Received: 18 September 2018 | Revised: 11 August 2019 | Accepted: 20 September 2019

DOI: 10.1111/mec.15261

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Urbanization impacts apex predator gene flow but not genetic diversity across an urban-rural divide

Daryl R. Trumbo[1] | Patricia E. Salerno[1] | Kenneth A. Logan[2] | Mathew W. Alldredge[3] | Roderick B. Gagne[4] | Christopher P. Kozakiewicz[5] | Simona Kraberger[4] | Nicholas M. Fountain-Jones[6] | Meggan E. Craft[6] | Scott Carver[5] | Holly B. Ernest[7] | Kevin R. Crooks[8] | Sue VandeWoude[4] | W. Chris Funk[1,9]

[1]Department of Biology, Colorado State University, Fort Collins, CO, USA

[2]Colorado Parks and Wildlife, Montrose, CO, USA

[3]Colorado Parks and Wildlife, Fort Collins, CO, USA

[4]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA

[5]Department of Biological Sciences, University of Tasmania, Hobart, TAS., Australia

[6]Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA

[7]Department of Veterinary Sciences, University of Wyoming, Laramie, WY, USA

[8]Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, CO, USA

[9]Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA

Correspondence
Daryl R. Trumbo, Department of Biology, Colorado State University, Fort Collins, CO, USA.
Email: daryl.trumbo@gmail.com

Funding information
Directorate for Biological Sciences, Grant/Award Number: 1413925 and 723676

Abstract

Apex predators are important indicators of intact natural ecosystems. They are also sensitive to urbanization because they require broad home ranges and extensive contiguous habitat to support their prey base. Pumas (*Puma concolor*) can persist near human developed areas, but urbanization may be detrimental to their movement ecology, population structure, and genetic diversity. To investigate potential effects of urbanization in population connectivity of pumas, we performed a landscape genomics study of 130 pumas on the rural Western Slope and more urbanized Front Range of Colorado, USA. Over 12,000 single nucleotide polymorphisms (SNPs) were genotyped using double-digest, restriction site-associated DNA sequencing (ddRAD-seq). We investigated patterns of gene flow and genetic diversity, and tested for correlations between key landscape variables and genetic distance to assess the effects of urbanization and other landscape factors on gene flow. Levels of genetic diversity were similar for the Western Slope and Front Range, but effective population sizes were smaller, genetic distances were higher, and there was more admixture in the more urbanized Front Range. Forest cover was strongly positively associated with puma gene flow on the Western Slope, while impervious surfaces restricted gene flow and more open, natural habitats enhanced gene flow on the Front Range. Landscape genomic analyses revealed differences in puma movement and gene flow patterns in rural versus urban settings. Our results highlight the utility of dense, genome-scale markers to document subtle impacts of urbanization on a wide-ranging carnivore living near a large urban center.

KEYWORDS
effective population size, gene flow, genetic diversity, landscape genomics, *Puma concolor*, urbanization

1 | INTRODUCTION

Urbanization is a major threat to biodiversity, and in particular to apex predators with broad home ranges (Cohen, 2003; Crooks et al., 2017; Theobald, 2005). Habitat fragmentation due to urbanization can have important impacts on predator movement, disease, and survival (Carver et al., 2016; Fountain-Jones et al., 2017; Markovchick-Nicholls et al., 2008). This reduced connectivity can

lead to smaller, more isolated populations, where less gene flow and genetic diversity, as well as smaller effective population sizes (Ernest, Vickers, Morrison, Buchalski, & Boyce, 2014; Riley et al., 2006; Vandergast, Bohanak, Weissman, & Fisher, 2007) ultimately cause local and regional extirpations through environmental and demographic stochasticity and inbreeding depression (Allendorf, Luikart, & Aitken, 2013). Moreover, increased human recreational activities in wildlife habitats associated with nearby urbanization can change wildlife movement patterns and habitat usage, exacerbating the impacts of fragmentation (Lewis et al., 2015; McKinney, 2002). As human populations continue to expand worldwide, urban areas are becoming larger and more extensive on the landscape. However, we do not fully understand how urbanization affects natural ecosystems near wildland-urban interfaces (Magle, Hunt, Vernon, & Crooks, 2012; Radeloff et al., 2005).

Large carnivores are important indicators of intact natural ecosystems, as they require an abundant and sustainable prey base, as well as high habitat connectivity to support their broad home ranges (Sergio et al., 2008; Sergio, Newton, Marchesi, & Pedrini, 2006). However, understanding the effects of urbanization on large carnivores is difficult due to their low population densities and secretive nature (Hornocker & Negri, 2009; Logan & Sweanor, 2001; Riley et al., 2006). Camera traps, radiotelemetry, and GPS collars provide valuable information on animal home ranges and population sizes (e.g., Blecha, Boone, & Alldredge, 2018; Lewis et al., 2015), but these studies are expensive, time consuming, and can only monitor a small fraction of the total population for limited time periods. Population and landscape genetics can provide additional, complementary techniques for a more detailed understanding of wildlife populations (Balkenhol, Cushman, Storfer, & Waits, 2016; Epps, Wehausen, Bleich, Torres, & Brashares, 2007; Lowe & Allendorf, 2010). Genetic studies provide an indicator of functional landscape connectivity through measures of gene flow, effective population sizes of breeding individuals, and cost-efficient monitoring of genetic diversity across broad geographic areas (McRae, Beier, Dewald, Huynh, & Keim, 2005; Solberg, Bellemain, Drageset, Taberlet, & Swenson, 2006). Moreover, recent high-throughput sequencing technologies enable the genotyping of many more thousands of loci than previously possible, providing higher power to detect the often subtle population genetic structure of wide-ranging species such as large carnivores (Holderegger, Kamm, & Gugerli, 2006; Luikart, England, Tallmon, Jordan, & Taberlet, 2003).

Pumas (*Puma concolor*; other common names include mountain lions, cougars, panthers, catamounts) are a large, apex predator with one of the broadest latitudinal ranges of any terrestrial carnivore, spanning western North America, Central America, and South America (Hornocker & Negri, 2009). Pumas are sensitive to urbanization, requiring broad-scale landscape connectivity to persist, and are thus useful indicators for monitoring the effects of urban fragmentation (Beier, 1995; Crooks, 2002; Maletzke et al., 2017). Given sufficient habitat area and landscape connectivity, however, pumas can still persist within and adjacent to urban systems (Blecha et al., 2018; Lewis et al., 2015; Riley et al., 2014; Wilmers et al., 2013; Zeller, Vickers, Ernest, & Boyce, 2017). Furthermore, the substantial area requirements of large carnivores such as pumas can enhance their role as umbrella species, whose protection also benefits co-occurring species through broadscale habitat preservation (Thorne, Cameron, & Quinn, 2006).

The southern Rocky Mountains in western Colorado, USA support natural habitats with high puma densities, as well as many rural and urban human developments (Hornocker & Negri, 2009). The Western Slope of the Rocky Mountains primarily consists of large areas of contiguous public wildlands with an abundant prey base for pumas, interspersed with small rural and exurban developments, including the Uncompahgre Plateau region near the town of Montrose (Western Slope Study Area; Figure 1). In contrast, the Front Range is a rapidly urbanizing, major metropolitan area on the Eastern Slope of the Continental Divide, where urbanization is spreading from lower elevation areas in and around the Denver Metropolitan Area into adjacent wildland habitats in the foothills of the Rocky Mountains. Pumas continue to persist near this wildland-urban interface, including adjacent to the city of Boulder on the western edge of the Denver Metropolitan Area (Front Range Study Area; Figure 1; Lewis et al., 2015; Moss, Alldredge, Logan, & Pauli, 2016). From 2010–2017, Colorado was the eighth fastest growing U.S. state by population (577,829 residents added) and the sixth fastest by percentage (11.5% population growth; US Census Bureau, 2017), with most of this growth occurring along the eastern edge of the Front Range. Thus, comparative studies of puma movement and gene flow in one of the most populous states in the midcontinental USA, which also supports a robust puma population, can provide insight into the effects of urbanization on this important apex predator.

Here, we tested how different landscape factors, including urbanization, enhance or restrict gene flow and genetic diversity in a large apex predator across an urban-rural divide in Colorado, USA. A large sample of pumas were utilized from (a) the rural Western Slope and (b) the more urbanized Front Range (n = 130; 76 in the Western Slope, 54 in the Front Range; Figure 1). We used double digest restriction site associated DNA sequencing (ddRADseq) to genotype pumas at 12,444 single nucleotide polymorphism (SNP) loci to evaluate the potential differences in gene flow, effective population sizes, genetic diversity, and population structure in these two different landscapes. We tested landscape genomic hypotheses by correlating key landscape factors with puma genetic distance measures. We hypothesized that pumas in the more urbanized Front Range would have (a) smaller effective population sizes, (b) lower levels of genetic diversity, and (c) more landscape factors related to urbanization that restrict gene flow, relative to the rural Western Slope landscape.

