

ORIGINAL ARTICLE

Influence of landscape features on the microgeographic genetic structure of a resident songbird

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Landscape features influence individual dispersal and as a result can affect both gene flow and genetic variation within and between populations. The landscape of British Columbia, Canada, is already highly heterogeneous because of natural ecological and geological transitions, but disturbance from human-mediated processes has further fragmented continuous habitat, particularly in the central plateau region. In this study, we evaluated the effects of landscape heterogeneity on the genetic structure of a common resident songbird, the black-capped chickadee (*Poecile atricapillus*). Previous work revealed significant population structuring in British Columbia that could not be explained by physical barriers, so our aim was to assess the pattern of genetic structure at a microgeographic scale and determine the effect of different landscape features on genetic differentiation. A total of 399 individuals from 15 populations were genotyped for fourteen microsatellite loci revealing significant population structuring in this species. Individual- and population-based analyses revealed as many as nine genetic clusters with isolation in the north, the central plateau and the south. Moreover, a mixed modelling approach that accounted for non-independence of pairwise distance values revealed a significant effect of land cover and elevation resistance on genetic differentiation. These results suggest that barriers in the landscape influence dispersal which has led to the unexpectedly high levels of population isolation. Our study demonstrates the importance of incorporating landscape features when interpreting patterns of population differentiation. Despite taking a microgeographic approach, our results have opened up additional questions concerning the processes influencing dispersal and gene flow at the local scale.

Heredity (2016) **117**, 63–72; doi:10.1038/hdy.2016.12; published online 24 February 2016

INTRODUCTION

Dispersal and gene flow are crucial for maintaining population connectivity and species persistence while also preventing population differentiation and species divergence. The heterogeneity and patchiness of landscapes can influence the ability of an individual to disperse between populations. If dispersal is restricted by barriers in the landscape, the resulting decrease in population connectivity can lead to discrete, isolated groups. Over time, these isolated groups may experience reduced genetic diversity and become genetically distinct (Baguette and Van Dyck, 2007). Landscape genetics offers new approaches to explicitly test the influence of landscape elements on genetic structure to identify barriers corresponding to structured populations (Manel *et al.*, 2003; Holderegger and Wagner, 2008; Sork and Waits, 2010; Manel and Holderegger, 2013).

Large physical structures (for example, mountain ranges and large water bodies) as well as stretches of unsuitable habitat are obvious barriers to dispersal and subsequent gene flow. The influence of barriers can vary within and among species, and hence it is important to be able to identify the specific factors influencing genetic differentiation of target groups before implementing management strategies (With *et al.*, 1997). For example, using a landscape genetics approach, Frantz *et al.* (2012) found that motorways influenced genetic structuring in red deer (*Cervus elaphus*), but not wild boars (*Sus scrofa*); as a result, considering fragmentation effects of

motorways would be primarily targeted at conservation efforts on only the former species. The effects of landscape features can also vary across a species range, as in the ornate dragon lizard (*Ctenophorus ornatus*), where land clearing was associated with genetic differentiation in one area, but not another (Levy *et al.*, 2012). Smaller, less conspicuous structures or environmental variables, such as microclimate, may also influence gene flow. Through landscape genetics, effects of multiple factors on contemporary patterns of genetic structure can be examined across different spatial scales and across species with varying dispersal capabilities, allowing us to better understand how organisms interact with their environment, and how they may respond to future environmental change.

In current landscapes, habitat fragmentation from natural and human-mediated processes can influence the potential for animals to disperse and thus affect the spatial distribution of genetic variation at both large and small geographical scales. Contemporary factors such as insect outbreaks (for example, mountain pine beetle *Dendroctonus ponderosae*) and habitat degradation (for example, forestry operations, agricultural conversion) have reduced population connectivity by removing suitable breeding/dispersal habitat (Martin *et al.*, 2006). For instance, a combination of the already-restricted range of the northern spotted owl (*Strix occidentalis caurina*) in the Pacific North West coupled with the removal of dense, late successional forest has

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Received 22 July 2015; revised 16 January 2016; accepted 18 January 2016; published online 24 February 2016

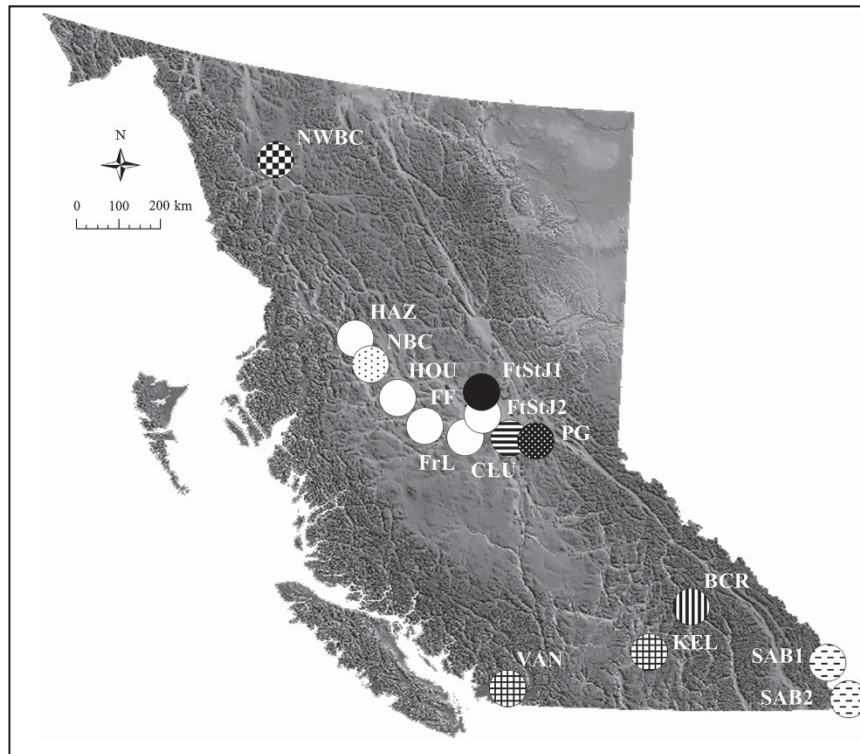


Figure 1 Sampling locations of the black-capped chickadee (*Poecile atricapillus*) in British Columbia (see Table 1 for abbreviations) with inferred clusters from GENELAND ($K=9$) denoted by the patterned circles (and colours in the online version). The nine genetic clusters are (1) NWBC, (2) NBC, (3) FtStJ1, (4) PG, (5) CLU, (6) HAZ, HOU, FF, FrL and FtStJ2, (7) BCR, (8) VAN and KEL and (9) SAB1 and SAB2.

left the species federally threatened (Blackburn and Godwin, 2003; Yezerinac and Moola, 2006; COSEWIC, 2008).

