POST-PLEISTOCENE DISPERSAL IN BLACK-CAPPED (*POECILE ATRICAPILLUS*) AND MOUNTAIN (*P. GAMBELI*) CHICKADEES, AND THE EFFECT OF SOCIAL DOMINANCE ON BLACK-CAPPED CHICKADEE WINTER RESOURCE ALLOCATION

John A Hindley
BSc Biological Sciences, California State University, Chico, 1996
MSc Biological Sciences, California State University, Chico, 2002

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfillment of the
Requirements for the Degree

[Doctorate of Philosophy]

Department of Biological Sciences
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

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Abstract

This study investigates the phylogeographic structure and population genetics of two non-migratory, congeneric species: the black-capped chickadee (*Poecile atricapillus*) and the mountain chickadee (*P. gambeli*). Mitochondrial DNA (control region) sequences for both species, as well as microsatellite data for mountain chickadee, revealed a pattern of recent expansion with subsequent genetic differentiation, and limited geographic structure. Results suggest multiple New World glacial refugia: Newfoundland; a southern; Pacific Northwest and southeast U.S. refugia for black-capped chickadee ; and a central Rockies, and two western refugium(a) (southern California and central California) for mountain chickadee. West of the Rocky Mountains, both chickadee species show evidence of more recent diversification and phylogeographic structure. Mechanisms that may differentially affect one species versus the other (e.g., geomorphological barriers, species dispersal/recolonization models, life history traits) are also discussed.

ACKNOWLEDGEMENTS

Acknowledgement

The pursuit of a Ph.D. is fraught with both pleasure and pain, and reminds me a bit of climbing a mountain; the beginning is full of excitement and anticipation of reaching your goal, the overall difficulty (or peak) is beyond one's sight, and the scenery is beautiful at times (isn't that why we do field work?). Eventually like climbing a mountain, the pursuit of a Ph.D. soon becomes more challenging and at times, frustrating. On a mountain, even though the peak is out of sight, you continue one step at a time, and when working on your PhD, when you have no idea what you are doing or how to get to the next part, you continue on as one wise person often said "one bird at a time." Keeping in mind the words of Albert Einstein, "If we knew what it was we were doing, it would not be called research, would it?"

First of all I would like to sincerely thank my advisor Theresa Burg for her patience, support, and especially her sage advice, even though sometimes I didn't realize it, yet she still patiently guided me in the right direction. I also want to thank Theresa for being willing to accept an American who, a few too many times, quoted one of the rare wise things George Bush ever said, "this job is hard." I would also like to thank the members of my Ph.D. committee Andy Hurly, John Bain and Sergio Pellis for all of their support, time and helpful suggestions.

On a personal note, I would like to thank my family and close friends back in California for always supporting and encouraging me; a special thank you goes to my partner, Alix, for all her support and love that has helped me in so may ways. Thank you Kiah and Lincoln for always asking "are you done yet?" because

eventually you got me there. I would also like to thank my grandfather, Angelo Ferrario, for being the man he was that made me want to be more and do more – you are missed. Last but not least, I would like to thank my father, Joseph Hindley, for inspiring me, supporting me, believing in me, and always being there when I needed him, without which none of this would have been possible; I love you Pop, you're still my hero, and this is for you.

I would like to thank the following institutions for providing additional samples: American Museum of Natural History, Burke Museum, Columbia University Museum, University of California, Berkeley Museum of Vertebrate Zoology, Chicago Field Museum, Louisiana State University Museum of Natural Sciences, University of Michigan Museum of Natural History, New Brunswick Museum, North Carolina Museum of Natural Science, Royal Alberta Museum, Royal British Columbia Museum, Royal Ontario Museum, Royal Museum of Saskatchewan, Smithsonian Museum of Natural History, University of Northern British Columbia, and Queen's University.

I would like to thank University of Cornell, Lab of Ornithology's Project Feederwatch, and their many participants who invited us into their yards to band birds and also provided us with food, shelter, and incredible hospitality. I thank the National Park Service, USFWS and Canada Wildlife Service for their assistance.

Finally, thank you to all the members of the Burg Lab at the University of Lethbridge for their assistance both in the field and lab, which include Kim Dohms, Brendan Graham, Linda Lait, Christine Mishra, Paulo Pulgarin, and numerous field assistants for their help with sample collection. I would also like to thank Brendan

Graham for being my primary sounding board and initial editor for many drafts, as well as a few well-timed gold stars. The research presented here was conducted under University of Lethbridge Animal Welfare Protocol No. 0614 in accordance with Canadian Council on Animal Care guidelines, and funded in part by Alberta Innovates Technologies Futures fund and Natural Science and Engineering Research Council of Canada.

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List of Abbreviations, Acronyms and Symbols

A - adenine
AB – Alberta
AIS – Alleles in Space
AK - Alaska
AKA – Anchorage population
AKF – Fairbanks population
AKW – Wrangell-St. Elias population
AMNH - American Museum of Natural History
AMOVA – analysis of molecular variance
AUC - area under curve
avg - average
AR - allelic richness
AZ - Arizona
BC - British Columbia
bp - base pairs
C - cytosine
CA - California
CAB - central Alberta
CBC - central British Columbia
CCA – central California
CeOR - central Oregon
CoOR - coastal Oregon
CO - Colorado
CG - campground
CR – control region
DNA - deoxyribonucleic acid
dNTP - deoxyribonucleotide triphosphate
EDTA - ethylenediaminetetraacetic acid
EMT – eastern MT population
EXO - exonuclease
FMC - Field Museum of Chicago
FS – full siblings
F_S - Fu's F_S
F_{ST} - Wright's fixation index
G - guanine
H - heavy strand of mitochondrial DNA, haplotype
HCl - hydrochloric acid
H_d - haplotype diversity
H_e - expected heterozygosity
H_o - observed heterozygosity
HS – half siblings
HWE – Hardy-Weinberg equilibrium
IBD – isolation by distance
ID - Idaho
K - cluster

km - kilometres
kya - thousand of years ago
LAB - Labrador
LETH - Lethbridge
LGM - last glacial maximum
LSUMZ - Louisiana State University Museum of Zoology
MCL – maximum composite likelihood
ML – maximum likelihood
MI - Michigan
MIROC - Model for Interdisciplinary Research on Climate
min - minute
MO - Missouri
msat - microsatellite
MT - Montana
mtDNA - mitochondrial DNA
Mtn - mountain
Mya - millions of years ago
MY - million years
N - data missing at site; sample size
Na - number of alleles
NaCl - sodium chloride
NBC - northern British Columbia
NC - North Carolina
NCMNS - North Carolina Museum of Natural Sciences
ND2 – Nicotinamide adenine dinucleotide dehydrogenase subunit 2
NEOR - northeast Oregon
NL - Newfoundland
NS - Nova Scotia
ON - Ontario
OR - Oregon
PA - private alleles
PCA - principle components analysis
PCR - polymerase chain reaction
PMIP2 - Paleoclimate Modeling Intercomparison Project Phase II
PO – parent/offspring
Pri – shared haplotypes found in only one population
Q – ancestry coefficient
QU - Queens University
r - raggedness index; mutation rate; correlation coefficient
R - related
r^2 - regression coefficient
R_2 - Ramos-Onsins-Rozas test
RABM - Royal Alberta Museum
RBCM - Royal British Columbia Museum
RNBM - Royal New Brunswick Museum
RSKM - Royal Saskatchewan Museum

s - second
SAB - southern Alberta
SAMOVA – spatial analysis of molecular variance
SAP - shrimp alkaline phosphatase
SCA - southern California
SCCA - south central California
SD - South Dakota
SDS - sodium dodecyl sulfate
SEBC – southeast British Columbia
SK - Saskatchewan
SMITH - Smithsonian Museum
SOR - southern Oregon
T - thymine
T_1 - annealing temperature 1
T_2 - annealing temperature 2
taq - DNA taq polymerase
TE - Tris-HCl EDTA buffer
Tris-HCl - tris(hydroxymethyl)aminomethane
U - unit
UMI - University of Michigan
UNBC - University of Northern British Columbia
UCOM - University of Columbia Museum
US - United States
UT - Utah
UWBM - University of Washington Burke Museum
VA – Virginia
WA - Washington
WI - Wisconsin
WMT – western MT population
WV – West Virginia
w/v - weight per volume
°C - degrees Celsius
π - nucleotide diversity (pi)
τ - estimated time since most recent population expansion (tau)
Φ_{ST} – F_{ST} analogue for sequence data

Chapter 1: General Introduction

1.1 Background

The discipline of biogeography investigates the distribution of biological diversity, and primarily involves identifying organisms' origins and where they live. Traditionally, biogeographers have compared species' geomorphometric measurements, geographic ranges, fossil records, and the richness of species or groups per region with regards to habitat availability, ecological constraints and presence/absence of barriers (e.g., islands, mountains, rivers) to investigate species' distributions through space and time. Phylogeography is a recent sub-discipline of biogeography, resulting from the development of molecular systematics and examines historical and phylogenetic components of the spatial distributions of gene lineages (Avise 2000).

The Pleistocene epoch began 2.6 millions years ago and has affected the dispersal, distribution and life history of species worldwide (Webb and Bartlein 1992). During Pleistocene ice ages, large ice sheets covered the Polar Regions and large parts of North America north of 48° N latitude, with the final glacial episode beginning approximately 126 kya (thousand years ago, Figure 1.1). The ice sheets created large areas of unsuitable habitat for many species, thereby confining populations to ice-free areas known as refugia(um) (Pielou 1991). Pleistocene glaciations are thought to be responsible for many recent avian speciation events (Johnson and Cicero 2004; Weir and Schluter 2004; Milá *et al.* 2007); however, the overall timing of many speciation events is still a topic of debate (Klicka and Zink 1997; Avise and Walker 1998; Zink *et al.* 2004; Milá *et al.* 2007; Zink and Barrowclough 2008). Despite this, paleoclimatic fluctuations during the

Pleistocene have been shown to result in the expansion and contraction of suitable habitat, and populations and species' physical ranges have expanded and contracted as a result (Avise 2000; Hewitt 2000; Johansen and Latta 2003; DeChaine and Martin 2005). Recent studies have sought to examine the effect that these climate fluctuations had on the genetic structure of a wide variety of plant and animal species following the Last Glacial Maximum (LGM) approximately 18 to 21 thousand years ago (kya; Taberlet *et al.* 1998; Lessa *et al.* 2003; Hewitt 2004).

Following the LGM, temperatures warmed, ice sheets receded and populations isolated in refugia expanded their geographic range as new habitat became available (Pielou 1991; Brunsfeld *et al.* 2001; Waltari *et al.* 2007; Galbreath *et al.* 2009). The source of colonizing populations (i.e., parent population, refugium) and colonization routes used by North American species have received considerable attention (Anderson 1948, 1953; Hewitt 1996; Klicka and Zink 1997; Hewitt 2000; Willis and Whittaker 2000) and the advent of new molecular techniques provides useful tools to investigate these biogeographic questions.

The earliest examples of phylogeographic models for North America were relatively straightforward and assumed that populations located in refugia south of the ice sheets slowly expanded north under a pioneer model, following the retreat of ice sheets. This resulted in decreased genetic diversity along a latitudinal gradient (Hewitt 1996; Zink 1996; Hewitt 2000; Hewitt 2004). However, these models did not take into account complex processes such as lineage mixing (Petit *et al.* 2003), refugia within refugia (Gomez and Lunt 2007) and cryptic refugia (Burg *et al.* 2006; Provan and Bennett 2008) that are now more widely recognized. More recently multiple modeling (e.g., ecological niche, coalescent paleodistribution; Richards *et al.* 2007) and statistical analyses (e.g., expansion estimates, divergence times, selective divergence, drift-induced change; for review see Knowles 2009) have become common practice to account for these often-multifaceted phylogeographies (Karlson 2002; Shafer *et al.* 2010).

When interpreting complex phylogenies, researchers should take into account all aspects of an organism's life history. In a comparative avian phylogeography study, Zink (1996) suggested that incongruent patterns between codistributed taxa were due to the effects of idiosyncratic histories including different historical events (e.g., dispersal routes, location of refugia, age of species), genetic factors (e.g., levels of genetic variation, gene flow), as well as ecological factors (e.g., range size, habitat). Dispersal is one of the most fundamental features of an organism and influences several of the effects identified by Zink. While the consequences of dispersal have been extensively discussed in ecological literature, investigations of "why" particular strategies evolve are lacking (Dieckmann and O'Hara 1998). The investigation of historical and ecological factors may provide possible mechanisms to explain certain species' phylogeographic histories.

1.2 Molecular Markers

Molecular techniques provide highly useful tools for investigating the life history, phylogeographic history, and taxonomy of species. Avise *et al.* (1980) were among the first to show that the increasing availability of molecular genetic data could provide common scales to compare a variety of taxonomic levels (e.g., populations, subspecies, species) and help with taxonomic classifications. In animals, mitochondrial DNA (mtDNA) provided the first extensive and readily accessible data for strong genealogical inference at the intraspecific level, and the field of taxonomy/phylogeography has only expanded over the last 20 years (Avise *et al.* 1987; Selkoe and Toonen 2006). Due to its circular structure, non-recombining, maternal inheritance, rapid pace of evolution and extensive intraspecific polymorphism, mtDNA is an excellent tool for phylogenetic analysis of microevolutionary (population genetics) and macroevolutionary (speciation) processes. The mtDNA control region (CR) is highly variable in birds, evolving at rates as high as 20% per million years (MY; Baker and Marshall 1997) and thus useful for studying population structure and exploring demographic events. My research used mtDNA data to answer questions about historical patterns of genetic variation and population structure for both black-capped and mountain chickadees by comparing sequence variation within the non-coding CR (Domains I and II; Figure 1.2).

Microsatellite markers are biparentally inherited, short tandem repeats (1-6 base pairs) of DNA sequences found throughout the genome. Microsatellites have a high mutation rate resulting in high levels of polymorphism, making them ideal for studying contemporary patterns where genetic differences (e.g., allele frequency differences between populations; Figure 1.2) have occurred over short periods of time (Primmer *et al.* 1996). The high mutation rate and biparental inheritance of microsatellites enables them to reveal more recent reductions in gene flow in comparison to mtDNA, making them useful for exploring contemporary genetic patterns (Jarne and Lagoda 1996; Selkoe and Toonen 2006). As a result, microsatellite markers have become one of the genetic markers of choice for studies of intraspecific variation (Feldman *et al.* 1999).

1.2 Study species

Chickadees (family Paridae) are widely distributed birds occurring in the Northern Hemisphere. North American chickadees are commonly found in a variety of habitats including forest (e.g., temperate, boreal, deciduous broadleaf, upper montane), woodland, parkland, open woods, disturbed areas, etc. It is hypothesized that North American chickadees are descended from an Old World ancestor thought to have arrived in North America from Asia during the Pliocene, approximately 3.5 million years ago (Mya; Gill *et al.* 2005). Subsequent divergence resulted in two phenotypically distinct groups: the black-caps (i.e., black-capped, *Poecile atricapillus*; Carolina, *P. carolinensis*; mountain, *P. gambeli*; and Mexican, *P. sclateri*); and the brown or grey-caps (i.e., chestnut-backed, *P. rufescens*; boreal, *P. hudsonicus*; and grey-headed chickadee [formerly Siberian tit], *P. cinctus*; Smith 2007).

The two species that I used for my dissertation research are both members of the black-cap group and considered sister species (Gill *et al.* 2005): the black-capped chickadee and the mountain chickadee. Both species are non-migratory and have limited altitudinal migration (Smith 1991; McCallum *et al.* 1999). The distribution of black-capped chickadees spans the entire width of North America covering much of Canada and the United States, while the mountain chickadee is restricted to the western portion of North America, within the Rocky, Cascade and Sierra Nevada Mountains, and adjacent areas (Figure 1.3).

The black-capped chickadee is a common resident, generalist species found primarily in deciduous and mixed deciduous/ coniferous woodlands (Foote *et al.* 2010). It is a primary nest excavator and often nests in birch (*Betula* sp.), aspen (*Populus* sp.) and maple (*Acer* sp.), but will also utilize knotholes and previously excavated holes in other tree species (Martin *et al.* 2004). The mountain chickadee is a common year-round resident, secondary nester (i.e., primarily utilizes previously excavated nest holes), and niche specialist of the high altitude dry, coniferous forests of western North America (McCallum *et al.* 1999; Martin *et al.* 2004).

1.4 Study Design

To investigate the phylogeographic structure of black-capped and mountain chickadees, I sampled populations across the contemporary ranges of both species, including individuals from known refugia (e.g., southern California for mountain chickadee), putative refugia (e.g., Newfoundland for black-capped chickadee), as well as areas previously covered by ice sheets (e.g., Pacific Northwest, central Canada). Sampling included paired sites on both sides of potential geophysical barriers such as the Rocky, Cascade and Sierra Nevada Mountain Ranges (Figure 1.4), as well as, the Gulf of St. Lawrence/Strait of Belle Isle to identify possible effects of physical barriers on genetic structure.

Using an integrative approach, I investigated post-glacial range expansion of both black-capped and mountain chickadees. Combining mtDNA molecular techniques with coalescent theory and ecological niche modeling allowed me to compare current genetic variation and species distribution with possible historic distribution and post-glacial dispersal patterns (Barker 2004). Coalescent models allow us to conduct rigorous statistical analyses and provide estimates of population size, growth and gene flow using genetic data (Barker 2004; Knowles 2009). By comparing population estimates (e.g., estimated time since last population expansion) with contemporary genetic patterns (e.g., haplotype and nucleotide diversities) and species distribution (both contemporary and paleodistribution), we can infer possible historic biogeographical patterns and identify putative glacial refugia during the LGM (Peterson *et al.* 2004; Beaumont 2005; Steele and Storfer 2006). As a result, conclusions are supported by both genetic analysis and paleoclimate data, rather than solely equating genealogical history with population history (i.e., statistical phylogeographic inferences rely on explicit models of historical scenarios such as isolation by distance or population expansion; Knowles and Maddison 2002; Barker 2004). Using these methods, the current study will identify putative glacial refugia (Figure 1.5), possible dispersal patterns, and potential barriers to gene flow (e.g., mountain ranges, large bodies of water) for black-capped and mountain chickadees.

1.5 Thesis Overview

The current research explores the effects of glaciation and barriers (both physical and nonphysical) to dispersal, and the resulting effects on genetic variation in high latitude bird species. While many studies focus on one species, this study will investigate phylogeographic patterns in two chickadee species. The first data chapter (Chapter 2) will focus on the role of social status on winter fat reserves in black-capped chickadees.

Increased fat reserves have been shown to negatively affect survival in small birds, therefore dominant birds should carry less overall body fat than subordinates. Using both behavioural and genetic (microsatellite; msat) analyses, we will investigate whether dominant birds are more willing to share resources (according to the prolonged brood care hypothesis or kin selection) with related than unrelated birds, which may influence overall dispersal due to increased resource availability for related birds. Avian dispersal in general has been shown to be influenced by several external factors (e.g., brood size, Nur 1988; hormonal change, Dufty and Belthoff 2000; genetic component, and Hansson *et al.* 2003). As a result, by identifying behavioral interactions that may influence resource availability, we may identify possible mechanisms affecting chickadee dispersal.

Chapter 3 focuses on the phylogeographic structure of black-capped chickadees in North America. Using mtDNA, we will look at the role of physical barriers such as mountain ranges (e.g., Rocky and Cascade Mountains) or large bodies of water (e.g., Strait of Belle Isle and the Cabot Strait) on gene flow. We compare contemporary genetic patterns with predicted paleodistribution models to infer possible glacial refugia and dispersal patterns, as well as look at how the colonization model used by black-capped chickadees has affected population genetic patterns.

Chapter 4 investigates the phylogeographic structure and population genetics of mountain chickadee in North America. Similar to Chapter 3, we compared contemporary genetic patterns with predicted paleodistribution models to infer possible glacial refugia and dispersal patterns, and role of physical barriers such as mountain ranges on gene flow using both mtDNA and microsatellites. This is the first study to use highly variable microsatellite markers to investigate the contemporary population structure of mountain chickadee. The use of both types of markers in my study allowed me to compare historical patterns between CR (this study) and ND2 (Spellman *et al.* 2007) as well as, identify contrasting patterns between mtDNA and microsatellites, and identify possible sex-biased dispersal in mountain chickadee.

The final chapter addresses the role of physical barriers as well as non-physical barriers (e.g., extreme philopatry, occasional irruptions, social status, and sex-biased dispersal) on gene flow within North America. I will explore how patterns in a generalist species, the black-capped chickadee, compare to other North American species, including the mountain chickadee. By comparing patterns observed in a generalist species (black-capped chickadee) with patterns of a more specialized habitat species (mountain chickadee) we can better understand if biogeographic patterns are localized to specific areas or if the whole area was affected similarly, as well as identify concordant barriers to gene flow. Alternately, if patterns are incongruent, we will be able to identify possible differences in historical events and life-history traits that may differentially affect dispersal patterns.

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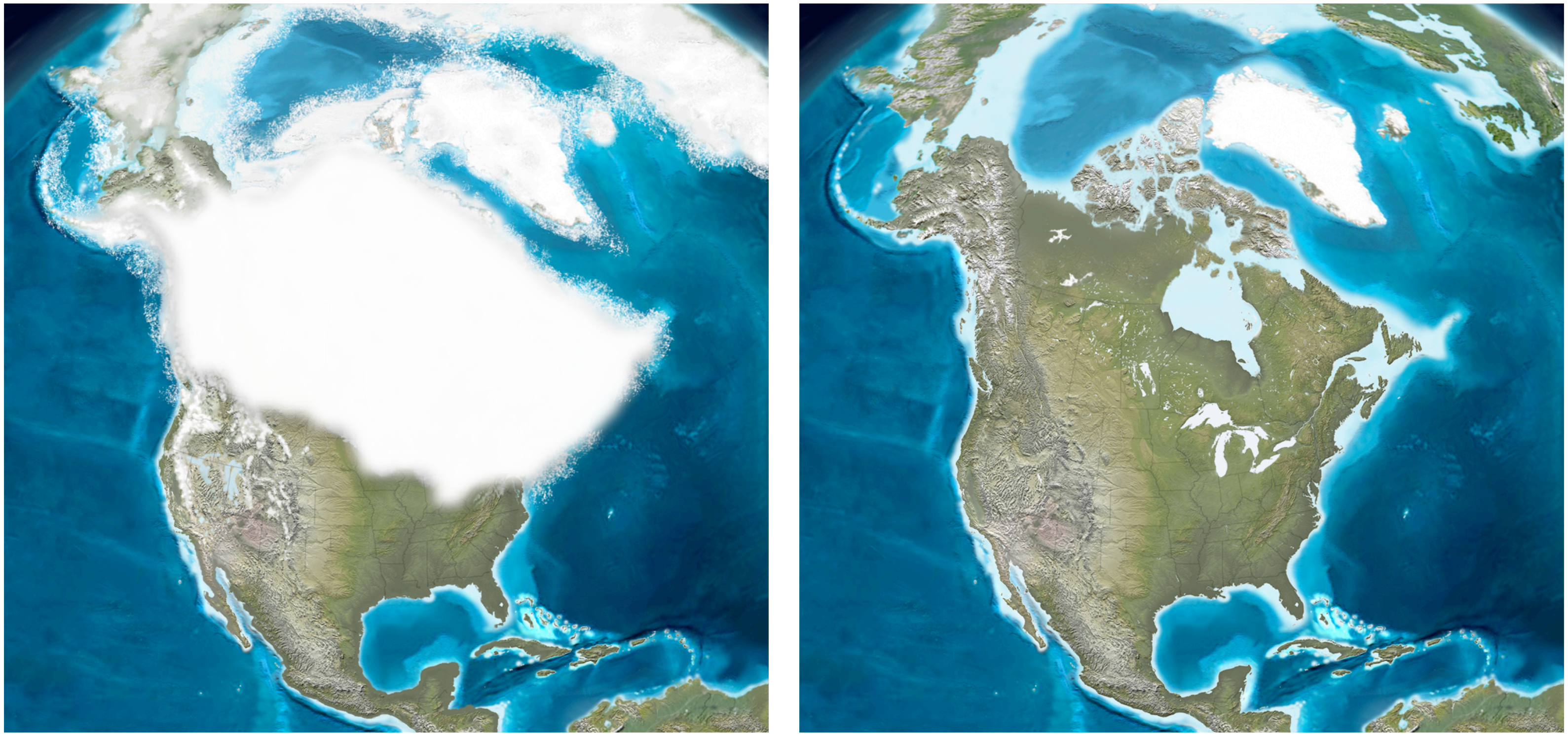

Figure 1.1. Extent of ice sheets at the Last Glacial Maximum ~18-21 kya (left) and present day (right). Maps reprinted with permission from Blakey (1999), with estimated extent of ice during the LGM (white) is based on Dyke *et al.* (2002).

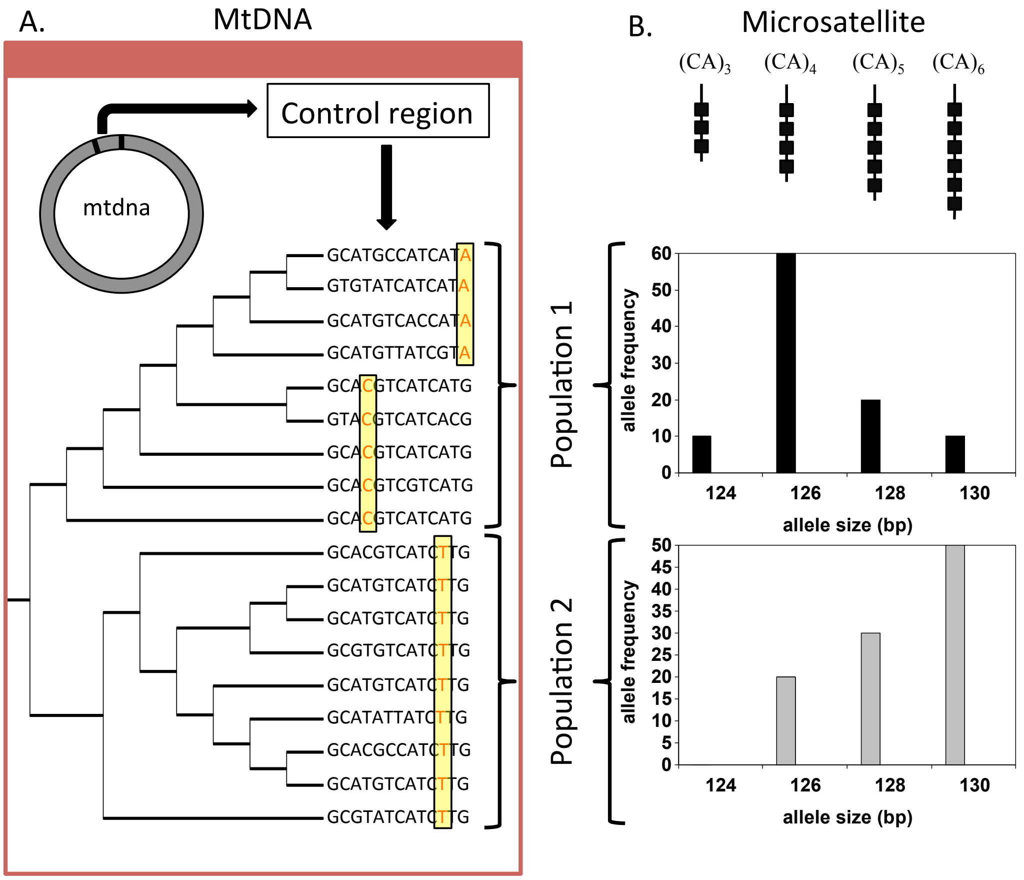
A.
MtDNA
Control region
mtdna
GCATGCCATCATA
GTGTATCATCATA
GCATGTCACCATA
GCATGTTATCGTA
GCACGTCATCATG
GTACGTCATCACG
GCACGTCATCATG
GCACGTCGTCATG
GCACGTCATCATG
GCACGTCATCTTG
GCATGTCATCTTG
GCATGTCATCTTG
GCGTGTCATCTTG
GCATGTCATCTTG
GCATATTATCTTG
GCACGCCATCTTG
GCATGTCATCTTG
GCGTATCATCTTG
B.
Microsatellite
(CA)3
(CA)4
(CA)5
(CA)6
Population 1
allele frequency
60
50
40
30
20
10
0
124
126
128
130
allele size (bp)
Population 2
allele frequency
50
45
40
35
30
25
20
15
10
5
0
124
126
128
130
allele size (bp)

Figure 1.2. Diagram of possible individual/population differences in mitochondrial (A) and microsatellite (B) markers for a simplified hypothetical scenario. A: Control region sequence analysis showing subgroup structure in population 1 and a fixed difference (T) separating population 2. B; microsatellites are biparentally inherited (one set of chromosomes from each parent) and differences between populations are based on allelefrequency differences.

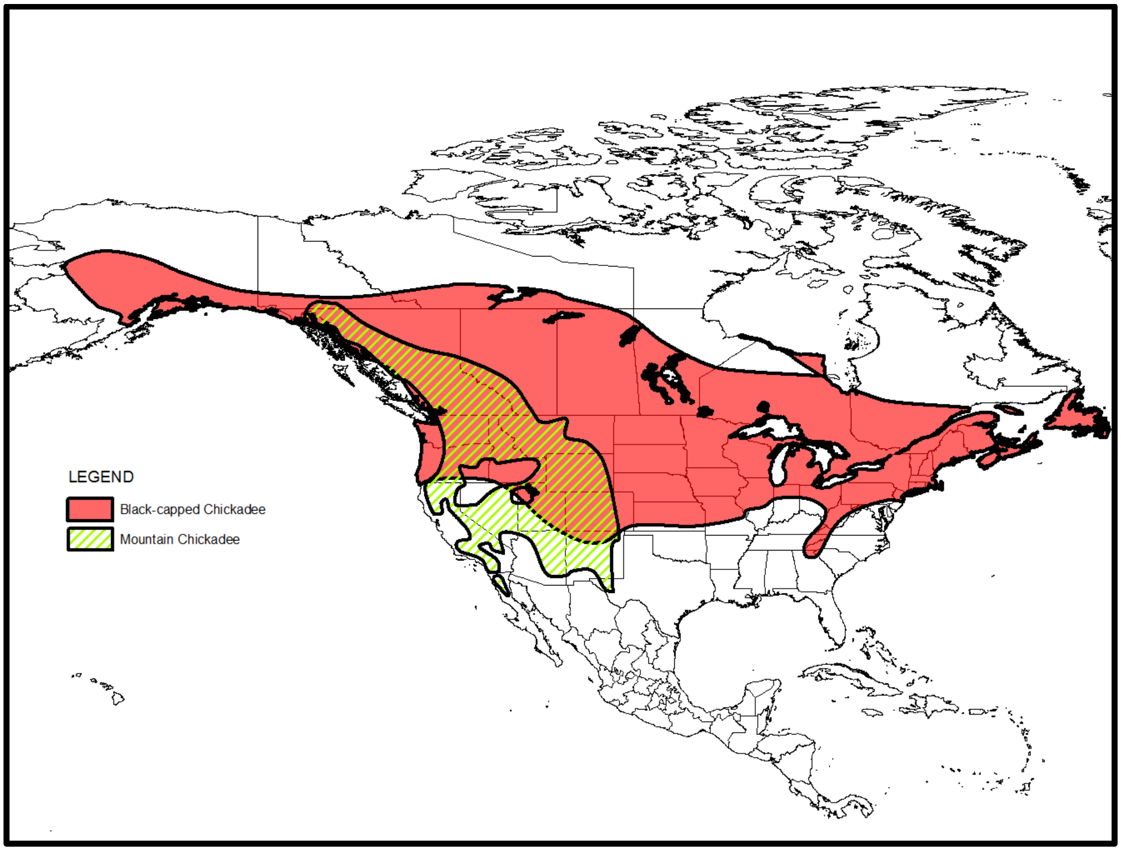
LEGEND
Black-capped Chickadee
Mountain Chickadee

Figure 1.3. Black-capped and mountain chickadee current distribution (Ridgely *et al.* 2007).

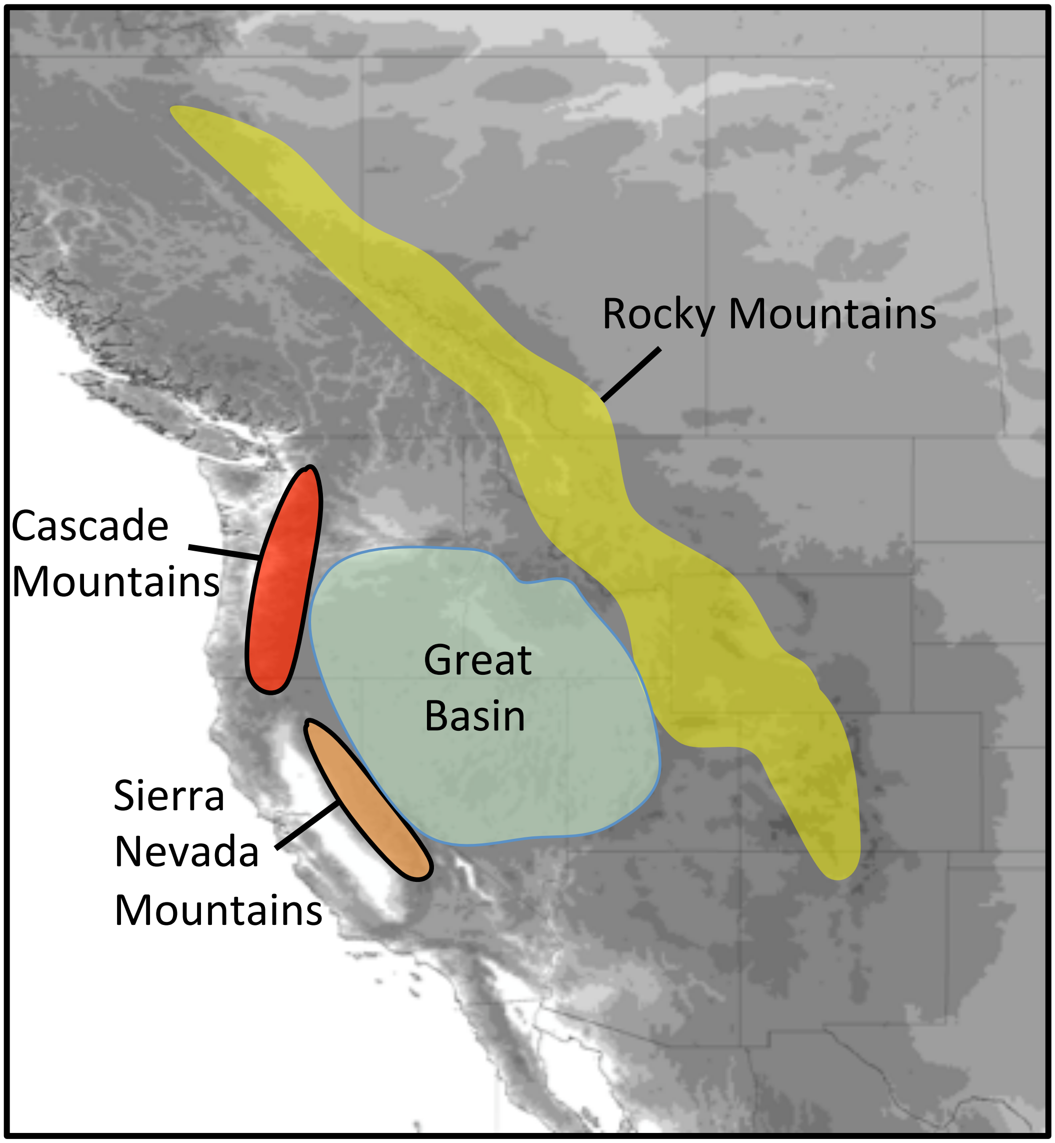
Rocky Mountains
Cascade Mountains
Great Basin
Sierra Nevada Mountains

Figure 1.4. Map of the Pacific Northwest including general locations of Rocky, Cascade, and Sierra Nevada Mountain Ranges as well as the Great Basin.

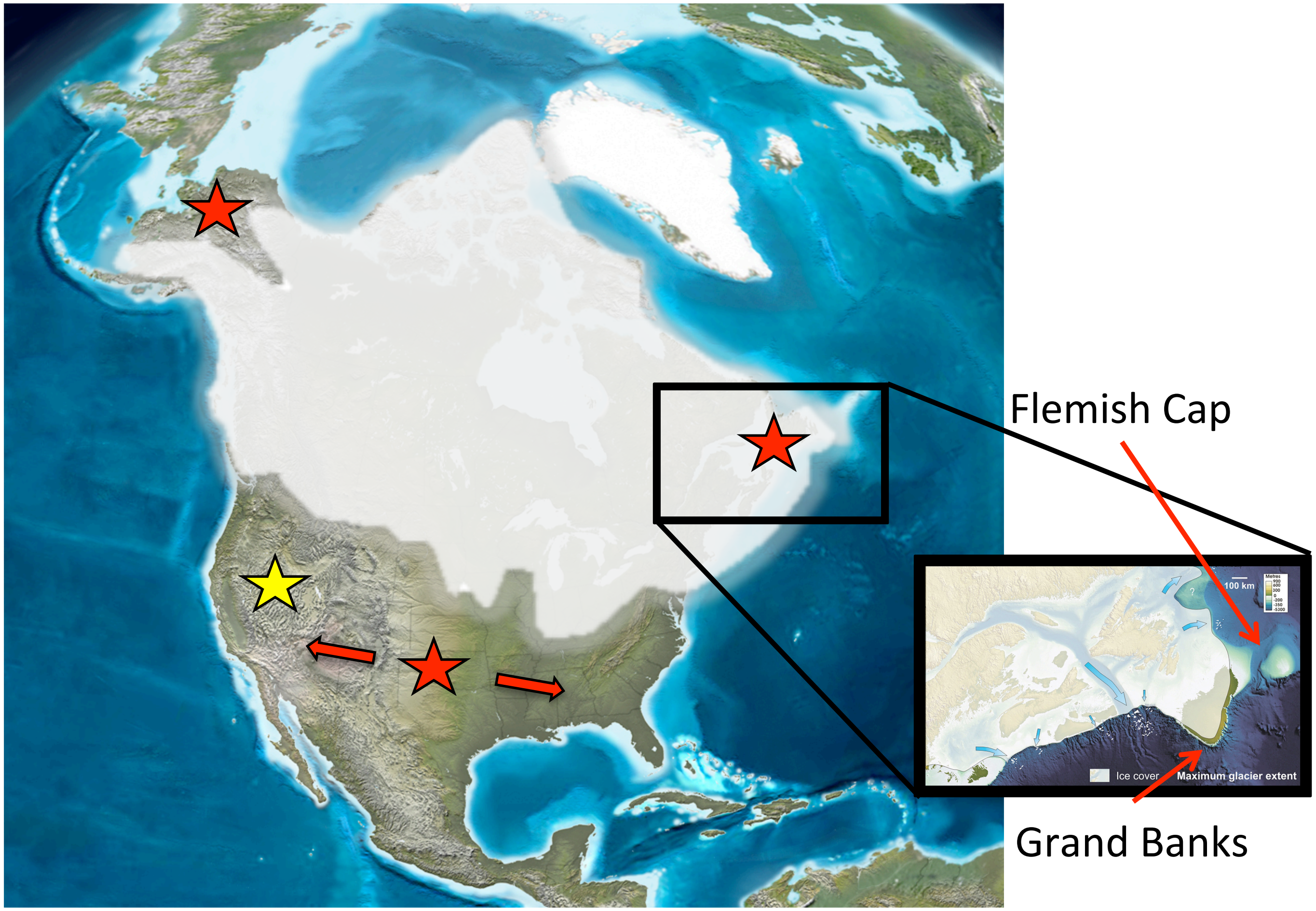
Flemish Cap
100 km
Metres
Ice cover
Maximum glacier extent
Grand Banks

Figure 1.5. Extent of ice sheets at the LGM (white) and general locations of possible chickadee glacial refugia (black-capped = red star, and mountain = yellow star). North America map and inset reprinted with permissions from Blakey (2011) and Shaw (2006), respectively. Estimated extent of ice during the LGM (white) is based on Dyke *et al.* (2002).

RHH: *Hindley* • CHICKADEE FAT RESERVES, STATUS AND RELATEDNESS

Chapter 2: The effect of fat reserves, sex and relatedness on winter flock behaviour in the black-capped chickadee

John A. Hindley[A]
Andrew Hurly[A]
Theresa M. Burg[A]

Prepared as manuscript for submission

[A]University of Lethbridge, Department of Biology

Correspondence: J. Hindley, University of Lethbridge, Department of Biology, 4401 University Dr., Lethbridge, AB, Canada T1K 3M4.

e-mail address: j.hindley@uleth.ca

Abstract

The effects of social status on winter fat reserves, and whether sex and/or relatedness affected the occurrence of agonistic encounters in the dominance-structured black-capped chickadee (*Poecile atricapillus*) were investigated using a combination of behavioural observations and molecular techniques. We observed feeder supplemented chickadees and assessed relatedness using seven microsatellite markers in a winter population located in Lethbridge, AB. Body fat was negatively correlated with dominance for all members within the population, which is consistent with optimal body mass theory. Males were involved more in agonistic interactions both as subordinates and as dominants, and females were involved in more solitary feeding consistent with a general Parid hierarchy that has been previously described. However, 16 out of 19 birds showed some relationship (i.e., half sibling or closer) with at least one other bird in the population that suggests winter flocks may be composed of more close relatives than previously thought.

Keywords: Black-capped chickadee, body fat, dominance, microsatellite, relatedness, winter, behaviour

2.1 Introduction

Acquiring food during winter is challenging for small-bodied passerines, especially where winter temperatures may drop below the lower critical temperature (i.e., minimum temperature that can be tolerated; Withers 1992). Such birds require energy reserves to survive challenges to energy budgets that are predictable such as long nights, as well as unpredictable challenges such as inclement weather and variable food availability and accessibility (Lack 1954; Pravosudova *et al*. 2001). For many years it was assumed that increasing winter body-fat reserves increased survival probability by insuring against possible starvation (Fretwell 1969; King & Mewaldt 1981; Gentle and Gosler 2001), as subcutaneous fat reserves increase in winter and decrease in summer (Lehikoinen 1987; Haftorn 1989; Krams *et al*. 2010). Growing evidence suggests increased fat reserves may decrease survival by decreasing predator avoidance (Lilliendahl 1997; Gentle and Gosler 2001; Creswell 2003). An increase in mass resulting from elevated fat reserves is associated with a reduction in velocity or acceleration, angle of ascent and maneuverability in flight, and therefore an increased risk of predation (Hedenstrom 1992; Witter *et al*. 1994; Metcalfe & Ure 1995; Kullberg *et al*. 1996; Lilliendahl 1997; Gentle & Gosler 2001; Krams *et al*. 2010). The optimal body mass hypothesis (Lima 1986) states individuals should maintain a body mass that minimizes the combined risks of predation and starvation (Houston *et al*. 1993; Rogers & Smith 1993; Krams 2000; Hedenström & Rosen 2001).

Fattening strategies may also be influenced by social status in dominance-structured groups (Haftorn 1989; Hake 1996; Verhulst & Hogstad 1996; Krams *et al*. 2010). Because dominant birds have more stable access to food, higher quality habitats, and safer habitats; dominants should be able to carry less fat without increasing risk of starvation (Lima 1986; Houston *et al*. 1993). Similarly, subordinates need to carry more reserves to cope with a higher risk of starvation through less stable food access (Clark & Ekman 1995). Essentially, both dominants and subordinates should minimize the combined risk of starvation and predation, but the optimal solution for each differs because of differential access to food. Several studies have shown subordinate individuals carry higher fat reserves than dominants (Gosler 1996; Gosler & Carruthers 1999; Gentle & Gosler 2001). Pravosudov *et al.* (1999) found dominant Carolina chickadee (*Poecile carolinensis*), tufted titmouse (*Baeolophus bicolor*), and white-breasted nuthatch (*Sitta carolinensis*) carried lower fat reserves than subordinates. Other studies failed to show any significant difference in fat reserves between dominant and subordinate birds (Lundberg 1985; Piper & Wiley 1990; Gentle & Gosler 2001). While Gentle and Gosler (2001) showed no difference in dominants' fat storage, they did find that dominant birds carried less body fat when under experimentally altered increased predation risk, and suggested there was a trend to carry less fat with increasing dominance status. Verhulst and Hogstad (1996) using an analytical model for willow tit (*P. montanus*) showed that dominants carried more body fat when social status affected predation risk but not food acquisition rate, suggesting that several factors influence optimal energy reserves.

