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Abstract

The amount of dispersal that occurs among populations can be heterogeneity, which is often due to both natural processes and ity leading to habitat loss or fragmentation. Understanding structured and mapping existing dispersal corridors among pop to both determining contemporary forces mediating population forming proper management of species with fragmented popu the contemporary processes mediating gene flow across heter on a large scale are understudied, particularly with respect to This study focuses on a widespread game bird, the Ruffed Gro for which we analyzed samples from the western extent of t types of genetic markers, we uncovered multiple factors acti responsible for mediating contemporary population connect Multiple genetically distinct groups were detected; microsate six groups, and a mitochondrial marker revealed four. Many p Grouse are genetically isolated, likely by macrogeographic barri addition of landscape genetic methods not only corroborated sults, but also uncovered compelling evidence that dispersal areas of unsuitable habitat is the most important factor medi nectivity among the sampled populations. This research has i for both our study species and other inhabitants of the early suc tat preferred by Ruffed Grouse. Moreover, it adds to a growing isolation by resistance is more prevalent in shaping populatio spread species than previously thought.

KEYWORDS
dispersal barriers, gene flow, isolation by resistance, landscape genetic ruffed grouse

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et al., 2005). For many species, the structure of the landscape is an important factor shaping contemporary distributions. Unsuitable habitat is a potential barrier to gene flow, but it is not necessarily an impermeable barrier. Habitat often varies in its degree of suitability (Cushman, McKelvey, Hayden, & Schwartz, 2006), resulting in a complex matrix of habitat types with varying dispersal costs or resistance to individuals moving across the landscape. With current landscape genetic methods, it is possible to identify areas of the landscape that are impeding or facilitating connectivity, and also identify the environmental factors that underlie patterns of contemporary gene flow (Keyghobadi, Roland, & Strobek, 1999; Manel, Schwartz, Luikart, & Taberlet, 2003; Storfer et al., 2007).

Differences in landscape resistance and physical distance can both dictate patterns of gene flow (Ruiz-Gonzalez, Cushman, Madeira, Randi, & Gómez-Moliner, 2015). When landscape heterogeneity exists between populations, suitable dispersal routes become more complex. For example, if one of two possible dispersal routes requires movement through habitat that is unsuitable for the study species, then it is likely to present more resistance to dispersing individuals than a route through suitable habitat even when geographic distances are the same. For this reason, landscape heterogeneity within a species' range means patterns of isolation by resistance (IBR) are more likely to occur (Fontaine et al., 2007; McRae & Beier, 2007; Ruiz-Gonzalez et al., 2015). Physical distance between populations can also act as a barrier by creating clinal genetic variation (Ruiz-Gonzalez et al., 2015), or isolation by distance (IBD), as species dispersal is as a function of geographic distance. IBD and IBR are not, however, mutually exclusive and sometimes a combination of the two best explains genetic structuring (Metzger, Espindola, Waits, & Sullivan, 2015; Piertney, MacColl, Bacon, & Dallas, 1998). Species that are widespread and relatively continuously distributed are expected to exhibit either panmixia or clinal patterns of genetic structure explained by IBD, particularly when comparing populations at a large scale (Alcaide et al., 2009; Purdue, Smith, & Patton, 2000; Ralston & Kirchman, 2012). A few studies have emerged where widespread, continuously distributed species exhibit unexpected patterns of IBR (Pease et al., 2009; Pilot et al., 2006), but the extent to which species with broad geographic ranges exhibit IBD or IBR is unclear (Basto et al., 2016; Frankham, Ballou, & Briscoe, 2010). Understanding the roles that distance and resistance play in structuring populations is dependent on studying both successful dispersal events.

Although Ruffed Grouse have been we
ecology and population dynamics (Atwater
1984; Rusch et al., 2000; Zimmerman & C
no published information on their populat
Grouse is one of the most extensively ma
heavy hunting pressure throughout most
2000). It is also considered an indicator sp
of early successional forest habitats (USD
Therefore, information on how macrogeog
tat limit gene flow has important implicatio
Ruffed Grouse, but also other early succes
American Woodcock [*Scolopax minor*], Mou
philadelphia], Golden-winged Warbler [*Ver*

The aims of this study were to quantify
assessing gene flow across a large sectio

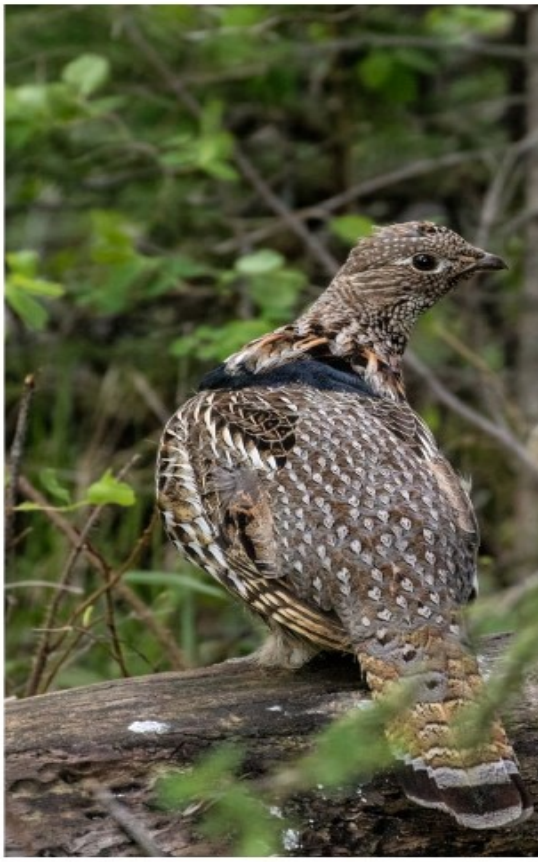


FIGURE 1 A Ruffed Grouse in the mixe
Crowsnest Pass, Alberta, Canada. Copyrigh

FIGURE 2 Map showing the current range of Ruffed Grouse (*Bonasa umbellus*), and sampling sites for this study. same label were pooled for analyses due to close proximity or lack of sufficient sampling at one or more of these sit available in Table 1. The data for the range distribution were taken from Birds of North America Online and were pr a digital elevation map of North America in ArcGIS® v10.2. Digital elevation map courtesy of ESRI®

of the species range and to identify geographic barriers and other landscape features that may be restricting or facilitating gene flow. We chose to focus on the western extent of the range because this is where macrogeographic barriers are most likely to be influencing population structure, based on studies in a range of other species (Adams, & Burg, 2015b; Pulgarín-R & Burg, 2012; Vonhof et al., 2015). Although widespread species with a continuous distribution are expected to show evidence of IBD, we predicted that Ruffed Grouse populations would exhibit patterns of IBR due to the heterogeneous distribution of suitable habitat throughout their range, their low dispersal ability, and their preference for early successional forest habitat. In addition, we predicted that Ruffed Grouse populations would show significant population genetic structuring, and of the extrinsic factors that may be affecting gene flow, both mountains and swaths of unsuitable habitat would be the most likely geographic features to act as barriers.

2 | MATERIALS AND METHODS

2.1 | Samples

Fieldwork was conducted from mid-April through May 2016, during the peak activity period of the male Ruffed Grouse's drumming display (Rusch et al., 2000). Birds were located aurally by drumming activity, and the location of each male's drumming log was marked with a handheld GPS unit. We caught birds with a lift net (Fischer, 1974), a carbon dioxide-powered net gun 1965), which were placed on males' drum drumming stage. Brachial venipuncture wa sample, which was stored in 99% ethanc lected 26 Ruffed Grouse samples at two Lake (52.91 N, 115.01 W), and Crowsnest An additional 49 samples were taken fro same two sites between 2010 and 2015 studies (Corfield, Harada, & Iwaniuk, 2013 Ligt, & Iwaniuk, 2013; Krilow & Iwaniuk, hered to the Canada Council for Animal C proved by the University of Lethbridge An and collected under research permits issue and Parks.

