# Pleistocene glacial cycles and physical barriers influence phylogeographic structure in black-capped chickadees, a widespread North American passerine

J. Hindley[1], B.A. Graham[1], and T. M. Burg[1]

[1]Department of Biological Sciences, University of Lethbridge, 4401 University Drive, Lethbridge AB, Canada T1K 3M4

Running title: Phylogeography of black-capped chickadees

Corresponding author email: b.graham001@gmail.com

**Abstract**

The non-migratory black-capped chickadee (*Poecile atricapillus*, Linnaeus 1766) has a continent-wide distribution extending across large parts of North America. To investigate the phylogeographic structure, and verify possible refugia during the last glacial maximum, we sequenced a 678 base pair region of the mitochondrial control region from 633 chickadees at 35 sites across North America, and performed paleoecological distribution modeling. Two genetically distinct groups were found using multiple analyses: one in Newfoundland and a widespread continental group, with additional substructure evident in western continental populations. While gene flow is low throughout the range, it is especially low in peripheral populations. The Newfoundland population has remained isolated from continental populations for at least 65,000 years and contains a number of fixed nucleotide differences. Within the continental populations, chickadees are subdivided into Pacific Coast, Alaska, southeast Rockies and main-northeast groups consistent with late Pleistocene vicariance events. Evidence of secondary contact was identified between the Pacific and main-northeast populations in northwest British Columbia and between the southeast Rockies and main-northeast group in Montana. Paleoecological distribution modeling predicted suitable habitat in Alaska, off the coast of Newfoundland and several locations across the southern United States during the last glacial maximum; whereas suitable habitat during the last interglacial was more similar to the contemporary distribution.

*Keywords*: black-capped chickadee, dispersal barriers, mitochondrial DNA, phylogeography, *Poecille atricapillus,* post-glacial colonization, refugia

## Introduction

Climate oscillations during the Quaternary had a profound effect on biodiversity, and are responsible for shaping the genetic structure of animal and plant communities globally (Hewitt 2000; Taberlet and Chiddari 2002). During Pleistocene glaciations, habitat became fragmented resulting in populations being isolated in ice-free refugia (Bennet and Provan 2008). The contraction of forest habitat and corresponding changes to species' ranges during this time are thought to be responsible for a number of recent speciation events (Weir and Schluter 2004; Levsen et al. 2012). Determining how species were distributed throughout the Pleistocene will help to improve our comprehension of how climate influences evolutionary processes (Keppel et al. 2012); this question is of critical importance given the growing threat that climate change poses to many species worldwide (Thomas et al. 2004; Pimm et al. 2014).

In North America, the biogeographical history is quite complex (Pielou 1991). During the Last Glacial Maximum (LGM), North American species were restricted to areas south of the ice sheets, or one of the putative refugia to the north such as Beringia, Haida Gwaii, or Newfoundland (Pielou 1991). Further, barriers to dispersal including mountain ranges, and large areas with unsuitable habitat for forest dwelling species may have restricted gene flow throughout the LGM and during recolonization. For this reason, North America offers an interesting region to investigate questions pertaining to barrier mediated dispersal and patterns of postglacial expansion.

In this study we examine phylogeographic patterns of the black-capped chickadee (*Poecile atricapillus*, Linnaeus 1766) using the highly variable mtDNA (mitochondrial DNA) control region (CR). The black-capped chickadee is a non-migratory species primarily inhabiting deciduous and mixed deciduous/coniferous woodlands (Foote et al. 2010). Their distribution

58 spans the entire width of North America, extending from the treeline in the north to Colorado
59 and North Carolina in the south (Figure 1). Given their broad distribution, black-capped
60 chickadees are an ideal species for investigating how Pleistocene glaciations and geographical
61 features have shaped genetic structure and diversity. Several previous studies have examined the
62 genetic structure of black-capped chickadees (Gill et al. 1993; Pravosudov et al. 2012; Adams
63 and Burg 2014). Gill et al. (1993) used 11 restriction enzymes to examine mtDNA variation
64 between black-capped chickadee populations, although relatively few sites were surveyed in this
65 study. Pravosudov et al. (2012) used mtDNA control region sequences and amplified fragment
66 length polymorphisms to examine the relationships between memory and hippocampal
67 morphology and population structure; in this study they noted strong population structure within
68 this species but again examined only part of the range of the black-capped chickadee. Finally,
69 Adams and Burg (2014) analyzed range-wide genetic patterns using microsatellites and found
70 high levels of contemporary population structure across the range. In this study Adams and Burg
71 (2014) determined that both ecological and geographical factors influence contemporary
72 population structure in this species. Despite these previous studies, questions still remain on
73 whether genetic patterns reflect recent processes (i.e. human mediated habitat changes) or events
74 pertaining to Pleistocene glaciations? In comparison to the nuclear markers used in these
75 previous studies, mtDNA control region sequences evolve at much slower rate, thereby
76 providing a greater resolution to explore the effects of historical processes, including Pleistocene
77 glaciations, on population structure. Further the greater sampling resolution used in this study
78 will build upon the previous study by Gill et al. (1993) which sampled considerably fewer
79 individuals and populations.

We analyzed mtDNA control region haplotypes to test two main hypotheses: 1) black-capped chickadees were isolated in multiple glacial refugia and 2) physical barriers restrict gene flow. Gill et al. (1993) proposed expansion out of a single, common refugium approximately 10 kya following the retreat of ice sheets, but the sample size was relatively limited (*n*=64). Despite this prediction by Gill et al. (1993), two of the nine black-capped chickadee haplotypes they found were restricted to Newfoundland suggesting the potential for genetic isolation of this island population. Therefore, we predict the presence of at least two glacial refugia, one main refugium on the North American mainland and a second one near Newfoundland. To answer this question we combined genetic analyses with ecological niche modeling to determine where suitable habitats may have existed for this species at the LGM. Large mountain ranges such as the Rockies and Cascades correspond with genetic breaks in other bird species (e.g., Barrowclough et al.. 2004; Ruegg 2008). Given black-capped chickadees are primarily restricted to lower elevations (<3200 m) and are year round residents, we predicted that physical barriers like mountain ranges may act as barriers to gene flow.

## Materials and methods

### *Sampling*

A total of 633 black-capped chickadees were used in this study. Most samples (*n*=588) were in the form of blood or feather samples taken from birds captured using mist nets (Adams and Burg 2014). The remaining samples (*n*=45) were from collections (Supplemental Table S1). An additional 26 Carolina chickadee (*Poecille carolinensus*, Audubon 1834) samples (Mike Braun and Brian Davidson) were included for outgroup comparison. The combined dataset represents 36 sampling sites (hereafter referred to as populations; Figure 1; 32 black-capped

chickadee, three of both species, and one Carolina chickadee) from across the contemporary black-capped chickadee range (Ridgely et al. 2007). Samples were collected during the breeding season, and all samples within each sample site were collected within a 50 $km^2$ radius. DNA was extracted from whole blood or tissue using a modified chelex protocol (Burg and Croxall 2001; Walsh et al. 1991).

*Ecological niche modeling*

In order to predict possible refugia during the LGM (~22 kya) and the last interglacial (LIG, ~120-140 kya), we reconstructed black-capped chickadee distribution (i.e., suitable conditions) through the use of ecological niche modeling (ENM) with the program MAXENT v3.3.3e (Phillips et al. 2006). Ecological niche models have been shown to be strong predictors of phylogeographic patterns, suggesting that the two methods are complementary (Waltari et al. 2007). Bioclimatic variables were obtained from the WorldClim dataset v1.4 with 2.5 arc-min (LGM) and 30 arc-seconds (LIG) resolution (Hijmans et al. 2005). We used 10 variables (annual mean temperature, mean diurnal temperature range, isothermality , temperature seasonality, mean temperature of wettest quarter, annual precipitation, precipitation of driest month, precipitation seasonality, precipitation of warmest quarter and precipitation of coldest quarter) to generate models in MAXENT with the default settings (regularization = 1, convergence threshold = 0.000001, iterations = 500) for 10 replicates. The nine other variables in the WorldClim dataset were removed from the analysis because they showed strong correlations ($r>0.90$) with one or more of the variables used in our analysis. We combined our banding locations with records downloaded from the Global Biodiversity Information Facility data portal (GBIF; http://data.gbif.org/, accessed on 28 March 2018). We removed all duplicate points and then used the rarefaction tool available in ENM tools (Warren et al. 2010) to account for sampling

