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RESEARCH ARTICLE

Genetic source–sink dynamics among naturally structured and anthropogenically fragmented puma populations

Kyle D. Gustafson[1,2] · Roderick B. Gagne[1] · T. Winston Vickers[3] · Seth P. D. Riley[4] · Christopher C. Wilmers[5] · Vernon C. Bleich[6] · Becky M. Pierce[7] · Marc Kenyon[8] · Tracy L. Drazenovich[9] · Jeff A. Sikich[4] · Walter M. Boyce[3] · Holly B. Ernest[1]

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Abstract

Fragmentation of wildlife populations is increasing on a global scale and understanding current population genetic structure, genetic diversity, and genetic connectivity is key to informing wildlife management and conservation. We genotyped 992 pumas (*Puma concolor*) at 42 previously developed microsatellite loci and identified 10 genetic populations throughout the states of California and Nevada, USA. Although some genetic populations had large effective population sizes, others were small and inbred. Genetic diversity was extremely variable (heterozygosity, uHe = 0.33–0.53), with some populations nearly as low as an endangered subspecies, the Florida Panther (*P. c. coryi*, uHe = 0.24). Specifically, pumas in the Sierra Nevada were genetically diverse and formed the largest genetic source population in the region. In contrast, coastal and southern populations surrounded by urbanization had low genetic diversity, fragmented gene flow, and tended to be genetic sinks. The strong population genetic structuring of pumas across California (F_{ST} = 0.05–0.39) is vastly different than other genetic studies in less-urbanized states, including our analysis in Nevada, where pumas had few barriers to gene flow and weak population differentiation. Our results have far-reaching conservation and management implications for pumas and indicate large-scale fragmentation in one of North America's most biodiverse and rapidly-urbanizing regions.

Keywords Mountain lion · Cougar · *Puma concolor* · Population genetics · Genetic structure

Introduction

Fragmentation of wildlife habitat and resultant impacts to populations are increasing worldwide and urbanization is one of the primary contributors (Crooks et al. 2017; Fahrig 2003; Haddad et al. 2015; Newbold et al. 2016). Unlike

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✉ Holly B. Ernest
holly.ernest@uwyo.edu

[1] Wildlife Genomics and Disease Ecology Laboratory, Department of Veterinary Sciences, University of Wyoming, Laramie, WY 82070, USA

[2] Department of Biology & Environmental Health, Missouri Southern State University, Joplin, MO 64870, USA

[3] Karen C. Drayer Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

[4] National Park Service, Santa Monica Mountains National Recreation Area, Thousand Oaks, CA 91360, USA

[5] Environmental Studies Department, University of California, Santa Cruz, CA 95064, USA

[6] Department of Natural Resources and Environmental Science, University of Nevada Reno, Reno, NV 89557, USA

[7] Bishop, CA 93515, USA

[8] Division of Fish, Wildlife, & Parks, Wisconsin Department of Natural Resources, Wisconsin Rapids, WI 54494, USA

[9] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

natural barriers that have impacts over a geological timescale (Albert et al. 2016), urbanization can have more immediate effects on gene flow among populations (Balkenhol and Waits 2009; Karlson et al. 2014). Gene flow is critically important to individual fitness and to the evolutionary potential of populations because successful migrants can diversify gene combinations (i.e., increase heterozygosity) and introduce new genetic material (i.e., increase allelic richness) (Caballero and García-Dorado 2013; Chapman et al. 2009; Frankham 2015). Without receiving gene flow, small populations are especially subject to inbreeding, genetic drift, and increased extinction risk (Carlson et al. 2014; Wootton and Pfister 2015).

Population fragmentation is increasingly evident for species located in the urbanized western United States (Buchalski et al. 2016; Delaney et al. 2010; Fisher and Shaffer 1996; Tuma et al. 2016), including the puma (*Puma concolor*) (Beier 1995; Gray et al. 2016), which is becoming a model for studying genetics of isolated populations (Ernest et al. 2014; Gustafson et al. 2017; Johnson et al. 2010; Riley et al. 2014). Despite the long-distance dispersal ability of pumas (Hawley et al. 2016; Newby et al. 2013; Pierce et al. 1999; Thompson and Jenks 2005), gene flow among adjacent puma populations has been nearly negated by freeways in densely populated Southern California (Ernest et al. 2014; Gustafson et al. 2017; Riley et al. 2014). Consequently, some California puma populations have become functionally isolated and have experienced rapid population divergence and inbreeding (Ernest et al. 2014; Gustafson et al. 2017; Riley et al. 2014; Vickers et al. 2015) with concerns for extinction (Benson et al. 2016). Given that *P. concolor* and other wide-ranging species serve as umbrella species (Carroll et al. 2001; Maehr et al. 2002; Thorne et al. 2006)—the conservation of which indirectly provides protection for many other species (Roberge and Angelstam 2004)—the low genetic diversity of puma populations in human-fragmented habitats suggests that a large-scale ecological problem may be occurring in some of the most biologically-diverse regions of North America (Calsbeek et al. 2003; Dobson et al. 1997).

During the late Pleistocene, pumas were extirpated from North America and repopulated by migrants from South America (Culver et al. 2000). As a result, pumas in North America compose a single phylogenetic group (based on mtDNA) and exhibit founder effects (i.e., reduced population genetic diversity based on mtDNA and microsatellites) compared to pumas in South America and Central America (Culver et al. 2000). Therefore, it is critical to understand effects of fragmentation on populations from this North American lineage. A previous genetic analysis along the west coast of the United States indicated that pumas in California did not exist as a single population and suggested urbanization may have led to genetically-depauperate, fragmented populations (Ernest et al. 2003). In addition, a population genetic analysis in Nevada indicated there were asymmetric migration rates between the two states, and that pumas from Nevada were a genetic source for genetic-sink populations in California (Andreasen et al. 2012). However, these previous reports relied on a limited number of genetic loci ($\leq$13 microsatellites) and investigators did not sample across the two states. In this study, we attempted to address these limitations and provide a more comprehensive view of puma genetic diversity and gene flow within and among California and Nevada.

