THE PHYLOGEOGRAPHY, POST-GLACIAL EXPANSION AND SYSTEMATICS OF THE PURPLE FINCH (*CARPODACUS PURPUREUS)*

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ABSTRACT

Using a combination of mitochondrial and z-linked sequence data, microsatellites and spatio-geographical modelling, I examine the historical and contemporary factors influencing the genetic structure of the purple finch (*Carpodacus purpureus*). The last glacial maximum likely forced the purple finch into multiple refugia: one on the Pacific Coast, one in south western United States, and one off the Atlantic shelf. The two subspecies remain distinct lineages, with little evidence of gene flow between *C. p. purpureus* and *C. p. californicus*. The contact zone between subspecies appears in south western British Columbia. As *C. p. purpureus* expanded from their refugia following a stepping-stone model, eastern and western populations came into secondary contact, resulting in extensive admixture. Contemporary levels of gene flow between populations are low with at least five genetic clusters, likely due to high site fidelity and morphological variation upon which mate selection occurs. Physical barriers do not appear to affect dispersal of the purple finch.

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LIST OF ABBREVIATIONS

AUC	Area Under the Curve
AMOVA	Analysis of Molecular Variance
ATP	Adenosine triphosphate
BAPS	Bayesian analysis of population structure
bp	Base pair
BC	British Columbia
CBC	central British Columbia
CCSM4	Community Climate System Model Four
CoOR	coastal Oregon
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphates
ENM	Ecological Niche Modeling
FDR	False Discovery Rate
GIS	Global Information System
h	Haplotype diversity
LAB	Labrador
LGM	Last Glacial Maximum
McMC	Markov chain Monte Carlo
$MgCl_2$	Magnesium Chloride
MIROC	Model for Interdisciplinary Research on Climate
mtDNA	Mitochondrial DNA
MI	Michigan
MYA	Million years ago
ND	North Dakota
NGS	Next Generation Sequencing
NL	Newfoundland
NS	Nova Scotia
PCoA	Principle Coordinate Analysis
PCR	Polymerase Chain Reaction
π	Nucleotide diversity
℃	Degrees celcius
s	Second
SCA	southern California
T_{m1}	Annealing temperature one
T_{m2}	Annealing temperature two
UTM	Universal Transverse Mercator
uL	Microlitre

uM	Micromolar
Van Is	Vancouver Island
WA	Washington

CHAPTER ONE: General Introduction

Geological and ecological processes play a fundamental role in determining a species' distribution and affect how individuals interact with one another. Intraspecific phylogeography examines how these interactions may have left genetic imprints on populations and the role of geological and ecological processes (Avise et al., 1987). Ultimately, these processes can result in populations becoming isolated, and may lead to speciation.

Speciation refers to the process by which new species are derived from existing ones. This process has been studied since Darwin examined the question in the mid-nineteenth century, and the term was first coined in 1906 by Cook (Cook, 1906). Despite this topic's long history of study, the drivers behind speciation and their relative importance are still debated today.. With the advances in genetic technology, we are continuously learning about historical and contemporary factors affecting speciation.

1.1: Physical Barriers

1.1.1: Mountain Ranges

Mountain ranges are an obvious example of a physical barrier leading to allopatric speciation, and species boundaries correspond to mountain ranges in a variety of taxa. Orogeny of the Andes in Peru correspond to patterns of speciation of fish (Ruzzante et al., 2006), while the Atlas mountain range acts as a barrier to dispersal for freshwater turtles (Fritz et al., 2006). In North America, the northern Rockies act as a barrier to dispersal for the Canada lynx (*Lynx canadensis*) (Rueness et al., 2003), while the southern Rockies and the Sierra Nevadas are

correlated with range limits of swift and kit foxes (genus *Vulpes*), with the Rockies limiting east-west dispersal, and the Sierra Nevadas limiting north-south dispersal (Mercure et al., 1993). The Rocky Mountains also appear to be a barrier for highly vagile species, such as the hairy woodpecker (*Picoides villosus*) (Graham & Burg, 2012) and Cooper's hawk (*Accipiter cooperii*) (Sonsthagen et al., 2012). Despite plenty of evidence that mountain ranges can act as physical barriers to dispersal, the efficiency by which they limit gene flow depends on the mountain range, and the organism involved. The same barrier for one species (hairy woodpecker and Cooper's hawk), appears to be a porous barrier for other species (boreal chickadee (*Poecile hudsonicus*)) (Lait & Burg, 2013).

1.1.2: Bodies of Water

The degree to which water acts as a barrier to dispersal will depend on the vagility of the organism, the distance between suitable habitats, the depth of the water body, and the mode of dispersal of the organism. To the terrestrial grey wolf (*Canis lupus*) and barren-ground caribou (*Rangifer tarandus groenlandicus*), the Mackenzie River is a barrier to dispersal (Carmichael et al., 2001). For some avian species such as the boreal chickadee the larger water barrier of the Strait of Belle Isle effectively disrupts gene flow (Lait & Burg, 2013), yet this strait has not been shown to disrupt gene flow for other avian species, such as the American redstart (*Setophaga ruticilla*) (Colbeck et al., 2008).

1.1.3: Unsuitable Habitat

In addition to these large-scale physical barriers to dispersal, less recognised, often smaller physical barriers also exist. Any area of unsuitable habitat can act as a barrier to dispersal,

provided this area is large enough to limit gene flow. A large scale example of unsuitable habitat acting as a barrier can be found in the Great Plains in central North America, where the lack of contiguous forest isolates populations of birds on either side of these grasslands (Graham & Burg, 2012; Milot et al., 2000; Sonsthagen et al., 2012).

1.1.4: Anthropogenic barriers

Along with natural physical barriers, anthropogenic activities can result in reduced gene flow. The over-harvest of *Taxus yunnanensis* has led to strong genetic differentiation between populations of this plant (Miao et al., 2014). Similarly, habitat fragmentation as the result of conversion of habitat type (clear-cutting for logging or agriculture), the building of major roads, railways or cities, and large agricultural development creates barriers to gene flow, which lead to genetic differentiation and, given time, speciation. For example, deforestation in northern Turkey has led to the genetic structuring of the edible dormouse (*Glis glis*) (Helvaci et al., 2012), while it is expected that the current deforestation on the Hawaiian Islands will lead to the genetic differentiation of the elepaio (*Chasiempis sandwichensis*), a flycatcher endemic to Hawaii (VanderWerf et al., 2010). Meanwhile, urban development has created genetically differentiated populations of coyote (*Canis latrans*) and bobcat (*Lynx rufus*) (Riley et al., 2006), big horned sheep (*Ovis canadensis*) (Epps et al., 2007) and the moor frog, *Rana arvalis* (Vos et al., 2001).

Although there are fewer examples of anthropogenic barriers acting on avifauna, likely due to their high vagility, large agricultural plots have been shown to limit gene flow among greater sage grouse (Bush et al., 2011). Roads and other anthropogenic barriers are also thought to affect the dispersal capabilities of some avifauna (Kociolek et al., 2011).

1.2: Pleistocene Glaciations

1.2.1: Speciation

Pleistocene glaciations have had a particularly strong effect on the evolutionary history of many high latitude species, including fish (Witt et al., 2011), mammals (Li et al., 2013), and birds (Weir & Schluter, 2004) (Hewitt, 2000). Extreme glaciation events, such as those during the Quaternary, displaced taxa through the expansion of large ice sheets and the conversion of temperate, boreal, or even rainforest habitat to tundra. Expansions of ice sheets and associated climatic changes are also linked with lower sea levels, and have resulted in the connection of otherwise isolated land masses, such as the Bering land bridge.

During the last glacial maximum (LGM), 21,000 years ago, much of northern North America was under ice, forcing most living organisms into glacial refugia, areas where climatic conditions were amenable to the survival of a species. Although the number and locations of refugia will differ by species, general refugia for multiple species are purported to have existed in Beringia, Queen Charlotte Islands (Haida Gwaii), off the Atlantic shelf, and in the southeastern United States (Pielou, 1991).

The displacement caused by ice sheet expansion has the potential to force individuals from different populations into refugia, shifting their natural ranges and affecting their evolutionary trajectory. As these ranges expand following the retreating ice sheets, previously isolated populations may come into secondary contact. The length of time these populations are isolated, along with the degree of natural selection, and competition, will all affect the degree to which these populations have diverged in allopatry. Two populations separated for 100 years,

with little selective pressure in either population will be much less divergent than two populations separated for 1,000 years, experiencing very different selective pressures.

Where a species survived the LGM plays an important role in its evolution. Species occupying a single refugium were unlikely to experience a reduction in gene flow (assuming no barriers within the refugium), and therefore little divergence would be expected. If, however, a species survived the LGM in heterogeneous, fragmented habitat or in multiple refugia, population divergence is more likely. Individuals sampled within a historic refugium may display unique haplotypes and higher diversity values (nucleotide and haplotype) than recently founded populations.

Vicariance during the LGM has led to divergence in plants, with two lineages of the herbaceous *Smilax* splitting around this time (Li et al., 2013). These lineages survived in two distinct refugia in the southern Appalachians (Hofreiter et al., 2004). The higher levels of divergence found in contemporary specimens suggest that the LGM played a significant role in reducing gene flow by forcing many species into multiple refugia, where speciation took place (Hofreiter et al., 2004).

In North America, the effects of the LGM on the avifauna have shown varied results. Although the changing climate did not result in speciation for tropical and sub-boreal species, the majority of boreal species showed effects from the LGM. Many endemic super-species, or species that are distinct, but closely related, display coalescence times linked to glaciation events from the Pleistocene (Weir & Schluter, 2004). Similarly, individual species show evidence of isolation, including the Steller's jay (*Cyanocitta stelleri*), which shows evidence of a refugium in in the Queen Charlotte Islands (Haida Gwaii) of northwestern North America (Burg et al., 2005).

1.2.2: Climatic changes

The effects of the LGM are not only found in areas where glaciers covered large portions of the continents, but also where the climate was significantly altered. While most non-boreal birds were able to maintain gene flow throughout most climate fluctuations, the much less vagile short-tailed viper (*Gloydius brevicaudus*) in southern Asia had to retreat to multiple refugia, causing limited speciation (Ding et al., 2011).

Although the formation of subspecies due to isolation associated with glaciation is more commonplace, the length of isolation is geologically and evolutionarily brief compared to speciation processes associated with mountain ranges and vicariance through continental drift. Because of this, often not enough time has passed for the speciation process to complete, leading to different populations, not yet reproductively isolated.

1.2.3: Post-glacial expansion

As the glaciers retreated, formerly allopatric populations expand into previously glaciated habitat. Populations can either advance in a slow, "phalanx" manner, resulting in the more even distribution of haplotypes and little loss of genetic variability. Populations may also recolonize following the "pioneer" model, whereby individuals from each refugial population will rapidly disperse long or short distances (Hewitt, 1996). Pioneer recolonization has the potential to result in a loss of genetic variability through the founder effect if the numbers of long distance colonizers are low enough, and the colonized populations see limited gene flow with the original population (Burg et al., 2006). A third colonization model, the stepping stone model, is characterised by stepwise, dispersal events, first from the founding population, and then consecutively from the most recently colonized population. This would lead to a gradual loss of

genetic diversity. These three models will display unique signatures when two isolated populations come into secondary contact. When both expanding populations follow the phalanx model, there will be an abrupt change in haplotypes, resulting in a sharp suture zone. Isolated haplotypes far from the original founding populations will be found if the pioneer model is followed, forming a mosaic suture zone. Finally, a gradual cline between the two populations will be found with the stepping stone model, with higher levels of diversity around the contact zone. Based on the narrow cline between subspecies of pine trees, with little to no mixing, Johansen and Latta (2003) determined that the predominant form of post-glacial expansion was the phalanx model, however, the presence of unique haplotypes far from the founding population shows that the pioneer model may also be possible for pollen (seen in chloroplast DNA) and even for less mobile seeds (mitochondrial DNA).

1.3: Nonphysical Barriers

In addition to physical barriers, nonphysical barriers can also act on populations and over time lead to sympatric speciation, or speciation despite the lack of physical barriers. Nonphysical barriers include any behaviour that may lead to a reduction in interactions between populations. Assortative mating, variation in vocalization and differing migratory behaviour can all act as non-physical barriers. The existence, prevalence, and causes of sympatric speciation have been widely debated. However, with the advancement of molecular studies, sympatric mechanisms for speciation are becoming more accepted (Via, 2001).

Ecology is suspected to be a main driver of sympatric speciation, with speciation processes emerging with the colonization of novel and heterogeneous habitats. One species can expand its range, occupying slightly different habitats. Over time this will lead to less interaction

and less gene exchange. As natural selection begins to act on different traits, it allows different habitat races, or genetically differentiated groups, to arise, even when these races are within dispersal distance of one another (Kawecki, 1997).

An example of this is courtship displays. Female white-crowned sparrows (*Zonotrichia leuchophrys*) have been found to select genetically similar males based on local dialects, with dialect explaining a significant amount of the genetic variation observed in sympatric populations in the Sierra Nevadas (MacDougall-Shackleton & MacDougall-Shackleton, 2001).

Multiple factors contribute to limited gene flow between sympatric populations. Despite breeding in close proximity, specific habitat preferences (nesting on buildings or in sandy lagoons) combined with slight differences in morphological differences (wing tip patterns), vocalizations and mating displays, can all play a role in maintaining genetic differentiation in populations of yellow-legged gulls (*Larus michahellis*) (Liebers et al., 2001).

1.4: Study Species

The purple finch (*Carpodacus purpureus*) is a songbird found across North America with a spectrum of morphological traits including size and plumage, as well as behavioural traits, including song and migratory behaviour that vary across its range (Wootton, 1996). Based on variability in these traits, as many as five subspecies have been described (Burleigh, 1948; Duvall, 1945; Sibley, 2011). The two currently accepted subspecies, *C. p. californicus* and *C. p. purpureus*, are morphologically distinct. Differences include a fourth primary that is shorter than its first in *C. p. californicus*, while the plumage of female *C. p. californicus* is generally more yellow-olive green with the flanks of the males more suffused with brown, and the rump a more dark, dull red than in *C. p. purpureus.* (Wootton, 1996). *C. p. purpureus* has an overall brighter,

more bubbly and varied song than *C. p. californicus* (Sibley, 2011), and tends to undertake longer migrations, with *C. p. californicus* displaying short altitudinal migrations. Although the majority of *C. p. purpureus* display long migrations, the migratory status of birds along the southern extent of the purple finch's breeding range is less certain. Purple finch are either resident in the winter or are migratory birds from further north (Figure 1.1). A Newfoundland subspecies (*C. p. nesophilus*) had been suggested based on its larger size and darker plumage than mainland *C. p. purpureus* (Burleigh, 1948). However, this was an artifact of small sample size (n=9), and it is now accepted that Newfoundland birds are on one end of a spectrum of *C. p. purpureus*, as opposed to being a unique subspecies (Wootton, 1996). Two subspecies are proposed to exist in the north-western United States, *C. p. rubidus* (Duvall, 1945), and in western Canada, (*C. p. taverneri*), however, others argue that these are clinal variants of *C. p. californicus* and *C. p. purpureus* respectively (Wootton, 1996). Combined with small sample sizes, these subspecies designations are based on morphological traits such as size and plumage, which can be influenced by elevation (James, 1986) and diet (Hill, 1993) respectively, meaning differences could be due to environmental factors.

The only genetic work conducted on the purple finch supports the current two subspecies taxonomy. Four of the 33 allozyme loci were variable between *C. p. purpureus* and *C. p. californicus.* Although sampling was extremely limited (n=17 *C. p. californicus* and n=4 *C. p. purpureus*) and was restricted to a single sampling location for each subspecies. The levels of variation at four loci was enough to lend support to the classification of *C. p. californicus* and *C. p. purpureus* as two separate subspecies (Marten & Johnson, 1986).

By using a more representative sampling regime that covers more of the purple finch's distribution and uses a number of molecular markers, it is the aim of this study to examine the genetic support for the current purple finch taxonomy, identify current and historical barriers to

gene flow, and examine how different behavioural patterns such as migration, habitat use, and vocalization might affect genetic patterns seen today.

1.5: Molecular Markers

Research questions dictate which molecular markers should be used in the course of any study. Because of this, it is important to use multiple markers with different modes of inheritance, to get a better picture of the processes behind the genetic structure found in target species.

1.5.1: Microsatellites

Microsatellites are short tandem repeats of DNA with repeats ranging from 1-6 base pairs. Microsatellite loci tend to have higher levels of mutation than other molecular markers. Mutations occur during DNA replication and are caused by slipped strand mispairing (Freeland, 2011). These mutations can occur in a stepwise mutation model, adding or subtracting one repeat (Kimura & Ohta, 1978), or in the infinite alleles model, which allows for the gain or loss of multiple repeats (Kimura & Crow, 1964).

While high mutation rates can limit the resolution of microsatellites when dealing with phylogenies, microsatellites have excellent resolution when determining contemporary population genetic events. High mutation rates mean that polymorphisms can quickly develop, allowing for comparisons of recently diverged lineages (Freeland, 2011). Such was the case with the recent radiation of cichlid species (Markert et al., 2001), where non-microsatellite loci with lower mutation rates revealed little variation, but microsatellite loci were able to resolve some *recent* evolutionary history.

1.5.2: Mitochondrial Markers

Unlike nuclear loci, which are biparentally inherited, mitochondria are maternally inherited in most animals. Mitochondrial DNA is relatively easy to work with as it is small, and its gene order is relatively conserved within taxa (Mindell et al., 1998). This conservation of gene order means that in many instances, primers can be used for multiple species, reducing development time and costs. Mitochondrial loci tend to have high mutation rates, due in part to the lack of a repair mechanism. High mutation rates can lead to relatively high levels of polymorphism, thus increasing the potential resolution of these loci (Wilson et al., 1985). Because mitochondrial DNA (mtDNA) is haploid and uniparentally inherited, it undergoes no recombination. This means that, aside from mutation, assuming no paternal leakage, the sequence inherited in the offspring should be identical to the maternal copy.

MtDNA has an effective population size one quarter of bi-parentally inherited nuclear markers. The smaller effective population size means that mtDNA is especially sensitive to genetic changes resulting from large demographic events, such as bottlenecks (Freeland, 2011). Being uniparentally inherited also allows for the investigation into female-biased dispersal, if examined in concert with bi-parentally inherited markers.

Finally, assumed overall neutrality of the mitochondrial genome means that none of the loci will be under selection, which would affect mutation rates. Any non-silent mutation that might function to affect cellular respiration, will either be deleterious, and selected against, or will be effectively neutral, and thereby retained. Although advantageous mutations are possible, they make up a negligible portion of mutations (Eyre-Walker & Keightley, 2007). Recent research is calling into question the neutrality of mitochondrial DNA (Castoe et al., 2009), as well

as the other purported benefits of this molecular marker type (Galtier et al., 2009). In addition to the presumed neutrality of the mitochondrial marker, exceptions to the clonal nature of mitochondria have been found with apparent recombination occurring in scorpions (*Buthidae* spp) (Gantenbein et al., 2005), lizard (*Chlamydosaurus kingie*) (Ujvari et al., 2007) and fish (*Platyicthys flesus*) (Hoarau et al., 2002) studies, while the mutation rate of the mitochondrial genome is variable in mammals and birds (Nabholz et al., 2008, 2009).

1.5.3: Z-linked markers

Reproductive isolation is often the final and necessary step to complete speciation, according to the biological species concept (Mayr, 1995). Recent work suggests that sex chromosomes play an important role in the final stages of reproductive isolation (Qvarnstrom & Bailey, 2009). Sex-linked traits in *Drosophila* spp, such as pheromone production (Gleason et al., 2005; Liimatainen & Jallon, 2007), and a variety of sexually isolating traits (Moehring et al., 2004) can lead to prezygotic isolation. Prezygotic reinforcement can be seen in avian species, as traits such as mate recognition (Saether et al., 2007), and male plumage characteristics (Saetre et al., 2003) are both sex-linked traits, found on the Z-chromosome of pied flycatcher (*Ficedula hypoleuca*), and collared flycatcher (*Ficedula albicollis*). Hybrid crosses of *F. hypoleuca* and *F. albicollis* experience sex-linked postzygotic barriers including female hybrid sterility and male hybrid unattractiveness (Svedin et al., 2008). Female hybrid sterility also occurs in female heterogametic *Heliconius* butterfly species (Naisbit et al., 2002) and among *Heliconius melpomone* geographic races (Jiggins et al., 2001).

In addition to strengthening reproductive isolation between subspecies, Z-linked chromosomes also have a stronger paternal heritance, which can be used, in combination with other markers, to examine the possibility of sex-biased dispersal (Freeland, 2011).

1.6: Ecological Niche Modeling

Ecological niche modeling (ENM), or predicting the distribution of a species based on climatic conditions and occurrence data, can be a powerful tool, especially when paired with genetic data. When trying to determine the historical distribution of a species genetic data alone can give clues as to which populations are differentiated from one another, making it possible to determine the number of refugia used during the LGM. However, assuming genetic splits correspond to the LGM, combining genetic data with ENM can elucidate the exact location of these refugia based on suitable climate, making ENM a powerful tool in evolutionary studies (Phillips et al., 2006).

1.7: Thesis Aims

Through the use of mitochondrial, z-chromosome and nuclear microsatellite markers, I studied the evolutionary history of the understudied passerine, the purple finch. By using multiple markers, I examine possible dispersal and breeding patterns at different evolutionary times, while the different modes of inheritance associated with these markers allows me to test for possible sex-linked dispersal.

I use ecological niche modeling, combined with historical climatic conditions, to examine potential glacial refugia for the purple finch. Combining these data with the results of genetic analysis, I determine which areas historically demonstrated conditions suitable for purple finch. Finally, I consider not only which refugia this species inhabited during the LGM, but also how these isolated populations might have re-colonized previously glaciated portions of North America.