British Columbia (Canada) has a complex climatic and vegetation history following the Last Glacial Maximum (26.5–19 thousand years ago). When this is combined with broad-scale climatic gradients (that is, moisture, temperature and topography; Meidinger and Pojar, 1991) in the province, the result is major regional transitions that create rich and heterogeneous landscapes (Gavin and Hu, 2013; Figure 1). The province contains 6 ecozones and 14 biogeoclimatic zones (see Figure 10 in Meidinger and Pojar, 1991). A major longitudinal moisture gradient formed by the Coastal Mountains is characterised by dominant maritime moist conifer forest in the west, transitioning to sagebush steppe in the rain shadow of the south central interior, to mixed conifer and pine forest in the east. The interior regions are further influenced by a latitudinal gradient with increasing summer moisture from south to north. This results in desert steppe in the south transitioning to subboreal and boreal spruce forest in the north. This natural heterogeneity is further increased by high levels of habitat fragmentation resulting from current forestry and agricultural practices occurring within the province.

To determine how these natural and anthropogenic factors influence population structure, we conducted a microgeographic landscape genetic assessment of a common resident songbird, the black-capped chickadee (*Poecile atricapillus*) in British Columbia. Our previous work identified population genetic structuring in central British Columbia, but the sampling regime and range-wide scale of the study meant that smaller geographical barriers were less noticeable (Adams and Burg, 2015). Here, a fine-scale transect sampling approach allowed for a more detailed examination of the landscape patterns and processes influencing population genetic structuring and a larger number of

microsatellite markers were used to better capture the spatial distribution of genetic variation (Runde and Capen, 1987; Selkoe and Toonen, 2006). The study area comprises a number of different habitats and environmental conditions, so studying genetic variability in a nonmigratory species with limited dispersal potential will allow us to investigate the role of habitat heterogeneity on the ecology and evolution of populations. The aims of the study were to identify where the genetic breaks occur and to evaluate the processes driving differentiation. This led to three main hypotheses: (1) fine-scale population genetic differentiation will be evident in the black-capped chickadee due to the inclusion of additional sampled populations and microsatellite loci; (2) given the level of topographical and climatic variability found within the province, dispersal and gene flow are influenced by landscape features and environmental variables; and (3) habitat fragmentation resulting from anthropogenic disturbance (for example, forestry and agriculture) isolates populations in central and southern British Columbia.

MATERIALS AND METHODS

Study species

The black-capped chickadee is a resident songbird, common throughout most of North America with a range that covers a large and complex geographical area. Black-capped chickadees are an important study species because they are generalists and thrive in a variety of environmental conditions, although they prefer mixed deciduous and coniferous woodland (Smith, 1993). If specific landscape processes are found to have a negative impact on chickadees, this would indicate that other species (particularly specialists) may also be affected. As primary cavity nesters, chickadees are dependent on advanced decaying trees or snags in mature forests. Their diet requirements also vary seasonally with a preference for mixed berries, seeds and insects in the winter in comparison with a completely insectivorous diet during the breeding season (Runde and Capen, 1987). Although chickadees do reside and breed in disturbed areas, studies have

found these low-quality habitats negatively affect reproduction (Fort and Otter, 2004a), territoriality (Fort and Otter, 2004b), song output (van Oort *et al.*, 2006), song consistency and perception (Grava *et al.*, 2013a) and song structure (Grava *et al.*, 2013b) in this species. Elevation and the presence of other chickadee species (for example, mountain chickadees) can also influence their distribution and habitat preference (Campbell *et al.*, 1997). Collectively, this information highlights the importance of a number of factors related to habitat quality (for example, mature, dense woodland) on species persistence.

Sample collection

We included samples from seven populations collected as part of our previous study (that is, FtStJ1, PG, NWBC, NBC, BCR, SAB1 and SAB2; Table 1; Adams and Burg, 2015). We collected additional samples during the 2012 breeding season using a transect-based approach along HWY 16, the main east–west corridor in north-central British Columbia. Birds were captured using mist nets and call playback, and samples of blood (<100 µl from the brachial vein) and/or feathers were obtained from each individual. This resulted in ~20 individual birds sampled from each of an additional 6 locations (that is, HAZ, HOU, FF, FrL, CLU and FtStJ2; Table 1). Where possible, sampling sites were confined to a 10 km radius. Feather samples were also obtained from two more populations: Vancouver (VAN) and Kelowna (KEL). With all individuals combined, sampling took place over ten breeding seasons (2003–2010, 2012 and 2013) and a total of 405 individuals from 15 populations were collected (Figure 1, Table 1 and Supplementary Table S1). Each bird was banded with a numbered metal band to prevent resampling and all blood/ feather samples were stored in 95% ethanol and, on return to the laboratory, stored at –80 °C.

DNA extraction and microsatellite genotyping

DNA was extracted from blood ethanol mix (10 µl) or feather samples using a modified Chelex protocol (Walsh *et al.*, 1991). Each individual was genotyped for 14 polymorphic microsatellite loci (Supplementary Table S2) and DNA was amplified for all loci (including new loci Pij02, VeCr05 and CTC101) using the same two-step annealing PCR conditions outlined in Adams and Burg (2015); the exception was for Pij02, where the two-step annealing temperatures were adjusted from 50 and 52 °C to 52 and 54 °C. All procedures following DNA amplification were conducted as in Adams and Burg (2015).

Most individuals were successfully genotyped for all 14 variable microsatellite loci. Seven populations were missing genotypes for locus PmanTAGAn45, four populations for Ppi2, two populations for Titgata02 and two populations for Pij02. All analyses were carried out with and without these four loci to determine whether missing data influenced levels of observed population differentiation. In addition, we conducted analyses with and without the feather-sampled populations (KEL and VAN) as the DNA extracted from

feathers were of lower quality that resulted in missing data and created the potential for genotyping errors from low amplification success for some loci.

