MAKING CONTACT: A POPULATION GENETIC ANALYSIS OF WARBLING VIREOS (*VIREO GILVUS*) IN WESTERN NORTH AMERICA

AMANDA MARIE CARPENTER
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AMANDA MARIE CARPENTER

Date of Defense: December 10, 2019

Dr. T. Burg Thesis Supervisor	Professor	Ph.D.
Dr. A. Iwaniuk Thesis Examination Committee Member	Associate Professor	Ph.D.
Dr. R. Laird Thesis Examination Committee Member	Associate Professor	Ph.D.
Dr. T. Russell Chair, Thesis Examination Committee	Associate Professor	Ph.D.

Abstract

This thesis examined range wide population genetic structure, morphological differences, and hybridization among three morphologically cryptic subspecies of warbling vireo (*Vireo gilvus*). MtDNA and microsatellite markers show genetic differences between the eastern, *gilvus*, and two western, *swainsonii* and *brewsteri*, subspecies, while genetic differences between the two western subspecies were detected only with microsatellite markers suggesting their separation is recent. Microsatellite genetic groups do not correspond to current taxonomic range distributions of the two morphologically similar western subspecies. A low frequency of hybridization between *gilvus* and *swainsonii* in Alberta suggest strong reproductive isolating barriers. A wide, unreported contact zone between *swainsonii* and *brewsteri* in southern British Columbia through western Montana had a high frequency of hybridization, where genetic composition of hybrids appears related to habitat and elevation. Molecular markers are a crucial tool in recognizing cryptic biodiversity for taxonomic and conservation implications.

Acknowledgments

Little did I know growing up that my frequent exposure to backyard bird watching with my grandmother would come full circle and have me eventually go down the path of studying the genetics of a songbird. During my undergraduate degree, it was not until my final semester that I finally knew a narrower field I wanted to involve myself in. I took an ornithology class with Dr. Jeff Huebschman, who opened my eyes to this fascinating field and the excitedness of bird watching – which, I thought was an old person hobby. When I saw my first bird in the field and positively IDed it based on our class (a white-breasted nuthatch), I was hooked. I explored other avenues of ornithology and came across bird banding, which greatly intrigued me. I took my first solo road trip across the United States, from Wisconsin to Idaho, to participate in a beginner's bird banding workshop. The first bird I held, unbeknown to me as the bird I would soon spend two years of my life studying, was a warbling vireo. I took away a lot of skills from the workshop and a sense of self growth from driving across the United States alone. My mom would tell you, however, that she didn't really want me to travel alone again. Well, I found myself doing grassland breeding bird surveys for the USGS in Saco, Montana, which required me to leave the home roost once more on my own.

Forward another year, I am once again traveling solo (unless you count my dog) to Lethbridge, Alberta to begin my MSc in Biology. I'd like to thank Theresa first and foremost for "taking me under her wing" in her lab with my little genetic background. Your patience with me over the last two years has been appreciated, and for all of the work she has put into teaching me to prepare me for defending this thesis. I would also like to thank my committee members, Dr. Andrew Iwaniuk and Dr. Rob Laird, for their input at meetings, their patience in my preparation of this thesis, and their thoroughness of examining my thesis. I am very grateful for other faculty members at the University of Lethbridge. Jennifer Burke and Katrina Mendez have been supportive of my time here, always welcoming me to pop by for a bit of chit chat. Many thanks to the rest of the lab, Kanishka Sevenvirathna, Dilini Abeyrama, and Ian Gazeley, for making it feel more than just lab, but a little unit of good friends helping each other to succeed.

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˚C	degrees Celsius
5x	five times
ANOVA	analysis of variance
ATPase 6 and 8	adenosine triphosphate synthase subunit 6 and 8
AUC	area under curve
bp	base pair
B-H	Benjamini-Hochberg
COII	cytochrome oxidase II
cyt b	cytochrome b
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
E	east
ENM	ecological niche model
FDR	false discovery rate
F_{ST}	fixation index
g	grams
H_E	expected heterozygosity
H_O	observed heterozygosity
K	genetic cluster
ka	thousand years ago
km	kilometers
LGM	last glacial maximum
McMC	Markov chain Monte Carlo
$MgCl_2$	magnesium chloride
mm	millimeters
mM	millimolar
mtDNA	mitochondrial DNA
Ma	million years ago
N	north
n	sample size
ng	nanogram
NE	northeast
NW	northwest
PCR	polymerase chain reaction
pers obs	personal observation
Q	ancestry coefficient
SE	southeast
SW	southwest
T_A	annealing temperature
µL	microliter
µM	micromolar
W	west

Populations

B Hills	Black Hills
CAB	Central Alberta
CBC	Central British Columbia
CCO	Central Colorado
CID	Central Idaho
CMT	Central Montana
CNV	Central Nevada
CoOR	Coastal Oregon
CWA	Central Washington
CWY	Central Wyoming
C Hills, CYP HILLS	Cypress Hills
IL	Illinois
MN	Minnesota
NAB	Northern Alberta
ND	North Dakota
NECA	Northeast California
NENV	Northeast Nevada
NEOR	Northeast Oregon
NEWA	Northeast Washington
NID	Northern Idaho
NM	New Mexico
NWBC	Northwest British Columbia
NWWA	Northwest Washington
NWWY	Northwest Wyoming
OH	Ohio
ON	Ontario
SEAB	Southeast Alberta
SECA	Southeast California
SEOR	Southeast Oregon
SESD	Southeast South Dakota
SK	Saskatchewan
SWBC	Southwest British Columbia
SWMT	Southwest Montana
SWNV	Southwest Nevada
UT	Utah
VI	Vancouver Island
WLNP	Waterton Lakes National Park

Chapter 1: General Introduction

1.1 Background

1.1.1. Contact zones and population genetics

Across the world, many ranges of paired species or subspecies meet in narrow or large zones of overlap called contact zones. Contact zones have long been recognized as “natural laboratories” for studying evolution because they give biologists the opportunity to ask questions about the mechanisms involved in divergence and reproductive isolation, precursors to the formation of new species (Hewitt 1988, 2001). There are three outcomes for paired taxa within contact zones: 1) if strong reproductive isolating barriers evolved, the two taxa remain distinct, 2) if moderate or partial reproductive isolating barriers evolved between paired taxa, a stable hybrid zone forms, or 3) if there are weak reproductive isolating barriers between paired taxa, the result can be widespread hybridization and introgression (Barton and Gale 1993; Barton and Hewitt 1989). Population genetics investigates the forces behind speciation, such as gene flow, genetic drift, and natural selection. Genetic variation is examined within and among populations by quantifying the changes in allele frequencies over time and space in order to understand population genetic structure (Moore 1977). Contemporary population genetic structure is greatly influenced by historical barriers, with many contact zones formed as the result of range expansion after a period of isolation during the last glacial maximum (LGM) of the Quaternary (Hewitt 2000; Hewitt 2001).

1.1.2. Pleistocene epoch

The Quaternary is divided into two epochs: the Pleistocene (2.5 Ma – 11.5 ka) and the Holocene (11.5 ka – present) (Gibbard et al. 2009). The role of climate change and glaciation during the Pleistocene epoch on the speciation of fauna and flora globally has been extensively debated (Rawlence et al. 2019; Carnaval and Bates 2007; Spellman et al. 2007; Klicka and Zink 1997; Lovette 2005; Johnson and Cicero 2004; Weir and Schluter 2004). The Pleistocene was characterized by generally cooler global temperatures with warming interglacial and cooling glacial periods (Pielou 1991). At the LGM, approximately 21 ka, the ice sheets were at their greatest extent in the northern hemisphere (Peltier 1994). Present day Canada and areas just south were covered by two main ice sheets: the Cordilleran (in the west) and the Laurentide (east of the Rocky Mountains) spanning 4,000 km across and up to 3 km thick (Figure 1.1) (Peltier 1994; Booth et al. 2003). The dynamic interaction of these two ice sheets changed the North American landscape, pushing high latitude populations to the peripheries of the ice sheets' extent into ice-free refugia (Pielou 1991). Within these refugia, populations diverged from each other genetically due to different local selection pressures, genetic drift, and a lack of gene flow (Avise 2000).

By the start of the Holocene epoch 11.5 ka, the Cordilleran ice sheet started to recede, followed by the Laurentide ice sheet by 7 ka (Peltier 1994; Mayewski et al. 2004). With the retreat of the ice sheets, newly diverged populations expanded their ranges from isolated refugia, some experiencing secondary contact. The impact of evolutionary forces and barriers on reproductive isolation between closely related taxa is highly variable, making the outcome of secondary contact unpredictable (Martin et al. 2017; Stelkens et al. 2010).

1.1.3. Hybridization

Barriers that once limited gene flow between populations may disappear, such as the ice sheets at the start of the Holocene. Without separation by a barrier, and in the presence of weak reproductive isolating barriers, populations may undergo hybridization in secondary contact (Gilman and Behm 2011; Wegener et al. 2019). The completion and importance of prezygotic (behavioral isolation, morphological incompatibilities) and postzygotic (hybrid inviability and sterility) reproductive isolating mechanisms vary across taxa (Wegener et al. 2019; Uy et al. 2018). For example, prezygotic mechanisms are thought to be a major barrier between closely related bird species because birds have diverse plumage and courtship displays (Price 2008). However, the fact that hybridization occurs between well-established bird species suggests prezygotic reproductive isolating barriers are likely imperfect in some cases, and that other isolating mechanisms are also important (Dobzhanksy 1937; Uy et al. 2018).

Two hypotheses have been proposed for the maintenance of hybrid zones: the tension zone or dynamic-equilibrium hypothesis and the bounded hybrid superiority model (Barton and Hewitt 1989; Moore 1977). Under the tension zone hypothesis, steep clines are reflective of selection against hybrids and the persistence of the zone is balanced by the movement of parental species into the zone (Barton and Hewitt 1989; Hewitt 1988). The Italian and house sparrow (*Passer italiae*, *P. domesticus*) hybrid zone in the Alps is an example of a tension zone because it is maintained by a steep cline of species-specific plumage traits and hybrids have lower fitness than parental species

(Hermansen et al. 2011). The other hypothesis, the bounded hybrid superiority model, states that hybrid zones are found in transitioning ecotones and hybrid individuals are selected for because they are better adapted to the environment within the zone than either parental species (Moore 1977). The width of the zone under the bounded hybrid superiority model reflects the width of the transitional ecotone (Moore and Price 1993).

Hybridization is not uncommon, occurring in 10% of all animal species and 25% of all plant species (Hendry 2009). Across the globe, patterns of post-Pleistocene secondary contact have been found in various biota, including pawpaw/sugar apple trees or shrubs (*Annona* spp.) in Brazil, fire-bellied toads (*Bombina* spp.) in central Europe, and seagrass (*Cymodocea nodosa*) at the Mediterranean-Atlantic transition (Ribeiro et al. 2016; Hofman et al. 2007; Alberto et al. 2008). In North America, clusters of contact zones, called suture zones, are hot spot locations where an abundance of pairs of closely related taxa meet (Remington 1968; Swenson and Howard 2005). Suture zones are often associated with present-day physiographic features, such as Say's and eastern phoebes (*Sayornis saya* and *S. phoebe*) at the Great Plains, while other suture zones reflect expansion out of Pleistocene glacial refugia (Swenson and Howard 2005; Schukman et al. 2011).

Hybrid zones are contact zones, but not all contact zones are hybrid zones. Toews and Irwin (2008) examined a contact zone between morphologically cryptic winter wrens (*Troglodytes t. pacificus* and *T. t. hiemalis*) and found that both subspecies were reproductively isolated genetically and behaviorally, with little to no evidence of hybridization. Black-headed and rose-breasted grosbeaks (*Pheucticus melanocephalus* and *P. ludovicianus*) on the other hand, hybridize at a contact zone along the Great Plains despite clear morphological differences, suggesting that phenotype is likely not an important reproductive isolating barrier between these species (Mettler and Spellman 2009). By studying contact zones and hybridization, we can better understand the independent roles of reproductive isolating mechanisms between closely related taxa, an important area in our comprehension of how biodiversity is generated and maintained.

1.2 Study species

The warbling vireo (*Vireo gilvus*: Aves: Vireonidae) is a Nearctic-Neotropical migrant with a large breeding distribution across North America (Phillips 1991; AOU 1998; Macmynowski et al. 2007) (Figure 1.2). The taxonomy of the warbling vireo complex has undergone a number of changes, with the most recent classification by Phillips (1991) and the American Ornithologists' Union (1998) separating five recognized subspecies into two groups: the *gilvus* group containing the single eastern subspecies *V. g. gilvus* and the *swainsonii* group comprised of four western subspecies *V. g. swainsonii*, *V. g. brewsteri*, *V. g. victoriae*, and *V. g. sympatrica* (Figure 1.2). There are a number of zones of sympatry, or contact zones, between the ranges of three of the subspecies: *gilvus*, *swainsonii, and brewsteri* in western North America and all three subspecies' ranges are reported to meet at a contact zone in northwest Montana (Figure 1.2). Contact zones in western North America occur in other taxa, including the spotted frog complex (*Rana pretiosa*) in the Pacific Northwest, the dusky shrew (*Sorex monticolus*) of the Cascade / Sierra Nevada mountains, and Douglas-fir (*Pseudotsuga menziesii*) in the northern and southern Rocky Mountains (Green et al. 1996; Demboski and Cook 2001; Li and Adams 1989).

The subspecies boundaries, or ranges, are based on geographical variation in very subtle plumage and morphological differences, warranting more study and support from molecular techniques to corroborate their distributions (Phillips 1991; AOU 1998). For example, recent study of warbling vireo (n=8) song in the lowlands of Boulder, CO (east of the foothills of the Rocky Mountains) found that songs were consistent with the eastern subspecies, *gilvus*, while within the foothills of the Rocky Mountains (~ 23 km west), songs were consistent with the western subspecies (Floyd 2013). This observation suggests that the range of *gilvus* is further west than originally proposed by Phillips (1991). It is plausible, however, that a range shift of *gilvus* has occurred in the past 20 years, as range shifts have been found in other bird species as a consequence of anthropogenic change (Vallender et al. 2007).

Genetic studies on warbling vireos have focused on limited populations or small sample sizes from the eastern subspecies, *gilvus*, and the nominate western subspecies, *swainsonii*. Large genetic differences in mitochondrial DNA (mtDNA) (2.6 – 4.0%) were found between the two subspecies (Murray et al. 1994; Lovell 2010; Slager et al. 2014) as well as some differences in song, molt, timing of migration, and habitat (Howes-Jones 1985; Voelker and Rohwer 1998; Semenchuk 1992).

To date, a single comprehensive study examined populations along and outside of a speculated contact zone between *swainsonii* and *gilvus* in Alberta using sequence data from mitochondrial DNA (mtDNA) cytochrome b (cyt b), morphology, and song (Lovell 2010). The study found three populations containing a mixture of both east and west mtDNA lineages in central Alberta, and steep morphological and acoustic clines suggest a hybrid tension zone between *swainsonii* and *gilvus* (Lovell 2010). The uniparental inheritance of mtDNA does not allow for the detection of hybrids; therefore, other molecular tools, such as biparentally inherited nuclear markers, are required to provide evidentiary support of hybridization at this contact zone (Avise 2004; Lovell 2010).

1.3 Molecular markers

MtDNA and neutral, nuclear markers (microsatellites) were used to answer questions about the influence of historical barriers and contemporary processes on the population genetic structure of warbling vireos. MtDNA is a widely used tool in population genetic studies for a number of reasons. Primers can be developed to amplify across a variety of taxa with little prior knowledge of the study species' mitochondrial genome, reducing time and costs. In addition, the lack of recombination allows for evolutionary biologists to track maternal lineages spatially and temporally (millions of years). The mtDNA genome has a high mutation rate, estimated to mutate five to ten times faster than nuclear DNA (Brown et al. 1979). This increases the potential for detecting distinct lineages by fixed nucleotides among individuals of even closely related species (Brown et al. 1979). Due to its maternal inheritance (uniparental), the use of mtDNA could be limiting in some studies as it only follows the demographic history of females. Among mtDNA genes, cyt b and ATPase 6 and 8 are commonly used in population genetic studies to examine historic genetic structure of populations. ATPase 6 and 8 is more variable than cyt b and can provide higher resolution of genetic differences between closely related taxa.

Microsatellites are tandem repeats of 1 – 6 base pairs in the nuclear genome of most taxa (Selkoe and Toonen 2006). The number of these repeats can vary, resulting in a large number of alleles and high allelic variation (Selkoe and Toonen 2006; Holderegger and Wagner 2008). Microsatellite primers can amplify different species within a taxonomic family and even between closely related taxonomic familial groups because the flanking regions of DNA surrounding them are generally conserved (Selkoe and Toonen 2006). The reported neutral nature of microsatellites, in addition to biparental inheritance and hypervariability, make them a good tool in population genetic studies to detect genetic differentiation in recently diverged taxa. Biparentally inherited markers can also be useful in detecting hybridization. The presence of fixed alleles in the parental species could aid in identifying a hybrid individual, whereby the hybrid’s genotype would be a composite of each parental species. Another method of identifying hybridization is cytonuclear disequilibrium. Cytonuclear disequilibrium (discordance) occurs when individuals have mtDNA characteristic of one taxa, but nuclear DNA of another (Arnold 1993). This is due to the maternal inheritance of the mtDNA and the biparental inheritance of nuclear DNA. Using a combination of molecular markers from different areas of the genome with different modes of inheritance (mtDNA vs nuclear DNA) is therefore good practice because markers are limited in the information they can provide about the complex evolutionary histories of taxa.

1.4 Thesis goals

The purpose of this research is to examine the cryptic biodiversity in three subspecies of the warbling vireo complex (*gilvus*, *swainsonii*, and *brewsteri*) by studying overall population genetic structure, morphology, and hybridization across their range. My three primary research questions are: 1) How many genetic groups exist, and do they correspond to current described range distributions? 2) Are the subspecies morphologically distinct? and 3) Is there evidence of hybridization between the subspecies at the contact zones?

1.4.1. Subspecies differences and overall population genetic structure

To address subspecies genetic differences, I used two markers from the mtDNA genome and 14 variable microsatellite loci to examine patterns of how historic barriers and contemporary processes have influenced population genetic structure across the range. Three studies have previously compared mtDNA between the nominate western subspecies, *swainsonii*, and the eastern subspecies, *gilvus* (see section 1.2), but sample sizes were small or included populations from a small fraction of their range. No study has yet examined range wide subspecies population genetic structure among all three subspecies, between the two western subspecies (*swainsonii* and *brewsteri*), or between the lesser known western subspecies, *brewsteri*, and the eastern subspecies, *gilvus*, to compare these genetic groups to their current range distributions.

I compared four morphological measurements between subspecies' populations (mass, wing chord, bill length, and bill depth) based on the genetic groups from our microsatellite data to add additional support of subspecies differences. I used a mixed model ANOVA to test for significance of these values. An ecological niche model (ENM) was projected for each subspecies to predict their range limits and extent of overlap based on climatic variables. I predict that differences will be found between the eastern and western subspecies based on differences found previously between the eastern subspecies, *gilvus*, and nominate western subspecies, *swainsonii* (Howes-Jones 1985; Voelker and Rohwer 1998; Semenchuk 1992; Lovell 2010; Murray et al. 1994; Slager et al. 2014). It is plausible for the subspecies' current range distributions to differ from our genetic data because they are based mainly on subtle morphological differences (Phillips 1991; AOU 1988).

1.4.2. Contact zones

I examined a previously reported contact zone between *swainsonii* and *gilvus* in central Alberta, and a contact zone between *swainsonii* and *brewsteri* and a contact zone among all three subspecies in northwest Montana that have not yet been confirmed. Using genetic data from 14 microsatellite loci, I determined where the contact zones were located and if there was any presence of hybridization. Subspecies specific mtDNA primers were also developed for the *swainsonii* – *gilvus* contact zone in central Alberta. These were used to screen individuals and assign each to a mtDNA lineage to compare to how each individual was assigned with microsatellite loci. Cytonuclear discordance (see section 1.3) is a phenomenon that is illustrated in other hybrid zone studies, including long-toed salamanders (*Ambystoma macrodactylum*) (Lee-Yaw et al. 2014).

1.5 Thesis organization

This thesis is composed of four chapters. Chapter two addresses the range wide population genetic structure of *swainsonii*, *brewsteri*, and *gilvus* to determine how many genetic groups exist in relation to their current range distributions, coupled with morphological and abiotic differences. In chapter three I examine the contemporary contact zones between *swainsonii* and *brewsteri*, *swainsonii* and *gilvus*, and among all three in northwest Montana. I determine the location of these contact zones and if hybridization is occurring. The fourth chapter summarizes the key findings of this thesis, discusses future research of this complex, and connects its utility back to the big picture for our understanding of hybridization and reproductive isolation among morphologically cryptic taxa.

Figure 1.1 A map of the North American continent illustrating the two primary ice sheets that covered northern latitudes during the last glacial maximum of the Pleistocene (Peltier 1994).

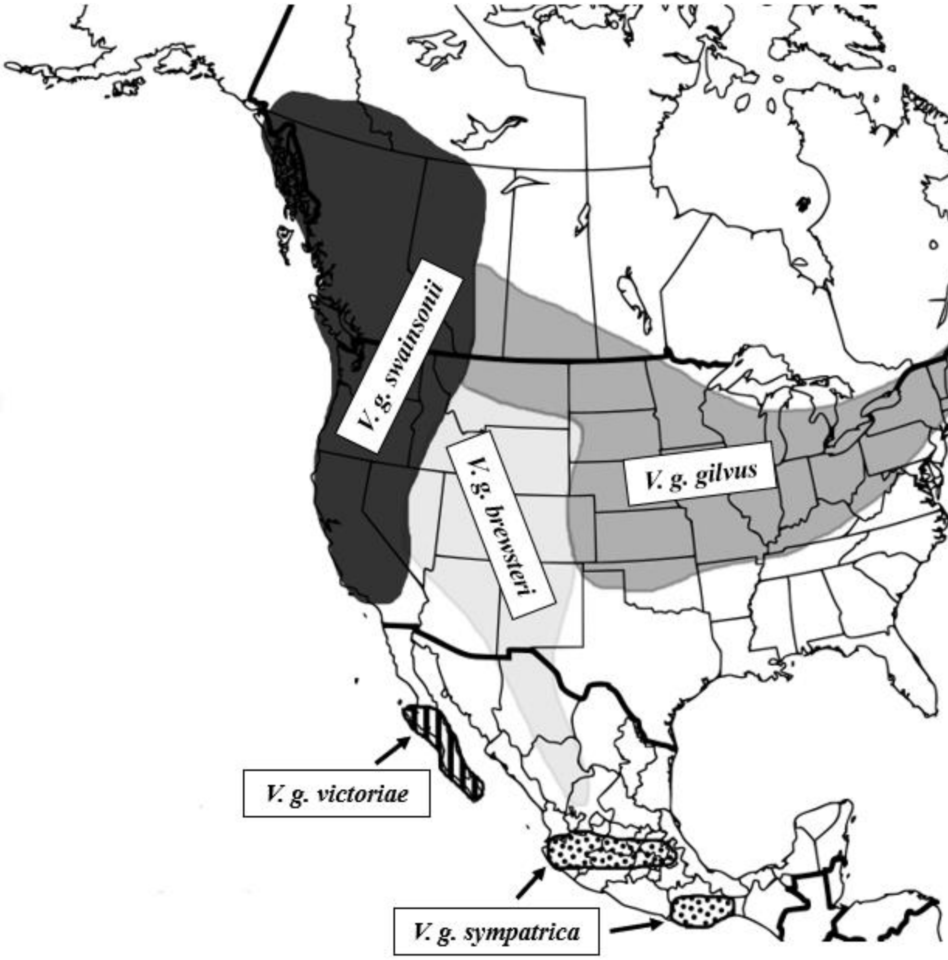

Figure 1.2 Breeding distributions of the five recognized subspecies of warbling vireo: the ***swainsonii* group**: *V. g. swainsonii* (SE Alaska, British Columbia, Alberta, NW Montana, NW Idaho, Oregon, Washington, California, and W Nevada), *V. g. brewsteri* (SW-SE Montana, SE Idaho, Wyoming, E Nevada, Utah, W Colorado, NE Arizona, New Mexico, and N Mexico), *V. g. victoriae* (Baja California), and *V. g. sympatrica* (Michoacán, Puebla, and Oaxaca in Mexico); the ***gilvus* group**: *V. g. gilvus* (Alberta, NE Montana, SC Saskatchewan, extending through the eastern states of the United States and into southern Ontario) (Phillips 1991; AOU 1998). The three subspecies of focus in this thesis are: *V. g. swainsonii*, *V. g. brewsteri*, and *V. g. gilvus*.