CHAPTER TWO

The phylogeography, post-glacial expansion and systematics of the purple finch (*Carpodacus purpureus)*

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2.1: Introduction

The ability of an organism to disperse and the genetic structure found in that species are intrinsically linked. Lower dispersal potential leads to reduced levels of gene flow, decreasing population connectivity and creating isolated populations (Clark et al., 2010). Both historical and contemporary barriers can limit gene flow between populations which not only affect population connectivity, but will also influence evolution, including speciation.

Ancient vicariance events as the result of geological processes such as plate tectonics (Georges et al., 2014; Sanmartin & Ronquist, 2004), island formation (Poulakakis et al., 2012), mountain formation (Apte et al., 2007; Cooper & Cooper, 1995) and volcanic activity (Brown et al., 2000; Carson et al., 1990) have all played a large role in evolution. Contemporary barriers to dispersal such as glaciations, mountain ranges, large bodies of water and anthropogenic effects have also played a role, particularly in more recent speciation events. Glaciation processes are one main driver of evolution, particularly in high latitude species. The advancing and retreating of ice sheet forced biota into small areas during which time fragmented populations evolved independently of one another (Holderegger & Thiel-Egenter, 2009, Hofreiter et al., 2004; Klicka & Zink, 1999; Krebes et al., 2010; Shafer et al., 2011). In North America there is support for Pleistocene refugia in Beringia (Brubaker et al., 2005; Lait & Burg, 2013), off the coast of Newfoundland (Colbeck et al., 2008; Lait & Burg, 2013; Sonsthagen et al., 2011), and large areas within contiguous United States and Mexico (Grus et al., 2009; Ralston & Kirchman, 2012). Refugial populations are characterized by higher levels of haplotype diversity, unique haplotypes, and high levels of nucleotide divergence between putative refugial populations.

Contemporary, physical barriers, such as mountain ranges and the Great Plains also act as barriers to dispersal for a variety of species (Kimura et al., 2002; Lovette et al., 2004; Mercure et al., 1993; Milot et al., 2000; Ruegg & Smith, 2002; Rueness et al., 2003; Ruzzante et al., 2006).

The presence of unique genetic groups in Newfoundland, suggests the Cabot Strait and the Strait of Belle Isle are barriers to dispersal (Holder et al., 2004; Khidas et al., 2013; Lait & Burg, 2013). Anthropogenic barriers also restrict dispersal. Roads correspond to genetic breaks in a range of species including snakes (Clark et al., 2010), frogs, (Vos et al., 2001), and large mammals (Hartmann et al., 2013; Riley et al., 2006). Large plots of agricultural land have decreased population connectivity in the endangered greater sage grouse (*Centrocersus urophasianus*) (Bush et al., 2011). Non-physical barriers include strong natal-site fidelity, migratory routes and associated overwintering sites, different foraging sites, and different sexual preferences (Friesen et al., 2007). The type and number of barriers influencing population connectivity and gene flow are ultimately determined by the distribution and characteristics of a species.

The purple finch (*Carpodacus purpureus*) is a songbird found across North America with two currently recognized subspecies, *Carpodacus purpureus purpureus* and *Carpodacus purpureus californicus*. There are morphological, plumage, vocal, and behavioural differences between these subspecies, and a range of traits within the subspecies (Wootton, 1996). The only genetic work conducted on the purple finch supports the current two subspecies taxonomy. The study used starch gel electrophoresis with 33 loci, 23 of which were variable in the Cardueline finches (Marten & Johnson, 1986). Four of these were variable between *C. p. purpureus* and *C. p. californicus*, hinting at genetic differentiation between subspecies. It should be noted, however, sampling was extremely limited (n=17 *C. p. californicus* and n=4 *C. p. purpureus*) and restricted to a single site for each subspecies (Marten & Johnson, 1986).

By using a more representative sampling regime, covering more of the purple finch's distribution, and using a number of molecular markers; it is the aim of this study to examine the genetic support for the current purple finch taxonomy, identify current and historical barriers to

gene-flow and examine how different behavioural patterns such as migration might affect genetic patterns seen today.

I have tested three hypotheses related to the systematics, refugia and contemporary barriers of the purple finch. Based on these, I predict the following. 1) Systematics: Given morphological and behavioural differences and relatively small contact zone found between subspecies, I predict very little gene flow to exist between subspecies, likely resulting in high, significant F_{ST} values between populations corresponding to each subspecies, and little to no haplotype sharing. 2) Glacial Refugia: Given their wide distribution across North America, it is likely the purple finch survived the LGM in multiple refugia. Beringia and the Atlantic shelf have been found to be a refugium for other species, including tree species that the purple finch rely on for their preferred habitat and as a source of food (Brubaker et al., 2005; Morris et al., 2010; Walter & Epperson, 2005), making these candidate refugia for the purple finch. And 3) Contemporary Barriers: Based on findings from other avian studies, I predict that the combination of the Rocky Mountains and Great Plains will isolate birds from CBC and all other *Carpodacus purpureus purpureus* populations. The straits separating Newfoundland and Vancouver from the mainland have also been found to isolate several bird populations, and may similarly isolate island populations of purple finch. Given the lack of obvious physical barriers for many of the populations sampled, isolation by distance may be detected.

2.2: Methods:

2.2.1: Sample Acquisition

Two hundred and sixty seven purple finches were captured from 10 populations (Table 2.1) with 12 m mist nets and play back recordings. A small (< 100 uL) blood sample was taken from the brachial vein and the birds were banded and released on site. Blood was stored in 99% ethanol. Purple finches were targeted across their breeding range from the months of May to July during the summers of 2007 to 2013 to limit the number of migrants caught (Figure 2.1).

2.2.2: DNA Extraction

Total genomic DNA was extracted from the blood samples using a modified Chelex extraction (Walsh et al., 1991). Following extraction, all samples were stored at -20 ^{o}C.

2.2.3: Mitochondrial and Z-Chromosome DNA Amplification

A 707 bp fragment of the ATP region of the mitochondrial genome, hereafter referred to as ATP6, containing both ATP 6 and ATP 8 was amplified using primers L8950 and H9694 (Table 2.2) for 243 samples. The thermal cycling profile was one cycle of 120 s at 94 ^{0}C, 45 s at 54 ^{0}C and 60 s at 72 ^{0}C; 37 cycles of 30 s at 94 ^{0}C, 45 s at 54 ^{0}C and 60 s at 72 ^{0}C; and one cycle of 5 minutes at 72 ^{0}C. The 25 uL PCR reaction contained: Green GoTaq® Flexi buffer (Promega), 0.2 mM dNTP, 2.5 mM $MgCl_2$,0.4 mM F and R primer, 1 UGoTaq® Flexi polymerase a M13 tag (0.5 uL) and genomic DNA(Table 2.3).

A 516 bp fragment of the AldB region of the Z-chromosome was amplified for 178 samples using primers AldB6-F and AldB8-R (Table 2.2, Hackett et. al, 2008). This region on the Z-chromosome was amplified using a similar thermal cycling program as the ATP6 reactions with the exception of a 50 ^{0}C annealing temperature. The 25 uL PCR reaction contained: Crimson buffer (New England Biolabs), 0.2 mM dNTP, 2.0 mM $MgCl_2$,0.4 mM F and R primer, 1U GoTaq® Flexi polymerase, 0.5 uL M13 tag and genomic DNA in the 25uL PCR mix can be found in (Table 2.3).

2.2.4: Sequencing

Successfully amplified samples were sent to NanuQ sequencing service at McGill University, Montreal, Quebec. Sequences were checked and aligned using MEGA version 5 (Tamura et al., 2011).

2.2.5: Microsatellite Amplification

To check for polymorphism and amplification success, a small set of samples were genotyped. Three purple finch samples (one CBC, NL and VanIs) were used to test 39 loci, designed for other avian species. Of these, nine loci worked consistently, and were screened for polymorphism with another 12 individuals (two SCA, two WA, two CBC, two NS, three NL, one CoOR). Six of the polymorphic loci: CE215 (Polakova et al., 2007), Ase18 (Richardson et al., 2000), PAT MP 43 (Otter et al., 1998), Titgata39 and Titgata 02 (Wang et al., 2005) and CTC 101 (Tarvin, 2006) were amplified using one cycle of 120 s at 94°C, 45 s at 50 °C and 60 s at 72°C; seven cycles of: 60 s at 94°C, 30 s at 50°C and 45 s at 72°C; 25 cycles of; 30 s at 94°C, 30 s at 52°C and 45 s at 72°C, and finishing with five minutes at 72°C. A seventh locus, Cuu28

(Gibbs et al., 1999), was amplified with the same program, except annealing T_{m1} and T_{m2} were 45°C and 48°C, respectively. The primer sequences can be found in Table 2.2, while the 25 uL PCR reaction contained: 0.2 mM dNTPs, 1.5-2.5 mM $MgCl_2$, 1.0 mM F and R primer, 1 U of GoTaq® Flexi, Crimson or Truin taq polymerase and0.5 uL M13 tag are in Table 2.3.

2.2.6: Genetic analyses

2.2.6.1 Sequence data

As AldB is Z linked, sequences from all known male birds were run through PHASE 2.0 (Stephens & Donnelly, 2003) DnaSP 5.1 (Librado & Rozas, 2009). PHASE reconstructs haplotypes, in this instance to account for the presence of two z-chromosome sequences in males. Four individuals from NS were removed due to very dissimilar z-linked sequences, although their mitochondrial sequences matched other purple finches. Haplotype (h) and nucleotide diversity (π) indices were calculated in DnaSP 5.1 (Librado & Rozas, 2009; Rozas & Rozas, 1999; Rozas et al., 2003) using the formulae $h = \left(\frac{n}{n-1}\right)\left(1 - \sum ix_i^2\right)$ (Nei & Tajima, 1981) where n is the sample size, and x_i is the frequency of the i^{th} haplotype, and $\pi = \sum_{ij} x_i x_j \pi_{ij}$, (Nei & Li, 1979), x_i and x_j are the frequencies of the i^{th} and j^{th} sequence, and π_{ij} is the pairwise divergence between the two sequences.

Genetic differentiation between sampling sites was examined using Φ_{ST}, a modified version of Wright's F_{ST} index (Wright, 1965). This index gives a measure of population differentiation, where zero represents complete panmixia, and one complete differentiation (no shared alleles). Pairwise Φ_{ST} values were calculated in GenAlEx 6.5 (Peakall & Smouse, 2006). Due to small sample size ($n \leq 6$), Labrador, coastal Oregon and Vancouver Island sampling sites were removed

from all pairwise F_{ST} analysis. Resulting values were corrected for multiple comparisons according to Narum (2006). Bonferroni corrections are very effective at controlling experiment α (Rice, 1989); however they are very conservative, and less likely to detect significant differentiation due to reduced power. False Discovery Rate (FDR) also corrects for multiple comparisons (Benjamini & Hochberg, 1995), and successfully corrects for multiple comparisons with very large data sets. Although less conservative than Bonferroni, this increased power makes this test more prone to type I errors–detecting significance where there is none (Narum, 2006). A modified FDR balances being conservative, and maintaining power to limit both Type I and Type II errors (Benjamini & Yekutieli, 2001). Given the number of tests run, and number of comparisons made, all multiple tests were corrected using the modified FDR.

AMOVAs (Analysis of Molecular Variance) were conducted in Arlequin v. 3.5.1.3, and AMOVAs partitioned the diversity found between the two subspecies, among the populations and within populations (Excoffier et al., 1992). Principle Coordinate Analysis (PCoA) was completed using Φ_{ST} values in GenAlEx for both mitochondrial DNA and z-linked loci. Principle component analyses attempt to explain the majority of variation found in the system in as few axes as possible. Conducting such analyses allow us to visualize the variance in our data.

A Bayesian clustering program, BAPs v5.2, was used to group individuals based on their sequence data. BAPs forms clusters with no *a priori* defined populations. The analysis was run with the linked loci option (Corander et al., 2003).

The relationship between all haplotypes was examined using statistical parsimony networks created in TCS v1.21 (Clement et al., 2000) for both mtDNA and z-linked loci. All connections were made with a 95% connection limit.

2.2.6.2 Molecular clock

Based on work by Fleischer et al. (1998) on Hawaiian honey-creepers, a divergence rate for passerines at the cytochrome *b* locus of mitochondrial DNA was established at 2.2% nucleotide differences per site per million years, and was assumed similar for the ND2 region used by Weir and Schluter (2004). By using this divergence rate, and calculating the percent divergence between the two subspecies of purple finch (# fixed differences between subspecies / total number of bp)*100 it is possible to determine how many years ago the subspecies diverged.

2.2.6.3 Microsatellite data

Amplified DNA was run on an acrylamide gel with a ladder and controls and visualized on the NEN Model 4300 DNA Analyzer (Licor Biosciences). Images were adjusted using SAGA Lite, and scored by hand. Individuals missing more than three of the seven loci were removed from the analyses (two ND, one WA sample). A total of 260 individuals from all 10 populations were successfully genotyped at the seven loci. Populations and loci were checked for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium with GENEPOP on the web (Raymond & Rousset, 1995; Rousset, 2008), and evidence of large allele dropout, null alleles, and genotyping errors were examined using Micro-checker (Van Oosterhout et al., 2004).

Genotype data from 260 individuals at seven loci were run through the Bayesian clustering program STRUCTURE v.2.3.4 to determine the number of clusters, or populations found in the data, assigning each individual Q values (ancestry coefficient), or the proportion of ancestry from each cluster for each individual (Pritchard et al., 2000). Because this type of analysis uses individuals and is not sensitive to levels of sampling within populations, LAB, CoOR and VanIs were included in the clustering analysis. The lengths of the burn-in and run

were determined by increasing both the burn-in, and run length until alpha and F_{ST} values converged. Increasing burn-in length minimizes the effects of the program start, which begins with a random configuration, while increasing the number of Markov Chain reactions ensures the results are as accurate as possible. A burn-in of 50,000 and MCMC length of 150,000 were used with admixture model and loc priors. Using sampling location (loc priors) to aid in assignment can assist with clustering when levels of differentiation are lower (Hubisz et al., 2009). Ten iterations were done for each cluster (K) 1-10 using the above conditions. Structure Harvester v.0.6.94 (Earl & Vonholdt, 2012) was used to calculate average likelihoods for each value of K, and implement the Evanno method (Evanno et al., 2005). ΔK was used to determine the optimal number of clusters that are present. By using Evanno, and looking for a "plateau in the likelihood of K", one can determine the likelihood of each K. Pritchard et al. (2000) suggest running hierarchical analysis by removing one group, and re-running the analysis with the remaining group(s), which can reveal further structure.

In addition to conducting cluster analysis, a measure of genetic differentiation between populations was calculated using Wright's F_{ST} Index (Wright, 1965) in GenAlEx v.6.5 (Peakall & Smouse, 2006). Using these F_{ST} values, an AMOVA and principal coordinate analysis was run. F_{ST} values and an AMOVA were conducted using Arlequin v.3.5 (Excoffier & Lischer, 2010).

When conducting pairwise comparisons, like F_{ST} analysis, on a microsatellite data set, it becomes important to have a large enough sample size for each population you are comparing. By using too few individuals, there is a risk of getting inaccurate allele frequencies, and diversity estimates, however, Hale et al. (2012) found the gains in accuracy minimal for sample sizes above 25-30 individuals. This number changes according to the resolution of the loci being used. Due to the presence of rare alleles in our data set, we felt justified using populations with more than 15 individuals, resulting in LAB, CoOR, and VanIs individuals being removed before

analysis. Discovery of potential barriers was conducted using Barrier v.2.2 (Manni et al., 2004) which takes geographic information in the form of coordinates, and genetic distance information in the form of pairwise F_{ST} matrices and maps putative barriers according to Monmonier's algorithm (Monmonier, 1973).

2.2.7: Isolation by distance

Often genetic distance corresponds to geographic distance. To clarify whether genetic differentiation is a result of geographic distance, Mantel tests were performed comparing genetic and geographic distances to test for Isolation by Distance. Tests were completed for all three datasets in GenAlEx v.6.5 using shortest geographic distances between sampling sites through suitable habitat.

2.2.8: Spatio-geographic modeling

Combining environmental data with presence / absence data of a species can be a powerful predictive tool, modeling a species' distribution based on the assumption that they will be present in a preferred set of environmental conditions, assuming species will favour a similar niche over time. Species distribution modeling combines current environmental conditions with occurrence data to predict the current distribution, while palaeo-distribution modelling uses environmental conditions from the past, along with occurrence data trained on current environmental conditions to predict historical distributions. Predictions of future climate conditions can be used to predict the future distribution of a species in the face of climate change (Phillips et al., 2006; Richards et al., 2007).

Distributions of the purple finch were predicted in MaxEnt, v.3.3.3, using presence data obtained from various museum records and Christmas bird counts. These coordinates are combined with GIS formatted environmental layers, which assign environmental values to pixels from the same geographic range covered by the presence data. For this study we used Bioclim layers from Global Climate Data developed through the University of California, Berkeley and the Museum of Vertebrate Zoology. The result is a fundamental niche, or a geographic area where the species is likely to occupy based solely on amenable environmental conditions. It is important to note that this niche is likely larger than the realized niche, which takes into account human influences, and geographic barriers (Pulliam, 2000). These climatic layers are based on different Global Climate models, and the layers used in this study were generated using the MIROC climate model (O'Ishi & Abe-Ouchi, 2013). The MIROC climate model takes into account factors in climate cooling during the LGM like vegetative feedback, which other models, such as CCSM4 model (Community Climate System Model) (Brady et al., 2013) do not take into account.

Occurrence information for the purple finch (>72,000 occurrences) was obtained from the Global Biodiversity Information Facility, an international open data infrastructure. However, points with no UTMs, potential outliers (outside of expected range), duplicated data, or any data from potentially unreliable sources (Great Backyard Bird Count) were removed. A file containing the UTMs of the remaining 5,275 purple finch locations was loaded into MaxEnt, along with nine Bioclim climatic layers (Table 2.4). The nine climate layers are those remaining after the original 19 layers were tested for correlation. Variables correlated above the 0.9 threshold were examined with ENMTools (Warren et al., 2008), and those that lent more support to the model, or were more biologically relevant were kept, and others were deleted. The climatic layers included averages, and extremes of precipitation and temperature. MaxEnt was run with the hinge only

function, which is recommended when a suitable number of occurrence points are used. To train the model, 25% of the occurrence locations were used.

2.3 Results

2.3.1 Genetic analyses

2.3.1.1 Sequence data

2.3.1.1.1 *ATP6 sequence data*

Diversity indices for the mitochondrial locus (ATP) showed a range, with a haplotype diversity ranging from 0.27 (SCA) to 0.90 (ND), with nucleotide diversity ranging from 0.0004 (SCA) to 0.0056 (NS), excluding populations with fewer than four individuals (Table 2.5).

For the mitochondrial locus, pairwise Φ_{ST} values ranged from 0.000 to 0.960, with the highest Φ_{ST} values between MI and SCA from different subspecies (Table 2.6). Between *C. p. californicus* populations of WA and SCA, Φ_{ST} was 0.092, while within the *C. p. purpureus* populations, Φ_{ST} values ranged from 0.000 to 0.194, for a within subspecies average of Φ_{ST}=0.070, while the between subspecies average was Φ_{ST}= 0.9193. All pairwise comparisons between subspecies were significant and Central British Columbia was significantly different from all other *C. p. purpureus* populations. Using the fixed differences in the mitochondrial data set between the two subspecies, a coalescence time of 1.1 MYA was calculated.

Principle Component Analysis (PCoA) for ATP6 showed coordinate one explained 87.3% of the variation, coordinate two explained 8.9% and coordinate three explained 3.2%. The

first two coordinates explain 96% of the variation and populations closest together geographically, clustered together based on genetic similarity (Figure 2.2). The Bayesian clustering program BAPS v.5.2 (Corander et al., 2003), identified three clusters for the mitochondrial locus. The two *C. p. californicus* populations clustered together and *C. p. purpureus* had predominantly eastern and western clusters (Figure 2.3) with these clusters representing an east-west differentiation within the *C. p. purpureus* subspecies.

The significant differentiation between subspecies was supported by AMOVAs conducted in both Arlequin and GenAlEx. The majority of the variance for the mitochondrial locus was explained by genetic differentiation between subspecies at 90%, while 9% was explained by differences between individuals, and just 1% of the variation was between populations (Table 2.7).

The parsimony haplotype network for the ATP6, mtDNA locus shows a total of 56 haplotypes (Table 2.9), and separates *C. p. californicus* and *C. p. purpureus* clusters, with no haplotype sharing between the two (Figure 2.4).

2.3.1.1.2 *AldB sequence data*

The final AldB dataset contained 306 sequences. The z-linked chromosome showed overall lower diversity values than the mitochondrial ATP6, with a range of haplotype diversity values for populations of n >4 from 0.086 for SCA to 0.837 for ND, while the nucleotide diversity ranged from 0.0005 to 0.0069 (Table 2.5).

The z-linked locus showed a range of Φ_{ST} values between 0.000 and 0.805 (Table 2.8). Higher Φ_{ST} values were seen in comparisons of populations from different subspecies (e.g. NL and SCA). The Φ_{ST} value from the comparison between the two *C. p. californicus* populations was 0.116, while comparisons between *C. p. purpureus* populations ranged from 0.000 to 0.060.

The average between and within subspecies Φ_{ST} values were 0.5937 and 0.0270 respectively. All pairwise Φ_{ST} values between subspecies were significant. The z-linked locus showed NL was significantly different from other *C. p. purpureus* populations, though it was only significantly different from CBC, moderately significantly different from ND and MI, and not different from NS.

The AMOVA from AldB showed 43%, 55%, and 2% of the variation is explained by differences between subspecies and within and between populations respectively (Table 2.7). PCoA for AldB had coordinates one and two explaining 89% and 7% percent of the variation (Figure 2.5). Populations in close geographic proximity clustered together with the PCoA. When analysed with BAPs, a total of four clusters were discovered. BAPS assigned at least one individual in all but one *C. p. purpureus* population to the *C. p. californicus* cluster (Figure 2.6).