Our aim was to identify the number and spatial structure of puma populations across California and Nevada and the extent of gene flow among the populations. In doing so, we were able to identify genetic source and sink populations as well as isolated populations with limited gene flow. We expected pumas would exhibit genetic structure associated with both natural geographic features and anthropogenic development. Given the complex structure of ecoregions and large human population in California (> 39 million people; 92.5/km^2; US Census Bureau 2016), we hypothesized pumas in California would exhibit more population divergence and less interpopulation gene flow relative to pumas in Nevada, which have access to more contiguous ecoregions with fewer humans (< 3 million people; 10.3/km^2; US Census Bureau 2016). To address these hypotheses, we genotyped 992 pumas at 42 microsatellite loci across California and Nevada. We then identified regional populations using population assignment models and evaluated functional connectedness of puma populations by modeling population divergence and computing bi-directional migration rate estimates.

Materials and methods

Sampling and extractions

We obtained tissue or blood samples from 992 pumas captured alive, found dead, or legally killed by authorized agencies for livestock depredation, public safety, or sport hunting (Nevada only) during 1992–2016 (Fig. 1). Approximately 49% of individuals sampled were legally killed, 31% were from captures, 11% were hit by vehicles, and the rest were found dead of other causes. We isolated genomic DNA using QIAGEN DNeasy Blood & Tissue kits (QIAGEN Inc., Valencia, CA, USA).

Genotyping

We genotyped each individual puma at 42 previously developed microsatellite loci, plus a single sex-linked locus (Ernest et al. 2003, 2014; Riley et al. 2014) and ran polymerase chain reactions on ABI 2720 thermocyclers (Life Technologies, Carlsbad, CA, USA) using QIAGEN

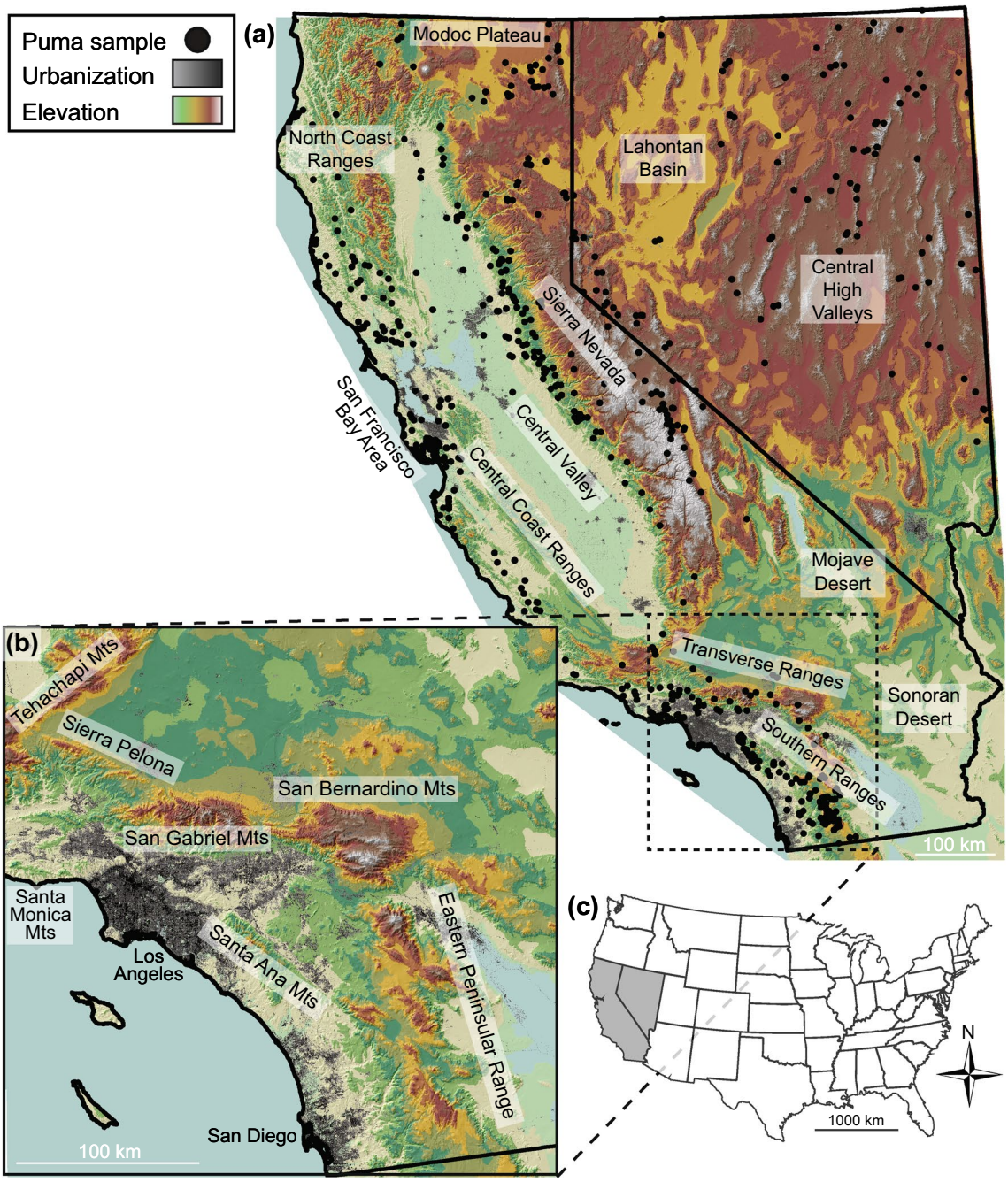

Fig. 1 Map of our study system, including **a** sampling locations of 992 pumas and ecoregions, **b** specific mountain ranges within the Transverse and Southern Ranges, and **c** an inset map of the United States of America showing the locations of California and Nevada. Elevation data source: USGS national elevation dataset (http://nationalmap.gov). Dark circles indicate locations where pumas were sampled, the gray to black scale indicates low to high urbanization, and the blue to white scale indicates 0 m elevation (sea level) to 4,421 m elevation

Multiplex PCR kits with Q solution (Table S1) following the protocols of Gustafson et al. (2017). We included negative and positive controls in each PCR run and visualized fragments with STRand version 2.3.69 (Toonen and Hughes 2001). For each locus, we confirmed heterozygous genotypes at least twice and homozygous genotypes at least three times.

