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RESEARCH ARTICLE

Genetic diversity, gene flow, and source-sink dynamics of cougars in the Pacific Northwest

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Abstract

Conservation and management of wide-ranging carnivores like cougars (*Puma concolor*), which occur across human-altered landscapes can benefit from an in-depth understanding of their genetic status. Here, we apply the largest collection of multi-locus genotypes currently available for cougars ($n = 1{,}903$) to provide a comprehensive assessment of genetic diversity, gene flow, and source-sink dynamics for cougars occurring across Washington, United States and south-central British Columbia, Canada. We found that cougars in the Olympic, Cascade, Kettle, Selkirk, and Blue Mountains ecosystems are genetically differentiated into two clusters with varying degrees of admixture, indicating moderate levels of gene flow across the area with the exception of the Olympic Peninsula and the Blue Mountains which form more distinct genetic groups. We detected several first-generation migrants confirming long-distance movements within our study system, but also observed that migration rates between areas were asymmetrical, which is an indication of genetic source-sink dynamics. Genetic diversity and inbreeding followed a clinal east-to-west pattern with Olympic Peninsula cougars having the lowest genetic diversity and highest inbreeding coefficients among all sites. Spatial autocorrelation results for cougars did not follow sex-specific patterns suggesting that anthropogenic pressures such as habitat fragmentation and/or mortality sources may have an impact on their spatial dynamics. As cougar habitat in the northwestern United States continues to be affected by rising levels of urbanization and anthropogenic activities, long-term regional genetic monitoring represents a critical decision-support tool for formulating effective cougar conservation and management actions to prevent further genetic decline and promote long-term persistence of cougar populations.

Keywords Cougar · Genetic connectivity · Genetic diversity · Inbreeding · *Puma concolor* · Source-sink dynamics

Introduction

Cougars (*Puma concolor*) are a top carnivore and as such, are a primary driver of the structure, function, and biodiversity of the ecosystems across their range (e.g., Beschta and Ripple 2009; Elbroch and Wittmer 2012; Sarasola et al. 2016; Hoeks et al. 2020). The benefits of conservation and management of large carnivores such as cougars are far-reaching in that their presence contributes to keeping prey populations physically healthy by removing older and weakened animals and keeping prey densities commensurate with habitat quality (Terborgh and Estes 2013). Cougars also exert other top-down effects on lower trophic levels and contribute vital nutrients to plants and animals within the ecosystems they occupy (Ripple and Beschta 2006; Suraci et al. 2016; Elbroch et al. 2017; Yovovich et al. 2021). The long-term viability of cougar populations can greatly benefit from maintaining natural landscape connectivity throughout their range including the northwestern United States (Cougar Management Guidelines Working Group 2005; Maletzke et al. 2017). Washington is one of the fastest-growing areas in western North America and the number of people living in just the Puget Sound area is projected to almost double by

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2050 (Puget Sound Regional Council, https://www.psrc.org/media/1749). As the human population of Washington continues to grow and development increases, natural areas may become more isolated and fragmented which can particularly impact connectivity of large carnivores such as cougars (e.g., Crooks 2002; Gustafson et al. 2019). Despite the adaptable nature of cougars and their capacity to travel long distances (e.g., Thompson and Jenks 2005; Stoner et al. 2008; Hawley et al. 2016), less contiguous habitat and intense human development can negatively impact cougar movement and ultimately cause population subdivision, reduction of population size, and genetic isolation (e.g., McRae et al. 2005; Loxterman 2011; Ernest et al. 2014; Wultsch et al. 2016a; Trumbo et al. 2019). Low levels of genetic diversity can lead to reduced reproductive success and fitness, increased susceptibility to infectious diseases and parasites, and a limited ability to adapt to changing environments (Roelke et al. 1993; Lacy 1997; Reed and Frankham 2003; Huffmeyer et al. 2022). In extreme cases, small population sizes and reduced levels of connectivity could cause loss of genetic diversity through genetic drift and inbreeding (e.g., Allendorf 1986; Frankham 1996), which was first documented in Florida panthers (Hedrick 1995; Culver et al. 2000; Johnson et al. 2010) but has more recently also been reported in California cougar populations (e.g., Ernest et al. 2014; Gustafson et al. 2017; Huffmeyer et al. 2022).

Previous studies reported that cougars occurring across the Olympic Peninsula underwent a genetic bottleneck and exhibited low levels of genetic diversity (Culver et al. 2000; Beier et al. 2010), but sample sizes may have been too small to be definitive. Later, a landscape genetics study identified four genetically differentiated groups in Washington with occasional exchange of individual cougars among the population clusters; one being the Olympic Peninsula (Warren et al. 2016). Warren et al. (2016) also reported that cougars on the Olympic Peninsula, while displaying a lower level of genetic diversity compared to other cougar populations in the State, did not appear to be a management concern. Nonetheless, the authors recommended expanding upon the statewide genetic assessment by increasing sample sizes and examining additional factors influencing cougar gene flow.

Given the impacts that habitat loss, fragmentation, and urbanization have on genetic diversity and connectivity of wildlife populations (e.g., Keyghobadi 2007; Crooks et al. 2011; Trumbo et al. 2019), effective conservation and management planning may require long-term monitoring of genetic connectivity, dispersal patterns, and source-sink dynamics of wildlife populations. This study expands on former genetic monitoring efforts in Washington, United States and south-central British Columbia, Canada and uses the largest genetic dataset currently available for cougars. First, we examined genetic diversity and inferred population structure from individual cougar genotypes at 18 microsatellite markers. Our aims were to characterize large-scale population structure and assess if cougars could be at risk of genetic isolation and/or inbreeding depression. Second, we assessed contemporary gene flow and genetic source-sink dynamics for cougars by identifying first-generation migrants and by estimating bi-directional migration rates between different geographic areas. Such analyses assist cougar conservation and management with the main goal of maintaining long-term viability of cougar populations across the region. We hypothesize that cougars exhibit population structure corresponding to anthropogenic developments and natural landscape features. We also predict asymmetrical gene flow with sources detected in more contiguous cougar populations across areas of lower human-caused mortality and sinks in less contiguous cougar populations with higher levels of mortality and human encroachment.

Materials and methods

Sample collection and study areas

Washington Department of Fish and Wildlife (WDFW) collected 3,355 cougar tissue samples from known cougar mortalities (hunting, agency removal, vehicle collision) and from some live-captured animals during research efforts across Washington between 2003 and 2018 (e.g., Beausoleil and Warheit 2015; Beausoleil et al. 2016; Maletzke et al. 2017). For all cougars that were physically captured or sampled via biopsy darts, animal handling protocols were performed in accordance with the American Society of Mammologists for the use of live animals in research (Sikes and Gannon 2011). Tissue samples were stored in 100% ethanol at room temperature. Sampling locations in Washington included the Olympic Peninsula, Cascade Mountain Range, Selkirk, and Kettle Ranges, and the Blue Mountains. In addition, the British Columbia Ministry of Forests, Lands and Natural Resource Operations contributed 55 cougar tissue samples from south-central British Columbia (Fig. 1).

