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Pleistocene and ecological effects on continental-scale genetic differentiation in the bobcat (*Lynx rufus*)

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Abstract

The potential for widespread, mobile species to exhibit genetic structure without clear geographic barriers is a topic of growing interest. Yet the patterns and mechanisms of structure—particularly over broad spatial scales—remain largely unexplored for these species. Bobcats occur across North America and possess many characteristics expected to promote gene flow. To test whether historical, topographic or ecological factors have influenced genetic differentiation in this species, we analysed 1 kb mtDNA sequence and 15 microsatellite loci from over 1700 samples collected across its range. The primary signature in both marker types involved a longitudinal cline with a sharp transition, or suture zone, occurring along the Great Plains. Thus, the data distinguished bobcats in the eastern USA from those in the western half, with no obvious physical barrier to gene flow. Demographic analyses supported a scenario of expansion from separate Pleistocene refugia, with the Great Plains representing a zone of secondary contact. Substructure within the two main lineages likely reflected founder effects, ecological factors, anthropogenic/topographic effects or a combination of these forces. Two prominent topographic features, the Mississippi River and Rocky Mountains, were not supported as significant genetic barriers. Ecological regions and environmental correlates explained a small but significant proportion of genetic variation. Overall, results implicate historical processes as the primary cause of broad-scale genetic differentiation, but contemporary forces seem to also play a role in promoting and maintaining structure. Despite the bobcat's mobility and broad niche, large-scale landscape changes have contributed to significant and complex patterns of genetic structure.

Keywords: bobcat, landscape genetics, *Lynx rufus*, phylogeography, Pleistocene, suture zone

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Introduction

In both terrestrial and aquatic environments, one can find numerous species that are vagile and distributed continuously across their ranges. Without geographic or habitat barriers to dispersal, unimpeded gene flow is expected to limit the development of population genetic structure and result in either (i) a genetically panmictic population, when dispersal is unbounded relative to the species' range and mating occurs at random (e.g., Als *et al.* 2011), or (ii) a simple pattern of isolation by distance, when dispersal is local and mating occurs more frequently among neighbours (e.g., Platt *et al.* 2010). Due in part to this assumption, many population genetic studies have concentrated on species that are habitat specialists or have fragmented distributions (Frankham *et al.* 2002), and abundant, widespread species have historically received less attention. There is increasing interest, however, in testing for genetic structure in wide-ranging organisms (Geffen *et al.* 2004; Hull *et al.* 2008; Sacks *et al.* 2008; Tammeleht *et al.* 2010). This line of research not only provides insight into the biology of ecologically and often economically important species, but also improves our general understanding of whether and how differentiation can emerge

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without the overriding element of geographic isolation (i.e., development of cryptic population structure).

Despite predictions of panmixia or isolation by distance, several recent studies of large mammals have provided evidence that ecological mechanisms such as natal habitat-biased dispersal can lead to discrete genetic subdivision within continuously distributed species. For example the genetic structure of coyotes (*Canis latrans*; Sacks *et al.* 2008) and mule deer (*Odocoileus hemionus*; Pease *et al.* 2009) in California, USA, closely parallel habitat subdivisions in the state, and habitat-related variables such as climate and vegetation types explain a significant portion of the cross-continental genetic variation observed in wolves in Europe and North America (*Canis lupus*; Geffen *et al.* 2004; Pilot *et al.* 2006; Carmichael *et al.* 2007). Naturally, these observations are somewhat limited, by either a regional scope or focus on social organisms, and additional work is needed to identify whether the phenomenon of ecological divergence applies more broadly. Furthermore, topographic features such as rivers and mountains, although unlikely to function as absolute dispersal barriers for mobile species, can nonetheless restrict gene flow (Rueness *et al.* 2003; Frantz *et al.* 2010). At continental scales, it is also important to consider that historical factors such as glaciation events can leave a lasting mark on current population genetic structure (Hewitt 1996). It is unclear, however, whether such events have had significant genetic effects in mobile generalist species, because (i) they may have experienced less restriction in their distributions and movements during the Pleistocene (Barton & Wisely 2012) and (ii) high levels of contemporary gene flow may have erased any evidence of earlier isolation or expansion events (Smith *et al.* 2011). Few studies have examined genetic variation both across broad spatial scales and at high resolution to evaluate comprehensively the influence of contemporary and historical processes in structuring widespread species.

Bobcats are one of the most common and broadly distributed species in North America (Fig. 1; Anderson & Lovallo 2003). These medium-sized felids are consummate habitat-generalists, thriving in a wide range of environments, and although they are strict carnivores, they have a diverse diet that can include lagomorphs, rodents, deer, birds, reptiles, fish and insects (Larivière & Walton 1997). Bobcats are solitary, territorial and have a polygynous mating system (Anderson & Lovallo 2003). They are capable of dispersing long distances (>150 km) (Knick & Bailey 1986; Gosselink *et al.* 2011), although dispersal distances vary greatly among individuals and study areas (Anderson & Lovallo 2003). Despite anthropogenic changes across North America, bobcats have retained the vast majority of their original

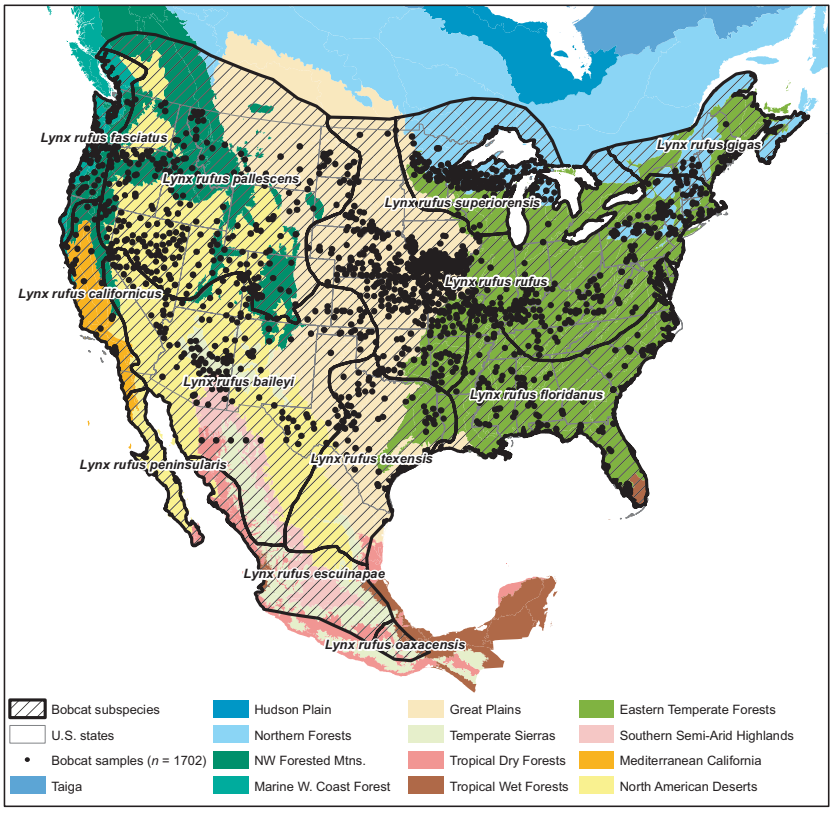

Fig. 1 Historic ranges of 12 bobcat (*Lynx rufus*) subspecies (Hall 1981), delineation of level 1 ecological regions of North America (Commission for Environmental Cooperation 1997) and locations of bobcat samples (n = 1702) used in this study.

range and appear to be increasing and expanding into once-extirpated areas, such as portions of the agricultural Midwest and heavily populated mid-Atlantic states (Deems & Pursley 1978; Woolf & Hubert 1998; Linde *et al.* 2012). Based on government surveys, Roberts & Crimmins (2010) estimate the total US bobcat population at approximately 2–3 million individuals. The species was also apparently abundant in the past, as it is commonly found in Pleistocene fossil deposits across much of the USA (Graham & Lundelius 2010).

