CHAPTER 2 - EFFECTS OF HABITAT DISCONTINUITIES ON GENETIC STRUCTURING AMONG PUMA POPULATIONS IN THE SOUTHWESTERN USA

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Abstract

I combined molecular genetic analyses with a simple habitat model to examine the effects of habitat discontinuities on genetic structuring in pumas (*Puma concolor*) in the southwestern USA. Using 16 microsatellite loci, I genotyped 540 pumas sampled throughout the states of Utah, Colorado, Arizona, and New Mexico, where a high degree of habitat heterogeneity provides for a wide range of connective habitat configurations between subpopulations. Individual- and population-based analyses revealed genetic structuring at two scales. First, strikingly strong differentiation between northern and southern portions of the study area suggests little migration between them, due to some combination of habitat and anthropogenic barriers. Second, within each region, gene flow appears to be strongly limited by distance, with greater effect of distance apparent when habitat barriers (e.g., low desert and grassland vegetation types) exist between samples. Genetic distances among sampled subpopulations were generally larger in the southern portion of the study area, where blocks of puma habitat were more disjunct. Significantly greater genetic diversity in the southern portion of the study area is consistent with previous hypotheses of a Pleistocene extinction of North American pumas, followed by a recolonization by a small number of founders from more southern populations. The results of this study complement those of previous studies, and begin to complete a picture of how different habitat types facilitate or impede gene flow among puma populations.

Introduction

The puma (*Puma concolor*) is a large and adaptable American felid, and the sole remaining large predator in many parts of the western United States and Canada. Pumas were once the most widely distributed mammal in the Western Hemisphere, but since European settlement, they have been eliminated from nearly all of eastern North America, as well as portions of their range in South America (Young and Goldman 1946; Anderson 1983). Pumas persist in western North America, but some populations are becoming isolated by habitat fragmentation (e.g., Beier 1996; Loxterman 2001; Ernest et al. 2003).

The effective conservation of a species requires reliable knowledge of its population structure and history, including levels of genetic diversity and variation in demographic parameters and barriers to movement throughout its geographic range (Schonewald-Cox et al. 1983; Avise 1989; O'Brien 1994; Haig 1998). Pumas and other large carnivores are

of particular conservation interest because of their important ecological roles, but also because they need large and well-connected habitat blocks (Beier 1993); as a result, movement of large carnivores has received considerable attention as a process to be conserved (Schonewald-Cox et al. 1991; Beier 1996; Noss et al. 1996; Weaver et al. 1996; Carroll et al. 2001). Empirical studies are needed to validate and parameterize models of dispersal and gene flow over large areas, and related population viability and metapopulation models (Levins 1970; Shaffer 1981; Gilpin and Hanski 1991; Hanski and Gilpin 1991; Beier 1993; Gilpin 1996).

Highly mobile species such as pumas often exhibit rates of gene flow sufficient to limit the accumulation of genetic differences between subpopulations (Wayne and Koepfli 1996). In the western United States, pumas have been shown to disperse long distances (e.g., Anderson et al. 1992), even in the presence of large discontinuities in habitat (Ruth et al. 1998; Logan and Sweanor 2001). Such observations would suggest high rates of gene flow, assuming dispersers successfully breed. However, genetic structure may exist if barriers to movement have been sufficient to prevent gene flow in prehistoric or contemporary times; genetic variation can be lost rapidly in small, isolated populations, and inbreeding in pumas and other large felids has been shown to result in reduced fertility and fitness (O'Brien et al. 1987a, b; Roelke et al. 1993; Barone et al. 1994; Driscoll et al. 2002).

The naturally patchy distribution of puma habitat in the southwestern United States provides an excellent opportunity to test relationships between landscape connectivity and gene flow using genetic data. Furthermore, several conservation organizations in the region have specifically focused on the puma's need for connectivity among habitat blocks in large-scale conservation planning efforts (e.g., Sky Islands Alliance 1992; Strittholt 1998), and rapid urbanization in the region necessitates the identification of habitat features that can provide connectivity between habitat patches that are becoming isolated. Finally, the region forms the only high elevation connection between large blocks of puma habitat in the USA and Mexico. The area thus provides an opportunity to investigate the intriguing hypothesis that North American pumas derived from a small number of South American founders within the last 10,000 - 12,000 years (Culver et al. 2001), because it covers the likely path of founding pumas during a northward range expansion.

Here I report results of a study of genetic variation in 540 pumas sampled across four states in the southwestern USA using 16 microsatellite loci. I address questions of fine-scaled phylogeography and population history differences across the study area relative to hypothesized gaps in puma habitat. I introduce a novel technique for grouping individuals into geographically delimited clusters without introducing investigator bias, and employ several complementary individual and population-based analyses. My results build on range-wide work by Culver et al. (2001), and complement similar fine-scaled studies elsewhere in North America. I draw conclusions about phylogeography, population history, and effects of habitat barriers on genetic structuring, and provide insight relevant for conservation and management of the species.

Methods

Sample collection and laboratory procedures

I collected muscle tissue samples of 540 legally hunted pumas from hunters, taxidermists, and state game management agencies in Arizona, Colorado, New Mexico, and Utah. All samples were of free-ranging pumas killed between 1999 and 2002. Samples were selected to exclude known relatives (e.g., mother-offspring pairs or siblings killed together) and mapped reported kill locations using ArcView GIS 3.3 (ESRI, Inc.). I used the Puregene Genomic DNA isolation Kit (Gentra Systems, Minneapolis, MN) to extract DNA from tissue samples. I amplified 16 microsatellite loci originally developed for the domestic cat (FCA026, FCA035, FCA043, FCA052, FCA057, FCA077, FCA082, FCA090, FCA096, FCA098, FCA132, FCA144, FCA176, FCA221, FCA229, and FCA290; Menotti-Raymond et al. 1999, 2003) by PCR using fluorescently labeled primers under conditions described in Menotti-Raymond et al. (1999). PCR products were electrophoresed using an ABI 377 sequencer and data were analyzed using GENESCAN and GENOTYPER software (ABI).

Delineation of sample groups and habitat mapping

I employed both individual-based and population-based analyses. For the latter, I chose to group individuals into local clusters of samples ("sample groups") based on geographic proximity alone (i.e. without knowledge of potential habitat barriers). Doing so allowed me to delineate clusters of samples without imposing my own notions of population boundaries on the data. I used a simple hierarchical clustering algorithm (UPGMC, Sneath and Sokal 1973) to group nearest individuals and nascent clusters based on Euclidean distance between individuals and cluster centroids. At each step the pair of individuals, pair of clusters, or individual-cluster pair that was closest geographically were combined. A new centroid location representing the geographic center of all individuals in the new, combined cluster was then calculated. I allowed individuals and clusters to merge at distances of 80 km or less because this distance is well within average dispersal distances reported in the literature for male pumas (e.g., an average of 167.2 km for three males in New Mexico tracked from independence to adulthood, Logan and Sweanor 2001). I did not assume resulting sample groups to necessarily be discrete populations, but instead treated them as local clusters of samples with allele frequencies assumed to be representative of those at cluster centroids.

To characterize the distribution of puma habitat within and adjacent to the study area, I merged USGS Gap Analysis Program land cover data for states from which it was available (Arizona, Colorado, New Mexico, Utah, California, Idaho, Oregon, Nevada and Wyoming) with USGS Eros Data Center Global Land Cover Characterization data (available at http://edcdaac.usgs.gov/glcc/glcc.html) for the remaining U.S. states, and a digitized version of the vegetation map of Brown and Lowe (1980) for Mexico. All land cover data were reprojected into a single equidistant conic projection and merged into a

single raster map in ArcView. I condensed each classification system into a common system with 28 land cover types (appendix A).
No puma habitat models were available for the study area, and few data are available on puma habitat use. To classify each of the 28 land cover types as puma habitat or nonhabitat, I developed a survey (appendix A) which I sent to six biologists who had extensive radio telemetry experience with pumas. I asked each expert to rate the density of pumas supported by each land cover type relative to the density supported by woodland, which was arbitrarily set to a standard value of 100. Respondents consistently rated woodland, forest, chaparral, montane shrub, sagebrush, forested wetland, vegetated riparian and tall shrub desert types (all of which received average scores $\geq$ 37) higher than low desert, grasslands, tundra, urban, agriculture, nonforested wetland and nonvegetated cover types (all of which scored $\leq$ 28.8; appendix A). I classified the former types as medium to high quality habitat ("habitat"), and the latter types as low quality habitat ("nonhabitat").

Data Analysis

Descriptive statistics. I used FSTAT 2.9.3 (Goudet 2001) to calculate descriptive statistics for sample groups including mean number of alleles per locus, expected and observed heterozygosity, and multilocus F_{IS}. Significance of F_{IS} values was tested by permutation using FSTAT; positive F_{IS} values indicate a heterozygote deficit within populations, and significant values provide support for population subdivision. I used GENEPOP version 3.3 (Raymond and Rousset 1995) to test for significant departure from Hardy-Weinberg equilibrium within sample groups using the Markov chain method of Guo and Thompson (1992) to estimate exact *P*-values for each sample group at each locus and across all loci. I also used GENEPOP to test for linkage disequilibrium among all pairs of loci using the Markov chain method and Fisher's exact test. Genetic distances between sample groups were measured by calculating pairwise F_{ST} values (Weir and Cockerham 1984) using FSTAT, Nei's standard genetic distance (D_S, Nei 1972) using MICROSAT (Minch 1997), and the log-likelihood ratio distance (D_{LR}, Paetkau et al. 1997) using the program available at http://www.biology.ualberta.ca/jbrzusto/doh.php. Statistical significance of pairwise F_{ST} values was tested using permutations in FSTAT; positive F_{ST} among two populations indicates a deficit of heterozygotes among the two populations taken as a whole, and significant values constitute evidence of population differentiation. I corrected for multiple comparisons in all statistical tests using a sequential Bonferroni adjustment (Rice 1989).

Population-based analyses. All genetic distance measures were used to construct neighbor joining (NJ) trees using the NEIGHBOR subroutine in PHYLIP version 3.5c (Felsenstein 1993). I included sample groups of $\geq$ 6 individuals for these analyses, because a higher cutoff would have eliminated sample groups of particular interest (e.g., the Kaibab Plateau, AZ, and Manzano Mountains, NM). I evaluated the robustness of the D_S NJ tree topology by generating 1000 bootstrap replicates. In each replicate, 16 loci were chosen randomly with replacement, resulting in the omission of some of the 16 loci and the duplication of others so that the resulting dataset had the same number of loci as the original dataset, the same number of individuals per sample group, and the same

allele frequencies (for loci included in the replicate) within each sample group. A new NJ tree was created for each replicate in NEIGHBOR and used to create a consensus tree using the CONSENSE subroutine. The proportion of replicates (out of 1000) in which a particular node was reproduced indicates the robustness of the node to the choice of loci. As an alternative to trees as a tool to describe relationships between sample groups, I used the same genetic distance data to perform nonmetric multidimensional scaling (NMDS) ordinations of the 36 sample groups using PRIMER version 5.2.8 (Primer-E Ltd., Plymouth, UK). For summarizing relationships among populations undergoing recurrent processes of gene flow and genetic drift, ordination may complement tree construction because it does not assume a bifurcating evolutionary history among populations (Paetkau et al. 1999).