Winter flocks of black-capped chickadees (*P. atricapillus*) form dominance hierarchies, and high ranking birds benefit from their status during both winter (e.g., over-winter survival) and summer (e.g., larger breeding territories; Desrochers *et al.* 1988). Schubert *et al.* (2007) using paired comparisons showed the highest ranked black-capped chickadee males within a flock were leaner than the lowest ranked males, which suggests a negative correlation between dominance level and fat reserves.

Living in flocks may reduce foraging efficiency due to competition among group members (Alexander 1974; Krause & Ruxton 2002; Tóth *et al.* 2009). Ficken *et al*. (1990) reported that dominant birds excluded subordinates from access to feeders. However, kin-selection theory (Hamilton 1964) predicts individuals may gain increased fitness through reduced aggression towards kin, thereby reducing their relative's cost. According to Ekman's (1994) prolonged brood care hypothesis, parental tolerance during winter may increase inclusive fitness if retained offspring experience relaxed competition and enhanced survival. Therefore, dominant birds that monopolize resources during winter should be more willing to share such resources with their own offspring than with unrelated birds.

In this study we investigated whether: (1) fat reserves of the dominance-structured black-capped chickadee in winter correlated with social status for all members of the flocks and tested the prediction that body fat is negatively correlated with dominance status; (2) agonistic encounters occur less frequently between males, females or between males and females; and (3) agonistic encounters occur less frequently between closely related birds than between unrelated birds.

2.2 Methods

We studied a feeder-supplemented winter population of black-capped chickadees at the Helen Schuler Nature Centre located along the Oldman River in Lethbridge, AB, Canada (N 49° 41’ 38”, W 112° 51’ 45”). In total 21 birds were trapped between 15

January and 1 February 2009 using Potter traps baited with black-oiled sunflower seed. All birds were banded with one numbered Canadian Wildlife Service aluminum leg band and three plastic colour leg bands in unique combination for individual identification. We measured mass, tarsus, uncompressed wing chord, and bill (depth, width, and length); assessed body fat using Kaiser's (1993) multi-category classification; and removed <100 µl of blood from the brachial vein from 19 of the 21 birds (two birds were trapped when conditions were too cold for blood sampling and were not subsequently re-trapped). Blood was stored in 95% ethanol for molecular analysis (see below).

To avoid feeding by more than one bird at a time, and to facilitate interactions between birds, the feeder was designed with two perches and one opening. Because feeding behaviour may vary according to time of day (Gosler 1996), all observations were made within a 4-hour block between 1200 and 1600 MST based on time before sunset (i.e., 3 hours before sunset to sunset). All observations were made from within the Helen Schuler Nature Centre at a distance of 4 m from the feeder and behind glass doors. Interactions were recorded using a modified four category system of Ekman *et al.* (1994): 0 (feeding) bird is allowed to feed with no interaction; 1 (non-feeding tolerance) subordinate bird allowed to stay at feeder but not feed; 2 (displacement) subordinate forced from feeder; and 3 (chase) after displacement dominant chases subordinate for a few meters. We noted the winner and loser whenever possible (i.e., most cases both winner and loser were identified, but some encounters happened too quickly to identify both individuals). Dominance was assessed by the proportion of wins for each individual with a win defined as either the displacement of another (score of 2), or chasing another bird away from the feeder and the winner being able to feed (score of 3).

2.2.1 Relatedness and Sexing

DNA was extracted from 5 µl of blood using modified chelex extraction (Walsh 1991). Seven microsatellite primer pairs isolated from black-capped chickadee or other passerine species were used for genotyping (Table 2.1). The primers were modified with the addition of M13 sequence to the 5' end to allow for direct incorporation of a fluorescently labeled M13 primer. PCR reactions consisted of approximately 100 ng of template DNA, 1 µM of each microsatellite primer and the M13 tag, 200 µM dNTPs, 1.0-2.0 mM $MgCl_2$ (Table 2.1), 0.5 unit of Taq DNA polymerase (Crimson) and the 10x PCR buffer (Promega) in a final volume of 10 µl. Samples were electrophoresed for 3 hours on a Li-COR 4300L. All microsatellite alleles were visually scored using the program Saga Lite (Build 1.0.2).

Sexing of birds was performed using PCR with the P2/P8 primers (Griffiths *et al.* 1998). The PCR mixture contained 100 ng of genomic DNA, 1 µM of each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 unit of Taq-polymerase (Crimson), and PCR buffer (Promega) in a final volume of 10 µl. The PCR was carried out in an Eppendorf Mastercycler under the following conditions: initial denaturation at 94°C for 90 s, and 30 cycles at 48°C for 45 s, 72°C for 45 s, and 94°C for 30 s, with final step at 48°C for 60 s and 72°C for 5 min. Amplification products were separated by horizontal electrophoresis in 3% agarose gels in 1×TBE. Males were identified by the presence of a single band (380 bp) and females by the presence of two bands (380 and 400 bp).

2.2.2 Body Fat Statistical Analysis

The primary focus of this study was to compare dominance, as measured by proportion of wins (wins/(wins + losses)), with body fat, and to identify behavioural differences according to relatedness and sex. Because time of day and mean daily temperature have been shown to significantly affect body fat (Gosler 1996, Pravosudov & Grubb 1998), both the initial body fat score at time of banding, as well as a standardized body fat (Gosler 1996) were used for analysis. Mean daily temperatures were obtained from Environment Canada, Daily Climate Data for Lethbridge, AB, and all data analyses were performed with SPSS Statistics version 17.0. Body fat was analyzed using one-tailed Spearman's rho partial correlations. One-tailed tests are justified because theory predicts the correlation between fat reserves and dominance is negative and the correlations between morphological measures are positive. Birds with fewer than five recorded observations (N = 4) were not included in analyses because dominance could not be determined with confidence. The effect of body size (i.e., larger birds require more fat reserves in general due to being larger) was controlled for using tarsus length after Garnett (1981) and Gosler (1996) and tested using standard partial correlation.

2.2.3 Relatedness and Sexing Analysis

We used GENEPOP version 4.0.10 (Raymond & Rousset 1995) to verify loci were in Hardy-Weinberg and linkage equilibrium following sequential Bonferroni correction (Rice 1989). ML-Relate (Kalinowski *et al*. 2006) and Pedigree 2.2 (Smith *et al*. 2001 & Herbinger 2005) with full-sibling constraint were both used to calculate maximum likelihood estimates of pair-wise relatedness. Because there were no prior genetic data for the study population, individual's genotypes were entered as if from a single population to estimate allele frequencies, pair-wise genetic relatedness, and to assign the following kinship categories (i.e., kin groups): U-unrelated, HS-half-siblings, FS-full-siblings, and PO-parent-offspring (PO were manually confirmed by comparing alleles and allowing for no allelic mismatches). Related pairs (i.e., those assigned as PO, FS, and HS) were pooled as an additional "R" group for analysis of all related bird interactions population wide. We analyzed aggressive interactions between birds based on kinship groups and sex, and compared the number of all agonistic encounters between close kin to encounters between unrelated birds.

Agonistic encounters were analyzed using the exact binomial goodness of fit test to determine whether the proportion of encounters by related birds was greater or lesser than expected by chance. Following Ficken *et al.* (1990), we assumed that the number of times individuals visited the feeder represented the number of possible chance encounters between any two individuals (e.g., probability of encounters between two individuals that both visit 50 times out of a total of 100 visits would be 0.25 or 25%). To determine the expected number of interactions for each kin group, we averaged the probability of encounters between each pair, multiplied by the number of pairs within each group (e.g. the average probability of expected encounters for all pairs of PO was 0.0025, multiplied by the number of PO ($N = 6$) is 0.015), and multiplied this by the total number of interactions observed ($N = 366$; $0.015 \times 366 = 5.5$ expected PO interactions). We used the same procedure to determine whether the proportion of male-male and male-female (or female-male) encounters was greater or less than expected by chance, but used the overall proportion of males and females within the population, respectively for expected proportion of “All Observations”. In order to assess whether a particular sex was allowed to solitarily feed more often, we included all observations of feeding, regardless of whether there was an interaction for both “All Observation” ($N = 3297$) and “Solitary Feeding” ($N = 2492$). Subordinate and dominant interactions include all observations where the respective participant was identified (e. g., subordinate bird was identified in a chase regardless of dominant identification, and vice versa).

2.3 Results

In total, 17 of the 21 individually marked birds were repeatedly observed over 23 days with 385 agonistic behavioural observations (i.e., displacement and/or chase due to lack of feeding tolerance observations) recorded out of 3297 feeder observations (average = 43 interactions per bird; Table 2.2). The maximum number of wins and losses observed was 83 and 42. There was a significant correlation for mass and wing length ($r_s = 0.51$, $N = 15$, $P = 0.03$), wing length and tarsus ($r_s = 0.46$, $N = 15$, $P = 0.04$), and bill depth and length ($r_s = 0.492$, $N = 15$, $P = 0.03$) and bill width and length ($r_s = 0.48$, $N = 15$, $P = 0.04$; Table 2.3). Both uncorrected body fat score and standardized body fat were negatively correlated with dominance (i.e., win percentage; $r_s = -0.47$ and -0.48, $N = 16$, $P = 0.03$ and 0.03, respectively), even when controlling for body size ($r_{11} = 0.617$, $N = 16$, $P = 0.025$, and $r_{11} = 0.558$, $P = 0.047$, respectively; data not presented).

2.3.1 Relatedness and Sex-specific Behaviour

Females were observed feeding solitarily more often than males ($P < 0.001$) in proportion to their number of overall visits to the feeder (Table 2.4). Significantly more males were involved in all observations (exact binomial test, P = 0.001) as compared to overall proportion in the population, respectively. There were significantly more males acting as dominants (exact binomial test, P < 0.001), and no significant difference in the number of males versus females observed as subordinates (exact binomial test, P = 0.284) in proportion to their number of overall visits to the feeder, respectively.

Examination of the number of microsatellite alleles, and observed and expected heterozygosity (Table 2.1) revealed that no locus deviated significantly from Hardy-Weinberg equilibrium (all P > 0.05) after Bonferroni correction, or showed evidence of linkage. There was no significant difference in dominance encounters between pairs of related PO (exact binomial test, N = 6, P = 0.827), and FS (exact binomial test, N = 1, P = 0.519) based on their average proportion of overall visits to the feeder within the population (Table 2.5). Because a large proportion of total interactions appeared to be between lower ranked birds, we further examined dominance relationships by restricting analyses to interactions involving the top five dominant ranked birds overall (i.e., win percentage ≥ 50%;), and the top four ranked dominant PO birds (only four PO had win percentage ≥ 50%), as the dominant participants. In this subset of the data, PO interactions were not significantly different from expected (exact binomial test, P = 0.721), but there were significantly more interactions among R pairs than expected (exact binomial test, N = 18, P = 0.003; Table 2.5).

2.4 Discussion

We explored winter fat reserves of individual chickadees in relation to social status, and behavioural interactions based on sex and relatedness within a winter black-capped chickadee population. Our study supports the hypothesis that subordinate individuals have larger fat reserves than dominants. Both measures of body fat (raw score and standardized for temperature and time of day) were negatively correlated with dominance level (i.e., percentage of wins), even when controlling for body size. This result is consistent with the previous study by Schubert *et al*. (2007) where the highest-ranking males were leaner and heavier than the lowest ranking males within flocks. However, this current study shows body fat correlates with dominance level across all individuals, and this relationship holds regardless of body size differences. Unfortunately, we could not identify individual flock membership and therefore, could not examine whether body fat correlated across all individuals within each flock.

These findings are consistent with the optimal body mass hypothesis that states dominant individuals should maintain a body mass that minimizes the combined risk of predation and starvation. The findings are also consistent with existing literature on winter fattening strategies that suggests subordinate individuals carry more fat in resource-limited environments (Clark & Ekman 1995; Gosler 1996; Hake 1996; Pravosudov *et al.* 1999; Schubert *et al.* 2007). A previous study on the great tit *(P. major*) showed significantly reduced fat reserves under experimentally increased predation risk, suggesting a cost to individuals carrying elevated fat reserves (Gentle & Gosler 2001). Therefore, dominant individuals with more stable access to food resources may have less dependence on internal energy stores, and thus can maintain lower levels of body fat, thereby increasing their maneuverability and reducing their risk of predation.

There was a significant difference between solitary feeding observations of female versus male individuals suggesting dominant birds may preferentially allow females to feed over males, or that more dominant birds feed first and subsequently the least dominant (i.e., female) feed last when solitary feeding is more likely. Males were significantly more involved as dominants during agonistic encounters than females ($P < 0.001$; Table 2.4), which is consistent with previous literature in which Parid dominance has been shown to follow a linear progression from adult males, followed by juvenile males, adult females and finally juvenile females (Smith 1976; Desrochers *et al.* 1988; Desrochers 1989; Gosler 1996; Lahti *et al*. 1998; Pravosudov *et al.* 1999). This suggests that individual interactions may be more important than overall male/female numbers.

Relatedness did not affect the proportion of interactions among PO and FS pairs ($P > 0.5$), but there were significantly more HS interactions than expected by chance ($P < 0.001$; Table 2.5). The number of FS was very low with only one pair present in the population and only one recorded interaction between the pair. Within all overall R birds, there were significantly more interactions than expected by chance ($P = 0.03$; Table 2.5). Upon further analysis, a large proportion of the interactions between related birds appeared to occur among more subordinate HS (11 out of 19 HS interactions involved three hatch year birds (data not presented), which may have still been establishing dominance/rank between the three; birds 3, 4, and 9; Table 2.5) and therefore may have inflated overall interactions within HS and R kin groups. If subordinate birds are being excluded by higher ranked birds, then individual energy demands may outweigh the sharing of resources between closely related birds (i.e., HS ranked 16th would not be in a position to share resources with a HS ranked 17th and instead would be competing for limited access to food), which could result in a nonlinear relationship between dominance and aggression towards related/unrelated. The most dominant birds however, would be in a better position to share resources with closely related subordinates (i.e., less aggression would result in more available resources, similar to sharing resulting in more available resources). Subsequent analysis of interactions involving only the top four dominant PO birds showed no significant difference from chance though, and the top five dominant birds within the flock showed significantly more interactions between R individuals. The increased number of agonistic interactions between HS and R groups, as compared to unrelated birds, suggests that distantly related birds may be treated more aggressively than close relatives (i.e., there was no significant difference in the number of agonistic interactions from expected for PO and FS suggesting more aggression for HS and R), but additional studies are needed to verify this finding.

Therefore, our results do not support the prolonged brood care hypothesis that dominant individuals should be more willing to share resources with offspring, but suggests dominants are more aggressive towards closely related individuals over more distantly related, due to the higher number of agonistic interactions within the HS and R groups. An alternative explanation may be that close relatives avoid direct competition when possible by avoiding the feeder when a dominant relative is in close proximity.

Our findings contradict results from Pravosudov *et al*. (1999) that showed dominant tufted titmice were more aggressive to unrelated birds, and there was no difference in the nutritional condition (i.e., body fat) between the offspring of dominant and unrelated birds. Verhulst and Hostad (1996) showed, theoretically, that when social dominance affects predation risk while foraging, but not food acquisition rate, the optimal energy level (i.e., body fat storage) for dominants was higher. Similarly, Gentle and Gosler (2001) found a significant effect of dominance on fat reserves only under increased risk of predation suggesting that resource allocation may be dependent on perceived risk. Because our study was conducted at a relatively constant food source, and investigated a flock of birds living under natural conditions (e.g., predation risk, starvation risk), we cannot interpret the effect perceived predation risk at our site had on overall body fat, and under an increased or decreased predation risk, optimal energy reserves may differ.

Interestingly, the presence of only one pair of full siblings within the 19 observed birds is consistent with previous literature that found winter flocks consisted of non-siblings (Smith 1976; Desrochers *et al*. 1988; Desrochers 1989). Although, 16 out of 19 birds showed some relationship (i.e., half sibling or closer) with at least one other bird in the population, we could not identify individual flocks with great accuracy over the 6-week study. Therefore we cannot rule out that closely related individuals are members of adjacent flocks rather than flockmates. The high number of related birds across all possible flocks however, suggests that at least some of the flocks were composed of related birds, and indicates that winter flocks may be composed of more close relatives than previously thought.

Acknowledgements

We would like to thank the Helen Schuler Nature Centre for allowing us to conduct this study at their facility. For help in the field we thank Heather Brule, Christine Mishra, Karma Tiberg, and Meg Martin. This research was funded in part by Alberta Ingenuity Fund and Natural Science and Engineering Research Council of Canada. We thank the members of the Burg Lab at the University of Lethbridge for their assistance, as well as Sandeep Mishra who provided helpful comments and discussions. The research presented here was conducted under University of Lethbridge Animal Welfare Protocol No. 0614, approved on 14 November 2008, in accordance with Canadian Council on Animal Care guidelines.

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Table 2.1 Number of alleles and observed (H_{obs}) and expected (H_{exp}) heterozygosity.

LOCUS	Number of Alleles	$MgCl_2$ (mM)	H_{obs}	H_{exp}
Escu6[1]	16	1.5	0.842	0.883
PAT2-14[2]	9	2	0.737	0.750
PAT2-43[2]	10	2	0.947	0.833
Pdo5[3]	6	1	0.684	0.731
Ppi2[4]	11	1.5	0.842	0.842
Titgata02[5]	8	2	0.842	0.826
Titgata39[5]	6	2	0.789	0.725

1 Hanotte *et al.* (1994); 2 Otter *et al.* (2001); 3 Griffith *et al.* (1999); 4 Martinez *et al.* (1999); 5 Wang *et al.* (2005)

Note: Linkage disequilibrium test was not significant for all loci (all P > 0.31); HWE for all loci P > 0.05 following Bonferroni correction

Table 2.2 Agonistic encounters between chickadees and dominance level as measured by percentage of winning encounters during displacements and chases (sorted by dominance level). A '+' indicates dominant outcome and '-' subordinate outcome.

	Interaction							
	Displacement		Chase					
Bird	(+)	(-)	(+)	(-)	Wins*	Losses*	Sex	Dominance Level
13	16	2	3	0	19	2	F	90.48
9	75	16	8	0	83	16	M	83.84
14	44	12	7	2	51	14	M	78.46
12	43	13	4	1	47	14	M	77.05
5	23	12	0	1	23	13	M	63.89
6	28	24	4	0	32	24	F	57.14
15	16	12	0	1	16	13	M	55.17
17	6	6	1	2	7	8	U**	46.67
4	12	15	1	1	13	16	M	44.83
11	10	16	2	0	12	16	F	42.86
7	24	31	0	5	24	36	M	40.00
8	19	32	1	2	20	34	F	37.04
10	9	22	2	2	11	24	F	31.43
2	13	28	2	5	15	33	M	31.25
1	7	19	1	2	8	21	F	27.59
16	4	20	1	3	5	23	F	17.86
3	1	38	0	4	1	42	M	2.33
Totals	350	318	37	31	387*	349*		

* wins ≠ losses because not every winner/loser was identified for each interaction

** sex unidentified

Table 2.3 Spearman's correlation (one-tailed) of fat score, dominance, and body measurements (Spearman's rho top right of diagonal, P value bottom left; asterisk = significant, P < 0.05).

	Fat	Std. Fat[A]	Wins (%)	Mass (g)	Wing (mm)	Tarsus (mm)	Bill Length (mm)	Bill Depth (mm)	Bill Width (mm)
Fat	-	0.94*	-0.47*	0.19	0.06	0.31	-0.42	-0.29	0.06
Std. fat[A]	0.00*	-	-0.48*	0.10	-0.02	0.27	-0.49*	-0.32	0.01
Wins	0.03*	0.03*	-	0.01	0.26	0.04	0.37	0.31	0.30
Mass	0.24	0.35	0.49	-	0.51*	0.24	-0.09	0.15	0.32
Wing	0.43	0.48	0.18	0.03*	-	0.46*	0.21	-0.02	0.23
Tarsus	0.14	0.17	0.45	0.20	0.04*	-	0.37	0.39	0.28
Bill length	0.06	0.03*	0.08	0.37	0.23	0.09	-	0.49*	0.48*
Bill depth	0.16	0.14	0.13	0.29	0.47	0.07	0.03*	-	0.20
Bill width	0.42	0.49	0.14	0.12	0.21	0.15	0.04*	0.24	-
Mean	2.19	3.83	48.81[B]	11.83	65.33	1.78	0.96	0.44	0.38
SD	0.74	0.88	24.22	0.87	2.09	0.21	0.05	0.11	0.03

A - Standardized for effect of daily mean temperature and time of day on body fat

B - Not all winners and losers were identified in each interaction. If clear loser/winner was identified, observation was included.

Table 2.4 Comparison of solitary and agonistic observations by sex.

	Observed	Expected	Proportion^{A}	P Value^{B}
All Observations				
Male	1813	1714	0.52	<0.001*
Female	1484	1583		
Total	3297			
Solitary Feeding				
Male	1294	1389	0.56	<0.001*
Female	1198	1103		
Total	2492			
Subordinate				
Male	228	217	0.56	0.284
Female	161	172		
Total	389			
Dominant				
Male	291	232	0.56	<0.001*
Female	125	184		
Total	416			

A = All Observations based on proportion in population; Solitary Feeding, Subordinate, and Dominant based on total proportion of individual male and female observations, respectively

B = exact binomial test; bold = significant at P = 0.05, two-tailed

Table 2.5. Exact binomial goodness of fit test by kin group agonistic observations as compared to unrelated. Expected values based on proportion of visits to feeder for each individual member of kin group, out of total observed visits at feeder for all birds, if interactions occurred randomly.

	Observed N	Expected N	Proportion	P Value[A]
PO	6	5.5	0.015	0.827
U	360	360.5		
Total	366			
FS	1	0.8	0.002	0.519
U	365	365.2		
Total	366			
HS	26	7.4	0.02	<0.001*
U	340	358.6		
Total	366			
R	33	13.5	0.037	<0.001*
U	333	352.5		
Total	366			
PO Top 4[B]	2	1.4	0.019	0.721
U	100	98.2		
Total	102			
R Top 5[C]	18	8.4	0.042	0.003*
U	184	193.6		
Total	202			

A = exact binomial test; asterisk = significant at P = 0.05, two-tailed

B = Interactions involving top 4 ranked PO birds (based on % win)

C = Interactions involving top 5 dominant birds (wins ≥ 50%) with related birds

PO = Parent/Offspring, FS = Full Siblings, HS = Half Siblings, R = includes PO, FS, and HS, and U = Unrelated

Chapter 3: East coast, west coast and in-between: Phylogeographic structure of black-capped chickadee

J. Hindley[1,2] and T. M. Burg[1]

Prepared as manuscript for submission

[1]Department of Biological Sciences, University of Lethbridge, 4401 University Drive, Lethbridge AB, T1K 3M4
[2]corresponding author: j.hindley@uleth.ca

Abstract

The non-migratory black-capped chickadee (*Poecile atricapillus*) has a continent-wide distribution extending from the northern half of the U.S. into central Canada, and in the west as far as the Northwest Territories and Alaska. To investigate the phylogeographic structure of black-capped chickadee, and verify possible refugium(a) during the Last Glacial Maximum (LGM), we used a 440 bp sequence of mitochondrial DNA (control region) from 439 chickadees across 28 populations in North America, and performed paleoecological distribution modeling (MAXENT) to identify locations of possible glacial refugia. Two main groups were found using multiple analyses: a monophyletic Newfoundland clade and a widespread polyphyletic continental group, with additional substructure evident in the western populations (OR, WA, AK).

Our results support a separate NL refugial population that has remained isolated from continental populations since at least 75 kya. Within the continental populations, black-capped chickadee shows typical East/West division between the Cascades (Pacific) and Rocky Mountains (all other continental groups) consistent with late Pleistocene vicariance events with results suggesting at least two refugia: one "south of the ice sheets" located east of the Rocky Mountains in the south-central U.S., and a separate Pacific refugium. Evidence of secondary contact between the Pacific and south-central U.S. refugial populations was identified in Northwest British Columbia, and a fourth refugium may have been located in the southern Rocky Mountains. Paleoecological distribution modeling predicted suitable habitat existed within similar possible refugia locations during the LGM. Finally, the effect of hybridization between black-capped and

Carolina chickadees (*P. carolinensis*) on species identification and possible extirpation of black-capped chickadee in the southeast U.S. are also discussed.

Keywords: black-capped chickadee, North America, Last Glacial Maximum, post-glacial colonization, refugia, dispersal barriers, mitochondria, control region, phylogeography

3.1 Introduction

Climate oscillations during the Quaternary influenced species' distributions (Hewitt 1996; Avise and Walker 1998; Hewitt 2000; Brunsfeld *et al.* 2001; Richardson *et al.* 2002 ; Barrowclough *et al.* 2004; Hewitt 2004a; Burg 2007; Milá *et al.* 2007) and isolated populations, resulting in subsequent radiations that were among the most remarkable in vertebrates (Schluter 2000; Coyne and Orr 2004). Pleistocene glaciations are thought to be responsible for many recent avian speciation events (Johnson and Cicero 2004; Weir and Schluter 2004; Milá *et al.* 2007), however, the overall timing of Pleistocene glaciations and their effect on species diversity is still a topic for debate (Klicka and Zink 1997; Avise and Walker 1998; Zink *et al.* 2004; Milá *et al.* 2007). Regardless of the timing of speciation events, paleoclimatic fluctuations have resulted in expansion and contraction of suitable habitat, and depending on individual species' niche requirements, populations have expanded and contracted as a result of these fluctuations (Avise 2000; Hewitt 2000; Johansen and Latta 2003; DeChaine and Martin 2005).

During unfavourable conditions (e.g., glacial periods), species' range distributions would have contracted and forced populations to survive in ice-free refugia. In contrast, during the interglacial periods, species underwent population and range expansion, as ice sheets receded (Avise 2000; Hewitt 2000; Hewitt 2004b), resulting in populations that had once been isolated in one or multiple refugia dispersing into newly available habitat (Pielou 1991; Waltari *et al.* 2007; Brunsfeld *et al.* 2001; Galbreath *et al.* 2009). This process has been documented for a multitude of plant and animal species following the Last Glacial Maximum (LGM) ~18 kya (thousand years ago) in Europe and North America (Taberlet *et al.* 1998; Lessa *et al.* 2003; Hewitt 2004a).

North America was strongly influenced by Pleistocene climatic oscillations (Pielou 1991) and several species show a strong east/west division as a result of Pleistocene associated habitat change and/ or the Rocky and Cascade Mountain Ranges acting as dispersal barriers (Barrowclough *et al.* 1981; Noonan 2001; Ruegg and Smith 2002; Barrowclough *et al.* 2004; Galbreath *et al.* 2009). The Rocky Mountains have provided glacial refugia for high-elevation plant (Brunsfeld *et al.* 2001; Brunsfeld and Sullivan 2005), invertebrate (DeChaine and Martin 2005), and vertebrate (Good and Sullivan 2001) species (for review see Shafer *et al.* 2010).

West of the Rocky Mountains, and within the Cascade Range region multiple molecular studies of both plant (Soltis *et al.* 1997; Soltis and Gitzendanner 1999) and animal species (Brown *et al.* 1997; Ostberg and Thorgaard 1999; Nielson *et al.* 2001) have suggested a recurrent pattern of genetic differentiation, resulting in two clades that often correspond to a northern clade (e.g., populations from Alaska to central and southern Oregon) and southern clade (e.g., populations from central Oregon southward to northern California; reviewed in Soltis *et al.* 1997). This pattern suggests the possibility of a western refugium, as well as the potential for separate northern and southern glacial refugia for species in the Pacific Northwest.

Hewitt (1996) proposed two models of recolonization from refugia; the pioneer and phalanx models. In the “phalanx” model, recolonization is slow and steady resulting in uniform distribution of refugial genetic diversity (i.e., no loss of genetic variation). In the “pioneer” or “leading edge” model, recolonization is rapid via both short- and long-distance dispersal resulting in some refugial genomes spread over large areas, with pockets of genetically isolated populations found within the larger, more homogenous metapopulation (Hewitt 1996; Johansen and Latta 2003).

Our study examines the contemporary population genetic structure and phylogeography of the black-capped chickadee (*Poecile atricapillus*) using the highly variable mtDNA control region (CR). The black-capped chickadee is a non-migratory species that primarily inhabits deciduous and mixed deciduous/coniferous woodlands (Foote *et al.* 2010). Their distribution spans the entire width of North America, extending from the treeline in the north to as far south as Colorado (Figure 3.1), and juveniles disperse a short distance (0.5-11 km) from their natal area (Weise and Meyer 1979). The relatively large continental distribution of black-capped chickadee provides an opportunity to investigate the presence/absence of previously identified east/west and north/south population subdivision within the same species. By sampling across the range, we can investigate not only population subdivision, but also identify the best model of recolonization for black-capped chickadee.

Gill *et al.* (1993) proposed that black-capped chickadees may have expanded out of a common refuge approximately 10 thousand years ago (kya) following the retreat of the Wisconsin ice shield (i.e., Laurentide and Cordilleran ice sheets), and found that all NL birds contained one of two haplotypes (out of nine total), which were restricted to NL (total samples n = 82 samples; Newfoundland n = 18, continental n = 64) from 10 provinces/states across North America. Additionally, Pravosudov *et al*. (2012) found population structure and differentiation among 10 continental populations of black-capped chickadee. However, sampling in both studies was limited to a small number of populations across the black-capped chickadee's range. Our study includes a more comprehensive sampling design that will allow us to verify and expand upon previous results. We expect to find evidence of glacial refugia, such as unique haplotypes, high haplotype/nucleotide diversity, and/or star-like haplotype patterns, for at least two possible refugia (continental and Newfoundland) as well as additional population structure.

We investigated the inferred evolutionary history of black-capped chickadee, focusing on the influence of the LGM during the late Pleistocene glaciation cycles on genetic structure. The four primary questions we examined were: is there evidence of one or more glacial refugia as previously suggested by Gill *et al.* (1993); is there evidence of physical or non-physical barriers to gene flow; does post-glacial range-expansion of black-capped chickadee follow a phalanx or pioneer model; and is there a strong east/west division?

We predict the Rocky Mountains will be a barrier to black-capped chickadee gene flow resulting in a typical east/west division across North America, as seen in other species (e.g., Barrowclough *et al.* 1981; Noonan 2001; Boulet and Gibbs 2006; Galbreath *et al.* 2009). Finally, dispersal is predicted to follow the phalanx model due to the non-migratory behaviour and typically short-distance dispersal by juvenile black-capped chickadees, and would be consistent with previously identified population structure (Pravosudov *et al*. 2012). The phalanx dispersal pattern would result in common refugial haplotypes, or alleles dispersed across the range, with little loss of genetic diversity, but occasional fall/winter irruptions may allow for long-distance dispersal and subsequent isolation of populations and unique haplotypes.

3.2 Methods

3.2.1 Sampling

Birds were captured using mist nets, and blood or feather samples were collected from 388 individuals during the summers of 2007 to 2010 and stored in 95% ethanol. An additional 51 tissue samples (IL, MI, WV, NC) collected within the last 20 years were obtained from museums. A total of 439 samples from 28 sampling sites (Figure 3.1) across the contemporary black-capped chickadee range (Ridgely *et al.* 2007) were used for analysis. Samples were collected during the summer, and all samples within each sample site were collected from as small an area as possible (typically within a 50-75 km radius). DNA was extracted from whole blood or tissue using a modified chelex protocol (Walsh *et al.* 1991; Burg and Croxall 2001).

3.2.2 MtDNA Amplification and Sequencing

We amplified the mitochondrial control region (CR) using polymerase chain reaction (PCR) primers HCRCBox (5′- CCACTTGTATCTGTGARGAGC -3′) and LbcchCR1 (5′- CCACCACCCCATAATAAGGA -3′). The PCR was carried out in an Eppendorf Mastercycler, and consisted of approximately 100 ng of template DNA, 1 μM of each primer, 200 μM dNTPs, 2.5 mM $MgCl_2$, 1 unit of Taq DNA polymerase (Crimson) and PCR buffer (Crimson or Promega) in a final volume of 25 μl. Amplification consisted of one cycle at 95°C for 2 min, 54°C for 45 s, and 72°C for 60 s; 37 cycles of 94°C for 30 s, 54°C for 45 s, and 72°C for 1 min; and one final cycle at 72°C for 5 min. We then sequenced a 440 bp section of the PCR product within Domain I and II of the CR using BigDye terminator mix in an Applied Biosystems 3130 Genetic

Analyzer following enzymatic clean up using 0.1 units of shrimp alkaline phosphatase (SAP) and 0.1 units of exonuclease I. MtDNA sequences were manually aligned using MEGA v5.0 (Tamura *et al.* 2011).

3.2.3 Phylogenetic Analyses and Genetic Diversity

Phylogenetic analyses were conducted using two different approaches, statistical parsimony and maximum likelihood, to investigate the phylogeographic relationship among the 439 chickadee samples. We used TCS v1.21 (Clement *et al.* 2000) to construct a statistical parsimony network with gaps treated as a fifth character state. MEGA v5.0 was used to select the model of sequence evolution that best fit the sequence data (K2 + G + I; BIC = 6161), and a maximum likelihood (ML) tree was constructed using the same substitution model (discrete gamma categories n = 4) and nearest neighbor interchange heuristic model with 1000 bootstrap replicates to evaluate robustness. The program DNASP v5.10 (Librado and Rozas 2009) was used to calculate the number of haplotypes (H), haplotype diversity (H_d), and private haplotypes (*Pri*; shared haplotypes found in a single population), and nucleotide diversity (π).

3.2.4 Population Structure and Gene Flow

Pairwise Φ_{ST} (mtDNA), a basic index of population differentiation, was calculated for the 25 populations with at least five samples (avg = 16.7). We used Arlequin v3.0 (Excoffier *et al.* 2005) to calculate Φ_{ST} and tested for significant differences using 10,000 permutations. All p-values were corrected for multiple tests using the Benjamini-Hochberg False Discovery Rate correction (Benjamini and Hochberg 1995). A principal coordinate analysis (PCA) was conducted for mtDNA (simple pairwise differences) both with and without the Newfoundland population using GenAlEx v6.41 (Peakall and Smouse 2006). NL was excluded from a separate analysis in case genetic differentiation within the continental group was masked by a large number of nucleotide substitutions between the continental and NL populations.

Using the program BAPS v5.3 (Corander and Tang 2007; Corander *et al.* 2008), we conducted a Bayesian cluster analysis to estimate the number of possible metapopulation groupings (K). BAPS estimates the optimal number of groupings without an *a priori* assumption of sampling location. We conducted 10 runs with a maximum number of possible clusters of 28 (total number of populations sampled).

Arlequin v3.0 (Excoffier *et al.* 2005) was used to perform an analysis of molecular variance (AMOVA) based on possible population groupings (i.e., clusters) to investigate population structure. Possible groupings were defined using clusters identified in BAPS, Φ_{ST}, and TCS as well as combining clusters into larger supergroups, based on geographic location.

A genetic landscape shape analysis was conducted by the program Alleles in Space (AIS; Miller 2005) using AIS-calculated pairwise genetic distances. We used residual genetic distances to account for any potential correlation between geographic and genetic distance (Manni and Gue 2004; Miller *et al.* 2006). The program assigns the genetic distances to midpoints between sampling locations (latitude/longitude) using the Delaunay triangulation-based connectivity network (Miller *et al.* 2006). The interpolation procedure in AIS was then used to infer residual genetic distances at sampling locations on a uniformly spaced grid. Finally, a three-dimensional surface plot was produced, where X and Y coordinates correspond to the geographic location of samples (latitude/longitude) and the *Z* coordinate depicts genetic distance; peaks and valleys correspond to higher and lower than expected genetic distances, respectively.

Isolation by distance (IBD) was evaluated using a Mantel test in GenAlEx v6.41 to identify any positive correlation between genetic distance ($F_{ST}/(1-F_{ST})$; Rousset and Raymond 1997) and straight-line geographic distance. Significance was tested with 9999 permutations. 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. We tested for IBD using all populations, and using only continental populations (i.e., excluding Newfoundland).

3.2.5 Phylogeographic History

We tested for deviations from neutrality using Fu and Li's F and D tests (Fu 1997). To test for recent population expansion, we performed Fu's F_S test and Ramos-Onsins and Rozas R_2 test (Fu 1997; Ramos-Onsins and Rozas 2002). Both tests have been shown to be the most powerful tests available for detecting population growth (Ramos-Onsins and Rozas 2002). Fu's F_S detects excess recent mutations based on the observed haplotype distribution and is negative when there has been population growth, background selection or genetic hitchhiking. R_2 compares the number of singleton mutations in a population to the average number of nucleotide differences. Populations that have experienced recent expansion should contain an increased number of singleton mutations, and therefore low R_2 values. Fu's F_S and R_2 were calculated using DnaSP v5.10 and significance was evaluated by comparing observed values to a distribution of values generated under 10,000 coalescent simulations.

The time since most recent population expansion was estimated by calculating the distribution of net pairwise nucleotide differences between populations using Arlequin v3.0 (Excoffier *et al.* 2005), and two different estimated mutation rates (% per million years (My)). We calculated the estimated time since most recent population expansion (see Rogers and Harpending 1992) using the formula $\tau = 2ut$; where t is the time between the current population size and its initial size at the start of last expansion, and $u = 2\mu k$; where μ = the mutation rate and k is the sequence length. We used both a traditional and a more conservative mutation rate. The first mutation rate was calculated using the avian mutation rate for Domains I (20% per My) and II (5% per My) of the mitochondrial CR (Baker and Marshall 1997), and adjusting for the proportion of each domain sequenced (i.e., 320 bp in Domain I, and 120 bp in Domain II; divergence rate = 15.9% per My; μ = 7.95% or 7.95×10^{-8} mutations/site/year/lineage). A conservative divergence rate of 3% per My was obtained using 1.2% per My for the genus *Poecile* (Päckert *et al.* 2007), the estimated split between Carolina/black-capped lineages ~2.5 Mya (million years ago; Gill *et al.* 1993; Gill *et al.* 2005) and control region sequence data for black-capped and Carolina chickadees.

3.2.6 Divergence Times

We estimated divergence times using both a strict molecular clock method and a Bayesian method. The mean genetic distances (maximum composite likelihood) among groups were calculated in MEGA v5.0 and a strict molecular clock (15.9% and 3%) was used to estimate divergence time. We used the program BEAST v1.6.1 (Drummond *et al.* 2006; Drummond and Rambaut 2007) with a relaxed lognormal 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 (Rambaut and Drummond 2007a), and a random generated tree was used with a constant population size prior and two different substitution rate priors for separate runs. The first substitution rate prior was based on a 15.9% divergence per My between two lineages (i.e., substitution rate = 7.95×10^{-8} mutations/site/year/lineage; this study) and the second substitution rate prior was 1.5% per My, based on a 3% divergence rate (see above). Both BEAST analyses were run for 10 million generations, sampled every 1000 generations. Output files were viewed with Tracer v1.5 (Rambaut and Drummond 2007b) to estimate time to most recent common ancestor (tmrca).

3.2.7 Ecological Niche Modeling

In order to predict possible refugia during the LGM, 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 spatially correlated with phylogeographic patterns, suggesting that the two methods are complementary (Waltari *et al.* 2007). Bioclimatic variables were obtained from the WorldClim dataset v1.4, with a resolution of 2.5 min (Hijmans *et al.* 2005). Eleven out of the 19 available variables were correlated with other variables ($r > 0.90$), of which seven were removed from analysis (i.e., when two variables were correlated, the variable that was more biologically relevant was retained and two or more variables were often correlated with the same variable). The remaining 12 variables (i.e., BIO1, BIO2, BIO3, BIO5, BIO6, BIO8, BIO12, BIO13, BIO14, BIO15, BIO18 and BIO19; Appendix 3.1) were used to generate models in MAXENT with the default settings (regularization = 1, convergence threshold = 0.000001, iterations = 500), 10 replicates and 25% of sample locations used for model training (cross validation method). A total of 554 chickadee locations were used for modeling, which includes all unique (i.e., non-duplicate, n = 232) sampling locations obtained in this study, as well as an additional 322 museum specimen locations downloaded from the Global Biodiversity Information Facility (GBIF) data portal. Museum samples were used assuming that species identification for these locations had a higher probability of being correct than observational sightings alone. Duplicate points were omitted to prevent sampling bias.

MAXENT uses a maximum entropy statistical model of presence-only occurrence data based on the current distribution's (i.e., known presence location) climate conditions to infer past distributions by identifying similar bioclimatic conditions during a particular time (e.g., LGM), assuming that present niche requirements reflect past and/or future requirements. The MIROC (a Model for Interdisciplinary Research on Climate) climate layers provided by the Paleoclimate Modelling Intercomparison Project Phase II (PMIP2; Waltari *et al.* 2007) were used for projecting past climatic conditions at the LGM (~21 kya).

3.3 Results

3.2.2 Sequence Analyses

We examined the 440 bp mtDNA CR sequences from 439 black-capped chickadees and found a total of 124 haplotypes (Hap), including 45 shared haplotypes (i.e., haplotypes found in more than one bird; Appendices 3.2 and 3.3), with a total of 67 variable sites (Appendix 3.4). BAPS analysis identified an optimal group cluster number of five (Figure 3.1) corresponding to the following population groups: Pacific (AKA, AKF, AKW, WA, SOR), Central North (Central N; CBC, CAB, SK, MB, LAB), Central (SEBC, ID, NEOR, LETH, SAB, MI, ON, NSNB, WV, MO), Southeast Rockies (SE Rockies; MT, CO, UT), and Newfoundland (NL) with mixed group assignment for NWBC (Pacific and Central N groups) and IL (Central, Central N, and SE Rockies; Figure 3.1). Similar results but with less sub-structure were obtained with TCS (Figure 3.2) showing an isolated NL group, a mostly Pacific group, a mostly SE Rockies group, and a widespread mixed Central/Central N group.

Within the Pacific populations (i.e., AKA, AKF, AKW, WA, SOR; see below) six of the 10 shared haplotypes are restricted to birds from the Pacific group (n = 7 with CoOR included). One haplotype (Hap 5) was widespread and found in 15 populations (Figure 3.2, Appendix 3.2) across North America, and Haps 4, 22, 24, and 25 were found in at least eight populations, but none of these haplotypes were found in NL.

The ML tree showed phylogenetic structure, but low bootstrap values for most nodes and one monophyletic (NL) group with a bootstrap value of 44% (Figure 3.3). Three haplotypes, two shared (Hap 36 found in IL only, and Hap 41 found in SE Rockies) and one unique (IL_11) were separated from all other continental haplotypes and the NL group.

Pairwise Φ_{ST} values revealed significant differentiation among black-capped chickadee populations (Table 3.1). Within the Pacific, all three AK populations (AKA, AKF, AKW) were significantly differentiated from all other non-AK populations ($\Phi_{ST} >$

0.061, $p < 0.032$). Within the SE Rockies, CO and UT were not significantly different from each other ($\Phi_{ST} = 0.046$, $p = 0.043$) following correction for multiple tests, but were significantly differentiated from all other populations ($\Phi_{ST} > 0.092$, $p < 0.013$). The NL population was significantly differentiated from all other populations ($\Phi_{ST} > 0.577$, $p < 0.001$).

