

**RANGEWIDE AND LOCAL BARRIERS TO GENE FLOW IN WHITE-
CROWNED SPARROWS (*ZONOTRICHIA LEUCOPHRYS*)**

CATHERINE ANNE WELKE

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CATHERINE A. WELKE

Date of Defence: November 7, 2018

Dr. Theresa Burg Thesis Supervisor	Associate Professor	Ph.D.
Dr. David Logue Thesis Examination Committee Member	Assistant Professor	Ph.D.
Dr. Cameron Goater Thesis Examination Committee Member	Professor	Ph.D.
Dr. Igor Kovalchuk Chair, Thesis Examination Committee	Professor	Ph.D.

ABSTRACT

Although white-crowned sparrows (*Z. leucophrys*) have been extensively researched for physiology and behaviour, surprisingly little recent work has been done to characterize the population genetic structure of this widespread North American passerine. This study used molecular markers and habitat modelling to test theories of Pleistocene refugia that could explain current subspecies delineations, then investigated rangewide and local barriers causing contemporary genetic differentiation across the geographical range of three subspecies: *Z. l. gambelii*, *Z. l. oriantha*, and *Z. l. pugetensis*. Phylogenetic analysis showed weak and inconclusive support for the genetic differentiation of subspecies and the theories of their Pleistocene origin. Mitochondrial, nuclear Z-linked, and microsatellite markers show a north-south genetic split caused by isolation by distance, and an east-west split caused by mountains as a barrier to gene flow between Alberta and British Columbia populations. Analysis revealed the importance of habitat ecotype and anthropogenic disturbance on contemporary population genetic structure.

Acknowledgements

There were two decisions I made for myself a few years before I began grad school: 1) Upon completing my third undergrad year, I swore I would never do genetics ever again, and 2) after one fateful day on Mt. Rainier, I met a white-tailed ptarmigan (*Lagopus leucura*) for the first time, I resolved to pursue research on that tenacious, alpine, red-browed master of camouflage. When grad school began I learned, and kept learning, that life seldom leads where you expect (hence the ‘white-crowned sparrow’ research as follows), that you can grow and accomplish things your past self would’ve never imagined, and that my goal-setting is a skill likely needs to carry on improving with age. I’m not the same person I was before grad school. The rigours of academia and research have a way of testing a person from every angle, and I am pleased to say that if anything, this Masters journey has polished to a brighter shine my love for biology, the wonder at every level of structure and function, the intricate ways that something as minute as nucleotides interact to influence a species’ continental distribution, behaviours, colours, and voices. It’s a privilege it is to share that knowledge and wonder with others.

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TABLE OF CONTENTS

Abstract	iii
Acknowledgements	iv
List of Tables	viii
List of Figures	ix
List of Abbreviations	x
CHAPTER 1: GENERAL INTRODUCTION	1
1.1 GENERAL OVERVIEW	1
1.2 BARRIERS TO GENE FLOW	2
1.3 LANDSCAPE GENETICS	4
1.4 PHYLOGEOGRAPHY	6
1.5 MOLECULAR MARKERS	8
1.5.1 Mitochondrial markers	9
1.5.2 Microsatellites	11
1.6 STATISTICAL METHODS	13
1.6.1 Methods in landscape genetics	14
1.6.2 Methods in phylogeography	16
1.7 STUDY SPECIES	17
1.7.1 <i>Z. leucophrys</i> subspecies	17
1.7.2 Phylogeographic history	20
1.8 THESIS GOALS	22
1.9 THESIS ORGANIZATION	23
1.10 REFERENCES	29
CHAPTER 2: RANGEWIDE AND LOCAL BARRIERS TO GENE FLOW IN WHITE-CROWNED SPARROWS (<i>ZONOTRICHIA LEUCOPHRYS</i>)	38
2.1 INTRODUCTION	38
2.2 MATERIALS AND METHODS	44
2.2.1 Sample collection	44
2.2.2 DNA extraction, amplification, and sequencing	45
2.2.3 Microsatellite genotyping	46
2.2.4 Genetic diversity analyses	47
2.2.5 Population genetic structure	49
2.2.6 Multivariate analyses	51
2.2.7 Barrier characterization	51
2.2.8 Species Distribution Models	52
2.2.9 Least-cost path models	54
2.2.10 Historical Species Distribution Models	55
2.3 RESULTS	55
2.3.1 Genetic diversity	55
2.3.2 Population genetic structure	56
2.3.3 Bayesian clustering analyses	59
2.3.4 Multivariate analyses	61
2.3.5 Species Distribution Models and dispersal routes	62
2.3.6 Historical Species Distribution Models	64

2.3.7	Isolation by distance or resistance	64
2.4	DISCUSSION	65
2.4.1	Evolutionary history of <i>Z. leucophrys</i> subspecies	65
2.4.2	Rangewide barriers to gene flow – distance, landscape, or subspecies?	67
2.4.2.1	Distance	67
2.4.2.2	Landscape barriers and resistance	69
2.4.2.3	Subspecies	71
2.4.3	Local barriers to gene flow	73
2.4.3.1	Ecosite type	73
2.4.3.2	Elevation	77
2.4	CONCLUSIONS	78
2.5	REFERENCES	95
CHAPTER 3: GENERAL DISCUSSION		105
3.1	INTRODUCTION	105
3.1.1	Historical processes	106
3.1.2	Rangewide genetic structure and the Rocky Mountains	107
3.1.3	Local genetic structure – microgeographic and elevational barriers	108
3.2	FUTURE DIRECTIONS	110
3.3	GENERAL CONCLUSION	115
3.4	REFERENCES	116
APPENDICES		
Appendix 1:	Information for all <i>Z. leucophrys</i> samples used	122
Appendix 2:	Microsatellite primer and PCR information	136
Appendix 3:	Pairwise F_{ST} and p-values for CR haplotypes	137
Appendix 4:	Pairwise F_{ST} and p-values for AldoB6 haplotypes	138
Appendix 5:	Pairwise F'_{ST} and p-values for microsatellite genotypes	139
Appendix 6:	Pairwise F'_{ST} and p-values for microsatellite genotypes in four ecosite types	140
Appendix 7:	AIC and AUC plots for subspecies SDM model fit	141
Appendix 8:	Least-cost paths for subspecies	142
Appendix 9:	Geographic distribution of AldoB6 haplotypes	143
Appendix 10:	Geographic distribution of CR haplotypes	144

LIST OF TABLES

Table 2.1:	<i>Z. leucophrys</i> subspecies breeding range distribution map	80
Table 2.2:	Example photos of <i>Z. leucophrys</i> subspecies for identification	81
Table 2.3:	Distribution of Pleistocene ice sheets and <i>Z. leucophrys</i> refugia	82
Table 2.4:	Theories of Pleistocene refugia and range expansion of <i>Z. leucophrys</i> subspecies	83

LIST OF FIGURES

Figure 1.1:	<i>Z. leucophrys</i> subspecies breeding range distribution map	25
Figure 1.2:	Example photos of <i>Z. leucophrys</i> subspecies for identification	26
Figure 1.3:	Distribution of Pleistocene ice sheets and <i>Z. leucophrys</i> refugia	27
Figure 1.4:	Theories of Pleistocene refugia and range expansion of <i>Z. leucophrys</i> subspecies	28
Figure 2.1:	Sample sites used in this study	84
Figure 2.2:	Phylogenetic tree of <i>Z. leucophrys</i> subspecies using CR data from this study and Weckstein et al. (2001)	85
Figure 2.3:	Haplotype networks and F_{ST} comparisons for CR data	86
Figure 2.4:	Haplotype networks and F_{ST} comparisons for AldoB6 data	87
Figure 2.5:	STRUCTURE and TESS results	88
Figure 2.6:	PCA of morphometric data and ecosite type	89
Figure 2.7:	Three-dimensional PCoA plot of microsatellite data	90
Figure 2.8:	BARRIER results plotted on sample sites map	91
Figure 2.9:	Species Distribution Models for three <i>Z. leucophrys</i> subspecies	92
Figure 2.10:	Least-cost corridors for <i>Z. l. gambelii</i> and <i>Z. l. oriantha</i>	93
Figure 2.11:	Species Distribution Model projections to Holocene, LGM, and LIG	94

LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

°C	degrees Celsius
μL	microliter
μM	micromolar
AB	Alberta
AICc	corrected Akaike's Information Criterion
AldoB6	aldolase B6 (z-chromosome DNA)
ATP	adenosine triphosphate
AUC	area under curve
BC	British Columbia
bp	base pair
BP	before present
CO	Colorado
CR	control region (mitochondrial DNA)
DEM	digital elevation model
DIC	deviance information criterion
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphates
ENM	ecological niche model
EROS	Earth Resources Observation and Science Center
ESRI	Environmental Systems Research Institute
F _{ST}	Wright's fixation index
F' _{ST}	standardized measure of genetic differentiation
GBIF	Global Biodiversity Information Facility
GIS	Global Imaging System
GWCS	Gambel's white-crowned sparrow (<i>Z. l. gambelii</i>)
Hd	haplotype diversity
He	expected heterozygosity
Ho	observed heterozygosity
HWE	Hardy-Weinberg equilibrium
IAM	infinite alleles model
IBD	isolation by distance
IBR	isolation by resistance
indel	insertion/deletion
K	number of clusters
LCC	least-cost corridor
LCP	least-cost path
LGM	last glacial maximum
LIG	last interglacial
M	molar
m	metre
McMC	Markov chain Monte Carlo
MEGA	Molecular Evolutionary Genetics Analysis
mg	milligram
MgCl ₂	magnesium chloride
mL	millilitre

mM	millimolar
mtDNA	mitochondrial DNA
MWCS	mountain white-crowned sparrow (<i>Z. l. oriantha</i>)
n	sample size
Na	number of alleles
NJ	neighbor joining
nuDNA	nuclear DNA
NY	New York
OR	Oregon
p	p-value (significance)
PA	private allele
PAUP*	Phylogenetic Analysis Using Parsimony *and other methods
PCA	principal component analysis
PCoA	principal coordinate analysis
PCR	polymerase chain reaction
PopART	Population Analysis with Relative Trees
PSWS	Puget Sound white-crowned sparrow (<i>Z. l. pugetensis</i>)
Q	ancestry coefficient
QC	Quebec
R ²	correlation coefficient
RAM	Royal Alberta Museum
RBCM	Royal British Columbia Museum
RNA	ribonucleic acid
s	second
SDM	species distribution model
SMM	stepwise mutation model
SNPs	single nucleotide polymorphism
T _{m1}	annealing temperature 1
T _{m2}	annealing temperature 2
U	units
USGS	United States Geological Survey
v	version
ΔK	delta K
Ψ	interaction parameter

Populations

BA	Banff National Park, Alberta
BV	Beaver Mines, Alberta
CNP	Crowsnest Pass, Alberta
JAS	Jasper National Park, Alberta
LE	Lethbridge, Alberta
WT	Waterton Lakes National Park, Alberta
FTSJ	Fort St. James, British Columbia
MK	Mackenzie, British Columbia
OK	Okanagan, British Columbia
RV	Revelstoke, British Columbia

CO-L	Colorado - low elevation
CO-M	Colorado - mid elevation
CO-H	Colorado - high elevation
OR-L	Oregon - low elevation
OR-H	Oregon - high elevation

Ecosite types

AC	alpine coniferous
RD	riparian deciduous
D-T	disturbed - townsite
D-G	disturbed - gas plant

Z. leucophrys subspecies

GWCS	Gambel's white-crowned sparrow (<i>Z. l. gambelii</i>)
MWCS	Mountain white-crowned sparrow (<i>Z. l. oriantha</i>)
PSWS	Puget Sound white-crowned sparrow (<i>Z. l. pugetensis</i>)

CHAPTER 1: General Introduction

1.1 General Overview

Population genetics is a branch of evolutionary biology that investigates genetic variation within populations using statistics and models to quantify the changes in allele frequencies in populations over space and time (Chen, 2015). Allele frequencies may vary due to forces of gene flow, genetic drift, selection, and mutation (Holderegger & Wagner, 2008; Slatkin, 1987). The intent of this thesis is to study the influence of these forces on genetic differentiation and allele frequencies, particularly the factors affecting gene flow. Gene flow within populations generally slows divergence and homogenizes the gene pool (DuBay & Witt, 2014; McNeilly & Antonovics, 1968). Small populations become vulnerable when genetic drift or bottleneck events cause fixation of alleles, jeopardizing their adaptive potential and survival (Slatkin, 1987). Larger populations maintain variation due to diversifying forces of mutation and local selection pressure (Cheviron & Brumfield, 2009; Slatkin, 1987; Wiens, 2004). Another important source of variation is when migration introduces new alleles to a population (Takagi, 2011). If migration is nonexistent, populations become reproductively isolated and acquire allelic differences via mutation and genetic drift. This lack of migration and the subsequent consequences of isolation and genetic divergence are precursors to speciation (Burney & Brumfield, 2009).

Much research in population genetics is devoted to elucidating the influence of geographic and ecological conditions on gene flow and the resulting population differentiation (Burney & Brumfield, 2009; Fjeldsâ et al., 2011; Gutiérrez-Pinto et al.,

2012; McNeilly & Antonovics, 1968). Depending on the species, different geographical and ecological factors will permit or inhibit gene flow caused by selection pressure and the movement of gametes, individuals, or populations (Slatkin, 1987). Whether this in turn causes isolation, local adaptation, divergence, or speciation is a question that needs to be asked for each species (Rissler, 2016). If a declining trend of gene flow and genetic diversity is discovered, researchers can identify vulnerable populations as targets for conservation (DuBay & Witt, 2014; Wiens, 2004).

1.2 Barriers to gene flow

In order for genetic differentiation to occur between two populations, reproductive isolation must occur (Via, 2001). This isolation can be caused by climatic (temperature, precipitation), geographical (bodies of water, mountain ranges), man-made (roads, deforestation) or behavioural (avoidance of crossing habitat gaps, song dialects) barriers (Fjeldsâ et al., 2011; Harris & Reed, 2002). For much of the early 1900's, allopatric speciation, where populations are geographically isolated, was embraced as the mechanism for species formation (Via, 2001). It was well established that speciation occurred in populations separated by large distances (Wright, 1943), or insurmountable physical barriers (Takagi, 2011). Of course, the severity of a barrier depends on the species and its dispersal capability. A river may be a barrier to an insect or mammal but not to a bird, whereas a mountain range or ocean could be a barrier to all three (Olah et al., 2017).

Eventually, cases were discovered where reproductive isolation occurred in the absence of physical barriers or in sympatry (Via, 2001). For example, in montane

systems, populations separated by elevation can be genetically differentiated even across short distances and contiguous habitat (DuBay & Witt, 2014; Gifford & Kozak, 2012; Kendeigh & Fawver, 1981). Increases in elevation bring lower temperatures, higher precipitation and frequency of snowfall, shorter growing seasons, and higher wind velocity (Kendeigh & Fawver, 1981). These environmental pressures can result in strong selection for local adaptation. Neutral genetic markers compared in *Festuca eskia*, a perennial alpine grass common to the Pyrenees Mountains of southwestern Europe, show very low gene flow between low and high altitude populations (Gonzalo-Turpin & Hazard, 2009). Additionally, phenotypic differentiation in traits related to fitness was detected along the altitudinal gradient. In *Festuca*, genotypic differentiation was reflected by phenotypic differences in entire bunch diameter, height, and reproductive traits along altitudinal gradients (Gonzalo-Turpin & Hazard, 2009). Elevation restricted gene flow and differentiation occurred within a single species inhabiting the same mountain slope.

Behavioural barriers also have a large role in reducing gene flow. Pillay and Rymer (2012) advocated in their review that the co-adaptation of male courtship rituals and female perception are a primary driver of behavioural divergence and subsequent reduction in gene flow. Studies have shown behavioural divergence in several types of communication including, but not restricted to, visual courtship displays, (Mathews et al., 2002; Ng et al., 2013; Zoppoth et al., 2013), auditory courtship signals (Fitzpatrick and Gray, 2001; Irwin 2000; Kingston et al., 2001; Lemmon, 2009), olfactory cues (Plenderleith et al., 2005, Pillay, 2000), and tactile courting rituals (Montanarin et al., 2011). Behavioural divergence in foraging strategies of killer whale populations resulted in at least two sympatric populations in northeastern Pacific having more genetic

differentiation than allopatric populations in the North Pacific and South Atlantic (Hoelzel & Dover, 1991).

In population genetics, signatures of genetic differentiation are detected between populations, and the reasons for the differentiation can only be inferred. Even if a lack of gene flow between populations was apparent, employing direct observation of individuals to identify physical barriers to movement, limits of dispersal distance, or behavioural barriers for many species is difficult, if not logistically infeasible (Storfer et al., 2010). In response to these limits of population genetics, two fields of evolutionary biology, landscape genetics and phylogeography, gained traction. These fields of study are concerned with genetic relationships within and among species as governed by their geographic distribution and the landscape features that facilitate gene flow (Storfer et al., 2010). These fields overlap in many goals and methods, but there are several important distinctions in choice of molecular marker and the time and spatial scales used (Freeland et al., 2012; Wang, 2010).

1.3 Landscape Genetics

Landscape genetics examines the contemporary landscape features and environmental variables affecting gene flow and influencing allele frequencies among populations (Wang, 2010). This field has rapidly evolved into a central topic in evolutionary biology over the last 30 years (Rissler, 2016). It integrates landscape ecology and population genetics with new capabilities in spatial statistical analyses (Manel et al., 2003; Storfer et al., 2007; Storfer et al., 2010). Before landscape genetics became established, population genetic research relied on models that assumed large

population sizes and habitats with high degrees of spatial symmetry (Segelbacher et al., 2010). Advancements in molecular genetic techniques and high resolution landscape data from Geographical Information System (GIS) technology have allowed researchers to address questions of gene flow, natural selection, behaviour, and evolutionary history with fewer assumptions and more realistic approaches (Charlesworth & Charlesworth, 2017; Manel & Holderegger, 2013; Storfer et al., 2010).

The greatest strength in landscape genetics is the ability to quantify the degree to which landscape features impede individual dispersal, not just detect the presence of barriers (Manel & Holderegger, 2013). Understanding how barriers influence dispersal is crucial for describing evolutionary history, but tracking it is notoriously difficult (Olah et al., 2017). Tracking either requires costly marking and continuous long-term monitoring of a large number of individuals (Selonen & Hanski, 2003) or making indirect estimates of number of migrants and gene flow from genetic data (Neigel, 1997). Landscape genetics assesses how structural connectivity, or continuity of certain landscape features, affects functional connectivity and gene flow between populations (Manel & Holderegger, 2013). Recent studies have shown that landscape genetics models using realistic landscape variables with isolation-by-resistance (IBR) models are better able to explain patterns of variance between some populations than the isolation-by-distance (IBD) models in classical population genetics (Petren, 2013; Storfer et al., 2010; van Strien et al., 2015). IBD assumes individuals are more likely to disperse to nearby sites, and as a result the rate of gene flow will decrease as the geographic distance between sites increases. It assumes the organism's movement depends only on physical distance between habitat patches (Freeland et al., 2012). In contrast, IBR predicts a positive

relationship between genetic distance and resistance distance, a theoretical distance measurement based on assigning resistance values to landscape features and how easy it is for a given species to traverse through the habitat using the path of least resistance (McRae, 2006).

1.4 Phylogeography

Complementary to landscape genetics, phylogeography investigates the historical processes that have led to the current geographical distribution of genealogical lineages (Avice, 2000). The term arose from the combination of phylogenetics and biogeography, and is a new discipline that combines molecular genetics with current geological theory. The temporal scale of phylogeography is typically on the order of tens of thousands of years versus thousands or hundreds of years for landscape genetics (Rissler, 2016). Phylogeography relies on paleogeographic and historical ecological conditions for testing hypotheses regarding population distribution and mechanisms of differentiation (Hickerson et al., 2010). Most commonly, organellar DNA (mitochondrial and chloroplast) is used to determine phylogenetic relationships among populations, species and subspecies, which are then plotted on their geographical distribution (Hewitt, 2001).

Many phylogeographic studies explore the profound effects that Pleistocene glaciations had on global species diversification (Avice, 2000; Barrowclough et al., 2005; Knowles, 2001; Lovette, 2005). The Pleistocene glaciations were the most recent climate cycles starting ~2.5 million years ago. During this time the global landscape was transformed by major climate oscillations between cold glacial and warmer interglacial periods. Multiple continental ice sheets advanced and receded, causing dramatic

fluctuations in species' distributions following habitat shifts, fragmentation and extinction events (Hewitt, 2004). The particular effects on species ranges varied with latitude, topography, and species dispersal capability. Ice and permafrost were dominant at high latitudes, shifting temperate and tropical regions towards the equator. Species living on mountains could survive the climate oscillations by altitudinal shifts (Hahn et al., 2004; Hewitt, 2004). In North America, species were pushed to glacial refugia on the southern front of the Laurentide ice sheet covering the east and center of the continent (from the Atlantic Coast, covering the Northwest Territories and south to 40°N, to the east of the Rocky Mountains), and Cordilleran ice sheet in the west (from the coast of British Columbia, covering Alaska to northern Washington, to the west of the Rocky Mountains) (Barendregt & Irving, 1998; Hewitt, 1996). Fossil records also show evidence of cold-tolerant species (trees, small mammals, birds) residing in more northerly refugia including Beringia (Freeland et al., 2012).

Many species are thought to have originated as a result of glacial vicariance during the Pleistocene, where once-contiguous populations were exposed to different selection pressures in isolated refugia and diverged (Rand, 1948). For avian taxa, this concept of Pleistocene speciation is somewhat contested. The combination of a depauperate avian fossil record and the popularity of the concept led to an almost universal agreement by the 1980s that most divergence of North American avian taxa occurred in the second half of the Pleistocene during the last glacial maximum (LGM) (Lovette, 2005). However, an influential study by Klicka and Zink (1997) used a mtDNA divergence rate of 2% per million years to show that the majority of avian species pairs split before the Pleistocene. Regardless of divergence occurring before or during the

Pleistocene, the oscillating glacial cycles were significant events that influenced the contemporary genetic structure of many species. Phylogeographic research uncovers signatures of historical processes on genetic structure and helps disentangle them from the contemporary influences on gene flow detected by landscape genetics.

1.5 Molecular Markers

Fundamental leaps in technology and methodology have broadened the capabilities of molecular genetics, allowing analysis of DNA sequence polymorphisms directly rather than only protein gene products or morphology (Cavalli-Sforza, 1998). Among the most important developments is the polymerase chain reaction (PCR) in the 1980s, which uses primer sets developed for specific molecular markers and amplifies those DNA segments of interest to usable concentrations, and the discovery of hypervariable microsatellite loci (Weber & May, 1989).

A molecular marker is a fragment of DNA that provides information about allelic variation at a given locus (Schlötterer, 2004). Polymorphisms in these sequences provide information such as genetic diversity between populations, dispersal patterns of individuals, and can be used to trace historical origins of species (Avise, 2000). The genetic patterns detected will be different depending on the mode of inheritance (i.e. uni- and bi-parental), location in genome (nuclear and organelle), effective population size and mutation rate of the molecular markers (Zink & Barrowclough, 2008).

No marker is able to provide all necessary information, so it is important to select the markers most suited to the temporal and spatial scale of the research question. A

major difference between phylogeography and landscape genetics is the class of markers most appropriate for each field (Wang, 2010). Phylogeography uses organellar (chloroplast and mitochondrial) and nuclear DNA sequences, while landscape genetics use microsatellite genotype data (hypervariable nuclear DNA) (Wang, 2010). However, relying too much on one type of marker may lead to biased results. Often the best approach is to use a combination of markers from different locations in the genome with different modes of inheritance. These multi-locus analyses will provide the most complete interpretation of evolutionary history.

1.5.1 Mitochondrial markers

Mitochondrial DNA (mtDNA) is one of the most widely used of the genetic markers. It occurs in a single circular genome, meaning that the loci along it are physically linked and transmitted as one haplotype (Cavalli-Sforza, 1998). Due to its highly conserved gene content among vertebrates, mtDNA can be easily amplified from a variety of taxa using the same primers, reducing time and cost of development (Harrison, 1989; Hurst & Jiggins, 2005). It contains genes coding for ribosomal RNA, transfer RNA, and proteins involved in electron transport and ATP synthesis, as well as a noncoding control region (CR) that contains sequences used to signal initiation of replication and transcription (Hurst & Jiggins, 2005).

Mitochondrial DNA is highly variable with a mutation rate in animals typically 5-10x faster than nuclear DNA (nuDNA) (Nabholz et al., 2009). The combination of a higher turnover rate than nuDNA in tissues and a lack of repair mechanisms allows for the accumulation of errors with more rounds of replication (Wan et al., 2004). Due to

mtDNA haploid and uni-parental inheritance, the effective population size is one quarter of bi-parentally inherited nuclear DNA (Sunnucks, 2000). This makes it especially sensitive to large demographic events like population bottlenecks and will show these genetic signatures from recent evolutionary history (Harrison, 1989). Since mtDNA is typically passed from the mother to offspring, genetic patterns in mtDNA markers compared with nuclear loci can trace matrilineal history and sex-biased colonization events in recent history (Harrison, 1989; Zhang & Hewitt, 2003).

There are a few limitations to using mtDNA due to the inherent properties previously mentioned. Since the mitochondrial genome is circular, all loci are physically linked and can only be considered one locus, and provide only one perspective of evolution (Zhang & Hewitt, 2003). In species with XX/XY chromosome sex determination, this perspective is only the matrilineal history which may differ a lot from the history of the overall species, and from the male-driven patterns shown by Y-chromosome markers (Z-linked in birds and some reptiles, amphibians, fish, crustaceans, and insects; Ellegren 2011). Species with other sex determination systems such as environmentally or hormonally determined gender and haplodiploidy (males develop from unfertilized eggs and are haploid, females develop from fertilized eggs and are diploid) would have different mtDNA patterns and must be carefully interpreted according to specific life histories (Borgia, 1980; Charnov & Bull, 1977). The small effective population size of mtDNA means lineages will sort faster, leading to oversimplified evolutionary relationships and underestimated genetic diversity (Zhang & Hewitt, 2003). To compensate for these limitations, it is best to use a multilocus approach combining both mtDNA and nuDNA when investigating questions in evolutionary biology.

1.5.2 Microsatellites

Microsatellites are short tandem repeats of nucleotide sequence found throughout the nuclear genome and in chloroplast DNA (Jarne & Lagoda, 1996). The most common repeat types used in population genetics studies are di- (AC)_n, tri- (GTG)_n, and tetra- (GATA)_n nucleotide repeats, with each microsatellite locus typically varying in length between 5-40 repeats (Eckert & Hile, 2009; Selkoe & Toonen, 2006). The DNA sequences flanking microsatellite regions are generally conserved across individuals of the same taxonomic family, so microsatellite primers can be used in closely related species (Selkoe & Toonen, 2006). These markers are typically neutral and have codominant alleles that are biparentally inherited, providing insight into both male and female histories (Jarne & Lagoda, 1996).

Due to polymerase slippage during DNA replication, microsatellites are susceptible to changes in number of repeats and as a consequence acquire length variations (Selkoe & Toonen, 2006). As a result, microsatellite loci are extremely polymorphic with high levels of allelic diversity. In population studies, alleles from each individual are sorted by length using high resolution gel electrophoresis to an accuracy of one base pair difference without requiring full sequencing (Jarne & Lagoda, 1996). The hypervariability of these markers allows detection of genetic differentiation after recent demographic events too recent for mtDNA markers to detect. It also makes them useful markers for observing distribution of genetic variation and estimating levels of gene flow at contemporary spatiotemporal scales.

A few problems with microsatellite marker use persist, however. PCR primers rarely work across broad taxonomic groups so they have to be developed for new species (Glenn & Schable, 2005). There is also continued debate over how microsatellite alleles mutate. While the mechanism of mutation is not always relevant, some statistics are based on estimates of allele frequencies that arise from explicit mutation models (Selkoe & Toonen, 2006). The default mutation model has often been the infinite alleles model (IAM), which explains that mutations give rise to new alleles independent of the size of previous forms (Jarne & Lagoda, 1996; Selkoe & Toonen, 2006). Another proposed model is the stepwise mutation model (SMM) where a single repeat is added or deleted with equal probability (Jarne & Lagoda, 1996). Alleles of different sizes may all differ equally from each other under the IAM, but for the SMM, alleles similar in size are more closely related than those very different in size (Jarne & Lagoda, 1996).

Finally, when interpreting results of microsatellite analysis, awareness of the phenomenon of homoplasy is necessary. It is a consequence of high mutation rates where alleles may appear identical by descent although they arose from different ancestral alleles. Disregarding homoplasy means both allelic diversity and actual divergence between populations will be underestimated (Selkoe & Toonen, 2006). Despite the above challenges when using microsatellites, they remain useful markers for studies of detecting fine scale spatial and landscape barriers to gene flow (Arnoux et al., 2014; Funk et al., 2005; Olah et al., 2017), resolving genetic distance of populations (Epps et al., 2007), and assigning individuals to populations for conservation (Coulon et al., 2004; Dubey et al., 2011). Using them in combination with other types of molecular markers provides tools

for comprehensive testing evolutionary hypotheses, compensating for any bias or shortcomings intrinsic to each marker type when used alone.

1.6 Statistical Methods

Once molecular data are acquired, several approaches must be used to establish genetic structure in a species. These approaches are found in numerous programs, formulae, and models available for study of population genetics, and must be chosen carefully to prevent statistical bias and errors in biological interpretation (Storfer et al., 2007; Zhang & Hewitt, 2003). Genetic diversity in populations of interest should first be established using measures of allele frequencies, allelic diversity, and observed heterozygosity, because the genetic diversity of a population determines its ability to adapt to change and indicates likelihood of long-term survival. A population with these previously mentioned measures that deviate from Hardy-Weinberg equilibrium (HWE) expectations of panmixia (random mating within a breeding population) is either being influenced by other forces like natural selection, inbreeding, and genetic drift (Slatkin, 1987), or there were null alleles in the dataset due to failed PCR amplification of marker loci (Storfer et al., 2010). Once natural intrapopulation genetic diversity is established, then genetic distance and levels of gene flow between populations can be found.

Wright (1943) developed a set of F -statistics for measuring allele frequencies and indicating levels of gene flow between two populations. Originally these F -statistics were designed under the assumption of having an infinite number of equal-sized populations that receive and give migrants to each of the other populations at the same rate (island model), using two alleles (Whitlock & McCauley, 1999). The most commonly used F -

statistic, the fixation value (F_{ST}), quantifies genetic distance between two populations by comparing allele frequencies of a biallelic marker under HWE (Storfer et al., 2010). If two populations have the same allele frequencies, they are not genetically differentiated and the F_{ST} value is zero. If they are completely fixed for the two different alleles and thus genetically differentiated, F_{ST} equals one.

Since these F -statistics were originally formulated to measure genetic distance using biallelic markers, the values of genetic distance between two populations could be a range between zero and one. However, with more than two alleles, this genetic distance value would never reach one even when no alleles are shared because there would always be some heterozygosity within populations. Analogs of Wright's F -statistics like F'_{ST} were thus created to account for molecular markers like microsatellites which have more than two alleles (Meirmans & Hedrick, 2011; Nei, 1973). The F'_{ST} value is acquired by standardizing all F_{ST} values by the maximum possible value obtainable with more than two alleles. Over the years, many statistical analyses have been modified to more accurately represent biological systems with fewer assumptions to help answer the complex evolutionary questions in landscape genetics and phylogeography.

1.6.1 Methods in landscape genetics

Using F -statistics to describe the genetic structure of a species can work well in situations where populations naturally occur in "island-like" spatial clusters (i.e. ponds or archipelagos). However, more often individuals of a species are distributed along a continuous range; in which case a landscape approach to statistical analyses is more appropriate (Manel et al., 2003). The data required for landscape genetics research are

samples of many loci across potential environmental selection gradients (temperature, soil composition, altitude, etc.). The following three statistical tools are a few exemplary methods used to identify genetic spatial patterns and correlations between genetic and environmental variables.

First, Mantel tests are a type of pairwise regression analysis used to measure the association between genetic distance of populations and an environmental variable. For example, Keyghobadi et al. (1999) used Mantel tests to show that more gene flow occurs in meadows compared to forests for the alpine butterfly *Parnassius smintheus*. A partial Mantel test can compare three or more environmental variables as it allows distance measurements to be made between two variables while controlling for the third (Scheiner & Gurevitch, 2001). Second, Bayesian clustering analyses use multilocus genotype data to assign individuals to population clusters without prior knowledge of boundaries or location (Manel et al., 2003). This analysis is popular in population genetics because it remains robust even when small numbers of loci are available, but can sometimes be limited by assumptions that populations have panmixia, preventing analysis of asexually reproducing species, and is unable to account for departures from HWE caused by small population size or inbreeding. A third method in landscape genetics for identifying spatial patterns are multivariate analyses such as principal component analyses (PCA) and principal coordinate analyses (PCoA) (Manel et al., 2003). These methods summarize all the genetic variation at multiple loci and plot the data into a two-dimensional scatterplot representing spatial genetic structure. PCoA displays the matrix of genetic distance (F_{ST}) and spatial distance between populations. Strengths of these analyses include that they are exploratory, do not require adherence to HWE, or for genotypes to be clustered into

discrete populations (Jombart et al., 2009). The summary of genetic variability plotted against data types like environmental (PCA) or spatial (PCoA) data can reveal clinal genetic structuring with freedom from requirements common to many other analyses and using low computational power (Jombart et al., 2009).

Once these types of analyses identify spatial genetic patterns, it is possible to use GIS technology in parallel to visualize potential genetic boundaries and generate hypotheses about the cause of genetic differentiation. Genetic data can be overlaid onto landscape variables for useful visualizations and maps (Manel et al., 2003). For example, Mantel tests used in concert with landscape types assigned with resistance values can create a ‘least-cost path’ model in the range of a particular species of interest (Holderegger & Wagner, 2008). This model calculates and visually displays dispersal corridors most likely to be used by the species on a map. Individuals of the species choose landscape types that afford them the least resistance, and the corridors used can be ranked according to predicted levels of gene flow (Epps et al., 2007).

1.6.2 Methods in phylogeography

Historical climate and environment variables (global oscillations in temperature, precipitation, and continental ice sheet coverage during the Pleistocene ice age) can be used to predict potential historical niches and glacial refugia where species’ habitats were pushed to ice sheet periphery. With prior knowledge of glacial refugia, the levels of connectivity and gene flow between populations originating from each refugium can be explored and used to inform contemporary genetic structure (Knowles, 2000; Manel & Holderegger, 2013). Maps that interpolate GIS climate and environment conditions with

known occurrence points of species are called ecological niche models (ENM). They first display ranges of suitable habitat for a species of interest, then can make predictions of historical suitable habitat ranges with paleoclimatic data (Rissler, 2016). Orsini et al. (2008) used this method and determined that genetic spatial structure of Glanville fritillary butterflies (*Melitaea cinxia*) was better explained by historical events than by current demographic structure of the species. Also, the observed heterozygosity was significantly explained by past metapopulation sizes (Orsini et al., 2008). This type of analysis has great utility for conservation and management because past and present barriers to gene flow can be determined and used to inform future decisions as global landscapes continue to change with climate and anthropogenic influence (Storfer et al., 2010).

1.7 Study species

1.7.1 Z. leucophrys subspecies

The genus *Zonotrichia* contains five species of sparrows that are found abundantly in the Americas from subarctic Canada and Alaska to Cape Horn in South America. Of these five species, white-crowned sparrows (*Zonotrichia leucophrys*) live solely in North America with both sedentary and migratory populations (Figure 1.1). From data on allozymes, morphometrics, and mtDNA profiles, Zink (1982) and Zink et al. (1991) concluded that speciation within *Zonotrichia* likely occurred during the Pleistocene, sometime before 140,000 years ago. *Z. leucophrys* have been widely used in lab and field investigations of avian biology due to their abundance and ease of maintenance in captivity.

Z. leucophrys are sexually monomorphic, with the exception of a slightly smaller body size in females (Dunn et al., 1995; Morton, 2002). Adults have a distinctive black and white striped head, with white eyebrow stripes bordering a pair of black stripes on the crown separated by a white median stripe (Figure 1.2). There are five subspecies mostly distinct in distribution with occasional small overlap of breeding areas. Subspecies can be categorized into two groups: (1) the *Z. l. pugetensis* and *Z. l. nuttalli* populations on the Pacific Coast from California to British Columbia and (2) the *Z. l. leucophrys-oriantha-gambelii* populations in the rest of North America (Figure 1.1). Only *nuttalli* is sedentary; *pugetensis* migrates the shortest distance and *oriantha*, *leucophrys*, and *gambelii* which display increasing migration distances, respectively (Morton, 2002). These sparrows occur at a variety of elevations, and are known to select nesting habitats in subalpine meadows as high as 3,500 m in the Sierra Nevada and Rocky Mountains, and down to sea level on the Pacific Coast (Dunn et al., 1995; Morton, 2002).

All *Z. leucophrys* subspecies prefer habitat with elements of thick shrubby cover mixed with open ground for both wintering and breeding habitat. These landscapes with patchwork shrubs and meadows are found in boreal forest, tundra, alpine meadows and coastal scrub (Chilton et al., 1995). They are an omnivorous species eating seeds, fruits, buds, flowers, grass, and terrestrial arthropods as the primary food source for dependent young. Subspecies can be distinguished from each other by a combination of bill colour, colour of the short feathers between the eyes and bill (the lores), and habitat range (Figures 1.1 and 1.2). Both *Z. l. pugetensis* and *Z. l. nuttalli* have yellow bills and pale grey lores, but occur in coastal Pacific Northwest versus coastal California, respectively. Both *Z. l. oriantha* (Rocky Mountains of western Canada and western US) and *Z. l.*

leucophrys (northeast Canada) have pink bills and black lores, and finally *Z. l. gambelii* (found in Alaska, west, and central Canada) have orange bills and pale lores (Dunn et al., 1995).

Field studies of *Z. leucophrys* have revealed subspecies variation in the form of vocal dialects. Males sing a single song type in their adult repertoire which is retained for their entire lives (Petrinovich, 1985). *Z. l. oriantha*, *nuttalli*, and *pugetensis* subspecies form dialects that vary greatly, in contrast to *Z. l. gambelii* which do not seem to form distinct dialects (Nelson, 1998). A number of studies have been published on either the weak or strong association of song dialects with population structure in *Z. leucophrys* subspecies (Baker, 1982; Chilton et al., 1990; MacDougall-Shackleton & MacDougall-Shackleton, 2001; Marler & Tamura, 1962; Morton, 2002; Nelson, 1998; Soha et al., 2004). Also found in the Rocky Mountains of Alberta was evidence of plumage hybridization of *Z. l. oriantha* and *Z. l. gambelii* in the subspecies contact zone (Lein and Corbin, 1990).

Although past research efforts were devoted to the identification of subspecies in extant species, subspecies do not always reflect patterns of genetic variation and are poor indicators of overall population structure (Zink & Barrowclough, 2008). Genetic differences between subspecies may or may not reflect detectable differences in appearance or song dialect, in the case of *Z. leucophrys*. Thus, *Z. leucophrys* are ideal for answering landscape genetics questions about gene flow because subspecies ranges overlap at some locations and span a wide variety of barrier types.

1.7.2 Phylogeographic history

Z. leucophrys are also ideal for answering phylogeographic questions because they occur in areas of North America previously covered by ice sheets. Rand (1948) speculated that the five *Z. leucophrys* subspecies' ranges correspond to four areas of refugia in North America during the Pleistocene (~ 18,000 years BP): 1) south of the ice sheets near the West Coast (*Z. l. nuttalli-pugetensis*), 2) south in the Rocky Mountain area (*Z. l. oriantha*), 3) northwest by the Yukon-Bering Sea (*Z. l. gambelii*), and 4) in the northeastern US (*Z. l. leucophrys*) (Figure 1.3). Rand's hypothesis is supported by the remarkably similar song dialects of some *Z. l. oriantha* and *Z. l. nuttalli-pugetensis* populations observed by Baptista and King (1980), as well as paleobotanical data supporting a continuum between coastal and boreal forest habitat around the Sierra Nevada mountain range during the Pleistocene that would have connected their habitat (Hubbard, 1969). According to Rand's hypothesis, in post-glacial times *Z. l. gambelii* travelled eastward to connect with *Z. l. leucophrys*, and the *Z. l. oriantha* and *Z. l. nuttalli-pugetensis* populations expanded northward in parallel, just as their breeding ranges do currently (Figure 1.1).