Across the study area, elevation ranges from sea level to 4,389 m and climate and vegetation vary greatly from the temperate evergreen rainforests of the Olympic Peninsula in the west to a semi-desert landscape that stretches from the Cascade Mountain range into the Columbia Plateau in the east. Human population densities are highest in the Puget Sound coastal region, which is home to over 75% of the residents in the study area. Regulated cougar hunting under WDFW's management authority occurs throughout much of the state (Washington Department for Fish and Wildlife 2015), with hunting pressure being generally heavier in eastern Washington and least in southwest Washington, not including the Olympic Peninsula. However, tribal governments authorize their own cougar hunting structures, so

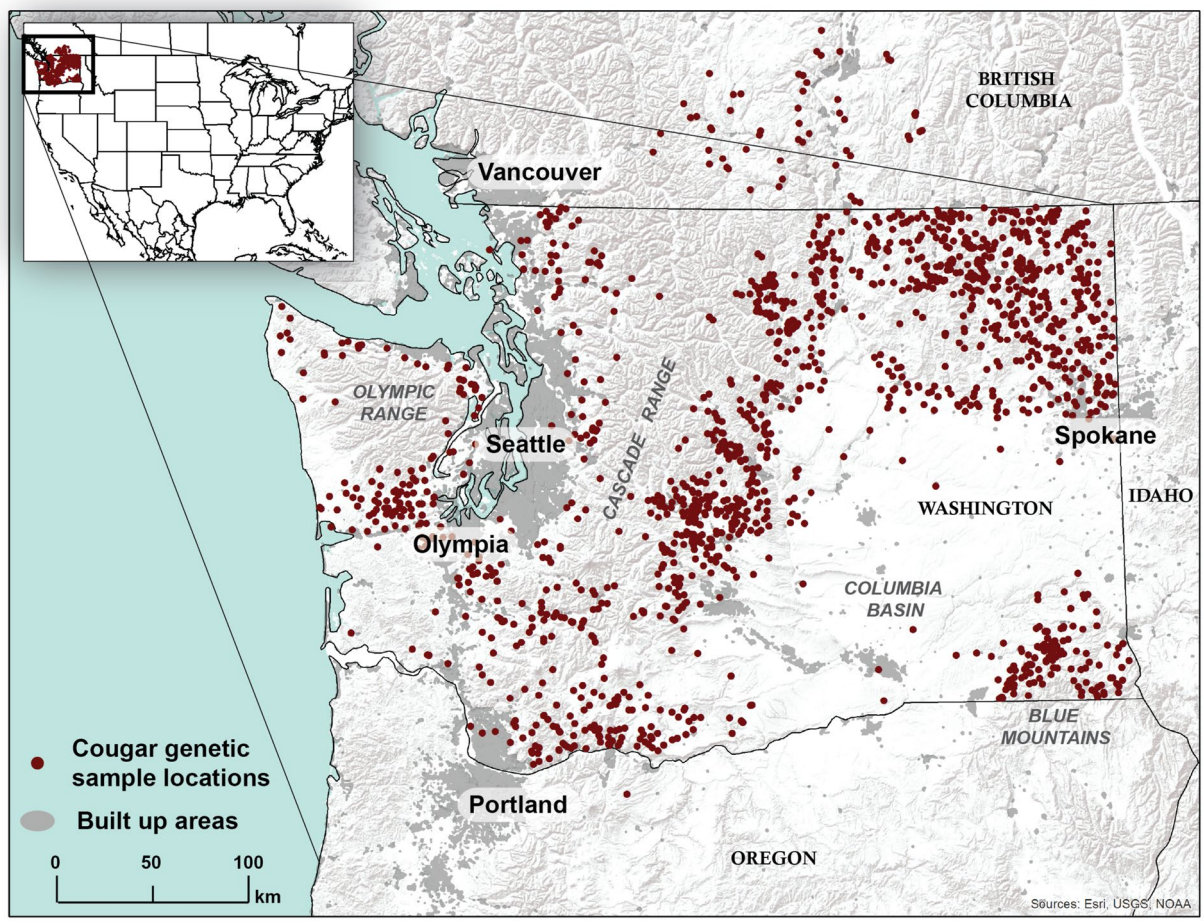

Fig. 1 Study area map depicting cougar (*Puma concolor*) DNA sampling locations across Washington, United States and south-central British Columbia, Canada, 2003–2018. Dots on the map indicate locations where cougar tissue samples assigned to cougar individuals ($n = 1{,}903$) were collected

those harvests are not represented here and likely affect the patterns we observed.

DNA extraction and microsatellite genotyping

Genomic DNA was extracted from cougar tissue (skin and muscle) samples using the DNeasy Blood and Tissue Kit (Qiagen Inc., Los Angeles, CA) or NucleoSpin Tissue Kit (Macherey–Nagel, Bethlehem, PA), as per the manufacturer's instructions. Individual cougars were genotyped at 18 previously identified polymorphic microsatellite loci (FCA008, FCA026, FCA035, FCA043, FCA057, FCA082, FCA090, FCA091, FCA096, FCA126, FCA132, FCA166, FCA176, FCA205, FCA254, FCA262, FCA275, FCA293) (Menotti-Raymond and O'Brien 1995; Menotti-Raymond et al. 1999; Culver et al. 2000). For sex identification, we simultaneously amplified sex-linked zinc-finger, ZF (Aasen and Medrano 1990; Woods et al. 1999) and SRY (Taberlet et al. 1993) loci. Protocols for multiplex polymerase chain reactions (PCR) and thermo-cycling conditions were described by Beausoleil and Warheit (2015) and Warren et al. (2016). Extraction and PCR negatives were added to all reactions to control for contamination. PCR products were visualized using Gene-Scan 500 LIZ sizing standard (Applied Biosystems™, Carlsbad, CA) and an ABI 3730 DNA analyzer (Applied Biosystems™, Carlsbad, CA). Alleles were scored using GENEMAPPER, version 3.7 (Applied Biosystems™, Carlsbad, CA). DNA extraction and genotyping were conducted at the WDFW Molecular Genetics Laboratory in Olympia, WA, US.

We used R package AlleleMatch, version 2.5.1 (Galpern et al. 2012) to identify individual multilocus genotypes and recaptures. We also confirmed unique identities of cougar genotypes by calculating probabilities of identity between siblings ($P_{(ID)sibs}$) using Gimlet, version 1.3.3 (Valière 2002), as recommended by Mills et al. (2000) and Waits et al. (2001). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed using exact tests and Markov chain Monte Carlo (MCMC) estimation (iterations per batch = 5,000; dememorization = 10,000;

batches = 5) in the R package *genepop*, version 1.1.4 (Rousset 2008). To test the random linkage or association of loci, we also calculated the standardized index of association (rD, Brown et al. 1980) using a permutation approach ($n = 999$). Significant levels of multiple comparisons were adjusted by applying a sequential Bonferroni correction (Rice 1989). In addition, we also screened all microsatellite loci for the occurrence of null alleles using MICROCHECKER, version 2.3.3 (Van Oosterhout et al. 2004).