Individual-based analyses. As an alternative to clustering individuals based on sample location, I used a Bayesian clustering method (STRUCTURE 2.1, Pritchard and Wen 2003) to infer numbers of populations and assign individuals to populations based only on multilocus genotype data (i.e., without knowledge of sample origin). For K population clusters, the program calculated the log likelihood probability of the data, $\ln(P|D)$, and the probability of individual membership in each cluster using a Markov chain Monte Carlo (MCMC) method under the assumption of Hardy-Weinberg equilibrium within each cluster. I conducted multiple runs using different numbers of population clusters (1-20) to estimate the number of true clusters with 1,000,000 MCMC cycles for burn-in (the number of simulation runs before collecting data in order to minimize the effect of the starting configuration, Pritchard and Wen 2003) and 1,000,000 cycles after burn-in. The value of K with the highest probability of the data indicates the most likely number of genetically distinct clusters under model assumptions.

I further examined structuring between regions using NMDS ordinations of individuals, with dissimilarity matrices created using shared allele distances ($1-D_{PS}$) calculated using MICROSAT. Because population-based analyses indicated a split between northern and southern regions, I also conducted assignment tests (Paetkau et al. 1995) between regions using the program available at http://www2.biology.ualberta.ca/jbrzusto/doh.php.

Isolation by distance. I used the "R Package" (Legendre and Vaudor 1991) to test for patterns of isolation by distance using Mantel tests (Mantel 1967). The goal of these analyses was to estimate the degree to which geographic distance explained variability in genetic differentiation among sample groups; unlike neighbor joining analyses and ordinations, including sample groups with allele frequency estimates that were as precise as possible was deemed more important than including individual sample groups of interest. Prior studies had indicated that increased variation due to small sample sizes may be a source of error in characterizing patterns of isolation by distance. For example, Sinclair et al. (2001) found no significant pattern of isolation by distance among ten Utah puma subpopulations with five individuals each, while Ernest et al. (2003) and Loxterman (2001) found significant patterns of isolation by distance in areas of similar size but among puma subpopulations with greater numbers of individuals. I chose a minimum sampling size of twice that used by Sinclair et al. (2001), including only sample groups with $\geq$ 10 individuals. Because a two-dimensional habitat model is

appropriate (i.e., puma habitat is not restricted to linear features but is arrayed across the study area), I log-transformed geographic distance before testing for correlations between geographic distance and $F_{ST}/(1 - F_{ST})$ as advocated by Rousset (1999). Based on indications of a split between northern and southern regions using NJ and Bayesian clustering analyses, I tested for a barrier by performing partial Mantel tests (Smouse et al. 1986).

Results

Sample groups and habitat mapping

Solely on the basis of geographic proximity, the UPGMC method allocated 500 individuals into 36 sample groups of $\geq$ 6 individuals each (Figure 1). The method resulted in intuitive local clustering of samples, and individuals were always within 150km of other sample group members (still within dispersal distances for male pumas reported in the literature, e.g., Logan and Sweanor 2001).

Although I did not consider sample location data when developing the habitat/nonhabitat classification, the classification system produced a map (Figure 1) in which samples were consistently associated with mapped habitat patches. The lack of samples in the considerable puma habitat in northeastern Arizona (Figure 1) is due to puma hunting being illegal on the Navajo Reservation. The map indicates that puma habitat in Utah and Colorado is fairly contiguous, while habitat in the southern two states appears to be more fragmented by desert grassland and low desert vegetation types. Sample groups tended to be within single habitat blocks, although nine sample groups straddled interstate highways. Six sample groups appear to be somewhat isolated from other sample groups by habitat discontinuities: CNM (Manzano Mountains), SENM (Sacramento Mountains), NAZ (Arizona Strip), and the three "Sky Island" region sample groups, SAZ1, 2, and 3 . The California portion of my habitat classification produced a map of puma habitat very similar to that produced by Torres et al. (1996), but at a finer scale (i.e., with smaller minimum mapping units).

Allele frequencies and genotypic equilibrium

No single locus deviations from Hardy-Weinberg equilibrium were detected after Bonferroni correction, and only one sample group (CAZ1) was found to be out of Hardy-Weinberg equilibrium using tests across all loci (Table 1). CAZ1 had a deficit of heterozygotes, suggesting possible subdivision within the sample group. No pair of loci exhibited linkage disequilibrium in any sample group after Bonferroni correction.

Table 2 shows pairwise F_{ST} and D_S values between the 36 geographically delineated sample groups. Pairwise F_{ST} values ranged from essentially zero to 0.24; 93% of the 630 pairwise comparisons were significant prior to Bonferroni correction, and 61% remained significant after correction.

Neighbor joining analyses and ordinations

Neighbor joining trees using the three genetic distance measures were all qualitatively similar in their topologies. Figure 2 shows NJ trees based on D_S and D_{LR}; all branches with bootstrap support > 60% in the D_S tree were shared among trees constructed using F_{ST} and D_{LR}. In all trees, northern sample groups and southern sample groups were separated into distinct clades, and NAZ and CNM appeared to be intermediate, suggesting that these samples may come from contact zones between northern and southern regions. When NAZ was removed from the analysis, bootstrap support for the branch separating northern and southern sample groups was 99.1%. Southern sample groups appeared to be more highly differentiated from one another than northern sample groups, as indicated by longer branch lengths in the southern clade. In each tree, SENM was separated from neighboring sample groups by longer branch lengths than any other single sample group, indicating that this sample group may be more divergent than others. The tree based on D_{LR} seemed to best reflect geographic relationships, with all SAZ and all SNM sample groups falling into single clades. CAZ2-5 also formed a single clade, while CAZ1 clustered closer to contact groups NAZ and CNM.

NMDS ordinations of sample groups (not shown) were similar to NJ analyses, with northern and southern sample groups clustering separately. As with NJ trees, ordinations based on D_S, D_{LR}, and F_{ST} were qualitatively similar. In all ordinations, NAZ, CAZ1, and CNM sample groups were intermediate, with CAZ1 and CNM more closely associated with the southern cluster. As in NJ analyses, northern sample groups clustered more tightly than did their southern counterparts, suggesting that southern sample groups may be more isolated from each other, and SENM appeared relatively divergent from neighboring sample groups. Within northern and southern regions, sample groups tended to cluster geographically. CAZ2-5 clustered together, as did all SNM and SAZ sample groups. In the north, NCO sample groups clustered together as did Utah sample groups. SCO and NNM sample groups were closely clustered as well.

Individual-based analyses

I used the Bayesian clustering algorithm to test for substructuring with different values of K (i.e., numbers of population clusters), ranging from a single panmictic population to 20 populations. Probabilities of the data given the model peaked at $K = 9$ populations, and again at $K = 12$ populations (Table 3). Alpha, a measure of admixture between populations, was lowest at $K = 9$. A problem with applying the model in this case is that it assumes panmixia within populations (although it also allows for population admixture), and departures from model assumptions can lead to overestimating K (Pritchard and Wen 2003). In such cases, the smallest value of K that captures the major structure in the data is likely the best choice. Because the model indicates radical improvement in $\text{Ln}(P|D)$ when two populations are assumed rather than one, but more modest improvement for higher population numbers, it is possible that there are as few as two relatively discrete populations, with further model improvement being due to the more subtle effects of restricted gene flow within populations. For the $K = 2$ and $K = 9$ cases, numbers of individuals from each of the 36 sample groups assigning to each

cluster are given in Table 4. Regardless of the "true" number of populations, a consistent north-south division of individuals indicates hierarchical structuring, with clusters nearly entirely residing in northern or southern portions of the study area. Grouping populations by their location at higher *K* values gives qualitative results very similar to the two-population case. As in NJ analyses and ordinations, NAZ, CNM and to a lesser degree CAZ1 appear to be admixed, with individuals assigning to northern and southern clusters.

For all values of $K > 2$, more clusters were identified in the northern portion of the study area than in the south. To determine whether the larger number of northern clusters was an artifact of the larger sample size in the north, I randomly discarded northern samples to achieve a sample size equal to that in the south (159). After discarding northern samples, $\text{Ln}(P|D)$ peaked at $K = 6$ populations, five of which consisted entirely or predominantly of samples from the south, and one which contained 156 northern samples and four southern samples.

To illustrate population differentiation at the sharpest interface between northern and southern clades, I performed NMDS ordinations based on individual genotypes in New Mexico using shared allele distances. I included animals from NNM and SNM (as single sample groups), and from SENM and CNM. I also included NCO individuals (as a single sample group) for comparison. As in other analyses, individuals clustered into strongly differentiated northern and southern groups (Figure 3). NNM and SNM sample groups were strongly differentiated (ANOSIM $r = 0.714$, $P < 0.01$). By comparison, NCO animals and NNM animals, although significantly differentiated, exhibited substantial overlap (ANOSIM $r = 0.135$, $P < 0.01$).

Although all sample groups included in the ordination were significantly differentiated ($P < 0.05$ after Bonferroni correction), the possibly admixed CNM animals (not plotted) were intermediate, with two appearing in the northern cluster and five in the southern cluster. SENM animals fell into the southern cluster and overlapped considerably with SNM animals ($r = 0.21$, $P < 0.01$).

Results from traditional assignment tests corroborate patterns observed in Bayesian clustering and NMDS analyses. Using northern (UT, CO, and NM North of Interstate 40, n = 353) and southern (AZ and NM south of Interstate 40 excluding CNM, n = 159) pumas to define nominal populations and calculating probability of assignment of all animals to each nominal population, only three southern pumas assigned to the northern region, and four northern pumas assigned to the southern region. Of 28 animals from possible contact sample groups (CNM; AZ north of Interstate 40), 15 assigned north and 13 assigned south. I observed a strong agreement between assignment to either the northern or southern region using assignment tests and assignment to the same respective region using STRUCTURE. Only one animal assigned to different regions based on the two methods; this animal, sampled from SNM2, assigned to the south using traditional assignment tests, but was marginally assigned to the northern region with a 62.3% probability by STRUCTURE.

Patterns of allelic richness

In addition to strong differentiation between pumas sampled in the northern and southern portions of the study area, I observed strong differences in genetic diversity between the two regions (Table 5). Excluding possible contact zones (AZ north of Interstate 40; CNM), southern pumas exhibited substantially greater numbers of alleles than those in the north; nearly all of the 107 alleles observed in the entire population were found in the south, whereas only 81 alleles were found in the north. Furthermore, there were six times as many unique alleles in the south as in the north. Given that more than twice as many pumas were sampled in the north than in the south, these results indicate that pumas in the southern region have substantially more alleles than those in the north, and also that genetic diversity in the northern region appears to consist mainly of a subset of genetic diversity in the south. That fewer alleles were found in the entire northern region than in portions of the southern region with much smaller sample sizes, e.g., SAZ1-3 (90 alleles, $n = 43$), CAZ1-5 (84 alleles, $n = 50$) and SNM1-3 (86 alleles, $n = 44$), further emphasizes these patterns. Heterozygosity levels were also higher in the south, as was the average range in allele sizes across all loci.