Richard Banks (1964) challenged this schema with another hypothesis raising doubts about the idea of a strong relationship between montane *Z. l. oriantha* and the coastal *Z. l. nuttalli-pugetensis*, and emphasizing the significance of phenotype: the reddish back plumage shared by *Z. l. oriantha* and *Z. l. leucophrys* and pale grey lores of *Z. l. gambelii* and *Z. l. nuttalli-pugetensis* (Figure 1.4). He suggested that at some point in history there were *Z. l. gambelii* and *Z. l. oriantha* ancestors forming a continuous red-backed population ranging from Alaska to the Mackenzie Mountains (Banks, 1964;

Baptista & King, 1980). Later there was selection for lore color between the high latitude individuals (pale-lored *Z. l. gambelii*) and low latitude (black-lored *Z. l. oriantha*). Banks also predicted that *Z. l. gambelii* spread south down the Pacific Coast, with subsequent isolation eventually forming the *Z. l. nuttalli-pugetensis* subspecies.

Through literature searches we found support for Rand's hypothesis in song dialect pattern. A recent study on song types and range shifts of *Z. leucophrys* notes interestingly that the *Z. l. leucophrys* and *Z. l. gambelii* subspecies share song structure described as a melodic line beginning mid-level, followed by low-then-high trills, and followed by descending notes (Hunn and Beaudette, 2014). Although *Z. l. nuttalli-pugetensis* have high dialect variation, they still have distinct structure in the opening phrases of their song. *Z. l. oriantha* sings both song patterns, with individuals from the Rocky Mountains and Great Basin most similar to *Z. l. gambelii*, but those in the Sierra Nevada singing the coastal *Z. l. nuttalli-pugetensis* pattern (Baptista & King, 1980). The ranges of *Z. l. oriantha* and *Z. l. nuttalli-pugetensis* were distinct until recent years where a 2014 study notes they "might" come into contact in the Cascade Mountains of northern Oregon (Hunn & Beaudette, 2014). The ability of *Z. l. oriantha* to replicate a song pattern typical of *Z. l. pugetensis* may be indicative of a time spent in a shared southern refugium. The song type shared by *Z. l. leucophrys* + *gambelii* is additional support for Rand's suggestion that these subspecies expanded east and westward and met post-glaciation.

In light of the ubiquity of Pleistocene speciation theories just like Rand's and Banks' for many avian taxa, Klicka and Zink's (1997) antithetical evidence of ancestral avian speciation events predating the Pleistocene, and a myriad of possible contemporary

influences, it is important to approach the explanation of *Z. leucophrys* subspecies origins with caution. Little work elucidating the evolutionary history of *Z. leucophrys* has been performed since the mid-1900s. It remains to be clarified how genetically related the subspecies are, if there is a higher likelihood of preferential mating within subspecies due to plumage or song dialect groups, and if Pleistocene refugia origins is correct. The many potential influences on genetic structure in white-crowned sparrows means that landscape genetics and phylogeographic insights will be invaluable for explaining current levels of gene flow, population genetic structure and evolutionary history. Molecular analyses of this widely distributed species can also give insight into processes causing divergence in other North American taxa.

1.8 Thesis goals

This study will focus on the genetic diversity of white-crowned sparrow populations in montane habitats of Alberta and British Columbia (central Rocky Mountains), Colorado (southern Rocky Mountains), and Oregon (Cascade Mountains). First, theories of post-Pleistocene expansion will be investigated using phylogenetic tree construction, historical ENM analyses, and findings from previous literature. Conclusions from these potential historical processes will be used to inform the current genetic structure and levels of gene flow established by our mtDNA and nuDNA analyses. A second goal is to use a landscape genetics approach to assess the influence of rangewide barriers on gene flow between populations of the three subspecies. Microsatellite and environmental data will be employed to determine dispersal corridors in the presence of the physical barriers of latitudinal distance and mountain ranges. A third goal is to assess the potential microgeographic barriers between the contrasting climate extremes of high

and low elevation using elevational transects to find genetic structure at local scales. Conclusions from these analyses and contemporary ENM will be supported by the suggestion of potential scenarios of phylogeographic history from historical ENM and phylogenetics.

1.9 Thesis Organization

The thesis has been written in three chapters. The first chapter provided the general background on how population genetics analyses combined with strategies of landscape genetics and phylogeography lead to more robust conclusions about processes influencing genetic structure of populations. Both historical and contemporary barriers affect individual dispersal and create certain genetic patterns at a population level. The second chapter is the data chapter which uses ecological niche modeling to propose historical and contemporary barriers to gene flow in white-crowned sparrows. It then looks at population structure using nuclear and mtDNA and addresses whether physical barriers (latitudinal distance and the Rocky Mountains) restrict dispersal and gene flow in this species, as well as climate barriers (climate extremes at high elevation versus low). Microsatellite analyses are used to highlight more contemporary patterns in genetic structure. Three hypotheses are tested: 1) genetic structure is present in white-crowned sparrows due to historical barriers, 2) physical barriers cause reproductive isolation resulting in changes in mtDNA and nuclear DNA, and 3) climate barriers reduce gene flow between low and high elevation populations and show patterns of isolation by resistance. The final chapter summarizes the results of chapter 2, suggests possible causes of patterns observed in the genetic markers, and proposes potential future research directions.

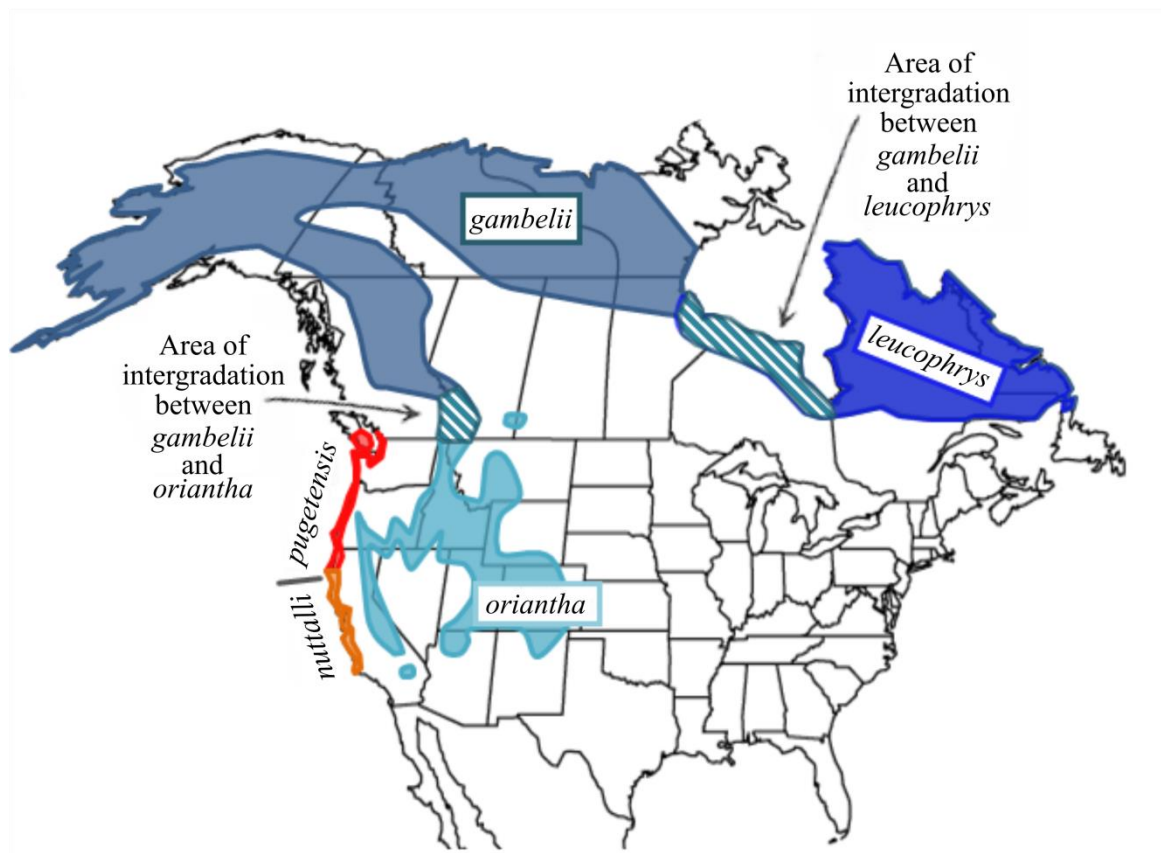
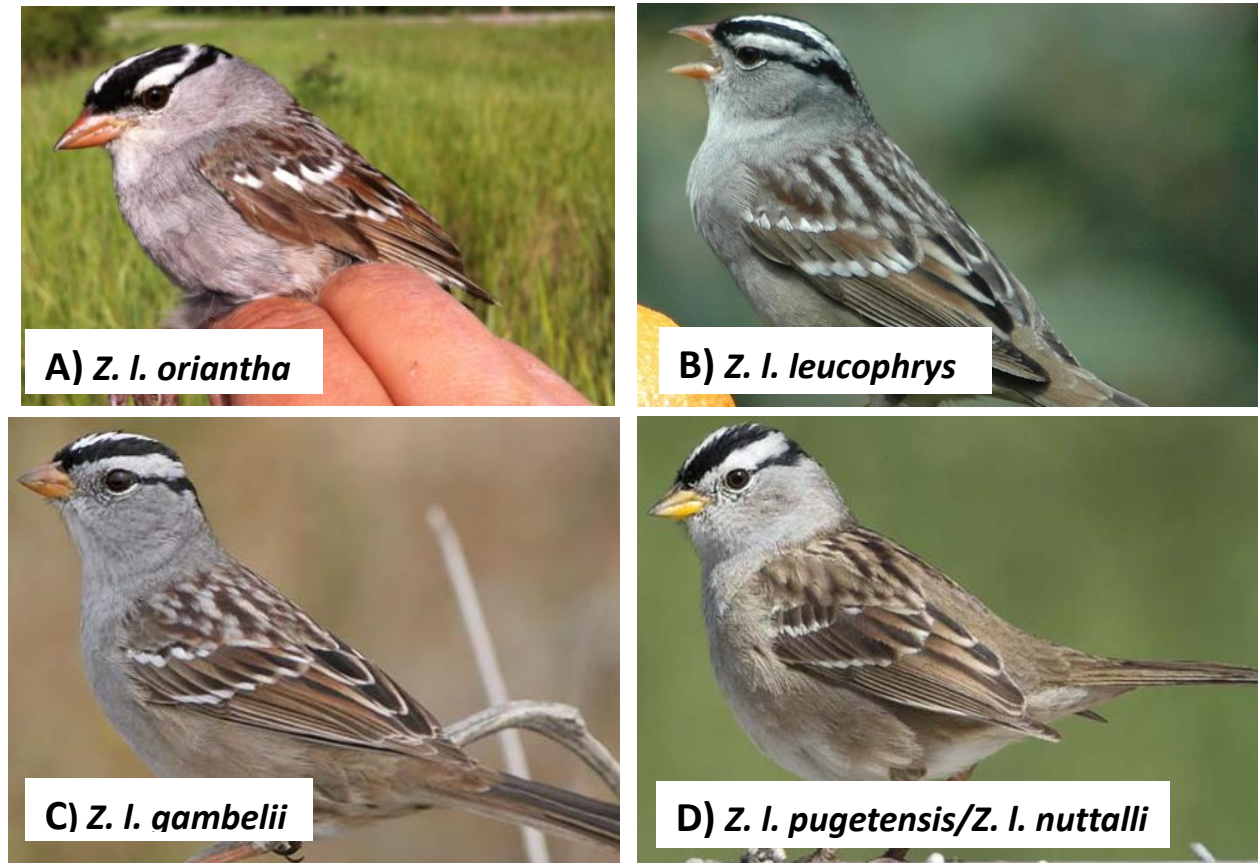


Figure 1.1: Breeding season range map of *Z. leucophrys* subspecies, *Z. l. gambelii*, and *Z. l. oriantha* in shades of blue, *Z. l. pugetensis* in red, and *Z. l. nuttalli* in orange. Areas with cross hatching indicate areas of overlap between subspecies. Adapted from Dunn et al. (1995).



A) *Z. l. oriantha*

B) *Z. l. leucophrys*

C) *Z. l. gambelii*

D) *Z. l. pugetensis/Z. l. nuttalli*

Figure 1.2: Example photos of *Zonotrichia leucophrys* subspecies. A) Distinguishing features of *Z. l. oriantha* include flared crown stripes and heavy black lores (small feathers between bill and eye), a dusky pink bill with darker red on culmen (upper bill). B) *Z. l. leucophrys* is very similar in appearance to *Z. l. oriantha*, but has more white encroaching into the black lores near the eye, a slightly lighter bill, and on average has a greater body mass, shorter wings, and longer tarsi. C) *Z. l. gambelii* has pale lores and orange bill. C) *Z. l. pugetensis* has pale lores and a yellow bill. *Z. l. nuttalli* is virtually indistinguishable in the field from *Z. l. pugetensis*. Photo credits to C. Welke (A) and © Don Robinson (B, C, and D; with permission).

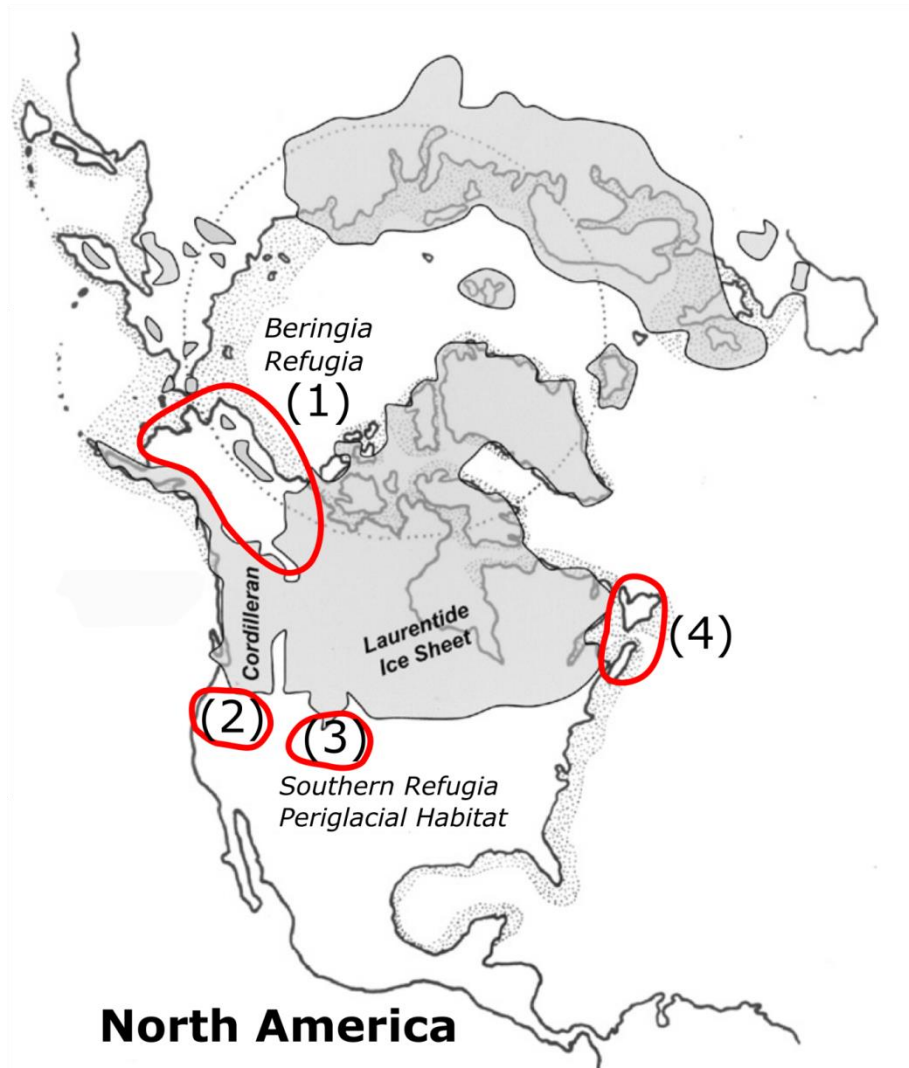


Figure 1.3: Polar projection of the geographic distribution of continental glaciers and glacial refugia during the last glacial maximum (circa 18,000 years BP). Major continental ice sheets are shown in grey, and the extent of exposed continental shelf indicated by stippling. Possible locations of *Z. leucophrys* subspecies refugia proposed by Rand (1984) are: (1) *Z. l. gambelii*, (2) *Z. l. pugetensis* and *Z. l. nuttalli*, (3) *Z. l. oriantha*, (4) *Z. l. leucophrys*. Adapted from Hoberg et al. (2012).

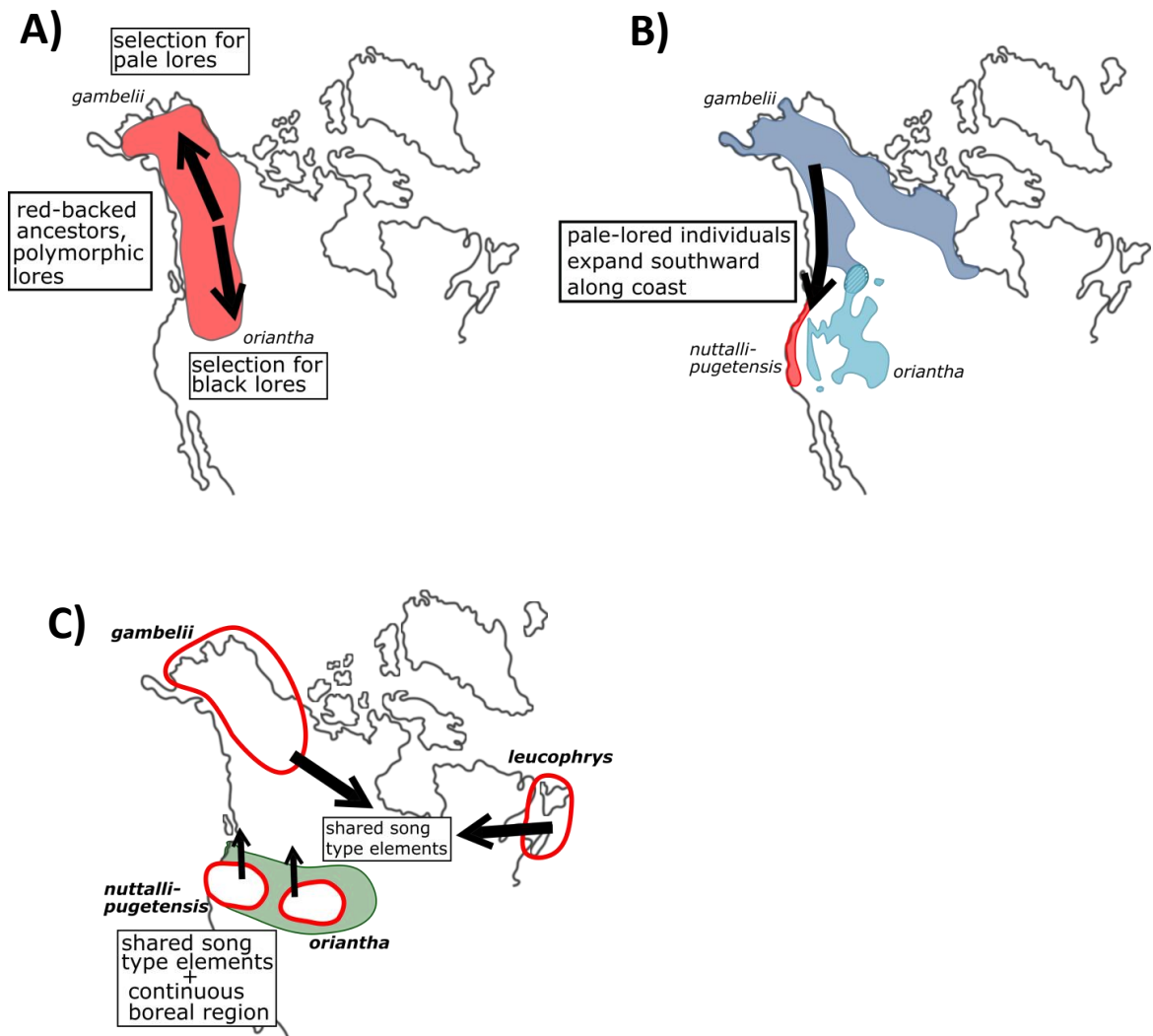


Figure 1.4: Two theories of post-glacial range expansion of *Z. leucophrys* subspecies. A two-step process was proposed by Banks (1964): first a continuous red-backed ancestral population (red) experienced selection for pale lores at high latitudes and black lores at low latitudes (A). Later, (B) some pale-lored *Z. l. gambelii* disperse southward along the coast and subsequent differentiation gives rise to *Z. l. nuttalli-pugetensis*. Rand (1948), Hubbard (1969), and Baptista & King (1980) suggest four Pleistocene refugia (in red): *Z. l. gambelii* + *leucophrys* acquire shared song elements after secondary contact. *Z. l. oriantha* + *nuttalli-pugetensis* survived in southern boreal refugia then expanded northward in parallel. They maintained very similar song types that can be observed in present-day (Hunn & Beaudette, 2014) (C). See Figure 1.1 for contemporary distributions of all five subspecies.

1.10 References

- Arnoux, E., Eraud, C., Navarro, N., Tougard, C., Thomas, A., Cavallo, F., Vetter, N., Faivre, B., & Garnier, S. (2014). Morphology and genetics reveal an intriguing pattern of differentiation at a very small geographic scale in a bird species, the forest thrush *Turdus lherminieri*. *Heredity*, *113*(6), 514-525. <https://doi.org/10.1038/hdy.2014.56>
- Avise, J. C. (2000). *Phylogeography : the history and formation of species*. Cambridge, MA: Harvard University Press.
- Baker, M. C. (1982). Vocal dialect recognition and population genetic consequences. *American Zoologist*, *22*(3), 561–569. <https://doi.org/10.1093/icb/22.3.561>
- Banks, R. C. (1964). Geographic variation in the white- crowned sparrow *Zonotrichia leucophrys*. *University of California Publications in Zoology*, *70*, 1–123.
- Baptista, L. F., & King, J. R. (1980). Geographical variation in song and song dialects of montane white-crowned sparrows. *The Condor*, *82*(3), 267–284. <https://doi.org/10.2307/1367392>
- Barendregt, R. W., & Irving, E. (1998). Changes in the extent of North American ice sheets during the late Cenozoic. *Canadian Journal of Earth Sciences*, *35*(5), 504–509. <https://doi.org/10.1139/e97-126>
- Barrowclough, G. F., Groth, J. G., Mertz, L. A., & Gutiérrez, R. J. (2005). Genetic structure, introgression, and a narrow hybrid zone between northern and California spotted owls (*Strix occidentalis*). *Molecular Ecology*, *14*(4), 1109–1120. <https://doi.org/10.1111/j.1365-294X.2005.02465.x>
- Borgia, G. (1980). Evolution of haplodiploidy: models for inbred and outbred systems. *Theories of Population Biology*, *17*(2), 103-128. [https://doi.org/10.1016/0040-5809\(80\)90001-5](https://doi.org/10.1016/0040-5809(80)90001-5)
- Burney, C. W., & Brumfield, R. T. (2009). Ecology predicts levels of genetic differentiation in neotropical birds. *The American Naturalist*, *174*(3), 358–368. <https://doi.org/10.1086/603613>
- Cavalli-Sforza, L. L. (1998). The DNA revolution in population genetics. *Trends in Genetics*, *14*(2), 60–65. [https://doi.org/10.1016/S0168-9525\(97\)01327-9](https://doi.org/10.1016/S0168-9525(97)01327-9)
- Charlesworth, B., & Charlesworth, D. (2017). Population genetics from 1966 to 2016. *Heredity*, *118*(1), 2–9. <https://doi.org/10.1038/hdy.2016.55>
- Charnov, E., & Bull, J. (1977). When is sex environmentally determined? *Nature*, *266*(5605), 828-830. <https://doi.org/10.1038/266828a0>
- Chen, H. (2015). Population genetic studies in the genomic sequencing era. *Zoological Research*, *36*(4), 223-232. <https://doi.org/10.13918/j.issn.2095-8137.2015.4.223>

- Cheviron, Z. A., & Brumfield, R. T. (2009). Migration-selection balance and local adaptation of mitochondrial haplotypes in rufous-collared sparrows (*Zonotrichia capensis*) along an elevational gradient. *Evolution*, *63*(6), 1593–1605. <https://doi.org/10.1111/j.1558-5646.2009.00644.x>
- Chilton, G., Baker, M. C., Barrentine, C. D., & Cunningham, M. A. (1995). White-crowned sparrow (*Zonotrichia leucophrys*). A. Poole & F. Gill (Eds.), *The Birds of North America Online* (Version 2.0). The Cornell Lab of Ornithology, Ithaca, NY. <https://doi.org/10.2173/bna.183>
- Chilton, G., Lein, M. R., & Baptista, L. F. (1990). Mate choice by female white-crowned sparrows in a mixed-dialect population. *Behavioral Ecology and Sociobiology*, *27*(3), 223–227. <https://doi.org/10.1007/bf00180307>
- Coulon, A., Cosson, J. F., Angibault, J. M., Cargnelutti, B., Galan, M., Morellet, N., Petit, E., Aulagnier, S., & Hewison, A. J. M. (2004). Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. *Molecular Ecology*, *13*(9), 2841–2850. <https://doi.org/10.1111/j.1365-294X.2004.02253.x>
- DuBay, S. G., & Witt, C. C. (2014). Differential high-altitude adaptation and restricted gene flow across a mid-elevation hybrid zone in Andean tit-tyrant flycatchers. *Molecular Ecology*, *23*(14), 3551–3565. <https://doi.org/10.1111/mec.12836>
- Dubey, S., Sumner, J., Pike, D. A., Keogh, J. S., Webb, J. K., & Shine, R. (2011). Genetic connectivity among populations of an endangered snake species from southeastern Australia (*Hoplocephalus bungaroides*, Elapidae). *Ecology and Evolution*, *1*(2), 218–227. <https://doi.org/10.1002/ece3.25>
- Dunn, J. L., Garrett, K. L., & Alderfer, J. (1995). White-crowned sparrow subspecies: identification and distribution. *Birding*, *27*, 182–201.
- Eckert, K. A., & Hile, S. E. (2009). Every microsatellite is different: Intrinsic DNA features dictate mutagenesis of common microsatellites present in the human genome. *Molecular Carcinogenesis*, *48*(4), 379–388. <https://doi.org/10.1002/mc.20499>
- Ellegren, H. (2011). Sex-chromosome evolution: recent progress and the influence of male and female heterogamety. *Nature Reviews Genetics*, *12*(3), 157–166. <https://doi.org/10.1038/nrg2948>
- Epps, C. W., Wehausen, J. D., Bleich, V. C., Torres, S. G., & Brashares, J. S. (2007). Optimizing dispersal and corridor models using landscape genetics. *Journal of Applied Ecology*, *44*(4), 714–724. <https://doi.org/10.1111/j.1365-2664.2007.01325.x>
- Fitzpatrick, M. J., & Gray, D. A. (2001). Divergence between the courtship songs of the field crickets *Gryllus texensis* and *Gryllus rubens* (Orthoptera, Gryllidae). *Ethology*, *107*(12), 1075–1085. <https://doi.org/10.1046/j.1439-0310.2001.00730.x>
- Fjeldsâ, J., Bowie, R., & Rahbek, C. (2011). The role of mountain ranges in the

- diversification of birds. *Annual Review of Ecology, Evolution, and Systematics*, 43(1), 249-265. <https://doi.org/10.1146/annurev-ecolsys-102710-145113>
- Freeland, J. R., Kirk, H., & Petersen, S. D. (2012). *Molecular Ecology* (Second Edition). Chichester, West Sussex, UK: Wiley-Blackwell.
- Funk, W. C., Blouin, M. S., Corn, P. S., Maxell, B. A., Pilliod, D. S., Amish, S., & Allendorf, F. W. (2005). Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology*, 14(2), 483–496. <https://doi.org/10.1111/j.1365-294X.2005.02426.x>
- Gifford, M. E., & Kozak, K. H. (2012). Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. *Ecography*, 35(3), 193–203. <https://doi.org/10.1111/j.1600-0587.2011.06866.x>
- Glenn, T. C., & Schable, N. A. (2005). Isolating microsatellite DNA loci. *Methods in Enzymology*, 395, 202–222. [https://doi.org/10.1016/S0076-6879\(05\)95013-1](https://doi.org/10.1016/S0076-6879(05)95013-1)
- Gonzalo-Turpin, H., & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*, 97(4), 742–751. <https://doi.org/10.1111/j.1365-2745.2009.01509.x>
- Gutiérrez-Pinto, N., Cuervo, A. M., Miranda, J., Pérez-Emán, J. L., Brumfield, R. T., & Cadena, C. D. (2012). Non-monophyly and deep genetic differentiation across low-elevation barriers in a Neotropical montane bird (*Basileuterus tristriatus*; Aves: Parulidae). *Molecular Phylogenetics and Evolution*, 64(1), 156–165. <https://doi.org/10.1016/j.ympev.2012.03.011>
- Hahn, T. P., Sockman, K. W., Breuner, C. W., & Morton, M. L. (2004). Facultative altitudinal movements by mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) in the Sierra Nevada. *The Auk*, 121(4), 1269–1281. <https://doi.org/10.2307/4090494>
- Harris, R. J., & Reed, J. M. (2002). Behavioral barriers to non-migratory movements of birds. *Annales Zoologici Fennici*, 39(4), 275–290.
- Harrison, R. G. (1989). Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology and Evolution*, 4(1), 6–11. [https://doi.org/10.1016/0169-5347\(89\)90006-2](https://doi.org/10.1016/0169-5347(89)90006-2)
- Hewitt, G. M. (1996). Some genetic consequences of ice ages, and their role, in divergence and speciation. *Biological Journal of the Linnean Society*, 58(3), 247–276. <https://doi.org/10.1006/bijl.1996.0035>
- Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography - or seeing genes in space and time. *Molecular Ecology*, 10(3), 537–549. <https://doi.org/10.1046/j.1365-294X.2001.01202.x>
- Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary.

- Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 359(1442), 183-195. <https://doi.org/10.1098/rstb.2003.1388>
- Hickerson, M. J., Carstens, B. C., Cavender-Bares, J., Crandall, K. A., Graham, C. H., Johnson, J. B., Rissler, L., Victoriano, P. F., & Yoder, A. D. (2010). Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution*, 54(1), 291–301. <https://doi.org/10.1016/j.ympev.2009.09.016>
- Hoberg, E. P., Galbreath, K. E., Cook, J. A., Kutz, S. J., & Polley, L. (2012). Northern host–parasite assemblages: history and biogeography on the borderlands of episodic climate and environmental transition. *Advances in Parasitology*, 79, 1–97. <https://doi.org/10.1016/B978-0-12-398457-9.00001-9>
- Hoelzel, A. R., & Dover, G. A. (1991). Genetic differentiation between sympatric killer whale populations. *Heredity*, 66, 191–195.
- Holderegger, R., & Wagner, H. H. (2008). Landscape Genetics. *BioScience*, 58(3), 199–207. <https://doi.org/10.1641/B580306>
- Hubbard, J. P. (1969). The relationships and evolution of the *Dendroica coronata* complex. *The Auk*, 86(3), 393–432. <https://doi.org/10.2307/4083405>
- Hunn, E. S., & Beaudette, D. (2014). Apparent sympatry of two subspecies of the white-crowned sparrow, *Zonotrichia leucophrys pugetensis* and *gambelii*, in Washington state. *Western Birds*, 45(2), 132–140.
- Hurst, G. D. D., & Jiggins, F. M. (2005). Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society B: Biological Sciences*, 272(1572), 1525–1534. <https://doi.org/10.1098/rspb.2005.3056>
- Irwin, D. E. (2000). Song variation in an avian ring species. *Evolution*, 54(3), 998-1010. <https://doi.org/10.1111/j.0014-3820.2000.tb00099.x>
- Jarne, P., & Lagoda, P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, 11(10), 424–429. [https://doi.org/10.1016/0169-5347\(96\)10049-5](https://doi.org/10.1016/0169-5347(96)10049-5)
- Jombart, T., Pontier, D., & Dufour, A. (2009). Genetic markers in the playground of multivariate analysis. *Heredity*, 102(4), 330–341. <https://doi.org/10.1038/hdy.2008.130>
- Kendeigh, S. C., & Fawver, B. J. (1981). Breeding bird populations in the Great Smoky Mountains, Tennessee and North Carolina. *The Wilson Bulletin*, 93(2), 218–242.
- Keyghobadi, N., Roland, J., & Strobeck, C. (1999). Influence of landscape on the population genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae). *Molecular Ecology*, 8(9), 1481–1495. <https://doi.org/10.1046/j.1365-294x.1999.00726.x>

- Kingston, T., Lara, M. C., Jones, G., Akbar, Z., Kunz, T. H., & Schneider, C. J. (2001). Acoustic divergence in two cryptic *Hipposideros* species: a role for social selection? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1474), 1381-1386. <https://doi.org/10.1098/rspb.2001.1630>
- Klicka, J.; & Zink, R. (1997). The importance of recent ice ages in speciation: a failed paradigm. *Science*, 277(5332), 1666-1669. <https://doi.org/10.1126/science.277.5332.1666>
- Knowles, L. L. (2000). Tests of pleistocene speciation in montane grasshoppers (*Genus melanoplus*) from the sky islands of western North America. *Evolution*, 54(4), 1337-1348. <https://doi.org/10.1111/j.0014-3820.2000.tb00566.x>
- Knowles, L. L. (2001). Did the pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshoppers. *Molecular Ecology*, 10(3), 691–701. <https://doi.org/10.1046/j.1365-294X.2001.01206.x>
- Lein, M. R., & Corbin, K. W. (1990). Song and plumage phenotypes in a contact zone between subspecies of the white-crowned sparrows (*Zonotrichia leucophrys*). *Canadian Journal of Zoology*, 68(12), 2625-2629. <https://doi.org/10.1139/z90-366>
- Lemmon, E. M. (2009). Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution*, 63(5), 1155–1170. <https://doi.org/10.1111/j.1558-5646.2009.00650.x>
- Lovette, I. J. (2005). Glacial cycles and the tempo of avian speciation. *Trends in Ecology & Evolution*, 20(2), 57–59. <https://doi.org/10.1016/j.tree.2004.11.011>
- MacDougall-Shackleton, E. A., & MacDougall-Shackleton, S. A. (2001). Cultural and genetic evolution in mountain white-crowned sparrows: song dialects are associated with population structure. *Evolution*, 55(12), 2568–2575. <https://doi.org/10.1111/j.0014-3820.2001.tb00769.x>
- MacRae, B. H. (2006). Isolation by resistance. *Evolution*, 60(8), 1551-1561. <https://doi.org/10.1111/j.0014-3820.2006.tb00500.x>
- Manel, S., & Holderegger, R. (2013). Ten years of landscape genetics. *Trends in Ecology & Evolution*, 28(10), 614–621. <https://doi.org/10.1016/j.tree.2013.05.012>
- Manel, S., Schwartz, M. K., Luikart, G., & Taberlet, P. (2003). Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, 18(4), 189–197. [https://doi.org/10.1016/S0169-5347\(03\)00008-9](https://doi.org/10.1016/S0169-5347(03)00008-9)
- Marler, P., & Tamura, M. (1962). Song “dialects” in three populations of white-crowned sparrows. *The Condor*, 64(5), 368–377. <https://doi.org/10.2307/1365545>
- Mathews, L. M., Schubart, C. D., Neigel, J. E., & Felder, D. L. (2002). Genetic, ecological, and behavioural divergence between two sibling snapping shrimp species (Crustacea: Decapoda: *Alpheus*). *Molecular Ecology*, 11(8), 1427–1437. <https://doi.org/10.1046/j.1365-294X.2002.01539.x>

- McNeilly, T., & Antonovics, J. (1968). Evolution in closely adjacent plant populations IV. Barriers to gene flow. *Heredity*, *23*, 205–218.
- Meirmans, P., & Hedrick, P. (2011). Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources*, *11*(1), 5-18. <https://doi.org/10.1111/j.1755-0998.2010.02927.x>
- Montanarin, A., Kaefer, I. L., & Lima, A. P. (2011). Courtship and mating behaviour of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. *Ethology Ecology & Evolution*, *23*(2), 141–150. <https://doi.org/10.1080/03949370.2011.554884>
- Morton, M. (2002). The mountain white-crowned sparrow: migration and reproduction at high altitude. *Studies in Avian Biology*, *24*, 1–236.
- Nabholz, B., Glémin, S., & Galtier, N. (2009). The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evolutionary Biology*, *9*(1), 54. <https://doi.org/10.1186/1471-2148-9-54>
- Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings from the National Academy of Sciences*, *70*(12), 3321–3323. <https://doi.org/10.1073/pnas.70.12.3321>
- Neigel, J. E. (1997). A comparison of alternative strategies for estimating gene flow From genetic markers. *Annual Review of Ecology and Systematics*, *28*(1), 105-128. <https://doi.org/10.1146/annurev.ecolsys.28.1.105>
- Nelson, D. A. (1998). Geographic variation in song of Gambel's white-crowned sparrow. *Behaviour*, *135*(3), 321–342. <https://doi.org/10.1163/156853998793066258>
- Ng, J., Landeen, E. L., Logsdon, R. M., & Glor, R. E. (2013). Correlation between *Anolis* lizard dewlap phenotype and environmental variation indicates adaptive divergence of a signal important to sexual selection and species recognition. *Evolution*, *67*(2), 573–582. <https://doi.org/10.1111/j.1558-5646.2012.01795.x>
- Olah, G., Smith, A. L., Asner, G. P., Brightsmith, D. J., Heinsohn, R. G., & Peakall, R. (2017). Exploring dispersal barriers using landscape genetic resistance modelling in scarlet macaws of the Peruvian Amazon. *Landscape Ecology*, *32*(2), 445–456. <https://doi.org/10.1007/s10980-016-0457-8>
- Orsini, L., Corander, J., Alasentie, A., & Hanski, I. (2008). Genetic spatial structure in a butterfly metapopulation correlates better with past than present demographic structure. *Molecular Ecology*, *17*(11), 2629–2642. <https://doi.org/10.1111/j.1365-294X.2008.03782.x>
- Petren, K. (2013). The evolution of landscape genetics. *Evolution*, *67*(12), 3383–3385. <https://doi.org/10.1111/evo.12278>
- Petrinovich, L. (1985). Factors influencing song development in the white-crowned

- sparrow (*Zonotrichia leucophrys*). *Journal of Comparative Psychology*, 99(1), 15–29. <https://doi.org/10.1037//0735-7036.99.1.15>
- Pillay, N. (2000). Female mate preference and reproductive isolation in populations of the striped mouse *Rhabdomys pumilio*. *Behaviour*, 137(11), 1431–1441. <https://doi.org/10.1163/156853900502655>
- Pillay, N., & Rymer, T. (2012). Behavioural divergence, interfertility and speciation: a review. *Behavioural Processes*, 91(3), 223–235. <https://doi.org/10.1016/j.beproc.2012.08.006>
- Plenderleith, M., Oosterhout, C., Robinson, R. L., & Turner, G. F. (2005). Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology Letters*, 1(4), 411–414. <https://doi.org/10.1098/rsbl.2005.0355>
- Rand, A. L. (1948). Glaciation, an isolating factor in speciation. *Evolution*, 2(4), 314–321. <https://doi.org/10.1111/j.1558-5646.1948.tb02749.x>
- Rissler, L. J. (2016). Union of phylogeography and landscape genetics. *Proceedings of the National Academy of Sciences*, 113(29), 8079–8086. <https://doi.org/10.1073/pnas.1601073113>
- Scheiner, S. M., & Gurevitch, J. (2001). *Design and analysis of ecological experiments*. New York, NY: Chapman & Hall.
- Schlötterer, C. (2004). The evolution of molecular markers — just a matter of fashion? *Nature Reviews Genetics*, 5(1), 63–69. <https://doi.org/10.1038/nrg1249>
- Segelbacher, G., Cushman, S. A., Epperson, B. K., Fortin, M.-J., Francois, O., Hardy, O. J., Holderegger, R., Taberlet, P., Waits, L. P., & Manel, S. (2010). Applications of landscape genetics in conservation biology: concepts and challenges. *Conservation Genetics*, 11(2), 375–385. <https://doi.org/10.1007/s10592-009-0044-5>
- Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters*, 9(5), 615–629. <https://doi.org/10.1111/j.1461-0248.2006.00889.x>
- Selonen, V., & Hanski, I. K. (2003). Movements of the flying squirrel *Pteromys volans* in corridors and in matrix habitat. *Ecography*, 26(5), 641–651. <https://doi.org/10.1034/j.1600-0587.2003.03548.x>
- Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science*, 236(4803), 787–792. <https://doi.org/10.1126/science.3576198>
- Soha, J. A., Nelson, D. A., & Parker, P. G. (2004). Genetic analysis of song dialect populations in Puget Sound white-crowned sparrows. *Behavioral Ecology*, 15(4), 636–646. <https://doi.org/10.1093/beheco/arh055>
- Storfer, A., Murphy, M. A., Evans, J. S., Goldberg, C. S., Robinson, S., Spear, S. F., Dezzani, R., Delmelle, E., Vierling, L., & Waits, L. P. (2007). Putting the

- 'landscape' in landscape genetics. *Heredity*, 98(3), 128–142.
<https://doi.org/10.1038/sj.hdy.6800917>
- Storfer, A., Murphy, M. A., Spear, S. F., Holderegger, R., & Waits, L. P. (2010). Landscape genetics: Where are we now? *Molecular Ecology*, 19(17), 3496–3514.
<https://doi.org/10.1111/j.1365-294X.2010.04691.x>
- Sunnucks, P. (2000). Efficient genetic markers for population biology. *Trends in Ecology and Evolution*, 15(5), 199–203. [https://doi.org/10.1016/S0169-5347\(00\)01825-5](https://doi.org/10.1016/S0169-5347(00)01825-5)
- Takagi, M. (2011). Vicariance and dispersal in the differentiation of vocalization in the Ryukyu Scops Owl *Otus elegans*. *Ibis*, 153(4), 779–788.
<https://doi.org/10.1111/j.1474-919X.2011.01163.x>
- van Strien, M. J., Holderegger, R., & van Heck, H. J. (2015). Isolation-by-distance in landscapes: considerations for landscape genetics. *Heredity*, 114(1), 27–37.
<https://doi.org/10.1038/hdy.2014.62>
- Via, S. (2001). Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology & Evolution*, 16(7), 381–390. [https://doi.org/10.1016/S0169-5347\(01\)02188-7](https://doi.org/10.1016/S0169-5347(01)02188-7)
- Wan, Q., Wu, H., Fujihara, T., & Fang, S. (2004). Which genetic marker for which conservation genetics issue? *Electrophoresis*, 25, 2165–2176.
<https://doi.org/10.1002/elps.200305922>
- Wang, I. J. (2010). Recognizing the temporal distinctions between landscape genetics and phylogeography. *Molecular Ecology*, 19(13), 2605–2608.
<https://doi.org/10.1111/j.1365-294X.2010.04715.x>
- Weber, J. L., & May, P. E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics*, 44(3), 388–396.
- Whitlock, M. C., & McCauley, D. E. (1999). Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. *Heredity*, 82, 117–125.
- Wiens, J. J. (2004). Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution*, 58(1), 193–197. <https://doi.org/10.1554/03-447>
- Wright, S. (1943). Isolation by Distance. *Genetics*, 28, 114–138.
- Zhang, D., & Hewitt, G. M. (2003). Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology*, 12(3), 563–584.
<https://doi.org/10.1046/j.1365-294X.2003.01773.x>
- Zink, R. M. (1982). Patterns of genic and morphologic variation among sparrows in the genera *Zonotrichia*, *melospiza*, *junco*, and *Passerella*. *The Auk*, 99(4), 632–649.
- Zink, R. M., & Barrowclough, G. F. (2008). Mitochondrial DNA under siege in avian

phylogeography. *Molecular Ecology*, 17(9), 2107–2121.
<https://doi.org/10.1111/j.1365-294X.2008.03737.x>

Zink, R. M., Dittmann, D. L., & Rootes, W. L. (1991). Mitochondrial DNA variation and the phylogeny of *Zonotrichia*. *The Auk*, 108(3), 578-584. <https://doi.org/10.2307/4088098>

Zoppoth, P., Koblmüller, S., & Sefc, K. M. (2013). Male courtship preferences demonstrate discrimination against allopatric colour morphs in a cichlid fish. *Journal of Evolutionary Biology*, 26(3), 577–586. <https://doi.org/10.1111/jeb.12074>

CHAPTER TWO: RANGEWIDE AND LOCAL BARRIERS TO GENE FLOW IN WHITE-CROWNED SPARROWS (*ZONOTRICHIA LEUCOPHRYS*)

2.1 Introduction

Dispersal of individuals from one habitat patch to another is perhaps a simple concept, but it can have vast consequences for gene flow, genetic diversity, adaptation, and spatial population dynamics of a species (Bowler & Benton, 2005; Olah et al., 2017; Ronce, 2007). Although dispersal is widely acknowledged as an important ecological process in evolution and the focus of many theoretical studies, simplistic assumptions about dispersal are still made (Bowler & Benton, 2005). It is essential to consider the unique dispersal capabilities and life histories of each species and determine what may act as dispersal barriers (Geffen et al., 2004). The influence of landscape on population connectivity and gene flow facilitated by individual dispersal is well-studied in the field of landscape genetics (Coulon et al., 2004; Holderegger & Wagner, 2008; Lee-Yaw et al., 2009; Rissler, 2016). Landscape features found in gaps between suitable habitat patches can be strong barriers to dispersal for organisms with low mobility or habitat specialization (Geffen et al., 2004). It was therefore often assumed that for organisms with high mobility, the landscape features in habitat gaps would hinder dispersal to a much smaller degree, especially organisms capable of flight (Olah et al., 2017).