Genetic analyses

Genetic diversity. A total of 399 individuals remained after removing those genotyped for ≤5 loci. Errors within the data (that is, input errors, allelic dropout, stutter and null alleles) were assessed in MICRO-CHECKER v2.2 (van Oosterhout *et al.*, 2004). Allelic richness was calculated in FSTAT v2.9.2.3 (Goudet, 2001) and tests for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium (LD) were performed in GENEPOP v4.0.10 (Raymond and Rousset, 1995; Rousset, 2008) using default Markov chain parameters (100 batches, 1000 iterations and 1000 dememorisation steps). Both observed and expected heterozygosities were calculated in GenAEx v6.5 (Peakall and Smouse, 2012) to determine the levels of population genetic diversity. Lastly, levels of significance were adjusted using the modified false discovery rate correction (Benjamini and Yekutieli, 2001).

Population genetic structure. We used multiple approaches to gain insight into the genetic structure of the black-capped chickadee. We used two clustering methods: GENELAND v4.0.0 (Guillot *et al.*, 2005a) and STRUCTURE v2.3.4 (Pritchard *et al.*, 2000). Both of these methods use Bayesian models to assign individuals to genetic clusters by maximising Hardy–Weinberg equilibrium and minimising LD, but differ in the way they use spatial information. STRUCTURE relies solely on genetic data (with the option of predefining populations with location priors), whereas GENELAND incorporates individual spatial coordinates.

Implemented in the program R v3.1.3 (R Development Core Team, 2015), GENELAND was run in two steps following the recommended protocol of Guillot *et al.* (2005a, b). First, we ran the program for 10 replicates for each *K* (1–10) using the correlated allele frequencies and null allele models and 100 000 Markov chain Monte Carlo iterations, a thinning interval of 100 and a maximum rate of Poisson process of 399 (equal to the sample size). The uncertainty attached to spatial coordinates was fixed to 20 km (that is, the precision of our sample locations; 10 km radius) and the maximum number of nuclei in the Poisson–Voronoi tessellation was fixed to 1197 (three times the sample size). The number of clusters (*K*) was inferred from the modal *K* and the run with the highest mean posterior probability. A second run was then conducted with the inferred *K* fixed and all parameters left unchanged to allow individuals to be assigned to clusters. To determine the robustness of this model, GENELAND was run multiple times with different parameters (for example, with and without the correlated allele frequencies and null allele models; and 50 000, 100 000 and 200 000 Markov chain Monte Carlo iterations).

Table 1 Sampling location information including site abbreviation (Abbrev.), geographical location (latitude (Lat) and longitude (Long)) and sample size (*N*)

Location	Abbrev.	Lat (°N)	Long (°W)	<i>N</i>	<i>H</i> _o	<i>H</i> _e	<i>PA</i>	<i>AR</i>
Revelstoke	BCR	50.9807	–118.1817	54	0.652	0.708	4	2.66
Northern BC	NBC	54.8883	–127.7665	43	0.647	0.690	5	2.68
Cluculz Lake	CLU	53.9102	–123.5496	20	0.654	0.703	4	2.70
Fort Fraser	FF	53.9629	–124.5331	11	0.644	0.672	0	2.79
Francois Lake	FrL	54.0488	–125.6988	20	0.622	0.668	1	2.64
Fort St James Town	FtStJ 2	54.4183	–124.2743	18	0.623	0.667	0	2.69
Hazelton	HAZ	55.2829	–128.0470	20	0.622	0.682	1	2.66
Houston	HOU	54.4043	–126.6433	18	0.620	0.666	1	2.72
Kelowna	KEL	49.9200	–119.3950	8	0.584	0.572	0	—
Northwest BC	NWBC	58.3003	–130.6677	17	0.658	0.689	2	2.63
Vancouver	VAN	49.2644	–123.0816	33	0.649	0.625	0	—
Fort St James John Prince Research Station	FtStJ 1	54.6453	–124.3949	61	0.666	0.717	11	2.79
Prince George	PG	53.8936	–122.8289	30	0.594	0.669	1	2.42
Southern Alberta 1	SAB1	49.3455	–114.4153	30	0.683	0.677	3	2.60
Southern Alberta 2	SAB2	49.0694	–113.8561	22	0.681	0.707	5	2.71

Microsatellite summary statistics for each population and all loci including: observed (*H*_o) and expected (*H*_e) heterozygosities, number of private alleles (*PA*) and allelic richness (*AR*).

STRUCTURE was run with the admixture model, correlated allele frequencies (Falush *et al.*, 2003) and locations as priors (locpriors). To determine the optimal number of clusters (K), we conducted ten independent runs (100 000 burn-in followed by 200 000 Markov chain Monte Carlo repetitions) for each value of K (1–10). Results were averaged using STRUCTURE HARVESTER v0.6.6 (Earl and vonHoldt, 2012) and both delta K (ΔK ; Evanno *et al.*, 2005) and LnPr(XIK) were used to determine the true K . Any populations with individuals showing mixed ancestry (for example, 50% Q to cluster 1, and 50% Q to cluster 2) were rerun individually with two populations representing each of the two clusters involved in the mixed ancestry to determine correct assignment. This is important to check because as K increases above the true K value, Q values will often decrease and split clusters (Pritchard *et al.*, 2000). This splitting of populations must be clarified before additional testing. Finally, if multiple populations were assigned to the same genetic cluster, these populations were rerun to test for additional substructure using the same parameters as the initial run, but only to a maximum of five runs for each K value.

Pairwise F_{ST} values were then calculated in GenALEX v6.5 to investigate the degree of genetic differentiation among the predefined populations. We also calculated D_{EST} (Jost, 2008) in SMOGD v1.2.5 (Crawford, 2010), an alternative measure of diversity that accounts for allelic diversity and is shown to measure genetic differentiation more accurately than traditional F_{ST} when using polymorphic microsatellite markers (Heller and Siegmund, 2009). We compared measures of D_{EST} and F_{ST} to determine the true level of genetic differentiation. As the theoretical maximum of 1 for F_{ST} is only valid when there are two alleles, population-wide F_{ST} , standardised by the maximum F_{ST} value, was calculated in GenALEX v6.5. To further assess genetic structure among populations, we carried out the principal coordinate analysis (PCoA) using both F_{ST} and D_{EST} in GenALEX v6.5.