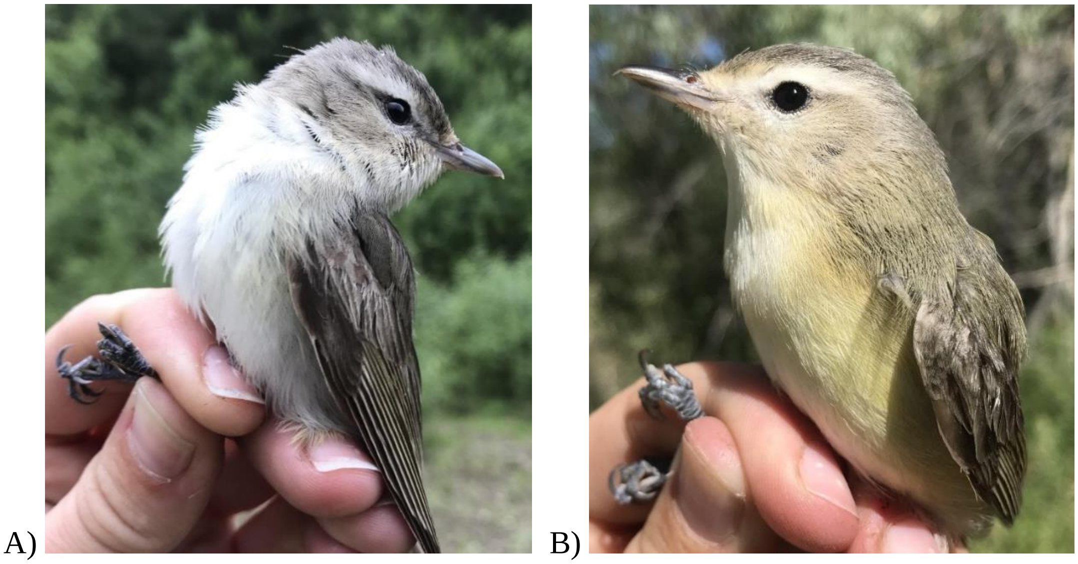

Figure 1.3 Male breeding plumages of the nominate taxa from each group: **A)** western warbling vireo, *swainsonii* and, **B)** eastern warbling vireo, *gilvus*, based on Pyle (1977).

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Chapter 2: Range wide microsatellite and mtDNA population genetic structure in a cryptic species complex, the warbling vireo (*Vireo gilvus*)

2.1 Introduction

The Pleistocene glaciations (2.5 Ma – 11.5 ka) played a major role in the divergence of many species across taxonomic groups (Klicka and Zink 1997; Lovette 2005; Johnson and Cicero 2004; Weir and Schluter 2004). A genetic pattern of west – east differentiation in many closely related pairs of taxa are byproducts of prolonged isolation during the Pleistocene, suggesting these groups occupied two, separate refugia (Hewitt 2000; Hewitt 2001; Peters et al. 2005; Toews and Irwin 2008; Burg et al. 2014; Beal and Pfau 2016; Lait and Burg 2013; Runck and Cook 2005). Some west – east forms are divergent morphologically, such as lazuli and indigo buntings (*Passerina amoena* and *P. cyanea*), while others may be morphologically cryptic and difficult to delimitate as species, such as carpet chameleons (*Furcifer lateralis*) (Baker and Boylan 1999; Florio et al. 2012). The identification of cryptic species is critically important for many reasons, including accurate assessments of biodiversity, taxonomy, and conservation efforts (Bickford et al. 2007). By studying populations of west – east forms, we can examine the importance of a historic barrier, glacial ice, along with contemporary barriers to better understand the evolutionary histories of these taxa and identify the processes driving speciation in morphologically divergent and cryptic taxa (Price 2008; Carnaval et al. 2009).

The genus *Vireo* contains 26 species, 11 of which occur in North America (AOU 1983). Underestimates of biodiversity based on morphology have been found in other *Vireo* complexes, including Hutton's vireo (*Vireo huttoni*) and blue-headed vireo (*Vireo solitarius*) (Johnson 1995; Cicero and Johnson 1992, 1998). Species of *Vireo* are an ideal model for analyzing questions of ecological divergence and reproductive isolation because certain features appear evolutionarily stable in the genus, including morphology (small body size) and foraging habits (Hamilton 1959, 1962). The warbling vireo (*Vireo gilvus*) is another species complex speculated to be comprised of multiple, cryptic species (AOU 1998). Occupying a large breeding distribution, there is a west – east subdivision among five subspecies based on subtle morphological differences, three of which are known to be migratory: two western (*V. g. swainsonii* and *V. g. brewsteri*) and one eastern (*V. g. gilvus*) (Figure 2.1) (AOU 1998; Phillips 1991). Studies to date have included small sample sizes or few populations from two of the five warbling vireo subspecies, *swainsonii* and *gilvus*. Large genetic differences in the mitochondrial DNA (mtDNA) genome were found in phylogenetic studies, and other studies on these two taxa also found differences in morphology, plumage color, song, habitat, and migratory behavior (Johnson et al. 1988; Murray et al. 1994; Slager et al. 2004; Phillips 1991; Lovell 2010; Howes-Jones 1985; Voelker and Rohwer 1998; Semenchuk 1992).

No study has yet examined range wide population genetic structure among all three migratory subspecies or compared these genetic groups to current range distributions that are based on cryptic variation in morphological characters (Phillips 1991; AOU 1988). Using a single, mtDNA locus and multiple nuclear (microsatellite) loci, we examined population genetic structure across each of the three subspecies' breeding ranges to study how historic barriers influenced contemporary population genetic structure in this species complex. A second, more variable mtDNA locus, ATPase 6 and 8, was used to provide more resolution on the historic genetic structure between the two western subspecies, *swainsonii* and *brewsteri* (Sorenson 2003). Utilizing a range wide sampling approach and markers with different modes of inheritance, mtDNA (uniparental) and nuclear (biparental), allows for the elucidation of the evolutionary histories and genetic patterns of taxa that might go unnoticed in studies that focus on a single marker.

Based on the genetic groups from our microsatellite data, we then tested for the significance of four morphometric measurements (mass, wing chord, bill length, and bill depth) between subspecies populations. An ecological niche model (ENM) was also projected for each subspecies to predict the extent of niche overlap based on abiotic (climatic) variables. This multimethod approach, the use of molecular data with morphological and ENM modelling data, is advantageous when adding support to overall differences in a cryptic species' complex.

Given the reported divergence times between *swainsonii* and *gilvus* implying probable isolation in different Pleistocene refugia, we hypothesize at least two mtDNA genetic groups (east and west) corresponding to the findings of other genetic studies (Murray et al. 1994; Slager et al. 2004; Lovell 2010), and that genetic differences in the nuclear genome will also be found. There are a number of geographic barriers separating the subspecies' current range distributions, including mountains and the Great Plains, that may limit gene flow between them (Figure 2.1). In the presence of these contemporary geographic barriers, we hypothesize at least three genetic groups that correspond to the three subspecies with microsatellite loci because microsatellites are able to detect more recent genetic events in populations.

2.2 Methods

2.2.1. Sample collection and DNA extraction

Blood or feather samples were collected from breeding birds during the field season (2007-2019). Additional tissue samples were obtained from museums to supplement field sampling. Birds sampled in the field were collected from the end of May to early July to reduce capture of family groups and to avoid catching migrants. Birds were caught using 12 m mistnets with song playbacks and each bird was banded to avoid resampling. A small (<50 µL) blood sample was taken from the brachial vein, along with a series of morphometric measurements (mass, wing chord, bill length, bill depth, bill width, and tarsus) and photographs. Blood samples were stored in 95% ethanol and all birds were released on site. We collected a total of 212 warbling vireo samples and 191 tissue samples were also obtained from museum collections (Denver Museum of Nature and Science, Burke Museum of Natural History and Culture, Natural History Museum University of Oslo, University of Michigan Museum, Field Museum of Natural History, Cleveland Museum of Natural History, and Royal Alberta Museum) from 37 sampling sites, herein referred to as populations. A population is defined in this chapter as samples collected within a 100 km distance.

Total genomic DNA was extracted from blood, tissue, or feather samples using a modified Chelex extraction (Walsh et al. 1991). All samples were stored at -20˚C following extraction.

2.2.2. MtDNA sequencing

A 1140 bp (base pair) region of the cytochrome b (cyt b) gene was amplified using primers H15944 and L14990 in 65 warbling vireos across all three subspecies populations (Lovell 2010; Sorenson et al. 1999) (Table 2.1). The 25 µL PCR (polymerase chain reaction) contained 5x Green GoTaq® Flexi buffer (Promega), 0.2 mM dNTP, 2 mM $MgCl_2$ (Promega), 0.5 µM primers H15944 and L14990, 0.5 U GoTaq® Flexi polymerase (Promega), and ~ 1 ng of genomic DNA (diluted 1:10 for blood or tissue, 1:5 for feathers). The thermocycling profile was one cycle for two minutes at 94°C, 45 seconds at 58°C, and one minute at 72°C; followed by 37 cycles for 30 seconds at 94°C, 45 seconds at 58°C, and one minute at 72°C; concluding with 72°C for five minutes.

A 503 bp region of the ATPase 6 and 8 gene was amplified using primers ATP H534ch and L8929 COII in 44 warbling vireos from only the western subspecies' populations, *swainsonii* and *brewsteri* (Lait and Burg 2013; Sorenson et al. 1999) (Table 2.1). The PCR conditions were the same as used in cyt b. Successfully amplified samples were sent to NanuQ at McGill University, Montreal, Quebec for sequencing. Sequences were checked and aligned using MEGA X v. 10.1 (Kumar et al. 2018). Cyt b sequences were aligned using a reference warbling vireo sequence (GenBank #: AY030111). Variable sites from 65 cyt b sequences and 44 ATPase 6 and 8 sequences were used to construct two, separate minimum spanning haplotype networks using Population Analysis with Reticulate Trees v. 1.7 (PopART) software (Bandelt et al. 1999).

2.2.3 Microsatellites

2.2.3.1. Genotyping

A subset of samples (n=6) from different geographical areas were screened with 57 passerine microsatellite loci. A total of 30 microsatellite loci were optimized in warbling vireos, 14 of which were polymorphic and used in our study to genotype 408 warbling vireos: BCV2-6, BCV4-5, BCV4-6New, Ck.1B5D, Ck.4B6D, Ck.2A5A, Ck.1B6G, Hofi5, Pocc1, ApCo46, Lox1, Ase18, Ppi2New, and CmAAAG30 (Table 2.2). Expected and observed heterozygosity and the average number of alleles per locus were calculated using a frequency-based analysis in GenAlEx 6.5 (Peakall and Smouse 2006, 2012) (Table 2.2). Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were checked with Genepop v4.7 (Raymond and Rousset 1995; Rousset 2008).

The 10 µL PCR contained FroggaBio buffer, 0.2 mM dNTP, 0.5 µM of forward primer, 1 µM of the reverse primer, 1 µM of the M13 fluorescently labeled primers, 1 U of FroggaBio Taq, and ~ 1 ng of genomic DNA (1:10 dilution for blood and tissue; 1:5 for feathers). The following thermocycling profile was used to amplify DNA: one cycle for one minute at 94°C, 45 seconds at T_1, and one minute at 72°C; seven cycles of one minute at 94°C, 30 seconds at T_1, and 45 seconds at 72°C; 25 cycles for 30 seconds at 94°C, 30 seconds at T_2, and 45 seconds at 72°C; concluding with one cycle of 72°C for five minutes. For DNA amplification, five loci (BCV4-6New, Hofi5, ApCo46, Lox1, and Ppi2New) were amplified using T_1=50°C and T_2=52°C, five loci (BCV2-6, BCV4-5, Ck.1B5D, Ck.2A5A, and Ck.4B6D) were amplified using T_1=52°C and T_2=54°C, and four loci (Pocc1, Ase18, CmAAAG30, and Ck.1B6G) using T_1=55°C and T_2=57°C.

Three loci (BCV4-5, ApCo46, and Ppi2New) required 31 cycles instead of 25 cycles (Table 2.2). Products were denatured and run on a 6% acrylamide gel on a Li-Cor 4300 DNA Analyzer. To maintain consistent sizing and scoring of alleles, the same three positive controls of known sizes were run with each load for each locus. All gels were scored independently by two people and a subset of samples from each gel was re-run to ensure consistent scoring across gels. A total of 377 samples from 37 populations were selected for this study after eliminating individuals that were missing genotypic information at more than seven loci (50% data) (Table 2.1) (Appendix 1.1).

2.2.3.2. Bayesian clustering

The program Structure v2.3.4 (Hubisz et al. 2009) uses a Bayesian clustering model and a Markov chain Monte Carlo (McMC) method to examine population structure and assign individuals to a genetic cluster (K) based on multi-locus genotype data (Pritchard et al. 2000). An individual's membership to a cluster is determined by the proportion of its genotype (Q) that belongs to any given K (Pritchard et al. 2000). For our study, we used correlated alleles, admixture model, and sampling locations as *locpriors*. The correlated allele frequency model can detect distinct populations that are closely related and when genetic data have weak hierarchal structure, the *locpriors* setting can improve the model by providing the knowledge of the sampling location (*locpriors*) for each individual (Hubisz et al. 2009; Porras-Hurtado et al. 2013). Initial Structure runs were performed with 50 000 burn-ins and 100 000 McMC repetitions for K=1 to 5. Methodology outlined in the Structure manual was used to determine the optimal number of genetic clusters (Pritchard et al. 2000). Following the initial Structure run, two genetic groups were detected. To test if additional genetic groups were present within these two groups, a second set of runs with the same settings for K=1 to 3 were done using individuals from each group separately. Subsequent runs were executed with the same methodology as the second run if additional genetic groups were found. Outputs from each Structure run were averaged and graphed using Structure Plot v2.0 (Ramasamy et al. 2014).

2.2.3.3. F_{ST}

A common *F*-statistic used in population genetic studies is the fixation index, or F_{ST}, which compares the frequency of alleles between two populations to see how different they are on a numerical scale from 0 to 1 (Hartl and Clark 1997). A value of zero means the two populations are not genetically different from one another, while a value of one means the two populations are genetically different. Pairwise F_{ST} values were calculated from microsatellite genotypes using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Populations with five or less individuals were omitted from the comparison. *P*-values were corrected *post hoc* with the Benjamini-Hochberg (B-H) method, which uses the equation of $(i/m)Q$, where i is the rank of each p-value, m is the total number of tests, and Q is your selected alpha value (or false discovery rate) (Benjamini and Hochberg 1995). The B-H method controls the false discovery rate (FDR) for multiple comparisons and lowers the critical value of significance to avoid type I errors (Benjamini and Hochberg 1995). Bonferroni corrections have been suggested as too severe, often underestimating the number of populations that are significantly different (Garamszegi 2006).

2.2.3.4. Morphology

Based on our genetic clusters from the microsatellite data, populations were selected in each subspecies' range to compare four morphometric measurements (mass, wing chord, bill length, and bill depth) (Table 2.1). A 30 gram scale with 0.5 gram increments was used to calculate the mass of the bird by taking the weight of the bird + bag and subtracting the weight of the bag. Wing chord (mm) was measured by placing a wing ruler underneath the wing, with the "shoulder" of the wing pressed against the stop of the ruler with no additional pressure that would otherwise flatten the wing; the measurement was to the tip of the longest primary feather. Bill length and bill depth (mm) were measured using 0.05 mm manual calipers; bill length was measured from the nares (nostril) to the tip of the bill, while bill depth was measured between the nares and the lower jaw of the mandible (Pyle 1997). A mixed model ANOVA (Analysis of Variance) was used to compare each morphological character in R using the *nlme* package, and Tukey tests were conducted *post hoc* to explore the significance of the values (Pinheiro et al. 2011). Morphometric measurements with *p*-values ≤ 0.05 were significant.

2.2.3.5. Ecological niche modeling

An ENM was projected for each subspecies to see the extent of overlap in their ranges based on shared or different climatic variables. We used the application Wallace v1.0.6 in R with WorldClim BioClim layers, which outputs predictive models that indicate suitable areas of habitat based on estimates of the species' response to environment conditions from occurrence datasets (Kass et al. 2017; Elith and Leathwick 2009). Occurrence data used for each subspecies' model was based off of our genetic groups from our microsatellite data. Points were obtained from the Global Biodiversity

Information Facility (GBIF, www.gbif.org), our data, and eBird (ebird.org) following screening of their location to ensure correctness. To restrict occurrences to breeding populations, entries for each subspecies were limited to those collected from May through August. All 19 WorldClim Bioclim layers were run with each subspecies' occurrence data to check for correlated variables (0.7 threshold); all but one correlated layer was removed. We used a combination of different background extents, background points, partitioning modules, regularization multipliers, environmental layers, and feature classes to test model suitability for each subspecies. The best fit model for each subspecies was selected by the highest area under curve (AUC) value; a value closer to one represents a more accurate model.

The final number of occurrence points used for each subspecies' model was: 268 points for *gilvus*, 207 points for *swainsonii*, and 228 points for *brewsteri*. Settings used for the final Maxent models in Wallace for all three subspecies included 10 km spatial thinning of occurrence points, 2.5 arcmin resolution of Bioclim variables, minimum convex polygon of one-degree buffer distance, 10000 background points, non-spatial partition of occurrence points by five folds, and a 1.0 regularization multiplier with a hinge feature class clamped to the area of data.

2.3 Results

2.3.1. Sequencing and mtDNA lineages

A 950 bp region of cyt b was sequenced in 65 warbling vireos from 22 populations across their range and aligned using MEGA X v. 10.1 (Kumar et al. 2018) (Table 2.1) (Figure 2.1). PopART identified two major mtDNA lineages, separating east (*gilvus*) and west (*swainsonii* / *brewsteri*) birds by 31 nucleotide differences (Figure 2.1, top inset photo). The SEAB population include samples from Medicine Hat and the Cypress Hills. Six birds (out of 22) group east (all five birds from Medicine Hat and one bird from the Cypress Hills), while the remaining 16 birds from the Cypress Hills group west. Thirty-five haplotypes were found, ten haplotypes exclusive to eastern (*gilvus*) birds (Bandelt et al. 1999).

A 503 bp region of ATPase 6 and 8 was sequenced in 44 western warbling vireos, 25 *swainsonii* and 19 *brewsteri*, and aligned using MEGA X v. 10.1 (Kumar et al. 2018). PopART identified a single, mtDNA lineage with no clear subspecies partitioning (Figure 2.1, bottom inset photo) (Bandelt et al. 1999).

2.3.2. Microsatellites

2.3.2.1. Bayesian clustering

A total of 377 birds were genotyped at 14 microsatellite loci from 37 populations to examine contemporary population genetic structure among subspecies (Table 2.1). No significant departures from Hardy-Weinberg equilibrium or linkage disequilibrium were detected with Genepop (Raymond and Rousset 1995; Rousset 2008).

The initial Structure run with all 37 populations indicated that the optimal number of clusters was K=2. At K=3, Structure indicated additional genetic differences within the west populations, but had a weak hierarchal signal with all 37 populations included. At K=2, we found a distinct west – east split that divided the western populations from the eastern populations along the Great Plains (Figure 2.2 A). Within the SEAB population, 6 of 7 birds from Medicine Hat grouped with the east genetic cluster and 16 of 17 birds from the Cypress Hills grouped with the west genetic cluster. One individual in Medicine Hat groups Q ≥ 60% west and one individual in the Cypress Hills groups Q ≥ 60% east. This bird from the Cypress Hills is the same individual that grouped with the eastern mtDNA lineage (see section 2.3.1). Populations that assigned to the eastern genetic cluster, including Medicine Hat and the single individual in the Cypress Hills (SEAB), were removed to test for additional structure among western populations.

Among the west populations, K=2 is the optimal number of genetic clusters, separating all western populations from the Black Hills (Figure 2.2 B). A subsequent run was done after removing the Black Hills, with two additional genetic clusters detected among remaining western populations of *swainsonii* and *brewsteri*. NWBC, CBC, JASPER, BANFF, CASTLE, WLNP, SWBC, and SEAB (Cypress Hills) populations group with *swainsonii*, while NEWA, NWWA, VI, CWA, NEOR, SEOR, CoOR, CoCA, NECA, SECA, SWNV, NENV, UT, NM, SWCO, CCO, CWY, NWWY, SWMT, and CMT populations group with *brewsteri* (Figure 2.2 C). The majority of individuals were assigned Q ≥ 60% to a specific west genetic cluster, and eleven populations within the described range of *swainsonii* group with *brewsteri* (SWNV, SECA, CoCA, NECA, SEOR, CoOR, NEOR, CWA, NEWA, NWWA, and VI). The CMT population in the described range of *gilvus* also groups with *brewsteri*. Altogether, Structure identified four genetic clusters: an east group, two west groups, and the Black Hills forming the fourth genetic group (Figure 2.2).

2.3.2.2. F_{ST}

Pairwise F_{ST} comparisons of 22 populations with 14 microsatellite loci found 145 significant values out of 231 comparisons (Table 2.3) (Appendix 1.2). Sixty-eight of the significant comparisons separated all eastern, *gilvus*, populations from both western, *swainsonii* and *brewsteri*, populations ($F_{ST} = \geq 0.20$), except for SEAB. Following a *post hoc* B-H correction (critical *p*-value = ≤ 0.03), all p-values between eastern, *gilvus*, and western, *swainsonii* and *brewsteri*, populations were significant ($p = < 0.001$), except for SEAB. Pairwise F_{ST} found little genetic differentiation within conspecific populations. Three *brewsteri* populations, NEOR, SWNV, and NM, were significantly different from all five *swainsonii* populations (CBC, JASPER, BANFF, CASTLE, WLNP) (Table 2.3). The Black Hills were also significantly different (Table 2.3) from all other west populations with the exception of CWY, the closest population to it, and NENV.

2.3.2.3. Morphology

Based on the genetic groups from our microsatellite data, 25 populations (8 *swainsonii*, 11 *brewsteri*, and 6 *gilvus*) were selected to compare four morphometric measurements (mass, wing chord, bill length, and bill depth) between subspecies (Table 2.1). A mixed model ANOVA and *post hoc* Tukey tests found significant differences between *gilvus* populations and both of the western subspecies populations at three out of the four morphological comparisons ($p = < 0.001$) (Figure 2.4). Wing chord was the only morphological character that was significantly different between the two western subspecies, *swainsonii* and *brewsteri*, ($p = < 0.001$) (Figure 2.4) (Appendix 1.3).

2.3.2.4. Ecological niche modeling

An ENM was projected for each subspecies using occurrence points based on our genetic groups from our microsatellite data to see the extent of overlap in their ranges corresponding to climatic variables. The number and specific Bioclim layers differed for each subspecies model: *swainsonii* (nine variables – 2, 6, 7, 9, 11, 14, 16, 17 and 18), *brewsteri* (eight variables – 1, 2, 3, 7, 8, 13, 17, and 18), and *gilvus* (twelve variables – 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 15, and 18) (Appendix 1.4). Differences were also found in which three BioClim layers contributed most to each subspecies model: *gilvus* (3 = 27.3%, 5 = 19.9%, 2 = 15.1%; all temperature variables), *swainsonii* (11 = 28.6%, 7 and 14 = 18%; temperature and precipitation of driest month), *brewsteri* (8 = 29%, 7 = 25.7%, 1 = 12%; precipitation of wettest quarter and temperature) (Appendix 1.3). Models for each subspecies were evaluated using the AUC value: *gilvus* AUC = 0.73, *swainsonii* AUC = 0.76, and *brewsteri* AUC = 0.66. Niche overlap appears greatest between the two western subspecies, *swainsonii* and *brewsteri*, while the eastern subspecies, *gilvus*, is confined east of the Rocky Mountains in the Great Plains (Figure 2.5).