It cannot be assumed that species capable of flight will have high population connectivity because they can cross or circumvent physical barriers like anthropogenic development, large bodies of water, or mountain ranges, which are insurmountable to flightless species (Holderegger & Wagner, 2008). Differentiation can also occur in

contiguous populations due to microclimates within a habitat area and behavioural differences unrelated to physical barriers. For example, mitochondrial DNA differentiation indicated a cessation of gene flow between rufous-collared sparrow (*Zonotrichia capensis*) populations locally adapted to low versus high elevation microclimates on one mountain slope (Cheviron & Brumfield, 2009). In another example, genetic differences in three South African gerbil species, one allopatric (*Tatera afra*) and two sympatric (*T. leucogaster* and *T. brantsii*), were a result of differentiation in physical courting behaviours (Dempster et al., 2010). Furthermore, differences in courting displays were much greater between the sympatric species than either of the comparisons with the allopatric species (Dempster et al., 2010). When the contemporary barriers have been identified at both rangewide and local scales, it is then important to distinguish them from the possible historical evolutionary processes responsible for pre-existing structure, and other stochastic forces unrelated to dispersal (e.g. mutation, genetic drift; Holderegger & Wagner, 2008).

Population genetics uses molecular markers to determine allele frequencies and how evolutionary forces are shaping the population (Avice, 1994; Epps et al., 2007). Barriers known to exist between populations can be disruptors of dispersal and result in genetic structure (Holderegger & Wagner, 2008). Combining genetic data with high-resolution GIS models allow for the identification of specific barriers (historically and contemporary) impeding dispersal and gene flow (Epps et al., 2007).

The white-crowned sparrow (*Z. leucophrys*) is an ideal model species for testing predictions that various barriers are responsible for current genetic structure. They are a

widespread North American passerine whose range encompasses areas of the continent once covered by ice sheets, spans multiple barrier types, and is composed of subspecies that display different song dialects and migration behaviours. Five subspecies form two groups: (1) the *Z. l. pugetensis* and *Z. l. nuttalli* populations breeding on the Pacific Coast from California to British Columbia and (2) the *Z. l. leucophrys-oriantha-gambelii* populations breeding elsewhere throughout North America (Figure 1.1). This study will focus on populations of mountain white-crowned sparrow (*Z. l. oriantha*) in the Rocky Mountains of Colorado, a contact zone with *Z. l. oriantha* and Gambel's white-crowned sparrows (*Z. l. gambelii*) in the Rocky Mountains area of Alberta, British Columbia, and Puget Sound white-crowned sparrows (*Z. l. pugetensis*) sampled in the Cascade Mountains of Oregon (Figure 2.1).

In white-crowned sparrows, song has a large impact on mate choice and ultimately on gene flow, though results vary by subspecies (Austen & Handford, 1991; Chilton et al., 1990; Lein & Corbin, 1990; Soha et al., 2004). Song dialects are associated with genetic structure in *Z. l. nuttalli*, but not *Z. l. gambelii* (MacDougall-Shackleton & MacDougall-Shackleton, 2001) suggesting a differential role among *Z. leucophrys* subspecies. Lein and Corbin (1990) report hybridization of *Z. l. gambelii* and *Z. l. oriantha* associated with a continuous shift in plumage which interestingly did not coincide with an abrupt change in song dialect type (Lein & Corbin, 1990). Consequently, biogeographic research will be important for understanding how differentiation is occurring in these *Z. leucophrys* subspecies if song dialect is not always restricting gene flow.

In addition to plumage and song, the population genetic structure of many avian species can be significantly influenced by migratory behaviour (Licona-Vera & Ornelas, 2017). Previous research of migratory birds has revealed genetic differences on either side of mountains in more than one species (Clegg et al., 2003; Hull et al., 2008; Ruegg, 2007); however, the cause of this can be difficult to distinguish from other factors such as mixing on wintering grounds or migratory pathways. Since many birds migrate to shared wintering grounds, it is possible for juveniles to mix with other populations or subspecies, follow them to different breeding sites, and create gene flow between sites (González-Solís et al., 2007; Ruegg & Smith, 2002). The circuitous migratory pathways employed by many North American passerines have been explained as the avoidance of taxing barrier crossings (e.g. mountain ranges and resource-poor habitat; Alerstam, 2001), but Ruegg and Smith (2002) counter that divergent migratory pathways may be artefacts of a species' post-glacial recolonization route instead of direct avoidance of contemporary barriers. Each *Z. leucophrys* subspecies displays different average migratory distances (Morton, 2002), so there is great potential in using this species to investigate and compare the influence of migration on genetic structure.

To date, most barrier studies on white-crowned sparrows are done with the non-migratory *Z. l. nuttalli* in California (Cortopassi & Mewaldt, 1965; Hafner & Petersen, 1985). *Z. l. oriantha* and *Z. l. gambelii* are migratory, but they display strong wintering and breeding site fidelity (Cortopassi & Mewaldt, 1965; Morton, 2002), and as a result we predict that populations may show similar levels of population structure to non-migratory species. Mountains are well known as a barrier to dispersal between populations on either side of a range for many species (Fjeldsâ et al., 2011; Hooge, 2003;

Robertson et al., 2009). However, if a species with breeding sites on either side of an impenetrable mountain range have mixing on wintering grounds and low philopatry, this could lead to the false conclusion that the mountains are not a barrier. Alternatively, if breeding populations occur on opposite sides of a mountain range simply due to different migratory pathways, wintering grounds, or strong breeding site fidelity, then mountains may falsely appear as a barrier. Our goal is to investigate the influence of the Rocky Mountains on the genetic structure in *Z. leucophrys*.

The Rocky Mountain range where both *Z. l. oriantha* and *Z. l. gambelii* are found is a broad north-south belt of mountains interspersed with rift valleys and glacier-carved basins (Cruden & Hu, 1999; Holland, 1976). To our knowledge, few studies have been undertaken to determine how dispersal across the Rockies and gene flow are affected by geographic and climatic barriers in *Z. leucophrys*. Using genetic data, we will first propose historical patterns of genetic structure caused by the Pleistocene glaciations, then test the hypotheses that: (1) rangewide genetic structure will be present due to distance between populations and mountains restricting dispersal, especially in *Z. l. oriantha*, since site fidelity is purported to be stronger in this subspecies as opposed to *Z. l. gambelii* (Nelson, 1998), and (2) genetic structure will be present at a local scale due to a reduction in gene flow along elevational transects, as has been reported in other mountain avian populations (Cheverson & Brumfield, 2009).

The evolutionary history of *Z. leucophrys* has had little attention since the mid 1900's when Rand (1948) proposed one hypothesis of the Pleistocene refugia of *Z. leucophrys* subspecies and Banks (1964) proposed another. Rand (1948) suggested that the

subspecies' ranges correspond to four North American refugia: 1) south of the ice sheets near the West Coast (*Z. l. nuttalli-pugetensis*), 2) south in the Rocky Mountain area (*Z. l. oriantha*), 3) northwest by the Yukon-Bering Sea (*Z. l. gambelii*), and 4) in the northeast (*Z. l. leucophrys*) (Figure 1.3). Later studies supported Rand's (1948) hypothesis with observations of shared song dialect patterns by *Z. l. oriantha* and *Z. l. nuttalli-pugetensis* (Baptista & King, 1980), and paleobotanical data supporting continuous boreal forest south of the ice sheets that would have connected their habitat (Hubbard, 1969). Banks (1964) suggested an alternate hypothesis that relied on a reddish back plumage phenotype shared by *Z. l. oriantha* and *Z. l. leucophrys* and pale lores of *Z. l. gambelii* and *Z. l. nuttalli-pugetensis* (Figure 1.4). He suggested that the ancestors of *Z. l. gambelii* and *Z. l. oriantha* once formed a continuous red-backed population ranging from Alaska to the Mackenzie Mountains which later split due to selection for lore colour between the high latitude (pale-lored *Z. l. gambelii*) and low latitude (black-lored *Z. l. oriantha*) (Banks, 1964). Despite the years that have passed and rise of new landscape genetics and phylogeography methods, to our knowledge, no study has attempted to compare these theories with contemporary genetic structure.

It is important to understand all the influences and barriers on population genetics in montane systems, since they are an important ecological zone for biodiversity (Fjeldsâ et al., 2011). Using a widespread and ubiquitous avian species can contribute to an understanding of barriers which could be affecting the gene flow in species that are of conservation concern. For populations of endangered species isolated after habitat fragmentation and degradation, understanding the degree of population isolation is critical for conservation and management decisions (Dubey et al., 2011).

2.2 Materials and Methods

2.2.1 Sample collection

Blood samples were collected from adult birds from 10 sampling areas total in the Rocky Mountains area of central and southern Alberta (JAS = Jasper, BA = Banff, BV = Beaver Mines, CNP = Crowsnest Pass, WT = Waterton Lakes National Park, and LE = Lethbridge), and central and southern British Columbia (MK = Mackenzie, FTSJ = Fort St. James, RV = Revelstoke, and OK = Okanagan). These birds were caught using 12 m mistnets with song playback over two breeding seasons (2015-2016). A small blood sample, < 100 μ L, was obtained from the brachial vein of each individual. Blood samples were added to 99% ethanol and stored at -20°C. Each bird was banded with a numbered metal band to prevent resampling. To supplement the field sampling, 11 feather samples from the Royal Alberta Museum and 17 tissues samples from the Royal British Columbia Museum were obtained from the same 10 sites used in this study (see Appendix 1).

We collected a total of 128 birds for our study from the museums and the field. An additional 85 blood samples were obtained from adult birds along a 415 m elevational transect north of the Rocky Mountain Biological Laboratory in Gunnison County, Colorado (38.9563802°N, -107.0106015°W) (CO) from Dr. Ross Conover (Paul Smith's College, NY). Another 121 blood samples from *Z. l. pugetensis* nestlings were obtained from four elevation transects averaging 400 m in the Coast Mountains of Oregon (OR) from Dr. Jim Rivers, Oregon State University. These additional samples from CO and OR added five populations to the study. The birds at the lowest and highest elevations in four OR transects were consolidated into two populations (OR-L and OR-H, for low and high

populations, respectively), and the CO birds categorized into low, moderate, and high elevation populations (CO-L, CO-M, and CO-H, respectively) (Figure 2.1).

2.2.2 DNA extraction, amplification, and sequencing

Genomic DNA was extracted from blood-ethanol mix (10 μ L), tissue (~1 μ g), or feather samples (basal portion of feather shaft) using a modified Chelex procedure (Burg & Croxall, 2001). Two fragments of nuclear and mitochondrial DNA were amplified using polymerase chain reaction (PCR). A 576 base pair (bp) fragment of the mitochondrial control region (CR) was amplified using the primers Finch Siskin CR L85 (5'-GGCACATCCTTGTTTCAGGT-3') and H807 (5'-CAGTGCCAAGTTTGMGACGA-3') for 66 samples. A 709 bp fragment of the Z-linked Aldolase B6 gene was amplified with forward and reverse primers (AldoB6F 5'-AAGATCACCAGCACAACACCCTCT-3' and AldoB6R 5'-AGGCTGCTGTGGAAAGACAGCTTA-3') for 54 samples including 31 males and 23 females.

Polymerase chain reactions (PCRs) were performed using a 25 μ L reaction volume containing 10X Green GoTaq® Flexi buffer (Promega), 0.2 mM dNTP (Fisher Scientific), 2 mM MgCl₂, 0.5 μ M primer, 0.5 U GoTaq® Flexi DNA polymerase (Promega) and 1 μ L of genomic DNA (diluted 1:5 for feathers, 1:10 for blood and tissue). The thermocycling profile for amplification of CR was an initial cycle of 2 mins at 94°C, 45 s at 50°C and 1 min at 72°C; then 31 cycles of 30 s at 94°C, 45 s at 50°C and 60 s at 72°C; and one final extension at 72°C for 5 mins followed by 4°C for 20 s. For AldoB6, the same conditions were used except 2.5 mM MgCl₂ was used and the annealing

temperature was 62°C. Three microliters of the PCR product were electrophoresed on a 0.8% agarose gel stained with ethidium bromide to visualize the PCR product. Amplified DNA was sent to Genome Quebec for Sanger sequencing (McGill University, QC).

The samples were returned as chromatograms and visually aligned and checked using MEGA 6.0 (Tamura et al., 2013). Haplotypes were reconstructed for each individual, accounting for the sex-linked nature of AldoB6.

2.2.3 Microsatellite genotyping

A subset of individuals were initially screened with 26 passerine loci, 10 of which are designed specifically for *Z. leucophrys* and the remaining which are known to cross-amplify in other passerines. In total, 12 loci produced PCR products, of which 9 were polymorphic: (ZoleA2, ZoleC11, ZoleH02 (Poesel et al., 2009); Escu6 (Hanotte et al., 1994); Gf01, Gf06 (Petren, 1998); Pocc2 (Bensch et al., 1997); VeCr05 (Stenzler et al., 2004); and YW16 (Dawson et al., 1997) (Appendix 2). PCR mixes were prepared in 10 µL reaction volumes containing Colourless GoTaq® Flexi buffer (Promega), 0.2 mM dNTP (Fisher Scientific), and 0.8 mM or 1 mM MgCl₂, 0.5 µM forward and reverse primer, 0.05 µM fluorescent M13 tag, 0.5 U GoTaq® Flexi DNA polymerase (Promega), and 1 µL of genomic DNA (diluted 1:5 for feathers, 1:10 for blood and tissue) (Appendix 2). The standard amplification profile consisted of a 2 minute denaturation at 94°C, 45 s at 50 °C and 1 min at 72°C; seven cycles of: 1 min at 94°C, 30 s at T_{m1} and 45 s at 72°C; 31 cycles of; 30 s at 94°C, 30 s at T_{m2} and 45 s at 72°C, and finishing with five minutes at 72°C (Appendix 2). For Escu6, the second step was decreased from 31 to 25 cycles. Products were denatured and run on a 6% polyacrylamide gel on a LI-COR 4300 DNA

Analyser (LI-COR Inc., Lincoln, NE, USA). Three positive controls of known sizes were amplified for each locus to aid in scoring and ensure consistent amplification. The scoring for each locus was checked by two separate people to reduce errors in the final dataset. A subset of each population was genotyped a second time to ensure consistent scores at each locus. In total, 334 DNA samples from 15 populations were chosen for this study, after suspected family groups and samples with more than 50% missing data were removed.

2.2.4 Genetic diversity analyses

To determine genetic diversity in AldoB6 and CR samples, DnaSP v5 (Rozas et al., 2017) was used to calculate haplotype diversity (H_d) for CR and AldoB6 sequences (Table 2.1).

To determine genetic relatedness of birds from all sampling locations and among subspecies of *Z. leucophrys*, a phylogenetic tree of CR haplotypes was created using the program PAUP* 4.0a152 (Swofford, 2002). The CR sequences used for this tree were a combination of this study's 66 CR sequences and 35 sequences from Weckstein et al. (2001; GenBank AF305744-AF305776), a study which compares genetic similarities between *Z. leucophrys* and closely related golden-crowned sparrow (*Z. atricapilla*) and is rooted with sequences from the Harris' sparrow (*Z. querula*) and white-throated sparrow (*Z. albicollis*). Using the same parameters as Weckstein et al. (2001), a tree was created using neighbor joining (NJ) Kimura-2-parameter model, validated with 500 bootstrap replicates.

The data from 66 CR and 85 AldoB6 sequences (after phasing the 54 AldoB6 sequences) were used to construct three sets of maximum likelihood haplotype networks with 500 iterations in PopART 1.7 (Bandelt et al., 1999). The first two networks compare overall haplotypes frequencies in 14 sampling areas for CR and 11 for AldoB6. Two more networks were made to show haplotype frequencies contained within the three *Z. leucophrys* subspecies, and the final networks were made to compare haplotypes on either side of the Rocky Mountains by grouping all populations on the east side of the range and comparing them to the group of populations on the west side. For population comparisons east and west of the Rocky Mountains, all five of the CO and OR populations were omitted due to the absence of a population at a similar latitude on the opposite side of the mountain range.

Pairwise F_{ST} values were calculated in Arlequin v3.5 and significance determined using 999 permutations (Excoffier & Lischer, 2010). The p-values were corrected for multiple tests by a modified False Discovery Rate (FDR) method, a multiplier accounting for the proportion of incorrectly rejected null hypotheses among all rejected nulls (Benjamini & Yekutieli, 2005).

For microsatellite diversity analysis, all samples were checked for errors and null alleles with MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004). Of the 334 samples genotyped for microsatellites, 328 remained for use in analysis after those with > 50% missing data were removed. GENEPOP v4.2 (Rousset, 2008) was used to detect deviations from Hardy-Weinberg equilibrium and linkage disequilibrium using default parameters. GenALEx v6.5 (Peakall & Smouse, 2006) was used to calculate observed and

expected heterozygosity, number of alleles per locus, and private alleles for all 15 populations. The pairwise F'_{ST} matrix and p-values were also calculated using the microsatellite data from all 15 populations. The F'_{ST} statistic was used because the theoretical maximum of traditional F_{ST} comparisons is one when there are two alleles (Wright, 1949). F'_{ST} values are modified F_{ST} values which are standardized by the maximum achievable F_{ST} when multiple alleles are present (Meirmans & Hedrick, 2011).

It was recognized that certain sparrows caught for this study originated from different habitat types, which we characterized as alpine coniferous (AC), riparian deciduous (RD), disturbed-gas plant (D-G), and disturbed-townsite (D-T) ecosite types. The AC ecosite includes the JAS, BA, RV, OK, and MK populations, RD includes BV, LE, some CNP and WT sparrows, and D-G includes the remaining CNP sparrows, and OK, FTSJ, and the remaining WT sparrows form the D-T ecosite (Figure 2.5). Samples were grouped into these four ecosites and pairwise F'_{ST} values were calculated.

2.2.5 Population genetic structure

STRUCTURE v2.3 is a non-spatial Bayesian clustering method which uses a Markov chain Monte Carlo (McMC) simulation to assign individuals to K clusters based on multilocus genotype data (Pritchard et al., 2000). The posterior probability values are used to infer the appropriate number of genetic clusters in the dataset. The program was used with correlated allele frequencies and the admixture model with sampling locations as *locpriors*. The *locpriors* option allows sampling location to aid the program in assigning individuals to clusters, without creating structure where it does not exist. Ten independent runs were performed with 50,000 burn-ins and 200,000 McMC repetitions

for K values from 1-10 for all samples. The output data of each of these runs were processed in STRUCTURE HARVESTER v0.6.94 (Earl & vonHoldt, 2012) to determine the highest delta K value which determines the optimal number of clusters. The seven out of ten northern populations showing admixture were run separately with the same settings to determine if additional structure was present. To determine if ecosite type causes genetic structure, we ran the ten northern populations separately since they allow for a comparison of genetic information across different ecosites with a more continuous sampling method. For the ecosite analysis, we set the four ecosites as *locpriors* and used the same number of burn-ins and MCMC repetitions previously mentioned for K = 1-6. Likewise, independent runs were executed to test for elevational differences in the three CO and two OR populations.

A second Bayesian clustering algorithm that incorporates spatial information, TESS v2.3, was also run (Chen et al., 2007). TESS assumes there are interactions between proximate individuals, so spatial dependencies are applied at the individual level. This model accounts for the fact that individuals from spatially continuous populations are more likely to share cluster associations with neighbours (Chen et al., 2007). STRUCTURE has been found to yield the best performance when testing for clinal variation, while TESS is useful in detecting migrants and very recent contact zones between weakly differentiated populations (Chen et al., 2007). TESS v2.3 was run for K values from 1-10 using 5000 burn-ins and 100,000 sweeps, with a spatial interaction parameter (Ψ) of 0.6. The optimal K was determined by choosing the K where the deviance information criterion (DIC) value began to plateau with no increases with higher numbers of clusters (Chen et al., 2007). As with STRUCTURE, the same groupings for

investigating admixture, ecosite types, and elevations in CO and OR were run separately for K values from 1 – 6 with the same burn-ins and sweeps previously mentioned to uncover possible substructure.

2.2.6 Multivariate analyses

To further assess genetic structure and test the ecosite substructure detected in previous Bayesian clustering analyses, a Principal Coordinate Analyses (PCoA) and a Principal Component Analysis (PCA), respectively, were executed in RStudio (RStudio Team 2015). For the PCoA, a matrix of multivariate genetic distances (F'_{ST} values) between all populations was plotted against their geographic coordinates. A three-dimensional PCoA graph was made in RStudio using the Scatterplot3D (Ligges & Mächler, 2003) to visualize the first three principal coordinates responsible for the most variation. For the PCA, morphometrics of bill length, depth, and width (mm), tarsus length (cm), wing length (cm), and total weight (g) of each bird sampled from the AC, RD, and D-G ecosite types were summarized with a standard multivariate ordination using the ggbiplot package in RStudio (Wickham, 2009).

2.2.7 Barrier characterization

To begin directly characterizing the specific barriers present among and between populations, BARRIER v2.2 software was used. This program uses a geometric approach known as a Delaunay triangulation and Monmonier's algorithm to calculate the location and direction of barriers across a landscape (Manni et al., 2004). Once the triangulation of all 15 populations' location is obtained using the latitude and longitude coordinates, the edges are associated with pairwise distance measures according to the F'_{ST} genetic

distance matrix calculated in GenAIEx v6.5 (Peakall & Smouse, 2006). The largest distances measured between pairs of populations determine the barrier location.

2.2.8 Species Distribution Models

Finally, GIS software was used to make a species distribution model (SDM) to predict the ecological niche of each *Z. leucophrys* subspecies which is required to construct least-cost path (LCP) and least-cost corridor (LCC) models to display their most likely dispersal routes. These models provide visualizations of potential barriers affecting dispersal across matrices of climate and landscape. The SDM was made in ArcMap 10.1 (ESRI, Redlands, CA) using SDMtoolbox v1.1, a landscape genetics toolbox for many pre- and post-processing steps of SDMs and other geospatial analyses (Brown, 2014). We acquired 552 occurrences of *Z. l. gambelii*, 762 of *Z. l. oriantha*, and 1285 of *Z. l. pugetensis* subspecies from the Global Biodiversity Information Facility (<http://data.gbif.org/>, accessed 13 January 2018). Occurrences were limited to breeding populations by filtering for data collected from mid-April through August. Any occurrences reported by non-academic institutions or reported before 1980 were excluded to ensure accuracy. Contemporary environmental data were downloaded from the WorldClim Global Climate Dataset (v2, <http://worldclim.org/version2>) from the standard set of 19 bioclimatic variables. This set of 19 variables includes different measures of precipitation and temperature averaged from the years 1970-2000. In addition to the BIOCLIM variables, a MODIS-based Global Land Cover layer was taken from the USGS Land Cover Institute which defines 16 land cover categories (Broxton et al., 2014). The final layer added was a global Digital Elevation Model (DEM) with a resolution of 1 km developed by the USGS Earth Resources Observation and Science Center (EROS)

(<http://cec.org/tools-and-resources/map-files/elevation-2007>). The land cover and DEM layers were included for more accuracy in predicting habitat for the *Z. leucophrys* subspecies in the SDM.

The SDMtoolbox was used to process the data before the final models were produced. First, the occurrence data were spatially rarefied to account for sampling bias and added to our study samples, leaving 216 occurrences of *Z. l. gambelii*, 285 of *Z. l. oriantha*, and 348 of *Z. l. pugetensis* to be used in the analyses. Next, the 19 environmental variables, land cover, and elevation layers were checked for autocorrelation with a 0.9 threshold, so that all but one of each autocorrelated pair could be removed from the analyses. All variables and layers were clipped to the extent of North America. Each subspecies' ecological niche was then modelled using Maxent v3.4 (Phillips et al., 2006). The settings used in Maxent to model *Z. l. gambelii* were a regularization multiplier of 5, 10 replicates of 500 iterations with cross-validation using the elevation, land cover, and the resulting 10 non-correlated environmental variables (variables 1, 2, 3, 4, 8, 12, 14, 15, 18, 19; see Table 2.3). The settings, layers and variables used for *Z. l. oriantha* and *Z. l. pugetensis* were the same, except both used a regularization multiplier of 2 instead of 5. The best fit model for each subspecies was selected by optimal corrected Akaike's Information Criterion (AICc) and the area under curve (AUC) (Warren & Seifert, 2011).

2.2.9 *Least-cost path models*

Once the SDM models were made for each subspecies, it was possible to model the most likely dispersal routes and dispersal costs. This allows a visual representation of how landscape and climate features may act as barriers and explain genetic differentiation between populations. The SDM was inverted to create a friction layer using the SDMtoolbox, and the geographic coordinates for each sampling site. The LCPs were calculated by mapping the routes between each population pair with the lowest friction values. An additional step is required to calculate the LCC; the resistance values of the LCPs are weighted into low, mid, and high classes (Brown, 2014). These classes were defined using the ‘percentage of LCP’ method where low, mid, and high were set as 1%, 2%, and 5% of LCP values, respectively. The output is a heat map of the dispersal network between all populations, with warm colors indicating low resistance and cool colors indicating high resistance to dispersal. Finally, the linear relationship between pairwise F'_{ST} values of sampling sites and geographic distance using a Mantel test procedure in GenAlEx to test for isolation by distance (IBD). These IBD results were compared with similarly calculated Mantel tests of pairwise F'_{ST} values and the along-path cost of the least-cost paths calculated in ArcMap 10.1. The along-path cost is the total sum of the friction values that characterize the LCP and allows for the testing of Isolation by Resistance (IBR) between sample sites (Etherington, 2011). LCPs were calculated for each subspecies so three sets of IBR calculations were compared with the IBD values between all populations, within each subspecies, east and west of the Rockies, and each ecosite type (provided the sample size was sufficient).

2.2.10 Historical Species Distribution Models

Once the SDMs were made using Maxent, it was possible to use each subspecies' preferred habitat conditions in combination with historical climate data to project suitable habitat during the mid-Holocene, LIG, and LGM periods. Different theories of Pleistocene refugia and post-glacial expansion for *Z. leucophrys* (Figures 1.3 and 1.4; Rand 1948; Banks 1964) were tested with our models. Climate data for mid-Holocene and LGM were sourced from MIROC-ESM Global Climate Models (Watanabe et al., 2011), and LIG data from Otto-Bliesner et al. (2006). Land cover and elevation layers were not available for these historical periods. Variables included in the historical climate datasets are monthly average minimum temperature ($^{\circ}\text{C} * 10$), monthly average maximum temperature ($^{\circ}\text{C} * 10$), monthly total precipitation (mm), and the 19 bioclimatic variables mentioned previously.

2.3 Results

2.3.1 Genetic diversity

The phylogenetic tree recreated from the Weckstein et al. (2001) study plus sequences from this study using a Kimura 2-parameter neighbor-joining model shows a high amount of paraphyly and sharing of identical sequences across multiple sample sites and across subspecies (Figure 2.2). The low bootstrap values indicate low phylogenetic support when *Z. leucophrys* subspecies are partitioned into distinct clades. The tree shows that the northern populations tend to be more genetically similar to each other than the southern populations. *Z. l. oriantha* can be found fairly uniformly throughout the tree, sharing sequence similarity with all other subspecies. The majority of *Z. l. gambelii* individuals share sequence similarity with *Z. l. leucophrys*, while *Z. l. pugetensis* and *Z. l.*

leucophrys do not. *Z. l. nuttalli* can only be found once in the tree sharing sequence similarity with *Z. l. pugetensis* and *Z. l. oriantha*.

2.3.2 Population genetic structure

The mitochondrial Control Region has a total of 17 haplotypes with six shared among multiple populations and the other 11 haplotypes restricted to a single population (Figure 2.3). Of these 11 haplotypes, one was from WT, RV, and CO-M each, two from OR-L, and three from BA and CO-H (Figure 2.3). Haplotype diversity was low in five populations ($H_d = 0$ in JAS, LE, FTSJ, OK, and MK) and high in five ($H_d > 0.7$ in BA, WT, RV, CO-H, and OR-L; Table 2.1). The Z-linked AldoB6 locus has a total of 30 haplotypes, with eight shared among multiple populations and 22 restricted to single populations. WT, CO-L, CO-M, and CO-H each have one unique haplotype, BA and MK each have two unique haplotypes, three from OR-H, four from OR-L, and seven from BV (Figure 2.4). Haplotype diversity is low in three populations ($H_d = 0$ in LE, RV, and OK) and high in eight ($H_d > 0.7$ in BA, BV, WT, CO-L, CO-M, CO-H, OR-L and OR-H). The *Z. l. gambelii* subspecies has the lowest H_d at both loci (CR: 0.56, AldoB6: 0.84), *Z. l. pugetensis* has the highest H_d for CR ($H_d = 0.83$), and both *Z. l. pugetensis* and *Z. l. oriantha* similarly high for AldoB6 ($H_d = 0.90$ and 0.91 , respectively; Table 2.1).

All 328 individuals from 15 sample sites were genotyped at nine microsatellite loci. The average number of different alleles per population ranged from 2.33 (OK) to 8.44 (OR-L & OR-H), observed heterozygosity (H_o) ranged from 0.43 (OK) to 0.75 (JAS & CO-H), and expected heterozygosity (H_e) from 0.39 (OK) to 0.73 (CO-L & CO-H; Table 2.1). Among the sample sites with the largest number of private alleles are WT and

CO-L with PA = 3, and OR-H the largest number with PA = 4. The CO elevations have the highest level of allelic richness with AR = 4.32, 4.56, and 4.53 for CO-L, -M, and -H respectively (Table 2.1).

The maximum likelihood haplotype networks constructed for 17 CR haplotypes show a similar segregation of haplotypes into the northern (AB and BC) and southern (OR and CO) populations (Fig 2.3a). Many of the northern individuals share the same CR haplotype (71% of the 35 total northern individuals), and most unique haplotypes from the northern populations originate from BA. One unique haplotype is shared by BA and RV on opposite sides of the Rocky Mountains. There is also one haplotype shared by most CO individuals, some OR and one WT individual, and two main haplotypes unique to OR. The same haplotype network showing subspecies groups indicates a nearly equal number of *Z. l. oriantha* and *gambelii* possessing the main northern haplotype (35% and 46%, respectively) and five haplotypes are restricted each to *Z. l. oriantha* and *Z. l. pugetensis*, but only two *Z. l. gambelii*.

The haplotype networks for AldoB6 were constructed with sequences from 11 out of 15 populations (Figure 2.4), the majority of which come from BV, MK, CO, and OR, and does not display a north and south pattern like the CR network. Out of 30 haplotypes, this network contains one main common haplotype found in 32% of 85 total sequences from eight populations (excluding BA, RV, and OR-H). The next most common haplotypes are one shared primarily by CO and BC sample sites, and one restricted to the OR elevations. The same network showing subspecies groups indicates that all three

subspecies share the main haplotype, but 15 out of 30 total haplotypes are restricted to *Z. l. oriantha*, eight to *Z. l. pugetensis*, and only three to *Z. l. gambelii*.

Pairwise F_{ST} values corresponding to CR haplotype data confirm the segregation of mitochondrial DNA into north and south groupings (Figure 2.3, Appendix 3). None of the 36 comparisons among the nine northern populations are significant. Out of all the CO or OR elevation comparisons with each other, only CO-H is significantly different from OR-H. In the 45 comparisons made between the southern and northern populations, 76% were significantly different and the three CO populations were not significantly different from BA, WT, OK, MK (except for CO-H with BA and WT). All comparisons between three subspecies are significant (Fig 2.3a). Unlike CR data, the F_{ST} values corresponding to AldoB6 haplotype data do not show any distinct north and south patterns (Figure 2.4, Appendix 4). In the 15 comparisons within northern populations, 40% are significantly different, and 20% are significantly different within the 10 comparisons between southern populations. BA, WT, CO-L and OR-H are the most similar to all other populations (BA has no significant comparisons and WT, CO-L and OR-H have one each), and MK is the least similar (eight significant comparisons). *Z. l. oriantha* and *Z. l. gambelii* are significantly different from *Z. l. pugetensis* but not from each other.

The total population-wide pairwise F_{ST} values for 328 microsatellite genotypes from all 15 populations ranged from -0.18 (JAS:RV) to 0.58 (BA:CO-M) (Appendix 5), and 94 out of 105 (89.5%) pairwise differences were significant after FDR correction for multiple tests (Table 2.2). The largest number of non-significant comparisons occur

within the Alberta populations (4 out of 15; 26%). Notably, JAS is not significantly different from RV, OK, and MK populations on the opposite side of the Rocky Mountain range but it is from WT, LE, CNP, and BA, its neighbours on the same side of the mountain range. However, aside from MK, the populations compared across the Rocky Mountains with non-significant values have $n \leq 7$ (RV, JAS, BA, and OK) (Table 2.1). These small sample sizes could be the reason the genotypes are not significantly different. Also notable is that the elevations are significantly different from each other within CO and within OR.

2.3.3 Bayesian clustering analyses

The optimized number of genetic clusters for microsatellite data from all 15 populations was determined to be $K = 4$ (Figure 2.5) by the highest delta K (ΔK) output (not shown) from the combined STRUCTURE runs. The four clusters are as follows: 1) JAS, RV, and MK, 2) BA, BV, CNP, WT, LE, FTSJ, and OK, 3) CO-L, CO-M, and CO-H, and 4) OR-L and OR-H. The first two clusters are not as clearly distinguished as the third and fourth. This admixture along with a bimodal ΔK plot prompted further hierarchical analyses to test for substructure by running the first and second clusters in STRUCTURE without CO and OR samples. When the seven admixed northern populations (BA, BV, CNP, WT, LE, FTSJ, and OK) were run on their own to test for substructure, $K = 2$ clusters were detected but no clear pattern emerged. No substructure was found within either the CO or OR elevations. Finally, analyses were run using all ten northern populations with the alpine coniferous (AC), riparian deciduous (RD), disturbed-gas plant (D-G), and disturbed-townsite (D-T) ecosite types as *locpriors*, the ΔK plot

indicated $K = 2$ clusters with substructure corresponding to ecotypes. The clusters were composed of 1) JAS, BA, RV, MK, and some OK, and 2) BV, CNP, WT, LE, FTSJ, and some OK, with the first cluster containing the AC ecosite, and the second combining the RD, D-G, and D-T ecosites. The D-G group shows a small portion of ancestry with the AC cluster (Figure 2.5a).