2 | MATERIALS AND METHODS

2.1 | Samples and sequences

Puma blood and tissue samples were collected as part of ongoing monitoring efforts by Colorado Parks and Wildlife in both the

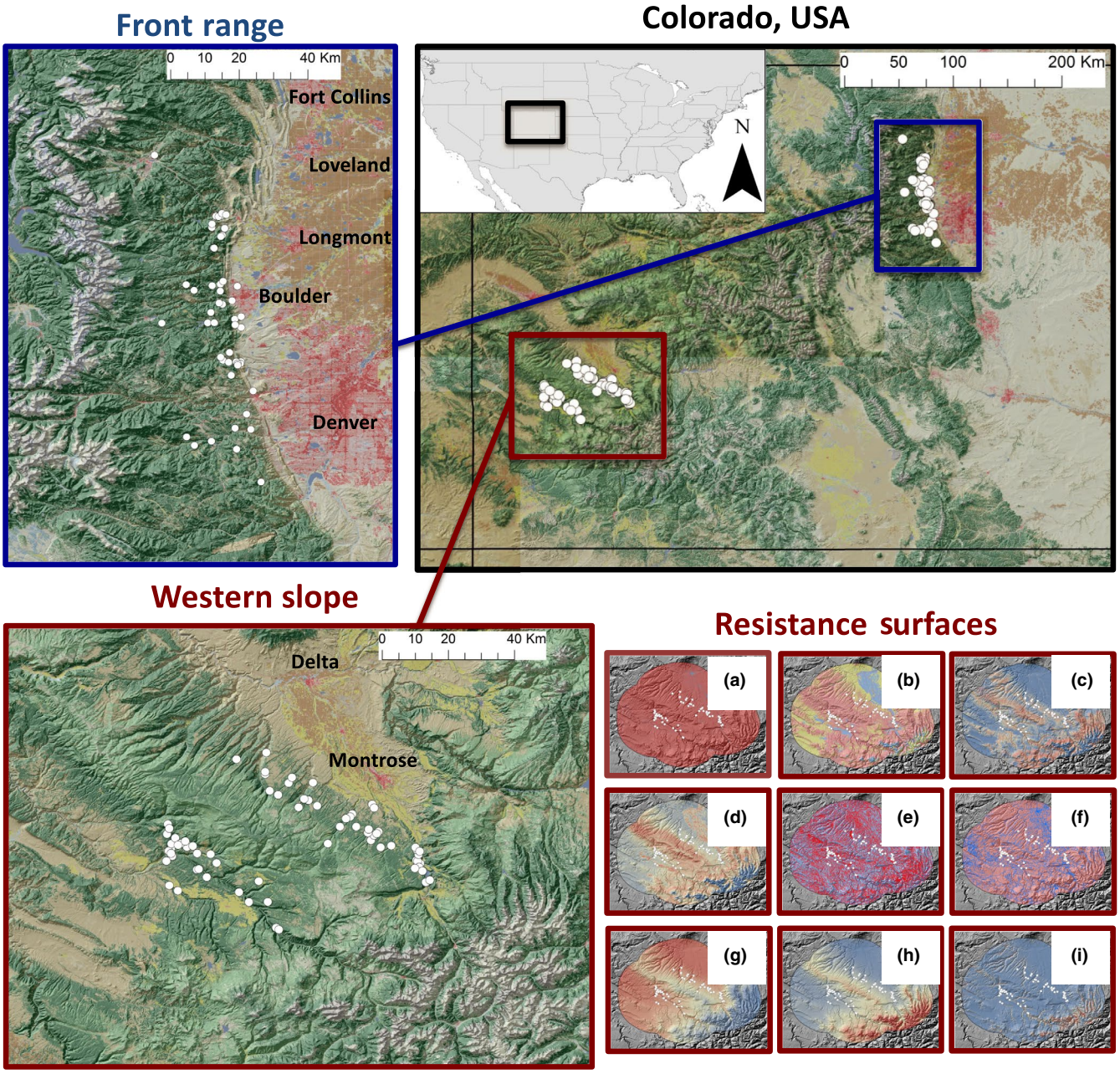

FIGURE 1 Study area in the Western Slope and Front Range of the southern Rocky Mountains of Colorado, USA. Landscape genomic analyses included 76 pumas from the Western Slope and 54 pumas from the Front Range (white circles). Resistance surfaces, shown for the Western Slope, represent alternative hypotheses of the effects of landscape variables on puma dispersal and gene flow (red = high gene flow, blue = low gene flow) for: (a) percent impervious surface cover (negative effect on gene flow), (b) land cover (forested, open-natural, and developed: positive, neutral, and negative effects on gene flow), (c) percent tree canopy cover (positive effect), (d) vegetation density (positive effect), (e) river and stream riparian corridors (positive effect), (f) roads (negative effect), (g) minimum temperature of the coldest month (negative effect), (h) annual precipitation (positive effect), and (i) topographic roughness (positive effect). We also tested isolation by geographic Euclidean distance. Land cover base maps show forests (green), shrub and grasslands (tan), urban areas (red), agriculture and ranchlands (brown and yellow), and alpine tundra (grey). Note that impervious surface shows very little spatial variation on the Western Slope (a) because there is very little impervious surface present in this rural region [Colour figure can be viewed at wileyonlinelibrary.com]

Western Slope and Front Range regions of the southern Rocky Mountains of Colorado, USA (Figure 1; Carver et al., 2016; Lewis et al., 2015). Samples were collected from 2005–2014 on the Western Slope and 2007–2013 on the Front Range. Western Slope samples consisted of 36 males and 40 females, and Front Range samples consisted of 23 males, 30 females, and one puma of unknown sex. Our sampling represents a large proportion of the resident pumas present in both regions during the sampling period, as Lewis et al. (2015) estimated 14.4 (*SE* 1.6) and 14.7 (*SE* 1.3) resident pumas occupying the Western Slope and Front Range study areas at a single time point, from motion camera and telemetry data collected in 2009 and 2010, using mark-resight analysis.

Genomic DNA was extracted from tissue or blood using QIAGEN DNeasy Blood &Tissue kits (QIAGEN Inc.). We genotyped a total of 76 individuals from the Western Slope and 54 individuals from the Front Range using the ddRADseq protocol described in Peterson, Weber, Kay, Fisher, and Hoekstra (2012) and sequenced on Illumina HiSeq2500 and 4000 machines (Illumina) using 100 bp single-end sequencing at the University of Oregon Genomics Facility (gc3f.uoregon.edu). We tested nine different combinations of restriction enzymes on puma samples for digestion efficiency and evaluated the size ranges of fragment distributions using an Agilent Tapestation 2200 (Agilent Genomics). We chose the digest enzymes EcoRI-HF (6b recognition) and NlaIII (4b recognition) and a target fragment size range of 300–400 bp (excluding adapters). We used a Blue Pippin with a 2%, internal standard, 100–600 bp gel cartridge (Sage Science) for size selection and a biotinylated P2 adapter with DynaBeads (Peterson et al., 2012) to purify the polymerase chain reaction (PCR) template for the final enrichment. PCR was performed for 12 cycles and five reactions were tested for each pool of individuals. We initially genotyped 16 individuals multiplexed into an Illumina 2500 HiSeq lane to estimate maximum multiplexing based on a target of >12× coverage per locus. After assessment of locus coverage, we proceeded to multiplex 48 and 66 individually-barcoded samples on Illumina 2500 and 4000 HiSeq lanes, respectively, using the Peterson et al. (2012) flex adaptors.

2.2 | Bioinformatics pipeline and filters

We evaluated read quality for each sequencing lane using FastQC (bioinformatics.babraham.ac.uk) and assembled our SNP data set de novo using Stacks v 1.41 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). Details on Stacks code and parameter settings used are on the GitHub repository; github.com/pesalerno/PUMAgenomics. We demultiplexed and filtered sequencing reads using the program process_radtags in Stacks. Due to sensitivity of downstream genotyping with different Stacks parameter settings (Mastretta-Yanes et al., 2015; Paris, Stevens, & Catchen, 2017), we incorporated individual sample replicates in library preparations. In each library, we included three within and three between library replicates, which were used for estimating genotyping error rates for different combinations of parameters used to construct loci with the denovo_map.pl pipeline in Stacks. We ran 11 different de novo assemblies, varying four different Stacks parameters one at a time while leaving the other parameters at their default values (Mastretta-Yanes et al., 2015), that affect locus, allele, and SNP error rates and the number of loci genotyped, consisting of (a) minimum number of identical, raw-reads required to create a stack (-m), (b) number of mismatches allowed between loci when processing a single individual (-M), (c) number of mismatches allowed between loci when building the catalog (-n), and (d) maximum number of stacks at a single de novo locus (-max_locus_stacks) (Table S1). Locus error rate was calculated as the number of loci present in only one of the samples of a replicate pair divided by the total number of loci, allele error rate was the number of allele mismatches between replicate pairs divided by the number of loci, and SNP error rate was the proportion of SNP mismatches between replicate pairs.

Locus, allele, and SNP error rates, as well as the number of SNPs retained, were largely consistent across Stacks parameter settings (Table S1), with the exception of the minimum number of identical, raw reads required to create a stack (–m). Increasing –m to seven reduced allele and SNP error rates, but not the locus error rate, while also reducing the number of SNPs retained by up to 40%. For our final de novo assembly, we balanced retaining SNPs with reducing locus, allele, and SNP error by using the parameter settings –m = 3, –M = 4, –n = 4, and max_locus_stacks = 3 (Table S1). In practice, the Pearson's correlation was very high between genetic distances calculated with the final parameter settings versus the most conservative (i.e., –m = 7) parameter settings (r = 0.997 for proportion of shared alleles). We then exported the SNP matrix with the populations program in Stacks (Catchen et al., 2013), retaining SNPs that were present in at least 20% of individuals by population, and retaining a single random SNP per locus. This matrix was further filtered for missing data in Plink v.1.07, first by locus, then by individual, and then by minor allele frequency (MAF) using multiple combinations of thresholds for reducing missing data in the matrix (see github.com/pesalerno/PUMAgenomics). After evaluating missing data from SNP matrices, we retained the matrix with a more stringent locus filter (excluding loci missing >25% individuals) and a less stringent filter on minor allele frequency (excluding loci with MAF < 0.01). We additionally filtered loci that were found at position 95 (the last position of our reads) due to a higher number of SNPs present in this position, suggesting increased error rates due to low sequence quality towards the end of the sequencing read. To compare landscape resistances with putatively neutral loci, we used a Principal Components Analysis (PCA) to identify loci showing strong signatures of selection relative to neutral background genomic variation with the program PCAdapt (Luu, Bazin, & Blum, 2016). We found 12, putatively adaptive, outlier loci using a false discovery rate of 10%, so we filtered these outliers out for downstream landscape genomic analyses to avoid confounding neutral demographic patterns with patterns generated by loci under selection.