Population structure and genetic diversity

We applied individual-based spatial Bayesian clustering using the locprior model in STRUCTURE, version 2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009) to infer population structure and obtain the optimal number of genetic clusters (K) for cougars across Washington and south-central British Columbia. We applied the admixture model assuming correlated allele frequencies with 10 independent runs of $K = 1$–10 using 1×10^6 MCMC iterations after a burn-in period of 100,000 replicates. We determined the most likely number of K by calculating the rate of change in the log probability of data between successive K values, ΔK (Evanno et al. 2005) in STRUCTURE HARVESTER, version 0.6.94 (Earl 2012) and R package POPHELPER, version 2.3.0 (Francis 2017). Individual membership assignments were averaged using CLUMPP, version 1.2.2 (Jakobsson & Rosenberg 2007) and 10,000 permutations. To complement these Bayesian clustering analyses, we also implemented discriminant analysis of principal components (DAPC) in R package *adegenet*, version 2.1.1 (Jombart 2008), which represents a powerful and flexible multivariate analysis approach for large genetic datasets and complex population structure inference (Jombart et al. 2010). We ran the functions '*find.cluster*' and '*optim.a.score*' to determine the best-supported number of genetic clusters based on the Bayesian Information Criterion (BIC) and the *a*-score (i.e. difference between observed discrimination and values obtained for random discrimination). To test for changes in genetic structure over time, we conducted additional DAPCs for cougar individuals grouped based on their time of collection (2003–2008, 2009–2013, 2014–2018).

To assess historical gene flow in cougars among geographic regions, we calculated pairwise F_{ST} values (Weir & Cockerham 1984) using 10,000 permutations in R package *hierfstat*, version 0.04.22 (Goudet 2005). To identify *a priori* spatial clusters of sampling locations across geographical regions, we ran a K-means clustering algorithm. The K-means approach finds clusters of points that are closer to each other than to other points. To select the number of clusters, we generated a scree plot and identified an elbow between 3 and 6 clusters. The elbow is the number of clusters that explain the most variation in the data. We generated 2–6 clusters with the K-means algorithm and selected 6 as our final number of clusters (Fig. S1) based on the locations of these clusters largely corresponding with genetic demarcations that were previously identified (Warren et al. 2016).

We examined relationships between genetic and geographic distances via Mantel tests (1,000 permutations) in GenAlEx, version 6.5 (Peakall and Smouse 2006) to evaluate if there was evidence for isolation by distance. In addition, we conducted spatial autocorrelation analysis for male and female cougars to assess the spatial extent of positive genetic structure and test for sex-specific differences. Spatial autocorrelation coefficients (r) between genetic and geographic distances and 95% confidence intervals were calculated by permutation (10,000 simulations) and bootstrapping (1,000 iterations) within several distance classes.

We calculated different genetic diversity indices for cougars, including the number of observed alleles (N_A), number of private alleles (N_P), observed heterozygosity (H_O), expected heterozygosity (H_E), Simpson's diversity index (1-D, Simpson 1949), evenness (E5, Grünwald et al. 2003), and the inbreeding coefficient (F) using GenAlEx, version 6.5 (Peakall and Smouse 2006) and R packages *adegenet*, version 2.1.1 (Jombart 2008), *poppr*, version 2.2.0 (Kamvar et al. 2015), and *hierfstat*, version 0.04.22 (Goudet 2005). In addition, we also computed rarified allelic richness (A_R) and private allelic richness (A_P) in HPRare, version 1.0 (Kalinowski 2005). Lastly, we assessed if a population size reduction and heterozygote excess as a consequence of recent bottleneck events occurred across any of the regions using program Bottleneck, version 1.2.02 (Piry et al. 1999). We applied a Two-Phase Mutation Model (TPM) incorporating 30% of an Infinite Allele Mutation Model (IAM), using 10,000 iterations. Wilcoxon rank-sum tests were used to test for heterozygosity excess.

To further examine genetic diversity in cougars, we conducted a comparative analysis of genetic diversity indices (N_A, H_E, F) to test for changes over time (2003–2008, 2009–2013, 2014–2018) and differences between sex groups. We also used R package sGD (Shirk & Cushman 2011) to map continuous gradients of genetic diversity indices across the region. We used a 200 km neighborhood size and required at least 20 observations in each neighborhood to calculate the indices. We selected 200 km as the window size as it was the distance identified from Mantel correlograms as the largest distance class with a positive correlation that was significant for all cougars of both sexes.

Gene flow, dispersal patterns, and genetic source-sink dynamics

To assess contemporary gene flow and genetic source-sink dynamics of cougars across this study system, we identified first-generation migrants (i.e. individuals born in a geographic area other than the one in which they were sampled) in a Bayesian framework (10,000 MCMC iterations; 1,000 simulated individuals; type I error threshold of 0.01) in GENECLASS, version 2.0 (Piry et al. 2004). Cougars with probabilities of < 0.01 were classified as dispersers, the remaining cougars represented residents.

Lastly, we applied a Bayesian inference framework in BayesAss, version 3.0.4.2 (Wilson and Rannala 2003) to estimate bidirectional contemporary gene flow rates (i.e., *m*, migration events that occurred within the last few generations) among cougars across different geographic regions. We adjusted the mixing parameters for genetic migration rates, allele frequency, and inbreeding coefficients to get MCMC state acceptance rates between 0.2 and 0.4. We used program TRACER, version 1.7.2 (Rambaut et al. 2018) to estimate effective sample size values and visually check MCMC outputs for adequate mixing and convergence. Once MCMC runs converged within an optimal acceptance rate, we conducted the analysis in ten independent runs using 25,000,000 MCMC iterations, 2,500,000 iterations burn-in, and a sampling frequency of 2,000. Bidirectional migration rates were visualized as Circos plot using R package *circlize*, version 0.3.4 (Gu et al. 2014). We identified source-sink populations based on differences between emigration and immigration rates. Source populations were classified as the main net exporters of cougar individuals.

Results

Microsatellite loci statistics

We identified 1,903 individual cougars (902 males, 902 females, 99 sex unknown) from 3,355 cougar tissue samples, which were collected by WDFW across Washington, USA and south-central British Columbia, Canada between 2003 and 2018. More specifically, 615 cougar individuals were sampled in the Northern Rocky Mountains, 314 in the northern Cascades, 366 in the Puget Sound and central Cascades, 202 on the Olympic Peninsula, 238 in the southern Cascades, and 168 in the Blue Mountains. We expanded upon the 667 samples used by Warren et al. (2016) by adding genotypes of 1,236 cougar individuals sampled between 2011 and 2018. The remaining proportion of tissue samples could not be included in the study due to DNA storage and amplification failures.

A cumulative $P_{(ID)sibs}$ value of 4.4×10^{-6} for all loci indicates high statistical power to differentiate between closely-related individuals. All loci (except FCA126) were under the null expectation of HWE. Tests for linkage disequilibrium indicated that some loci may be linked (Fig. S2; $r_D = 0.048$, $P = 0.001$, 999 permutations), but the standardized index of association r_D was small and close to zero. Linkage disequilibrium of microsatellite loci may be caused by several other factors, including population structure and genetic drift. We detected null alleles associated with loci FCA090, FCA166, and FCA262. Given the low null allele frequencies (Table S1), we assume that our analysis is not biased by their presence.