126 bias (Syfret et al. 2013; Fourcade et al. 2014 ); samples were rarified at a 10 km distance. Overall
127 we retained 8726 points; we randomly chose a total of 1726 chickadee records (20% of all the
128 retained records) to train the dataset and then used the remaining 7000 chickadee locations for
129 the models (Figure S1). MAXENT uses a maximum entropy statistical model of presence-only
130 occurrence data based on the current distribution's (i.e., known presence location) climate
131 conditions to infer past distributions by identifying similar bioclimatic conditions during a
132 particular time (e.g., LGM), assuming that present niche requirements reflect past and/or future
133 requirements. The MIROC (Model for Interdisciplinary Research on Climate) climate layers
134 provided by the Paleoclimate Modelling Intercomparison Project Phase II were used for
135 projecting past climatic conditions both at the LGM (~21 kya; Hasumi & Emori, 2004) and the
136 Last Interglacial Maximum (LIM; Otto- Bliesner et al. 2006).
137 *MtDNA amplification and sequencing*
138 We amplified a 678 bp segment of the mitochondrial control region (CR; Domain I and
139 II) using two polymerase chain reaction (PCR) primers HCRCBox (5′-
140 CCACTTGTATCTGTGARGAGC -3′) and LbcchCR1 (5′- CCACCACCCCATAATAAGGA -
141 3′) designed for this species. The PCR was carried out in an Eppendorf Mastercycler, and
142 consisted of approximately 100 ng of template DNA, 1 μM of each primer, 200 μM dNTPs, 2.5
143 mM $MgCl_2$, 1 unit of Taq DNA polymerase and PCR buffer in a final volume of 25 μl.
144 Amplification consisted of one cycle at 95°C for 2 min, 54°C for 45 s, and 72°C for 60 s; 37
145 cycles of 94°C for 30 s, 54°C for 45 s, and 72°C for 60 s; and one final cycle at 72°C for 5 min.
146 PCR products were sequenced using BigDye terminator mix in an Applied Biosystems 3130
147 Genetic Analyzer following enzymatic clean up using 0.1 units of shrimp alkaline phosphatase

148 (SAP) and 0.1 units of exonuclease I. MtDNA sequences were manually aligned using MEGA
149 v5.0 (Tamura et al. 2011).
150 *Population genetic structure and variation*
151 We used DNASP v5.10 (Librado and Rozas 2009) to calculate the number of haplotypes (*Hn*),
152 haplotype diversity ($H_d$), and nucleotide diversity ($\pi$). Next, we used TCS v1.21 (Clement et al.
153 2000) to construct a statistical parsimony network and examine the relationship among
154 haplotypes. In addition, we constructed a maximum likelihood tree in MEGA v5.0. Prior to
155 running our tree we used MEGA to determine the model of sequence evolution that best fit our
156 data. The program chose a Kimura 2 parameter model plus gamma and invariants (BIC = 6161),
157 In addition the maximum likelihood tree was constructed using the nearest neighbor interchange
158 heuristic model with 1000 bootstrap replicates to evaluate robustness. We used four Carolina
159 chickadee sequences as the outgroup for our maximum likelihood tree.

160 Additionally we conducted a principal coordinate analysis (PCoA) in GenAlEx v6.41
161 (Peakall and Smouse 2006) to further examine the relationship among populations. For this
162 analysis we calculated the mean Nei's genetic distance between population pairs. In our first
163 analysis we included the four Carolina chickadee populations to examine population clustering
164 between the two species. We then ran a second analysis with only black-capped chickadee
165 populations to determine if there was greater population structure among populations.
166 We calculated pairwise $\Phi_{ST}$ values, a basic index of population differentiation, for the 33
167 black-capped chickadee populations with at least five samples (mean = 19 ±1.7 SE). We used
168 Arlequin v3.0 (Excoffier et al. 2005) to calculate $\Phi_{ST}$ and tested for significant differences using
169 10,000 permutations. All p-values were corrected for multiple tests using the Benjamini-
170 Hochberg False Discovery Rate (FDR) correction (Benjamini and Hochberg 1995). To

171 investigate hierarchical population structure and determine whether genetic breaks correspond
172 with refugia locations, we performed an analysis of molecular variance (AMOVA) in Arlequin
173 v3.0 (Excoffier et al. 2005). We defined possible groupings based on potential refugia locations
174 that were identified using ENM predictions, analyzing the following potential groupings: a) a
175 single panmictic population; b) two groups: Newfoundland and all continental populations; c)
176 three groups: Newfoundland, Pacific Coast populations, and the remaining continental
177 populations; d) four groups: Newfoundland, Pacific Coast populations, southeast Rocky
178 Mountain populations, and the remaining continental populations.
179 To visualize genetic differentiation across the entire range, we used the genetic landscape
180 GIS toolbox (Vandergast et al. 2011) in ArcGIS. We used the single species divergence tool and
181 pairwise $\Phi_{ST}$ values to create landscape surfaces and depict gradients of genetic variation across
182 the complete distribution of black-capped chickadees. This analysis is particularly useful when
183 analyzing large numbers of populations, and uses an inverse distance weighted interpolation
184 algorithm to produce the surface file. Values range between 0 and 1, with zero representing areas
185 of little genetic differentiation and one representing strong genetic differentiation. To
186 complement this analysis, we used the program Barrier 2.2 (Manni et al. 2004) to identify
187 potential barriers to dispersal. The program uses a pairwise genetic distance matrix (based on
188 pairwise $\Phi_{ST}$ values for this study) to determine the magnitude and location of barriers dispersal
189 by employing Delauney triangulation to connect populations (based on the latitiude and
190 longitude) utilizing Monmonier's algorithm to identify the paired populations with the greatest
191 genetic distance. The program will identify up to 10 barriers, with the lowest barriers
192 representing those barriers where gene flow is lowest between population pairs. For the purpose
193 of this study, we retained the first five barriers identified by the program.

Additionally, we explored if populations exhibited an isolation by distance (IBD) signal. We tested for IBD using two different analyses: the first analysis included all populations, while for the second analyses we included all continental populations and excluded Newfoundland. We transformed our pairwise $\Phi_{ST}$ values into a genetic distance using the $\Phi_{ST}/(1-\Phi_{ST})$ transformation recommended by Rousset (1996); the central location for each population was estimated by mapping the mid-point for all samples collected at each sampling site (e.g., AKA) and calculating the straight-line distance between pairs of sampling sites. Mantel tests were performed in GenAlEx and significance was tested with 9999 permutations.

*Divergence times*

We estimated divergence times using a strict molecular clock with both a genetic distance and a Bayesian method. The mean genetic distances (maximum composite likelihood; MCL) among groups (main-northeast, SE Rockies, Alaska, Pacific Coast, and Newfoundland) were calculated in MEGA v5.0 using a strict molecular clock (12.1% and 3%; see below). We used the program BEAST v1.6.1 (Drummond and Rambaut 2007; Drummond et al. 2005) with a strict molecular clock to estimate the coalescence time of the Newfoundland and continental populations. A BEAST .xml input file was created using BEAUti v1.6.1 (Drummond and Rambaut, 2007) and a random generated tree was used with a skyline coalescent population prior and two different substitution rate priors for each run.

The first substitution rate prior was based on a 12.1% divergence / million years between two lineages (substitution rate = $6.05 \times 10^{-8}$ mutations/site/year/lineage). The weighting was determined using standard avian mutation rates for each domain and the proportion of the domain sequenced; 320 bp in Domain I (20% mutation rate) and 358 bp in Domain II (5% mutation rate; Baker and Marshall 1997). The second substitution rate prior was 1.5% / million

years and based on three separate lines of evidence; a conservative 3% mutation rate based on 1.2% / million years for the genus *Poecile* (Päckert et al. 2007), the estimated split between Carolina/black-capped lineages ~2.5 million years (Gill et al. 1993; Gill et al. 2005), and control region sequence data for black-capped and Carolina chickadees. The 3% subsitution rate is similar to the 3.7% rate used by Lerner et al. (2011) in Hawaiian honeycreepers.

BEAST analyses were run for 10 million generations, sampled every 1000 generations, and had a burn-in of 100,000 generations. Output files were viewed with Tracer v1.5 (Rambaut and Drummond 2007) to estimate time to most recent common ancestor.

## Results

*Ecological niche modeling*

The model distributions had an area under curve value of 0.756 (LGM) and 0.757 (LIG), and both training and test sample omission curves were close to the predicted value. The high area under the curve values and training/omission curves indicate the models performed well (Elith 2000). Present day black-capped chickadee distribution as predicted by MAXENT using location information and present day environmental variables matched the current distribution (Ridgely et al. 2007; Figure 2a). MAXENT predicted a large range contraction for black-capped chickadees 21 kya (Figure 2b), and identified four primary areas of suitable climate: Newfoundland, the central and southeastern United States, western United States (specifically California and Oregon), and Alaska. By comparison, MAXENT predicted greater tracts of suitable climate for black-capped chickadees ~120-140 kya (Figure 2b), and the range of black-capped chickadees at this time was comparable to its current range (Figure 2c). For example, southeastern Rocky Mountain populations were relatively isolated from the west coast and eastern United States.

Further the highest MAXENT predictions for suitable black-capped chickadee climate in the LIG were along the west coast, the central plains of North America, and Alaska.

*Genetic diversity*

Black-capped chickadees exhibited high levels of mtDNA genetic diversity. Haplotype diversity ranged from 0.56 (Alaska-Fairbanks) to 1.00 (coastal Oregon, West Virginia and North Carolina) and nucleotide diversity ranged from 0.001 (Alaska-Fairbanks) to a high of 0.026 (North Carolina; Table 1). Overall, we found 198 black-capped chickadee haplotypes, including 65 shared haplotypes (i.e., haplotypes found in more than one individual), with a total of 118 variable sites in 633 black-capped chickadees. Outside of these 198 haplotypes, we identified 9 additional individuals with Carolina chickadee haplotypes. Putative hybrids were collected in Missouri (n = 3), West Virginia (n = 4), and North Carolina (n = 2); all of which are located in known hybrid zones and were removed from all black-capped chickadee analyses.