AMOVA results revealed the highest among group variation for two groups (Φ_{CT} = 38.67%, $F = 0.387$, $p = 0.032$; Table 3.2) separating NL from the rest of the continental populations. NWBC and IL showed mixed grouping assignments in BAPS, but using a possible group number of five identified in BAPS, AMOVA results showed the highest among group variation when combining NWBC in the Central N group and IL in the Central group (Φ_{CT} = 36.4%, $F = 0.364$, $p < 0.001$).

PCA revealed that 93% of the variation was explained in the first two axes and coordinate 1 separated NL from all other populations (Figure 3.4A). When NL was removed from analysis, 57% of the variation was explained by separating the Central, Central N, and SE Rockies groups from the Pacific group (Figure 3.4B, coordinate 1).

Genetic landscape shape interpolation analysis (AIS) revealed three major peaks/ridges (Figure 3.5). One peak isolated NL from all other populations; a second ridge between the Pacific and Central N/Central, with the strongest peak near AK; and a large ridge east and north of the SE Rockies group. AIS did not find any large genetic breaks between the Central N and Central groups.

3.3.2 Genetic diversity

Within the Pacific, AKA and AKF showed lower haplotype and nucleotide diversities ($H_d < 0.54$, $\pi < 0.003$; Table 3.3), while AKW had relatively higher diversities ($H_d = 0.92$, $\pi = 0.007$). The highest haplotype diversities were found in WV ($H_d = 0.97$), MI ($H_d = 0.97$) and MB ($H_d = 0.96$). NC and CoOR had $H_d = 1.0$, but were not included in population comparisons due to sample size (n < 5). The highest nucleotide diversities (excluding populations with n < 5) were found in NWBC ($\pi = 0.0098$) and MB ($\pi = 0.010$). One population (UT) was significant for deviation from neutrality (D = -3.26, p < 0.02, F = -3.44, p < 0.02; all other populations were not significant, data not presented). Eight populations had significant Fs and R_2 values (NEOR, MI, ON, NSNB, WV, CO, UT, NL; $F_S < -2.83$, $p < 0.019$; $R_2 < 0.09$, $p < 0.036$) suggesting recent population expansion.

Mantel tests found a significant correlation between straight-line geographic and genetic distances (IBD) among all populations ($r = 0.209$, p = 0.010) and among all continental populations (i.e., excluding NL; $r = 0.115$, p = 0.020; Figure 3.6).

3.3.3 Divergence and Diversification

The estimated time since the last population expansion began (τ), based on average nucleotide differences between individuals within each population, was calculated using both 3.0% and 15.9% divergence rates (Table 3.4). All three AK populations show population expansion times between ~4.3-8.6 kya and 22.7-45.5 kya, but SOR shows the longest times between 42.1 and 223.5 kya using 15.9% and 3% mutation rates, respectively. The estimated time since last expansion for NL is between

~7.9-41.7 kya. Overall, NL and AK populations suggest post-Pleistocene expansion times, while SOR suggests late Pleistocene or earlier. The Central, Central N and SE Rockies show varying expansion times from as little as 3.6-18.9 kya (UT) to as high as 47.9-253.8 kya (NWBC: expansion time for NWBC may be inflated due to secondary contact between Pacific and Central N groups in this area that would increase average number of nucleotide differences between individuals: see below).

Divergence time estimates varied across populations and groups (e.g., Central, Central N), and was heavily contingent on the relative mutation rate used for the analysis. MCL distances ranged from a maximum of 0.017 (Pacific and NL) to a minimum of 0.008 (Pacific and SE Rockies) which corresponds to divergence times between ~267-566 kya and ~50.3-106.9 kya with 3.0% and 15.9% divergence rate, respectively (Table 3.5). Divergence time estimates between NL and all continental populations ranged between ~94.3 kya (15.9%) and 500 kya (3.0%). BEAST results from the relaxed lognormal clock analysis for both 3% and 15.9% divergence revealed a tmrca between the NL and all other black-capped chickadee populations at 27 and 220 kya, respectively (Table 3.6).

3.3.4 Ecological Niche Modeling

Present day black-capped chickadee distribution as predicted by MAXENT using location information and present day environmental variables matched the current known distribution of this species (Ridgely *et al.* 2007; data not presented). The potential black-capped chickadee distribution predicted by MAXENT 21 kya (Figure 3.7) showed a large range contraction with four primary areas of suitable habitat; Newfoundland area; central and southeast U.S.; southwestern and western (CA and OR) U.S.; and Alaska. MAXENT predicted LGM model distribution had an AUC (area under curve) value of 0.944, and both training and test sample omission curves were close to the predicted value. The high AUC value and training/omission curves that are close to expected both indicate the model performed well.

Potential habitat was identified along the exposed continental shelf on the southeastern portion of Newfoundland (Grand Banks), as well as, the exposed Flemish Cap, both of which were ice free at the end of the LGM (Shaw 2006). In the southeastern U.S. suitable habitat was predicted 21 kya that extended from the eastern New Mexico/western Texas area east to the Georgia/South Carolina coastline. The southwestern portion of the U.S. supported suitable habitat for black-capped chickadee that included the southern portion of the Rocky Mountains (primarily in UT) and extended west across Arizona and Nevada, into the Sierra Nevada Mountains of California, and continued north to the Coast/Cascade Ranges of the Pacific Northwest (OR and WA). The fourth potential refugial area is located in southern/central Alaska and extended from the present day Anchorage area, including mountains within the Alaska Range, extending north of the Yukon River into the Arctic Circle and west of the present day Yukon-Kuskokwim Delta area.

3.3.5 Hybridization

A total of eight samples originally included in this study and identified as black-capped chickadee (museum samples) were excluded due to presence of Carolina chickadee mtDNA that was identified by fixed differences and unique insertion/deletion(s) among the samples, as well as comparing with Carolina chickadee samples. Excluded samples were collected in MO (n = 2), WV (n = 4), and NC (n = 2) population locations; all of which are located in known hybrid zones (Rising 1968; Braun and Robbins 1986; Robbins *et al.* 1986; Johnston 1971; Sattler and Braun 2000; Curry 2005).

3.4 Discussion

Mitochondrial analyses of black-capped chickadee populations revealed evidence of two main North American groups: a Newfoundland group and a widespread continental group, and analyses suggest different Pleistocene refugia for each. Within the continental group, there is a minimum of three subgroups including the Pacific, central continental (Central and Central N groups), and SE Rockies identified in multiple analyses (BAPS, TCS, AMOVA, PCA).

Our results are consistent with a previous study by Gill *et al.* (1993) that found a widespread continental group and a Newfoundland group, with no sharing of haplotypes between the two groups (total of 10 populations sampled). However, our study area included comprehensive sampling from across the black-capped chickadee range (total populations sampled, n = 25), and our results identified substructure within continental populations consistent with results from Pravosudov *et al*. (2012) who found structure and differentiation across 10 continental black-capped chickadee populations.

3.4.1 Pleistocene Refugium

Ecological niche modeling predicted a considerable reduction in suitable habitat availability for black-capped chickadee (Figure 3.7), with most of the habitat located in the southern mid-latitude portion of the continent, consistent with historically proposed glacial refugia south of the North American ice sheets (Pielou 1991). East of the Rocky Mountains results suggest that black-capped chickadees were confined to two main areas, the southeast Gulf States region (from the eastern corner of present day New Mexico through the Gulf States east to Georgia/Carolina coast) and NL. These results are consistent with previous studies that have identified refugia in the southeast portion of the continent (Boulet and Gibbs 2006; Colbeck *et al.* 2008; Ralston and Kirchman 2012) and Newfoundland (Holder *et al.* 1999; Lait 2011; Ralston and Kirchman 2012).

The NL group shows evidence of long term isolation (over 75 kya) and no secondary contact with continental populations due to significant pairwise Φ_{ST} with all other populations, the presence of private and divergent haplotypes, and no sharing of haplotypes between NL and continental groups, as well as the estimated divergence times (i.e., all continental populations show the highest divergence times with NL as compared to any other continental population; Table 3.5). The long-term isolation of NL suggests that large expanses of water such as Strait of Belle Isle and the Cabot Strait, which separate NL from the mainland, provide a substantial barrier to black-capped chickadee dispersal. The absence of reciprocal monophyly between NL and all continental populations could be due to recent separation and incomplete lineage sorting (Avise *et al.* 1983; Maddison and Knowles 2006).

Previous studies have suggested the presently submerged coastal shelf of Newfoundland served as a refugium for plants (Boys *et al.* 2005), insects (Berlocher and Dixon 2004), fish (Bernatchez 1997), mammals (Paetkau and Strobeck 1996; Kyle and Strobeck 2003) and frogs (Lee-Yaw *et al.* 2008). The role of Newfoundland as a possible Pleistocene refugium for birds has been suggested previously for black-capped chickadee (Gill *et al.* 1993), as well as rock ptarmigan (*Lagopus mutus*; Holder *et al.* 2000), song sparrow (*Melospiza melodia*; Zink and Dittmann 1993), and boreal chickadee (*P. hudsonicus*; Gill *et al.* 1993; Paige *et al.* 2006; Lait 2011), and our results add to the evidence supporting a Newfoundlad refugium during the LGM. Both the American redstart (*Setophaga ruticilla*) whose range overlap portions of the Central, Central N (excluding IL, MO), part of MT, and NL groups, and the blackpoll warbler (*S. striata*) whose range overlaps the Central N and Pacific (AK) groups, show similar patterns of a second Atlantic coast refugium such as Newfoundland and/or a previously exposed continental shelf, as well as dispersal across North America from a possible southeastern U.S. glacial refugium, with (Colbeck *et al.* 2008; Ralston and Kirchman 2012).

Within the western portion of the continent, multiple refugia may have been present along the Pacific Coast and in the southern Rocky Mountains (Figure 3.7), contrary to previously suggested dispersal out of a common refugium for continental populations by Gill *et al.* (1993). Significant differentiation (Φ_{ST}) between the Pacific group and Central/Central N groups, and high haplotype and nucleotide diversities within the AKW population, suggests a Pacific Northwest and/or Alaska/Beringia refugium, while the high nucleotide and haplotype diversities in SOR, combined with the longest estimated time since last population expansion, suggests isolation and a possible Pacific

Northwest refugium. Several plant and animal species show evidence of refugia within the AK/Beringia area (Fedorov and Stenseth 2002; Galbreath and Cook 2004; Brubaker *et al.* 2005; Waltari and Cook 2005; Anderson *et al.* 2006; Burg *et al.* 2006; Weksler *et al.* 2010), and the Pacific Northwest region (Smith and Sawyer 1988; Byun *et al.* 1997; Soltis *et al.* 1997; Demboski *et al.* 1999; Arbogast *et al.* 2001; Steele and Storfer 2006; Gugger *et al.* 2010; Pravosudov *et al.* 2012); for review of northwestern refugia see Shafer *et al.* (2010).

Within the SE Rockies geographic clustering of haplotypes and fewer shared haplotypes with other groups suggest long term isolation and restricted gene flow, possibly indicating a SE Rockies refugium. Previous studies have suggested refugia for both plant and animal species in the northern (Nielson *et al.* 2001; Carstens *et al.* 2004; Brunsfeld and Sullivan 2005) and southeast (Gugger *et al.* 2010) Rocky Mountains. Specifically, a southern UT area refugium has been suggested by both fossil and molecular data for Douglas-fir (*Pseudotsuga menziesii;* Gugger *et al.* 2010), a primary species component of mixed coniferous forest and a species that provides suitable habitat for black-capped chickadee. A similar refugium has also been identified for another Douglas-fir inhabitant, the mountain chickadee (Spellman *et al.* 2007; unpublished data).

3.4.2 Patterns of Population Expansion

As the ice sheets receded, black-capped chickadees expanded their range colonizing previously glaciated areas from Pleistocene refugia. Individuals in the Newfoundland area show no postglacial dispersal outside of NL, while those in the SE

Rockies show limited dispersal. In contrast, the Pacific and central continental groups (Central and Central N) show strong evidence of dispersal and secondary contact.

Within the Pacific group, we see low haplotype and nucleotide diversities (AKA, AKF, WA and SOR) due to possible genetic bottleneck and/or founder effect following long distance dispersal (Pravosudov *et al.* 2012), as well as the observance that six of the 10 shared Pacific haplotypes (seven if CoOR included) are not found in any other continental group. AKW shows above-average genetic distance (AIS) suggesting isolation. However, the AKW population has much higher haplotype and nucleotide diversities (over 1.6x higher than AKA and AKF) and has two shared haplotypes between each AKA and AKF (and an additional haplotype shared in all three populations; Appendix 3.2), suggesting recent contact between AKA and AKF within the AKW area.

AKA is separated from AKW by the Chugach Mountains (highest peak at 4,016 m), but both AKW and AKA are separated from AKF by the Alaskan Range that includes Denali (highest peak at 6194 m; Gesch 2009). This suggests that while the mountain ranges may be a barrier, at least some long distance dispersal has occurred between AKA and AKF. Dispersal may have occurred across mountain ranges, or more probably through low elevation valleys (e.g., Mentasta Pass connecting Copper River Basin (AKW) and Tanana River Valley (AKF), and Matanuska River Valley connecting Copper River Basin and Cook Inlet/Anchorage (AKA) area). Therefore, two dispersal scenarios are possible: 1) individuals from both AKA and AKF colonized the AKW area; 2) individuals dispersed out of AKW into AKA and AKF, but further studies are needed.

The Cascade Range appears to be an important barrier to dispersal due to significant differentiation (Φ_{ST}) between western populations (e.g., between both WA and SOR, and populations located on the eastern side of the Cascades (e.g., NEOR and ID). Additionally, both SOR and WA contain haplotypes not found in neighboring NEOR and ID, located east of the Cascades. The east-west division corresponds to similar patterns found in mountain chickadee (Spellman *et al.* 2007; unpublished data), as well as several other species including birds (Johnson and Cicero 2002; Ruegg and Smith 2002; Barrowclough *et al.* 2004), mammals (Demboski and Cook 2001), and plants (Soltis *et al.* 1997; Jaramillo-Correa *et al.* 2009). Additionally, the east-west phylogeographic split between Pacific Northwest coastal (Cascade) populations and the inland Rocky Mountains has been identified in a comparative framework for several species (Carstens *et al.* 2005; Albach *et al.* 2006).

Overall, post-glacial dispersal via a phalanx model of recolonization with occasional long distance dispersal appears to be the best explanation for the current population structure of black-capped chickadee. Population expansion following the LGM across large areas and from a common refugium under a phalanx model would be characterized by common widespread refugial haplotypes (Hewitt 2000; Milá *et al.* 2007), consistent with the observed widepsread haplotype disritbution within the continental groups (e.g., haplotypes 4, 5, 11, 12, 22, 25). Similarly, under a phalanx model, we expect populations to be more homogenous (than under a pioneer model), and adjacent populations to show similar genetic patterns (Hewitt 2000), which we see within major groups due to evidence of IBD and patterns of non-significant Φ_{ST} differentiation within groups, especially within the Central and Central N groups. The presence of a distinct contact zone (i.e., suture zone) between Pacific and Central N population in NWBC (see Secondary Contact) is also consistent with the phalanx recolonization model

(Johansen and Latta 2003). Based on Φ_{ST}, some geographically proximal populations were genetically similar (e.g., CAB and SK, SK and MB, MI and IL) consistent with a phalanx model, while others were not (e.g., CAB differentiated from SAB but not SK, LETH differentiated from SEBC but not MB) suggesting either a barrier to dispersal was present and/or long distance dispersal may have occurred. Under a phalanx model, we would expect a uniform (i.e., flat) AIS distribution between genetic and geographic distances as well as possible decrease in genetic variation as colonization proceeded northward from a southern refugium (Pruett and Winker 2005). AIS distribution generally shows a flat distribution across the Central and Central N groups consistent with a phalanx model. However, one area (CO/UT) shows lower than expected genetic distances contrary to the expected phalanx pattern, suggesting long distance dispersal, genetic bottleneck, and/or dispersal from a separate refugium. The presence of multiple populations with private haplotypes, and widespread group-specific haplotypes (i.e., found solely within each group; haplotypes 1-4, 6-8 in Pacific, for example) is consistent with what would be expected under a phalanx model of recolonization out of separate refugia, as well (Ibrahim *et al.* 1996; Johansen and Latta 2003).

Reduced gene flow due to geographic distance (IBD) is expected due to the life history of black-capped chickadee, which is known to disperse short distances from their natal area (median distance = 1.1 km; Weise and Meyer 1979) and exhibit extreme site fidelity (Smith 1991; van Oort and Otter 2005) with occasional fall and winter irruptions (band returns have shown dispersal distances over 1000 km; Brooks 1987) outside of the “typical” dispersal area. As a result, although black-capped chickadees are widely distributed across North America, gene flow may be limited within local regions, but occasional irruptions may facilitate gene flow between populations. For example CAB and SAB are separated by over 400 km with no obvious physical barriers, yet they share only 4 haplotypes (Appendix 3.2) supporting limited local gene flow.

Within the Central N region, boreal forest tree species quickly expanded north and eastward out of an eastern U.S. refugium (Soltis *et al.* 2006), and westward as the Laurentide ice sheet retreated, with contact between eastern and western forests ~ 6 kya (Webb *et al.* 1983; Williams 2002). Rapid dispersal by black-capped chickadee following boreal forest colonization, may provide one possible mechanism for the similar estimated times since last population expansion for four of the five Central N population (SK being the exception) and explain the weak, but significant differentiation between the Central and Central N groups. Several species show distinct boreal clades, from lower latitude clades, associated with recent colonization of northern boreal habitat including boreal chickadee (Lait 2011), gray jay (*Perisoreus canadensis*; van Els *et al.* 2012), fox sparrow (*Passerella iliaca*; Zink 1994), and mammals such as the flying squirrel (*Glaucomys sabrinus*; Arbogast 1999) and black bear (*Ursus americana*; Byun *et al.* 1997).

Evidence suggests that the northern and central Rocky Mountains may be a barrier to gene flow due to significant (Φ_{ST}) differentiation (Table 3.1) between populations located on opposite sides of the mountains (e.g., NEOR and MT, MT and ID, SAB and CBC). However, some populations on opposite sides are not differentiated (e.g., CAB and CBC) suggesting that the Rocky Mountains are porous and gene flow has occurred. Similarly, long-tailed vole (*Microtus longicaudus*), found in montane forest habitats, has been shown to have dispersed across prominent geographic features such as the central Rocky Mountain Continental Divide (Spaeth *et al.* 2009), with the same haplotypes (i.e., northern clade) found on both sides of the Rocky Mountains.

3.4.3 Secondary Contact

Following the LGM, an ice-free corridor developed between the Cordilleran and Laurentide ice sheets (~13 kya), with re-forestation within the corridor occurring ~9.3 kya (Pielou 1991; Rohwer *et al.* 2001; Dyke *et al.* 2002). If black-capped chickadees occupied separate coastal and inland refugia, then as re-forestation occurred within the ice free corridor, dispersal from both the Pacific and continental refugia would have occurred with subsequent contact between the two resulting. Evidence of secondary contact between Pacific and Central/Central N groups in NWBC, and the SE Rockies and central group in MT are evident through high haplotype and nucleotide diversity and sharing of haplotypes from two distinct groups in both NWBC and MT. NWBC in particular is a well-studied area for secondary contact between coastal and interior populations (Rohwer *et al.* 2001; Burg *et al.* 2006; Ruegg 2007a, 2007b; Krosby *et al.* 2009). The presence of Pacific group haplotypes within the NWBC population that are absence in adjacent Central N populations supports secondary contact between the Pacific and Central N groups.

A similar pattern of separate coastal Pacific and inland British Columbia refugia has been observed in chestnut-backed chickadee (*P. rufescens*; Burg *et al.* 2006; Lait *et al.* 2012), as well as introgression occurring in northern British Columbia between Townsend's warbler (*Dendroica townsendi*) dispersing out of an inland refugium and hermit warblers (*D. occidentalis*) out of a coastal refugium (Rohwer *et al.* 2001). Our results are consistent with a general pattern of an east-west split between the Rockies and the more coastal Cascade Range areas (Brunsfeld *et al.* 2001; Shafer *et al.* 2010), but also include the addition of our Alaska population within the western portion of the east-west split.

Another area of secondary contact may have occurred in IL, due to high haplotype diversity, mixed BAPS cluster assignment, and because IL contains shared haplotypes that are found in Central, Central N, and SE Rockies populations. The southern Illinois area has been identified as a significant suture zone in a comparative framework (Swenson and Howard 2005), as well as been previously identified as a hybrid contact zone between black-capped and Carolina chickadees (see Hybridization below). Additionally, according to Hewitt's (1996; 2000) phalanx model, refugial populations located near the glacial maximum will expand north, but the refugial population will obstruct southern populations from expanding northward (i.e., birds in a refugium expand north, but birds located south of the refuge cannot expand into already occupied refugium). This will result in a clustering of contact zones near the glacial maximum. Therefore, a contact zone within the southern Great Lakes region is consistent with the proposed limits of the Laurentide ice sheet (Dyke and Prest 1987; Dyke *et al.* 2002), and suitable habitat predicted at the LGM (Figure 3.7).

3.4.4 Hybridization

Present day black-capped chickadee dispersal may be influenced by competition with the parapatrically distributed Carolina chickadee. Once regarded as sister species, the two are now considered congeneric, are phenetically similar, and have long been known to hybridize (Rising 1968; Braun and Robbins 1986; Curry 2005). Carolina chickadee is restricted to the southeast United States and as a result, the two species come into contact along a narrow zone from Texas to New Jersey, with hybrid zones occurring in Kansas (Rising 1968), Missouri (Braun and Robbins 1986; Robbins *et al.* 1986), Virginia (Johnston 1971; Sattler and Braun 2000), Illinois (Brewer 1963), Pennsylvania (Ward and Ward 1974), and Ohio (Bronson *et al.* 2003a; Bronson *et al.* 2003b, 2005). In several studies, dominance by male Carolina chickadees has been shown to be a possible mechanism driving female mate choice in both (Bronson *et al.* 2003a; Bronson *et al.* 2003b, 2005), thereby reducing the fitness of male black-capped chickadees, and possibly resulting in the currently observed northern expansion of Carolina chickadee (Curry 2005; Reudink *et al.* 2007). Therefore, the possibility exists that genetically distinct black-capped chickadee populations previously occupying present day Carolina chickadee habitat, may have already been extirpated.

3.5 Conclusion

The black-capped chickadee shows an east-west and north-south division consistent with late Pleistocene vicariance events and evidence suggests at least two glacial refugia; one in NL and at least one refugium located in the southern U.S. A third potential refugium probably occurred along the Pacific Coast either in the Pacific Northwest (SOR) and/or within Alaska, and a possible fourth may have been located in the southeast U.S., but additional studies are needed to verify the locations of these refugia l.

Geographic substructure was evident within continental populations that clustered and were differentiated, indicating that gene flow is limited, but evidence of long-distance dispersal within the Pacific Northwest was evident. Therefore, due to the observed patterns, dispersal in black-capped chickadee appears to primarily follow the phalanx model with limited gene flow, but evidence suggests occasional long-distance dispersal, consistent with black-capped chickadee life history traits.

Large expanses of open water (e.g., Strait of Belle Isle, Cabot Strait) appear to be a barrier to black-capped chickadee dispersal due to significant differentiation between NL and all other populations. The Rocky Mountains are either not a significant barrier to dispersal for black-capped chickadee, or dispersal has occurred along both sides out of a southern refugium due to shared haplotypes on both the east and west sides. Alternatively, the Cascade Range appears to have been a significant barrier that is supported by differentiation among western (WA and SOR), and eastern populations (NEOR, and ID), as well as evidence of secondary contact between Pacific and Central N groups observed in NWBC. The Alaskan Range is also a significant barrier due to differentiation between AKW and AKF populations, but limited gene flow due to possible secondary contact is evident within the AKW area.

Acknowledgements

We are grateful to the following museums for providing additional samples for this study: samples 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. We thank Project FeederWatch, the National Park Service, USFWS and Canada Wildlife Service for their help and assistance. This research was funded in part by Alberta Innovates Technologies Futures fund and Natural Science and Engineering Research Council of Canada. 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, Brendan Graham, Linda Lait, Paulo Pulgarin, and numerous field assistants for their help with sample collection. The research presented here was conducted under University of Lethbridge Animal Welfare Protocol No. 0614 in accordance with Canadian Council on Animal Care guidelines.

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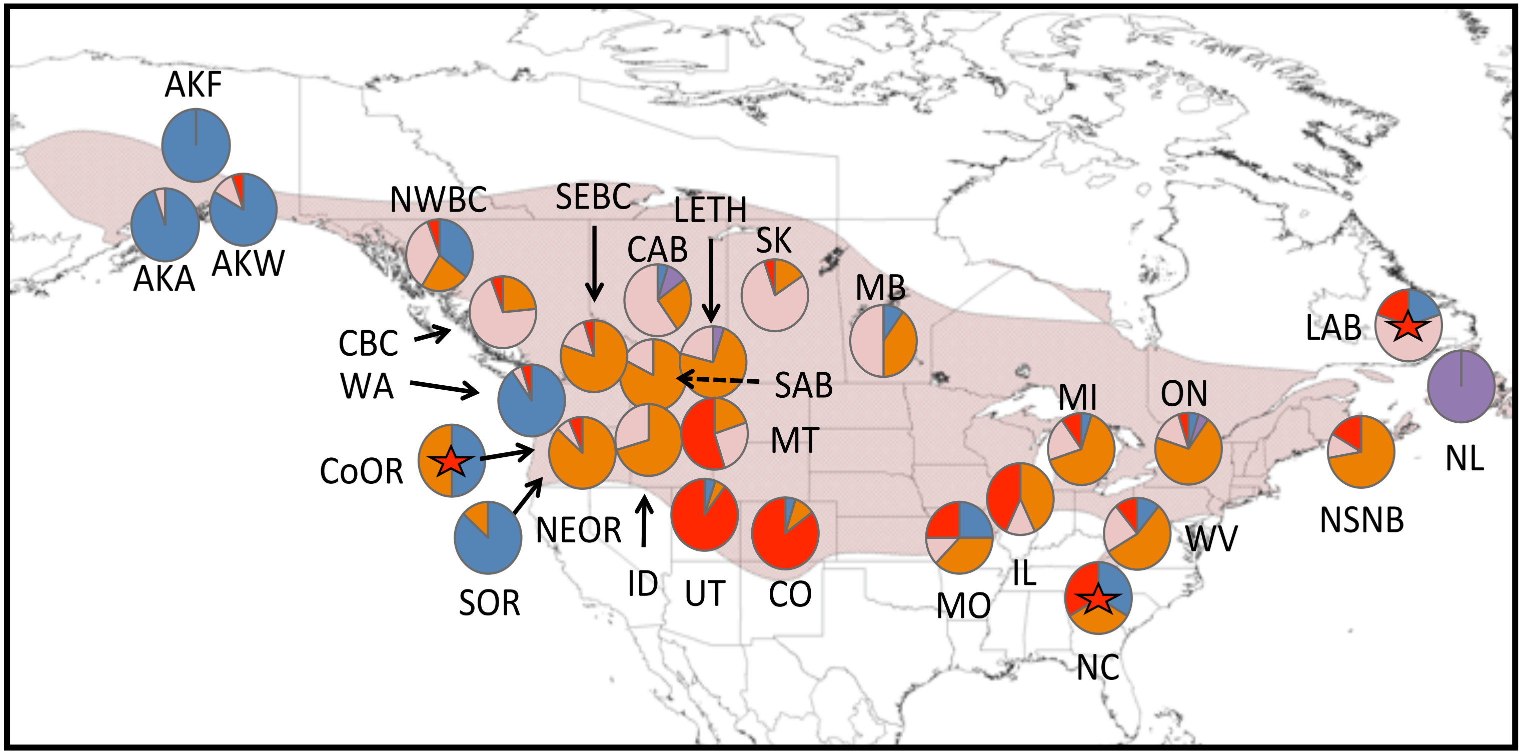

Figure 3.1. BAPS 95% CI cluster assignment (K = 5) of mtDNA haplotypes with each pie-chart centered over approximate mid-point sampling location for each population (excluding SOR, CoOR – see arrows); dark blue – Pacific, light pink – Central N, red – Southeast Rockies, orange – Central, purple – Newfoundland. Contemporary black-capped chickadee distribution is outlined in red crosshatch. Populations include Anchorage, Alaska (AKA), Fairbanks, AK (AKF), Wrangell-St. Elias Wilderness, AK (AKW), southern Oregon (SOR), northeast OR (NEOR), western Washington (WA), northwest British Columbia (NWBC), central BC (CBC), southeast BC (SEBC), central Alberta (CAB), Lethbridge, AB (LETH), Manitoba (MB), Saskatchewan (SK), Idaho (ID), southern AB (SAB), Michigan (MI), Missouri (MO), West Virginia (WV), Illinois (IL), Colorado (CO), Montana (MT), Utah (UT), coastal Oregon (CoOR), Ontario (ON), North Carolina (NC), Nova Scotia/New Brunswick (NSNB), Labrador (LAB), and Newfoundland (NL). Note: populations with n < 5 (NC, CoOR and LAB; red star) not included in population analyses.

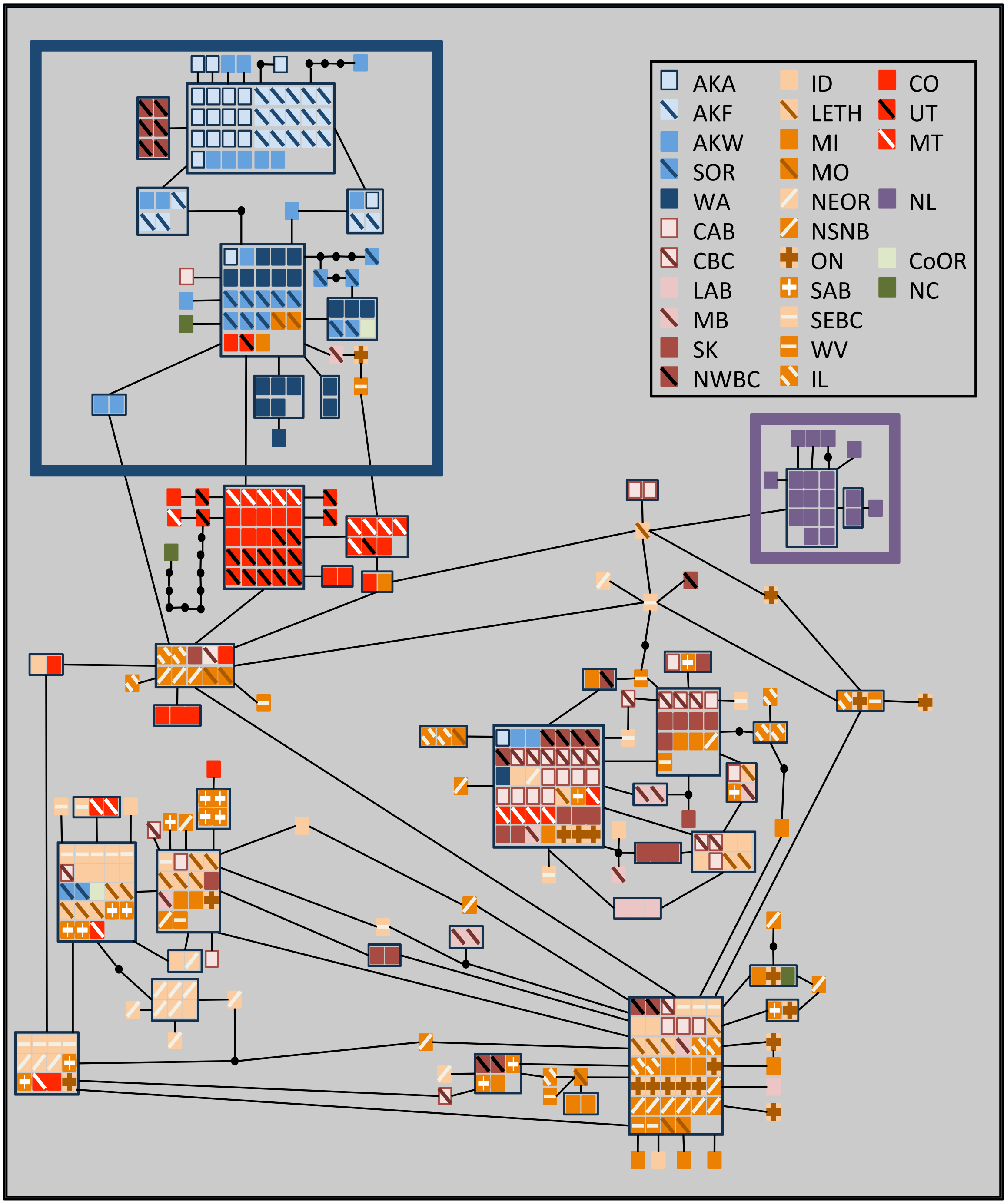
AKA
AKF
AKW
SOR
WA
CAB
CBC
LAB
MB
SK
NWBC
ID
LETH
MI
MO
NEOR
NSNB
ON
SAB
SEBC
WV
IL
CO
UT
MT
NL
CoOR
NC

Figure 3.2. Statistical parsimony network (TCS) of black-capped chickadee mitochondrial haplotypes. Each square represents a single individual and black boxes indicate inferred haplotypes. Refer to Figure 3.1 for location of sampling sites and abbreviations; Purple box indicates NL group and blue box outlines Pacific group haplotypes, with remaining haplotypes forming a widespread mixed continental group. Note NC, CoOR, LAB individuals are shown here, but not included in population analyses, and hybrids are not shown.

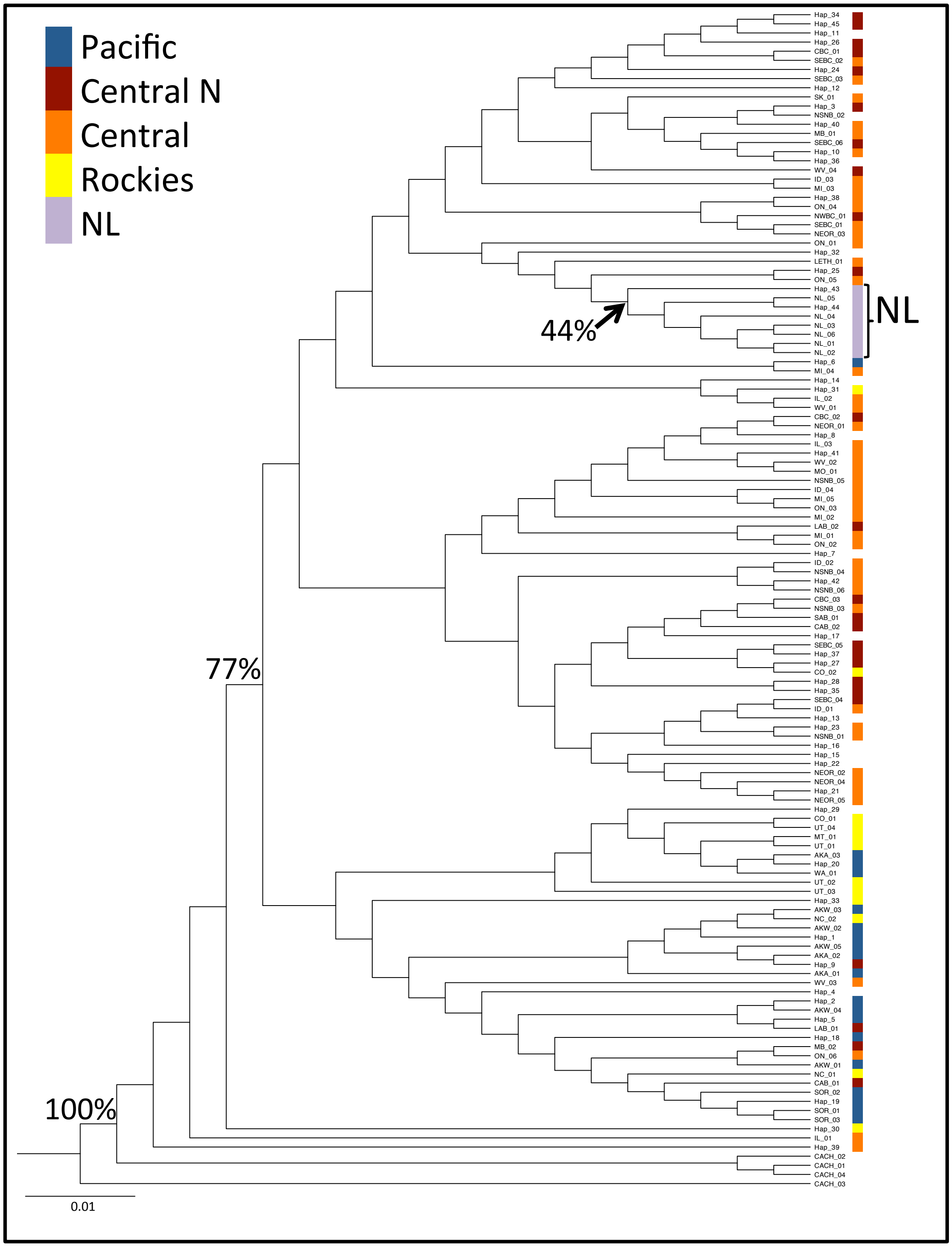

Figure 3.3. Maximum likelihood (ML) tree of black-capped chickadee haplotypes with bootstrap values greater than 40% shown (1000 bootstraps). NL is the only monophyletic group (44% bootstrap support). Carolina chickadee (CACH) sequences were used as outgroup, with 100% bootstrap separation from black-capped chickadee. (Note: * = shared haplotypes; all others are unique. Haplotypes are coloured according to BAPS/TCS/AMOVA grouping; mixed assignment is white). The bar indicates the number of substitutions per site, n = 0.01.

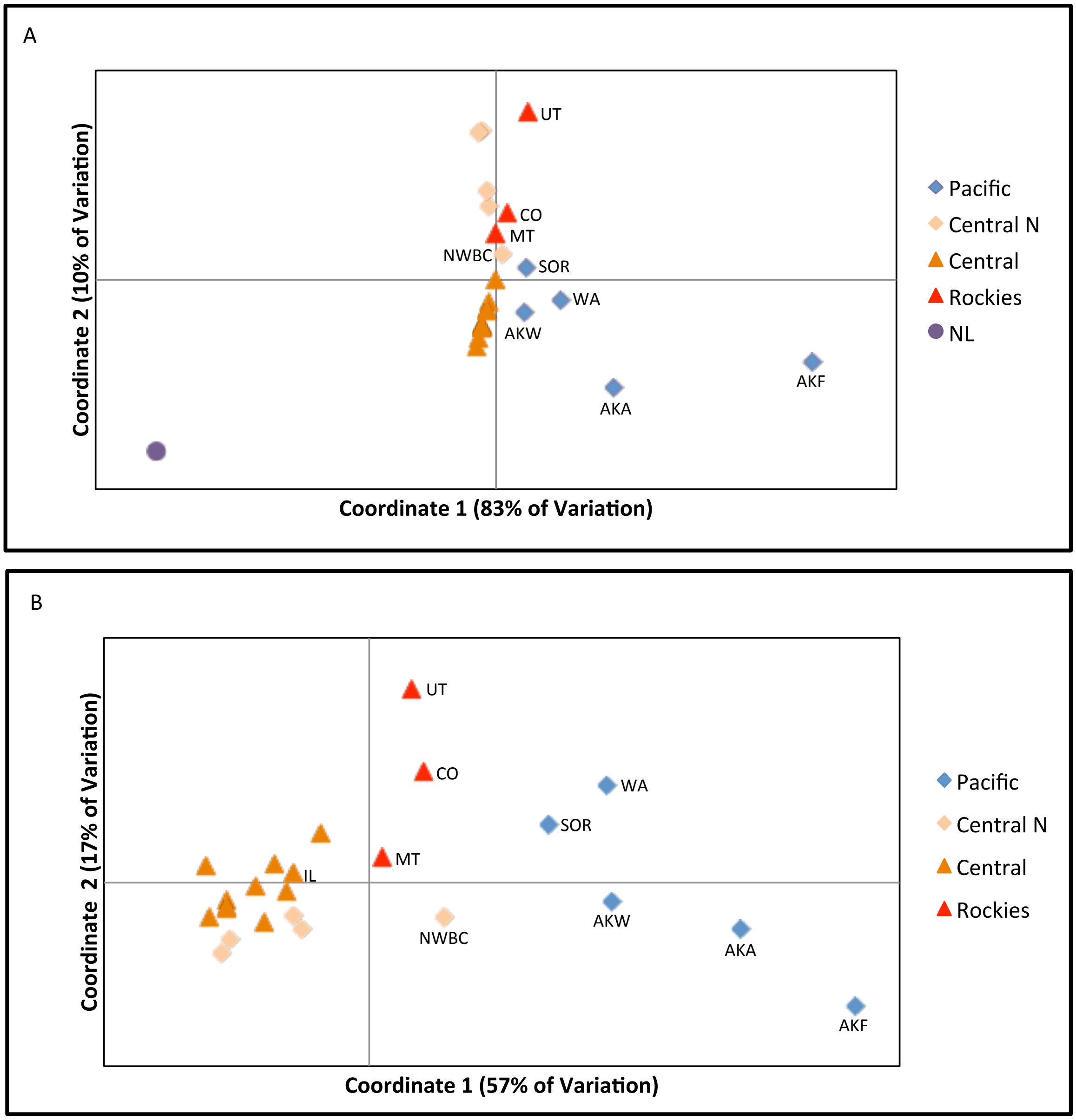

Figure 3.4. A: Principle coordinates analysis (PCA) of mtDNA sequences ($F_{ST}/1\text{-}F_{ST}$) for all populations based on population location; coordinate 1 explains 83% of the variation and coordinate 2 explains 10%. B: PCA of mtDNA sequences (continental populations only; NL excluded), coordinate 1 explains 57% of the variation and coordinate 2 explains 17%.

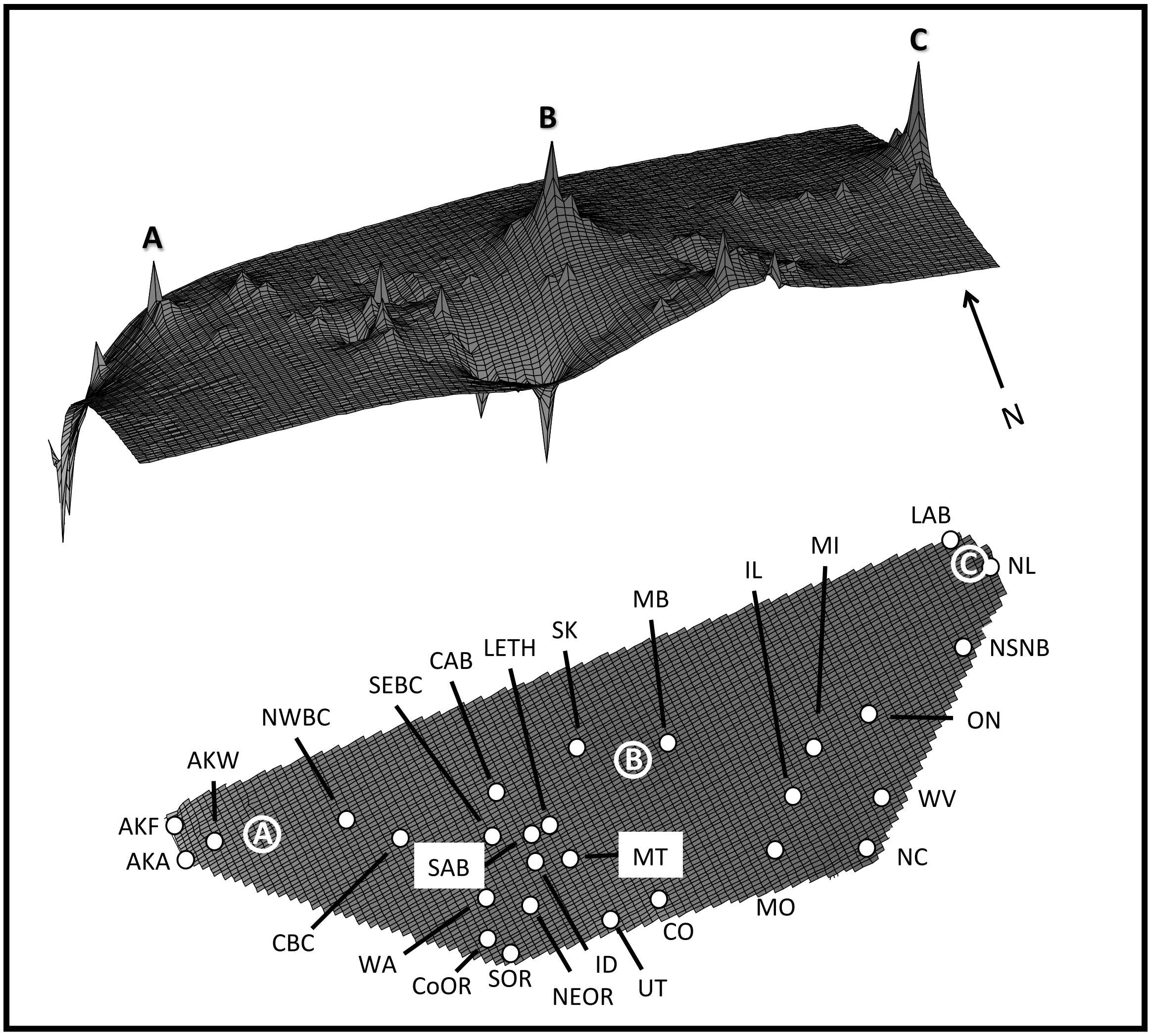

Figure 3.5. Top: Genetic landscape interpolation analysis (AIS) using residual genetic distances and a 100 x 100 grid with a distance weighting of 0.5. The X and Y coordinates refer to geographic location within the landscape based on individual sample latitude and longitude coordinates, and the Z axis (surface plot heights) corresponds to genetic distances. Large peaks indicate higher than expected genetic distances in relation to scaled geographic distance, suggesting possible genetic barriers (e.g., **C**: NL), while valleys indicate lower than expected and suggest possible genetic bottleneck or founder effect (e.g., **A**: AKA and AKF). Bottom: Sampling locations indicated by white dots (see Figure 3.1 for population abbreviations).