The z-linked locus shows more haplotype sharing between the two subspecies, with no distinct clusters (Figure 2.7). A total of 47 haplotypes were found at this locus, including a number of unique and private haplotypes (Table 2.10. Private haplotypes are those only exist in one population, while unique haplotypes are those that are only found in one individual.

2.3.1.2: Genotype data

After removing individuals with missing data, 260 individuals were genotyped at seven loci, and the resulting genotypes were run through Genepop v.4.0 (Rousset, 2008). None of the loci showed significant effects of large allele drop-out, or stuttering causing scoring errors for any of the populations; however, CETC215 showed a homozygote excess, suggesting null alleles for four (NS, NL, CBC and SCA) of the populations, while Microchecker v.2.2.3(Van Oosterhout et al., 2004) suggested possible null alleles for Titgata 39 with NL and ND samples, and for Titgata

02 among NL and CBC samples. Finally, Ase18 had possible null alleles among CBC samples. Analyses were run with and without CETC 215; however, the results were not different, so CETC 215 was kept in the analysis.

Deviations from Hardy-Weinberg equilibrium were found in six of the 10 population/loci comparisons: Titgata 39 (NL, CBC, WA, ND and MI), CETC 215 (NS and CBC) and Ase18 (CBC and LAB), Titgata 02 (SCA), Pat 43 (NS) and CTC 101 (CBC).

Pairwise F_{ST} comparisons between populations ranged from 0.028 to 0.174, with all comparisons significant (p=0.001-0.003) (Table 2.11). The between and within subspecies F_{ST} values were 0.0861 and 0.0800 respectively. AMOVA analysis revealed that the majority of variance found in the samples is due to differences within populations (92%), 8% of the variance was due to differences among populations, and there was a negligible contribution of variation from comparisons between regions (subspecies) at 0.5% (Table 2.7.

Coordinates one and two explained a total of 70% of the variation (45% and 25% respectively), while coordinate three explained an additional 16% (Figure 2.8) Coordinates one and two clustered populations in similar geographic areas together; plotting NL and NS, WA and SCA, and ND and MI in close proximity. CBC fell within the WA and SCA quadrant.

Bayesian clustering using STRUCTURE initially revealed two clusters, separating NL and NS from all other populations. Hierarchical analysis excluding NS and NL, divided the remaining populations into four groups:1) central populations of ND and MI, 2) VanIs and CBC, 3) SCA and CoOR , and 4) WA. LAB showed mixed ancestry, assigning to the central populations of ND and MI, and the CBC and VanIs cluster (Figures 2.9 and 2.10). BARRIER v2.2 only discovered one barrier, located between NS and MI, around where James Bay cuts into the Canadian Shield (Fig 2.11).

Isolation by distance proved to be moderately significant using mtDNA (Figure 2.12), significant using z-linked data (Figure 2.13), and not significant when microsatellite data was used (Figure 2.14). Despite the significant findings for z-linked and mitochondrial data, the correlation values were weak, and no strong trends were observed.

2.3.2: Spatio-geographic modeling

The model resulting from running nine climatic layers and over 5000 occurrence locations performed significantly better than random, as demonstrated by the analysis of omission curve (Figure 2.15). The average omission rate for this model closely resembled the predicted omission rate, meaning few occurrences were in unsuitable areas. The area under the curve (AUC) was 0.874 (±0.004), where 0.5 is random and values closer to one denote a greater ability to distinguish between suitable and unsuitable habitat (Carstens 2007) (Figure 2.16).

The model predicted a distribution of *C. purpureus* which closely resembles those found in current bird guides (Sibley, 2000) (Figure 2.17). All of the correlated climatic layers above the accepted 0.9 threshold were examined, and layers not playing a significant role in the model were deleted. Bioclim layers one (annual mean temperature) and 19 (precipitation of the coldest month) explained 39.5 and 44.3% of the distribution of the purple finch. However, the importance of each, measured by the decrease in predictability when each variable is removed, showed that temperature was more important than precipitation during the coldest quarter (importance ratings of 56.2 and 23.1 respectively (Figure 2.18)). When the paleo-environmental layers were used on trained data, the model highlighted three major areas potentially acting as refugia for purple finch (Figure 2.19). The Pacific Coast shows suitable habitat for the purple finch, as do large areas at the southern extent of the ice sheets – south of the Appalachians, and further west, in central

United States, where current-day Texas would be, as well as in the Arizona region. In addition to the large potential refugia south of the ice sheets, MaxEnt identified suitable habitat off the coast of Newfoundland.

2.4 Discussion

2.4.1: Species systematics

While examining genetic differentiation between populations of separate subspecies, wide support for the current classification of two subspecies was evident. All of the loci used provided various levels of support, with the clearest support coming from the mitochondrial ATP6 locus. All pairwise Φ_{ST} comparisons between populations belonging to different subspecies were highly significant (Table 2.6), and analysis of molecular variance found 90% of the variance was between *C. p. purpureus* and *C. p. californicus* (Table 2.7). Principle Coordinate Analysis and the Bayesian Clustering program BAPs both supported this classification separating the two subspecies (Figures 2.2 and 2.3). Finally, the mitochondrial haplotype network (Figure 2.6) shows clear distinction with no haplotype sharing between *C. p. purpureus* and *C. p. californicus*. Although not as strong, genetic support for the two subspecies can be seen with the z-linked locus. All pairwise Φ_{ST} comparisons were significant (Table 2.8), and the majority of molecular variance was partitioned to different subspecies (Table 2.7). Principle Coordinate Analysis also shows the greatest distance between the two subspecies along coordinate one which explained 89.5% of the variance (Figure 2.4). BAPs (Figure 2.5) and the haplotype network (Figure 2.7) both show distinction between the two subspecies; however, some mixing is suggested at all but one sampling site. Contemporary levels of differentiation between subspecies, measured using

microsatellites, provide limited support for the current classification of purple finch (Table 2.7). STRUCTURE analyses (Fig 2.9) first clustered individuals not by subspecies, but geography separating NL and NS from all other populations. The same dataset at K=3 did separate *C. p. californicus* from *C. p. purpureus* with the exception of birds from CBC who contained ancestry from both subspecies; some with high Q values to *C. p. californicus*. Another surprising result is the clustering of VanIs (*C. p. californicus*) individuals with *C. p. purpureus* birds from CBC (Figures 2.9, 2.10). CBC and VanIs populations, while from different subspecies, are geographically close in proximity. A number of factors could explain the genetic patterns: male-biased dispersal, mixing on overwintering grounds/flyways or incomplete lineage sorting.

While our sampling focused on breeding birds, with late migrants/migration, it is possible a few migrating individuals were sampled. The CoOR individuals displaying z-haplotypes more common among *C. p. purpureus* were caught in early May, and may be *C. p. purpureus* individuals who were caught mid-migration. However, the mtDNA haplotype network shows they cluster with *C. p. californicus*. Although the two subspecies should be distinguishable in the field by their variable plumage, plumage characteristics were not noted in the field, and photographs have proved unreliable. The single SCA individual might be an overwintering *C. p. purpureus* bird that was unable to build enough fat reserves to make the migration north. The presence of *C. p. californicus* z-haplotypes north and east of the *C. p. californicus* distribution cannot be explained through migration routes as *C. p. californicus* are year round residents. Rather, this lends support to limited gene flow between the two subspecies, possibly through hybridization at a contact zone, and subsequent male biased dispersal.

Sex-biased dispersal could explain the discordance between mtDNA and nuclear data. If males are the only birds dispersing, and mating with the other subspecies, z-linked genes and microsatellite alleles would be exchanged between subspecies, while mitochondrial genomes

would remain differentiated. Similar patterns are found in the spectacled eider (*Anatidae*; Scribner et al., 2001). In purple finch, juvenile males are known to disperse to new breeding sites creating the potential for gene flow among subspecies.

Some northern populations of *C. p. purpureus* overwinter in SCA where *C. p. californicus* is resident year round. Events occurring on wintering grounds can have significant effects on what happens during the breeding season (Webster and Marra 2005, Marra et al. 2006, Newton 2008). If pair bond formation occurs on wintering grounds, it opens the possibility for gene exchange between these otherwise allopatric subspecies. Despite this possibility, our results indicate no evidence of substantial historical or contemporary gene flow between *C. p. purpureus* and *C. p. californicus* at mtDNA loci. A similar pattern was seen with citril finch (*Serinus citronella*) in western Europe (Borras et al., 2011) where multiple, genetically distinct subpopulations overwinter at a common site. Despite this opportunity for gene flow between allopatrically breeding populations of citril finch, Borras et al. (2011) found none, suggesting the presence of non-physical barriers. They suggest that although pair bonds are formed in the winter, because individual flocks are generally formed at the end of the breeding season, at the overwintering site birds from the same breeding grounds are contained within smaller flocks thereby reducing the opportunity for gene flow. Though there seems to little be consensus on when purple finch form pair bonds, many other avian species form pair bonds while overwintering (Borras et al., 2011). Given that *C. p. purpureus* and *C. p. californicus* diverged 1.1 MYA, the two subspecies could share overwintering grounds, and minimize gene flow through pre-zygotic reproductively isolating barriers. With current morphological vocal and plumage differences between subspecies (Sibley, 2011; Wootton, 1996), it is possible that mate selection is occurring based on these traits, as has been seen in *Ficedula* flycatchers (Qvarnstrom et al., 2010).

Incomplete lineage sorting could explain these patterns of two distinct lineages in mtDNA and limited mixing at z chromosome loci. With a larger effective population size, lineage sorting in the z chromosome will take longer than for the mtDNA (Wang et al., 2014).

Despite these unexpected results, there is enough evidence to support the current classification of subspecies; especially when combined with morphological and vocal characters. The only population not conforming to their current designation is VanIs. Given the close proximity of this population to a potential contact zone and the small sample size, additional samples are required from this site. While all VanIs birds were collected at the same site, on the same day, and display only one haplotype for multiple loci; we can eliminate the possibility of a family group based on the patterns at microsatellite loci.

2.4.2: Multiple Refugia

Genetic evidence suggests *C. purpureus* survived the last glacial maximum in three different refugia: one for *C. p. californicus* and two for *C. p. purpureus*. Within *C. p. purpureus* an east-west difference is noticeable with mtDNA data and ENM shows the presence of suitable habitat during the LGM in at least four locations: in the west within much of the current *C. p. californicus* range and others to the east of the Sierra Nevada Mountains, SE US north of the Gulf of Mexico and off the Grand Banks of Newfoundland (Figure 2.9).

Within *C. p. purpureus* the east-west differentiation lends support to a western refugium for *C. p. purpureus* (Figures 2.6, 2.19). It is possible that individuals from this location descended from a western refugium, perhaps in the southern US. Although Beringia would be the most logical refugium for populations currently inhabiting CBC, based on findings from species with similar distributions to the purple finch (Brubaker et al., 2005; Flagstad & Roed,

2003; Lait & Burg, 2013) the lack of suitable habitat (Figure 2.9) suggests that perhaps western *C. p. purpureus* populations survived elsewhere during the LGM. Cryptic refugia and nunatuks were used by thin horned sheep (Loehr et al., 2006), and very cold tolerant plant species (Marr et al., 2013). Survival in CBC during the LGM is supported by the high diversity values found here; however, the lack of evidence of less cold-tolerant species, including trees, or any avian species suggests another refugium might be more likely for CBC birds. High diversity values can also be apparent in populations undergoing secondary contact, which given CBC's proximity to a potential contact zone with *C. p. californicus* populations may be a more likely explanation than survival in cryptic refugia.

Although not the most proximal area of suitable climate during the LGM, a refugial area for western *C. p. purpureus* birds might be in the south-western United States. Contemporary purple finch populations in CBC use this area as their overwintering grounds and migratory behaviour has been linked to post-glacial expansion from a refugium in other avian species (Ruegg & Smith, 2002). While limited by our sampling sites, microsatellite data (Figure 2.9) show genetic differentiation within Atlantic Canada and mtDNA data contain high levels of haplotype diversity (Table 2.5), similar to what was found in the boreal chickadee (Lait & Burg, 2013), rock ptarmigan (*Lagopus muta*)(Holder et al., 2004), and Canada lynx (*Lynx canadensis*) (Khidas et al., 2013). Based on this, a second eastern refugium around the Atlantic shelf for *C. p. purpureus* is likely.

C. p. californicus likely survived the LGM in smaller refugia found along the western coast of North America similar to their current range (Figures 2.17, 2.19). The lower genetic diversity (Table 2.5) in SCA suggests the refugium was more northern although the reduced genetic diversity could have been caused by a population bottleneck. Surviving the LGM in a north western refugium and expanding south to SCA would be supported by nucleotide and

haplotype diversity values, with WA having high values for both. This is congruent with source, or founder, populations, however, this would be the first evidence supporting a refugium that far south along the western coast of North America. Previous studies have purported a refugium in the vicinity of the Haida Gwaii (Burg et al., 2005; Byun et al., 1997), so there is precedence for a coastal refugium on the west coast of North America.

2.4.3: Postglacial expansion

While *C. p. purpureus* and *C. p. californicus* populations were able to maintain strong genetic differentiation, even as postglacial expansion lead to potential secondary contact, the same cannot be said of populations of *C. p. purpureus* that survived the LGM in eastern and western refugia. High levels of haplotype sharing with both mtDNA (Figure 2.6) and z-linked loci (Figure 2.7), and overall lower pairwise Φ_{ST} comparison values (Tables 2.6, 2.8) indicate that there was extensive gene flow upon secondary contact. Whether from cryptic refugia in British Columbia, a refugium along the coast of Washington or in south-western US, western *C. p. purpureus* birds would have followed the retreating ice sheets east, before meeting purple finch from the eastern refugium off the Atlantic shelf, which also has suitably high diversity values. The area where they met, would have overall higher diversity values (Table 2.5), and would contain haplotypes from both refugial populations, as is the case in ND. This east-west split is also apparent in the haplotype network for the mitochondrial locus (Figure 2.6), which shows a clear east-west gradient in the distribution of haplotypes. The gradient apparent in the haplotype network, along with the high levels of diversity at the secondary contact zone is clear evidence the eastern and western *C. p. purpureus* populations both followed the stepping-stone expansion model from their putative refugia. This pattern was not obvious in the z-linked locus AldB, which also fails to separate CBC from other *C. p. purpureus* populations (Figure 2.7).

As discussed in section 2.4.2, the most likely refugium for *C. p. californicus* was along the western coast of North America, likely in the area around WA. From there nucleotide and haplotype diversity values suggest a north-south expansion into southern California. SCA has the lowest haplotype and nucleotide diversity values of all populations (with the exception of the under-sampled VanIs) (Table 2.5). Low diversity indices are often the result of bottlenecks, or founder effects, and are more common in the periphery of a population, than the centre (Bush et al., 2011; Eckert et al., 2008; Grus et al., 2009). From the refugium, potentially in WA, *C. p. californicus* birds could have expanded south through a series of long distance dispersals, which would explain the lower nucleotide and haplotype diversity values found in southern California. The San Bernardino Mountains likely restricted this north-south expansion, further reducing the genetic diversity of birds currently found south of this barrier.

2.4.4 Contemporary barriers

Microsatellites, with a higher mutation rate, show significant pairwise F_{ST} comparisons between all populations (Table 2.11) and as many as five genetic groups in the 10 sampling sites (Figure 2.9). The high differentiation might be expected for the *C. p. californicus* populations, given their relative sedentary nature, but not for the more migratory *C. p. purpureus*.

High site fidelity can maintain genetic differentiation. Natal site fidelity was responsible for maintaining genetic differentiation between populations of sympatric northern pike (*Esox lucius*) (Miller et al., 2001), and green turtles (*Chelonia mydas*) (Encalada et al., 1996), which display breeding and non-breeding areas, with seasonal migration between them, much like many migratory bird species. Based on studies in Michigan (Magee, 1924) and New York (Yunick, 1983, 1987), *C. p. purpureus* populations show 56.8% and 23.6% (n=1770) of banded birds

returning to breed in the same area. Wilson and Brown (2012) also found evidence of strong breeding site fidelity.

Most *C. p. purpureus* populations along the southern edge of the breeding range in eastern half of their range contain birds year round. Unlike the *C. p. californicus* populations, which are resident year round; it is not clear if birds are permanent resident or birds from further north are migrating south into these areas. Permanent residency could help explain the extremely high differentiation found between populations (Table 2.11). The only widely accepted migratory population of purple finch we sampled is CBC, however, no evidence of admixture was detected between the birds from CBC and SCA (the overwintering grounds for CBC birds) for reasons outlined in section 2.4.1.

A number of potential barriers are present throughout the purple finch range potentially leading to restrictions in gene flow. One such barrier would be physical distance, however, no significant correlation was found between geographic and genetic distance (Figure 2.14). Furthermore, some populations that are geographically close to one another are genetically differentiated such as WA and CoOR (Figure 2.19). Soltis et al. (1997) found a number of plant species whose division into northern and southern groups was also found in central Oregon. Contemporary gene flow could be restricted between these two populations due in part to plumage differences noted by Duvall (1945).

The only barrier found in the program BARRIER using microsatellite data does not correspond to major mountain ranges, or bodies of water, but instead separates populations from the east coast (NL and NS) from the continental populations (Figure 2.11). Though no obvious barrier is present, there is evidence of this region acting as a barrier for both animal and plant species. Rueness et al. (2003) found the area south of James Bay impeded gene flow between eastern and western populations of Canadian lynx (*Lynx canadensis*), despite a lack of clear

physical barrier, while a barrier also exists in this area for the boreal chickadee (*Poecile hudsonicus*). Lait (2011) attributed this barrier to the post-glacial expansion of pine forests, boreal chickadee's preferred habitat. These jack pines (*Pinus banksiana*) also show an eastern and western lineage which meet south of Hudson Bay (Jaramillo-Correa et al., 2009).

Multiple bodies of water are present across the range of the purple finch, however, few appear to impede gene flow. Although the Strait of Georgia limits gene flow between WA and VanIs, there is no differentiation between VanIs and CBC populations (Figure 2.19). Likewise, Cabot Strait is not a barrier to gene flow, given NL and NS populations are not genetically differentiated.

The Rocky Mountain range may also be limiting gene flow between populations of purple finch, with genetic differentiation occurring between CBC and all other *C. p. purpureus* populations. Birds on either side of the Rocky Mountains also have very different overwintering sites, with those east of the Rockies migrating south-east, to overwinter in the southern United States, and those west of the Rockies overwintering in southern California and Arizona (Salt, 1952). The San Bernardino Mountains likely restricted gene flow within the *C. p. californicus* subspecies, leading to a population bottleneck, as can be seen in the mtDNA and z-linked haplotype networks (Figures 2.6, 2.7), and low haplotype and nucleotide diversity values ($h=0.270$, $\pi=0.0004$ for mtDNA and $h=0.086$, $\pi=0.0005$ for the z-linked locus respectively). The San Bernardino Mountains have also affected the genetic structure of the mountain chickadee (*Poecile gambeli*) (Hindley, 2013; Spellman et al., 2007)

2.4.5: Population structure

Overall five groups were identified by STRUCTURE. These were isolated by various mechanisms. Some such as WA and Atlantic Canadian populations show distinct plumage differences such that they were once considered a different subspecies (Burleigh, 1948; Duvall, 1945), while others, such as the CoOR and SCA birds are isolated by their sedentary, non-migratory behaviour. The continental cluster (ND, MI and LAB) is likely isolated from CBC birds by the Rocky Mountains and Great Plains, and from the eastern cluster by plumage variation.

CHAPTER THREE

General Discussion

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3.1: Conclusions

Through the combination of genetic work and paleo-ecological support, we concluded that three refugia were found: off the coast of Newfoundland, along the Pacific Coast (around Washington and Oregon), and in the southern United States, around Arizona. Following postglacial expansion into northern areas, purple finch maintained the presence of two purple finch subspecies and gene flow is now restricted between subspecies and within the range of each subspecies. It appears there may be a contact zone within the west and/or male-biased dispersal which warrants further investigation.

3.2: Future Directions

3.2.1: Refugia

With plenty of genetic support for the Atlantic shelf refugium and both behavioural and genetic support for the south-eastern United States refugium, more work could be directed at elucidating the exact location of the refugium used by *C. p. californicus* populations. Although a refugium around WA is most likely based on the genetic data available, SCA is the only other population with enough samples to allow for direct comparisons. Increasing the sample size for the VanIs and CoOR sites, and adding another site in central/northern California would create a transect, more able to detect putative refugia. Areas of high haplotype and nucleotide diversity most likely acted as refugia in the past. Creating a haplotype network and locating populations

with a star-shaped network and a high degree of haplotype sharing could also help identify putative refugia.

3.2.2: Purple Finch Systematics

There is clear evidence of two distinct subspecies with significant differentiation occurring between all F_{ST} and Φ_{ST} comparisons among populations from different subspecies. Having established genetic support for the current systematics, more work should be spent looking at the putative contact zone in south western British Columbia and northern Washington. Increasing sample size and sampling along a transect, at a finer scale in this area could detect the actual contact zone, where gene flow between the two subspecies begins to occur, and things like haplotype sharing is common. Sampling in this area is complicated by lack of road access in a south western British Columbia, and coordination with programs such as Project Feeder Watch would likely be needed capture appropriate sample sizes from each site.

Analysis of the pigment of each bird sampled, obtained by assigning a value to the red or brown hue of each bird sampled could elucidate the relationship between geographic location, colouration and genetic assignment. Recording songs and calls of the birds in each sampling location and looking at corresponding sonograms in combination with colour analysis could clarify whether vocalization and plumage act as reproductively isolating barriers, or if they are simply artefacts of environment and diet. This is especially important around the putative contact zone, where reproductively isolating barriers are needed to limit gene flow between two subspecies in close proximity to one another.