Population genetic structure

The spatial arrangement of sample locations can confound population genetic analyses (Meirmans 2012; Schwartz and McKelvey 2009). Thus, we used spatially-explicit hierarchical Bayesian clustering programs TESS 2.3 (Durand et al. 2009a) and GENELAND 4.0 (Guillot et al. 2005b). We tested for consistency among programs because TESS has been shown to identify finer-scale hierarchical puma population genetic structure compared to GENELAND (Gustafson et al. 2017). In general, TESS outperforms GENELAND in the presence of isolation-by-distance (Safner et al. 2011) whereas GENELAND outperforms TESS at detecting genetic barriers to dispersal (Blair et al. 2012; Safner et al. 2011).

In TESS, the number of populations (K) must be specified and tested over a range of possible values. Model selection must be used to determine the K with the best fit to the data. We followed developer instructions for determining K and population assignments. First, we ran 10 non-admixture models for each K from 2 to 20. For model comparisons, TESS computes a deviance information criterion (DIC). We ran 10 spatially-conditional auto-regressive admixture models for each K to the DIC plateau of non-admixture models (Figs. S1, S2). All models included pairwise great circle geographic distances for weighting the Voronoi neighborhood, 100,000 iterations, and a 25,000 iteration burn-in period. We retained 20% of the models exhibiting the lowest DIC scores and used CLUMPP 1.1.2 to perform model-averaging (Jakobsson and Rosenberg 2007).

In GENELAND, K is optimized by the model. We followed developer recommendations for determining K and individual population assignments (Guillot et al. 2005a). First, we identified a distribution of K from initial models, and then we ran correlated allele frequency models allowing K to vary within its distribution from the initial models (Fig. S1). Finally, we ran 5 spatial, correlated allele frequency models with K fixed at the mode and selected the model with the highest negative log-likelihood value for further inference. Each run included an uncertainty on GPS coordinates of 0.1 decimal degrees (~ 11 km), 1,000,000 iterations, a thinning interval of 10,000, and a 25% burn-in period prior to extracting model output. We assigned individuals to populations based on their highest assignment probability. To visualize the probability of population membership across the study area, we used package POPSutilities 1.0 in R 3.3.0, which interpolates admixture coefficients using geospatial kriging (Jay et al. 2012).

Temporal variation in sampling can bias spatial population genetic analyses; however, spatially-explicit Bayesian clustering models should account for most temporal variation (Durand et al. 2009b; François and Durand 2010). Populations did not group based on sampling date in TESS or GENELAND. Additionally, isolation-by-distance was significant across our study area ($R^2 = 0.15$, $P < 0.001$). Although TESS and GENELAND showed nearly identical results, we used TESS admixture models for analyses and inferences because TESS outperforms GENELAND in the presence of isolation-by-distance.

Genetic diversity

We tested for linkage disequilibrium, deviations from Hardy–Weinberg proportions, and null alleles in GENEPOP 4.5.1 (Rousset 2008). For each identified population, we calculated standard measures of genetic diversity and used 1000 permutations to test for significant genetic isolation-by-distance in GenAlEx 6.502 (Peakall and Smouse 2006, 2012). To measure the number of alleles, we calculated allelic richness using rarefaction methods which correct for sample size in FSTAT 2.9.3.2 (Goudet 1995). To assess inbreeding, we calculated internal relatedness using package Rhh 1.0.2 in Program R 3.3.0 (Alho et al. 2010). We calculated effective population size (N_e) for each population using NeEstimator 2.01 using the linkage disequilibrium method assuming random mating (Do et al. 2014). Because the inclusion of low-frequency alleles can upwardly bias estimates of N_e (Waples and Do 2010), we ran two separate models including alleles with frequencies $\geq 5\%$ or $\geq 1\%$. To test for evidence of recent reductions in N_e (i.e., genetic bottlenecks), we used program BOTTLENECK 1.2.02 to determine if a population exhibited a significant number of loci with heterozygote excess (Piry et al. 1999). For each population identified by assignment models, we performed bottleneck analyses using two-phase (70:30 step-wise:infinite-alleles) microsatellite mutation models for 100,000 iterations.

We used biotools 3.1 (da Silva et al. 2017) in R to obtain spatial unbiased genetic diversity estimates [uHe: unbiased expected heterozygosity; (Nei 1978)] based on the interpolation of individual estimates (Manel et al. 2007). We minimized spatial extrapolation by using a radius of 500 m and reduced bias by setting the neighborhood size (i.e., minimum number of individuals used to calculate uHe) to 2. The mean size of each neighborhood was 14.6 and 42.5% of the neighborhoods contained at least 10 individuals.

Population differentiation and genetic source–sink dynamics

We used three complementary approaches to assess functional population connectivity, including a discriminant analysis of principal components (DAPC), pairwise estimates of population divergence (F_{ST}), and pairwise estimates of bi-directional migration rates (m). The DAPC uses linear combinations of alleles to maximize between-population genetic variation and provides a graphical representation

of functional connectivity among genetic clusters (Jombart et al. 2010).

We implemented the DAPC in program R using package adegenet 2.0.1 (Jombart 2008). The identified number of genetic clusters in adegenet agreed with TESS and GENELAND (Fig. S3). Because the algorithm for individual assignments in adegenet is not as powerful as Bayesian population assignment algorithms (Jombart et al. 2010), we defined populations in the DAPC using results from the Bayesian population assignments. Because we were not assigning individual membership probabilities in the DAPC, we retained all information (i.e., 344 PCA axes and all 9 discriminant functions) in the analysis. Our results from retaining all information did not differ from results when only retaining an estimated optimal number of PCA axes using the α-score method. Pairwise population divergence estimates (F_{ST}) were calculated in GenAlEx using 999 permutation tests for significance. To conform to the expectations of genetic isolation-by-distance, rather than an island model, we also calculated Rousset's $F_{ST}/(1-F_{ST})$ (Rousset 1997).