Landscape genetics

Parameterisation of landscape variables. To assess the functional connectivity among populations, we evaluated four competing models: (1) the null model of isolation by geographical distance (Wright, 1943), (2) isolation by elevation resistance, (3) isolation by land cover resistance and (4) isolation by combined elevation and land cover resistance (that is, both land cover and elevation raster layers were combined into one resistance layer, termed 'land-elevation' herein). Pairwise resistance distances were calculated among all sampling sites using spatial data sets and an eight-neighbour connection scheme in CIRCUITSCAPE v4.0 (McRae, 2006). This method is based on circuit theory and uses resistance distances to assess all possible pathways between two focal points (or populations) to better map gene flow across the landscape and measure isolation by resistance.

Categorised land cover and digital elevation maps, circa 2000, were obtained from GEOBASE (www.geobase.ca) and resistances to habitat types were assigned using ArcMap, ESRI (Table 2). Land cover data were categorised into six cover types. The lowest resistance values were assigned to suitable chickadee habitat known to facilitate dispersal (that is, forest cover, particularly broadleaf and mixed forests), whereas other land cover types were classified as being moderately permeable (that is, coniferous forest, shrubland and grassland), or completely impermeable (that is, unsuitable habitat that included agricultural land and water) to dispersal (Table 2). For elevation, five different ranges were assigned resistance values based on elevations where chickadees have previously been observed. For example, low resistances were given to low elevation ranges (<1500 m), whereas higher resistance values were given to higher elevations where chickadees are rarely observed (>1500 m) (Table 2). The program outputs a cumulative 'current map' to portray the areas where resistance to gene flow is either high or low. Populations SAB1 and SAB2 were excluded from these analyses as geo-referenced coordinates were outside the spatial extent of the data. Given the size of our study area, all resistance surfaces were based on a 2 × 2 km resolution.

Landscape effects. We implemented a linear mixed-effect modelling approach based on the maximum likelihood population effects (MLPE) model (Clarke *et al.*, 2002) using the 'lmer' function in the package 'lmer4' v1.1.8 (Bates *et al.*, 2015) in R v3.1.3 (R Development Core Team, 2015). This approach is superior to the Mantel test to identify the landscape variable(s) that best explain population genetic differentiation. This is because Mantel tests are often described as having low statistical power (Legendre and Fortin, 2010) and, more importantly, fail to account for nonindependence of each pairwise observation within the distance matrix (Yang, 2004).

Nine predefined models were used to test for effects of different landscape variables on both estimates of pairwise genetic distance (that is, F_{ST} and D_{EST}). When fitting MLPE models, the 'lmer' function was modified so the random factor would account for multiple memberships (that is, two individual populations for each pairwise distance) following van Strien *et al.* (2012). Explanatory variables were centred around their mean, and parameter estimation was performed with the residual maximum likelihood criterion (Clarke *et al.*, 2002). For each parameter estimate, 95% confidence intervals were calculated. Models satisfied the assumptions of normality and constant variance, and showed no evidence of multicollinearity. In landscape genetics, a common technique is to use the Akaike information criterion for model selection (Storfer *et al.*, 2007). However, use of residual maximum likelihood precludes the use of Akaike information criterion; therefore, we used the marginal R^2 statistic developed by Nakagawa and Schielzeth (2013) in the R package, MuMIn v 1.14.0 (Barton, 2015), to select the best fitting and most parsimonious model (cf, van Strien *et al.*, 2012).

Table 2 Landscape models and corresponding resistance values assigned to each cover type or range in CIRCUITSCAPE analyses

Model	Resistance values assigned to cover types/ranges	Hypothesis
Null	Uniform landscape (all cells given a value of 1)	Isolation by distance
Land cover	Mixed forest = 1 Broadleaf forest = 2 Coniferous forest = 5 Shrubland = 10 Grassland/wetland/bryoids = 100 Unsuitable habitat (incl. agricultural land) = 1000	Isolation by resistance
Elevation	0–800 m = 1 801–1000 m = 5 1001–1500 m = 10 1501–2300 m = 100 2301–3454 m = 1000	Isolation by resistance
Land elevation	Combined land cover and elevation resistance maps using 'raster calculator'	Isolation by resistance

Model hypotheses are also provided.

RESULTS

Genetic structure

Genetic diversity. Among all loci and populations, the total number of alleles ranged from 3 to 46 alleles (Supplementary Table S2). Observed heterozygosity at each site and across all loci ranged from 0.584 (KEL) to 0.683 (SAB1, followed closely by SAB2 at 0.681), and expected heterozygosity ranged from 0.572 (KEL) to 0.717 (FtStJ1; Supplementary Table S3). Accounting for differences in sample size, allelic richness ranged from 2.42 (PG) to 2.79 (FtStJ1 and FF; Table 1). Of the 15 populations, 11 contained at least one private allele (Table 1); FtStJ1 contained the highest number of private alleles (PA=11) followed by NBC and SAB2 (PA=5). Null alleles were detected at a low frequency for a number of loci and were not consistent across populations with the exception of two loci: VeCr05 (0–25%) and Cuμ28 (31–71%). We found a large difference between observed and expected heterozygosities across populations for locus VeCr05 (H_o : 0.185, H_e : 0.306), but not for Cuμ28 (H_o : 0.485, H_e : 0.502, Supplementary Table S3). Exclusion of VeCr05 and/ or Cuμ28 did not alter the results, and hence all 14 loci were included in the final data set. Thirteen deviations from Hardy–Weinberg equilibrium (Supplementary Table S3) and two pairs of loci in disequilibrium were identified after corrections for multiple tests. All deviations were the result of a heterozygote deficit. Significant LD was found between loci Titgata02 and CTC101 ($P \leq 0.001$) within FtStJ1 and between loci Escu6 and Titgata02 ($P \leq 0.001$) within SAB1. As LD was not consistent across populations and genotypes showed no association, it is possible that LD is the result of a type 1 error. Results were not substantially affected after removing either the underrepresented loci or the feather-sampled populations (see summary statistics in Supplementary Table S4).

Population genetic structure. The two clustering analyses failed to converge on the total number of genetic clusters (K), but a number of groupings were similar across analyses. As GENELAND produced more distinct and biologically meaningful clustering of populations, we have focused our interpretation on GENELAND’s results and included the results of STRUCTURE as Supplementary Material for comparison.