Isolation by distance

Patterns of isolation by distance were evident at several scales. Among the 24 sample groups with ≥ 10 individuals across the study area, genetic and geographic distance were strongly correlated ($R^2 = 0.51$, $P < 0.001$) (Figure 4). Such analyses assume that recurrent gene flow and genetic drift, rather than historical events, are the primary causes of differentiation. However, Figures 1, 2, and 3 indicate a strong division into two regions, and partial Mantel tests indicate a significant barrier between northern and southern sample groups ($r = 0.85$, $P < 0.001$). Furthermore, diversity differences between northern and southern portions of the study area suggest differences in population histories between the two regions. Among seven sample groups with $n \geq 10$ in the southern region, correlations between genetic and geographic distance remained strong ($R^2 = 0.54$, $P = 0.003$), as they did among 17 northern sample groups ($R^2 = 0.46$, $P < 0.001$). Furthermore, I observed significant relationships between genetic and geographic distance within each of the three states that had enough sample groups with ≥ 10 individuals to conduct Mantel tests (UT, $n = 6$, $R^2 = 0.60$, $P = 0.011$; CO, $n = 7$, $R^2 = 0.16$, $P = 0.035$; NM, $n = 8$, $R^2 = 0.75$, $P = 0.001$). I also observed significant isolation by distance among all nine sample groups with ≥ 6 individuals in Arizona ($R^2 = 0.27$, $P = 0.002$).

Discussion

Delineating sample groups

In studies of natural populations in heterogeneous landscapes, potential bias introduced in identifying populations can be problematic, particularly when individuals are relatively continuously distributed or do not naturally fall into obvious populations. Although

using the individual as the operational unit of study is one way to avoid this problem (Manel et al. 2003), clustering individuals into populations allows analyses based on allele frequencies, which can increase resolving power and provide convenient ways to analyze and summarize patterns of genetic structuring. I took both individual and population-based approaches. Strong agreement between conclusions drawn using population- and individual-based approaches (e.g., differentiation of pumas sampled in northern and southern portions of the study area indicated by NJ trees, ordinations, the Bayesian clustering algorithm, and assignment tests) supports this approach.

All five previous studies of genetic structuring in pumas at scales similar to this one (Walker et al. 2000; Loxterman 2001; Sinclair et al. 2001; Anderson 2003; Ernest et al. 2003) grouped individuals into populations based on investigator judgment, although Ernest et al. (2003) also used the Bayesian clustering technique described above to form an initial foundation for their choices. Although the ad-hoc delineation of populations may be reasonable when samples form natural clusters or exist in obvious habitat patches, the approach is problematic in cases like this study in which samples are continuously distributed over large areas. The UPGMC method allows the user to specify a biologically reasonable maximum geographic distance within clusters, and delineates local sample groups without introducing further subjectivity or bias. The method resulted in sample groups of reasonable geographic extent, the allele frequencies of which could be assumed to approximate average allele frequencies at sample group centroids, an assumption supported by nonsignificant F_{IS} values within all sample groups except CAZ1.

Spatial scale and pattern of genetic differentiation

Nonsignificant F_{IS} values within 35 of the 36 sample groups support the notion that subdivision is minimal and movement is often unrestricted within the scale at which the sample groups were delineated. Two mechanisms may be responsible for significant F_{IS} values in CAZ1. First, CAZ1 is the only sample group that is bisected by a metropolitan area (Flagstaff, AZ), and barriers due to development and/or the Interstate 40 transportation corridor may be subdividing the area. Alternatively, neighbor joining trees and NMDS ordinations suggest that CAZ1 may be a contact population containing individuals from divergent northern and southern clades. In either case, I would be sampling individuals from separate populations, resulting in a deficit of heterozygotes because of a Wahlund effect (Wahlund 1928).

Although little subdivision was observed within sample groups, significant pairwise F_{ST} values between 93% of sample group pairs and significant patterns of isolation by distance within states shows that within a few hundred kilometers, significant population structuring occurs throughout the study area. This indicates that although state agencies may be managing relatively panmictic populations within individual hunting units (all < 15,000 km^2 in Arizona), populations cannot be considered panmictic in areas much larger than about 20,000 km^2 (the area of an 80-km radius circle). Most nonsignificant pairwise F_{ST} values before Bonferroni adjustment (Table 2) involved adjacent sample groups or groups with small sample sizes.

The most striking pattern of subdivision was a north-south division of pumas, confirmed by all three population-based analyses and all three methods based on individual genotypes. Excluding the three possible contact groups, pairwise F_{ST} values averaged 0.0414 among the 22 northern sample groups (range -0.0160 - 0.127), 0.0522 among the 11 southern sample groups (range 0.00900 - 0.110), and 0.152 in 242 comparisons (all significant) between 11 northern and 22 southern sample groups (range 0.0740 - 0.235).

STRUCTURE indicated a greater number of clusters in the northern portion of the study area than in the south (Table 4). This would seem to contradict the results of population-based analyses (e.g., Figure 2), which indicate less population subdivision in the north. However, repeating the analyses with equal numbers of samples in both portions of the study area indicated that the larger number of northern clusters is an artifact of the greater number of samples in the northern portion of the study area. This suggests that results from this technique should be interpreted with caution with regard to the number of populations inferred when isolation by distance plays a strong role in population structuring and populations are apparently not discrete. What is important is that the procedure indicated a hierarchical pattern of structuring, with clusters residing nearly entirely in the north or south, regardless of the value of K or the number of samples analyzed.

Pairwise F_{ST} values between northern and southern regions and among some subdivided puma populations in California (Ernest et al. 2003) are high compared to those reported in other studies of microsatellite variation in large carnivores conducted within single subspecies. Among five Canadian lynx (*Lynx canadensis*) populations spanning from Alaska to eastern Canada, Rueness et al. (2003) reported pairwise F_{ST} values of 0.0017 - 0.0244. Although these values may be artificially low due to the large geographic extent of individual populations, Schwartz et al. (2002) found pairwise F_{ST} values that did not exceed 0.07 among 17 lynx populations of smaller geographic extent sampled from Alaska to Wyoming. Among polar bear (*Ursus maritimus*) populations sampled across the species' circumpolar range (Paetkau et al. 1999), pairwise F_{ST} values (0.002-0.108) were also lower than in this study. Similarly, among seven mainland populations of grey wolves (*Canis lupus*), Carmichael et al. (2001) reported pairwise F_{ST} values from 0.015 - 0.097. All of these studies sampled populations across study areas larger than my own.

Strong differentiation at many spatial scales within the study area is surprising given the strong dispersal capabilities of pumas. In a classic example of long distance movement ability, Ruth et al. (1998) translocated thirteen pumas from the San Andres Mountains in southern New Mexico to Northern New Mexico. Two of the pumas, both adult males, returned to their original home ranges, a distance of 465-490 airline km. Although pumas can make the journey from northern to southern New Mexico (and presumably the reverse), strong differentiation between pumas sampled in northern and southern New Mexico suggest that either this is a rare event or that dispersers between the two regions do not often breed. This may be an example of the reverse of "Slatkin's Paradox" (Slatkin 1985), the observation that direct methods of estimating dispersal rates often underestimate the potential for gene flow between populations.

The patterns of genetic structuring observed here echo those reported in other puma studies. Using 431 puma samples and 11 loci, Ernest et al. (2003) found significant pairwise F_{ST} values in 97% of comparisons among twelve California populations (average pairwise F_{ST} = 0.117, range = 0.01 – 0.37). Ernest et al. (2003) concluded that the Central Valley, San Francisco Bay and Delta, Los Angeles Basin, and Mojave and Sonoran Deserts were likely responsible for subdivision between puma populations, as well as reduced heterozogysities in small populations surrounded by these barriers. Similarly, Walker et al. (2000) found evidence for substructure between a pair of populations in western and southern Texas (F_{ST} = 0.107) based on 10 loci. These populations are separated by > 350 km, with much of the intervening habitat composed of grassland and desert scrub.

Loxterman (2001) found greater structuring among populations inhabiting mountain ranges in southern Idaho that are isolated by agriculture and other human uses than in more contiguously forested areas in northern Idaho. For example, populations in northern and southern Idaho, which were separated by agricultural development on the Snake River Plain, were strongly differentiated from each other (average pairwise F_{ST} = 0.111, range = 0.049 - 0.188). Similarly, pumas which inhabit more fragmented habitats in the southern portion of my study area exhibit greater genetic structuring than those in the more contiguous northern portion.

Two studies found little differentiation among puma populations sampled across large areas. Anderson (2003) found relatively low levels of genetic structuring among puma populations in Wyoming and western South Dakota. Using nine microsatellite loci and 297 puma samples, Anderson (2003) reported maximum pairwise F_{ST} values of 0.051 between geographically distinct populations separated by the Wyoming Basin. Furthermore, STRUCTURE suggested a single puma population across his study area, even when 15 samples from a distant population in southern Colorado were included. Similarly, Sinclair et al. (2001) found relatively little differentiation in Utah. The results of both studies are similar to my own for Utah and Colorado. Although I found significant patterns of isolation by distance in both states, sample groups within Utah (average pairwise F_{ST} = 0.033, range = 0.005 - 0.073) and Colorado (average pairwise F_{ST} = 0.030, range = -0.005 - 0.095) were not as strongly differentiated as elsewhere in my study area.

When combined with the results of these recent studies, my results begin to complete a picture of effects of landscape connectivity on genetic structuring in pumas. That puma populations appear to be well-connected in Utah and Colorado indicates that large areas of forest and woodland that are unbisected by desert, grassland, or human development apparently facilitate high rates of gene flow. Low levels of differentiation among populations in northern Idaho, portions of California, and along the Mogollon Rim in Arizona and New Mexico also support this conclusion. In Wyoming, large areas of sagebrush in the Wyoming Basin appear not to act as barriers to dispersal among mountain ranges. Greater genetic structuring among populations between northern Arizona and Utah, between northern and southern New Mexico, between the Sacramento Mountains and other mountain ranges in New Mexico, and in portions of California,

southern Idaho, and Texas appear to indicate that low desert, grasslands, agriculture and other human development may act as barriers to dispersal. In some portions of the study area (e.g., northern Arizona and New Mexico), such discontinuities may have been greater prior to recent invasions of grasslands by woodland types (Johnsen 1962; Archer 1994; Miller and Wigand 1994).

North-south differences in genetic diversity and evidence for a post-Pleistocene range expansion

Differences in allelic diversity between northern and southern regions suggest that the north-south split between pumas in the study area is not due solely to contemporary dispersal barriers, but also reflects historical processes. Not only are northern pumas less diverse, but alleles detected in the northern portion of the study area appear to be a subset of those detected in the south.