We used program BayesAss 3.0 to estimate migration rates (*m*) among populations identified by population assignment models (Wilson and Rannala 2003). We used 10 randomly-seeded runs each with 5,000,000 iterations, a burnin of 1,000,000, and thinning interval of 1000. Posterior mean parameter estimates were nearly identical among runs, and all trace files indicated convergence of model parameters (Meirmans 2014). We tested the hypothesis of Andreasen et al. (2012) that Nevada pumas were a genetic source for California pumas by summing emigration rates and subtracting the sum of immigration rates for each population (Andreasen et al. 2012). Positive numbers indicate the population was a genetic source whereas negative numbers indicate a sink. We used package circlize 0.3.7 in program R to visualize bi-directional migration rates estimated in BayesAss (Gu et al. 2014).

Results

Population genetic structure and diversity

Our analyses revealed that pumas in California exhibited strong population genetic structure and some California populations had extremely low levels of genetic diversity. We identified nine genetic clusters in California and one genetic cluster in Nevada (Figs. 2, S1, S2, S4). We classified these 10 genetic clusters as genetic populations, including the Nevada (NV), Eastern Sierra Nevada (ESN), Western Sierra Nevada (WSN), North Coast (NC), Northern section of the Central Coast (CC-N), Central section of the Central Coast (CC-C), Southern section of the Central Coast (CC-S), San Gabriel/San Bernardino (SGSB), Santa Ana (SA), and Eastern Peninsular Range (EP) populations (Fig. 2).

The genetic diversity of California puma populations exhibited a large amount of variation with some populations having estimates similar to other large populations and some exhibiting estimates nearly as low as the endangered Florida Panther. The NV, ESN, and WSN populations had the highest estimates of genetic diversity compared to other populations (Table 1). Regionally, the Modoc Plateau and Sierra Nevada contained individuals that had consistently high genetic diversity (Fig. 3). Although the NV population had high genetic diversity, the individual-based analysis indicated spatially-heterogenous genetic diversity across Nevada with low levels occurring near the Lahontan Basin (Fig. 3). The CC-C population had relatively intermediate levels of genetic diversity (Table 1). The SA population had the lowest genetic diversity observed across all estimates, followed by the SGSB, NC, CC-S, and CC-N populations. SA also had the highest measure of internal relatedness. WSN had the largest effective population size (N_e), followed by NV, NC, and CC-C (Table 2). All other populations had an N_e of < 50 (often given as a desirable minimum from a conservation genetics point of view; Frankham 1995; Mace et al. 2008), and CC-S and SGSB had extremely low effective population sizes (≤ 5). All populations except NV and NC exhibited evidence of a prior genetic bottleneck (Table 2).

Population differentiation and genetic source–sink dynamics

Our discriminant analysis of principal components (DAPC) revealed that puma populations in California had low connectivity compared to pumas in Nevada which were composed of a single genetic population that exhibited high connectivity with several California populations. The first axis (x-axis; 33.3% of total variation) of the DAPC broadly corresponded to a latitudinal population separation with north to the left and south to the right (Fig. 4a). The second axis (y-axis; 24.4%) separated populations longitudinally and primarily separated central coast populations from southern populations (Fig. 4a). The NV, ESN, WSN, and NC populations grouped together, as did the CC-N, CC-C, and CC-S populations. The SA and EP populations grouped slightly but were separated from all other populations (Fig. 4a). Lastly, the SGSB was intermediate relative to all other populations, but was most closely-related to the WSN population (Fig. 4a).

Bi-directional migration rate models indicated there were 5 genetic source populations (i.e., ESN, WSN, CC-N, CC-C, EP) and 5 genetic sink populations (i.e., NV, NC, CC-S, SGSB, SA), however, there was only weak evidence indicating CC-N and NC were source and sink populations, respectively. Bi-directional migration