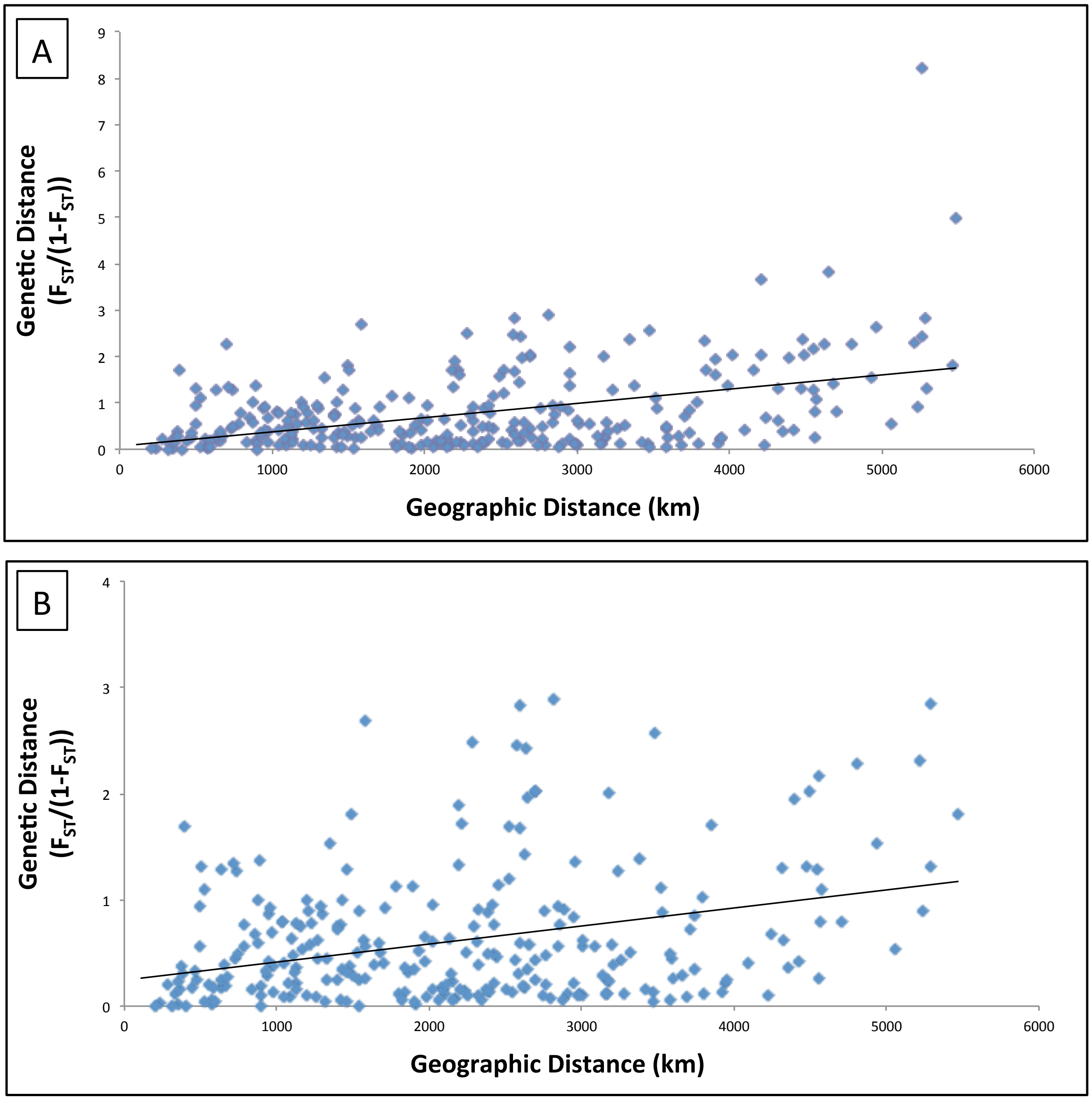

Figure 3.6. Isolation by distance (IBD) for all populations (A: $R^2 = 0.209$, $p = 0.010$) and for continental populations (i.e., excluding Newfoundland population; B: $R^2 = 0.115$, $p = 0.020$).

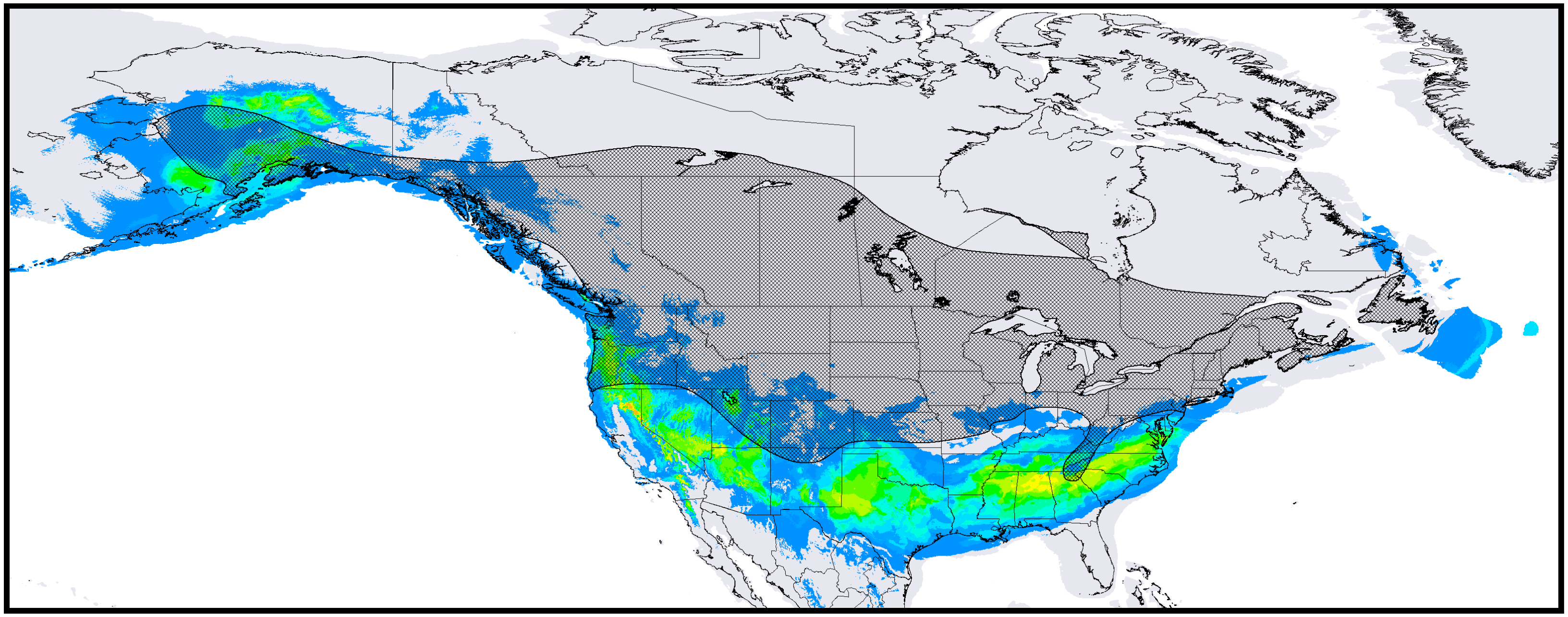

Figure 3.7. Average Ecological Niche Model (ENM) of possible black-capped chickadee refugia at the LGM ~21kya. Warmer colours (yellow and orange respectively) indicate higher habitat suitability. Black cross-hatch denotes present-day distribution.

Table 3.1. MtDNA pairwise Φ_{ST} values (bottom left) for comparison among black-capped chickadee populations (populations with n < 5 omitted; *italics* = non-significant, bold = significant after Benjamini-Hochberg correction (new critical p = 0.039); p value top right; * = for populations not shown all p ≤ 0.01, ** = p < 0.005. See Figure 3.1 for population abbreviations.

	1	2	3	4	5
AKA (1)*		0.14	0.20	**	**
AKW(2)*	0.03		**	**	**
AKF(3)*	0.02	**0.11**		**	**
WA(4)*	**0.38**	**0.19**	**0.53**		**0.03**
SOR(5)*	**0.33**	**0.15**	**0.46**	**0.06**	

	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
NWBC(6)		**0.01**	0.04	**0.00**	0.10	**	**	**	**	**	**0.01**	**	**	0.09	0.21	**0.03**	**0.02**	**	**
CBC(7)	**0.15**		0.70	0.50	0.29	**	**0.01**	**	**	**	**	**	**	**0.01**	**0.01**	**	**	**	**
CAB(8)	0.08	-0.02		0.28	0.66	**	**0.01**	**	**	**	**0.01**	**	**	0.05	0.04	**0.02**	**0.01**	**	**
SK(9)	**0.21**	-0.01	0.01		0.14	**	**	**	**	**	**	**	**	**	**	**	**	**	**
MB(10)	0.07	0.01	-0.03	0.05		**0.01**	0.06	**	0.04	**0.01**	0.06	**0.03**	**0.01**	0.14	0.17	0.16	0.07	**	**
SEBC(11)	**0.27**	**0.31**	**0.27**	**0.37**	**0.20**		0.36	**0.01**	0.36	0.38	**0.01**	**0.01**	**0.01**	**0.03**	**0.01**	**	**	**	**
ID(12)	**0.20**	**0.18**	**0.16**	**0.23**	0.09	0.00		**0.01**	0.64	0.35	**0.02**	**0.02**	**0.02**	0.06	**0.04**	**0.01**	**	**	**
NEOR(13)	**0.38**	**0.44**	**0.41**	**0.48**	**0.34**	**0.14**	**0.14**		**	**	**	**	**	**	**	**	**	**	**
LETH(14)	**0.23**	**0.24**	**0.19**	**0.28**	0.12	0.00	-0.03	**0.20**		0.60	0.06	0.06	0.08	0.14	**0.03**	**0.03**	**	**	**
SAB(15)	**0.25**	**0.29**	**0.25**	**0.33**	**0.17**	0.00	0.00	**0.17**	-0.02		**0.02**	**0.01**	**0.02**	0.05	**0.01**	**0.01**	**	**	**
MI(16)	**0.14**	**0.20**	**0.13**	**0.27**	0.08	**0.11**	**0.09**	**0.30**	0.06	**0.09**		0.73	0.55	0.89	0.42	0.61	**	**	**
ON(17)	**0.18**	**0.26**	**0.18**	**0.33**	**0.11**	**0.11**	**0.11**	**0.30**	0.07	**0.11**	-0.02		0.35	0.53	0.13	0.38	**	**	**
NSNB(18)	**0.21**	**0.30**	**0.22**	**0.36**	**0.16**	**0.11**	**0.10**	**0.29**	0.05	**0.08**	-0.01	0.00		0.39	0.10	0.10	**	**	**
WV(19)	0.09	**0.19**	**0.11**	0.25	0.05	**0.11**	0.09	**0.28**	0.06	0.09	-0.04	-0.01	0.00		0.72	0.74	0.06	**	**
MO(20)	0.04	**0.22**	**0.13**	0.30	0.06	**0.18**	**0.13**	**0.33**	**0.14**	**0.17**	0.00	0.05	0.06	-0.05		0.50	0.15	**0.01**	**
IL(21)	**0.11**	**0.19**	**0.12**	**0.26**	0.04	**0.14**	**0.12**	**0.32**	**0.10**	**0.13**	-0.01	0.00	0.04	-0.04	-0.01		0.04	**	**
MT(22)	**0.11**	**0.20**	**0.14**	**0.25**	0.09	**0.22**	**0.17**	**0.36**	**0.19**	**0.23**	**0.15**	**0.17**	**0.21**	0.09	0.06	0.08		**0.01**	**
CO(23)	**0.20**	**0.43**	**0.34**	**0.47**	**0.31**	**0.39**	**0.35**	**0.50**	**0.37**	**0.39**	**0.29**	**0.33**	**0.34**	**0.23**	**0.13**	**0.24**	**0.09**		0.04
UT(24)	**0.28**	**0.53**	**0.43**	**0.56**	**0.42**	**0.47**	**0.44**	**0.57**	**0.47**	**0.48**	**0.39**	**0.43**	**0.46**	**0.37**	**0.28**	**0.34**	**0.15**	0.05	
NL(25)*	**0.58**	**0.70**	**0.61**	**0.70**	**0.62**	**0.67**	**0.63**	**0.70**	**0.66**	**0.67**	**0.63**	**0.63**	**0.70**	**0.62**	**0.69**	**0.61**	**0.58**	**0.70**	**0.79**

Table 3.2. Analyses of molecular variance (AMOVA) of potential black-capped chickadee groupings. All F values were significant ($p < 0.001$ for all groupings except $n = 2$; $p = 0.032$) based on 1000 random permutations of the DNA sequences using ARLEQUIN v3.11. Populations with mixed assignment (NWBC and IL) were included subsequent groupings tested to identify the group assignment that explained the highest variance (i.e., NWBC was excluded from the first two groupings tested, IL was excluded from the first seven groupings tested).

Grouping Tested*	Variation Source	% of variance	*F*
(NL) (Continental)	Among groups (Φ_{CT})	38.67	0.387
	Among populations (Φ_{SC})	21.67	0.353
	Within populations (Φ_{ST})	39.67	0.603
(NL) (Pacific)	Among groups (Φ_{CT})	37.67	0.376
(Central, Central N, SE Rockies)	Among populations (Φ_{SC})	14.69	0.236
	Within populations (Φ_{ST})	47.64	0.524
(Pacific+NWBC) (Central N)	Among groups (Φ_{CT})	36.12	0.361
(Central) (SE Rockies) (NL)	Among populations (Φ_{SC})	8.86	0.139
	Within populations (Φ_{ST})	55.02	0.450
(Pacific) (Central N+NWBC)	Among groups (Φ_{CT})	37.05	0.371
(Central) (SE Rockies) (NL)	Among populations (Φ_{SC})	7.97	0.127
	Within populations (Φ_{ST})	54.98	0.450
(Pacific) (Central N+NWBC+Central)	Among groups (Φ_{CT})	35.87	0.359
(SE Rockies) (NL)	Among populations (Φ_{SC})	12.63	0.196
	Within populations (Φ_{ST})	51.50	0.485
(Pacific) (Central N+NWBC)	Among groups (Φ_{CT})	34.75	0.348
(SE Rockies+Central) (NL)	Among populations (Φ_{SC})	12.32	0.189
	Within populations (Φ_{ST})	52.93	0.471
(Pacific) (Central)	Among groups (Φ_{CT})	33.83	0.338
(SE Rockies+Central N+NWBC) (NL)	Among populations (Φ_{SC})	11.99	0.181
	Within populations (Φ_{ST})	54.18	0.458
(Pacific) (Central N, NWBC)	Among groups (Φ_{CT})	35.35	0.354
(Central) (SE Rockies+IL) (NL)	Among populations (Φ_{SC})	8.54	0.132
	Within populations (Φ_{ST})	56.11	0.438
(Pacific) (Central N+NWBC)	Among groups (Φ_{CT})	36.40	0.364
(Central+IL) (SE Rockies) (NL)	Among populations (Φ_{SC})	7.99	0.126
	Within populations (Φ_{ST})	55.61	0.444
(Pacific) (Central N, NWBC+IL)	Among groups (Φ_{CT})	35.47	0.355
(Central) (SE Rockies) (NL)	Among populations (Φ_{SC})	8.53	0.132
	Within populations (Φ_{ST})	56.00	0.440

Table 3.3. Genetic diversity within populations of black-capped chickadee: number of samples (*N*), Haplotypes (*H*), unique haplotypes (*U*), private haplotypes (*Pri*), haplotype diversity (*Hd*) and its standard deviation (*SD*), nucleotide diversity (π) and its SD, F_S and R_2 tests (bold denotes significance after Benjamini-Hochberg correction). * = populations with n < 5 not included in populations analyses.

	N	*H*	*U*	*Pri*	*Hd*	*Hd* ±*SD*	π	π ±*SD*	F_S	*p*	R_2	*p*
AKA	19	7	3		0.5439	0.1360	0.003	0.001	-2.878	0.039	0.1010	0.087
AKW	18	11	5	1	0.9150	0.0500	0.007	0.001	**-4.730**	**0.007**	0.1004	0.098
AKF	20	3			0.4263	0.1220	0.001	<0.001	-0.377	0.261	0.1145	0.134
WA	20	6	1	2	0.7790	0.0650	0.003	0.001	-1.093	0.145	0.1285	0.148
SOR	15	6	3		0.7143	0.1160	0.006	0.002	-0.193	0.217	0.1099	0.154
NWBC	17	6	1		0.8015	0.0650	0.010	0.001	1.672	0.173	0.1930	0.926
CBC	17	9	3		0.8603	0.0680	0.006	0.001	-2.569	0.049	0.1510	0.600
CAB	20	10	2	1	0.8316	0.0750	0.008	0.001	-2.279	0.059	0.1141	0.284
SK	19	8	1		0.8597	0.0490	0.006	0.001	-1.348	0.118	0.1420	0.556
MB	10	8	2	2	0.9556	0.0590	0.010	0.001	-2.333	0.071	0.1607	0.417
SEBC	20	11	6		0.9000	0.0440	0.006	0.001	-4.244	0.011	0.1022	0.152
ID	17	11	4		0.9265	0.0450	0.008	0.001	-3.795	0.017	0.1353	0.459
NEOR	15	9	5		0.8762	0.0700	0.007	0.002	**-2.830**	**0.040**	**0.0897**	**0.013**
LETH	19	7	1		0.8421	0.0470	0.006	0.001	-0.292	0.193	0.1531	0.671
SAB	17	9	1	1	0.8971	0.0480	0.007	0.001	-2.619	0.067	0.1303	0.388
MI	20	15	5	1	0.9684	0.0250	0.007	0.001	**-10.783**	**<0.001**	**0.0897**	**0.016**
ON	20	13	6		0.9053	0.0540	0.006	0.001	**-7.656**	**<0.001**	**0.0897**	**0.006**
NSNB	18	10	6		0.8431	0.0900	0.005	0.001	**-3.669**	**0.019**	**0.0897**	**0.005**
WV	9	8	4		0.9722	0.0640	0.007	0.001	**-4.034**	**0.016**	**0.0897**	**0.036**
MO	8	5	1		0.8929	0.0860	0.006	0.002	-0.552	0.237	0.0897	0.391
IL	14	8	3	1	0.9011	0.0580	0.007	0.001	-0.818	0.171	0.1258	0.224
MT	20	7	1		0.8368	0.0450	0.007	0.001	0.229	0.208	0.1754	0.858
CO	20	10	2	2	0.8316	0.0750	0.004	0.001	**-5.639**	**0.004**	**0.0853**	**0.020**
UT	19	8	4	2	0.6140	0.1300	0.002	0.001	**-4.948**	**0.006**	**0.0819**	**0.001**
NL	19	8	6	2	0.6725	0.1190	0.002	0.001	**-5.105**	**0.005**	**0.0897**	**<0.001**
CoOR*	2	2			1.0000	0.2500	0.014	0.007	1.792	0.857	0.5000	1.000
NC*	3	3	2		1.0000	0.2720	0.021	0.007	1.066	0.744	0.0897	0.231
LAB*	4	2	1	1	0.7000	0.2180	0.009	0.003	1.775	0.342	0.0897	0.376
Total	439	124	79	17	0.9635	0.0030	0.010	0.001				

Table 3.4. Estimated time since last population expansion (kya) based on average number of nucleotide differences between individuals using 3% and 15.9% divergence rates.

Pacific	AKA	AKW	AKF	SOR	WA
τ	1.0	1.2	0.6	5.9	1.2
15.9%	7.1	8.6	4.3	42.1	8.6
3%	37.9	45.5	22.7	223.5	45.4

Central N	NWBC	CBC	CAB	SK	MB
τ	6.7	5.9	5.8	2.4	5.4
15.9%	47.9	42.1	41.4	17.1	38.6
3%	253.8	223.5	219.7	90.9	204.6

Central	SEBC	ID	NEOR	LETH	SAB	MI	ON	NSNB
τ	0.9	2.0	2.9	6.7	2.1	5.9	1.0	1.0
15.9%	6.4	14.3	20.7	47.9	15.0	20.7	7.1	7.1
3%	34.1	75.8	109.8	253.8	79.5	109.9	37.9	37.9
	WV	MO	IL					
τ	3.6	1.2	4.6					
15.9%	25.7	8.6	32.9					
3%	136.4	45.5	174.3					

SE Rockies	MT	CO	UT
τ	5.3	1.9	0.5
15.9%	37.9	13.6	3.6
3%	200.8	72.0	18.9

NL	NL
τ	1.1
15.9%	7.9
3%	41.7

Table 3.5. Mitochondrial control region sequence divergence of black-capped chickadee groups, and black-capped (BCCH)/Carolina (CACH) chickadee using pairwise differences (MCL distance). Upper right diagonal = MCL distance; lower left diagonal = divergence (kya).

15.90%	Pacific	Central N	Central	SE Rockies	NL	Continental*
Pacific		0.013	0.013	0.008	0.017	–
Central N	81.8		0.011	0.010	0.016	–
Central	81.8	69.1		0.009	0.014	–
SE Rockies	50.3	62.9	57.6		0.012	–
NL	106.9	100.6	88	75.4		0.015
Continental*	–	–	–	–	94.3	
3%						
Pacific						
Central N	433					
Central	433	366				
SE Rockies	267	333	300			
NL	566	533	467	400		
Continental*	–	–	–	–	500	

15.9%	CACH	BCCH
CACH		0.077
BCCH	484	
3%		
CACH		
BCCH	2,567	

* = all continental populations (i.e., excluding NL)

Table 3.6. BEAST estimated time (kya) to most recent common ancestor (tmrca) for Newfoundland (NL) and continental populations of black-capped chickadee using both 15.9% and 3% divergence rate.

	15.9%	3%
tmrca NL	27.9	153
tmrca continental	220	1,150

Appendix 3.1. Bioclimatic variables used for ecological niche modeling of black-capped chickadee habitat. Variables obtained from WorldClim dataset (Hijmans *et al.* 2005).

Bioclimatic Variable	Description	Removed*
BIO1	Annual mean temperature	
BIO2	Mean diurnal temperature range	
BIO3	Isothermality (mean diurnal range/temperature annual range	
BIO4	Temperature seasonality	X
BIO5	Maximum temperature of warmest month	
BIO6	Minimum temperature of coldest month	
BIO7	Temperature annual range	X
BIO8	Mean temperature of wettest quarter	
BIO9	Mean temperature of driest quarter	X
BIO10	Mean temperature of warmest quarter	X
BIO11	Mean temperature of coldest quarter	X
BIO12	Annual precipitation	
BIO13	Precipitation of wettest month	
BIO14	Precipitation of driest month	
BIO15	Precipitation seasonality	
BIO16	Precipitation of wettest quarter	X
BIO17	Precipitation of driest quarter	X
BIO18	Precipitation of warmest quarter	
BIO19	Precipitation of coldest quarter	

* removed from analysis due to correlation with one or more other variables

Appendix 3.2. Geographic distribution of shared haplotypes (H) in black-capped chickadee populations. Private haplotypes (i.e., found in single population) are in red font. Note: NC, CoOR, and LAB were not included in population analyses due to small sample size.

	Pacific						Central N						Central												SE Rockies			NL
H	AKA	AKW	AKF	WA	SOR	CoOR	NWBC	CBC	CAB	SK	MB	LAB	SEBC	ID	NEOR	LETH	SAB	MI	ON	NSB	WV	MO	IL	NC	MT	CO	UT	NL
1	13	5	15																									
2	1	1	2																									
3		2	3																									
4	1	1		8	8													1				2				1	1	
5	1	2		1			5	6	8	5	1			1	1	1	1	1	3						5			
6		2																										
7				2																								
8				5																								
9				3	2	1																						
10							6																					
11								2	1					3		2												
12								3	1	5								2		1	1							
13								1		1										3		2	2			1		
14									1	1							1											
15									2																			
16									1		1					1	1											
17										3																		
18										2																		
19												3																
20											2																	
21											2																	
22									1	1	1		1			5		2	1	1	1							
23													4		3		2		1						1		1	
24					2	1		1					5	4		5	4								1			
25							2	1	3		1		3	2		4		3	6	7	2	2	4					
26													1												2			
27														1	5													

	Pacific						Central N						Central												SE Rockies			NL
H	AKA	AKW	AKF	WA	SOR	CoOR	NWBC	CBC	CAB	SK	MB	LAB	SEBC	ID	NEOR	LETH	SAB	MI	ON	NSNB	WV	MO	IL	NC	MT	CO	UT	NL
28														1	1													
29							2										2	1										
30							1											1										
31																		2										
32																	4											
33																	1		1									
34																		1	1					1				
35																			1		1		1					
36																							2					
37																						1	2					
38														1												1		
39																		1								1		
40																									5	8	12	
41																									5	1	1	
42																										3		
43																										2		
44																												11
45																												2

Appendix 3.3. Black-capped chickadee sample locations, band number, identification (ID), haplotype, latitude/longitude, and museum collection (where applicable).

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Anchorage, AK (AKA)						
Eagle River Campground, AK	2540-22801	AKA001	1		61° 18' 24"	149° 34' 15"
Eagle River Campground, AK	2540-22803	AKA002	1		61° 18' 20"	149° 34' 13"
Eagle River Campground, AK	2540-22804	AKA003	1		61° 18' 20"	149° 34' 13"
Eagle River Campground, AK	2540-22805	AKA004	1		61° 18' 20"	149° 34' 13"
Eagle River Campground, AK	2540-22806	AKA005	1		61° 18' 20"	149° 34' 13"
Eagle River Campground, AK	2540-22807	AKA006	1		61° 18' 23"	149° 34' 15"
Eagle River Campground, AK	2540-22808	AKA007	1		61° 18' 22"	149° 34' 19"
Eagle River Campground, AK	2540-22809	AKA008	1		61° 18' 21"	149° 34' 2"
Eagle River Campground, AK	2540-22810	AKA009	1		61° 18' 21"	149° 34' 2"
Eagle River Campground, AK	2540-22813	AKA010	1		61° 18' 26"	149° 34' 9"
Eklutna Rd, AK	2540-22815	AKA011	1		61° 25' 23"	149° 12' 8"
Eklutna Rd, AK	2540-22816	AKA012	1		61° 24' 38"	149° 9' 21"
Eagle River Rd x Roop Rd, AK	2540-22817	AKA013	2		61° 16' 39"	149° 22' 40"
Eagle River Rd x Vantage Av, AK	2540-22818	AKA014	3		61° 16' 32"	149° 22' 28"
Eagle River Rd x Vantage Av, AK	2540-22819	AKA015	1		61° 16' 32"	149° 22' 28"
Eagle River Rd x Vantage Av, AK	2540-22821	AKA017	AKA017		61° 16' 32"	149° 22' 28"
Eagle River Rd x "Fill site", AK	2540-22825	AKA018	4		61° 16' 6"	149° 20' 53"
Eagle River Rd x Clemens Cres, AK	2540-22827	AKA019	AKA019		61° 16' 56"	149° 23' 21"
Eagle River Rd x Clemens Cres, AK	2540-22828	AKA020	AKA020		61° 16' 56"	149° 23' 21"
Fairbanks, AK (AKF)						
Old Nanana Rd, AK	2540-22845	AKF001	1		64° 48' 59"	148° 11' 15"
Standard Crk Rd, AK	2540-22847	AKF002	2		64° 48' 42"	148° 12' 30"
Standard Crk Rd, AK	2540-22848	AKF003	5		64° 48' 42"	148° 12' 30"
Murphy Dome Rd, AK	2540-22849	AKF019	1		64° 55' 25"	148° 59' 18"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Murphy Dome Rd, AK	2540-22850	AKF020	1		64° 55' 25"	148° 59' 18"
Spinach Crk Rd, AK	2540-22851	AKF004	1		64° 55' 44"	148° 0' 35"
Miller Hill Rd, AK	2540-22852	AKF005	1		64° 52' 5"	147° 52' 51"
Miller Hill Rd, AK	2540-22853	AKF006	1		64° 52' 5"	147° 52' 51"
Miller Hill Rd, AK	2540-22858	AKF007	1		64° 52' 5"	147° 52' 51"
Spinach Crk Rd, AK	2540-22859	AKF008	1		64° 56' 29"	148° 5' 15"
Spinach Crk Rd, AK	2540-22860	AKF009	1		64° 56' 29"	148° 5' 15"
Tanana Valley Campground, AK	2540-22864	AKF010	1		64° 51' 55"	147° 45' 33"
Birch Hill Rec Area, AK	2540-22869	AKF011	1		64° 52' 16"	147° 38' 48"
Two Rivers Road, AK	2540-22875	AKF012	1		64° 52' 40"	147° 2' 33"
Two Rivers Road, AK	2540-22876	AKF013	1		64° 52' 40"	147° 2' 33"
Two Rivers Road, AK	2540-22877	AKF014	1		64° 52' 13"	147° 2' 34"
Steese Hwy, AK	2540-22879	AKF015	5		64° 12' 25"	147° 12' 39"
Steese Hwy, AK	2540-22880	AKF016	2		64° 12' 25"	147° 12' 39"
Nordale Rd, AK	2540-22882	AKF017	1		64° 51' 29"	147° 24' 18"
Tanana Valley Campground, AK	2540-22883	AKF018	5		64° 51' 51"	147° 45' 38"
Wrangell-St. Elias, AK (AKW)						
Old Edgerton HWY, AK	2540-23169	AKW001	AKW001		61° 45' 3"	144° 59' 23"
Old Edgerton HWY, AK	2540-23171	AKW002	AKW002		61° 45' 3"	144° 59' 23"
Old Edgerton HWY, AK	2540-23174	AKW003	2		61° 46' 1"	145° 1' 18"
Old Edgerton HWY, AK	2540-23176	AKW005	3		61° 46' 1"	145° 1' 18"
Old Edgerton HWY, AK	2540-23177	AKW006	1		61° 46' 1"	145° 1' 18"
Old Edgerton HWY, AK	2540-23178	AKW007	1		61° 46' 1"	145° 1' 18"
Old Edgerton HWY, AK	2540-23179	AKW008	AKW008		61° 46' 30"	145° 2' 11"
Old Edgerton HWY, AK	2540-23180	AKW009	5		61° 46' 30"	145° 2' 11"
Old Edgerton HWY, AK	2540-23182	AKW010	5		61° 46' 30"	145° 2' 11"
Old Edgerton HWY, AK	2540-23184	AKW011	AKW011		61° 46' 30"	145° 2' 11"
Old Edgerton HWY, AK	2540-23187	AKW012	1		61° 47' 39"	145° 4' 17"
Old Edgerton HWY, AK	2540-23188	AKW013	6		61° 47' 39"	145° 4' 17"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Old Edgerton HWY, AK	2540-23191	AKW014	6		61° 47' 39"	145° 4' 17"
Old Edgerton HWY, AK	2540-23193	AKW015	4		61° 49' 8"	145° 8' 29"
Richardson HWY x Old Edgerton HWY, AKW	2540-23197	AKW017	3		61° 49' 28"	145° 13' 9"
WISE headquarters, AK	2540-23200	AKW018	1		61° 48' 14"	145° 5' 35"
WISE headquarters, AK	2540-22901	AKW019	1		61° 48' 14"	145° 5' 35"
Old Edgerton HWY, AK	2540-22907	AKW020	AKW020		61° 49' 18"	145° 10' 17"
Central AB (CAB)						
Olds, AB	3111-48301	CAB001	17		51° 47' 29"	114° 17' 10"
Olds, AB	2520-38902	CAB002	7		51° 48' 22"	114° 35' 35"
Olds, AB	2520-39804	CAB004	12		51° 48' 25"	114° 35' 35"
Innisfail, AB	2520-39805	CAB005	24		52° 1' 38"	113° 56' 49"
Innisfail, AB	2520-39806	CAB006	25		52° 1' 38"	113° 56' 49"
Innisfail, AB	2520-39807	CAB007	7		52° 1' 38"	113° 56' 49"
Innisfail, AB	2520-39808	CAB008	25		52° 1' 38"	113° 56' 49"
Innisfail, AB	2520-39809	CAB009	3		52° 1' 38"	113° 56' 49"
Innisfail, AB	2520-39810	CAB010	26		52° 1' 38"	113° 56' 49"
Innisfail, AB	2520-39811	CAB011	7		52° 1' 38"	113° 56' 49"
Innisfail, AB	2520-39812	CAB012	3		52° 1' 38"	113° 56' 49"
Buck Lake, AB	2520-39814	CAB014	3		52° 57' 9"	114° 45' 1"
Buck Lake, AB	2520-39815	CAB015	3		52° 57' 9"	114° 45' 1"
Buck Lake, AB	2520-39816	CAB016	CAB016		52° 57' 9"	114° 45' 1"
Buck Lake, AB	2520-39817	CAB017	3		52° 57' 9"	114° 45' 1"
Buck Lake, AB	2520-39818	CAB018	3		52° 57' 9"	114° 45' 1"
Buck Lake, AB	2520-39819	CAB019	CAB019		52° 57' 9"	114° 45' 1"
Hinton, AB	2520-39822	CAB021	3		53° 24' 1"	117° 34' 44"
Hinton, AB	2520-39823	CAB022	3		53° 23' 11"	117° 35' 24"
Edmonton, AB	2520-39827	CAB024	11		53° 31' 46"	113° 33' 14"
Central British Columbia (CBC)						