A hierarchical STRUCTURE analysis inferred seven genetic clusters (Supplementary Figure S1a) using both mean log likelihood ($\text{Pr}(\text{XIK}) = -17544.9$) and ΔK (Supplementary Figure S1b). A larger number of groupings was found in GENELAND; eight runs suggested $K=9$ and two runs suggested $K=10$. As the highest posterior probability was for $K=9$ (–958) we took this as being the true estimation of K . For population membership and boundary graphs see Supplementary Figure S2. The genetic clusters included single populations (BCR, CLU, FtStJ1, NBC, NWBC and PG) as well as groups of populations (KEL+VAN, SAB1+SAB2, and all remaining populations; Figure 1). Five of the groupings were identical to those identified in STRUCTURE (BCR, NWBC, PG, FtStJ1 and VAN+KEL; Supplementary Figure S1). The distinction of PG and FtStJ1 is concordant with patterns observed in our previous study (Adams and Burg, 2015).

Pairwise F_{ST} and D_{EST} values showed a significant positive correlation ($R^2 = 0.692$, $P = 0.003$). Pairwise F_{ST} values ranged from 0.009 to 0.316 (Table 3), and after corrections for multiple tests, 86 of the 105 tests were significant, indicating a high level of genetic differentiation among populations. Similar levels of population structure were detected using D_{EST} that ranged from 0.005 to 0.329 (Table 3). Overall F'_{ST} was 0.240 (Supplementary Table S5).

Distinct clustering of populations in PCoA was only found using D_{EST} values. The first principal coordinate analysis with all 15 populations resulted in clear separation of populations KEL and VAN from all other populations, as well as differentiation from each other, with the first two axes explaining 50.59% and 17.04% of the variation (Figure 2a) respectively. Isolation of KEL and VAN is concordant with GENELAND. It is important to note that these two populations contained some missing genotype information. As PCoA is sensitive to missing data, we removed KEL and VAN from analyses to identify additional structure. Concordant with some of the patterns observed in GENELAND, we see separation of PG as well as NWBC and BCR (coordinate 1 = 31.05%, coordinate 2 = 19.93%; Figure 2b).

Landscape genetics

MLPE models were ranked based on marginal R^2 (Table 4). For F_{ST} , the best fitting model included land-elevation ($R^2(\text{mar}) = 0.879$;

Table 3 Pairwise F_{ST} values (below diagonal) and harmonic mean estimates of D_{EST} (above diagonal) for 15 black-capped chickadee populations based on 14 microsatellite loci

	BCR	NBC	CLU	FF	FtL	FtStJ2	HAZ	HOU	KEL	NWBC	VAN	FtStJ1	PG	SAB1	SAB2
BCR	–	0.045	0.032	0.038	0.040	0.056	0.035	0.062	0.224	0.041	0.149	0.037	0.106	0.031	0.021
NBC	0.014	–	0.015	0.018	0.030	0.043	0.029	0.048	0.217	0.043	0.162	0.043	0.091	0.015	0.009
CLU	0.017	0.020	–	0.017	0.019	0.042	0.023	0.037	0.239	0.051	0.184	0.017	0.063	0.019	0.008
FF	0.056	0.054	0.058	–	0.034	0.039	0.010	0.011	0.218	0.070	0.190	0.020	0.096	0.026	0.010
FtL	0.087	0.097	0.097	0.130	–	0.048	0.040	0.052	0.202	0.070	0.167	0.049	0.098	0.030	0.024
FtStJ2	0.088	0.094	0.100	0.129	0.159	–	0.050	0.063	0.166	0.066	0.123	0.046	0.103	0.033	0.038
HAZ	0.057	0.063	0.058	0.094	0.136	0.135	–	0.044	0.202	0.052	0.157	0.029	0.094	0.039	0.018
HOU	0.065	0.063	0.072	0.096	0.140	0.140	0.116	–	0.279	0.059	0.211	0.046	0.102	0.048	0.021
KEL	0.195	0.204	0.207	0.235	0.264	0.253	0.226	0.262	–	0.243	0.168	0.240	0.329	0.243	0.222
NWBC	0.018	0.019	0.025	0.065	0.101	0.099	0.069	0.067	0.212	–	0.175	0.050	0.103	0.048	0.043
VAN	0.172	0.183	0.189	0.217	0.246	0.234	0.218	0.234	0.316	0.188	–	0.164	0.237	0.156	0.178
FtStJ1	0.011	0.014	0.012	0.053	0.092	0.090	0.057	0.064	0.200	0.017	0.177	–	0.091	0.013	0.025
PG	0.035	0.033	0.034	0.080	0.118	0.121	0.087	0.081	0.237	0.036	0.211	0.031	–	0.073	0.043
SAB1	0.014	0.014	0.017	0.055	0.095	0.094	0.062	0.066	0.208	0.022	0.187	0.009	0.037	–	0.005
SAB2	0.013	0.013	0.019	0.056	0.092	0.094	0.061	0.065	0.201	0.021	0.183	0.013	0.030	0.012	–

Bold values indicate significance after corrections for multiple tests.