In addition, 159 samples were collecte hunters throughout Alberta in the 2016 hu supplied by the Royal Alberta Museum, f originating in Alberta (Figure 2). Outside 100 samples from various western sites v populations that are likely to be affected b ers, such as mountain ranges. This include Yukon Fish and Game, 32 from the Univer Museum, and 25 from University of Alas tained 30 samples in the Great Lakes area of Natural History to represent a populat of the range. All of the museum samples w

Edson area	EA	5	3	0.700	0.00457
Lloydminster	LM	5	3	0.900	0.00609
Minnesota	MN	12	9	0.970	0.02300

2.2 | DNA extraction and amplification

Genomic DNA was isolated from each blood sample with a modified chelex extraction method (Walsh, Metzger, & Higuchi, 1991). Samples were screened at intron 6 of Aldolase B on the Z-chromosome (Cheviron & Brumfield, 2009), a 394 bp portion of the mitochondrial control region (domain I and II), and an intron of the nuclear *SLC45a2* gene (involved in the melanin production pathway; Gunnarsson et al., 2007). We chose these markers because they are presumed to be neutrally evolving, and this diversity of marker types represents different modes of inheritance and different rates of mutation. Samples were amplified with polymerase chain reaction (PCR) in a 25 µl reaction containing Green GoTaq® Flexi buffer (Promega), 1.5 mM $MgCl_2$, 0.08 mM dNTP, 0.4 µM of each primer and 0.5 U GoTaq® Flexi DNA polymerase (Promega) for Aldolase B. These reaction mixes were the same for control region and *SLC45a2*,

upstream from the indel, while the other tw
to bind to the insertion and deletion regio
tag was added to the 5′ end of the reverse p
increase the size difference between the f
products were 161 bp for the insertion and
All 351 samples were screened on a 3% aga

A total of 25 microsatellite loci isolated
lated to Ruffed Grouse (Burt et al., 2003; Cai
Mondor, & Raspluss, 2001; Cheng & Critt
Dallas, 1997; Piertney & Hoglund, 2001;
Höglund, 2008; Segelbacher, Paxton, Steinb
2000; Taylor, Oyler-McCance, & Quinn, 20
checked for variation. For the 10 polymor
was amplified in 10 µl reactions containing
buffer (Promega), 2.5 mM $MgCl_2$, 0.2 mM
ward primer, 1.0 µM of the reverse primer,
tag, and 0.5 U GoTaq® Flexi DNA polymera
SGCA5 were amplified using 2 mM $MgCl_2$
2.3 mM $MgCl_2$. All forward primers were
sequence on the 5′ end of the primer sequ
tion of the fluorescent M13 tag and visualiza

TABLE 2 Sampling site or group of sampling sites used in microsatellite analyses (population), sample size per site (*n*), sampling site abbreviation (ID), number of different alleles occurring at a frequency of ≥ 5% (Na), private alleles (PA), allelic richness, (A_R), observed heterozygosity (Ho), and expected heterozygosity (He)

Population	ID	*n*	Na	PA	A_R
Alaska	AK	22	5.25	0	3.29
Yukon	YT	13	5.62	2	3.56
Washington	WA	23	5.05	2	3.43
Crowsnest Pass	CP	36	6.87	2	3.69
Cochrane area	COA	12	5.50	0	3.78
Buck Lake	BL	29	7.50	5	3.87
Edson area	EA	63	9.25	3	3.81
Grande Prairie	GP	18	5.87	1	3.85
Peace River	PR	8	5.12	–	–
Athabasca area	AT	29	7.12	2	3.55
Fort McMurray	FM	11	5.62	1	3.68
Bonnyville area	BV	19	6.62	5	3.77
Lloydminster	LM	13	5.62	0	3.64
Minnesota	MN	21	6.88	1	4.06
Wisconsin	WI	7	5.38	–	–

Notes. Statistics that may be sensitive to low sample sizes were exclud
$N < 10$.

ensure consistent scoring. A second person scored all gels to reduce scoring error. As an additional measure against potential errors, a subset of samples from each population were genotyped a second time at each locus.

2.3 | Genetic diversity analyses

All sequences were checked, manually aligned and assessed for variation using MEGAv6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). DnaSP v5.1 (Rozas, Sánchez-DelBarrio, Messeguer, & Rozas, 2003) was used to calculate shared haplotypes, nucleotide diversity (π), and haplotype diversity (H_d) for control region sequences. The *SLC45a2* sequence data were not variable enough to be informative and were not included in the remainder of our analyses.

Genetic diversity was measured at the population level using microsatellite loci by calculating observed and expected heterozygosities, the number of alleles per locus, and number of private alleles in GenAlEx v6.5 (Peakall & Smouse, 2012). FSTAT v2.3.1.0 (Goudet, 1995) was used to calculate allelic richness (A_R). Genotypes at the microsatellite loci were checked for linkage disequilibrium and deviations from Hardy–Weinberg equilibrium using GENEPOP v4.2 (Raymond & Rousset, 1995) with default parameters. MICRO-CHECKER v2.2.3 (van Oosterhout, Hutchinson, Wills, & Shipley, 2004) was used to check for errors in the genotyping data including allelic dropout and null alleles. The resulting significance levels were corrected for multiple tests using a modified False Discovery Rate (FDR; Benjamini & Yekutieli, 2001). Two loci, ADL230 and TTD2, were removed due to significant deviation from Hardy–Weinberg equilibrium. The SGCA5 locus had a significant probability of null alleles for a few sampling sites, so analyses were performed with and without this marker to determine if the potential presence of null alleles was biasing the data. SGCA5 was retained in the final analyses because its exclusion did not cause noticeable variation in the results. SGCA5 had more missing data than the other markers (>25% for some sampling sites) and had to be excluded from *F*-statistic calculations. Of the 351 genotyped samples, 324 were used for analyses after removing samples that amplified at fewer than six loci. Samples collected in the same area on the same day (i.e., hunter-donated or museum collection samples harvested on the same day) were checked for shared ancestry that would indicate multiple individuals from the same family group; none were found. For analyses that required a priori population

& Yekutieli, 2001), and control region hapl
ate a statistical parsimony network in Pop
2015).

Individuals were sexed prior to comp
Aldolase B to determine if each individual
or homozygous (males). The allele frequen
significant pairwise population differentia
test (Fisher, 1922).

Genetic structure was quantified for
all populations at microsatellite loci using
2011) calculations in GenAlEx v6.5 (Peak
p-values were corrected for multiple tes
method (Benjamini & Yekutieli, 2001).

2.5 | Bayesian clustering analyse

Three Bayesian clustering analyses were
v2.3.4, TESS v2.3, and GENELAND v4.0.
use geographic coordinates as a paramete
structure, and the use of multiple Bayesia
help elucidate complex patterns, and aid in
Miller, McRae, Fortin, & Manel, 2011).

The data were analyzed with STRUC
Stephens, & Donnelly, 2000) using correl
the admixture model, and sampling locatio
ors option allows sampling location inform
model, but will not create population st
none. Ten independent runs were perfor
repetitions and a 10,000 burn-in period fo
to 10. After these initial runs, values from
and delta *K* (ΔK; Evanno, Regnaut, & Goude
STRUCTURE HARVESTER v0.6.94 (Earl &
mine the most likely value of *K* or number c
optimal *K*, any clusters that included more
run through the program independently u
test for additional substructure.

TESS v2.3 (Chen, Durand, Forbes, & F
mented for *K* values from 2 to 10 using 100
burn-in, and Ψ (value determining how muc
influence clustering) was set to 0.6. *K* was s
with the highest posterior probability and
tion criterion (DIC). As with STRUCTURE,

runs were conducted at this fixed *K* value.

2.6 | Principal coordinates analysis

To examine genetic structure from a multivariate perspective, we ran a principal coordinates analysis (PCoA) using GenAlEx v6.5 (Peakall & Smouse, 2012). Because it does not make any assumptions about the input data (e.g., Hardy–Weinberg Equilibrium), PCoA is well suited for genetic data (Jombart, Pontier, & Dufour, 2009). Furthermore, patterns revealed by multivariate analyses of genetic data are increasingly being used to further validate Bayesian clustering patterns (Basto et al., 2016). The PCoA analysis was conducted on the matrix of F_{ST}' values for the microsatellite data, and the three axes containing the most variation were retained. A three-dimensional plot was made in R using the 3D Scatter Plot package (R Core Team, 2016) to visualize the first three principal coordinates.

2.7 | Species distribution modeling

We constructed a species distribution model (SDM) using 53,145 Ruffed Grouse occurrences from the Global Biodiversity Information Facility (GBIF; http://data.gbif.org/). Observations from nonscientific institutions that were not reviewed or moderated were removed, and we further excluded any occurrences prior to 1980 to ensure accuracy of georeferencing. Environmental data were obtained from the WORLDCLIM dataset (v1.4, http://www.worldclim.org/) at a resolution of 2.5 min, and we used the 19 variables in the BIOCLIM layers (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) for the most recent time period (1960–1990). The MODIS-based Global Land Cover Climatology layer (Broxton et al., 2014) from the USGS Land Cover Institute (https://landcover.usgs.gov/) contains high-resolution data on global land cover types from 2001 to 2010. We added this to the SDM for more accuracy in predicting suitable habitat for Ruffed Grouse. The BIOCLIM and MODIS layers were clipped to the extent of North America and then projected in World Geodetic System 1984 using ArcMap v10.2 (ESRI®).