2.4. Discussion

2.4.1. Historic population genetic structure

Analysis of mtDNA cyt b sequences from all three subspecies indicated two major lineages, a division of west – east separated by 31 nucleotide differences (Figure 2.1). Our data support previous studies that have found mtDNA differences between *swainsonii* and *gilvus* (Murray et al. 1994; Lovell 2010; Slager et al. 2014). Analyses of sequences at an additional variable mtDNA gene, ATPase 6 and 8, did not reveal support for genetic differences between populations of the western subspecies, *swainsonii* and *brewsteri*. The west – east split along the Great Plains suggest the eastern subspecies was isolated in a separate glacial refugium from the two western subspecies during the LGM (Rising 1983). A GIS-based niche study done on Bullock's orioles (*Icterus bullocki*, west form) and Baltimore orioles (*I. galbula*, east form) found support that the western and eastern forms occupied different refugia during the Pleistocene, where the Baltimore oriole adapted to a cooler environment in its east refugium, thus restricting them from expanding beyond their Great Plains range into the arid west (Swenson 2006). Little differences found in both cyt b and ATPase 6 and 8 mtDNA genes between the two western subspecies suggest that they may have occupied the same glacial refugium (Colbeck et al. 2008). If the western subspecies were in separate refugia, a greater number of fixed nucleotide differences, and partitioning of haplotypes, would be observed because of a lack of gene flow and genetic drift (Yang and Kenagy 2009).

One population in the study, SEAB, does not follow the west – east Great Plains divide. Six individuals (all five from Medicine Hat and one from the Cypress Hills) group with the east mtDNA lineage, while the remaining 16 individuals from the Cypress Hills group with the west mtDNA lineage (Figure 2.1). The Cypress Hills are the highest point east of the Rocky Mountains, surrounded by xeric grasslands in the southeast corner of Alberta and southwest corner of Saskatchewan (Strong and Hills 2005). During the LGM (21 ka), pollen data suggest that the Cypress Hills were on the fringe of the western extent of the boreal forest and the eastern extent of the Cordilleran forest at the edge of the Cordilleran ice sheet (Strong and Hills 2005). Species found in the Cordilleran forest included lodgepole pine (*Pinus contorta*), white spruce (*Picea glauca*), black spruce (*Picea mariana*), and fir species (*Abies* spp.), while the boreal forest contained a mosaic of coniferous and deciduous tree species (Strong and Hills 2005). After the LGM, the Cypress Hills became surrounded by grassland vegetation as temperatures warmed, restricting any further mixture of boreal (Rocky Mountain) or Cordilleran vegetation (Strong and Hills 2005). The predominance of coniferous species found in the Cypress

Hills is typical of contemporary *swainsonii* habitat, while *gilvus* has been found to prefer mature deciduous tree stands, specifically aspen (Semenchuk 1992).

Other animal species have disjunct populations in the Cypress Hills including tree-roosting big brown bats (*Eptesicus fuscus*), spruce budworm (*Choristoneura fumiferana*), white-crowned sparrows (*Zonotrichia leucophrys oriantha*), and ovenbirds (Seiurus *aurocapilla*) (Metheny et al. 2008; Lumley and Sperling 2011; Chilton 2003; Haché et al. 2017). Our data for the Cypress Hills support findings by Lovell (2010) with the exception of the one individual that groups with the east mtDNA lineage. All nine birds sampled by Lovell (2010) grouped west, while our study included 17 new samples perhaps highlighting the importance of sample size in certain locations.

2.4.2. Contemporary population genetic structure

Microsatellite analyses revealed population genetic structure across the North American range of warbling vireos. We found a main west – east division, with additional population genetic structure within the west group using Structure. The west group can be divided further into two genetic clusters suggesting a north – south split: a southwestern cluster (including Vancouver Island), and a cluster that occupies mainland British Columbia extending east to the Canadian Rockies with a disjunct population in the Cypress Hills (SEAB) (Figure 2.3). Pairwise F_{ST} comparisons support the west – east split found with Structure, also differentiating the eastern subspecies' populations from both western subspecies' populations (Table 2.3). There are two populations that stand out in the pairwise F_{ST} comparisons and in Structure: SEAB and the Black Hills.

The SEAB population includes birds from Medicine Hat and the Cypress Hills, locations that are approximately 60 km apart, but differ in elevation and habitat. The Cypress Hills are isolated highlands of montane forest surrounded by grassland (see section 2.4.1) with its highest point at 1,468 m, while Medicine Hat is situated in the Great Plains at an elevation of 650 m. Data from microsatellites and mtDNA show that all but one (out of 17) Cypress Hills individuals' group with the west genetic cluster and, more specifically using microsatellites, *swainsonii*. The presence of an individual with a *gilvus* haplotype and nuclear DNA suggests migration of *gilvus* in the Cypress Hills (Malmos et al. 2008). While habitat of the Cypress Hills is similar to the boreal forest of the Rocky Mountains, it does have some deciduous species that are found in typical *gilvus* habitat with some areas of lower elevation (Semenchuk 1992). One individual from Medicine Hat had a Q value ≥ 60% to the west genetic cluster. All individuals from Medicine Hat grouped with the east mtDNA lineage (see section 2.4.1.). The presence of cytonuclear discordance, assignment to one mtDNA lineage and grouping with another in the nuclear genome, suggest the presence of hybridization between the western subspecies, *swainsonii*, and eastern subspecies, *gilvus*, in Medicine Hat, and presumably from the disjunct population in the Cypress Hills.

The Black Hills, on the western edge of South Dakota and eastern edge of Wyoming, was found to be its own genetic group from both western subspecies and the eastern subspecies using Structure (Figure 2.2 B). Pairwise F_{ST} comparisons support the findings of Structure, in that significant differences were found between the Black Hills and all other populations included, except for the most nearby population, CWY, and NENV (Table 2.3). An explanation for this genetically diverged population is if there is restricted gene flow to the Black Hills and if the population size is small, changes in its genetic composition is likely due to genetic drift, a pattern also found in American dippers (*Cinclus mexicanus*) (Anderson et al. 2008). Comparisons of allele frequencies from both surrounding *brewsteri* (SWCO, CCO, CWY, and NWWY) and *gilvus* (SK, ND, MN, and IL) populations shows that the Black Hills' alleles are more similar to *brewsteri*, and that it is not a hybrid population of *brewsteri* and *gilvus*. Additionally, observed heterozygosity between the Black Hills, *brewsteri*, and *gilvus* populations does not show a loss in genetic diversity, thus excluding the explanation of a population bottleneck or genetic drift due to a bottleneck event.

Ecologically, the Black Hills are an isolated group of mountains that have average elevations of 1,372 meters above the surrounding Great Plains; an elevation not typical of *gilvus* but suitable for *brewsteri* (Froiland 1990). There is no evidence of glaciation in the Black Hills or the immediate surrounding plains, but areas of north and east were covered by the Laurentide ice sheet, affecting the climate of the Black Hills (Froiland 1990). During the LGM, there is evidence that the boreal forest and Cordilleran forest extended south into the Black Hills and may explain why endemic species to the boreal forest (Rocky Mountains), such as lodgepole pine (*Pinus contorta*), are found here (Froiland 1990; Strong and Hills 2005). Species across several taxonomic groups, including land snails (*Oreohelix* spp.), stone fly (Plecoptera), northern flying squirrel (*Glaucomys sabrinus*), and red-bellied snake (*Storeria occipitomaculata*) have genetically distinct populations in the Black Hills compared to all other populations (Weaver et al. 2006; Huntsman et al. 1999; Hough and Dieter 2009; Smith 1963).

2.4.3. Delineation of subspecies ranges

The current range distributions of all three warbling vireo subspecies in this study are based on cryptic variation in morphological differences and plumage coloration (AOU 1988; Phillips 1991). Our mtDNA data agree with these distributions, with the exception of western mtDNA haplotypes in the Cypress Hills (SEAB) (Figure 2.1). Our microsatellite data overall disagree with the two western subspecies distributions (Phillips 1991; AOU 1988) (Figure 2.3).

V. g. brewsteri is described to occupy the central portion of the western range which includes the Black Hills, the Rocky Mountains in the United States, and areas of the Great Basin (Figure 2.1). The Black Hills, on the periphery of *brewsteri's* range, forms an independent genetic cluster from all other populations in this study. Our microsatellite data also group eleven populations within the *swainsonii* range (SWNV, SECA, CoCA, NECA, SEOR, CoOR, NEOR, CWA, NWWA, NEWA, and VI) and one population within the *gilvus* range (CMT) with *brewsteri* (Figure 2.3). This suggests that *brewsteri's* range is much larger than previously reported, extending all the way to the West Coast and north to United States – Canada border. The CMT population was sampled from the Little Belt Mountains, an isolated range east of the Rocky Mountains situated in the Great Plains of central Montana (Johnson 1996). This population, along with the other eleven in *swainsonii's* range, are found at a broad range of elevations, from 200 – 3000 m, with predominant dense coniferous tree – deciduous bush habitat and shrub-steppe grasses (Comer et al. 2003; Noy-Meir 1973; Sala et al. 1988).

V. g. swainsonii's range is smaller based on our microsatellite genetic groups, restricted to mainland British Columbia and east to the Canadian Rocky Mountains, with a potential disjunct population in the Cypress Hills. These populations are found at a narrower range of elevations, from 400 – 1500 m, with some different, prominent coniferous tree species in the boreal forest, and deciduous tree species (Meidinger and Pojar 1991; Semenchuk 1991).

The north – south split at nuclear loci of the two western subspecies, *swainsonii* and *brewsteri*, with our range wide sampling method does not appear to show a divide by any one contemporary geographic barrier. Based on these genetic groups, our data suggest *swainsonii* and *brewsteri's* ranges meet in southern British Columbia (Figure 2.3). A possible explanation for the location of the contact zone is southern British Columbia was located at the maximum extent of the Pleistocene ice sheets, and *swainsonii* rapidly expanded its range north as the ice sheets retreated limiting *brewsteri's* range expansion north (Hewitt 1996, 2001). Other high latitude bird species, including Audubon and myrtle warblers (*Setophaga auduboni* and *S. coronata*), MacGillivray and mourning warblers (*Oporornis tolmiei* and *O. philadelphia*), winter and pacific wrens (*Troglodytes hiemalis* and *T. pacificus*), and Townsend's and black-throated green warblers (*Dendroica townsendi* and *D. virens*) have ranges that meet in central or southern British Columbia as a byproduct of the Pleistocene ice sheets (Toews et al. 2014; Irwin et al. 2009; Toews and Irwin 2008; Toews et al. 2011; Weir and Schluter 2004). The cyt b haplotype network shows a demographic pattern of one large, main haplotype shared by the majority of individuals from both western subspecies' populations, with many haplotypes branching off suggesting rapid, postglacial range expansion in western warbling vireos (Figure 2.1) (Hindley et al. 2018). The greater genetic partitioning found with microsatellite loci than mtDNA genes suggest that ecological contemporary processes are separating *swainsonii* and *brewsteri*.

Our mtDNA and microsatellite data support the distribution of *gilvus* in the eastern portion of the range and being the most different genetically from the two western subspecies (Figures 2.1, 2.3). *V. g. gilvus* populations are found at elevations < 700 m and prefer mature, open deciduous tree stands, particularly aspen (*Populus tremuloides*), along riparian areas (Semenchuk 1991; Lovell 2010; pers obs). On average, *gilvus* is a larger bird in three out of the four morphological characters that were compared (mass, wing chord, and bill depth) between the two western subspecies (Figure 2.4). Larger body size was also found in pied flycatchers (*Ficedula hypoleuca*) that inhabited deciduous habitats compared to those found in coniferous habitats (Alatalo and Lundberg 1986). Aspen understory and canopy support greater invertebrate abundance than coniferous trees, and therefore *gilvus's* larger body size may be attributed to a greater quantity of high quality food and the less compactness of deciduous stands (Rumble et al. 2001). While *gilvus* is described to be an overall brighter bird with yellower flanks (Pyle 1977; Phillips 1991; Lovell 2010), a lot of plumage variation exists among conspecific populations geographically (pers obs) (Appendix 1.5). Noticeable differences in song have been found between *gilvus* and *swainsonii*, where eastern songs are a lot longer with more syllables compared to the shorter, choppier song of *swainsonii* (Lovell 2010). Song of the second western subspecies, *brewsteri*, has yet been studied. In a morphologically cryptic complex, song could be an important distinguishing character among eastern and western subspecies' populations and a barrier to reproductive isolation.

2.5 Conclusions

Distinct population genetic structure is present in warbling vireos within their North American range. Our genetic data at both mtDNA and microsatellites support our hypothesis of finding a west – east split, with the observed genetic patterns in western and eastern warbling vireos similar to patterns found in other birds (Lait and Burg 2013) and mammals (Aubry et al. 2009). Data from two, evolutionarily different mtDNA genes revealed a single lineage for the western subspecies; however, population genetic structure revealed by microsatellite loci suggests the separation between *swainsonii* and *brewsteri* is relatively recent and may be influenced by contemporary processes, such as elevation and habitat. This study highlights the risk of relying on a single method, molecular or morphological, to distinguish recently diverged, morphologically cryptic taxa. Using multiple molecular markers with morphological data can more powerfully describe discrete taxa or populations for the importance of accurate biodiversity estimates, taxonomy, and conservation implications.

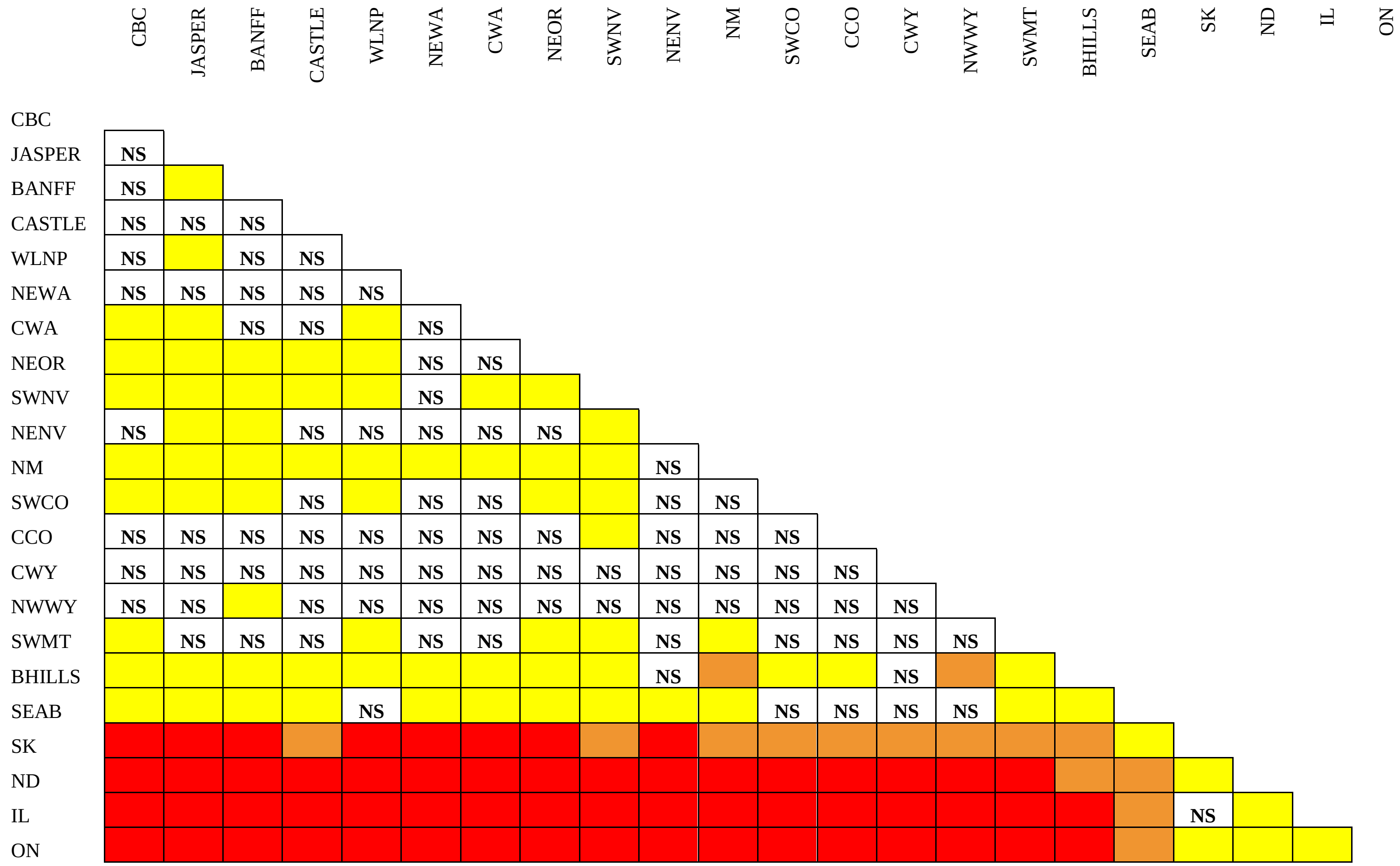
Table 2.1 Population ID and the number of individuals (n) used in cyt b, ATPase 6 and 8, and microsatellite analyses. Populations with a ^{M} were included in the morphological comparisons. See Appendix 1.1 for additional details by individual.

Population ID	**cyt b**	**ATPase 6 and 8**	**Microsatellites**
NWBC (Northwest British Columbia)^{M}	3	5	5
CBC (Central British Columbia) ^{M}	2	4	24
JASPER (Jasper National Park, Alberta) ^{M}	2	2	13
BANFF (Banff National Park, Alberta) ^{M}	-	-	15
CASTLE (Castle Provincial Park, Alberta) ^{M}	1	8	17
WLNP (Waterton Lakes National Park, Alberta) ^{M}	-	4	9
NEWA (Northeast Washington) ^{M}	3	-	14
SWBC (Southwest British Columbia) ^{M}	2	-	5
NWWA (Northwest Washington)	-	-	1
VI (Vancouver Island)	1	-	4
CWA (Central Washington)	-	-	20
NEOR (Northeast Oregon) ^{M}	3	3	10
SEOR (Southeast Oregon)	-	-	1
CoOR (Coastal Oregon) ^{M}	1	2	2
CoCA (Coastal California)	-	-	3
NECA (Northeast California) ^{M}	-	-	3
SECA (Southeast California)	-	-	4
SWNV (Southwest Nevada)	1	-	10
NENV (Northeast Nevada)	-	-	10
UT (Utah) ^{M}	1	4	4
NM (New Mexico) ^{M}	1	-	8
SWCO (Southwest Colorado)	1	1	13
CCO (Central Colorado) ^{M}	2	3	13
CWY (Central Wyoming) ^{M}	1	-	6
NWWY (Northwest Wyoming) ^{M}	1	1	10
SWMT (Southwest Montana) ^{M}	-	7	29
CMT (Central Montana) ^{M}	-	-	4
B HILLS (Black Hills, South Dakota)	9	-	34
SEAB (Southeast Alberta) ^{M}	22	-	24
CAB (Central Alberta) ^{M}	1	-	1
SK (Saskatchewan) ^{M}	-	-	10
ND (North Dakota) ^{M}	4	-	16
SESD (Southeast South Dakota) ^{M}	1	-	1
MN (Minnesota)	-	-	4
IL (Illinois)	-	-	8
OH (Ohio) ^{M}	-	-	5
ON (Ontario)	2	-	17

Table 2.2 Microsatellite primer names and sequences, average number of alleles per locus (n), range (bp size), annealing temperature (T_A), average expected and observed heterozygosity (H_E/H_O), and sources with the species the primers were originally designed for. Sequences marked with an asterisk were modified from the original source.

Locus	Primers	Alleles (n)	Range (bp)	T_A (°C)	H_E	H_O	Source and species
BCV2-6	F 5' CTTGGATAGAGCAGCCTCTG 3' R 5' CAGTCTTATCATTGCAGGATAGC 3'	4	204-220	52/54	0.50	0.44	Barr et al. 2007 *Vireo atricapilla*
BCV4-5	F 5’ TACGAGGATGTCACCGTATG 3’ R 5’ CGGTTACTCAACACAGACCTC 3’	13	257-350	52/54	0.88	0.87	Barr et al. 2007 *V. atricapilla*
BCV4-6 New	*F 5’ GCGCAGACAAAAGGATCTA 3’ R 5’ ATGGAGGTGGGAGGGAATA 3’	7	174-220	50/52	0.71	0.59	Barr et al. 2007 *V. atricapilla*
Ck.1B5D	F 5’ ACTGCGTGTCCTGAATACGC 3’ R 5’ ACAAGACCAGAGCTTCAGCT 3’	3	116-124	52/54	0.34	0.36	Tarr & Fleischer 1998 *Corvus kubaryi*
Ck.4B6D	F 5’ TTGCATCCCTGATTTATGGC 3’ R 5’ CTAGGAAGCAATCCAGAGTC 3’	2	162-171	52/54	0.36	0.40	Tarr & Fleischer 1998 *C. kubaryi*
Ck.2A5A	F 5’ TGCTAAGCACAGTTAGAGAC 3’ R 5’ GAAGACAGGCAGGAGAGTTG 3’	4	155-171	52/54	0.30	0.30	Tarr & Fleischer 1998 *C. kubaryi*
Hofi5	F 5’ ACTGCAGAAAGCGATCACATTACG 3’ R 5’ GGCCCTCATTCCTCCTGCTTAC 3’	10	348-378	50/52	0.85	0.82	Hawley 2005 *Carpodactus mexicanus*
Pocc1	F 5’ TTCTGTGCTGCAATCACACA 3’ R 5’ GCTTCCAGCACCACTTCAAT 3’	9	226-250	55/57	0.77	0.80	Bensch et al. 1997 *Phylloscopus occipitalis*
ApCo46	F 5’ GGGAGCCTAGTATGTTAAGATGCT 3’ R 5’ TTCCAGGTGAGGTGATTCAGC 3’	4	143-175	50/52	0.52	0.57	Stenzler & Fitzpatrick 2002 *Aphelocoma coerulescens*
Lox1	F 5’ ATGATGGTAAGTCTAATGAAAGC 3’ R 5’ CCACACACATTCACTCTATTG 3’	4	274-286	50/52	0.31	0.28	Piertney et al. 1998 *Loxia scotica*
Ase18	F 5’ ATCCAGTCTTCGCAAAAGCC 3’ R 5’ TGCCCCAGAGGGAAGAAG 3’	4	210-230	55/57	0.54	0.56	Richardson et al. 2000 *Acrocephalus sechellensis*
CmAAAG30	F 5’ GGGATCTGTATTCATAGCATAAC 3’ R 5’ AATGGTAGAAACAGGAATTAGTG 3’	15	250-400+	55/57	0.90	0.93	Williams et al. 2004 *Cyanocorax morio*
Ppi2New	F 5’ CACAGACCATTCGAAGCAGA 3’ *R 5’ GGCTGTGAGCAGTGTCTGAA 3’	5	192-202	50/52	0.59	0.58	Martinez et al. 1999 *Pica pica*
Ck.1B6G	F 5’ ATGGAGTGGAGGGAGAGATG 3’ R 5’ AGTACTACCAGTTCACCTGC 3’	3	150-160	55/57	0.35	0.37	Tarr and Fleischer 1998 *C. kubaryi*

Table 2.3 Pairwise F_{ST} comparions for 22 populations (n > 5) based on 14 microsatellite loci. Following the F_{ST} classes outlined by Hartl and Clark (1997): NS = < 0.5, yellow = 0.05 – 0.15, orange = 0.15 – 0.25, and red = > 0.25. See Appendix 1.2 for F_{ST} values and *p*-values.