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Smithers, BC	2520-39893	CBC001	11		54° 47' 7"	127° 9' 2"
Smithers, BC	2529-39882	CBC002	3		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39883	CBC003	3		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-29884	CBC004	12		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39885	CBC005	3		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39886	CBC006	CBC006		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39887	CBC007	CBC007		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39888	CBC008	3		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39890	CBC010	13		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39891	CBC011	11		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39892	CBC012	CBC012		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39898	CBC013	12		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39894	CBC014	3		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39899	CBC015	12		54° 47' 7"	127° 9' 2"
Smithers, BC	2520-39900	CBC016	14		54° 47' 7"	127° 9' 2"
Smithers, BC	2490-57761	CBC017	7		54° 47' 7"	127° 9' 2"
Smithers, BC	2490-57763	CBC019	3		54° 47' 7"	127° 9' 2"
Colorado (CO)						
Rist Canyon, CO	2540-23101	CO001	4		40° 37' 34"	105° 13' 21"
Boulder (Jone's), CO	2540-23102	CO002	CO002		39° 59' 44"	105° 16' 11"
Boulder (Jone's), CO	2540-23103	CO003	29		39° 59' 44"	105° 16' 11"
Rollands Pass Road (FS 149), CO	2540-23104	CO004	31		39° 54' 28"	105° 36' 28"
Central City (graveyard), CO	2540-23105	CO005	29		39° 48' 41"	105° 31' 49"
Central City (graveyard), CO	-	CO006	29		39° 48' 41"	105° 31' 49"
Pickle Gulch, CO	2540-23106	CO007	22		39° 50' 31"	105° 31' 19"
N of Cottonwood, CO	2540-23107	CO008	32		39° 46' 58"	105° 23' 31"
N of Cottonwood, CO	2540-23108	CO009	29		39° 46' 29"	105° 22' 31"
N of Cottonwood, CO	2540-23109	CO010	33		39° 46' 11"	105° 24' 7"
Cottonwood, CO	2540-23110	CO011	29		39° 47' 0"	105° 23' 54"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Pickle Gulch Campground, CO	2540-23111	CO012	14		39° 50' 32"	105° 31' 25"
N of Central City (HWY 119), CO	2540-23112	CO013	29		39° 51' 1"	105° 28' 54"
N of Central City, CO	2540-23113	CO014	29		39° 46' 43"	105° 22' 7"
N of Central City, CO	2540-23114	CO015	33		40° 2' 30"	105° 30' 4"
Fort Collins, CO	2540-23115	CO016	29		40° 37' 56"	105° 11' 9"
Fort Collins, CO	2540-23116	CO017	30		40° 37' 56"	105° 11' 9"
Fort Collins, CO	2540-23117	CO018	31		40° 37' 56"	105° 11' 9"
Fort Collins, CO	2540-23118	CO019	CO019		40° 37' 56"	105° 11' 9"
Fort Collins, CO	2540-23119	CO020	31		40° 37' 56"	105° 11' 9"
Coastal Oregon (CoOR)						
Toledo, OR 510 Strdevant DR.	2540-23001	CoOR001	19		44° 37' 57"	123° 55' 13"
Toledo, OR 510 Strdevant DR.	2540-23002	CoOR002	13		44° 37' 57"	123° 55' 13"
Idaho (ID)						
1037 Showalter Rd, Moscow ID	-	ID001	21		46° 46' 26"	115° 8' 16"
1358 4 Mile Rd, Moscow, ID	2540-23054	ID002	22		46° 50' 22"	115° 2' 7"
1358 4 Mile Rd, Moscow, ID	2540-23055	ID003	13		46° 50' 22"	115° 2' 7"
6341 1300 Rd, Coeur d'Alene ID	2540-23056	ID004	7		47° 37' 14"	115° 12' 5"
6341 1300 Rd, Coeur d'Alene ID	2540-23057	ID005	3		47° 37' 14"	115° 12' 5"
6341 1300 Rd, Coeur d'Alene ID	2540-23058	ID006	23		47° 37' 14"	115° 12' 5"
6341 1300 Rd, Coeur d'Alene ID	2540-23059	ID007	13		47° 37' 14"	115° 12' 5"
2136 Roop Rd Cocolalla, ID	2540-23060	ID008	13		48° 7' 54"	115° 20' 18"
2136 Roop Rd Cocolalla, ID	2540-23061	ID009	ID009		48° 7' 54"	115° 20' 18"
2136 Roop Rd Cocolalla, ID	2540-23062	ID010	ID010		48° 7' 54"	115° 20' 18"
2136 Roop Rd Cocolalla, ID	2540-23063	ID011	13		48° 7' 54"	115° 20' 18"
2136 Roop Rd Cocolalla, ID	2540-23065	ID013	ID013		48° 7' 54"	115° 20' 18"
2136 Roop Rd Cocolalla, ID	2540-23066	ID014	ID014		48° 7' 54"	115° 20' 18"
2136 Roop Rd Cocolalla, ID	2540-23067	ID015	11		48° 7' 54"	115° 20' 18"
2136 Roop Rd Cocolalla, ID	2540-23068	ID016	11		48° 7' 54"	115° 20' 18"
2136 Roop Rd Cocolalla, ID	2540-23069	ID017	11		48° 7' 54"	115° 20' 18"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Garfield Recreation Area, Sandpoint, ID	2540-23070	ID018	7		48° 16' 35"	115° 26' 48"
Illinois (IL)						
Tinley Park, Cook Co, IL	Specimen # 351136 Field #S90-007	IL001	14	FMC	41° 34' 24"	87° 47' 4"
Palos Park, Cook Co, IL	Specimen #351137 Field #S90-008	IL002	38	FMC	41° 40' 2"	87° 49' 49"
Chicago, Lincoln Park Zoo, Cook Co, IL	Specimen #434418 Field #LPZ-171	IL003	14	FMC	41° 52' 41"	87° 37' 47"
Glen Ellyn, DuPage Co, IL	Specimen #435597 Field #WWH-343	IL004	IL004	FMC	41° 52' 39"	88° 4' 1"
Wheaton, DuPage Co, IL	Specimen #435598 Field #WWH-266	IL005	7	FMC	41° 52' 3"	88° 6' 25"
Lisle, DuPage Co, IL	Specimen #435599 Field #WWH-258	IL006	39	FMC	41° 48' 4"	88° 4' 29"
Lake Forest, Lake Co, IL	Specimen #436104 Field #S02-082	IL007	7	FMC	42° 15' 31"	87° 50' 26"
Glen Ellyn, DuPage Co, IL	Specimen #440305 Field #WWH-565	IL008	7	FMC	41° 52' 39"	88° 4' 1"
Warrenville, DuPage Co, IL	Specimen #440306 Field #WWH-535	IL009	7	FMC	41° 49' 4"	88° 10' 24"
Warrenville, DuPage Co, IL	Specimen #440308 Field #WWH-541	IL010	40	FMC	41° 49' 4"	88° 10' 24"
West Chicago, DuPage Co, IL	Specimen #443459 Field #WWH-736	IL011	IL011	FMC	41° 53' 5"	88° 12' 14"
Oak Brook Terrace, DuPage Co, IL	Specimen #449034 Field #WWH-850	IL012	IL012	FMC	41° 51' 0"	87° 57' 52"
Lake Forest, Shaw Woods, Lake Co, IL	Specimen #460034 Field #S08-920	IL013	40	FMC	40° 37' 59"	89° 23' 54"
Wheaton, DuPage Co, IL	Specimen #471531 Field #WWH-2637	IL014	39	FMC	41° 52' 3"	88° 6' 25"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Labrador (LAB)						
Birch Island Road, Happy Valley-Goose Bay, Lab	2500-94857	LAB001	45		53° 17' 26"	60° 19' 4"
416 Hamilton River Road, Happy Valley-Goose Bay, Lab	2500-94863	LAB003	45		53° 18' 55"	60° 22' 55"
Blind Hill' Road, Happy Valley-Goose Bay, Lab	2500-94871	LAB004	45		53° 22' 34"	60° 25' 38"
Birch Island Road, Happy Valley-Goose Bay, Lab	2500-94879	LAB005	LAB05		53° 17' 42"	60° 18' 39"
Lethbridge, Alberta (LETH)						
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57738	LETH001	13		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57739	LETH002	7		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57740	LETH003	7		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57741	LETH004	17		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57742	LETH005	7		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57743	LETH006	LETH006		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57744	LETH007	7		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57745	LETH008	11		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57746	LETH009	13		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57747	LETH010	17		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57748	LETH011	13		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57749	LETH012	13		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57750	LETH013	13		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57751	LETH014	17		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57752	LETH015	17		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57753	LETH016	17		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57754	LETH017	11		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57755	LETH018	3		49° 41' 38"	112° 51' 45"
Helen Schuler Nat. Cntr., Lethbridge AB	2490-57757	LETH019	26		49° 41' 38"	112° 51' 45"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Manitoba (MB)						
Aggassiz, MB (RMNP vicinity)	3510-63176	MB002	MB02		50° 46' 39"	99° 39' 6"
Aggassiz, MB (RMNP vicinity)	2060-41988	MB003	36		50° 46' 19"	99° 39' 39"
Edward's Creek, MB (RMNP vicinity)	2060-41989	MB004	MB04		51° 1' 21"	100° 2' 20"
Ostenfeld, MB	2060-41368	MB005	17		49° 47' 0"	96° 30' 7"
Edward's Creek, MB (RMNP vicinity)	3510-63164	MB006	26		50° 59' 55"	100° 3' 54"
Edward's Creek, MB (RMNP vicinity)	3510-63169	MB007	3		51° 0' 44"	100° 4' 18"
Dawson Road, MB	2060-41365	MB008	36		49° 38' 56"	96° 14' 30"
Edward's Creek, MB (RMNP vicinity)	2060-41953	MB009	7		50° 59' 22"	100° 3' 57"
Edward's Creek, MB (RMNP vicinity)	3510-63189	MB010	37		51° 0' 39"	100° 4' 9"
Vermillion Creek, MB (RMNP vicinity)	2060-41966	MB011	37		50° 58' 19"	100° 15' 56"
Michigan (MI)						
Rapid River, Delta Co., MI	240966	MI001	41	UMI	45° 42' 16"	86° 56' 10"
Whitefish Pt Bird Observatory, MI	240978	MI002	8	UMI	44° 18' 53"	85° 36' 8"
Rapid River, Delta Co., MI	240965	MI003	MI003	UMI	45° 42' 16"	86° 56' 10"
Dearborn, U. Mich Dearborn, MI	240960	MI004	MI004	UMI	42° 19' 20"	83° 10' 34"
Commerce Twp., 2000 Marble Ct, MI	240716	MI005	4	UMI	42° 33' 53"	83° 27' 49"
Hancock, Houghton Co., MI	240890	MI006	MI006	UMI	47° 7' 36"	88° 34' 51"
Waterloo Twp, Sec 24, Jackson Co., MI	240793	MI007	17	UMI	42° 22' 54"	84° 8' 17"
Dexter, 2 mi NW, Washtenaw Co., MI	240595	MI008	12	UMI	42° 20' 18"	83° 53' 18"
Joyfield Twp, Benzie Co., MI	239393	MI009	12	UMI	44° 32' 26"	86° 6' 50"
Sands Twp, Marquette Co., MI	239368	MI010	10	UMI	46° 25' 47"	87° 25' 55"
Sylvan Twp, Hayes Rd, Washtenaw Co., MI	238975	MI011	MI011	UMI	42° 18' 0"	84° 4' 48"
Ann Arbor, Washtenaw Co., MI	238705	MI012	3	UMI	42° 16' 15"	83° 43' 34"
Whitefish Point, Chippewa Co., MI	238245	MI013	32	UMI	45° 58' 14"	84° 12' 54"
Albee, Saginaw Co., MI	238223	MI014	42	UMI	43° 16' 6"	83° 59' 44"
Colfax Twp., Mecosta Co., MI	238189	MI015	17	UMI	43° 47' 24"	83° 3' 27"
Austin Twp., Mecosta Co., MI	238188	MI016	7	UMI	43° 36' 6"	85° 22' 32"
Austin Twp., Mecosta Co., MI	238186	MI017	41	UMI	43° 36' 6"	85° 22' 32"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Austin Twp., Mecosta Co., MI	238185	MI018	7	UMI	43° 36' 6"	85° 22' 32"
Ontonagon Co., MI	238163	MI019	7	UMI	46° 52' 15"	89° 18' 50"
Ontonagon Co., MI	238162	MI020	MI020	UMI	46° 52' 15"	89° 18' 50"
Missouri (MO)						
Grand pass conservation area, MO	2580-47064	MO003	MO03		39° 18' 27"	93° 19' 42"
Grand pass conservation area, MO	2580-47099	MO004	7		39° 18' 27"	93° 19' 42"
Grand pass conservation area, MO	2580-47276	MO005	14		39° 18' 27"	93° 19' 42"
Grand pass conservation area, MO	2580-47280	MO006	4		39° 18' 27"	93° 19' 42"
Ashland, state road Y, MO	2540-23158	MO007	14		38° 45' 33"	92° 8' 38"
U. of Missouri research area, MO	2540-23160	MO009	40		38° 45' 24"	92° 12' 4"
U. of Missouri research area, MO	2540-23161	MO010	7		38° 45' 24"	92° 12' 4"
U. of Missouri research area, MO	2540-23162	MO011	4		38° 45' 24"	92° 12' 4"
Montana (MT)						
Helena National Forest, Helena, MT	2540-22891	MT001	29		46° 29' 0"	111° 50' 53"
Helena National Forest, Helena, MT	2540-22892	MT002	3		46° 29' 0"	111° 50' 53"
Helena National Forest, Helena, MT	2540-22893	MT003	13		46° 28' 56"	111° 50' 35"
Helena National Forest, Helena, MT	2540-22894	MT004	MT004		46° 28' 56"	111° 50' 35"
Helena National Forest, Helena, MT	2540-22895	MT005	29		46° 28' 56"	111° 50' 35"
Orofino, Helena, MT	2530-19201	MT006	29		46° 33' 16"	112° 4' 0"
Orofino, Helena, MT	2530-19209	MT007	30		46° 31' 27"	112° 6' 43"
Road to Park Lake, Helena, MT	2530-19219	MT008	30		46° 28' 5"	112° 9' 33"
Road to Park Lake, Helena, MT	2530-19220	MT009	29		46° 28' 5"	112° 9' 33"
Orofino, Helena, MT	2530-19221	MT010	30		46° 33' 43"	112° 3' 54"
Orofino, Helena, MT	2530-19224	MT011	29		46° 33' 43"	112° 3' 54"
Orofino, Helena, MT	2530-19225	MT012	15		46° 33' 43"	112° 3' 54"
Road to Park Lake, Helena, MT	2530-19226	MT013	3		46° 31' 27"	112° 6' 42"
Road to Park Lake, Helena, MT	2530-19228	MT014	3		46° 31' 27"	112° 6' 42"
Road to Park Lake, Helena, MT	2530-19229	MT015	3		46° 31' 27"	112° 6' 42"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Road to Park Lake, Helena, MT	2530-19230	MT016	3		46° 31' 27"	112° 6' 42"
Road to Park Lake, Helena, MT	2530-19231	MT017	16		46° 31' 18"	112° 7' 4"
Road to Park Lake, Helena, MT	2530-19232	MT018	16		46° 31' 18"	112° 7' 4"
Road to Park Lake, Helena, MT	2530-19233	MT019	30		46° 31' 18"	112° 7' 4"
Twin Peaks Rd, Helena, MT	2530-19234	MT020	30		46° 45' 3"	112° 13' 40"
North Carolina (NC)						
Purchase Knob, NC	2540-23155	NC001	42		35° 35' 10"	83° 4' 24"
North Carolina	Catalog #15207	NC003	NC03	NCMNS	35° 25' 58"	83° 27' 51"
North Carolina	Catalog #15248	NC005	NC05	NCMNS	35° 25' 58"	83° 27' 51"
Northeast Oregon (NEOR)						
Morgan Lake, OR	2540-23028	NEOR001	15		45° 18' 3"	118° 8' 8"
Catherine Creek State Park, OR	2540-23031	NEOR002	21		45° 9' 8"	117° 44' 30"
Catherine Creek State Park, OR	2540-23032	NEOR003	NEOR003		45° 9' 8"	117° 44' 30"
Bird Track Springs Trail, OR	2540-23034	NEOR004	NEOR004		45° 18' 10"	118° 18' 30"
Bird Track Springs Trail, OR	2540-23035	NEOR005	21		45° 18' 10"	118° 18' 30"
Bird Track Springs Trail, OR	2540-23036	NEOR006	NEOR006		45° 18' 10"	118° 18' 30"
Bird Track Springs Trail, OR	2540-23037	NEOR007	21		45° 18' 10"	118° 18' 30"
Bird Track Springs Trail, OR	2540-23038	NEOR008	15		45° 18' 10"	118° 18' 30"
Hilgard junction State Park, OR	2540-23040	NEOR009	21		45° 20' 35"	118° 14' 20"
Red Bridge State Park, OR	2540-23041	NEOR010	15		45° 17' 22"	118° 19' 58"
Red Bridge State Park, OR	2540-23042	NEOR011	NEOR011		45° 17' 22"	118° 19' 58"
Hilgard Junction State Park, OR	2540-23043	NEOR012	3		45° 20' 35"	118° 14' 20"
Bird Track Springs Trail, OR	2540-23033	NEOR013	23		45° 18' 10"	118° 18' 30"
Hilgard Junction State Park, OR	2540-23039	NEOR014	21		45° 20' 35"	118° 14' 20"
Hilgard Junction State Park, OR	2540-23044	NEOR015	NEOR015		45° 20' 35"	118° 14' 20"
Newfoundland (NL)						
Richard Squires PP, NL	2490-57579	NL001	43		49° 10' 58"	57° 25' 36"
Richard Squires PP, NL	2490-57582	NL002	NL002		49° 10' 58"	57° 25' 36"
Richard Squires PP, NL	2490-57585	NL003	NL003		49° 10' 58"	57° 25' 36"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Richard Squires PP, NL	2490-57586	NL004	43		49° 10' 58"	57° 25' 36"
Richard Squires PP, NL	2490-57587	NL005	NL005		49° 10' 58"	57° 25' 36"
Barachois PP, NL	2490-57588	NL006	43		48° 29' 7"	58° 17' 14"
Barachois PP, NL	2490-57589	NL007	43		48° 29' 7"	58° 17' 14"
Barachois PP, NL	2490-57590	NL008	NL008		48° 29' 7"	58° 17' 14"
Barachois PP, NL	2490-57591	NL009	43		48° 29' 7"	58° 17' 14"
Barachois PP, NL	2490-57593	NL010	NL010		48° 29' 7"	58° 17' 14"
Passadena, NL	2490-57594	NL011	43		49° 0' 46"	57° 35' 21"
Passadena, NL	2490-57595	NL012	43		49° 0' 46"	57° 35' 21"
Passadena, NL	2490-57599	NL013	NL013		49° 0' 46"	57° 35' 21"
Passadena, NL	2490-57600	NL014	44		49° 0' 46"	57° 35' 21"
Passadena, NL	2490-57604	NL015	44		49° 0' 46"	57° 35' 21"
Passadena, NL	2490-57606	NL016	43		49° 0' 46"	57° 35' 21"
Passadena, NL	2490-57610	NL017	43		49° 0' 46"	57° 35' 21"
Passadena, NL	2490-57611	NL018	43		49° 0' 46"	57° 35' 21"
Deer Lake, NL	2490-57612	NL019	43		49° 10' 58"	57° 25' 36"
Nova Scotia/New Brunswick (NSNB)						
Margaretsville, NS	2490-57501	NSNB001	7		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57511	NSNB002	7		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57512	NSNB004	NSNB004		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57505	NSNB005	14		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57506	NSNB006	7		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57507	NSNB007	7		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57508	NSNB008	NSNB008		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57509	NSNB009	7		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57510	NSNB010	14		45° 2' 52"	65° 3' 53"
Economy Lake, NS	2490-57561	NSNB011	12		45° 28' 47"	63° 51' 34"
Margaretsville, NS	2490-57513	NSNB012	NSNB012		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57514	NSNB013	NSNB013		45° 2' 52"	65° 3' 53"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Margaretsville, NS	2490-57515	NSNB014	17		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57516	NSNB015	7		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57517	NSNB016	7		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57518	NSNB017	NSNB017		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57520	NSNB019	14		45° 2' 52"	65° 3' 53"
Margaretsville, NS	2490-57521	NSNB020	NSNB020		45° 2' 52"	65° 3' 53"
Northwest British Columbia (NWBC)						
Telegraph Creek, BC	2520-39865	NWBC001	7		58° 24' 2"	131° 12' 43"
Telegraph Creek, BC	2520-39866	NWBC002	3		58° 24' 2"	131° 12' 43"
Telegraph Creek, BC	2520-39867	NWBC003	3		57° 54' 30"	131° 13' 27"
Telegraph Creek, BC	2520-39868	NWBC004	8		57° 54' 30"	131° 13' 27"
Dease Lake, BC	2520-39874	NWBC005	3		58° 30' 24"	130° 1' 23"
Dease Lake, BC	2520-39875	NWBC006	9		58° 25' 49"	129° 59' 12"
Dease Lake, BC	2520-39876	NWBC007	9		58° 25' 49"	129° 59' 12"
Dease Lake, BC	2520-39877	NWBC008	9		58° 25' 49"	129° 59' 12"
Dease Lake, BC	2520-39878	NWBC009	3		58° 25' 49"	129° 59' 12"
Dease Lake, BC	2520-39879	NWBC010	9		58° 25' 49"	129° 59' 12"
Dease Lake, BC	2520-39880	NWBC011	NWBC011		58° 25' 49"	129° 59' 12"
Dease Lake, BC	2520-39881	NWBC012	9		58° 25' 49"	129° 59' 12"
Telegraph Creek, BC	2520-39859	NWBC013	10		57° 54' 45"	131° 12' 34"
Telegraph Creek, BC	2520-39860	NWBC014	9		57° 54' 45"	131° 12' 34"
Telegraph Creek, BC	2520-39861	NWBC015	3		57° 54' 45"	131° 12' 34"
Telegraph Creek, BC	2520-39862	NWBC016	7		57° 54' 45"	131° 12' 34"
Telegraph Creek, BC	2520-39863	NWBC017	8		57° 54' 45"	131° 12' 34"
Ontario (ON)						
QUBS near Kingston ON	11-2005	ON001	28	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	112-2005	ON002	42	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	116-2005	ON003	ON003	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	119-2005	ON004	ON004	Foote *et al.*	44° 32' 18"	76° 22' 0"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
QUBS near Kingston ON	120-2005	ON005	17	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	121-2005	ON006	38	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	123-2005	ON007	7	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	124-2005	ON008	ON008	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	126-2005	ON009	7	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	127-2005	ON010	ON010	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	128-2005	ON011	3	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	129-2005	ON012	7	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	131-2005	ON013	3	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	133-2005	ON014	3	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	137-2005	ON015	7	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	145-2005	ON016	ON016	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	147-2005	ON017	15	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	172-2005	ON018	7	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	173-2005	ON019	7	Foote *et al.*	44° 32' 18"	76° 22' 0"
QUBS near Kingston ON	51-2005	ON020	ON020	Foote *et al.*	44° 32' 18"	76° 22' 0"
Southern Alberta (SAB)						
West Castle, AB	2490-57633	SAB001	13		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57634	SAB002	15		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57635	SAB003	SAB003		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57636	SAB004	15		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57637	SAB005	8		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57638	SAB006	13		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57639	SAB007	26		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57646	SAB008	13		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57647	SAB009	24		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57649	SAB010	13		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57650	SAB011	27		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57651	SAB012	27		49° 34' 10"	114° 22' 35"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
West Castle, AB	2490-57652	SAB013	27		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57653	SAB014	8		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57654	SAB015	3		49° 34' 10"	114° 22' 35"
West Castle, AB	2490-57655	SAB016	27		49° 34' 10"	114° 22' 35"
Syncline Ski Area, AB	2490-57659	SAB018	28		49° 34' 10"	114° 22' 35"
Southeast British Columbia (SEBC)						
Revelstoke, BC	2490-57684	SEBC001	SEBC001		50° 58' 50"	118° 10' 54"
Revelstoke, BC	2490-57685	SEBC002	13		50° 58' 50"	118° 10' 54"
Revelstoke, BC	-	SEBC003	7		50° 58' 58"	118° 10' 44"
Mt Revelstoke Ski Chalet, BC	2490-57686	SEBC004	15		51° 0' 23"	118° 11' 28"
Mt Revelstoke Ski Chalet, BC	2490-57687	SEBC005	SEBC005		51° 0' 23"	118° 11' 28"
Mt Revelstoke Ski Chalet, BC	2490-57688	SEBC006	7		51° 0' 50"	118° 12' 10"
Mt Revelstoke Ski Chalet, BC	2490-57689	SEBC007	13		51° 0' 50"	118° 12' 10"
Mt Revelstoke Ski Chalet, BC	2490-57690	SEBC008	SEBC008		51° 0' 22"	118° 10' 55"
Revelstoke field, BC	2490-57691	SEBC009	SEBC009		50° 58' 56"	118° 10' 49"
Revelstoke Resort, BC	2490-57692	SEBC010	15		50° 58' 12"	118° 10' 20"
Mount MacPherson Revelstoke, BC	2490-57695	SEBC013	13		50° 56' 31"	118° 13' 22"
9 mile Revelstoke, BC	2490-57696	SEBC014	13		50° 53' 49"	118° 6' 49"
Frisby Rd Revelstoke, BC	2490-57699	SEBC017	16		51° 3' 58"	118° 11' 38"
Frisby Rd Revelstoke, BC	2490-57700	SEBC018	13		51° 3' 7"	118° 13' 8"
Frisby Ridge Rd Revelstoke, BC	2490-57701	SEBC019	15		51° 3' 33"	118° 12' 21"
Frisby Ridge Rd Revelstoke, BC	2490-57702	SEBC020	17		51° 3' 33"	118° 12' 21"
Frisby Ridge Rd Revelstoke, BC	2490-57703	SEBC021	7		51° 8' 26"	118° 12' 30"
Frisby Ridge Rd Revelstoke, BC	2490-57706	SEBC024	SEBC024		51° 3' 43"	118° 13' 24"
Frisby Ridge Rd Revelstoke, BC	2490-57707	SEBC025	15		51° 3' 43"	118° 13' 24"
Frisby Ridge Rd Revelstoke, BC	2490-57708	SEBC026	SEBC026		51° 3' 52"	118° 13' 34"
Saskatchewan (SK)						
Narrows Campground, Campsite 67, Prince Albert NP, SK	2500-94893	SK001	34		53° 58' 55"	106° 17' 31"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Narrows Campground, Campsite 67, Prince Albert NP, SK	2500-94894	SK002	34		53° 58' 55"	106° 17' 31"
Narrows Campground, Campsite 67, Prince Albert NP, SK	2500-94895	SK003	3		53° 58' 55"	106° 17' 31"
South Bay, Prince Albert NP, SK	2500-94898	SK004	35		53° 53' 57"	106° 9' 31"
South Bay, Prince Albert NP, SK	2500-94899	SK005	12		53° 53' 57"	106° 9' 31"
South Bay, Prince Albert NP, SK	-	SK006	3		53° 53' 57"	106° 9' 31"
South Bay, Prince Albert NP, SK	2500-94900	SK007	17		53° 53' 57"	106° 9' 31"
South Bay, Prince Albert NP, SK	2500-94898	SK008	35		53° 53' 57"	106° 9' 31"
57 Trail, Prince Albert NP, SK	2490-57777	SK009	24		53° 56' 40"	106° 13' 42"
57 Trail, Prince Albert NP, SK	2490-57778	SK010	3		53° 56' 40"	106° 13' 42"
57 Trail, Prince Albert NP, SK	2490-57779	SK011	3		53° 56' 40"	106° 13' 42"
Fisher Trail, Prince Albert NP, SK	2490-57780	SK012	3		53° 55' 23"	106° 3' 59"
Fisher Trail, Prince Albert NP, SK	2490-57781	SK013	12		53° 55' 23"	106° 3' 59"
Fisher Trail, Prince Albert NP, SK	2490-57782	SK014	SK14		53° 55' 23"	106° 3' 59"
Treebeard Trail, Prince Albert NP, SK	2490-57784	SK016	14		53° 58' 21"	106° 17' 25"
Narrows Campground, Campsite 82, Prince Albert NP, SK	2500-94942	SK017	12		53° 58' 50"	106° 17' 37"
Narrows Campground, Campsite 82, Prince Albert NP, SK	2500-94943	SK018	12		53° 58' 50"	106° 17' 37"
Fisher Trail, Prince Albert NP, SK	2490-57785	SK019	12		53° 55' 23"	106° 3' 59"
Narrows Campground, Campsite 74, Prince Albert NP, SK	2500-94894	SK020	34		53° 58' 50"	106° 17' 37"
Southern Oregon (SOR)						
N. Mtn. Nature Center, Ashland, OR	2510-51352	SOR001	13		42° 12' 2"	122° 41' 7"
N. Mtn. Nature Center, Ashland, OR	2540-23045	SOR002	4		42° 12' 2"	122° 41' 7"
N. Mtn. Nature Center, Ashland, OR	2510-19834	SOR003	4		42° 12' 2"	122° 41' 7"
N. Mtn. Nature Center, Ashland, OR	2540-23046	SOR004	19		42° 12' 2"	122° 41' 7"
N. Mtn. Nature Center, Ashland, OR	2540-23047	SOR005	19		42° 12' 2"	122° 41' 7"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
N. Mtn. Nature Center, Ashland, OR	2440-87440	SOR006	4		42° 12' 2"	122° 41' 7"
N. Mtn. Nature Center, Ashland, OR	2510-52793	SOR007	4		42° 12' 2"	122° 41' 7"
N. Mtn. Nature Center, Ashland, OR	2540-23048	SOR008	4		42° 12' 2"	122° 41' 7"
N. Mtn. Nature Center, Ashland, OR	2560-67576	SOR009	4		42° 12' 2"	122° 41' 7"
N. Mtn. Nature Center, Ashland, OR	2460-25547	SOR010	4		42° 12' 2"	122° 41' 7"
Central Point, Medford, OR	2540-23049	SOR011	4		42° 22' 2"	122° 53' 7"
Central Point, Medford, OR	2540-23050	SOR012	13		42° 22' 2"	122° 53' 7"
Central Point, Medford, OR	2540-23051	SOR013	SOR013		42° 22' 2"	122° 53' 7"
Central Point, Medford, OR	2540-23052	SOR014	SOR014		42° 22' 2"	122° 53' 7"
Central Point, Medford, OR	2540-23053	SOR015	SOR015		42° 22' 2"	122° 53' 7"
Utah (UT)						
NE Huntsville (Reservoir), UT	2540-23120	UT001	29		41° 17' 23"	111° 34' 57"
Magpie campground, UT	2540-23121	UT002	29		41° 15' 21"	111° 39' 56"
Magpie campground, UT	2540-23122	UT003	UT003		41° 15' 21"	111° 39' 56"
W of Woodruff (Cache Forestry Rd.), UT	2540-23123	UT004	15		41° 26' 8"	111° 28' 47"
W of Woodruff (Cache Forestry Rd.), UT	2540-23124	UT005	30		41° 26' 8"	111° 28' 47"
Boots campground, UT	2540-23125	UT006	29		41° 17' 40"	111° 39' 29"
Boots campground, UT	2540-23126	UT007	4		41° 17' 40"	111° 39' 29"
Boots campground, UT	2540-23127	UT008	29		41° 17' 40"	111° 39' 29"
Boots campground, UT	2540-23128	UT009	29		41° 17' 40"	111° 39' 29"
Snowbasin Road, UT	2540-23129	UT010	29		41° 13' 35"	111° 51' 3"
Snowbasin Road, UT	2540-23130	UT011	29		41° 13' 35"	111° 51' 3"
Snowbasin Road, UT	2540-23131	UT012	29		41° 13' 35"	111° 51' 3"
Snowbasin Road, UT	2540-23132	UT013	29		41° 13' 35"	111° 51' 3"
Snowbasin Road, UT	2540-23133	UT014	UT014		41° 13' 35"	111° 51' 3"
Snow Basin Rd., UT	2540-23135	UT016	UT016		41° 16' 46"	110° 39' 13"
Snow Basin Rd., UT	2540-23136	UT017	29		41° 16' 46"	110° 39' 13"
Snow Basin Rd., UT	2540-23137	UT018	UT018		41° 16' 46"	110° 39' 13"
Snow Basin Rd., UT	2540-23138	UT019	29		41° 16' 46"	110° 39' 13"

Location	Band Number*	ID	Haplotype	Museum**	Latitude (°N)	Longitude (°W)
Snow Basin Rd., UT	2540-23139	UT020	29		41° 16' 46"	110° 39' 13"
Washington (WA)						
206 23 Ave SE Puyallup, WA	2540-23003	WA001	3		47° 10' 9"	122° 17' 29"
206 23 Ave SE Puyallup, WA	2540-23004	WA002	18		47° 10' 9"	122° 17' 29"
206 23 Ave SE Puyallup, WA	2540-23005	WA003	19		47° 10' 9"	122° 17' 29"
206 23 Ave SE Puyallup, WA	2540-23006	WA004	20		47° 10' 9"	122° 17' 29"
Auburn 2535 26 St, WA	2540-23007	WA005	4		47° 17' 10"	122° 11' 42"
Auburn 2535 26 St, WA	2540-23008	WA006	18		47° 17' 10"	122° 11' 42"
Lake Tapps 16318 37St. Cr. E., WA	2540-23009	WA007	4		47° 13' 22"	122° 12' 46"
Lake Tapps 16318 37St. Cr. E., WA	2540-23010	WA008	19		47° 13' 22"	122° 12' 46"
Puyallup, WA	2540-23011	WA009	20		47° 6' 47"	122° 12' 46"
Lake Tapps 16318 37St. Cr. E., WA	2540-23012	WA010	20		47° 13' 22"	122° 12' 46"
Puyallup 12009 64th Ave E., WA	2540-23013	WA011	19		47° 8' 50"	122° 20' 39"
Forest Park 15815 34th Ave NE, WA	2550-23014	WA012	20		47° 44' 35"	122° 17' 35"
Seattle South Othello St., WA	2540-23015	WA013	20		47° 32' 11"	122° 15' 47"
Seattle South Othello St., WA	2540-23016	WA014	4		47° 32' 11"	122° 15' 47"
Seattle South Othello St., WA	2540-23017	WA015	WA015		47° 32' 11"	122° 15' 47"
Seattle South Othello St., WA	2540-23018	WA016	4		47° 32' 11"	122° 15' 47"
Seattle South Othello St., WA	2540-23019	WA017	4		47° 32' 11"	122° 15' 47"
Seattle Shoreline Ashworth Ave., WA	2540-23020	WA018	4		47° 45' 51"	122° 20' 27"
Seattle Shoreline Ashworth Ave., WA	2540-23021	WA019	4		47° 45' 51"	122° 20' 27"
Seattle Shoreline Ashworth Ave., WA	2540-23022	WA020	4		47° 45' 51"	122° 20' 27"
West Virginia (WV)						
Monterey, Highland, VA	tissue#B08984 voucher#587440	WV001	17	SMITH	38° 35' 8"	79° 38' 13"
Reddish Knob, Augusta, VA	tissue#B09005 voucher#587441	WV002	38	SMITH	38° 27' 16"	79° 15' 6"
Ryder Gap, Bath, VA	tissue#B12081 voucher#601417	WV003	WV03	SMITH	38° 11' 7"	79° 55' 17"

Location	**Band Number***	**ID**	**Haplotype**	**Museum****	**Latitude (°N)**	**Longitude (°W)**
Warm Springs, Bath, VA	tissue#B12110 voucher#601401	WV004	WV04	SMITH	38° 8' 57"	79° 45' 55"
Trout Dale, Grayson (SW), VA	tissue#B13208 voucher#601580	WV005	7	SMITH	36° 40' 10"	81° 29' 12"
Monterey, Highland, VA	tissue#B17866 voucher#634200	WV010	WV10	SMITH	38° 34' 58"	79° 38' 13"
Monterey, Highland, VA	tissue#B17867 voucher#634201	WV011	WV11	SMITH	38° 34' 58"	79° 38' 13"
Paddy Knob, Pocahontas, WV	tissue#B08865 voucher#586253	WV012	7	SMITH	38° 16' 5"	79° 47' 35"
Paddy Knob, Pocahontas, WV	tissue#B08870 voucher#586255	WV013	12	SMITH	38° 16' 5"	79° 47' 35"

* Band number or specimen number/other identification number (where museum is listed)

** Museum or other tissue collection/collector (e.g., Foote *et al.* = J. Foote, L. Ratcliffe and D. Mennill)

Appendix 3.4: Variable sites for black-capped chickadee mtDNA control region haplotypes (hap). Table shows the 67 variable sites for a 440 bp sequence. Reverse compliment of sequence begins at site 61 in Kvist *et al.* 2001 black-capped chickadee sequence; Genbank accession no. AF354496.

	Variable Site																																																																
	2	5	6	17	20	26	32	34	50	51	62	66	74	75	90	91	109	113	126	132	133	134	136	137	182	192	204	205	210	211	219	221	223	229	232	235	244	245	248	254	263	264	265	266	270	271	274	290	292	293	294	304	307	336	352	355	370	377	378	390	392	393	398	400	418	428	433
H	G	C	A	G	A	C	T	G	G	C	T	G	T	C	T	T	C	A	G	G	G	T	T	T	T	A	C	T	G	G	G	A	T	G	G	T	T	A	C	T	C	C	C	G	C	T	G	T	C	A	A	A	T	G	G	T	G	A	A	G	T	A	G	T	G	C	A
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Chapter 4. Post-glacial colonization patterns in mountain chickadee

J. Hindley[1,2] and T. M. Burg[1]

Prepared as manuscript for submission

[1]Department of Biological Sciences, University of Lethbridge, 4401 University Drive,
Lethbridge AB, T1K 3M4
[2]corresponding author: j.hindley@uleth.ca

Abstract

Postglacial recolonization of North America was influenced by several factors including the number and size of refugia, patterns of glacial retreat and physical barriers such as mountain ranges. Using both mitochondrial DNA (control region) and microsatellite data, we investigated the phylogeography and population genetics of the mountain chickadee (*Poecile gambeli*). We analyzed 268 samples from 17 sites across the mountain chickadee's contemporary range. Two main groups were found (California/southern Cascade and Rocky Mountain/northern Cascade) in both mitochondrial and microsatellite data, with additional substructure in the SOR/CA. Microsatellite data revealed a fourth group in Idaho.

The presence of distinct mtDNA groups suggests isolation in at least two Pleistocene refugia: a Rocky Mountain refugium, and a Sierra Nevada Mountain refugium. Findings from our study show contrasting patterns between mtDNA and microsatellite data for several populations. The Washington population clustered with the Rocky Mountain populations using mtDNA and with southern OR and CA using microsatellite data, indicating possible male-biased dispersal or different population histories. Within the California/southern Oregon Cascades group mtDNA data show two distinct monophyletic groups (southern California separated from all other populations) whereas microsatellite data (STRUCTURE) show some clustering of individuals from south-central California (SCCA) with southern California (SCA).

Keywords: Pleistocene, barrier-mediated dispersal, microsatellite, mitochondrial DNA, barriers, Last Glacial Maximum, Rocky Mountains

4.1 Introduction

In North America, populations isolated during Pleistocene glacial cycles diverged, resulting in subsequent radiations that were among the most remarkable in vertebrates (Coyne and Orr 2004; Schluter 2000). Glacial cycles profoundly affected the distributions, in both latitude and elevation, of temperate organisms as ranges have expanded and contracted (Delcourt and Delcourt 1991; Webb 1995). As a result, Pleistocene glaciations are thought to be responsible for many recent avian speciation events (Johnson and Cicero 2004; Milá et al. 2007; Weir and Schluter 2004). Similarly, following the Last Glacial Maximum (LGM) ~18-21 thousand years ago (kya), temperatures warmed, ice sheets receded and populations that had been isolated in one or multiple refugia expanded their geographic distributions as new habitat became available (Pielou 1991; Waltari *et al.* 2007; Brunsfeld *et al.* 2001; Galbreath *et al.* 2009).

Due to these climatic oscillations, large arid areas within western North America such as the Columbia, Wyoming and Great Basins now separate coniferous forest habitat. Several sub-alpine species exhibit concordant genetic breaks associated with habitat fragmentation and isolation across these arid, low elevation barriers (Galbreath *et al.* 2009; Noonan 2001; DeChaine and Martin 2004, 2005). The effect on genetic diversity (e.g., population differentiation, substructure) due to the east/west barriers between the Cascade/Sierra Nevada and Rocky Mountain regions, combined with Pleistocene associated habitat changes has been demonstrated in several bird species including blue grouse (*Dendragapus obscurus*; Barrowclough *et al.* 1981; Barrowclough *et al.* 2004), Swainson's thrush (*Catharus ustulatus*; Ruegg and Smith 2002), and white-breasted nuthatch (*Sitta carolinensis*; Spellman and Klicka 2007), and mammals such as American marten (*Martes americana*; Stone *et al.* 2002), northern flying squirrel *(Glaucomys sabrinus*; Arbogast 1999), as well as plants such as ponderosa pine (*Pinus ponderosa*; Latta and Mitton 1999) and whitebark pine (*P. albicaulis*; Richardson *et al.* 2002).

The mountain chickadee, *Poecile gambeli*, is a common year-round resident of dry, coniferous forests in western North America, from northwest British Columbia to Baja California and Texas following the various mountain ranges (Figure 4.1). The mountain chickadee is thought to have limited natal dispersal, extreme philopatry, limited winter altitudinal migration, a patchy distribution, and marked geographic variation, all of which make this species well suited for phylogeographic study (Behle 1956; Dixon and Gilbert 1964; McCallum *et al.* 1999; Spellman *et al.* 2007).

Previous work by Spellman *et al.* (2007) used the mitochondrial (mtDNA) ND2 gene to assess the impact of late Pleistocene (12 kya – 1.8 million years ago (Mya)) glacial cycles on population structure and the evolutionary history of the mountain chickadee. Their analysis revealed two well-supported groups, an eastern (Rocky Mountains and Great Basin) and a western (Sierra Nevada and Cascades) clade, consistent with two of the three major groups described by Behle (1956), and partially supported by previous genetic data (Gill *et al.* 1993; Gill *et al.* 2005). Recently, the American Ornithologists' Union's Classification and Nomenclature Committee (2010) recommended splitting the mountain chickadee into two species, *P. gambeli* (Rocky Mountain and Great Basin populations) and *P. baileyae* (coastal California, Sierra Nevada and Cascade Range populations), based on molecular (mtDNA), morphological and acoustic data. This suggestion was rejected by the Classification and Nomenclature Committee due to lack of published data, particularly within potential contact zones (e.g., Pacific Northwest). In fact, no phylogeographic genetic analysis of mountain chickadee to date has included an extensive number of samples from southern Oregon east of the Cascades into western Washington that represents approximately 30% of the *P. g. baileyae* range. Therefore, genetic affinity of mountain chickadees in large parts of Oregon and Washington, a potential contact zone between two subspecies, is unknown.

The purpose of this study was to evaluate the population genetic structure and phylogeography of the mountain chickadee using the highly variable mtDNA control region (CR) and nuclear microsatellite markers. We investigated how the inferred evolutionary history of mountain chickadee populations was influenced by Pleistocene glaciation cycles; assessed if mtDNA and nuclear data show concordant phylogenetic patterns; and determined the genetic affinity of birds from the central and northern Cascade Mountain Range.

4.2 Methods

4.2.1 Sampling

Birds were captured using mist nets, and blood and/or feather samples were collected from 202 individuals during the summer of 2008 to 2010 and stored in ethanol (95%). Sixty-six samples (SCCA, CCA, SCA, WA) collected within the last 20 years were obtained from the Burke Museum of Natural History, University of Michigan Museum of Natural History, Museum of Vertebrate Zoology (Berkeley), Louisiana State University Museum of Natural Science, and Smithsonian Museum of Natural Science. Field samples were collected during the summer, and all samples within each population were collected from as small an area as possible (typically within a 50 km radius). A total of 268 samples from 17 sampling sites (Figure 4.1; Appendix 4.1) across the contemporary mountain chickadee range (Ridgely *et al.* 2007) were used for analyses. Two samples from MT were excluded due to being captured with probable closely related individual(s) in the same net. DNA was extracted from whole blood or tissue using a modified chelex method (Walsh *et al.* 1991; Burg and Croxall, 2001).

4.2.2 MtDNA Amplification and Sequencing

Two polymerase chain reaction (PCR) primers, H1015 (5′-CGCGGGTTTAACGAATGTGG-3′) and LmochCR1 (5′-CAGGGTATGTATGTCTTTGCATTC-3′), were used to amplify a 765 bp fragment within Domains I and II of the control region for 190 samples (a maximum of 20 samples from each population). The PCR was carried out in an Eppendorf Mastercycler. PCR consisted of approximately 100 ng of template DNA, 1 µM of each primer, 200 µM dNTPs, 2.5 mM $MgCl_2$, 1 unit of Taq DNA polymerase (Crimson) and the PCR buffer (Crimson or Promega) in a final volume of 25 µl. Amplification consisted of one cycle at 95°C for 2 min, 54°C for 45 s, and 72°C for 60 s; 37 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. The PCR products were sequenced using an Applied Biosystems 3130 Genetic Analyzer following enzymatic clean up using 0.1 units of shrimp alkaline phosphatase (SAP) and 0.1 units of exonuclease I. MtDNA sequences were manually aligned using MEGA v5.0 (Tamura *et al.* 2011).

4.2.3 Microsatellite Genotyping

Seven microsatellite primer pairs isolated from other passerine species were used for genotyping (see Appendix 4.2). The forward primer of each primer pair was modified with the addition of M13 sequence to the 5’ end to allow for direct incorporation of a fluorescently labeled M13 primer. PCR reactions consisted of approximately 100 ng of template DNA, 1 μM of each microsatellite primer and the M13 tag, 200 μM dNTPs, 1-2 mM $MgCl_2$ (see below), 0.5 units of Crimson Taq DNA polymerase (New England BioLabs) and PCR buffer in a final volume of 10 μl (Appendix 4.2). $MgCl_2$ concentration varied depending on the locus (2 mM for Escu4, Titgata02, Titgata39 and Pat14, 1.5 mM for Escu6 and Ppi2, and 1 mM for Pdo5) and 1% formamide was added to PCR for Escu4 and Ppi2. All loci were amplified using a two-step annealing procedure: one cycle for 2 min at 94°C, and 45 s at T_{A1}, 1 min at 72°C; 7 cycles of 1 min at 94°C, 30 s at T_{A1}, 45 s at 72°C; 31 cycles of 30 s at 94°C, 30 s at T_{A2}, 45 s at 72°C; and one final cycle of 5 min at 72°C. For loci Escu4 and Pdo5 T_{A1} = 45°C and T_{A2} = 48°C, and for the other five loci T_{A1} = 50°C and T_{A2} = 52°C. The PCR was carried out in an Eppendorf Mastercycler and PCR products were run on a 6% acrylamide gel using a Li-COR 4300 (Li-COR Inc.). All microsatellite genotypes were visually scored using the program Saga Lite (Build v1.0.2).

4.2.4 Phylogenetic Analyses

Two different phylogenetic approaches, statistical parsimony and maximum likelihood, were used to determine the phylogeographic relationship among the 190 chickadee mtDNA samples. A statistical parsimony network was constructed using the program TCS v1.21 (Clement *et al.* 2000), with gaps treated as a fifth state. The program jModeltest (Guindon and Gascuel 2003; Posada 2008) was used to select the model of sequence evolution that best fit the sequence data (HKY + G + I; Akaike Information Criteria (AIC) = 3960), and a maximum likelihood (ML) tree was constructed in MEGA v5.0 using the same substitution model (discrete gamma categories n = 4) and nearest neighbor interchange heuristic model, with 1000 bootstrap replicates to evaluate robustness.

4.2.5 Genetic Diversity

We tested for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium of microsatellite data using the program GENEPOP v4.0.10 (Raymond and Rousset 1995; Rousset 2008), and calculated observed and expected heterozygosity, and number of alleles using GenAlEx v6.41 (Peakall and Smouse 2006). For mtDNA, we calculated the number of haplotypes (H), haplotype diversity (H_d), number of private haplotypes (*Pri*; haplotypes found in a single population), and nucleotide diversity (π) using the program DnaSP v5.10 (Librado and Rozas 2009). Allelic richness (AR) was calculated using FSTAT v2.9.3 (Goudet 2001).

4.2.6 Phylogeographic History

To test for recent population expansions, we performed Fu's F_S test of neutrality (Fu 1997) and Ramos-Onsins and Rozas R_2 test (Ramos-Onsins and Rozas 2002) using mtDNA data. Both of these tests have been shown to be the most powerful tests available for detecting population growth (Ramos-Onsins and Rozas 2002). Fu's F_S detects excess recent mutations based on the observed haplotype distribution and is negative when there has been population growth, background selection or genetic hitchhiking. R_2 compares the number of singleton mutations in a population to the average number of nucleotide differences in that population. Populations that have experienced a recent expansion should contain an increased number of singleton mutations, and therefore low values of R_2. Fu's F_S and R_2 were calculated using DnaSP v5.10 and significance was evaluated by comparing observed values to a distribution of values generated under 10,000 coalescent simulations.

We estimated time since most recent population expansion using the distribution of net number of nucleotide differences between populations (τ) and an estimated mutation rate (Rogers and Harpending 1992). The estimated time since most recent expansion was calculated using the formula $\tau = 2ut$; where t is the time between the current population size and its initial size at the start of last expansion, and $u = 2\mu k$; where μ = the mutation rate and k is the sequence length. The mutation rate was calculated using two different methods. The first method was based on an avian mutation rate for Domains I (20% per My) and II (5% per My) of the mtDNA control region (Baker and Marshall 1997), and adjusting for the proportion of each domain in the 765 bp sequence (i.e., 340 bp in Domain I, and 425 bp in Domain II; divergence rate = 11.67% per My; μ = 5.8% or 5.8×10^{-8} mutations/site/year/lineage). The second rate was a conservative 3% per MY divergence rate, based on the Päckert *et al.* (2007) rate they estimated at 1.2% for the genus *Poecile* as well as a comparison of the same mtDNA sequence fragments of Carolina chickadee (*P. carolinensis*) and black-capped chickadee (*P. atricapillus*), adjusted to an estimated split between the Carolina/black-capped lineages at ~2.5 My (Gill *et al.* 1993; Gill *et al.* 2005).

4.2.7 Population Structure

Pairwise Φ_{ST} (mtDNA) and F_{ST} (microsatellite) values, basic indices of population differentiation, were calculated for all populations with at least eight samples (avg = 19.8 samples/population). For mtDNA data we used Arlequin v3.0 (Excoffier *et al.* 2005) and tested significance using 10,000 permutations. Although R_{ST} was developed specifically for microsatellites, F_{ST} has been shown to perform better when sample sizes are less than 50 per population and/or the number of loci is less than 20 (Gaggiotti *et al.* 1999). Therefore, pairwise F_{ST} was calculated from microsatellite data using GENODIVE v2.0b20 (Meirmans and Van Tienderen 2004), with p-values obtained after 10,000 permutations. All Φ_{ST} and F_{ST} p-values were corrected for multiple tests using the Benjamini-Hochberg False Discovery Rate (FDR) correction (Benjamini and Hochberg 1995). A principal coordinate analysis (PCA) was conducted for both mtDNA (individual pairwise distances) and microsatellite data (pairwise F_{ST}) in GenAlEx v6.41.

Both mtDNA and microsatellite data were analyzed using Bayesian cluster analyses to estimate the number of possible metapopulation groupings (K). STRUCTURE v2.3 (Pritchard *et al.* 2000) was used for microsatellite data with sampling locations, admixture and correlated alleles as priors. To estimate the most likely number of clusters (K), we conducted 10 runs each of K = 1-10 for all populations, using a burn-in of 100,000 and a run of 300,000 steps (Evanno *et al.* 2005). We identified K using Bayes factor as described in Pritchard *et al.* (2000) and ΔK using Structure Harvester (Earl 2009). Individuals were assigned to the cluster with the highest average *Q* value (ancestry coefficient) for the runs at the optimal K. Individuals with similar *Q* values (or overlapping standard deviation) to two or more clusters were not assigned to a cluster. A second Bayesian analysis was performed using the program BAPS v5.3 (Corander *et al.* 2008; Corander and Tang 2007) to estimate the optimal number of clusters for mtDNA, without using the sampling location as a prior. We conducted 10 runs with a maximum number of possible clusters set at 17.