Data were prepared for ecological niche modeling using the SDMtoolbox v1.1c (Brown, 2014) for ArcGIS. All duplicate occurrence records were removed and rarified at a resolution of 30 km^2 to reduce sampling bias toward human settlements and roads; 2,421 occurrences were retained. Due to the similarity of some of the climatic variables used in the layers, we tested for layer autocorrelation

the Gaussian kernel density bias file to cr
appropriate settings were determined using
in ENMTools v1.3 (Warren, Glor, & Turelli,
Akaike's information criterion (AIC), area
Bayesian information criterion (BIC) value
follows: hinge features only, regularization
run type of 10 cross-validations, maximum
points = 10,000, 500 maximum iterations
gence threshold. For training the model,
points were used and the SDM displayed us

2.8 | Dispersal route analyses

To evaluate whether the intervening landsc
lation differentiation by influencing dispe
costs (i.e., IBR), we conducted least cost p
corridor (LCC) analyses using SDMtoolbox
ArcGIS v10.2 (ESRI®). The SDM was inve
layer, and coordinates for each sampling s
mal degrees. LCPs and LCCs were calculat
populations using the friction values. LCPs
persal paths of least resistance between ea
based on the resistance surface. To calcul
weighted by resistance values based on th
categorized using a "percentage of LCP" m
clusion into high-, mid-, and low-classes set
LCP value, respectively. The weighted and
then summed to create a LCC dispersal net

2.9 | Isolation by distance and re

We tested for IBD with a Mantel test in
Smouse, 2012) using pairwise F_{ST}' (Meirma
geographic distance. We calculated geograp
line between populations, where lines were
ries of the species' geographic distributio
conducted using least cost path distance
tance. To explicitly test for IBR, we used
Matrices of genetic distance (F_{ST}') and res
sessed for correlations using a paired Ma
GenAlEx v6.5 (Peakall & Smouse, 2012).
values was created from the LCC map by

each LCC by the resistance values along the corridor. After calculating the Mantel tests for all population pairs, the same tests were performed on subsets of populations to examine patterns at a regional scale (if the number of sampling sites permitted).

Because Mantel tests can be prone to Type I error (Legendre & Fortin, 2010; Legendre, Fortin, & Borcard, 2015), the matrices described above were also analyzed using distance-based Moran's eigenvector map analysis in the MEMGENE package (Galpern, Peres-Neto, Polfus, & Manseau, 2014) for R. This metho
tors in the spatial data (input as geographi
distance) using principal coordinates analysi
sis is used to select a reduced set of vectors b
as predictors of the response variable (gen
added to the model in a stepwise procedur
improvement of model fit. This method has
pared to other methods like the Mantel tes

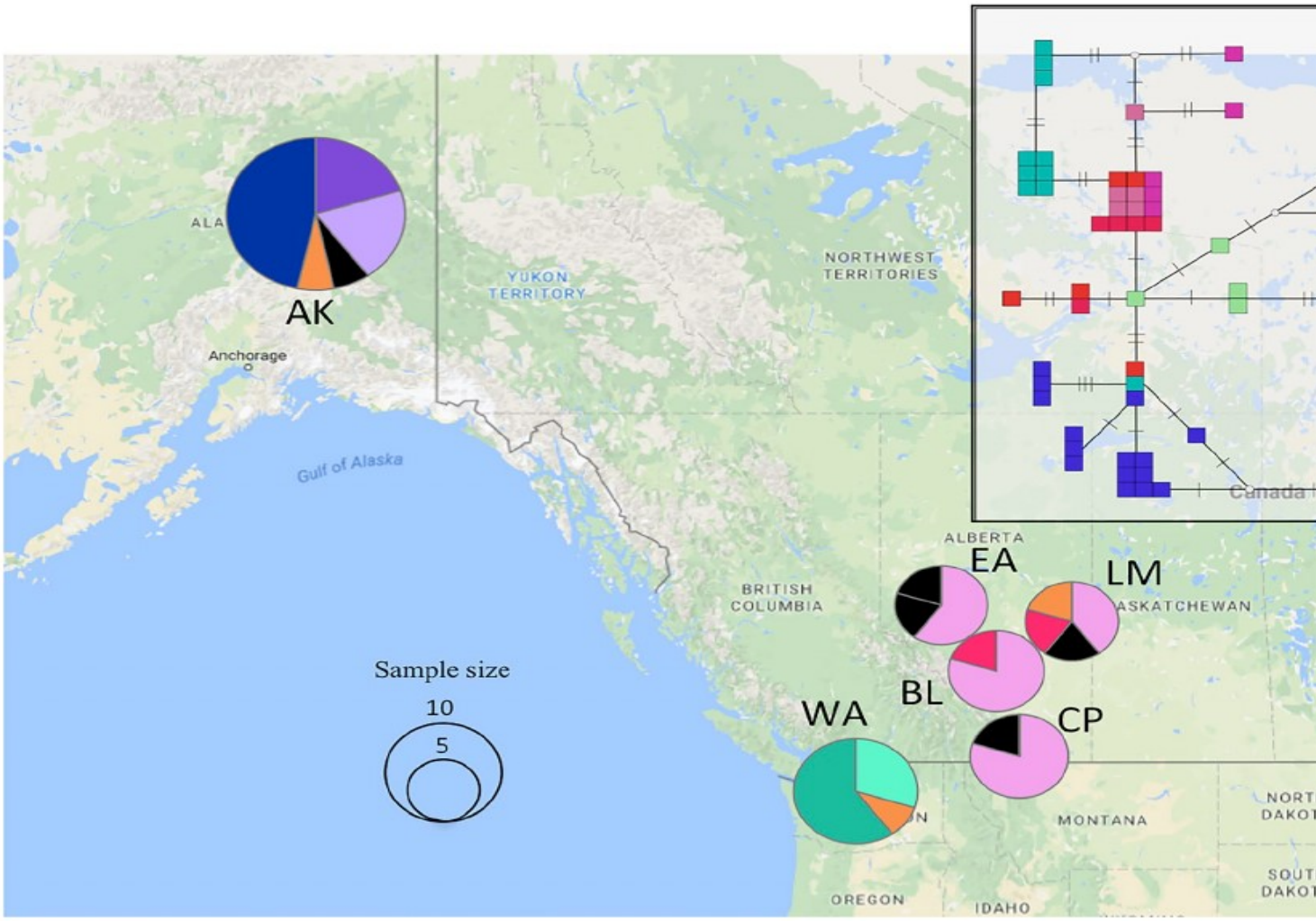


FIGURE 3 The statistical parsimony network from PopArt v1.7 (Leigh & Bryant, 2015) using control region sequ
inset. Each individual is a box, and individuals sharing haplotypes are grouped. On the lines that connect haplotypes
a line represents a mutational step, and nodes with inferred haplotypes are denoted by open circles. The geographi
haplotypes can be seen on the map. On the map, each haplotype is represented by a different color, singletons are c
charts are sized based on the number of samples (*n*)

TABLE 4 Significance values of Fisher's exact test (Fisher, 1922) for allele frequency pairwise populations comparisons of the biallelic Aldolase B SNP above the diagonal. Below the diagonal, pairwise F'_{ST} comparisons of data from seven microsatellites