	CBC	JASPER	BANFF	CASTLE	WLNP	NEWA	CWA	NEOR	SWNV	NENV	NM	SWCO	CCO	CWY	NWWY	SWMT	BHILLS	SEAB	SK	ND	IL	ON
CBC																						
JASPER	NS																					
BANFF	NS	🟨																				
CASTLE	NS	NS	NS																			
WLNP	NS	🟨	NS	NS																		
NEWA	NS	NS	NS	NS	NS																	
CWA	🟨	🟨	NS	NS	🟨	NS																
NEOR	🟨	🟨	🟨	🟨	🟨	NS	NS															
SWNV	🟨	🟨	🟨	🟨	🟨	NS	🟨	🟨														
NENV	NS	🟨	🟨	NS	NS	NS	NS	NS	🟨													
NM	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	NS												
SWCO	🟨	🟨	🟨	NS	🟨	NS	NS	🟨	🟨	NS	NS											
CCO	NS	NS	NS	NS	NS	NS	NS	NS	🟨	NS	NS	NS										
CWY	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS									
NWWY	NS	NS	🟨	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS								
SWMT	🟨	NS	NS	NS	🟨	NS	NS	🟨	🟨	NS	🟨	NS	NS	NS	NS							
BHILLS	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	NS	🟧	🟨	🟨	NS	🟧	🟨						
SEAB	🟨	🟨	🟨	🟨	NS	🟨	🟨	🟨	🟨	🟨	🟨	NS	NS	NS	NS	🟨	🟨					
SK	🟥	🟥	🟥	🟧	🟥	🟥	🟥	🟥	🟧	🟥	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟨				
ND	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟧	🟧	🟨			
IL	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟧	NS	🟨		
ON	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟧	🟨	🟨	🟨	

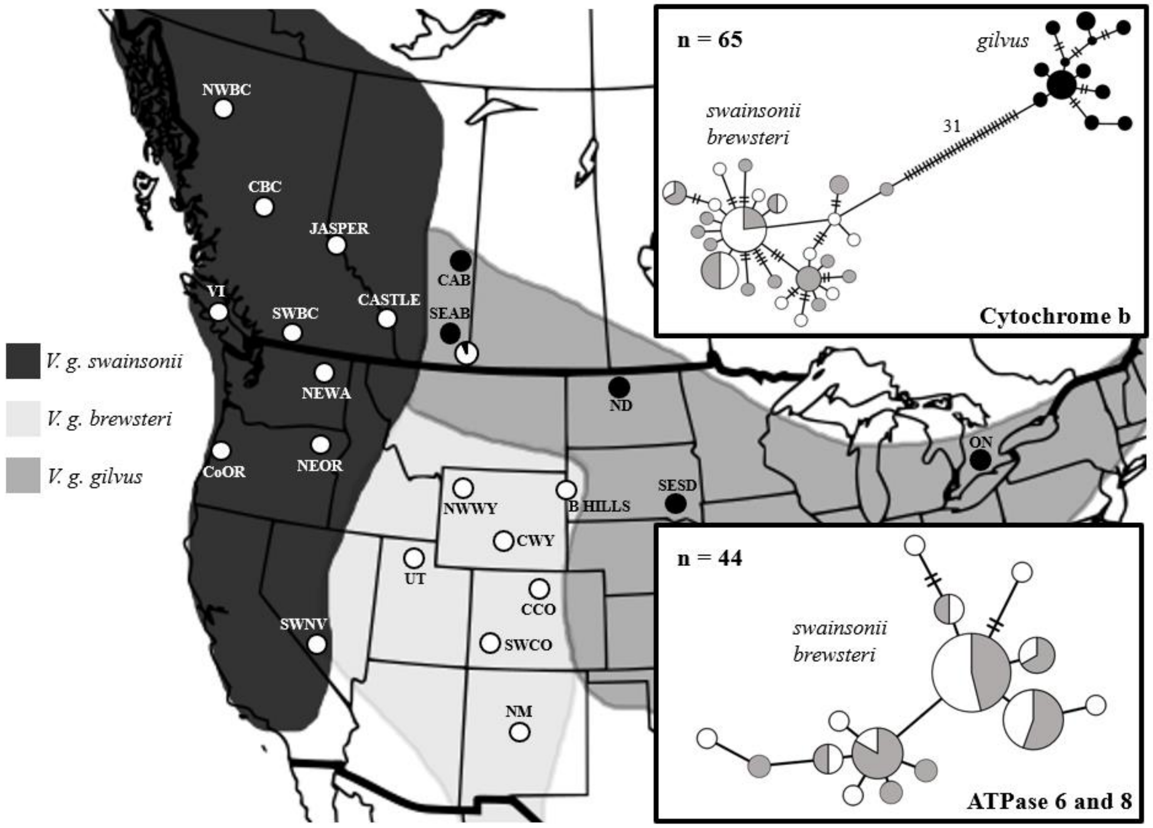


Figure 2.1 Range distributions of the three warbling vireo subspecies in this study based on Phillips (1991) and AOU (1988). Circles on the map are sampling locations included in the cyt b analysis (top inset photo). Black represents *gilvus* haplotypes, while white represents *swainsonii* and *brewsteri* haplotypes. The ATPase 6 and 8 results are not on the map (bottom inset photo) and do not include *gilvus*. Gray represents *swainsonii* haplotypes, while white represents *brewsteri* haplotypes. The size of the circle in both minimum spanning haplotype networks indicate how many individuals share a haplotype, while each cross hatch indicates more than one nucleotide difference between haplotypes. The total number of individuals included in each analysis is represented by n. Refer to Table 2.1 for sample size by population.

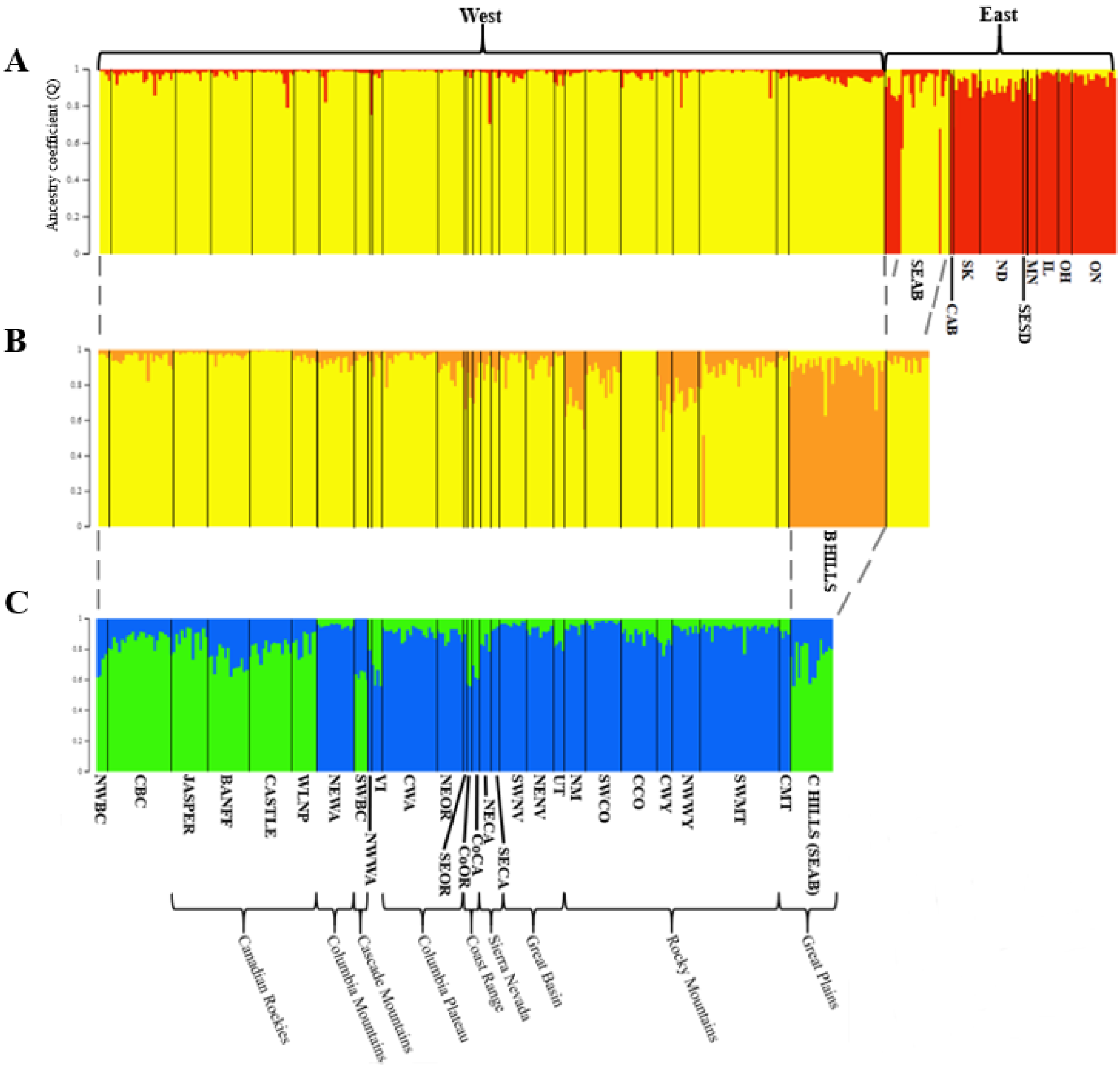


Figure 2.2 Hierarchical Structure plots (K=2) based on 14 microsatellite loci. **A)** All subspecies' populations (red cluster = *gilvus*), **B)** Western subspecies' populations and the Black Hills (orange cluster), **C)** Remaining western subspecies' populations (green cluster = *swainsonii*, blue cluster = *brewsteri*).

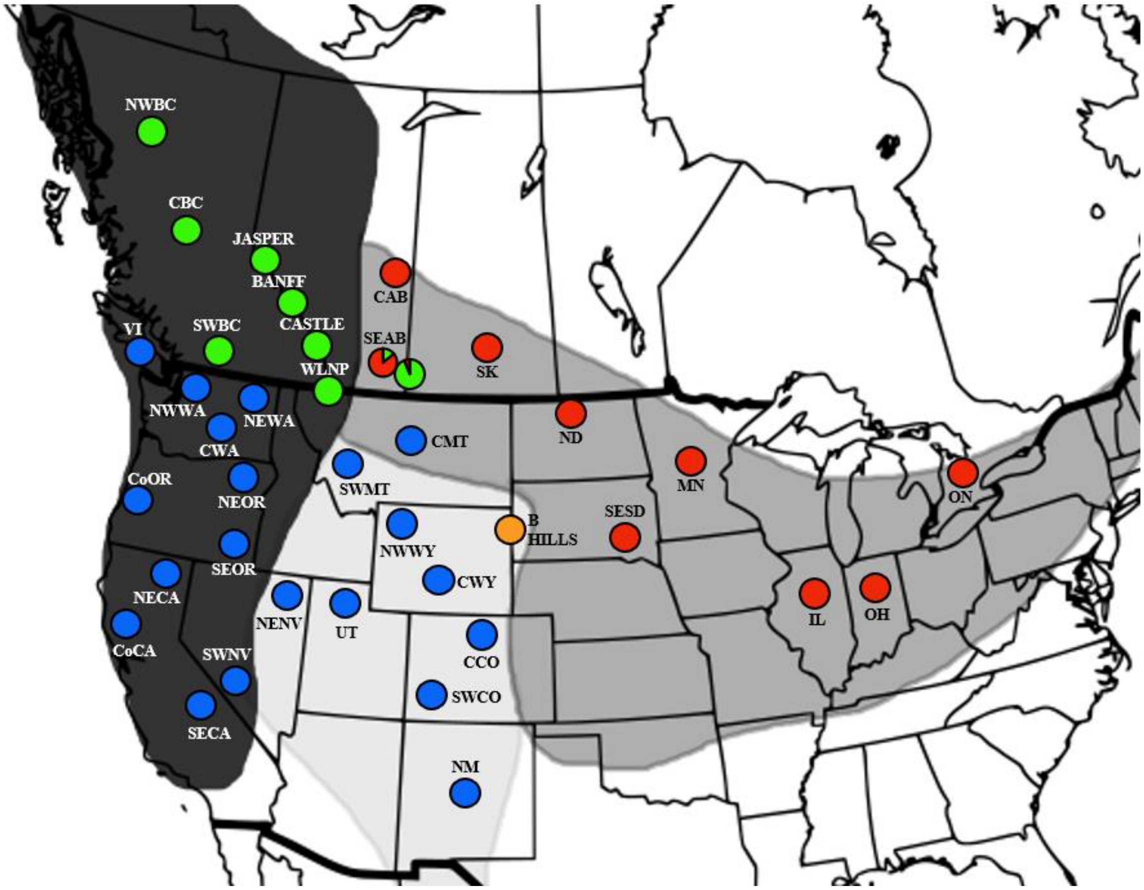


Figure 2.3 Location of populations genotyped at 14 microsatellite loci and colored based on the output from Structure: red (*gilvus*), green (*swainsonii*), and blue (*brewsteri*) (see Fig 2.2). Refer to Table 2.1 for sample sizes by population.

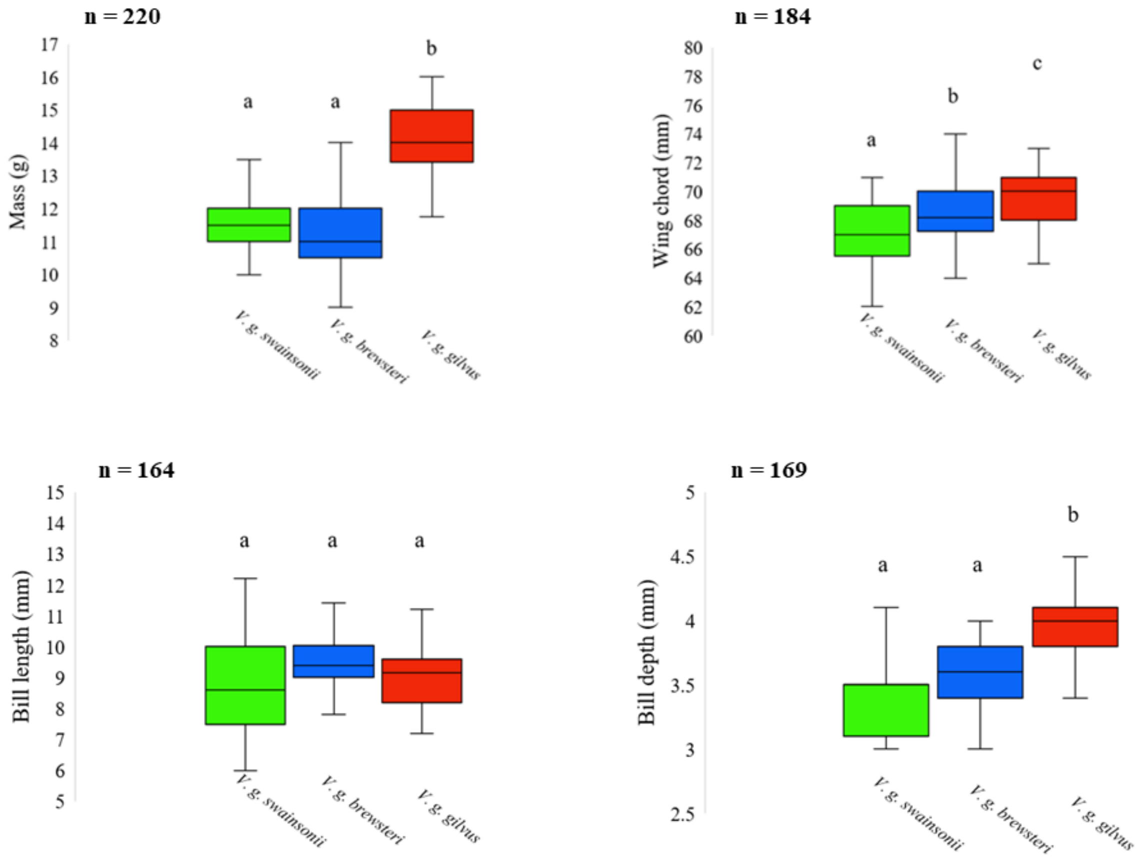


Figure 2.4 Mixed model ANOVA results from four morphological comparisons between subspecies populations: red (*gilvus*), green (*swainsonii*), and blue (*brewsteri*). Characters that were significant after *post hoc* Tukey tests are represented by different lowercase letters and n is the number of individuals compared for each model. Whiskers represent the lowest and highest value range for that morphometric character, and the line inside of each boxplot represents the morphometric character's mean value. See Appendix 1.3 for *p*-values, *F*-values, standard deviation, and degrees of freedom.

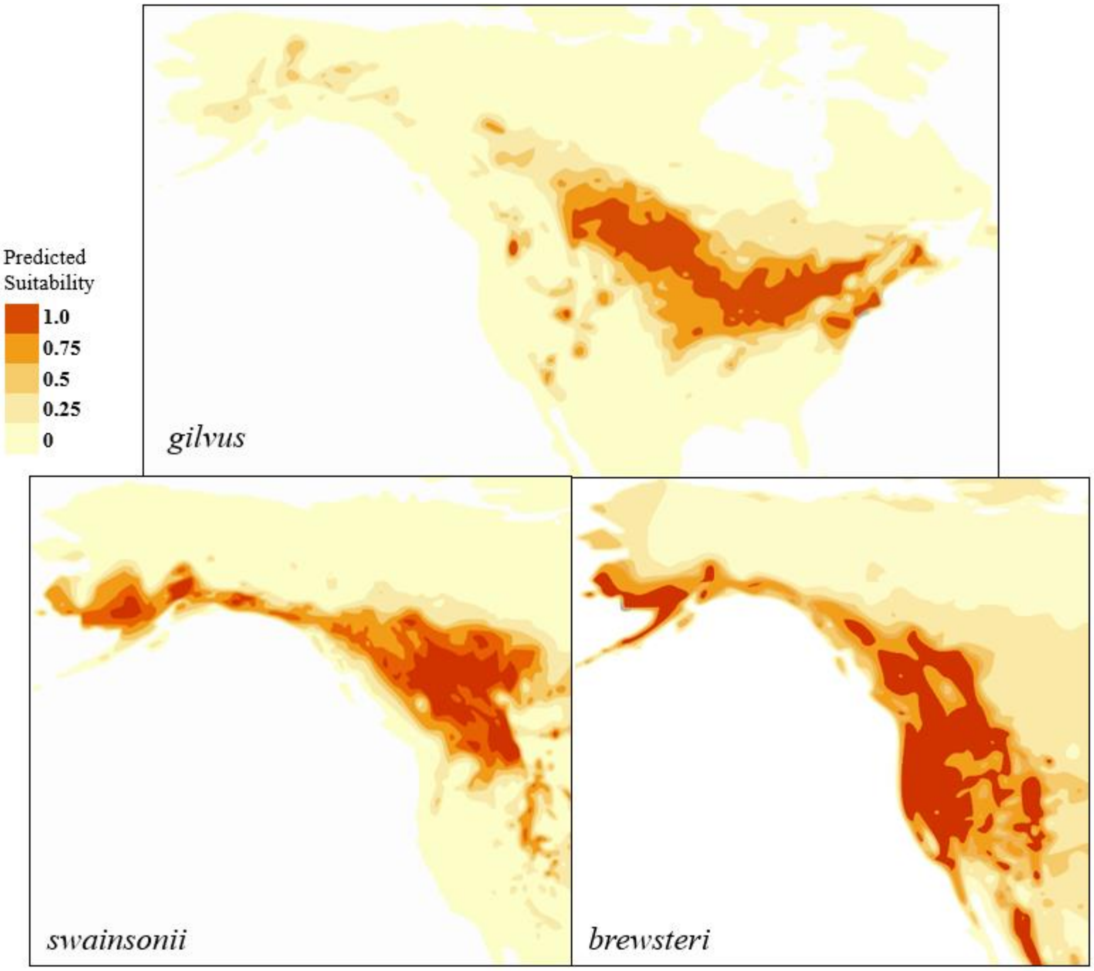


Figure 2.5 Ecological niche models for *gilvus*, *swainsonii*, and *brewsteri*. The logarithmic scale depicts the percent likelihood of suitability based on climatic variables (see Appendix 1.4).

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Chapter 3: Making contact: examining hybridization in populations of warbling vireo (*Vireo gilvus*) in a previously reported contact zone in Alberta and an unreported contact zone in western North America

3.1 Introduction

Reproductive isolation occurs when barriers between populations accrue to such that gene flow between them is greatly reduced or absent (Coyne and Orr 2004). Barriers to gene flow are often geographic, but they can also be behavioral, including assortative mating or differences in migratory timing (Weir and Schluter 2004; Uy et al. 2018). Geographic barriers may be ephemeral to restricting gene flow between populations. With the retreat of the ice sheets during the last glacial maximum (LGM), isolated populations expanded their ranges from ice-free refugia, some experiencing secondary contact in areas known as contact zones. Genetic patterns seen in populations at contact zones are byproducts of prolonged isolation during the LGM coupled with interactions of contemporary processes (Graham and Burg 2012; Hewitt 2000; Hewitt 2001). The completion of reproductive isolation is highly variable across taxa, making the outcome of secondary contact between closely related species or subspecies unpredictable (Martin et al. 2017; Stelkens et al. 2010).

Three scenarios have been described for paired taxa that meet within contact zones: 1) if strong reproductive isolating barriers evolved in isolation, the two taxa remain distinct, 2) if moderate or partial reproductive isolating barriers evolved between paired taxa, a stable hybrid zone forms, or 3) if there are weak reproductive isolating barriers between paired taxa, the result can be widespread hybridization and introgression (Barton and Gale 1993; Barton and Hewitt 1989). Studying hybridization in natural populations is advantageous to our understanding of speciation, in how it affects biodiversity.

There is evidence to suggest that the warbling vireo complex (*Vireo gilvus*) diverged during the Pleistocene, with their secondary contact zone in western North

America as a result of range expansion following the LGM (Chapter 2; Lovell 2010). These three subspecies of warbling vireo, two western subspecies (*V. g. swainsonii* and *V. g. brewsteri*) and one eastern subspecies (*V. g. gilvus*), are thought to have a large south – north contact zone from the southwestern United States through northern Alberta, with all three subspecies meeting at a contact zone in northwest Montana (Figure 3.1). Their current range distributions and taxonomic status are based on subtle morphological and plumage differences, but lack molecular evidence (AOU 1998; Phillips 1991).

A previous study by Lovell (2010) examined a contact zone between the nominate western subspecies, *swainsonii*, and the eastern subspecies, *gilvus*, in central Alberta using a single mtDNA molecular marker with morphological and song data. Due to the uniparental inheritance of mtDNA, assessing the presence of hybrids is not possible, but steep clines found between the subspecies in song and morphology suggest a tension zone model for this contact zone (Lovell 2010). MtDNA coupled with a biparentally inherited marker, i.e. nuclear DNA, can detect hybridization because an individual will either have mixed assignment between both parental populations at nuclear loci, or have the nuclear DNA of one parental population and mtDNA ancestry of the other, a phenomenon known as cytonuclear discordance (Arnold 1993). Based on the need for a nuclear marker to confirm the findings in Lovell's study (2010), we used microsatellite loci to examine the presence of hybridization in and outside of the contact zone in Alberta between *swainsonii* and *gilvus*. Subspecies specific primers were also developed from mtDNA cytochrome b (cyt b) sequences to screen individuals and compare their mtDNA assignment to their nuclear assignment at microsatellite loci. Based on Lovell's study (2010) and data from our range wide subspecies genetic structure (Chapter 2), we hypothesized a similar pattern of nuclear haplotypes outside of and within the contact zone to mtDNA haplotypes, with little evidence of hybridization between these well differentiated subspecies.

To date, no study has examined nor confirmed that the western subspecies meet along a contact zone in the western United States, nor that all three subspecies meet in a contact zone in northwest Montana. Because we found a single western mtDNA lineage in our range wide subspecies genetic structure (Chapter 2), the use of a mtDNA marker was excluded between the western subspecies as it would not provide any resolution of cytonuclear discordance. Microsatellites were used to detect hybridization between *swainsonii* and *brewsteri* on the premise that a hybrid individual would exhibit a mixture of genetic assignment between both parental populations. Concordant to genetic and habitat patterns found among all three subspecies in Chapter 2, we hypothesized that the eastern subspecies, *gilvus*, does not meet at the contact in northwest Montana with the two western subspecies based on habitat typical of *gilvus*. While the delineation of the western subspecies is more unclear based on geographic barriers, we hypothesized that higher levels of hybridization will be found between *swainsonii* and *brewsteri* at their contact zone because of the more recent separation and similarity of these two subspecies.