*Population structure*

The TCS network illustrates the high genetic diversity in black-capped chickadees and the close genetic relationship amongst continental populations (Figure 3). The maximum likelihood tree produced similar results to our TCS network (Figure 4); the only monophyletic group in our analysis was Newfoundland as all other groups shared one or more haplotypes with another group. Despite the close relationship, we do see some clustering of populations in western North America; a pattern further depicted by our PCoA (Figure 5). For example southwest Rocky, Pacific Northwest, and Alaska populations all clustered apart from other

262 populations. Both TCS and PCoA indicate that Newfoundland is distinct from continental
263 populations.

264 Pairwise $\Phi_{ST}$ values revealed significant differentiation among black-capped chickadee
265 populations (Supplemental Table 2). Of the 528 pairwise comparisons, all but 80 pairwise
266 comparisons were significant following FDR correction and 77 of these comparisons that were
267 insignificant involved populations within the main-northeast group. The remaining three non-
268 significant $\Phi_{ST}$ values were within the Alaska populations (Alaska-Anchorage and-Alaska-
269 Wrangler and Alaska-Anchorage and Alaska-Fairbanks) and between Kansas (one of the
270 southeast Rocky group populations) and the adjacent Missouri population. The highest $\Phi_{ST}$
271 values involved comparisons with NL and other populations ($\Phi_{ST}$ = 0.63-0.87, $p < 0.001$). High
272 levels of genetic differentiation were also detected in the Pacific Northwest, southeastern
273 Rockies, and near the Alaska panhandle defining three of the four continental groups (Pacific,
274 southeast Rockies, and Alaska). Differences among the southeast Rockies and the main-northeast
275 groups were also present, though relatively lower than among the other groups.

276 Hierarchical AMOVA analyses of all populations indicated the presence of two main
277 groups (Newfoundland and all remaining continental populations; $\Phi_{CT} = 0.50$, $p$=0.04). The next
278 highest grouping suggested one panmictic population ($\Phi_{CT} = 0.32$, $p$=0.001), but this grouping
279 explained almost 20% less of the variation than our two group model. Our analysis of continental
280 populations indicated four separate groups ($\Phi_{CT} = 0.27$, $p$<0.001): Alaska, Pacific Coast,
281 southeast Rocky Mountain, and the main-northeast.

282 Analysis using the GIS toolbox supported the presence of multiple groups (Figure S2).
283 The heat map produced from pairwise $\Phi_{ST}$ values show the isolation of Newfoundland, Alaska
284 and the Pacific. The southeast Rockies populations (Utah, Colorado and Kansas) also show

moderate isolation. Mantel tests found a significant correlation between geographic and genetic distances for our analysis of all populations ($r = 0.152$, $p = 0.01$) and our analysis among all continental populations (i.e. excluding Newfoundland; $r = 0.115$, $p = 0.01$). Two of the five barriers identified by BARRIER separated Newfoundland from the two adjacent continental populations (Labrador and Nova Scotia/New Brunswick). The next two barriers separated southeast Rocky populations (Colorado, Utah, and Kansas) from the Pacific Coast (Washington and Southern Oregon) and main-northeast populations. The final barrier identified, separated Pacific Coast populations from interior British Columbia populations and populations east of the Cascade Mountains (Figure 1).

*Divergence estimates*

Divergence estimates between Newfoundland and all continental populations ranged from ~10 kya (using a 12.1% substitution rate) to 331 kya (using a 3% substitution rate; Table 2). BEAST results from Bayesian skyline analysis for both substitution rates revealed a time to most recent common ancestor of 19.1 kya (HPD 12.7-26.4 kya using the 12.1% substitution rate) and 76.5 kya (HPD 51.5-107.7 kya using the 3% substitution rate).

## Discussion

Ecological niche modeling predicted four potential areas of suitable habitat for black-capped chickadees following extensive range retractions during the LGM. Combined with genetic analyses, these results support the multiple refugia hypothesis. In combination with Pleistocene glaciations, our study suggests physical barriers restrict gene flow among populations.

*Pleistocene refugia and postglacial expansion*

Paleodistribution modeling predicted a considerable reduction in suitable habitat availability for black-capped chickadee at the LGM. The majority of habitat was located in the southern mid-latitude portion of the continent, consistent with the hypothesis that glacial refugia persisted south of the North American ice sheets, although some habitat appears to have been available in Beringia, and off the coast of Newfoundland. Genetic data also suggest prolonged isolation of the continental and Newfoundland chickadee populations and more recent divergence among continental populations. Our results indicate that the present-day Newfoundland lineage has been isolated for at least 19-66 ky and contains diverse and unique haplotypes absent from the continental populations. While the exact location of the refugium is debatable, previous studies have suggested the now submerged coastal shelf off Newfoundland as a refugium. Studies on the American redstart (*Setophaga ruticilla*, Linnaeus 1758), boreal chickadee (*Poecile hudsonicus*, Forster 1772) and blackpoll warbler (*Setophaga striata,* Forster 1772) whose ranges overlap extensively with black-capped chickadees suggest the presence of an Atlantic Coast refugium (Colbeck et al. 2008; Lait and Burg 2013; Ralston and Kirchman 2013). These results are in contrast to the single refugium hypothesis for black-capped chickadees proposed by Gill et al. (1993).

Within the continental populations, the origins of the four groups (Pacific, Alaska, southeast Rockies and main-northeast) are less certain. Refugia existed in each of these areas and while it is possible individuals survived in multiple refugia, the divergence among these four groups is more recent than the split observed between continental populations and Newfoundland. Patterns of genetic differentiation are consistent with multiple refugia along the Pacific Coast, in the southern Rocky Mountains, and in the eastern United States (Figure 1), and

suitable climate for black-capped chickadee was present in each of these areas during the LGM (Figure 2a). Postglacial dispersal of a few individuals from a single continental refugium could have been responsible for establishing new populations in some areas along with the reduced genetic variation; however, we believe recolonization from a single continental refugium is unlikely for several reasons. First, of the 49 haplotypes in southeast Rockies, Alaska, and Pacific groups, 39 are restricted to these three groups (14 AK, seven Pacific, and 18 southeast Rockies). With the extensive sampling (n = 444 individuals from the main-northeast group), if they were common in other parts of the range, they should have been detected. Second, most of the haplotypes in these three groups cluster together, not with haplotypes from the main-northeast group, suggestive of long-term isolation. Predictions for the LIG (Figure 2b) indicate suitable climate for black-capped chickadee was present, with large areas of suitable climate in Alaska, western North America, eastern North America, and Newfoundland. Descendants of the Pacific, Alaska, and southeast Rockies groups could have originated from a second common continental refugium with subsequent bottlenecks or lineage sorting explaining the low number of shared haplotypes. A number of other studies have found similar patterns of population structure particularly in western North America and suggest multiple western refugia including Alaska/Beringia and the Pacific Northwest (Boulet and Gibbs 2006; Spellman and Klicka 2007; Graham and Burg 2012; Lait et al. 2013; Dohms et al. 2017).

*Population structure and modern barriers to gene flow*

Rangewide patterns of population genetic structure in the black-capped chickadee suggest a number of factors are influencing population structure including modern barriers to gene flow. The divergent population of black-capped chickadees on the island of Newfoundland is likely the

result of prolonged isolation and restricted dispersal. The mainland population is ~ 20 km away on Labrador in marginal black-capped chickadee habitat, and the next closest "mainland" point is Cape Breton Island, Nova Scotia ~100 km across the Cabot Strait. In combination with previous studies examining microsatellite variation (Adams and Burg 2014), the absence of shared haplotypes and restricted geographic distribution of Newfoundland haplotypes indicate limited maternal gene flow between Newfoundland and continental populations.

Among continental populations we found evidence of four genetic clusters: Alaska, Pacific, southeast Rockies, and the remaining continental populations (main-northeast group). Landscape patterns as revealed by GIS toolbox indicate low to moderate levels of gene flow among all four groups. The landscape genetic patterns reveled for mtDNA patterns mirror those found by Adams and Burg (2014) in their analysis of range-wide genetic structure in black-capped chickadee using microsatellites. The maintenance of genetic structure in both studies suggests limited dispersal among black-capped chickadee populations. For example the location of the Cascade Mountains in the Pacific Northwest corresponds to the isolation of the Pacific group (Washington, central Oregon and southern Oregon) from the main-northeast (including northeast Oregon and Idaho) mtDNA groups. Similarly a number of tall mountains surround the three genetically distinct Alaskan populations in the northwest portion of the range. Despite the correspondence between genetic breaks and the location of mountains for Alaska and the Pacific group, the Rocky Mountains do not appear to restrict gene flow among northern populations separated by mountain ranges (i.e. interior British Columbia). In this area, low valleys exist such as the northern and southern Rocky Mountain trench, which could facilitate dispersal.

We observed a significant isolation by distance signal. Although the non-migratory behaviour and limited dispersal exhibited by black-capped chickadees contributes to this pattern

(Desrochers and Hannon 1997), other environmental factors also contribute to the observed genetic boundaries. In particular habitat fragmentation appears to significantly reduce gene flow for black-capped chickadees. For example, Adams and Burg (2014) found that large gaps in forested habitat associated with Great Plains and Basins separating southern Rocky Mountain populations from northern populations reduced range-wide gene flow. Similar to our study, previous research has attributed the genetic breaks in this region with habitat fragmentation by these two modern barriers to gene flow (Hafner and Sullivan 1995; Hull et al. 2008; Galbreath et al. 2010; Graham and Burg 2012; Dohms et al. 2017). Despite being viewed as a generalist, small-scale habitat changes also influence gene flow for black-capped chickadees. This species relies on woodland corridors to disperse between areas and the removal or fragmentation of these corridors increases genetic differentiation (Adams et al. 2014; Adams and Burg 2015).