Genetic diversity, inbreeding, and recent genetic bottlenecks

Cougars had moderate levels of genetic diversity ($N_A = 6.00$, $A_R = 4.77$, $H_E = 0.59$; Table S1) when measured across all loci and samples. Mean observed heterozygosity ($H_O = 0.52$) was significantly lower (Paired t-test, $t = 8.33$, $df = 8$, $P = 1.631e\text{-}05$) than mean expected heterozygosity ($H_E = 0.59$), which is an indicator of population differentiation or inbreeding. Comparative diversity analysis of the geographic regions revealed that the genetic diversity was highest for cougars sampled in the Northern Rocky Mountains region ($H_E = 0.58$) and lowest for cougars on the Olympic Peninsula ($H_E = 0.47$) (Table 1), but differences between sites were not statistically significant (Kruskal Wallis Test, $H = 2.34$, $df = 5$, $P = 0.800$). Inbreeding coefficients assessed for each study area ranged from 0.05 to 0.12 (mean $F = 0.07$, Table 1), and differed significantly across sites (Kruskal Wallis Test, $H = 12.86$, $df = 5$, $P = 0.025$), with Olympic Peninsula cougars having the highest level of inbreeding ($F = 0.12$) and Northern Rocky Mountains and Blue Mountains cougars not being significantly different from zero (Table 1).

Additional comparative analysis suggested that genetic diversity indices did not significantly change when tracked over time, but we observed small increases and decreases in N_A and H_E in some areas (Table S2A). Inbreeding coefficients slightly increased at most sites, except for the northern Cascades, Puget Sound, and central Cascades (Table S2A). Consistently across all sites, male cougars had higher inbreeding coefficients than females, but the differences were not statistically significant except for the Blue Mountains cougars (Table S2B). We could also detect these sex-based differences when gradients of average inbreeding coefficients were mapped spatially (Fig. 2). Lastly, the bottleneck analysis did not find genetic signatures of a recent genetic bottleneck for cougars in any of the study areas.

Table 1 Genetic diversity indices for cougars (*Puma concolor*, $n = 1{,}903$) across six geographic regions in Washington, United States and south-central British Columbia, Canada, 2003–2018

Regions	*n*	N_A	A_R	N_P	A_P	H_O	H_E	*F* (95% CI)
Northern Rocky Mountains	615	5.28	4.52	0.06	0.06	0.55	0.58	0.049 (− 0.02–0.06)
Northern Cascades	314	5.44	4.64	0.09	0.09	0.51	0.56	0.098 (0.05–0.12)
Puget Sound & central Cascades	366	5.11	4.52	0.09	0.06	0.51	0.54	0.065 (0.04–0.09)
Olympic Peninsula	202	5.06	4.49	0.08	0.11	0.42	0.47	0.120 (0.08–0.16)
Southern Cascades	238	5.11	4.65	0.08	0.09	0.53	0.56	0.051 (<0.00–0.08)
Blue Mountains	168	4.61	4.33	0.00	0.02	0.53	0.56	0.058 (− 0.04–0.07)
Grand mean		5.10	4.53	0.07	0.07	0.51	0.55	0.074
SE		0.18	0.05	0.01	0.01	0.02	0.02	0.012

Diversity indices were averaged across all loci for each site and include the number of observed alleles (N_A), rarified allelic richness (A_R), number of private alleles (N_P), rarified private allelic richness (A_P), observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (*F*) with 95% confidence intervals (CI) intervals using 1,000 bootstrap iterations. SE, standard error; *n*, number of individual cougars per site

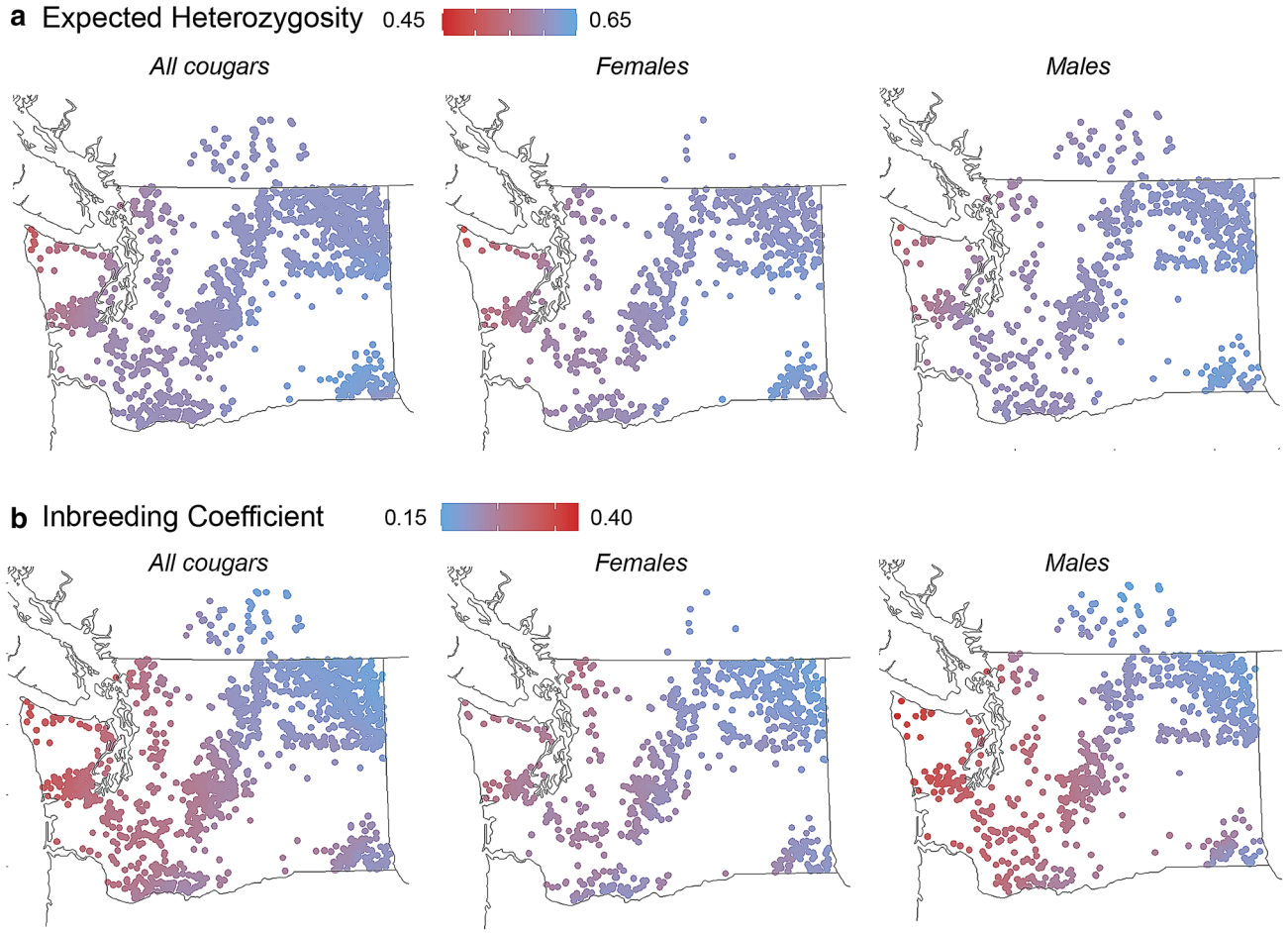

Fig. 2 Spatially explicit evaluation of genetic diversity indices for cougars (*Puma concolor*, $n = 1{,}903$) studied across Washington, United States and south-central British Columbia, Canada, 2003–2018, using R package sGD (Shirk & Cushman 2011). **a** average expected heterozygosity across all loci/individuals within a 200 km neighborhood; **b** average inbreeding coefficient across all loci/individuals within a 200 km neighborhood