	AK	YT	WA	CP	COA	BL	EA	GP	PR	AT	FM	BV
AK	•	**0.001**	**0.001**	**0.027**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.00**
YT	**0.392**	•	**0.001**	**0.025**	0.334	0.316	0.605	0.072	0.253	0.747	0.472	1.00
WA	**0.481**	**0.324**	•	**0.001**	0.060	**0.003**	**0.001**	0.147	0.331	**0.001**	**0.024**	**0.00**
CP	**0.340**	**0.179**	**0.210**	•	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.00**
COA	**0.378**	**0.319**	**0.198**	**0.094**	•	0.874	0.472	0.868	0.824	0.566	1.000	0.28
BL	**0.252**	**0.271**	**0.284**	**0.130**	0.033	•	0.472	0.389	0.635	0.586	1.000	0.16
EA	**0.335**	**0.251**	**0.239**	**0.127**	0.008	0.005	•	0.089	0.361	1.000	0.772	0.47
GP	**0.288**	**0.242**	**0.193**	**0.097**	−0.016	0.042	**0.027**	•	1.000	0.130	0.472	**0.02**
PR	**0.234**	**0.196**	**0.147**	0.017	−0.001	−0.083	−0.044	−0.028	•	0.472	0.824	0.17
AT	**0.335**	**0.266**	**0.268**	**0.124**	0.098	0.024	0.046	**0.081**	−0.014	•	0.874	0.51
FM	**0.375**	**0.233**	**0.296**	**0.208**	0.045	0.007	0.024	0.096	−0.019	0.022	•	0.40
BV	**0.336**	**0.266**	**0.200**	**0.110**	0.018	−0.013	0.010	**0.065**	−0.060	0.051	0.017	•
LM	**0.288**	**0.463**	**0.332**	**0.175**	0.027	0.008	0.058	0.065	−0.016	0.061	0.090	0.01
MN	**0.387**	**0.292**	**0.186**	**0.203**	**0.206**	**0.225**	**0.164**	**0.067**	0.019	**0.284**	**0.248**	**0.14**
WI	**0.526**	**0.333**	**0.405**	**0.200**	0.079	**0.180**	**0.092**	**0.042**	0.046	**0.235**	**0.174**	**0.14**

Notes. Values that were significant after False Discovery Rate correction for multiple testing are bolded.

MK604036) from seven populations showed 11 shared haplotypes and 11 singletons. Haplotype diversity (H_d) ranged from 0.400 (BL, CP) to 0.970 (MN), and π values from 0.00102 (CP) to 0.02300 (MN; Table 1).

Of the 19 microsatellite loci that successfully amplified, nine loci were monomorphic (LLST1, LLSD4, TTD1, TUD1, TUD4, TUT1, TUT3, ADL184, RHT0094) and 10 were polymorphic (LLSD7, TTD2, TTD6, TUT2, TUT4, SGCA5, BG15, BG18, BG20, and ADL230). TTD2 and ADL230 were removed due to significant deviations from Hardy–Weinberg equilibrium. For the eight remaining polymorphic loci, the number of alleles per locus ranged from 5 to 28. Observed

3.2 | Genetic structure

Pairwise Φ_{ST} values for the CR locus ran
CP:EA comparison to 0.660 for the AK:W
Furthermore, all 11 significant Φ_{ST} values
statistical parsimony network for CR ex
structure (Figure 3). The samples from th
ter together within the network, as do m
Washington. These two populations are t
are significantly different from other pop

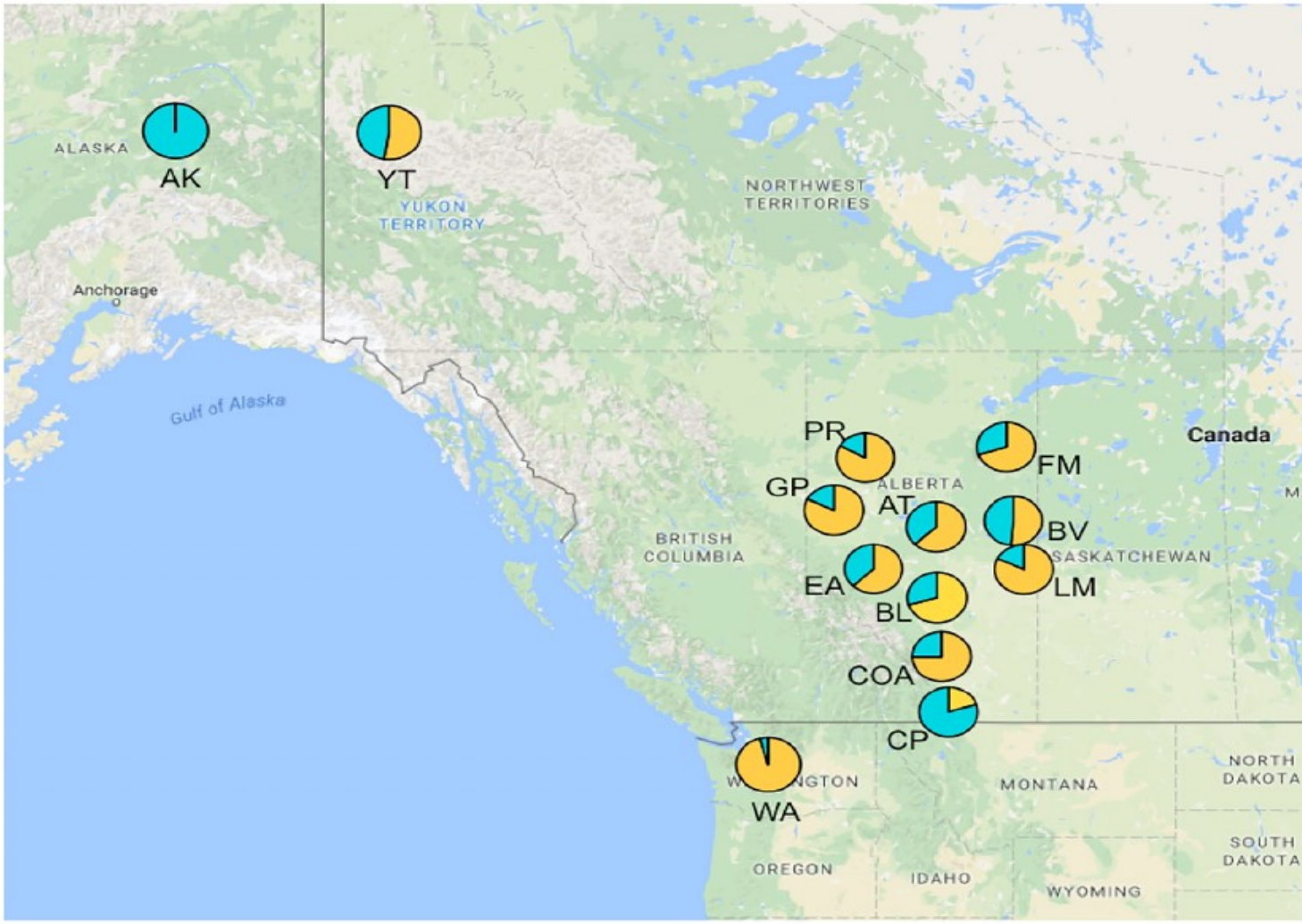


FIGURE 4 Allele frequencies of a SNP on the Z-linked Aldolase B gene for Ruffed Grouse from 15 populations. T
the proportions of the two possible alleles inferred from the screening data at each population. Pairwise comparison
testing for significant differences among the populations can be seen in Table 4

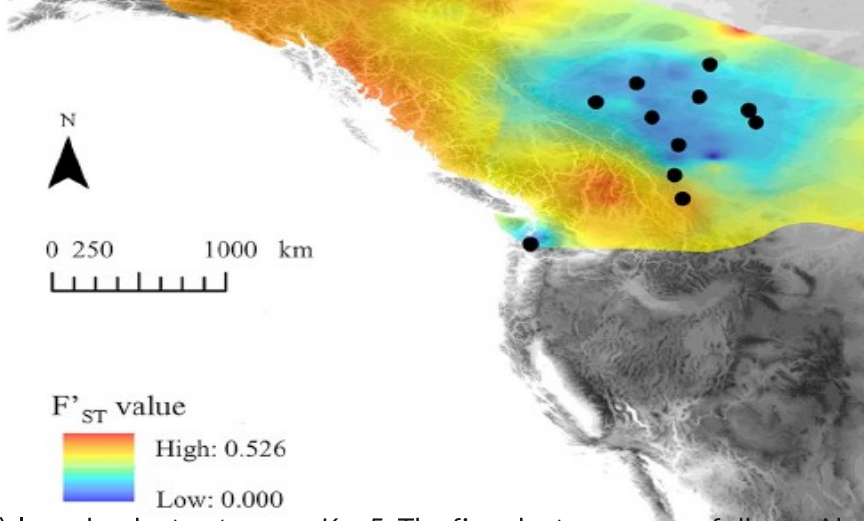


FIGURE 5 The species divergence map made using the Landscape Genetics toolbox (Vandergast, Perry, Lugo, & Hathaway, 2011) in ArcGIS®. Pairwise F'_{ST} values (Table 4) were color-coded and interpolated across a geographic map of the sampling sites. Colors that are yellow or warmer are statistically significant F'_{ST} values

values. Samples from Alberta (CP, BL, EA, LM) loosely cluster together on the network, but also share haplotypes with other populations (Figure 3). The Minnesota (MN) samples also show a slight geographic pattern with two clusters, but the most noticeable characteristic of this population is the large diversity of haplotypes present (H_d = 0.97; Figure 3).