Other studies corroborate the observed diversity differences. Culver et al. (2001) found individuals sampled from the range of *Puma concolor azteca* (the historically defined subspecies to which most of the southern pumas belong) to have greater numbers of alleles and greater average range in allele sizes than other historically defined North American subspecies. Using allozymes, Roelke et al. (1993) also found high levels of genetic diversity in samples from the southern portion of the study area (Arizona, $n = 6$) relative to populations they sampled in Oregon ($n = 5$), California ($n = 4$), Texas ($n = 12$), Colorado ($n = 3$), and Utah ($n = 2$). Of 41 loci screened, seven were polymorphic in Arizona pumas, compared with two to four loci in samples from each of the other states. Even if samples from Oregon, California, Colorado and Utah were combined (total $n = 14$), only six loci were polymorphic across these four states, still fewer than in six samples from Arizona. Similarly, heterozygosity levels in Arizona were 6.7%, compared with 2.0 - 4.1% in samples from other states.

Taken together, these patterns echo the larger patterns found by Culver et al. (2001), in which substantially lower levels of microsatellite and mitochondrial diversity were detected in pumas sampled from North America than in those sampled from South America. Although there is fossil evidence of pumas in North America spanning 300,000 years, Culver et al. (2001) hypothesized that pumas in North America were eliminated at the end of the Pleistocene (along with other large mammal species) and replaced by a handful of immigrants from South America within the last 10,000 - 12,000 years. If this is the case, then patterns observed in my study area may reflect the recolonization event, with a historical range expansion from the south resulting in decreasing diversity in more northern populations due to serial founder events (Mayr 1942). Just as a small number of South American animals may have acted as founders of the North American population as a whole, pumas in the northern portion of the study area would be derived from founders to the south. Other studies have documented a loss of genetic diversity in populations newly established as a result of range expansions in humans and other animals (e.g., Rendine et al. 1986; Sokal et al. 1991; Hewitt 1993; Barbujani et al. 1995; Hansson et al. 2000), especially when dispersal distributions include rare long distance dispersal events (Nichols and Hewitt 1994; Ibrahim et al. 1996). A handful of

documented dispersal events > 400 km (e.g., Ruth et al. 1998; Logan and Sweanor 2000) suggest this may be the case in pumas.

Although the data presented here are consistent with the extinction-recolonization hypothesis of Culver et al. (2001), at least two other mechanisms may be responsible for the observed patterns. First, gene flow from more diverse populations in Mexico may explain elevated diversity in the southern portion of the study area. Second, widespread persecution of pumas in the last century could have resulted in reduced population sizes and a genetic bottleneck (Nei et al. 1975) in the north. However, invoking the first explanation would assume substantially higher genetic diversity in Mexico, which would still be consistent with the extinction-recolonization hypothesis. Invoking the second explanation would assume a reduction of northern populations severe enough to result in a rapid loss of diversity, which would have also resulted in greater levels of differentiation among northern populations through the increased effects of genetic drift—an expectation inconsistent with the relatively low levels of differentiation observed among northern sample groups. There is also little reason to believe that exploitation due to hunting and predator control in the northern portion of the study area has been more severe than in the south, although data on trends in puma densities over time (or even currently) are scant. Furthermore, the data of Roelke et al. (1993) and Culver (1999) suggest that the pattern observed in this study area may be more general, with pumas sampled in other portions of the United States and Canada consistently exhibiting lower levels of allozyme and microsatellite diversity than pumas sampled in Arizona.

If the observed pattern is indeed due to events that occurred thousands of years in the past, the persistence of differences between northern and southern populations may be due to a combination of persistent habitat barriers (primarily grassland and desert areas as discussed above) and possibly ecological factors that limit gene flow between the two regions. For example, natal habitat-biased dispersal behaviors ("habitat imprinting;" Vogl et al. 2002) could limit gene flow and reinforce differentiation between regions with differences in vegetation characteristics or prey species (Sacks et al. 2004).

If the extinction-recolonization hypothesis is further borne out as the mechanism behind the patterns observed here, then the sharp gradient in genetic diversity between northern and southern regions may provide fertile ground for future research on the history of the recolonization. Studies of vegetation and prey distributions in the Holocene may provide clues as to how and when pumas likely expanded north of central Arizona and southern New Mexico.

Conservation implications and puma management units

This study is unique in that it examines structuring at spatial scales large enough to contrast situations in which high rates of gene flow occur across large areas (Utah, Colorado, and Northern New Mexico), and also at which gene flow is restricted within smaller regions (Arizona and New Mexico). Like Ernest et al. (2003), Loxterman (2001) and Walker et al. (2000), this study supports the conclusion that habitat barriers result in

significant population structuring in pumas, but like Sinclair et al. (2001) and Anderson (2003), it also supports the notion that when habitat is well connected, high rates of gene flow occur at scales larger than individual states.

I agree with the argument of Sinclair et al. (2001) that observations of gene flow at large scales mean that conservation of pumas will require an integrated approach, involving agencies across a number of states, as opposed to current management practices involving individual units within states. However, evidence for structuring and dispersal barriers within states also means that populations in some states may not be connected by frequent dispersal and should be divided into separate management units. These would define populations with divergence of allele frequencies significant enough to indicate such low levels of gene flow that they may be functionally independent (Moritz 1994). The delineation of management units should not be taken to justify land use practices that will further divide populations, as even low levels of movement will still be important for maintaining genetically healthy populations. Rather, because population dynamics within management units are determined primarily by birth and death rather than immigration and emigration (Paetkau 1999), management units would define areas between which demographic rescue cannot be assumed. A case in point is New Mexico, where pumas in the northern portion of the state actually share closer genetic ties with pumas in Colorado and Utah than with pumas in southern New Mexico.

Logan and Sweanor (2001) have recommended partitioning New Mexico into zones with different management objectives, including some zones where no hunting would be allowed, and which would function as refuges to ensure population resilience. They suggested that at least two refuge zones would be appropriate, one in the north and one in the south. My finding of strong genetic partitioning between these two areas supports their conclusion. Reserve areas in one region would do little to stabilize populations in another if there is little dispersal between the two. Although appropriate genetic criteria for defining management units remain under debate (Paetkau 1999), separate management units have been proposed for populations showing lower levels of genetic differentiation than in this study. For example, Waits et al. (2000) proposed that four populations of Scandinavian brown bears (*Ursos arctos*) with pairwise F_{ST} values ranging from 0.0151 - 0.1393 be treated as four separate management units. In general, patterns of genetic structuring documented in this study and others should inform planning for the number and juxtaposition of zones with different management objectives within each state, and hopefully across state borders as well.

The results of this study suggest priority areas for conservation of habitat and connectivity. First, limited gene flow between northern and southern portions of the study area indicates that travel corridors between the two areas are few. Although natural habitat barriers have undoubtedly played a role in limiting gene flow in the past, the consistent genetic subdivision along Interstate 40 in Arizona and New Mexico suggests that highways and urban development may have become potent factors restricting movement on the contemporary landscape. Although the data presented here are not fine-scaled enough to determine the extent to which highways and other human development are responsible for observed genetic structuring, future studies could focus

along this genetic split in Arizona and in New Mexico, to more precisely determine the proportion of genetic structuring attributable to anthropogenic and natural habitat barriers. In the meantime, known connective areas like the Manzano Mountains (from which CNM samples were taken) should receive priority for protection to maintain connectivity for pumas and other species; these mountains have already been identified as an important north-south corridor by conservation organizations, and my results support their choice. The Manzano Mountains are bisected by a section of highway (Interstate 40) with high traffic volumes, and rapid development in areas east of Albuquerque is isolating this mountain range. A section of Interstate 40 near Santa Rosa, NM, was apparently a partial barrier to pumas attempting to move south after their translocation to northern New Mexico from the San Andres Mountains in southern New Mexico (Ruth et al. 1998).

Second, my study area included a portion of the Madrean Archipelago, or "Sky Islands" region of southern Arizona, southern New Mexico, and northern Sonora. The region is home to mountain ranges that form "islands" of forest and woodland surrounded by "seas" of desert scrub and semidesert grassland (Brown 1994; Warshall 1995), and forms the only high elevation connection between major floristic and faunal realms of the USA and Mexico (Walter 1979; Sky Islands Alliance 1992; Warshall 1995). The area supports a remarkably high diversity of species, reflecting contributions from Northern Rocky Mountain flora and fauna to the north, and from Madrean flora and fauna to the south (Gottfried et al. 1995; Warshall 1995). My data indicate that the region also appears to be an area of high genetic diversity for pumas, possibly because of gene flow from large blocks of habitat to the north and south. Habitat blocks are naturally disjunct in the Sky Island region, and connectivity between them is threatened by human development. Currently there is no evidence of loss of genetic diversity in pumas due to isolation of mountain ranges in the Sky Islands region, but Loxterman (2001) found decreased genetic diversity in similar mountain ranges in southern Idaho that are more strongly isolated by agriculture and other human development. Isolated populations in southern California studied by Ernest et al. (2003) also showed lower levels of diversity than did populations in larger, more contiguous habitat blocks. To avoid the loss of genetic diversity observed in isolated mountain ranges elsewhere, preservation of connectivity in the Sky Islands region should be a high priority. More research on puma movement in this region could inform efforts to maintain dispersal routes between mountain ranges.

Appendix A: A survey of experts to develop a simple, predictive habitat model for pumas based on land cover types

To classify each of the 28 land cover types as puma habitat or nonhabitat, I developed a survey which I sent to six biologists who had extensive radio telemetry experience with pumas: Paul Beier, Brett Dickson, Lisa Haynes, Ken Logan, Harley Shaw, and Linda Sweanor. I asked each expert to rate the density of pumas supported by each land cover type relative to the density supported by woodland, which was arbitrarily set to a standard value of 100. The survey included the following text asking experts to rate population densities supported by each land cover type:

> We are not asking for estimates of actual population densities, but instead would like you to score each habitat type with a *relative* density index. Within our study area, some of the highest densities of pumas are found within open woodland vegetation types (pinyon-juniper, oak, and similar woodland types). We have scored these vegetation types with a 100. If you believe vegetation type X should support half the density generally found in woodlands, score it with a 50. If you believe twice the density would be supported by X, score it with a 200.
>
> If you believe density or permeability will vary between summer and winter, please average your seasonal scores.
>
> For all classes but agriculture and urban, please consider each habitat type to be undisturbed, and do not consider anthropogenic factors like differences in hunting pressures or road development.
>
> We realize this exercise will produce an overly simplified model of puma density, and that many other factors will affect real densities. However, we are modeling across a very large area and over long time periods. Our main goal is not to produce a map of puma densities *per se*, but to approximately characterize variation in densities in order to test our hypotheses about gene flow.
>
> If you have no knowledge on which to base a rating for a particular habitat type, just leave the column blank for that type.