One of the findings of this study was the coalescent time for the two subspecies. The coalescence time of 1.1MYA was based on the assumption of mitochondrial neutrality, which has been questioned lately (Castoe et al., 2009, Galtier et al., 2009). If selection can act on loci within

the mitochondrial genome, any estimates of coalescence based on neutrality will be overestimated. The ATP6 region of the mitochondrial genome plays a crucial role in the formation of Adenosine Triphosphate, and energy production within the mitochondria. The majority of *C. p. californicus* and *C. p. purpureus* individuals display very different migratory strategies, which require very different energy regulation strategies. Given ATP6's crucial role in energy production, it is possible that selection might act on this locus, thereby rendering this locus non-neutral. Sequencing the control region (less likely to be under selection), will allow comparison with ATP6, and could give another, potentially more precise estimate of coalescence times.

3.2.3: Contemporary Barriers

The clustering of VanIs birds with those from CBC using microsatellite data was unexpected given that they belong to different subspecies, and sequence data from both mitochondrial and z-linked loci places them clearly with other *C. p. californicus* populations. With only four individuals sampled from VanIs, it wasn't possible to conduct pairwise tests on these individuals. Given their proximity to a putative contact zone, and unusual clustering, increasing sampling effort in this area, in order to clarify the degree and direction of gene flow is justified.

Individuals sampled in SCA also showed unexpected results. Although they clustered with the appropriate subspecies when both sequence and microsatellite loci were used, diversity values were much lower than all other populations, when examined with all loci. Previous work has shown the San Bernardino mountain range is a barrier to gene flow for the mountain chickadee (Hindley, 2013; Spellman et al., 2007). Through conducting finer-scale sampling along a transect on crossing the San Bernardino Mountains, one could determine the degree to which this mountain range is limiting gene flow. If diversity values show no major fine scale

differentiation, the location of SCA at the extreme southern extent of the purple finch's range could explain the lower diversity values through geographic isolation and smaller effective population size (Eckert et al., 2008), as was the case with black-throated blue warblers (*Setophaga caerulescens*) (Grus et al., 2009), and the North American mountain goat (*Oreamnos americanus*).

3.2.4: Additional Directions

With many journals following the mantra that more is better, the temptation exists to increase the number of loci, or to invest in Next Generation Sequencing in order to increase resolution. Although the resolution is usually improved, often this increase is very slight, and disproportionate to the increased financial investment. A potential advantage to NGS, however, is its ability to detect loci under selection. This could be especially beneficial with the purple finch, where it appears the ATP6 locus is under selection.

In addition to clarifying the genetic story of the purple finch, the behavioural traits which could be affecting gene flow need to be examined. A study elucidating migratory behaviour of the purple finch, which is poorly understood currently, could reveal genetic differentiation occurring along different migratory routes. This could be done with geo-locators, stable isotope analysis, or a combination of both. Furthermore, analysing of sonograms of vocalizations and quantifying plumage traits in the field, putative reproductively isolating traits could be revealed.

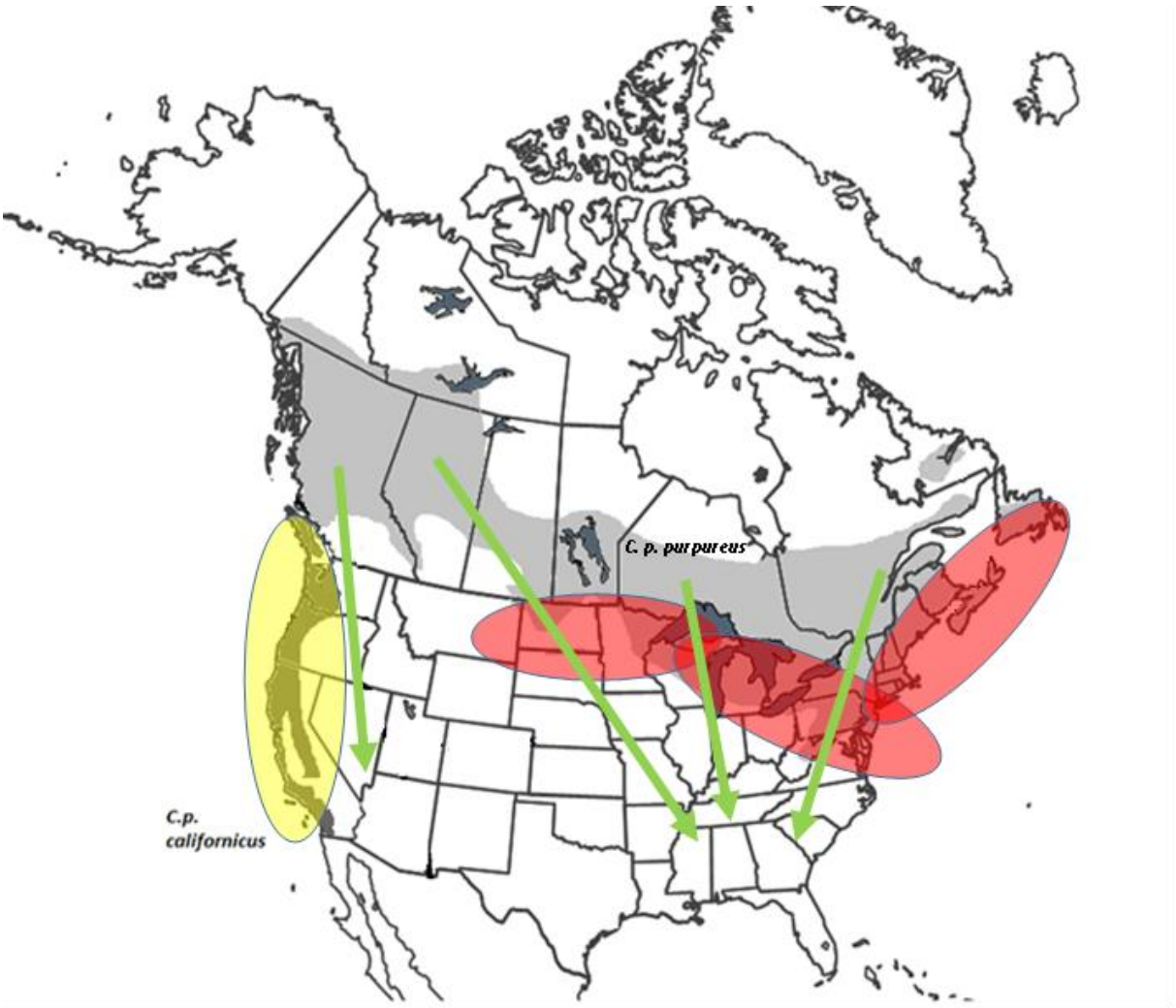

Figure 1.1: Map depicting migratory behaviour and migration routes (green arrows) of the purple finch. Most populations are migratory with the exception of populations on the West Coast which are resident year round (yellow). The migratory behaviour of some eastern populations (red) is unknown.

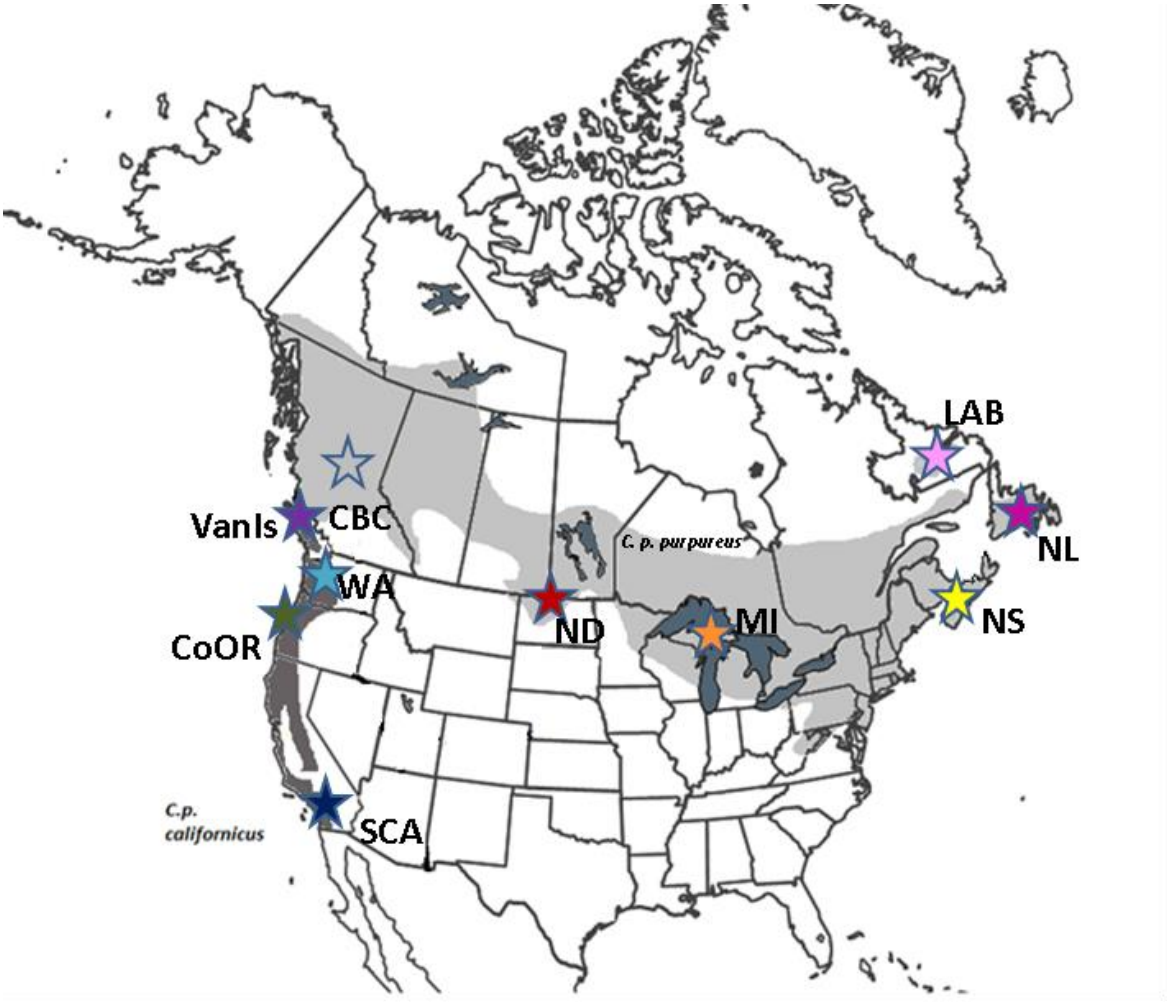

Figure 2.1: Distribution of breeding and year round resident *C. p. purpureus* (light gray) and *C. p. californicus* (dark gray). Modified from Sibley (2000). Sample locations denoted by coloured stars: VanIs (Vancouver Island), CoOR (Coastal Oregon), SCA (Southern California), WA (Washington), CBC (Central British Columbia), ND (North Dakota), MI (Michigan), NS (Nova Scotia), LAB (Labrador) and NL (Newfoundland). Colour scheme maintained throughout document.

Table 2.1: Populations sampled and their abbreviations

abbrev	location
CBC	Central British Columbia
CoOR	Coastal Oregon
LAB	Labrador
MI	Michigan
ND	North Dakota
NL	Newfoundland
NS	Nova Scotia
SCA	Southern California
VanIs	Vancouver Island
WA	Washington

Table 2.2: Sequence, orientation and reference for forward and reverse primers and M13 Tag: Forward (F), Reverse (R), Light (L) and Heavy (H) strand.

Primer	Primer Orientation	Sequence	**Reference**
ATP6	L	CCAACCACAGCTTCATACCA	Developed in Burg Lab
	H	GCTAGTGGGCGGATGAGTAG	
AldB 6	F	GAGCCAGAAGTCTTACCTGAYGG	**(**Hackett et al., 2008)
	R	GCTCKCCCGTATGAGAAGGTCAGYTT	
CE215	F	ACCTGACCTAGCTGATGTTC	(Polakova et al., 2007)
	R	GCTGCCTGGCAGTAATAA	
Ase 18	F	ATCCAGTCTTCGCAAAAGCC	(Richardson et al, 2000)
	R	TGCCCCAGAGGGAAGAAG	
Cuu 28	F	GAGGCACAGAAATGTGAATT	(Gibbs et al., 1999)
	R	TAAGTAGAAGGACTTGATGGCT	
CTC101	F	GTCCAGTAGGTAGGTGTGATG	(Tarvin, 2006)
	R	TTATTTAGGTGCCAGAGAGATG	
Titgata 02	F	ATTGCTTGATATTTGAAAGCATA	(Wang et al., 2005)
	R	TTGTCTTTTGGGTTGCCTGA	
Titgata 39	F	CATGTATTTTCCAAAAGTAAATAT	(Wang et al., 2005)
	R	CTGCTATTCTGCAAACTTGTGG	
Pat 43	F	CACGACGTTGTAAAACGAC	(Otter et al., 1998)
	R	GTATCCAGAGTCTTTGCTGATG	
M13 Tag		CACGACGTTGTAAAACGAC	

Table 2.3: Concentration of reagents used in Polymerase Chain Reactions for the mitochondrial, z-linked and seven microsatellite loci used, including buffers: Truin (T), Flexi (F), and Crimson (C).

Primer	**Buffer**	**F/R Primer (mM)**	**taq**	**MgCl2 (mM)**
ATP6	F	0.4	F	2.5
AldB	C	0.4	F	2
CE215	T	1	T	2.5
Ase 18	T	1	T	2
Cuu 28	T	1	T	2
CTC101	T	1	T	1.5
Titgata 02	F	1	F	2
Titgata 39	F	1	F	2
Pat 43	C	1	C	1.5

Table 2.4: BioClim Layer Code and the corresponding climatic condition for the climatic layers used in analysis.

Bio Clim Layer Code	Climatic Condition
Bio 1	Annual Mean Temperature
Bio 2	Mean Diurnal Range (Mean of monthly (max temp-min temp))
Bio 4	Temperature Seasonality (standard deviation *100
Bio 8	Mean Temperature of Wettest Quarter
Bio 14	Precipitation of Driest Month
Bio 15	Precipitation Seasonality (Coefficient of Variation)
Bio 16	Precipitation of Wettest Quarter
Bio 18	Precipitation of Warmest Quarter
Bio 19	Precipitation of Coldest Quarter

Table 2.5: Haplotype (h) and nucleotide (π) diversity values, and sample size (N) at the mitochondrial and z-linked loci.

Pop	mtDNA ATP			Z chromosome AldB		
	h	π	N	h	π	N
VanIs	0.000	0.0000	4	0.000	0.0000	4
CoOr	1.000	0.0029	2	0.786	0.0062	8
WA	0.679	0.0015	38	0.486	0.0017	39
SCA	0.270	0.0004	28	0.086	0.0005	46
NL	0.889	0.0026	34	0.464	0.0024	50
CBC	0.858	0.0024	53	0.816	0.0052	56
NS	0.857	0.0056	35	0.604	0.0038	47
ND	0.899	0.0028	24	0.837	0.0069	26
MI	0.661	0.0018	19	0.754	0.0045	26
LAB	0.733	0.0014	6	0.500	0.0058	4
All	0.896	0.0113	243	0.788	0.0057	306

Table 2.6: Mitochondrial pairwise Φ_{ST} comparisons from Arlequin (Excoffier and Lischer, 2010) (below diagonal) and corresponding significance scores (above diagonal). Significance values have been corrected for multiple comparisons using the modified FDR calculator and significant comparisons are bolded.

Subspecies		WA	SCA	NL	CBC	NS	ND	MI
C. p. californicus	WA	*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	SCA	0.092	*****	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
C. p. purpureus	NL	0.898	0.932	*	**<0.0001**	0.820	0.099	0.306
	CBC	0.903	0.928	0.180	*	**<0.0001**	**<0.0001**	**<0.0001**
	NS	0.898	0.932	0.000	0.194	*	0.06	0.306
	ND	0.896	0.936	0.019	0.131	0.017	*	0.234
	MI	0.910	0.960	0.005	0.113	0.006	0.014	*

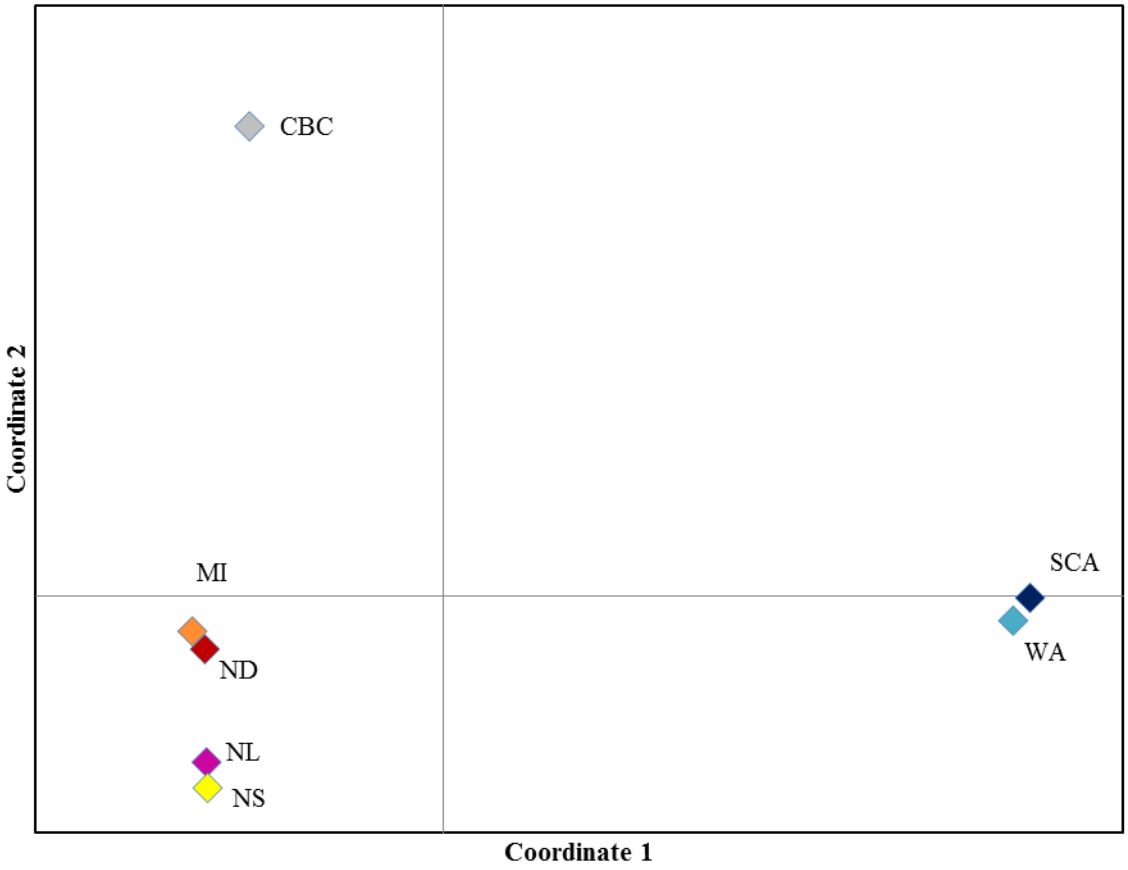

Figure 2.2: Principle Coordinate Analysis of the mtDNA ATP sequence data. Coordinates one and two explain 87.3% and 8.9% of the variation respectively. Refer to Figure2.1 for sampling locations.

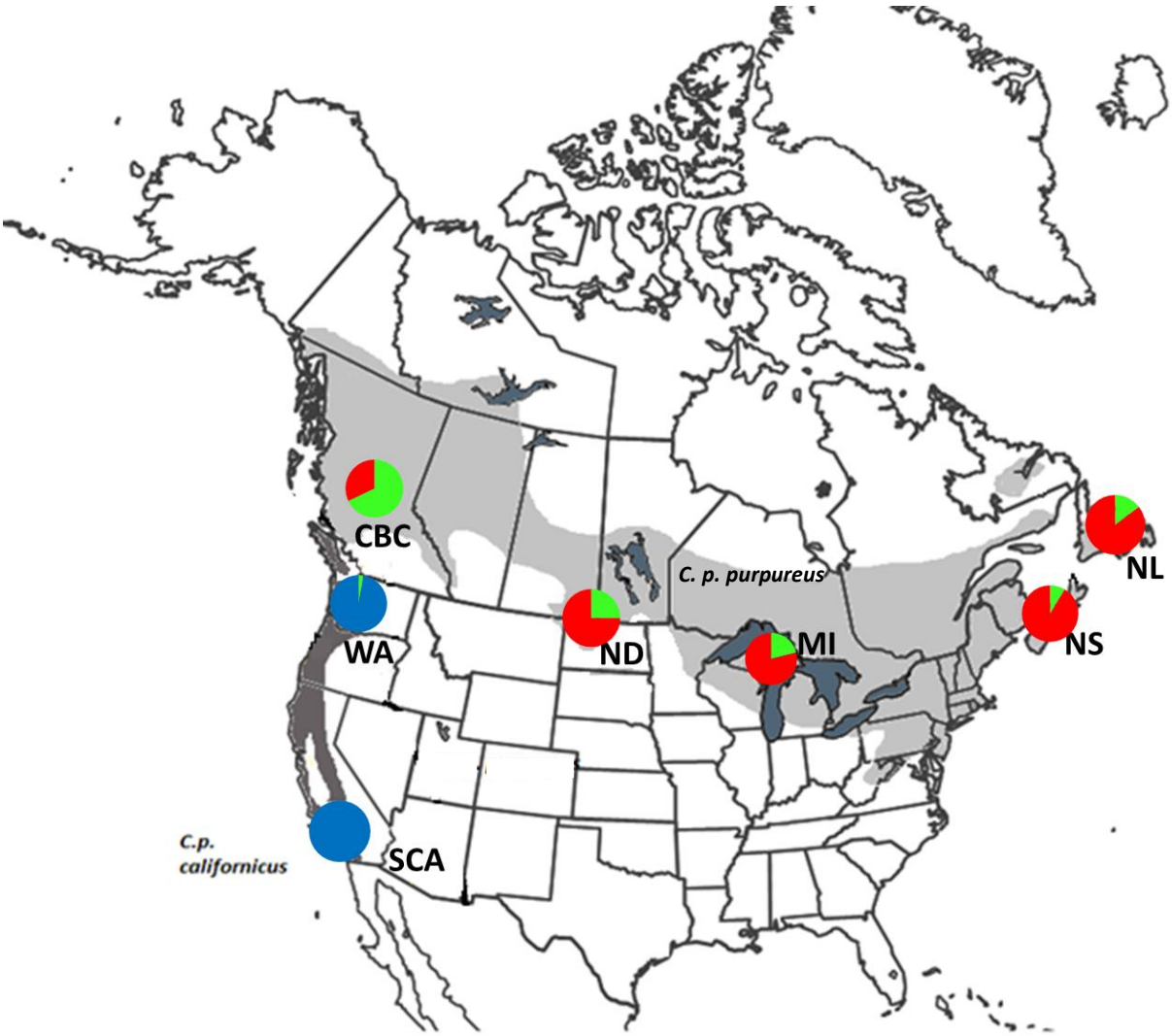

Figure 2.3: Based on BAPs analysis of the mitochondrial locus ATP 6, a map depicting assignment of individuals from seven populations. Three clusters corresponding to a *C. p. californicus* group (blue), and a predominately western (green) and eastern (red) *C. p. purpureus* group.