The Fisher's exact tests performed on the Aldolase B SNP resulted in statistically significant comparisons for all population pairs including AK or CP (Table 4; Figure 4). Comparisons between WA and other populations were statistically significant for all but four pairs (COA, GP, PR, and WI). Of all other remaining population comparisons, only three were significant; EA:WI, BV:GP, and BV:WI (Table 4; Figure 4). Like the control region locus, the Aldolase B SNP reveals divergence of the Washington population (Table 4; Figure 4).

Pairwise F'_{ST} values of microsatellite loci ranged from −0.083 (BL:PR) to 0.526 (AK:WI; Table 4). After FDR corrections, 67 out of 105 comparisons were significant. Three populations (AK, YT, and WA) were significantly differentiated from all other populations, while CP was significantly differentiated from all but PR, and MN was differentiated from all but WI and PR. WI was differentiated from all but three populations (MN, PR, and COA). The population divergence map displaying the interpolated pairwise F'_{ST} values clearly shows the low differentiation among all northern and central Alberta populations, and differentiation of the AK, YT, and WA populations (Figure 5).

3.3 | Bayesian clustering analyses

Plots of delta K (ΔK) and mean log likelihood (LnPr($X|K$); Supporting Information Figure S1) from the initial STRUCTURE analyses showed K = 5. The five clusters were as follows: Alas the Great Lakes, Crowsnest Pass, and all r (Figure 6a). To investigate additional popu lyzed each cluster independently. The AK dence of additional structure (K = 2; Figure cluster in two creates a total of six cluste analyses which is concordant with the pairw

Both DIC and log likelihood plots of analysis performed in TESS showed the mo ters as K = 4 with potential substructure Figure S2). The DIC plot was bimodal with (Supporting Information Figure S2a); howe Q plots for K = 7 showed clear oversplittin fore concluded that once hierarchical analy veal substructure, the true number of cluste Information Figure S3), which is concordan and F'_{ST} results.

The GENELAND analysis showed evid the highest frequency over the MCMC ch ment with the highest value for the avera ties of the initial set of runs. Five of the se by GENELAND corroborated the clusters and TESS: AK, YT, WA, CP and Great Lak five, GENELAND split GP from the remaini (Figure 6c; Supporting Information Figure S

3.4 | Principal coordinates analys

The PCoA using F'_{ST} values showed dist with the first and second axes accountin of the variation, respectively, and the thi

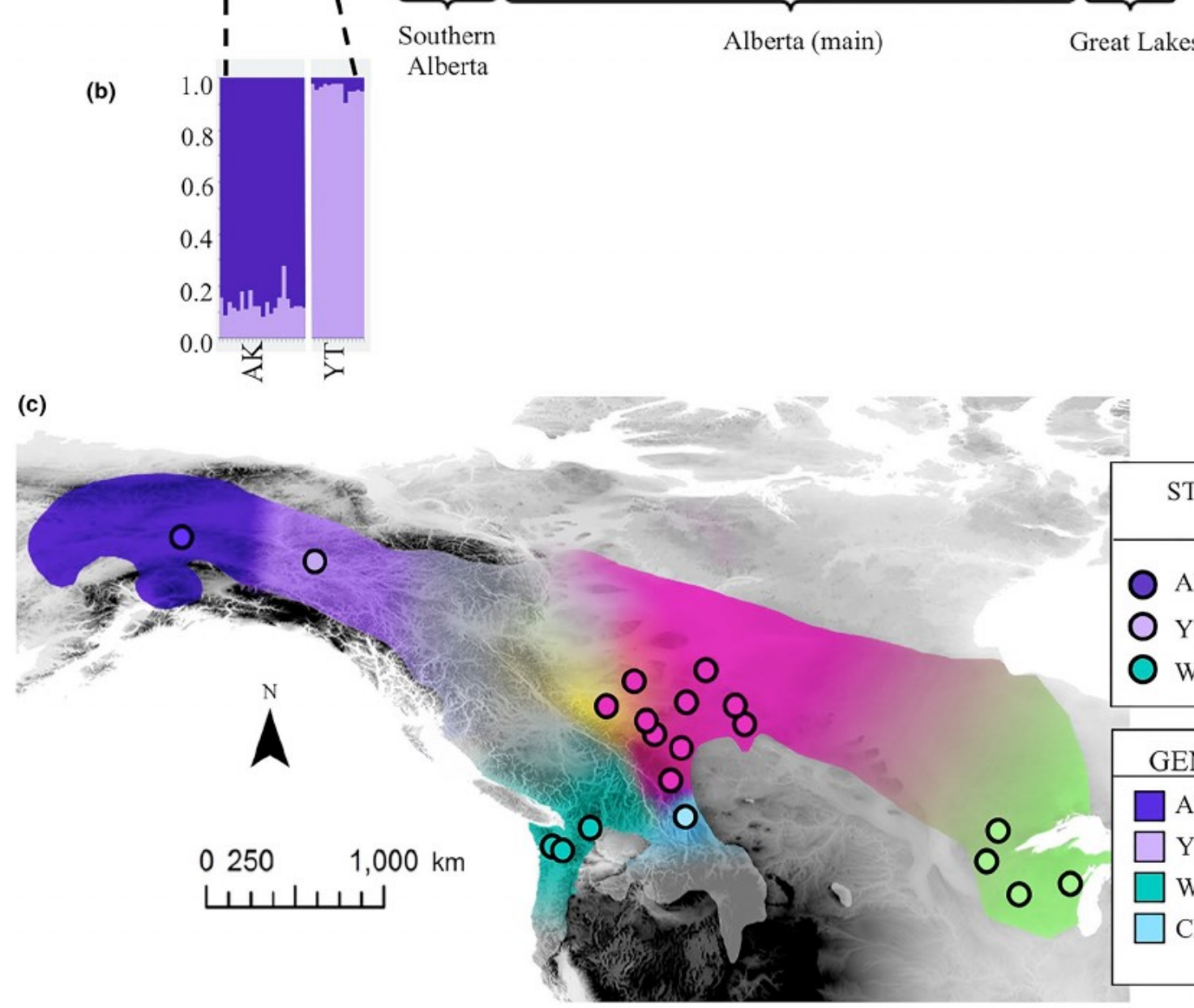


FIGURE 6 Ruffed Grouse population structure as inferred by hierarchical runs in STRUCTURE v2.3.4 (Pritchard microsatellite loci. At (a) *K* = 5, and further substructure was apparent when samples from Alaska and Yukon were a which resulted in (b) *K* = 2. No further substructure was found when the remaining clusters were analyzed indepen multiple Bayesian clustering programs where GENELAND clusters have been color-coded, mapped in geographic sp limits of the species' range, with the gradient of colors representing clines created by the contour lines of the poste in GENELAND (Supporting Information Figure S3). Circles represent sampling sites, and circle color corresponds to TESS consensus cluster assignments. There was only one instance of discordance between the programs: additiona GENELAND

of the variation. When all three axes are examined together as a three-dimensional plot, it is clear that AK, YT, WA, CP, MN, and WI are separated from all other populations (Figure 7). The majority of the Alberta sampling sites (COA, BL, EA, AT, FM, BV, LM) cluster together as they do in all other analyses, and the GP and PR sites clustered together. Although GP and PR were separated from other Alberta sampling sites, they w imity to these Alberta sites than to the groupings of the PCoA confirm grouping STRUCTURE and provide evidence of G from the rest of Alberta as indicated by (Figure 6b).