Table A1 shows descriptions provided to experts and average expert ratings for each cover type. Figure A1 shows ratings for each type, with cover types ranked in order of their ratings.

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Table 1. Descriptive statistics for 36 geographically delineated puma sample groups, including population identification (ID), geographic features associated with sample groups (Locale; see also Fig. 1), sample size (*n*), mean number of alleles per locus (Alleles), average expected heterozygosity (H_E), average observed heterozygosity (H_O), and F_{IS} (values in bold differ significantly from Hardy-Weinberg equilibrium).

ID	Locale	*n*	Alleles	H_E	H_O	F_{IS}
NAZ	Kaibab plateau	8	4.13	0.70	0.66	0.056
CAZ1	Flagstaff, AZ area	9	4.06	0.67	0.52	**0.237**
CAZ2	Prescott National Forest	12	4.00	0.66	0.69	-0.039
CAZ3	W. Mogollon Rim	9	4.31	0.68	0.69	-0.004
CAZ4	Pinal Mountains	14	4.19	0.62	0.61	0.024
CAZ5	San Carlos Indian Res.	7	3.50	0.61	0.63	-0.031
SAZ1	Baboquivari/Sierrita Mts.	9	3.50	0.59	0.60	-0.015
SAZ2	Huachuca/Dragoon Mts.	9	4.31	0.68	0.70	-0.032
SAZ3	Chiricahua/Peloncillo Mts.	25	5.06	0.66	0.67	-0.013
NCO1	Northeastern CO	6	3.13	0.59	0.60	-0.025
NCO2	Elkhead/Williams Fork Mts.	11	3.50	0.60	0.59	0.019
NCO3	Book Cliffs	25	4.06	0.61	0.60	0.018
NCO4	White River National Forest	29	4.19	0.63	0.63	0.012
NCO5	Vail Pass area	20	3.75	0.61	0.57	0.061
CCO	Uncompahgre Plateau	13	4.06	0.63	0.64	-0.010
SCO1	Sangre de Cristo Mts.	12	3.44	0.61	0.59	0.021
SCO2	W. San Juan Mts.	10	3.63	0.61	0.57	0.076
SCO3	E. San Juan Mts.	6	3.13	0.55	0.50	0.108
SCO4	Sangre de Cristo Mts.	8	3.38	0.58	0.61	-0.054
NNM1	Navajo Lake area	12	3.88	0.60	0.59	0.028
NNM2	Abiquiu Lake area	18	3.56	0.57	0.61	-0.085
NNM3	Raton, NM area	25	4.13	0.63	0.65	-0.017
NNM4	Sangre de Cristo Mts.	21	4.06	0.58	0.60	-0.030
CNM	Manzano Mts.	7	3.88	0.68	0.71	-0.049
SNM1	Reserve, NM area	10	4.00	0.66	0.63	0.039
SNM2	San Mateo/Gallinas Mts.	21	5.00	0.65	0.66	-0.020
SNM3	Black Range	12	4.44	0.64	0.63	0.012
SENM	Sacramento Mts.	15	4.38	0.61	0.61	-0.008
NUT1	Grouse Creek Mts.	8	3.25	0.55	0.60	-0.102
NUT2	Wasatch/Monte Cristo Ranges	18	3.81	0.62	0.61	0.011
NEUT	Flaming Gorge area	13	3.81	0.59	0.66	-0.120
CUT1	S. Wasatch Range	27	4.06	0.63	0.66	-0.058
CUT2	Price, UT area	12	3.63	0.64	0.63	0.026
CUT3	Pahvant Range/Sevier Plateau	8	3.75	0.63	0.69	-0.101
SUT1	Beaver, UT area	10	3.44	0.57	0.57	-0.003
SUT2	Southeastern UT	21	4.00	0.61	0.62	-0.016

Table 2. Pairwise F_{ST} (upper diagonal) and D_S values between 36 sample groups. Underlined F_{ST} values were significant before Bonferroni correction ($P < 0.05$), and bold values remained significant after correction.

	NAZ	CAZ1	CAZ2	CAZ3	CAZ4	CAZ5	SAZ1	SAZ2	SAZ3	NCO1	NCO2	NCO3	NCO4	NCO5	CCO	SCO1	SCO2	SCO3
NAZ		0.013	0.056	0.032	0.053	0.062	0.066	0.049	**0.060**	0.043	0.039	0.062	0.046	0.063	0.045	0.071	0.081	0.123
CAZ1	0.049		0.002	0.004	0.039	0.070	0.073	0.022	0.043	0.095	**0.091**	**0.107**	**0.081**	**0.091**	0.067	**0.100**	**0.115**	0.123
CAZ2	0.132	0.013		0.013	**0.073**	**0.078**	**0.086**	**0.060**	**0.061**	0.146	**0.147**	**0.159**	**0.129**	**0.146**	**0.120**	**0.154**	**0.157**	**0.168**
CAZ3	0.080	0.022	0.023		0.023	0.024	**0.087**	0.035	0.038	0.130	**0.133**	**0.139**	**0.113**	**0.126**	**0.110**	**0.145**	**0.156**	0.179
CAZ4	0.108	0.083	0.149	0.040		0.013	**0.098**	**0.050**	**0.037**	0.148	**0.130**	**0.167**	**0.127**	**0.145**	**0.111**	**0.144**	**0.156**	**0.191**
CAZ5	0.129	0.163	0.156	0.040	0.022		**0.092**	0.050	0.049	0.181	0.162	**0.196**	**0.165**	**0.186**	**0.153**	**0.189**	0.184	0.223
SAZ1	0.126	0.153	0.166	0.172	0.185	0.160		0.034	**0.064**	0.160	**0.151**	**0.166**	**0.137**	**0.162**	**0.110**	0.166	**0.159**	0.182
SAZ2	0.121	0.061	0.132	0.077	0.096	0.092	0.052		0.009	0.147	**0.117**	**0.129**	**0.099**	**0.122**	**0.086**	**0.126**	**0.138**	0.136
SAZ3	0.138	0.097	0.134	0.079	0.070	0.095	0.122	0.016		**0.152**	**0.134**	**0.152**	**0.119**	**0.142**	**0.090**	**0.136**	**0.150**	**0.170**
NCO1	0.081	0.226	0.342	0.305	0.319	0.394	0.318	0.353	0.370		0.028	0.010	0.011	0.031	0.022	0.050	0.046	0.050
NCO2	0.068	0.200	0.347	0.313	0.270	0.350	0.303	0.255	0.312	0.045		0.028	0.017	0.024	0.027	0.030	0.033	0.047
NCO3	0.116	0.234	0.390	0.330	0.388	0.479	0.360	0.293	0.372	0.018	0.047		0.002	0.018	0.029	**0.039**	0.045	0.033
NCO4	0.090	0.180	0.314	0.271	0.285	0.402	0.295	0.225	0.283	0.021	0.030	0.004		0.006	0.003	0.012	0.029	0.036
NCO5	0.123	0.196	0.348	0.291	0.320	0.445	0.348	0.274	0.337	0.055	0.040	0.030	0.010		0.020	0.016	0.034	0.053
CCO	0.092	0.152	0.284	0.263	0.236	0.350	0.215	0.189	0.199	0.037	0.045	0.050	0.006	0.034		0.014	0.010	0.037
SCO1	0.143	0.227	0.376	0.355	0.312	0.444	0.352	0.287	0.321	0.084	0.049	0.066	0.021	0.029	0.023		-0.005	0.008
SCO2	0.183	0.285	0.399	0.416	0.359	0.442	0.340	0.339	0.379	0.082	0.058	0.080	0.054	0.061	0.020	-0.004		0.000
SCO3	0.277	0.288	0.393	0.452	0.435	0.516	0.363	0.295	0.411	0.078	0.077	0.055	0.063	0.092	0.062	0.017	0.009	
SCO4	0.181	0.288	0.405	0.366	0.319	0.463	0.363	0.308	0.360	0.137	0.072	0.110	0.055	0.112	0.076	-0.003	0.035	0.065
NNM1	0.146	0.193	0.320	0.306	0.303	0.411	0.277	0.240	0.297	0.087	0.071	0.052	0.036	0.056	0.001	0.015	-0.020	0.020
NNM2	0.206	0.284	0.377	0.349	0.369	0.446	0.339	0.329	0.375	0.081	0.080	0.063	0.038	0.075	0.040	0.021	0.018	0.030
NNM3	0.123	0.194	0.284	0.268	0.288	0.351	0.253	0.230	0.297	0.047	0.042	0.036	0.025	0.045	0.022	0.011	0.006	-0.005
NNM4	0.196	0.265	0.379	0.369	0.368	0.454	0.334	0.310	0.365	0.134	0.067	0.095	0.078	0.085	0.031	0.015	0.006	0.023
CNM	0.076	0.128	0.173	0.138	0.127	0.107	0.097	0.131	0.171	0.180	0.160	0.233	0.198	0.218	0.132	0.179	0.128	0.209
SNM1	0.095	0.113	0.152	0.049	0.043	0.018	0.142	0.097	0.067	0.248	0.175	0.296	0.219	0.261	0.157	0.230	0.262	0.333
SNM2	0.092	0.113	0.170	0.059	0.051	0.027	0.126	0.119	0.090	0.295	0.293	0.370	0.325	0.331	0.224	0.320	0.312	0.418
SNM3	0.111	0.109	0.174	0.083	0.055	0.053	0.102	0.092	0.086	0.273	0.285	0.340	0.292	0.362	0.218	0.316	0.332	0.350
SENM	0.160	0.204	0.225	0.213	0.217	0.124	0.149	0.165	0.158	0.322	0.262	0.409	0.315	0.418	0.222	0.300	0.277	0.363
NUT1	0.128	0.331	0.498	0.443	0.412	0.527	0.427	0.432	0.507	0.058	0.051	0.081	0.073	0.119	0.168	0.113	0.146	0.176
NUT2	0.121	0.299	0.405	0.391	0.412	0.454	0.339	0.310	0.371	0.042	0.037	0.026	0.025	0.093	0.071	0.079	0.068	0.081
NEUT	0.137	0.267	0.436	0.426	0.447	0.555	0.427	0.367	0.453	0.002	0.035	0.021	0.036	0.081	0.080	0.078	0.091	0.065
CUT1	0.111	0.285	0.413	0.356	0.381	0.449	0.396	0.343	0.396	0.012	0.030	0.012	0.021	0.067	0.076	0.088	0.091	0.085
CUT2	0.140	0.289	0.423	0.373	0.412	0.541	0.448	0.399	0.420	0.047	0.096	0.027	0.033	0.091	0.090	0.109	0.113	0.152
CUT3	0.123	0.248	0.406	0.367	0.440	0.509	0.419	0.336	0.425	0.031	0.055	0.035	0.047	0.089	0.102	0.110	0.136	0.088
SUT1	0.100	0.221	0.387	0.343	0.408	0.539	0.383	0.364	0.433	0.060	0.076	0.081	0.064	0.131	0.124	0.129	0.165	0.163
SUT2	0.058	0.169	0.289	0.283	0.372	0.467	0.334	0.296	0.381	0.074	0.027	0.070	0.065	0.092	0.124	0.098	0.117	0.133

Table 2 (Continued).