3.2 Methods

3.2.1. Sample collection and acquisition

With no previous studies examining the contact zone between populations of the western subspecies or among all three subspecies of *V. gilvus* in northwest Montana, we sampled populations on either side of the subspecies' distributions in order to examine where pure and admixed populations occurred (i.e. the location of the contact zone). Based on our range wide subspecies population genetic structure (see Chapter 2), we found that *swainsonii* and *brewsteri* may also come into contact in southern British Columbia. Based on this information, we sampled six populations in the contact zone around northwest Montana, eight populations in southern British Columbia, and three additional populations at the contact zone in the current range distributions of the two western subspecies. Populations sampled for the Alberta contact zone were based on a transect previously reported between *swainsonii* and *gilvus* (Lovell 2010). We acquired samples from five of those populations in that study and added eight additional populations of our own (Table 3.1). Birds sampled in the field were caught during the early months of the breeding season, end of May to early July, to reduce the capture of family groups and to avoid catching migrants. Birds were caught using 12 m mistnets with song playbacks and each bird was banded to avoid resampling. A small (<50 μL) blood sample was taken from the brachial vein, along with a series of morphometric measurements (mass, wing chord, bill length, bill depth, bill width, and tarsus) and photographs. Blood samples were stored in 95% ethanol and all birds were released on site. We collected a total of 402 warbling vireo samples, and 199 tissue or blood samples were obtained from museum collections (Denver Museum of Nature and Science, Burke Museum of Natural History and Culture, University of Michigan Museum, and Royal Alberta Museum) from 48 sample sites, herein referred to as populations (Appendix 2.1). A population in this chapter is defined as a cluster of samples, and is not dependent on distance, because we wanted to examine how population genetic structure changed on small spatial scales in the contact zones.

3.2.2. MtDNA screening

A 1140 bp (base pair) region of the cyt b gene was amplified using primers H15944 and L14990 in six eastern (*gilvus*) and seven western (*swainsonii*) warbling vireos (Lovell 2010; Sorenson et al. 1999). Successfully amplified samples were sent to NanuQ at McGill University, Montreal, Quebec for sequencing. Sequences were checked and aligned using MEGA X v. 010.1 and sequences were aligned using a reference warbling vireo cyt b sequence (GenBank #: AY030111) (Kumar et al. 2018). We examined fixed nucleotide sites in these *gilvus* and *swainsonii* sequences to develop subspecies specific primers to screen samples along and outside of the contact zone in Alberta. Paired with the L14990 primer, we developed the H15469 Gilvus primer (5' ACGAAGGGTAGTAG**C**AAA 3') and H15634 West (5' GAGAATAGGGCTAGG**T**G 3') introduced with mismatches in the sequences (underlined and bolded) (Sorenson et al. 1999). With these subspecies' specific primers, a total of 131 individuals were screened from outside of and in the contact zone between *gilvus* and *swainsonii* in Alberta and Saskatchewan (Table 3.1). The 25 µL PCR (polymerase chain reaction) contained Green GoTaq® Flexi buffer (Promega), 0.2 mM dNTP, 2 mM $MgCl_2$ (Promega), 0.5 µM H strand primers (H15469 Gilvus or H15634 West), 0.5 µM L14990 primer, 0.5 U GoTaq® Flexi polymerase (Promega), and ~ 1 ng of genomic DNA (diluted 1:10 for blood or tissue, 1:5 for feathers). The thermocycling profile for both H15469 Gilvus and H15634 West was one cycle for two minutes at 94°C, 45 seconds at 48°C, and one minute at 72°C; followed by 37 cycles for 30 seconds at 94°C, 45 seconds at 48°C, and one minute at 72°C; concluding with 72°C for five minutes. PCR products were run on a 0.8% agarose gel and identified based on size to a standard DNA ladder: H15469 Gilvus ~ 550 bp and H15634 West ~ 600 bp. Samples that amplified artifacts or had a faint band were sequenced to ensure correctness of identification (all 17 birds from the Cypress Hills, two birds from Swan Hills, and one bird from W Edmonton).

3.2.3 Microsatellites

3.2.3.1. Genotyping

A subset of samples (n=6) from different geographical areas were screened with 57 passerine loci. A total of 30 loci could be optimized in warbling vireos, 14 of which were polymorphic and used in our study to genotype 601 warbling vireos from outside of and in the contact zones: BCV2-6, BCV4-5, BCV4-6New (Barr et al. 2007; modified BCV4-6 F), Ck.1B5D, Ck.4B6D, Ck.2A5A, Ck.1B6G (Tarr and Fleischer 1998), Hofi5 (Hawley 2005), Pocc1 (Bensch et al. 1997), ApCo46 (Stenzler and Fitzpatrick 2002), Lox1 (Piertney et al. 1998), Ase18 (Richardson et al. 2000), Ppi2New (Martinez et al. 1999; modified R), and CmAAAG30 (William et al. 2004).

The 10 µL PCR contained FroggaBio buffer, 0.2 mM dNTP, 0.5 µM of forward primer, 1 µM of the reverse primer, 0.05 µM of the M13 fluorescently labeled primers, 1 U of FroggaBio Taq, and ~ 1 ng of genomic DNA (1:10 dilution for blood and tissue; 1:5 for feathers). The following thermocycling profile was used to amplify DNA: one cycle for one minute at 94°C, 45 seconds at T_1, and one minute at 72°C; seven cycles of one minute at 94°C, 30 seconds at T_1, and 45 seconds at 72°C; 25 cycles for 30 seconds at 94°C, 30 seconds at T_2, and 45 seconds at 72°C; concluding with one cycle of 72°C for five minutes. For DNA amplification, five loci (BCV4-6New, Hofi5, ApCo46, Lox1, and Ppi2New) were amplified using T_1=50°C and T_2=52°C, five loci (BCV2-6, BCV4-5, Ck.1B5D, Ck.2A5A, and Ck.4B6D) were amplified using T_1=52°C and T_2=54°C, and four loci (Pocc1, Ase18, CmAAAG30, and Ck.1B6G) using T_1=55°C and T_2=57°C. Three loci (BCV4-5, ApCo46, and Ppi2New) required 31 cycles instead of 25 cycles. Products were denatured and run on a 6% acrylamide gel on a Li-Cor 4300 DNA Analyzer. To maintain consistent sizing and scoring of alleles, the same three positive controls of known sizes were run with each load for each locus. All gels were scored independently by two people and a subset of samples from each gel was re-run to ensure consistent scoring across gels. A total of 528 samples from 48 populations were selected for this study after eliminating individuals that were missing genotypic information at more than seven loci (50% data) (Table 3.1).

3.2.3.2. Bayesian clustering

The program Structure v2.3.4 (Hubisz et al. 2009) uses a Bayesian clustering model and a Markov chain Monte Carlo (McMC) method to examine population structure and assign individuals to a genetic cluster (K) based on multi-locus genotype data (Pritchard et al. 2000). An individual's membership to a cluster is determined by the proportion of its genotype (Q) that belongs to any given K (Pritchard et al. 2000). For our analyses, we used correlated alleles, admixture model, and sampling locations as *locpriors*. The correlated allele frequency model can detect distinct populations that are closely related and when genetic data have weak hierarchal structure, the *locpriors* setting can improve the model by providing the knowledge of the sampling location (*locpriors*) for each individual (Hubisz et al. 2009; Porras-Hurtado et al. 2013). Initial Structure runs were performed with 50 000 burn-ins and 100 000 McMC repetitions for K=1 to 5. Methodology outlined in the Structure manual was used to determine the optimal number of genetic clusters (Pritchard et al. 2010). Following the initial Structure run, three genetic groups (K=3) were detected. Subsequent runs were executed using individuals from each group separately at K=2 to provide better resolution of hierarchal structure. Outputs from each Structure run were averaged and graphed using Structure Plot V2.0 (Ramasamy et al. 2014).

3.3 Results

3.3.1. MtDNA screening

Using subspecies specific primers developed for this study, H15469 Gilvus and H15634 West, 131 individuals from sixteen populations across the province of Alberta and a population in Saskatchewan were screened and assigned to a mtDNA lineage (Table 3.1). Three populations (BARRHEAD, W EDMONTON, and CYP HILLS) had individuals that assigned to both mtDNA lineages, while all other populations were all *swainsonii* or *gilvus* (Figure 3.2). BARRHEAD had the greatest mix of mtDNA haplotypes, with 16 individuals grouping east and 8 individuals grouping west. In the CYP HILLS, all individuals except for one (16 of 17) grouped west and in W EDMONTON, all but one individual (8 of 9) grouped east (Figure 3.2).

3.3.2. Microsatellites

A total of 529 birds were genotyped at 14 microsatellite loci from 48 populations outside of and in their contact zones to determine the size of the contact zones and examine the presence of hybridization between subspecies (Table 3.1).

The initial run with all 48 populations indicated that the optimal number of genetic clusters was K=3. We found that the populations included to examine the west contact zone and the contact zone in northwest Montana among all three subspecies formed two genetic clusters, *swainsonii* and *brewsteri* (herein referred to collectively as the west contact zone) (Figure 3.3 A). Within the west contact zone, ten populations included hybrid individuals and individuals from one or both parental populations (REVELSTOKE, ENDERBY, KELOWNA, OK FALLS, OSOYOOS, CID, NELSON, WHITEFISH, CHOTEAU, and RED LODGE), while all other populations were typical of *swainsonii* or *brewsteri* with a Q value of $\geq$ 60% (Figure 3.4). These data further support the findings of our range wide subspecies' genetic groups (Chapter 2), where the contact zone between the two western subspecies does not match its current location in their range. Populations included to examine the Alberta contact zone were also from two genetic clusters, representing the western subspecies s*wainsonii* and the eastern subspecies *gilvus* (Figure 3.3 B). Two populations in Alberta included hybrids individuals and individuals from both parental populations (BARRHEAD and CYP HILLS), while all other populations were typical of *swainsonii* or *gilvus* with a Q value of $\geq$ 60% (Figure 3.4). Five individuals had cytonuclear discordance in three populations (BARRHEAD, W EDMONTON, and CYP HILLS) (Figure 3.3 B). Our findings of Barrhead County being the most admixed (3 hybrids, 6 *swainsonii*, and 16 *gilvus*) support findings in a previous study (Lovell 2010).

3.4 Discussion

3.4.1. West contact zone

3.4.1.1. Western United States and British Columbia

A primary objective of this chapter was to investigate the speculated contact zone among all three subspecies in northwest Montana and the contact zone between the two western subspecies, s*wainsonii* and *brewsteri*, using molecular evidence. Previous research has emphasized the importance of historical events on the genetic composition of contemporary populations and where they come into contact (Avise 2000). Based on our range wide subspecies population genetic structure (Chapter 2), we found no genetic separation in mtDNA between the two western subspecies, but detected substructure in nuclear DNA suggesting that contemporary processes may be causing genetic differences between them. The genetic pattern had us further hypothesize that s*wainsonii* and *brewsteri*'s ranges may also meet somewhere in southern British Columbia where there was an abrupt transition in genetic groups, rather than in the Sierra Nevada and through the Rocky Mountains (Figure 3.1).

Our microsatellite data revealed six populations in southern British Columbia and one population in Idaho (CID) with hybrid individuals. Five out of six populations in southern British Columbia had an abundance of pure s*wainsonii* individuals and genetic composition of hybrid individuals had a greater Q value to *swainsonii* than *brewsteri*. Within these populations (REVELSTOKE, ENDERBY, KELOWNA, OK FALLS, and OSOYOOS), habitat is more typical to *swainsonii* including white spruce (*Picea glauca*) and trembling aspen (*Populus tremuloides*); however, some tree and grass species typical of *brewsteri* habitat including Douglas-fir (*Pseudotsgua menziesii*), mountain hemlock (*Tsuga mertensiana*), and sagebrush-steppe, are also found in these areas (Meidinger and Pojar 1991; Demarchi 2011). Lower elevation may better explain the greater abundance of pure *swainsonii* and s*wainsonii* hybrid individuals, as these populations range from approximately 400 – 1300 m. Based on our range wide subspecies population genetic structure (Chapter 2), we found that s*wainsonii* occupies an elevation of 400 – 1500 m, while *brewsteri* occupies a broader and higher elevation of 200 – 3000 m. In ENDERBY specifically, we found that all three hybrid individuals occupy the points of higher elevation (1200 m), while all three pure *swainsonii* individuals occupy the points of lower elevation (800 m). This pattern has also been observed in the Andes with recently diverged flycatchers (*Anairetes reguloides* and *A. nigrocristatus*) where *A. reguloides* are adapted to occupy a broad elevation range, *A. nigrocristatus* are more restricted to higher elevations, and their hybrid zone is found in between (DuBay and Witt 2014).

The sixth population in British Columbia, NELSON, contains the greatest number of hybrid individuals in this region (8 out of 9). NELSON is similar to REVELSTOKE in habitat; however, all birds were caught at higher elevation (1700 m) compared to the REVELSTOKE population (400 – 1100 m) and may explain why a higher genetic composition from *brewsteri* is seen in these hybrids (Figure 3.3 A and 3.4). While one hybrid was found in CID, the other three CID individuals' group with *brewsteri*, following the trend of *brewsteri* birds found in higher elevation areas (2000 m). One additional population, CNV, located in the current described ranges of the western subspecies as a contact zone, clustered genetically with *brewsteri* only, indicating that the two western subspecies do not meet at this area in the southwestern United States. This agrees with habitat (mixed conifer / sagebrush steppe) and more specifically elevation (1800 – 2000 m) typical of *brewsteri*. These data add support to our overall, range wide subspecies genetic structure found in Chapter 2.

3.4.1.2. Montana

Among our seven populations included to examine the contact zone among all three subspecies in northwest Montana, we did not find genetic evidence of *gilvus*. We did, however, find an extension of *swainsonii* and *brewsteri's* contact zone into Montana (Figure 3.4). Three populations are admixed, with greater abundance of s*wainsonii* and *swainsonii* hybrids in WHITEFISH and CHOTEAU, and greater abundance of *brewsteri* and *brewsteri* hybrids in RED LODGE.

WHITEFISH contains a mosaic of tree species found in typical *swainsonii* habitat, including the boreal white spruce and white bark pine (*Pinus albicaulis*), but also has mountain hemlock and Douglas-fir that are typical of *brewsteri* (Vance et al. 2017). At an elevational scale, our sampling site in WHITEFISH is at 900 m, which corresponds greater to s*wainsonii's* preference. CHOTEAU has similar tree species as WHITEFISH, with the exclusion of mountain hemlock, but birds were collected at 1200 m and this location is in the transition of the continental divide of the Rocky Mountains. This slight increase in elevation, or differences in climatic variables compared to WHITEFISH on the west side of the divide, may explain why a few (3 of 10) individuals have greater genetic composition from *brewsteri*. RED LODGE contains an abundance of pure *brewsteri* (13 of 30) individuals and the most hybrids (15 of 30) in our Montana sampling, with hybrids having a greater genetic composition from *brewsteri* than *swainsonii* (Figure 3.3 A). Pockets of typical habitat of *swainsonii* are found here (coniferous and deciduous mix), however, this area also contains a greater mix of conifer species including Douglas-fir, lodgepole pine (*Pinus contorta*), and single-leaf pinyon (*Pinus monophyla*) typical of *brewsteri* habitat (Vance et al. 2017). The elevation of RED LODGE falls outside of *swainsonii's* elevational range based on our range wide sampling (Chapter 2), but is typical for *brewsteri* (2000 m). This may explain why we see hybridization of *brewsteri* into *swainsonii*, but *brewsteri* hybrids are difficult to explain given that habitat is suitable from an elevation perspective. Further research on the relationship between elevation and habitat in RED LODGE could help resolve this genetic pattern. In a study on Canada Jays (*Perisoreus canadensis*), one mtDNA clade (Transcascades) was found in the Columbia Plateau, northern Idaho, and northern Rockies, while the other clade (Boreal) was found in the central Rocky Mountains of Montana, revealing a close relationship between populations, elevation, and habitat (Els et al. 2012).

Other populations we examined in and outside of the contact zones grouped entirely with *swainsonii* or *brewsteri* ≥ 60% assignment. These populations included preferred habitat typical of *swainsonii* or *brewsteri* overall, but the observation of elevational differences was the most compelling distinction among them: Kamloops, Vernon, and NID (1000 - 1200 m) (s*wainsonii*), Bozeman (1600 m) (s*wainsonii*); Helena and Lewistown (1900 – 2300 m) (*brewsteri*).

3.4.2. Alberta contact zone

Our second objective of this chapter was to examine a contact zone examined previously between *swainsonii* and *gilvus* in central Alberta (Lovell 2010). Using mtDNA, Lovell (2010) reported the greatest admixture of haplotypes in Barrhead County where a transitional ecotone occurs between parkland and boreal forest habitat (Semenchuk 1992). Our genetic data from mtDNA cyt b and microsatellites support the findings in Lovell's study (2010) of BARRHEAD containing the greatest admixture of hybrid individuals and parental individuals, however our CYP HILLS and W EDMONTON populations also show evidence of cytonuclear discordance (Figure 3.3 B). The W EDMONTON population was newly sampled in this study and we collected seventeen samples in the Cypress Hills, whereas the previous study included nine (Lovell 2010).

These three discordant populations occur in different natural regions of Alberta with habitat typical of both *swainsonii* and *gilvus* (Semenchuk 1992). Situated 1,468 m above the surrounding fescue plains at its highest point, habitat of the CYP HILLS resemble the foothill region with predominant coniferous vegetation from Rocky Mountain and boreal forest species, but also some, sparse deciduous woodland species (Semenchuk 1992). Based on previous work by Lovell (2010) and our data, the disjunct ecology and elevation of the Cypress Hills Interprovincial Park is more suitable to the western subspecies, *swainsonii*, than the eastern subspecies, *gilvus*, with 15 out of 17 individuals clustering genetically with *swainsonii* (Figure 3.4). Asymmetries in *swainsonii's* range could provide a possible explanation for the *gilvus* bird and hybrid found in the Cypress Hills because this population is isolated within the range of the eastern subspecies (Figure 3.4). This pattern of cytonuclear discordance occurs in toads (*Bufo woodhousii* and *B. microscaphus*), where mtDNA from *woodhousii* introgressed into *microscaphus* because part of its range was nested within *woodhousii's* range (Malmos et al. 2008). There is no evidence of gene flow outside of the Cypress Hills in either our MEDICINE HAT or SK populations that surround it, while the proximity of Medicine Hat to the Cypress Hills is a short distance, around 60 km. Behavioral barriers or patches of unsuitable habitat between these two areas may suggest why gene flow of *swainsonii* is not found outside of the Cypress Hills.

The other two populations, W EDMONTON and BARRHEAD, are located on the edge of Parkland habitat, a transitional area between the drier grasslands of the plains and the coniferous dominated forests of the boreal forest; the latter (BARRHEAD) located on the southern cusp of the boreal forest (Semenchuk 1992). All four hybrids found in these two populations have cytonuclear discordance with west mtDNA ancestry and east nuclear DNA, with the exception of one individual showing equal assignment to the west and east genetic groups at nuclear DNA (Figure 3.3 B). We also found ≥ 60% assignment of 23 out of 33 birds to *gilvus* and 6 out of 33 birds to *swainsonii* in these two populations (Figure 3.4). An abrupt transition in song from *swainsonii* in northwest Barrhead to *gilvus* in southeast Barrhead was found by Lovell (2010), with no evidence of mixed singers in this contact zone.

While sampling birds in W EDMONTON, we caught individuals using a mix of eastern song with west calls. This call is a type of high-pitched screech that appears to occur more often in the west call than the east call, based on sonograms provided with the sounds on the Cornell Lab Merlin Bird ID app. Birds caught in SE EDMONTON (~ 31 km), for comparison, only responded to song and calls of the eastern subspecies, *gilvus*. A recent finding of *gilvus* song type along the Rocky Mountain front range west of Denver, Colorado led to a subsequent study of behavioral response to like and unlike song in this newly found contact zone (Floyd 2014; Spellman, pers comm.). Males of the western and eastern subspecies were found to respond equally to like and unlike song, no difference in male aggression (Spellman, pers comm.). A study on six species of Darwin's ground finches (*Geospiza*) found a similar pattern of males reacting equally to conspecific songs, suggesting other visual cues may be important for species recognition (Ratcliffe and Grant 1985).

While our evidence of using a combination of east song and west call playbacks in W EDMONTON is purely anecdotal, a more comprehensive study of behavioral response of female warbling vireos to like and unlike song would provide more resolution on the importance of song as a reproductive isolating barrier between *swainsonii* and *gilvus*. Given that the contact zone in Barrhead County and W Edmonton occurring at an ecotone transition follows the model of the bounded hybrid superiority hypothesis, we would then expect to see a greater number of hybrids than parental individuals (Barton and Hewitt 1985). The small number of hybrids found (4 of 33), lack of strong gene flow outside of this contact zone, and narrowness of the zone (~ 116 km) suggest a tension zone, or bimodal hybrid zone, between *swainsonii* and *gilvus*, a conclusion also suggested by Lovell (2010) (Barton and Hewitt 1985; Jiggins and Mallet 2000). Both the tension and bimodal hybrid zone models attest to the greater presence of parental individuals than hybrids inside of the zone, invariably due to some form of natural or sexual selection against hybrids (Jiggins and Mallet 2000).

3.4.4. General conclusions

Finding hybrids between divergent avian species is not uncommon, as studies have found that postzygotic reproductive isolating barriers (hybrid sterility and inviability) take a lot longer to develop than prezygotic reproductive isolating mechanisms (Price and Bouvier 2002). Other avian systems, including *Ficedula* flycatchers, have been found to maintain species differences in the wake of low frequency hybridization (Saetre et al. 2003). The small number (4 of 33) of hybrids between *gilvus* and *swainsonii* suggest strong reproductive isolation between the two, while a higher occurrence of hybrids between the western subspecies suggest the presence of weak reproductive isolating barriers and that *swainsonii* and *brewsteri* are in the early stages of speciation.

3.5 Conclusions

We have provided the first description of a contact zone and hybrid zone between the two western subspecies of warbling vireo, *swainsonii* and *brewsteri*, and supported a previous report of a contact zone between *swainsonii* and *gilvus* in central Alberta. We accept our hypothesis that our mtDNA and microsatellite data would reflect similar patterns in the Alberta contact zone and that the frequency of hybridization between s*wainsonii* and *gilvus* is low. Our data also support the location of the contact zone between *swainsonii* and *gilvus* found in a previous study (Lovell 2010); however, we did find cytonuclear discordances in two other populations not previously recognized, which highlights the importance of sampling and sample sizes in unique regions such as the Cypress Hills. Our hypothesis of a greater number of hybrids between s*wainsonii* and *brewsteri* was also supported, in addition to the western subspecies ranges coming into contact in southern British Columbia. Further ecological characterization between *swainsonii* and *brewsteri* is needed to reveal what is driving the separation of the two, as well as the potential role of hybridization in admixed populations. More thorough sampling of eastern Montana and eastern Colorado will show the western extent of *gilvus's* range and sampling the gap between eastern Idaho and western Montana would provide better resolution of the two western subspecies' contact zone and connectivity.

Table 3.1 Population ID/number (corresponds to Figure 3.4) with the number of individuals (n) used in the cyt b and microsatellite analyses. Populations with a L were used in a previous study. See Appendix 2.1 for details by individual.