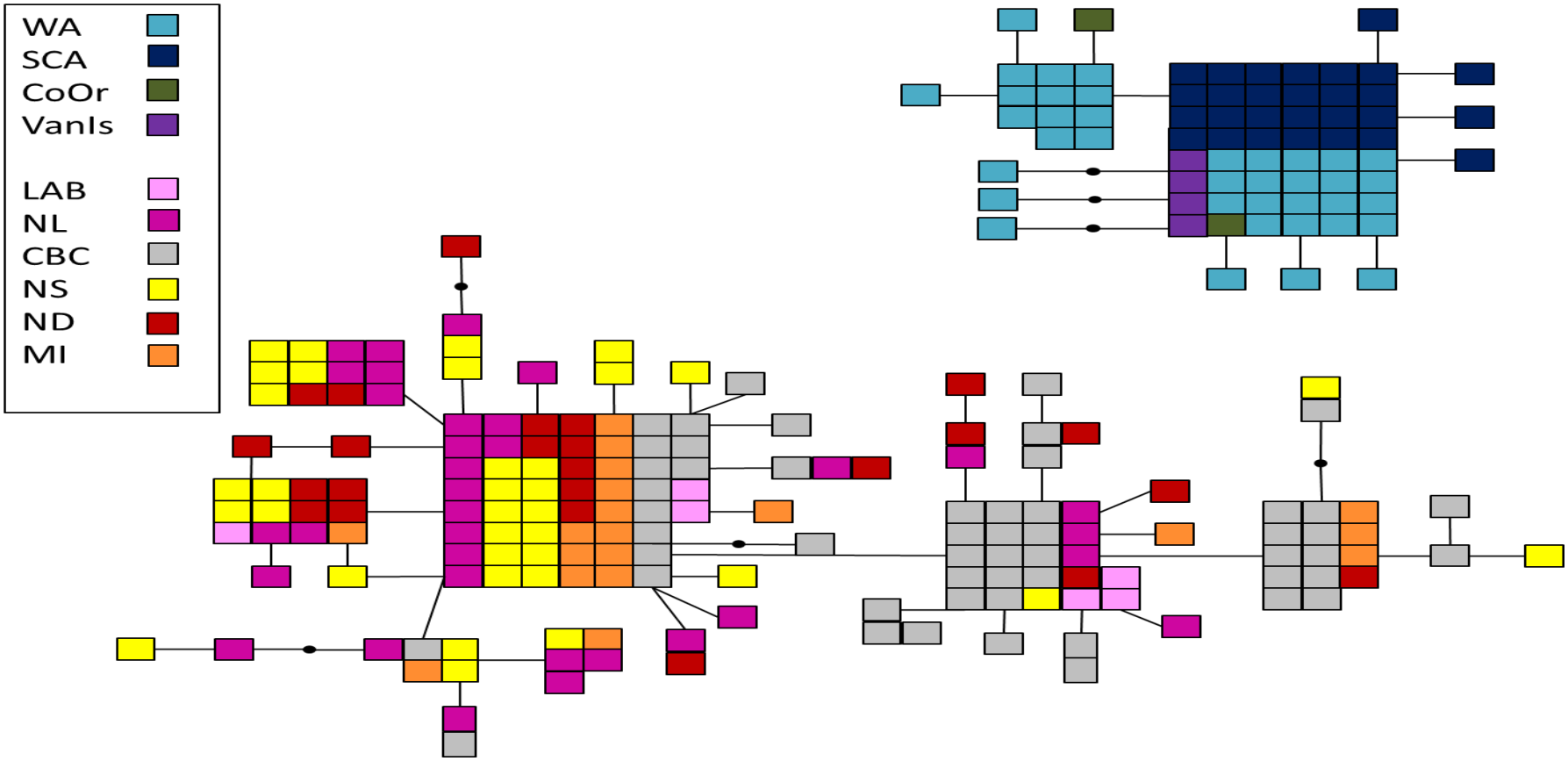

Figure 2.4: Parsimony haplotype network from the mitochondrial locus. Each square represents an individual, a group of squares are individuals that share a haplotype, lines connecting squares represent a one base pair differences, and black dots are unsampled or missing haplotypes (see legend). *C. p. californicus* and *C. p. purpureus* individuals are represented by cool and warm colours respectively, while individuals from CBC are gray.

Table 2.7: Results of AMOVAs from three loci tested. All values are the percent of total variation found in each partition, with associated p-value in parentheses. P-values in bold are significant.

Partition	Locus		
	mitochondrial	z-linked	microsatellite
Among Subspecies	90 (**0.001)**	55 (**0.001**)	0 (**0.002**)
Among Populations	1 (**0.001**)	2 (**0.006**)	8 (**0.001**)
Within Populations	9 (**0.001**)	43 (**0.001**)	92 (**0.001**)

Table 2.8: Pairwise Φ_{ST} comparisons from Arlequin (Excoffier and Lischer, 2010) (below diagonal) and corresponding P values (above diagonal) for AldB data. Values in bold remained significant following FDR corrections.

Subspecies		WA	SCA	NL	CBC	NS	ND	MI
C. p. californicus	WA	*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	SCA	0.116	*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
C. p. purpureus	NL	0.621	0.805	*	**0.009**	0.405	0.054	0.099
	CBC	0.438	0.558	0.060	*	0.189	0.135	0.847
	NS	0.535	0.707	0.000	0.007	*	0.144	0.522
	ND	0.462	0.649	0.048	0.020	0.017	*	0.414
	MI	0.467	0.695	0.034	0.000	0.000	0.000	*

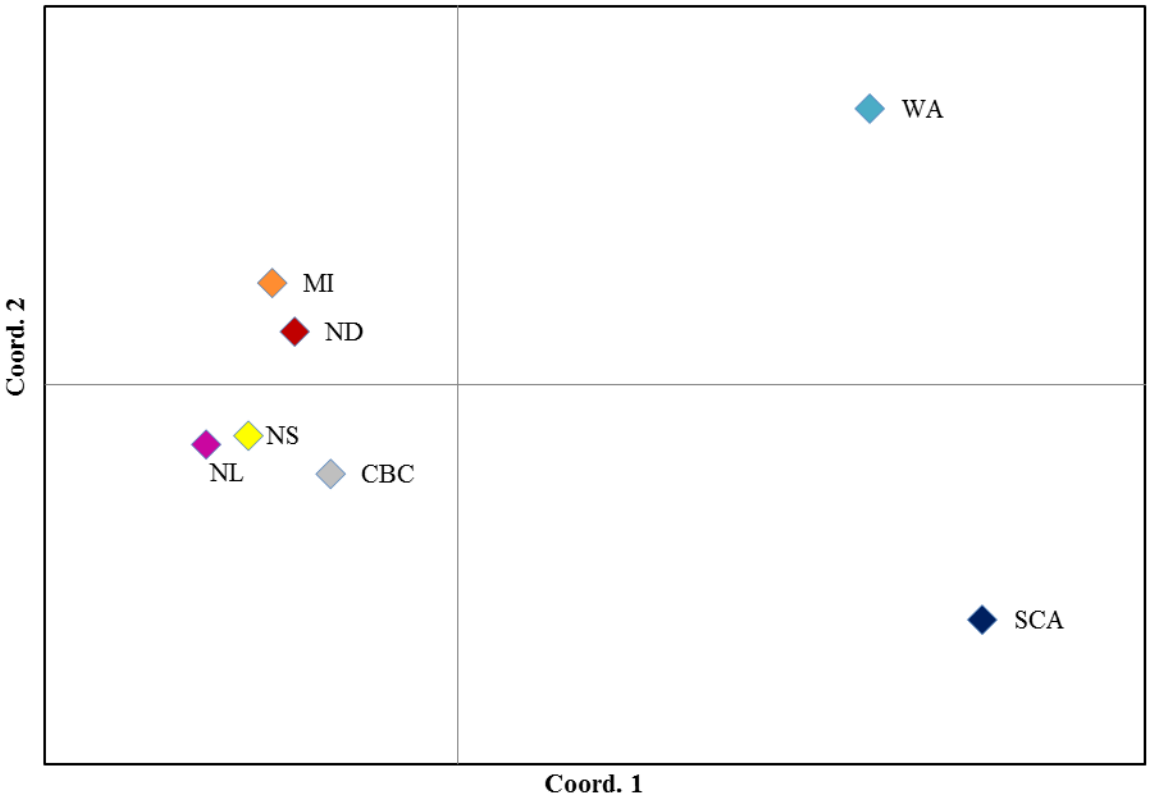

Figure 2.5: Principle Coordinate Analysis of the sequence data from the z-linked locus. Coordinates one and two explain 89.5% and 6.7% of the variation respectively.

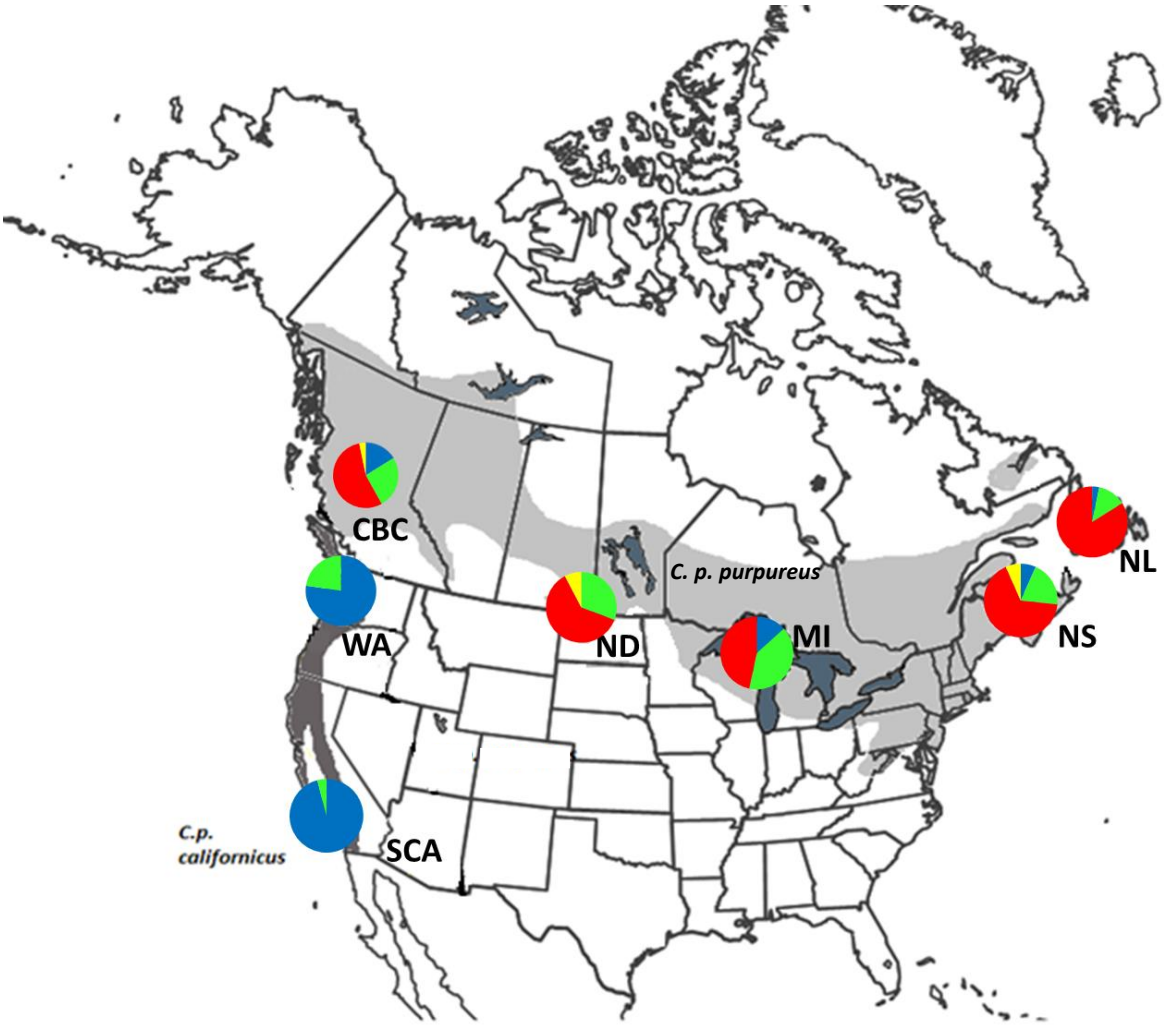

Figure 2.6: Based on the z-linked locus AldB, a map of assignment of individuals from seven populations showing four clusters corresponding to a predominantly *C. p. californicus* group (blue), a widespread (green) and *C. p. purpureus* (red) group. A forth cluster (yellow) shows no obvious geographic centralization

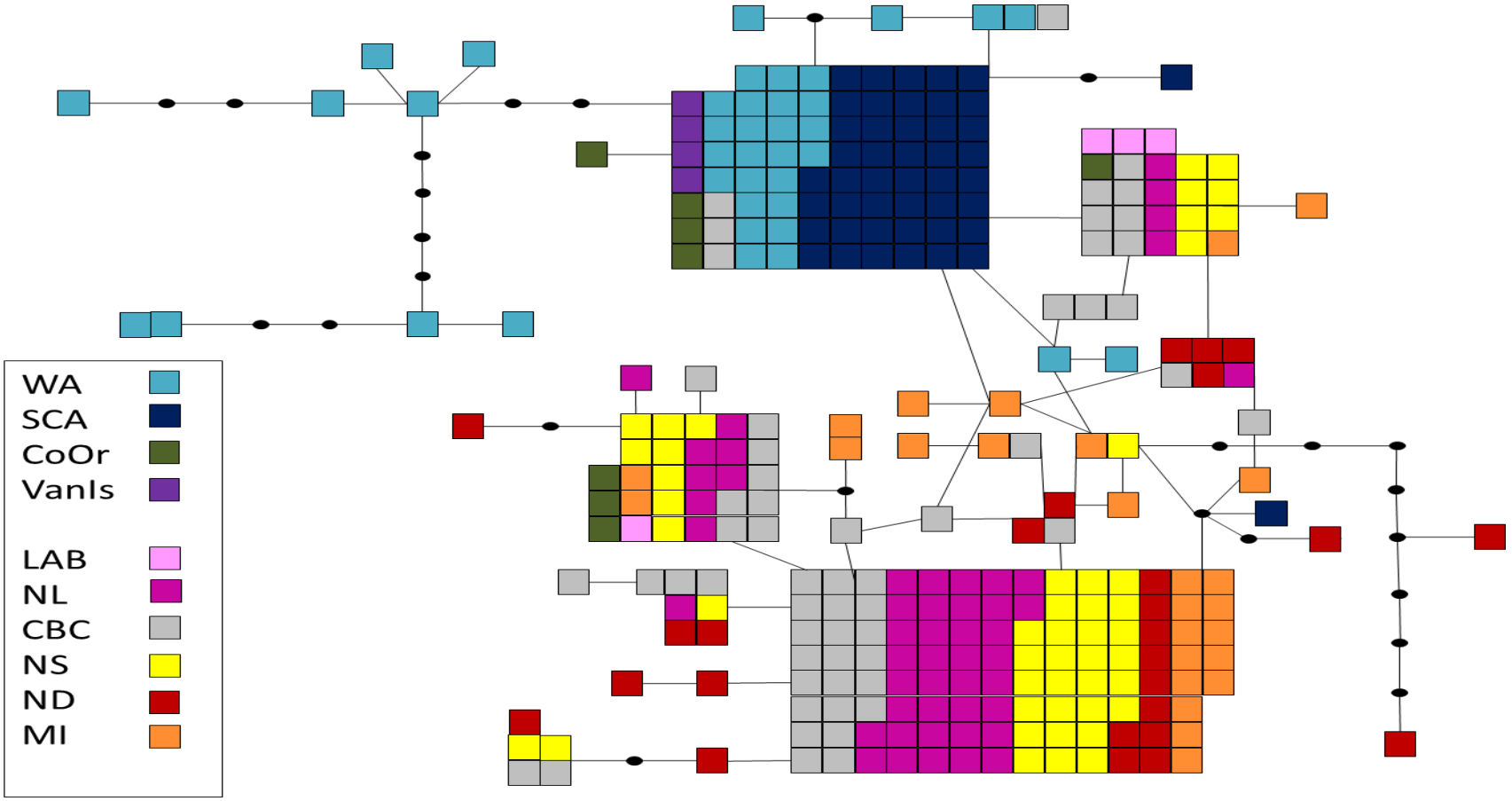

Figure 2.7: Parsimony haplotype network from the Z-linked locus. Each square represents an individual, a group of squares are individuals that share a haplotype, lines connecting squares represent a one base pair differences, and black dots are unsampled, or missing haplotypes (see legend). *C. p. californicus* and *C. p. purpureus* individuals are represented by cool and warm colours respectively, while individuals from CBC are gray.

Table 2.9: List of all haplotypes, broken down by population for the mitochondrial locus ATP6.

Haplotype	VanIs	CoOR	SCA	WA	CBC	ND	MI	NS	LAB	NL	Total
A				11							**11**
B	4	1	24	19							**48**
C					14	1		1	3	3	**22**
D					10	1	3				**14**
E					2	1					**3**
F					2						**2**
G					3						**3**
H					1		1	2		1	**5**
I					1			1			**2**
J					11	7	11	12	2	10	**53**
K						4	1	4	1	2	**12**
L						1				1	**2**
M						2		5		5	**12**
N							1	1		3	**5**
O								2		1	**3**
P					1	1				1	**3**
Q					1					1	**2**
R						1				1	**2**
S								2			**2**
# unique	**0**	**1**	**4**	**8**	**7**	**5**	**2**	**5**	**0**	**5**	**37**
# private	**0**	**1**	**4**	**9**	**9**	**5**	**2**	**6**	**0**	**5**	**41**
# haplotypes	**1**	**2**	**5**	**10**	**17**	**14**	**7**	**14**	**3**	**16**	**56**
sample size	**4**	**2**	**28**	**38**	**53**	**24**	**19**	**35**	**6**	**34**	**243**

Table 2.10: List of all haplotypes, broken down by population for the z-linked locus AldB.

Haplotype	VanIs	CoOR	SCA	WA	CBC	ND	MI	NS	LAB	NL	Total
A	4	3	44	24	3						**78**
B		3			7		2	8	1	7	**28**
C		1			7		1	7	3	4	**23**
D					22	10	13	28		36	**109**
E							2				**2**
F					1	4				1	**6**
G					2	1		2			**5**
H					3	2		1		1	**7**
I					1	2					**3**
J					3						**3**
K							1	1			**2**
L					1		1				**2**
M				2	1						**3**
N				2							**2**
# unique	**0**	**1**	**2**	**11**	**5**	**7**	**6**	**0**	**0**	**1**	**33**
# private	**0**	**1**	**2**	**12**	**6**	**7**	**7**	**0**	**0**	**1**	**36**
# haplotypes	**1**	**4**	**3**	**14**	**16**	**12**	**12**	**6**	**2**	**6**	**47**
sample size	**4**	**8**	**46**	**39**	**56**	**26**	**26**	**47**	**4**	**50**	**306**

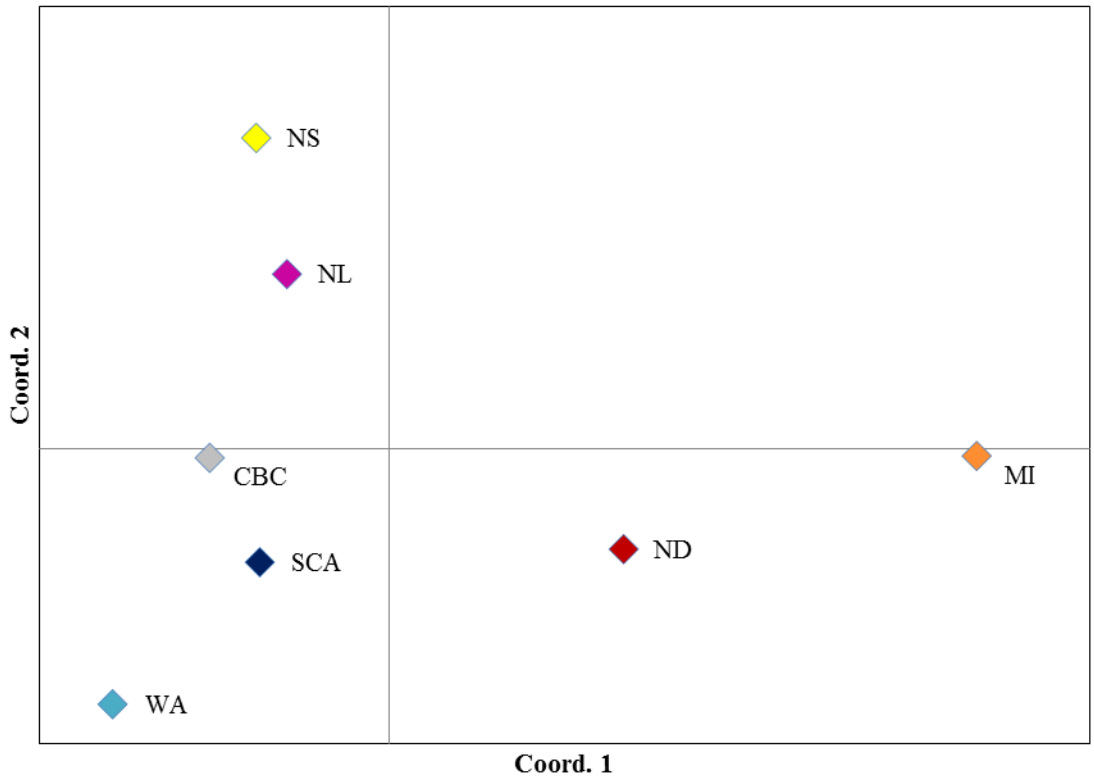

Figure 2.8: Principle Coordinate Analysis of the genotype data from the seven microsatellite loci. Coordinates one and two explain 44.7% and 24.5% of the variation.