	SCO4	NNM1	NNM2	NNM3	NNM4	CNM	SNM1	SNM2	SNM3	SENM	NUT1	NUT2	NEUT	CUT1	CUT2	CUT3	SUT1	SUT2
NAZ	0.090	0.072	**0.112**	**0.060**	0.103	0.032	0.040	0.044	0.051	0.079	0.077	0.061	0.077	0.058	0.061	0.059	0.061	0.036
CAZ1	0.125	0.088	**0.139**	**0.086**	**0.128**	0.048	0.045	**0.051**	0.047	**0.094**	0.150	**0.125**	**0.123**	**0.121**	**0.112**	0.101	0.107	**0.082**
CAZ2	0.169	**0.138**	**0.172**	**0.120**	**0.167**	0.076	**0.068**	**0.077**	**0.080**	**0.107**	0.204	**0.160**	**0.179**	**0.161**	**0.156**	**0.157**	**0.169**	**0.129**
CAZ3	0.155	**0.130**	**0.162**	**0.113**	**0.162**	0.059	0.023	0.030	0.041	**0.101**	0.187	**0.153**	**0.173**	**0.143**	**0.138**	0.142	**0.153**	**0.125**
CAZ4	0.152	**0.141**	**0.177**	**0.129**	**0.172**	0.064	0.023	0.028	0.030	**0.110**	**0.191**	**0.172**	**0.192**	**0.161**	**0.164**	**0.177**	**0.185**	**0.162**
CAZ5	0.205	0.179	**0.208**	**0.151**	**0.203**	0.059	0.010	0.016	0.030	0.071	0.235	**0.187**	0.227	**0.184**	**0.199**	0.201	0.230	**0.194**
SAZ1	0.180	**0.140**	**0.177**	**0.122**	**0.169**	0.058	0.075	**0.067**	**0.058**	**0.086**	**0.211**	**0.158**	**0.198**	**0.174**	**0.183**	0.184	0.190	**0.159**
SAZ2	**0.140**	0.110	**0.158**	**0.101**	**0.146**	0.059	**0.044**	**0.057**	0.045	**0.084**	**0.187**	**0.131**	**0.160**	**0.141**	**0.147**	0.136	**0.162**	**0.130**
SAZ3	**0.153**	**0.129**	**0.166**	**0.124**	**0.159**	**0.075**	0.033	**0.044**	**0.042**	**0.078**	**0.200**	**0.149**	**0.179**	**0.155**	**0.155**	**0.160**	**0.178**	**0.154**
NCO1	0.086	0.052	**0.059**	0.027	**0.084**	0.092	0.115	**0.132**	**0.128**	**0.156**	0.048	0.025	0.007	0.009	0.026	0.024	0.043	0.045
NCO2	0.047	0.042	**0.055**	0.025	**0.044**	0.082	**0.087**	**0.131**	**0.131**	**0.132**	0.038	0.022	0.025	0.019	**0.053**	0.035	0.050	0.017
NCO3	**0.065**	**0.031**	**0.042**	**0.021**	**0.058**	**0.110**	**0.132**	**0.155**	**0.148**	**0.177**	0.052	0.016	0.014	0.008	0.016	0.022	**0.050**	**0.041**
NCO4	0.032	0.020	**0.026**	0.014	**0.047**	**0.090**	**0.099**	**0.135**	**0.126**	**0.141**	**0.045**	0.015	0.023	**0.013**	0.018	0.026	0.038	**0.037**
NCO5	0.065	0.032	**0.049**	**0.026**	**0.053**	**0.103**	**0.119**	**0.143**	**0.155**	**0.180**	0.073	**0.052**	0.050	**0.039**	**0.049**	0.051	**0.077**	**0.053**
CCO	0.046	0.001	**0.030**	0.013	0.022	0.065	**0.074**	**0.102**	**0.101**	**0.110**	**0.098**	0.040	0.050	**0.043**	0.047	0.056	**0.073**	**0.067**
SCO1	-0.002	0.009	0.017	0.007	0.011	0.089	**0.108**	**0.139**	**0.141**	**0.144**	0.074	**0.046**	0.050	**0.051**	**0.058**	0.063	**0.078**	**0.057**
SCO2	0.021	-0.016	0.015	0.003	0.005	0.064	0.115	**0.134**	**0.142**	**0.134**	0.089	0.038	**0.057**	**0.051**	**0.058**	0.073	**0.095**	**0.065**
SCO3	0.045	0.010	0.024	-0.005	0.016	0.108	0.150	**0.177**	0.160	0.176	0.117	0.048	0.047	0.052	0.082	0.057	0.103	**0.078**
SCO4		0.030	0.020	0.032	0.036	0.130	**0.121**	**0.163**	0.150	**0.144**	0.062	**0.049**	**0.078**	**0.070**	**0.080**	0.087	0.061	**0.060**
NNM1	0.046		0.003	-0.008	0.004	0.064	**0.115**	**0.126**	**0.126**	**0.126**	0.101	**0.046**	0.047	**0.057**	**0.069**	0.071	0.083	**0.059**
NNM2	0.022	0.001		0.008	0.012	**0.113**	**0.143**	**0.167**	**0.159**	**0.148**	**0.099**	**0.054**	**0.053**	**0.056**	**0.080**	**0.092**	**0.089**	**0.074**
NNM3	0.053	-0.012	0.008		0.011	0.051	**0.096**	**0.115**	**0.112**	**0.124**	**0.082**	**0.032**	0.028	**0.033**	**0.047**	**0.043**	**0.067**	**0.047**
NNM4	0.051	0.004	0.014	0.015		**0.087**	**0.124**	**0.145**	**0.153**	**0.144**	**0.127**	**0.073**	**0.068**	**0.072**	**0.105**	**0.106**	**0.111**	**0.084**
CNM	0.268	0.118	0.198	0.097	0.148		0.041	0.019	0.049	0.082	0.146	**0.102**	0.120	**0.106**	0.116	0.125	0.140	**0.105**
SNM1	0.251	0.249	0.284	0.211	0.246	0.088		0.013	0.020	**0.052**	0.155	**0.118**	**0.154**	**0.116**	**0.128**	**0.118**	**0.147**	**0.122**
SNM2	0.381	0.279	0.367	0.264	0.309	0.032	0.025		0.013	**0.081**	**0.180**	**0.158**	**0.185**	**0.162**	**0.172**	**0.166**	**0.187**	**0.156**
SNM3	0.326	0.271	0.326	0.249	0.321	0.099	0.040	0.023		**0.060**	**0.178**	**0.143**	**0.179**	**0.151**	**0.165**	**0.166**	**0.178**	**0.153**
SENM	0.282	0.249	0.277	0.264	0.278	0.158	0.094	0.160	0.011		**0.188**	**0.139**	**0.179**	**0.160**	**0.189**	**0.182**	**0.177**	**0.146**
NUT1	0.081	0.167	0.144	0.141	0.211	0.283	0.319	0.411	0.385	0.383		0.035	0.057	0.050	0.068	0.033	0.037	0.029
NUT2	0.081	0.079	0.083	0.056	0.123	0.218	0.263	0.390	0.330	0.293	0.051		0.025	0.013	**0.036**	0.029	0.062	**0.042**
NEUT	0.118	0.072	0.073	0.043	0.105	0.236	0.340	0.461	0.419	0.387	0.075	0.037		0.012	0.031	0.012	0.036	0.034
CUT1	0.120	0.101	0.087	0.058	0.123	0.229	0.257	0.407	0.361	0.363	0.077	0.020	0.016		0.005	0.012	0.038	**0.036**
CUT2	0.147	0.132	0.133	0.089	0.194	0.284	0.319	0.471	0.438	0.486	0.110	0.066	0.047	0.006		0.007	0.040	**0.049**
CUT3	0.144	0.127	0.146	0.078	0.188	0.292	0.272	0.433	0.418	0.432	0.035	0.048	0.007	0.016	0.010		0.023	0.021
SUT1	0.088	0.137	0.133	0.116	0.183	0.285	0.311	0.450	0.399	0.365	0.046	0.102	0.048	0.058	0.063	0.027		0.011
SUT2	0.099	0.102	0.118	0.085	0.143	0.218	0.267	0.373	0.353	0.309	0.040	0.072	0.052	0.060	0.088	0.031	0.015	

Table 3. Results of Bayesian clustering analysis for 540 pumas, including number of clusters tested (K), probabilities of the data for each value of K (Ln($P|D$)), variance of the probability (Var), and admixture value for $K > 1$ (Alpha).

K	Ln($P|D$)	Var	Alpha
1	-23003.1	45.4	-
2	-21338.7	207.0	0.0474
3	-21084.5	350.4	0.0450
4	-21017.4	508.1	0.0436
5	-20954.5	670.2	0.0435
6	-20810.0	773.8	0.0418
7	-20766.6	928.1	0.0412
8	-20707.9	1020.1	0.0411
9	-20610.7	1095.1	0.0406
10	-20643.5	1316.1	0.0408
11	-20678.4	1556.1	0.0410
12	-20576.6	1527.9	0.0409
13	-20591.9	1676.3	0.0412
14	-20638.3	1872.7	0.0414
15	-20648.9	1994.1	0.0417
20	-20983.0	3081.1	0.0420

Table 4. Number of individuals from each sample group assigning to clusters identified by STRUCTURE for $K = 2$ and $K = 9$ cases. For the $K = 2$ case, membership in northern (*N*) and southern (*S*) clusters are listed. For $K = 9$ case, membership in each of six northern clusters and three southern clusters are listed. Purported northern, contact, and southern sample groups are sorted separately, as are purported northern and southern clusters. Highest proportion of membership assigned to sample groups is in bold, and boxes indicate individuals assigning to clusters within their region of origin. The larger number of northern clusters is apparently due to the larger sample size from this region.