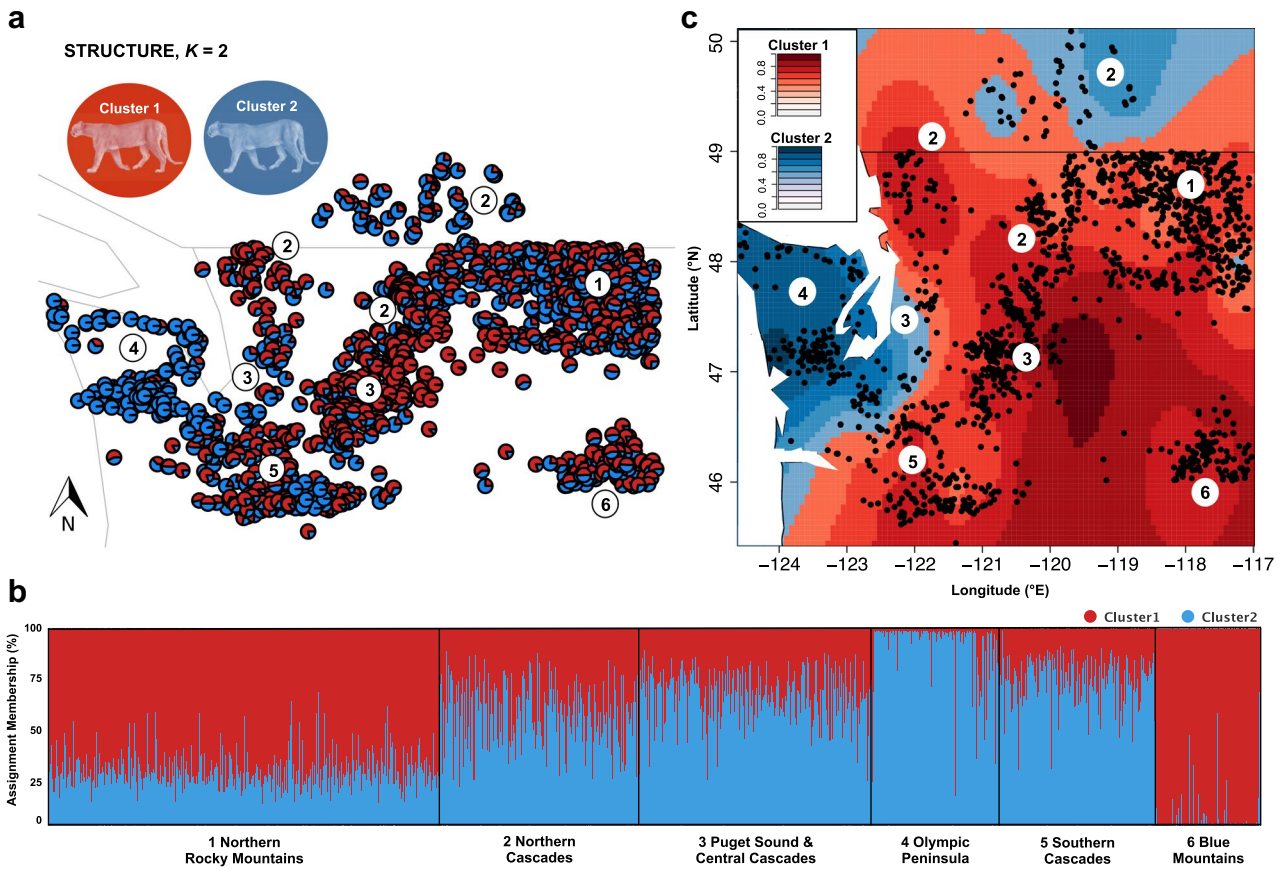

Fig. 3 Bayesian clustering analysis for cougars (*Puma concolor*, $n = 1{,}903$) studied across Washington, United States and south-central British Columbia, Canada, 2003–2018. The analysis was implemented using the locprior admixture model assuming correlated allele frequencies in program STRUCTURE, version 2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009). Geographic regions included in the study are (1) northern Rocky Mountains, (2) northern Cascades, (3) Puget Sound and central Cascades, (4) Olympic Peninsula, (5) southern Cascades, and (6) Blue Mountains. STRUCTURE results for $K = 2$ (cluster 1 in red, cluster 2 in blue) were visualized in three different ways. **a** STRUCTURE assignment proportions displayed as pie charts at the sampling location for each individual cougar. **b** STRUCTURE barplot displaying assignment membership (%) for $K = 2$. Vertical bars represent cougar individuals and the color of each bar visualizes the % of the assignment membership (y-axis) the individual belongs to the genetic clusters (K) identified. **c** Assignment memberships for cougar clusters ($K = 2$) were spatially interpolated and displayed across all study areas. The different shades of red and blue for both genetic clusters represent varying probabilities of membership assignment indicating different degrees of genetic admixture for cougars across sites

Population structure and genetic connectivity

Bayesian clustering analysis in STRUCTURE suggested a subdivision of 1,903 cougars into two genetic clusters ($K = 2$) with varying degrees of genetic admixture (Fig. 3, Fig. S3). At $K = 2$, Blue Mountains cougars were primarily assigned to the genetic cluster in red (average Q, membership assignment = 0.96) and Olympic Peninsula cougars were primarily assigned to the genetic cluster in blue (average $Q = 0.88$) (Fig. 3). Cougars sampled across the northern and central Cascades, including the Seattle metropolitan area, showed a high degree of admixture between both genetic clusters, whereas cougars from the southern Cascades were also admixed to some degree but genetically more similar to Olympic Peninsula cougars. Cougars from the northern Rocky Mountains were also admixed but probabilities of population assignment were highest for the red genetic cluster (average $Q = 0.73$; Fig. 3). DAPC corroborated the STRUCTURE results and showed that cougars form 2–3 genetic groups with some degree of overlap (Fig. 4). DAPC over time revealed that at the beginning of the study (2003–2008), cougars in the southern Cascades and to a lesser extent cougars from the Puget Sound and central Cascades formed more distinct genetic clusters. DAPC for the following years (2009–2013, 2014–2018) suggested beginning separation between cougars from the Puget Sound and central Cascades, the Olympic Peninsula, and all remaining sites (Fig. S4). Pairwise F_{ST} values calculated between sites (Table 2) also supported our findings from the DAPC and STRUCTURE analyses.