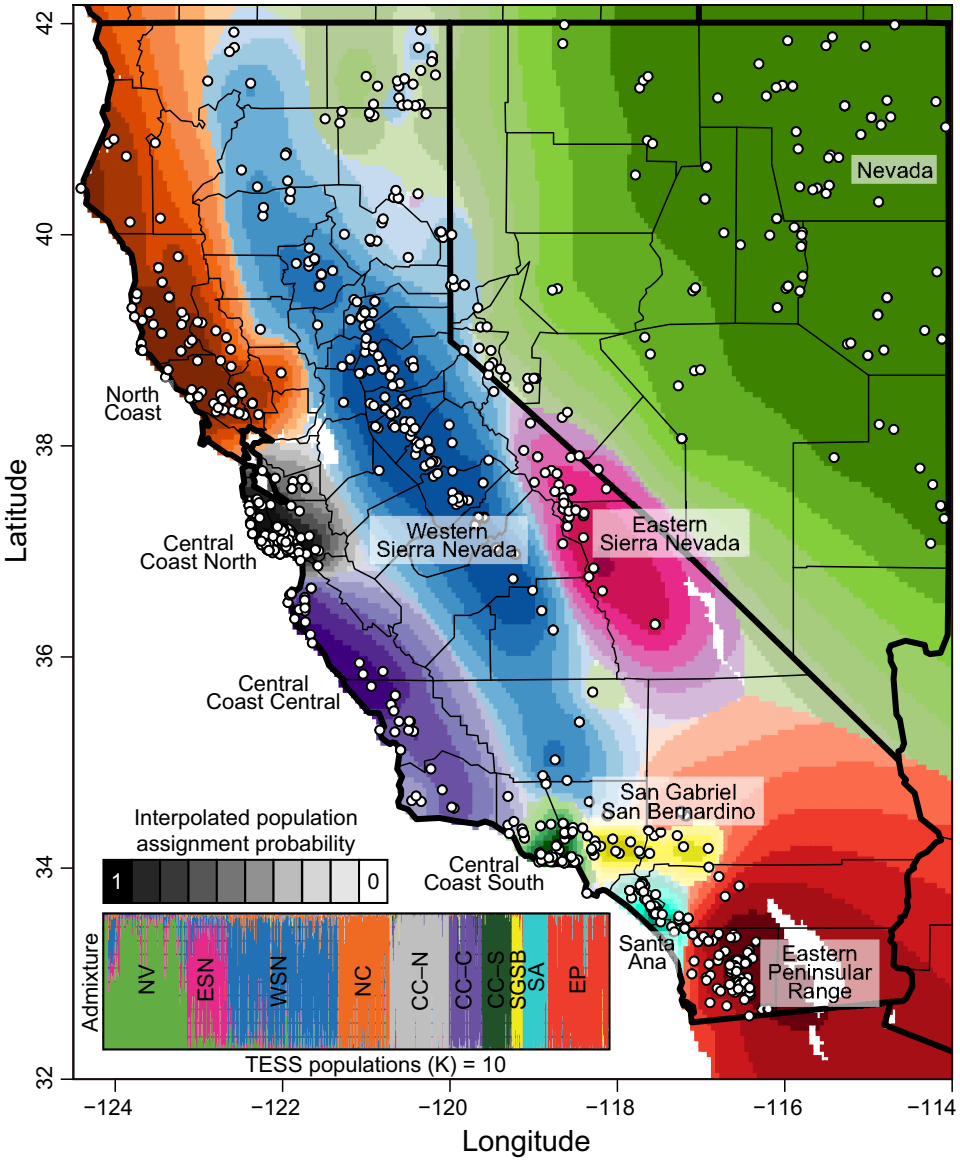

Fig. 2 Population genetic structure of pumas across California and Nevada. Individual admixture proportions from TESS (inset barplot) were spatially-interpolated. Each color represents a genetic population. The decay in color intensity on the map represents lower probabilities of population assignment and indicates areas with admixture between populations. State and county borders are displayed for reference. *NV* Nevada, *ESN* Eastern Sierra Nevada, *WSN* Western Sierra Nevada, *NC* North Coast, *CC-N* Northern section of the Central Coast, *CC-C* Central section of the Central Coast, *CC-S* Southern section of the Central Coast, *SGSB* San Gabriel/San Bernardino, *SA* Santa Ana, *EP* Eastern Peninsular Range

rate estimates showed connectivity patterns similar to the DAPC (Fig. 4). Although there was gene flow among the NV, ESN, and WSN populations based on bi-directional migration rates, the NC population primarily exchanged migrants with the ESN and WSN populations (Fig. 4b). The populations in the Sierra Nevada (ESN, WSN) were the greatest genetic source populations but exhibited limited gene flow with the populations along the central coast of California (CC-N, CC-C, CC-S), and neither NV nor NC exhibited appreciable gene flow with central coast populations (Fig. 4b; Table S2). The SA population exhibited gene flow only with the EP population, and the EP population had low connectivity with the SGSB population (Fig. 4b). The puma population in the Transverse Ranges (SGSB) was the largest genetic sink but exchanged some genetic material with the WSN, CC-C, and EP populations (Fig. 4b). Populations in the Southern Ranges (SA, EP) were largely disconnected from all other populations (Fig. 4b).

Table 1 Allelic and genetic diversity of puma populations, including sample-size corrected allelic richness, the number of private alleles, the percent of polymorphic loci, observed heterozygosity, unbiased expected heterozygosity, and average internal relatedness (a measure of inbreeding)

Population	N	Allelic richness	Private alleles	Polymorphic Loci (%)	Observed heterozygosity	Expected heterozygosity	Internal relatedness
NV	166	3.47 (0.09)	9	100	0.50 (0.03)	0.52 (0.03)	0.15 (0.01)
ESN	79	3.46 (0.13)	5	100	0.52 (0.03)	0.53 (0.03)	0.11 (0.01)
WSN	217	3.63 (0.08)	5	100	0.51 (0.03)	0.52 (0.03)	0.09 (0.01)
NC	101	3.06 (0.10)	5	97.6	0.40 (0.03)	0.41 (0.03)	0.28 (0.01)
CC-N	116	2.62 (0.08)	1	97.6	0.41 (0.03)	0.42 (0.03)	0.27 (0.01)
CC-C	63	3.00 (0.12)	1	95.2	0.45 (0.03)	0.46 (0.03)	0.19 (0.02)
CC-S	60	2.63 (0.13)	1	92.9	0.41 (0.04)	0.41 (0.03)	0.27 (0.02)
SGSB	22	2.75 (0.17)	0	95.2	0.40 (0.03)	0.42 (0.03)	0.29 (0.03)
SA	48	2.27 (0.12)	0	85.7	0.34 (0.03)	0.33 (0.03)	0.39 (0.02)
EP	120	3.07 (0.11)	3	100	0.44 (0.03)	0.44 (0.03)	0.21 (0.01)

NV Nevada, *ESN* Eastern Sierra Nevada, *WSN* Western Sierra Nevada, *NC* North Coast, *CC-N* Northern section of the Central Coast, *CC-C* Central section of the Central Coast, *CC-S* Southern section of the Central Coast, *SGSB* San Gabriel/San Bernardino, *SA* Santa Ana, *EP* Eastern Peninsular Range. Standard errors are presented in parentheses

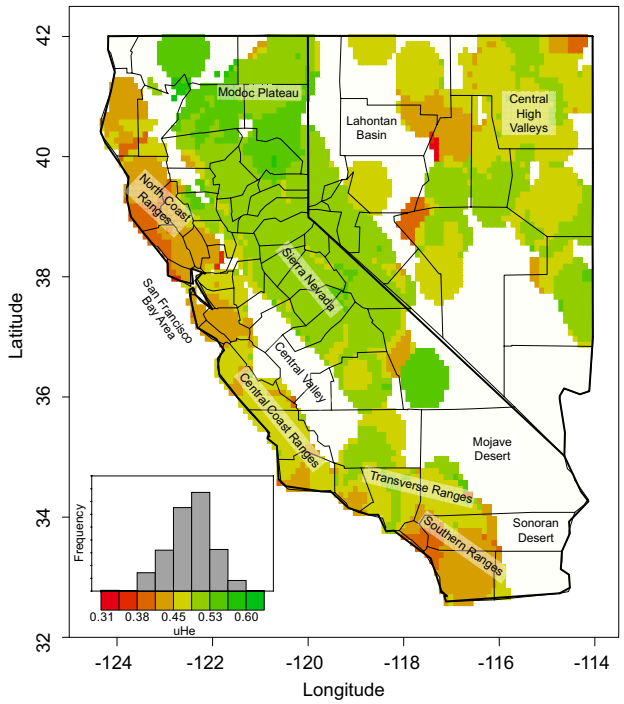

Fig. 3 Gene diversity (uHe: unbiased expected heterozygosity) heat map of pumas in California and Nevada. Neighborhood size was not significantly related to uHe ($R^2 = 0.005$, $P < 0.001$)