Population ID	**cyt b**	**Microsatellites**
1. CBC (Central British Columbia)	-	24
2. JASPER (Jasper National Park, Alberta)	-	13
3. BANFF (Banff National Park, Alberta)	7	15
REVELSTOKE (British Columbia)	-	24
ENDERBY (British Columbia)	-	6
KAMLOOPS (British Columbia)	-	7
VERNON (British Columbia)	-	14
KELOWNA (British Columbia)	-	6
OK FALLS (British Columbia)	-	6
OSOYOOS (British Columbia)	-	15
4. SWBC (Southwest British Columbia)	-	5
5 / 6. CWA (Central Washington)	-	20
7. NEOR (Northeast Oregon)	-	10
8. SEOR (Southeast Oregon)	-	1
9. NECA (Northeast California)	-	4
10. SECA (Southwest California)	-	3
11. SWNV (Southwest Nevada)	-	10
12. CNV (Central Nevada)	-	9
13. NENV (Northeast Nevada)	-	10
CID (Central Idaho)	-	4
NID (Northern Idaho)	-	8
14. NEWA (Northeast Washington)	-	14
NELSON (British Columbia)	-	9
15. CASTLE (Castle Provincial Park, Alberta)	9	16
16. WLNP (Waterton Lakes National Park, Alberta)	7	9
WHITEFISH (Montana)	-	18
CHOTEAU (Montana)	-	10
HELENA (Montana)	-	29
BOZEMAN (Montana)	-	31
17. NWWY (Northwest Wyoming)	-	10
18. UT (Utah)	-	4
19. SWCO (Southwest Colorado)	-	13
20. CWY (Central Wyoming)	-	6
RED LODGE (Montana)	-	30
LEWISTOWN (Montana)	-	5
LETHBRIDGE (Alberta)	4	6
RED DEER (Alberta)	9	9
ALDER FLATS (Alberta)^L	5	6
W EDMONTON (Alberta)	9	9
BARRHEAD (Alberta)^L	24	25
SWAN HILLS (Alberta)^L	11	9
SLAVE LAKE (Alberta)	4	4
NAB (Northern Alberta)^L	2	2
COLD LAKE (Alberta)	1	1
SE EDMONTON (Alberta)^L	5	5
MEDICINE HAT (Alberta)	7	7
CYP HILLS (Cypress Hills Interprovincial Park, Alberta)	17	17
21. SK (Saskatchewan)	10	10

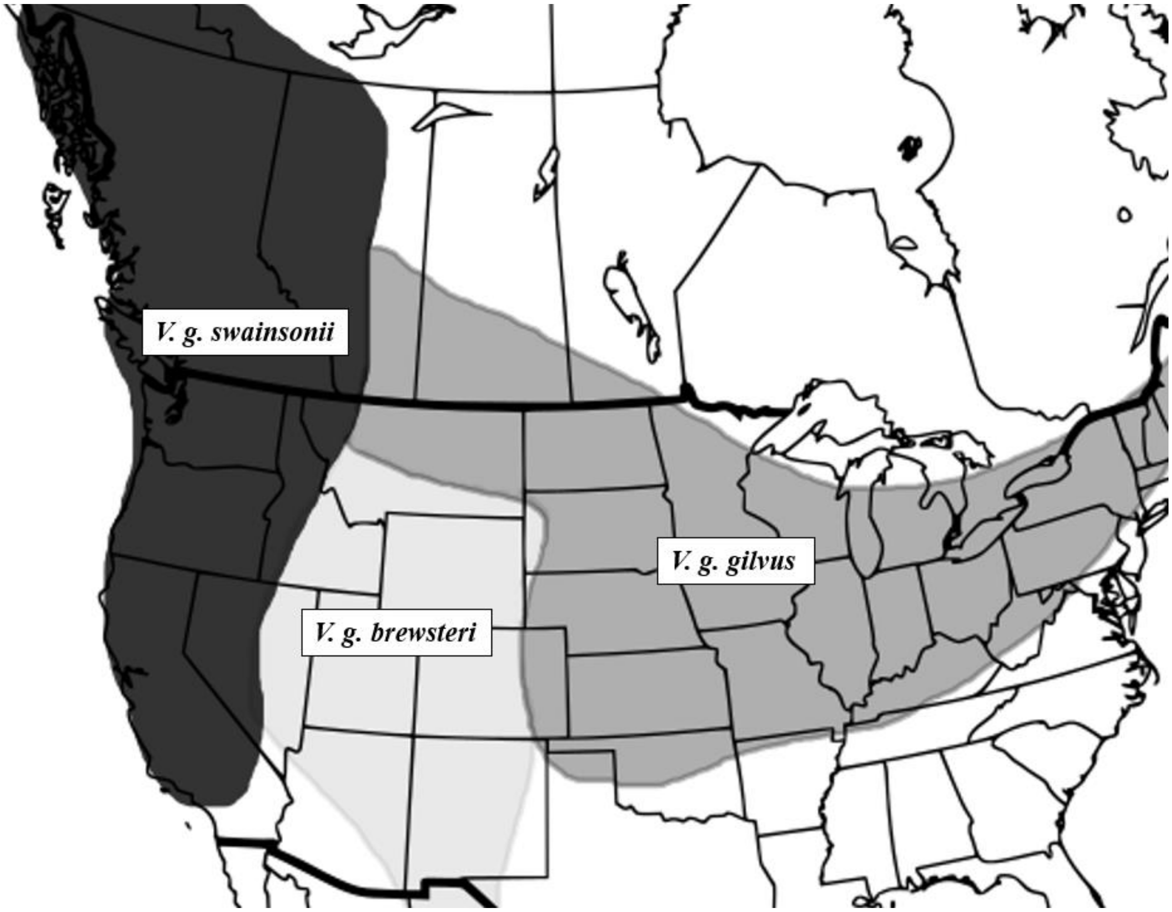

Figure 3.1 Range distributions and contact zones of the three warbling vireo subspecies in this study based on Phillips (1991) and AOU (1988).

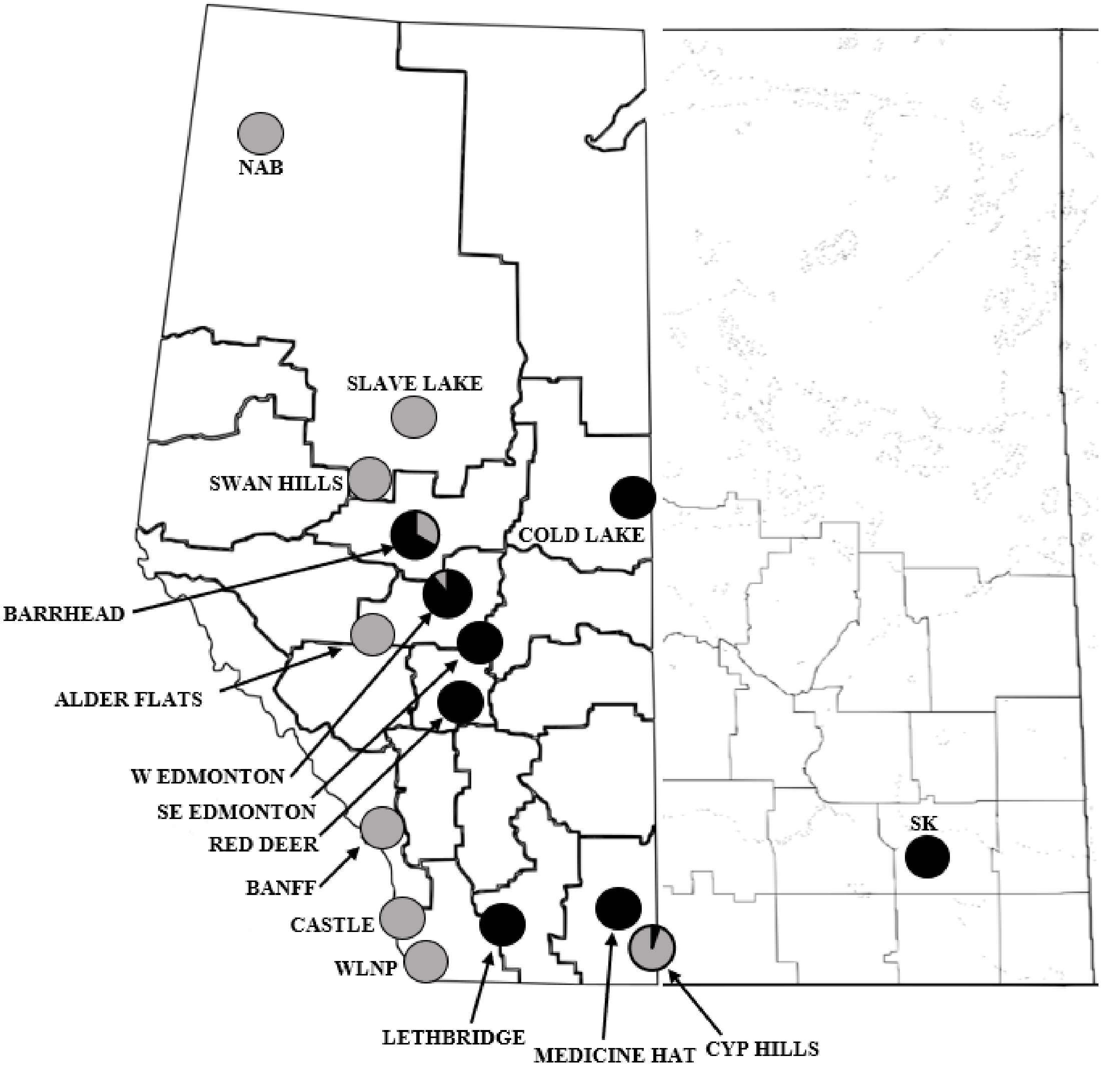

Figure 3.2 Map of Alberta and Saskatchewan with the populations included in the cyt b screening. Black corresponds to the east *gilvus* mtDNA lineage and gray corresponds to the west mtDNA lineage.

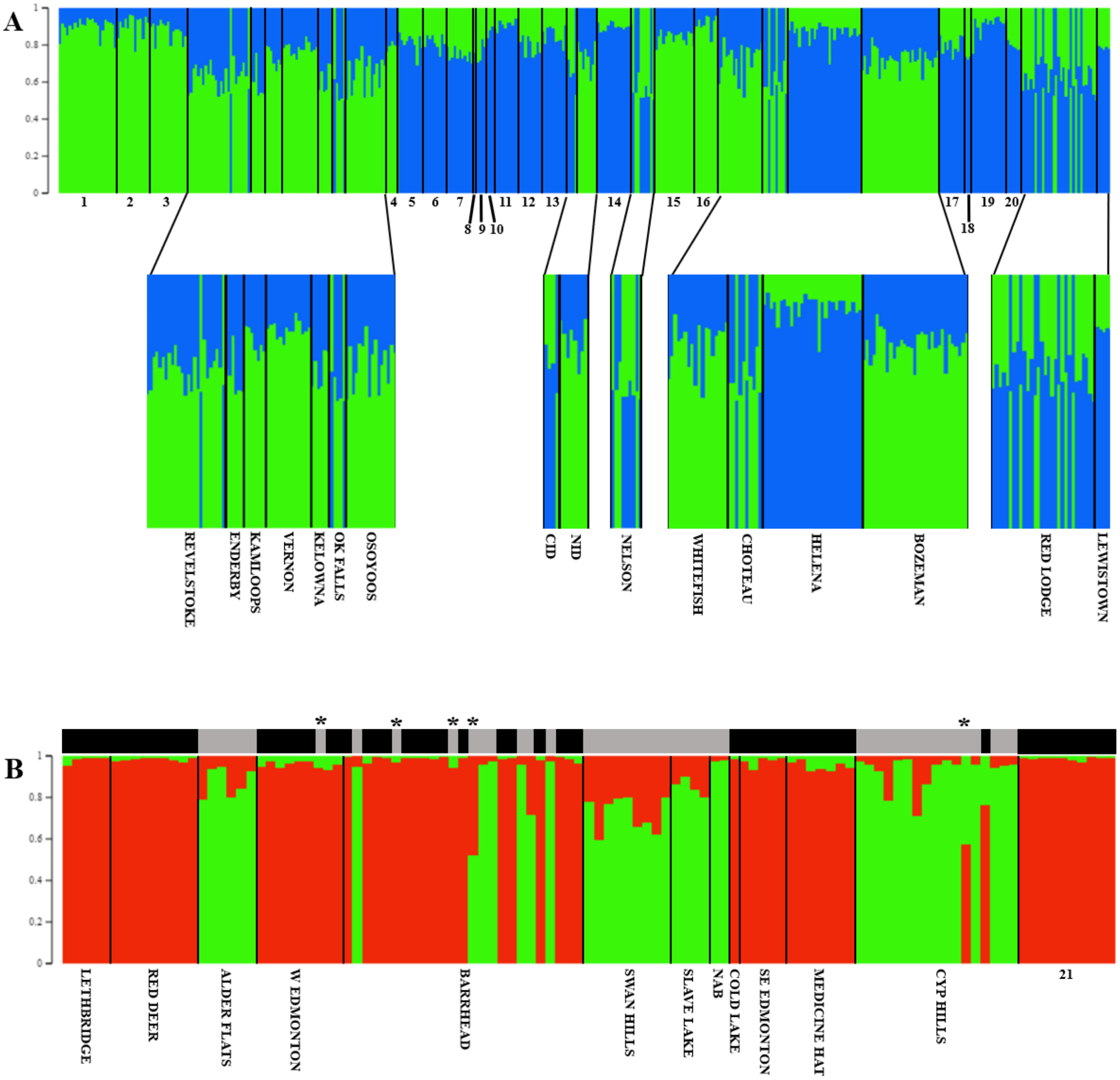

Figure 3.3 Hierarchical Structure plots (K=2) based on 14 microsatellite loci: green (*swainsonii*), blue (*brewsteri*), and red (*gilvus*). Names of numeric populations can be found in Table 3.1. **A)** Populations included in the analysis of the west contact zone and the contact zone in northwest Montana. Zoomed in populations focus on the admixed populations between *swainsonii* and *brewsteri*. **B)** Populations included in the analysis of a contact zone between *swainsonii* and *gilvus* in Alberta. Bars above the Structure plot correspond to the mtDNA lineage that individual assigned to, black (*gilvus*) and grey (west). Asterisks represent individuals with cytonuclear discordance in mtDNA and nuclear DNA.

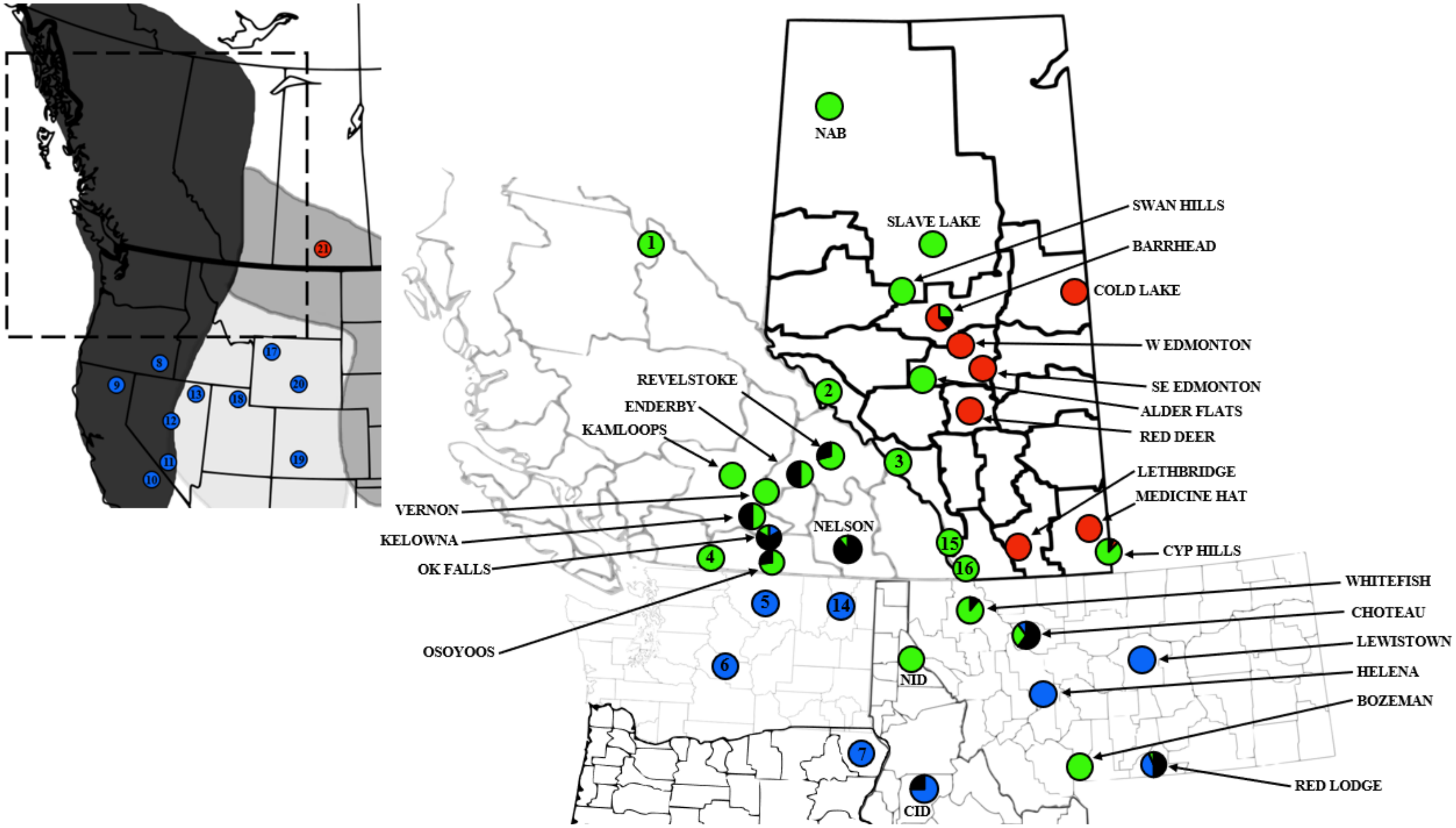

Figure 3.4 Location of populations included in the examination of the contact zones colored based on the out of Structure (see Figure 3.3): green (*swainsonii*), blue (*brewsteri*), red (*gilvus*), and black (hybrid). Population samples sizes, and the names of numbered populations, can be found in Table 3.1.

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Chapter 4: General Discussion

4.1 Subspecies distributions

Differences in eastern and western forms of species or subspecies have been recognized across taxa in North America (Peters et al. 2005; Toews and Irwin 2008; Burg et al. 2014; Beal and Pfau 2016; Lait and Burg 2013; Runck and Cook 2005). The importance of Pleistocene glaciations have been thoroughly examined and played a major role in divergence of these forms through isolation in separate ice-free refugia (Klicka and Zink 1997; Lovette 2005; Johnson and Cicero 2004; Weir and Schluter 2004). The warbling vireo's (*Vireo gilvus*) current taxonomy recognizes five subspecies based on subtle variation in cryptic morphological differences, three of which were included in this thesis: two western (*V. g. swainsonii*, *V. g. brewsteri)*, and one eastern (*V. g. gilvus*) (Phillips 1991; AOU 1998). Data presented in this thesis from mtDNA and nuclear (microsatellite) loci found a divide between the western and eastern subspecies, with additional substructure detected between the two western subspecies only with microsatellite loci. East – west differentiation mostly follows the western extent of the Great Plains for both microsatellite and mtDNA data. While this study did not specifically examine the locations of Pleistocene refugia for warbling vireos, the large genetic differences found between western and eastern populations of warbling vireos at mtDNA and microsatellite loci, coupled with a division along the Great Plains – Rocky Mountain interface, suggest evidence of separate refugia, a pattern seen in other species pairs that occur at this suture zone (Swenson 2006).

Populations that do not follow the east – west genetic pattern along the Great Plains are a disjunct west population found in the Cypress Hills Interprovincial Park of southeastern Alberta and southwestern Saskatchewan, and a genetically distinct population found in the Black Hills of western South Dakota. There is evidence of historic habitat connectivity between the Cypress Hills and the Black Hills, with boreal-type forest extending east from the Rocky Mountains along the periphery of the Cordilleran ice sheet (Strong and Hills 2005). An increase in temperature by the end of the Pleistocene disrupted this continuous habitat, as areas surrounding the Cypress Hills and the Black Hills were replaced by grassland, subsequently isolating them. Other animal and plant species have genetically isolated or disjunct populations in the Black Hills and the Cypress Hills, including mountain sucker (*Pantosteus jordani*), dragonflies (Odonata), bloodroot (*Sanguinaria canadensis*), southern maidenhair fern (*Adiantum capillus-veneris*), and stream orchid (*Epipactis gigantea*) (Bertrand et al. 2016; Hilton 1985; Hornbeck et al. 2003, 2003).

One of the primary conclusions of our study is that the current range distributions of the western subspecies do not match with our genetic groups found with microsatellite loci. Rather, *brewsteri* occupies the majority of the western range and *swainsonii* is confined to most of mainland British Columbia, areas of the Canadian – US Rocky Mountains, and parts of central to northern Alberta. A single, mtDNA lineage shared between the western subspecies suggests insufficient time for lineage sorting. The misidentification of the western subspecies range limits could be due to the greater cryptic morphological differences between them (Phillips 1991; AOU 1988). Our study highlights that relying on a single method, traditional morphology or molecular, to describe the evolutionary histories of taxa can be misleading or biased. Molecular techniques have aided to reveal morphologically cryptic species in other species complexes, including skipper butterflies (*Astraptes fulgerator*), black salamander (*Aneides flavipunctatus*), and a South American catfish (*Pimelodella chagresi*) (Hebert el al. 2004; Rissler and Apodaca 2007; Martin and Bermingham 2000). It is advantageous to integrate multiple molecular tools with different modes of inheritance and evolutionary rates, along with morphology, behavior, and ecology if available, in order to distinguish discrete morphological pairs of taxa and accurate estimates of biodiversity.

4.2 Contact zones

Many contact and hybrids zones in North America are thought to be the result of secondary contact after periods of isolation during the glacial cycles of the Pleistocene (Price 2008; Hewitt 2001). Cytonuclear discordance, the genetic pattern of having mtDNA from one lineage and nuclear DNA of another, has been used to identify hybrids in populations that were separated during the Pleistocene (Arnold 1993). Discordances between mtDNA and nuclear genomes have been found across other animal taxa, including two-banded killifish (*Fundulus diaphanus diaphanus* and *F. d. menona*) and North American red squirrels (*Tamiasciurus* spp.), that have strong evidence of secondary contact (April and Turgeon 2006; Hope et al. 2016).

Genetic data from our range wide sampling approach across the distribution of all three subspecies to compare overall subspecies genetic structure in Chapter 2 led us to hypothesize another potential area of contact between the western subspecies, *swainsonii* and *brewsteri*, in southern British Columbia. The results presented in Chapter 3 of this thesis found that a large contact zone occurs between *swainsonii* and *brewsteri* in southern British Columbia, with an extension through the Rocky Mountains of western Montana. While we were unable to examine cytonuclear discordance between mtDNA and nuclear DNA of the western subspecies, the biparental inheritance of microsatellite loci are able to detect hybrid individuals. With this tool, we found strong evidence of hybridization between the western subspecies in ten populations along the contact zone suggesting that there are weak reproductive isolating barriers between them. The genetic composition of hybrids appear to be associated with elevation of the population, where a greater proportion of *swainsonii* was found in lower elevations and *brewsteri* in higher elevations. Given that the two western subspecies are unable to be separated by mtDNA but are different in the nuclear genome, we infer that contemporary processes are responsible for the genetic differences found between *swainsonii* and *brewsteri*.

Data from mtDNA and microsatellite analyses in Chapter 3 support a previous study of a contact zone between *swainsonii* and *gilvus* in Barrhead County, Alberta (Lovell 2010); however, our study also found cytonuclear discordances in a W Edmonton population south of Barrhead County and in the Cypress Hills. The width of the contact zone is narrow (~ 116 km) and follows a transitional ecotone from parkland to boreal forest habitat, with more pure *gilvus* found in southeast Barrhead County and more pure *swainsonii* found in northwest Barrhead County, a distance of approximately 6 km (Semenchuk 1992). With an underwhelming number of hybrid individuals (4 of 33), our data indicates that hybrids have lower fitness than parental populations, therefore suggesting a tension zone or bimodal hybrid zone between s*wainsonii* and *gilvus* and selection against hybrids, possibly through assortative mating. Further research on this behavior, or sex-biased dispersal, will provide additional evidence of selective pressures on hybrid or parental populations and its role in maintaining reproductive isolation between *swainsonii* and *gilvus*. Assortative mating has been found to be an important selective mechanism in the maintenance of other interspecific avian, white-headed gull (*Larus argentatus* and *L. cachinnans*), and non-avian, strawberry poison-dart frog (*Dendrobates pumilio*) hybrids (Gay et al. 2007; Reynolds and Fitzpatrick 2007). Intraspecific contact and hybrid zones are far less documented in literature, with one example between two subspecies of winter wren (*Troglodytes troglodytes*) that were found to be divergent from one another in the presence of weak morphological variation (Toews and Irwin 2008). The contact and hybrid zones between warbling vireos (*V. gilvus*) are a system of novelty from an intraspecific perspective and highlights the importance of examining biodiversity at all levels of classification.