		$K = 2$		$K = 9$								
	Pop	*N*	*S*	*N1*	*N2*	*N3*	*N4*	*N5*	*N6*	*S1*	*S2*	*S3*
Northern sample groups	NCO1	**6**	0	1				2	**3**			
	NCO2	**11**	0	**3**	**3**	1	1	1	2			
	NCO3	**25**	0	1	2	2	3	**12**	5			
	NCO4	**28**	1		4	9	2	**9**	4	1		
	NCO5	**20**	0		5	**6**	4	4	1			
	CCO	**12**	1	1	**4**	2		1	**4**		1	
	SCO1	**12**	0		1	3	**7**		1			
	SCO2	**10**	0		2	3	**5**					
	SCO3	**6**	0			2	**4**					
	SCO4	**8**	0			1	**7**					
	NNM1	**12**	0	1	2	**4**	**4**					1
	NNM2	**18**	0		4	4	**7**		3			
	NNM3	**24**	1	1	5	6	**8**	1	2			2
	NNM4	**20**	1		7	1	**12**			1		
	NUT1	**8**	0					1	**7**			
	NUT2	**18**	0		2	2	1	4	**9**			
	NEUT	**13**	0	2	1		1	3	**6**			
	CUT1	**27**	0		2		1	**17**	7			
	CUT2	**12**	0					**9**	3			
	CUT3	**8**	0	2				1	**5**			
	SUT1	**10**	0	**6**			1		3			
	SUT2	**21**	0	**14**			2	1	4			
Contact	NAZ	**5**	3	**4**	1			1		1		1
	CAZ1	3	**6**	3							**5**	1
	CNM	2	**5**	1						**3**		**3**
Southern sample groups	CAZ2	1	**11**	1						1	**10**	
	CAZ3	0	**9**							1	**7**	1
	CAZ4	0	**14**							2	4	**8**
	CAZ5	0	**7**							**3**	1	**3**
	SAZ1	0	**9**							**7**		2
	SAZ2	0	**9**							**7**	2	
	SAZ3	0	**25**							**16**	9	
	SNM1	0	**10**							2	2	**6**
	SNM2	1	**20**				1			4	1	**15**
	SNM3	0	**12**							3		**9**
	SENM	0	**15**	1						**12**	1	1

Table 5. Summary of allelic diversity patterns for northern and southern regions, including sample size (*n*), number of alleles detected out of 107 total (Alleles), number of unique alleles (Unique), average observed heterozygosity (H_O), average number of alleles per locus (*A*), average variance in allele size (Var), and average range in allele size (Range). Northern region includes all animals from Utah, Colorado, and New Mexico sampled north of Interstate 40. Southern region includes all individuals from Arizona and New Mexico sampled south of Interstate 40 except for CNM pumas. * = significantly greater than northern samples based on Wilcoxon signed rank test, $P < 0.05$.

Region	*n*	Alleles	Unique	H_O	*A*	Var	Range
Northern	353	81	4	0.611	5.06	4.55	6.63
Southern	159	103	26	0.643	6.44*	6.01*	7.69*

Table A1. Numeric vegetation code assigned to each cover type in classification (Code), cover type description and characteristic species provided to respondents (Description), and average rating assigned by experts (Rating) for 28 cover types. Ratings indicate estimated carrying capacity relative to that of woodland, which was fixed at 100.

Code	Description	Rating
140	**Subalpine forest:** Subalpine fir, bristlecone pine, whitebark pine, upper elevation lodgepole pine and Engelmann spruce	44.0
151	**Upper montane and mesic coastal forest**: Douglas-fir, spruce/fir, hemlock, red cedar, lodgepole pine, redwood forest, grand fir	66.3
152	**Lower montane and xeric coastal forest:** Ponderosa pine, Jeffrey pine, Sierra yellow pine	71.3
210	**Woodland- general:** Pinyon-juniper, oak-pine woodlands, live oak woodlands, Madrean oak woodlands	100.0
310	**Misc. Upper montane shrub:** Mesic montane shrub including maple, serviceberry, *Prunus*; drier montane shrub including mountain mahogany, oak shrub	90.0
321	**Bitterbrush:** Bitterbrush (*Purshia*)	75.0
322	**Mountain sagebrush:** Mountain big sage and mountain low sage	62.5
323	**Sagebrush:** Big sage, black sage, low sage, silver sage	48.0
330	**Interior and mesic California chaparral:** Manzanita, mixed evergreen sclerophyll, shin oak, ceanothus	80.0
340	**Xeric California chaparral:** Semi-desert chaparral, chamise, red shank	65.0
350	**Costal scrub:** Dune scrub, coastal bluff scrub	58.3
540	**Desert shrub- low shrub:** Greasewood, creosote, shadscale/saltbush, bursage	23.0
551	**Desert shrub- tall shrub- Mojave:** Joshua tree	37.0
552	**Desert shrub- tall shrub- Sonoran:** Palo verde, saguaro, mixed cacti	56.0
553	**Desert shrub- tall shrub- Chihuahuan:** Whitethorn acacia	48.8
620	**Alpine tundra:** Herbaceous tundra- grasses, forbs, sedges	16.7
630	**Upper and mid-elevation grasslands:** Montane parklands, meadows, plains grasslands, foothill-piedmont grasslands	28.8
640	**Semidesert grassland and grassland/scrub:** Sacaton, bunchgrass, scrub mixed with grasses	21.7
710	**Riparian- vegetated, nonforested:** Low elevation riparian areas dominated by grasses, herbs, and shrubs	45.0
720	**Riparian- forested:** Broadleaf (e.g. cottonwood-willow, mesquite) and needleleaf riparian areas	74.0
730	**Riverine\lacustrine:** Nonvegetated riparian areas lacking trees, shrubs, persistent emergents	10.0
740	**Wetland- vegetated nonforested:** Wetlands dominated by grasses, herbs and shrubs	26.3
750	**Wetland-forested:** Broadleaf (e.g. cottonwood-willow, mesquite) and needleleaf wetlands	58.8
760	**Playa:** Unvegetated playa	3.3
810	**Barren:** Salt flats, bare rock, bare soil, sandy areas, etc.	2.5
820	**Permanent snow:** Permanent snow and glaciers	1.3
910	**Agriculture:** Cropland, not including rangeland	6.0
920	**Urban:** Heavily developed residential and commercial areas	1.2

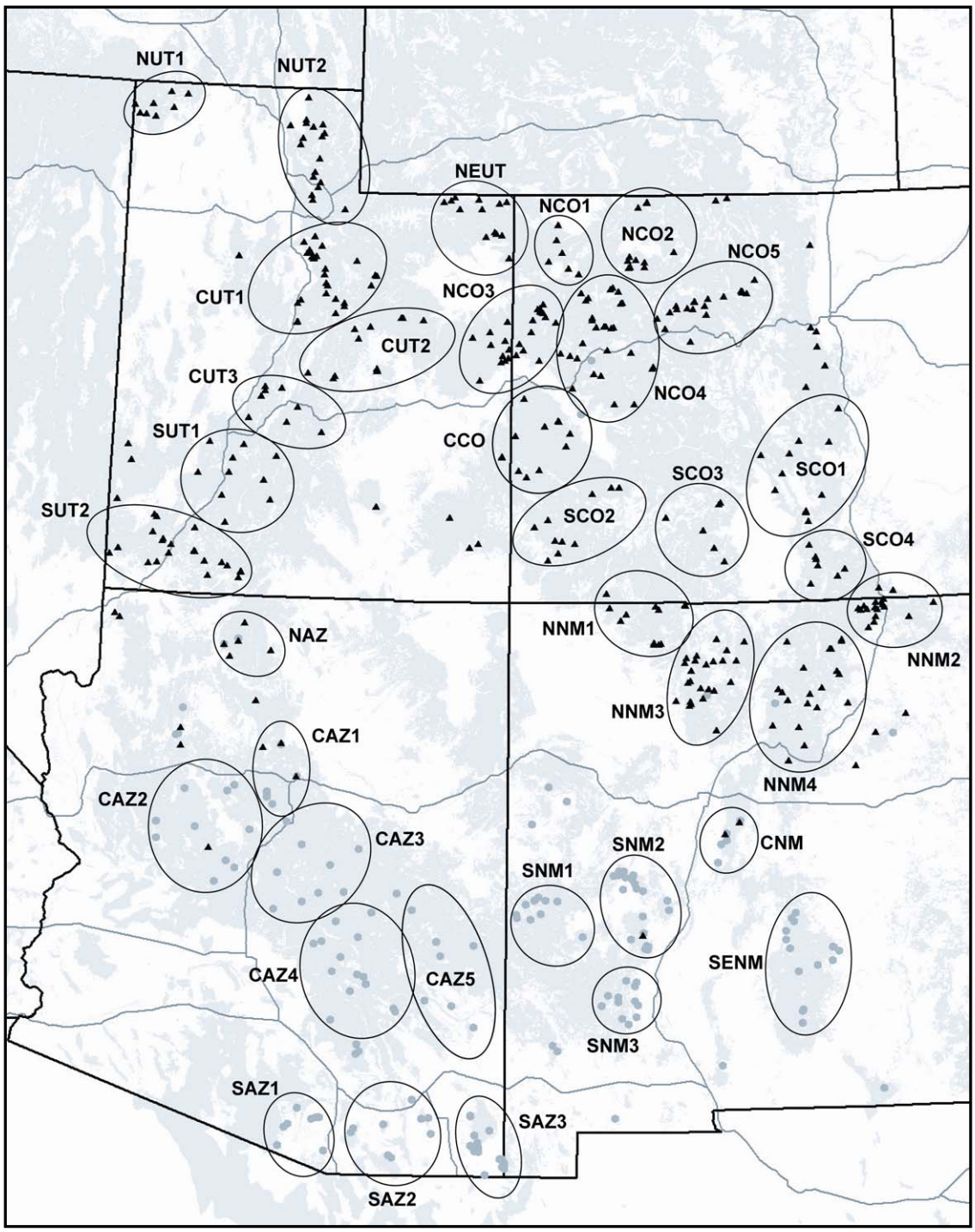

Figure 1. Map of study area and locations of 540 puma samples**.** Ellipses indicate sample membership in 36 geographically delimited sample groups for population-based analyses (i.e., the ellipses do not reflect any genetic information). Shading indicates puma habitat based on classification described in text. Interstate highways are also shown. Black triangles denote samples assigned to northern cluster using STRUCTURE with $K = 2$; grey circles denote samples assigned to southern cluster.