Discussion

We identified 10 genetically-distinct puma populations within California and Nevada that varied considerably in genetic diversity (uHe range 0.33–0.53) and effective population size (N_e range 5–157). Some of our previous analyses identified family-level genetic structure which was not observed here (Ernest et al. 2014; Gustafson et al. 2017; Riley et al. 2014), indicating these genetic populations are not the result of sampling related individuals. The large number of populations (N = 9) and the strong genetic differences among neighboring puma populations in California differed from other studies at similar spatial scales (Anderson et al. 2004; Holbrook et al. 2012; Loxterman 2011; McRae et al. 2005), including Nevada (Andreasen et al. 2012). Most state-wide studies have been conducted in less-developed locations with more continuous habitat and showed that geographic distance and natural landscape components were the most common factors associated with the broad-scale genetic structure of puma populations (Anderson et al. 2004; Holbrook et al. 2012; Loxterman 2011; McRae et al. 2005; Wright 1943). In contrast, mountain ranges in California are variable in size and arrangement and there are vast areas of inter-mountain anthropogenic development throughout the state. Previous local studies in California have identified individual roadways and associated human development as major barriers to puma movements (Ernest et al. 2014; Gustafson et al. 2017; Riley et al. 2014; Vickers et al. 2015), and our study confirms, on a broad geographic scale, strong population structure among adjacent puma populations. The considerable variation in genetic diversity and effective population size among California and Nevada populations is likely attributable to the variation in the amount of suitable habitat and their degree of isolation. The Western Sierra Nevada population had the largest effective size and was closely related (i.e., lowest F_{ST} values) to every population except for the Northern Central Coast population and populations south of Los Angeles (Santa Ana, Eastern Peninsular Range), suggesting puma populations form a

Table 2 Summary of effective population size and bottleneck analyses for each population

Population	Sample size	Effective population size (N_e) N_e with AFs ≥ 0.05	Bottleneck N_e with AFs ≥ 0.01	P-value
NV	166	92.2 (84.2–101.4)	107.2 (98.5–117.1)	0.123
ESN	79	22.6 (20.8–24.5)	26.5 (24.7–28.5)	< **0.001**
WSN	217	157.5 (141.2–176.8)	180.6 (164.1–199.7)	**0.038**
NC	101	82.5 (71.3–96.8)	66 (59.3–73.9)	0.256
CC-N	116	16.6 (15.1–18.2)	15.5 (14.2–16.8)	**0.001**
CC-C	63	56.6 (47.4–69.0)	63 (53.3–75.8)	**0.018**
CC-S	60	2.7 (2.5–2.9)	3.6 (3.4–3.9)	**0.008**
SGSB	22	5 (3.3–6.4)	7.5 (6.2–9.1)	**0.046**
SA	48	15.6 (13–18.7)	21.7 (18–26.4)	**0.007**
EP	120	31.6 (29.1–34.4)	37.4 (34.5–40.5)	**0.021**

AF allele frequencies, *NV* Nevada, *ESN* Eastern Sierra Nevada, *WSN* Western Sierra Nevada, *NC* North Coast, *CC-N* Northern section of the Central Coast, *CC-C* Central section of the Central Coast, *CC-S* Southern section of the Central Coast, *SGSB* San Gabriel/San Bernardino, *SA* Santa Ana, *EP* Eastern Peninsular Range. Parametric 95% confidence intervals are presented in parentheses. Bottleneck P-values from standardized differences tests are presented