4.3 Future considerations

The limited amount of research on warbling vireos yield numerous avenues of opportunity to understand how reproductive isolating mechanisms arise in a phenotypically cryptic complex. Phylogeographic analysis of the warbling vireo complex to include the other two subspecies, *V. g. sympatrica* and *V. g. victoriae*, would address additional questions regarding population genetic structure, subspecies boundaries, and the evolutionary histories of these taxa. Genetic sequencing, such as NGS or SNPs, could further differentiate these subspecies and specifically, verify my findings in Chapters 2 and 3 about the genetic distribution of the western subspecies *swainsonii* and *brewsteri*. A revision of morphological and plumage characters between the western subspecies should be done in light of our molecular evidence disagreeing with current range distributions. Further sampling and genetic, ecological, and behavioral characterization of populations in the contact zone between the two western subspecies could shed light on the contemporary processes mediating or restricting gene flow between them, as this thesis presents the first description of this large contact zone between s*wainsonii* and *brewsteri*. Further sampling needs to be done to find the western extent of *gilvus* in eastern Montana and Colorado.

While distinct migratory pathways and wintering areas of warbling vireos (*V. gilvus*) are poorly understood, differences in migratory routes and wintering areas have been found to influence population genetic structure in other avian species (Rolshausen et al. 2009). Anecdotal evidence of eBird sightings in April showed two main groups of birds, one along the Pacific Flyway and the other along the Mississippi Flyway, with sparse individuals in the Central Flyway. A more comprehensive examination of tracking the movements of warbling vireos, in addition to recording the arrival of individuals on their breeding territories, could better define migratory strategies among each of the subspecies and provide resolution of the connective pathways between the western subspecies and their range distributions.

4.4 Closing statement

Cryptic diversity is the type of biological variation that can be difficult to identify, but with the use of molecular techniques, we can more accurately estimate levels of biodiversity that are otherwise challenging based on superficial morphological characters.

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Appendix 1: Supplementary Information for Chapter 2

Range wide microsatellite and mtDNA population genetic structure in a cryptic species complex, the warbling vireo (*Vireo gilvus*)

Appendix 1.1 Sample ID, location, coordinates, source, and band/museum ID for each individual used in Chapter 2 listed by population. All individuals were used in the microsatellite analysis unless marked with an asterisk. Individuals that were also sequenced are identified by ^{C} = cyt b and ^{A} = ATPase 6 and 8. Samples caught for this study are left blank, while samples that were donated from a museum and/or collaborator are listed as follows: University of Washington Burke Museum (UWBM), Denver Museum of Nature and Science (DMNS), Royal Alberta Museum (RAM), Field Museum of Natural History (FMNH), University of Michigan Museum (UMMZ), Cleveland Museum of Natural History (CMNH), and Natural History Museum Oslo (NHMO).

Sample ID	**Location**	**Lat (˚N)**	**Long (˚W)**	**Source**	**ID**
waviNWBC001$^{C, A}$	Dease Lake, BC	58.30	-130.12		wavi 1
waviNWBC002$^{C, A}$	Dease Lake, BC	58.30	-130.12		wavi 2
waviNWBC003$^{C, A}$	Dease Lake, BC	58.30	-130.12		wavi 3
waviNWBC004	Dease Lake, BC	58.30	-130.12		wavi 4
waviNWBC005*^{A}	Telegraph Creek, BC	57.84	-131.12		wavi 5
waviNWBC006^{A}	Telegraph Creek, BC	57.84	-131.12		wavi 6
waviCBC002^{A}	Prince George, BC	53.55	-122.44		wavi 2
waviCBC003^{A}	Prince George, BC	53.55	-122.44		wavi 3
waviCBC004^{A}	Smithers, BC	54.48	-127.93		wavi 4
waviCBC001^{A}	Prince George, BC	53.53	-122.49		wavi 1
waviCBC005	Lily Lake Rd, Fort Fraser, BC	53.57	-124.32		wavi 5
waviCBC006	Morfee Mt. Rd., Mackenzie, BC	55.37	-123.10		2710-78244
waviCBC007	Morfee Mt. Rd., Mackenzie, BC	55.37	-123.10		2710-78245
waviCBC008	Clearcut near boat launch, Mackenzie, BC	55.38	-123.17		2710-78246
waviCBC009	Industrial area 1, Mackenzie, BC	55.33	-123.17		2710-78247
waviCBC010	Industrial area 2, Mackenzie, BC	55.32	-123.15		2710-78248
waviCBC011	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78249
waviCBC012	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78250
waviCBC013	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78251
waviCBC014	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78252
waviCBC015	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78254
waviCBC016	Forestry Road, Mackenzie, BC	55.30	-123.01		2710-78255
waviCBC017	Forestry Road, Mackenzie, BC	55.30	-123.01		2710-78256
waviCBC018	Forestry Road, Mackenzie, BC	55.30	-123.01		2710-78257
waviCBC019	Mugaha Marsh 1, Mackenzie, BC	55.38	-123.20		2710-78258
waviCBC020	Mugaha Marsh 1, Mackenzie, BC	55.38	-123.20		2710-78259
waviCBC021^{C}	Mugaha Marsh 2, Mackenzie, BC	55.38	-123.20		2710-78261
waviCBC022^{C}	Mugaha Marsh 2, Mackenzie, BC	55.38	-123.20		2710-78262
waviCBC023	Mugaha Marsh 2, Mackenzie, BC	55.38	-123.20		2710-78264
waviCBC024	Mugaha Marsh 2, Mackenzie, BC	55.38	-123.20		2710-78263
waviCAB001^{A}	Edson, AB	53.37	-116.49		wavi 1
waviCAB002$^{C, A}$	Edson, AB	53.37	-116.49		wavi 2
waviCAB003^{C}	Patricia Lake, Jasper, AB	52.91	-118.09		2710-78241
waviCAB004	Pyramid Beach 1, Jasper, AB	52.91	-118.10		2710-78268
waviCAB005	Pyramid Beach 1, Jasper, AB	52.91	-118.10		2710-78269
waviCAB006	Pyramid Beach 1, Jasper, AB	52.91	-118.10		2710-78270

waviCAB007	Pyramid Beach 2, Jasper, AB	52.91	-118.10		2710-78272
waviCAB008	Pyramid Beach 2, Jasper, AB	52.91	-118.10		2710-78273
waviCAB009	Pyramid Beach 2, Jasper, AB	52.91	-118.10		2710-78274
waviCAB010	MAPS station, Jasper, AB	52.55	-118.65		2340-81929
waviCAB011	MAPS station, Jasper, AB	52.55	-118.65		2340-81933
waviCAB012	Moab Lake, Jasper, AB	52.67	-117.96		2710-78277
waviCAB013	Mushroom Patch, Jasper, AB	53.06	-118.07		2710-78280
waviSEBC048	Field Cemetery, Yoho National Park	51.23	-116.29		2710-78573
waviSAB014	Vermillion Lakes Road, Banff, AB	51.10	-115.35		2710-78576
waviSAB015	Cave and Basin, Banff, AB	51.10	-115.35		2710-78577
waviSAB016	Cave and Basin, Banff, AB	51.10	-115.35		2710-78578
waviSAB017	Cave and Basin, Banff, AB	51.10	-115.35		2710-78579
waviSAB018	Vermillion Lakes 2, Banff, AB	51.10	-115.36		2710-78580
waviSAB019	Vermillion Lakes 3, Banff, AB	51.10	-115.37		2710-78581
waviSAB020	Vermillion Lakes 4, Banff, AB	51.11	-115.35		2710-78582
waviCAB014	Quarry Lake, AB	51.08	-115.37		2221-85084
waviCAB015	Quarry Lake, AB	51.08	-115.37		2221-85085
waviCAB016	Quarry Lake, AB	51.08	-115.37		2221-85086
waviCAB017	Quarry Lake, AB	51.08	-115.37		2221-85087
waviCAB018	Quarry Lake, AB	51.08	-115.37		2221-85088
waviCAB019	Quarry Lake, AB	51.08	-115.37		2221-85089
waviCAB020	Quarry Lake, AB	51.08	-115.37		2221-85090
waviSAB001*[A]	West Castle, AB	49.20	-114.25		wavi 1
waviSAB002[A]	West Castle, AB	49.20	-114.25		wavi 2
waviSAB003[A]	West Castle, AB	49.20	-114.25		wavi 3
waviSAB004[A]	West Castle, AB	49.20	-114.25		wavi 4
waviSAB005[A]	West Castle, AB	49.20	-114.25		wavi 5
waviSAB006[A]	West Castle, AB	49.20	-114.25		wavi 6
waviSAB007[A]	West Castle, AB	49.20	-114.25		wavi 7
waviSAB008*[A]	West Castle, AB	49.20	-114.25		wavi 8
waviSAB013	River Valley Park, Fort Macleod (811 Bridge)	49.44	-113.24		2430-38995
waviSAB021[C]	West Castle Wetlands, AB	49.22	-114.22		2710-78585
waviSAB022	West Castle Wetlands, AB	49.20	-114.26		2710-78586
waviSAB023	Lynx Creek Road, near Beaver Mines, AB	49.27	-114.22		2710-78587
waviSAB024	Lynx Creek Road, near Beaver Mines, AB	49.27	-114.22		2710-78588
waviSAB025	Beaver Mines Lake Recreation Area, AB	49.22	-114.19		2710-78589
waviSAB026	Beaver Mines Lake Recreation Area, AB	49.22	-114.19		2710-78590
waviSAB027	Beaver Mines Lake Recreation Area, AB	49.22	-114.19		2710-78591
waviSAB028	Beaver Mines Lake Recreation Area, AB	49.24	-114.20		2710-78592
waviSAB029	Beaver Mines Lake Recreation Area, AB	49.24	-114.20		2710-78593
waviSAB035	Castle River, AB	49.31	-114.30	RAM	Z06.4.3
waviSAB009[A]	Hay Barn, Waterton, AB	49.04	-113.51		wavi 9
waviSAB010[A]	Belly River Campground Waterton, AB	49.12	-113.41		A
waviSAB011[A]	Belly River Campground Waterton, AB	49.12	-113.41		B
waviSAB012[A]	Belly River Campground Waterton, AB	49.12	-113.41		C

waviSAB030	Road to Cameron Lake, Waterton Lakes, AB	49.42	-113.59		2710-78594
waviSAB031	Road to Cameron Lake, Waterton Lakes, AB	49.42	-113.59		2710-78595
waviSAB032	Canyon Road, Waterton Lakes, AB	49.54	-113.50		2710-78596
waviSAB033	Canyon Road, Waterton Lakes, AB	49.54	-113.50		2710-78597
waviSAB034	Stables Road, Waterton, AB	49.40	-113.53		2710-78611
waviWA001	Big Meadow Lake, WA	48.43	-117.33		2540-22996
waviWA002	Big Meadow Lake, WA	48.43	-117.33		2540-22997
waviWA013	Walker Valley ORV Trailhead, WA	48.22	-117.10		2580-49307
waviWA014	Amazon Creek, Steven's County, WA	48.32	-117.36		2580-49324
waviWA003	Amazon Creek, Steven's County, WA	48.32	-117.36		2580-49326
waviWA004[C]	Power Lake, Steven's County, WA	48.15	-117.23		2580-49329
waviWA005[C]	Twin Lakes, Steven's County, WA	48.34	-117.38		2580-49332
waviWA006	Twin Lakes, Steven's County, WA	48.34	-117.38		2580-49333
waviWA007[C]	Twin Lakes, Steven's County, WA	48.34	-117.38		2580-49334
waviWA008	Starvation Lake, WA	49.29	-117.42		2580-49335
waviWA009	Gillette Lake Road, Steven's County, WA	48.36	-117.33		2580-49336
waviWA010	Gillette Lake Road, Steven's County, WA	48.36	-117.33		2580-49337
waviWA011	Gillette Lake Road, Steven's County, WA	48.36	-117.33		2580-49340
waviWA012	Gillette Lake Road, Steven's County, WA	48.36	-117.33		2580-49343
waviSWBC001	Lightning Lakes 1, Manning, BC	49.06	-120.84		2710-78281
waviSWBC002	Lightning Lakes 1, Manning, BC	49.06	-120.84		2710-78282
waviSWBC003	PCT Trailhead, Manning, BC	49.06	-120.80		2710-78286
waviSWBC005[C]	Cambie Creek Trailhead, Manning, BC	49.11	-120.85		2710-78288
waviSWBC006[C]	Cambie Creek Trailhead, Manning, BC	49.11	-120.85		2710-78289
waviWA035	Skagit, WA			UWBM	119647
waviVI001	Port Renfrew, Vancouver Island, BC	48.40	-124.30	UWBM	110836
waviVI002[C]	Port Renfrew, Vancouver Island, BC	48.40	-124.30	UWBM	110839
waviVI003	Comox Lake, Vancouver Island, BC	49.30	-125.00	UWBM	110837
waviVI004	Lake Elsie, Vancouver Island, BC	49.30	-125.00	UWBM	110838
waviWA015	Naneum Creek, Kittitas, WA			UWBM	121335
waviWA016	Naneum Creek, Kittitas, WA			UWBM	121383
waviWA017	1801 North B Street, Kittitas, WA			UWBM	89898
waviWA018	Whiskey Dick Creek, Kittitas, WA			UWBM	66875
waviWA019	Whiskey Dick Creek, Kittitas, WA			UWBM	66876
waviWA020	Whiskey Dick Creek, Kittitas, WA			UWBM	66877
waviWA021	Iron Creek, Kittitas, WA			UWBM	120123
waviWA022	Iron Creek, Kittitas, WA			UWBM	118370
waviWA023	South Fork Manastash Creek, Kittitas, WA			UWBM	119559
waviWA024	Whiskey Dick Creek, Kittitas, WA			UWBM	66828
waviWA025	Twisp River, Okanogan, WA			UWBM	118280
waviWA026	Winthrop, Bear Creek, Okanogan, WA	48.48	-120.10	UWBM	113779
waviWA027	Winthrop, Bear Creek, Okanogan, WA	48.48	-120.10	UWBM	113780
waviWA028	Winthrop, Bear Creek, Okanogan, WA	48.48	-120.10	UWBM	113778
waviWA029	Bear Creek, Okanogan, WA			UWBM	113782
waviWA030	Winthrop, Bear Creek, Okanogan, WA	48.48	-120.10	UWBM	113781

waviWA031	Twisp River, Okanogan, WA			UWBM	118297
waviWA032	Benson Creek, Okanogan, WA			UWBM	118332
waviWA033	Little Buck Mountain, Okanogan, WA			UWBM	86319
waviWA034	Okanogan, WA			UWBM	85954
waviNEOR001[C, A]	Catherine Creek St. park, OR	45.98	-117.44		wavi 1
waviNEOR002[C, A]	Bird Track Springs Trail, OR	45.18	-118.18		wavi 2
waviNEOR003[C, A]	Hilgard junction state park, OR	45.20	-118.14		wavi 3
waviNEOR004	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112650
waviNEOR005	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112649
waviNEOR006	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112648
waviNEOR007	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112647
waviNEOR008	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112646
waviNEOR009	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112645
waviNEOR010	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112644
waviSEOR001	Steens Mountain Wilderness, OR	42.64	-118.76	DMNS	48097
waviCoOR001[C, A]	Fogarty State Park, OR	44.50	-124.25		wavi 1
waviCoOR002[A]	Fogarty State Park, OR	44.50	-124.25		wavi 2
waviCA003	Mendocino Pass Road, Mendocino, CA			UWBM	112790
waviCA004	Mendocino Pass Road, Mendocino, CA			UWBM	112792
waviCA006	Mendocino Pass Road, Mendocino, CA			UWBM	113895
waviCA007	Emerson Cr., Warner Mountains, Modoc, CA			UWBM	117496
waviCA008	Emerson Cr., Warner Mountains, Modoc, CA			UWBM	117794
waviCA010	Emerson Cr., Warner Mountains, Modoc, CA			UWBM	117448
waviCA011	Emerson Cr., Warner Mountains, Modoc, CA			UWBM	117518
waviCA001	Wyman Creek; White Mountains, Inyo, CA			UWBM	117576
waviCA002	Wyman Creek; White Mountains, Inyo, CA			UWBM	117762
waviCA005	Bishop, White Mountains, Inyo, CA	37.24	-118.14	UWBM	100552
waviNV001[C]	Bonanza Trail, Spring Mountains, Clark, NV	36.22	-115.45	UWBM	109683
waviNV002	Bonanza Trail, Spring Mountains, Clark, NV	36.22	-115.45	UWBM	109684
waviNV003	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111568
waviNV004	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111569
waviNV005	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111584
waviNV006	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111581
waviNV007	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111582
waviNV008	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	106733
waviNV009	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	106734
waviNV010	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111585
waviNV011	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	112109
waviNV012	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	112107
waviNV013	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	112111
waviNV014	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	107130
waviNV015	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	107126
waviNV016	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	107124
waviNV017	Jarbidge, 0.5 mi N, Elko, NV	41.54	-115.25	UWBM	107133
waviNV018	Mountain City, 15 mi ESE, Elko, NV	41.47	-115.42	UWBM	107128

waviNV019	Mountain City, 15 mi ESE, Elko, NV	41.47	-115.42	UWBM	107117
waviNV020	Mountain City, 15 mi ESE, Elko, NV	41.47	-115.42	UWBM	107116
waviUT001[A]	W of Woodruff (Hwy 39), UT	41.27	-111.31		wavi 1
waviUT002[A]	W of Woodruff (Hwy 39), UT	41.27	-111.31		wavi 2
waviUT003[C, A]	Cache National Forest (Forestry Rd.), UT	41.30	-111.30		wavi 3
waviUT004[A]	South Fork campground, UT	41.16	-111.39		wavi 4
waviNM002	Nogal Canyon Road, Lincoln, NM	33.30	-105.47	UWBM	111656
waviNM003[C]	Nogal Canyon Road, Lincoln, NM	33.30	-105.47	UWBM	111666
waviNM004	Nogal Canyon Road, Lincoln, NM	33.30	-105.47	UWBM	111670
waviNM005	Nogal Canyon Road, Lincoln, NM	33.30	-105.47	UWBM	111671
waviNM006	Nogal Canyon Road, Lincoln, NM	33.30	-105.47	UWBM	111668
waviNM007	Nogal Canyon Road, Lincoln, NM	33.30	-105.47	UWBM	106765
waviNM008	Nogal Canyon Road, Lincoln, NM	33.30	-105.47	UWBM	114924
waviNM009	Nogal Canyon Road, Lincoln, NM	33.30	-105.47	UWBM	106764
waviCO005	Fremont, CO			UWBM	70334
waviCO006	Off highway 9, Fremont, CO			UWBM	113803
waviCO007	Off highway 9, Fremont, CO			UWBM	113802
waviCO008	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112082
waviCO009	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112077
waviCO010	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112076
waviCO011	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112080
waviCO012[C]	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112078
waviCO013	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	107090
waviCO014	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	107091
waviCO015	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	107093
waviCO016	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	107089
waviCO017	Missionary Ridge Road, La Plata, CO	37.26	-107.45	UWBM	114990
waviCO001[C, A]	Cottonwood (Ed and Susan Dentry), CO	39.46	-105.23		wavi 1
waviCO002[A]	Cottonwood (Ed and Susan Dentry), CO	39.46	-105.23		wavi 2
waviCO003[C, A]	N of Central City (HWY 119), CO	39.51	-105.29		wavi 3
waviCO004[A]	Lime Creek Rd, San Juan NF, CO	37.39	-107.48		wavi 4
waviCO018	Glendevey, Larimer, CO	40.47	-105.55	UWBM	108755
waviCO019	Livermore, 20 mi W, Larimer, CO	40.44	-105.36	UWBM	112208
waviCO020	Livermore, 20 mi W, Larimer, CO	40.44	-105.36	UWBM	112205
waviCO021	Livermore, 20 mi W, Larimer, CO	40.44	-105.36	UWBM	112204
waviCO022	Livermore, 20 mi W, Larimer, CO	40.44	-105.36	UWBM	112209
waviCO023	Livermore, 20 mi W, Larimer, CO	40.44	-105.36	UWBM	107174
waviCO024	Livermore, 20 mi W, Larimer, CO	40.44	-105.36	UWBM	107178
waviCO026	Livermore, 20 mi W, Larimer, CO	40.44	-105.36	UWBM	114992
waviCO027	Livermore, 20 mi W, Larimer, CO	40.44	-105.36	UWBM	114993
waviWY002	Ferris Mountains, WY	42.29	-107.32	DMNS	46028
waviWY003[C]	Ferris Mountains, WY	42.29	-107.31	DMNS	46026
waviWY004	Green Mountains, WY	42.34	-107.68	DMNS	45973
waviWY005	Ferris Mountains, WY	42.29	-107.32	DMNS	46021
waviWY006	Laramie Mountains near Esterbrook, WY	42.42	-105.34	DMNS	46041

waviWY019	Fremont County - Green Mountains, WY	42.34	-107.68	DMNS	45973
waviWY007	Ferris Mountains, WY	42.29	-107.32	DMNS	46029
waviWY008	18 miles south of Lander, WY	42.51	-108.81	DMNS	45043
waviWY009	18 miles south of Lander, WY	42.51	-108.81	DMNS	45044
waviWY010	18 miles south of Lander, WY	42.57	-108.73	DMNS	45018
waviWY011	18 miles south of Lander, WY	42.57	-108.73	DMNS	44979
waviWY014	18 miles south of Lander, WY	42.57	-108.73	DMNS	44968
waviWY016	18 miles south of Lander, WY	42.57	-108.73	DMNS	44996
waviWY017	19 miles south of Lander, WY	42.57	-108.73	DMNS	45003
waviWY018	20 miles south of Lander, WY	42.57	-108.73	DMNS	45001
waviWY001[C, A]	Aspen Trailhead, Bridger-Teton NF, WY	43.42	-110.36		wavi 1
waviMT001[A]	Helena National Forest, Helena, MT	46.29	-111.51		wavi 1
waviMT002[A]	Orofino, Helena, MT	46.34	-112.40		wavi 2
waviMT003[A]	Road to Park Lake, Helena, MT	46.31	-112.07		wavi 3
waviMT004[A]	Road to Park Lake, Helena, MT	46.31	-112.07		wavi 4
waviMT005[A]	Road to Park Lake, Helena, MT	46.31	-112.07		wavi 5
waviMT009[A]	Canyon off Highway 2, Butte, MT	45.53	-112.28		wavi 9
waviMT010	Cottonwood Creek, Beartooth NWMA, MT	46.56	-111.56		2580-49347
waviMT011	Cottonwood Creek, Beartooth NWMA, MT	46.56	-111.56		2580-49348
waviMT012	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49349
waviMT014	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49351
waviMT016	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49353
waviMT018	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49355
waviMT019	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49356
waviMT020	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49357
waviMT021	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49358
waviMT022	Lowland Creek Rd, Elk Park, MT	46.11	-112.27		2580-49360
waviMT023	Lowland Creek Rd, Elk Park, MT	46.11	-112.27		2580-49361
waviMT024	Lowland Creek Rd, Elk Park, MT	46.11	-112.27		2580-49362
waviMT025	Lowland Creek Rd, Elk Park, MT	46.11	-112.27		2580-49363
waviMT026	Lowland Creek Rd, Elk Park, MT	46.11	-112.27		2580-49365
waviMT027	Lowland Creek Rd, Elk Park, MT	46.11	-112.27		2580-49366
waviMT028	Lowland Creek Rd, Elk Park, MT	46.11	-112.27		2580-49367
waviMT029	Thunderbolt Creek Trailhead, Basin, MT	46.17	-112.28		2580-49368
waviMT030	Thunderbolt Creek Trailhead, Basin, MT	46.17	-112.28		2580-49369
waviMT031	Thunderbolt Creek Trailhead, Basin, MT	46.17	-112.28		2580-49370
waviMT032	Moose Creek Campground, Helena, MT	46.32	-112.15		2580-49372
waviMT033	Moose Creek Campground, Helena, MT	46.32	-112.15		2580-49374
waviMT034	Rimini Rd, Helena, MT	46.32	-112.15		2580-49376
waviMT035	Rimini Rd, Helena, MT	46.32	-112.15		2580-49377
waviMT042	33 miles south of Lewistown, MT	46.71	-109.33	DMNS	45334
waviMT047	30 miles south of Lewistown, MT	46.71	-109.33	DMNS	45335
waviMT053	30 miles south of Lewistown, MT	46.71	-109.33	DMNS	45341
waviMT055	32 miles south of Lewistown, MT	46.71	-109.33	DMNS	45349
waviWY013	4 miles NW Sundance, WY [Black Hills]	44.45	-104.42	DMNS	45148