2.3 | Population genomics and structure

Population genomic statistics were calculated for the two sampling regions, the Western Slope and Front Range (Figure 1). Observed and expected heterozygosity (H_{obs} and H_{exp}), nucleotide diversity (π), inbreeding coefficient (F_{IS}), and population genetic differentiation (F_{ST}) were calculated using the populations program in Stacks with SNP loci that passed previous filters, excluding a single individual (sample_1382) that did not pass the 75% missing data threshold. We estimated allelic richness (A_r) using hp-rare 1.0 (Kalinowski, 2005), which corrects for variance in sample sizes using rarefaction. Two complementary, individual-based genetic distances were calculated: proportion of shared alleles distance (D_{ps}; Bowcock et

al., 1994) using the ADEGENET R v. 3.3.3 package and relatedness distance (*r*; Smouse & Peakall, 1999) using the POPGENREPORT R package. We then calculated mean genetic distance among individuals for each region, corrected for geographic distance (i.e., genetic distance per km), as individuals that are further apart are expected to have higher genetic distances due to neutral isolation by distance population processes (Balkenhol et al., 2016; Wright, 1942). Effective population sizes (N_e) were estimated using the linkage disequilibrium method in NEESTIMATORV.2.01 (Do et al., 2014), using the correction for chromosome number (Waples, Larson, & Waples, 2016), which has been shown to be a robust method for inferring N_e using SNP data sets and large sample sizes (Waples, 2016; Waples et al., 2016). We evaluated overall genetic structure as well as genetic differentiation among the two sampling sites (Western Slope and Front Range) using PCA and Discriminant Analysis of Principal Components (DAPC) in the R package ADEGENET (Jombart, 2008; Jombart, Devillard, & Balloux, 2015) and ADMIXTURE ancestry analysis (Alexander, Novembre, & Lange, 2009). Individual membership probabilities of DAPC were based on the first 30 principal components and the first discriminant function. We used the function assignplot identify individuals that were putative migrants or admixed based on the individual DAPC assignment probabilities. We used the find.clusters command in ADEGENET and minimized cross validation error in ADMIXTURE to estimate the number of populations (i.e., *K*).

2.4 | Landscape genomics

Geographic Information Systems (GIS) data were collected for different landscape factors that we hypothesized would affect puma dispersal and gene flow in Colorado. Table 1 provides details on GIS data sources, spatial resolution, and ecological justification for each landscape factor. Study area extents were calculated and landscape variables were compared across regions by buffering individual data points by a typical female puma dispersal distance of 34.6 km (Logan & Sweanor, 2001), dissolving overlapping buffers, and calculating zonal statistics within each region (Western Slope and Front Range) using ARCGISV. 10.1 (ESRI, Redlands, California). Landscape data were converted into resistance surfaces using the Reclassify and Raster Calculator tools in ARCGIS. The following hypothesized relationships of landscape factors with puma gene flow were modeled: percent impervious surface cover (negative effect on gene flow), land cover (forested, open-natural, and developed: positive, neutral, and negative effects on gene flow), percent tree canopy cover (positive effect), vegetation density (positive effect), river and stream riparian corridors (positive effect), roads (negative effect), minimum temperature of the coldest month (negative effect), annual precipitation (positive effect), topographic roughness (positive effect), and elevation (positive effect). Additionally, we included an isolation by geographic distance model, which would be supported if none of the landscape variables had an effect on gene flow except for straight line, Euclidean distance between individuals (Balkenhol et al., 2016; Wright, 1942). Table S2 describes methods and justification for converting raw landscape variables to resistance surfaces.

Two genetic distance measures were used as response variables in landscape genomic analyses: proportion of shared alleles distance (D_{ps}; Bowcock et al., 1994) and relatedness distance (*r*; Smouse & Peakall, 1999). Environmental resistances among individuals were calculated using CIRCUITSCAPE (McRae, 2006) for each landscape resistance surface (McRae, 2006; Row, Knick, Oyler-McCance, Lougheed, & Fedy, 2017). CIRCUITSCAPE resistances are a useful tool in landscape genetics because they summarize all potential movement pathways simultaneously, as opposed to least cost paths that evaluate only a single idealized pathway, and thus assume the study organism has complete knowledge of the landscape and always chooses the ideal pathway (Balkenhol et al., 2016; McRae, 2006). Landscape variables were tested for multicollinearity, both prior to and after calculating environmental resistances in CIRCUITSCAPE, to ensure Pearson's *r* correlations < .7 and variance inflation factor (VIF) scores <5 in final landscape genomics models, as collinearity can cause instability in parameter estimation in regression models (Tables S3 and S4; Dormann et al., 2012; Row et al., 2017; Warren, Glor, & Turelli, 2010).

Two complementary methods were used to estimate the effects of environmental resistances on genetic distances: multiple regression on distance matrices (MRDM; Legendre, Lapointe, & Casgrain, 1994) using PERMUTE V.3.4 and maximum likelihood of population effects (MLPE; Clarke, Rothery, & Raybould, 2002; Row et al., 2017; van Strien, Keller, & Holderegger, 2012) using the LME4 R package. MRDM is a permutational, distance matrix-based approach that has been traditionally used in landscape genetic analyses, whereas MLPE is a newer linear mixed effects modelling technique that models pairwise comparisons as a random effect and environmental resistances as fixed effects (Balkenhol et al., 2016). Recent evaluations of landscape genetic approaches found linear mixed effects modelling using MLPE to be more accurate, although both approaches performed well (Shirk, Landguth, & Cushman, 2017). Therefore, we included the traditional MRDM approach as well as MLPE in order to utilize multiple, complementary techniques for inferring associations between landscape features and gene flow. For MRDM and MLPE, genetic distances were the response variable and environmental resistances were explanatory variables. Additionally for MLPE, a random effect matrix of individual comparisons was included to control for the nonindependent, pairwise structure of the data, and landscape resistances were standardized to units of standard deviation centered on the mean (Row et al., 2017; van Strien et al., 2012). Models were ranked using the Bayesian information criterion (BIC), and top models within five BIC units are reported (Richards, 2015).

3 | RESULTS

3.1 | Genotyping and filtering SNP matrices

Initial STACKS processing retained a single random SNP per 95 bp read and SNPs present in at least 20% of individuals by population, resulting in a matrix of 98,813 SNPs. These SNPs were further filtered in

TABLE 1 Environmental variables used for landscape genomic analyses, data sources, spatial resolution, and ecological justification

Category	Landscape variable	Code	Description	Data source, spatial resolution	Calculation	Ecological justification
Distance	Isolation by geographic distance	Geo. dist.	Euclidean, straight-line distance between individuals	No environmental data; model assumes only distance affects gene flow, 30 m	ArcGIS Reclassify tool, Circuitscape	Model of isolation by straight-line distance (Wright, 1942)
Land cover	Land cover: forested, open-natural, and developed	Land cover	Multiple land cover categories collapsed into 3 costs of movement: forested (lowest), open natural areas (medium), and developed (highest)	National Land Cover Database (mrlc.gov/nlcd2011.php; Homer et al., 2004), 30 m	ArcGIS Spatial Analyst	Forested habitats provide the most cover for hunting and dispersal, open natural areas are intermediate, and developed areas are the least suitable habitat for dispersal (Crooks, 2002; Lewis et al., 2015)
	Percent impervious surface cover	Imperv.	Percentage of impervious surface	National Land Cover Database (mrlc.gov/nlcd2011.php; Homer et al., 2004), 30 m	ArcGIS Spatial Analyst	Human development results in increased noise, lights, and hunter access, limiting dispersal (Ernest et al., 2014; Maletzke et al., 2017; Riley et al., 2006)
	Road corridors	Roads	Roads, with 50 m buffers on each side	Colorado Department of Transportation (dtdapps.coloradodot.info/otis), 30 m	ArcGIS Analysis Tools, Spatial Analyst	Roads increase mortality, noise, lights, and hunter access, limiting dispersal (Maletzke et al., 2017; Newby et al., 2013; Riley et al., 2006)
	River and stream riparian corridors	Riparian	River and stream riparian corridors, with 50 m buffers on each side	National Hydrography Data set (nhd.usgs.gov), 30 m	ArcGIS Analysis Tools, Spatial Analyst	River and stream riparian corridors provide vegetative and topographical cover for dispersal, as well as water sources attracting prey species (Dickson et al., 2005; Hilty & Merenlender, 2004; Naiman, Decamps, & Polluck, 1993)
Vegetation	Percent tree canopy cover	Tree cover	Percentage of tree canopy cover	National Land Cover Database (mrlc.gov/nlcd2011.php; Homer et al., 2004), 30 m	ArcGIS Spatial Analyst	Low tree canopy limits cover for ambush predation and concealment, and restricts dispersal (Blecha et al., 2018; Logan & Sweanor, 2001; Sweanor et al., 2000; Warren et al., 2016)
	Enhanced vegetation index	Veg. density	Density of vegetation calculated from chlorophyll reflectance in visual and near-infrared spectra	Moderate Resolution Imaging Spectroradiometer (modis.gsfc.nasa.gov), 250 m	ArcGIS Spatial Analyst	Low vegetation density limits cover for ambush predation and concealment, and restricts dispersal (Blecha et al., 2018; Hilty & Merenlender, 2004; Sweanor et al., 2000; Warren et al., 2016)
Climate	Minimum temperature of the coldest month	Min. temp.	Mean annual minimum temperature of the coldest month (°C) calculated from 1970–2000 weather station data, interpolated between stations	Global Climate Data (worldclim.org/bioclim; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005), 1 km	ArcGIS Spatial Analyst	Low minimum temperatures and high snowfall, found at high elevation mountain ridgelines (e.g., alpine tundra habitats) restrict hunting, breeding, and dispersal (Hornocker & Negri, 2009)
	Mean annual precipitation	Ann. precip.	Mean annual precipitation accumulation (mm) calculated from 1970–2000 weather station data, interpolated between stations	Global Climate Data (worldclim.org/bioclim; Hijmans et al., 2005), 1 km	ArcGIS Spatial Analyst	Dry habitats with low precipitation accumulation limit prey species for hunting and vegetative cover, restricting dispersal (Logan & Sweanor, 2001; McRae et al., 2005)