Collectively, these characteristics—habitat and prey generalist, solitary, vagile, currently and historically abundant and widespread—predict little genetic differentiation across the range of this species. Several local and regional genetic studies, however, support the potential for population structuring in this species because of topographic barriers including major waterways (Straits of Mackinac in Michigan; Williams 2006; Millions & Swanson 2007), intensive row crop agriculture (Midwest Corn Belt; Reding 2011) and mountains (Cascade Range in Oregon; Reding *et al.* 2011). In addition, the boundaries of 12 morphologically defined bobcat subspecies often correspond with transitions between major ecological regions (i.e. ecotones) or steep clines in climatic variables (Fig. 1; Hall 1981), suggesting that environmental and ecological factors may play a prominent role in differentiating this species across its range. Croteau (2009) found limited phylogeographic structure at mtDNA control region with samples from 14 states and two Canadian provinces, but suggested a potential historical subdivision between western bobcats (California, Wyoming, Nevada and North Dakota) and those from the rest of the sampled range. Unfortunately, the lack of a broad-scale, comprehensive genetic study makes it difficult to evaluate the general importance of topographic, ecological and historical factors in shaping genetic patterns.

In this study, we present a phylogeographic and population genetic analysis of *Lynx rufus* across the entire USA, its primary range. Our goal was to assess quantitatively the spatial genetic patterns in this widespread species to test whether it exhibits a simple pattern of isolation by distance, or whether more complex patterns emerge from historical and contemporary processes. To achieve this goal, we sampled over 1700 geo-referenced individuals collected from throughout the majority of the bobcat range, including nine of the 12 recognized subspecies, and assessed genetic variation at ~1 kb of mtDNA sequence and 15 microsatellite loci using both individual and population-based analyses. We performed several analyses to test for potential influences of Pleistocene glaciation events on current genetic patterns. We quantified how well major ecological regions explain genetic variation and tested for specific environmental correlates (elevation, temperature and precipitation measurements) to contemporary patterns of genetic structure, as these variables are important determinants of relevant ecological differences such as habitat. We also evaluated the influence of two prominent topographic features within the bobcat's range: the Mississippi River and Rocky Mountains. Through this continental-scale survey, we provide insight into the potential mechanisms involved in establishing and maintaining population divergence in this common North American carnivore.

Methods

Sample collection

The sample set consists of tissue and DNA (n = 1702) from live and dead bobcats collected between 1994 and 2011 (Fig. 1; Appendix S1, Supporting information). These samples include data from a stratified random subset of those used in regional examinations in Oregon (n = 108 of 250 total; Reding *et al.* 2011) and the Midwest (n = 755 of 1447 total; Reding 2011), as well as additional samples obtained and analysed specifically for this study (n = 839). In total, the sample represents 45 states and includes five samples from Mexico. See Supporting information for details on processing location data. The Mexico samples did not have location data and thus were assigned spatial coordinates for visualization purposes only and omitted from spatial analyses. Although we primarily employed individual-based approaches that do not require a priori populations, we grouped individuals into 52 'sample groups' based on geographic proximity and topography to permit the use of several population-based approaches (Fig. S1, Supporting information). We used ArcGIS 10 (ESRI) to calculate the geographic mean center of individuals assigned to each sample group, which we used as the spatial coordinates for the given group.

Laboratory analysis

We extracted DNA using DNeasy (Qiagen) or IDPure (IDLabs) purification kits. We amplified and sequenced a 949-bp portion (excluding the primers) of the mtDNA NADH dehydrogenase subunit 5 (ND5) gene (see Supporting information) and used Sequencher 4.6 (Gene Codes Corporation) to edit, assemble and align the sequences. We genotyped individuals at 15 autosomal microsatellite markers developed from domestic cat (*Felis catus*): FCA008, FCA031, FCA043, FCA077, FCA082, FCA090, FCA096, FCA132, FCA149, FCA391, FCA559 (Menotti-Raymond *et al.* 1999) and FCA740 (Menotti-Raymond *et al.* 2005); Canada lynx (*Lynx canadensis*):

Lc109 and Lc111 (Carmichael *et al.* 2000) and bobcat: BCE5T (Faircloth *et al.* 2005). See Table S1, Supporting information, for polymerase chain reaction (PCR) and electrophoresis conditions. Alleles were scored using the software GENEMAPPER 4.0 (Applied Biosystems).

mtDNA analysis

To investigate historical population structure, we examined relationships among unique mtDNA haplotypes using the Bayesian phylogenetic method of MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001; see Supporting information for details). As intraspecific genealogies are often not well represented by bifurcating trees (Posada & Crandall 2001), we also constructed a median joining network using program NETWORK 4.5 (Bandelt *et al.* 1999). The haplotypes were nested into hierarchical clades to visualize higher order patterns of association (Templeton & Sing 1993).

To test for evidence of demographic expansion resulting from postglacial recolonization, we used ARLEQUIN 3.5 (Excoffier *et al.* 2005) to calculate Fu's F_S (Fu 1997) for different phylogenetic subunits indicated by the phylogenetic tree and haplotype network. Recent demographic expansions lead to significant negative values for Fu's F_S. We further evaluated evidence for sudden population growth in each identified clade by constructing mismatch distributions of pairwise nucleotide differences between haplotypes. Mismatch distributions are usually ragged in populations at demographic equilibrium, but unimodal in populations having experienced a recent expansion (Slatkin & Hudson 1991; Rogers & Harpending 1992). We estimated the parameters of a demographic expansion: $\tau = 2ut$, $\theta_0 = 2uN_0$ and $\theta_1 = 2uN_1$ (Schneider & Excoffier 1999). The parameters θ_0 and θ_1 describe the population sizes before and after the expansion, whereas τ reflects the time to expansion. We employed a parametric bootstrap method (1000 permutations) to obtain confidence intervals for the parameters and to test the validity of the sudden expansion model, using the sum of squared deviations between the observed and expected mismatch distributions as the test statistic (Schneider & Excoffier 1999). We estimated the time since expansion from the equation $t = \tau/2u$, where τ is estimated from the mismatch distribution, u is mutation rate per generation for the entire sequenced region and t is measured in units of generations. We used a generation time of 2.3 years that was estimated from demographic data (Gosselink *et al.* 2011) and $u = 6.467 \times 10^{-5}$ cumulative substitutions/generation (see Supporting information). As several sources of error could affect our estimate of u, we also considered a lower (2.345×10^{-5}) and higher (1.217×10^{-4}) estimate (see Supporting information).

We used ARLEQUIN to calculate basic estimates such as haplotype (h) and nucleotide (π) diversities within the total sample and each sample group. To quantify whether genetic diversity decreased with increasing latitude, a pattern consistent with postglacial expansion (Hewitt 2000), we performed regression analyses in SAS 9.2 (SAS Institute Inc.) and included sample size and sample group area as covariates. To measure genetic differentiation, we used ARLEQUIN to estimate pairwise Φ_{ST} values between the sample groups and to conduct a Mantel test (999 permutations; Mantel 1967) for isolation by distance. To further evaluate patterns of mtDNA population structure, we performed spatial analysis of molecular variance using the program SAMOVA 1.0 (Dupanloup *et al.* 2002). This approach identifies the structure that maximizes the total genetic variance attributable to differences among groups of populations (Φ_{CT}). We tested a range for the number of groups (K) from 2 to 10.

Microsatellite data analysis: descriptive statistics

For all data analysis procedures using the microsatellite data, we omitted samples with fewer than eight loci genotyped. With the remaining samples ($n = 1680$), we calculated for each locus the total number of alleles, observed (H_O) and expected (H_E) heterozygosity and Weir & Cockerham's (1984) F_{IS}, and tested for deviations from Hardy–Weinberg (HW) and linkage equilibrium using GENEPOP 4.0 (Rousset 2008). To account for multiple tests, we applied the sequential Bonferroni correction (Rice 1989) to significance values (nominal $\alpha = 0.05$). In addition, we used FSTAT 2.9.3 (Goudet 2001) to calculate allelic richness (AR) within sample groups, corrected for differences in sample size. We used ARLEQUIN to calculate pairwise F_{ST} as a measure of differentiation between the sample groups, and to conduct a Mantel test (999 permutations) to test for a positive relationship between genetic ($F_{ST}/(1-F_{ST})$) and *ln*-transformed geographic distance. In addition, we used GENALEX 6.41 (Peakall & Smouse 2006) to calculate pairwise genetic and geographic distance between all individuals, and again applied a Mantel test with 999 permutations.