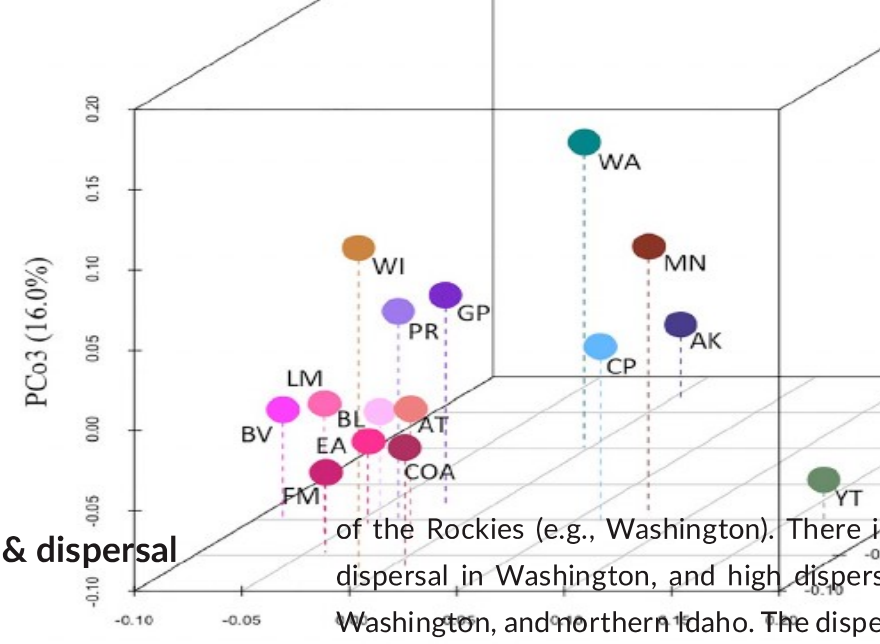


FIGURE 7 A three-dimensional plot of the first three axes of the PCoA as calculated in GenAlEx v6.5 (Peakall & Smouse, 2012). Populations are labeled, and principal components are labeled on their respective axes including the amount of variation captured by each in R (R Core Team 2016)

3.5 | Species distribution modeling & dispersal route analyses

The most suitable SDM identified by ENMTools v1.3 (Warren et al., 2010) performed significantly better than expected at random with an AUC of 0.799, where 0.5 is when the fit of the model is no better than random, and as values get closer to one, the model approaches a perfect fit. The SDM approximately matches the species' known distribution (Figure 2) indicating that the environmental variables used to build the model were sufficient to accurately reflect the species' habitat preferences (Figure 8a). The layers that contributed most to the model were land cover, annual mean temperature, and isothermality at 36.1%, 22.2%, and 21.9%, respectively.

When the dispersal routes are examined across the LCC corridor map, it is clear that some population pairs appear to have one or more low-resistance dispersal routes between them, while for others, the only route revealed by the analysis has relatively high resistance. This pattern could change with the addition of sampling sites in the intervening areas (Figure 8b; Supporting Information Figure S5). The LCC revealed high niche connectivity among most of the Alberta populations, particularly those in the center of the province, and a dispersal route with low-resistance stretching across the parkland between eastern Alberta and the Great Lakes area (Figure 8b). The LCC (Figure 8b) implies high-elevation mountains may act as barriers to Ruffed Grouse dispersal. There is low niche suitability in much of the high-elevation mountains (Figure 8a), with one corridor through the Intermountain West, and another along the Peace River valley, which is the only river valley to penetrate the entire width of the Rocky Mountains (Figure 8b; Cannings, Nelson, & Cannings, 2011). The corridor through the Intermountain West appears to provide connectivity between south-central Alberta and populations west of the Rockies (e.g., Washington). There i
dispersal in Washington, and high dispers
Washington, and northern Idaho. The dispe
Yukon to populations south of it has mode
passes between the Rocky and Coast Mou
with the corridor through the Peace River v

3.6 | Isolation by distance

The results of the Mantel test for IBD sh
lation between Euclidean distance and p
all sampling sites were compared (R^2 = 0
Figure 9). MEMGENE results were similar
test, although a lower proportion of geneti
by Euclidean distance = 0.211; p = 0.
sets of data were tested for IBD using Ma
sis containing populations east of the Ro
Lakes clusters) provided evidence that geo
nificant predictor of pairwise genetic dista
Table 5). Analysis including only western p
YT) yielded a significant correlation (R^2 =
the analysis of the Alberta, Alaska, and Yu
p = 0.002). The only nonsignificant compar
CP and remaining Alberta sampling sites (
the analysis of sites within the Alberta clus
AT, FM, BV, LM; R^2 = 0.082, p = 0.063).

3.7 | Isolation by resistance

When a Mantel test was performed to
tween LCP distance and genetic distance

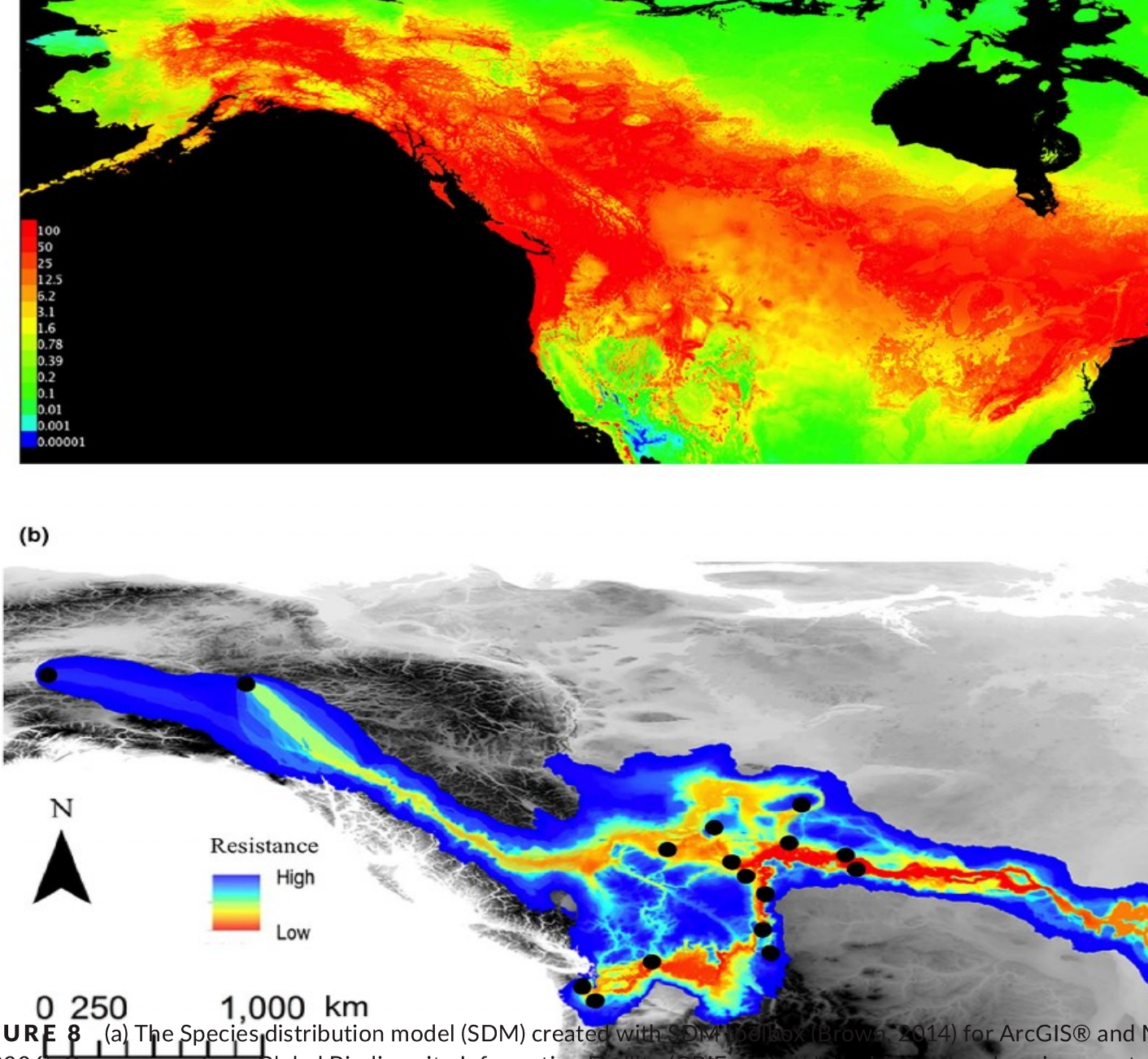


FIGURE 8 (a) The Species distribution model (SDM) created with SDM Toolbox (Brown, 2014) for ArcGIS® and M al., 2006). Occurrences from Global Biodiversity Information Facility (GBIF; http://data.gbif.org/) and environmenta vegetation data) were input into the model. The SDM shows areas where the environmental conditions are suitable occur (i.e., ecological niche). The scale depicted is cumulative and represents the percent likelihood of habitat suitab based on the model variables. Using a resistance layer created from inverse of the SDM, (b) least cost corridors (LCC the 15 sampled populations of Ruffed Grouse. The LCC provides more information than least cost paths (Supportin S4) and shows the most likely dispersal routes among populations as corridors instead of paths. It also provides disp corridors coded by color; red representing areas where there is low resistance (i.e., low dispersal cost), and blue rep resistance

considerably higher (R^2= 0.649, p = 0.01; Table 5) than the value calculated for IBD (R^2 = 0.370, p = 0.01). The correlation between resistance distance and genetic distance was higher yet (R^2 = 0.674, p = 0.001). MEMGENE analysis using least cost distances provided an adjusted R^2 value (R_{adj^2} = 0.189; p = 0.01; Table 5) similar to that of the IBD analysis. However, using resistance distances, both the MEMEGENE and Mantel tests were simila siderably more of the variation in the ge two distance measures (R_{adj^2} = 0.487; p = Mantel tests were performed on subsets model explained more of the variation in the IBD or LCP models for all subsets (Tab