To cross-reference the STRUCTURE results, a spatial Bayesian clustering analysis was performed in TESS (Figure 2.5). The optimal number of clusters was $K = 3$, as determined by the highest DIC value. The clusters were similar except it grouped OR with some northern populations: 1) JAS, RV, OK, and MK, 2) BA, BV, CNP, WT, LE, FTSJ, OR-L, and OR-H, and 3) CO-L, CO-M, and CO-H. When running the first two clusters independently (excluding CO) to test for substructure, the DIC plots (not shown) indicated $K = 2$ clusters each: 1) JAS, RV, OK and 2) MK for the first run, and 3) BA, BV, CNP, WT, LE, and FTSJ, and 4) OR for the second run. TESS also uncovered substructure when testing for elevational differences in the CO sample sites. When the three CO elevations were run independently, the DIC plot showed no increases in K after $K = 2$ clusters, with CO-M forming its own cluster apart from CO-L and CO-H. No substructure was found between OR-L and OR-H (Figure 2.5b). The lack of clustering within OR populations is not surprising given the low but significant F'_{ST} values (Appendix 5) as STRUCTURE and TESS are not as sensitive as F'_{ST} tests. A run of the northern populations sorted by ecosite types uncovered $K = 2$ clusters, with one comprised of the AC ecosite (JAS, RV, OK, and MK) and the second containing the RD, D-G, and D-T ecosites (BA, BC, CNP, WT, LE, WT, OK and FTSJ). In TESS, BA

clusters with the second group instead of the AC ecosite as it did in STRUCTURE analyses.

2.3.4 Multivariate analyses

The PCA using morphometric data from all northern populations (AB and BC) showed two distinct ecosite groupings similar to the STRUCTURE and TESS output with the first and second axes accounting for 32.8% and 18.7% of the variation, respectively. The six phenotypic traits of the sparrows caused individuals to group into the AC or RD/D-G ecosites. The samples from the disturbed group only include the D-G population in CNP (Figure 2.6).

The PCoA using the F'_{ST} values (Table 2.2) shows the first and second axes accounting for 34.5% and 23.6% of the variation, with the third axis explaining 16.1% (Figure 2.7). When all three axes are displayed in a three-dimensional plot, groupings observed in the other analyses are reiterated. The southern populations, CO and OR, group apart from the northern populations, similar to the CR haplotype network, STRUCTURE, and TESS outputs. JAS is separated from all the other AB populations and aligned closest with OK, MK, RV, FTSJ and CO-M on axes 2 and 3 (except RV on axis 3). This is reflected in TESS and STRUCTURE results, which group JAS with RV and MK. The non-significant F'_{ST} comparisons indicate that JAS, OK, RV, and MK have similar genetic composition.

The BARRIER program used a combination of genetic and geographic distance matrices to detect ten discontinuities between populations (Figure 2.8). If directed, the

program's algorithm could compute as many barriers as there are populations, therefore the barriers displayed are the first ten possible barriers. Barriers isolate the CO and OR locations from the northern sites and each other which is supported by CR haplotype data, Bayesian clustering, and PCA (Figures 2.3a, 2.5, and 2.7). Two barriers separate all three CO elevations from each other which is supported by significant F'_{ST} values between all three CO elevations (Table 2.2), but not STRUCTURE or TESS. A barrier separates JAS from the other Alberta populations of BA, BV, CNP, WT, and LE, yet leaves it connected with the RV and MK populations on the west side of the Rocky Mountains in British Columbia. This pattern is also supported by the F'_{ST} values, STRUCTURE, TESS, and the PCoA (Table 2.2, Figures 2.5 and 2.7). BARRIER predicted that CNP and FTSJ are each entirely isolated from all other populations, which corresponds to the fact that both are significantly different in 100% of their pairwise F'_{ST} comparisons (Table 2.2).

2.3.5 Species Distribution Models and dispersal routes

The contemporary SDMs made for each subspecies closely follow their known distributions in North America (Figure 1.1). The layers that made the top contributions to model construction (after which the remaining variables show a large drop in influence for model prediction) were annual mean temperature (29.4%), isothermality (15.7%), elevation (14.3%), temperature seasonality (12.6%), and precipitation of warmest quarter (12.2%) for *Z. l. gambelii*; elevation (71.7%), precipitation of warmest quarter (10.6%), isothermality (8.2%) for *Z. l. oriantha*; and precipitation of coldest quarter (61.9%), precipitation seasonality (10.5%), precipitation of warmest quarter (10.5%), and elevation (8.2%) for *Z. l. pugetensis* (Table 2.3). The layers contributing to the SDM for *Z. l. gambelii* yielded an AUC value of 0.828, where 0.5 means the model fit is no better than

random, and values close to one are a good fit. The layers contributing to the SDMs for *Z. l. oriantha* and *Z. l. pugetensis* yielded high AUC values of 0.996 and 0.995, respectively.

The contemporary SDMs were used to calculate the least-cost path (LCP; Appendix 8) and least-cost corridor (LCC; Figure 2.10) models for visualization of dispersal connectivity between sites within the breeding ranges of *Z. l. gambelii* and *Z. l. oriantha*. The *Z. l. gambelii* model shows the strongest dispersal routes on the east edge of our sampling area in British Columbia, and highlights pathways through the Rockies between the British Columbia and Alberta populations. In the *Z. l. gambelii* model, the southern Alberta populations of LE, WT, CNP, and BV have stronger dispersal routes through the mountains to OK than they do to reach each other on the same side. FTSJ and MK also have paths of low resistance connecting to OK, but not to each other. JAS is more strongly connected to the corridors in BC than to other Alberta sites. The *Z. l. oriantha* model shows low north-south routes between CO, through the southern Alberta sites and extending up to MK. Unlike the *Z. l. gambelii* model, JAS is more connected to BA than any other population. The three CO populations have low-cost dispersal routes to travel northwards along the Rockies, but resistance increases as the route nears the southern Alberta sites of WT, LE, CNP, and BV (Figure 2.10), and then southern Alberta sites have a low-cost route extending north to MK. There are higher resistance connections between the east and west populations, with FTSJ having a slight connection to MK. The LE, OK, and FTSJ populations do not have much lower dispersal potential to other populations, which was also detected by the isolating barriers surrounding these sites (Figure 2.8).

2.3.6 Historical Species Distribution Models

The historical SDMs tested hypotheses of glacial refugia for *Z. leucophrys* subspecies by Rand (1948) (Figure 2.11). *Z. l. gambelii* had potential suitable habitat in the Beringian refugium area (Alaska and northern coastal BC) during the LIG, then in the Pacific Northwest during the LGM and Holocene. *Z. l. gambelii* habitat is now restricted to the Rockies and northwest Canada (Figure 1.1) even though the SDM marks most of North America as suitable habitat (Figure 2.11). *Z. l. oriantha* had the highest likelihood of suitable habitat in the refugia found in the southern Rocky Mountain range during the LIG, expanded to northern U.S. and Canada and south to Mexico in the LGM, were restricted to western mountains in the Holocene, and now inhabit western U.S. and Canadian mountain ranges. *Z. l. pugetensis* maintains its high likelihood of a west coast refugium for all three time periods and now can be found in the mountains of western U.S. and along the Pacific Coast (Figure 2.12).

2.3.7 Isolation by distance or by resistance

Comparison of F'_{ST} values with Euclidean distances between populations, and F'_{ST} values with the least-cost path (LCP) distances as determined by landscape resistance values were made (Table 2.3). IBD showed the strongest correlations when explaining genetic differentiation involving *Z. l. gambelii*. IBD had significant and high R^2 values for all sites within *Z. l. gambelii* breeding range ($R^2 = 0.54$, $p = < 0.01$) and the sites where *Z. l. gambelii* were sampled in this study ($R^2 = 0.72$, $p = 0.01$). IBD was also best for explaining the genetic differences between populations on the east versus the west sides of the Rocky Mountains ($R^2 = 0.35$, $p = < 0.01$). *Z. l. oriantha* appear to be more affected by landscape resistance. While IBD has significant correlation values between sites

within *Z. l. oriantha* breeding range ($R^2 = 0.64$, $p = 0.01$) and all sites where *Z. l. oriantha* were sampled in this study ($R^2 = 0.72$, $p = 0.01$), the significant LCP correlation values show IBR also contributed to genetic differentiation between *Z. l. oriantha* populations within their breeding range and between sample sites in this study ($R^2 = 0.17$, $p < 0.01$, and $R^2 = 0.25$, $p = 0.02$, respectively). When testing for what determines connectivity between individuals sampled in different ecosites, the results show that the overall genetic differentiation between alpine coniferous (AC) sites is caused by IBD ($R^2 = 0.56$, $p < 0.01$), but *Z. l. gambelii* connectivity between ecosite types containing coniferous forest (AC and D-G) is much better explained by landscape resistance ($R^2 = 0.87$, $p = 0.04$). The results for explaining differentiation between the riparian deciduous (RD) ecosite sites is unclear, as neither IBD nor IBR yielded significant values for subspecies. There were not enough sites within the *Z. l. pugetensis* subspecies (OR-L and OR-H) to perform this analysis, or to test between the disturbed – gas plant (D-G) or disturbed – townsite (D-T) populations.

2.4 Discussion

2.4.1 Evolutionary history of Z. leucophrys subspecies

The first objective of our study was to investigate the influences of historical processes during and after the Pleistocene on the genetic composition of three subspecies of white-crowned sparrows. Previous research has emphasized the important role of historical processes in shaping the genetic architecture in contemporary taxa (Avice, 2000; Bermingham et al., 1992; Hewitt, 2004; Knowles, 2001; Rand, 1948). From data on allozymes, morphometrics, and mitochondrial DNA, Zink et al. (1991) concluded that speciation within the *Zonotrichia* (one of the five species being *Z. leucophrys*) occurred in

the Pleistocene, but before 140,000 years ago (Morton, 2002). Little recent work has been done to elucidate the subsequent evolutionary history and glacial refugia of white-crowned sparrows since the two theories were debated in the mid-1900s by Rand (1948) and Banks (1964) (Figure 1.4). We combined analyses from our study and other literature to provide a more robust investigation of these two possible evolutionary histories. We found varying levels of support for Rand's (1948) hypothesis of four North American refugia for *Z. leucophrys* subspecies, including *Z. l. gambelii* in the northwestern Beringia and *Z. l. leucophrys* in the northeast with post-Pleistocene expansion east and westward to come into contact, and *Z. l. nuttalli-pugetensis* with their Pacific Coast refugium and later expanding northward in parallel with *Z. l. oriantha* from its refugium in the southern Rockies (Rand, 1948). The shared CR haplotypes between *Z. l. leucophrys* and the majority of *Z. l. gambelii* in the phylogenetic tree follow Rand's suggestion that these subspecies expanded west and eastward from their refugia (Figure 1.4). The CR haplotype data from Weckstein et al., (2001) used to make a phylogenetic tree (Figure 1.4) showed that *Z. l. pugetensis* and *Z. l. nuttalli* share one haplotype with *Z. l. oriantha* but never with *Z. l. leucophrys*, also following Rand's suggestion of a shared southern boreal refugia during the Pleistocene. If Bank's hypothesis is correct, we would have expected *Z. l. gambelii* to be more similar to *Z. l. nuttalli-pugetensis* (not *Z. l. leucophrys*) since he proposed that the coastal subspecies diverged directly from *Z. l. gambelii*. Bank's hypothesis would also result in the two coastal subspecies having a subset of *Z. l. gambelii* haplotypes and microsatellite alleles, but in our microsatellite data *Z. l. gambelii* never clustered with *Z. l. pugetensis* in STRUCTURE or TESS (Figures 2.5A and B). We can postulate these ideas, but the low bootstrap values in the tree, and lack of microsatellite pattern lead us to conclude that our study is inconclusive to support Banks

or Rand. It also reiterates how in some species, subspecies are not always good indicators of overall population structure (Zink & Barrowclough, 2008). It is possible that the *Z. leucophrys* subspecies did not spend enough time in Pleistocene refugia to diverge significantly in mitochondrial CR DNA. It would be a benefit to repeat this analysis with more samples (especially *Z. l. leucophrys* and *Z. l. nuttalli*), and with other molecular markers like nuclear internal transcribed spacer 2 (ITS2) which is commonly used in tandem with a mtDNA marker to differentiate between species and detect introgression, incomplete lineage sorting, and hybridization (Hebert et al., 2003). Alternatively, our historical SDMs support the idea of southern refugia shared by *Z. l. pugetensis* and *Z. l. oriantha* and the use of Beringia refugium by *Z. l. gambelii*, but these are models based on occurrence data not genetic data.

2.4.2 Rangewide barriers to gene flow – distance, landscape, or subspecies?

Mantel tests were used to compare the influences of IBD and IBR at various spatial scales for our study's second objective to determine the rangewide barriers between populations. We hypothesized that distance and landscape barriers (i.e. the Rocky Mountains) influence *Z. l. gambelii* and *Z. l. oriantha* genetic structure. The Mantel tests confirmed that distance has a large influence on gene flow in these subspecies. We also found that habitat variability is causing resistance to dispersal (IBR) in addition to the Rocky Mountain range.

2.4.2.1 Distance

IBD is more influential than IBR when describing the genetic differentiation between sites within the breeding ranges of *Z. l. gambelii* and *Z. l. oriantha*, even though

IBR values account for the many different vegetation types and montane extremes of elevation and climate which have often proved highly restrictive in other species (Coulon et al., 2004; DuBay & Witt, 2014; Dubey et al., 2011; Funk et al., 2005; Gonzalo-Turpin & Hazard, 2009; Lee-Yaw et al., 2009; Olah et al., 2017). This result is not entirely unique. Even though dispersal corridors exist between the majority of extant populations of grey wolves, a highly vagile species, the effect of IBD was more significant than IBR at a continental scale (Geffen et al., 2004). Like Geffen et al. (2004), we performed smaller scale Mantel tests between sites where *Z. l. gambelii* and *Z. l. oriantha* occur to ensure that patterns of IBD versus IBR were not confounded by corresponding large-scale climate or habitat zones. Both *Z. l. gambelii* and *Z. l. oriantha* showed the genetic differentiation best explained by IBD as well as some influence by IBR at smaller spatial scales in *Z. l. oriantha* that was previously undetected in total rangewide tests (Table 2.5).

It is possible that IBD is playing a large role in describing genetic differentiation in *Z. leucophrys* because the habitat between populations is largely homogenous, and the small-but-significant influence of IBR is present because of the genetic structure of the FTSJ population. Although it is the most western population of *Z. l. gambelii*, both STRUCTURE and TESS analyses grouped FTSJ with the populations east of the Rockies. Huge clearcut operations in the FTSJ area have been conducted to recover pine beetle-infested forest in British Columbia since the 1950s (Adams, et al., 2015; Proulx & Kariz, 2005). Since it is known that *Z. leucophrys* establish territories quickly in clearcuts (Hunn & Beaudette, 2014), a population could have colonized the FTSJ area and subsequently experience a bottleneck when continuous habitat disturbance created fragmented central BC habitats and restricted gene flow with other populations, leaving a

pocket of eastern microsatellite alleles. The restricted gene flow between FTSJ and other eastern populations is reinforced by the strong north-south pathways in the *Z. l. gambelii* ENM. The landscape resistance between FTSJ and other populations could be driving the slight IBR patterns.

2.4.2.2 *Landscape barriers and resistance*

When testing our second hypothesis of mountains as a rangewide barrier, CR and AldoB6 haplotype data from the ten northern populations did not show clear structure corresponding to location on either side of the Rockies, but the higher resolution microsatellite data in F'_{ST} and Bayesian analyses showed that most population comparisons on the east versus west of the Rocky Mountains are significant, except in three (JAS with RV, OK, and MK, and BV with RV). BARRIER analysis also shows isolation of LE, CNP, and BA from their proximate neighbours of BV and WT but not between JAS on the eastern of the Rockies and RV or MK to the west (Figure 2.8). Dispersal is restricted between most populations by the Rockies in AB and BC. It is possible that the genetic similarity of JAS to RV, OK, and MK is due to the Yellowhead Pass, a major low elevation corridor through the Rocky Mountains used for dispersal by multiple other species including mountain pine beetles (Robertson et al., 2009) and mountain caribou (Hooge, 2003). The genetic similarity of BV and RV could potentially be explained by dispersal through the Rocky Mountain Trench, but more sampling would be needed in areas between these populations along this route for verification. The environmental data that contributed to the subspecies SDMs and LCPs also confirm that mountains are a barrier to *Z. leucophrys* (Table 2.3). While temperature is the strongest contributor for the *Z. l. gambelii* SDM, and precipitation is for *Z. l. pugetensis*, elevation

has an important influence on the distribution models of all three subspecies, especially *Z. l. oriantha*.

Mountains are a landscape barrier affecting rangewide genetic structure in *Z. leucophrys*, but according to Mantel tests, landscape resistance (IBR) is also important. IBR is correlated with genetic structure at a rangewide scale in *Z. l. oriantha* but not in *Z. l. gambelii*, perhaps because *Z. l. gambelii* is more of a generalist and *Z. l. oriantha* is more of a habitat specialist. Multiple studies note that there are multiple factors influence *Z. l. oriantha* life history that do not influence *Z. l. gambelii* life history. *Z. l. gambelii* show a negligible response to many environmental cues except for photoperiod which triggers gonadal development in highly predictable breeding season conditions and timing (Maney et al., 1999; Wingfield et al., 1996). In contrast, *Z. l. oriantha* shows robust responses to temperature and snowpack conditions in addition to photoperiod due to consistently unpredictable breeding season in montane habitats (Wingfield et al., 2003). Hahn et al., (1995) proposes that *Z. l. gambelii* should be categorized as “spatial opportunists” because banding data shows they have low site-fidelity and after spring migration will continue to search a wide area until they discover suitable nesting sites, while *Z. l. oriantha* have strong site-fidelity and at the end of migration will wait until specific conditions are ideal for nesting. The expansive suitable habitat across North America in the contemporary SDM of *Z. l. gambelii* compared to those of the other two subspecies supports this proposition (Figure 2.9). In sympatric species of butterflies, the genetic structure of generalist species was unaffected by the landscape matrix, but the specialist species was highly sensitive to fine scale habitat features (Engler et al., 2014). Since *Z. l. oriantha* migrate a shorter distance than *Z. l. gambelii*, and inhabit more

extreme montane niches, we could expect *Z. l. oriantha* to be more specialized and thus affected by landscape features.

2.4.2.3 *Subspecies*

The subspecies delineations in *Z. leucophrys* are supported by distribution, migratory strategy, and phenotype (Cortopassi & Mewaldt, 1965; Morton, 2002; Rand, 1948), but our genetic analyses and some song dialect studies do not always support strong subspecies differences. Our northern samples originate mostly from a known contact zone of *Z. l. oriantha* and *Z. l. gambelii* in the Rocky Mountains of southern AB and BC (Lein & Corbin, 1990). In addition to Lein & Corbin's (1990) data, we have evidence that hybridization is occurring in this area by the three phenotypic hybrids we captured in OK, CNP, and MK; however, the small sample sizes do not allow for analysis of genetic patterns of hybrids. For better investigation of *Z. leucophrys* subspecies hybridization patterns, more samples need to be collected from outside the known contact zone. The fact that our CO samples are significantly different in microsatellite data compared to all the northern individuals may be due to the sampling gap, or because the CO sparrows breed outside of the hybrid zone and have the genetics of the pure parental form of *Z. l. oriantha*. Further research on assortative mating behaviour and more thorough sampling should be performed to find patterns of introgression or clinal variation in environment that could be creating selection pressure on hybrid or parental forms (Barton & Hewitt, 1989).

Behaviour (namely song dialect for *Z. leucophrys*; Marler and Tamura, 1962; Baptista and King, 1980) may be an additional potential rangewide barrier to gene flow in

both contact zones and hybrid zones. According to Nelson (1999), *Z. l. gambelii* have an observed lack of song dialects unlike all other subspecies. This could influence or reflect the generalist nature of this subspecies' SDM and facilitate the observed hybridization with *Z. l. oriantha* in the contact zone of the Rocky Mountain area of AB and BC (Lein & Corbin, 1990). To tease apart the effect of song dialect on population connectivity and genetic structure for *Z. l. gambelii* and *Z. l. oriantha*, we would have to do further tests. Lipshutz et al. (2017) did exactly this with *Z. l. pugetensis* and *Z. l. nuttalli*. They characterized song divergence by measuring behavioural responses to dialect playback experiments within and outside the contact zone and compared this data to genomic data. They found that song acts as a reproductively isolating mechanism reducing gene flow between admixed populations of these subspecies (Lipshutz et al., 2017). This type of analysis is outside the scope of our study but could be informative future work for contemporary population structure and gene flow in these subspecies. According to our subspecies SDMs and LCPs (*Z. l. pugetensis* LCP not shown), each subspecies' distribution is affected differently by environmental variables (Table 2.3) and displays different population connectivity and dispersal patterns through the landscape matrix (Figures 2.9 and 2.10). The question remains whether the current subspecies distributions are the result of ancient range expansion patterns (Rand, 1948), or just a consequence of recently diverged regions of a ubiquitous species due to local adaptation (Richardson et al., 2014). Although their evolutionary histories differ, with the low bootstrap support for subspecies in our phylogenetic tree, mixed subspecies representation in microsatellite-based Bayesian clustering analyses, and varied influence of song dialect in literature, it may be worthwhile to perform more analyses with different molecular markers, additional sample sites, and larger sample sizes of each subspecies to see if there are distinguishing

genetic signatures of subspecies we have missed in this study. In the absence of novel genetic signatures of subspecies, it is possible that subspecies divergence was too recent to detect, or that they are not genetically distinct.

2.4.3 Local barriers to gene flow

2.4.3.1 Ecosite type

When performing rangewide Bayesian analyses, we also tested for substructure on a smaller scale according to the characterization of habitat type at each sampling site. We found clustering that corresponds to alpine coniferous and riparian deciduous ecosite types and that disturbed habitats fell into the riparian deciduous cluster (Figure 2.5). This was substantiated by multivariate PCA, using combined measures of body size and weight (Figure 2.6). Morphological differences have been observed in many other species when distributed across heterogeneous landscapes, including plants (Gram and Sork, 2001), insects (Hamer et al., 2003), aquatic invertebrates (Etter et al., 2005), and terrestrial vertebrates (Barley et al., 2015). This shows that local environmental conditions and landscape type play an important role in determining population genetic structure of *Z. leucophrys*.

The same phenomenon of larger body size in deciduous compared to coniferous habitats was observed in male pied flycatchers, interpreted as the consequence of competition (Lundberg et al., 1981). The distinct clustering of *Z. leucophrys* genetic and morphological characteristics into coniferous or deciduous habitats is surprising because not much data is available for *Z. leucophrys* in deciduous habitats. Most descriptions of *Z. leucophrys* habitat include thickets and streamside shrubbery (*Z. l. gambelii*), meadows of

high sagebrush near the conifer timberline (*Z. l. oriantha*), and brushy clearcuts in coniferous forest (*Z. l. pugetensis*) (Dunn et al., 1995; Morton, 2002). We found that sparrows from deciduous habitats have a larger body type observed in multiple measurements (wing, bill size, tarsus length, and weight), while sparrows from coniferous habitats have smaller body size in all the same metrics. Since aspen understory and canopy support greater invertebrate abundance than conifers (Rumble et al., 2001), it is possible that sparrow body size is greater in deciduous habitats due to a greater abundance of high quality food. Insects are the primary food source utilized by parents to feed their offspring during the critical growth period of young birds (Dunn et al., 1995, Rumble et al., 2001). There has been increasing evidence of the importance of hybrid poplar zones in influencing arthropod abundance and genetic differentiation and movement decisions of dependent insectivorous avian species (i.e. black-capped chickadees, *Poecile atricapillus* display genetic structure according to zones of riparian poplar species and their hybrids; Adams & Burg, 2015).

Another possibility is that white-crowned sparrows experience different predation pressures in each habitat. LaManna et al. (2015) found that the predation risk of chipping sparrows decreased as coniferous vegetation increased, despite an increase in predator density (Martin et al., 1993). The sparrows could offset a greater predation risk because of improved camouflage for nests and improved ease of thwarting predators (LaManna et al., 2015, Martin et al., 1993). In the more open arrangement of vegetation in deciduous forest understory, perhaps predation risk is greater and the stronger, large-bodied *Z. leucophrys* have greater survival success, or alternatively that the greater food availability

allows the maintenance of larger body sizes despite greater predation risk (Seitz et al., 1993).

More research must be conducted on our body size and habitat correlation, as there are other contradicting observations of species maintaining lower body mass in high-resource habitats. One example of this is due to a trade-off of starvation versus predation risk. Blackbirds (*Turdus merula*) maintained body mass below maximum because spending less time foraging reduced predation risk, and lower body mass allowed faster take-off and more agility to avoid predators (Macleod et al., 2005). Another example is when birds make trade-offs of brood size versus offspring quality. When average body mass found in house sparrow (*Passer domesticus*) populations in high-resource urban habitats had lower body size, Shochat (2004) suggested either overexploitation of food reduced foraging success of less competitive individuals, or selection favoured large broods at the expense of offspring quality because even low-quality offspring had high chances of survival in the high-resource habitat.

Something to be considered in our study is that the smaller *Z. leucophrys* sparrow morphometrics in conifer habitats could be confounded somewhat by the fact that the majority of our conifer habitat samples were also from high elevations. The greater energy costs of living in harsher climates of the high elevation sites could explain the lower body size of sparrows sampled from those areas. Extra-pair paternity rates in Emberizid sparrows decrease with increasing elevation as males invest more parental effort and resources into raising their offspring and less to maintaining sexually selected traits for seeking extra-pair copulations (Bonier et al., 2014). This could explain why

body size is smaller at our high elevation conifer habitats. Contrary to our expectation, individuals from habitats affected by anthropogenic disturbance (D-G and D-T populations) did not cluster differently from the other habitats. Multiple studies have observed anthropogenic disturbance events leading to population declines by increasing predation risk perception, reducing survival and reproductive success and decreased access to food and nesting sites (Gill et al., 1996; Kendeigh & Fawver, 1981; McCleery et al., 2006; Selonen & Hanski, 2003). However, anthropogenic disturbance does not seem to be a detriment to *Z. leucophrys*. Hunn & Beaudette (2014) reported that *Z. l. pugetensis* individuals were capitalizing on disturbed habitats, using clearcuts to expand their range from the western side of the Cascade Mountains across to the lower eastern slopes just in the last 35 years. A large number of our own samples came from breeding territories in clearcuts (29 from MK, 4 from RV, 121 from OR).

Interestingly, the individuals sampled from habitats disturbed by construction with the gas plant and townsites clustered with the riparian deciduous ecosite type in STRUCTURE and TESS (Figure 2.5), but gas plant individuals clustered with the alpine coniferous ecosite according to morphology (Figure 2.6). The gas plant construction opened an area of dense coniferous forest allowing deciduous species to advance, but it is still surrounded by dense coniferous forest with coniferous clearcuts nearby where we sampled other sparrows. These clearcut sparrows also grouped with the deciduous ecotype but showed a larger portion of ancestry shared with the coniferous ecotype group (Figure 2.5). Perhaps the individuals with deciduous microsatellite genotypes colonized this disturbed area by identifying familiar habitat type, but the local montane environmental conditions cause selection for an alpine coniferous body phenotype.

Without the morphological data for townsite individuals, we cannot make a similar comparison between morphology and genetic structure. Maybe the birds sampled from D-G and D-T have not lived with disturbance long enough for the effects to be reflected in genetic structure and morphology that differentiated from the other riparian deciduous and alpine coniferous ecosites. It is also possible that, although the D-G individuals group with riparian deciduous genotypes are displaying phenotypic plasticity to develop a larger body type for the cooler alpine coniferous ecotype (as is known to happen in multiple species inhabiting cooler areas; Ashton, 2002).

2.4.3.2 Elevation

With regards to our final hypothesis on elevation, only a few of the most sensitive analyses using F'_{ST} comparisons from microsatellite data (BARRIER, pairwise F'_{ST} , and TESS) were able to detect significant differentiation between the proximate populations along elevational transects. TESS was able to detect CO elevation substructure, where the CO-M population had some individuals forming a unique cluster (Figure 2.5). The clustering is weak, but noticeable compared to STRUCTURE which detected no differences. The cause for CO-M to have separate genetic structure from both the CO-L and CO-H populations is not clear, but could possibly be caused by the unique sample site. To access CO-M from *Z. leucophrys*-dense CO-L requires passage from a river valley of rich biodiversity and plentiful shrub nesting habitat, through a long, narrow, rocky, and barren mountain pass. The CO-M site is a unique and isolated meadow of short willow and open grassy areas surrounded by steep and densely forested mountain slopes. The CO-H sites for sampling *Z. l. oriantha* are in neighbouring alpine meadows far upslope, often separated by more dense coniferous forest unsuitable for *Z. leucophrys*

habitat. The patchiness of habitat among the CO elevations could explain why CO-M sparrows are beginning to show either signs of microgeographic adaptation or reproductive isolation, especially since this subspecies shows sensitivity to landscape resistance. This local adaptation could also explain why the CO populations differentiated from northern populations in microsatellite data and not as much with sequence data. Microsatellite analyses are much more sensitive to short term changes in genetic structure, such as the selection pressure on populations which recently colonized an area (Cheviron & Brumfield, 2009). The OR elevations only appear to have genetic differentiation in pairwise F'_{ST} analysis (Appendix 5), which may be due to the small elevational difference (430 m), or because all samples were from disturbed habitat (clearcuts). To detect if elevation causes microgeographic structure as we hypothesized, it would be much better to have larger elevational differences.

2.4 Conclusions

Our study shows the importance of considering the combination of evolutionary history and barriers at multiple spatial scales to fully understand contemporary population genetic structure of widespread species. High levels of genetic differentiation were found in white-crowned sparrow populations of three subspecies in western North America using nuclear and mitochondrial sequence data and microsatellite markers. Understanding the evolutionary history of the *Z. l. gambelii*, *Z. l. oriantha*, and *Z. l. pugetensis* subspecies is important for discerning the effects of contemporary barriers from genetic signatures of isolating mechanisms initiated thousands of years ago. From a rangewide perspective, it is clear that geographic distance is an isolating mechanism for white-crowned sparrows, but using high resolution microsatellite data with landscape genetic

analyses revealed barriers on the smaller scales of ecosite type and elevational transects. Bayesian cluster analysis and resistance modelling revealed genetic structure corresponding to the degree of habitat specialization shown by each subspecies. The research presented here is one of few using a comprehensive approach to the historical and contemporary barriers to gene flow at rangewide and local scales. Further study is necessary to detect possible structure due to microgeographic differences along larger elevation transects, particularly in the montane *Z. l. oriantha* subspecies and habitat specialist *Z. l. pugetensis*.

Table 2.1: Number of samples sequenced (n) at each sampling site and for each subspecies (ID) for control region (CR) and Aldolase B (AldoB6) sequences and microsatellite genotypes. The number of haplotypes (Hn) and haplotype diversity (Hd) were calculated in DnaSP v5. Microsatellite samples were screened at nine loci with average number of different alleles (Na), number of private alleles (PA), allelic richness (AR), observed heterozygosity (Ho), and expected heterozygosity (He) reported. For AldoB6 number of sequences, n* refers to the number of chromosomes.

Population	ID	CR sequences			AldoB6 sequences			Microsatellites					
		n	Hn	Hd	n*	Hn	Hd	n	Na	PA	AR	Ho	He
Jasper National Park	JAS	4	1	0.00	-	-	-	6	3.89	1	3.10	0.75	0.64
Banff National Park	BA	7	5	0.90	2	2	1.00	7	4.56	0	3.32	0.71	0.64
Beaver Mines	BV	4	1	0.00	12	11	0.98	13	5.67	2	3.93	0.58	0.69
Crowsnest Pass	CNP	-	-	-	-	-	-	10	5.33	2	3.71	0.60	0.62
Waterton Lakes National Park	WT	4	3	0.83	4	3	0.83	19	6.44	3	4.13	0.60	0.70
Lethbridge	LE	3	1	0.00	4	1	0.00	21	7.22	2	4.05	0.68	0.67
Fort St. James	FTSJ	4	1	0.00	-	-	-	11	3.44	0	2.70	0.47	0.52
Revelstoke	RV	5	4	0.90	2	1	0.00	4	3.33	0	3.02	0.59	0.54
Okanagan	OK	2	1	0.00	1	1	0.00	4	2.33	0	2.14	0.43	0.39
Mackenzie	MK	2	1	0.00	18	6	0.68	29	7.44	2	4.21	0.64	0.68
Colorado - Low elevation	CO-L	4	2	0.50	7	5	0.83	23	7.44	3	4.32	0.74	0.73
Colorado - Mid elevation	CO-M	4	2	0.50	6	3	0.73	32	7.22	2	4.56	0.67	0.70
Colorado - High elevation	CO-H	7	5	0.90	9	5	0.86	28	7.33	0	4.53	0.75	0.73
Oregon - Low elevation	OR-L	11	6	0.84	16	7	0.81	61	8.44	2	4.01	0.65	0.71
Oregon - High elevation	OR-H	5	2	0.60	4	4	1.00	60	8.44	4	4.01	0.61	0.71
Population total:		66	17	0.80	85	30	0.93	328	5.90	23	3.72	0.63	0.64
<i>Z. l. gambelii</i>	GWCS	15	5	0.56	15	6	0.84	67	10.11	5	7.72	0.64	0.71
<i>Z. l. oriantha</i>	MWCS	24	9	0.76	47	19	0.91	115	11.44	13	7.76	0.70	0.76
<i>Z. l. pugetensis</i>	PSWS	16	8	0.83	20	10	0.90	121	9.78	12	7.80	0.63	0.71
Subspecies total:		55	17	0.83	82	30	0.92	303	10.44	30	7.76	0.66	0.73

Table 2.2: Pairwise F'_{ST} results from nine microsatellite loci for 15 populations. Significant p-values after FDR correction for multiple tests are in red ($p < 0.001$), orange ($p = 0.002-0.009$), and yellow ($p = 0.01 - 0.05$) and non-significant comparisons (NS) are white. See Appendix 5 for all F'_{ST} values.

	JAS	BA	BV	CNP	WT	LE	FTSJ	RV	OK	MK	CO-L	CO-M	CO-H	OR-L	OR-H
JAS															
BA	•														
BV	NS	NS													
CNP	•	•	•												
WT	•	NS	NS	•											
LE	•	•	•	•	•										
FTSJ	•	•	•	•	•	•									
RV	NS	•	NS	•	•	•	•								
OK	NS	•	•	•	•	•	•	NS							
MK	NS	•	•	•	•	•	•	NS	•						
CO-L	•	•	•	•	•	•	•	•	•	•					
CO-M	•	•	•	•	•	•	•	•	•	•	•				
CO-H	•	•	•	•	•	•	•	•	•	•	•	•			
OR-L	•	•	•	•	•	•	•	NS	•	•	•	•	•		
OR-H	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Table 2.3: Table of the land cover (LC), elevation (Elv), and 10 non-correlated WorldClim environmental variables (Layer numbers) and their percent contribution to the SDM made for each subspecies. Sample sizes are indicated by n.

<i>Z. l. gambelii</i> SDM (n = 216)		
Layer	Variable	Contribution (%)
1	Annual mean temperature	29.4
3	Isothermality	15.7
Elv	Digital elevation model (DEM) layer 1 km resolution	14.3
4	Temperature seasonality (standard deviation *100)	12.6
18	Precipitation of warmest quarter	12.2
8	Mean temperature of wettest quarter	4.9
15	Precipitation seasonality (coefficient of variation)	4.7
14	Precipitation of driest month	4.3
12	Annual precipitation	1.9
2	Mean diurnal range (mean of monthly (max temp – min temp))	0
LC	MODIS land cover	0
19	Precipitation of coldest quarter	0
<i>Z. l. oriantha</i> SDM (n = 285)		
Elv	Digital elevation model (DEM) layer 1 km resolution	71.7
18	Precipitation of warmest quarter	10.6
3	Isothermality	8.2
8	Mean temperature of wettest quarter	4.0
19	Precipitation of coldest quarter	3.8
4	Temperature seasonality (standard deviation *100)	1.0
2	Mean diurnal range (mean of monthly (max temp - min temp))	0.5
15	Precipitation seasonality (coefficient of variation)	0.5
1	Annual mean temperature	0.2
LC	MODIS land cover	0.1
14	Precipitation of driest month	0
12	Annual precipitation	0
<i>Z. l. pugetensis</i> SDM (n = 348)		
19	Precipitation of coldest quarter	61.9
15	Precipitation seasonality (coefficient of variation)	10.5
18	Precipitation of warmest quarter	10.5
Elv	Digital elevation model (DEM) layer 1 km resolution	8.2
8	Mean temperature of wettest quarter	3.8
LC	MODIS land cover	1.7
3	Temperature seasonality (standard deviation *100)	1.3
14	Isothermality	0.7
1	Annual mean temperature	0.7
4	Precipitation of driest month	0.7
2	Annual precipitation	0
12	Mean diurnal range (mean of monthly (max temp - min temp))	0

Table 2.4: Mantel tests comparing genetic distance with both geographic distance and dispersal resistance. F^2_{ST} values are compared against Euclidian distances between populations for a test of isolation by distance (IBD), and against the least-cost path resistance values for a test of isolation by resistance (IBR). Significant p-values are in bold. Comparisons with only two populations could not be performed (-). Tests of IBR were calculated from the relevant subspecies' SDM friction layer.