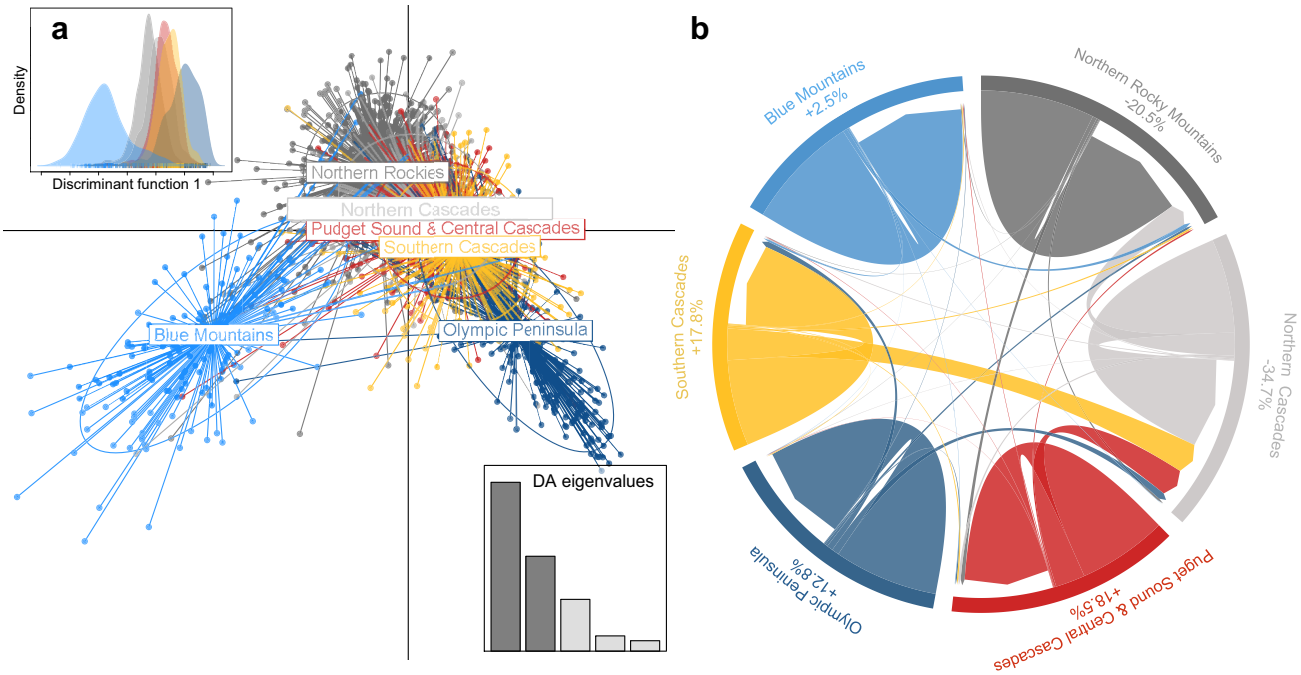

Fig. 4 Population structure and source-sink gene flow dynamics assessed for cougars (*Puma concolor*, $n = 1{,}903$) across Washington, United States and south-central British Columbia, Canada, 2003–2018. **a** Discriminant analysis of principal components (DAPC) using R package *adegenet*, version 2.1.1 (Jombart 2008) identifies genetic clusters. Results are displayed as scatter plot, points representing individual cougars and colors denote sampling sites (Northern Rocky Mountains, northern Cascades, Puget Sound and central Cascades, Olympic Peninsula, southern Cascades, Blue Mountains) and 95% inertia ellipses. Insets display density distributions (top-left) and eigenvalues for the discriminant analysis (bottom-right). **b** Bidirectional contemporary gene flow rates among cougars studied across different geographical regions were estimated using BayesAss, version 3.0.4.2 (Wilson & Rannala 2003) and visualized as circos plot using R package *circlize*, version 0.3.4 (Gu et al. 2014). The direction of arrows represents in- or outward gene flow from one region to another. The width of the arrows corresponds to the relative amount of gene flow between sites. Source and sink populations were identified via net migration rates (listed below region names), calculated as differences between emigration and immigration rates (positive net values indicate a net genetic source, whereas negative values represent a genetic sink population)

Across the entire study system, we detected evidence for isolation by distance when tested for all cougars ($r = 0.216$,

Table 2 Pairwise F_{ST} values for cougars (*Puma concolor*, $n = 1{,}903$) with 95% confidence intervals for six geographic regions in Washington, United States and south-central British Columbia, Canada, 2003–2018, using 10,000 permutations in R package *hierfstat*, version 0.04.22 (Goudet 2005)

Region	Northern Rocky Mountains	Northern Cascades	Puget Sound & central Cascades	Olympic Peninsula	Southern Cascades
Northern Cascades	0.021 (0.013–0.028)				
Puget Sound & central Cascades	0.045 (0.032–0.057)	0.015 (0.008–0.023)			
Olympic Peninsula	0.142 (0.106–0.188)	0.099 (0.071–0.140)	0.096 (0.075–0.119)		
Southern Cascades	0.057 (0.038–0.082)	0.020 (0.009–0.034)	0.016 (0.009–0.022)	0.084 (0.066–0.108)	
Blue Mountains	0.088 (0.062–0.121)	0.115 (0.085–0.149)	0.145 (0.108–0.189)	0.229 (0.173–0.298)	0.162 (0.117–0.222)

All F_{ST} estimates were significantly different from zero

$P = 0.010$), males ($r = 0.213$, $P = 0.010$), and females ($r = 0.206$, $P = 0.010$). Spatial autocorrelation analysis for females and males showed significant positive correlation between individuals within the first 8–9 distance classes (females, 25–225 km, $r = 0.003$–0.060, $P = 0.001$–0.023; males, 25-200 km, $r = 0.007$–0.051, $P = 0.001$), after which r decreased indicating that spatially distant cougars become genetically less similar. The x-intercept of r was at ~ 245 km for females and at ~ 225 km for males, defining the extent of positive genetic structure ("genetic neighborhood") for each sex (Fig. S5).