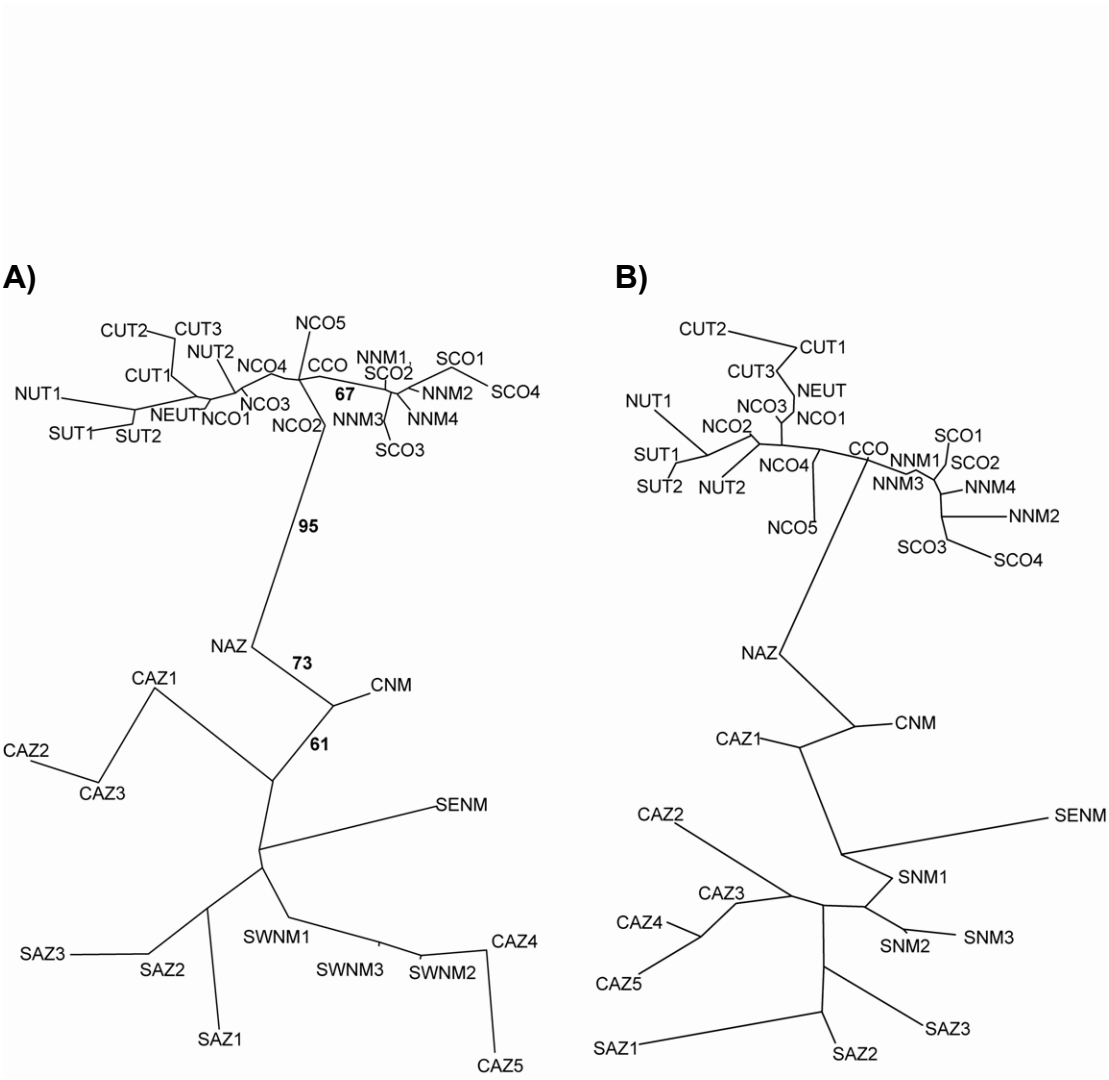

Figure 2. Unrooted neighbor joining trees based on genetic distances between 36 sample groups**.** A) Tree based on Nei's D_S, with bootstrap values over 60% shown in bold. B) Tree based on D_{LR}. Branch lengths reflect magnitudes of genetic distances.

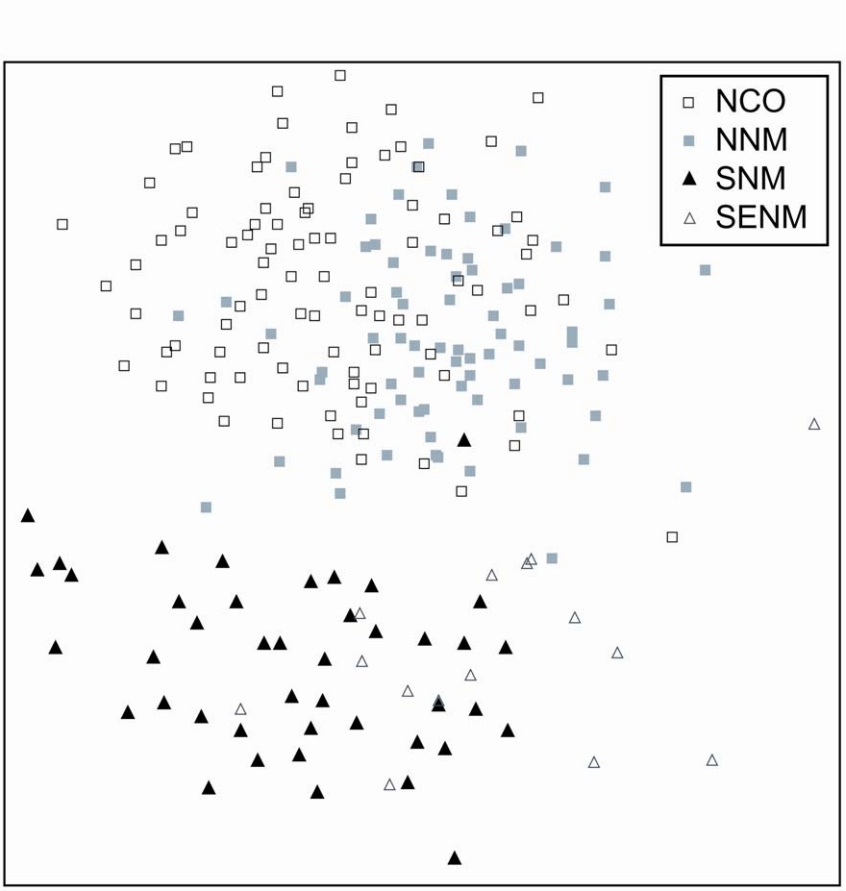

Figure 3. Nonmetric multidimensional scaling ordination of individuals from NCO, NNM, CNM, SNM, and SENM sample groups**.** Ordination is based on genetic distances (1-D_{PS}) between individuals. Northern and southern pumas formed distinct clusters, while CNM individuals (not shown) fell in both northern and southern clusters.

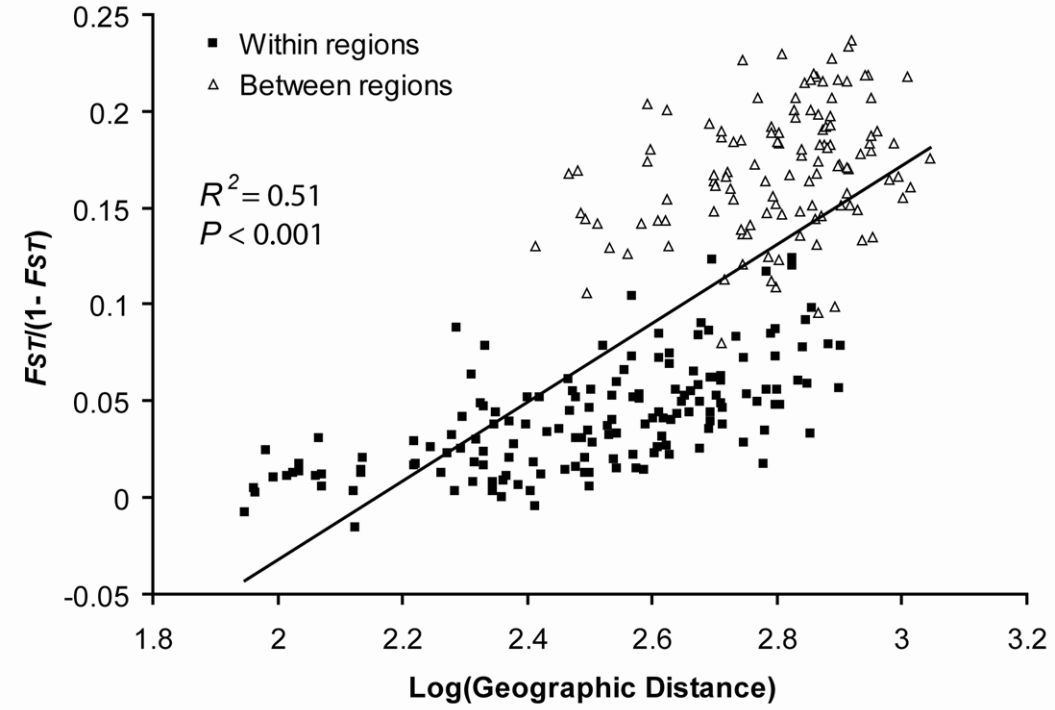

Figure 4. Genetic distance plotted against $\log_{10}$(geographic distance) for 24 sample groups with > 10 individuals. Within region points represent distances between pairs of populations sampled within northern or southern region, while between region points represent pairs of populations falling across boundaries separating the regions.

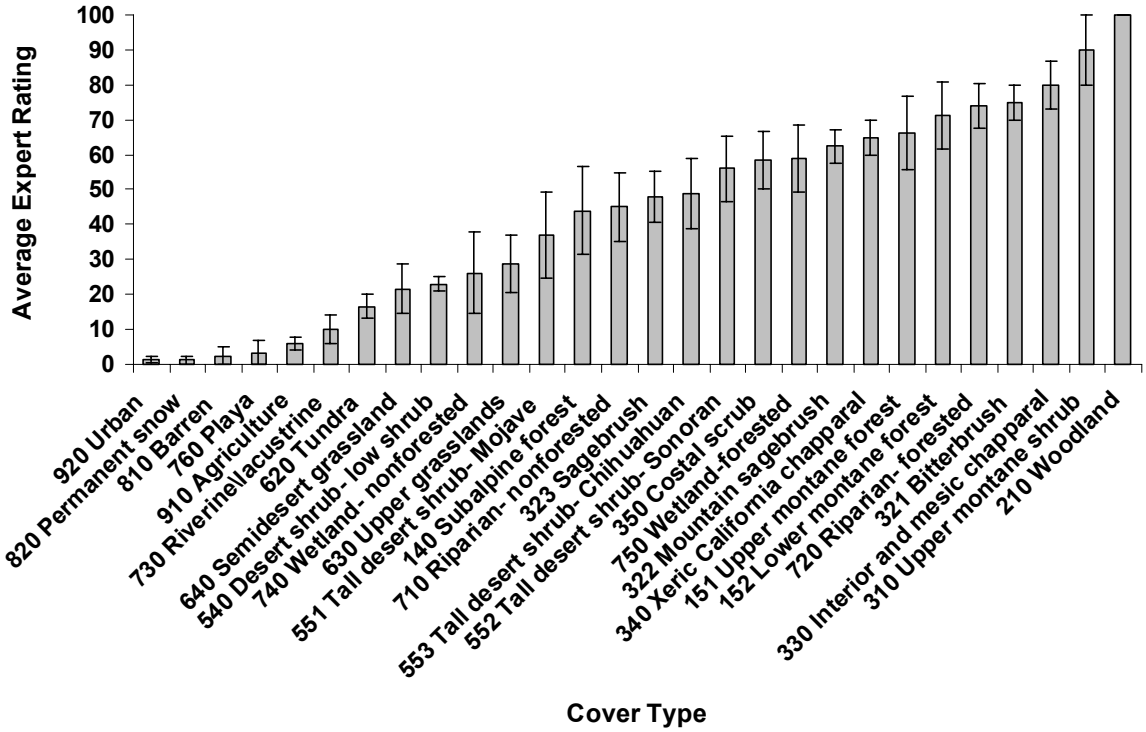

Figure A1. Average ratings (± standard error) assigned to 28 cover types by six experts with extensive experience tracking pumas using radio telemetry. Ratings indicate estimated carrying capacity relative to that of woodland, which was fixed at 100.