Spatial principal components analysis

Multivariate analyses, such as principal components analysis, offer several advantages for genetic analysis in that they do not rely on assumptions of HW and linkage equilibrium, and they can reveal genetic clines as well as discrete populations (Jombart *et al.* 2009). We performed spatial principal components analysis (sPCA) on the microsatellite data to examine contemporary

genetic structure. This reduced space ordination method, developed by Jombart *et al.* (2008), takes into account not only the genetic variation between individuals, but also their spatial autocorrelation, by finding synthetic variables that optimize the product of allele frequency variance and Moran's *I*, an index of spatial autocorrelation. Highly positive eigenvalues, therefore, reflect axes with both a large variance and a global (i.e., positive spatial autocorrelation) structure. We used the *adegenet* package (Jombart 2008) of the software R (R Development Core Team) to perform sPCA as well as a permutation procedure to test for a significant global pattern in the data. We used a distance-based connection network such that individuals separated by ≤250 km (which resulted in all samples having at least one connection) were considered neighbours.

Bayesian clustering analysis

Geographic patterns of genetic structure can often entail complex combinations of clines, clusters and patterns of isolation by distance (Francois & Durand 2010), and multiple analysis methods can provide complimentary information regarding such patterns (Balkenhol *et al.* 2009). Thus, as another means of examining patterns of microsatellite genetic structure, we employed a spatial Bayesian clustering method using the program BAPS 5 (Corander *et al.* 2008). We first performed a mixture analysis using the 'spatial clustering of individuals' model. The analysis consisted of five iterations of each value of K_{max} (the maximum number of populations) for the range K_{max} = 1–20. This step determines the optimum number of genetic clusters in the sample based on the partition with the maximum likelihood [L(*K*)] and highest posterior probability (*p*), and then assigns each individual to a cluster. In the second step, we performed an admixture analysis conditional on the assignments from the previous step. We used 500 simulations from the observed allele frequencies to estimate admixture coefficients (*q*), which provide the proportion of an individual's genotype attributed to each of the identified populations. To provide support for the clusters inferred from the spatial method, we also performed aspatial Bayesian clustering using program STRUCTURE (Pritchard *et al.* 2000; see Supporting information). For each of the populations inferred by BAPS, we calculated standard genetic estimates following the same procedures outlined for the total sample and sample groups.

Topographic barriers

As a first approach to evaluate genetic boundaries, we visually examined the individual cluster memberships from BAPS to determine if the patterns coincided with topographic features (Safner *et al.* 2011). To evaluate formally the influence of two prominent features—the Mississippi River and the Rocky Mountains (i.e. Continental Divide)—we used partial distance-based redundancy analyses (dbRDA), a form of multivariate multiple regression (Legendre & Anderson 1999; McArdle & Anderson 2001). To focus specifically on the effects of these potential barriers, we limited our analysis to include only individuals within 300 km (approximately the longest recorded dispersal distance; Johnson *et al.* 2010) on either side of the given linear feature. We also omitted samples from Minnesota and Wisconsin when evaluating the Mississippi River as the headwaters are located in this region. We coded the given barrier variable with a 0 if the animal was located west of the feature and 1 if east, and calculated pairwise genetic distance between individuals using GENALEX. We then used DISTLM v.5 (Anderson 2004) to test the relationship between genetic differentiation among individuals (dependent variable) and the topographic feature (predictor variable), having first fit *X* and *Y* coordinates as covariables to examine the extent to which the feature explains genetic differentiation above and beyond that explained by geographic distance alone. Statistical significance was tested using 999 permutations of the multivariate residual matrix under the reduced model (Anderson & Legendre 1999).

Ecological and environmental correlates

To examine whether ecological and environmental factors may be important in differentiating this species across its range, we conducted a series of distance-based redundancy analyses to test for a relationship between genetic differentiation among individuals (dependent variable) and several geographic, ecological and climate variables (predictor variables) (Geffen *et al.* 2004; Pilot *et al.* 2006; Carmichael *et al.* 2007). The predictor variable sets include (i) *X* and *Y* coordinates (i.e. longitude and latitude); (ii) elevation; (iii) temperature (annual mean temperature, mean diurnal range, temperature seasonality (standard deviation*100), maximum temperature of the warmest month and minimum temperature of the coldest month); (iv) precipitation (annual precipitation, precipitation seasonality (standard deviation*100), precipitation of the wettest quarter, precipitation of the driest quarter, precipitation of the warmest quarter and precipitation of the coldest quarter) and (v) ecoregion (coded as nine dummy variables). The temperature and precipitation variables represent 11 of the 19 BIOCLIM variables from WORLDCLIM (version 1.4; Hijmans *et al.* 2005), interpolated to 1-km spatial resolution. The elevation data were also at 1-km resolution from WorldClim. We used the level 1

ecological regions of North America (Commission for Environmental Cooperation 1997). The relationship between the genetic distance matrix for $n = 1675$ individuals and each of the five sets of predictor variables was first analysed separately using dbRDA marginal tests with 999 unrestricted permutations of the genetic distance matrix. We also tested the relationship to each of the individual predictor variables, except for the ecoregion dummy variables which we only examined as a set. Next, partial dbRDA was performed for the elevation, temperature, precipitation and ecoregion predictor variable sets, having first fit X and Y coordinates as covariables as previously described. We repeated this analysis on the individual predictor variables. Both the marginal and partial dbRDAs were conducted in DISTLM v.5. Finally, we performed a stepwise forward selection procedure to identify the subset of variables or sets of variables that provide the best model explaining genetic differences among individuals (Pilot *et al.* 2006; Carmichael *et al.* 2007). We used program DISTLM forward 1.3 (Anderson 2003), with significance assessed with 999 permutations of the multivariate residual matrix under the reduced model. Several of the predictor variables are themselves correlated, but this sequential approach allowed us to control for these correlations as the proportion of variation explained by each variable is conditional on the variables already included in the model.

Results

mtDNA

Among 1128 individuals sequenced, we identified 73 unique haplotypes, encompassing 79 substitutions (75