Genetic differentiation observed in black-capped chickadees resembles that observed for other forest dwelling resident North American bird species with broad distributions (Spellman and Klicka 2007; Manthey et al 2011; Graham and Burg 2012, Dohms et al. 2017, although see Pulgarin and Burg 2013). In comparison to resident species with broad distributions, migratory species appear to exhibit lower levels of population genetic structure (Ball et al. 1988; Milá et al. 2006; 2007; Colbeck et al. 2008), although there are exceptions (Milot et al. 2008; Macfarlane et al. 2016). These differences reflect the different life histories exhibited by North American avian species (Zink 1996). For example migratory behaviour has been shown to increase gene flow (Arguedas and Parker 2000). Second genetic patterns reflect patterns of isolation during the LGM; the majority of migratory species were isolated in a single refugium while many resident species show evidence of being isolated in multiple refugia during the LGM and genetic patterns reflect post-glacial expansion from these refugia (Weir and Schluter 2004).

*Conclusions*

Patterns of population genetic differentiation in the black-capped chickadee are consistent with late Pleistocene vicariance events and isolation in at least two refugia; Newfoundland and at least one refugium in the southern United States. In the west, the potential for black-capped chickadees being isolated in three additional refugia is also likely: Pacific, southeast Rockies, and Alaska. The high levels of population substructure in this species suggest that physical barriers limit gene flow across the range, and highlight the complex biogeographic factors that shape genetic patterns in natural populations.

**Funding**

This work was supported by Alberta Innovates Technologies Futures New Faculty Award and Natural Science and Engineering Research Council Discovery Grant.

**Acknowledgments**

We are grateful to the following institutions for providing additional samples for this study: University of Michigan Museum of Natural History, Smithsonian Museum of Natural Science, Chicago Field Museum, North Carolina Museum of Natural Science, Queens University Biological Station. Michael Braun, Brian Davidson, Jenn Foote, Angelique Grava, Ken Otter, and Tim Roth III kindly provided Carolina chickadee samples. We thank Mark Miller, Project FeederWatch, the National Park Service, US Fish and Wildlife Service and Canadian Wildlife Service for their help. We thank the members of the Burg Lab at the University of Lethbridge for their assistance both in the field and lab, including Kim Dohms, Linda Lait, Paulo Pulgarin, and numerous field assistants who helped with sample collection. Kim Dohms provided helpful

advice on the ENM. The research presented here was conducted under University of Lethbridge Animal Welfare Protocol No. 0614 in accordance with Canadian Council on Animal Care guidelines.

## References


Adams, R. V, and Burg, T.M. 2014. Influence of ecological and geological features on rangewide patterns of genetic structure in a widespread passerine. Heredity, **114**(2): 143–154. doi:10.1038/hdy.2014.64.

Adams, R. V, and Burg, T.M. 2015. Gene flow of a forest-dependent bird across a fragmented landscape. PLoS One, **10**(11): 1–22. doi:10.1371/journal.pone.0140938.

Arguedas, N., and Parker, P.G. 2000. Seasonal migration and genetic population structure in house wrens. Condor, **102**: 517–528.

Baker, A.J., and Marshall, H.D. 1997. Mitochondrial control region sequences as tools for understanding evolution. *In* Avian Molecular Evolution and Systematics. *Editted by* D.P. Mindell. New York, Academic Press. pp. 51-82.

Ball, R.M., Freeman, S., James, F.C., Bermingham, E., and Avise, J.C. 1988. Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. Proc. Natl. Acad. Sci. U. S. A. **85**(5): 1558–1562.

Barrowclough, G.F., Groth, J.G., Mertz, L.A., and Gutiérrez, R.J. 2004. Phylogeographic structure, gene flow and species status in blue grouse (*Dendragapus obscurus*). Mol. Ecol. **13(7)**: 1911–1922.

Benjamini, Y., and Yekutiele, D. 2001. The control of the false discovery rate in multiple testing under dependency. Ann. Stat. **29**(4): 1165–1188.

Bennett, K.D., and Provan, J. 2008. What do we mean by 'refugia'?. Q. Sci. Rev. ***27***(27): 2449-2455.

Boulet, M., and Gibbs, H.L. 2006. Lineage origin and expansion of a Neotropical migrant songbird after recent glaciation events. Mol. Ecol. **15**(9): 2505–25. doi:10.1111/j.1365-294X.2006.02956.x.

Burg, T.M., and Croxall, J.P. 2001. Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. Mol. Ecol. **10**(11): 2647–60.

Clement, M., Posada, D., and Crandall, K.A. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9**:1657-1659.

Colbeck, G.J., Gibbs, H.L., Marra, P.P., Hobson, K., and Webster, M.S. 2008. Phylogeography of a widespread North American migratory songbird (*Setophaga ruticilla*). J. Hered. **99**(5): 453–463. doi:10.1093/jhered/esn025.

459 Desrochers, A., and Hannon, S.J. 1997. Gap crossing decisions by forest songbirds during the
460 post-fledgling period. Conserv. Biol. **11**(5): 1204–1210.

461 Dohms, K.M., Graham, B.A., and Burg, T.M. 2017. Multilocus genetic analyses and spatial
462 modeling reveal complex population structure and history in a widespread resident North
463 American passerine (*Perisoreus canadensis* ). Ecol. Evol. **7**(23) 9869–9889.
464 doi:10.1002/ece3.3478.

465 Drummond, A J., Rambaut, A, Shapiro, B., and Pybus, O.G. 2005. Bayesian coalescent inference
466 of past population dynamics from molecular sequences. Mol. Biol. Evol. **22**(5): 1185–
467 1192. doi:10.1093/molbev/msi103.

468 Drummond, A.J., Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees.
469 BMC Evol. Biol. **7**:214.

470 Elith, J. 2000. Quantitative methods for modeling species habitat: Comparative performance and
471 an application to Australian plants. *In* Quantitative Methods for Conservation Biology.
472 Springer, New York, NY Excoffier, pp. 39-58.

473 Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin (version 3.0): An integrated software
474 package for population genetics data analysis. Evol. Bioinform. Online **1**: 47–50.
475 doi:10.1111/j.1755-0998.2010.02847.x.

476 Foote, J.R., Mennill, D.J., Ratcliffe, L.M., and Smith, S.M. 2010. Black-capped Chickadee
477 (*Poecille atricapillus*), version 2.0. *In* the Birds of North America. *Editted by* P.G.
478 Rodewald. Cornell Lab of Ornithology, Ithaca, New York, USA.
479 https://doi.org/10.2173/bna.39.
480
481 Fourcade, Y., Engler, J.O., Rödder, D., and Secondi, J. 2014. Mapping species distributions with
482 MAXENT using a geographically biased sample of presence data: a performance
483 assessment of methods for correcting sampling bias. PLoS One 9: e97122.
484
485 Galbreath, K.E., Hafner, D.J., Zamudio, K.R. and Agnew, K. 2010. Isolation and introgression in
486 the Intermountain West: contrasting gene genealogies reveal the complex biogeographic
487 history of the American pika (*Ochotona princeps*). J. Biogeogr. **37**(2): 344-362.

488 Gill, F.B., Mostrom, A.M., and Mack, A.L. 1993. Speciation in North American chickadees: I.
489 patterns of mtDNA genetic divergence. Evolution. **47**: 195–212.

490 Gill, F.B., Slikas, B. and Sheldon, F.H., 2005. Phylogeny of titmice (Paridae): II. Species
491 relationships based on sequences of the mitochondrial cytochrome-b gene. Auk ***122***(1):
492 121-143.

493 Graham, B.A., and Burg, T.M. 2012. Molecular markers provide insights into contemporary and
494 historic gene flow for a non-migratory species. J. Avian Biol. **43**(3): 198–214.
495 doi:10.1111/j.1600-048X.2012.05604.x.

Hafner, D.J. and Sullivan, R.M. 1995. Historical and ecological biogeography of Nearctic pikas (Lagomorpha: Ochotonidae). *J. Mammal*. **76**(2): 302–321.

Hasumi, H., and Emori, S. 2004. K-1 coupled GCM (MIROC) description. Tech. Report 1. Tokyo: Center for Climate System Research, University of Tokyo.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature. **405**(6789): 907–913. http://doi.org/10.1038/35016000.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., and Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. **25**(15): 1965–1978. doi:10.1002/joc.1276.

Hull, J.M., Hull, A.C., Sacks, B.N., Smith, J.P., and Ernest, H.B. 2008. Landscape characteristics influence morphological and genetic differentiation in a widespread raptor (*Buteo jamaicensis*). Mol. Ecol. **17**(3): 810–824. doi:10.1111/j.1365-294X.2007.03632.x.

Keppel, G., Van Niel, K.P., Wardell-Johnson, G.W., Yates, C.J., Byrne, M., Mucina, L., Schut, A.G.T., Hopper, S.D., and Franklin, S.E. 2012. Refugia: Identifying and understanding safe havens for biodiversity under climate change. Glob. Ecol. Biogeogr. **21**(4): 393–404. doi:10.1111/j.1466-8238.2011.00686.x.