Gene flow and source-sink dynamics

We identified 47 first-generation migrants (16 females, 30 males, 1 sex unknown) between sites, meaning that 2.5% of all cougar individuals were classified as first-generation migrants (Table S3). The BayesAss analysis revealed that most cougars remained within their putative natal population or were killed before they reached another area (Fig. 4, Table 3). Our results also suggested that most sites exchanged migrants to some degree during the last few generations with an average migration rate (i.e., fraction of population i that originates from population j) of 0.04 (range 0.002 – 0.257). The highest migration rates within our study system were detected for cougars moving from the southern Cascades to the northern Cascades ($m = 0.26$), Puget Sound and the central Cascades to the northern Cascades ($m = 0.24$), northern Cascades to the northern Rocky Mountains ($m = 0.19$), and Olympic Peninsula to the northern ($m = 0.06$) and the southern Cascades ($m = 0.05$). The migration rates at these sites were asymmetrical and were ≤ 0.02 in the opposite direction, indicating that genetic source-sink dynamics occur for cougars in this study system. All other pairwise migration rates were low ($m \leq 0.06$) and mostly directional (Fig. 4, Table 3). Cougars from the Puget Sound, central Cascades, and the southern Cascades were the largest net providers of immigrants within this study system, whereas cougars from the northern Cascades had the highest net immigration. We also found that to a lesser extent the Olympic Peninsula and the Blue Mountains had more emigration than immigration, whereas the northern Rocky Mountains had more immigration than emigration (Fig. 4, Table 3).

Discussion

In our Pacific northwest study area, genetic diversity and inbreeding levels of cougars followed a clinal east-to-west pattern with genetic diversity being lowest and inbreeding being highest for cougars in the western coastal populations which occupy or are adjacent to areas with rising levels of urbanization and habitat fragmentation. Expected heterozygosity and inbreeding coefficients for Washington's mainland cougars were overall comparable to other cougar populations in the western United States (e.g., Murphy 1998; Holbrook et al. 2012; Ernest et al. 2014). We corroborate the findings of Warren et al. (2016) that northwestern cougars are not a single, panmictic population. Both studies revealed similar patterns of admixture, but Warren et al. (2016) ran a spatial clustering analysis in program Geneland in contrast to our locprior model in program STRUCTURE and concluded $K = 4$. We detected strong evidence for moderate genetic differentiation with cougars experiencing an east–west subdivision into two main genetic clusters with a high degree of genetic admixture for most areas with the exception of the Olympic Peninsula and the Blue Mountains, which were the

Table 3 Genetic source-sink dynamics of cougars (*Puma concolor*, $n = 1{,}903$) studied across Washington, United States and south-central British Columbia, Canada, 2003–2018. Bidirectional migration rates were calculated using BayesAss, version 3.0.4.2 (Wilson & Rannala 2003) between different geographic areas

Region	To					
From	Northern Rocky Mountains	Northern Cascades	Puget Sound & central Cascades	Olympic Peninsula	Southern Cascades	Blue Mountains
Northern Rocky Mountains	**0.934 (0.009)**	0.018 (0.004)	0.035 (0.007)	0.002 (0.001)	0.004 (0.002)	0.008 (0.002)
Northern Cascades	0.190 (0.013)	**0.768 (0.012)**	0.020 (0.007)	0.005 (0.002)	0.010 (0.004)	0.006 (0.003)
Puget Sound & central Cascades	0.013 (0.005)	0.242 (0.012)	**0.731 (0.010)**	0.003 (0.002)	0.004 (0.003)	0.006 (0.003)
Olympic Peninsula	0.020 (0.007)	0.059 (0.010)	0.012 (0.006)	**0.855 (0.012)**	0.050 (0.010)	0.005 (0.003)
Southern Cascades	0.016 (0.007)	0.257 (0.012)	0.012 (0.006)	0.006 (0.003)	**0.702 (0.009)**	0.007 (0.004)
Blue Mountains	0.033 (0.009)	0.002 (0.003)	0.004 (0.004)	0.002 (0.002)	0.016 (0.006)	**0.943 (0.011)**

Migration rates m represent the fraction of individuals in population i that are migrants derived from source population j, along with 95% confidence intervals (in parentheses). Bolded values along the diagonal describe the fraction of non-migrant individuals or residents within each geographic region

most divergent groups identified in this study. Besides the difference in Bayesian clustering algorithms applied by both studies, sample sizes also varied significantly ($n = 667$, Warren et al. 2016; $n = 1{,}903$, current study) which may have also directly influenced these updated research findings. Part of the genetic structure detected could be explained through a simple isolation-by-distance pattern, but genetic clustering also corresponded to patterns associated with habitat loss, fragmentation, and urbanization, which was most evident for cougars inhabiting the Puget Sound and Olympic Peninsula areas during the last decade confirming findings of other northwestern cougar studies (Warren et al. 2016; Zeller et al. 2023). The effect of geographic isolation within Washington was most noticeable for cougars in the Blue Mountains, an area surrounded by suboptimal, arid lowland habitat and agriculture in Washington known as the Columbia River Basin. Within our study system, Blue Mountain cougars formed a genetic cluster with high membership assignment values, but also had moderate levels of heterozygosity and low levels of inbreeding, suggesting that they were sufficiently connected to cougar populations in the neighboring jurisdictions of Idaho and Oregon. Therefore, cross-state efforts may be needed to fully understand their genetic status and source-sink dynamics with other populations. Signals of restricted gene flow in this study were most pronounced in Olympic Peninsula cougars. Warren et al. (2016) and Zeller et al. (2023) also reported depressed functional connectivity for Peninsula cougars, especially for males indicating that the Peninsula may pose a higher level of management concern than previously reported by Warren et al. (2016). Our analyses showed that Olympic Peninsula cougars were not completely isolated but experienced asymmetric gene flow with emigration to the Cascades, and immigration rates were among the lowest measured in this study. Olympic Peninsula cougars are separated from the mainland by the Pacific Ocean to the west, the Strait of Juan de Fuca to the north, the Columbia River to the south, and Puget Sound to the northeast. While there is land connectivity to the southeast, there is a large amount of human development including the Interstate 5 highway and urban and agricultural areas, which essentially reduces functional connectivity (Zeller et al. 2023). The observed genetic differentiation in Olympic Peninsula cougars is most likely also impacted by the peripheral location of the Peninsula and historical processes such as the recolonization of cougars after the Pleistocene glaciation (Culver et al. 2000). Since Washington, especially the coastal areas, represent some of the fastest-growing regions within the western United States, it is likely that genetic connectivity of Olympic Peninsula cougars will continue to erode.

Olympic Peninsula cougars had the lowest genetic diversity and highest inbreeding coefficient when compared with other areas in Washington. Warren et al. (2016) reported slightly lower levels of genetic diversity ($H_E = 0.35$ versus $H_E = 0.47$) and inbreeding ($F = 0.08$ versus $F = 0.12$) for Olympic Peninsula cougars, which may be an artifact of the smaller sample size used in that earlier study. Culver et al. (2000) stated that Olympic Peninsula cougars underwent a bottleneck event in the past and consequently had one of the lowest genetic diversity values measured in North American cougars. Olympic Peninsula cougars still had higher levels of genetic diversity and lower levels of inbreeding when compared to cougars in the Santa Ana Mountains of California ($H_E = 0.32$; Ernest et al. 2014) and in southern Florida, which both represent cougar populations of high conservation concern suffering from negative effects of inbreeding depression, including poor sperm quality, low fecundity, cryptorchidism, distal kinked tails, and cowlicks at some point (Roelke et al. 1993; Huffmeyer et al. 2022). Since Olympic Peninsula cougars have a mean inbreeding coefficient above $F = 0.10$, they are at a potentially higher risk to be negatively impacted by inbreeding depression in the future (Ralls et al. 2018). For example, an inbreeding coefficient of 0.10 means that any particular locus has a probability of 10% to be homozygous, which increases the risk of a population being predisposed to genetic disorders. We suspect that the completion of Interstate 5, which vertically bisects Washington from Oregon to the Canadian border, and the concurrent completion of Highway 12 south of the Olympic Peninsula, led to further development of the Chehalis River valley, which may have increased the geographic isolation of Olympic Peninsula cougars in recent decades. That reduced genetic connectivity to and from mainland cougars likely influenced our reported levels of inbreeding and genetic diversity. In summary, symptoms of inbreeding depression have not yet been observed in northwestern cougars, but current findings for Olympic Peninsula cougars highlight the importance of enhancing the genetic connectivity of Peninsula cougars with those of the mainland.