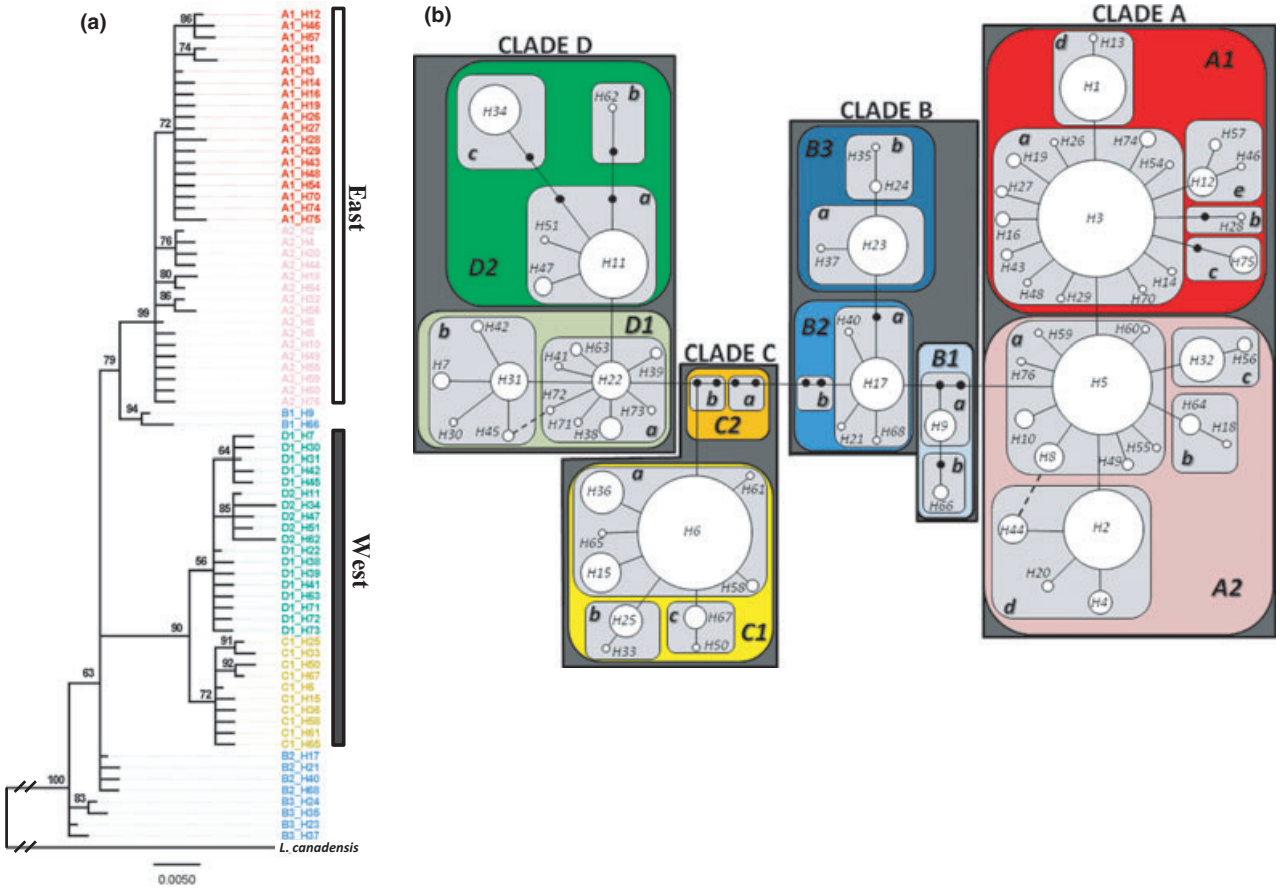

Fig. 2 (a) Bayesian tree showing genealogical relationships among 73 unique mtDNA haplotypes found among 1128 bobcat samples. Labels at nodes indicate posterior probability values. Labels at tips reflect name of first-level haplotype subclade, followed by haplotype name. A Canada lynx sequence was used as the outgroup. (b) Median-joining network illustrating relationships among the 73 haplotypes. Each white circle represents a unique haplotype, with size proportional to frequency. Haplotype names (e.g. H2) are provided in or near each circle. Small black circles represent missing haplotypes not present in the data set. Lines connecting haplotypes are one mutation long. Haplotypes are nested into hierarchical clades, with the highest level grouped by dark grey boxes (clades A to D), the second level grouped in coloured boxes and labelled in upper case letter–number combinations (subclades A1 to D2) and the first level grouped by light grey boxes and labelled in lower case letters (subclades A1a to D2c).

transitions and four transversions) observed at 78 polymorphic sites across the 949-bp region. Overall, haplotype diversity = 0.917, nucleotide diversity = 0.007 and mean number of pairwise differences = 6.67. Both the Bayesian tree (Fig. 2a) and median-joining network (Fig. 2b) supported two main clades (Eastern: subclade A; Western: subclades C and D) that diverged from more ancestral haplotypes (subclade B). Haplotypes from subclades C and D on the network are separated by more than seven substitutions from any others. These C and D haplotypes also group together on the Bayesian tree with moderately high support (BPP = 0.90), and geographically are only found in the western USA (Fig. 3). Although subclades C and D each form monophyletic groups, they have BPP < 0.8 as well as overlapping geographic ranges in the west. Haplotypes from subclade A also form a well-supported group (BPP = 0.99), and they are found primarily in the eastern USA, although some are also scattered across the west. Haplotypes from subclade B are basal on the tree and do not form a monophyletic clade. These ancestral haplotypes are less common and are primarily restricted to either the northwest or the southeast parts of the country (Fig. 3).

For individuals falling into the Eastern clade (subclade A; $n = 594$), F_S was significantly negative (Table 1) and the mismatch distribution showed a distinct unimodal peak (Fig. 4a), indicative of recent demographic expansion. The test statistic, however, rejected the expansion model, although this is likely attributed to the large number of samples resulting in high power to detect even slight deviations from the model. Furthermore, the analysis estimated a significant change in population size from θ_0 to θ_1, as 95% CIs around θ_0 always included 0, whereas CIs around θ_1 did not. For individuals in the Western clade (subclades C and D; $n = 424$), F_S was also significantly negative (Table 1), and the mismatch distribution did not differ signifi-

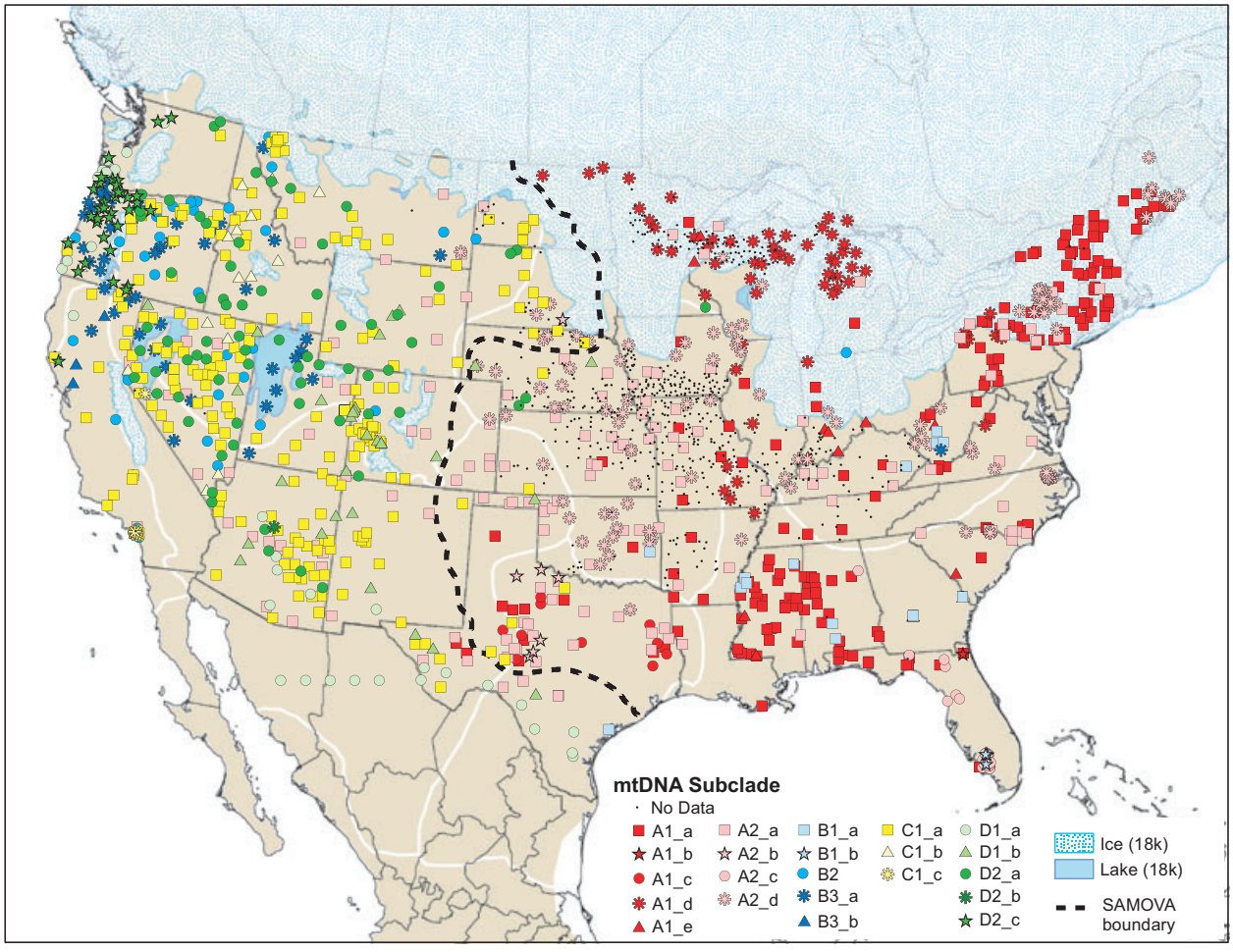

Fig. 3 Distribution of bobcat mtDNA haplotype subclades. Names of subclades are as in Fig. 2. The dashed black line represents the approximate location of the genetic boundary indicated by SAMOVA. The map also illustrates the location of ice sheets and lakes during the last glacial maximum (Dyke *et al.* 2003), and white lines reflect bobcat subspecies (Hall 1981).