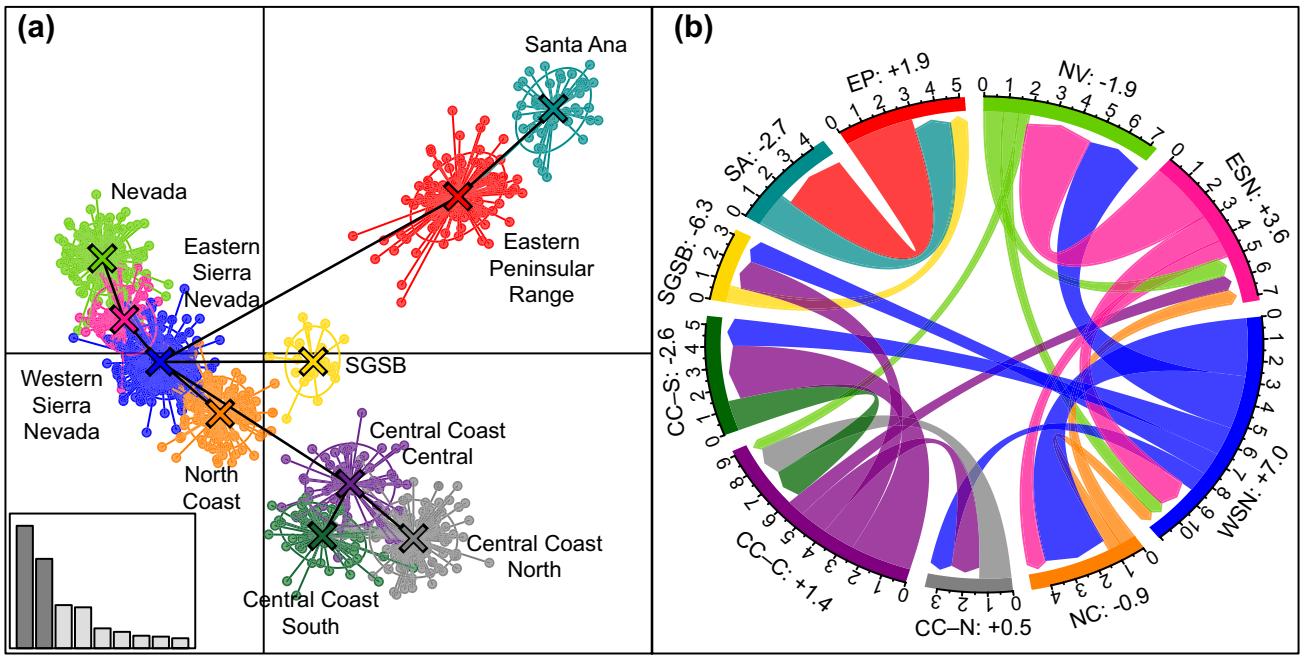

Fig. 4 Functional connectedness of puma populations, based on **a** a discriminant analysis of principal components and **b** bi-directional migration rate estimates (multiplied by 100 for visualization). Each dot represents an individual (**a**). Each color **a, b** represents a population. Black lines **a** indicate the most closely-related population based on genetic dissimilarities. The inset barplot **a** shows which axes are being displayed (i.e., discriminant functions 1 and 2) and the relative proportion of variation explained by each of the 9 discriminant functions. Two-thirds of the individuals in each population are contained within the corresponding ellipsoid. For a biologically meaningful interpretation, only estimates of interpopulation migration rates with 95% confidence intervals that do not cross 0 are presented (b; Table S2). Net genetic source–sink migration rates are presented next to population names with positive values indicating a net genetic source and negative values indicating a net genetic sink (e.g., WSN exported 9% of migrants and received 2%, so its net rate is +7.0). *NV* Nevada, *ESN* Eastern Sierra Nevada, *WSN* Western Sierra Nevada, *NC* North Coast, *CC-N* Northern section of the Central Coast, *CC-C* Central section of the Central Coast, *CC-S* Southern section of the Central Coast, *SGSB* San Gabriel/San Bernardino, *SA* Santa Ana, *EP* Eastern Peninsular Range

"horseshoe" network around the Central Valley with San Francisco Bay acting as a major barrier along the coast (Hooper 1944). The large National Parks and National Forests (e.g., Sequoia–Kings Canyon and Yosemite National Parks) in the Sierra Nevada provide contiguous habitat for pumas with minimal anthropogenic infrastructure (Ernest et al. 2000).

Our results are consistent with a previous report (Andreasen et al. 2012) indicating pumas from Nevada form a single genetic cluster and are distinct from pumas in the Sierra Nevada of California, but our results contrast with their suggestion that pumas from Nevada are a genetic source for pumas in California. There are several differences between the studies that could explain the inconsistencies. Andreasen et al. (2012) used considerably fewer genetic markers than the present study (9 microsatellites vs. 42). Because the number of loci used in bi-directional migration rate models has the largest effect on the accuracy of the estimates (Faubet and Gaggiotti 2008; Wilson and Rannala 2003), we expect the differences are driven by the different number of loci. Although we sampled fewer pumas from Nevada and more pumas from California, sample size differences generally only affect the variance and not the accuracy of the bi-directional migration rate estimates (Faubet and Gaggiotti 2008; Wilson and Rannala 2003). Further, sample size alone likely does not explain the contrasting results and the multiple lines of evidence supporting the Sierra Nevada populations as a genetic source for the surrounding populations, including Nevada.

Both our population-level and individual-based analyses clearly indicated that the Western Sierra Nevada population had the highest genetic diversity, which is likely being maintained by the large effective population size and not via migrants from the Nevada population, which had lower genetic diversity estimates. Further, instead of testing migration rates among the two populations (K = 2) which had the highest model support in their study, Andreasen et al. (2012) tested among five genetic clusters (K = 5) which had average within-cluster migration estimates of only 54% (and a large SD of 8.4%) compared to our within-population migration estimates of 94% (± 1.9%). Thus, their examination of genetic source–sink dynamics was based on significantly less distinct genetic units (F_{ST} = 0.05–0.09 compared to our study where Rousset's F_{ST} = 0.05–0.39), which is computationally problematic with a small number of loci (Faubet and Gaggiotti 2008; Wilson and Rannala 2003). Additionally, puma hunting is legal in Nevada but not California, and puma densities that have been reduced regionally from hunter harvest are known to be compensated by higher immigration rates from neighboring populations (Cooley et al. 2009; Robinson et al. 2008), which is biologically consistent with our observations.

The North Coast and inland populations (Nevada, Eastern Sierra Nevada, Western Sierra Nevada) appear to be large (i.e., high N_e), genetically diverse, and well-connected, and may form an evolutionary significant unit (ESU: a group of populations that have accumulated adaptive differences from other populations in part from reproductive isolation; Palsbøll et al. 2007). However, genome-wide data and gene–environment correlation studies will be needed to evaluate whether these population are exhibiting adaptations to specific habitats or ecoregions. Within this group of populations, we detected evidence for bottlenecks in the Eastern Sierra Nevada population and Western Sierra Nevada population. The bottleneck in the Eastern Sierra Nevada population is not surprising given that the puma abundance in this region may have been reduced by 50% after a severe decline in mule deer (Pierce and Bleich 2014; Pierce et al. 2000; Villepique et al. 2011). Besides the North Coast and Nevada populations, all of the other populations also exhibited evidence of genetic bottlenecks; however, we do not know if this was caused by urbanization, a decrease in prey abundance, or some other factor, because the demographic and genetic histories of these populations are not well-documented.