(Continues)

TABLE 1 (Continued)

Category	Landscape variable	Code	Description	Data source, spatial resolution	Calculation	Ecological justification
Topography	Topographic roughness	Topo. rough.	Topographic complexity based on variance in elevation within a moving window	National Elevation Data set (lta.cr.usgs.gov/ned) National Map Tool (viewer.natio nalmap.gov), 30 m	Geomorphometric and Gradient Metric Toolbox (Cushman, Gutzweiler, Evans, & McGarigal, 2010), ArcGIS Spatial Analyst	Steep, topographically-complex canyons and mountain slopes provide cover for hunting and dispersal (Dickson et al., 2005; Hornocker & Negri, 2009)
	Elevation	Elev.	Elevation calculated from digital elevation models	National Elevation Data set (lta.cr.usgs.gov/ned) National Map Tool (viewer.natio nalmap.gov), 30 m	ArcGIS Spatial Analyst	Increased forest cover and reduced human development found at higher elevations enhances hunting, breeding, and dispersal (Hornocker & Negri, 2009)

TABLE 2 Study areas (km^2), number of individuals genotyped (N_{gen}), and population genomic parameter estimates from the Western Slope and Front Range of Colorado

Region	Area (km^2)	N_{gen}	H_{obs}	H_{exp}	π	A_r	F_{IS}	F_{ST}	D_{PS}/km (*SE*)	*r*/km (*SE*)	N_e (95% CI)
Western Slope	11,889	76 indiv.	0.241	0.272	0.0029	1.94	0.118	0.024	0.29 (0.06)	0.10 (0.02)	81.2 (77.6–84.8)
Front Range	11,958	54 indiv.	0.243	0.263	0.0028	1.89	0.084		0.51 (0.18)	0.17 (0.07)	48.1 (46.3–49.9)

Note: Population genomic measures are observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}), nucleotide diversity (π), allelic richness (A_r), inbreeding coefficient (F_{IS}), genetic differentiation among populations (pairwise F_{ST}), mean genetic distance among individuals corrected for geographic distance (D_{PS} and *r* per km) with standard errors (*SE*), and effective population size (N_e) with 95% confidence intervals (CI) based on parametric bootstrapping.

PLINK by removing loci that were present in less than 75% of individuals, which resulted in a matrix of 20,355 SNPs. Only a single individual was excluded based on our >75% missing loci per individual threshold. After excluding SNPs present in the 95th sequencing base position and with minor allele frequency <0.01, we retained 12,456 SNPs. PCADAPT detected twelve outlier loci, putatively under selection, while accounting for population structure (K = 2). After removing these putatively adaptive loci, the final neutral data set contained 12,444 SNPs (Table S1; github.com/pesalerno/PUMAgenomics).

3.2 | Population genomics and structure

The two study areas encompass similar geographic extents: 11,889 km^2 for the Western Slope and 11,958 km^2 for the Front Range (Table 2). Measures of genetic diversity (H_{obs}, H_{exp}, π, A_r) and inbreeding (F_{IS}) were similar for the Western Slope and Front Range (Table 2). However, the effective population size (N_e) was smaller, mean genetic distances among individuals (D_{PS}/km and r/km) were higher, and there was more admixture in the more urbanized Front Range (Table 2, Figure 2). We also calculated N_e using subsets of individuals (i.e., pre- and post-2010 individuals in the Front Range, pre- and post-2011 individuals in the Western Slope), because multiple overlapping generations may bias effective population size estimates low or high (Waples, 2016; Waples et al., 2016). N_e remained consistently higher in the Western Slope, although it differed between the earlier and later sampling periods, indicating the population may be expanding (Table S5). We were able to differentiate between the Western Slope and Front Range regions based on PCA, DAPC, and ADMIXTURE ancestry analysis, and K = 2 was the best supported value of K by minimizing cross validation error (Figure 2; Alexander et al., 2009). The proportion of correct individual assignment to populations based on DAPC was high for most individuals in both the Western Slope (0.98) and the Front Range (0.96), and the assign plot identified putative migrants and admixed individuals in both regions (Figure 2b). However, the ADMIXTURE ancestry analysis showed more admixed individuals, particularly in the Front Range (Figure 2c). We also analyzed both regions separately for population substructure (Figure S1), and there was no signature of population differentiation within the Western Slope or Front Range.

3.3 | Landscape genomics

The Front Range has more urban development than the Western Slope, with more impervious surface cover and a higher density of roads (Figure 1, Table 3, Table S2). The Front Range also has more tree canopy cover, higher vegetation density, and higher annual

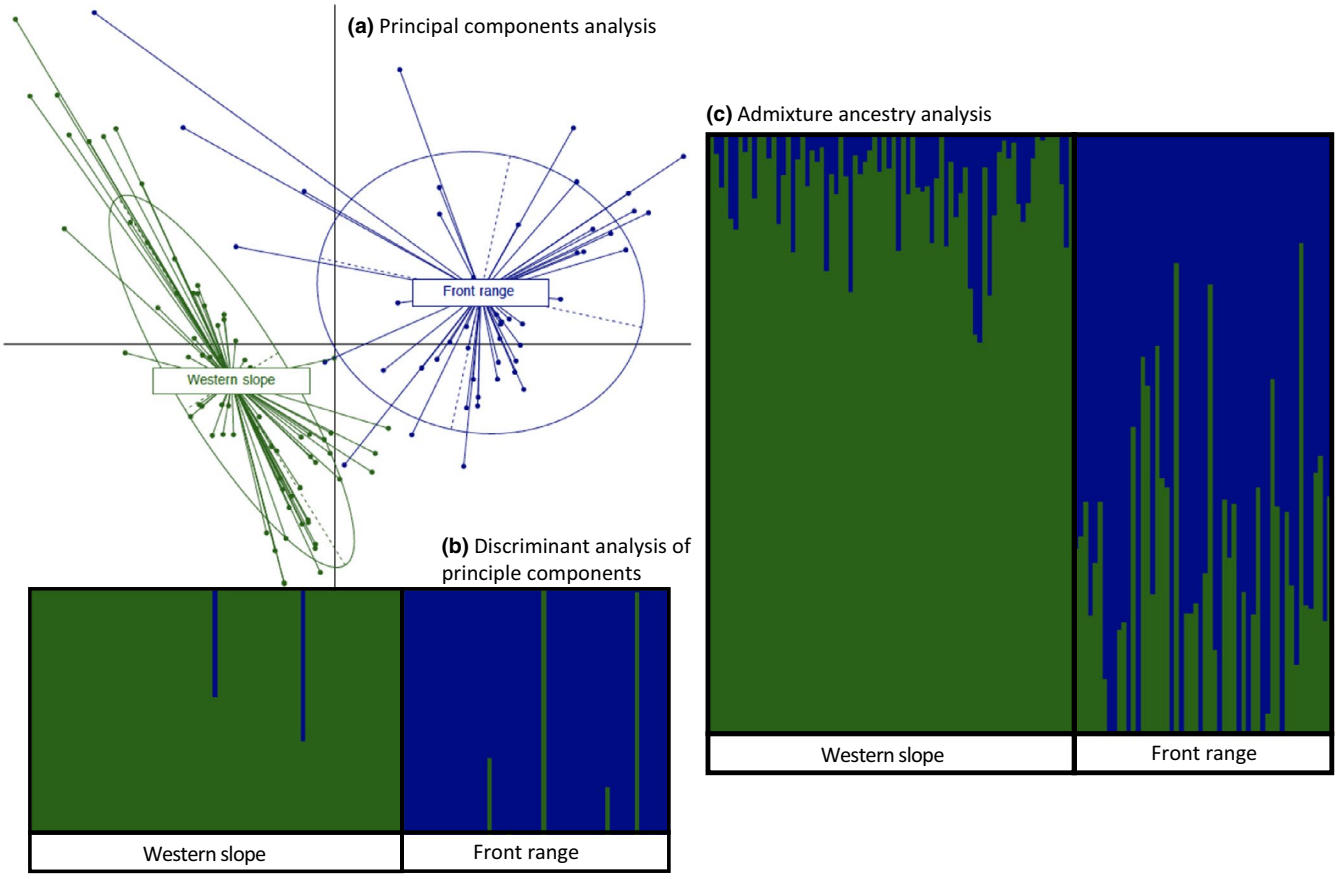

FIGURE 2 Population structure from (a) Principal components analysis (PCA), (b) Discriminant analysis of principal components (DAPC), and (c) ADMIXTURE ancestry analysis. Individuals assigned to the Western Slope and Front Range are green and blue, respectively. K = 2 was most supported in ADMIXTURE ancestry analysis using cross validation error [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Habitat differences between the Western Slope and Front Range of Colorado