Table 1 Summary of diversity and demographic statistics for an eastern and western clade of bobcat mtDNA haplotypes

Estimates	Eastern (subclade A)	Western (subclades C/D)
Sample size	594	424
Haplotypes	35	28
h	0.822 ± 0.009	0.783 ± 0.017
π	0.0016 ± 0.0010	0.0025 ± 0.0015
Mean no. pairwise differences	1.485 ± 0.899	2.416 ± 1.314
Fu's F_S (P-value)	−27.23 (<0.001)	−10.42 (0.015)
SSD (P-value)	0.0046 (0.015)	0.0185 (0.331)
τ (95% CI)	1.605 (1.424–1.980)	3.793 (0.598–7.168)
θ_0 (95% CI)	0 (0–0.069)	0 (0–1.067)
θ_1 (95% CI)	∞ (19.469–∞)	4.314 (2.168–∞)
Expansion time (95% CI)	28 540 ybp (25 321–35 208)	67 447 ybp (10 634–127 461)
Expansion time (95% CI)*	15 165 ybp (13 455–18 708)	35 838 ybp (5650–67 726)
Expansion time (95% CI)†	78 717 ybp (69 840–97 109)	186 027 ybp (29 329–351 554)

h – haplotype diversity; π – nucleotide diversity; SSD – sum of squared deviations between observed and expected mismatch distributions; τ – parameter reflecting time to expansion; θ_0 – parameter reflecting population size prior to expansion; θ_1 – parameter reflecting population size after expansion; ybp – years before present.
*Based on the high estimate of u.
†Based on the low estimate of u.

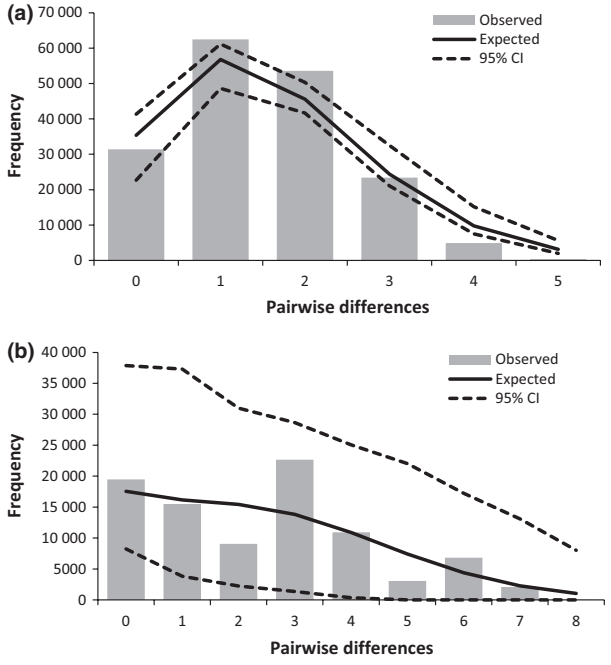

Fig. 4 Mismatch distributions for the Eastern clade (a), which consists of all individuals with mtDNA haplotypes from subclade A, and the Western clade (b), which consists of all individuals with mtDNA haplotypes from subclades C and D.

cantly from the expected distribution under the stepwise expansion model (Fig. 4b). Again, estimates of θ_0 and θ_1 differed significantly. Based on estimates of τ, the time of expansion was dated at approximately 28 500 ybp for the Eastern clade, and 67 000 ybp for the Western clade (Table 1). Using a lower estimate for u pushed the dates back to approximately 80 000 and 185 000 ybp for Eastern and Western clades respectively, whereas a higher estimate of u moved the dates to 15 000 and 36 000 ybp.

Haplotype diversity (h) tended to decrease with increasing latitude (Fig. S2; Table S2, Supporting information), but the relationship was significant only when considering sample groups from the eastern USA ($P < 0.05$). Nucleotide diversity estimates did not show a strong latitudinal pattern overall or in either region ($P > 0.05$), but were notably smaller in the eastern USA (Fig. S2; Table S2, Supporting information). Estimates of pairwise Φ_{ST} values between the sample groups ranged from 0 to 1 and demonstrated a significant pattern of isolation by distance ($r = 0.658$, $P = 0.001$; Fig. S3a, Supporting information). Results of the SAMOVA indicated that the grouping with the highest Φ_{CT} value (0.600, $P < 0.001$) differentiated eastern and western sample groups ($K = 2$; Fig. 3 and Fig. S1, Supporting information). For $K > 2$, the final genetic structure did not change except that with each increase in K, a single sample group was distinguished from the others; although Φ_{CT} values were still significant, they declined with increasing K. Thus, we consider two populations (eastern vs. western sample groups) as the best characterization of mtDNA structure.

Microsatellite descriptive statistics

We expected the total data set to exhibit signs of the Wahlund effect (i.e. reduction of heterozygosity caused by population structure) if population structure existed

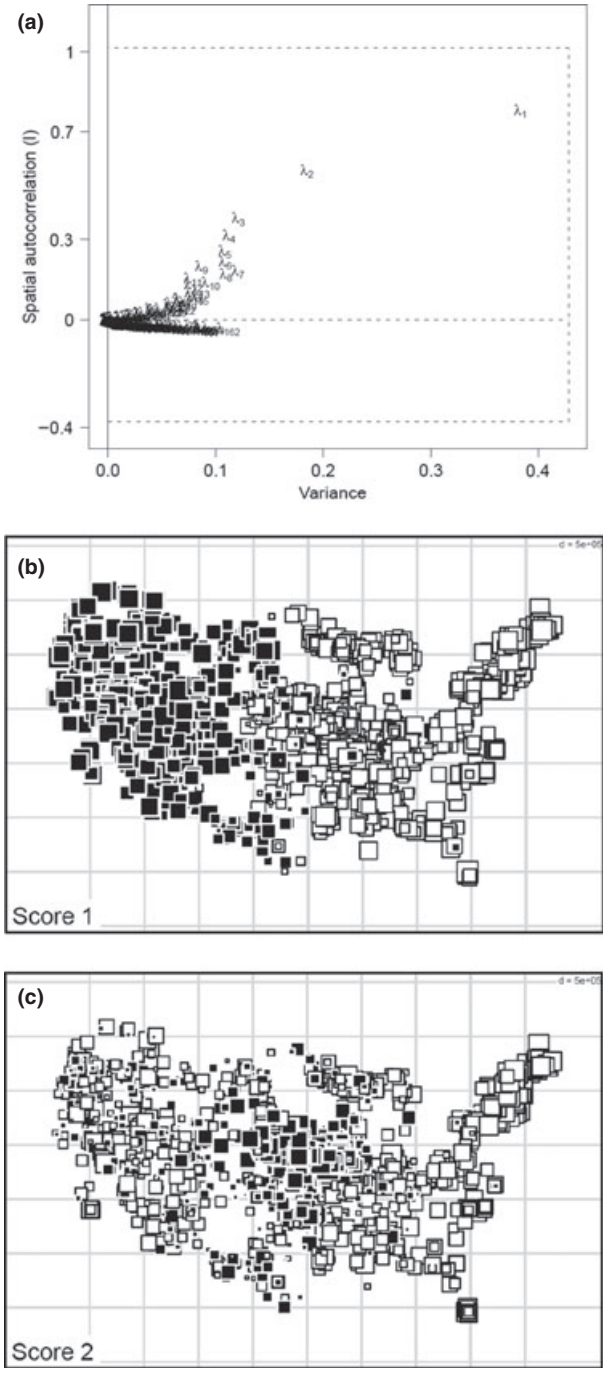

Fig. 5 Results of spatial PCA analysis. Plot (a) shows each spatial PCA eigenvalue, decomposed into a variance and spatial autocorrelation component. The vertical dashed line indicates the maximum attainable variance, defined as the one from ordinary PCA. The horizontal dashed lines above and below 0 indicate the range of variation of Moran's I components. Plots (b) and (c) show the scores for the first and second principal components respectively. Each square represents the score of a genotype (white indicates negative and black positive values, and larger squares reflect greater absolute values) and is positioned by its spatial coordinates.

within the study area. Indeed, all 15 microsatellite markers deviated from HW equilibrium, each showing a significant deficit of heterozyogotes relative to random-mating expectations (Table S1, Supporting information). In addition, 50 of the 105 locus pairs deviated from linkage equilibrium after sequential Bonferroni adjustments and all possible pairs had P-values < 0.05, despite the fact that these loci are unlikely to be physically linked (Table S1, Supporting information). Thus, this nonrandom association of alleles may also be attributed to population structure. In contrast to the total sample, the sample groups did not deviate significantly from HWE or LE. Measures of microsatellite genetic diversity (H_E and AR) within sample groups decreased with increasing latitude ($P < 0.05$; Fig. S2; Table S2, Supporting information). Estimates of pairwise F_{ST} values ranged from 0 to 0.188 and demonstrated a significant pattern of isolation by distance ($r = 0.716$, $P = 0.001$; Fig. S3b, Supporting information). The individual-based genetic distances also demonstrated a weak but statistically significant pattern of isolation by distance ($r = 0.243$, $P = 0.001$).