		<i>Z. l. gambelii</i>	<i>Z. l. oriantha</i>
Comparisons	IBD	IBR	IBR
Sites within <i>Z. l. gambelii</i> breeding range (10 sites excluding OR and CO)	$R^2 = 0.54$ p < 0.01	$R^2 = 0.06$ p = 0.10	- -
Sites within <i>Z. l. oriantha</i> breeding range (13 sites excluding OR)	$R^2 = 0.64$ p = 0.01	- -	$R^2 = 0.17$ p < 0.01
<i>Z. l. gambelii</i> sampled in this study (BA, WT, LE, FTSJ, RV, OK, MK)	$R^2 = 0.72$ p = 0.01	$R^2 < 0.01$ p = 0.42	- -
<i>Z. l. oriantha</i> sampled in this study (BA, BV, CNP, WT, MK, CO-L, CO-M, CO-H)	$R^2 = 0.57$ p < 0.01	- -	$R^2 = 0.25$ p = 0.02
East of Rockies (JAS, BA, BV, CNP, WT, LE)	$R^2 = 0.15$ p = 0.17	$R^2 = 0.19$ p = 0.11	$R^2 = 0.19$ p = 0.11
West of Rockies (FTSJ, RV, OK, MK)	$R^2 = 0.92$ p = 0.09	$R^2 < 0.01$ p = 0.54	$R^2 = 0.01$ p = 0.294
East and West of Rockies (JAS, BA, BV, CNP, WT, LE, FTSJ, RV, OK, MK)	$R^2 = 0.35$ p < 0.01	$R^2 = 0.08$ p = 0.06	$R^2 = 0.07$ p = 0.08
CO Elevations (CO-L, CO-M, CO-H)	$R^2 = 0.35$ p = 0.18	- -	$R^2 = 1.0$ p = 0.34
Coniferous Ecosite (AC) (BA, RV, OK, MK)	$R^2 = 0.56$ p < 0.01	$R^2 = 0.87$ p = 0.04	$R^2 = 0.73$ p = 0.08
Deciduous Ecosite (RD) (BV, WT, LE)	$R^2 = 0.02$ p = 0.50	$R^2 = 0.61$ p = 0.33	$R^2 = 0.61$ p = 0.33

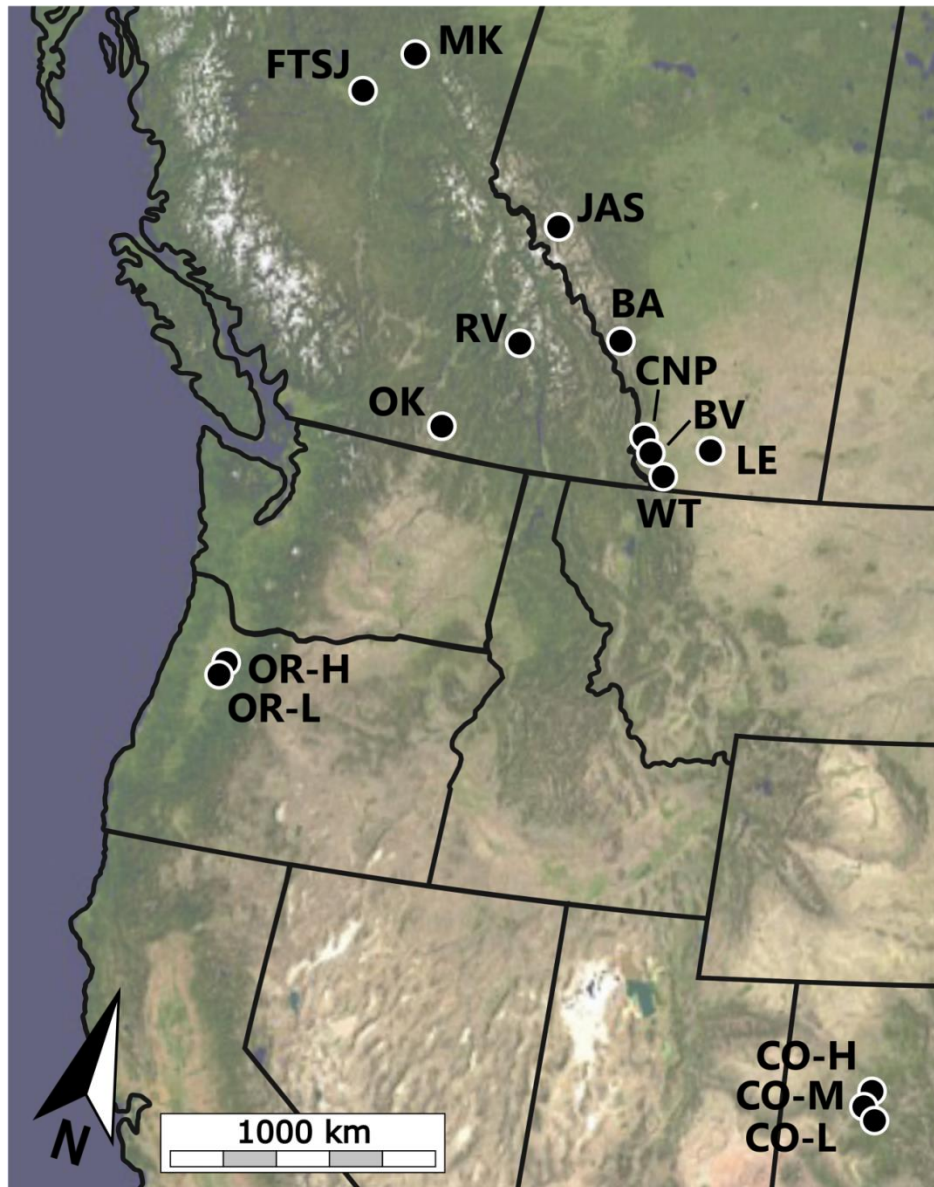
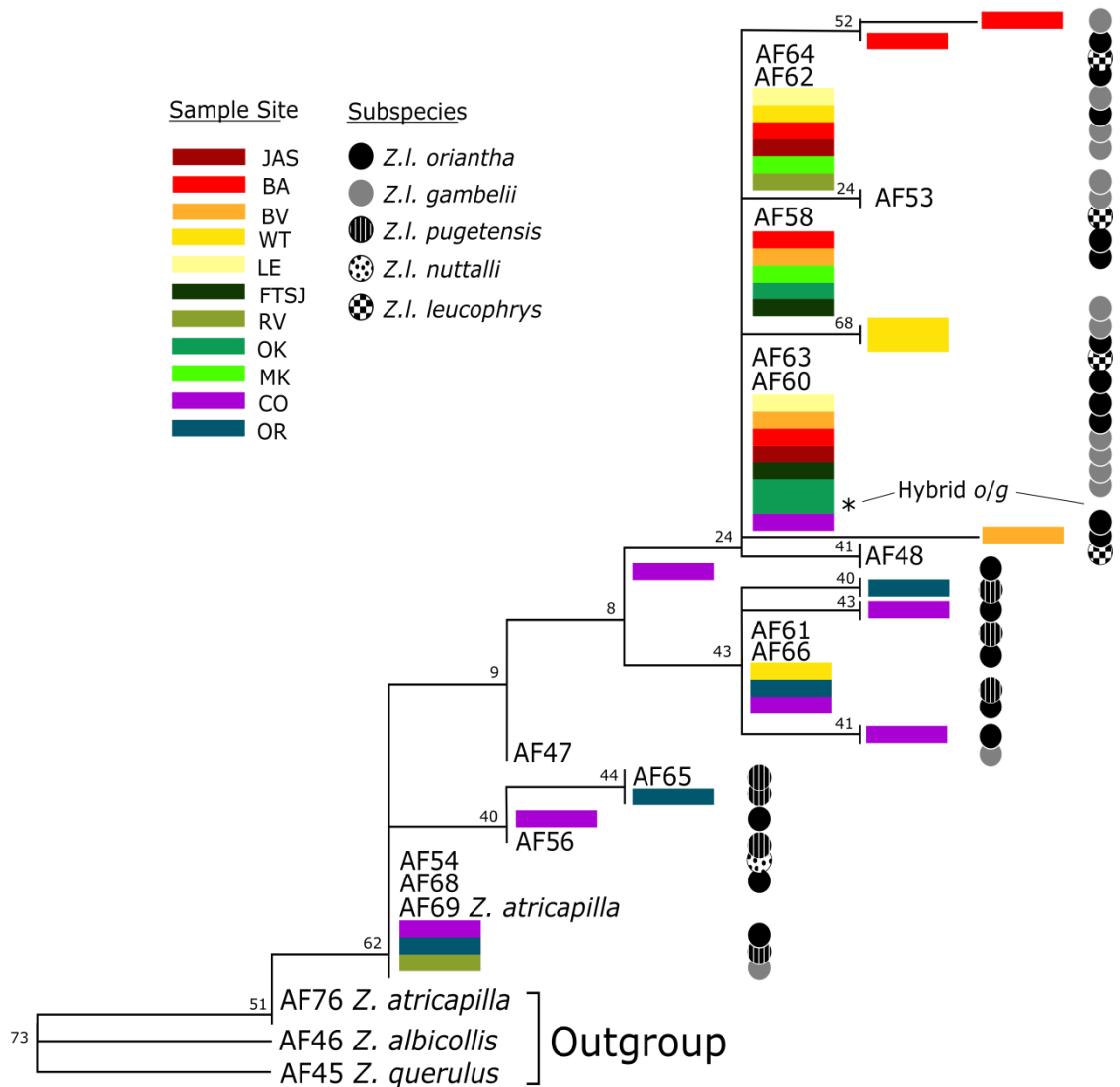
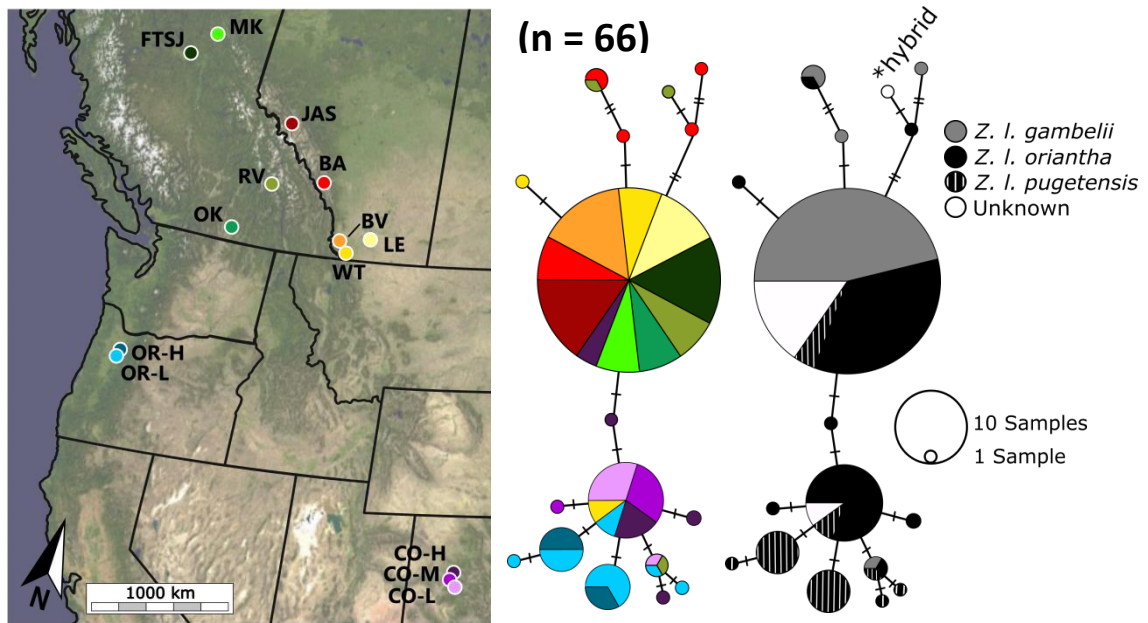


Figure 2.1: Overview of the 15 sampling sites used in this study. Oregon (OR) has four elevation transects (~390 m elevation gain), and the highest and lowest elevation sparrows from each transect were grouped into OR-H (~ 600 m; n = 60) and OR-L (~ 210 m; n = 61) respectively. Colorado (CO) has one transect with an elevation gain of 430 m grouped into low (CO-L: ~ 2900 m; n = 23), mid (CO-M: ~ 3100 m; n = 32), and high (CO-H: ~ 3330 m; n = 28) populations.



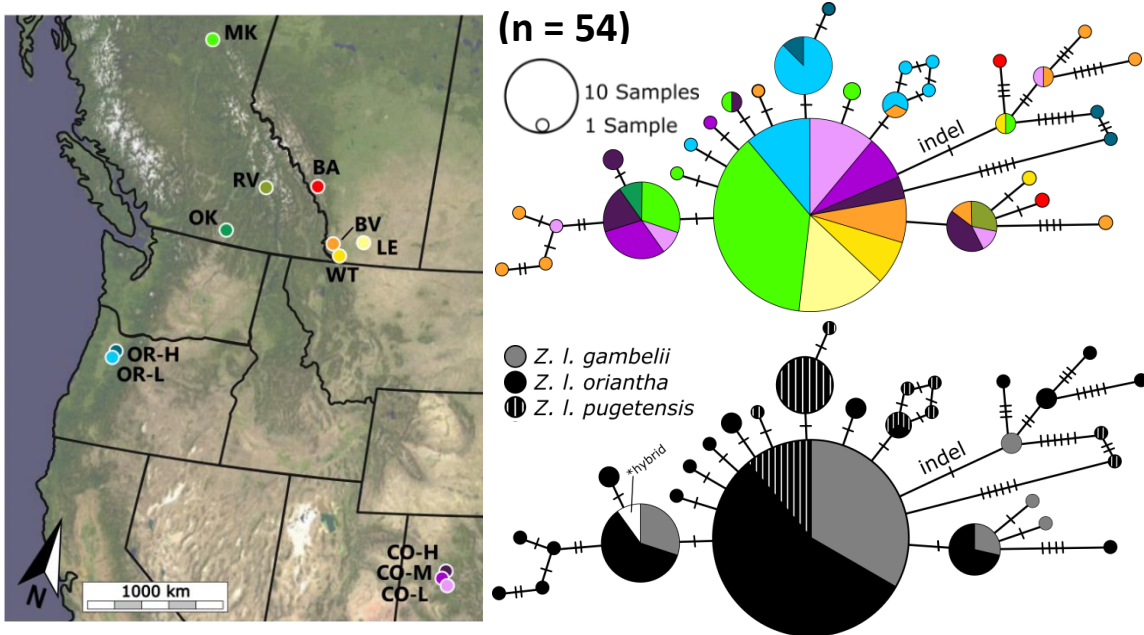
-0.0005 substitutions/site

Figure 2.2: Maximum likelihood tree of CR haplotypes from this study and Weckstein et al., (2001) (samples labelled as “AF##”). *Z. querulus* and *Z. albicollis* were used as outgroups. Sample sites are indicated by colors on the tree, and *Z. leucophrys* subspecies are indicated by circle symbols with grayscale shades and patterns to the right. Labels with no color or symbol are unknown sampling location or subspecies type. Branch lengths are proportional to average substitutions per site and bootstrap values based on 1000 replicates of the Kimura 2-parameter model are shown above branches.



	JAS	BA	BV	WT	LE	FTSJ	RV	OK	MK	CO-L	CO-M	CO-H	OR-L	OR-H	Subspecies
JAS		NS													
BA	NS														
BV	NS	NS													
WT	NS	NS	NS												
LE	NS	NS	NS	NS											
FTSJ	NS	NS	NS	NS	NS										
RV	NS	NS	NS	NS	NS	NS									
OK	NS	NS	NS	NS	NS	NS	NS								
MK	NS	NS	NS	NS	NS	NS	NS	NS							
CO-L	•	NS	•	NS	•	•	•	NS	NS						
CO-M	•	NS	•	NS	•	•	•	NS	NS	NS					
CO-H	•	•	•	•	•	•	•	NS	NS	NS	NS				
OR-L	•	•	•	•	•	•	•	•	•	NS	NS	NS			
OR-H	•	•	•	•	•	•	•	•	NS	NS	NS	•	NS		

Figure 2.3: Minimum spanning CR haplotype networks using sequences from 14 sites and 66 birds. Each circle represents a haplotype, and the number of dashes across lines represents number of base pair differences. Size of the circle is proportional to the number of individuals sharing a haplotype. Colours correspond to population of origin as shown on the map to the left and grayscale patterns correspond with subspecies. One individual marked with an asterisk is a phenotypic hybrid of *Z. l. oriantha* and *Z. l. gambelii*. Significant pairwise F_{ST} comparisons corresponding to each network shown below after FDR correction for multiple tests are shown in red. See F_{ST} values in Appendix 3.



	BA	BV	WT	LE	RV	MK	CO-L	CO-M	CO-H	OR-L	OR-H
BA											
BV	NS										
WT	NS	NS									
LE	NS	•	NS								
RV	NS	•	NS	NS							
MK	NS	•	•	•	•						
CO-L	NS	NS	NS	NS	NS	•					
CO-M	NS	•	NS	NS	NS	•	NS				
CO-H	NS	NS	NS	•	NS	•	NS	NS			
OR-L	NS	NS	NS	•	•	•	NS	•	•		
OR-H	NS	NS	NS	•	NS	NS	NS	NS	NS	NS	

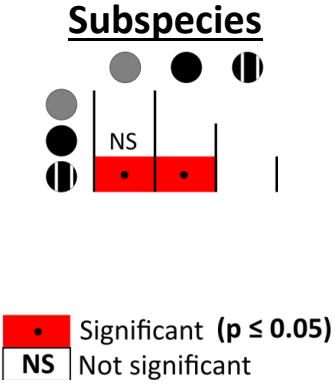


Figure 2.4: Minimum spanning AldoB6 haplotype networks using sequences from 12 sites. Out of 54 birds, 31 were male and after phasing the data there were 85 alleles. Each circle represents a haplotype, each dash across a line represents a single mutation, and a 9 bp insertion linked to a 19 bp deletion in some haplotypes is indicated by “indel”. Size of the circle is proportional to the number of individuals sharing a haplotype. Colours correspond to population of origin as shown on the map to the left and grayscale patterns correspond with subspecies. One individual marked with an asterisk is a phenotypic hybrid of *Z. l. oriantha* and *Z. l. gambelii*. Significant pairwise F_{ST} comparisons (red) after FDR correction for multiple tests corresponding to each network are below. Since OK only has one sample so it is displayed in the network, but omitted from F_{ST} comparisons. See F_{ST} values in Appendix 4.

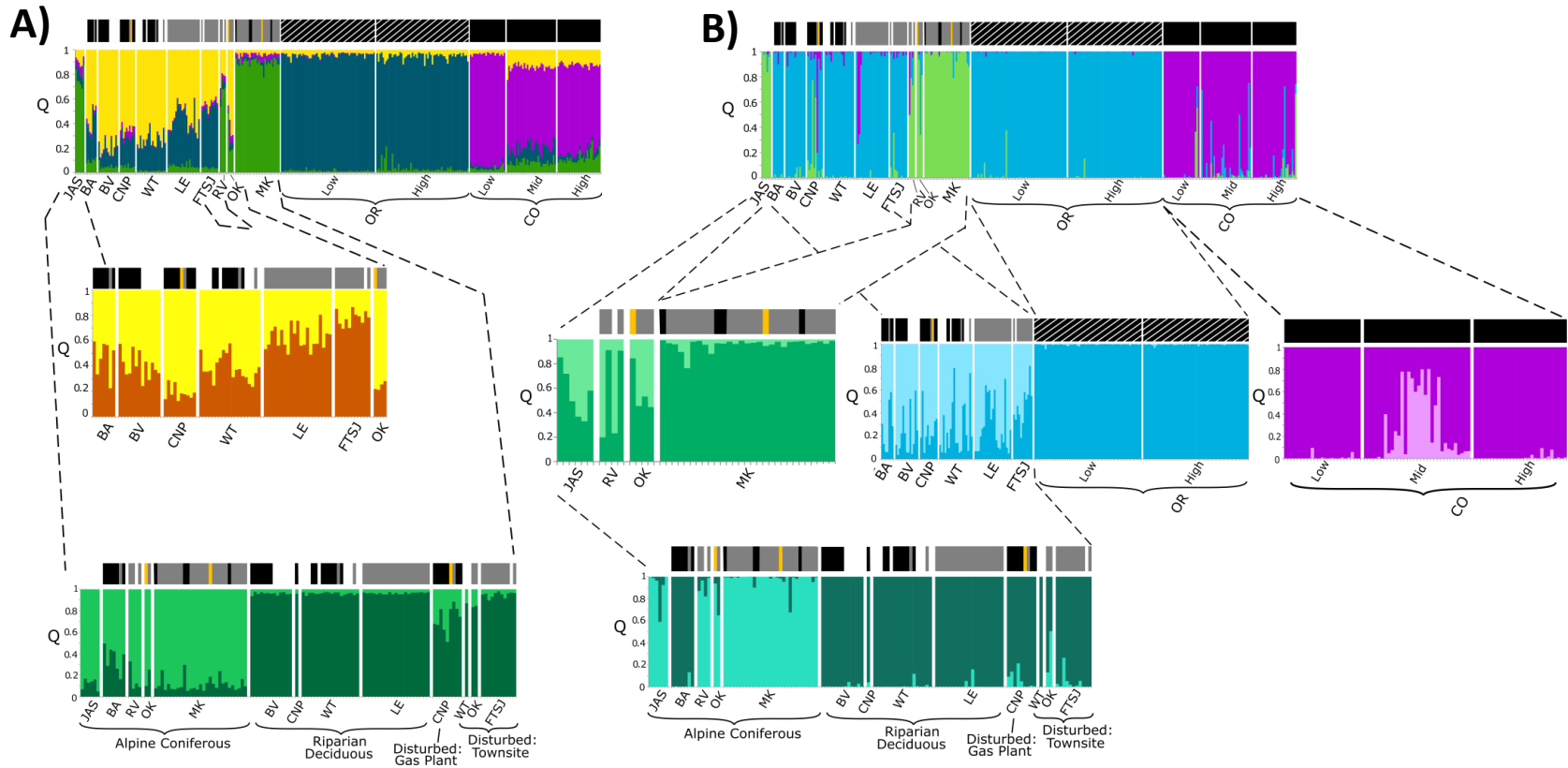


Figure 2.5a: **A)** STRUCTURE plot of all populations with optimal number of genetic clusters of $k = 4$ as determined by highest log probability for 328 samples. Inset below the first histogram is the hierarchical analysis of the first group (yellow) which had an optimal number of clusters $k = 2$. The final histogram is analysis of substructure according to ecosite type which also had $K = 2$ clusters (dark green). **B)** A similar Bayesian analysis (TESS v2.3) for comparison, which yields an optimal $k = 3$ in the overall clustering, $k = 2$ for cluster 1 (light green), cluster 2 (light blue), Colorado elevation groups (purple), and ecosite groups (teal). Above each plot is a bar showing subspecies of each individual as *Z. l. gambelii* (grey) *Z. l. oriantha* (black), *Z. l. pugetensis* (striped), or hybrid *gambelii-oriantha* (yellow). Unknown subspecies are uncoloured.

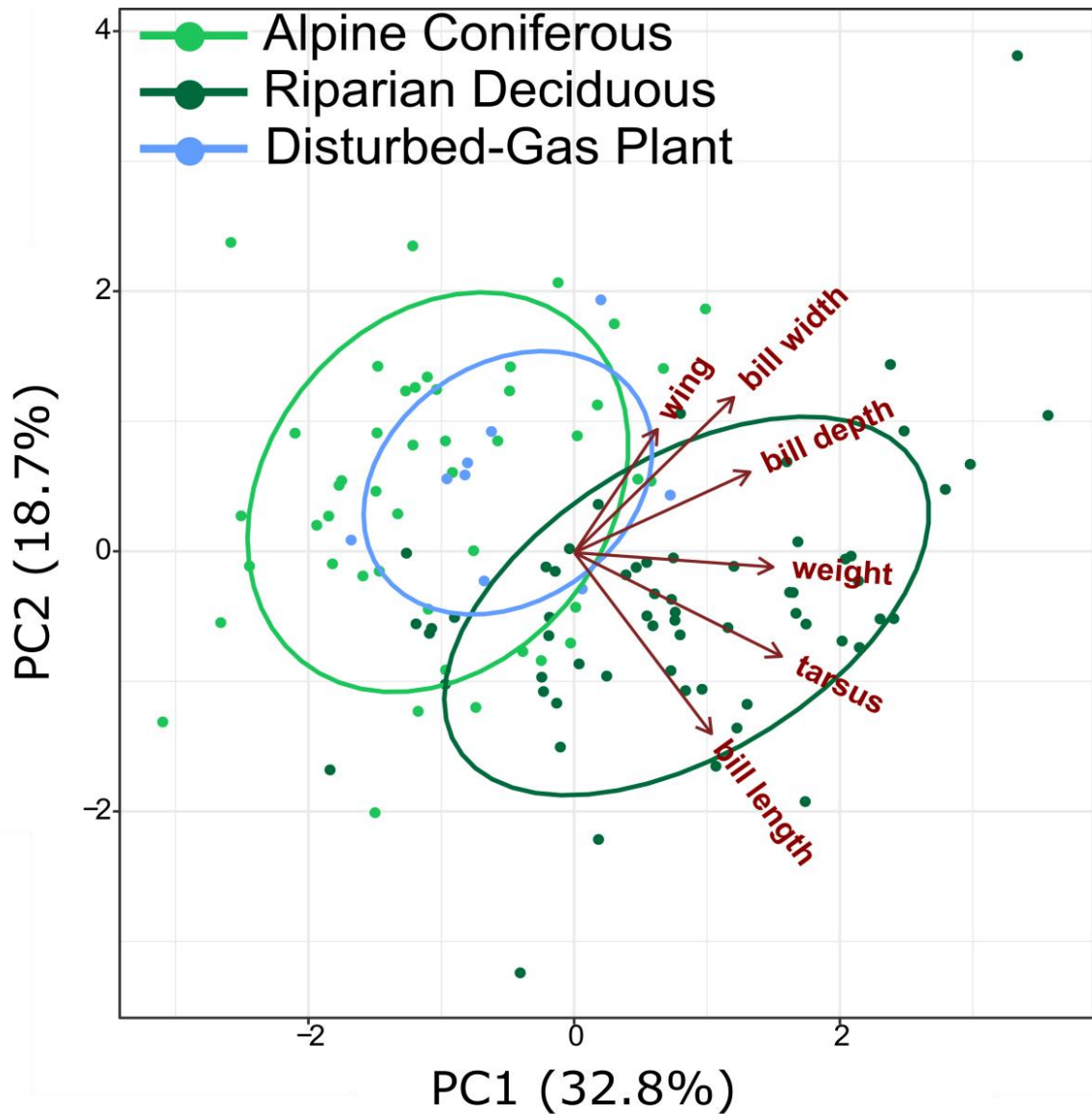


Figure 2.6: A standard principal component analysis of 113 individuals with multivariate analysis of six morphological features (wing length; bill width, length, and depth; tarsus length; and body weight). Ellipses show individuals from alpine coniferous, riparian deciduous, and disturbed habitat ecosites as compared in TESS and STRUCTURE plots. Disturbed habitat individuals are from the disturbed-gas plant site only, as no morphological data were available for the 11 townsite individuals, and CO and OR populations were omitted. Ordination was executed in RStudio using the ggbiplot package.

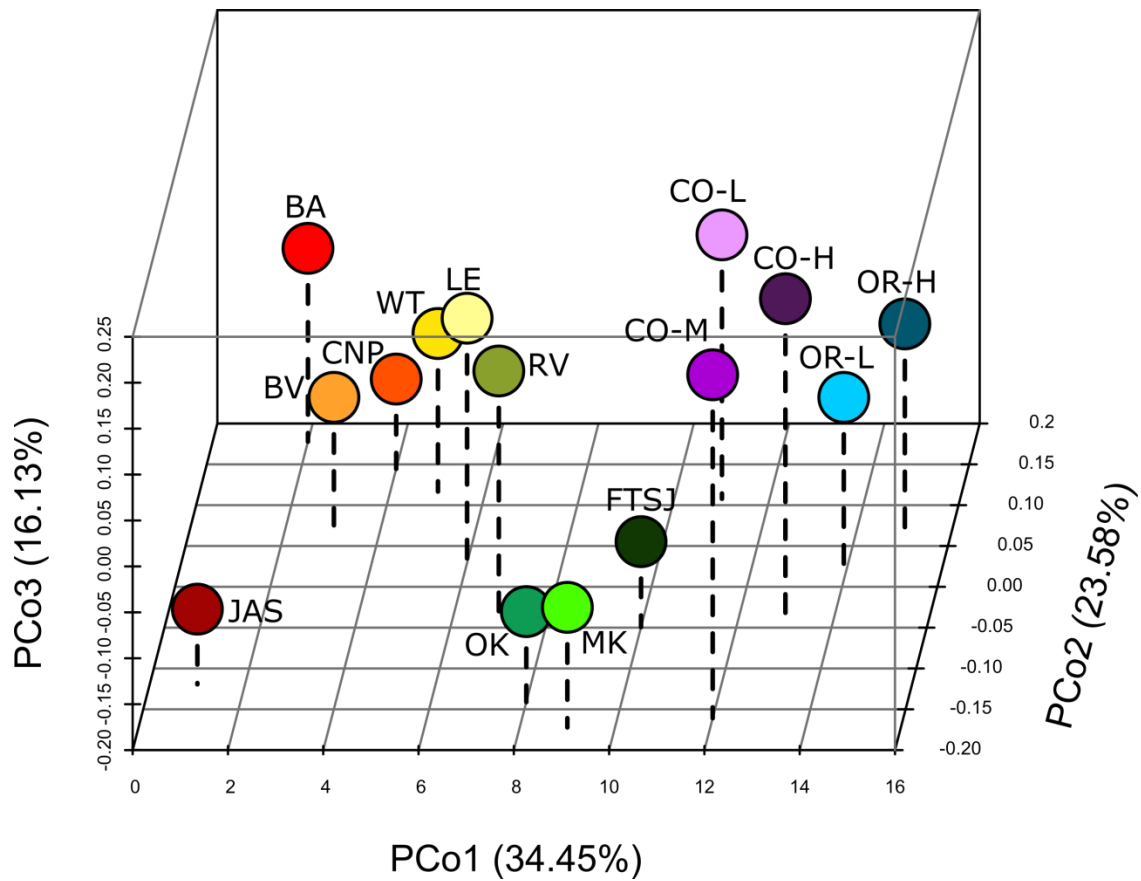


Figure 2.7: A three-dimensional plot of the first three axes of the principal coordinate analysis of all 15 populations using multilocus genotype data.

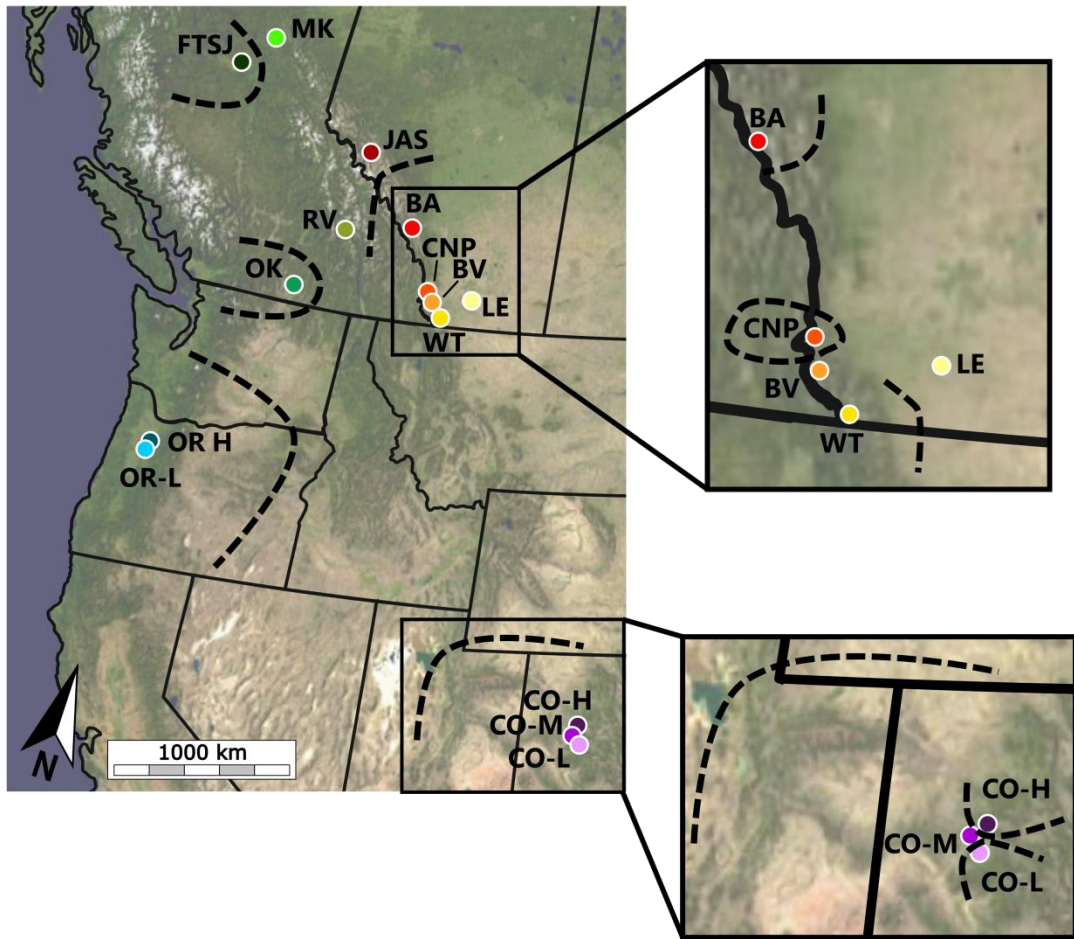
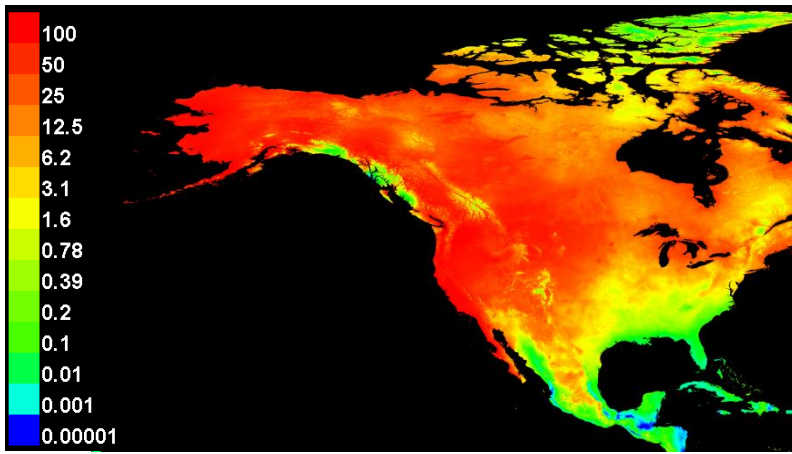
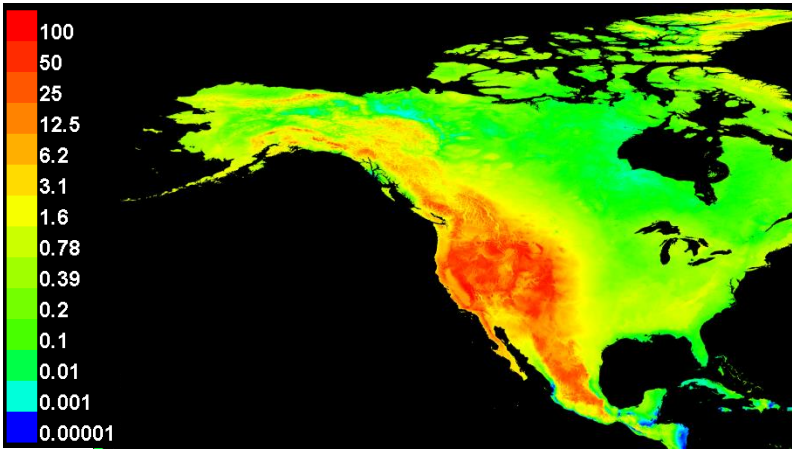


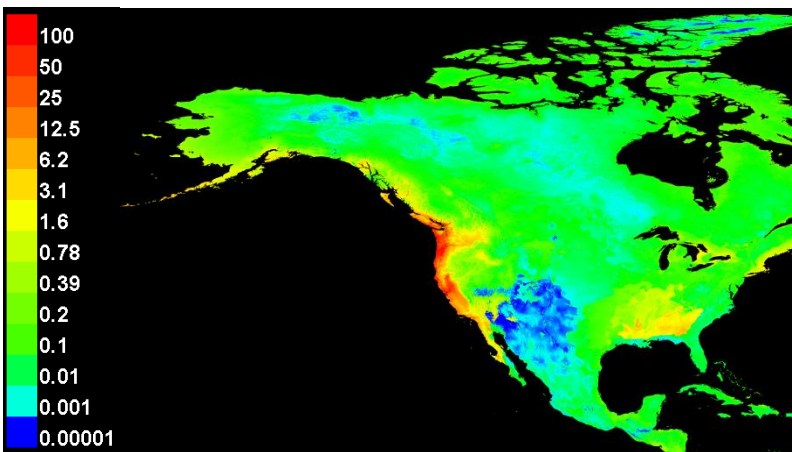
Figure 2.8: BARRIER analysis using Monmonier's (1973) maximum difference algorithm to compute locations of barriers corresponding to genetic differences between populations based on Delaunay triangulation of geographic coordinates and pairwise F'_{ST} values.



Z. l. gambelii



Z. l. oriantha



Z. l. pugetensis

Figure 2.9 Contemporary Species Distribution Models created using occurrence data from three subspecies of *Z. leucophrys* and environmental variables, a vegetation cover layer and elevation layer. The model was created using the SDM toolbox (Brown 2014) in Arc GIS® and MaxEnt (Phillips et al., 2006). The model shows areas of the most suitable environmental and habitat conditions (i.e. ecological niche) for each subspecies in warmer colours.

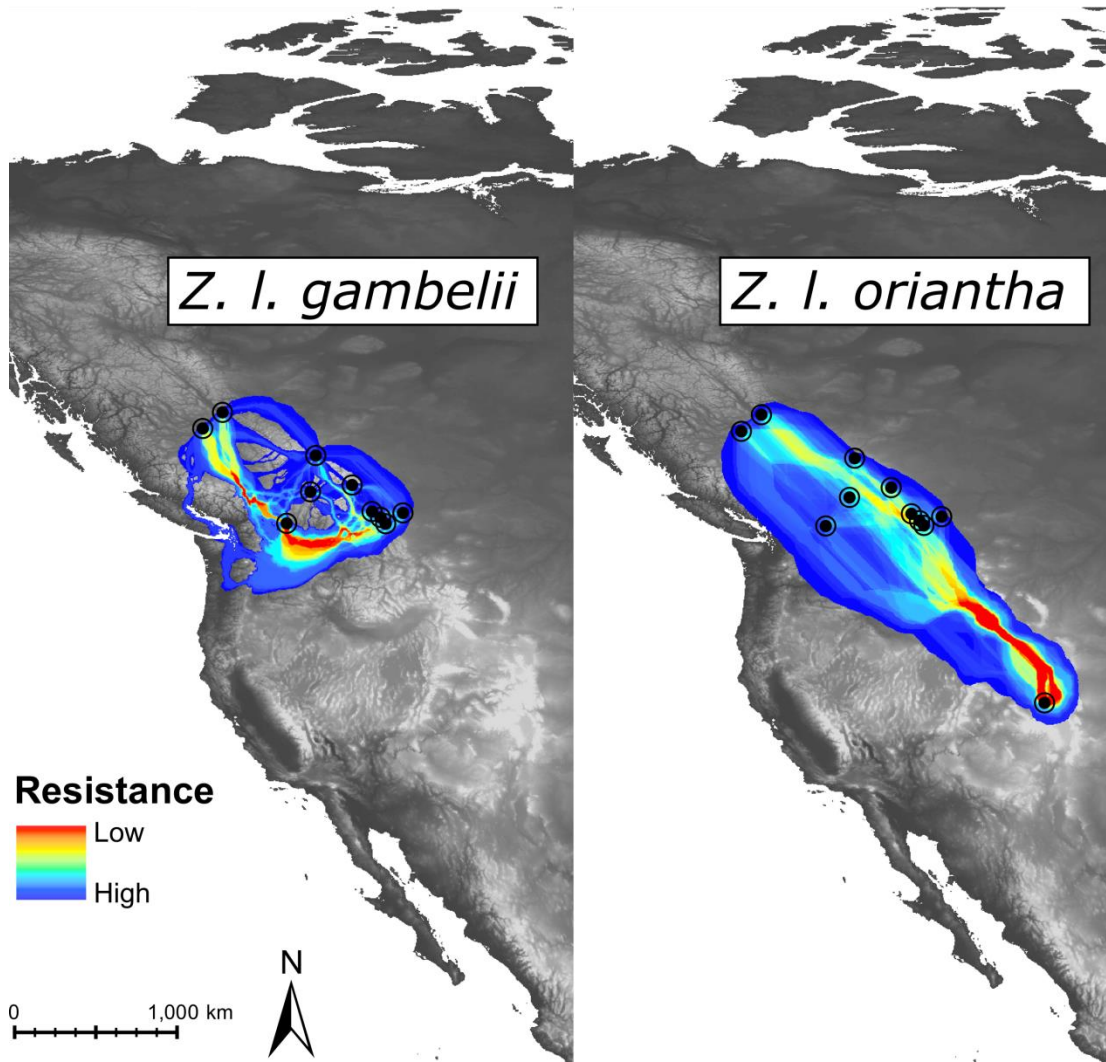


Figure 2.10: Least-cost corridor projections of dispersal routes between sites within the breeding ranges of *Z. l. gambelii* and *Z. l. oriantha* based on the preferred environmental conditions as inferred by the species distribution model (Fig 2.9). Dispersal costs are coded by color, red representing areas with low resistance (i.e. low dispersal cost), and blue representing areas of high resistance. See Appendix 8 for least-cost paths drawn between populations according to the lowest total dispersal cost along these corridors.