A spatial analysis of molecular variance was conducted using SAMOVA (Dupanloup *et al.* 2002) for mtDNA to identify groups of sampling sites that are geographically homogenous and maximally differentiated from each other. SAMOVA uses a simulated annealing procedure that maximizes the proportion of genetic variance between groups, but unlike AMOVA does not require groups to be defined *a priori*.

Isolation by distance (IBD) was evaluated using a Mantel test in GenAlEx v6.41 for both mtDNA and microsatellite data to identify any positive correlation between genetic distance ($F_{ST}/(1-F_{ST})$) and straight-line geographic distance. Significance was tested with 9999 permutations. The central geographic location of each population was estimated by mapping the mid-point for all samples collected at each sampling site (e.g., SAB) and calculating the straight-line distance between each pair of sampling sites. For mtDNA we tested for IBD using all populations and populations within each of the two major groups identified by both BAPS and the ML tree. Similarly, microsatellite data were tested for IBD using all populations and within each of the two main groups identified at K = 4 in STRUCTURE (i.e., Rockies plus ID and SOR/CA plus SCA, see below).

4.2.8 Divergence Times

We estimated divergence times using both a strict molecular clock method and a Bayesian method. The average pairwise genetic distances between populations and groups were calculated in DnaSP and a strict molecular clock based on both 11.67% per My and 3% per My (see above) were used to estimate divergence time. We used the program BEAST v1.6.1 (Drummond *et al.* 2006; Drummond and Rambaut 2007) with a relaxed lognormal molecular clock to estimate the coalescence time of the Rockies and SOR/CA groups. BEAUti v1.6.1 (Rambaut and Drummond 2007a) was used to create the BEAST .xml input file and a user specified tree (ML, this study) was used with a constant population size prior and a substitution rate prior of 11.67% divergence per million years between two lineages (i.e., 5.8% or 5.8×10^{-8} mutations/site/year/lineage; this study), and a second more conservative substitution rate of 1.5%/My/lineage (see above). BEAST analysis was run for 10 million generations, sampled every 1000 generations. Output files were viewed with Tracer v1.5 (Rambaut and Drummond 2007b) to estimate divergence times (i.e., time to most recent common ancestor).

4.2.9 Ecological Niche Modeling

We constructed ecological niche modeling using the program MAXENT v3.3.3e (Phillips *et al.* 2006) to predict possible refugia during the LGM. Ecologial niche models have been shown to be spatially correlated with phylogeographic patterns suggesting both methods are complementary (Waltari *et al.* 2007). MAXENT uses a maximum entropy statistical model based on the current distribution’s (i.e., known presence-only occurrence data) climate conditions to infer past distributions by identifying similar bioclimatic conditions during a particular time (e.g., LGM), assuming that present niche requirements reflect past and/or future requirements.

Bioclimatic variables were obtained from the WorldClim dataset v1.4, with a resolution of 2.5 min (Hijmans *et al.* 2005). Thirteen out of the 19 available variables were correlated with at least one other variable ($r > 0.90$), of which five were removed from analysis based on biological relevance (i.e., when two variables were correlated, the variable that was more biologically relevant was retained) as well as removing only correlated variables with low (< 0.2) regularized training gain based on jackknife analysis for all variables. We used AIC, as implemented in ENMTools (Warren *et al.* 2010) to select the best model (i.e., lowest AIC score, and ΔAIC of > 35 for all model comparisons). The remaining 14 variables (i.e., BIO1, BIO2, BIO3, BIO4, BIO5, BIO6, BIO7, BIO8, BIO9, BIO11, BIO12, BIO15, BIO18 and BIO19; Appendix 4.3) were used to generate models in MAXENT with the default settings (regularization = 1, convergence threshold = 0.000001, iterations = 500), 10 replicates and 25% of sample locations used for model training (cross validation method). We used the MIROC (a Model for Interdisciplinary Research on Climate) climate layers provided by the Paleoclimate Modelling Intercomparison Project Phase II (PMIP2; Waltari *et al.* 2007) were used for projecting past climatic conditions at the LGM (~21 kya). A total of 1200 unique (i.e., non-duplicate) chickadee locations were used for modeling, which includes all sampling locations obtained in this study (n = 69), as well as an additional 1131 random non-duplicate location observations downloaded from the Global Biodiversity Information Facility data portal.

4.3 Results

4.3.1 MtDNA Sequence Analysis

We examined the 765 bp mtDNA CR sequences from 190 mountain chickadees. A total of 80 haplotypes were present including 23 shared haplotypes (i.e., haplotypes found in more than one bird), 19 within Rocky Mountain populations (i.e., populations east of the Cascade Range; Rockies group) and four within the SOR and/or CA populations (i.e., within and west of the Cascade Range; SOR/CA group), with none shared between the two groups (Appendix 4.4), and a total of 68 variable sites (Appendix 4.5). Similarly, none of the haplotypes were shared between SCA and birds from SOR, CCA or SCCA. Pairwise Φ_{ST} values revealed significant differentiation in mountain chickadees (Table 4.1). Within the Rockies group, CeOR and NEOR were not significantly different from each other, but CeOR was different from all other Rockies populations, and NEOR was different from all other Rockies populations except SAB ($F_{ST} = 0.042$, $p = 0.042$). CO and UT were also significantly different from all other populations with the exception of ID. Within the SOR/CA group, the SCA population was significantly different from all other populations based on pairwise Φ_{ST} values and SOR, CCA and SCCA from all other populations. Similar population groupings (e.g., Rockies and SOR/CA groups) were evident in BAPS (Figure 4.1), statistical parsimony network (Figure 4.2A), and PCA (Figure 4.2B); all three analyses differentiate SCA from the other SOR/CA populations.

The ML tree showed shallow phylogenetic structure and low bootstrap support (21%) within the Rockies clade, but showed 99% bootstrap support for the SOR/CA clade, and 93% bootstrap for the node between SCA and SOR, SCCA and CCA (Figure

4.1). Principal coordinate analysis revealed that 73% of the total variation was explained between the two major clades (Principal Coordinate 1), and 12% explained between SCA and all the other populations (Principal Coordinate 2). SAMOVA revealed the highest among group variation (i.e., maximally differentiated from each other) with three groups: Rockies, SOR/CA and SCA (Table 4.2).

4.3.2 Microsatellite Analyses

We found no significant linkage disequilibrium between loci ($p > 0.77$), but five populations deviated from HWE at one or two loci; WMT at locus Pdo5 ($p < 0.001$), SOR at Ppi2 ($p < 0.001$), WA at Titgata02 ($p = 0.03$) and SCCA at Pat14 ($p = 0.003$; Appendix 4.2). One population, SCA, was found to be out of HWE ($p < 0.001$; Appendix 4.2) for two loci, Pdo5 and Titgata39 ($p < 0.017$). The presence of one shared mtDNA haplotype within a subset of the SCA samples (8 of 15 birds), and the fact that all eight of these birds were sampled from the same location on the same day (Smithsonian samples), suggests the potential for at least one family group. However, based on the number of microsatellite alleles observed (4-9 per locus); it is unlikely that these birds are first order relatives. CO had the highest number of private alleles ($n = 5$) followed by NEOR ($n = 4$) and CCA ($n = 3$). Microsatellite pairwise F_{ST} showed significant differences between each of ID, CCA and SCA and all or all but one of the other 13 populations (Table 4.1). The remaining populations showed no clear pattern of differentiation with F_{ST}. Bayesian analysis of population structure with the program STRUCTURE (Figure 4.3) revealed an optimal cluster number of $K = 4$ (Pr Ln $(X|K) = -7541.27$, Bayes factor = 0.99) with the four clusters corresponding to the mtDNA Rockies group (with the exception of WA which clusters with the SOR/CA group), ID, WA/SOR/CA and SCA.

4.3.3 Genetic diversity

Five populations had significant F_S and R_2 values (SAB, CO, UT, NEOR, and SOR; $F_S < -2.72$, $p < 0.01$; $R_2 < 0.10$, $p < 0.03$; Table 4.3) indicative of recent population expansion. The mismatch distribution was not significant for the Rockies group ($p = 0.99$), SOR/CA group ($p = 0.63$), or for all populations combined ($p > 0.08$). Mismatch distributions were unimodal for both the Rockies and SOR/CA groups and the raggedness index was not significant for all groups/populations (data not presented). Therefore, we failed to reject the null hypothesis of demographic expansion. Mantel tests failed to find any correlation between geographic and genetic distances among all populations ($r = 0.0003$, $p = 0.42$), Rockies populations ($r = 0.0014$, $p = 0.55$), and SOR/CA populations ($r = 0.1400$, $p = 0.23$).

The seven microsatellite loci showed variable levels of genetic diversity within populations (Table 4.3). The highest number of alleles was found in CO (n = 91) for the Rockies group, and SOR (n = 66; data not presented) for the SOR/CA group. Pdo5 and Pat14 had the highest allelic richness (*AR* = 4.86, 4.79, respectively) with 2-20 alleles per population. The highest average *AR* in the Rockies group was found in WMT and CO (avg *AR* = 4.4), and in the SOR/CA group within SCCA (avg *AR* = 4.5; Table 4.3, Appendix 4.2). Observed heterozygosity across all loci ranged from 0.668 (SCA) to 0.829 (SAB; Table 4.3). Overall, the average number of alleles was 19.7 per population

(Table 4.3), average number of alleles per locus was 8.24, and the average allelic richness was 4.38 (Appendix 4.2).

4.3.4 Divergence and Diversification

Estimated divergence times varied across populations and are heavily contingent on the estimated mutation rate, therefore actual times should be viewed with caution. The average number of pairwise nucleotide differences between the Rockies group and the SOR/CA group was 16.3. Divergence times between groups are estimated at ~180-710 kya with 11.67% and 3% per MY divergence rate, respectively (Table 4.4). The average number of pairwise differences between SCA and the rest of the SOR/CA group was 9.054 and provides a divergence time estimate of ~100-395 kya using the two divergence rates. BEAST results from the relaxed lognormal clock analysis for both 11.67% and 3% divergence revealed a time to most recent common ancestor between the Rockies and SOR/CA groups as between ~442 kya and ~1.6 My. Within the SOR/CA lineage, SCA split from the rest of that group approximately ~220 kya and ~765 kya.

The estimated time since the population(s) last began expanding was based on the average number of nucleotide differences between individuals (τ) and using both a 5.8% and 1.5% mutation rate. The estimated time since last expansion was ~14.6-56.6 kya for the Rockies group, ~12.9-50.1 kya for the SOR/CA group, and ~5.3-20.9 kya for SCA (Table 4.5). The Rockies populations showing the most recent time since last expansion include CO (~6-23.3 kya) and SAB (~11.9-46.3 kya). ID, WMT and CBC have the highest estimated time since last expansion of ~23.7-32.4 kya (5.8%) and ~92.6-126.3 kya (1.5%). Within the SOR/CA populations, estimated times since last expansion are generally lower at between ~5.3-15.9 kya (5.8%) and ~20.9-62.9 kya with CCA showing the longest time since last population expansion.

4.3.5 Ecological Niche Modeling

MAXENT predicted mountain chickadee distribution using current known occurrences of mountain chickadee, revealed two highly suitable refugia locations within the Sierra Nevada Mountain Range (central) and San Gabriel Mountains (southern) of California (Figure 4.4), and multiple locations within the Intermountain West (i.e., between Rocky Mountains and Cascade/Sierra Nevada), consistent with our genetic results. The AIC score for the selected MAXENT model was 54509, and the model distribution had an AUC (area under curve) value of 0.881, with both training and test sample omission curves close to the predicted value. The AUC value and training/omission curves both indicate the model performed well. MAXENT results indicate mountain chickadee distribution 21 kya experienced a moderate range contraction, primarily within the northern and central Rocky Mountains including the eastern WA, MT, WY, AB and BC, with expanded habitat in the southern Rocky Mountains and Great Basin areas.

4.4 Discussion

We found two well-supported mountain chickadee groups: a Rockies group (with an Idaho subgroup) and a western southern Oregon/California group (with a SCA subgroup). The Rockies group shows evidence of gene flow, although microsatellite loci show evidence of some isolation within the group. Evidence of restricted gene flow within the southwest group (southern California) is more pronounced. Our results are consistent with previous mtDNA studies (Gill *et al.* 1993; Spellman *et al.* 2007) that identified a major east/west division in mountain chickadees, but our results indicate different genetic and geographic substructure within the northern Rockies populations suggesting a possible northern refugium. Ecological niche modeling at the LGM showed suitable habitat may have occurred in multiple locations (e.g., northern California, southern California, Cascades, the central (UT and CO) and southern (NM) Rocky Mountains, and the Great Basin) suggesting mountain chickadees could have survived the LGM in multiple refugia.

4.4.1 Population Genetic Structure

MtDNA (PCA, BAPS, SAMOVA) and microsatellite data (STRUCTURE) both show three consistent groups: Rockies, SOR/CA, and SCA. Differences between the two types of markers are apparent in SCA, ID, WA and the SE Rockies (CO and UT). In the case of SCA and SE Rockies, the mtDNA data support significant genetic differences (Φ_{ST}), isolating those populations from adjacent ones. Different patterns can be attributed to sex-biased dispersal or inherent differences in the markers themselves. For example, in the case of sex-biased dispersal in species where females are philopatric and males disperse, genetic differentiation between populations is expected to be higher using mtDNA (or another maternal marker) than when using a bi-parental marker (e.g., microsatellites), due to females remaining in the natal area and males dispersing farther away (for review see Prugnolle and Meeus 2002). The higher resolution of the microsatellites may explain why nuclear markers are detecting differences where mtDNA are not (i.e., ID) because the higher mutation rate of the microsatellites enables them to reveal more recent reductions in gene flow, making them useful in detecting contemporary patterns (Jarne and Lagoda 1996). Recent isolation provides a probable explanation for the discordant pattern between mtDNA and microsatellites within ID, while male sex-biased dispersal best explains the significant mtDNA differentiation and contrasting lack of nuclear differentiation in CO and UT, as well as WA (see Pacific Northwest).

With mtDNA results, the resolution and subsequent inferences are heavily contingent on the specific regions of the genome that are used for analysis. For example, the overall mitochondrial substitution rate has been estimated at ~2% per My (Fleischer *et al.* 1998), cytochrome *b* at between 1.6-2% per My (Päckert *et al.* 2007), and ND2 at ~ 2.5% (Spellman *et al.* 2007; Manthey *et al.* 2011), while divergence rate (2x substitution rate) estimates within the control region range from 5-20% per My for most birds (Baker and Marshall 1997) to less than 3% per My for Paridae (Päckert *et al.* 2007). The different substitution rates between ND2 and control region may explain the contrasting results in our study with previous work (Spellman *et al.* 2007) regarding both south-central California and Washington populations. The higher mutation rate of the control region would require less time for reciprocal monophyly to occur within a lineage and therefore could explain why we found two monophyletic clades in contrast to Spellman *et al.* (2007), who found only a single clade using the more slowly evolving ND2.

4.4.2 Refugia and Colonization Patterns

4.4.2.1 Rockies

The central Rocky Mountains is a topographically complex region that has been heavily influenced by Pleistocene glacial cycles (Spaeth 2009), and has provided glacial refugia for plant (Brunsfeld *et al*. 2001; Brunsfeld and Sullivan 2005), invertebrate (DeChaine and Martin 2005) and vertebrate (Good and Sullivan 2001) species (for review see Shafer *et al.* 2010). Consistent with these studies, our data suggest a possible northern mountain chickadee refugium, as well as the previously identified possible southern refugium by Spellman *et al.* 2007. The shallow population structure in the Rockies clade and lack of significant pairwise Φ_{ST} and F_{ST} values among multiple Rockies populations support a Rockies refugium for mountain chickadee, while distribution of the two most common widespread shared haplotypes (B and I) and separate southern (CO/UT) and northern (all other populations) Rockies clusters (BAPS) suggests a northern refugium, rather than dispersal out of a southern Rockies refugium. High genetic diversity within refugia is expected due to a reduction in the geographic range and subsequent isolation, as well as high dissimilarity between refugia (Hewitt 1996; 2000). As a result, the high haplotype and nucleotide diversities found in EMT, WMT, ID, as well as BAPS clustering and significant Φ_{ST} differentiation between CO/UT and all other Rockies populations excluding ID, further support a northern/central refugium, possibly within the Cascades or intermountain west region. Ecological niche modeling showed very limited suitable habitat within Montana at the LGM, but was consistent with a possible southern ID, southern WA, and/or OR refugium. Additional suitable habitat occurred within the southern Rocky Mountains, Great Basin and southwest areas.

A possible northern refugium location is consistent with previous mountain chickadee studies by Spellman *et al.* (2007) who found significant differentiation between northern Rocky Mountain and Great Basin populations, and ENM results by Waltari *et al.* (2007) that showed suitable habitat for mountain chickadee east of the Rocky Mountains to the Cascade Range. Similar population structure/patterns have been observed in many other North America taxa (Milá *et al.* 2000; Zink 1994; Zink *et al.* 2000), suggesting a possible northern refugium(a) may have been present within the northern Rocky Mountain area. Microsatellite data support a recent shared population history (F_{ST}, STRUCTURE) among SAB, WMT, CO, and UT due to the lack of differentiation, indicative of recent gene flow because the high mutation rate in microsatellites would otherwise likely result in rapid differentiation. However, gene flow is restricted within the Rockies group (see Barriers) due to significant differentiation among both CO and UT and the rest of the Rockies populations, as well as, contrasting differentiation (F_{ST}) between CO/UT and NEOR/CeOR. The overall separation of the more southern Rockies populations (CO and UT) from northern populations is consistent with expected patterns of dispersal from multiple glacial refugia.

Source populations, in this case refugial population(s), are expected to have high genetic diversity and contain most of the alleles present in recently dispersed subpopulations (Hewitt 1996, 2000; Burg and Croxall 2001; Abbott and Double 2003). Our results however, do not clearly indicate the location of the central/northern refugium (i.e., no single population has all haplotypes present in all other populations, highest allelic richness), and large areas of eastern OR, WA, southern ID and northwest Nevada include suitable habitat predicted at the LGM that are absent from the current distribution of mountain chickadee (Figure 4.4). Therefore, the possibility exists that we did not sample within the refugium location, the refugial population was located in areas that are currently not within the range of mountain chickadee and has since been extirpated, or refugial populations were not separated long enough for differentiation to be evident. Additionally, conflicting results between mtDNA and microsatellite data within the CO/WMT populations for example, suggest that sex-biased dispersal may also affect genetic diversity within central and southern Rockies populations, as well.

The overall genetic pattern though, suggests late or post-Pleistocene dispersal out of multiple glacial refugia, with evidence of a central Rockies/intermountain west refugium. Although we cannot determine exact dispersal patterns, evidence suggests possible northern dispersal into MT and SAB, and west into ID and WA, followed by either a southern dispersal into NEOR and CeOR, or a more recent northern expansion from a Cascade or intermountain west population both south into CeOR and northeast into WA, ID, WMT and SAB, or a combination thereof. Estimated expansion times for many northern mountain chickadee populations (WMT, CBC, ID, WA) are between 17-29 kya (5.8% mutation rate) and support dispersal out of a northern refugium. Phylogenetic splits between eastern Rockies and western Cascades have been observed in American pika (*Ochotona princepsi;* Galbreath *et al.* 2009), blue grouse (Barrowclough *et al.* 2004), and Swainson's thrush (Ruegg and Smith 2002). Additionally, both blue grouse and American pika show differentiation between southern Colorado groups and more northern populations. Refugial/dispersal patterns in mountain chickadee are also consistent with other high elevation species such as lodge-pole pine (Godbout *et al.* 2008) and white-bark pine (Richardson *et al.* 2002), two important foraging species for mountain chickadees (Hutchins and Lanner 1982; McCallum *et al.* 1999), that both show evidence of northern rockies refugia and dispersal out of separate east/west refugia.

4.4.2.2 California

The California landscape has been heavily influenced by Pleistocene events, which resulted in several cycles of montane glaciers covering most of the Sierra Nevada Mountains and isolated coniferous forest habitats (Williams *et al.* 1999), as well as the presence of several large shallow seas and lakes within the Central Valley, all of which have shaped the California landscape (Yanev 1980; Hall 2002). California mountain ranges (e.g., Sierra Nevada, Cascade, Transverse, and Coast) are separated by large basins (e.g., Central Valley), and several taxa show concordant genetic breaks across these areas, presumably following common vicariance events (Calsbeek *et al.* 2003; Lapointe and Rissler 2005; Rissler *et al.* 2006; Chatzimanolis and Caterino 2007) and/or dispersal from glacial refugia (Soltis *et al*. 1997; Brunsfeld *et al*. 2001; Calsbeek *et al*. 2003; Thompson and Calsbeek 2005). Additionally, several species of amphibians (Macey *et al*. 2001; Kuchta and Tan 2006; Kuchta 2007), reptiles (Rodriguez-Robles *et al.* 2001; Feldman and Spicer 2006), mammals (Matocq 2002), birds (Sgariglia and Burns 2003; Alexander and Burns 2006; Spellman *et al.* 2007), and invertebrates (Sandoval *et al*. 1998; Law and Crespi 2002; Starrett and Hedin 2007, Rich *et al*. 2008) exhibit genetic patterns consistent with a possible Sierra Nevada glacial refugium. Consistent with these previous studies, the central Sierra Nevada population (i.e., CCA) has the highest number of private alleles (n = 3), haplotype diversity, and nucleotide diversity within the SOR/CA group, all of which suggest this area could have served as a possible Pleistocene refugium for mountain chickadee. However, SCCA shows the highest average allelic richness (*AR* = 4.46; Appendix 4.2), suggesting that the exact refugium location may not have been sampled, and more detailed studies area needed. Ecological niche modeling also supports both a CCA and/or SCCA predicted LGM refugia.

Pairwise divergence estimates suggest the Rockies and SOR/CA groups have been separated for at least ~180 ky indicating that Pleistocene glacial cycles have influenced population demography. Both mtDNA (SAMOVA, Φ_{ST}, PCA) and microsatellite (STRUCTURE, F_{ST}) data support the separation of a southern Oregon/California group from the Rockies populations, with an additional southern group (i.e., SCA) that has been isolated and evolving independently from the rest of the group for over 100,000 years.

Within the southern OR/CA group, birds north and south of the Transverse Mountains form two monophyletic mtDNA clades. However, microsatellite data (F_{ST}, STRUCTURE) show evidence of unidirectional gene flow from the southern California birds to the nearest adjacent population north of the Transverse Ranges (SCCA; Fig 2; see Molecular Markers) suggesting male-biased dispersal.

4.4.2.3 Pacific Northwest

Within the Pacific Northwest populations of WA, NEOR, and CeOR, the pattern is less clear and results from mtDNA and microsatellite data differ (Table 4.1). Our mtDNA data clearly show the WA population clustering with the Rockies group (Φ_{ST}, BAPS, SAMOVA, TCS, ML), which contrasts the previous study by Spellman *et al.* (2007). Spellman *et al.'s* WA population was comprised of two samples from Ferry County (Supplemental Data) in the northeastern portion of the state that grouped most closely with their CA and central OR populations. WA population samples from our current study are from the central (Yakima County) and western (Mt. Rainier) portions of the state and include 18 and 20 individuals, respectively. While mtDNA groups WA with the Rockies, microsatellite analysis (STRUCTURE) groups WA with the SOR/CA group suggesting possible male-biased dispersal (see Molecular Markers). A similar pattern of dispersal across eastern Washington has also been observed in the hermit (*Dendroica occidentalis*) and Townsend's (*D. townsendi*) warblers, where Rohwer and Martin (2007) suggested that Rocky Mountain populations dispersed through the forested Okanogan Highlands in the northeast corner of Washington and into the northern Cascades resulting in a contact zone (Rohwer and Martin 2007). Additionally, both the chestnut-backed chickadee (*P. rufescens;* Burg *et al*. 2006; Lait *et al*. 2012) and Steller's jay (*Cyanocitta stelleri;* Burg *et al.* 2005) show high levels of population substructure and differentiation between coastal and inland Pacific Northwest populations.

If mountain chickadees from the central Rockies expanded into central Washington, with subsequent male dispersal from southern OR and CA populations north into Washington we would expect to find some birds with mtDNA from eastern populations and at least some nuclear DNA from OR and CA populations, consistent with our results. A similar pattern of introgression between eastern and western subspecies of blue grouse has been observed in eastern Washington where haplotypes from both subspecies are present but restricted to the contact area (Barrowclough *et al.* 2004). Additionally, more than one possible refugium and multiple potential dispersal routes have been shown in mitochondrial and chloroplast analyses of white-bark pine (Richardson *et al.* 2002) where two contrasting patterns of possible post-Pleistocene dispersal from a central Rocky Mountain refugium have been identified: 1) northward expansion into Montana, Idaho and the central Canadian Rockies as early as ~10 kya, as well as westward expansion through Washington, and south along the Cascades; 2) a western expansion from Utah, through Idaho and into northeast and central Oregon (Richardson *et al.* 2002). Similarly, mountain chickadee results support a pattern of complex refugium(a) and post-Pleistocene dispersal within the Pacific Northwest and intermountain west regions.

4.4.4 Barriers

The lack of significant results for IBD suggests that distance is not a barrier to dispersal for mountain chickadee that contrasts previous studies by Spellman *et al.* (2007) who found significant IBD for Rocky Mountain populations (excluding Great Basin populations), but not for all populations within their Eastern Clade. One possible explanation for the different results may be the inclusion of additional CO, UT, EMT and SAB populations within our Rockies group that were not sampled previously, and therefore reduced the overall correlation of geographic distance with genetic distance.

Within this study, samples were collected from areas that would allow us to identify if the Rocky Mountains were a significant barrier to mountain chickadee dispersal. The presence of multiple shared haplotypes within populations located in both western and eastern portions of the Rocky Mountains suggests gene flow among populations. The presence of gene flow indicates the Rocky Mountains may have not been a barrier to dispersal for this species, but as one migrant per generation is sufficient to prevent differentiation (Wright 1931), the Rocky Mountains may still prevent at least some gene flow. Discontinuous habitat appears to affect gene flow and has resulted in differentiation between CO and UT and between both CO/UT and other Rockies populations. The northern central Rockies (MT, ID and SAB) coniferous forest habitat for example, is separated from the southern Rockies (CO and UT) by the upper Colorado River Basin (specifically, the Green River Basin separates MT from CO and UT, and the Uinta and Piceance Creek Basins separate CO from UT), which could explain the lack of mtDNA gene flow between these two areas. Several high-elevation species show similar discordant north/south genetic breaks across the Upper Colorado River Basin (Noonan 2001; DeChaine and Martin 2004, 2005).

In the Pacific Northwest, results suggest discontinuous forest habitat across the western portion of the Columbia Basin may provide a barrier to gene flow. Previous work by Spellman *et al.* (2007) found a common haplotype (11) in two western Oregon populations (Mt. Hood and Deschutes National Forests) located on the eastern side of the Cascade Range that was shared with northern California populations, as well as two other Oregon haplotypes (12 and 14) within their western clade (Spellman *et al.* (2007) Figure 1 and supplemental data). As a result, they included both western Oregon populations (see Figure 4.1 this study), along with Washington populations (see Pacific Northwest) within their western clade (eastern Oregon sample in Mt. Baker was included in their eastern clade and located near our NEOR population; Spellman *et al.* (2007)). The CeOR population in this study however, was located in the Ochoco National Forest, approximately 120 km east of Spellman *et al.'s* Deschutes National Forest location, and was included within the eastern group and significantly differentiated from our SOR populations. In both studies, the low elevation basin in the western portion of the Columbia Basin separates the east/west populations, respectively.

The late-Pleistocene uplift of the Transverse Ranges within California began approximately 5 Mya (Atwater 1998) and resulted in the separation of the area from the northern Sierra Nevada by both the Central Valley and Mojave Desert. Subsequently, several species show a discordant north/south break that may be a result of vicariance events associated with the Transverse uplift (Wake 1997; Tan and Wake 1995; Rodriquez-Robles *et al.* 2001; Smith 1979; Cicero 1996; Sgariglia and Burns 2003). Our results are consistent with long-term isolation and separation of southern California populations within the Transverse Ranges from the Sierra Nevada as evident in both mtDNA and microsatellite data, divergence estimates, and ecological niche modeling. While our data support past vicariance events and historic separation, recent gene flow from SCA northward is evident in microsatellite data suggesting isolation is not complete for mountain chickadee.

4.4.5 Subspecies

Regarding possible species/subspecies designations, we found no evidence of differentiation between SOR and the central and northern CA populations, thereby supporting the subspecies designation for SOR of *P. g. baileyae* based on morphological features (Behle 1956) and previous molecular studies (Gill *et al.* 1993; Gill *et al.* 2005;

Spellman *et al.* 2007). Spellman *et al.* (2007) suggested that the CA populations were not differentiated from the central OR and WA populations, which has been cited as support for splitting the mountain chickadee into two species (N&MA Classification Committee 2010). As evident in our results though, there is a clear division between WA and SOR, and WA shows introgression (see Pacific Northwest) whereas SOR shows a distinct east-west split across the Columbia Basin (Figure 4.1; see Barriers).

Regardless of conflicting patterns, our results suggests the inclusion of Washington and western Oregon populations in either the baileyae supergroup as described in Behle (1956) and outlined in Spellman *et al.* (2007), or as the separate species *P. baileyae* as described by the AOU Classification Committee (2010), is not warranted. Mitochondrial data clearly indicate WA populations group with Rockies populations and microsatellite data show evidence of gene flow between WA (STRUCTURE), NEOR, and CeOR (F_{ST}) each and other SOR/CA and Rockies populations. This is further supported by Q values for numerous WA individuals with similar assignment to both Rockies and SOR/CA clusters. While most WA individuals had the highest Q values for the SOR/CA cluster (avg = 48%), the next highest was always the Rockies cluster (avg = 30%), further suggesting gene flow between the Rockies and SOR/CA groups. Additionally, OR populations east of the Cascade Range, including the eastern slope, should not be included in the *P. g. baileyae* group. The current *P. g. baileyae* subspecies designations which includes OR and WA populations should be revised, and additional studies to identify the limits of the WA population, both mtDNA and microsatellite (south and east, respectively) should be conducted, as well as additional studies to identify the physical/genetic barriers between the western and eastern Oregon groups.

4.5 Conclusions

The mountain chickadee shows an east-west (Cascade) and north/south (California) division consistent with late Pleistocene vicariance events and expansion from multiple refugia located in west of the central Rockies, the southern Rockies, the central Sierra Nevada, and the Transverse Ranges of southern California. Central and eastern Oregon populations cluster with the Rockies group using both mtDNA and microsatellite data, while Washington populations show differing patterns based on the molecular marker used. Idaho appears to have been recently isolated from the rest of the Rockies, and southern California has been isolated since ~100 kya.

A comparison of the patterns observed between different molecular markers (mtDNA and microsatellite) in this study indicates male-biased dispersal provides the best explanation for contrasting mtDNA and microsatellite patterns for both southern California (into southern Sierra Nevada) and northern California/southern Oregon (into Washington), and possibly within central Rockies. The contrasting mtDNA and microsatellite patterns highlight the importance of using multiple loci for phylogenetic studies.

While the Rocky Mountains are not a significant barrier to dispersal for mountain chickadee, evidence suggests that discontinuous habitat associated with the low elevation Columbia Basin has been a significant barrier to dispersal. Additionally, discontinuous habitat associated with the upper Colorado River Basin appears to limited dispersal as well. Several taxa with distributions extending across the Pacific Northwest and into California often exhibit a distinct genetic break in Northern California or Central Oregon, presumably due to range expansion out of glacial refugia (Soltis *et al.* 1997; Brunsfeld *et al.* 2001; Calsbeek *et al.* 2003; Thompson and Calsbeek 2005; Rich *et al.* 2008) with the southern Oregon population often differentiated from more inland populations, which has also been interpreted as evidence for refugia during or even before the Pleistocene glaciations (Nielson *et al*. 2001; Carstens *et al*. 2005; Burg *et al.* 2006). Our results suggest discontinuous habitat across the western Columbia Basin may also provide a barrier to dispersal, and are consistent with range expansion out of separate refugia. The addition of SOR samples in this study allowed us to confirm both a connection between California and southern Cascade populations, evident by the lack of mtDNA differentiation between SOR and both CCA and SCCA, and the presence of two common haplotypes shared among all three populations, as well as differentiation between southern Oregon populations (SOR) and inland populations (CeOR, NEOR) consistent with a California refugium and subsequent dispersal north into southern Oregon.

Finally, the current *P. g. baileyae* subspecies designations of mountain chickadee should be revised to reflect inclusion of central and eastern Oregon populations with the eastern Rockies group. Additional studies should be conducted to more accurately delineate the east/west split along the Cascade Range and/or Columbia Basin with eastern Oregon populations, and Columbia Plateau within central Washington, as well as, identify specific patterns of gene flow within Washington.

Acknowledgements

We are grateful to the following museums for providing additional samples for this study: Burke Museum of Natural History, University of Michigan Museum of Natural History, University of California, Berkeley Museum of Vertebrate Zoology, Louisiana State University Museum of Natural Science, and Smithsonian Museum of Natural Science. We thank the National Park Service, USFWS and Canada Wildlife Service for their help and assistance. This research was funded in part by Alberta Innovates Technologies Futures fund and Natural Science and Engineering Research Council of Canada. 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, Brendan Graham, Paulo Pulgarin, and numerous field assistants for their help with sample collection. Additionally, we thank Linda Lait for all her help in the lab. The research presented here was conducted under University of Lethbridge Animal Welfare Protocol No. 0614 in accordance with Canadian Council on Animal Care guidelines.

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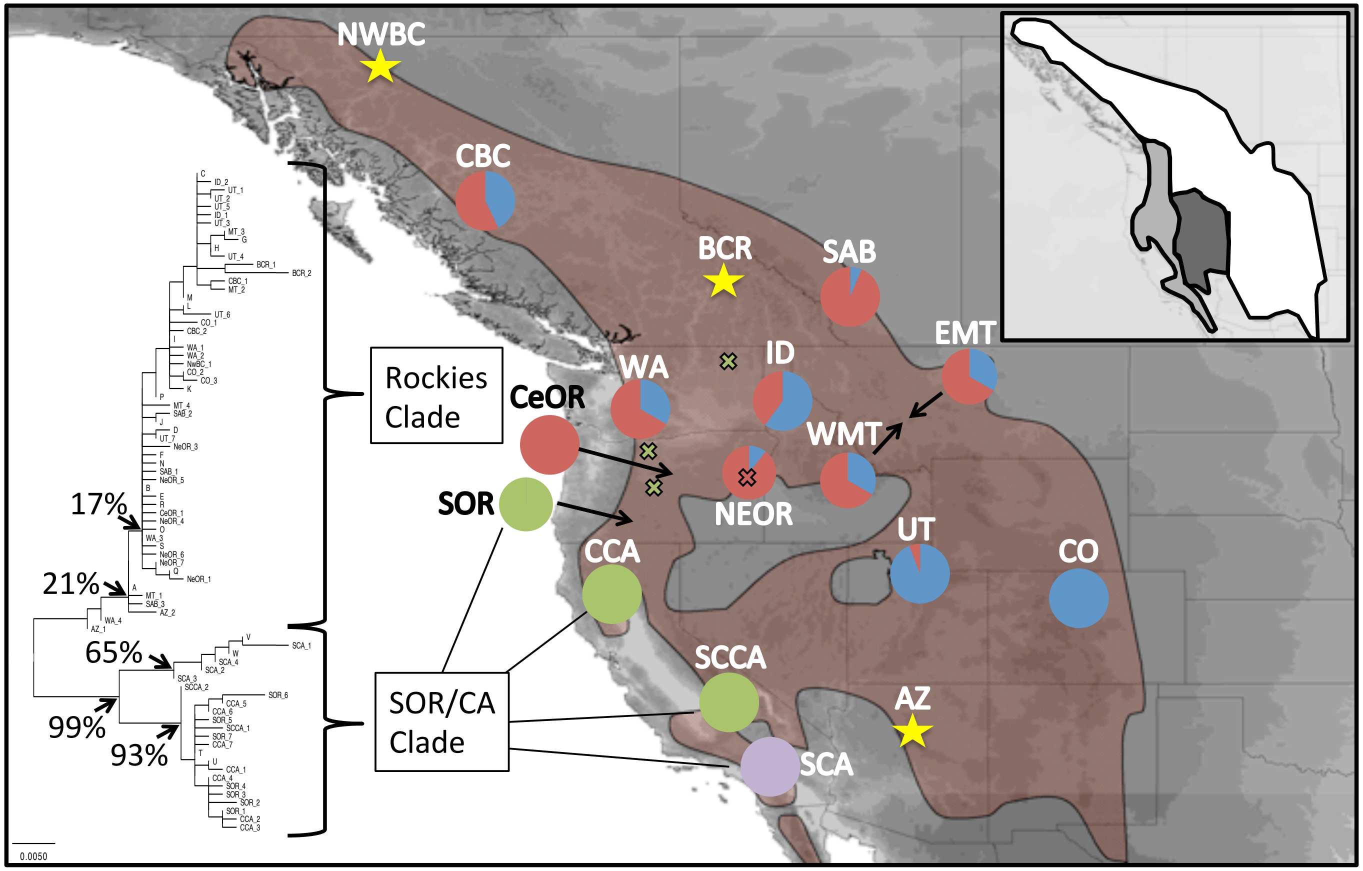
NWBC
CBC
BCR
SAB
EMT
WA
ID
CeOR
WMT
NEOR
SOR
CCA
UT
CO
SCCA
AZ
SCA
Rockies Clade
SOR/CA Clade
17%
21%
65%
99%
93%
C
ID_2
UT_1
UT_2
UT_5
ID_1
UT_3
MT_3
G
H
UT_4
BCR_1
BCR_2
CBC_1
MT_2
M
L
UT_6
CO_1
CBC_2
I
WA_1
WA_2
NwBC_1
CO_2
CO_3
K
P
MT_4
SAB_2
J
D
UT_7
NeOR_3
F
N
SAB_1
NeOR_5
B
E
R
CeOR_1
NeOR_4
O
WA_3
S
NeOR_6
NeOR_7
Q
NeOR_1
A
MT_1
SAB_3
AZ_2
WA_4
AZ_1
V
SCA_1
W
SCA_4
SCA_2
SCA_3
SCCA_2
SOR_6
CCA_5
CCA_6
SOR_5
SCCA_1
SOR_7
CCA_7
T
U
CCA_1
CCA_4
SOR_4
SOR_3
SOR_2
SOR_1
CCA_2
CCA_3
0.0050

Figure 4.1. Unrooted ML tree with bootstrap values (left) and BAPS 95% CI cluster assignment (K = 4; coloured circles) of mountain chickadee mtDNA (scale bar indicates number of substitutions per site = 0.005). Contemporary mountain chickadee distribution is outlined (red shading), populations with n < 8 sequences (star) and WA and OR sampling locations from Spellman *et al.* (2007; x, green western clade and red eastern clade) are shown. Inset shows three major morphological groups described in Behle (1956): *gambeli* –white, *inyoensis* – dark grey, *baileyae* – light grey. Sampling sites include central British Columbia (CBC), northwest BC (NWBC), Revelstoke, BC (BCR), southern Alberta (SAB), western Montana (WMT), eastern Montana (EMT), Colorado (CO), Utah (UT), Arizona (AZ), Washington (WA), Idaho (ID), northeast Oregon (NEOR), central OR (CeOR), southern Oregon (SOR), central California (CCA), south central CA (SCCA), and southern CA (SCA); arrows indicate location for SOR, CeOR, WMT, and EMT sampling sites.

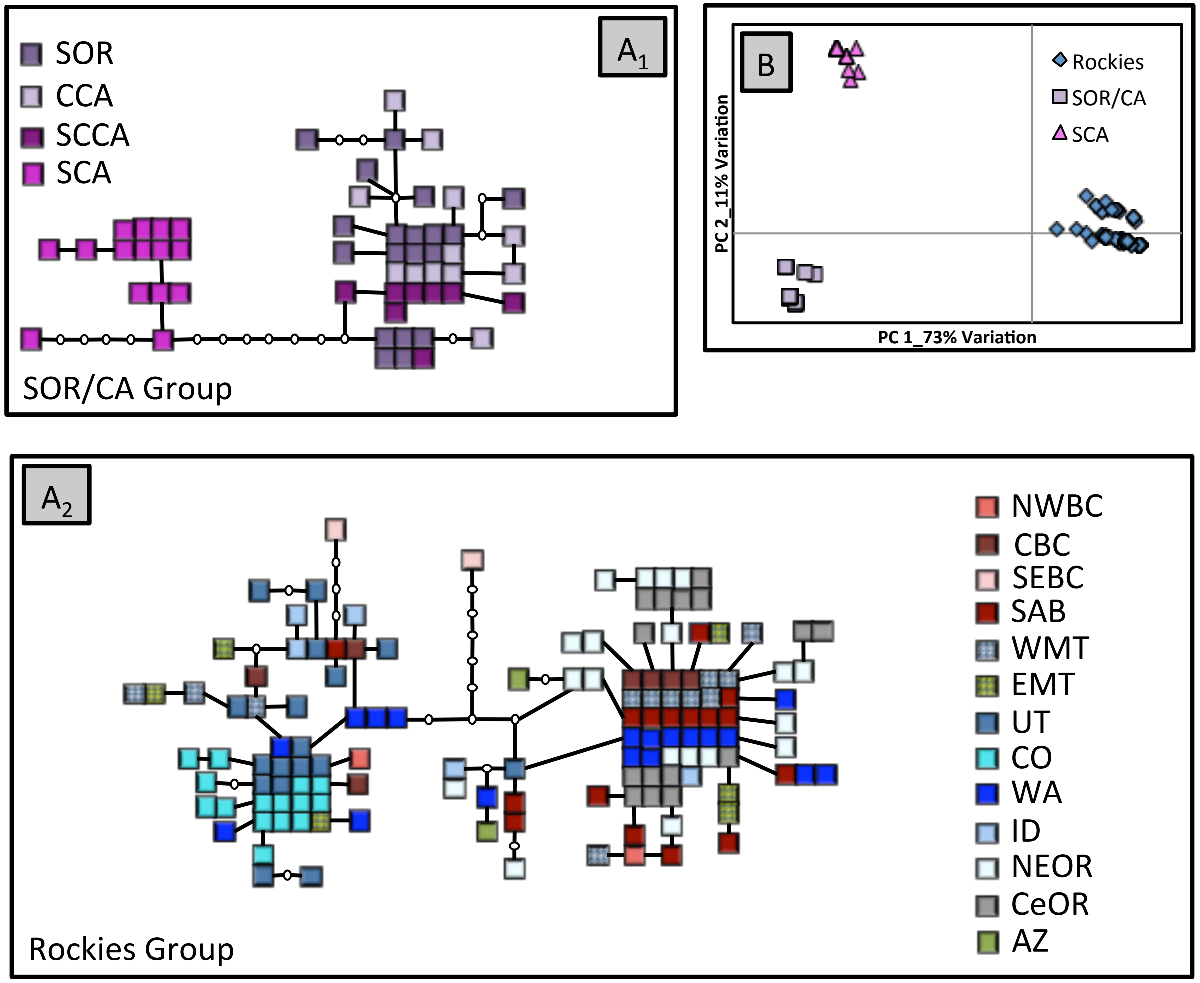

Figure 4.2. A. Statistical parsimony network (TCS) of mountain chickadee mtDNA haplotypes: A_1 SOR/CA group; A_2 Rockies group. Each square represents a single individual; open circles indicate inferred haplotypes; separate groups indicate >95% probability, or for connections n > 12. Refer to Figure 4.1 for location of sampling sites. B. Principal coordinates analysis of mtDNA sequences based on population location. Principal coordinate (PC) 1 explains 73% of the variation and PC 2 12%.