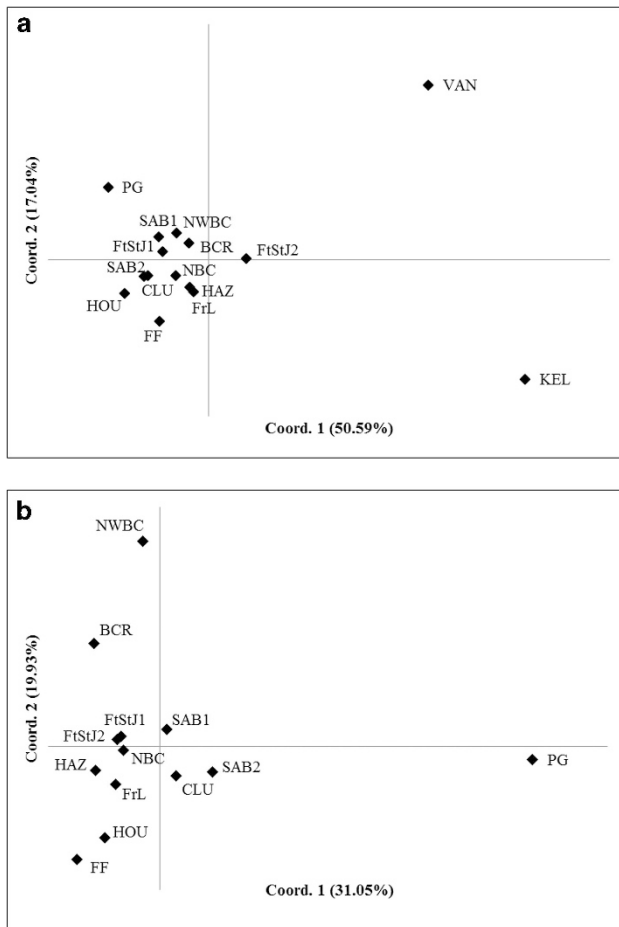


Figure 2 Principal coordinate analysis conducted in GenAlEx based on pairwise D_{EST} values for (a) all 15 populations (coordinates 1 and 2 explained 50.59% and 17.04% of the variation respectively) and (b) after removal of populations KEL and VAN (coordinates 1 and 2 explained 31.05% and 19.93% of the variation respectively).

Table 4a), whereas for D_{EST} the model with the highest R^2 included both land-elevation and geographical distance (R^2 (mar)=0.711; Table 4b). All variables in the best models had a positive effect on genetic distance. Over all models, those including either land cover or land elevation as explanatory variables produced consistently high R^2 values for both F_{ST} (≥ 0.874) and D_{EST} (≥ 0.660). The effects of geographical distance and elevation varied across all models. Only once was there a significant effect of the parameter elevation (model 7 for D_{EST}), and although geographical distance was significant in all models for D_{EST} (including the top two models), for F_{ST} geographical distance was significant in only two of the nine models. This may be explained by the different properties of the response variables (that is, F_{ST} is based on allele frequencies whereas D_{EST} is based on allelic diversity) and emphasises the importance of comparing measures of genetic distance. D_{EST} corrects for sampling bias and as the sample sizes varied between sites, this may explain the differences between the two. The effect of geographical distance on D_{EST} was consistent across all models, and suggests an isolation-by-distance effect. Meanwhile, land cover and land-elevation had a clear significant effect on all models and across both measures of genetic distance. This suggests that although the combined effect of both land cover and elevation resistances on genetic distance is significant, ultimately land cover

resistance is the largest factor contributing to variation in population genetic differentiation.

DISCUSSION

Fine-scale genetic structure of the black-capped chickadee

Populations of black-capped chickadees in British Columbia are spatially structured from restricted population connectivity as supported by individual-based (Bayesian clustering analyses), population-based (F_{ST} , PCoA) and landscape-based analyses (CIRCUITSCAPE and MLPE modelling). Intensive sampling and additional microsatellite loci used in this study resulted in a finer resolution of observed genetic structure. Here, nine genetic clusters were inferred in comparison with four clusters in our previous study (Adams and Burg, 2015), and population genetic differentiation was observed in all regions of British Columbia from the north (NWBC) to the interior (CLU, NBC, FtStJ1, PG) and in the south (VAN, KEL and BCR).

Despite their vagility and generalist behaviour, black-capped chickadees are a highly sedentary species, showing strong aversion to crossing gaps in suitable habitat and this characteristic appears to have a significant impact on dispersal across fragmented landscapes (Desrochers and Hannon, 1997). Population genetic structure is an expected evolutionary consequence of species inhabiting fragmented landscapes (Shafer *et al.*, 2010), especially in species with restricted dispersal (Unfried *et al.*, 2013) like black-capped chickadees. Spontaneous and highly irregular, large-distance movements (that is, irruptions) are observed in juveniles (Weise and Meyer, 1979), and occasionally in adults (Brewer *et al.*, 2000), and adults will sometimes move down from high-altitude localities in response to severe weather conditions or food availability (Campbell *et al.*, 1997). However, black-capped chickadees rarely disperse long distances; although a maximum dispersal of 2000 km was recorded for one bird in a recapture study on 1500 individuals, <2% of birds dispersed >50 km from banding locations, and >90% remained in the location they were initially banded (Brewer *et al.*, 2000). Distances between adjacent populations in this study are within the potential dispersal range, yet genetic differentiation was observed between populations separated by both small (for example, ~30 km between FtStJ1 and FtStJ2) and large (for example, ~390 km between PG and HAZ) distances (Figure 1). The observed patterns suggest that at smaller geographical distances, other factors such as habitat heterogeneity and fragmentation resulting from both natural and anthropogenic causes may be influencing dispersal and gene flow.

Effects of landscape features on genetic differentiation

A landscape genetic approach revealed the complexity of black-capped chickadee population structuring from just two spatial data sets (elevation and land cover), highlighting the importance of incorporating landscape-level data into studies of gene flow in addition to using traditional measures of isolation by distance. Despite the relatively weak resolution of model-based analyses, both land cover (suitable forest cover) and elevation (low–mid-elevation valleys) appear to be important factors in explaining the observed patterns of genetic differentiation in black-capped chickadees. The models that included land cover combined with elevation (land-elevation) best explained genetic differentiation for F_{ST} and D_{EST} in two separate analyses, but it is likely that land cover is the most influential factor (Table 4). As forest generalists, dispersal for black-capped chickadees is largely dependent upon the availability of woodland corridors (Bélisle and Desrochers, 2002; Desrochers and Bélisle, 2007). For example, differences in forest cover can be observed between genetically differentiated populations in Fort St James (FtStJ1 and FtStJ2). Timber

Table 4 Results of maximum likelihood population effects (MLPE) model selection after accounting for non-independence of pairwise distance observations