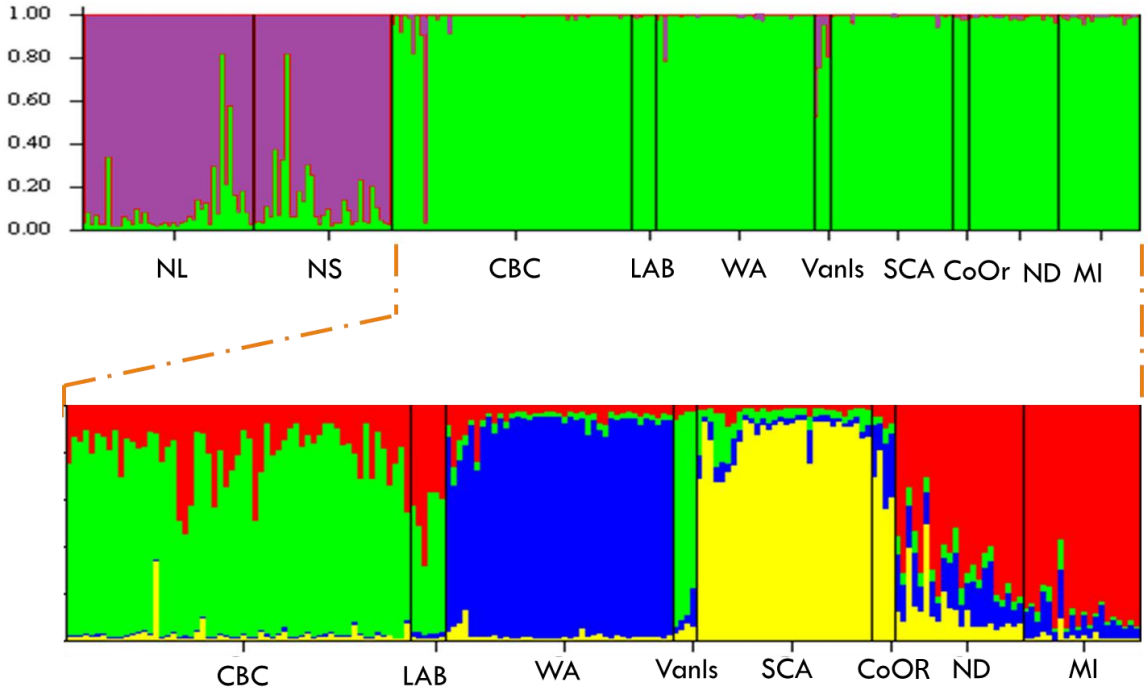

Figure 2.9: Hierarchical runs from STRUCTURE. Ancestry coefficient values on the y-axis, while different colours represent different clusters. After the initial run (top plot), NS and NL populations were excluded to examine additional structure (bottom plot).

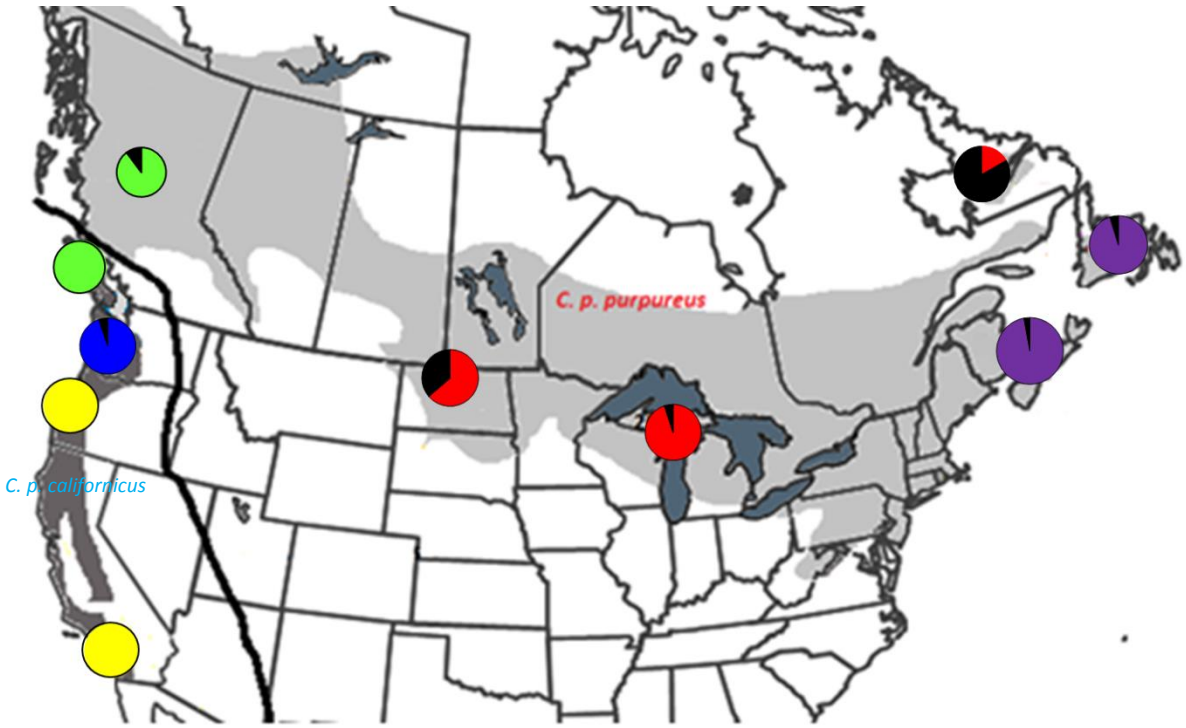

Figure 2.10: Map of the *Carpodacus purpureus* range, with the proportion of individuals assigned by STRUCTURE to each cluster represented as a pie chart. A maritime cluster (purple), central continental cluster (red), western *C. p. p.* cluster (green) (includes VanIs), and two *C. p. c.* clusters (blue and yellow) were all discovered using microsatellite data. Individuals with a Q value (ancestry coefficient <XXX were unassigned (black).

Table 2.11: Microsatellite pairwise F_{ST} comparisons from Arlequin (Excoffier & Lischer, 2010) (below diagonal) and corresponding P values (above diagonal). Values in bold remained significant following FDR corrections.

Subspecies		WA	SCA	NL	CBC	NS	ND	MI
C. p. californicus	WA	*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	SCA	0.066	*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
C. p. purpureus	NL	0.095	0.080	*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	CBC	0.045	0.028	0.053	*	**<0.0001**	**<0.0001**	**<0.0001**
	NS	0.109	0.083	0.076	0.049	*	**<0.0001**	**<0.0001**
	ND	0.070	0.047	0.065	0.045	0.090	*	**0.003**
	MI	0.174	0.130	0.137	0.125	0.140	0.029	*

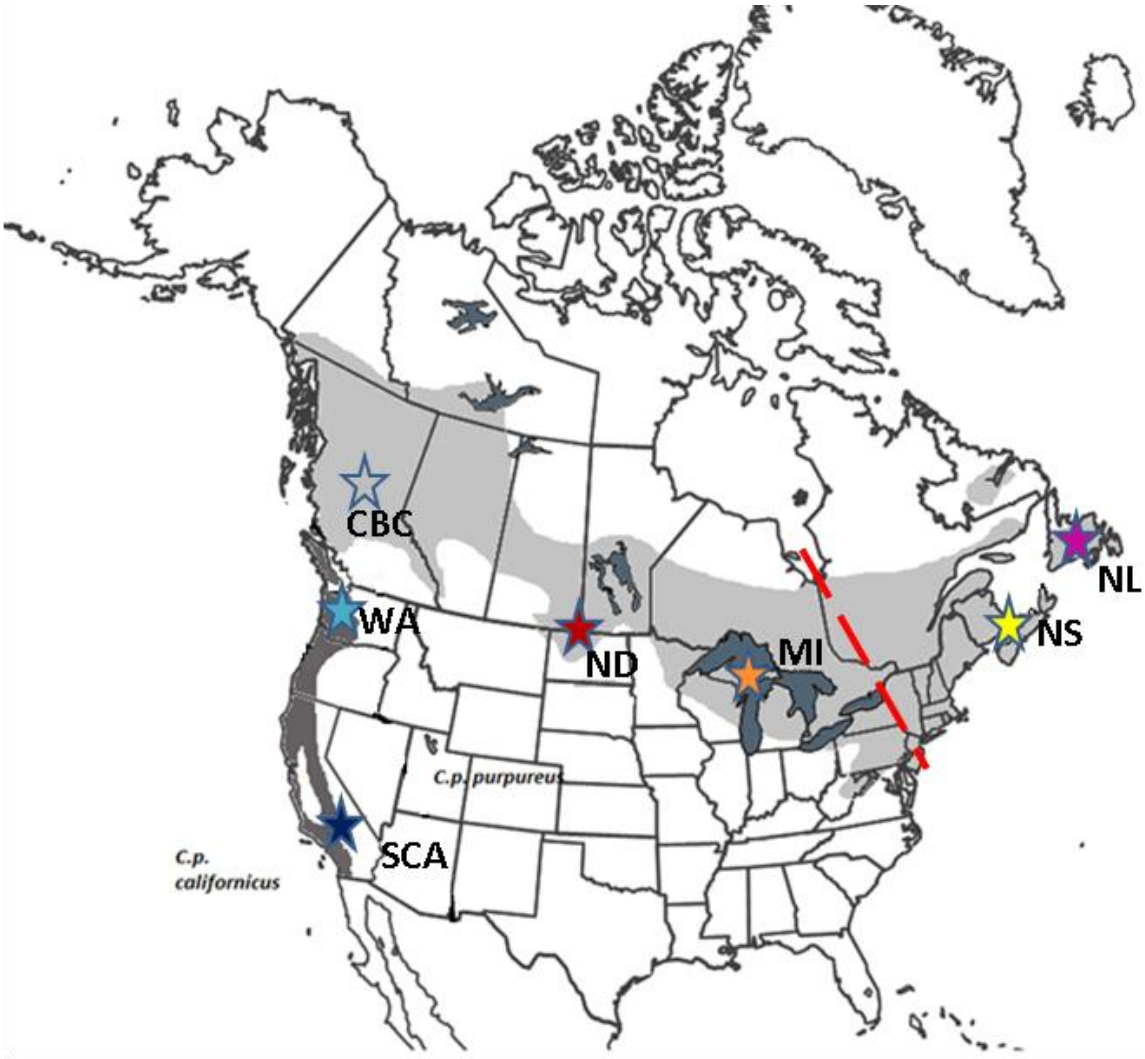

Figure 2.11: Lone physical barrier to gene flow based on microsatellite F_{ST} values (red dashed line), separating Nova Scotia and Newfoundland populations from all other populations, according to the barrier discovery program BARRIER v.2.2 (Manni et al., 2004).

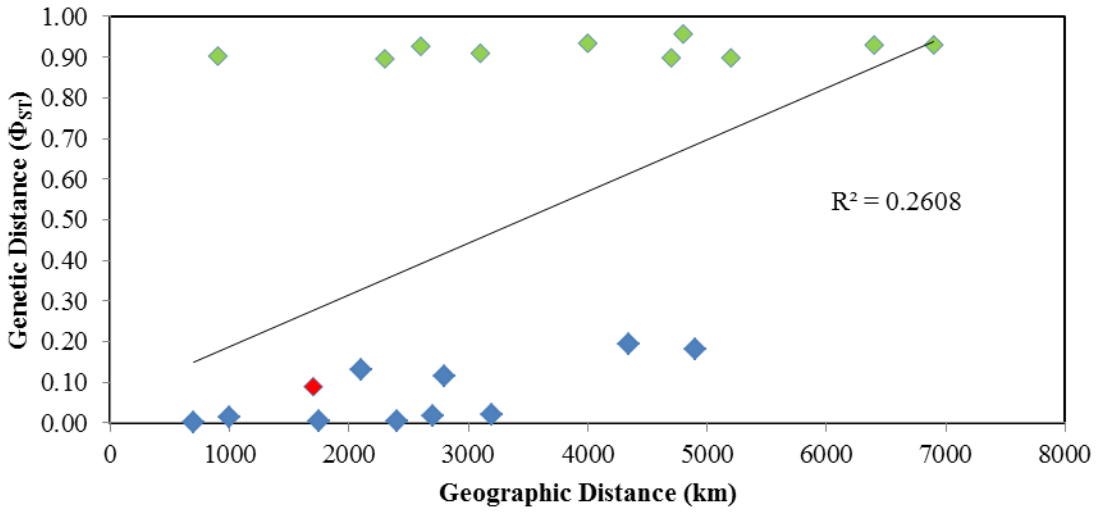

Figure 2.12: Mantel test depicting relationship between geographic (x-axis), and genetic (y-axis) distance for the mitochondrial locus, ATP6. R^2 shows a moderately significant relationship (p=0.05). Green markers are comparisons between subspecies and blue and red markers are comparisons within *C. p. purpureus* and *C. p. californicus* respectively.

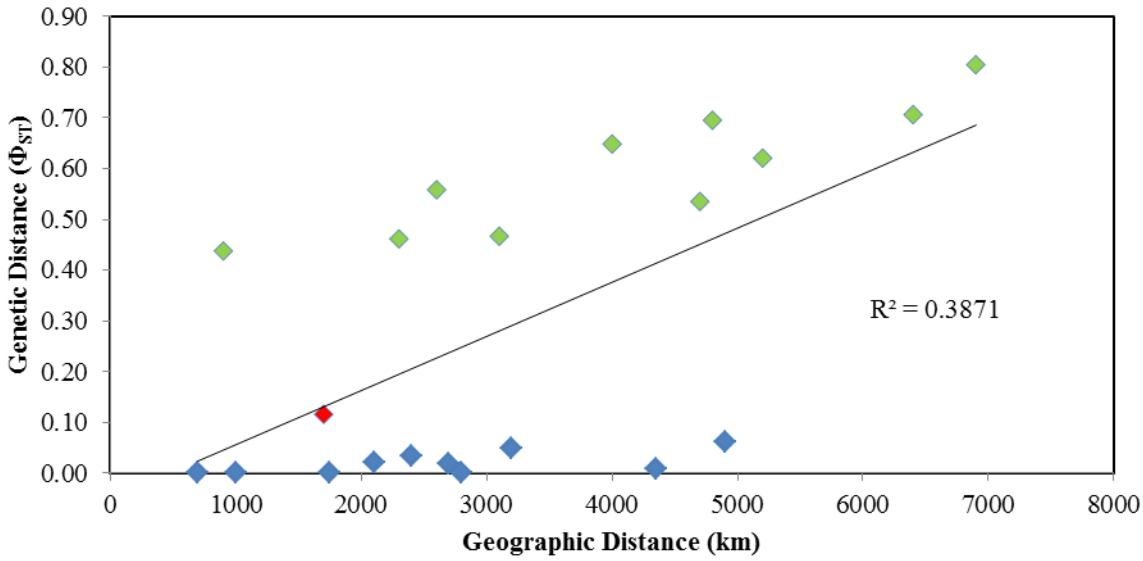

Figure 2.13: Mantel test depicting relationship between geographic (x-axis), and genetic (y-axis) distance for the z-linked locus. R^2 shows a significant relationship (p=0.01). Green markers are comparisons between subspecies and blue and red markers are comparisons within *C. p. purpureus* and *C. p. californicus* respectively.

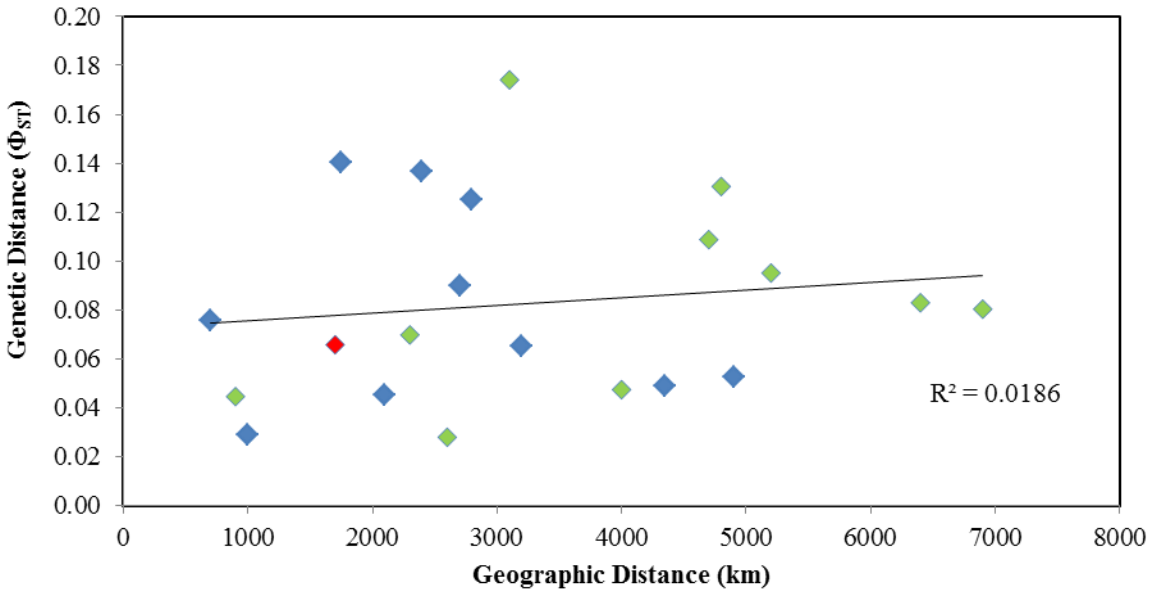

Figure 2.14: Mantel test depicting relationship between geographic (x-axis), and genetic (y-axis) distance for the seven microsatellite loci. R^2 shows no correlation. Green markers are comparisons between subspecies and blue and red markers are comparisons within *C. p. purpureus* and *C. p. californicus* respectively.

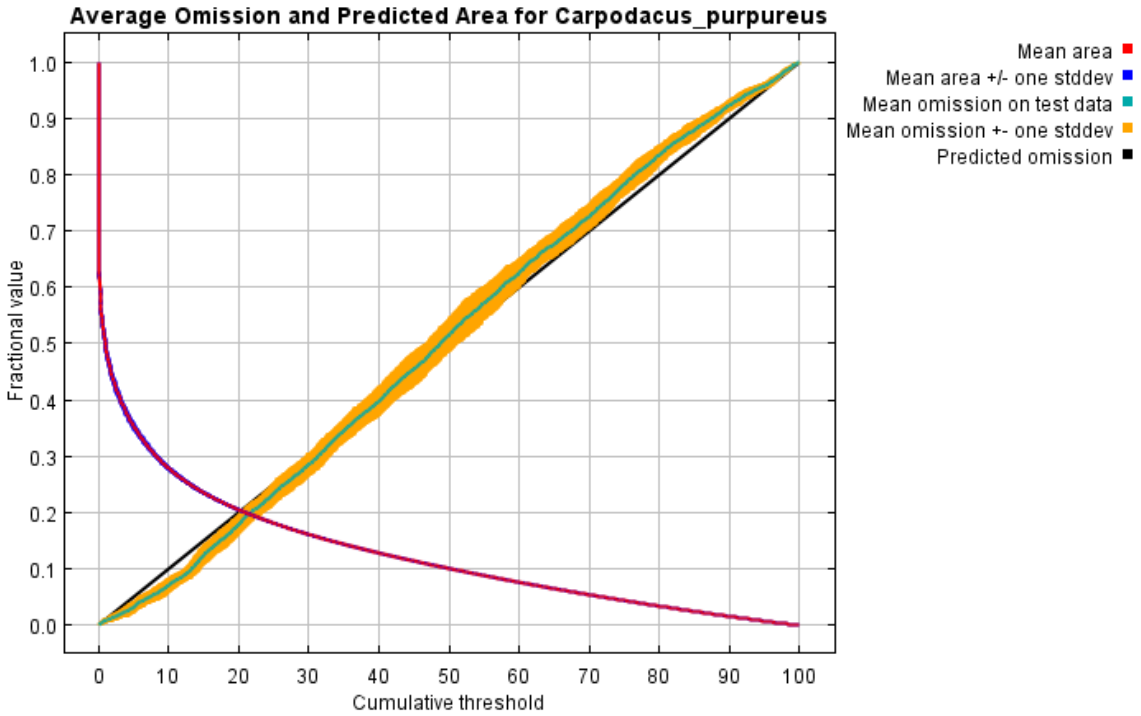

Figure 2.15: Comparison of predicted and test omission rates. The turquoise line represents the test omission rate, the yellow on either side of the test omission rate is +/- 1 standard deviation, and the black line is the predicted omission rate.