	Western Slope					Front Range				
Landscape data	Min	Max	Median	Mean	Std Dev	Min	Max	Median	Mean	Std Dev
Elevation (m)	1,453.5	4,362.9	2,354	2,418.0	552.5	1,474.5	4,347.1	2,365	2,374.9	629.3
Tree canopy cover (%)	0	100	20	29.9	31.4	0	100	32	35.0	33.6
Impervious surface (%)	0	100	0	0.5	4.1	0	100	0	4.0	13.5
Minimum temp. coldest month (°C)	−20.2	−9.5	−13.3	−13.9	2.8	−19.9	−8.3	−12.7	−12.6	2.8
Annual precipitation (mm)	208	1,137	458	483.3	171.5	359	1,006	452	496.4	121.9
Enhanced vegetation index	−1,806	8,955	4,634	4,434.9	1,858.3	−1,969	9,132	5,416	4,957.8	1,800.9
Topographic roughness	0	27,924.6	11	53.1	129.6	0	20,067.0	25	56.2	100.8
Landcover	1	10	1	3.2	3.0	1	10	1	4.2	3.7
Roads	1	10	1	1.7	2.5	1	10	1	2.7	3.6
Riparian areas	1	10	10	9.4	2.3	1	10	10	9.4	2.3

Note: Units are percent cover for impervious surface and tree canopy cover; resistance values for landcover, river and stream riparian corridors, and roads; °C for temperature; millimeters for precipitation; meters for elevation; and unitless measurements based on chlorophyll reflectance and variance in elevation, respectively, for enhanced vegetation index and topographic roughness.

precipitation than the Western Slope (Table 3), probably due to the high desert habitats (i.e., the Colorado Plateau ecoregion) in the Western Slope being drier than the grassland and shrub habitats found at lower elevations of the Front Range (i.e., the Great Plains ecoregion; McMahon et al., 2001).

Prior to running Circuitscape, landscape raster surfaces were largely uncorrelated (i.e., Pearson's $r < .7$), with the exception of elevation, which was positively correlated with annual precipitation and negatively correlated with minimum temperature of the coldest month in both regions, and vegetation density, which was negatively correlated with annual precipitation in the Front Range (Table S3). After Circuitscape analyses, environmental resistance variables showed more collinear relationships than raw raster surfaces (Table S4), probably due to Circuitscape resistances being higher for individuals separated by larger geographic distances (McRae, 2006). Therefore, we removed landscape variables from both regions that were strongly correlated with many other variables, until all VIF scores were less than 10 (Row et al., 2017). Variables retained were geographic distance, river and stream riparian corridors, roads, impervious surface cover, tree canopy cover, vegetation density, and minimum temperature of the coldest month. However, vegetation density was still correlated with geographic distance in both regions, and impervious surface was correlated with geographic distance in the Western Slope (Table S4). We removed these variables as well, resulting in Pearson's *r* correlations less than .7 for all explanatory variables, and final model VIF scores of 4.07 in the Western Slope and 3.54 in the Front Range. Thus final MRDM and MLPE models for the Western Slope included geographic distance, tree canopy cover, stream and river riparian corridors, roads, and minimum temperature of the coldest month; and for the Front Range included the same landscape variables plus impervious surface cover.

Landscape genomic patterns of pumas were different in the rural Western Slope compared to the more urbanized Front Range, with the exception of geographic distance being supported in both regions (Tables 4 and 5). In the Western Slope, tree canopy cover was consistently positively correlated with gene flow in MRDM and MLPE models. In addition, low minimum temperatures of the coldest month were negatively correlated gene flow, and riparian habitats were positively correlated with gene flow, in three of the top MLPE models (Tables 4 and 5). In contrast, in the Front Range, tree canopy cover and percent impervious surface cover were negatively associated with gene flow in the top MLPE models (Table 5). Because the relationship between tree cover and gene flow was the opposite of what we hypothesized in the Front Range, we also inverted the tree cover resistance surface (i.e., higher tree cover = higher resistance), reran Circuitscape and MLPE analyses, and higher tree cover still showed significant negative correlations with gene flow in this region.

4 | DISCUSSION

The apex predator puma (*P. concolor*) persists in many urbanized regions throughout its range, yet the localized effects of recent urban sprawl remain unclear. Here, we compared patterns of landscape genomic connectivity and genetic diversity of pumas

across two regions that span an urban-rural divide in Colorado, USA. Landscape genomic connectivity patterns differed between regions, such that genetic distances were higher and urbanization (i.e., percent impervious surface cover) restricted gene flow in the more urbanized Front Range, whereas forest and riparian cover were most important for enhancing gene flow on the rural Western Slope. Despite finding reductions in gene flow associated with urbanization on the Front Range, population-level genetic diversity and inbreeding measures were similar to those on the rural Western Slope. This suggests that recent urban sprawl in the Colorado Front Range has not yet had a large impact on the genetic diversity of pumas. This is in contrast to more isolated puma populations in other highly urbanized landscapes such as southern California and Florida, which exhibit reduced genetic diversity

TABLE 4 Multiple regression on distance matrices (MRDM) landscape genomic results from the Western Slope and Front Range of Colorado

Region	Genetic distance	Landscape factors	Direction of effect	r^2	*p*
Western Slope	D_{ps}	tree cover	+	.07	0.001
	r	geo. dist.	−	.03	0.001
Front Range	D_{ps}	geo. dist.	−	.05	0.001
	r	geo. dist.	−	.04	0.001

Note: Response variables were individual-based genetic distances, i.e., proportion of shared alleles (D_{ps}) and relatedness (*r*). Explanatory variables, after removing correlated variables, were the geographic (Euclidean) distance model (geo. dist.), percent impervious surface cover, percent tree canopy cover, river and stream riparian corridors, roads, and minimum temperature of the coldest month. Forward selection followed by backward elimination was performed, with 1,000 random permutations of the dependent distance matrix per step, using Bonferroni-corrected p-to-enter and p-to-remove alpha values of .05. Standardized beta coefficients were used to assess the direction of effect of each landscape variable on gene flow. Only univariate models were supported.

TABLE 5 Maximum likelihood of population effects (MLPE) landscape genomic results from the Western Slope and Front Range of Colorado

Region	Genetic distance	Landscape factors	Direction of effect	r^2	ΔBIC
Western Slope	D_{ps}	Tree cover	+	.13	0
		Tree cover	+	.14	2.5
		Min. Temperature	−		
	r	Tree cover	+	.17	0
		Geo. Dist.	−		
		Tree cover	−	.16	3.4
		Tree cover	+	.16	3.4
		Riparian areas	+		
		Geo. Dist.	−	.16	3.7
		Tree cover	+	.16	3.8
		Min. Temperature	−		
Front Range	D_{ps}	Geo. Dist.	−	.12	0
		Tree cover	−		
		Geo. Dist.	−	.13	4.1
		Tree cover	−		
		Impervious surface	−		
	r	Geo. Dist.	−	.14	0
		Tree cover	−		
		Geo. Dist.	−	.14	4.9
		Tree cover	−		
		Impervious surface	−		

Note: Response variables were individual-based genetic distances, i.e., proportion of shared alleles (D_{ps}) and relatedness (*r*). Pairwise comparisons of individuals were controlled as a random effect. Fixed effects, after removing correlated variables, were the geographic (Euclidean) distance model (geo. dist.), percent impervious surface cover, percent tree canopy cover, vegetation density, river and stream riparian corridors, roads, and minimum temperature of the coldest month. Standardized beta coefficients were used to assess the direction of effect of each landscape variable on gene flow. Models reported are within the top 5 BIC units. Landscape factors are in order of standardized beta coefficients (largest to smallest).

and strong evidence of inbreeding compared to Colorado pumas (Ernest et al., 2003, 2014; Johnson et al., 2010; Riley et al., 2014). However, a smaller effective population size and higher among-individual genetic distances in the recently urbanized Front Range suggest habitat fragmentation has already impacted this population and could cause further reductions of genetic diversity as urbanization continues to expand in Colorado (Theobald, 2005; US Census Bureau, 2017). Extensive losses of genetic diversity could eventually cause puma populations to decline, which in turn could have important cascading effects into lower trophic levels, such as overgrazing of vegetation by ungulate herbivores (Markovchick-Nicholls et al., 2008).

4.1 | Population genomics and structure

Using our genomic dataset of over 12,000 SNPs, we were able to distinguish the Western Slope and Front Range regions (i.e., $K = 2$; Figures 1 and 2). Minimum temperature of the coldest month was also negatively associated with gene flow in one of the top landscape genomic models on the Western Slope (Table 5), suggesting there may be restricted gene flow through high elevation, alpine tundra habitats (McMahon et al., 2001). However, potential immigrants and admixed individuals were identified moving in both directions (Figure 2), particularly using ADMIXTURE ancestry analysis, and DAPC group assignments may be overfit given the method's approach to minimize within population distances and maximize between population distances (Jombart et al., 2015). In addition, overall genetic differentiation between the two populations was low (pairwise $F_{ST} = 0.02$; Table 2). Because our sample archive consisted of opportunistically collected samples, our analyses were restricted to populations in two distinct regions, whereas pumas occur throughout the southern Rocky Mountains in Colorado. Therefore, potential immigrants and admixed individuals are not necessarily moving between our specific Western Slope and Front Range study areas, but may originate from other unsampled populations that share genetic ancestry with our two study regions. Nevertheless, results from our study suggest pumas maybe somewhat limited in dispersing across the high elevation peaks of the Continental Divide, and future studies should attempt to sample more intensively across the entire region to further investigate this trend.