Model	Regression slope Est. \pm 95% Confidence Intervals				Marginal R^2
	Distance	Elevation	Land cover	Land-elevation	
<i>(a)</i>					
4	—	—	—	0.83 \pm 0.04	0.879
9	0.01 \pm 0.01	—	—	0.83 \pm 0.04	0.878
7	—	2.05 \pm 5.42	0.83 \pm 0.04	—	0.875
6	0.01 \pm 0.01	—	0.83 \pm 0.04	—	0.875
3	—	—	0.89 \pm 0.04	—	0.875
8	0.03 \pm 0.02	-7.38 \pm 10.72	0.81 \pm 0.04	—	0.874
5	0.23 \pm 0.11	94.38 \pm 45.94	—	—	0.158
1	0.05 \pm 0.06	—	—	—	0.022
2	—	-12.00 \pm 27.41	—	—	0.008
<i>(b)</i>					
9	0.08 \pm 0.05	—	—	0.59 \pm 0.14	0.711
6	0.08 \pm 0.05	—	0.59 \pm 0.14	—	0.710
7	—	24.19 \pm 20.31	0.65 \pm 0.14	—	0.709
8	0.12 \pm 0.10	-17.46 \pm 42.29	0.56 \pm 0.16	—	0.694
4	—	—	—	0.06 \pm 0.37	0.667
3	—	—	0.64 \pm 0.15	—	0.660
5	0.27 \pm 0.11	-80.24 \pm 48.16	—	—	0.173
1	0.11 \pm 0.07	—	—	—	0.129
2	—	16.96 \pm 29.46	—	—	0.022

For all models, pairwise (a) F_{ST} and (b) D_{EST} values were the response variable. Models are ranked based on marginal R^2 value (high to low) with the best fitting model having the highest R^2 value. For each explanatory variable included in the model, values ($\times 10^{-4}$) are presented as regression slopes \pm 95% confidence interval. Values in bold indicate significant parameter estimates (that is, 95% confidence intervals that do not overlap zero). Explanatory variables not included in the model are indicated by '—'.

harvesting of the abundant lodgepole pine (*Pinus contorta*) significantly reduces the amount of suitable forest in the south (FtStJ2) in comparison with the north (FtStJ1) where the forest is managed and protected from logging (Fondahl and Atkinson, 2007).

Populations were sampled on either side of a distinct mountain (Pope Mountain; \sim 1400 m elevation) and large water body (Stuart Lake) that may act as connectivity barriers. Elevation may therefore be a significant factor, as black-capped chickadees are often associated with low-elevation riparian corridors in British Columbia, and tend to be replaced ecologically at higher elevations by mountain chickadees (*Poecile gambeli*) (Foote *et al.*, 2010). Low-resistance dispersal routes also corresponded to areas of low elevation (that is, within the central plateau and to the south; Figure 3). Black-capped chickadees frequently breed between 270 and 1500 m elevation with the highest elevation recorded at 2300 m in British Columbia (Campbell *et al.*, 1997). As black-capped chickadees are forest dependent and found at lower elevations, it is not surprising that the lack of forest cover and high elevations would impede gene flow. The same two landscape features are important in facilitating black bear (*Ursus americanus*) dispersal in northern Idaho (Cushman *et al.*, 2006).

Differences in land cover and elevation may reflect multiple biogeoclimatic zones across the region, characterised by variation in climate, topography and vegetation. As our populations are distributed across a number of these zones, it is possible that habitat discontinuity is playing a bigger role in genetic differentiation than physical geographical barriers. For example, genetic differentiation in the north (NWBC) could be explained by local environmental conditions. NWBC is situated within the boreal-black and white spruce biogeoclimatic zone, characterised by long, extremely cold winters and short, warm summers, and is isolated from other sampling sites by the

Skeena and Omineca Mountains. To the south of NWBC, there is a sharp transition from boreal-black and white spruce to Engelmann spruce-subalpine fir to interior cedar-hemlock (Parish and Thomson, 1994). The Engelmann spruce-subalpine fir zone occupies the highest forested elevations in British Columbia. Our landscape analyses revealed high pairwise resistance values (results not shown) between NWBC and nearby populations for both elevation and land cover, suggesting limited dispersal. This is also evident from both CIR-CUITSCAPE (Figure 3), where there are little to no connections between NWBC and nearby populations, and the effect of isolation by distance on pairwise D_{EST} values (Table 4b). Our resistance map of elevation (Supplementary Figure S3a) supports isolation of NWBC. Therefore, high variability in habitat and climatic conditions combined with high elevations and large geographic distances may explain the genetic differentiation of this population, as when gene flow is low, isolated populations may adapt to local environmental conditions as a result of divergent selection pressures (Cheviron and Brumfeld, 2009). However, it is important to note that many neighbouring populations to NWBC have not been sampled and hence these observations could be a function of sampling regime rather than specific landscape effects. To confirm these speculations, more robust sampling in and around this area is necessary.

Genetic clustering of KEL and VAN was supported by high yet nonsignificant pairwise F_{ST} (0.316). Black-capped chickadee subspecies delimitations by size and colouration might explain this grouping: VAN birds are grouped within the Oregon subspecies (*P. a. occidentalis*); KEL birds within the Columbian subspecies (*P. a. fortuitus*) and all other populations in this study within the larger-sized long-tailed subspecies (*P. a. septentrionalis*) (Smith, 1991). Although we expected to see reduced gene flow between KEL and

