were detected, with circles providing the number of migrants found.
land-cover types than are found in the heavily forested northerly portions of the Cascade Range. The mixture of mountains and valleys and ecotypes may therefore permit higher levels of gene flow across southern Oregon.

In addition to the main east–west division, our data suggested higher levels of genetic distinctiveness of bobcats from sampling districts 1 and 2, in the far northwestern portion of the state. These 2 districts showed the highest microsatellite pair-wise \( F_{ST} \)-values, and little evidence for admixture based on the clustering results. Bobcats in this area may be relatively isolated due to recent human-induced gene flow barriers, such as extensive development and agriculture in the Willamette Valley. A follow-up study using an individual-based landscape genetics approach, in which the sampling units are geo-referenced individuals rather than pre-defined populations, would be beneficial to provide a detailed understanding of how specific landscape features affect the connectivity of bobcats across Oregon (Manel et al. 2003).

Currently there are regulatory differences between the east and west regions that were established by Oregon Department of Fish and Wildlife based on the presumed subspecific boundaries. Our findings validate these regions as reflecting the actual structure of the bobcat population. The impetus behind implementing 2 management regions for bobcats was to manage for sustainable harvest levels under 2 different management situations. Management of high-value furbearers is often based on data that show that prevailing market demand influences harvest rate by an increase in the number of fur-takers and an increase in individual fur-taker effort (Erickson 1981). Pelt values of bobcats in eastern Oregon have frequently exceeded US$ 300/pelt in recent years and the number of bobcats harvested has generally been greatest in southeastern Oregon (Hiller 2011). Alternatively, genetic sampling at the management-unit level for bobcats has been used to inform decision-making based on genetic isolation of a single subspecies (e.g., MI; Millions and Swanson 2007) and could be used in other states with >1 subspecies of bobcat (e.g., WA; or similarly for other furbearers), particularly under conditions of relatively liberal harvest regulations and high harvest levels.

Population growth rate and density of bobcats in most jurisdictions is not well-known, but most state agencies assume a maximum harvest rate of 20%, which was originally estimated for populations in Idaho, USA (Knick 1990). Harvest is largely viewed as an additive source of mortality (Anderson and Lovallo 2003) and it appears to be independent of population density, which makes bobcat populations vulnerable to overharvest. Nearly all mortality is human-caused and some studies have revealed significant mortality related to poaching or incidental harvest when trapping for

Figure 4. Genetic assignments, based on mitochondrial DNA, of 250 bobcats collected in Oregon, USA, 2009–2010. (a) Median-joining network illustrating relationships among mtDNA ND5 haplotypes. Unique haplotypes are displayed as circles, with size proportional to haplotype frequency. The small white circle represents a missing or ancestral haplotype not present in the data set. Lines connecting haplotypes are one mutation long unless otherwise denoted by the number of hash marks, and haplotypes are labeled with their respective names. (b) Spatial distribution of haplotypes, color-coded as in the median-joining network. Sample from each county is shown as a pie chart, indicating the number of individuals with particular haplotypes.

Figure 5. Log-likelihood values and the \( \Delta K \) measure at each number of subpopulations (\( K \)) of STRUCTURE analysis of bobcats in Oregon, USA, 2009–2010, with (a) no location prior and (b) with location prior.
other species (Gosselink et al. 2011). With these factors in mind, many states have adopted relatively conservative harvest, especially where there is concern of over harvest of low-density populations (Rolley 1987) pressured by high harvest demand. This study documents the fact that bobcat populations in Oregon are demographically distinct units and that genetic differentiation should be taken into account in conservation planning to preserve the adaptive potential of the species, thus possibly mitigating whatever future landscape changes occur in Oregon.

**MANAGEMENT IMPLICATIONS**

The identification of distinct subpopulations of bobcats in Oregon provides a number of benefits to sustainable management of this natural resource. Bobcats are generally relatively susceptible to harvest and, given the substantial differences in pelt values of the subspecies and the associated differential regional demand for harvest opportunity, our findings allow for continued and defensible relative simplicity in the regulatory process. Given confirmation of relatively low rates of dispersal across the Cascade Mountain Range, each subspecies may essentially be considered a closed population for harvest management and population modeling purposes. However, this also implies that changes in ecological conditions within either region could affect the long-term viability of one or the other subpopulation. The genetic analysis identified that weaker barriers to dispersal across the Cascades occur in the southern part of the state. Future management decisions may be affected if climate change allows for increased connectivity between subspecies through habitat corridors, such as decreased snow depths and snow persistence, or increased high-elevation abundance and persistence of bobcats in the Cascade Mountain Range.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


Read, J. A. 1981. Geographic variation in the bobcat (Felicis rufus) in the southcentral United States. Thesis, Texas A&M University, College Station, USA.


Reid, A. E. 2006. Spatial genetic structure of four bobcat populations in the southeastern US. Thesis, University of Georgia, Athens, USA.


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