Lait, L.A., and Burg, T.M. 2013. When east meets west: population structure of a high-latitude resident species, the boreal chickadee (*Poecile hudsonicus*). Heredity: 1–9. doi:10.1038/hdy.2013.54.

Lerner. H. L. Meyer, M., James, H.F., Horfreiter, M., Fleischer, R.C. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. Curr. Biol. **21**(21): 1838–1846.

Levsen, N.D., Tiffin, P., and Olson, M.S. 2012. Pleistocene speciation in the genus *Populus* (salicaceae). Syst. Biol. **61**(3): 401–412. doi:10.1093/sysbio/syr120.

Librado, P., and Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25**(11): 1451–1452. doi:10.1093/bioinformatics/btp187.

Macfarlane, C.B.A., Natola, L., Brown, M.W., and Burg, T.M. 2016. Population genetic isolation and limited connectivity in the purple finch (*Haemorhous purpureus*). Ecol. Evol. **6**(22): 8304–8317. doi:10.1002/ece3.2524.

Manni, F., Guéard, E., and Heyer, E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: How barriers can be detected using Monmomier's Algorithm. Hum. Biol. **76**(2): 173–190.

Manthey, J.D., Klicka, J., and Spellman, G.M. 2011. Cryptic diversity in a widespread North American songbird: phylogeography of the brown creeper (*Certhia americana*). Mol. Phylogenet. Evol. **58**(3): 502–512. doi:10.1016/j.ympev.2010.12.003.

Milá, B., Smith, T.B., and Wayne, R.K. 2006. Postglacial expansion drives the evolution of long-distance migration in a songbird. Evolution (N. Y). **60**(11): 2403–2409.

Milá, B., McCormack, J.E., Castañeda, G., Wayne, R.K., and Smith, T.B. 2007. Recent postglacial range expansion drives the rapid diversification of a songbird lineage in the genus *Junco*. Proc. Biol. Sci. **274**(1626): 2653–60. doi:10.1098/rspb.2007.0852.

Milot, E., Gibbs, H.L., and Hobson, K.A. 2000. Phylogeography and genetic structure of northern populations of the yellow warbler (*Dendroica petechia*). Mol. Ecol. **9**(6): 667–681.

Otto-Bliesner, B. L., Marshall, S. J., Overpeck, J. T., and Miller, G. H. 2006. Simulating polar amplification of orbital forcing for the last interglacial. Science. 311: 1751–1753.

Pielou EC. 1991. After the Ice Age: the Return of Life to Glaciated North America. Chicago: University of Chicago Press.

Peakall, R., and Smouse, P.E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes **6**(1): 288–295. doi:10.1111/j.1471-8286.2005.01155.x.

Phillips, S.J., Anderson, R.P., and Schapire, R.E. 2006. Maximum entropy modeling of species geographic distributions. Ecol. Modell. **190**: 231–259. doi:10.1016/j.ecolmodel.2005.03.026.

Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., Raven, P.H., Roberts, C.M., and Sexton, J.O. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science **344**(6187): 1246752–1246752. doi:10.1126/science.1246752.

Pravosudov, V. V., Roth, T.C., Forister, M.L., LaDage, L.D., Burg, T.M., Braun, M.J., and Davidson, B.S. 2012. Population genetic structure and its implications for adaptive variation in memory and the hippocampus on a continental scale in food-caching black-capped chickadees. Mol. Ecol. **21** (18): 4486-4497. doi:10.1111/j.1365-294X.2012.05721.x.

Ralston, J., and Kirchman, J.J. 2013. Predicted range shifts in North American boreal forest birds and the effect of climate change on genetic diversity in blackpoll warblers (*Setophaga striata*). Conserv. Genet. **14**(2): 543–555. doi:10.1007/s10592-012-0418-y.

Rambaut, A. and Drummond, A.J. 2007. Tracer Version 1.5. [online]. *Available from* http.beast.bio.ed.ac.uk/Tracer.

Ridgley, R.S., Allnut, T.F., Brooks, T. McNicol, K.D., Mehlman, D.W., Young, B.E., and Zook, J.R. 2007. Digital distribution maps of the birds of the western hemisphere, version 3.0. Natureserve. Arlington, Virginia. www.natureserve.org/getData/birdMaps.jsp

Rousset, F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics. **142**:1357-1362.

Ruegg, K.C. 2008. Genetic, morphological, and ecological characterization of a hybrid zone that spans a migratory divide. Evolution (N. Y). **62**(2): 452–466.

Spellman, G.M., and Klicka, J. 2007. Phylogeography of the white-breasted nuthatch (*Sitta carolinensis*): diversification in North American pine and oak woodlands. Mol. Ecol. **16**(8): 1729–1740. doi:10.1111/j.1365-294X.2007.03237.x.

Syfert M.M., Smith, M.J., and Coomes, D.A. 2013. The effects of sampling bias and model complexity on the predictive performance of MaxEnt species distribution models. PLoS ONE 8: e55158.

Taberlet, P., and Cheddadi, R. 2002. Quaternary refugia and persistence of biodiversity. Science. **297**(5589): 2009.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. **28**(10): 2731–2739. doi:10.1093/molbev/msr121.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., De Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M. A, Peterson, A.T., Phillips, O.L., and Williams, S.E. 2004. Extinction risk from climate change. Nature **427**(6970): 145–148. doi:10.1038/nature02121.

Vandergast, A.G., Perry, W.M., Lugo, R.V., Hathaway, S.A. 2011. Genetic landscapes GIS Toolbox: tools to map patterns of genetic divergence and diversity. Mol. Ecol. Res. **11**:158-161.

Walsh, P.S., Metzger, D.A. and Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**(4): 506-513.

Waltari, E., Hijmans, R.J., Peterson, A.T., Ban, L., Perkins, S.L., and Guralnick, R.P. 2007. Locating Pleistocene refugia : Comparing phylogeographic and ecological niche model predictions. PLoS One **2**(7): e563. doi:10.1371/journal.pone.0000563.

Warren, D. L., Glor, R. E., and Turelli, M. 2010. ENMTools: A toolbox for comparative studies of environmental niche models. Ecography. **33**: 607–611.

599 Weir, J.T., and Schluter, D. 2004. Ice sheets promote speciation in boreal birds. Proc. R. Soc.
600 London. Ser. B Biol. Sci. **271**(1551): 1881-1885. doi:10.1098/rspb.2004.2803.

601 Zink, R.M. 1996. Comparative Phylogeography in North American Birds. Evolution (N. Y).
602 **50**(1): 308–317.

603

## Figure Captions and Table Legends

**Figure 1:** Map depicting the contemporary distribution (red shading) of black-capped chickadees (*Poecille atricapillus*) in in North America (Ridgely 2007). Pie-charts represent the distribution of haplotypes from each mtDNA groups at a given population. The five genetic groups are depicted with the following coulours: purple (Alaska), blue (Pacific Coast), red (southeast Rockies), white (main-northeast) and yellow (Newfoundland). Dark blue lines on the figure represent the five first-order barriers to dispersal identified using BARRIER. Two barriers separated Newfoundland from continental populations in Labrador and Nova Scotia/ New Brunswick. Additionally two barriers separate Pacific Coast populations; the first separates Pacific Coast populations from northern populations in Canada and the second separates Pacific Coast populations from populations east of the Cascade Mountain range. Abbreviations for sampling sites (grey circles) are provided in Table 1.

**Figure 2:** Map of paleodistribution model showing suitable black-capped chickadee (*Poecille atricapillus*) habitat at the LGM ~21 kya (a) and at the LIM ~120-140 kya (b). (c) contemporary distribution. Warmer colours (red, orange and yellow) indicate higher climate suitability.

**Figure 3:** Statistical parsimony network (TCS) of chickadee mitochondrial haplotypes. Small open circles represent haplotypes found in a single individual, small black circles are inferred haplotypes and ovals are shared haplotypes. The size of the oval is proportional to the number of individuals sharing that haplotype. Haplotypes are colour-coded corresponding to genetic clusters as determined by PCoA (see Figure 4). Both Carolina chickadees (*Poecille carolinensis*; orange) and black-capped (*Poecille atricapillus*; main-northeast: white, southeast Rockies: red, Pacific: blue, Alaska: purple, and Newfoundland: yellow) chickadee haplotypes are shown. Refer

to Figure 1 for location of sampling sites and abbreviations. Lines representing more than one mutational step are indicated with a double slash and number. Lines show the shortest number of steps between haplotypes; for these three connections, alternate connections are possible.

**Figure 4:** Principal coordinates analysis (PCoA) of mtDNA sequences (pairwise $F_{ST}$) for all black-capped chickadee (*Poecille atricapillus*) populations (n > 5); coordinate 1 explains 37.7% of the variation, coordinate 2 18.1% and coordinate 3 15.9%. Colours correspond to major groups: NL (yellow), SE Rockies (red), Pacific (blue), AK (purple), and main-northeast (white).

**Figure 5:** Maximum likelihood tree of black-capped chickadee (*Poecille atricapillus*) haplotypes with bootstrap values greater than 40% shown (1000 bootstraps). Colours match those coulours used in the minimum spanning network in Figure 3. Newfoundland is the only monophyletic group (44% bootstrap support). Carolina chickadee (green rectangles) sequences were used as the outgroup with 100% bootstrap separation from black-capped chickadee. Black rectangles denote shared haplotypes (all other haplotypes were unique to a specific group). Bottom line indicates the number of substitutions per site, $n = 0.01$.