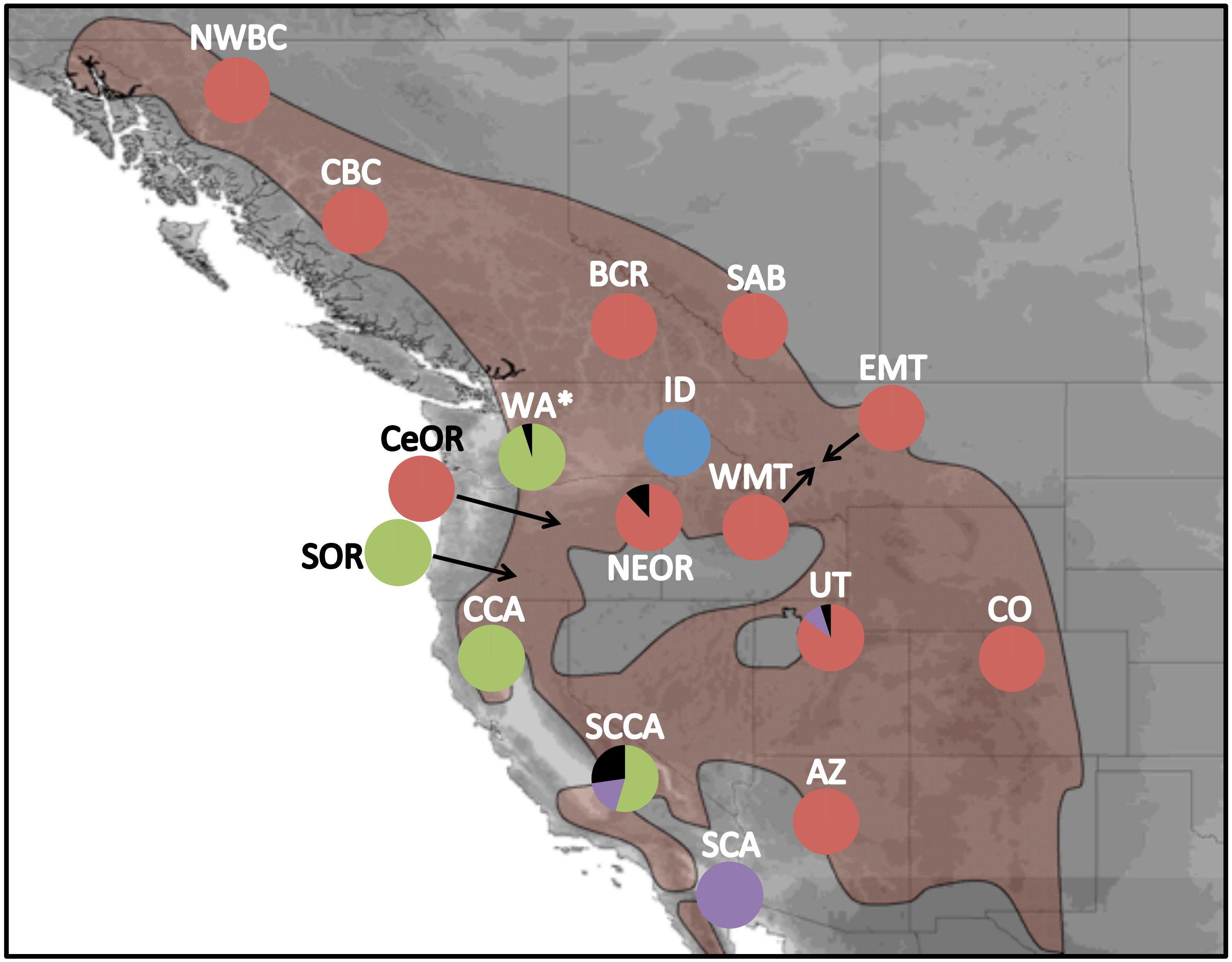

Figure 4.3. Proportion of mountain chickadee populations assigned to one of the four clusters by STRUCTURE (k = 4; Bayes factor = 0.99) from microsatellite data. Individual birds were assigned to the cluster (each cluster indicated by different colour) with the highest *Q* value (ancestry coefficient). Individuals in black could not be assigned to one cluster. *Note at K = 4, all assignable WA birds group with SOR/CA cluster (avg *Q* value = 48%) but second highest *Q* value was Rockies cluster (avg Q = 30%). Arrows indicate location for SOR, CeOR, WMT, and EMT sampling sites.

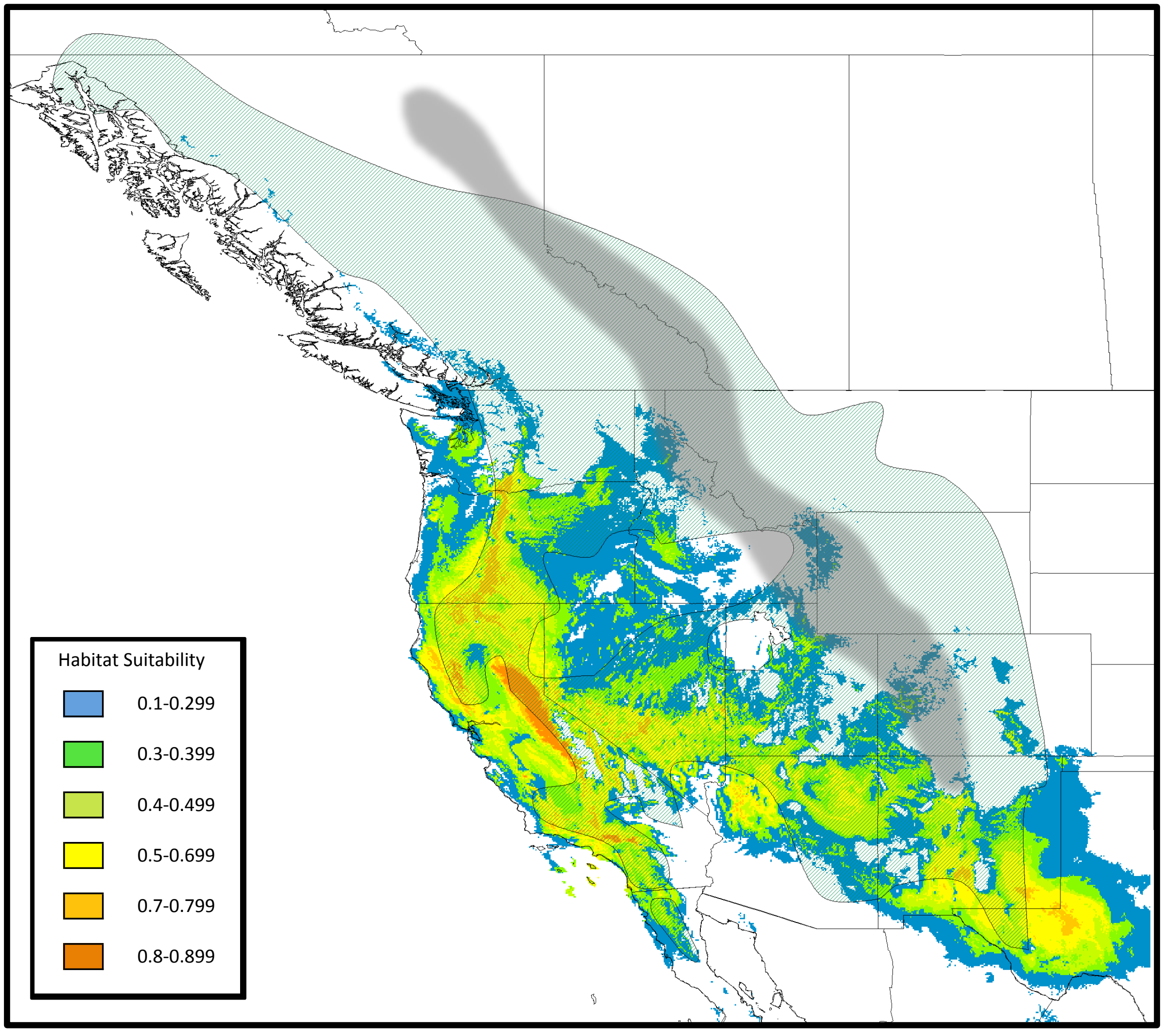
Habitat Suitability
0.1-0.299
0.3-0.399
0.4-0.499
0.5-0.699
0.7-0.799
0.8-0.899

Figure 4.4. Average ecological niche model of possible mountain chickadee refugia following the LGM, ~21kya. Warmer colours indicate higher habitat suitability (yellow indicates habitat suitability > 0.5, orange/red 0.79 - 0.99, respectively). Black outline with green crosshatch indicates present-day distribution and dark shaded area indicates approximate location of Rocky Mountains.

Table 4.1. MtDNA Φ_{ST} (bottom left) and microsatellite F_{ST} (upper right) values for pairwise comparisons among mountain chickadee populations (bold = significant after Benjamini-Hochberg correction). AZ, NWBC, BCR populations were excluded due to small sample size (n < 8). Lines separate Rockies, SOR/CA, and SCA groups.

	CBC	SAB	WMT	EMT	CO	UT	WA	ID	NEOR	CeOR	SOR	CCA	SCCA	SCA
CBC	*	0.002	0.005	0.046	**0.022**	**0.018**	**0.030**	**0.055**	**0.018**	**0.037**	**0.062**	**0.095**	**0.021**	**0.076**
SAB	0.088	*	0.001	**0.028**	0.004	0.008	0.011	**0.054**	0.002	0.008	0.033	**0.077**	0.012	**0.057**
WMT	-0.076	0.061	*	0.022	0.009	0.007	**0.020**	**0.076**	**0.015**	0.013	**0.030**	**0.070**	0.005	**0.056**
EMT	-0.050	0.040	-0.043		0.008	0.013	0.017	**0.111**	**0.023**	0.016	0.010	**0.064**	0.005	0.036
CO	**0.322**	**0.538**	**0.346**	**0.364**	*	0.007	**0.014**	**0.060**	**0.001**	0.012	**0.019**	**0.061**	0.003	**0.033**
UT	**0.140**	**0.424**	**0.201**	**0.224**	**0.111**	*	0.011	**0.078**	0.014	**0.019**	0.027	**0.061**	0.009	**0.033**
WA	-0.022	0.047	0.014	0.011	**0.357**	**0.272**	*	**0.061**	**0.002**	**0.004**	**0.016**	**0.062**	0.002	**0.056**
ID	0.009	**0.313**	0.046	0.113	**0.385**	0.063	0.176	*	**0.041**	**0.083**	**0.095**	**0.110**	0.075	**0.123**
NEOR	**0.145**	0.042	**0.124**	**0.107**	**0.538**	**0.446**	**0.107**	**0.351**	*	**0.005**	0.018	**0.060**	-0.003	**0.041**
CeOR	**0.257**	**0.119**	**0.204**	**0.216**	**0.653**	**0.522**	**0.192**	**0.459**	-0.006	*	0.009	**0.070**	0.009	**0.042**
SOR	**0.899**	**0.901**	**0.882**	**0.891**	**0.929**	**0.893**	**0.899**	**0.891**	**0.901**	**0.919**	*	**0.035**	0.012	**0.048**
CCA	**0.880**	**0.889**	**0.863**	**0.868**	**0.922**	**0.879**	**0.887**	**0.867**	**0.890**	**0.911**	0.008	*	**0.057**	**0.098**
SCCA	**0.912**	**0.910**	**0.882**	**0.902**	**0.950**	**0.897**	**0.906**	**0.900**	**0.907**	**0.936**	-0.002	0.022	*	0.018
SCA	**0.898**	**0.899**	**0.878**	**0.891**	**0.933**	**0.890**	**0.898**	**0.885**	**0.900**	**0.922**	**0.835**	**0.824**	**0.856**	*

Table 4.2. Spatial analysis of molecular variance (SAMOVA) among Rockies, SOR/CA, and SCA groups.

Source of Variation	% Variation	Statistic	P
Among groups	85.43	$F_{CT} = 0.85432$	< 0.0001
Among populations within groups	3.86	$F_{SC} = 0.26525$	< 0.0001
Within populations	10.70	$F_{ST} = 0.89296$	< 0.0001

Table 4.3. Mitochondrial (top) and microsatellite (bottom) genetic diversity within populations of mountain chickadee; number of mtDNA samples (N^1), segregating sites (*S*), haplotypes (*H*), private haplotypes (*Pri*), unique haplotypes (*U*), haplotype diversity (*Hd*) and its standard deviation (SD), nucleotide diversity (π) and its SD, F_S and R_2 tests (bold denotes significance; $p < 0.05$), number of microsatellite samples (N^2), average number of alleles (*A*), average allelic richness (*AR*), and heterozygosity (observed – H_O, expected – H_E). AZ, NWBC and BCR were excluded from theses analyses due to small sample size (n<8).

	Rockies									SOR/CA				
	CBC	SAB	WMT	EMT	CO	UT	ID	NEOR	CeOR	WA	SOR	CCA	SCCA	SCA
N^1	7	16	12	6	15	17	5	19	15	18	19	12	8	15
S	6	13	10	7	6	12	6	11	4	8	12	8	4	8
H	2	9	7	5	6	11	4	11	4	7	9	8	4	6
Pri	0	1	0	1	0	1	0	3	0	1	0	0	0	1
U	2	3	4	0	3	7	2	6	1	4	7	7	2	4
Hd	0.714	0.817	0.773	0.933	0.648	0.846	0.900	0.930	0.695	0.739	0.819	0.848	0.643	0.705
HdSD	0.181	0.095	0.128	0.122	0.089	0.134	0.126	0.030	0.007	0.099	0.069	0.104	0.184	0.114
π	0.0033	0.0026	0.0040	0.0038	0.0014	0.0032	0.0047	0.0027	0.0018	0.0026	0.0024	0.0026	0.0013	0.0023
πSD	0.0008	0.0006	0.0009	0.0011	0.0006	0.0004	0.0011	0.0003	0.0003	0.0005	0.0005	0.0006	0.0005	0.0009
F_S	0.281	**-4.298**	-1.335	-1.327	**-2.726**	**-5.936**	-1.901	**-7.841**	0.311	-1.434	**-3.924**	-4.045	-1.236	-1.208
R_2	0.158	**0.085**	0.1272	0.1949	**0.104**	**0.089**	0.185	**0.074**	0.162	0.114	**0.075**	0.113	0.177	0.17
N^2	9	23	23	8	38	20	10	25	18	20	25	12	11	15
A	8.0	10.0	10.9	7.0	13.0	9.7	7.4	11.4	10.0	9.3	9.4	8.4	7.6	7.7
AR	4.1	4.2	4.4	4.3	4.4	4.3	3.6	4.3	4.2	4.1	4.0	3.9	4.5	3.9
H_O	0.804	0.829	0.780	0.704	0.821	0.810	0.763	0.780	0.825	0.764	0.767	0.797	0.740	0.668
H_E	0.810	0.820	0.856	0.746	0.852	0.827	0.796	0.826	0.831	0.810	0.815	0.786	0.802	0.792

Table 4.4. Estimated divergence times based on pairwise differences between groups and BEAST analysis, using both 3% and 11.67 % divergence rates.

Rate	Method	Rockies and SOR/CA	SCA
11.67%	Pairwise	180 kya	100 kya
	BEAST	442 kya	220 kya
3%	Pairwise	710 kya	395 kya
	BEAST	1.6 Mya	765 kya

Table 4.5. Estimated time since last population expansion (kya) of mountain chickadee populations based on the net number of nucleotide differences between populations (τ) using both 1.5% and 5.8% mutation rates. AZ, NWBC and BCR excluded due to small sample size (n < 8).

Rockies Clade	CBC	SAB	WMT	EMT	CO	UT	ID	NEOR	CeOR	WA	Overall
t	4.25	2.13	5.34	2.77	1.07	3.15	5.8	2.27	2.18	3.11	2.6
Time (5.8%)	23.7	11.9	29.8	15.5	6.0	17.6	32.4	12.7	12.2	17.4	14.6
Time (1.5%)	92.6	46.3	116.4	60.5	23.3	68.7	126.3	49.3	47.4	67.8	56.6

SOR/CA Clade	SOR	CCA	SCCA	SCA	Overall
t	2.13	2.85	1.06	0.96	2.3
Time (5.8%)	11.8	15.9	5.9	5.3	12.9
Time (1.5%)	46.3	62.1	23.1	20.9	50.1

Appendix 4.1. Mountain chickadee sample locations, band number, identification (ID), haplotype, latitude/longitude, and museum collection (where applicable).

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Southern Alberta (SAB)						
West Castle, AB	2490-57632	mochSAB001	B		49° 20' 42"	114° 24' 55"
West Castle, AB	2490-57640	mochSAB002	B		49° 20' 42"	114° 24' 55"
West Castle, AB	2490-57641	mochSAB003	D		49° 20' 42"	114° 24' 55"
West Castle, AB	2490-57642	mochSAB004	-		49° 20' 42"	114° 24' 55"
West Castle, AB	2490-57643	mochSAB005	-		49° 20' 42"	114° 24' 55"
West Castle, AB	2490-57644	mochSAB006	-		49° 20' 42"	114° 24' 55"
West Castle, AB	2490-57645	mochSAB007	E		49° 20' 42"	114° 24' 55"
West Castle, AB	2490-57648	mochSAB008	-		49° 20' 42"	114° 24' 55"
Syncline Ski Area, AB	2490-57657	mochSAB009	-		49° 23' 27"	114° 20' 23"
Syncline Ski Area, AB	2490-57658	mochSAB010	-		49° 23' 27"	114° 20' 23"
Syncline Ski Area, AB	2490-57665	mochSAB011	B		49° 23' 27"	114° 20' 23"
Syncline Ski Area, AB	2490-57666	mochSAB012	B		49° 23' 27"	114° 20' 23"
Beaver Mines Prov. Prk Rd., AB	2490-57667	mochSAB013	F		49° 23' 60"	114° 19' 60"
Beaver Mines Prov. Prk Rd., AB	2490-57668	mochSAB014	A		49° 22' 21"	114° 18' 32"
Field station cabin, AB	2490-57669	mochSAB015	B		49° 20' 57"	114° 24' 39"
Field station cabin, AB	2490-57670	mochSAB016	D		49° 20' 57"	114° 24' 39"
Field station cabin, AB	2490-57671	mochSAB017	SAB017		49° 20' 57"	114° 24' 39"
Field station cabin, AB	2490-57672	mochSAB018	SAB018		49° 20' 57"	114° 24' 39"
Field station cabin, AB	2490-57674	mochSAB019	SAB019		49° 20' 57"	114° 24' 39"
Lynx Creek, AB	2490-57675	mochSAB020	B		49° 28' 2"	114° 25' 1"
Lynx Creek, AB	2490-57676	mochSAB021	-		49° 28' 2"	114° 25' 1"
North Lost Creek Rd, AB	2490-57681	mochSAB022	B		49° 26' 49"	114° 29' 36"
Crandall Lk. CG, Waterton, AB	2490-57720	mochSAB023	B		49° 05' 50"	113° 57'19"
Montana (MT)						
Helena National Forest, Helena, MT	2540-22890	mochMT001	B		46° 28' 59"	111° 51' 43"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Helena National Forest, Helena, MT	2540-22896	mochMT002	B		46° 29' 5"	111° 52' 2"
Grizzly Gulch, Helena, MT	2540-22897	mochMT003	MT003		46° 32' 32"	112° 6' 40"
Grizzly Gulch, Helena, MT	2540-22898	mochMT004	MT004		46° 32' 32"	112° 6' 40"
Grizzly Gulch, Helena, MT	2540-22899	mochMT005	B		46° 32' 32"	112° 6' 40"
Orofino, Helena, MT	2540-22900	mochMT006	-		46° 33' 16"	112° 4' 0"
Orofino, Helena, MT	2530-19203	mochMT007	-		46° 33' 16"	112° 4' 0"
Orofino, Helena, MT	2530-19205	mochMT008	B		46° 32' 32"	112° 5' 55"
Orofino, Helena, MT	2530-19204	mochMT009	MT009		46° 32' 32"	112° 5' 55"
Orofino, Helena, MT	2530-19206	mochMT010	MT010		46° 32' 32"	112° 5' 55"
Orofino, Helena, MT	2530-19207	mochMT011	B		46° 32' 32"	112° 5' 55"
Orofino, Helena, MT	2530-19208	mochMT012	G		46° 32' 32"	112° 5' 55"
Orofino, Helena, MT	2530-19210	mochMT013	H		46° 31' 27"	112° 6' 43"
Black Sands, Helena, MT	2530-19211	mochMT014	E		46° 44' 44"	111° 53' 2"
Black Sands, Helena, MT	2530-19212	mochMT015	B		46° 44' 44"	111° 53' 2"
Black Sands, Helena, MT	2530-19215	mochMT016	I		46° 44' 44"	111° 53' 2"
Black Sands, Helena, MT		mochMT017	G		46° 44' 44"	111° 53' 2"
Canyon Ferry Rd, Helena, MT	2530-19213	mochMT018	J		46° 39' 32"	111° 43' 43"
Canyon Ferry Rd, Helena, MT	2530-19216	mochMT019	-		46° 39' 32"	111° 43' 43"
Canyon Ferry Rd, Helena, MT	2530-19217	mochMT020	-		46° 39' 32"	111° 43' 43"
Canyon Ferry Rd, Helena, MT	2530-19218	mochMT021	J		46° 39' 32"	111° 43' 43"
Orofino, Helena, MT	2530-19222	mochMT022	-		46° 33' 44"	112° 3' 55"
Orofino, Helena, MT	2530-19223	mochMT023	-		46° 33' 44"	112° 3' 55"
Rd to Park Lake, Helena, MT	2530-19227	mochMT024	-		46° 31' 27"	112° 6' 43"
Rd to Park Lake, Helena, MT	2530-19237	mochMT025	-		46° 31' 27"	112° 6' 43"
Orofino, Helena, MT	2530-19238	mochMT026	-		46° 33' 17"	112° 4' 4"
Rd to Park Lake, Helena, MT	2530-19240	mochMT027	-		46° 27' 56"	112° 7' 47"
Bridger Woods Rd, Bozeman, MT	2530-19245	mochMT028	-		45° 41' 38"	110° 54' 16"
Helena, MT	2530-19249	mochMT030	-		46° 32' 32"	112° 6' 39"
Helena, MT	2530-19250	mochMT031	-		46° 32' 32"	112° 6' 39"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Helena, MT	2530-19251	mochMT032	-		46° 9' 31"	111° 42' 60"
Helena, MT	2530-19252	mochMT033	-		46° 9' 31"	111° 42' 60"
Washington (WA)						
Mt. Rainier Paradise, WA	2550-40402	mochWA001	F		46° 47' 5"	121° 44' 31"
Mt. Rainier Paradise, WA	2550-40403	mochWA002	WA002		46° 47' 5"	121° 44' 31"
Mt. Rainier Paradise, WA	2550-40404	mochWA003	B		46° 47' 5"	121° 44' 31"
Mt Rainier Visitor Centre, WA	2550-40472	mochWA004	I		46° 54' 3"	121° 38' 7"
Mt Rainier Visitor Centre, WA	2550-40473	mochWA005	B		46° 54' 3"	121° 38' 7"
Mt Rainier Visitor Centre, WA	2550-40474	mochWA006	B		46° 54' 3"	121° 38' 7"
Mt Rainier Visitor Centre, WA	2550-40475	mochWA007	B		46° 54' 3"	121° 38' 7"
Mt Rainier Visitor Centre, WA	2550-40476	mochWA008	B		46° 54' 3"	121° 38' 7"
Mt Rainier Visitor Centre, WA	2550-40477	mochWA009	M		46° 54' 3"	121° 38' 7"
MT. Rainier Paradise, WA	2550-40401	mochWA010	WA010		46° 47' 5"	121° 44' 31"
Naches, Yakima Co. WA	UWBM# 72671 SAR 6311	mochWA011	M	UWBM	46° 43' 52"	120° 41' 58"
Ellensburg, Yakima Co. WA	UWBM# 74543 DRF 177	mochWA012	M	UWBM	47° 10' 30"	120° 55' 55"
Naches, Yakima Co. WA	UWBM# 79037 SAR 6312	mochWA013	F	UWBM	46° 43' 52"	120° 41' 58"
Naches, Yakima Co. WA	UWBM# 87352 SAR 6314	mochWA014	WA014	UWBM	46° 43' 52"	120° 41' 58"
Naches, Yakima Co. WA	UWBM# 72672 SAR 6313	mochWA015	WA015	UWBM	46° 43' 52"	120° 41' 58"
Ellensburg, Yakima Co. WA	UWBM# 57140 GAV 292	mochWA016	B	UWBM	47° 10' 30"	120° 55' 55"
Ellensburg, Yakima Co. WA	UWBM# 62628 RAP 23	mochWA017	B	UWBM	47° 10' 30"	120° 55' 55"
Ellensburg, Yakima Co. WA	UWBM# 57160 JMB 1569	mochWA018	-	UWBM	47° 10' 30"	120° 55' 55"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Ellensburg, Yakima Co. WA	UWBM# 57128 DAB 453	mochWA019	-	UWBM	47° 10' 30"	120° 55' 55"
Mt. Rainier Paradise, WA	2550-40405	mochWA020	B		46° 47' 5"	121° 44' 31"
Northwest British Columbia (NWBC)						
Dease Lake, BC	2520-39854	mochNWBC001	A		58° 30' 25"	130° 1' 23"
Dease Lake, BC	2520-39858	mochNWBC002	NWBC002		58° 30' 25"	130° 1' 23"
Central British Columbia (CBC)						
Smithers, BC	2520-39895	mochCBC001	CBC001		54° 45' 11"	127° 15' 3"
Smithers, BC	2520-39896	mochCBC002	B		54° 45' 11"	127° 15' 3"
Smithers, BC	2520-39897	mochCBC003	C		54° 45' 11"	127° 15' 3"
Smithers, BC	2490-57771	mochCBC004	CBC004		54° 45' 34"	127° 21' 42"
Smithers, BC	2490-57772	mochCBC005	B		54° 45' 13"	127° 21' 4"
Smithers, BC	2490-57773	mochCBC006	B		54° 45' 13"	127° 21' 4"
Hudson Bay Mtn., Smithers BC	2500-94925	mochCBC07			54° 46' 1"	127° 16' 25"
Hudson Bay Mtn., Smithers BC	2500-94926	mochCBC08	B		54° 46' 1"	127° 16' 25"
Hudson Bay Mtn., Smithers BC	2500-94927	mochCBC09	-		54° 46' 1"	127° 16' 25"
Revelstoke, British Columbia (BCR)						
Mt Revelstoke, Revelstoke, BC	2500-94936	mochBCR001	-		51° 2' 24"	118° 7' 57"
Revelstoke, BC	2500-94938	mochBCR002	-		51° 2' 24"	118° 8' 52"
Whitewater Ski Hill, Nelson BC	2500-94934	mochBCR003	BCR003		49° 26' 44"	117° 8' 54"
Whitewater Ski Hill, Nelson BC	2500-94935	mochBCR004	BCR004		49° 26' 44"	117° 8' 54"
Northeast Oregon (NEOR)						
Mt. Howard Summit, OR	2540-23029	mochNEOR001	B		45° 15' 45"	117° 10' 49"
Mt. Howard Summit, OR	2540-23030	mochNEOR002	O		45° 15' 45"	117° 10' 49"
Anthony Lakes CG, OR	2550-40407	mochNEOR003	NEOR003		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40408	mochNEOR004	O		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40409	mochNEOR005	P		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40410	mochNEOR006	-		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40411	mochNEOR007	P		44° 57' 47"	118° 13' 32"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Anthony Lakes CG, OR	2550-40412	mochNEOR008	Q		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40413	mochNEOR009	NEOR009		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40414	mochNEOR010	B		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40415	mochNEOR011	Q		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40416	mochNEOR012	NeOR012		44° 57' 47"	118° 13' 32"
Anthony Lakes CG, OR	2550-40417	mochNEOR013	R		44° 57' 47"	118° 13' 32"
Anthony Lakes Ski Resort, OR	2550-40418	mochNEOR014	NeOR014		44° 57' 50"	118° 13' 60"
Anthony Lakes Ski Resort, OR	2550-40419	mochNEOR015	B		44° 57' 50"	118° 13' 60"
Anthony Lakes Ski Resort, OR	2550-40420	mochNEOR016	NeOR016		44° 57' 50"	118° 13' 60"
Anthony Lakes Ski Resort, OR	2550-40421	mochNEOR017	Q		44° 57' 50"	118° 13' 60"
Anthony Lakes Ski Resort, OR	2550-40422	mochNEOR018	R		44° 57' 50"	118° 13' 60"
Spout Springs Ski Resort., OR	2550-40423	mochNEOR019	N		45° 45' 16"	118° 3' 5"
Spout Springs Ski Resort., OR	2550-40424	mochNEOR020	NeOR020		45° 45' 16"	118° 3' 5"
Mt. Howard Summit, OR	2550-40425	mochNEOR021	-		45° 15' 45"	118° 10' 49"
Two colors CG, OR	2550-40426	mochNEOR022	-		45° 2' 15"	117° 26' 39"
Anthony Lakes Ski Resort., OR	2550-40427	mochNEOR023	-		44° 57' 50"	118° 13' 60"
Anthony Lakes Ski Resort., OR	2550-40428	mochNEOR024	-		44° 57' 50"	118° 13' 60"
Anthony Lakes CG, OR	2550-40406	mochNEOR025	-		44° 57' 47"	118° 13' 32"
Central Oregon (CEOR)						
Walton Lake CG, OR	2550-40429	mochCeOR001	Q		44° 26' 3"	120° 19' 7"
Walton Lake CG, OR	2550-40430	mochCeOR003	CEOR003		44° 26' 3"	120° 19' 7"
Barnhouse Springs CG, OR	2550-40431	mochCeOR002	Q		44° 26' 3"	119° 56' 7"
Barnhouse Springs CG, OR	2550-40432	mochCeOR004	S		44° 26' 3"	119° 56' 7"
Walton Lake CG, OR	2550-40433	mochCeOR005	B		44° 26' 3"	120° 19' 7"
Walton Lake CG, OR	2550-40434	mochCeOR006	B		44° 26' 3"	120° 19' 7"
Walton Lake CG, OR	2550-40435	mochCeOR007	Q		44° 26' 3"	120° 19' 7"
National Forest Rd 12, OR	2550-40436	mochCeOR008	Q		44° 26' 3"	119° 55' 7"
National Forest Rd 12, OR	2550-40437	mochCeOR009	B		44° 26' 3"	119° 55' 7"
National Forest Rd 12, OR	2550-40438	mochCeOR010	B		44° 26' 3"	119° 55' 7"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
National Forest Rd 12, OR	2550-40439	mochCeOR011	-		44° 26' 3"	119° 55' 7"
National Forest Rd 12, OR	2550-40440	mochCeOR012	-		44° 26' 3"	119° 55' 7"
National Forest Rd 12, OR	2550-40441	mochCeOR013	B		44° 26' 3"	119° 55' 7"
National Forest Rd 12, OR	2550-40442	mochCeOR014	B		44° 26' 3"	119° 55' 7"
Walton Lake CG, OR	2550-40443	mochCeOR015	B		44° 26' 3"	120° 19' 7"
Walton Lake CG, OR	2550-40444	mochCeOR016	S		44° 26' 3"	120° 19' 7"
Walton Lake CG, OR	2550-40445	mochCeOR017	Q		44° 26' 3"	120° 19' 7"
7 Mile Creek Bridge, OR	2550-40446	mochCEOR18	-		44° 26' 3"	120° 19' 7"
Southern Oregon (SOR)						
Crystal Springs, OR	2550-40447	mochSOR002	T		42° 42' 3"	122° 4' 7"
Crystal Springs, OR	2550-40448	mochSOR003	T		42° 42' 3"	122° 5' 7"
Crystal Springs, OR	2550-40449	mochSOR004	SOR004		42° 42' 3"	122° 5' 7"
Crystal Springs, OR	2550-40450	mochSOR005	T		42° 42' 3"	122° 5' 7"
Crystal Springs, OR	2550-40451	mochSOR006	SOR006		42° 42' 3"	122° 5' 7"
Mazama CG, Crater Lake, OR	2550-40460	mochSOR007	T		42° 42' 3"	122° 9' 7"
Crystal Springs, OR	2550-40453	mochSOR008	T		42° 42' 3"	122° 5' 7"
Crystal Springs, OR	2550-40454	mochSOR009	SOR009		42° 42' 3"	122° 5' 7"
Crystal Springs, OR	2550-40455	mochSOR010	SOR010		42° 42' 3"	122° 5' 7"
Collier Memorial State Park, OR	2550-40456	mochSOR011	T		42° 42' 3"	121° 52' 7"
Crater Lake Rim Village, OR	2550-40457	mochSOR012	SOR012		42° 42' 3"	122° 8' 7"
S&L Cabin, Crater Lake, OR	2550-40458	mochSOR013	SOR013		42° 42' 3"	122° 8' 7"
Mazama CG, Crater Lake, OR	2550-40462	mochSOR014	U		42° 42' 3"	122° 9' 7"
Mazama CG, Crater Lake, OR	2550-40463	mochSOR015	U		42° 42' 3"	122° 9' 7"
Mazama CG, Crater Lake, OR	2550-40464	mochSOR016	SOR016		42° 42' 3"	122° 9' 7"
Mazama CG, Crater Lake, OR	2550-40465	mochSOR017	U		42° 42' 3"	122° 9' 7"
Mazama CG, Crater Lake, OR	2550-40467	mochSOR018	U		42° 42' 3"	122° 9' 7"
Mazama CG, Crater Lake, OR	2550-40468	mochSOR019	U		42° 42' 3"	122° 9' 7"
Mazama CG, Crater Lake, OR	2550-40469	mochSOR020	T		42° 42' 3"	122° 9' 7"
S&L Cabin, Crater Lake, OR	2550-40471	mochSOR021	-		42° 42' 3"	122° 8' 7"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Crystal Springs, OR	2550-40452	mochSOR022	-		42° 42' 3"	122° 5' 7"
Mazama CG, Crater Lake, OR	2550-40459	mochSOR023	-		42° 42' 3"	122° 9' 7"
Mazama CG, Crater Lake, OR	2550-40461	mochSOR024	-		42° 42' 3"	122° 9' 7"
Mazama CG, Crater Lake, OR	2550-40466	mochSOR025	-		42° 42' 3"	122° 9' 7"
S&L Cabin, Crater Lake, OR	2550-40470	mochSOR026	-		42° 42' 3"	122° 8' 7"
Colorado (CO)						
Virginia Dale, CO	2530-19253	mochCO001	K		40° 43' 60"	105° 24' 19"
Virginia Dale, CO	2530-19254	mochCO002	I		40° 43' 60"	105° 24' 19"
Virginia Dale, CO	2530-19255	mochCO003	I		40° 43' 60"	105° 24' 19"
20 km W of Solder, CO	2530-19256	mochCO004	I		39° 46' 58"	105° 21' 37"
20 km W of Solder, CO	2530-19257	mochCO005	CO005		39° 46' 58"	105° 21' 37"
20 km W of Solder, CO		mochCO006	CO006		39° 46' 58"	105° 21' 37"
20 km W of Solder, CO	2530-19258	mochCO007	I		39° 46' 58"	105° 21' 37"
Boulder, CO	2530-19259	mochCO008	CO008		39° 59' 45"	105° 16' 11"
Arapahoe CG, CO		mochCO009	L		39° 50' 32"	105° 16' 11"
10 km NE of BlackHawk, CO		mochCO010	I		39° 49' 0"	105° 23' 31"
10 km NE of BlackHawk, CO	2530-19261	mochCO011	-		39° 49' 0"	105° 23' 31"
Cottonwood, CO	2530-19262	mochCO012	I		39° 45' 8"	105° 23' 21"
Cottonwood, CO	2530-19263	mochCO013	I		39° 45' 8"	105° 23' 21"
Cottonwood, CO	2530-19264	mochCO014	K		39° 45' 8"	105° 23' 21"
Cottonwood, CO	2530-19265	mochCO015	I		39° 45' 8"	105° 23' 21"
Cottonwood, CO		mochCO016	I		39° 45' 8"	105° 23' 21"
Central City (Graveyard), CO	2530-19266	mochCO017	-		39° 48' 41"	105° 31' 50"
Cottonwood, CO		mochCO018	-		39° 45' 8"	105° 23' 21"
Central City (Graveyard), CO	2530-19267	mochCO019	-		39° 48' 41"	105° 31' 50"
Central City (Graveyard), CO	2530-19268	mochCO020	-		39° 48' 41"	105° 31' 50"
Central City (Columbine CG), CO	2530-19270	mochCO021	-		39° 48' 51"	105° 32' 57"
Pickle Gulch, CO	2530-19273	mochCO022	-		39° 50' 32"	105° 31' 20"
Pickle Gulch, CO		mochCO023	-		39° 50' 32"	105° 31' 20"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Pickle Gulch, CO	2530-19271	mochCO024	-		39° 50' 32"	105° 31' 20"
N of Cottonwood, CO	2530-19274	mochCO025	-		39° 46' 58"	105° 23' 31"
N of Cottonwood, CO	2530-19275	mochCO026	-		39° 46' 58"	105° 23' 31"
N of Cottonwood, CO	2530-19276	mochCO027	-		39° 46' 29"	105° 22' 32"
N of Cottonwood, CO		mochCO028	-		39° 46' 29"	105° 22' 32"
N of Cottonwood, CO	2530-19277	mochCO029	-		39° 46' 29"	105° 22' 32"
N of Cottonwood, CO	2530-19278	mochCO030	-		39° 46' 29"	105° 22' 32"
N of Cottonwood, CO	2530-19279	mochCO031	-		39° 46' 29"	105° 22' 32"
N of Cottonwood, CO	2530-19280	mochCO032	-		39° 46' 29"	105° 22' 32"
N of Cottonwood, CO	2530-19281	mochCO033	-		39° 46' 29"	105° 22' 32"
N of Cottonwood, CO	2530-19282	mochCO034	-		39° 46' 20"	105° 22' 47"
N of Cottonwood, CO	2530-19284	mochCO035	-		39° 46' 20"	105° 22' 47"
N of Cottonwood, CO	2530-19285	mochCO036	-		39° 46' 20"	105° 22' 47"
N of Cottonwood, CO		mochCO037	-		39° 46' 20"	105° 22' 47"
Pickle Gulch CG, CO		mochCO038	-		39° 50' 33"	105° 31' 25"
Utah (UT)						
Cache Forest (Service Rd), UT	2530-19286	mochUT001	I		41° 24' 24"	111° 32' 34"
Cache Forest (Service Rd), UT	2530-19287	mochUT002	H		41° 24' 24"	111° 32' 34"
Cache Forest (Logging Rd.), UT	2530-19288	mochUT003	I		41° 24' 25"	111° 29' 29"
Rd to Mt. McKinnon, UT	2530-19289	mochUT004	UT004		41° 27' 16"	111° 30' 15"
Mill Creek Rd, UT	2540-23151	mochUT005	I		40° 54' 59"	110° 46' 23"
Rd to Mt. McKinnon, UT	2530-19290	mochUT006	UT006		41° 27' 16"	111° 30' 15"
Rd to Mt. McKinnon, UT	2530-19291	mochUT007	I		41° 27' 16"	111° 30' 15"
W of Woodruff (Forestry Rd), UT	2530-19292	mochUT008	L		41° 31' 5"	111° 28' 12"
W of Woodruff (Forestry Rd), UT	2530-19293	mochUT009	UT009		41° 31' 45"	111° 28' 12"
W of Woodruff (Forestry Rd), UT	2530-19294	mochUT010	I		41° 32' 49"	111° 26' 34"
W of Woodruff (Forestry Rd), UT	2530-19295	mochUT011	UT011		41° 31' 8"	111° 28' 16"
W of Woodruff (Forestry Rd), UT	2530-19296	mochUT012	C		41° 31' 38"	111° 27' 29"
W of Woodruff (Forestry Rd), UT	2530-19297	mochUT013	I		41° 31' 38"	111° 27' 29"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
W of Woodruff (Forestry Rd), UT	2530-19298	mochUT014	I		41° 31' 38"	111° 27' 29"
W of Woodruff (Forestry Rd), UT	2530-19299	mochUT015	UT015		41° 31' 56"	111° 26' 52"
Lost Creek CG, UT	2530-19300	mochUT016	UT016		40° 40' 46"	110° 56' 6"
Lost Creek CG, UT	2540-23150	mochUT017	-		40° 40' 46"	110° 56' 6"
Wasatche-Cache, FR 73, UT	2540-23152	mochUT018	-		40° 56' 2"	110° 23' 58"
Wasatche-Cache, FR 73, UT	2540-23153	mochUT019	-		40° 56' 2"	110° 23' 58"
Bridger Lake CG, UT	2540-23154	mochUT020	UT020		40° 57' 59"	110° 28' 15"
Idaho (ID)						
1358 4 Mile Rd, Moscow, ID		mochID01	-		46° 50' 23"	116° 57' 52"
1358 4 Mile Rd, Moscow, ID		mochID02	-		46° 50' 23"	116° 57' 52"
1358 4 Mile Rd, Moscow, ID		mochID03	-		46° 50' 23"	116° 57' 52"
1358 4 Mile Rd, Moscow, ID		mochID04	ID004		46° 50' 23"	116° 57' 52"
1358 4 Mile Rd, Moscow, ID		mochID05	-		46° 50' 23"	116° 57' 52"
1358 4 Mile Rd, Moscow, ID		mochID06	ID006		46° 50' 23"	116° 57' 52"
1358 4 Mile Rd, Moscow, ID		mochID07	C		46° 50' 23"	116° 57' 52"
1358 4 Mile Rd, Moscow, ID		mochID08	-		46° 50' 23"	116° 57' 52"
1358 4 Mile Rd, Moscow, ID		mochID09	N		46° 50' 23"	116° 57' 52"
6341 1300 Rd, Coeur d'Alene, ID		mochID10	B		47° 37' 15"	116° 47' 55"
Arizona (AZ)						
XYRanch RD 1		mochAZ01	-		35° 9' 14"	111° 39' 8"
XYRanch RD 2		mochAZ02	-		35° 9' 26"	111° 38' 58"
XYRanch RD 2		mochAZ03	-		35° 9' 26"	111° 38' 58"
Lake Mary Rd 1		mochAZ04	AZ004		35° 8' 53"	111° 39' 0"
Lake Mary Rd 1		mochAZ05	AZ005		35° 8' 53"	111° 39' 0"
South Central California (SCCA)						
Sequoia National Forest, CA	Tissue #B21411 Voucher #636851	mochSCCA001	SCCA001	SMITH	35° 53' 49"	118° 20' 15"
Sequoia National Forest, CA	Tissue #B21433 Voucher #636873	mochSCCA002	SCCA02	SMITH	35° 51' 32"	118° 19' 46"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Sequoia National Forest, CA	Tissue #B21435 Voucher #636875	mochSCCA003	T	SMITH	35° 51' 32"	118° 19' 46"
Sequoia National Forest, CA	Tissue #B21516 Voucher #636891	mochSCCA004	T	SMITH	35° 53' 49"	118° 20' 15"
Sequoia National Forest, CA	Tissue #B21517 Voucher #636892	mochSCCA005	T	SMITH	35° 53' 49"	118° 20' 15"
Quail Flat, Pinehurst, Tulare, CA	UWBM #66155 CSW 6224	mochSCCA006	U	UWBM	36° 43' 14"	118° 8' 52"
Quail Flat, Pinehurst, Tulare, CA	UWBM #66154 CSW 6223	mochSCCA007	T	UWBM	36° 43' 14"	118° 8' 52"
Sierra National Forest, Fresno, CA	UWBM #80690 SEZ 032	mochSCCA008	-	UWBM	36° 59' 44"	119° 1' 1"
Sierra National Forest, Fresno, CA	UWBM #80682 EVL 826	mochSCCA009	T	UWBM	36° 59' 44"	119° 1' 1"
Stanislaus Nat Forest, CA	239537	mochSCCA010		UMICH	38° 0' 55"	120° 14' 59"
Central California (CCA)						
North Yolla Bolly Mt, CA	MVZ #175794	mochCCA001	CCA001	MVZ	40° 18' 44"	123° 5' 49"
North Yolla Bolly Mt, CA	MVZ #175795	mochCCA002	CCA002	MVZ	40° 18' 44"	123° 5' 49"
North Yolla Bolly Mt, CA	MVZ #175796	mochCCA003	CCA003	MVZ	40° 18' 44"	123° 5' 49"
North Yolla Bolly Mt, CA	MVZ #175797	mochCCA004	T	MVZ	40° 18' 44"	123° 5' 49"
North Yolla Bolly Mt, CA	MVZ #175798	mochCCA005	T	MVZ	40° 18' 44"	123° 5' 49"
North Yolla Bolly Mt, CA	MVZ #175799	mochCCA005	T	MVZ	40° 18' 44"	123° 5' 49"
North Yolla Bolly Mt, CA	MVZ #175802	mochCCA007		MVZ	40° 18' 44"	123° 5' 49"
North Yolla Bolly Mt, CA	MVZ #167871	mochCCA008	CCA008	MVZ	40° 18' 44"	123° 5' 49"
North Yolla Bolly Mt, CA	MVZ #167872	mochCCA009	CCA009	MVZ	40° 18' 27"	123° 5' 12"
North Yolla Bolly Mt, CA	MVZ #167873	mochCCA010	CCA010	MVZ	40° 18' 27"	123° 5' 12"
North Yolla Bolly Mt, CA	MVZ #167874	mochCCA011	CCA011	MVZ	40° 18' 27"	123° 5' 12"
North Yolla Bolly Mt, CA	MVZ #167875	mochCCA012	T	MVZ	40° 18' 27"	123° 5' 12"
Mendocino National Forest, CA	UWBM #80687 MNG 172	mochCCA0013	T	UWBM	36° 36' 43"	122° 56' 41"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Southern California (SCA)						
Hwy 38, San Bernardino, CA	Tissue #B00840 Voucher #100421	mochSCA001	V	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00841 Voucher #100422	mochSCA002	SCA002	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00842 Voucher #100423	mochSCA003	SCA003	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00843 Voucher #100418	mochSCA004	W	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00844 Voucher #100419	mochSCA005	SCA005	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00845 Voucher #100420	mochSCA006	W	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00846 Voucher #100417	mochSCA007	W	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00847 Voucher #100424	mochSCA008	W	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00848 Voucher #100425	mochSCA009	W	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00849 Voucher #100426	mochSCA010	V	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00850 Voucher #100416	mochSCA011	V	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00851 Voucher #100428	mochSCA012	W	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00852 Voucher #100429	mochSCA013	W	SMITH	34° 9' 36"	116° 47' 59"
Hwy 38, San Bernardino, CA	Tissue #B00853 Voucher #100430	mochSCA014	W	SMITH	34° 9' 36"	116° 47' 59"

Location	Band Number*	ID	Hap**	Museum	Latitude (°N)	Longitude (°W)
Hwy 38, San Bernardino, CA	Tissue #B00854 Voucher #100427	mochSCA015	SCA015	SMITH	34° 9' 36"	116° 47' 59"

* Band number or specimen number/other identification number (where museum is listed)

** - not sequenced; microsattelite analysis only

Appendix 4.2. Microsatellite allelic diversity of mountain chickadee including number of alleles (*A*), allelic richness (*AR*), number of private alleles (*PA*), and heterozygosity (observed – H_O, expected – H_E) of mountain chickadee. None of the tests for linkage disequilibrium were significant (all p >0.77). Populations with n < 8 were excluded from population analyses (only included in STRUCTURE analysis) and H_O, H_E, and *AR*.