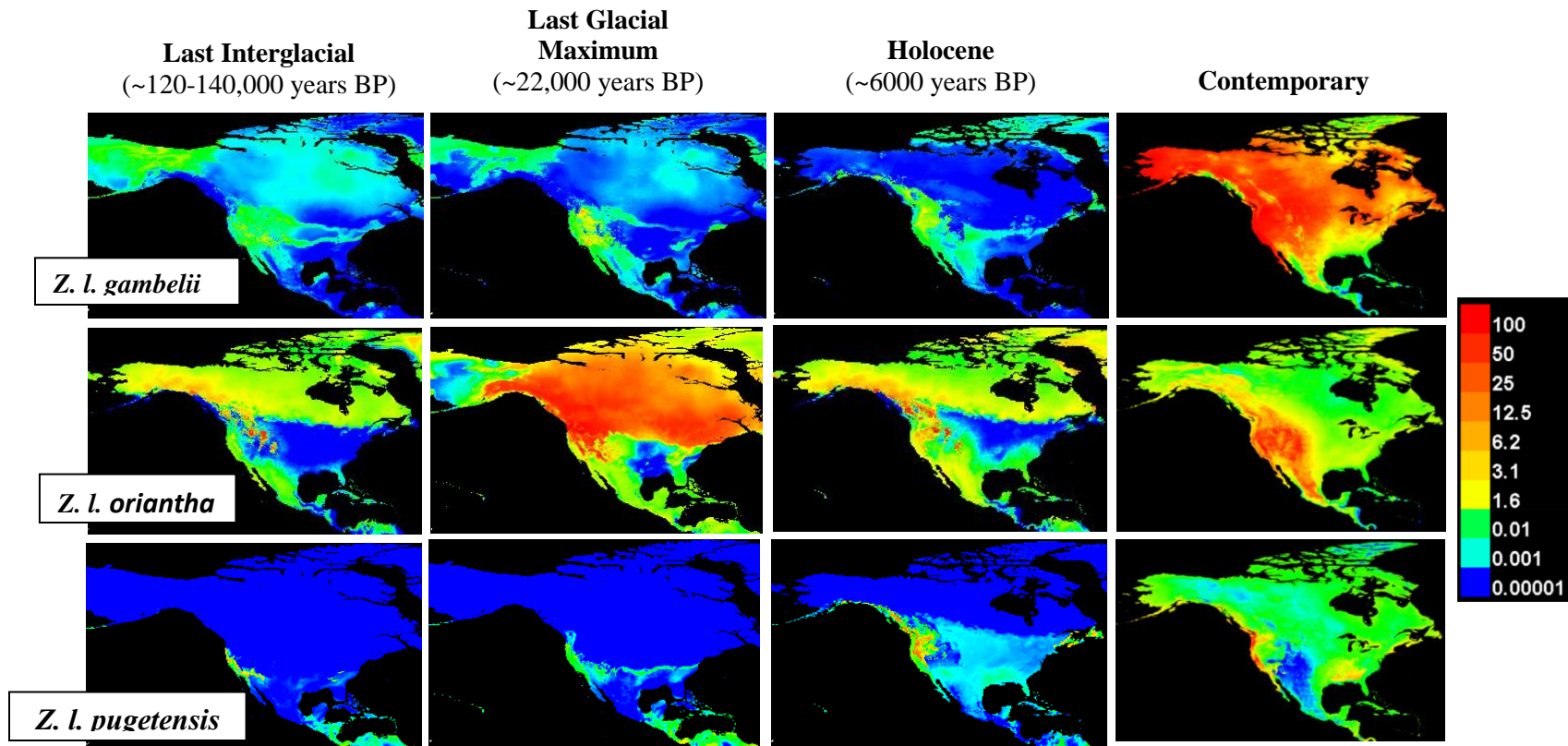


Figure 2.11 Historical species distribution model (SDM) projections of potential suitable habitat for each *Z. leucophrys* subspecies in North America. The contemporary SDMs for each subspecies are placed on the right for comparison. The species distribution models made with Maxent were projected to mid-Holocene and last glacial maximum (LGM) periods using historical climate data from MIROC-ESM Global Climate Models (Watanabe et al., 2011), and from the last inter-glacial (LIG) period (Otto-Bliesner et al., 2006). The colors correspond to the percent likelihood of environment and habitat suitability for each subspecies.

2.5 References

- Adams, R. V., & Burg, T. M. (2015). Gene flow of a forest-dependent bird across a fragmented landscape. *PLoS ONE*, *10*(11), 1-22. <https://doi.org/10.1371/journal.pone.0140938>
- Alerstam, T. (2001). Detours in bird migration. *Journal of Theoretical Biology*, *209*(3), 319–331. <https://doi.org/10.1006/JTBI.2001.2266>
- Ashton, K. G. (2002). Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography*, *11*(6), 505–523. <https://doi.org/10.1046/j.1466-822X.2002.00313.x>
- Austen, M. J. W., & Handford, P. T. (1991). Variation in the songs of breeding Gambel's white-crowned sparrows near Churchill, Manitoba. *The Condor*, *93*(1), 147–152. <https://doi.org/10.2307/1368616>
- Avise, J. (1994). *Molecular markers, natural history and evolution* (1st edition). Boston, MA: Springer US. <https://doi.org/10.1007/978-1-4615-2381-9>
- Avise, J. C. (2000). *Phylogeography : the history and formation of species*. Cambridge, MA: Harvard University Press.
- Bandelt, H. J., Forster, P., & Rohlf, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*(1), 37–48. <https://doi.org/10.1093/oxfordjournals.molbev.a026036>
- Banks, R. C. (1964). Geographic variation in the white- crowned sparrow *Zonotrichia leucophrys*. *University of California Publications in Zoology*, *70*, 1–123.
- Baptista, L. F., & King, J. R. (1980). Geographical variation in song and song dialects of montane white-crowned sparrows. *The Condor*, *82*(3), 267–284. <https://doi.org/10.2307/1367392>
- Barley, A. J., Monnahan, P. J., Thomson, R. C., Grismer, L. L., & Brown, R. M. (2015). Sun skink landscape genomics: assessing the roles of micro-evolutionary processes in shaping genetic and phenotypic diversity across a heterogeneous and fragmented landscape. *Molecular Ecology*, *24*(8), 1696-1712. <https://doi.org/10.1111/mec.13151>
- Barton, N. H., & Hewitt, G. M. (1989). Adaptation, speciation and hybrid zones. *Nature*, *341*(6242), 497-503. <https://doi.org/10.1038/341497a0>
- Benjamini, Y., & Yekutieli, D. (2005). False discovery rate-adjusted multiple confidence intervals for selected parameters. *Journal of the American Statistical Association*, *100*(469), 71–81. <https://doi.org/10.1198/016214504000001907>
- Bensch, S., Price, T., & Kohn, J. (1997). Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Molecular Ecology*, *6*(1), 91–92. <https://doi.org/10.1046/j.1365-294X.1997.00150.x>

- Bermingham, E., Rohwer, S., Freeman, S., & Wood, C. (1992). Vicariance biogeography in the Pleistocene and speciation in North American wood warblers: a test of Mengel's model. *Proceedings of the National Academy of Sciences of the United States of America*, 89(14), 6624–6628. <https://doi.org/10.1073/PNAS.89.14.6624>
- Bonier, F., Eikenaar, C., Martin, P. R., & Moore, I. T. (2014). Extrapair paternity rates vary with latitude and elevation in Emberizid sparrows. *The American Naturalist*, 183(1), 54–61. <https://doi.org/10.1086/674130>
- Bowler, D. E., & Benton, T. G. (2005). Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews*, 80(2), 205–225. <https://doi.org/10.1017/S1464793104006645>
- Brown, J. L. (2014). SDMtoolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *Methods in Ecology and Evolution*, 5(7), 694–700. <https://doi.org/10.1111/2041-210X.12200>
- Broxton, P. D., Zeng, X., Sulla-Menashe, D., & Troch, P. A. (2014). A global land cover climatology using MODIS data. *Journal of Applied Meteorology and Climatology*, 53(6), 1593–1605. <https://doi.org/10.1175/JAMC-D-13-0270.1>
- Burg, T. M., & Croxall, J. P. (2001). Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. *Molecular Ecology*, 10(11), 2647–2660. <https://doi.org/10.1046/j.0962-1083.2001.01392.x>
- Chen, C., Durand, E., Forbes, F., & Francois, O. (2007). Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, 7(5), 747–756. <https://doi.org/10.1111/j.1471-8286.2007.01769.x>
- Cheviron, Z. A., & Brumfield, R. T. (2009). Migration-selection balance and local adaptation of mitochondrial haplotypes in rufous-collared sparrows (*Zonotrichia capensis*) along an elevational gradient. *Evolution*, 63(6), 1593–1605. <https://doi.org/10.1111/j.1558-5646.2009.00644.x>
- Chilton, G., Lein, M. R., & Baptista, L. F. (1990). Mate choice by female white-crowned sparrows in a mixed-dialect population. *Behavioral Ecology and Sociobiology*, 27(3), 223–227. <https://doi.org/10.1007/BF00180307>
- Clegg, S. M., Kelly, J. F., Kimura, M., & Smith, T. B. (2003). Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). *Molecular Ecology*, 12(4), 819–830. <https://doi.org/10.1046/j.1365-294X.2003.01757.x>
- Cortopassi, A. J., & Mewaldt, L. R. (1965). The circumannual distribution of white-crowned sparrows. *Bird-Banding*, 36(3), 141. <https://doi.org/10.2307/4511181>
- Coulon, A., Cosson, J. F., Angibault, J. M., Cargnelutti, B., Galan, M., Morellet, N., Petit, E., Aulagnier, S., & Hewison, A. J. M. (2004). Landscape connectivity influences

- gene flow in a roe deer population inhabiting a fragmented landscape: An individual-based approach. *Molecular Ecology*, 13(9), 2841–2850.
<https://doi.org/10.1111/j.1365-294X.2004.02253.x>
- Cruden, D. M., & Hu, X. (1999). The shapes of some mountain peaks in the Canadian Rockies. *Earth Surface Processes and Landforms*, 24(13), 1229–1241.
[https://doi.org/10.1002/\(SICI\)1096-9837\(199912\)24:13<1229::AID-ESP42>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1096-9837(199912)24:13<1229::AID-ESP42>3.0.CO;2-1)
- Dawson, R. J., Gibbs, H. L., Hobson, K. A., & Yezerinac, S. M. (1997). Isolation of microsatellite DNA markers from a passerine bird, *Dendroica petechia* (the yellow warbler), and their use in population studies. *Heredity*, 79 (5), 506–514.
- Dempster, E., Dempster, R., & Perrin, M. (2010). Behavioural divergence in allopatric and sympatric gerbil species (Rodentia: Gerbillinae). *Ethology*, 93(4), 300–314.
<https://doi.org/10.1111/j.1439-0310.1993.tb01211.x>
- DuBay, S. G., & Witt, C. C. (2014). Differential high-altitude adaptation and restricted gene flow across a mid-elevation hybrid zone in Andean tit-tyrant flycatchers. *Molecular Ecology*, 23(14), 3551–3565. <https://doi.org/10.1111/mec.12836>
- Dubey, S., Sumner, J., Pike, D. A., Keogh, J. S., Webb, J. K., & Shine, R. (2011). Genetic connectivity among populations of an endangered snake species from southeastern Australia (*Hoplocephalus bungaroides*, Elapidae). *Ecology and Evolution*, 1(2), 218–227. <https://doi.org/10.1002/ece3.25>
- Dunn, J. L., Garrett, K. L., & Alderfer, J. (1995). White-crowned sparrow subspecies: identification and distribution. *Birding*, 27, 182–201.
- Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Engler, J. O., Balkenhol, N., Filz, K. J., Habel, J. C., & Rödder, D. (2014). Comparative landscape genetics of three closely related sympatric Hesperid butterflies with diverging ecological traits. *PLoS ONE*, 9(9), 1–13.
<https://doi.org/10.1371/journal.pone.0106526>
- Epps, C. W., Wehausen, J. D., Bleich, V. C., Torres, S. G., & Brashares, J. S. (2007). Optimizing dispersal and corridor models using landscape genetics. *Journal of Applied Ecology*, 44(4), 714–724. <https://doi.org/10.1111/j.1365-2664.2007.01325.x>
- Etherington, T. R. (2011). Python based GIS tools for landscape genetics: visualising genetic relatedness and measuring landscape connectivity. *Methods in Ecology and Evolution*, 2(1), 52–55. <https://doi.org/10.1111/j.2041-210x.2010.00048.x>
- Etter, R., Rex, M. A., Chase, M. R., & Quattro, J. M. (2005). Population differentiation decreases with depth in deep-sea bivalves. *Evolution*, 59(7), 1479–1491.
<https://doi.org/10.1111/j.0014-3820.2005.tb01797.x>

- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- Fjeldsâ, J., Bowie, R., & Rahbek, C. (2011). The role of mountain ranges in the diversification of birds. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 249-265. <https://doi.org/10.1146/annurev-ecolsys-102710-145113>
- Funk, W. C., Blouin, M. S., Corn, P. S., Maxell, B. A., Pilliod, D. S., Amish, S., & Allendorf, F. W. (2005). Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology*, *14*(2), 483–496. <https://doi.org/10.1111/j.1365-294X.2005.02426.x>
- Geffen, E., Anderson, M., & Wayne, R. (2004). Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular Ecology*, *13*(8), 2481–2490. <https://doi.org/10.1111/j.1365-294X.2004.02244.x>
- Gill, J. A., Sutherland, W. J., & Watkinson, A. R. (1996). A method to quantify the effects of human disturbance on animal populations. *The Journal of Applied Ecology*, *33*(4), 786-792. <https://doi.org/10.2307/2404948>
- González-Solís, J., Croxall, J. P., Oro, D., & Ruiz, X. (2007). Trans-equatorial migration and mixing in the wintering areas of a pelagic seabird. *Frontiers in Ecology and the Environment*, *5*(6), 297–301.
- Gonzalo-Turpin, H., & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*, *97*(4), 742–751. <https://doi.org/10.1111/j.1365-2745.2009.01509.x>
- Gram, W., & Sork, V. (2001). Association between environmental and genetic heterogeneity in forest tree populations. *Ecology*, *82*(7), 2012-2021.
- Hafner, D. J., & Petersen, K. E. (1985). Song dialects and gene flow in the white-crowned sparrow, *Zonotrichia leucophrys nuttalli*. *Evolution*, *39*(3), 687-694. <https://doi.org/10.2307/2408663>
- Hahn, T. P., Wingfield, J. C., Mullen, R., & Deviche, P. J. (1995). Endocrine bases of spatial and temporal opportunism in Arctic-breeding birds. *American Zoologist*, *35*(3), 259–273. <https://doi.org/10.1093/icb/35.3.259>
- Hamer, K., Hill, J., Benedick, S., Mustaffa, N., Sherratt, T. N., Maryati, M., & Chey, V. K. (2003). Ecology of butterflies in natural and selectively logged forests of northern Borneo: the importance of habitat heterogeneity. *Journal of Applied Ecology*, *40*(1), 150-162. <https://doi.org/10.1046/j.1365-2664.2003.00783.x>
- Hanotte, O., Zanon, C., Pugh, A., Greig, C., Dixon, A., & Burke, T. (1994). Isolation and

- characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoeniclus*. *Molecular Ecology*, 3(5), 529–530. <https://doi.org/10.1111/j.1365-294X.1994.tb00133.x>
- Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1512), 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 359(1442), 183–195. <https://doi.org/10.1098/rstb.2003.1388>
- Holderegger, R., & Wagner, H. H. (2008). Landscape genetics. *BioScience*, 58(3), 199. <https://doi.org/10.1641/B580306>
- Holland, S. S. (1976). Bulletin 48: Landforms of British Columbia, A Physiographic Outline. In *BC Geological Survey* (pp. 1–138). K.M. MacDonald, Queen's Printer.
- Hooge, J. (2003). *Population Characteristics and Habitat Use of Mountain Caribou in the Wood Arm Area: Annual Report 2002-2003*. B.C. Ministry of Forests. Revelstoke, BC.
- Hubbard, J. P. (1969). The relationships and evolution of the *Dendroica coronata* complex. *The Auk*, 86(3), 393–432. <https://doi.org/10.2307/4083405>
- Hull, J. M., Hull, A. C., Sacks, B. N., Smith, J. P., & Ernest, H. B. (2008). Landscape characteristics influence morphological and genetic differentiation in a widespread raptor (*Buteo jamaicensis*). *Molecular Ecology*, 17(3), 810–824. <https://doi.org/10.1111/j.1365-294X.2007.03632.x>
- Hunn, E. S., & Beaudette, D. (2014). Apparent sympatry of two subspecies of the white-crowned sparrow, *Zonotrichia leucophrys pugetensis* and *gambelii*, in Washington State. *Western Birds*, 45(2), 132–140.
- Kendeigh, S. C., & Fawver, B. J. (1981). Breeding bird populations in the Great Smoky Mountains, Tennessee and North Carolina. *The Wilson Bulletin*, 93(2), 218–242.
- Knowles, L. L. (2001). Did the pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshoppers. *Molecular Ecology*, 10(3), 691–701. <https://doi.org/10.1046/j.1365-294X.2001.01206.x>
- LaManna, J., Hemenway, A., Boccadori, V., & Martin, T. (2015). Bird species turnover is related to changing predation risk along a vegetation gradient. *Ecology*, 96(6), 1670–1680. <https://doi.org/10.1890/14-1333.1>
- Lee-Yaw, J. A., Davidson, A., McRae, B. H., & Green, D. M. (2009). Do landscape processes predict phylogeographic patterns in the wood frog? *Molecular Ecology*, 18(9), 1863–1874. <https://doi.org/10.1111/j.1365-294X.2009.04152.x>

- Lein, M. R., & Corbin, K. W. (1990). Song and plumage phenotypes in a contact zone between subspecies of the white-crowned sparrow (*Zonotrichia leucophrys*). *Canadian Journal of Zoology*, *68*(12), 2625–2629. <https://doi.org/10.1139/z90-366>
- Licona-Vera, Y., & Ornelas, J. F. (2017). The conquering of North America: dated phylogenetic and biogeographic inference of migratory behavior in bee hummingbirds. *BMC Evolutionary Biology*, *17*(126), 1–17. <https://doi.org/10.1186/s12862-017-0980-5>
- Ligges, U., & Mächler, M. (2003). Scatterplot3d - an R package for visualizing multivariate data. *Journal of Statistical Software*, *8*(11), 1–20.
- Lipshutz, S. E., Overcast, I. A., Hickerson, M. J., Brumfield, R. T., & Derryberry, E. P. (2017). Behavioural response to song and genetic divergence in two subspecies of white-crowned sparrows (*Zonotrichia leucophrys*). *Molecular Ecology*, *26*(11), 3011–3027. <https://doi.org/10.1111/mec.14002>
- Lundberg, A., Alatalo, R. V., Carlson, A., & Ulfstrand, S. (1981). Biometry, habitat distribution and breeding success in the pied flycatcher *Ficedula hypoleuca*. *Ornis Scandinavica*, *12*(1), 68–69. <https://doi.org/10.2307/3675907>
- MacDougall-Shackleton, E. A., & MacDougall-Shackleton, S. A. (2001). Cultural and genetic evolution in mountain white-crowned sparrows: song dialects are associated with population structure. *Evolution*, *55*(12), 2568–2575. <https://doi.org/10.1111/j.0014-3820.2001.tb00769.x>
- Macleod, R., Barnett, P., Clark, J. A., Cresswell, W. (2005). Body mass changes strategies in blackbirds (*Turdus merula*): the starvation-predation risk trade-off. *Journal of Animal Ecology* *74*, 292–302. <https://doi.org/10.1111/j.1365-2656.2005.00923.x>
- Martin, T. (1993). Nest predation and nest sites. *BioScience*, *43*(8), 523–532. <https://doi.org/10.2307/1311947>
- Maney, D. L., Hahn, T. P., Schoech, S. J., Sharp, P. J., Morton, M. L., & Wingfield, J. C. (1999). Effects of ambient temperature on photo-induced prolactin secretion in three subspecies of white-crowned sparrow, *Zonotrichia leucophrys*. *General and Comparative Endocrinology*, *113*, 445–456. <https://doi.org/10.1006/gcen.1998.7219>
- Manni, F., Guérard, E., & Heyer, E. (2004). Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by “Monmonier’s algorithm”. *Human Biology*, *76*(2), 173–190.
- Marler, P., & Tamura, M. (1962). Song “dialects” in three populations of white-crowned sparrows. *The Condor*, *64*(5), 368–377. <https://doi.org/10.2307/1365545>
- McCleery, R. a, Lopez, R. R., Silvy, N. J., Frank, P. a, & Klett, S. B. (2006). Population status and habitat selection of the endangered Key Largo woodrat. *The American Midland Naturalist*, *155*(1), 197–210.

- Meirmans, P., & Hedrick, P. (2011). Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources*, *11*(1), 5–18.
<https://doi.org/10.1111/j.1755-0998.2010.02927.x>
- Monmonier, M. S. (1973). Maximum-difference barriers: An alternative numerical regionalization method*. *Geographical Analysis*, *5*(3), 245–261.
<https://doi.org/10.1111/j.1538-4632.1973.tb01011.x>
- Morton, M. (2002). The mountain white-crowned sparrow: migration and reproduction at high altitude. *Studies in Avian Biology*, *24*, 1–236.
- Nelson, D. A. (1998). Geographic variation in song of Gambel's white-crowned sparrow. *Behaviour*, *135*(3), 321–342. <https://doi.org/10.1163/156853998793066258>
- Nelson, D. A. (1999). Ecological influences on vocal development in the white-crowned sparrow. *Animal Behaviour*, *58*(1), 21–36. <https://doi.org/10.1006/anbe.1999.1118>
- Olah, G., Smith, A. L., Asner, G. P., Brightsmith, D. J., Heinsohn, R. G., & Peakall, R. (2017). Exploring dispersal barriers using landscape genetic resistance modelling in scarlet macaws of the Peruvian Amazon. *Landscape Ecology*, *32*(2), 445–456.
<https://doi.org/10.1007/s10980-016-0457-8>
- Otto-Bliesner, B. L., Marshall, S. J., Overpeck, J. T., Miller, G. H., & Hu, A. (2006). Simulating Arctic climate warmth and icefield retreat in the last interglaciation. *Science*, *311*(5768), 1751–1753.
- Peakall, R., & Smouse, P. E. (2006). GenAEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*(1), 288–295.
<https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Petren, K. (1998). Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches. *Molecular Ecology*, *7*(12), 1782–1784.
<https://doi.org/10.1046/j.1365-294x.1998.00518.x>
- Phillips, S. J., Dudík, M., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*, 231–259.
<https://doi.org/10.1016/j.ecolmodel.2005.03.026>
- Poesel, A., Gibbs, H., & Nelson, D. (2009). Twenty-one novel microsatellite DNA loci isolated from the Puget Sound white-crowned sparrow, *Zonotrichia leucophrys pugetensis*. *Molecular Ecology Resources*, *9*(3), 795–798.
<https://doi.org/10.1111/j.1755-0998.2008.02182.x>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.
- Proulx, G., & Kariz, R. M. (2005). Winter habitat use by moose, *Alces alces*, in central interior British Columbia. *The Canadian Field-Naturalist*, *119*(2), 186–191.
<https://doi.org/10.22621/cfn.v119i2.104>

- Rand, A. L. (1948). Glaciation, an isolating factor in speciation. *Evolution*, 2(4), 314–321. <https://doi.org/10.1111/j.1558-5646.1948.tb02749.x>
- Richardson, J. L., Urban, M. C., Bolnick, D. I., & Skelly, D. K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution*, 29(3), 165–176. <https://doi.org/10.1016/J.TREE.2014.01.002>
- Rissler, L. J. (2016). Union of phylogeography and landscape genetics. *Proceedings of the National Academy of Sciences*, 113(29), 8079–8086. <https://doi.org/10.1073/pnas.1601073113>
- Robertson, C., Nelson, T. A., Jelinski, D. E., Wulder, M. A., & Boots, B. (2009). Spatial–temporal analysis of species range expansion: the case of the mountain pine beetle, *Dendroctonus ponderosae*. *Journal of Biogeography*, 36, 1446–1458. <https://doi.org/10.1111/j.1365-2699.2009.02100.x>
- Ronce, O. (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology, Evolution, and Systematics*, 38(1), 231–253. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095611>
- Rousset, F. (2008). GENEPOP'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, 8(1), 103–106. <https://doi.org/10.1111/j.1471-8286.2007.01931.x>
- Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 34(12), 3299–3302. <https://doi.org/10.1093/molbev/msx248>
- Ruegg, K. (2007). Genetic, morphological, and ecological characterization of a hybrid zone that spans a migratory divide. *Evolution*, 62(2), 452–466. <https://doi.org/10.1111/j.1558-5646.2007.00263.x>
- Ruegg, K. C., & Smith, T. B. (2002). Not as the crow flies: a historical explanation for circuitous migration in Swainson's thrush (*Catharus ustulatus*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1498), 1375–1381. <https://doi.org/10.1098/rspb.2002.2032>
- Rumble, M. A., Flake, L., Mills, T., & Dystra, B. (2001). Do pine trees in aspen stands increase bird diversity? WD Shepperd et al. (compilers). Sustaining Aspen in Western Landscapes: Symposium Proceedings. Proceedings RMRS-P-18. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. Fort Collins, CO.
- Seitz, L., & Zegers, D. (1993). An experimental study of nest predation in adjacent deciduous, coniferous and successional habitats. *The Condor*, 95(2), 297–304. <https://doi.org/10.2307/1369352>
- Selonen, V., & Hanski, I. K. (2003). Movements of the flying squirrel *Pteromys volans* in corridors and in matrix habitat. *Ecography*, 26(5), 641–651.

<https://doi.org/10.1034/j.1600-0587.2003.03548.x>

- Shochat, E. (2004). Credit or debit? Resource input changes population dynamics of city-slicker birds. *Oikos*, *106*(3), 622–626. <https://doi.org/10.1111/j.0030-1299.2004.13159.x>
- Soha, J. A., Nelson, D. A., & Parker, P. G. (2004). Genetic analysis of song dialect populations in Puget Sound white-crowned sparrows. *Behavioral Ecology*, *15*(4), 636–646. <https://doi.org/10.1093/beheco/arh055>
- Stenzler, L., Fraser, R., & Lovette, I. (2004). Isolation and characterization of 12 microsatellite loci from golden-winged warblers (*Vermivora chrysoptera*) with broad cross-taxon utility in emberizine songbirds. *Molecular Ecology Notes*, *4*(4), 602–604. <https://doi.org/10.1111/j.1471-8286.2004.00750.x>
- Swofford, D. (2002). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Team, Rs. (2015). RStudio: Integrated development for R. Boston, MA: RStudio, Inc.
- Van Oosterhout, C., Hutchinson, W., Wills, D., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>
- Warren, D. L., & Seifert, S. N. (2011). Ecological niche modeling in Maxent : the importance of model complexity and the performance of model selection criteria. *Ecological Applications*, *21*(2), 335–342. <https://doi.org/10.1890/10-1171.1>
- Watanabe, S., Hajima, T., Sudo, K., Nagashima, T., Takemura, T., Okajima, H., Nozawa, T., Kawase, H., Abe, M., Yokohata, T., Ise, T., Sato, H., Kato, E., Takata, K., Emori, S., & Kawamiya, M. (2011). MIROC-ESM 2010: model description and basic results of CMIP5-20c3m experiments. *Geoscientific Model Development*, *4*(4), 845–872. <https://doi.org/10.5194/gmd-4-845-2011>
- Weckstein, J. D., Zink, R. M., Blackwell-Rago, R. C., & Nelson, D. A. (2001). Anomalous variation in mitochondrial genomes of white-crowned (*Zonotrichia leucophrys*) and golden-crowned (*Z. atricapilla*) sparrows: pseudogenes, hybridization, or incomplete lineage sorting? *The Auk*, *118*(1), 231–236. <https://doi.org/10.2307/4089773>
- Wickham, H. (2009). ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag.
- Wingfield, J. C., Hahn, T. P., Maney, D. L., Schoech, S. J., Wada, M., & Morton, M. L. (2003). Effects of temperature on photoperiodically induced reproductive

development, circulating plasma luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha*. *General and Comparative Endocrinology*, 131(2), 143–158. [https://doi.org/10.1016/S0016-6480\(02\)00648-2](https://doi.org/10.1016/S0016-6480(02)00648-2)

Wingfield, J. C., Hahn, T. P., Wada, M., Astheimer, L. B., & Schoech, S. (1996). Interrelationship of day length and temperature on the control of gonadal development, body Mass, and fat score in white-crowned sparrows, *Zonotrichia leucophrys gambelii*. *General and Comparative Endocrinology*, 101(3), 242–255. <https://doi.org/10.1006/GCEN.1996.0027>

Wright, S. (1949). The genetical structure of populations. *Annals of Eugenics*, 15(1), 323–354. <https://doi.org/10.1111/j.1469-1809.1949.tb02451.x>

Zink, R. M., & Barrowclough, G. F. (2008). Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, 17(9), 2107–2121. <https://doi.org/10.1111/j.1365-294X.2008.03737.x>

Zink, R. M., Dittmann, D. L., & Rootes, W. L. (1991). Mitochondrial DNA variation and the phylogeny of *Zonotrichia*. *The Auk*, 108(3), 578-584. <https://doi.org/10.2307/4088098>

CHAPTER THREE: General Discussion

3.1 Introduction

Over the years, a multitude of strategies and statistics have been used to detangle the effects of different evolutionary processes on population genetic structure. Improved technologies have allowed for collections of global datasets, high throughput gene sequencing, and high resolution modelling capabilities with the aim of quantifying genetic connectivity, discerning the origins of reproductive isolation and speciation in natural populations (Charlesworth & Charlesworth, 2017). These advances allow for multi-faceted studies, like ours, to describe both the local and rangewide genetic dynamics of a species across time with greater comprehensiveness than historically possible. By combining data from previous studies on white-crowned sparrows with the patterns observed from our multi-locus genetic markers and landscape genetics models, the species-specific barriers to dispersal can be identified with greater clarity, especially when informed by the influences of Pleistocene isolation. This strategy has huge potential for more accurate predictions of natural populations and their responses to continuing environmental change and natural or anthropogenic disturbance. In this study, our mitochondrial and nuclear DNA data revealed that three subspecies of white-crowned sparrow show rangewide genetic structure best explained by IBD with underlying signatures of IBR, as well as fine-scale structure due to local habitat ecotype differences and elevation.

3.1.1 Historical processes

The signatures of ancient vicariance from the retreat into refugia during the Pleistocene era have been detected in many species (Awise, 1994; Hamilton et al., 2013; Hewitt, 2004; Knowles, 2001; Ruegg, 2007), though the timing and pace of diversification has been especially contentious in avian taxa (Klicka & Zink, 1997; Lovette, 2005). This historic allopatry has been used to explain the origin of subspecies delineations in plants (Soltis et al., 1997; Tremblay & Schoen, 1999), fish (Perdices et al., 2003), mammals (Martin et al., 2003; Van Hooft et al., 2002), and birds (Ruegg & Smith, 2002). Secondary contact of these closely related subspecies groups remains the subject of a multitude of studies on the recolonization strategies used and dynamics of hybrid zones between the refugial lineages (Albaladejo & Aparicio, 2007; Hewitt, 2001; Zamudio & Savage, 2003). Little work has been done on investigating the origins of subspecies and recolonization phenomena in *Z. leucophrys* subspecies since the mid-1900s (Banks, 1964; Rand, 1948), making our study a unique contribution in this field.

Using multi-locus genetic analyses, ecological niche modeling, and previous song dialect literature (Hunn & Beaudette, 2014), we detected patterns corresponding to the partition of *Z. leucophrys* subspecies into the known refugia mentioned above. Our data correspond to Rand's hypothesis (1948) with *Z. l. leucophrys* from the eastern refugium, *Z. l. gambelii* from Beringia, and *Z. l. nuttalli-pugetensis* and *Z. l. oriantha* in two separate refugia south of the ice sheets in the USA. Our phylogenetic tree of mtDNA revealed that *Z. l. leucophrys* and *Z. l. gambelii* group together, while *Z. l. oriantha*, *Z. l. pugetensis*, and *Z. l. nuttalli* group separately, the same groupings of subspecies reported to have shared song dialect elements (Baptista & King, 1980; Hunn & Beaudette, 2014).

The subspecies ENMs in our study support the possibility of Beringia refugium for *Z. l. gambelii* and a shared southern refugium for *Z. l. oriantha* and, *Z. l. pugetensis*. This is the first study in many years to directly test refugia hypotheses for *Z. leucophrys* against DNA patterns and climate models.

3.1.2 Rangewide genetic structure and the Rocky Mountains

Although white-crowned sparrows have been the subject of a multitude of studies on physiology of circadian and hormone cycles (Benoit, 2006; Menaker & Eskin, 1967; Wingfield & Farner, 1978), migratory dynamics (King et al., 1963), breeding behaviour (Baker et al., 1987; Bonier et al., 2014), and especially song dialect learning and responses (Baker, 1983, 2017; Maney et al., 2003; Nelson, 1999), few studies have directly explored the multiple causal factors of contemporary rangewide population genetic structure among and within the subspecies. Each subspecies displays a different migratory strategy, but most genetic studies have compared the non-migratory *Z. l. nuttalli* and/or *Z. l. pugetensis* (a short-distance migrant) and how diverse song dialects affect their regional population structure (Baker et al., 1981 and 1982; Baptista & King, 1980; Chilton & Lein, 1996; Hafner & Petersen, 1985; Macdougall-Shackleton et al., 2002; Nelson, 1999; Soha et al., 2004). Our study is unique because we compared multiple subspecies with various migratory strategies across large geographic distances with a greater emphasis on landscape and climate influences on population genetic structure. We found high levels of genetic differentiation that coincides with IBD for *Z. l. gambelii* and IBD and some IBR for *Z. l. oriantha* at a rangewide scale.

Z. l. oriantha and *Z. l. gambelii* have a capacity for high connectivity due to strong migratory behaviours (Morton, 2002), but the homogenizing effect of high gene flow throughout their ranges is obstructed by IBD in a north-south split, and by mountains in an east-west split. This east-west pattern is common in many genera of North American birds, attributed to the allopatric speciation before and/or during the Pleistocene which created sister taxa with eastern and western forms (Klicka & Zink, 1997; Lovette, 2005). After *Z. l. oriantha* and *Z. l. gambelii* ranges expanded to the interior west of North America post-Pleistocene, we hypothesized that the Rocky Mountains would inhibit gene flow within and between populations on alternate sides of the range. Our high resolution microsatellite data supported our hypothesis that the Rocky Mountains are a barrier causing east-west genetic structure between Alberta and British Columbia populations with a few exceptions. The populations of JAS, RV, MK, and OK were connected by the Yellowhead Pass, a major corridor through the mountains facilitating dispersal and preventing reproductive isolation, but were not genetically distinct. This mountain pass has played a similar role for insects (Robertson et al., 2009) and mammals (Hooge et al., 2003).

3.1.3 Local genetic structure - microgeographic and elevational barriers

In our analyses to characterize rangewide genetic structure, we uncovered local substructure among the northern populations that corresponds to habitat ecotype. The individuals from these northern populations grouped distinctly into alpine coniferous or riparian deciduous habitats, the latter which also included anthropogenically disturbed habitats. Many studies have shown negative relationships between adaptive divergence (when populations diverge genetically due to environmental selection) and both dispersal

and gene flow (Räsänen & Hendry, 2008). However, a growing body of empirical work is showing populations also diverge in the absence of strong physical barriers to gene flow, and furthermore, strong natural selection can lead to divergence in spite of high levels of gene flow (Frachon et al., 2018; Jordan et al., 2005; Zellmer, 2018). To our knowledge, our study is the first to use landscape genetics and bioclimatic niche modelling to show fine-scale effects of environmental conditions on *Z. l. oriantha* and *Z. l. gambelii* distribution and population genetic structure in areas of their distribution not affected by physical barriers.

We also found genetic divergence between populations along an elevational transect could only be detected by the most sensitive microsatellite analyses. Strong clines in mitochondrial DNA have been observed in rufous-collared sparrows (*Zonotrichia capensis*) along much larger elevational transects of ~ 3800 m, attributed to the role of mitochondria in oxidative phosphorylation being a target of natural selection in cold, hypoxic environments (Cheviron & Brumfield, 2009). If we had larger transects with more extreme selection between low and high elevations we might have more likelihood of detecting mtDNA divergence and be able to discuss the implications of local adaptation on *Z. leucophrys* distribution. Instead we observed microsatellite divergence between the CO and OR elevations allowing us to predict that there is some level of reproductive isolation occurring between elevations but we can only speculate that it is caused by climatic differences at different elevations. It has long been known that differentiation in neutral markers is driven by stochastic processes and not always directly by environmental selection (Kirk & Freeland, 2011). Although microsatellites are considered neutral with no effects on phenotype, Kashi and King (2006) noted case

studies of microsatellite divergence with quantitative phenotypic effects and correlations with natural selection: circadian rhythm patterns associated with cold temperature microclimates had longer alleles in *Drosophila* (Zamorzaeva et al., 2005) and *Hordeum spontaneum* grass (Nevo et al., 2005), tandem repeat lengths in a protein-coding region that expresses in vole forebrains (*Microtus montanus* and *M. pennsylvanicus*) were associated with monogamy and social bonding behaviours (Hammock & Young, 2004), and polymorphisms consistently linked to polydactyly in dogs (Fondon & Garner, 2004). It is clear that further investigation is required to gain an understanding of environmental selection pressures and how they manifest in neutral marker divergence patterns for *Z. leucophrys*.

3.2 Future directions

We have identified some of the barriers that influence the genetic structure of three *Z. leucophrys* subspecies at rangewide and local scales, but there are many aspects of this study that could be strengthened and further questions to be investigated. Our study focused on the potential historical origins and phylogenetics of *Z. l. gambelii*, *Z. l. oriantha*, and *Z. l. pugetensis* and their current genetic structure within and between populations due to distance, mountains as physical barriers, and microgeographic barriers.

Sampling additional areas in gaps between current sample sites would allow for more focused tests of genetic structure caused by barriers versus isolation by distance. For example, we could have increased sampling in the gaps between the northern, OR, and CO populations. It is possible that there are other physical or climatic barriers causing the north-south split in genetic structure and not just IBD as we found in our analyses. Since

there are *Z. l. oriantha* in northern populations that cluster apart from the CO *Z. l. oriantha*, additional samples between these sites could determine if there is a gradual change of DNA or if the CO populations are unique. An important improvement in sampling design for testing our hypothesis that gene flow is restricted across elevation would be to use additional transects on multiple mountain ranges with larger elevation gains for comparison. Our microsatellite analyses detected some weak genetic structure across transects that were a ~ 400 m change in elevation, but transects with a greater elevation change could reveal more information about climatic barriers to gene flow. Having multiple mountain ranges to compare could reveal how *Z. leucophrys* adapt to the different areas of heterogeneous landscape within their distribution. Another valuable sample site would be in Cypress Hills Provincial Park where *Z. l. oriantha* inhabitants are separated from all other *Z. leucophrys* breeding populations by 250 km of unsuitable grassland habitat and “cultural isolation” has caused a fixed song element difference compared to other subspecies (Chilton, 2003). This isolated plateau in southern Alberta and Saskatchewan is known to harbour unique haplotype frequencies in lodgepole pine (*Pinus contorta*) after range expansions and contractions throughout historical glacial cycles (Godbout et al., 2008). It is also sometimes the case that island populations are more vulnerable to extinction because of inbreeding and genetic homogenization if there is a lack of immigration from neighbouring populations (Frankham, 1997). It is possible the *Z. l. oriantha* in Cypress Hills could also show genetic signatures from the unique history of this landform or low genetic diversity as compared to the populations in the Rocky Mountains.

To more accurately test the theories of evolutionary history in *Z. leucophrys*, it would be beneficial to incorporate the two other subspecies (*Z. l. nuttalli* and *Z. l. leucophrys*). To best distinguish between Rand's (1948) and Bank's (1964) theories of *Z. leucophrys* refugia and post-Pleistocene expansion, the sequence relatedness between *Z. l. gambelii* and *Z. l. leucophrys* should be tested against relatedness between *Z. l. gambelii* and *Z. l. nuttalli-pugetensis*. If the former pair shows higher similarity, this would provide support for Rand's (1948) hypothesis. Having samples from all the subspecies would also allow for a better comparison of how different migratory strengths affect gene flow patterns within and between populations (especially because *Z. l. nuttalli* is the only sedentary subspecies, and *Z. l. leucophrys* are the furthest east-dwelling subspecies with long distance migratory behaviour and have had much less attention in research than the other four subspecies; Dunn et al., 1995; Morton, 2002).

Many studies have used hybrid zones as natural settings to explore evolution and speciation (Rohwer et al., 2001; Smadja et al., 2004). They are ideal locations to study historical reproductive isolation and range expansion, followed by possible genetic patterns of homogenization through continued interbreeding and introgression, or incipient speciation from local adaptation (e.g. hybrid superiority; Good et al., 2000) and other reproductive barriers (Grant & Grant, 2016). Apart from our study in which we sampled within a hybrid zone between *Z. l. gambelii* and *Z. l. oriantha*, there are other possible hybrid zones to investigate using population and landscape genetics methods, especially any study of *Z. l. leucophrys* for which there is a great paucity of genetic research (Dunn et al., 1995). An interesting location to sample would be the contact zone between *Z. l. leucophrys* and *Z. l. gambelii* near the Hudson and James Bay area where

phenotypic intergradation of black and pale lores are present, and compare to recent evidence of black lore intergradation for the first time in 2015 at Mackenzie Nature Observatory's Mugaha Marsh Banding Station, BC where in the past only pale-lored *Z. l. gambelii* have been predominate. Genetic analyses will be able to answer the question whether there was northward range expansion of *Z. l. oriantha*, or a westward expansion of *Z. l. leucophrys*.