Spatial principal components analysis

In sPCA, Jombart *et al.* (2008) recommend using an abrupt decrease in the eigenvalues to indicate the boundary between strong and weak structures. For this data set, the first eigenvalue was considerably larger than the others, and the second eigenvalue also stood out (Fig. 5a). The scores from the first principal component revealed a longitudinal (east–west) cline, differentiating bobcats in the eastern USA from those in the western half of the country, but with genotypes in the center of the range having less extreme scores than those nearer the coasts (Fig. 5b). This pattern exhibited a strong signal of positive spatial autocorrelation ($I = 0.778$) and represented a considerable proportion of the entire genetic variability (var = 0.383). The scores of the second principal component differentiated bobcats in the central USA from others (Fig. 5c) ($I = 0.555$; var = 0.185). Subsequent scores were more challenging to interpret (results not shown). The permutation test confirmed the existence of at least one global pattern ($P = 0.001$).

Bayesian clustering analysis

In the BAPS analysis, $K = 10$ was the optimal solution [$L(K) = -87041.8$; $P = 0.927$)] (Fig. 6). Similar to the sPCA, bobcats in eastern and western USA were differentiated, with a third population detected between them. BAPS recognized several additional populations in the northern USA, and clusters of individuals in southern Florida and southern California were also identified. The 10 inferred populations include coastal Oregon; western and southern

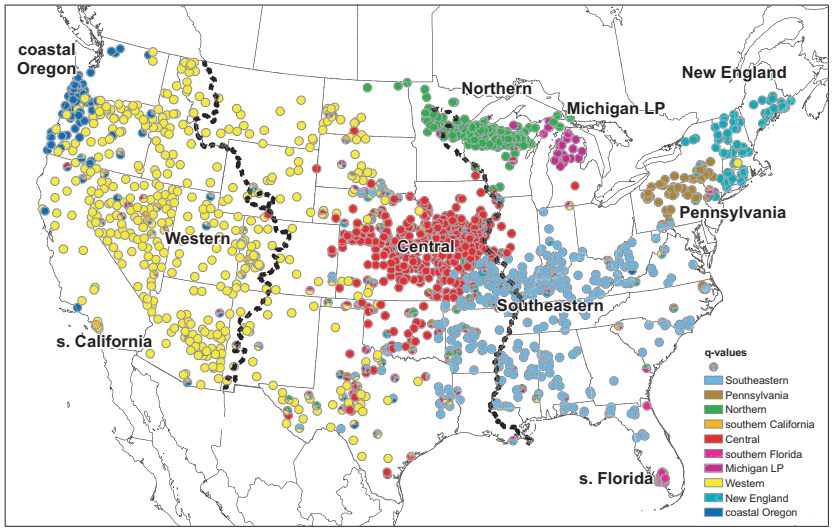

Fig. 6 Results of BAPS admixture analysis, showing each individual depicted as a pie chart reflecting its ancestry coefficients (q) for each of the 10 inferred populations. The black dashed lines depict two prominent topographic features: the Continental Divide in the west and the Mississippi River in the east.

Table 2 Summary of microsatellite genetic variation, averaged across 15 loci, for the total bobcat sample and 10 populations inferred from BAPS mixture analysis.

Group	N	H_O (SD)	H_E (SD)	F_{IS}	AR
Total	1680	0.736 (0.047)	0.802 (0.050)	0.082*	11.78
Coastal Oregon	58	0.692 (0.094)	0.722 (0.078)	0.041	5.35
Western	541	0.750 (0.061)	0.778 (0.066)	0.035	6.56
Central	418	0.750 (0.059)	0.771 (0.053)	0.028	6.14
Northern	126	0.718 (0.086)	0.745 (0.077)	0.038	5.52
LP of Michigan	22	0.652 (0.098)	0.655 (0.105)	−0.006	4.30
Southeastern	356	0.736 (0.075)	0.775 (0.075)	0.051	6.49
Southern Florida	25	0.730 (0.098)	0.724 (0.111)	−0.016	5.52
Pennsylvania	50	0.734 (0.105)	0.744 (0.074)	0.016	5.58
Upper New England	71	0.661 (0.103)	0.679 (0.091)	0.025	4.86
Southern California	13	0.677 (0.168)	0.656 (0.127)	−0.031	4.73

H_O, observed heterozygosity; H_E, expected heterozygosity; SD, standard deviation; F_{IS}, Weir & Cockerham's (1984) inbreeding coefficient, with values significantly different from zero marked with an asterisk; AR, allelic richness, adjusted to a minimum sample size of 13; LP, lower peninsula.

California; central, northern and lower peninsula (LP) of Michigan; southeastern, southern Florida; Pennsylvania and upper New England (Fig. 6). These populations did not deviate significantly from HWE or LE (Table 2), and all were significantly differentiated from one another based on pairwise F_{ST} values (Table S3, Supporting information). Results of the STRUCTURE analysis revealed patterns concordant with those detected by the other methods; they suggested hierarchical structure with two clusters at the first level corresponding to the east–west divergence observed through sPCA, and subsequent clusters largely matching those detected by BAPS (Figs S4–S6, Supporting information).

Hypothesized topographic barriers

Our examination of the influence of the Rocky Mountains included $n = 221$ samples, and the Mississippi River included $n = 367$. Results of the partial dbRDAs indicated that neither the Rocky Mountains ($F = 2.76$, $P = 0.063$) nor the Mississippi River ($F = 2.21$, $P = 0.122$) was significantly associated with genetic differentiation after accounting for the influence of geographic distance. Visual examination of the BAPS cluster memberships also did not support either feature as a significant genetic barrier, as individuals located on either side of the given feature were often assigned to the same genetic cluster with high probability (Fig. 6). The BAPS clusters did, however, confirm the findings of earlier studies showing the influence of the Corn Belt (which separates Northern and LP Michigan populations from populations to the south), Straits of Mackinac (which separates the Northern and LP Michigan populations) and Cascade Range (which separates the coastal Oregon population from populations east of the mountains).

Table 3 Tests for relationships between genetic differentiation among individual bobcats and several environmental factors using the dbRDA multivariate *F*-statistic. We analysed five predictor sets individually (marginal), with spatial coordinates as covariables (conditional), and with a forward selection procedure to obtain a combined model (sequential). Analysis was repeated, examining the geographic and climate predictor variables individually