We identified similar levels of genetic diversity on the rural Western Slope compared to the more urbanized Front Range, although H_{exp}, A_r, and F_{IS} were slightly higher on the Western Slope (Table 2). This suggests urbanization is not yet having a major impact on the genetic diversity of pumas in Colorado. One potential explanation is that urbanization in the Front Range is primarily occurring on the eastern edge of the region, possibly creating a relatively impermeable urban boundary on the eastern border, but not isolating pumas in fragments or limiting their connectivity to wildland habitat to the west (Figure 1; Blecha et al., 2018; Lewis et al., 2015). Another possibility is that many of the SNPs we sampled may not have high enough mutation rates to show a strong genomic signature of the relatively recent effects of rapid urbanization occurring in the Front Range (Allendorf et al., 2013; Haasl & Payseur, 2011). As the human population continues to expand, future urbanization could result in more fragmented populations and reductions in genetic diversity, as has been detected in other more urbanized landscapes like southern California and Florida (Ernest et al., 2003, 2014; Johnson et al., 2010; Riley et al., 2014).

Despite similar geographic extents and levels of genetic diversity in the Western Slope and Front Range, mean genetic distances among individuals were higher in the urban Front Range (Table 2), suggesting that fragmentation due to urbanization may be limiting puma dispersal and gene flow. In addition, a larger effective population size (N_e) of pumas was detected on the rural Western Slope ($N_e = 81.2$) compared to the urban Front Range ($N_e = 48.1$; Table 2), with the caveat that some assumptions of this estimator are violated in both regions (e.g., closed populations with no immigration, non-overlapping generations). The effect of non-overlapping generations on N_e is difficult to predict (Waples et al., 2016), and this assumption is expected to be violated similarly in both the Western Slope and Front Range populations. Immigration, however, is expected to downwardly bias N_e by creating linkage disequilibrium through a multilocus Wahlund effect (Wahlund, 1928; Waples & England, 2011). Thus, it is possible that the Front Range may be showing a lower N_e due to having more immigrants from outside populations than the Western Slope. This is possible, and perhaps likely, given the presence of more admixed individuals in the Front Range (Figure 2), which could indicate more potential immigrants into this region. On the other hand, if immigration rates are similar for both regions, the relatively smaller Front Range N_e may be due to: (a) urbanization and fragmentation impacting and limiting population size, and/or (b) species range limit theory (Abundant Center Hypothesis) predicting that smaller population sizes are likely to occur at the edge of the geographic range relative to core areas (Brown, 1984; Sagarin & Gaines, 2002). These potential underlying factors are not mutually exclusive and may both be acting together. However, the lack of difference in most genetic diversity measures, in addition to slightly lower allelic richness in the Front Range, which is the most sensitive metric to recent bottlenecks (Allendorf et al., 2013), suggests lower effective population size on the Front Range may be more consistent with recent urbanization impacts than historical range boundary effects.

4.2 | Landscape genomics

With regard to general landscape genomics methodology, we found MRDM to be a much more conservative approach that adds fewer explanatory variables to the models than MLPE (Tables 4 and 5). Therefore only the strongest landscape genomic relationships were identified using MRDM, consisting of isolation by geographic distance in both regions, as well as tree cover in the Western Slope. Conversely, MLPE results in more complex models with more explanatory variables and higher r^2 values (genetic variation explained) than MRDM (Tables 4 and 5). The different genetic distance measures we used (D_{PS} and r) showed largely consistent relationships with landscape variables, but still provided a few different insights,

particularly using MLPE (Tables 4 and 5). Overall r^2 values were somewhat low (r^2 = .03–.07 for MRDM, r^2 = .12–.17 for MLPE), but this is expected for a large carnivore with extreme long distance dispersal abilities (e.g., Balkenhol et al., 2016; Short Bull et al., 2011).

On the rural Western Slope, tree canopy cover was most important for enhancing gene flow, suggesting pumas prefer to disperse through forests rather than more open shrub and grassland habitats in this landscape (Table 5). In addition, riparian areas were an important predictor in one top model (Table 5), further supporting the importance of tree cover for enhancing gene flow in this region. Forests and riparian areas provide more tree cover for concealment and ambush predation (Hornocker & Negri, 2009; Logan & Sweanor, 2001; Warren, Wallin, Beausoleil, & Warheit, 2016). Use of open areas may also increase susceptibility to mortality by hunters and ranchers (Newby et al., 2013), which are both more prevalent in the rural Western Slope than the more urbanized Front Range. In addition, non-forested and non-riparian areas on the Western Slope are dry, high elevation desert habitats (i.e., the Colorado Plateau ecoregion; McMahon et al., 2001), which may provide less prey and water resources, and thus be poorer habitats for hunting and dispersal (Dickson, Roemer, McRae, & Rundall, 2013; McRae et al., 2005; Sweanor, Logan, & Hornocker, 2000).

In the more urbanized Front Range, impervious surface cover restricted gene flow (Table 5). This suggests urbanization is limiting gene flow, despite high levels of genetic diversity (Table 2). Similarly, Lewis et al. (2015) found pumas were less likely to be detected in habitats with residential development, even low-density exurban developments, which are increasingly encroaching into the foothills of the Front Range region. Genetic studies on pumas from more urbanized and fragmented populations in southern California and Florida have detected strong inbreeding and isolation associated with urbanization (Ernest et al., 2003, 2014; Johnson et al., 2010; Riley et al., 2014). Our study detected more subtle impacts of urbanization in a less fragmented landscape, within mountainous wildland habitats adjacent to a major metropolitan center, which experiences high levels of human outdoor recreation activities such as hiking and skiing (Figure 1). In addition, in contrast with the rural Western Slope and contrary to our initial hypotheses, forest cover was negatively associated with gene flow on the Front Range (Table 5). This pattern suggests pumas are more willing to disperse through open shrub and grassland habitats in this region. The reasons for this are unclear, but pumas living in the more developed Front Range may be more acclimated to human activities and thus more willing to travel outside of forested habitats, demonstrating that pumas have a range of adaptable behaviors and will use and move through different types of habitat (Blecha et al., 2018; Dickson, Jenness, & Beier, 2005). Pumas may also be hunting more urban mesopredators, domestic, and agricultural animals in these open habitats on the more developed Front Range, which was shown in a prior study using stable isotope analysis of Front Range puma diets (Moss, Alldredge, & Pauli, 2016). There is also less hunting of pumas in the Front Range compared to the rural Western Slope, so pumas may be less wary of open areas, although this effect would be expected to be counteracted in part by higher traffic mortality in the more urbanized region (Beier, 1995; Crooks, 2002).

It is important to note that unsampled landscape variables for which we have no data, as well as correlated landscape variables removed from the final models (Table S4), may also be contributing to landscape genomic patterns. For example, we don't have data on snowpack or prey (e.g., mule deer) abundance, and elevation and annual precipitation were removed from final models because they were strongly correlated with several other landscape variables, including tree cover (Table S4). Thus the unexpected negative relationship of puma gene flow with tree cover on the Front Range may also be due to pumas utilizing lower elevation areas with less snowpack and higher prey abundance for dispersal and hunting during the late fall, winter, and early spring months. Moreover Row et al. (2017) found that MLPE models were able to distinguish the true underlying dispersal models in approximately 75% of their simulations when multiple, correlated landscape predictor variables influenced dispersal. This highlights the inherent difficulty of identifying the underlying landscape drivers of gene flow in empirical systems, while also underscoring the importance of choosing landscape genomic hypotheses judiciously and interpreting the resulting landscape genomic associations carefully.

In conclusion, our findings are consistent with prior comparative landscape genetic studies that have revealed varying effects of landscape factors on movement and gene flow across different portions of a species' geographic range (e.g., Short Bull et al., 2011; Trumbo, Spear, Baumsteiger, & Storfer, 2013; Vandergast et al., 2007). We found that in the rural Western Slope with high hunting pressure, forests and riparian areas with high tree canopy cover are most important for conserving puma genetic connectivity. In contrast, in the more urbanized Front Range, non-forested habitats such as shrubland and grassland habitats are utilized more for dispersal and gene flow, effective population sizes are smaller, genetic distances among individuals are higher, and gene flow is being restricted by urbanization (Tables 2, 4, and 5). Next generation sequencing techniques can provide dense, genome-scale SNP data sets of thousands of putatively neutral markers, which gives researchers increased power to detect the often subtle effects of landscape factors, such as urbanization, on gene flow (Allendorf et al., 2013; Lowe & Allendorf, 2010; Luikart et al., 2003). This is particularly important for wide-ranging species with broad geographic distributions, as landscape effects on gene flow occur at broader geographic scales and may be weaker and more difficult to detect compared to more dispersal-limited species with smaller home ranges (Balkenhol et al., 2016; Epps et al., 2007; Holderegger et al., 2006). Indeed, prior work on pumas using 16 microsatellites found no population structure across the southern Rocky Mountains of Colorado and northern New Mexico (McRae et al., 2005). Our results demonstrate that large SNP data sets can allow researchers to identify impacts of urbanization on gene flow, effective population sizes, and patterns of population genetic structure of wide-ranging species, even before fragmentation is extensive enough to cause substantial declines in genetic diversity. Maintaining genetic connectivity in these umbrella species

can have outsized benefits towards conserving biodiversity, as preserving broad swathes of contiguous habitats that are necessary for their persistence also benefits many other species with smaller home ranges and narrower habitat requirements (Sergio et al., 2008, 2006; Thorne et al., 2006).

ACKNOWLEDGEMENTS

Funding was provided by the National Science Foundation, Ecology of Infectious Disease Program (NSF-EID 1413925 and 723676). Samples were collected by Colorado Parks and Wildlife. We also thank Michael Antolin, Kelly Pierce, and Jill Gerberich at Colorado State University for assistance in the laboratory.

AUTHOR CONTRIBUTIONS

D.R.T. performed laboratory work, analyzed landscape and population genomic data, and wrote the manuscript; P.S., R.B.G., C.P.K, S.K., and N.F.J. performed laboratory work and analyzed landscape and population genomic data; K.L., and M.A. directed fieldwork and collected field data; M.E.C., S.C., H.B.E., K.C., S.V., and W.C.F. conceived of study questions and directed research; and all authors contributed input to draft and final versions of the manuscript.