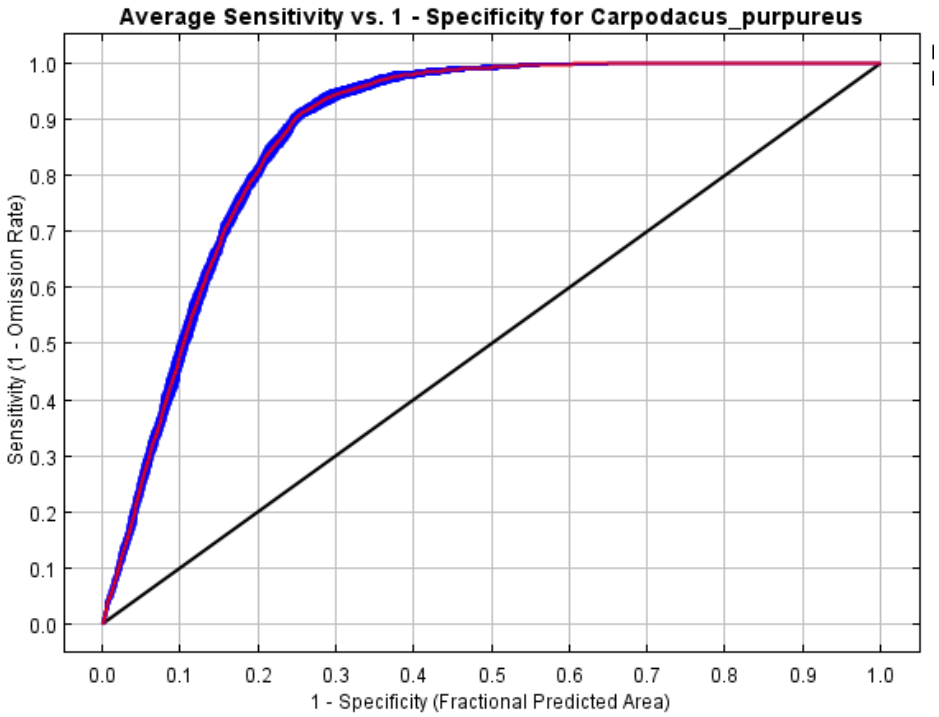

Figure 2.16: This figure depicts the mean Area Under the Curve (AUC) in red (0.874), while the blue represents the mean AUC ± one standard deviation. A random prediction would have the AUC represented by the black line.

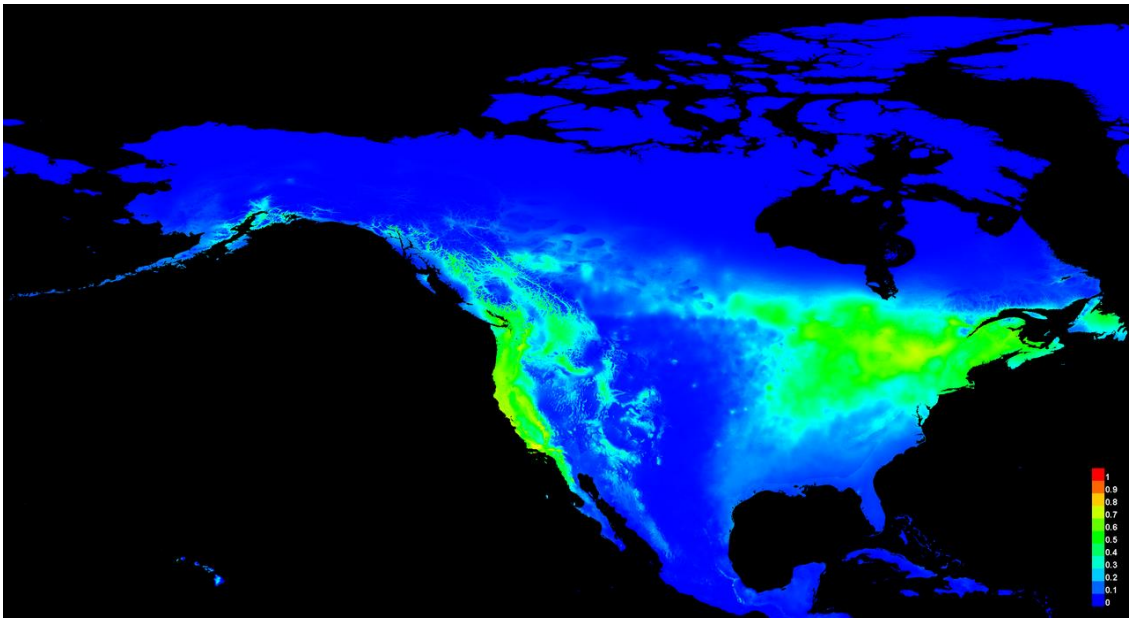

Figure 2.17: Estimation of purple finch current distribution based on suitable climatic conditions using nine current climate models and the UTMs from 5,400 occurrence values.

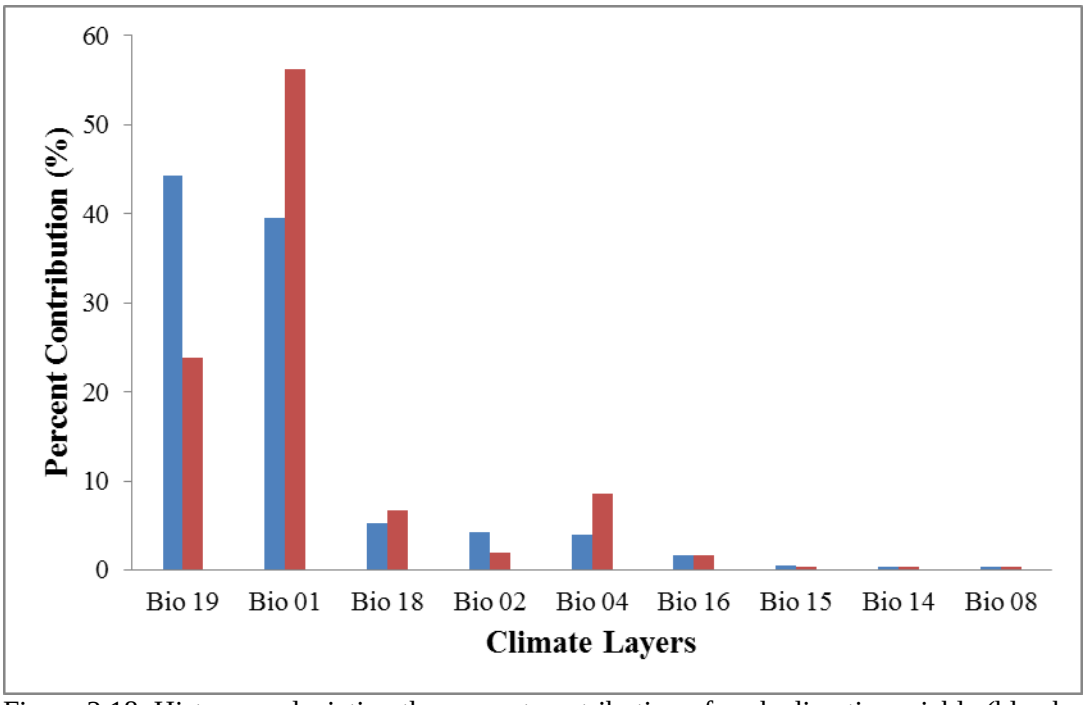

Figure 2.18: Histogram depicting the percent contribution of each climatic variable (blue bars), and the importance of each variable- as measured by the decrease in predictability when that variable is removed (red bars). The same layers contributed and were similarly important for both predicting the current distribution and the distribution of 21,000 years ago.

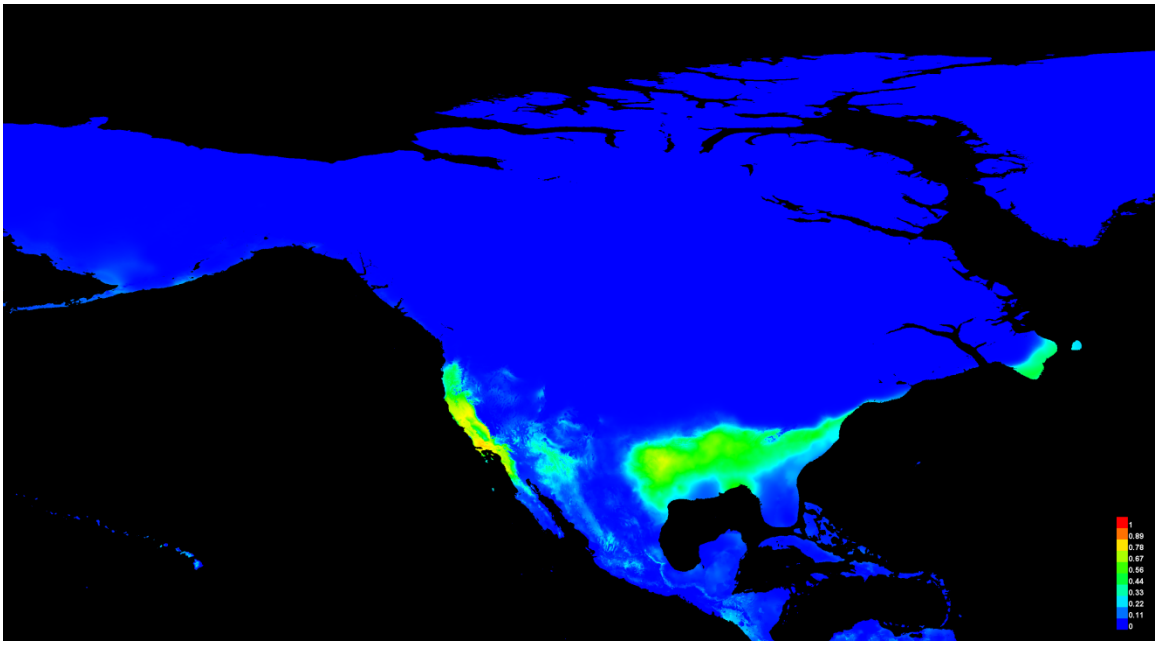

Fig.2.19: Projection of suitable climatic conditions for purple finch from 21,000 years ago, during the LGM. Areas of suitable conditions are in light blue and green and is based on trained data from 5,400 presence occurences, nine uncorelated climate layers and the MIROC-LJP Global Climate Model.

REFERENCES

Apte, S., Smith, P. J., & Wallis, G. P. (2007). Mitochondrial phylogeography of New Zealand freshwater crayfishes, *Paranephrops* spp. *Molecular Ecology, 16*(9), 1897-1908. doi: 10.1111/j.1365-294X.2007.03224.x

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., & Saunders, N. C. (1987). Intraspecific phylogeography - the mitochondrial-DNA bridge between population-genetics and systematics. *Annual Review of Ecology and Systematics, 18*, 489-522. doi: 10.1146/annurev.ecolsys.18.1.489

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological, 57*(1), 289-300.

Benjamini, Y., & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics, 29*(4), 1165-1188.

Borras, A., Cabrera, J., Colome, X., Cabrera, T., & Senar, J. C. (2011). Patterns of connectivity in citril finches *Serinus citrinella*: sympatric wintering of allopatric breeding birds? *Bird Study, 58*(3), 257-263. doi: 10.1080/00063657.2011.587107

Brady, E. C., Otto-Bliesner, B. L., Kay, J. E., & Rosenbloom, N. (2013). Sensitivity to glacial forcing in the CCSM4. *Journal of Climate, 26*(6), 1901-1925. doi: 10.1175/jcli-d-11-00416.1

Brown, R. P., Campos-Delgado, R., & Pestano, A. (2000). Mitochondrial DNA evolution and population history of the Tenerife skink *Chalcides viridanus. Molecular Ecology, 9*(8), 1061-1067. doi: 10.1046/j.1365-294x.2000.00962.x

Brubaker, L. B., Anderson, P. M., Edwards, M. E., & Lozhkin, A. V. (2005). Beringia as a glacial refugium for boreal trees and shrubs: new perspectives from mapped pollen data. *Journal of Biogeography, 32*(5), 833-848. doi: 10.1111/j.1365-2699.2004.01203.x

Burg, T. M., Gaston, A. J., Winker, K., & Friesen, V. L. (2005). Rapid divergence and postglacial colonization in western North American Steller's jays (*Cyanocitta stelleri*). *Molecular Ecology, 14*(12), 3745-3755. doi: 10.1111/j.1365-294X.2005.02710.x

Burg, T. M., Gaston, A. J., Winker, K., & Friesen, V. L. (2006). Effects of Pleistocene glaciations on population structure of North American chestnut-backed chickadees. *Molecular Ecology, 15*(9), 2409-2419. doi: 10.1111/j.1365-294X.2006.02957.x

Burleigh, T. D. a. H. S. P. (1948). Geographic variation in Newfoundland birds. *Proceedings of the Biological Society of Washington, 61*, 111-126.

Bush, K. L., Dyte, C. K., Moynahan, B. J., Aldridge, C. L., Sauls, H. S., Battazzo, A. M., Walker, B. L., Doherty, K. E., Tack, J., Carlson, J., Eslinger, D., Nicholson, J., Boyce, M. S., Naugle, D. E., Paszkowski, C. A., & Coltman, D. W. (2011). Population structure and genetic diversity of greater sage-grouse (*Centrocercus urophasianus*) in fragmented

landscapes at the northern edge of their range. *Conservation Genetics, 12*(2), 527-542. doi: 10.1007/s10592-010-0159-8

Byun, S. A., Koop, B. F., & Reimchen, T. E. (1997). North American black bear mtDNA phylogeography: Implications for morphology and the Haida Gwaii glacial refugium controversy. *Evolution, 51*(5), 1647-1653. doi: 10.2307/2411216

Carmichael, L. E., Nagy, J. A., Larter, N. C., & Strobeck, C. (2001). Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. *Molecular Ecology, 10*(12), 2787-2798. doi: 10.1046/j.0962-1083.2001.01408.x

Carson, H. L., Lockwood, J. P., & Craddock, E. M. (1990). Extinction and recolonization of local-populations on a growing shield-volcano. *Proceedings of the National Academy of Sciences of the United States of America, 87*(18), 7055-7057. doi: 10.1073/pnas.87.18.7055

Castoe, T. A., de Koning, A. P. J., Kim, H. M., Gu, W. J., Noonan, B. P., Naylor, G., Jiang, Z. J., Parkinson, C. L., & Pollock, D. D. (2009). Evidence for an ancient adaptive episode of convergent molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America, 106*(22), 8986-8991. doi: 10.1073/pnas.0900233106

Clark, R. W., Brown, W. S., Stechert, R., & Zamudio, K. R. (2010). Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. *Conservation Biology, 24*(4), 1059-1069. doi: 10.1111/j.1523-1739.2009.01439.x

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology, 9*(10), 1657-1659. doi: 10.1046/j.1365-294x.2000.01020.x

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

Cook, O. F. (1906). Factors of species formation. *Science, 23*, 506-507. doi: 10.1126/science.23.587.506

Cooper, A., & Cooper, R. A. (1995). The oligocene bottleneck and New-Zealand biota - genetic record of a past environmental crisis. *Proceedings of the Royal Society B-Biological Sciences, 261*(1362), 293-302. doi: 10.1098/rspb.1995.0150

Corander, J., Waldmann, P., & Sillanpaa, M. J. (2003). Bayesian analysis of genetic differentiation between populations. *Genetics, 163*(1), 367-374.

Ding, L., Gan, X. N., He, S. P., & Zhao, E. M. (2011). A phylogeographic, demographic and historical analysis of the short-tailed pit viper (*Gloydius brevicaudus*): evidence for early divergence and late expansion during the Pleistocene. *Molecular Ecology, 20*(9), 1905-1922. doi: 10.1111/j.1365-294X.2011.05060.x

Duvall, A. (1945). Variation in *Carpodacus purpureus* and *C. cassinii. Condor, 47*, 202-205.

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. doi: 10.1007/s12686-011-9548-7

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology, 17*(5), 1170-1188. doi: 10.1111/j.1365-294X.2007.03659.x

Encalada, S. E., Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Miyamoto, M. M., & Bowen, B. W. (1996). Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: A mitochondrial DNA control region sequence assessment. *Molecular Ecology, 5*(4), 473-483.

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. doi: 10.1111/j.1365-2664.2007.01325.x

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology, 14*(8), 2611-2620. doi: 10.1111/j.1365-294X.2005.02553.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. doi: 10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes - application to human mitochondrial-DNA restriction data. *Genetics, 131*(2), 479-491.

Eyre-Walker, A., & Keightley, P. D. (2007). The distribution of fitness effects of new mutations. *Nature Reviews Genetics, 8*(8), 610-618. doi: 10.1038/nrg2146

Flagstad, O., & Roed, K. H. (2003). Refugial origins of reindeer (*Rangifer tarandus L.*) inferred from mitochondrial DNA sequences. *Evolution, 57*(3), 658-670.

Fleischer, R. C., McIntosh, C. E., & Tarr, C. L. (1998). Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Molecular Ecology, 7*(4), 533-545. doi: 10.1046/j.1365-294x.1998.00364.x

Freeland, J. R., Kirk H., Petersen, S. D. (2011). *Molecular Ecology* (2nd ed.). West Sussex, , UK: Wiley-Blackwell.

Friesen, V. L., Burg, T. M., & McCoy, K. D. (2007). Mechanisms of population differentiation in seabirds. *Molecular Ecology, 16*(9), 1765-1785. doi: 10.1111/j.1365-294X.2006.03197.x

Fritz, U., Barata, M., Busack, S. D., Fritzsch, G., & Castilho, R. (2006). Impact of mountain chains, sea straits and peripheral populations on genetic and taxonomic structure of a freshwater turtle, *Mauremys leprosa* (Reptilia, Testudines, Geoemydidae). *Zoologica Scripta, 35*(1), 97-108. doi: 10.1111/j.1463-6409.2005.00218.x

Galtier, N., Nabholz, B., Glemin, S., & Hurst, G. D. D. (2009). Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology, 18*(22), 4541-4550. doi: 10.1111/j.1365-294X.2009.04380.x

Gantenbein, B., Fet, V., Gantenbein-Ritter, I. A., & Balloux, F. (2005). Evidence for recombination in scorpion mitochondrial DNA (*Scorpiones : Buthidae*). *Proceedings of the Royal Society B-Biological Sciences, 272*(1564), 697-704. doi: 10.1098/rspb.2004.3017

Georges, A., Zhang, X. W., Unmack, P., Reid, B. N., Le, M., & McCord, W. P. (2014). Contemporary genetic structure of an endemic freshwater turtle reflects Miocene orogenesis of New Guinea. *Biological Journal of the Linnean Society, 111*(1), 192-208. doi: 10.1111/bij.12176

Gibbs, H. L., Tabak, L. M., & Hobson, K. (1999). Characterization of microsatellite DNA loci for a neotropical migrant songbird, the Swainson's thrush (*Catharus ustulatus*). *Molecular Ecology, 8*(9), 1551-1552.

Gleason, J. M., Jallon, J. M., Rouault, J. D., & Ritchie, M. G. (2005). Quantitative trait loci for cuticular hydrocarbons associated with sexual isolation between *Drosophila simulans* and *D. sechellia*. *Genetics, 171*(4), 1789-1798. doi: 10.1534/genetics.104.037937

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

Grus, W. E., Graves, G. R., & Glenn, T. C. (2009). Geographic variation in the mitochondrial control region of black-throated blue warblers (*Dendroica caerulescens*). *Auk, 126*(1), 198-210. doi: 10.1525/auk.2009.07159

Hale, M. L., Burg, T. M., & Steeves, T. E. (2012). Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. *Plos One, 7*(9), 10. doi: 10.1371/journal.pone.0045170

Hartmann, S. A., Steyer, K., Kraus, R. H. S., Segelbacher, G., & Nowak, C. (2013). Potential barriers to gene flow in the endangered European wildcat (*Felis silvestris*). *Conservation Genetics, 14*(2), 413-426. doi: 10.1007/s10592-013-0468-9

Helvaci, Z., Renaud, S., Ledevin, R., Adriaens, D., Michaux, J., Colak, R., Kankilic, T., Kandemir, I., Yigit, N., & Colak, E. (2012). Morphometric and genetic structure of the edible dormouse (*Glis glis*): a consequence of forest fragmentation in Turkey. *Biological Journal of the Linnean Society, 107*(3), 611-623. doi: 10.1111/j.1095-8312.2012.01952.x

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. doi: 10.1111/j.1095-8312.1996.tb01434.x

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

Hill, G. E. (1993). Geographic-variation in the carotenoid plumage pigmentation of male house finches (*Carpodacus-mexicanus*). *Biological Journal of the Linnean Society, 49*(1), 63-86. doi: 10.1111/j.1095-8312.1993.tb00685.x

Hindley, J. A. (2013). *Post-Pleistocene dispersal in chickadees.* (Ph.D), University of Lethbridge.

Hoarau, G., Holla, S., Lescasse, R., Stam, W. T., & Olsen, J. L. (2002). Heteroplasmy and evidence for recombination in the mitochondrial control region of the flatfish *Platichthys flesus*. *Molecular Biology and Evolution, 19*(12), 2261-2264.