Population	**Escu4**[1]	**Escu6**[1]	**Pdo5**[2]	**Ppi2**[3]	**Titgata 02**[4]	**Titgata 39**[4]	**Pat14**[5]
Rockies Group							
NWBC (n = 2)							
A	3	4	2	3	4	3	3
AR	--	--	--	--	--	--	--
PA	0	0	0	0	0	0	0
H_O	1.000	1.000	0.000	1.000	1.000	0.500	1.000
H_E	0.625	0.750	0.500	0.625	0.750	0.625	0.625
CBC (n = 9)							
A	5	7	9	11	7	7	10
AR	3.32	4.90	5.01	3.73	3.43	4.11	3.88
PA	0	0	0	0	0	0	0
H_O	0.889	0.750	0.875	1.000	0.556	0.667	0.889
H_E	0.741	0.789	0.859	0.840	0.778	0.809	0.852
BCR (n = 4)							
A	1	3	3	4	3	3	3
AR	--	--	--	--	--	--	--
PA	0	0	1	0	0	0	0
H_O	0.000	1.000	0.333	1.000	1.000	1.000	1.000
H_E	0.000	0.625	0.611	0.719	0.625	0.594	0.625
SAB (n = 23)							
A	7	10	9	13	9	8	14
AR	3.73	4.41	4.69	3.93	3.70	4.33	4.76
PA	0	0	2	1	0	0	0
H_O	0.810	1.000	0.667	0.700	0.857	0.857	0.913
H_E	0.773	0.849	0.865	0.759	0.772	0.846	0.879
WMT (n = 23)							
A	7	14	10	14	9	7	15
AR	3.72	4.41	4.70	4.48	4.28	4.29	5.02
PA	0	0	0	0	0	0	0
H_O	0.750	0.688	0.417	0.870	0.870	0.870	1.000
H_E	0.808	0.889	0.872	0.894	0.801	0.824	0.907
EMT (n = 8)							
A	6	7	2	7	10	7	10
AR	4.14	5.46	2.00	5.07	4.14	4.55	5.04
PA	1	0	0	0	0	1	0
H_O	0.750	0.857	0.000	0.571	0.875	0.875	1.000

H_E	0.727	0.786	0.500	0.704	0.875	0.773	0.859
CO (n = 38)							
A	6	15	15	20	9	9	17
AR	3.83	4.96	4.70	4.57	3.66	4.21	4.88
PA	0	2	0	2	1	0	0
H_O	0.750	1.000	0.781	0.722	0.757	0.771	0.944
H_E	0.794	0.903	0.872	0.869	0.783	0.840	0.900
UT (n = 20)							
A	7	11	10	15	5	8	12
AR	3.77	4.43	4.66	4.55	3.41	4.17	4.95
PA	0	0	0	0	0	0	1
H_O	0.737	0.941	0.643	0.842	0.667	0.947	0.895
H_E	0.798	0.849	0.829	0.849	0.744	0.845	0.874
AZ (n = 5)							
A	4	5	3	5	4	6	3
AR	--	--	--	--	--	--	--
PA	0	0	0	0	0	0	0
H_O	0.500	0.500	1.000	0.800	0.600	1.000	0.400
H_E	0.563	0.750	0.594	0.740	0.660	0.800	0.340
ID (n = 10)							
A	4	8	8	6	6	10	10
AR	2.12	2.93	4.46	3.97	3.86	3.49	4.17
PA	0	0	0	0	0	0	1
H_O	0.833	0.700	0.833	0.571	0.800	0.900	0.700
H_E	0.653	0.795	0.847	0.745	0.805	0.875	0.850
NEOR (n = 25)							
A	6	13	16	16	7	9	13
AR	3.11	4.46	4.87	4.68	3.57	4.37	4.92
PA	0	0	1	2	1	0	0
H_O	0.625	0.900	0.905	0.792	0.600	0.720	0.957
H_E	0.701	0.861	0.875	0.869	0.718	0.842	0.899
CeOR (n = 18)							
A	6	10	11	17	7	8	11
AR	3.48	3.65	4.37	5.10	3.96	4.22	4.70
PA	0	0	0	0	0	0	0
H_O	0.750	0.857	0.667	0.938	0.857	0.941	0.824
H_E	0.762	0.814	0.826	0.916	0.791	0.817	0.865
WA (n = 20)							
A	6	9	11	12	7	9	11
AR	3.05	4.31	3.91	4.47	3.82	4.31	4.55
PA	1	0	1	0	0	0	0
H_O	0.533	0.714	0.824	0.895	0.643	0.850	0.889
H_E	0.631	0.847	0.798	0.871	0.796	0.854	0.870

SOR/CA Group							
SOR (n = 25)							
A	6	7	12	14	6	8	13
AR	3.48	3.87	4.08	4.64	3.76	4.05	4.33
PA	1	0	0	0	0	0	0
H_O	0.700	0.563	0.875	0.583	0.778	0.920	0.880
H_E	0.775	0.813	0.797	0.853	0.776	0.815	0.855
CCA (n = 12)							
A	3	8	11	12	8	10	7
AR	3.17	3.69	4.55	4.68	3.82	4.36	2.77
PA	0	1	1	1	0	0	0
H_O	0.455	0.900	0.900	0.833	0.800	0.917	0.778
H_E	0.483	0.800	0.860	0.875	0.835	0.854	0.796
SCCA (n = 10)							
A	5	7	8	10	7	7	9
AR	4.00	4.12	4.52	4.67	4.08	4.84	5.00
PA	1	0	0	0	1	0	0
H_O	0.571	0.900	0.818	0.545	0.800	0.857	0.818
H_E	0.735	0.770	0.835	0.876	0.790	0.796	0.855
SCA[6] (n = 15)							
A	6	6	8	10	5	7	12
AR	3.83	4.35	3.05	3.79	3.44	4.03	4.77
PA	0	0	0	0	0	0	1
H_O	0.583	0.714	0.308	0.800	0.714	0.667	0.786
H_E	0.740	0.765	0.778	0.824	0.758	0.818	0.898
Total A	11	20	25	31	14	11	24
Avg A	5.18	8.47	8.71	11.12	6.65	7.41	10.18
Overall AR	3.61	4.59	4.79	4.72	3.81	4.27	4.86
Avg H_O	0.656	0.822	0.677	0.802	0.769	0.837	0.854
Avg H_E	0.622	0.804	0.787	0.819	0.763	0.805	0.806

1 Hannote 1994
2 Martinez *et al.* 1999
3 Wang *et al.* 2005
4 Otter *et al.* 1998
5 Griffith *et al.* 1999
6 Population not in HWE ($p < 0.001$) for Pdo5 and Titgata39

Appendix 4.3. Bioclimatic variables used for ecological niche modeling of mountain chickadee chickadee habitat. Variables obtained from WorldClim dataset (Hijmans *et al.* 2005).

Bioclimatic Variable	Description	Removed*
BIO1	Annual mean temperature	
BIO2	Mean diurnal temperature range	
BIO3	Isothermality (mean diurnal range/temperature annual range	
BIO4	Temperature seasonality	
BIO5	Maximum temperature of warmest month	
BIO6	Minimum temperature of coldest month	
BIO7	Temperature annual range	
BIO8	Mean temperature of wettest quarter	
BIO9	Mean temperature of driest quarter	
BIO10	Mean temperature of warmest quarter	X
BIO11	Mean temperature of coldest quarter	
BIO12	Annual precipitation	
BIO13	Precipitation of wettest month	X
BIO14	Precipitation of driest month	X
BIO15	Precipitation seasonality	
BIO16	Precipitation of wettest quarter	X
BIO17	Precipitation of driest quarter	X
BIO18	Precipitation of warmest quarter	
BIO19	Precipitation of coldest quarter	

* removed from analysis due to correlation with one or more other variables

Appendix 4.4. Geographic distribution of shared haplotypes (A - W) in mountain chickadee populations. Refer to Table 4.3 for diversity measures. AZ and BCR populations had no shared haplotypes (each with 2 unique haplotypes) and are not included.

H	NWBC	CBC	SAB	WMT	EMT	UT	CO	ID	NEOR	CeOR	WA	SOR	CCA	SCCA	SCA
A	1		1												
B		4	7	6	1			1	3	7	8				
C		1	1			1		1							
D			2												
E			1		1										
F			1								2				
G				1	1										
H				1		1									
I					1	7	9				1				
J					2										
K							2								
L						1	1								
M											3				
N								1	1						
O									2						
P									2						
Q									3	5					
R									2						
S										2					
T												7	5	5	
U												5		1	
V															3
W															8

Appendix 4.5. Variable sites for mountain chickadee mtDNA control region haplotypes (hap). Table shows the 68 variable sites for a 765 bp sequence. Reverse compliment of sequence begins at site 51 in Kvist *et al*. (2001) black-capped chickadee sequence; Gen Bank accession no. AF354496

	Variable Site																																																																			
							1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7
	2	3	3	4	7	9	5	5	8	2	2	3	3	3	4	4	9	0	2	4	8	8	9	0	0	1	2	3	4	4	4	4	5	9	0	2	2	3	3	4	4	4	5	6	6	7	7	8	9	9	0	2	3	4	4	6	6	7	8	8	9	0	1	1	2	3	5	6
	4	5	9	2	1	3	6	8	1	1	6	1	3	6	3	6	4	5	5	1	7	8	1	3	4	9	2	9	5	6	7	9	3	2	5	0	4	0	3	4	5	8	6	0	6	4	5	2	4	7	4	2	6	1	5	6	7	8	2	9	0	2	2	8	7	5	4	1
Hap	G	T	C	T	G	G	G	G	G	G	G	A	G	G	T	A	T	T	T	G	T	T	T	C	T	T	T	G	A	G	T	T	G	G	A	A	G	G	A	A	A	T	T	C	C	T	T	T	C	T	G	G	G	A	T	G	A	G	G	G	A	G	A	T	T	T	A	G
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SCA015	.	.	-	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	C	.	G	.	.	.	.	A	.	G	.	.	.	.	.	A	.	T	T	.	C	C	.	.	A	.	.	.	C	.	T	.	.	.	G	A	.	.	.	.	.	.

Chapter 5: General Discussion

5.1. Phylogeographic Patterns

The overall phylogeographic pattern observed in both black-capped and mountain chickadees based on mtDNA control region sequences is one of recent expansion with subsequent genetic differentiation, and limited geographic structure. Excluding NL, the black-capped chickadee shows weak genetic structure east of the Rocky Mountains (Great Plains, southeast/eastern U.S., central and eastern Canada). These results are consistent with a general pattern of no mtDNA differentiation within the southeast and midwestern U.S. that has been observed for several species including prairie grouse (*Tympanuchus* spp; Ellsworth *et al.* 1994), common grackle (*Quiscalus quiscala*; Zink *et al.* 1991), hairy woodpecker (Graham and Burg 2012), and downy woodpecker (*Dendrocopos pubescens*; Zink *et al.* 1991, Pulgarín-R and Burg 2012; see Zink 1996 for review). This type of pattern suggests recent common ancestors in these areas with high levels of gene flow and/or insufficient time for lineage sorting.

Within western North America (i.e., Rocky Mountains west to the Pacific Coast), both chickadee species show evidence of more recent diversification and phylogeographic structure with a general east-west split between the Rockies and the Cascade/Sierra/Coast Ranges, consistent with general patterns identified within the area (Brunsfeld *et al.* 2001; Shafer *et al.* 2010). Microsatellite analysis of mountain chickadee was generally consistent with mtDNA, revealing a Rockies and an Oregon/California group, with the addition of an isolated Idaho group not seen in mtDNA. Additionally, there was evidence of male-biased dispersal between the Oregon/California group and western Rockies group within the eastern Washington area (i.e., no substructure identified with microsatellite, but present in mtDNA), which is consistent with similar patterns of dispersal observed in Townsend's and hermit warblers (Rohwer and Martin 2007).

5.2 Refugia

Both species show evidence of multiple glacial refugia (Figures 5.1 and 5.2; Table 5.1): an Atlantic coast (NL) refugium for black-capped chickadee; a southern refugium (central Rockies for mountain chickadee, and south central/southeast U.S. for black-capped chickadee); and a western refugium(a) (a southern California and central California refugium for mountain chickadee, and a AK and/or Pacific Northwest refugium for black-capped). In black-capped chickadee, I found evidence of allopatric divergence between the Newfoundland (possibly including the now submerged Grand Banks/Flemish Cap and/or submerged Atlantic Coastal Shelf) and all continental populations. The NL black-capped chickadee population is a separate subspecies (*P. a. bartletti)*, that has been isolated for ~75 kya, presently by the Strait of Belle Isle and Cabot Strait. Similar studies have identified an Atlantic refugium, separated from an interior continental population for multiple species (e.g., rock-ptarmigan (Holder *et al.* 1999), American redstart (Colbeck *et al.* 2008), yellow warbler (Boulet and Gibbs 2006), and boreal chickadee (Lait 2011)).

The possible Pacific Northwest refugia for black-capped chickadee, based on high genetic diversity AK and SOR populations and paleoecological modeling, is supported by previous work that suggested conditions along the Pacific Northwest coast were relatively wet during the last glacial maximum, creating a large refugium for mesic temperate forests south of glaciation (Brunsfeld *et al.* 2001) that include tree species known to be utilized by black-capped chickadee, such as western hemlock (*Tsuga heterophylla*) and red-cedar (*Thuja plicata*; Sullivan 1995). Several studies have identified glacial refugia in the southern Siskiyou-Klamath Mountains (Smith and Sawyer 1988; Steele and Storfer 2006) and northern Olympic Peninsula (Soltis *et al.* 1997; Demboski *et al.* 1999; Steele and Storfer 2006), as well as within the AK/Beringia area (Fedorov and Stenseth 2002; Galbreath and Cook 2004; Brubaker *et al.* 2005; Waltari and Cook 2005; Anderson *et al.* 2006; Burg *et al.* 2006; Weksler *et al.* 2010).

5.3 Chickadee Patterns in the West: Barriers and Gene Flow

The possible Pacific Northwest black-capped chickadee refugium and different east/west splits within the Pacific Northwest/Cascade Range region between black-capped and mountain chickadees suggests different phylogeographic histories and leads to the question: why do these species' patterns differ, or what possible mechanism is different between the two species? In a comparative study of codistributed North American species, Zink (1996) suggested that the lack of phylogeographic congruence can be attributed to idiosyncratic connections. Potential underlying mechanisms that may differentially affect one species of chickadee in this study (versus the other) might include alternate dispersal mechanism (pioneer versus phalanx), different life history traits (e.g., natal philopatry, winter irruptions, habitat requirements), location of source refugium(a), and/or nest site availability (Hill and Lein 1989).

By comparing codistributed species, we can identify whether they exhibit congruent phylogeographic patterns, indicative of similar phylogeographic histories (e.g., vicariance event, genetic/dispersal barriers, timing of colonization). Alternatively, incongruent or idiosyncratic phylogeographic patterns are indicative of different histories (Lamb *et al.* 1992). Black-capped and mountain chickadees' ranges overlap within the western portion of North America (Figure 1.1), and so provide the opportunity to compare phylogeographic histories between the two species, and possibly identify alternate barriers/mechanisms to gene flow.

Western North America consists of essentially two north-south mountain ranges (Rocky Mountains to the east and the Coast Ranges, including Cascades and Sierra Nevada, to the west). The orogeny of the Cascade/Sierra Mountains has been used to explain detected impacts to current species distribution, and vicariance events (Graham 1999; Brunsfeld *et al.* 2001). The formation has been estimated between 2-5 Mya, which roughly correlates with speciation of black-capped and mountain chickadee (~2.5 Mya from Gill *et al.* 1993; Gill *et al.* 2005), but estimated divergence times in this study, between 81-433 kya for black-capped and 180-710 kya for mountain chickadee populations (based on pairwise distances; Tables 3.4 and 4.4), and time since last population expansions (maximum 42 and 46 kya for black-capped and mountain chickadee SOR populations, respectively) suggests more recent events have influenced both species. Previous studies have also identified non-orogeny events that have influenced allopatric diversification within this area (e.g., historic glacial advances; Johnson and Cicero 2004; Weir and Schluter 2004).

Both black-capped and mountain chickadees show differentiation (Φ_{ST}) between SOR and NEOR (CeOR as well for mountain chickadee) populations, suggesting that the mountains may be a barrier to gene flow for both species. However, both species show evidence of gene flow across mountain ranges. For example, black-capped chickadee shows a lack of differentiation (Φ_{ST}, BAPS, TCS) between both NEOR and ID, and SAB and LETH, as well as between CBC and CAB. Similarly, mountain chickadee shows a lack of differentiation between ID and WMT/EMT (Φ_{ST}, BAPS, TCS), and between both NEOR and CEOR and multiple populations (e.g., SAB, WMT, EMT; BAPS, TCS). All of which suggests that while mountain ranges are a barrier to gene flow, possibly limiting overall dispersal, some other mechanism or barrier is also involved and/or mountains are porous through valleys allowing for gene flow.

One prominent difference between black-capped and mountain chickadees based on mtDNA analysis is the inclusion of the western, Cascade Range population (WA) of mountain chickadee within the Rocky Mountain clade. In the Pacific Northwest, an east-west split between Cascade/Coast Ranges and northern Rocky Mountain phylogroups has been identified for numerous plant and animal species (see Shafer *et al.* (2010) and Swenson and Howard (2005) for reviews). Black-capped chickadee results are consistent with this east-west split (Table 5.2) and show a separate Pacific group (located west of the Coast/Cascade Ranges) that is differentiated from northern Rockies populations (and all populations east). My results for mountain chickadee however, do not clearly separate the east from the west in WA, due to the WA population being included in the eastern Rockies group in mtDNA, but microsatellite show mixed results (e.g., BAPS assigns WA to Rockies group, STRUCTURE assigns WA to SOR/SCA group).

The Cascade Range formation began around 5 Mya, and by the end of the Tertiary (~2.5 Mya) coniferous forests already dominated substantial portions of the Cascade/Sierra and Rocky Mountains (Brunsfeld *et al.* 2001). Black-capped chickadee is a generalist species found in mixed deciduous (Foote *et al.* 2010) and coniferous forests, while mountain chickadee is a specialist found in high elevation coniferous forests (McCallum *et al.* 1999). Therefore, in the case of incongruence between mountain ranges (Cascade and Rocky) as barriers, the ability of mountain chickadee to occupy higher elevation, and lack thereof in black-capped chickadee suggests that mountains should be more of a barrier to black-capped chickadee, than to mountain chickadee. Consequently, due to the mountain chickadee's higher-elevation distribution, we would expect low elevation areas that do not support suitable habitat to provide a more substantial barrier to mountain chickadee, as compared to the generalist, lower elevation black-capped chickadee.

The Cascade Range appears to be a significant barrier for black-capped chickadees. In the case of the Rocky Mountains, results suggest that either the mountains are porous (i.e., valleys provide possible dispersal routes) or dispersal in both species has occurred from a southern refuge, northward on both sides of the mountains. Support for dispersal on both sides of the Rocky Mountains is evident in both species due to CO/UT haplotypes present in NEOR. Both species also show evidence of gene flow across the Rocky Mountains due to lack of differentiation in northern populations (e.g., SAB and ID for black-capped chickadee, and WMT and ID for mountain chickadee).

However, when we compare the location of population differentiation between species, one interesting pattern emerges; mountain chickadee show phylogeographic breaks (mtDNA) across low elevation basins/plateaus that lack suitable habitat (distribution of suitable tree species based on Critchfield and Little 1966; Little 1971, 1976; basin/subbasin delineations and habitat types obtained from Natural Resources

Conservation Service (NRCS) 2012a,b). For example, CO and UT are differentiated (Φ_{ST}) and separated by the Uinta and Piceance Creek Basins, and SCA is differentiated (Φ_{ST}) and separated from SCCA by the Mojave Desert, lower elevation portions of the Transverse Ranges (Sierra Pelona Mountains, Tehachapi Mountains) and the Central Valley of California. Alternatively, in black-capped chickadee CO and UT are not differentiated, and although SOR and WA are differentiated (Φ_{ST}) and physically separated by the Willamette Subbasin, they share haplotypes (mountain chickadee do not share any haplotypes between SOR and WA) and are not differentiated by TCS or BAPS. NEOR/CEOR populations of mountain chickadee are differentiated (Φ_{ST} and F_{ST}) from WA populations and separated by the low elevation Deschutes Subbasin. Overall, this suggests that habitat availability and connectedness may differentially influence mountain chickadee population structure/dispersal. In particular, a central Oregon population (Deschutes National Forest) in a previous mtDNA study by Spellman *et al.* (2007) grouped with their California/Oregon haplotypes, but was located on the eastern side of the Cascade Range and separated from their eastern populations (Whitman National Forest) by the low elevation Columbia Plateau. Similarly, my CEOR mountain chickadee population (Ochoco National Forest) is separated from the SOR population (Crater Lake area) by the discontinuous habitat across the Columbia Plateau, primarily by the Deschutes Subbasin (Critchfield and Little 1966; Little 1971, 1976; NRCS 2012a), rather than the Columbia Crest of the Cascade Range, consistent with habitat availability and connectedness being important to mountain chickadee dispersal and gene flow.

The identification of a possible coastal Pacific Northwest refugium for black-capped chickadee, but not mountain chickadee also suggests a high probability that black-capped chickadee was present in the Pacific Northwest during the LGM. This leads to the possibility that either the habitat was not suitable for mountain chickadee or that suitable habitat occurred within the Pacific Northwest, but black-capped chickadee excluded mountain chickadee from preferred habitats. Both of these species often occupy different habitats, but some overlap occurs (Hill and Lein 1989) and black-capped chickadees have been shown to dominate mountain chickadees (Minock 1972; Grava *et al.* 2012). However, Hill and Lien (1988) showed that black-capped and mountain chickadees may compete for nest sites occasionally, but they differentially used habitats within the same general area. Therefore, competitive exclusion is unlikely and the most plausible reason for the discordant pattern is dispersal out of different refugia.

5.4 Possible Mechanisms of Dispersal

Dispersal is one of the most fundamental features of an organism and is involved with patterns of geographical distribution, abundance of species, dynamics and persistence of populations, and population structure (Dieckmann *et al.* 1999; Walters 2000). Within ecological and evolutionary literature, the consequences of dispersal have been extensively discussed, but there is a paucity of research on why/how particular dispersal strategies evolve (Dieckmann *et al.* 1999). One particular problem is that much of the work on dispersal was theoretical (Walters 2000). The consequences of a phalanx dispersal model in black-capped chickadee for example, may result in the observed isolation by distance and broadly dispersed haplotypes, but dispersal itself does not explain the specific mechanisms that influence movement. What possible mechanism(s) results in isolation by distance in black-capped chickadee or sex-biased dispersal in mountain chickadee?

Dispersal is generally thought to be costly (i.e., risks of leaving a familiar environment, energetic expense of searching for new habitat, and the competition in new area for space and resources; Roff 1984; Alberts and Altmann 1995; Zera 1997; Duckworth 2008). The primary reasoning was that males within resource defence systems will benefit from remaining near their natal area where they are familiar with the resources present, and consequently females would benefit from dispersal (Greenwood 1980). Conversely, in mate defence systems, males seeking females should be more willing to disperse. Greenwood (1980) confirmed a more common general pattern of female biased dispersal among birds, which was later supported by Clarke *et al.* (1997). Dobson (1982) predicted that intrasexual competition would lead to the dispersal of the sex that is most involved in competition. Dobson's argument has been questioned though because it does not account for or explain female biased dispersal observed in many monogamous birds, or variation in dispersal patterns among polygynous species (see Greenwood 1980; Clarke *et al.* 1997).

Dominance hierarchy has been shown to be similar in both black-capped and mountain chickadee with males dominant over females (McCallum *et al.* 1999; Ratcliffe *et al.* 2007; Grava *et al.* 2012), and between species back-capped is dominant over mountain chickadee (Minock 1972; Hill and Lein 1989; Grava *et al.* 2012). Reproductive strategy in both species involves monogamy with extra-pair copulations. In a study on the effects of social rank and fitness, Schubert *et al.* (2007) showed that males with higher average rank over their lifespan had higher reproductive success, and Otter *et al.* (1998)

showed that higher ranking males had higher success as a result of extra-pair copulations (i.e., females chose higher ranking males for extra-pair copulations, and higher ranking males had more offspring).

Since males of both species are in direct competition with other males in terms of dominance rank, based on Dobson's (1982) prediction, male black-capped and mountain chickadees should disperse more often than females. Studies on black-capped (Weise and Meyer 1979) as well as the closely related blue tit (Zeh *et al.* 1985) showed that females dispersed more often than males, which contradicts Dobson's prediction. However, the definition of dispersal/philopatry is often ambiguous, and while both studies determined females dispersed more, they defined dispersal differently (total number moving out of a 2.4 km area for black-capped, or moving over 1 km away for blue tit).

Avian dispersal has been shown to be influenced by several external factors including brood size (Nur 1988), hormonal changes (Dufty and Belthoff 2000), genetics (Hansson *et al.* 2003), etc. In western bluebirds, Duckworth (2008) showed that aggressive males were more dispersive than nonaggressive males, resulting in more aggressive populations at the edge of their range, but nonaggressive males performed best (i.e., fitness consequences of aggression) in an older population. All of which suggests that there may not be one specific dispersal pattern for each and every species, but rather multiple adaptive strategies. In Chapter 2, I showed that male black-capped chickadees were involved in significantly more aggressive interactions than females, and distantly related individuals were involved in significantly more interactions, but many of the individuals were low ranking. The higher aggression between distantly related males is consistent with Dobson's prediction, and may be a result of aggressive males being more dispersive, as seen in bluebirds.

Evidence of male-biased dispersal in an expanding southern Oregon/northern California mountain chickadee clade would also be consistent with higher aggression on the edge, leading to dispersal across the fragmented forests of northeast Oregon and central Washington. The discrepancy between mtDNA and microsatellite results in WA and Idaho mountain chickadee populations also suggests male-biased dispersal into newly available habitats. The maximum southern extent of Cordilleran Ice Sheets east of the Cascade Ranges was around 15-14 kya, but the glacial chronology of eastern Washington and Idaho is unknown (Whitlock 1992). Regardless of the specific chronology, patterns within this area have led researchers to formalize hypotheses relating to the origin of disjunct mesic forests found in northern Idaho (Brunsfeld *et al.* 2001; Brunsfeld and Sullivan 2005; Carstens *et al.* 2005; Brunsfeld *et al.* 2007), which include both vicariance resulting from orogeny of the Cascades as well as, by more recent dispersal via a northern or southern route (see Brunsfeld *et al.* (2001) for more detailed description of hypotheses). Within the Clearwater Range of northern Idaho, Brunelle and Whitlock (2003) showed that parkland and alpine meadow dominated the area at the LGM, with subsequent transitions between *Pinus* species beginning around 14 kya, followed by *Pseudotsuga* forest development around 9.5 kya. The cycle of forest development with recent *Pseudotsuga* forest, a component of mountain chickadee coniferous habitat, is consistent with evidence of recent dispersal into Idaho by mountain chickadees. Mountain chickadee results are consistent with complex patterns of glacial chronology, forest distribution and multiple refugia within the Pacific Northwest and Intermountain West regions.

Limited dispersal across fragmented habitats in mountain chickadee may also be explained by mountain chickadee being a habitat specialist. As mentioned above, individuals involved in resource defence systems would benefit from remaining in their natal area where they are most familiar with the resources. If mountain chickadee females choose the nest site location as female black-capped chickadees usually do, and the older, large diameter trees that mountain chickadee use for nests are limited (Hill and Lein 1988), then females should have a tendency to remain within their natal area. Black-capped chickadee is a primary nest excavator, while mountain chickadee is primarily a secondary nest excavator (Hill and Lein 1988; Martin *et al.* 2004). Hill and Lien (1988), showed that both species may compete for nest sites, and in fact nest site reuse was higher in mountain chickadee (e.g., in 1984, eight of 17 were reused by mountain chickadee and zero of eight for black-capped) suggesting that nest sites may be limiting. Black-capped chickadees therefore do not need to reuse nest sites since they are able to create new nest holes, when needed. As such, nest sites would then be a limited resource for mountain chickadee, and females would benefit from remaining in their natal territory under resource defence systems. Therefore, nest availability (as a component of habitat availability) may influence gene flow in mountain chickadee by reducing female dispersal and contribute to different dispersal strategies in male and female mountain chickadee.

5.5 Future Considerations

Future considerations include verifying whether nuclear markers support the mtDNA results of my black-capped chickadee study in Chapter 3. Based on the lack of differentiation within the eastern region, I would not expect different results from nuclear DNA analysis, but think it is highly probable that analysis of the western populations may provide additional insight. Within Alaska for example, the low haplotype diversity in the AKA and AKF suggests a possible bottleneck, and analysis of nuclear markers may identify a genetic bottleneck and/or male biased dispersal. Additionally, nuclear markers may also support a contact zone between the Pacific group and the rest of the continental populations identified in NWBC. The addition of nuclear data within the Pacific Northwest for black-capped chickadee would be particularly useful in identifying whether there is a discordant pattern between black-capped and mountain chickadee or whether incongruences are more a result of different glacial refugia.

Additional studies of mountain chickadee within the Cascade Range could also shed light on the contact zone between the western and eastern groups. The inconsistencies between my study and previous work by Spellman *et al.* (2007) especially within the Washington area would benefit from a more thorough analysis of gene flow within and around the Colorado Basin; which populations dispersed into Idaho; is there evidence of dispersal across the Okanogan Highlands of WA and/or Okanagan Highlands of BC; does gene flow occur over the Cascades or has dispersal occurred from the south and along both sides. Within southern California, no detailed genetic analysis has been conducted on any of the Transverse Mountains besides the San Bernardino Mountains leaving several areas of research: is there evidence of long term isolation in the more northern portions of the Transverse Ranges; do populations on the northern portion of the Transverse Mountains show gene flow across or around the mountains; etc.

Overall, the presence of SOR/WA refugium for black-capped chickadee and a central/northern California refugium for mountain chickadee best explains the discordant patterns between these species. Additionally, conditions along the Pacific Northwest coast were considered to be much wetter (Brunsfeld *et al.* 2001) and therefore more suitable for black-capped chickadee. Mountain chickadee dispersed northward out of a southern refugium, along both sides of the Cascades into central Washington, while at the same time populations dispersed out of the central Rockies, possibly across the Okanogan Highlands, with subsequent introgression between the clades within eastern Washington (Figure 5.1). A similar dispersal pattern of both western (along the Cascades) and eastern (along the central Rockies and in central/northeast Oregon) haplogroups has been observed in whitebark pine, a species which mountain chickadee forages upon. Black-capped chickadee on the other hand, may have dispersed out of a more northern SOR/CA and/or AK refugium (Figure 5. 2), and out of a common southern refugium within western North America. Both black-capped and mountain chickadees evidence of dispersal out of a possible Rockies refugium, as well.

Post-glacial dispersal and phylogeography of both species has been influenced by the location of glacial refugia, barriers, varying dispersal strategies, and behavioural interactions, and supports the conclusion by Zink (1996) that “codistributed species reached their current distributions at different times and possibly via different historical routes, and were subject to different historical events”. The black-capped and mountain chickadee show similar phylogeographic histories, but provide evidence that both historical process and species-specific attributes have independently shaped each species' current phylogeographic structure.

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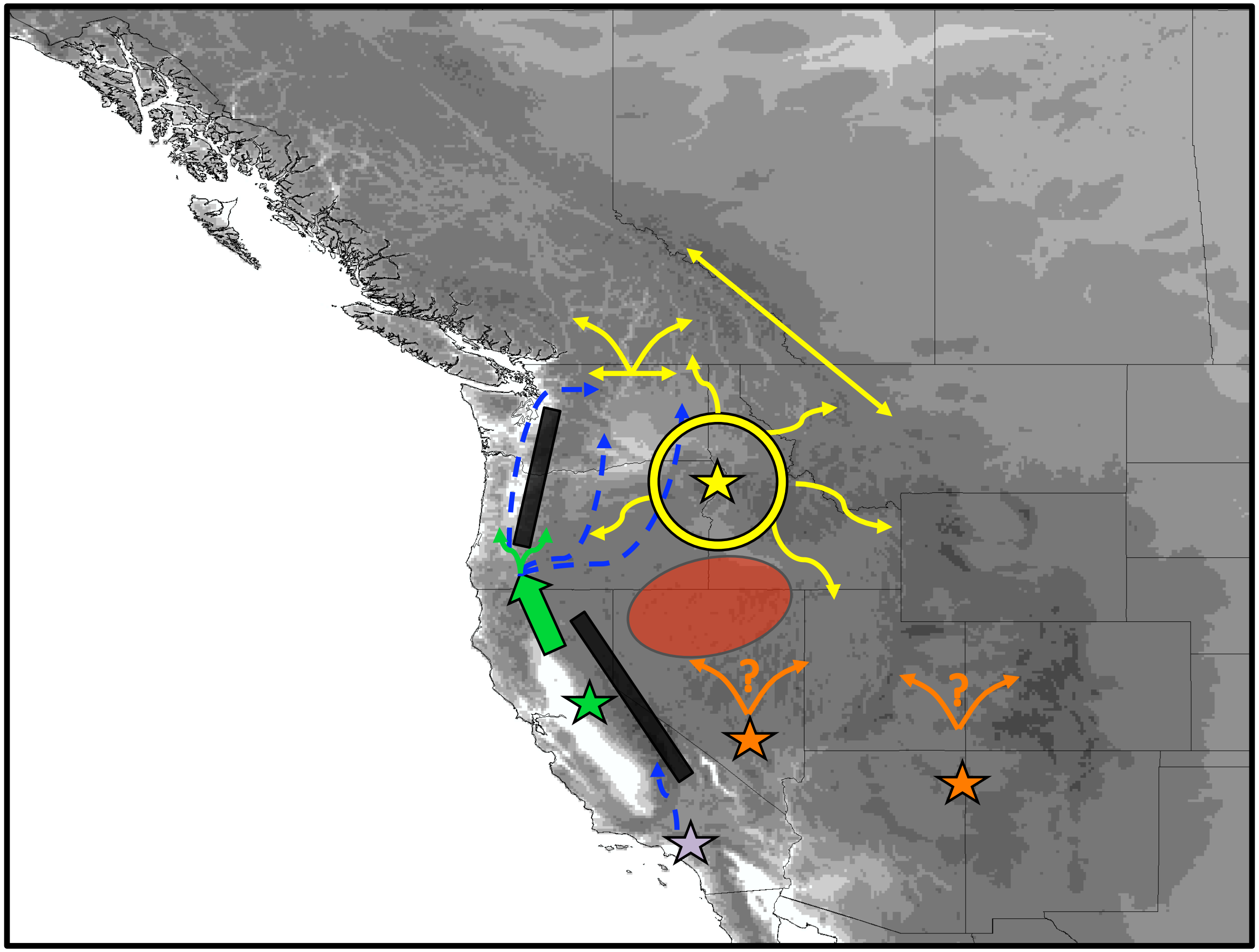
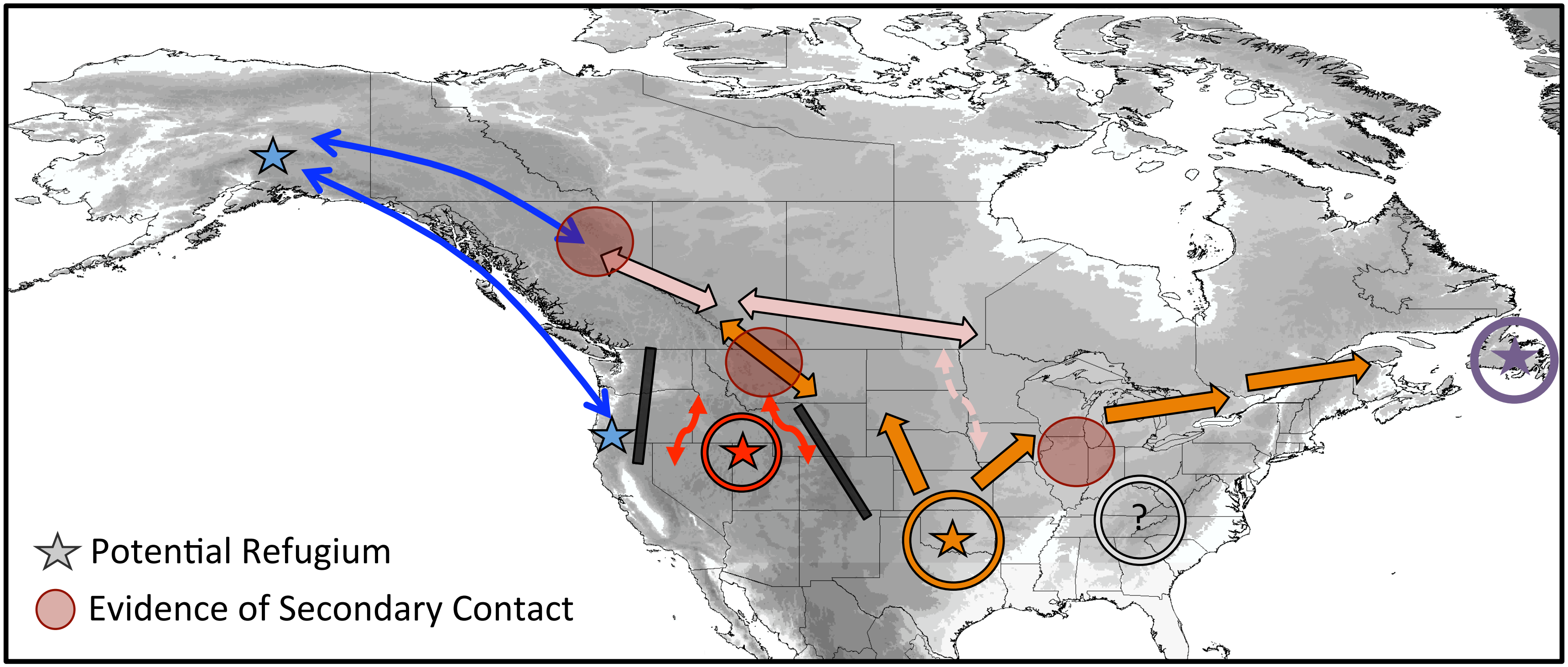


Figure 5.1 Possible mountain chickadee refugia (stars) and dispersal patterns (lined arrows; thicker lines indicate major routes; black lines indicate the Cascade and Sierra Nevada Mountain Ranges). Dashed arrows indicate possible male-biased dispersal and large circle indicates general refugium location. Transparent red oval indicates an area currently outside the mountain chickadee range, but was predicted to have suitable habitat at LGM. Orange stars in southwest from Spellman *et al.* (2007).



Potential Refugium
Evidence of Secondary Contact
?

Figure 5.2 Possible black-capped chickadee refugia (stars; blue = Pacific, red = Rockies, Orange = Central, pink = Central N) and dispersal patterns (arrows; thicker lines indicate major routes). Large circle indicates general refugium location and black bars represent physical barriers. Dashed arrow indicates possible separate dispersal into northern areas (Central N) out of southeast refugium, as compared to Central (orange) group.

Table 5.1. Possible refugia for black-capped and mountain chickadees.

Refuge	Black-capped	Mountain*
East (Atlantic)		
NL	Y	--
South of Ice Sheets		
Central Rockies**	Y	Y
South central/east U.S.	Y	--
West (Pacific)		
Southern CA	--	Y
Central CA	--	Y
Coastal Pacific Northwest	Y	N
Alaska	Y	--

* previously identified refugium(a) by Spellman *et al*. (2007) include Great Basin, Southern & Central California

** including Intermountain West Region

-- range does not include geographic area

Table 5.2. Population-specific differentiation across discontinuous habitat (within BAPS/ STRUCTURE clusters) using mitochondrial DNA (mtDNA, Φ_{ST}; both species) and microsatellites (msat, F_{ST}; mountain chickadee only)

	Black-capped	Mountain		
Populations	mtDNA	mtDNA	msat	Separating Geophysical Feature
CO & UT	N	Y	N	Green River Basin (Uinta & Piceance Subbasins)
WA & NEOR	Y*	Y	Y*	Yakima and Middle Columbia Subbasin
SOR & WA	Y**	Y**	N	Willamette Subbasin

* different BAPS/ STRUCTURE cluster(s)

** no haplotypes shared between populations in mountain chickadee, but two shared haplotypes (n = 21) in black-capped (Appendix II and V)