With the advent of new high-throughput molecular techniques like next generation sequencing (NGS), researchers are able to study hybridization and speciation using a genome-wide approach (Davey et al., 2011). Using NGS, Toews et al. (2016) were able to distinguish for the first time two wood warbler species known to have perplexed biologists since the 1800s due to their distinct plumages yet incredibly similar genetics, ecology, distribution, and song. These warblers were indistinguishable with traditional molecular markers, but whole-genome comparisons revealed divergence at six small genomic regions involved in feather development or pigmentation. With this high resolution method it is possible that the subspecies delineation in *Z. leucophrys* can be clarified, after we observed weak differentiation in phylogenetics and other traditional marker analyses in our study. This also begs the question if the plumage and pigment differences between *Z. leucophrys* subspecies correspond to strong genetic divergence at small genomic regions as was found in the wood warblers.

NGS could also be used to find which regions of the genome diverge due to local adaptation at microgeographic scales. After combining NGS with fine-tuned ENMs that employ more specific layers (such as vegetation species distributions, water sources, and

anthropogenic development), we can identify the role that both neutral and non-neutral markers play in population divergence due to barriers corresponding to habitat heterogeneity. For example, Halliday and Brown (1943) noted that the *Z. leucophrys* subspecies distributions correlate “surprisingly well” with the distributions of closely related white spruce, Engelmann spruce, and Sitka spruce. We could apply these species distributions to the *Z. leucophrys* ENMs in addition to the more general categories of our land cover layer for a more ecologically meaningful model. Adding anthropogenic disturbance into models will also be increasingly important as landscape alteration, development, and climate change continue. The effects of these disturbances on gene flow and genetic diversity in wild populations vary with species, as disturbances can introduce novel stressors to which some species are maladapted, or some species can respond with adaptive phenotypic plasticity (Crispo et al., 2010). For *Z. leucophrys*, there is evidence that individuals move to colonize clearcut areas which over time may have significant genetic consequences, such as range shifts that put *Z. l. pugetensis* in contact with *Z. l. oriantha* for the first time (Hunn & Beaudette, 2014). With the vast majority of past and present research on *Z. leucophrys* focused on behaviour and physiology, genetic analyses with this widespread species with various dispersal propensities and unique communication behaviour will provide valuable case study work for questions in population genetics and comparisons with other species that share any of these life history traits.

3.3 General conclusions

The population genetic structure of *Z. leucophrys* is in need of a more comprehensive rangewide, local, and subspecies-inclusive study employing updated multi-locus genetic analyses and landscape modelling methods to supplement the vast array of existing physiological and behavioural research on this species. First we outlined the most current theories of glacial refugia and found with mitochondrial and nuclear DNA that there is weak structure between *Z. l. gambelii*, *Z. l. oriantha*, and *Z. l. pugetensis* subspecies. Although we also used historical ecological niche modelling to suggest suitable Pleistocene refugia for each subspecies, it is premature timing to make strong claims on the historical evolution of these subspecies without further research. Clearer genetic structure occurs between northern populations of Alberta and British Columbia versus southern populations of Oregon and Colorado as a consequence of isolation by distance, and between the eastern populations of Alberta versus western populations of British Columbia caused by the Rocky Mountains as a barrier to dispersal and gene flow. At a local scale we detected genetic structure associated with local adaptation to alpine coniferous and riparian deciduous habitat ecotypes that was supported by high resolution microsatellite analyses and least-cost path models. Our research gives insight into how *Z. leucophrys* are affected by their environment and prompts further investigation into how future environmental changes and increasing anthropogenic influence may impact this species and others with similar life history traits. With such a wide-ranging species of varied behaviour, phenotypically distinct subspecies, and well-researched physiology, there is huge potential for investigating countless more questions of the factors affecting population genetic structure at many spatial and temporal scales to further the quest to understand evolution.

3.4 References

- Albaladejo, R. G., & Aparicio, A. (2007). Population genetic structure and hybridization patterns in the Mediterranean endemics *Phlomis lychnitis* and *P. crinita* (Lamiaceae). *Annals of Botany*, *100*(4), 735–746. <https://doi.org/10.1093/aob/mcm154>
- Adams, R.V., Lazerte, S.E., Otter, K.A., & Burg, T.M. (2016). Influence of landscape features on the microgeographic genetic structure of a resident songbird. *Heredity*, *117*(2), 63-72.
- Avise, J. (1994). *Molecular markers, natural history and evolution* (1st edition). Boston, MA: Springer US. <https://doi.org/10.1007/978-1-4615-2381-9>
- Baker, M. C. (1983). The behavioral response of female Nuttall's white-crowned sparrows to male song of natal and alien dialects. *Behavioral Ecology and Sociobiology*, *12*(4), 309–315. <https://doi.org/10.1007/BF00302898>
- Baker, M. C. (2017). Dialect discrimination by white-crowned sparrows : reactions to near and distant dialects, *100*(2), 452–460.
- Baker, M. C., Spittler-Nabors, K. J., Thompson, A. D., & Cunningham, M. A. (1987). Reproductive behaviour of female white-crowned sparrows: effect of dialects and synthetic hybrid songs. *Animal Behaviour*, *35*(6), 1766–1774. [https://doi.org/10.1016/S0003-3472\(87\)80069-6](https://doi.org/10.1016/S0003-3472(87)80069-6)
- Baker, M. C., Thompson, D. B., Sherman, G. L., & Cunningham, M. A. (1981). The role of male vs male interactions in maintaining population dialect structure. *Behavioral Ecology and Sociobiology*, *8*(1), 65–69. <https://doi.org/10.1007/BF00302845>
- Baker, C. M., Thompson, D. B., Sherman, G. L., & Cunningham, M. A., & Tomback, D. F. (1982). Allozyme frequencies in a linear series of song dialect populations. *Evolution*, *36*(5), 1020–1029. <https://doi.org/10.1111/j.1558-5646.1982.tb05470.x>
- Banks, R. C. (1964). Geographic variation in the white- crowned sparrow *Zonotrichia leucophrys*. *University of California Publications in Zoology*, *70*, 1–123.
- Baptista, L. F., & King, J. R. (1980). Geographical variation in song and song dialects of montane white-crowned sparrows. *The Condor*, *82*(3), 267–284. <https://doi.org/10.2307/1367392>
- Benoit, J. (2006). The structural components of the hypothalamo-hypophyseal pathway, with particular reference to photostimulation of the gonads in birds. *Annals of the New York Academy of Sciences*, *117*(1), 23–34. <https://doi.org/10.1111/j.1749-6632.1964.tb48157.x>
- Bonier, F., Eikenaar, C., Martin, P. R., & Moore, I. T. (2014). Extrapair paternity rates vary with latitude and elevation in Emberizid sparrows. *The American Naturalist*, *183*(1), 54-61. <https://doi.org/10.1086/674130>

- Charlesworth, B., & Charlesworth, D. (2017). Population genetics from 1966 to 2016. *Heredity*, *118*(1), 2–9. <https://doi.org/10.1038/hdy.2016.55>
- Cheviron, Z. A., & Brumfield, R. T. (2009). Migration-selection balance and local adaptation of mitochondrial haplotypes in rufous-collared sparrows (*Zonotrichia capensis*) along an elevational gradient. *Evolution*, *63*(6), 1593–1605. <https://doi.org/10.1111/j.1558-5646.2009.00644.x>
- Chilton, G. (2003). Cultural evolution and song stereotypy in an isolated population of montane white-crowned sparrows (*Zonotrichia leucophrys oriantha*). *Bird Behavior*, *15*(2), 53–63.
- Chilton, G., & Lein, M. R. (1996). Songs and sexual responses of female white-crowned sparrows (*Zonotrichia leucophrys*) from a mixed-dialect population, *133*(3), 173–198.
- Crispo, E., Dibattista, J. D., Correa, C., Thibert-Plante, X., Mckellar, A. E., Schwartz, A. K., Berner, D., De León, L. F., Hendry, A. P., & Crispo, E. (2010). The evolution of phenotypic plasticity in response to anthropogenic disturbance. *Evolutionary Ecology Research*, *12*, 47–66.
- Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., & Blaxter, M. L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, *12*(7), 499–510. <https://doi.org/10.1038/nrg3012>
- Dunn, J. L., Garrett, K. L., & Alderfer, J. (1995). White-crowned sparrow subspecies: identification and distribution. *Birding*, *27*, 182–201.
- Fondon, J. W., & Garner, H. R. (2004). Molecular origins of rapid and continuous morphological evolution. *Proceedings of the National Academy of Sciences*, *101*(52), 18058–18063. <https://doi.org/10.1073/pnas.0408118101>
- Frachon, L., Bartoli, C., Carrère, S., Bouchez, O., Chaubet, A., Gautier, M., Roby, D., & Roux, F. (2018). A genomic map of climate adaptation in *Arabidopsis thaliana* at a micro-geographic scale. *Frontiers in Plant Science*, *9*, 1–15. <https://doi.org/10.3389/fpls.2018.00967>
- Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity*, *78* (3), 311–327.
- Godbout, J., Fazekas, A., Newton, C. H., Yeh, F. C., & Bousquet, J. (2008). Glacial vicariance in the Pacific Northwest: evidence from a lodgepole pine mitochondrial DNA minisatellite for multiple genetically distinct and widely separated refugia. *Molecular Ecology*, *17*(10), 2463–2475. <https://doi.org/10.1111/j.1365-294X.2008.03761.x>
- Good, T. P., Ellis, J. C., Annett, C. A., & Pierotti, R. (2000). Bounded hybrid superiority in an avian hybrid zone: effects of mate, diet, and habitat choice. *Evolution*, *54*(5), 1774–1783. <https://doi.org/10.1111/j.0014-3820.2000.tb00721.x>

- Grant, P. R., & Grant, B. R. (2016). Introgressive hybridization and natural selection in Darwin's finches. *Biological Journal of the Linnean Society*, *117*(4), 812–822. <https://doi.org/10.1111/bij.12702>
- Hafner, D. J., & Petersen, K. E. (1985). Song dialects and gene flow in the white-crowned sparrow, *Zonotrichia leucophrys nuttalli*. *Evolution*, *39*(3), 687–694. <https://doi.org/10.2307/2408663>
- Halliday, W., & Brown, A. (1943). The distribution of some important forest trees in Canada. *Ecology*, *24*(3), 353–373. <https://doi.org/10.2307/1930537>
- Hamilton, J. A., Lexer, C., & Aitken, S. N. (2013). Genomic and phenotypic architecture of a spruce hybrid zone (*Picea sitchensis* × *P. glauca*). *Molecular Ecology*, *22*(3), 827–841. <https://doi.org/10.1111/mec.12007>
- Hammock, E. A. D., & Young, L. J. (2004). Functional microsatellite polymorphism associated with divergent social structure in vole species. *Molecular Biology and Evolution*, *21*(6), 1057–1063. <https://doi.org/10.1093/molbev/msh104>
- Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography - or seeing genes in space and time. *Molecular Ecology*, *10*(3), 537–549. <https://doi.org/10.1046/j.1365-294X.2001.01202.x>
- Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1442), 183–195. <https://doi.org/10.1098/rstb.2003.1388>
- Hooge, J. (2003). *Population Characteristics and Habitat Use of Mountain Caribou in the Wood Arm Area: Annual Report 2002-2003*. B.C. Ministry of Forests. Revelstoke, BC.
- Hunn, E. S., & Beaudette, D. (2014). Apparent sympatry of two subspecies of the white-crowned sparrow, *Zonotrichia leucophrys pugetensis* and *gambelii*, in Washington State. *Western Birds*, *45*(2), 132–140.
- Jordan, M. A., Snell, H. L., Snell, H. M., & Jordan, W. C. (2005). Phenotypic divergence despite high levels of gene flow in Galápagos lava lizards (*Microlophus albemarlensis*). *Molecular Ecology*, *14*, 859–867. <https://doi.org/10.1111/j.1365-294X.2005.02452.x>
- Kashi, Y., & King, D. (2006). Simple sequence repeats as advantageous mutators in evolution. *Trends in Genetics*, *22*(5), 253–259. <https://doi.org/10.1016/j.tig.2006.03.005>
- King, J. R., Barker, S., & Farner, D. S. (1963). A comparison of energy reserves during autumnal and vernal migratory periods in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Ecology*, *44*(3), 513–521. <https://doi.org/10.2307/1932530>
- Kirk, H., & Freeland, J. R. (2011). Applications and implications of neutral versus non-neutral markers in molecular ecology. *International Journal of Molecular Sciences*,

- 12(6), 3966–3988. <https://doi.org/10.3390/ijms12063966>
- Klicka, J.; & Zink, R. (1997). The importance of recent ice ages in speciation: a failed paradigm. *Science*, 277(5332), 1666–1669. <https://doi.org/10.1126/science.277.5332.1666>
- Knowles, L. L. (2001). Did the pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshoppers. *Molecular Ecology*, 10(3), 691–701. <https://doi.org/10.1046/j.1365-294X.2001.01206.x>
- Lovette, I. J. (2005). Glacial cycles and the tempo of avian speciation. *Trends in Ecology & Evolution*, 20(2), 57–59. <https://doi.org/10.1016/j.tree.2004.11.011>
- Macdougall-Shackleton, E. A., Derryberry, E. P., & Hahn, T. P. (2002). Nonlocal male mountain white-crowned sparrows have lower paternity and higher parasite loads than males singing local dialect. *Behavioral Ecology*, 13(5), 682–689. <https://doi.org/10.1093/beheco/13.5.682>
- Maney, D. L., MacDougall-Shackleton, E. A., MacDougall-Shackleton, S. A., Ball, G. F., & Hahn, T. P. (2003). Immediate early gene response to hearing song correlates with receptive behavior and depends on dialect in a female songbird. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, 189(9), 667–674. <https://doi.org/10.1007/s00359-003-0441-z>
- Martin, R. A., Hurt, R. T., Honey, J. G., & Peláez-Campomanes, P. (2003). Late Pliocene and early Pleistocene rodents from the northern Borchers Badlands (Meade County, Kansas), with comments on the Blancan-Irvington boundary in the Meade Basin. *Journal of Paleontology*, 77(5), 985–1001.
- Menaker, M., & Eskin, A. (1967). Circadian clock in photoperiodic time measurement: a test of the Bunning Hypothesis. *Science*, 157(3793), 1182–1185. <https://doi.org/10.1126/science.157.3793.1182>
- Morton, M. (2002). The mountain white-crowned sparrow: migration and reproduction at high altitude. *Studies in Avian Biology*, 24, 1–236.
- Nelson, D. A. (1999). Ecological influences on vocal development in the white-crowned sparrow. *Animal Behaviour*, 58(1), 21–36. <https://doi.org/10.1006/anbe.1999.1118>
- Nevo, E., Beharav, A., Meyer, R. C., Hackett, C. A., Forster, B. P., Russell, J. R., & Powell, W. (2005). Genomic microsatellite adaptive divergence of wild barley by microclimatic stress in ‘Evolution Canyon.’ *Biological Journal of the Linnean Society*, 84(2), 205–224. <https://doi.org/10.1111/j.1095-8312.2005.00425.x>
- Perdices, A., Doadrio, I., Economidis, P. S., Bohlen, J., & Bănărescu, P. (2003). Pleistocene effects on the European freshwater fish fauna: double origin of the cobitid genus *Sabanejewia* in the Danube Basin (Osteichthyes: Cobitidae). *Molecular Phylogenetics and Evolution*, 26(2), 289–299. [https://doi.org/10.1016/S1055-7903\(02\)00334-2](https://doi.org/10.1016/S1055-7903(02)00334-2)

- Rand, A. L. (1948). Glaciation, an isolating factor in speciation. *Evolution*, 2(4), 314–321. <https://doi.org/10.1111/j.1558-5646.1948.tb02749.x>
- Räsänen, K., & Hendry, A. P. (2008). Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters*, 11, 624–636. <https://doi.org/10.1111/j.1461-0248.2008.01176.x>
- Robertson, C., Nelson, T. A., Jelinski, D. E., Wulder, M. A., & Boots, B. (2009). Spatial–temporal analysis of species range expansion: the case of the mountain pine beetle, *Dendroctonus ponderosae*. *Journal of Biogeography*, 36, 1446–1458. <https://doi.org/10.1111/j.1365-2699.2009.02100.x>
- Rohwer, S., Bermingham, E., & Wood, C. (2001). Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. *Evolution*, 55(2), 405–422. <https://doi.org/10.1111/j.0014-3820.2001.tb01303.x>
- Ruegg, K. (2007). Genetic, morphological, and ecological characterization of a hybrid zone that spans a migratory divide. *Evolution*, 62(2), 452–466. <https://doi.org/10.1111/j.1558-5646.2007.00263.x>
- Ruegg, K. C., & Smith, T. B. (2002). Not as the crow flies: a historical explanation for circuitous migration in Swainson’s thrush (*Catharus ustulatus*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1498), 1375–1381. <https://doi.org/10.1098/rspb.2002.2032>
- Smadja, C., Catalan, J., & Ganem, G. (2004). Strong premating divergence in a unimodal hybrid zone between two subspecies of the house mouse. *Journal of Evolutionary Biology*, 17, 165–176. <https://doi.org/10.1046/j.1420-9101.2003.00647.x>
- Soha, J. A., Nelson, D. A., & Parker, P. G. (2004). Genetic analysis of song dialect populations in Puget Sound white-crowned sparrows. *Behavioral Ecology*, 15(4), 636–646. <https://doi.org/10.1093/beheco/arh055>
- Soltis, D. E., Gitzendanner, M. A., Strenge, D. D., & Soltis, P. S. (1997). Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution*, 206(1–4), 353–373. <https://doi.org/10.1007/BF00987957>
- Toews, D. P. L., Taylor, S. A., Vallender, R., Brelsford, A., Butcher, B. G., Messer, P. W., & Lovette, I. J. (2016). Plumage genes and little else distinguish the genomes of hybridizing warblers. *Current Biology*, 26, 2313–2318. <https://doi.org/10.1016/j.cub.2016.06.034>
- Tremblay, N. O., & Schoen, D. J. (1999). Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. *Molecular Ecology*, 8(7), 1187–1198. <https://doi.org/10.1046/j.1365-294x.1999.00680.x>
- Van Hooft, W. F., Groen, A. F., & Prins, H. H. T. (2002). Phylogeography of the African buffalo based on mitochondrial and Y-chromosomal loci: Pleistocene origin and population expansion of the Cape buffalo subspecies. *Molecular Ecology*, 11(2),

267–279. <https://doi.org/10.1046/j.1365-294X.2002.01429.x>

Wingfield, J. C., & Farner, D. S. (1978). The annual cycle of plasma irLH and steroid hormones in feral populations of the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Biology of Reproduction*, *19*(5), 1046–1056.
<https://doi.org/10.1095/biolreprod19.5.1046>

Zamorzaeva, I., Rashkovetsky, E., Nevo, E., & Korol, A. (2005). Sequence polymorphism of candidate behavioural genes in *Drosophila melanogaster* flies from “Evolution canyon.” *Molecular Ecology*, *14*(10), 3235–3245.
<https://doi.org/10.1111/j.1365-294X.2005.02616.x>

Zamudio, K. R., & Savage, W. K. (2003). Historical isolation, range expansion, and secondary contact of two highly divergent mitochondrial lineages in spotted salamanders (*Ambystoma maculatum*). *Evolution*, *57*(7), 1631–1652.
<https://doi.org/10.1554/02-342>

Zellmer, A. (2018). Microgeographic morphological variation across larval wood frog populations associated with environment despite gene flow. *Ecology and Evolution*, *8*(5), 2504–2517. <https://doi.org/10.1002/ece3.3829>

Appendix 1: Information for all white-crowned sparrow samples used in this study including sample ID with population label before the number (JAS, BA, BV, CNP, WT, LE, FTSJ, FL, RV, OK, MK, OR, CO), Band or museum ID number, and subspecies ID for *Z. l. gambelii* (GWCS), *Z. l. oriantha* (MWCS), *Z. l. pugetensis* (PSWS), or unknown (UNK). If samples were donated from a museum or a collaborator, the label is listed under “Source”, while samples caught for this study are blank. Latitude and longitude are recorded in decimal degrees, except for the OR samples which are UTM. Dr. JR refers to Dr. Jim Rivers (Oregon State University) and Dr. RC refers to Dr. Ross Conover (Paul Smith’s College, NY)

Sample ID	Location	Band/Museum ID	Subspecies ID	Source	Latitude	Longitude
JAS001	Jasper Park	14954	GWCS	RBCM	52.10000	-121.93333
JAS002	Medicine Lake Burn, Jasper, AB	1451-65788	GWCS		52.87244	-117.817944
JAS003	Medicine Lake Burn, Jasper, AB	1451-65791	GWCS		52.87244	-117.817944
JAS004	Wilcox Pass Trailhead, Jasper, AB	1451-65792	GWCS		52.21822	-117.185028
JAS005	Wilcox Pass Trailhead, Jasper, AB	1451-65793	GWCS		52.21822	-117.185028
JAS006	Wilcox Pass Trailhead, Jasper, AB	1451-65794	GWCS		52.21822	-117.185028
BA001	Cave and Basin, Banff, AB	1451-65760	GWCS		51.17058	-115.587472
BA002	Cave and Basin, Banff, AB	1451-65761	MWCS		51.17058	-115.587472
BA003	Cave and Basin, Banff, AB	1451-65762	MWCS		51.17058	-115.587472
BA004	Cave and Basin, Banff, AB	1451-65763	MWCS		51.17058	-115.587472
BA005	MAPS Station, Bow Valley Parkway, Banff, AB	1391-86780	MWCS		51.20358	-115.750028
BA006	MAPS Station, Bow Valley Parkway, Banff, AB	1391-86781	GWCS		51.20358	-115.750028
BA007	MAPS Station, Bow Valley Parkway, Banff, AB	1391-86782	MWCS		51.20358	-115.750028
BV001	West Castle Wetlands 1, near Castle Mountain and Beaver Mines, AB	1451-65764	MWCS		49.37661	-114.378389
BV002	West Castle Wetlands 1, near Castle Mountain and Beaver Mines, AB	1451-65765	MWCS		49.37661	-114.378389
BV003	Lynx Creek Road, near Beaver Mines, AB	1451-65766	MWCS		49.45925	-114.372111
BV004	Mill Creek Road, near Beaver Mines, AB	1451-65770	MWCS		49.34667	-114.147306
BV005	Mill Creek Road, near Beaver Mines, AB	1451-65767	MWCS		49.34667	-114.147306
BV006	Mill Creek Road, near Beaver Mines, AB	1451-65768	MWCS		49.34667	-114.147306

BV007	Mill Creek Road, near Beaver Mines, AB	1451-65769	MWCS		49.34667	-114.147306
BV008	Lynx Creek	Z06.4.15	UNK	RAM	49.46140	-114.42567
BV010	Vicary Creek	Z12.2.15	UNK	RAM	49.80000	-114.46655
BV011	Vicary Creek	Z12.2.17	UNK	RAM	49.79996	-114.46646
BV012	Skyline Trail, (Porcupine Hills)	Z96.18.22	UNK	RAM	49.92000	-114.02000
BV013	Beaver Creek, (Porcupine Hills)	Z96.18.14	UNK	RAM	49.82000	-113.95000
BV009	3 miles North West of Beaver Mines Lake	Z99.12.5	UNK	RAM	49.41305	-114.34055
BV014	Bow Crow Forest, Drywood Creek, SAB	WCSP5	UNK		-	-
CNP001	Allison Creek Road 1 Crowsnest Pass, AB	1451-35763	MWCS		49.73077	-114.60774
CNP002	Allison Creek Road 1 Crowsnest Pass, AB	1451-35764	MWCS		49.73077	-114.60774
CNP003	Allison Creek Road 2 Crowsnest Pass, AB	1451-35765	MWCS		49.73077	-114.60774
CNP004	Allison Creek Road 2 Crowsnest Pass, AB Allison Creek Road 3 (Gas plant) Crowsnest Pass, AB	1451-35766	MWCS		49.73077	-114.60774
CNP005	Allison Creek Road 4 Crowsnest Pass, AB	1451-35767	MWCS		49.73077	-114.60774
CNP006	Allison Creek Road 4 Crowsnest Pass, AB	1451-35768	MWCS		49.73077	-114.60774
CNP007	Allison Creek Road 5 Crowsnest Pass, AB	1451-35769	MWCS		49.73077	-114.60774
CNP008	Allison Creek Road 5 Crowsnest Pass, AB	1451-35770	GWCS		49.73077	-114.60774
CNP009	Allison Creek Road 6 Crowsnest Pass, AB	1451-35772	HYWC		49.73077	-114.60774
CNP010	Allison Creek Road 6 Crowsnest Pass, AB	1451-35773	MWCS		49.73077	-114.60774
WT001	Hay Barn, Waterton, S AB	wcsp 1	WCSP		49.08003	-113.85100
WT002	Hwy 6, Waterton, S AB	1791-23986	WCSP		49.10564	-113.82100
WT003	Hwy 6 further, Waterton, S AB	wcsp 3	WCSP		49.09658	-113.80900
WT004	Marquis Hole, Waterton, S AB	wcsp 4	WCSP		49.07119	-113.86100
WT005	Haybarn, Waterton, AB	1451-65771	MWCS		49.07961	-113.85930
WT006	Haybarn, Waterton, AB	1451-65772	MWCS		49.07961	-113.85930
WT007	Haybarn, Waterton, AB	1451-65773	WCSP		49.07961	-113.859306
WT008	Haybarn II, Waterton, AB	1451-65774	MWCS		49.07881	-113.8615
WT009	Haybarn II, Waterton, AB	1451-65775	MWCS		49.07881	-113.8615
WT010	Haybarn II, Waterton, AB	1451-65776	MWCS		49.07881	-113.8615

WT011	Haybarn II, Waterton, AB	1451-65777	MWCS	49.07881	-113.8615
WT012	Haybarn II, Waterton, AB	1451-65778	MWCS	49.07881	-113.8615
WT013	Stables and Camp Columbus, Waterton, AB	1451-65779	GWCS	49.06286	-113.887278
WT014	Stables and Camp Columbus, Waterton, AB	1451-65780	MWCS	49.06286	-113.887278
WT015	Stables and Camp Columbus, Waterton, AB	1451-65781	WCSP	49.06286	-113.887278
WT016	Stables and Camp Columbus, Waterton, AB	1451-65782	WCSP	49.06286	-113.887278
WT017	Stables and Camp Columbus, Waterton, AB	1451-65783	WCSP	49.06286	-113.887278
WT018	Stables Road, Waterton, AB	1451-65785	GWCS	49.06286	-113.887278
WT019	Stables Road, Waterton, AB	1451-65784	WCSP	49.06286	-113.887278
LE001	Lethbridge, AB Helen Schuler Coulee Center	wcsp 1 leth09	WCSP	-	-
LE002	Linda's, Lethbridge	WCSP 2	GWCS	49.69158	-112.837
LE003	Linda's, Lethbridge	WCSP 3	GWCS	49.69158	-112.837
LE004	Linda's, Lethbridge	WCSP 4	GWCS	49.69158	-112.837
LE005	Popson Park (Cam's place), AB	WCSP 5	GWCS	49.55639	-112.872
LE006	Popson Park (Cam's place), AB	WCSP 6	GWCS	49.55639	-112.872
LE007	Popson Park (Cam's place), AB	WCSP 7	GWCS	49.55639	-112.872
LE008	Popson Park (Cam's place), AB	WCSP 8	GWCS	49.55639	-112.872
LE009	Popson Park (Cam's place), AB	WCSP 9	GWCS	49.55639	-112.872
LE010	Popson Park (Cam's place), AB	WCSP 10	GWCS	49.55639	-112.872
LE011	Popson Park (Cam's place), AB	WCSP 11	GWCS	49.55639	-112.872
LE012	Popson Park (Cam's place), AB	WCSP 12	GWCS	49.55639	-112.872
LE013	Popson Park (Cam's place), AB	WCSP 13	GWCS	49.55639	-112.872
LE014	Popson Park (Cam's place), AB	WCSP 14	GWCS	49.55639	-112.872
LE015	Popson Park (Cam's place), AB	WCSP 15	GWCS	49.55639	-112.872
LE016	Popson Park (Cam's place), AB	WCSP 16	GWCS	49.55639	-112.872
LE017	Popson Park (Cam's place), AB	WCSP 17	GWCS	49.55639	-112.872
LE018	Popson Park (Cam's place), AB	WCSP 18	GWCS	49.55639	-112.872
LE019	Cottonwood Park, Lethbridge	WCSP19	UNK	-	-

FTSJ001	Necoslie Road, Fort St James, BC	WCSP 1	GWCS		54.41603	-124.22
FTSJ002	Necoslie Road, Fort St James, BC	WCSP 2 CBC249	GWCS		54.41603	-124.22
FTSJ003	Necoslie Road, Fort St James, BC	WCSP 3 FTSTJ250	WCSP		54.41603	-124.22
FTSJ004	Necoslie Road, Fort St James, BC	WCSP 4	WCSP		54.41603	-124.22
FTSJ005	Necoslie Road, Fort St James, BC	WCSP 5	GWCS		54.41603	-124.22
FTSJ006	Necoslie Road, Fort St James, BC	WCSP 6	GWCS		54.41603	-124.22
FTSJ007	Necoslie Road, Fort St James, BC	WCSP 7	GWCS		54.41603	-124.22
FTSJ008	Necoslie Road, Fort St James, BC	WCSP 8 CBC266	GWCS		54.41603	-124.22
FTSJ009	Necoslie Road, Fort St James, BC	WCSP 9 CBC267	GWCS		54.41603	-124.22
FTSJ010	4712 Sowchea Road, Fort St James, BC	WCSP 10	GWCS		54.42586	-124.317
FTSJ011	4712 Sowchea Road, Fort St James, BC	WCSP 11	GWCS		54.42586	-124.317
FTSJ012	Hanceville	3603	GWCS		51.91667	-123.033333
FTSJ013	Williams Lake	15910	GWCS		52.141673	-122.141688
FL001	17224 Colleymount Rd, Francois Lake, BC	WCSP 12 CBC321	GWCS		54.04006	-125.991
FL002	17224 Colleymount Rd, Francois Lake, BC	WCSP 13	GWCS		54.04006	-125.991
FL003	Ootsa Lake	5355	GWCS	RBCM	53.806821	-126.04532
RV001	Blanket Creek Forestry Road, high elevation	1451-65757	GWCS		50.82672	-118.121722
RV002	Blanket Creek Forestry Road, mid elevation	1451-65758	GWCS		50.82672	-118.121722
RV003	Blanket Creek Forestry Road, mid elevation	1451-65759	WCSP		50.82672	-118.121722
RV004	Sproat Mountain		GWCS	RBCM	49.03333	-119.45
RV005	Shuswap River	18227	GWCS	RBCM	50.71667	-119.05
RV006	Vernon	18677	GWCS	RBCM	50.26667	-119.266667
OK001	McDiarmid Meadows, Manning, BC	1451-65795	HYWC		49.12875	-120.623944
OK002	McDiarmid Meadows, Manning, BC	1451-65796	GWCS		49.12875	-120.623944
OK003	Okanagan Falls	3593	GWCS	RBCM	49.35	-119.566667
OK004	Okanagan Landing		GWCS	RBCM	51.91667	-123.033333
OK005	Okanagan Landing		GWCS	RBCM	51.91667	-123.033333
OK006	Okanagan Landing		GWCS	RBCM	51.91667	-123.033333

OK007	Okanagan		GWCS	RBCM	51.91667	-123.033333
OK008	Okanagan		GWCS	RBCM	51.91667	-123.033333
OK009	Okanagan Landing	7599	GWCS	RBCM	51.91667	-123.033333
OK010	Manning Provincial Park	9141	GWCS	RBCM	49.03333	-115.2
OK011	Osoyoos Lake	11926	GWCS	RBCM	52.13333	-122.15
OK012	Osoyoos	15143	GWCS	RBCM	52.16667	-121.9
OK013	Osoyoos	15144	GWCS	RBCM	52.16667	-121.9
CR001	Cranbrook	10940	GWCS	RBCM	49.03333	-119.45
CR002	Cranbrook	10941	GWCS	RBCM	49.03333	-119.45
CR003	Cranbrook	10942	GWCS	RBCM	49.03333	-119.45
CR004	Newgate	14216	GWCS	RBCM	49.025716	-115.197493
CR005	Newgate	14217	GWCS	RBCM	49.025716	-115.197493
CR006	Windermere Lake	18472	GWCS	RBCM	50.45	-116
MK001	Mackenzie, BC Municipal RV Park Clearcut	1451-35774	MWCS		55.32492	-123.095861
MK002	Mackenzie, BC Municipal RV Park Clearcut	1451-35775	GWCS		55.32492	-123.095861
MK003	Mackenzie, BC Municipal RV Park Clearcut	1451-35776	GWCS		55.32492	-123.095861
MK004	Mackenzie, BC Municipal RV Park Clearcut	1451-35777	GWCS		55.32492	-123.095861
MK005	Mackenzie, BC Municipal RV Park Clearcut	1451-35778	GWCS		55.32492	-123.095861
MK006	Mackenzie, BC Municipal RV Park Clearcut	1451-35779	GWCS		55.32492	-123.095861
MK007	Clearcut off highway 39, Mackenzie, BC	1451-35780	GWCS		55.38839	-123.139111
MK008	Clearcut off highway 39, Mackenzie, BC	1451-35781	GWCS		55.38839	-123.139111
MK009	Clearcut 2 off highway 39, Mackenzie, BC	1451-35782	GWCS		55.38839	-123.139111
MK010	Clearcut near boat launch, Mackenzie, BC	1451-35783	MWCS		55.38839	-123.139111
MK011	Clearcut near boat launch, Mackenzie, BC	1451-35784	MWCS		55.38839	-123.139111
MK012	Industrial area 1, Mackenzie, BC	1451-35785	GWCS		55.33419	-123.167028
MK013	Industrial area 1, Mackenzie, BC	1451-35786	GWCS		55.33419	-123.167028
MK014	Industrial area 1, Mackenzie, BC	1451-35787	GWCS		55.33419	-123.167028
MK015	Industrial area 2, Mackenzie, BC	1451-35788	GWCS		55.33419	-123.167028

MK016	Industrial area 2, Mackenzie, BC	1451-35789	GWCS		55.33419	-123.167028
MK017	Mackenzie, BC Municipal RV Park Clearcut	1451-35790	GWCS		55.32492	-123.095861
MK018	Mackenzie, BC Municipal RV Park Clearcut	1451-35791	HYWC		55.32492	-123.095861
MK019	Industrial area 2, Mackenzie, BC	1451-35792	GWCS		55.33419	-123.167028
MK020	Industrial area 2, Mackenzie, BC	1451-35793	GWCS		55.33419	-123.167028
MK021	Industrial area 2, Mackenzie, BC	1451-35794	GWCS		55.33419	-123.167028
MK022	Industrial area 2, Mackenzie, BC	1451-35795	GWCS		55.33419	-123.167028
MK023	Industrial area 2, Mackenzie, BC	1451-35796	GWCS		55.33419	-123.167028
MK024	Industrial area 3, Mackenzie, BC	1451-35797	MWCS		55.2855	-123.147139
MK025	Industrial area 3, Mackenzie, BC	1451-35798	GWCS		55.2855	-123.147139
MK026	Industrial area 3, Mackenzie, BC	1451-35799	GWCS		55.2855	-123.147139
MK027	Industrial area 3, Mackenzie, BC	1451-35800	GWCS		55.2855	-123.147139
MK028	Industrial area 3, Mackenzie, BC	1451-65789	GWCS		55.2855	-123.147139
MK029	Industrial area 3, Mackenzie, BC	1451-65790	GWCS		55.2855	-123.147139
WA001	Lake Tapps 16318 37St. Cr. E., WA	wesp 1 WA30	UNK		47.22295	-122.213
WA002	Lake Tapps 16318 37St. Cr. E., WA	wesp 2 WA36	UNK		47.22295	-122.213
OR001	Pacific City, OR	wesp 1 OR coast	PSWS		45.20652	-123.964
OR002	Pacific City, OR	wesp 2 OR coast	PSWS		45.20652	-123.964
OR003	Pacific City, OR	wesp 3 OR coast	PSWS		45.20652	-123.964
OR004Low	Black Rock SAGA11	2541-57466	PSWS	Dr. JR	468792	4980089
OR005Low	Black Rock SAGA15	2541-57329	PSWS	Dr. JR	468788	4979730
OR006Low	Black Rock SAPA61	1501-30561	PSWS	Dr. JR	466883	4979713
OR007Low	Black Rock SAPA7	1421-12573	PSWS	Dr. JR	466761	4979501
OR008Low	Luckiamute NWRO15	1501-30631	PSWS	Dr. JR	460764	4961877
OR009Mid	Luckiamute NWRO5	1501-30603	PSWS	Dr. JR	467852	5027370
OR010Mid	Luckiamute TEAS3	1421-12945	PSWS	Dr. JR	467233	5026242
OR011Mid	Luckiamute TEAS5	1131-88634	PSWS	Dr. JR	466981	5026503
OR012Mid	Trask TOL719	1421-12552	PSWS	Dr. JR	462607	5003319

OR013Mid	Trask TOL725	1421-12885	PSWS	Dr. JR	462532	5003605
OR014Mid	Trask TOPI5	1131-88718	PSWS	Dr. JR	461639	5004252
OR020Low	Black Rock SAGA_1Low	2541-57466	PSWS	Dr. JR	468675	4979894
OR021Low	Black Rock SAGA_1Low	1421-12890	PSWS	Dr. JR	468762	4979948
OR022Low	Black Rock SAGA_1Low	1421-12891	PSWS	Dr. JR	468828	4979963
OR023Low	Black Rock SAGA_1Low	2541-57467	PSWS	Dr. JR	468694	4979823
OR024Low	Black Rock SAGA_1Low	1421-12868	PSWS	Dr. JR	468920	4979955
OR025Low	Black Rock SAGA_1Low	1421-12869	PSWS	Dr. JR	468855	4979948
OR026Low	Black Rock SAGA_1Low	1501-30844	PSWS	Dr. JR	468866	4979948
OR027Low	Black Rock SAGA_1Low	2541-57468	PSWS	Dr. JR	468930	4979950
OR028Low	Black Rock SAGA_1Low	1501-30661	PSWS	Dr. JR	468793	4979883
OR029Low	Black Rock SAGA_1Low	2541-57451	PSWS	Dr. JR	468745	4979820
OR030Low	Black Rock SAGA_1Low	2541-57333	PSWS	Dr. JR	468779	4979820
OR031Low	Black Rock SAGA_1Low	1421-12871	PSWS	Dr. JR	463425	4976190
OR032Low	Black Rock SAGA_1Low	1501-30662	PSWS	Dr. JR	463612	4976344
OR033Low	Black Rock SAGA_1Low	1421-12875	PSWS	Dr. JR	463606	4976285
OR034Low	Black Rock SAGA_1Low	1421-12899	PSWS	Dr. JR	463626	4976367
OR050Low	Luckiamute SCRE_3Low	1501-30558	PSWS	Dr. JR	463488	4959848
OR051Low	Luckiamute SCRE_3Low	1421-12929	PSWS	Dr. JR	463359	4959942
OR052Low	Luckiamute SCRE_3Low	1421-12910	PSWS	Dr. JR	463536	4939844
OR053Low	Luckiamute SCRE_3Low	1421-12733	PSWS	Dr. JR	463418	4959848
OR054Low	Luckiamute SCRE_3Low	1501-30731	PSWS	Dr. JR	463443	4959811
OR055Low	Luckiamute SCRE_3Low	1421-12789	PSWS	Dr. JR	463393	4959862
OR056Low	Luckiamute SCRE_3Low	1131-88204	PSWS	Dr. JR	463390	4959847
OR057Low	Luckiamute SCRE_3Low	1501-30779	PSWS	Dr. JR	460613	4961960
OR058Low	Luckiamute SCRE_3Low	1501-30785	PSWS	Dr. JR	460611	4961968
OR059Low	Luckiamute SCRE_3Low	1131-88808	PSWS	Dr. JR	460698	4962009
OR060Low	Luckiamute SCRE_3Low	2541-57254	PSWS	Dr. JR	460531	4961875