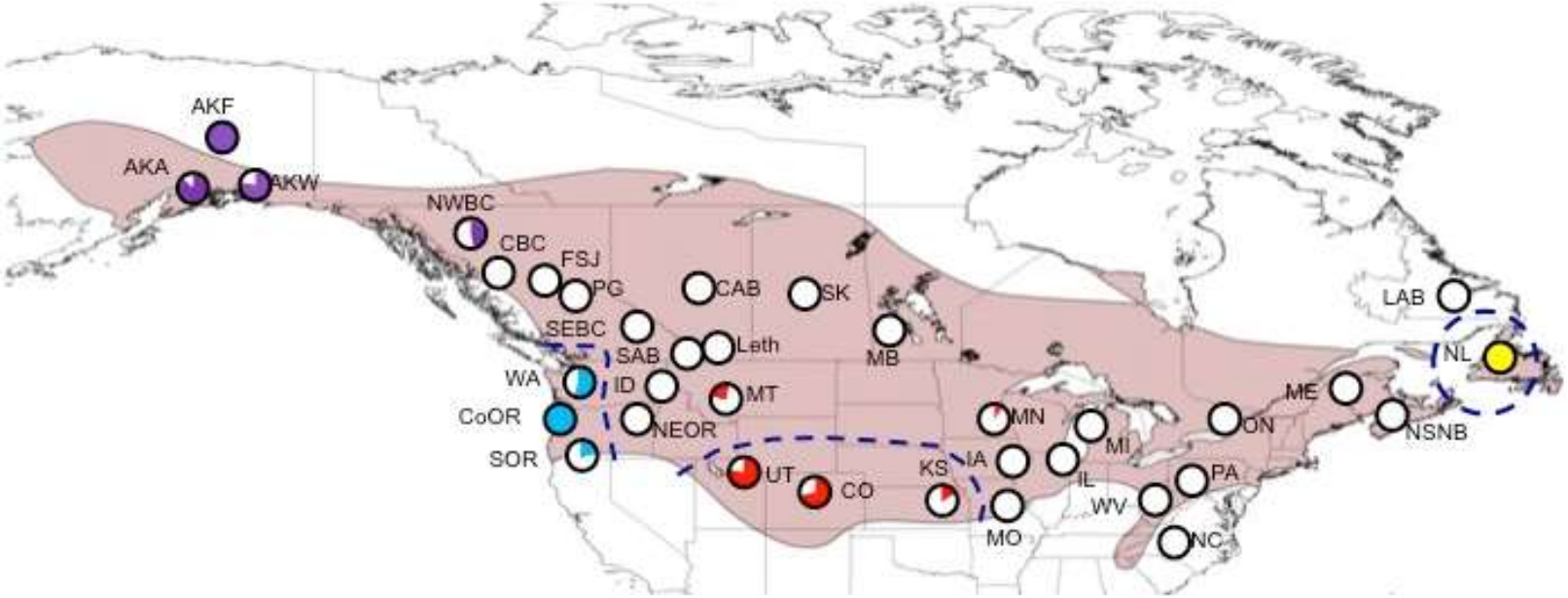
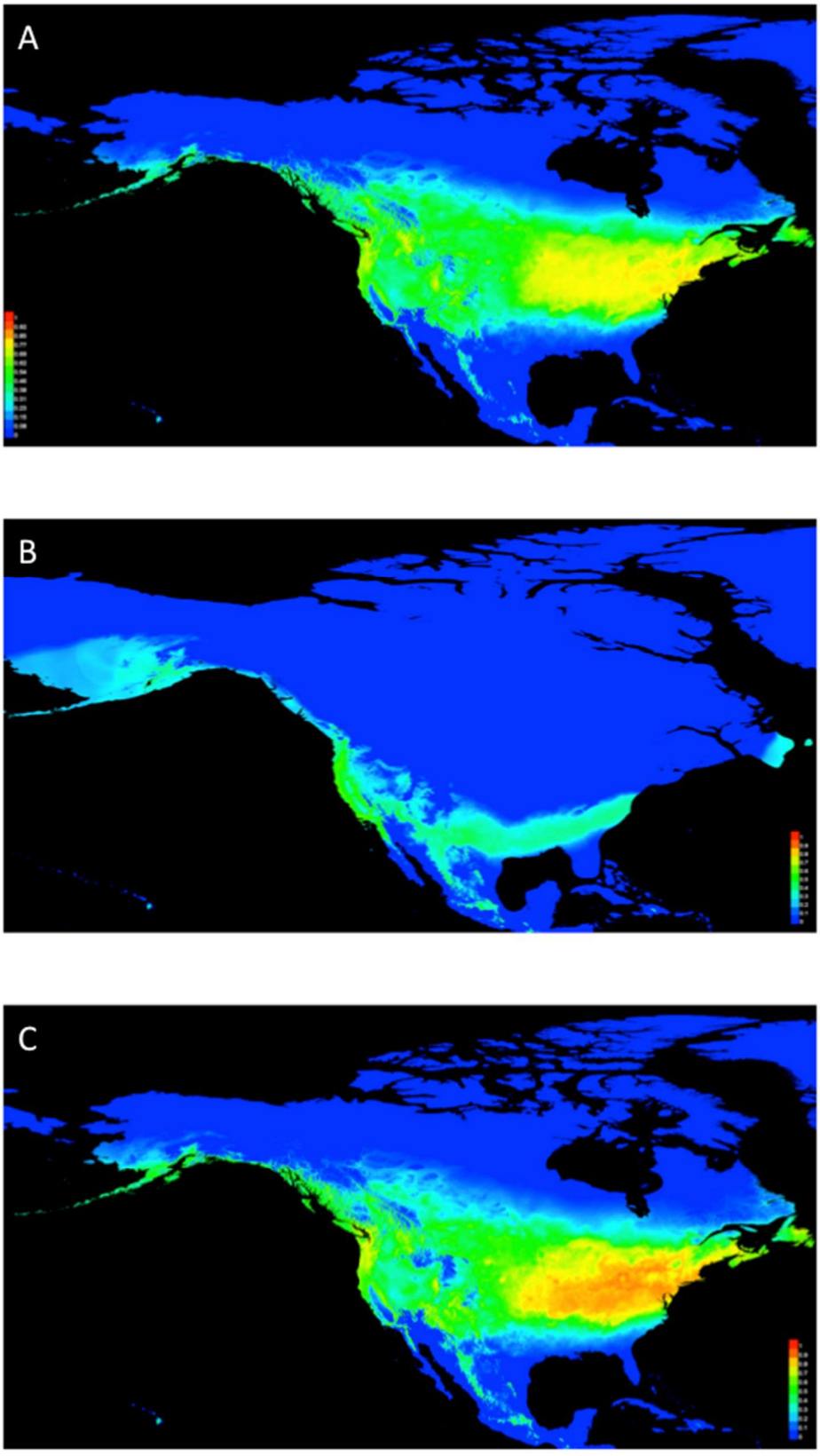
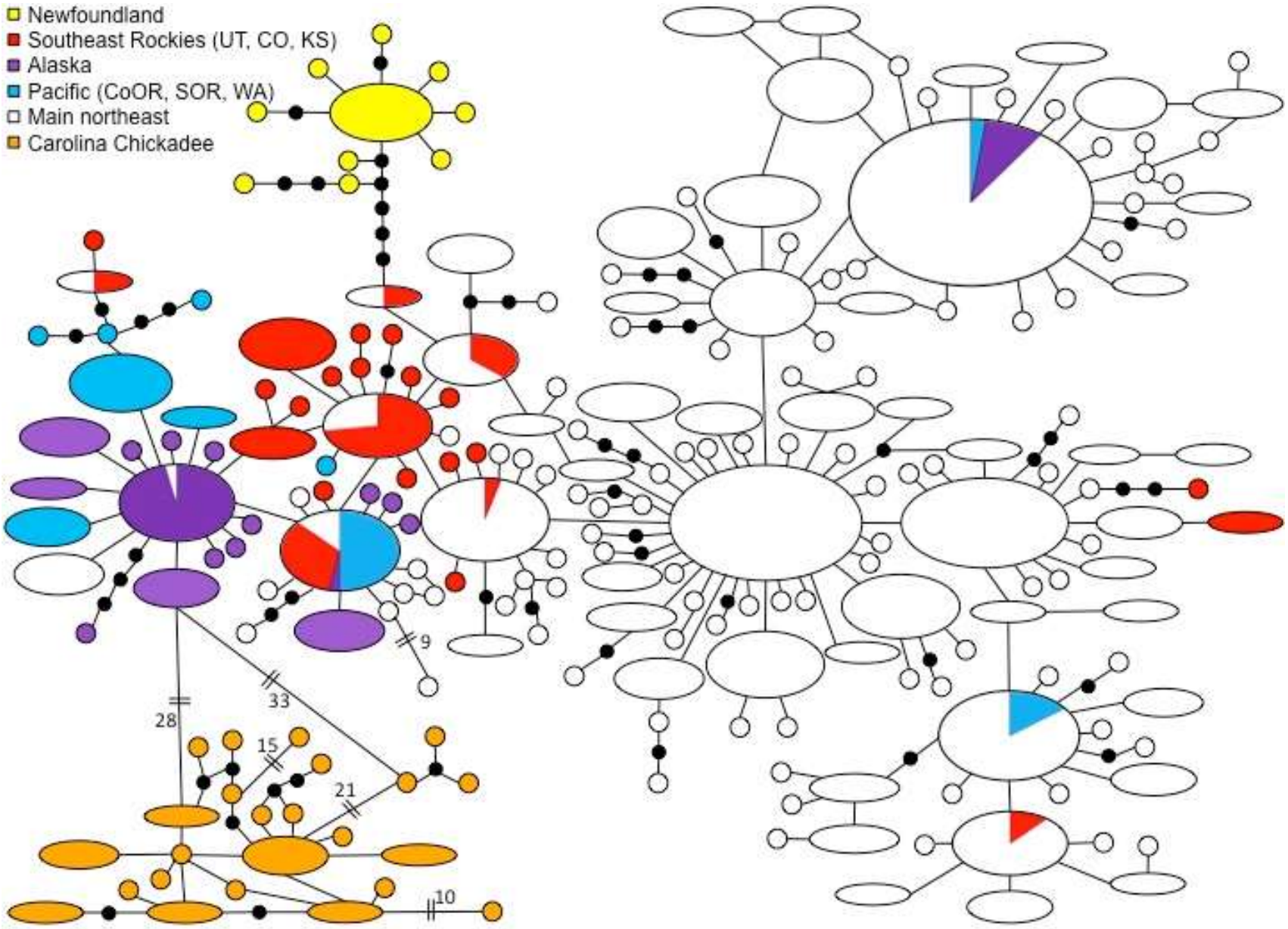
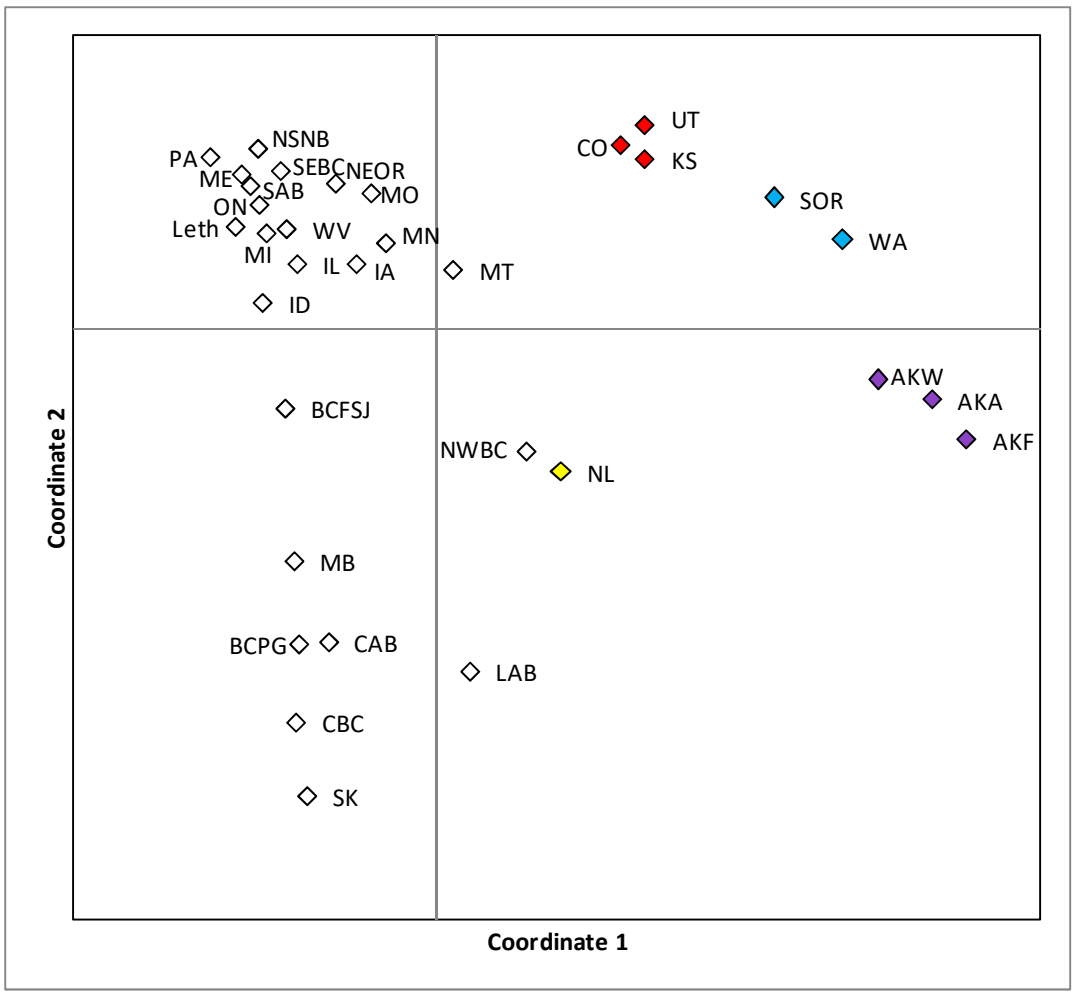