Hofreiter, M., Serre, D., Rohland, N., Rabeder, G., Nagel, D., Conard, N., Munzel, S., & Paabo, S. (2004). Lack of phylogeography in European mammals before the last glaciation. *Proceedings of the National Academy of Sciences of the United States of America, 101*(35), 12963-12968. doi: 10.1073/pnas.0403618101

Holder, K., Montgomerie, R., & Friesen, V. L. (2004). Genetic diversity and management of Nearctic rock ptarmigan (*Lagopus mutus*). *Canadian Journal of Zoology-Revue Canadienne De Zoologie, 82*(4), 564-575. doi: 10.1139/z04-018

Holderegger, R., & Thiel-Egenter, C. (2009). A discussion of different types of glacial refugia used in mountain biogeography and phylogeography. *Journal of Biogeography, 36*(3), 476-480. doi: 10.1111/j.1365-2699.2008.02027.x

Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources, 9*(5), 1322-1332. doi: 10.1111/j.1755-0998.2009.02591.x

James, D. A. & Neal, J. C. (1986). *Arkansas birds: their distribution and abundance*. Fayetteville: Univ. of Arkansas Press.

Jaramillo-Correa, J. P., Beaulieu, J., Khasa, D. P., & Bousquet, J. (2009). Inferring the past from the present phylogeographic structure of North American forest trees: seeing the forest for the genes. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 39*(2), 286-307. doi: 10.1139/x08-181

Jiggins, C. D., Linares, M., Naisbit, R. E., Salazar, C., Yang, Z. H., & Mallet, J. (2001). Sex-linked hybrid sterility in a butterfly. *Evolution, 55*(8), 1631-1638.

Johansen, A. D., & Latta, R. G. (2003). Mitochondrial haplotype distribution, seed dispersal and patterns of postglacial expansion of ponderosa pine. *Molecular Ecology, 12*(1), 293-298. doi: 10.1046/j.1365-294X.2003.01723.x

Kawecki, T. J. (1997). Sympatric speciation via habitat specialization driven by deleterious mutations. *Evolution, 51*(6), 1751-1763. doi: 10.2307/2410998

Khidas, K., Duhaime, J., & Huynh, H. M. (2013). Morphological divergence of continental and island populations of Canada lynx. *Northeastern Naturalist, 20*(4), 587-608. doi: 10.1656/045.020.0413

Kimura, M., Clegg, S. M., Lovette, I. J., Holder, K. R., Girman, D. J., Mila, B., Wade, P., & Smith, T. B. (2002). Phylogeographical approaches to assessing demographic connectivity between breeding and overwintering regions in a Nearctic-Neotropical warbler (*Wilsonia pusilla*). *Molecular Ecology, 11*(9), 1605-1616. doi: 10.1046/j.1365-294X.2002.01551.x

Kimura, M., & Crow, J. F. (1964). Number of alleles that can be maintained in finite population. *Genetics, 49*(4), 725-738.

Kimura, M., & Ohta, T. (1978). Stepwise mutation model and distribution of allelic frequencies in a finite population. *Proceedings of the National Academy of Sciences of the United States of America, 75*(6), 2868-2872. doi: 10.1073/pnas.75.6.2868

Klicka, J., & Zink, R. M. (1999). Pleistocene effects on North American songbird evolution. *Proceedings of the Royal Society B-Biological Sciences, 266*(1420), 695-700.

Kociolek, A. V., Clevenger, A. P., Clair, C. C. S., & Proppe, D. S. (2011). Effects of road networks on bird populations. *Conservation Biology, 25*(2), 241-249. doi: 10.1111/j.1523-1739.2010.01635.x

Krebes, L., Blank, M., Jurss, K., Zettler, M. L., & Bastrop, R. (2010). Glacial-driven vicariance in the amphipod *Gammarus duebeni*. *Molecular Phylogenetics and Evolution, 54*(2), 372-385. doi: 10.1016/j.ympev.2009.07.034

Lait, L. A. (2011). *Phylogeography of the boreal chickadee.* (Master Science), University of Lethbridge.

Lait, L. A., & Burg, T. M. (2013). When east meets west: population structure of a high-latitude resident species, the boreal chickadee (*Poecile hudsonicus*). *Heredity, 111*(4), 321-329. doi: 10.1038/hdy.2013.54

Li, P., Li, M. M., Shi, Y., Zhao, Y. P., Wan, Y., Fu, C. X., & Cameron, K. M. (2013). Phylogeography of north american herbaceous *smilax* (smilacaceae): Combined AFLP and cpDNA data support a northern refugium in the driftless area. *American Journal of Botany, 100*(4), 801-814. doi: 10.3732/ajb.1200250

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

Liebers, D., Helbig, A. J., & De Knijff, P. (2001). Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* group (Aves : Charadriiformes). *Molecular Ecology, 10*(10), 2447-2462. doi: 10.1046/j.0962-1083.2001.01370.x

Liimatainen, J. O., & Jallon, J. M. (2007). Genetic analysis of cuticular hydrocarbons and their effect on courtship in *Drosophila virilis* and *D. lummei*. *Behavior Genetics, 37*(5), 713-725. doi: 10.1007/s10519-007-9158-z

Loehr, J., Worley, K., Grapputo, A., Carey, J., Veitch, A., & Coltman, D. W. (2006). Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. *Journal of Evolutionary Biology, 19*(2), 419-430. doi: 10.1111/j.1420-9101.2005.01027.x

Lovette, I. J., Clegg, S. M., & Smith, T. B. (2004). Limited utility of mtDNA markers for determining connectivity among breeding and overwintering locations in three neotropical migrant birds. *Conservation Biology, 18*(1), 156-166. doi: 10.1111/j.1523-1739.2004.00239.x

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. doi: 10.1111/j.0014-3820.2001.tb00769.x

Magee, M. J. (1924). Notes on the Purple Finch (*Carpodacus purpureus purpureus*). *Auk, 41*(4), 606-610.

Manni, F., Guerard, E., & Heyer, E. (2004). Geographic patterns of (genetic, morphologic, linguistic) variation: How barriers can be detected by using Monmonier's algorithm. *Human Biology, 76*(2), 173-190. doi: 10.1353/hub.2004.0034

Markert, J. A., Danley, P. D., & Arnegard, M. E. (2001). New markers for new species: microsatellite loci and the East African cichlids. *Trends in Ecology & Evolution, 16*(2), 100-107. doi: 10.1016/s0169-5347(00)02064-4

Marr, K. L., Allen, G. A., Hebda, R. J., & McCormick, L. J. (2013). Phylogeographical patterns in the widespread Arctic-alpine plant *Bistorta vivipara* (Polygonaceae) with emphasis on western North America. *Journal of Biogeography, 40*(5), 847-856. doi: 10.1111/jbi.12042

Marten, J. A., & Johnson, N. K. (1986). Genetic-relationships of North-American cardueline finches. *Condor, 88*(4), 409-420. doi: 10.2307/1368266

Mercure, A., Ralls, K., Koepfli, K. P., & Wayne, R. K. (1993). Genetic subdivisions among small canids - mitochondrial-DNA differentiation of swift, kit, and arctic foxes. *Evolution, 47*(5), 1313-1328. doi: 10.2307/2410150

Miao, Y. C., Lang, X. D., Zhang, Z. Z., & Su, J. R. (2014). Phylogeography and genetic effects of habitat fragmentation on endangered *Taxus yunnanensi*s in southwest China as revealed by microsatellite data. *Plant Biology, 16*(2), 365-374. doi: 10.1111/plb.12059

Miller, L. M., Kallemeyn, L., & Senanan, W. (2001). Spawning-site and natal-site fidelity by northern pike in a large lake: Mark-recapture and genetic evidence. *Transactions of the American Fisheries Society, 130*(2), 307-316. doi: 10.1577/1548-8659(2001)130<0307:ssansf>2.0.co;2

Milot, E., Gibbs, H. L., & Hobson, K. A. (2000). Phylogeography and genetic structure of northern populations of the yellow warbler (*Dendroica petechia*). *Molecular Ecology, 9*(6), 667-681. doi: 10.1046/j.1365-294x.2000.00897.x

Mindell, D. P., Sorenson, M. D., & Dimcheff, D. E. (1998). Multiple independent origins of mitochondrial gene order in birds. *Proceedings of the National Academy of Sciences of the United States of America, 95*(18), 10693-10697. doi: 10.1073/pnas.95.18.10693

Moehring, A. J., Li, J. A., Schug, M. D., Smith, S. G., deAngelis, M., Mackay, T. F., & Coyne, J. A. (2004). Quantitative trait loci for sexual isolation between *Drosophila simulans* and *D. mauritiana*. *Genetics, 167*(3), 1265-1274. doi: 10.1534/genetics.103.024364

Monmonier, M. S. (1973). Maximum-Difference Barrier: An Alternative Numerical Regionalization Method. . *Geographical Analysis, 5*, 245-261.

Morris, A. B., Graham, C. H., Soltis, D. E., & Soltis, P. S. (2010). Reassessment of phylogeographical structure in an eastern North American tree using Monmonier's algorithm and ecological niche modelling. *Journal of Biogeography, 37*(9), 1657-1667. doi: 10.1111/j.1365-2699.2010.02315.x

Nabholz, B., Glemin, S., & Galtier, N. (2008). Strong variations of mitochondrial mutation rate across mammals - the longevity hypothesis. *Molecular Biology and Evolution, 25*(1), 120-130. doi: 10.1093/molbev/msm248

Nabholz, B., Glemin, 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*, 13. doi: 10.1186/1471-2148-9-54

Naisbit, R. E., Jiggins, C. D., Linares, M., Salazar, C., & Mallet, J. (2002). Hybrid sterility, Haldane's rule and speciation in *Heliconius cydno* and *H. melpomene*. *Genetics, 161*(4), 1517-1526.

Narum, S. R. (2006). Beyond Bonferroni: Less conservative analyses for conservation genetics. *Conservation Genetics, 7*(5), 783-787. doi: 10.1007/s10592-005-9056-y

Nei, M., & Li, W. H. (1979). Mathematical-model for studying genetic-variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America, 76*(10), 5269-5273. doi: 10.1073/pnas.76.10.5269

Nei, M., & Tajima, F. (1981). DNA polymorphism detectable by restriction endonucleases. *Genetics, 97*(1), 145-163.

O'Ishi, R., & Abe-Ouchi, A. (2013). Influence of dynamic vegetation on climate change and terrestrial carbon storage in the Last Glacial Maximum. *Climate of the Past, 9*(4), 1571-1587. doi: 10.5194/cp-9-1571-2013

Otter, K., Ratcliffe, L., Michaud, D., & Boag, P. T. (1998). Do female black-capped chickadees prefer high-ranking males as extra-pair partners? *Behavioral Ecology and Sociobiology, 43*(1), 25-36. doi: 10.1007/s002650050463

Peakall, R. O. D., & Smouse, P. E. (2006). genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes, 6*(1), 288-295. doi: 10.1111/j.1471-8286.2005.01155.x

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling, 190*(3-4), 231-259. doi: 10.1016/j.ecolmodel.2005.03.026

Pielou, E. C. (1991). *After the Ice Age: The Return of Life to Glaciated North America* (Vol. 1991:19). Chicago: University of Chicago Press.

Polakova, R., Vyskocilova, M., Martin, J. F., Mays, H. L., Hill, G. E., Bryja, J., & Albrecht, T. (2007). A multiplex set of microsatellite markers for the scarlet rosefinch (*Carpodacus erythrinus*). *Molecular Ecology Notes, 7*(6), 1375-1378. doi: 10.1111/j.1471-8286.2007.01892.x

Poulakakis, N., Russello, M., Geist, D., & Caccone, A. (2012). Unravelling the peculiarities of island life: vicariance, dispersal and the diversification of the extinct and extant giant Galapagos tortoises. *Molecular Ecology, 21*(1), 160-173. doi: 10.1111/j.1365-294X.2011.05370.x

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Pulliam, H. R. (2000). On the relationship between niche and distribution. *Ecology Letters, 3*(4), 349-361. doi: 10.1046/j.1461-0248.2000.00143.x

Qvarnstrom, A., & Bailey, R. I. (2009). Speciation through evolution of sex-linked genes. *Heredity, 102*(1), 4-15. doi: 10.1038/hdy.2008.93

Qvarnstrom, A., Rice, A. M., & Ellegren, H. (2010). Speciation in *Ficedula* flycatchers. *Philosophical Transactions of the Royal Society B-Biological Sciences, 365*(1547), 1841-1852. doi: 10.1098/rstb.2009.0306

Ralston, J., & Kirchman, J. J. (2012). Continent-scale genetic structure in a boreal forest migrant, the blackpoll warbler (*Setophaga striata*). *Auk, 129*(3), 467-478. doi: 10.1525/auk.2012.11260

Raymond, M., & Rousset, F. (1995). GENEPOP (version 1.2) - Population-genetics software for exact tests and ecumenicism. *Journal of Heredity, 86*(3), 248-249.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution, 43*(1), 223-225. doi: 10.2307/2409177

Richards, C. L., Carstens, B. C., & Knowles, L. L. (2007). Distribution modelling and statistical phylogeography: an integrative framework for generating and testing alternative biogeographical hypotheses. *Journal of Biogeography, 34*(11), 1833-1845. doi: 10.1111/j.1365-2699.2007.01814.x

Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J., & Burke, T. (2000). Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology, 9*(12), 2226-2231.

Riley, S. P. D., Pollinger, J. P., Sauvajot, R. M., York, E. C., Bromley, C., Fuller, T. K., & Wayne, R. K. (2006). A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology, 15*(7), 1733-1741. doi: 10.1111/j.1365-294X.2006.02907.x

Rousset, F. (2008). GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources, 8*(1), 103-106. doi: 10.1111/j.1471-8286.2007.01931.x

Rozas, J., & Rozas, R. (1999). DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics, 15*(2), 174-175. doi: 10.1093/bioinformatics/15.2.174

Rozas, J., Sanchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics, 19*(18), 2496-2497. doi: 10.1093/bioinformatics/btg359

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 B-Biological Sciences, 269*(1498), 1375-1381. doi: 10.1098/rspb.2002.2032

Rueness, E. K., Stenseth, N. C., O'Donoghue, M., Boutin, S., Ellegren, H., & Jakobsen, K. S. (2003). Ecological and genetic spatial structuring in the Canadian lynx. *Nature, 425*(6953), 69-72. doi: 10.1038/nature01942

Ruzzante, D. E., Walde, S. J., Cussac, V. E., Dalebout, M. L., Seibert, J., Ortubay, S., & Habit, E. (2006). Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism. *Molecular Ecology, 15*(10), 2949-2968. doi: 10.1111/j.1365-294X.2006.03010.x

Saether, S. A., Saetre, G. P., Borge, T., Wiley, C., Svedin, N., Andersson, G., Veen, T., Haavie, J., Servedio, M. R., Bures, S., Kral, M., Hjernquist, M. B., Gustafsson, L., Traff, J., & Qvarnstrom, A. (2007). Sex chromosome-linked species recognition and evolution of reproductive isolation in flycatchers. *Science, 318*(5847), 95-97. doi: 10.1126/science.1141506

Saetre, G. P., Borge, T., Lindroos, K., Haavie, J., Sheldon, B. C., Primmer, C., & Syvanen, A. C. (2003). Sex chromosome evolution and speciation in *Ficedula* flycatchers. *Proceedings of the Royal Society B-Biological Sciences, 270*(1510), 53-59. doi: 10.1098/rspb.2002.2204

Salt, G. W. (1952). The relation of metabolism to climate and distribution in 3 finches of the genus *Carpodacus*. *Ecological Monographs, 22*(2), 121-152. doi: 10.2307/1943514

Sanmartin, I., & Ronquist, F. (2004). Southern Hemisphere biogeography inferred by event-based models: Plant versus animal patterns. *Systematic Biology, 53*(2), 216-243. doi: 10.1080/10635150490423430

Scribner, K. T., Petersen, M. R., Fields, R. L., Talbot, S. L., Pearce, J. M., & Chesser, R. K. (2001). Sex-biased gene flow in spectacled eiders (Anatidae): Inferences from molecular markers with contrasting modes of inheritance. *Evolution, 55*(10), 2105-2115.

Shafer, A. B. A., Cote, S. D., & Coltman, D. W. (2011). Hot spots of genetic diversity descended from multiple Pleistocene refugia in an alpine ungulate. *Evolution, 65*(1), 125-138. doi: 10.1111/j.1558-5646.2010.01109.x

Sibley, D. A. (2000). *The Sibley Guide to Birds*: Alfred A. Knopf.

Sibley, D. A. (2011, March 24th, 2011). Distinguishing the subspecies of Purple Finch. Retrieved June 30 2014, from http://www.sibleyguides.com/2011/03/distinguishing-the-subspecies-of-purple-finch/

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. doi: 10.1007/bf00987957

Sonsthagen, S. A., Rosenfield, R. N., Bielefeldt, J., Murphy, R. K., Stewart, A. C., Stout, W. E., Driscoll, T. G., Bozek, M. A., Sloss, B. L., & Talbot, S. L. (2012). Genetic and morphological divergence among Cooper's hawk (*Accipiter cooperii*) populations breeding in north-central and western North America. *Auk, 129*(3), 427-437. doi: 10.1525/auk.2012.11166

Sonsthagen, S. A., Talbot, S. L., Scribner, K. T., & McCracken, K. G. (2011). Multilocus phylogeography and population structure of common eiders breeding in North America and Scandinavia. *Journal of Biogeography, 38*(7), 1368-1380. doi: 10.1111/j.1365-2699.2011.02492.x

Spellman, G. M., Riddle, B., & Klicka, J. (2007). Phylogeography of the mountain chickadee (*Poecile gambeli*): diversification, introgression, and expansion in response to Quaternary climate change. *Molecular Ecology, 16*(5), 1055-1068. doi: 10.1111/j.1365-294X.2006.03199.x

Stephens, M., & Donnelly, P. (2003). A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics, 73*(5), 1162-1169. doi: 10.1086/379378

Svedin, N., Wiley, C., Veen, T., Gustafsson, L., & Qvarnstrom, A. (2008). Natural and sexual selection against hybrid flycatchers. *Proceedings of the Royal Society B-Biological Sciences, 275*(1635), 735-744. doi: 10.1098/rspb.2007.0967

Tarvin, K. A. (2006). Polymorphic microsatellite loci from the American goldfinch (*Carduelis tristis*) and their cross-amplification in a variety of passerine species. *Molecular Ecology Notes, 6*(2), 470-472. doi: 10.1111/j.1471-8286.2006.01277.x

Ujvari, B., Dowton, M., & Madsen, T. (2007). Mitochondrial DNA recombination in a free-ranging Australian lizard. *Biology Letters, 3*(2), 189-192. doi: 10.1098/rsbl.2006.0587

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes, 4*(3), 535-538. doi: 10.1111/j.1471-8286.2004.00684.x

VanderWerf, E. A., Young, L. C., Yeung, N. W., & Carlon, D. B. (2010). Stepping stone speciation in Hawaii's flycatchers: molecular divergence supports new island endemics within the elepaio. *Conservation Genetics, 11*(4), 1283-1298. doi: 10.1007/s10592-009-9958-1

Via, S. (2001). Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology & Evolution, 16*(7), 381-390. doi: 10.1016/s0169-5347(01)02188-7

Vos, C. C., Antonisse-De Jong, A. G., Goedhart, P. W., & Smulders, M. J. M. (2001). Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity, 86,* 598-608. doi: 10.1046/j.1365-2540.2001.00865.x

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

Walter, R., & Epperson, B. K. (2005). Geographic pattern of genetic diversity in *Pinus resinosa*: Contact zone between descendants of glacial refugia. *American Journal of Botany, 92*(1), 92-100. doi: 10.3732/ajb.92.1.92

Wang, M. T., Hsu, Y. C., Yao, C. T., & Li, S. H. (2005). Isolation and characterization of 12 tetranucleotide repeat microsatellite loci from the green-backed tit (*Parus monticolus*). *Molecular Ecology Notes, 5*(2), 439-442. doi: 10.1111/j.1471-8286.2005.00955.x

Wang, W. J., Dai, C. Y., Alstrom, P., Zhang, C. L., Qu, Y. H., Li, S. H., Yang, X. J., Zhao, N., Song, G., & Lei, F. M. (2014). Past hybridization between two East Asian long-tailed tits (*Aegithalos bonvaloti* and *A. fuliginosus*). *Frontiers in Zoology, 11,* 13. doi: 10.1186/1742-9994-11-40

Warren, D. L., Glor, R. E., & Turelli, M. (2008). Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution, 62*(11), 2868-2883. doi: 10.1111/j.1558-5646.2008.00482.x

Weir, J. T., & Schluter, D. (2004). Ice sheets promote speciation in boreal birds. *Proceedings of the Royal Society B-Biological Sciences, 271*(1551), 1881-1887. doi: 10.1098/rspb.2004.2803

Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Gyllensten, U. B., Helmbychowski, K. M., Higuchi, R. G., Palumbi, S. R., Prager, E. M., Sage, R. D., & Stoneking, M. (1985). Mitochondrial-DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society, 26*(4), 375-400. doi: 10.1111/j.1095-8312.1985.tb02048.x

Wilson, H. W., & Brown, B. (2012). Fidelity and interseasonal movements of purple finches (*Carpodacus purpureus* (Gmelin)): Analysis of band re-encounter data. *The Open Ornithology Journal, 5*, 61-72.

Witt, J. D. S., Zemlak, R. J., & Taylor, E. B. (2011). Phylogeography and the origins of range disjunctions in a north temperate fish, the pygmy whitefish (*Prosopium coulterii*), inferred from mitochondrial and nuclear DNA sequence analysis. *Journal of Biogeography, 38*(8), 1557-1569. doi: 10.1111/j.1365-2699.2011.02497.x

Wootton, J. T. (1996). Purple Finch (*Haemorhous purpureus*). *The Birds of North America Online.* Retrieved February 2, 2014, from http://bna.birds.cornell.edu/bna/species/208

Wright, S. (1965). The interpretation of population-structure by f-statistics with special regard to systems of mating. *Evolution, 19*(3), 395-420. doi: 10.2307/2406450

Yunick, R. P. (1983). Winter site fidelity of some northern finches (fringillidae). *Journal of Field Ornithology, 54*(3), 254-258.

Yunick, R. P. (1987). Seven multiple-recapture encounters with banded birds. *North American Bird Bander, 12*, 60-63.