	Marginal tests			Conditional tests			Sequential tests		
Variable	*F*	*P*	% var	*F*	*P*	% var	*F*	*P*	% var
(a) Results from predictor sets									
Ecoregion	24.20	0.001	11.57	9.51	0.001	4.48	24.20	0.001	11.57
Precipitation	34.73	0.001	11.11	14.05	0.001	4.40	7.73	0.001	13.97
Coordinates	77.85	0.001	8.52	–	–	–	10.95	0.001	15.10
Temperature	34.11	0.001	9.27	12.06	0.001	3.19	2.67	0.001	15.78
Elevation	82.29	0.001	4.69	12.76	0.001	0.69	4.83	0.001	16.02
(b) Results from single predictors									
X coordinate	142.96	0.001	7.87	–	–	–	142.96	0.001	7.87
Precip of warmest quarter	116.16	0.001	6.49	21.91	0.001	1.18	22.15	0.001	9.08
Precip of driest quarter	79.00	0.001	4.51	17.43	0.001	0.94	16.26	0.001	9.95
Y coordinate	16.25	0.001	0.96	–	–	–	11.00	0.001	10.54
Min temp of coldest month	12.26	0.001	0.73	13.66	0.002	0.74	9.37	0.001	11.04
Elevation	82.29	0.001	4.69	12.76	0.001	0.69	19.45	0.001	12.07
Annual precip	53.35	0.001	3.09	10.54	0.002	0.57	6.95	0.001	12.43
Precip of coldest quarter	12.30	0.001	0.73	13.28	0.001	0.72	11.59	0.001	13.04
Precip of wettest quarter	22.26	0.001	1.31	10.58	0.002	0.58	7.19	0.001	13.41
Max temp of warmest month	12.55	0.001	0.74	9.31	0.003	0.51	5.23	0.001	13.68
Precip seasonality	32.07	0.001	1.88	12.25	0.002	0.67	3.38	0.001	13.86
Annual mean temp	15.63	0.001	0.93	4.70	0.031	0.26	3.42	0.001	14.03
Mean diurnal range	55.89	0.001	3.23	10.43	0.001	0.57	3.80	0.001	14.23
Temp seasonality	12.60	0.001	0.75	14.04	0.001	0.76	2.38	0.001	14.35

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

Ecological and environmental correlates

Each of the predictor variable sets we tested with dbRDA showed a significant relationship to genetic differentiation, explaining 4.69–11.57% of the total genetic variation, and all remained significantly associated even after accounting for the influence of geographic distance (i.e. by including *X* and *Y* coordinates as covariables; Table 3). Ecoregion accounted for the largest portion of genetic variation, followed closely by precipitation, and it was the first predictor set fit in the sequential model (Table 3). In total, the five predictor sets explained 16.02% of the total genetic variation. In testing the geographic and climate variables individually, longitude was the best single predictor (7.87%). Latitude, however, only accounted for 0.96% of the variation in the response matrix. Summer precipitation (precipitation of the warmest quarter) was the best predictor based on the conditional tests, accounting for an additional 1.18% of the variation on top of that already explained by spatial coordinates. In the sequential test, the *X* coordinate was the first variable fit, followed by two precipitation variables and the *Y* coordinate (Table 3).

Discussion

Despite the potential for high levels of historical and contemporary gene flow in the bobcat, our data revealed significant substructure in this widespread generalist. The broad, underlying spatial pattern we observed was a longitudinal cline, with a sharp transition zone occurring along the Great Plains in the central USA. This pattern is evident in both mtDNA and microsatellite data, and is supported by different statistical approaches including SAMOVA, sPCA and Bayesian clustering analysis. Although an overall pattern of isolation by distance is also present, evidence indicates that the observed cline cannot be solely attributed to this process. For example although longitude was one of the most important predictor variables in the dbRDAs, latitude contributed very little to the models. If isolation by distance is the driving mechanism, variation in both spatial variables should contribute equally, given that

the sampling region covers a broad area in terms of both longitudinal and latitudinal distances. Furthermore, the mtDNA haplotype network and phylogenetic tree support two monophyletic and geographically structured lineages, primarily corresponding to eastern and western bobcats. Collectively, our results implicate historical climate oscillations as a significant factor shaping patterns of bobcat genetic variation.

Pleistocene refugia

Genetic clines, such as we observed, often result from genetic admixture at secondary contact zones, and postglacial recolonization from Pleistocene refugia is a common cause of secondary contact (Barton & Hewitt 1985). Hall & Kelson (1959) pointed out that a common zoogeographic pattern in North America is a distinction between eastern and western sister taxa. They postulated that this pattern stems from the aridification of the mid-continent region during Pleistocene interglacial periods, which separated species (particularly forest specialists) into disjunct eastern and western refugia from which they subsequently expanded. Indeed, phylogeographic evidence indicates that the Great Plains region is a common 'suture zone' for North American biota (Remington 1968; Swenson & Howard 2004).

Our data are consistent with the hypothesis of a historical mid-continent barrier and subsequent expansion from separate eastern and western refugia. In addition to the general east–west division we observed in the genetic data, the ancestral haplotypes (subclade B) are restricted to the western and southeastern USA, and are notably absent from the central USA. Following range expansions, ancestral haplotypes are generally less widespread than derived haplotypes and are likely to be centered on the origins of expansion (Templeton 2006), potentially pinpointing locations of refugia. Further supporting a Great Plains barrier during the Pleistocene, bobcat fossils from the range 35–10 ka have been found in several eastern and western states, as well as eastern Wyoming and southern Texas, but have not been found in the Great Plains (Graham & Lundelius 2010). Although bobcats are not a forest specialist per se, they do favor structure in their habitat, such as trees, shrubs or rocky outcroppings (Anderson & Lovallo 2003). Thus, widespread arid grasslands devoid of such structure (a landscape that no longer exists today) may have functioned as a historical barrier for this species.

Results of the demographic analyses, including significantly negative F_S values and unimodal mismatch distributions, further support a scenario of expansion from glacial refugia. Our estimates of time since expansion are recent (measured in tens of thousands of years and not hundreds of thousands of years) and indicate a more recent expansion in the eastern USA than the western. In addition, nucleotide diversity estimates were notably smaller in the eastern USA, consistent with a more recent expansion in this region (Zink *et al.* 2000) or with the existence of multiple refugia in the west (Fry & Zink 1998). We acknowledge that our estimates of time since expansion are sensitive to estimates of mutation rate, but we have interpreted them cautiously and find that they are consistent with, and do not rule out, the hypothesis of recent, postglacial expansion. Ideally, our results should be followed up with additional sequence data including nuclear markers to provide a more precise account of the sources, timing and routes of historical dispersal.

Founder effects

In addition to the east–west cline, we observed a number of putative genetic clusters in the northern portions of the bobcat's range which may at least partially result from founder effects. The Great Lakes region and New England both were covered by ice during the last glacial maximum (LGM), which persisted until ~10–15 ka. Once appropriate habitat became available, postglacial recolonization likely happened relatively quickly, as long-distance dispersers at the leading edge expanded into the region (i.e. the pioneer model; Hewitt 1996). Such a founding event leaves a signature of reduced genetic diversity (Hewitt 2000), which we observed in both the mtDNA and microsatellite data from these areas. Thus, contemporary gene flow has not erased the genetic signature of postglacial expansion in bobcats, although such homogenization has been observed in other vagile species (Smith *et al.* 2011).

Another distinct region was coastal Oregon/Washington. Based on mtDNA and microsatellite patterns, bobcats along the Pacific Northwest coast seem to have a unique evolutionary history from their more continental relatives. Here, persistent glacial ice in high elevation areas may have worked to isolate populations well after the LGM (18 ka), and the mountains may continue to restrict gene flow (Latch *et al.* 2009; Reding et al. 2011). However, the pattern may also be attributed to post-Pleistocene recolonization of the Pacific Northwest coastal areas from a postulated glacial refugium on the Haida Gwaii archipelago (Shafer *et al.* 2010).

Anthropogenic and topographic effects

Superimposed on the longer term founder/glacial effects, divergence of bobcat populations in the Midwest and Northeast could be reinforced by recent habitat loss and extirpation. Although historically bobcats occurred throughout the USA, these predators were

extirpated from portions of the agricultural Midwest and heavily populated mid-Atlantic coastal region for most of the 20th century, and have only recently increased in abundance and distribution in these areas (Deems & Pursley 1978; Woolf & Hubert 1998). Anthropogenic influences, which appear to be most dramatic in these two eastern areas, may help to explain our finding that western bobcat populations are more homogeneous than eastern populations.

The BAPS clusters also confirmed the previously identified influence of the Corn Belt (separating the Northern and LP of Michigan bobcats from populations south of the agricultural zone), Straits of Mackinac (separating the Northern and the LP of Michigan populations) and Cascade Range (separating the coastal Oregon population from bobcats west of the mountains). However, we found no evidence for the role of the Mississippi River or Rocky Mountains in structuring this species at a broad scale.