AKF
AKA
AKW
NWBC
CBC
FSJ
PG
CAB
SK
MB
LAB
NL
SEBC
SAB
Leth
WA
ID
MT
CoOR
NEOR
SOR
UT
CO
KS
IA
MN
MI
IL
WV
MO
PA
ON
ME
NSNB
NC

Figure 1


A
B
C


Newfoundland
Southeast Rockies (UT, CO, KS)
Alaska
Pacific (CoOR, SOR, WA)
Main northeast
Carolina Chickadee
9
33
28
15
21
10


UT
CO
KS
SOR
WA
NSNB
PA
SEBC
NEOR
ME
SAB
MO
ON
Leth
WV
MN
MI
IL
IA
MT
ID
AKW
AKA
AKF
BCFSJ
NWBC
NL
MB
BCPG
CAB
LAB
CBC
SK
Coordinate 2
Coordinate 1

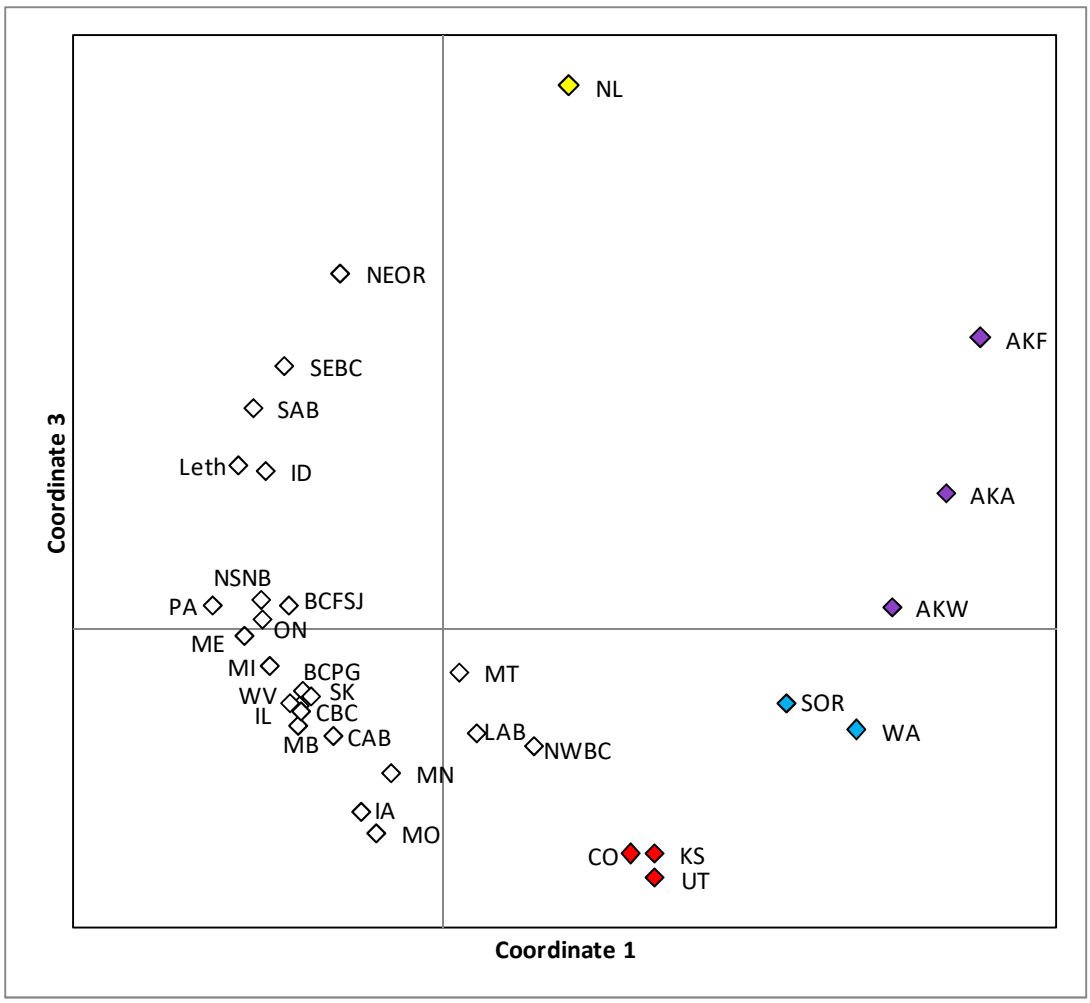

NL
NEOR
AKF
SEBC
SAB
Leth
ID
AKA
NSNB
PA
BCFSJ
ON
AKW
ME
MI
BCPG
MT
WV
SK
SOR
IL
CBC
LAB
WA
MB
CAB
NWBC
MN
IA
MO
CO
KS
UT
Coordinate 3
Coordinate 1