DATA AVAILABILITY STATEMENT

ddRADseq data used in genomic analyses are on Dryad (https://doi.org/10.5061/dryad.12jm63xsr).

ORCID

Daryl R. Trumbo https://orcid.org/0000-0002-8438-2856
Christopher P. Kozakiewicz https://orcid.org/0000-0002-4868-9252
Nicholas M. Fountain-Jones https://orcid.org/0000-0001-9248-8493
Holly B. Ernest https://orcid.org/0000-0002-0205-8818
W. Chris Funk https://orcid.org/0000-0002-6466-3618

REFERENCES

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*, 1655–1664. https://doi.org/10.1101/gr.094052.109

Allendorf, F. W., Luikart, G., & Aitken, S. N. (2013). *Conservation and the genetics of populations*. Chichester, UK: Wiley-Blackwell.

Balkenhol, N., Cushman, S. A., Storfer, A. T., & Waits, L. P. (2016). *Landscape genetics: Concepts, methods, applications*. Chichester, UK: Wiley-Blackwell.

Beier, P. (1995). Dispersal of juvenile cougars in fragmented habitat. *Journal of Wildlife Management*, *59*, 228–237. https://doi.org/10.2307/3808935

Blecha, K. A., Boone, R. B., & Alldredge, M. W. (2018). Hunger mediates apex predator's risk avoidance response in wildland–urban interface. *Journal of Animal Ecology*, *87*, 609–622. https://doi.org/10.1111/1365-2656.12801

Bowcock, A. M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J. R., & Cavalli-Sforza, L. L. (1994). High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, *368*, 455–457. https://doi.org/10.1038/368455a0

Brown, J. H. (1984). On the relationship between abundance and distribution of species. *American Naturalist*, *124*, 255–279. https://doi.org/10.1086/284267

Carver, S., Bevins, S. N., Lappin, M. R., Boydston, E. E., Lyren, L. M., Alldredge, M., … Vandewoude, S. (2016). Pathogen exposure varies widely among sympatric populations of wild and domestic felids across the United States. *Ecological Applications*, *26*, 367–381.

Catchen, J. M., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, *22*, 3124–3140. https://doi.org/10.1111/mec.12354

Clarke, R. T., Rothery, P., & Raybould, A. F. (2002). Confidence limits for regression relationships between distance matrices: Estimating gene flow with distance. *Journal of Agricultural Biological and Environmental Statistics*, *7*, 361–372. https://doi.org/10.1198/108571102320

Cohen, J. E. (2003). Human population: The next half century. *Science*, *302*, 1172–1175. https://doi.org/10.1126/science.1088665

Crooks, K. R. (2002). Relative sensitivities of mammalian carnivores to habitat fragmentation. *Conservation Biology*, *16*, 488–502. https://doi.org/10.1046/j.1523-1739.2002.00386.x

Crooks, K. R., Burdett, C. L., Theobald, D. M., King, S. R. B., Di Marco, M., Rondini, C., & Boitani, L. (2017). Quantification of habitat fragmentation reveals extinction risk in terrestrial mammals. *Proceedings of the National Academy of Sciences*, *114*, 7635–7640. https://doi.org/10.1073/pnas.1705769114

Cushman, S. A., Gutzweiler, K., Evans, J. S., & McGarigal, K. (2010). The gradient paradigm: A conceptual and analytical framework for landscape ecology. In S. A. Cushman, & F. Huettman (Eds.), *Spatial complexity, informatics, and wildlife conservation*. New York, NY: Springer.

Dickson, B. G., Jenness, J. S., & Beier, P. (2005). Influence of vegetation, topography, and roads on cougar movement in southern California. *Journal of Wildlife Management*, *69*, 264–276. https://doi.org/10.2193/0022-541X(2005)069<0264:IOVTAR>2.0.CO;2

Dickson, B. G., Roemer, G. W., McRae, B. H., & Rundall, J. M. (2013). Models of regional habitat quality and connectivity for pumas (*Puma concolor*) in the southwestern United States. *PLoS ONE*, *8*, 1–11. https://doi.org/10.1371/journal.pone.0081898

Do, C., Wapels, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2; re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*, *14*, 209–214.

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., … Lautenbach, S. (2012). Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, *36*, 27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

Epps, C. W., Wehausen, J. D., Bleich, V. C., Torres, S. G., & Brashares, J. S. (2007). Optimizing dispersal and corridor models using landscape genetics. *Journal of Applied Ecology*, *44*, 714–724. https://doi.org/10.1111/j.1365-2664.2007.01325.x

Ernest, H. B., Boyce, W. M., Bleich, V. C., May, B., Stiver, S. J., & Torres, S. G. (2003). Genetic structure of mountain lion (*Puma concolor*) populations in California. *Genetics*, *4*, 353–366.

Ernest, H. B., Vickers, T. W., Morrison, S. A., Buchalski, M. R., & Boyce, W. M. (2014). Fractured genetic connectivity threatens a southern California puma (*Puma concolor*) population. *PLoS ONE*, *9*, 1–12. https://doi.org/10.1371/journal.pone.0107985

Fountain-Jones, N. M., Craft, M. E., Funk, W. C., Kozakiewicz, C., Trumbo, D. R., Boydston, E. E., … Carver, S. (2017). Urban landscapes can change virus gene flow and evolution in a fragmentation-sensitive

carnivore. *Molecular Ecology*, *26*, 6487–6498. https://doi.org/10.1111/mec.14375

Haasl, R. F., & Payseur, B. J. (2011). Multi-locus inference of population structure: A comparison between single nucleotide polymorphisms and microsatellites. *Heredity*, *106*, 158–171. https://doi.org/10.1038/hdy.2010.21

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*, 1965–1978. https://doi.org/10.1002/joc.1276

Hilty, J. A., & Merenlender, A. M. (2004). Use of riparian corridors and vineyards by mammalian predators in northern California. *Conservation Biology*, *18*, 126–135. https://doi.org/10.1111/j.1523-1739.2004.00225.x

Holderegger, R., Kamm, U., & Gugerli, F. (2006). Adaptive vs. neutral genetic diversity: Implications for landscape genetics. *Landscape Ecology*, *21*, 797–807. https://doi.org/10.1007/s10980-005-5245-9

Homer, C., Huang, C., Yang, L., Wylie, B. K., & Coan, M. (2004). Development of a 2001 national land cover database for the United States. *Photogrammetric Engineering and Remote Sensing*, *70*, 829–840. https://doi.org/10.14358/PERS.70.7.829

Hornocker, M., & Negri, S. (2009). *Cougar: Ecology and conservation*. Chicago, IL: University of Chicago Press.

Johnson, W. E., Onorato, D. P., Roelke, M. E., Land, E. D., Cunningham, M., Belden, R. C., … O'Brien, S. J. (2010). Genetic restoration of the Florida panther. *Science*, *329*, 1641–1645. https://doi.org/10.1126/science.1192891

Jombart, T. (2008). ADEGENET: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., Devillard, S., & Balloux, F. (2015). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(94), 1–15.

Kalinowski, S. T. (2005). HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, *5*, 187–189. https://doi.org/10.1111/j.1471-8286.2004.00845.x

Legendre, P., Lapointe, F. J., & Casgrain, P. (1994). Modeling brain evolution from behavior: A permutational regression approach. *Evolution*, *48*, 1487–1499. https://doi.org/10.1111/j.1558-5646.1994.tb02191.x

Lewis, J. S., Logan, K. A., Alldredge, M. W., Bailey, L. L., Vandewoude, S., & Crooks, K. R. (2015). The effects of urbanization on population density, occupancy, and detection probability of wild felids. *Ecological Applications*, *25*, 1880–1895. https://doi.org/10.1890/14-1664.1

Logan, K. A., & Sweanor, L. L. (2001). *Desert puma: Evolutionary ecology and conservation of an enduring carnivore*. Washington, DC: Island Press.

Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology*, *19*, 3038–3051. https://doi.org/10.1111/j.1365-294X.2010.04688.x

Luikart, G., England, P. R., Tallmon, D., Jordan, S., & Taberlet, P. (2003). The power and promise of population genomics: From genotyping to genome typing. *Nature Reviews Genetics*, *4*, 981–994. https://doi.org/10.1038/nrg1226

Luu, K., Bazin, E., & Blum, M. G. B. (2016). PCADAPT: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, *17*, 67–77.

Magle, S. B., Hunt, V. M., Vernon, M., & Crooks, K. R. (2012). Urban wildlife research: Past, present, and future. *Biological Conservation*, *155*, 23–32. https://doi.org/10.1016/j.biocon.2012.06.018

Maletzke, B., Kertson, B., Swanson, M., Koehler, G., Beausoleil, R., Wielgus, R., & Cooley, H. (2017). Cougar response to a gradient of human development. *Ecosphere*, *8*, 1–14. https://doi.org/10.1002/ecs2.1828

Markovchick-Nicholls, L., Regan, H. M., Deutschman, D. H., Widyanata, A., Martin, B., Noreke, L., & Hunt, T. A. (2008). Relationships between human disturbance and wildlife land use in urban habitat fragments. *Conservation Biology*, *22*, 99–109. https://doi.org/10.1111/j.1523-1739.2007.00846.x

Mastretta-Yanes, A., Arrigo, N., Alvaraz, N., Jorgensen, T. H., Piñero, D., & Emerson, B. C. (2015). Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. *Molecular Ecology*, *15*, 28–41.