Results for the Bayesian clustering analysis were also corroborated by our migration rate assessment, which suggested that most sites exchanged migrants to some degree during the last few generations, but migration rates were also asymmetrical between some areas, indicating the presence of source-sink dynamics. Variable levels of human-caused mortality (e.g., hunter harvest lethal removal, vehicle collisions) across the landscape can lead to source-sink dynamics in cougar populations (e.g., Logan & Sweanor 2001; Robinson et al. 2008; Cooley et al. 2009; Andreasen et al. 2012). We observed a similar pattern in our study system as cougars from the Puget Sound and central and southern Cascades, where human-caused mortality tends to be low compared to other areas in Washington, had the highest number of emigrants. Conversely in areas with higher human-caused mortality such as the northern Cascades and northern Rocky Mountains, cougar genetics were archetypal of having more

immigration than emigration. Delibes et al. (2001) and Robinson et al. (2008) described this kind of site as an 'attractive sink' that has high-quality habitat and abundant resources, but also increased levels of human-caused mortality. To fill unoccupied territories after the numbers of resident cougars are reduced, dispersing subadults emigrate from adjacent areas into these vacant areas (e.g., Logan & Sweanor 2001; Robinson et al. 2008). More insight regarding source-sink dynamics in these areas is likely to be gleaned from genetic information in the surrounding unsampled landscapes of Idaho and British Columbia.

This study also provides insights into fine-scale spatial dynamics and broad-scale dispersal patterns of female and male cougars which both exhibited strong genetic associations up to 200–225 km with large extents of positive genetic structure ("genetic neighborhood") of ~ 245 km for females and ~ 225 km for males. Similar patterns of spatial autocorrelation were observed in other cougar (Holbrook et al. 2012), jaguar (*Panthera onca*; Wultsch et al. 2016b), snow leopard (*Panthera uncia*; Janecka et al. 2017), and African leopard (*Panthera pardus*; Naude et al. 2020) populations, but most studies did not describe patterns for males and females separately. We also detected several long-distance dispersal movements between different geographical areas for both sexes. In felids and other polygamous mammals, males typically disperse over large distances for inbreeding avoidance and females stay closer to their natal sites (i.e. philopatry) or disperse over shorter distances than males (e.g., Waser & Jones 1983; Logan & Sweanor 2001). These sex-specific behaviors and associated spatial patterns can exhibit plasticity and change in areas impacted by habitat loss, fragmentation, hunting pressure, and/or other human-caused mortalities (e.g., Onorato et al. 2011; Naude et al. 2020; de Oliveira et al. 2022). This is most likely the case in this study system since we found evidence for male philopatry, which has been also observed in cougar populations in Florida and California where habitat fragmentation led to constrained or unsuccessful dispersal attempts (i.e. 'frustrated dispersal') (Sweanor et al. 2000; Riley et al. 2006, 2014). Fattebert et al. (2015), Fattebert et al. (2016), and Naude et al. (2020) found that high hunting pressure on leopards increased the rate of female philopatry and caused a disruption of dispersal patterns in male leopards, which ultimately led to opportunistic male philopatry and localized inbreeding. In our study, male cougars had higher inbreeding coefficients than females across all sites, and although differences were not statistically significant with the exception of the Blue Mountains cougars, we observed an east-to-west gradient, which was particularly pronounced for males in the coastal regions, especially on the Olympic Peninsula. This may be an indication that gene flow of male cougars is more limited across these human-altered landscapes, which corroborates the findings of Zeller et al. (2023) reporting that male cougars had a higher resistance to movement across developed, built-up areas when compared to their female counterparts. Onorato et al. (2011) also described higher relatedness among male than female cougars in a managed population in western Montana, which differed from patterns hypothesized to occur under male-biased dispersal theories for cougars. That study recommended combining demographic with genetic data when cougar harvest strategies are determined.

Conservation and management implications for northwestern cougars

Cougar conservation and management across human-impacted landscapes is challenging, often requiring policy-level decision-making with little information on a low-density and wide-ranging carnivore species (e.g., Beausoleil et al. 2021; Murphy et al. 2022). This study highlights the importance of large-scale genetic monitoring efforts for broadly distributed wildlife species such as cougars as it provides novel insights into evolutionary, ecological, and demographic processes that are difficult or impossible to obtain using other traditional population monitoring methods (e.g., Schwartz et al. 2007).

Our results suggest that the Olympic Peninsula may be an area to consider focused management attention since cougars are exhibiting moderate to high levels of inbreeding and low immigration rates. An in-depth assessment of human-caused mortalities and dispersal patterns using GPS collars may prove informative for wildlife and land development managers to ensure and, if needed, to restore functional connectivity, even if by human-made structures such as highway under- and overpasses. In the northern Cascades and northern Rocky Mountains, our findings suggest cougar emigration rates to other areas of Washington are low and efforts to enhance genetic exchange with other areas may be warranted. Additionally, understanding the role of human-caused mortality and how it influences asymmetric migration patterns we reported for these two areas warrants further investigation as it may be of value to wildlife managers. Finally, the collection of cougar DNA samples across other jurisdictions during mandatory inspections of hunting removals, and from all known mortalities (Beausoleil and Warheit 2015) may be beneficial for current conservation and management planning. In jurisdictions where funds may not yet be available for genetic analysis, DNA from biological samples can be extracted and safely archived in a minus 80 °C freezer for many years. Genetic data can contribute critical knowledge on how carnivores respond to environmental changes and anthropogenic impacts, as we have demonstrated, but it can also help inform harvest guidelines, identify appropriately-sized and objective management units, and identify areas that may be acting as

sources or sinks. The expansion of genetic monitoring of cougars across jurisdictional borders would allow for larger-scale cougar conservation and management objectives and intra- and inter-agency partnerships. For example, in the Blue Mountains, cougars are most likely more connected to populations from neighboring states than within Washington, and an inter-agency genetic sampling effort to assess genetic status would be informative. If such a collaborative effort was to be undertaken, the use of standardized DNA storage and a centralized genetic laboratory conducting the analysis, and the establishment of formal data-sharing agreements would enhance cross-jurisdictional genetic monitoring so that the data can be of maximum benefit.

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Declarations

Competing interests The authors have no conflict of interest to disclose.

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