The Central population of the Central Coast exhibited intermediate levels of genetic diversity, and maintaining gene flow from this population to the genetically-depauperate Northern and Southern Central Coast populations is critically important for their long-term viability (Benson et al. 2016; Gray et al. 2016; Riley et al. 2014). A previous report examined the southern area of the central coast region specifically and observed extremely low genetic diversity in the Santa Monica Mountains, south of Highway 101 in the Los Angeles Area (Riley et al. 2014). At a statewide level, we found pumas in the Santa Monica Mountains to be part of a larger genetic population including pumas in the Simi Hills and Santa Susana Mountains; however, our larger sample from the Southern Central Coast population revealed only slightly higher estimates of genetic diversity than pumas sampled from the Santa Monica Mountains alone (Riley et al. 2014). Road-isolated pumas in the Santa Monica Mountains only receive rare migrants from the Simi Hills and Santa Susana Mountains and are at a high risk of extirpation from isolation and subsequent demographic and genetic stochasticity (Benson et al. 2016). These results emphasize the need to conserve within-population connectivity, specifically from the Coast Ranges and the Sierra Nevada through the Santa Susana Mountains and Simi Hills to the Santa Monica Mountains.

Despite being very close geographically, the puma populations around Los Angeles (Southern Central Coast, San Gabriel/San Bernardino, Santa Ana) are highly diverged. For example, the Santa Ana and Southern Central Coast population are among the closest populations geographically

(~ 100 km apart) but are among the most genetically distant populations we observed (Rousset's $F_{ST} = 0.32$). Additionally, the Southern Range populations (Santa Ana, Eastern Peninsular Range) are largely disconnected from all other populations in this study, including those just to the north of the Los Angeles Basin. These observations are consistent with the hypothesis of reduced connectivity from habitat fragmentation by human development (i.e., the Los Angeles metropolitan area), including major roads (i.e., I-10, I-15, I-210, etc.) (Ernest et al. 2003). The San Gabriel/San Bernardino population was most genetically similar to the Western Sierra Nevada, Central region of the Central Coast, and Eastern Peninsular Range populations, indicating it is an area of intersection between multiple populations. We suggest the small mountain ranges in this area (i.e., Tehachapi, Sierra Pelona, San Gabriel, and San Bernardino Mountains) are necessary for contiguous statewide genetic connectivity and that pumas occupying those ranges, and the wildlands habitat in those ranges, should be considered conservation priorities (Beier et al. 2009; Ernest et al. 2003; Wildlands 2008).

The Santa Ana population exhibited the lowest measures of genetic diversity and the highest measures of inbreeding among all populations, with levels nearing those of Florida panthers (most recent estimates of $He = 0.24$), which nearly went extinct from genetic factors prior to artificial genetic rescue (Johnson et al. 2010). It is important to note, however, that out of the 42 microsatellite loci used in this study, only 4 were shared with the 23 microsatellite loci used in the Florida panther study. A set of shared markers would be most appropriate for direct interpopulation comparisons (e.g., Culver et al. 2000). A single immigrant from the Eastern Peninsular Range recently enhanced the genetic diversity of Santa Ana pumas and is likely responsible for the higher effective population size than previously observed (Ernest et al. 2014). Nevertheless, genetic diversity of Santa Ana pumas will decline without additional immigration (Gustafson et al. 2017). The Eastern Peninsular Range population had the highest genetic diversity and effective size among the populations in the Los Angeles—San Diego area (Southern Central Coast, San Gabriel/San Bernardino, Santa Ana, Eastern Peninsular Range). Restoring connectivity with the Eastern Peninsular Range and reducing further impacts from development on gene flow among the adjacent populations, including pumas from Arizona and Mexico (Gustafson et al. 2017), are critically important to avoiding extirpation of genetically-depauperate populations (Benson et al. 2016).

By identifying puma populations and measuring gene flow among them, our analyses can help guide and inform puma conservation and management. Whenever possible, government agencies and other stakeholders should consider population connectivity and prevent further fragmentation by human development both within and among populations. In contrast to other studies in 7 western states that generally indicated weak puma genetic structure (Anderson et al. 2004; Holbrook et al. 2012; Loxterman 2011; McRae et al. 2005), our study showed strong genetic structure. Although puma habitat in California is aggregated and separated by valleys, it is unlikely these valleys would have been such strong barriers to gene flow pre-development given that pumas have been documented to move across the entire Central Valley post-development (Ernest et al. 2003; McClanahan et al. 2017). Further, similar geographic features, such as the Wyoming Basin, have not been reported to structure puma populations (Anderson et al. 2004). Instead, we hypothesize that human-associated infrastructure within the valleys are artificially isolating pumas beyond what they would naturally experience among ecoregions.

Population-level conservation strategies are needed to reintegrate fragmented, at-risk populations into a connected multi-state, multi-landscape population network (Zeller et al. 2017). Gene flow via maintenance of existing occupied habitat combined with improved and additional networks of wildlife corridors (Bennett 2017; Gloyne and Clevenger 2001; Johnson et al. 2010; Sawaya et al. 2013) will ultimately be necessary to promote the long-term persistence of isolated populations (Benson et al. 2016; Ernest et al. 2014; Gustafson et al. 2017; Riley et al. 2014). Without such measures, it is likely too late to expect a natural increase in genetic connectivity or selection for increased dispersal (Burdett et al. 2010; Cheptou et al. 2017), and assisted gene flow may be needed in perpetuity for several populations to remain viable (Benson et al. 2011, 2016; Ernest et al. 2014; Gustafson et al. 2017; Johnson et al. 2010; Vickers et al. 2015).

In some of these populations, individual migrants are of immediate conservation importance, and human-induced mortality should be avoided to the extent possible. The effects of fragmentation on multiple populations of this umbrella species are likely indicative of a larger ecological problem in one of the most biologically diverse regions of North America (Calsbeek et al. 2003; Dobson et al. 1997; Thorne et al. 2006). We strongly encourage land owners and managers to proactively consider broad-scale wildlife connectivity in future development proposals. However, in the absence of maintaining habitat of a spatial scale grand enough to ensure the persistence of prey and predator populations, the issue of connectivity will become a moot point.

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Data availability Through agreements with non-profit organizations, private landowners, and Native American Tribes, exact GPS locations of puma samples are not to be publicly shared. Thus, puma GPS locations are referenced to the nearest town or city. Sampling locations and microsatellite genotypes are available on Dryad: https://doi.org/10.5061/dryad.j76c4k4.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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