Alberta, Alaska, and Yukon (COA, BL, EA, GP, PR, AT, FM, BV, LM, AK, YT)	R^2 = 0.806 p = 0.002	R^2 p
Alberta, S. Alberta, and Washington (COA, BL, EA, GP, PR, AT, FM, BV, LM, CP, WA)	R^2 = 0.575 p = 0.001	R^2 p
Alberta and Washington (COA, BL, EA, GP, PR, AT, FM, BV, LM, WA)	R^2 = 0.594 p = 0.010	R^2 p
Alberta and S. Alberta (COA, BL, EA, GP, PR, AT, FM, BV, LM, CP)	R^2 = 0.190 p = 0.090	R^2 p
Alberta (within cluster comparison) (COA, BL, EA, GP, PR, AT, FM, BV, LM)	R^2 = 0.082 p = 0.063	R^2 p
Distance-based Moran's Eigenvector Map analysis		
Overall	R_{adj^2} = 0.211 p = 0.020	R_{adj} p

Notes. To examine multiple spatial scales, an overall correlation was run
Mantel tests and dbMEM), as well as subsets of the different sampling sit
correlation value for each comparison is reported (R^2 or adjusted R^2), a
level of each test.

did not yield a significant correlation were the Alberta populations (excluding CP). No other groups had enough sampling sites to perform within region comparisons.

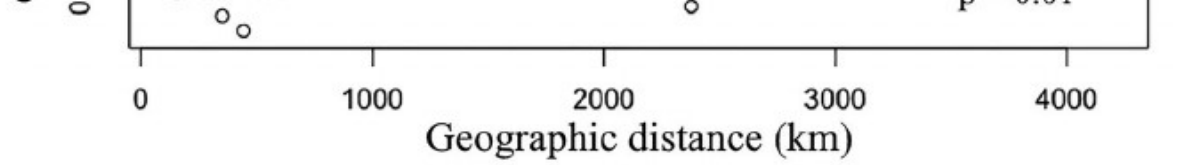
4 | DISCUSSION

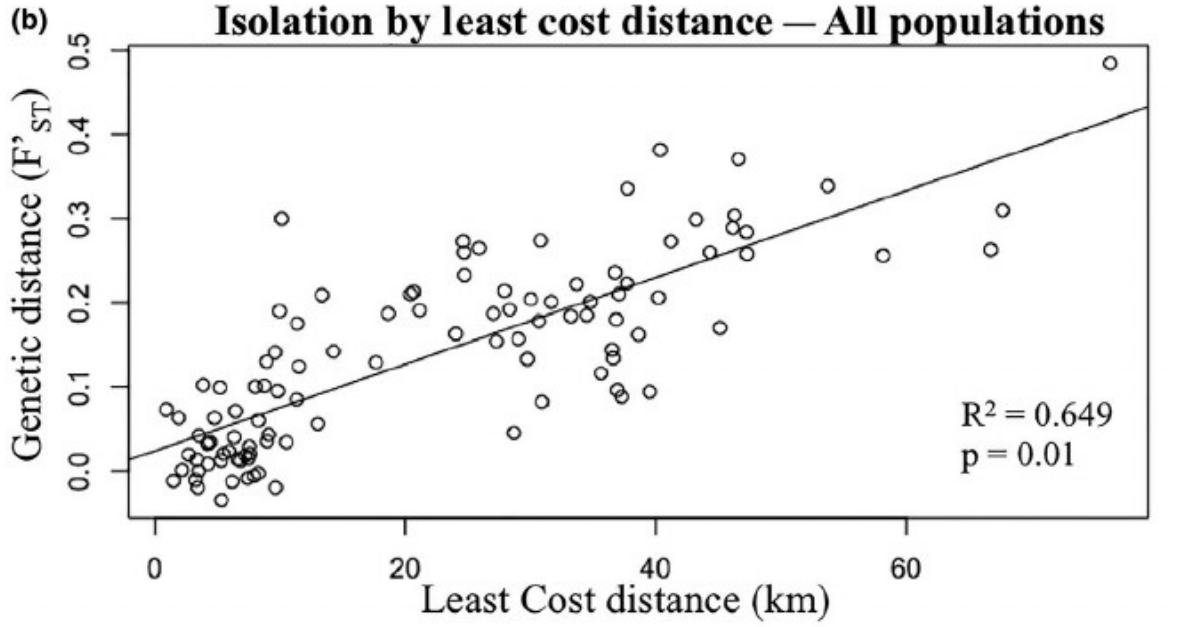
Using multi-locus genetic data and environmental variables, we found significant genetic differentiation and limited connectivity among western populations of Ruffed Grouse. Macrogeographic barriers, tracts of unsuitable habitat, and the species' preference for aspen-dominated mixed forest are likely playing important roles in creating genetically structured populations.

4.1 | Contemporary population genetic structure and macrogeographic barriers

Data from multiple neutral genetic markers show structuring of Ruffed Grouse populations across their range at multiple spatial scales. Aside from the most highly differentiated populations, AK and WA, at least four other distinct genetic groups exist: Yukon, southwest Alberta (CP), a large group including most of central/northern Alberta, and one group near the
Although these results support our hypotl
tiation due to low dispersal ability, the de
somewhat unexpected.

A number of landscape features co-occ
genetic clusters for Ruffed Grouse across
The Columbia River basin (southeast of W
tent of the Great Plains (southeast of Albe
Great Lakes) impose sharp limits on the
and mountain ranges appear to be a pron
western extent of the range. The Alaska, W
Mountains effectively isolate the Alaska p
by divergence of this population at the mi
SNP, and control region. In addition, the mit
shows very little haplotype sharing with a
sites (Figure 3). The Yukon population is isc
tain ranges preventing connectivity with th
by the Mackenzie Range potentially restr
populations to the east. Mountains also c
restricted populations in other parts of the
present between the Washington populati
lations suggesting restricted movement acr





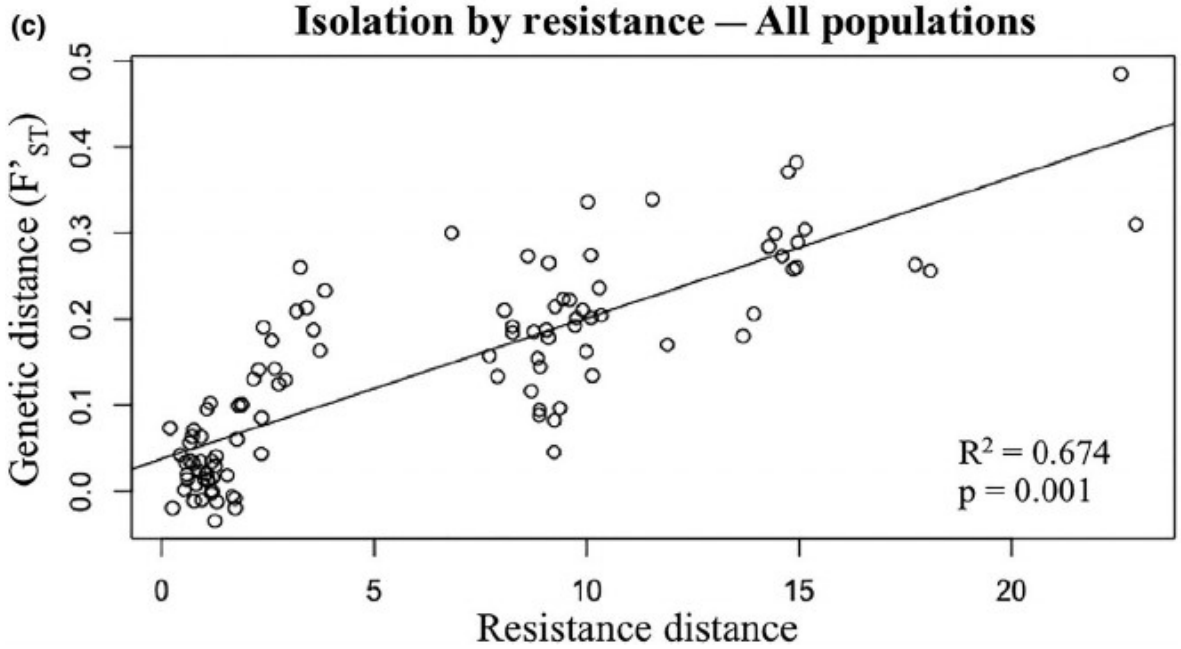


FIGURE 9 Plo
tests of (a) isolati
comparing geneti
by F'_{ST} and geogra
populations, (b) is
distance using lea
and (c) isolation b
resistance-weigh
from the LCC ma
and *p*-values of e
for each respecti
GenAlEx v6.5 (Pe
Plots shown are
15 sampled popu