Climate and ecological effects

An intriguing question is whether the bobcat suture zone in the Great Plains is merely the midpoint of glacial refugia, or if ongoing factors are involved in its formation and maintenance (Swenson 2006). In addition to secondary contact, genetic clines can result from adaption along an environmental gradient (Endler 1977). Today, the Great Plains region represents a major shift in climatic types, particularly with regard to a sharp precipitation gradient that separates the moist east from the arid west. Our dbRDA results indicated that climate variables—particularly precipitation—explained a small but significant portion of bobcat genetic differentiation. Thus, the observed genetic cline in bobcats may be maintained by niche divergence along an environmental gradient. We examined neutral genetic variation, however, and inferences about selective pressures would benefit from future examination of potentially adaptive genes.

We observed distinct genetic clusters in southern Florida and southern California. These areas may indeed be unique given their ecological distinctness, and the occurrence of some unique haplotypes in these areas supports such a hypothesis. However, sampling was aggregated in these areas, and clustering algorithms may be picking up on family structure (Anderson & Dunham 2008), or reflect population structure at a finer scale. We detected other congruencies between genetic clusters and ecoregions (e.g. divergence between the Central and Southeastern populations, and distinction of coastal Oregon bobcats), and ecoregions explained a significant portion of the genetic variation in the dbRDAs. Natal habitat-biased dispersal, whereby individuals preferentially disperse into habitat similar to that in which they were born, may be a possible mechanism contributing to bobcat genetic structure (Davis & Stamps 2004). Although bobcats do not form social groups, which might facilitate behavioural divergences among individuals from different ecotypes, offspring do stay with their mothers for an extended period of time (9 months to 2 years; Anderson & Lovallo 2003), perhaps developing preferences for specific habitat or prey. The potential for habitat-specific divergence in this species should be further explored, for example by focusing radiotelemetry studies along ecotones to test whether dispersal movements are biased toward natal habitat type (e.g., Sacks *et al.* 2005).

Conclusions

This broad-scale survey of spatial genetic variation clearly indicates that despite possessing many characteristics predicted to limit genetic differentiation, bobcat population genetic structure exhibits complex temporal and spatial patterns. Our results not only suggest that historical processes are responsible for the main, underlying pattern, but they also indicate that environmental and anthropogenic forces have played a role in more recent time. With large sample sizes collected over a broad area, this study provides one of the most comprehensive pictures of neutral genetic patterns for a widespread North American species, providing a firm foundation for future work testing specific hypotheses related to, for example divergent selection across environmental gradients, comparative phylogeography or genetic impacts of climate change.

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References

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

Anderson MJ (2003) *DISTLM Forward: A FORTRAN Computer Program to Calculate a Distance-based Multivariate Analysis for a Linear Model Using Forward Selection*. Department of Statistics, University of Auckland, New Zealand.

Anderson MJ (2004) *DISTLM v.5: A FORTRAN Computer Program to Calculate a Distance-based Multivariate Analysis for a Linear Model*. Department of Statistics, University of Auckland, New Zealand.

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

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

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

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

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

Barton NH, Hewitt GM (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, **16**, 113–148.

Barton HD, Wisely SM (2012) Phylogeography of striped skunks (*Mephitis mephitis*) in North America: pleistocene dispersal and contemporary population structure. *Journal of Mammalogy*, **93**, 38–51.

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

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

Commission for Environmental Cooperation (1997) *Ecological Regions of North America: Toward a Common Perspective*. Commission for Environmental Cooperation, Montreal, Canada.

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

Croteau EK (2009) *Population Genetics and Phylogeography of Bobcats (*Lynx rufus*) Using Microsatellites and Mitochondrial DNA*. PhD dissertation, Southern Illinois University, Carbondale.

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

Deems Jr EF, Pursley D (1978) *North American Furbearers: Their Managment, Research, and Harvest Status in 1976*. International Association of Fish and Wildlife Agencies, Washington, District of Columbia.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.

Dyke AS, Moore A, Robertson L (2003) Deglaciation of North America. Geological Survey of Canada Open File 1574.

Endler JA (1977) *Geographic Variation, Speciation and Clines*. Princeton University Press, Princeton, New Jersey.

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

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

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

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

Frantz AC, Pop LC, Etherington TR, Wilson GJ, Burke T (2010) Using isolation-by-distance-based approaches to assess the barrier effect of linear landscape elements on badger (*Meles meles*) dispersal. *Molecular Ecology*, **19**, 1663–1674.

Fry AJ, Zink RM (1998) Geographic analysis of nucleotide diversity and song sparrow (Aves: Emberizidae) population history. *Molecular Ecology*, **7**, 1303–1313.

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

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

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

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.html.

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

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

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

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

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

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

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

Hull JM, Hull AC, Sacks BN *et al.* (2008) Landscape characteristics influence morphological and genetic differentiation in a widespread raptor (*Buteo jamaicensis*). *Molecular Ecology*, **17**, 810–824.

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

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

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

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

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

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

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

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

Linde SA, Roberts SD, Gosselink TE, Clark WR (2012) Habitat modeling used to predict relative abundance of bobcats in Iowa. *The Journal of Wildlife Management*, **76**, 534–543.

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

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

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

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

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

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

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

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

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

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

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

Reding DM (2011) *Patterns and Processes of Spatial Genetic Structure in a Mobile and Continuously Distributed Species, the Bobcat (*Lynx rufus*)*. PhD dissertation, Iowa State University, Ames, Iowa.

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

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

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

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

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

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

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

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

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

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

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

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

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

Smith BT, Escalante P, Hernández Baños BE *et al.* (2011) The role of historical and contemporary processes on phylogeographic structure and genetic diversity in the Northern Cardinal, *Cardinalis cardinalis*. *BMC Evolutionary Biology*, **11**, 136.

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

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

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

Templeton AR (2006) *Population Genetics and Microevolutionary Theory*. John Wiley & Sons Inc., Hoboken, New Jersey.

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

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

Williams BW (2006) *Applications of Genetic Methods in Furbearer Management and Ecology: Case Studies of Fishers, American Martens and Bobcats*. MS thesis, Michigan State University, East Lansing, Michigan.

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

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

D.R. is interested in landscape genetics, phylogeography, and conservation genetics. This study was part of her dissertation research on the patterns and processes of genetic structure in bobcats at different spatial scales. A.B. is interested in the evolution, environmental drivers, and physiological mechanisms of life histories in vertebrates. W.J. specializes in the comparative genomics, molecular ecology, conservation genetics, and evolutionary biology of felids. W.C.'s research is centered on population dynamics of vertebrates in response to large-scale effects of landscape changes.

Data accessibility

mtDNA haplotype sequences: GenBank accessions JN230433–JN230483, JN230486–JN230500, JN230502–JN230508.

Sample locations, microsatellite data and DNA alignment files: DRYAD entry doi:10.5061/dryad.d3t16pd2.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Locations of 52 a priori 'sample groups' of bobcat (*Lynx rufus*) DNA samples.

Fig. S2 Estimates of genetic diversity for sample groups, as measured by: (a) mtDNA haplotype diversity (h); (b) mtDNA nucleotide diversity (π); (c) expected heterozygosity (H_E), averaged across 15 microsatellite loci; and (d) allelic richness (AR), averaged across 15 microsatellite loci and corrected for sample size differences.

Fig. S3 (a) Matrix of mtDNA pairwise Φ_{ST} values, depicted as a heat map, for 49 sample groups with eight or more samples. (b) Matrix of microsatellite pairwise F_{ST} values, depicted as a heat map, for all 52 sample groups.

Fig. S4 Plots of structure log-likelihood values, LnP(D), and the ΔK measure for each value of K = 1–20.

Fig. S5 Results of structure analysis, with map showing proportions of ancestry for each individual for K = 2 as miniature pie charts.

Fig. S6 Results of structure analysis, with maps showing proportions of ancestry for each individual for K = 3–10 (a–h) as miniature pie charts.

Table S1 Properties of the 15 microsatellite loci used in this study.

Table S2 Summary of mtDNA and microsatellite genetic variation for the 52 a priori sample groups.

Table S3 Pairwise F_{ST} values based on data from 15 microsatellites for 10 bobcat populations inferred from BAPS mixture analysis.

Appendix S1 Details on the samples used in this study.

Appendix S2 Methods, results, and acknowledgements.

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