OR061Low	Luckiamute SCRE_3Low	1131-88287	PSWS	Dr. JR	460585	4961950
OR062Low	Luckiamute SCRE_3Low	1501-30782	PSWS	Dr. JR	460540	4961929
OR063Low	Luckiamute SCRE_3Low	1131-88957	PSWS	Dr. JR	460546	4961935
OR064Low	Luckiamute SCRE_3Low	1501-30525	PSWS	Dr. JR	460510	4961818
OR080Low	Trask FAIR_3Low	1131-88836	PSWS	Dr. JR	470236	5028435
OR081Low	Trask FAIR_3Low	1421-12949	PSWS	Dr. JR	470306	5028324
OR082Low	Trask WEFL_2Low	1421-12941	PSWS	Dr. JR	468680	5028939
OR083Low	Trask FAIR_3Low	2541-57418	PSWS	Dr. JR	470290	5028343
OR084Low	Trask FAIR_3Low	1421-12887	PSWS	Dr. JR	470278	5028347
OR085Low	Trask FAIR_3Low	1131-88835	PSWS	Dr. JR	470227	5028464
OR086Low	Trask FAIR_3Low	2541-57421	PSWS	Dr. JR	470183	5028422
OR087Low	Trask FAIR_3Low	1421-12581	PSWS	Dr. JR	470198	5028316
OR088Low	Trask FAIR_3Low	2541-57407	PSWS	Dr. JR	470081	5028452
OR089Low	Trask FAIR_3Low	1131-88209	PSWS	Dr. JR	470046	5028685
OR090Low	Trask FAIR_3Low	1131-88824	PSWS	Dr. JR	470014	5028368
OR091Low	Trask FAIR_3Low	1131-88340	PSWS	Dr. JR	470013	5028446
OR092Low	Trask FAIR_3Low	2541-57461	PSWS	Dr. JR	469959	5028451
OR093Low	Trask FAIR_3Low	1131-88825	PSWS	Dr. JR	469920	5028522
OR094Low	Trask FAIR_3Low	2541-57405	PSWS	Dr. JR	469973	5028504
OR111Low	Willamina CORN_2Low	1501-30650	PSWS	Dr. JR	464182	5004963
OR112Low	Willamina CORN_2Low	1421-12797	PSWS	Dr. JR	464320	5005004
OR113Low	Willamina CORN_2Low	2541-57305	PSWS	Dr. JR	464164	5004974
OR114Low	Willamina CORN_2Low	1501-30644	PSWS	Dr. JR	464183	5004915
OR115Low	Willamina CORN_2Low	1501-30647	PSWS	Dr. JR	464150	5004893
OR116Low	Willamina CORN_2Low	1501-30540	PSWS	Dr. JR	464154	5004812
OR117Low	Willamina CORN_2Low	2541-57322	PSWS	Dr. JR	464170	5004886
OR118Low	Willamina CORN_2Low	1501-30589	PSWS	Dr. JR	464199	5004845
OR119Low	Willamina CORN_2Low	1501-30851	PSWS	Dr. JR	464217	5004871

OR120Low	Willamina CORN_2Low	1501-30653	PSWS	Dr. JR	464168	5004811
OR121Low	Willamina CORN_2Low	2541-57309	PSWS	Dr. JR	464306	5004938
OR122Low	Willamina CORN_2Low	2541-57320	PSWS	Dr. JR	464234	5004864
OR123Low	Willamina CORN_2Low	1131-88272	PSWS	Dr. JR	464318	5004917
OR124Low	Willamina CORN_2Low	1131-88675	PSWS	Dr. JR	464209	5004787
OR015High	Trask TOPI9	1131-88804	PSWS	Dr. JR	466981	5026503
OR016High	Willamina GLOW12	1131-88742	PSWS	Dr. JR	462607	5003319
OR017High	Willamina GLOW8	1501-30542	PSWS	Dr. JR	462532	5003605
OR018High	Willamina TSUN14	1501-30572	PSWS	Dr. JR	461639	5004252
OR019High	Willamina TSUN28	1131-88350	PSWS	Dr. JR	461763	5004480
OR035High	Black Rock MIDI_2High	1501-30584	PSWS	Dr. JR	463425	4976190
OR036High	Black Rock MIDI_2High	1131-88262	PSWS	Dr. JR	463612	4976344
OR037High	Black Rock MIDI_2High	1501-30552	PSWS	Dr. JR	463606	4976285
OR038High	Black Rock MIDI_2High	1131-88414	PSWS	Dr. JR	463626	4976367
OR039High	Black Rock MIDI_2High	1131-88263	PSWS	Dr. JR	463645	4976303
OR040High	Black Rock MIDI_2High	1131-88401	PSWS	Dr. JR	463665	4976316
OR041High	Black Rock MIDI_2High	1421-12770	PSWS	Dr. JR	463668	4976317
OR042High	Black Rock MIDI_2High	1501-30774	PSWS	Dr. JR	463550	4976200
OR043High	Black Rock MIDI_2High	1421-12775	PSWS	Dr. JR	463685	4976309
OR044High	Black Rock MIDI_2High	1131-88422	PSWS	Dr. JR	463592	4976197
OR045High	Black Rock MIDI_2High	1421-12769	PSWS	Dr. JR	463713	4976262
OR046High	Black Rock MIDI_2High	1421-12784	PSWS	Dr. JR	463721	4976211
OR047High	Black Rock MIDI_2High	1421-12777	PSWS	Dr. JR	463676	4976209
OR048High	Black Rock MIDI_2High	1421-12787	PSWS	Dr. JR	463710	4976239
OR049High	Black Rock MIDI_2High	1501-30577	PSWS	Dr. JR	463772	4976290
OR065High	Luckiamute NWRO_4High	1501-30546	PSWS	Dr. JR	460613	4961960
OR066High	Luckiamute NWRO_4High	1421-12774	PSWS	Dr. JR	460611	4961968
OR067High	Luckiamute NWRO_4High	1421-12724	PSWS	Dr. JR	460698	4962009

OR068High	Luckiamute NWRO_4High	1421-12762	PSWS	Dr. JR	460531	4961875
OR069High	Luckiamute NWRO_4High	1131-88809	PSWS	Dr. JR	460585	4961950
OR070High	Luckiamute NWRO_4High	1131-88894	PSWS	Dr. JR	460540	4961929
OR071High	Luckiamute NWRO_4High	1421-12713	PSWS	Dr. JR	460546	4961935
OR072High	Luckiamute NWRO_4High	1131-88997	PSWS	Dr. JR	460510	4961818
OR073High	Luckiamute NWRO_4High	1421-12745	PSWS	Dr. JR	460479	4961862
OR074High	Luckiamute NWRO_4High	1131-88884	PSWS	Dr. JR	460506	4961933
OR075High	Luckiamute NWRO_4High	1131-88790	PSWS	Dr. JR	460633	4962027
OR076High	Luckiamute NWRO_4High	1421-12928	PSWS	Dr. JR	460462	4961920
OR077High	Luckiamute NWRO_4High	1501-30603	PSWS	Dr. JR	460555	4962004
OR078High	Luckiamute NWRO_4High	1131-88922	PSWS	Dr. JR	460646	4962044
OR079High	Luckiamute NWRO_4High	1131-88904	PSWS	Dr. JR	460542	4962036
OR095High	Trask TOL7_1High	1131-88646	PSWS	Dr. JR	467807	5027567
OR096High	Trask TOL7_1High	2541-57425	PSWS	Dr. JR	467844	5027416
OR097High	Trask TOL7_1High	2541-57414	PSWS	Dr. JR	467748	5027547
OR098High	Trask TOL7_1High	1131-88366	PSWS	Dr. JR	467796	5027597
OR099High	Trask TOL7_1High	1131-88815	PSWS	Dr. JR	467805	5027583
OR100High	Trask TOL7_1High	1131-88823	PSWS	Dr. JR	467746	5027628
OR101High	Trask TOL7_1High	1421-12600	PSWS	Dr. JR	467737	5027656
OR102High	Trask TOL7_1High	1131-88822	PSWS	Dr. JR	467736	5027559
OR103High	Trask TOL7_1High	1131-88383	PSWS	Dr. JR	467719	5027609
OR104High	Trask TOL7_1High	1501-30637	PSWS	Dr. JR	467719	5027608
OR105High	Trask TOL7_1High	1131-88715	PSWS	Dr. JR	467747	5027726
OR106High	Trask TOL7_1High	1421-12881	PSWS	Dr. JR	467682	5027640
OR107High	Trask TOL7_1High	1501-30629	PSWS	Dr. JR	467757	5027774
OR108High	Trask TOL7_1High	1501-30622	PSWS	Dr. JR	467769	5027762
OR109High	Trask TOL7_1High	1421-12552	PSWS	Dr. JR	467718	5027790
OR110High	Willamina CORN_2High	1131-88210	PSWS	Dr. JR	464084	5004825

OR125High	Willamina CHEF_3High	1131-88747	PSWS	Dr. JR	465059	5006968
OR126High	Willamina CHEF_3High	1131-88726	PSWS	Dr. JR	465045	5006984
OR127High	Willamina CHEF_3High	1501-30581	PSWS	Dr. JR	464938	5006858
OR128High	Willamina CHEF_3High	1131-88752	PSWS	Dr. JR	464916	5006976
OR129High	Willamina CHEF_3High	2541-57245	PSWS	Dr. JR	464927	5006955
OR130High	Willamina CHEF_3High	1131-88343	PSWS	Dr. JR	464902	5006900
OR131High	Willamina CHEF_3High	1501-30738	PSWS	Dr. JR	464889	5006928
OR132High	Willamina CHEF_3High	1421-12795	PSWS	Dr. JR	464905	5006979
OR133High	Willamina CHEF_3High	2541-57318	PSWS	Dr. JR	464889	5006900
OR134High	Willamina CHEF_3High	1421-12796	PSWS	Dr. JR	464929	5007001
OR135High	Willamina CHEF_3High	1501-30724	PSWS	Dr. JR	464864	5006872
OR136High	Willamina CHEF_3High	1501-30535	PSWS	Dr. JR	464865	5006979
OR137High	Willamina CHEF_3High	1131-88326	PSWS	Dr. JR	464903	5007042
OR138High	Willamina CHEF_3High	1131-88967	PSWS	Dr. JR	464827	5006899
OR139High	Willamina CHEF_3High	1421-12675	PSWS	Dr. JR	464883	5007036
CO005Low	Colorado Site 1_Lower Parking	2331-96089	MWCS	Dr. RC	38.96582	-106.99402
CO006Low	Colorado Site 1_Whorehouse	2661-01488	MWCS	Dr. RC	38.96734	-106.99413
CO007Low	Colorado Site 1_Whorehouse	2661-01492	MWCS	Dr. RC	38.96734	-106.99413
CO008Low	Colorado Site 1_Tame	2331-96081	MWCS	Dr. RC	38.96569	-106.99323
CO009Low	Colorado Site 2_Mudbar	2331-96077	MWCS	Dr. RC	38.9753	-106.99825
CO010Low	Colorado Site 2_Mudbar	2331-96080	MWCS	Dr. RC	38.9753	-106.99825
CO011Low	Colorado Site 2_Mudbar	2541-37969	MWCS	Dr. RC	38.9753	-106.99825
CO012Low	Colorado Site 2_Mudbar	2661-01271	MWCS	Dr. RC	38.9753	-106.99825
CO013Low	Colorado Site 2_Mudbar	2541-37940	MWCS	Dr. RC	38.9753	-106.99825
CO014Low	Colorado Site 1_Behind the Bushes	2341-30622	MWCS	Dr. RC	38.96927	-106.99553
CO015Low	Colorado Site 1_Yellow	2331-96087	MWCS	Dr. RC	38.97016	-106.99571
CO032Low	Colorado Site 1_Lower Parking	2661-01389	MWCS	Dr. RC	38.96582	-106.99402
CO033Low	Colorado Site 1_Cut Stump	2661-01483	MWCS	Dr. RC	38.96804	-106.99439

CO034Low	Colorado Site 2_No Parking	2331-96091	MWCS	Dr. RC	38.97698	-107.00065
CO035Low	Colorado Site 2_No Parking	2541-37963	MWCS	Dr. RC	38.97698	-107.00065
CO036Low	Colorado Site 2_Small Spruce	2541-37957	MWCS	Dr. RC	38.97594	-106.99929
CO037Low	Colorado Site 2_Small Spruce	2541-37959	MWCS	Dr. RC	38.97594	-106.99929
CO038Low	Colorado Site 2_Small Spruce	2661-01340	MWCS	Dr. RC	38.97594	-106.99929
CO039Low	Colorado Site 2_Bridge	2661-01417	MWCS	Dr. RC	38.97765	-107.00243
CO040Low	Colorado Site 2_Bridge	2541-37993	MWCS	Dr. RC	38.97765	-107.00243
CO041Low	Colorado Site 2_Cliff	2281-70965	MWCS	Dr. RC	38.97776	-107.00313
CO042Low	Colorado Site 2_Ohio	2661-01465	MWCS	Dr. RC	38.97577	-106.99876
CO043Low	Colorado Site 2_Next to Avery	2661-01276	MWCS	Dr. RC	38.97636	-106.99739
CO044Low	Colorado Site 2_3 Amigos	2661-01477	MWCS	Dr. RC	38.97649	-106.999
CO001Mid	Colorado Site 4_Fetch	1301-50806	MWCS	Dr. RC	38.99457	-107.01405
CO002Mid	Colorado Site 4_Fetch	2331-96079	MWCS	Dr. RC	38.99457	-107.01405
CO003Mid	Colorado Site 4_Fetch	2661-01497	MWCS	Dr. RC	38.99457	-107.01405
CO004/031Mid	Colorado Site 4_Fetch	2661-01226	MWCS	Dr. RC	38.99457	-107.01405
CO045Mid	Colorado Site 4_Scrub Slope	2331-96075	MWCS	Dr. RC	38.99292	-107.01314
CO046Mid	Colorado Site 4_Scrub Slope	2661-01437	MWCS	Dr. RC	38.99292	-107.01314
CO047Mid	Colorado Site 4_Scrub Slope	2661-01480	MWCS	Dr. RC	38.99292	-107.01314
CO048Mid	Colorado Site 4_Scrub Slope	2331-96082	MWCS	Dr. RC	38.99292	-107.01314
CO049Mid	Colorado Site 4_Scrub Slope	2661-01406	MWCS	Dr. RC	38.99292	-107.01314
CO050Mid	Colorado Site 4_Scrub Slope	1301-50812	MWCS	Dr. RC	38.99292	-107.01314
CO051Mid	Colorado Site 4_Tea Stick	2661-01426	MWCS	Dr. RC	38.99318	-107.01367
CO053Mid	Colorado Site 4_Dorothy	2661-01423	MWCS	Dr. RC	38.99409	-107.01385
CO054Mid	Colorado Site 4_The Beav	2541-37986	MWCS	Dr. RC	38.994	-107.01469
CO055Mid	Colorado Site 4_Stubby Spruce	2331-96078	MWCS	Dr. RC	38.99557	-107.01424
CO056Mid	Colorado Site 4_Stubby Spruce	2661-01409	MWCS	Dr. RC	38.99557	-107.01424
CO057Mid	Colorado Site 4_Box	2661-01411	MWCS	Dr. RC	38.99594	-107.01431
CO058Mid	Colorado Site 4_Box	2661-01402	MWCS	Dr. RC	38.99594	-107.01431

CO059Mid	Colorado 4N_Box	2661-01455	MWCS	Dr. RC	38.99594	-107.01431
CO060Mid	Colorado Site 4_Higher Ground	2661-01405	MWCS	Dr. RC	38.99689	-107.01474
CO061Mid	Colorado Site 4_Higher Ground	2661-01407	MWCS	Dr. RC	38.99689	-107.01474
CO083Mid	Colorado Site 4_Alamo	2331-96076	MWCS	Dr. RC	38.99	-107.01
CO084Mid	Colorado Site 4_Edge of Sea	2661-01470	MWCS	Dr. RC	38.99	-107.01
CO085Mid	Colorado Site 4_Edge of Sea	2541-37946	MWCS	Dr. RC	38.99	-107.01
CO086Mid	Colorado Site 4_Edge of Sea	2661-01434	MWCS	Dr. RC	38.99	-107.01
CO087Mid	Colorado Site 4_Edge of Sea	2341-30498	MWCS	Dr. RC	38.99	-107.01
CO088Mid	Colorado Site 4_Edge of Sea	2661-01475	MWCS	Dr. RC	38.99	-107.01
CO089Mid	Colorado Site 4_Pylon	2331-96074	MWCS	Dr. RC	38.99	-107.01
CO090Mid	Colorado Site 4_Pylon	2661-01341	MWCS	Dr. RC	38.99	-107.01
CO091Mid	Colorado Site 4_Quagmire	2661-01408	MWCS	Dr. RC	38.99	-107.01
CO092Mid	Colorado Site 4_Sea View	2331-96071	MWCS	Dr. RC	38.99	-107.01
CO093Mid	Colorado Site 4_Sea View	2661-01479	MWCS	Dr. RC	38.99	-107.01
CO094Mid	Colorado Site 4_Sea View	2661-01343	MWCS	Dr. RC	38.99	-107.01
CO095Mid	Colorado Site 4_Skunk Island Pier	2661-01268	MWCS	Dr. RC	38.99	-107.01
CO096Mid	Colorado Site 4_Skunk Island Pier	2661-01433	MWCS	Dr. RC	38.99	-107.01
CO016High	Colorado NPB_Alpine Lawn	2661-01387	MWCS	Dr. RC	39.02291	-107.08385
CO017High	Colorado NPB_Spruce Graveyard	2661-01333	MWCS	Dr. RC	39.02292	-107.08498
CO018High	Colorado NPB_Generic	2661-01489	MWCS	Dr. RC	39.02495	-107.08555
CO019High	Colorado NPB_Rock Garden	2661-01331	MWCS	Dr. RC	39.02411	-107.08573
CO020/082High	Colorado NPB_Oxidized	2661-01493	MWCS	Dr. RC	39.02321	-107.08519
CO021/100High	Colorado Paradise_Raggeds View	1301-50811	MWCS	Dr. RC	38.99	-107.05
CO022High	Colorado Paradise_Slope Spruce	1301-50807	MWCS	Dr. RC	38.99	-107.05
CO023/101High	Colorado Paradise_Slope Spruce	1301-50809	MWCS	Dr. RC	38.99	-107.05
CO024/102High	Colorado VB_Base of Slope	2661-01472	MWCS	Dr. RC	38.98	-106.98
CO025/104High	Colorado VB_Base of Slope	2661-01498	MWCS	Dr. RC	38.98	-106.98
CO026High	Colorado VB_Central Meadow	2661-01460	MWCS	Dr. RC	38.98	-106.98

CO027/105High	Colorado VB_Central Meadow	2661-01464	MWCS	Dr. RC	38.98	-106.98
CO028High	Colorado VB_Tentacle Stump	2661-01440	MWCS	Dr. RC	38.98	-106.98
CO029/103High	Colorado VB_Base of Slope	2661-01459	MWCS	Dr. RC	38.98	-106.98
CO030High	Colorado VB_Undercover	2661-01441	MWCS	Dr. RC	38.98	-106.98
CO062High	Colorado Schofield_Southcentral	2661-01328	MWCS	Dr. RC	39.02629	-107.05093
CO063High	Colorado Schofield_Swampview	1301-50835	MWCS	Dr. RC	39.02897	-107.0527
CO064High	Colorado Schofield_UNO	1301-50820	MWCS	Dr. RC	39.02885	-107.05412
CO065High	Colorado Schofield_UNO	1301-50829	MWCS	Dr. RC	39.02885	-107.05412
CO066High	Colorado Schofield_Medussa Spruce	2661-01450	MWCS	Dr. RC	39.02819	-107.05379
CO067High	Colorado Schofield_Ant Mound	1301-50815	MWCS	Dr. RC	39.02713	-107.05232
CO068High	Colorado Schofield_Ant Mound	1301-50821	MWCS	Dr. RC	39.02713	-107.05232
CO069High	Colorado Schofield_TH View	2661-01427	MWCS	Dr. RC	39.02602	-107.05149
CO070High	Colorado Schofield_TH View	2661-01428	MWCS	Dr. RC	39.02602	-107.05149
CO071High	Colorado Schofield_TH View	2661-01431	MWCS	Dr. RC	39.02602	-107.05149
CO072High	Colorado Schofield_Streamside	1301-50830	MWCS	Dr. RC	39.02711	-107.05058
CO073High	Colorado Schofield_Streamside	2661-01436	MWCS	Dr. RC	39.02711	-107.05058
CO074High	Colorado Schofield_High Perch	1301-50826	MWCS	Dr. RC	39.02819	-107.05202
CO075High	Colorado Schofield_High Perch	2661-01319	MWCS	Dr. RC	39.02819	-107.05202
CO076High	Colorado Schofield_Quarry	1301-50834	MWCS	Dr. RC	39.02731	-107.05122
CO077High	Colorado Schofield_West Cove	1301-50819	MWCS	Dr. RC	39.02649	-107.0518
CO078High	Colorado Schofield_Esker Isle	1301-50827	MWCS	Dr. RC	39.02814	-107.05297
CO079High	Colorado Schofield West_Esker Isle	1301-50816	MWCS	Dr. RC	39.02814	-107.05297
CO080High	Colorado NPB_Grey Rock	2661-01490	MWCS	Dr. RC	39.02526	-107.0856
CO081High	Colorado NPB_Grey Rock	2661-01334	MWCS	Dr. RC	39.02526	-107.0856

Appendix 2: Microsatellite primer sequences, unique PCR conditions, sources, and species primers were originally designed for. Sequences marked with an asterisk were labelled with the fluorescent M13 tag (5'- caccgacgttgtaaaccgac -3') on the 5' end.

Locus	Primer Sequences	MgCl₂ (mM)	Annealing T_{m1}/T_{m2} (°C)	Source and Species
ZoleA2	*ZoleA2F 5'- GCAGCCATTTTGCTGTCATTC -3' ZoleA2R 5'- CCATCTGTCTGTCTTTCTGTCTG -3'	1	55/57	Poesel et al., 2009 <i>Z. leucophrys</i>
ZoleC11	*ZoleC11F 5'- TCCATGCTTCTGAACTGCC -3' ZoleC11R 5'- ACACCTGCTTTTCCTGACTG-3'	1	60/62	Poesel et al., 2009 <i>Z. leucophrys</i>
ZoleH02	*ZoleH02F 5'- ACTGTTCTTTTCTCCACCCAC -3' ZoleH02R 5'- GGTTGAATCCCAGGTGGA AAC -3'	1	50/52	Poesel et al., 2009 <i>Z. leucophrys</i>
Escu6	*Escu6F 5'- CATAGTGATGCCCTGCTAGG -3' Escu6R 5'- GCAAGTGCTCCTTAATATTTGG -3'	0.8	60/62	Hanotte, 1994 <i>P. atricapillus</i>
Gf01	*Gf01F 5'- TAGCATTCTATGTAGTGTTATTTTAA -3' Gf01R 5'- TTTATTTATGTTTCATATAAACTGCATG -3'	1	55/57	Petren, 1998 <i>G. fortis</i>
Gf06	Gf06F 5'- GCTATTGAGCTAACTAAATAAACA ACT -3' Gf06R 5'- CACAAATAGTAATTA AAAAGGAAGTACC -3'	0.8	45/47	Petren, 1998 <i>G. fortis</i>
Pocc2	*Pocc2F 5'- AACCACACTGAGTAAGCTGCTG -3' Pocc2R 5'- TTTAGCTCACCTTGCAAATGG -3'	1	45/47	Bensch, 1997 <i>P. atricapillus</i>
VeCr05	VeCr05F 5'- ACACACTTATGTGCATGGGCT -3' VeCr05R 5'- ATATTT CAGGTATGGGTTTGGTTC -3'	1	45/47	Stenzler, et al. 2004 <i>B. cedrorum</i>
YW16	*YW16F 5'- ACAGCAAGGTCAGAAATAAA -3' YW16R 5'- AACTGTTGTGTCTGAGCCT -3'	1	60/62	Dawson et al., 1997 <i>Z. leucophrys</i>

APPENDIX 3: Pairwise F_{ST} values (above diagonal) and corresponding p-values (below diagonal) for CR sequences from 14 sites and three subspecies corresponding to matrices in Figure 2.4. Significant p-values after FDR correction ($p < 0.05$) are marked in red.

	JAS	BA	BV	WT	LE	FTSJ	RV	OK	MK	CO-L	CO-M	CO-H	OR-L	OR-H
JAS	-	0.22	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.89	0.89	0.48	0.61	0.78
BA	0.17	-	-0.10	-0.02	0.15	0.22	0.04	0.02	0.02	0.27	0.27	0.37	0.49	0.32
BV	0.99	0.63	-	-0.13	-0.09	0.00	0.09	-0.26	-0.26	0.09	0.09	0.19	0.34	0.17
WT	0.99	0.30	0.99	-	-0.09	0.00	0.19	-0.26	-0.26	0.11	0.11	0.16	0.33	0.19
LE	0.99	0.37	0.99	0.99	-	0.00	0.21	0.00	0.00	0.87	0.87	0.43	0.59	0.74
FTSJ	0.99	0.17	0.99	0.99	0.99	-	0.30	0.00	0.00	0.89	0.89	0.48	0.61	0.78
RV	0.14	0.32	0.26	0.26	0.26	0.14	-	0.06	0.06	0.30	0.30	0.44	0.55	0.36
OK	0.99	0.63	0.99	0.99	0.99	0.99	0.33	-	0.00	0.84	0.84	0.36	0.54	0.69
MK	0.99	0.63	0.99	0.99	0.99	0.99	0.33	0.99	-	0.84	0.84	0.36	0.54	0.55
CO-L	0.03	0.06	0.03	0.14	0.03	0.03	0.03	0.07	0.07	-	0.00	-0.04	0.28	0.30
CO-M	0.03	0.05	0.03	0.13	0.03	0.03	0.03	0.07	0.07	0.99	-	0.01	0.08	0.30
CO-H	0.03	< 0.01	0.01	0.04	0.04	0.03	< 0.01	0.11	0.11	0.55	0.40	-	0.09	0.19
OR-L	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01	0.01	0.33	0.15	0.08	-	-0.08
OR-H	0.01	0.01	0.03	0.01	0.02	< 0.01	0.01	0.04	0.05	0.10	0.10	0.04	0.80	-

	<i>Z. l. oriantha</i>	<i>Z. l. gambelii</i>	<i>Z. l. pugetensis</i>
<i>Z. l. oriantha</i>	-	< 0.01	< 0.01
<i>Z. l. gambelii</i>	0.15	-	< 0.01
<i>Z. l. pugetensis</i>	0.40	0.18	-

APPENDIX 4: Pairwise F_{ST} values (above diagonal) and corresponding p-values (below diagonal) for AldoB6 sequences from 11 sites and three subspecies corresponding to matrices in Figure 2.3. Significant p-values after FDR correction ($p < 0.05$) are marked in red.

	BA	BV	WT	LE	RV	MK	CO-L	CO-M	CO-H	OR-L	OR-H
BA	-	0.01	-0.02	0.72	0.50	0.02	0.10	0.19	0.10	0.13	0.00
BV	0.74	-	0.01	0.30	0.18	0.02	-0.01	0.10	0.04	0.05	0.00
WT	0.72	0.48	-	0.17	0.25	0.08	-0.12	0.07	0.01	0.08	0.08
LE	0.07	< 0.01	0.43	-	1.00	0.34	0.14	0.37	0.39	0.31	0.50
RV	0.33	0.04	0.39	0.07	-	0.24	0.26	0.45	0.06	0.33	0.31
MK	0.27	0.03	0.04	< 0.01	0.03	-	0.08	0.13	0.08	0.10	0.02
CO-L	0.51	0.50	0.99	0.19	0.17	< 0.01	-	-0.01	0.02	0.08	0.08
CO-M	0.25	0.03	0.32	0.11	0.08	< 0.01	0.42	-	0.06	0.16	0.15
CO-H	0.20	0.11	0.43	< 0.01	0.44	< 0.01	0.36	0.22	-	0.14	0.08
OR-L	0.16	0.06	0.14	0.01	0.01	< 0.01	0.08	0.02	< 0.01	-	0.04
OR-H	0.99	0.60	0.43	0.03	0.06	0.20	0.24	0.10	0.12	0.29	-

	<i>Z. l. oriantha</i>	<i>Z. l. gambelii</i>	<i>Z. l. pugetensis</i>
<i>Z. l. oriantha</i>	-	0.15	< 0.01
<i>Z. l. gambelii</i>	0.04	-	< 0.01
<i>Z. l. pugetensis</i>	0.12	0.06	-

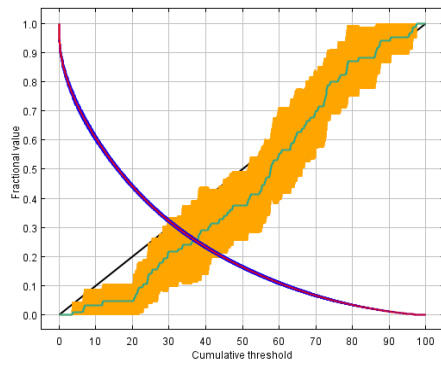
APPENDIX 5: Pairwise F'_{ST} values (above diagonal) and corresponding p-values (below diagonal) from nine microsatellite loci for 15 populations (corresponding to Table 2.2). Significant p-values after FDR correction are red ($p < 0.001$), orange ($p = 0.002 - 0.009$), and yellow ($p = 0.01 - 0.05$). Nonsignificant F'_{ST} values are in bold above diagonal.

	JAS	BA	BV	CNP	WT	LE	FTSJ	RV	OK	MK	CO-L	CO-M	CO-H	OR-L	OR-H
JAS	-	0.351	0.089	0.417	0.299	0.311	0.390	0.178	0.038	0.023	0.334	0.372	0.304	0.169	0.300
BA	0.001	-	- 0.014	0.268	0.073	0.200	0.495	0.382	0.532	0.239	0.100	0.583	0.278	0.160	0.139
BV	0.109	0.436	-	0.125	0.001	0.092	0.303	0.070	0.169	0.114	0.131	0.459	0.226	0.106	0.109
CNP	0.001	0.001	0.011	-	0.065	0.396	0.383	0.387	0.445	0.295	0.372	0.712	0.533	0.360	0.332
WT	0.002	0.087	0.435	0.04	-	0.190	0.266	0.254	0.264	0.255	0.195	0.503	0.277	0.179	0.212
LE	0.001	0.002	0.011	0.001	0.001	-	0.216	0.151	0.267	0.231	0.178	0.385	0.171	0.111	0.075
FTSJ	0.001	0.001	0.001	0.001	0.001	0.001	-	0.277	0.259	0.344	0.411	0.382	0.243	0.330	0.355
RV	0.426	0.002	0.205	0.001	0.003	0.015	0.001	-	- 0.145	- 0.016	0.352	0.297	0.251	0.054	0.165
OK	0.309	0.001	0.034	0.001	0.003	0.001	0.002	0.451	-	0.109	0.448	0.254	0.270	0.204	0.360
MK	0.313	0.001	0.001	0.001	0.001	0.001	0.001	0.444	0.024	-	0.325	0.464	0.271	0.171	0.227
CO-L	0.001	0.024	0.005	0.001	0.001	0.001	0.001	0.001	0.001	0.001	-	0.442	0.194	0.218	0.187
CO-M	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	-	0.174	0.366	0.459
CO-H	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.007	0.004	0.001	0.001	0.001	-	0.229	0.274
OR-L	0.002	0.005	0.002	0.001	0.001	0.001	0.001	0.216	0.001	0.001	0.001	0.001	0.001	-	0.016
OR-H	0.001	0.003	0.002	0.001	0.001	0.001	0.001	0.008	0.001	0.001	0.001	0.001	0.001	0.043	-

APPENDIX 6: Pairwise F'_{ST} values from nine microsatellite loci for sparrows from alpine coniferous (AC), riparian deciduous (RD), disturbed – gas plant (D-G), and disturbed-townsite (D-T) ecosites below the diagonal. P-values are above the diagonal, showing that all comparisons are significant after FDR correction.

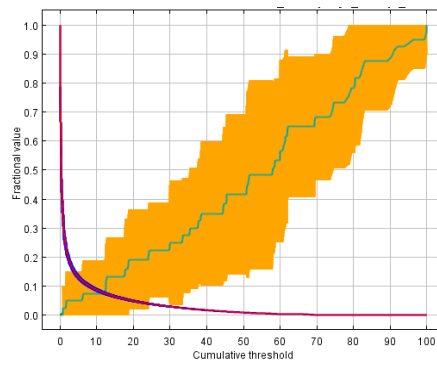
	AC	RD	D-G	D-T
AC	-	0.001	0.001	0.001
RD	0.241	-	0.001	0.001
D-G	0.289	0.200	-	0.001
D-T	0.457	0.344	0.429	-

Z. l. gambelii

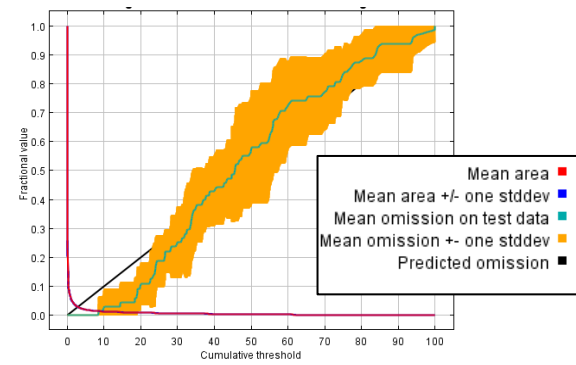


Z. l. oriantha

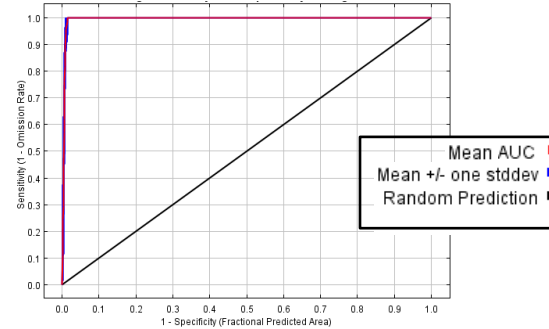
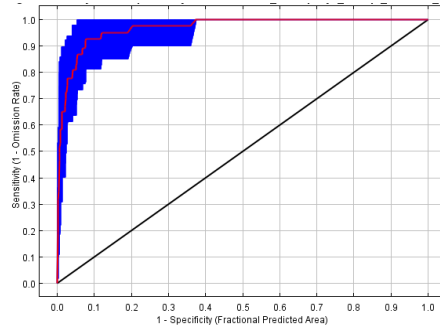
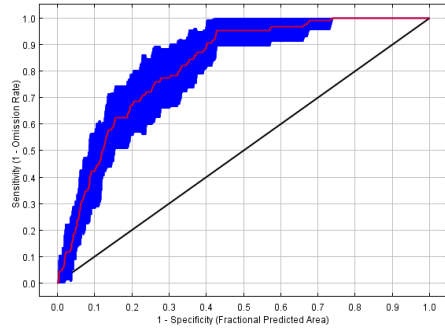
Average omission and predicted area



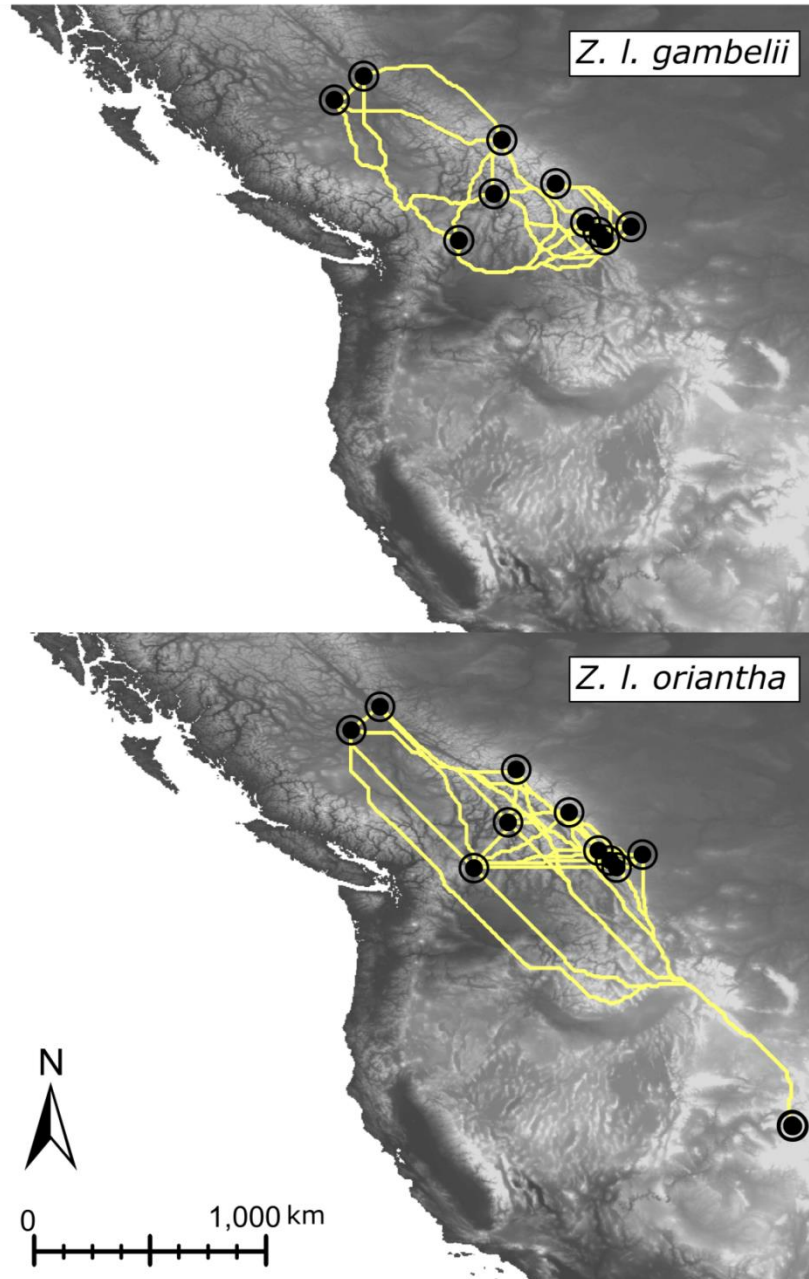
Z. l. pugetensis



Average sensitivity vs. 1 – specificity



APPENDIX 7: Graphs of Akaike's Information Criterion (AIC) and area under curve (AUC) to show best fit models of each subspecies contemporary SDM.



APPENDIX 8: Least-cost paths (yellow) connecting 10 sites with *Z. l. gambelii* individuals and the 13 sites with *Z. l. oriantha*. Paths were calculated from the friction layer created from corresponding subspecies' SDMs.

APPENDIX 9: Geographic distribution of AldoB6 haplotypes across 12 sample sites.
For site location refer to Figure 2.1.

Haplotype	Total	BA	BV	WT	LE	RV	OK	MK	OR-L	OR-H	CO-L	CO-M	CO-H
1	27		2	2	4			10	3		3	2	1
2	1	1											
3	7		1			2					1		3
4	1	1											
5	3		1						2				
6	1		1										
7	2			1				1					
8	1		1										
9	1		1										
10	1		1										
11	1		1										
12	2		1								1		
13	10						1	3			1	3	2
14	1							1					
15	1									1			
16	1									1			
17	2							1					1
18	2							2					
19	8								7	1			
20	1								1				
21	1								1				
22	1								1				
23	1								1				
24	1									1			
25	1											1	
26	1										1		
27	2												2
28	1		1										
29	1			1									
30	1		1										
Total	85	2	12	4	4	2	1	18	16	4	7	6	9

APPENDIX 10: Geographic distribution of CR haplotypes across 14 sample sites. For site location refer to Figure 2.1.

Haplotype	Total	JAS	BA	BV	WT	LE	FTSJ	RV	OK	MK	CO-L	CO-M	CO-H	OR-L	OR-H
1	26	4	2	4	2	3	4	2	2	2			1		
2	1		1												
3	1		1												
4	1		1												
5	3		2					1							
6	1				1										
7	10				1						3	3	2	1	
8	1							1							
9	3							1			1			1	
10	1													1	
11	6													3	3
12	6													4	2
13	1													1	
14	1											1			
15	2												2		
16	1												1		
17	1												1		
Total	66	4	7	4	4	3	4	5	2	2	4	4	7	11	5