The Washington population is genetically distinct, as supported by the microsatellite data (Table 4), Z-linked marker data (Table 4), and minimal haplotype sharing in the control region (Figure 3). If the Cascade Range is acting as a barrier, substructure should have been detected within the WA cluster through Bayesian analyses because the sampling sites were on both sides of the Cascades. Bayesian methods do not use a priori population assignments, so any potential substructure should be apparent in the an samples were grouped (Figure 6a,b; Suppo S3 and S4). The Cascade Range contains est habitat (Broxton et al., 2014; Pater, B Chapell, 1998), and generally lower elevati ern Rockies (Franklin & Dyrness, 1973). Sin tiation occur in a widespread generalist pa

CP does not show significant differentiation at the control region (Figure 3), it is unlikely that divergence of this population reflects historical isolation. Instead, the differentiation of the CP population likely arose due to contemporary barriers to gene flow. In some species, southwest Alberta populations are divergent from individuals sampled throughout the rest of Alberta, and instead group with either British Columbia populations (Adams, & Burg, 2015a) or Intermountain West (i.e., Montana, Idaho, Wyoming) populations (Dohms, Graham, & Burg, 2017; Pulgarín-R & Burg, 2012) implying that the geography of the Rocky Mountains may affect the genetic structure of species differently depending on their life history.

The presence of unsuitable habitat may also be restricting gene flow, particularly for the CP population. Only a narrow swath of suitable Ruffed Grouse habitat presently connects southwestern Alberta and the rest of the province; most of the southeast part of the province is open grassland, which this species is reluctant to disperse through (Yoder, 2004), and the Rocky Mountains run along the western edge of the province. The habitat in the Rocky Mountains consists mainly of contiguous coniferous forest, with suitable mixed forest habitat occurring mostly on low elevation slopes and valleys (Broxton et al., 2014; Natural Regions Committee, 2006). Although Ruffed Grouse are more likely to disperse through coniferous forests than grasslands, their short dispersal distances (approx. 2–4 km; Yoder, 2004) suggest that dispersal through vast expanses of coniferous forest are likely to be infrequent. Because the CP population is in close spatial proximity to some of the other populations sampled in Alberta, geographic distance is unlikely to be a causal factor for population differentiation and this is corroborated by IBD analysis (Table 5). Therefore, the combination of the Rocky Mountains as a physical barrier, as well as the northwest corner of the Great Plains (where they meet the Rocky Mountains) are likely the main factors isolating the CP population. However, this assertion would be strengthened by the addition of sampling locations west of the Rockies, such as sites in Montana, Idaho, and British Columbia.

While there is not complete consensus across our analyses for the GP cluster, there is certainly evidence of differentiation of this population, which could be due to its proximity to the Peace River valley. The Peace River is the only river to cut a continuous valley through the entire width of the Rocky Mountain range (Feinstein, 2010). It is possible that genotype frequencies at GP are subject to an influx of genes from British Columbia through

Toews, Brelsford, & Irwin, 2011). Furthern
a mountain barrier may range from porou
impermeable (Irwin, Irwin, & Smith, 2011
cies. Sampling of Ruffed Grouse in British
further test the extent to which the nor
are a permeable barrier.

4.2 | Landscape genetics: isolati
resistance?

Aside from mountain ranges, the presenc
the most prevalent potential barrier betw
Due to the seemingly high degree of habi
the landscape, we incorporated environm
analyses to test their effects on the gen
and to help further test the presence of p
ers discussed previously. LCC analysis rev
varies across the landscape and confirms o
mountain ranges are likely to impede dispe
Mountain ranges in Alaska, as well as the
high levels of resistance to Ruffed Grouse d
tion of two corridors through the mountain
River valley, and one through the Intermo
south-central Alberta and northeast Wash
corridors are lower elevation areas with sl
a higher proportion of mixed forest than th
slopes (Broxton et al., 2014; Hijmans et al.,
sal routes could be identified among Yukon
populations, and the only dispersal route
other sampled populations has high resista
be noted that the lack of sampling from wit
have prevented the identification of dispe
population. That said, the LCCs occurred m
of mesic, mixed forest, which implies that
forest type across the landscape may be im
lation structuring.

Across all populations, IBR explained sig
netic differentiation than IBD at most spatia
exception being the comparisons within Al
Alberta and the Great Lakes had low resi
the parkland/boreal forest in a direct path (
of the landscape between these two regio

moderate to high resistance. The patterns lend support to the idea that the higher heterogeneity of habitat types west of the Rocky Mountains are restricting dispersal, but sampling of BC populations is required to rule out the effects of small sample sizes.

Due to large gaps among our sampling sites outside of Alberta, it is difficult to be certain whether genetic boundaries between populations are gradual genetic clines or genetic breaks. IBD manifests as smooth, clinal gradients between genetic clusters (Mims et al., 2016), whereas distinct boundaries among genetic clusters are more likely to occur in populations mediated by IBR (Coulon et al., 2006). The high pairwise F'_{ST} values and relatively steep genetic cluster boundaries (Table 4; Figure 5; Supporting Information Figure S3) provide further evidence that Ruffed Grouse populations are distinct genetic clusters mediated by IBR. Furthermore, the patterns of IBR among Alberta populations and those west of the Rockies point to the Rocky Mountain range as a barrier. The conifer-dominated habitat characterizing high-elevation mountains has high dispersal costs and would explain patterns of reduced connectivity in Ruffed Grouse found in the SDM and LCC (Figure 8a,b).

Although IBR performed consistently better than IBD, both of these models only explained a small to moderate proportion of the genetic differentiation between CP and adjacent populations (Table 5). CP might therefore have additional factors affecting connectivity with other Ruffed Grouse populations. The environmental variables used in the LCC analysis explained much of the differentiation present among the populations sampled for this study (Table 5), but it is possible that additional, unsampled environmental factors are contributing to the genetic break at the CP population. In some species, genetic structure may not be evident at a broad spatial scale and is only detected when populations are assessed at a finer scale (Adams, & Burg, 2015a). Sampling in other areas such as British Columbia, Saskatchewan, and Manitoba could also provide additional insight into Ruffed Grouse population structure. Therefore, a smaller scale analysis with more environmental variables and higher resolution sampling may aid in identifying the factors underlying the genetic differentiation of the CP population. Furthermore, differences in the vegetation communities of the Intermountain West (including southwestern Alberta) compared to the boreal region (including central-northern Alberta) may be important. The shift in *Populus* species (e.g., *P. trichocarpa*, *P. angustifolia*, *P. balsamifera*) between these regions is an example of how the environmental conditions in these areas differ and may be contributing to genetic

strongest genetic breaks co-occurring with
ranges and habitat. Resistance modeling
ture in Ruffed Grouse is primarily influenc
habitat mosaic of the western North Amer
routes appear to be restricted to areas w
supporting our hypothesis of IBR in Ruffed
spread and relatively continuous distributi
represents one of relatively few contemp
studies focusing on broadly distributed or
fewer revealing patterns of IBR in widesp
distributed organisms that may be expec
Finnegan, Manseau, & Wilson, 2010; Peas
2006). As such, we should no longer assur
and distribution are the only factors drivi
that IBR is only likely to affect species at
the growing body of work highlighting the
the role of environmental variables in pop
ing (McRae & Beier, 2007; Vergara et al., 2
the need for more landscape genetic stu
distributed taxa because they may be expe
regardless of their relatively ubiquitous dis

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CONFLICT OF INTEREST

None declared.

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