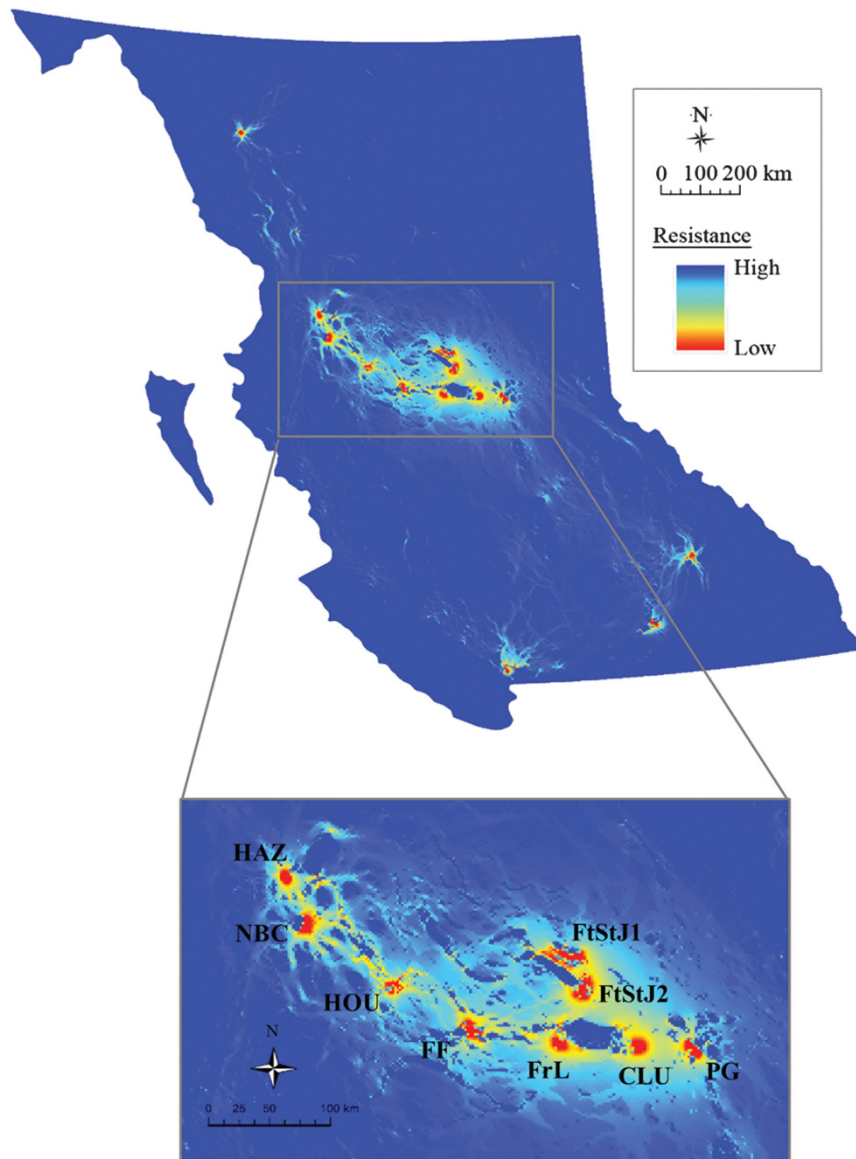


Figure 3 Map showing the resistance grid output from CIRCUITSCAPE analyses for the resistance surface of land cover and elevation combined (land-elevation) as this variable best explained genetic differentiation in other analyses. A close-up of the central plateau region is included (bottom).

VAN because of the presence of two prominent north–south mountain ranges bisecting the two sampling sites, there were inconsistencies among analyses (that is, differentiation was indicated by F_{ST} and PCoA analyses, but not by Bayesian clustering analyses). It is possible that low valleys within the Coastal Range act as important corridors to dispersal between these two populations. The genetic status of KEL and VAN, however, will require validation with additional sampling.

Dispersal in fragmented landscapes

Loss of genetic diversity from habitat loss can impede a species' ability to adapt to changes in their environment, and lead to reductions in reproductive fitness and population size (Frankham, 1995; Haag *et al.*, 2010; Woltmann *et al.*, 2012; Finger *et al.*, 2014). As such, loss of forests within low- to mid-elevation areas from both natural and anthropogenic processes could have a significant impact on chickadee dispersal, and thus on the health of chickadee populations. One reason

for reduced dispersal in fragmented habitats is predation risk. Both St. Clair *et al.* (1998) and Desrochers and Hannon (1997) found that black-capped chickadees are less willing to cross gaps of > 50 m of unsuitable habitat. In areas of central British Columbia where logging and other activities have fragmented chickadee habitat, dispersal would be restricted. The size and abundance of cut blocks from forestry activities may be restricting dispersal; however, explicit testing at an even smaller spatial scale is required. Unexpectedly, our resistance map (Figure 3) displayed a large area in the central plateau (between FrL and CLU) where movement is impeded. This area corresponds to an area of increased agriculture that could explain differentiation of CLU in GENELAND analyses as well as lower observed allelic diversity and observed heterozygosities (FF, FrL and FtStJ2; Table 1 and Supplementary Table S3).

Natural contributors to habitat fragmentation may also explain patterns of genetic structure observed here. Bark beetle outbreaks have been observed in western Canada since the 1900s (Swaine, 1918).

Current outbreaks are spreading quickly with warmer/milder winters facilitating their expansion across western Canada. The mountain pine beetle outbreak has destroyed huge portions of mature pine forests throughout British Columbia, particularly in the central plateau region within elevations of 800 and 1400 m (Safranyik and Wilson, 2006). Habitat loss could be leading to high levels of population isolation here, particularly in low–mid-elevation forested valleys that serve as dispersal corridors. In fact, a number of populations within this region are showing signs of reduced genetic diversity, particularly the PG population ($H_0=0.594$, $H_e=0.669$; Table 1), suggesting that some populations may be experiencing a bottleneck as a result of restricted gene flow. Thus, despite being common, widely distributed and of little conservation concern (IUCN Red List), isolated chickadee populations may be undergoing microevolutionary processes that may eventually lead to local adaptation.

CONCLUSIONS

Weak population genetic differentiation is expected for common and widespread species with the ability to disperse among habitat patches (that is, bird flight), but our findings suggest that variation and/or changes in the environment can affect genetic differentiation in mobile species, resulting in microgeographic population structuring.

Dispersal and gene flow among black-capped chickadee populations appear to be affected by variation in landscape topography and forest cover, features critical to chickadee survival and reproductive success. Climatic differences among sampling sites may also create differential selective pressures. The importance of including landscape features when assessing connectivity and population differentiation is particularly relevant when identifying vulnerable populations and management units, as over time isolated populations may diverge through local adaptation or inbreeding. In the face of climate change, biogeographic zones will change and forest tree species are under threat of shifting and narrowing distributions (Hebda, 1997; Hamann and Wang, 2006; Wang *et al.*, 2012) that could in turn, have an impact on black-capped chickadee populations. Changes in precipitation and winter temperature have already driven shifts in the geographic patterns of abundance of bird populations in western North America (Illán *et al.*, 2014).

Overall, when assessing patterns of genetic differentiation of populations, a smaller sampling scale and the inclusion of more loci can provide additional patterns of genetic structure. In addition, incorporating both landscape features and environmental variables when explaining patterns can significantly improve our understanding of how species evolve in response to changes in their environment.

DATA ACCESSIBILITY

Genotype data are available from the Dryad Digital Repository: doi:10.5061/dryad.gs228.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Natural Science and Engineering Research Council (NSERC) Discovery Grant and Alberta Innovates (AI) New Faculty Award. We also thank P Pulgarin-Restrepo, B Graham, K Gohdes, C MacFarlane, A Martin and volunteers from Project Feederwatch and BC Bird Breeding Atlas for helping with sample collection for this project. Finally, we thank Dr Maarten J van Strien for his expertise in the R programming environment.

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