Figure 4

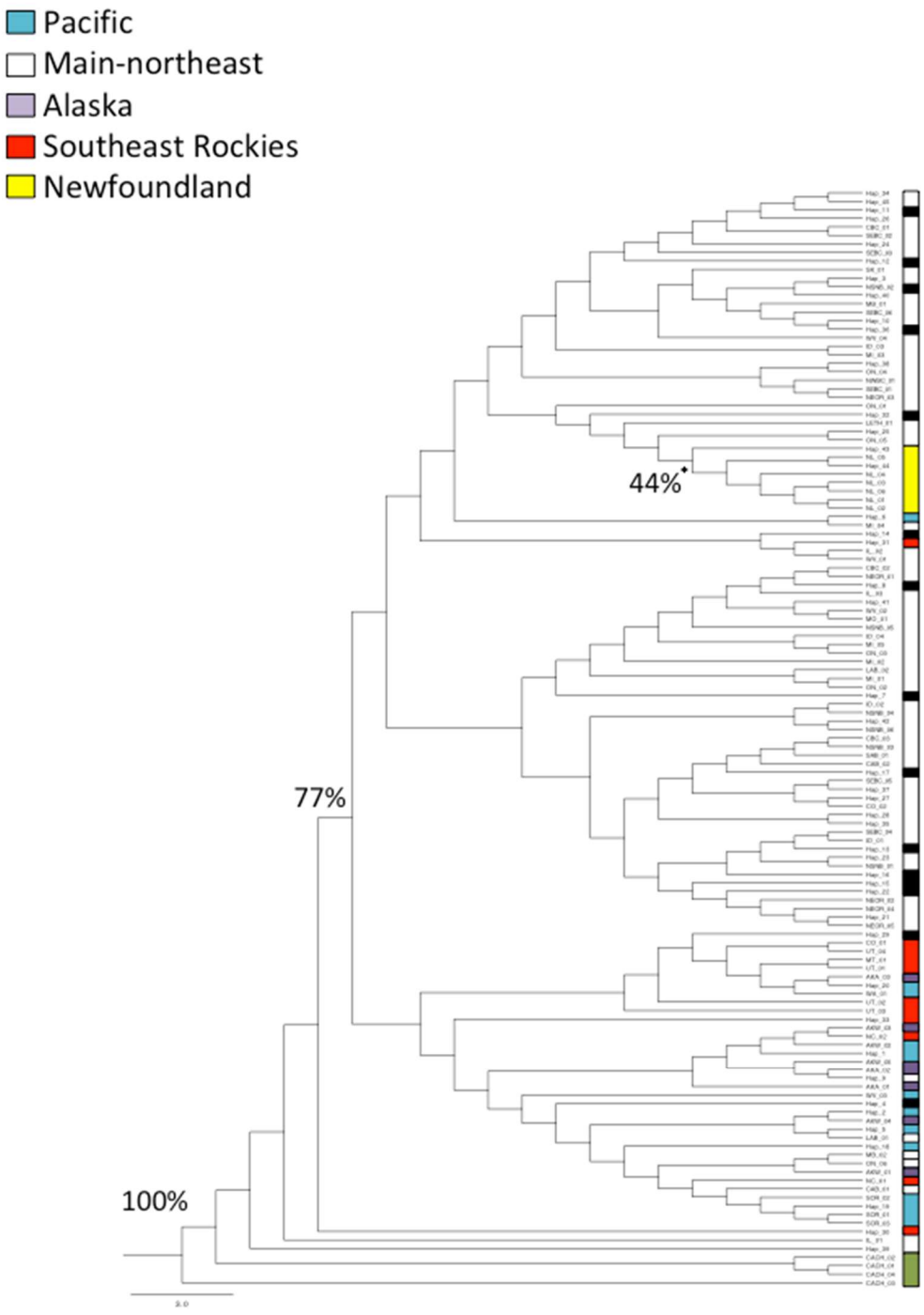

Pacific
Main-northeast
Alaska
Southeast Rockies
Newfoundland
44%
77%
100%

**Table 1**: Sampling site information for black-capped chickadee (*Poecille atricapillus*) and Carolina chickadee (*Poecille carolinensis;* CACH) chickadee samples. Sample size (N), number of haplotypes (Hn), haplotype diversity (Hd) and nucleotide diversity ($\pi$) for each sampling site as well as group identity (mt group). Nine black-capped chickadees with Carolina chickadee mtDNA (MO, $n = 3$, WV, $n = 4$; and NC, $n = 2$) are not included.

| sampling site | abbrev. | N | Hn | Hd | $\pi$ | mt group |
|---|---|---|---|---|---|---|
| Anchorage, AK | AKA | 20 | 8 | 0.647 | 0.0028 | AK |
| Wrangell-St Elias, AK | AKW | 18 | 11 | 0.915 | 0.0042 | AK |
| Fairbanks, AK | AKF | 20 | 4 | 0.563 | 0.0010 | AK |
| northwest BC | NWBC | 17 | 7 | 0.831 | 0.0069 | main-northeast |
| Prince George, BC | BCPG | 30 | 13 | 0.828 | 0.0051 | main-northeast |
| Ft St James, BC | BCFSJ | 65 | 24 | 0.939 | 0.0055 | main-northeast |
| central BC | CBC | 17 | 10 | 0.919 | 0.0050 | main-northeast |
| Revelstoke, BC | SCBC | 20 | 14 | 0.958 | 0.0055 | main-northeast |
| Washington | WA | 20 | 6 | 0.779 | 0.0022 | Pacific |
| Idaho | ID | 17 | 12 | 0.956 | 0.0063 | main-northeast |
| northeast OR | NEOR | 15 | 11 | 0.952 | 0.0056 | main-northeast |
| coastal OR | CoOR | 2 | 2 | 1.000 | 0.0104 | Pacific |
| southern OR | SOR | 15 | 6 | 0.714 | 0.0042 | Pacific |
| central AB | CAB | 20 | 14 | 0.937 | 0.0060 | main-northeast |
| Lethbridge, AB | Leth | 19 | 7 | 0.842 | 0.0051 | main-northeast |
| southern AB | SAB | 17 | 10 | 0.919 | 0.0055 | main-northeast |
| Montana | MT | 32 | 13 | 0.905 | 0.0061 | main-northeast |
| Colorado | CO | 32 | 14 | 0.881 | 0.0034 | SE Rockies |
| Utah | UT | 19 | 8 | 0.614 | 0.0017 | SE Rockies |
| Saskatchewan | SK | 19 | 8 | 0.860 | 0.0040 | main-northeast |
| Manitoba | MB | 10 | 8 | 0.956 | 0.0071 | main-northeast |
| Illinois | IL | 14 | 9 | 0.934 | 0.0055 | main-northeast |
| Michigan | MI | 20 | 16 | 0.974 | 0.0048 | main-northeast |
| Ontario | ON | 20 | 16 | 0.968 | 0.0049 | main-northeast |
| Iowa | IA | 12 | 6 | 0.848 | 0.0040 | main-northeast |
| Kansas | KS | 24 | 9 | 0.804 | 0.0033 | SE Rockies |
| Maine | ME | 12 | 9 | 0.939 | 0.0041 | main-northeast |
| Minnesota | MN | 12 | 8 | 0.848 | 0.0047 | main-northeast |
| Pennsylvania | PA | 12 | 8 | 0.848 | 0.0035 | main-northeast |
| Nova Scotia/New Brunswick | NSNB | 19 | 15 | 0.959 | 0.0045 | main-northeast |
| Newfoundland | NL | 19 | 10 | 0.737 | 0.0024 | NL |
| Labrador | Lab | 5 | 3 | 0.700 | 0.0056 | main-northeast |
| West Virginia | WV | 9 | 9 | 1.000 | 0.0056 | main-northeast |
| Missouri | MO | 8 | 5 | 0.893 | 0.0039 | main-northeast |
| North Carolina | NC | 3 | 3 | 1.000 | 0.0264 | main-northeast |

**Table 2**: MtDNA divergence of black capped chickadee (*Poecille atricapillus*) population groups (in thousands of years +/- SE) using pairwise differences (MCL distance) and 3.0% (upper) and 12.1% (lower) divergence rates.

| | AK | Main-northeast | Pacific | SE Rockies | NL |
|---|---|---|---|---|---|
| AK | | 126.7 ± 68.3 | 38.3 ± 33.3 | 63.0 ± 43.7 | 364.0 ± 134.0 |
| Main-northeast | 31.7 ± 16.9 | | 96.3 ± 52.7 | 46.0 ± 22.0 | 266.7 ± 100.0 |
| Pacific | 9.6 ± 8.4 | 24.1 ± 13.1 | | 31.7 ± 24.0 | 331.0 ± 123.3 |
| SE Rockies | 15.8 ± 10.8 | 11.5 ± 5.5 | 7.9 ± 6.0 | | 264.7 ± 103.0 |
| NL | 91.0 ± 33.2 | 66.7 ± 24.8 | 82.8 ± 30.6 | 66.2 ± 25.5 | |