McKinney, M. L. (2002). Urbanization, biodiversity, and conservation. *BioScience*, *52*, 883–890. https://doi.org/10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2

McMahon, G., Gregonis, S. M., Waltman, S. W., Omernik, J. M., Thorson, T. D., Freeouf, J. A., … Keys, J. E. (2001). Developing a spatial framework of common ecological regions for the conterminous United States. *Environmental Management*, *28*, 293–316. https://doi.org/10.1007/s0026702429

McRae, B. H. (2006). Isolation by resistance. *Evolution*, *60*, 1551–1561. https://doi.org/10.1111/j.0014-3820.2006.tb00500.x

McRae, B. H., Beier, P., Dewald, L. E., Huynh, L. Y., & Keim, P. (2005). Habitat barriers limit gene flow and illuminate historical events in a wide-ranging carnivore, the American puma. *Molecular Ecology*, *14*, 1965–1977. https://doi.org/10.1111/j.1365-294x.2005.02571.x

Moss, W. E., Alldredge, M. W., Logan, K. A., & Pauli, J. N. (2016). Human expansion precipitates niche expansion for an opportunistic apex predator (*Puma concolor*). *Scientific Reports*, *6*, 1–5. https://doi.org/10.1038/srep39639

Moss, W. E., Alldredge, M. W., & Pauli, J. N. (2016). Quantifying risk and resource use for a large carnivore in an expanding urban-wildland interface. *Journal of Applied Ecology*, *53*, 371–378. https://doi.org/10.1111/1365-2664.12563

Naiman, R. J., Decamps, H., & Polluck, M. (1993). The role of riparian corridors in maintaining regional biodiversity. *Ecological Applications*, *3*, 209–212. https://doi.org/10.2307/1941822

Newby, J. R., Scott Mills, L., Ruth, T. K., Pletscher, D. H., Mitchell, M. S., Quigley, H. B., … DeSimone, R. (2013). Human-caused mortality influences spatial population dynamics: Pumas in landscapes with varying mortality risks. *Biological Conservation*, *159*, 230–239. https://doi.org/10.1016/j.biocon.2012.10.018

Paris, J. R., Stevens, J. R., & Catchen, J. M. (2017). Lost in parameter space: A road map for Stacks. *Methods in Ecology and Evolution*, *8*, 1360–1373.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for denovo SNP discovery and genotyping in model and nonmodel species. *PLoS ONE*, *7*, 1–11. https://doi.org/10.1371/journal.pone.0037135

Radeloff, V. C., Hamer, R. B., Stewart, S. I., Fried, J. S., Holcomb, S. S., & McKeefry, J. F. (2005). The wildland-urban interface in the United States. *Ecological Applications*, *15*, 799–805. https://doi.org/10.1890/04-1413

Richards, S. A. (2015). Likelihood and model selection. In G. A. Fox, S. Negrete-Yankelevich, & V. J. Sosa (Eds.), *Ecological statistics: Contemporary theory and application* (pp. 58–80). Oxford, UK: Oxford University Press.

Riley, S. P., Pollinger, J. P., Sauvajot, R. M., York, E. C., Bromley, C., Fuller, T. K., & Wayne, R. K. (2006). A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology*, *15*, 1733–1741. https://doi.org/10.1111/j.1365-294X.2006.02907.x

Riley, S. P., Serieys, L. E. K., Pollinger, J. P., Sikich, J. A., Dalbeck, L., Wayne, R. K., & Ernest, H. B. (2014). Individual behavior dominates the dynamics of a mountain lion population in a highly fragmented urban landscape. *Current Biology*, *24*, 1989–1994.

Row, J. R., Knick, S. T., Oyler-McCance, S. J., Lougheed, S. C., & Fedy, B. C. (2017). Developing approaches for linear mixed modeling in landscape genetics through landscape-directed dispersal simulations. *Ecology and Evolution*, *7*, 3751–3761. https://doi.org/10.1002/ece3.2825

Sagarin, R. D., & Gaines, S. D. (2002). The abundant centre distribution: To what extent is it a biogeographical rule? *Ecology Letters*, *5*, 137–147. https://doi.org/10.1046/j.1461-0248.2002.00297.x

Sergio, F., Caro, T., Brown, D., Clucas, B., Hunter, J., Ketchum, J., … Hiraldo, F. (2008). Top predators as conservation tools: Ecological rationale, assumptions, and efficacy. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 1–19. https://doi.org/10.1146/annurev.ecolsys.39.110707.173545

Sergio, F., Newton, I., Marchesi, L., & Pedrini, P. (2006). Ecologically justified charisma: Preservation of top predators delivers biodiversity conservation. *Journal of Applied Ecology*, *43*, 1049–1055. https://doi.org/10.1111/j.1365-2664.2006.01218.x

Shirk, A. J., Landguth, E. L., & Cushman, S. A. (2017). A comparison of regression methods for model selection in individual-based landscape genetic analysis. *Molecular Ecology Resources*, *18*, 55–67. https://doi.org/10.1111/1755-0998.12709

Short Bull, R. A., Cushman, S. A., Mace, R., Chilton, T., Kendall, K. C., Landguth, E. L., … Luikart, G. (2011). Why replication is important in landscape genetics: American black bear in the Rocky Mountains. *Molecular Ecology*, *6*, 1092–1107. https://doi.org/10.1111/j.1365-294X.2010.04944.x

Smouse, P. E., & Peakall, R. (1999). Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, *82*, 561–573. https://doi.org/10.1038/sj.hdy.6885180

Solberg, K. H., Bellemain, E., Drageset, O. M., Taberlet, P., & Swenson, J. E. (2006). An evaluation of field and non-invasive genetic methods to estimate brown bear (*Ursus arctos*) population size. *Biological Conservation*, *128*, 158–168. https://doi.org/10.1016/j.biocon.2005.09.025

Sweanor, L. L., Logan, K. A., & Hornocker, M. G. (2000). Cougar dispersal patterns, metapopulation dynamics, and conservation. *Conservation Biology*, *14*, 798–808. https://doi.org/10.1046/j.1523-1739.2000.99079.x

Theobald, D. M. (2005). Landscape patterns of exurban growth in the USA from 1980 to 2020. *Ecological Society*, *10*, 1–34.

Thorne, J. H., Cameron, D., & Quinn, J. F. (2006). A conservation design for the central coast of California and the evaluation of mountain lion as an umbrella species. *Natural Areas Journal*, *26*, 137–148. https://doi.org/10.3375/0885-8608(2006)26[137:ACDFTC]2.0.CO;2

Trumbo, D. R., Spear, S. F., Baumsteiger, J., & Storfer, A. (2013). Rangewide landscape genetics of an endemic Pacific northwestern salamander. *Molecular Ecology*, *22*, 1250–1266. https://doi.org/10.1111/mec.12168

U.S. Census Bureau (2017). *Cumulative estimates of resident population change for the United States, regions, states, and Puerto Rico and region and state rankings: April 1, 2010 to July 1, 2017, Table 2 (NST-EST2017-02)*. Source: U.S. Census Bureau, Population Division; Release date: December 2017. Retrieved from www.census.gov/programs-surveys/popest.html

van Strien, M. J., Keller, D., & Holderegger, R. (2012). A new analytical approach to landscape genetic modelling: Least-cost transect analysis and linear mixed models. *Molecular Ecology*, *21*, 4010–4023. https://doi.org/10.1111/j.1365-294X.2012.05687.x

Vandergast, A. G., Bohanak, A. J., Weissman, D. B., & Fisher, R. N. (2007). Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: Phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: *Stenopelmatus*). *Molecular Ecology*, *16*, 977–992. https://doi.org/10.1111/j.1365-294X.2006.03216.x

Wahlund, S. (1928). Zuzammensetzung von populationen und korrelation-serscheiunungen von standpunkt der vererbungslehre aus betrachtet. *Hereditas*, *11*, 65–106.

Waples, R. S. (2016). Making sense of genetic estimates of effective population size. *Molecular Ecology*, *25*, 4689–4691. https://doi.org/10.1111/mec.13814

Waples, R. S., & England, P. R. (2011). Estimating contemporary effective population size on the basis of linkage disequilibrium in the face of migration. *Genetics*, *189*, 633–644. https://doi.org/10.1534/genetics.111.132233

Waples, R. K., Larson, W. A., & Waples, R. S. (2016). Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity*, *117*, 233–240. https://doi.org/10.1038/hdy.2016.60

Warren, D. L., Glor, R. E., & Turelli, M. (2010). ENMTools: A toolbox for comparative studies of environmental niche models. *Ecography*, *33*, 607–611. https://doi.org/10.1111/j.1600-0587.2009.06142.x

Warren, M. J., Wallin, D. O., Beausoleil, R. A., & Warheit, K. I. (2016). Forest cover mediates genetic connectivity of northwestern cougars. *Conservation Genetics*, *17*, 1011–1024. https://doi.org/10.1007/s10592-016-0840-7

Wilmers, C. C., Wang, Y., Nickel, B., Houghtaling, P., Shakeri, Y., Allen, M. L., … Williams, T. (2013). Scale dependent behavioral responses to human development by a large predator, the puma. *PLoS ONE*, *8*, 1–11. https://doi.org/10.1371/journal.pone.0060590

Wright, S. (1942). Isolation by distance. *Genetics*, *28*, 114–138.

Zeller, K. A., Vickers, T. W., Ernest, H. B., & Boyce, W. M. (2017). Multi-level, multi-scale resource selection functions and resistance surfaces for conservation planning: Pumas as a case study. *PLoS ONE*, *12*, 1–20. https://doi.org/10.1371/journal.pone.0179570

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Trumbo DR, Salerno PE, Logan KA, et al. Urbanization impacts apex predator gene flow but not genetic diversity across an urban-rural divide. *Mol Ecol*. 2019;28:4926–4940. https://doi.org/10.1111/mec.15261