waviWY015	4 miles NW Sundance, WY [Black Hills]	44.45	-104.42	DMNS	45157
waviSD001	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45240
waviSD002	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45485
waviSD003	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45501
waviSD004	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45461
waviSD005	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45474
waviSD006	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45483
waviSD007	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45484
waviSD008	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45457
waviSD009	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45458
waviSD010	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45460
waviSD011	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45499
waviSD012	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45473
waviSD013	1.5 miles SW of Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45187
waviSD014	1.5 miles SW of Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45179
waviSD015	1.5 miles SW of Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	45180
waviSD016	0.4 mile south of Spearfish, SD [Black Hills]	44.45	-103.84	DMNS	44958
waviSD017	0.4 mile south of Spearfish, SD [Black Hills]	44.45	-103.84	DMNS	44954
waviSD018	0.4 mile south of Spearfish, SD [Black Hills]	44.45	-103.84	DMNS	44945
waviSD019	0.4 mile south of Spearfish, SD [Black Hills]	44.45	-103.84	DMNS	44957
waviSD020	0.4 mile south of Spearfish, SD [Black Hills]	44.45	-103.84	DMNS	44965
waviSD021	0.4 mile south of Spearfish, SD [Black Hills]	44.45	-103.84	DMNS	44953
waviSD022	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	44859
waviSD023	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	44862
waviSD024	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	44865
waviSD025[C]	Big Hills, south of Spearfish, SD [Black Hills]	44.45	-103.91	DMNS	44887
waviSD026[C]	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	44664
waviSD027[C]	13 miles south of Spearfish, SD [Black Hills]	44.41	-103.96	DMNS	44908
waviSD028[C]	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	44656
waviSD029[C]	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	44863
waviSD030[C]	Big Hills, south of Spearfish, SD [Black Hills]	44.45	-103.91	DMNS	44885
waviSD031	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	44861
waviSD032[C]	Higgins Gulch, Spearfish, SD [Black Hills]	44.48	-103.93	DMNS	44665
waviSD033	Big Hills, south of Spearfish, SD [Black Hills]	44.45	-103.91	DMNS	44879
waviSD035	Big Hills, south of Spearfish, SD [Black Hills]	44.45	-103.91	DMNS	44886
waviSEAB001[C]	Police Point Park, Medicine Hat, AB	50.04	-110.64		1691-93363
waviSEAB002[C]	Police Point Park, Medicine Hat, AB	50.04	-110.64		1691-93364
waviSEAB003[C]	Strathcona Island Park, Medicine Hat, AB	50.04	-110.65		1691-93384
waviSEAB004[C]	Strathcona Island Park, Medicine Hat, AB	50.04	-110.65		1691-93385
waviSEAB005[C]	Strathcona Island Park, Medicine Hat, AB	50.04	-110.65		1691-93386
waviSEAB006	Police Point Park, Medicine Hat, AB	50.04	-110.65		1691-93387
waviSEAB007	Police Point Park, Medicine Hat, AB	50.04	-110.65		1691-93388
waviCYHILLS001	Cypress Hills, AB	49.66	-110.08		1691-93365
waviCYHILLS002[C]	Cypress Hills, AB	49.66	-110.08		1691-93366
waviCYHILLS003[C]	Cypress Hills, AB	49.66	-110.04		1691-93367

waviCYHILLS004[C]	Cypress Hills, AB	49.64	-110.03		1691-93368
waviCYHILLS005[C]	Cypress Hills, AB	49.64	-110.03		1691-93369
waviCYHILLS006[C]	Cypress Hills, AB	49.64	-110.03		1691-93370
waviCYHILLS007[C]	Cypress Hills, AB	49.64	-110.03		1691-93371
waviCYHILLS008[C]	Cypress Hills, AB	49.64	-110.03		1691-93372
waviCYHILLS009[C]	Cypress Hills, AB	49.64	-110.03		1691-93373
waviCYHILLS010[C]	Cypress Hills, AB	49.66	-110.05		1691-93374
waviCYHILLS011[C]	Cypress Hills, AB	49.66	-110.05		1691-93375
waviCYHILLS012[C]	Cypress Hills, AB	49.66	-110.10		1691-93376
waviCYHILLS013[C]	Cypress Hills, AB	49.66	-110.10		1691-93377
waviCYHILLS014*[C]	Cypress Hills, AB	49.66	-110.10		1691-93378
waviCYHILLS015*[C]	Cypress Hills, AB	49.66	-110.10		1691-93379
waviCYHILLS016[C]	Cypress Hills, AB	49.66	-110.10		1691-93380
waviCYHILLS017[C]	Cypress Hills, AB	49.66	-110.10		1691-93381
waviCYHILLS018	Cypress Hills, AB	49.66	-110.10		1691-93382
waviCYHILLS019[C]	Cypress Hills, AB	49.66	-110.10		1691-93383
waviNAB003[C]	Cold Lake, AB	54.60	-110.20	UMMZ	244792
waviSK002	Wascana Park, Regina, SK	50.43	-104.61		1691-93396
waviSK003	Wascana Park, Regina, SK	50.43	-104.61		1691-93397
waviSK004	Wascana Park, Regina, SK	50.43	-104.61		1691-93398
waviSK005	Wascana Park, Regina, SK	50.43	-104.61		1691-93399
waviSK006	Wascana Lake Display Pond, Regina, SK	50.43	-104.59		1691-93400
waviSK008	North Wascana Lake, Regina, SK	50.43	-104.59		2091-71902
waviSK009	North Wascana Lake, Regina, SK	50.43	-104.59		2091-71903
waviSK010	North Wascana Lake, Regina, SK	50.43	-104.59		2091-71904
waviSK011	North Wascana Lake, Regina, SK	50.43	-104.59		2091-71905
waviSK012	Les Sherman Park, Regina, SK	50.49	-104.64		2091-71906
waviND001[C]	1124 73rd St NE Towner, ND	48.28	-100.20		2580-49391
waviND002	1124 73rd St NE Towner, ND	48.28	-100.20		2580-49392
waviND003[C]	1124 73rd St NE Towner, ND	48.28	-100.20		2580-49393
waviND004*[C]	124 73rd St NE Towner, ND	48.28	-100.20		2580-49395
waviND005	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2580-49396
waviND006	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2580-49397
waviND007[C]	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2580-49398
waviND008	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2580-49399
waviND009	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2580-49400
waviND017	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2540-23071
waviND010	Hans Bay Campground, ND	48.58	-100.22		2540-23078
waviND011	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2540-23095
waviND012	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2540-23096
waviND013	Sandhill, J. Clark Salyer NWR, ND	48.32	-100.31		2540-23097
waviND014	1124 73rd St NE Towner, ND	48.28	-100.20		2540-23098
waviND015	1124 73rd St NE Towner, ND	48.28	-100.20		2540-23099
waviND016	1124 73rd St NE Towner, ND	48.28	-100.20		2540-23100
waviSD034[C]	20 miles west of Platte, SD	43.41	-99.17	DMNS	44701

waviMN001	Olmstead Co., MN	44.02	-92.38	FMNH	442528
waviMN002	Olmstead Co., MN	46.44	-93.36	FMNH	395898
waviMN003	Appleton Towers, MN [window collision]			FMNH	463124
waviMN005	Appleton Towers, MN [window collision]			FMNH	461725
waviIL001	Cook Co., Chicago IL [window collision]	41.88	-87.64	FMNH	489075
waviIL002	Cook Co., Chicago IL [window collision]	41.85	-87.61	FMNH	380960
waviIL004	Cook Co., Chicago IL [window collision]	41.89	-87.62	FMNH	500270
waviIL005	Warrenville, Herrick Lake, IL			FMNH	502741
waviIL006	West Chicago, Hawthorne and Arbor, IL			FMNH	478616
waviIL007	Wheaton, Herrick Lake, IL			FMNH	454720
waviIL008	Warrenville Rd, IL			FMNH	514966
waviIL009	IL			FMNH	495477
waviOH001	Winous Point, OH	41.46	-83.01	CMNH	71716
waviOH002	Ottawa National Wildlife Refuge, OH	41.64	-83.22	CMNH	73538
waviOH003	Winous Point, OH	41.46	-83.01	CMNH	71665
waviOH004	Winous Point, OH	41.46	-83.01	CMNH	71717
waviOH005	Winous Point, OH	41.46	-83.01	CMNH	71751
waviON001[C]	Curtis Marsh/QUBS, Ontario			NHMO	BI-42030
waviON002[C]	Massassauga Rd., Frontenac, Ontario			NHMO	BI-41604
waviON003	Massassauga Rd., Frontenac, Ontario			NHMO	BI-41605
waviON004	Lindsay Lake Stream, Frontenac, Ontario			NHMO	BI-41747
waviON005	QUBS, Leeds and Grenville, Ontario			NHMO	BI-42748
waviON006	SRB/QUBS, Ontario			NHMO	BI-9464
waviON007	SRB/QUBS, Ontario			NHMO	BI-9465
waviON008	D. Pond/QUBS, Ontario			NHMO	BI-9466
waviON009	HU/QUBS, Ontario			NHMO	BI-9467
waviON010	Lindsay Lake Stream/QUBS, Ontario			NHMO	BI-9468
waviON011	BG/QUBS, Ontario			NHMO	BI-9469
waviON012	Bedford Rd., Ontario			NHMO	BI-9470
waviON013	Bracken, Ontario			NHMO	BI-9471
waviON014	Bracken, Ontario			NHMO	BI-9472
waviON015	SRB/QUBS, Ontario			NHMO	BI-9473
waviON016	SP/QUBS, Ontario			NHMO	BI-9475
waviON017	C. Trail/M. Rd., Ontario			NHMO	BI-9570

Appendix 1.2 Pairwise F_{ST} comparions for 22 populations (n > 5) based on 14 microsatellite loci (Table 2.3). Significant *p*-values after a Benjamini-Hochberg correction, $p = \leq 0.03$, are bolded and values $p \leq 0.004$ have been replaced with an asterisk.

	CBC	JASPER	BANFF	CASTLE	WLNP	NEWA	CWA	NEOR	SWNV	NENV	NM	SWCO	CCO	CWY	NWWY	SWMT	BHILLS	SEAB	SK	ND	IL	ON
CBC	--	0.29	**0.02**	0.17	0.19	0.19	*	*	*	0.06	*	**0.01**	0.20	0.13	0.07	*	*	*	*	*	*	*
JASPER	0.01	--	**0.03**	0.24	**0.03**	0.13	**0.01**	*	**0.01**	**0.03**	*	**0.01**	0.15	0.16	0.08	0.07	*	**0.02**	*	*	*	*
BANFF	0.04	**0.05**	--	0.19	0.60	0.43	**0.02**	*	*	**0.03**	**0.01**	0.04	0.14	0.33	0.06	**0.03**	*	*	*	*	*	*
CASTLE	0.02	0.02	0.02	--	0.35	0.09	0.15	**0.01**	**0.01**	0.27	**0.01**	0.13	0.54	0.33	0.42	0.08	*	**0.02**	*	*	*	*
WLNP	0.02	**0.05**	-0.01	0.01	--	0.20	**0.01**	**0.01**	*	0.06	*	**0.02**	0.20	0.15	0.23	**0.01**	*	0.11	*	*	*	*
NEWA	0.02	0.03	0.00	0.04	0.03	--	0.40	0.13	0.64	0.56	**0.03**	0.38	0.69	0.39	0.22	0.41	*	**0.01**	*	*	*	*
CWA	**0.05**	**0.06**	0.04	0.02	**0.06**	0.00	--	0.12	**0.03**	0.74	**0.01**	0.69	0.49	0.77	0.30	0.04	*	*	*	*	*	*
NEOR	**0.09**	**0.10**	**0.10**	**0.08**	**0.09**	0.03	0.02	--	0.06	0.08	**0.02**	**0.03**	0.17	0.43	0.16	0.05	*	*	*	*	*	*
SWNV	**0.13**	**0.10**	**0.13**	**0.09**	**0.15**	-0.02	**0.06**	**0.10**	--	0.19	0.08	0.11	0.08	0.23	0.18	**0.01**	*	*	*	*	*	*
NENV	0.04	**0.05**	**0.05**	0.01	0.04	-0.01	-0.02	**0.05**	**0.05**	--	0.16	0.72	0.77	0.58	0.83	0.35	**0.02**	0.05	*	*	*	*
NM	**0.10**	**0.15**	**0.09**	**0.08**	**0.09**	**0.09**	**0.07**	**0.09**	**0.12**	0.03	--	0.31	0.33	0.71	0.23	0.05	**0.02**	0.06	*	*	*	*
SWCO	**0.06**	**0.07**	**0.05**	0.03	**0.07**	0.01	-0.01	**0.06**	**0.06**	-0.02	0.02	--	0.91	0.79	0.49	0.57	**0.02**	0.05	*	*	*	*
CCO	0.01	0.02	0.02	0.00	0.02	-0.02	0.00	0.03	**0.06**	-0.03	0.01	-0.03	--	0.90	0.25	0.79	**0.01**	0.06	*	*	*	*
CWY	0.04	0.04	0.02	0.01	0.04	0.01	-0.02	0.00	0.04	-0.02	-0.03	-0.03	-0.04	--	0.89	0.64	0.93	0.46	*	*	*	*
NWWY	0.04	**0.05**	**0.05**	0.00	0.03	0.02	0.01	0.04	0.04	-0.04	0.03	0.00	0.02	-0.04	--	0.28	**0.01**	0.17	*	*	*	*
SWMT	**0.05**	0.03	0.04	0.02	**0.06**	0.00	0.03	**0.05**	**0.10**	0.01	**0.05**	-0.01	-0.02	-0.01	0.01	--	*	**0.01**	*	*	*	*
BHILLS	**0.09**	**0.09**	**0.08**	**0.08**	**0.07**	**0.06**	**0.07**	**0.08**	**0.14**	0.04	**0.19**	**0.15**	**0.05**	-0.04	**0.23**	**0.05**	--	*	*	*	*	*
SEAB	**0.07**	**0.06**	**0.09**	**0.05**	0.00	**0.09**	**0.09**	**0.14**	**0.11**	**0.05**	**0.05**	0.04	0.04	0.00	0.02	**0.07**	**0.06**	--	**0.01**	*	*	*
SK	**0.29**	**0.26**	**0.30**	**0.25**	**0.34**	**0.28**	**0.31**	**0.36**	**0.25**	**0.26**	**0.21**	**0.23**	**0.19**	**0.18**	**0.19**	**0.23**	**0.19**	**0.09**	--	**0.02**	0.30	0.05
ND	**0.32**	**0.33**	**0.33**	**0.31**	**0.35**	**0.29**	**0.37**	**0.44**	**0.28**	**0.35**	**0.31**	**0.31**	**0.30**	**0.29**	**0.28**	**0.32**	**0.25**	**0.17**	**0.07**	--	**0.01**	0.04
IL	**0.40**	**0.32**	**0.41**	**0.35**	**0.47**	**0.42**	**0.42**	**0.50**	**0.36**	**0.40**	**0.36**	**0.37**	**0.33**	**0.34**	**0.35**	**0.35**	**0.33**	**0.17**	0.02	**0.09**	--	0.10
ON	**0.37**	**0.37**	**0.39**	**0.35**	**0.43**	**0.35**	**0.40**	**0.49**	**0.34**	**0.35**	**0.31**	**0.30**	**0.28**	**0.32**	**0.32**	**0.34**	**0.29**	**0.17**	**0.09**	**0.05**	**0.07**	--

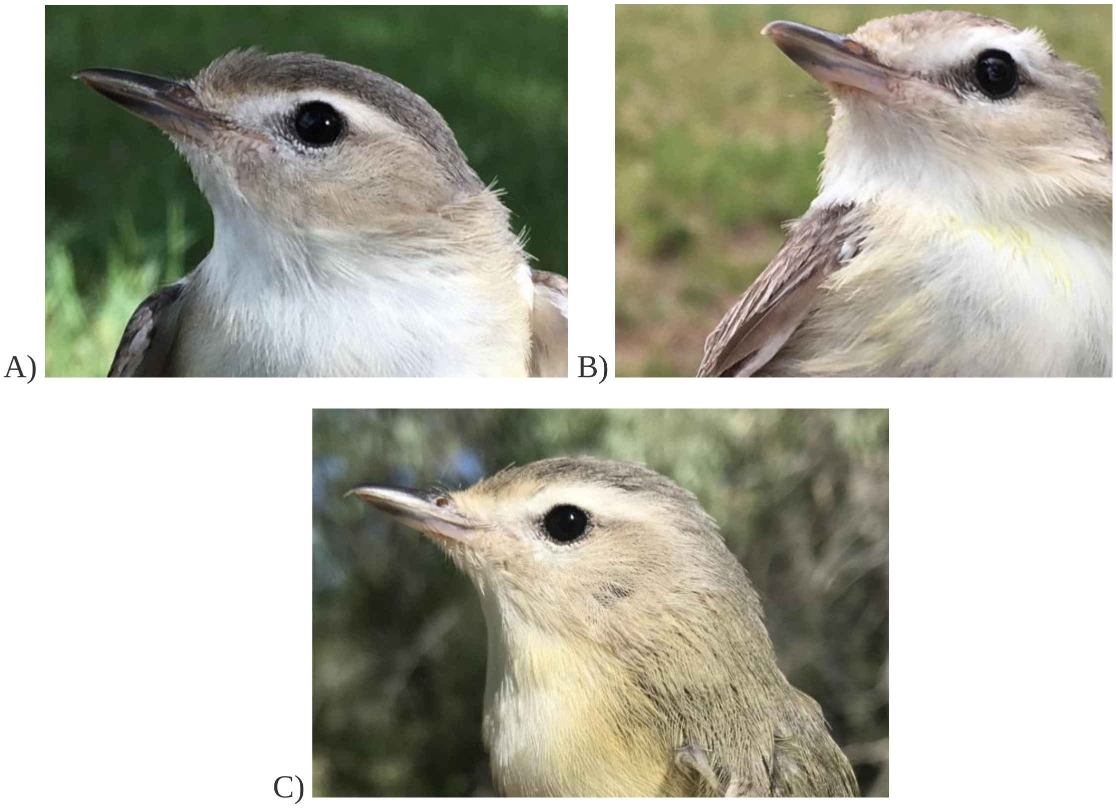
Appendix 1.3 Statistical results from a mixed model ANOVA comparing four morphometric measurements among all three subspecies of warbling vireo. Reported are degrees of freedom (*df*), *F*-values, standard deviation (StdDev), and *p*-values of each model. *P*-values that were significant ($p \leq 0.05$) are bolded.

Character	*df*	*F*-value	StdDev	*p*-value
Mass (g)	221	78.26	± 0.3	**< 0.001**
Wing chord (mm)	185	14.32	± 0.6	**< 0.001**
Bill length (mm)	165	2.63	± 1.5	0.07
Bill depth (mm)	170	25.51	± 0.2	**< 0.001**

Appendix 1.4 Complete list of variables available from WorldClim. Variables used to model *V. g. gilvus* (*), *V. g. swainsonii* (†), and *V. g. brewsteri* (•).

Layer Number	**BioClim Variable**
BIO1•	Annual mean temperature
BIO2*†•	Mean diurnal range (monthly mean (maximum temp – minimum temp))
BIO3*•	Isothermality (BIO2 / BIO7) *100
BIO4	Temperature seasonality
BIO5*	Maximum temperature of the warmest month
BIO6*†	Maximum temperature of the coldest month
BIO7*†•	Annual temperature range (BIO5 – BIO6)
BIO8*•	Mean temperature of wettest quarter
BIO9*†	Mean temperature of driest quarter
BIO10*	Mean temperature of warmest quarter
BIO11*†	Mean temperature of coldest quarter
BIO12*	Annual precipitation
BIO13•	Precipitation of wettest month
BIO14†	Precipitation of driest month
BIO15*	Precipitation seasonality
BIO16†	Precipitation of wettest quarter
BIO17†•	Precipitation of driest quarter
BIO18*†•	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter

Appendix 1.5 Three individuals of eastern warbling vireo (*Vireo gilvus gilvus*) caught in this study from different populations to illustrate the observed, geographic plumage variation: **A)** Regina, Saskatchewan (50.428096, -104.605937), **B)** Lethbridge, Alberta (49.7097473, -112.8657618), **C)** Medicine Hat, Alberta (50.0402676, -110.6490666).



Appendix 2: Supplementary Information for Chapter 3

Making contact: examining hybridization in populations of warbling vireo (*Vireo gilvus*) in a previously reported contact zone in Alberta and an unreported contact zone in western North America

Appendix 2.1 Sample ID, location, coordinates, source, and band/museum ID for each individual used in Chapter 3 listed by population. All individuals were used in the microsatellite analysis, while individuals that were also screened with cyt b primers are indicated by ^{C}. Samples caught for this study are left blank, while samples that were donated from a museum and/or collaborator are listed as follows: University of Washington Burke Museum (UWBM), Denver Museum of Nature and Science (DMNS), Royal Alberta Museum (RAM), and University of Michigan Museum (UMMZ).

Sample ID	Location	Lat (˚N)	Long (˚W)	Source	ID
waviCBC002	Prince George, BC	53.55	-122.44		wavi 2
waviCBC003	Prince George, BC	53.55	-122.44		wavi 3
waviCBC004	Smithers, BC	54.48	-127.93		wavi 4
waviCBC001	Prince George, BC	53.53	-122.49		wavi 1
waviCBC005	Lily Lake Rd, Fort Fraser, BC	53.57	-124.32		wavi 5
waviCBC006	Morfee Mt. Rd., Mackenzie, BC	55.37	-123.10		2710-78244
waviCBC007	Morfee Mt. Rd., Mackenzie, BC	55.37	-123.10		2710-78245
waviCBC008	Clearcut near boat launch, Mackenzie, BC	55.38	-123.17		2710-78246
waviCBC009	Industrial area 1, Mackenzie, BC	55.33	-123.17		2710-78247
waviCBC010	Industrial area 2, Mackenzie, BC	55.32	-123.15		2710-78248
waviCBC011	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78249
waviCBC012	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78250
waviCBC013	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78251
waviCBC014	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78252
waviCBC015	Industrial area 3, Mackenzie, BC	55.29	-123.15		2710-78254
waviCBC016	Forestry Road, Mackenzie, BC	55.30	-123.01		2710-78255
waviCBC017	Forestry Road, Mackenzie, BC	55.30	-123.01		2710-78256
waviCBC018	Forestry Road, Mackenzie, BC	55.30	-123.01		2710-78257
waviCBC019	Mugaha Marsh 1, Mackenzie, BC	55.38	-123.20		2710-78258
waviCBC020	Mugaha Marsh 1, Mackenzie, BC	55.38	-123.20		2710-78259
waviCBC021	Mugaha Marsh 2, Mackenzie, BC	55.38	-123.20		2710-78261
waviCBC022	Mugaha Marsh 2, Mackenzie, BC	55.38	-123.20		2710-78262
waviCBC023	Mugaha Marsh 2, Mackenzie, BC	55.38	-123.20		2710-78264
waviCBC024	Mugaha Marsh 2, Mackenzie, BC	55.38	-123.20		2710-78263
waviCAB001	Edson, AB	53.37	-116.49		wavi 1
waviCAB002	Edson, AB	53.37	-116.49		wavi 2
waviCAB003	Patricia Lake, Jasper, AB	52.91	-118.09		2710-78241
waviCAB004	Pyramid Beach 1, Jasper, AB	52.91	-118.10		2710-78268
waviCAB005	Pyramid Beach 1, Jasper, AB	52.91	-118.10		2710-78269
waviCAB006	Pyramid Beach 1, Jasper, AB	52.91	-118.10		2710-78270
waviCAB007	Pyramid Beach 2, Jasper, AB	52.91	-118.10		2710-78272
waviCAB008	Pyramid Beach 2, Jasper, AB	52.91	-118.10		2710-78273
waviCAB009	Pyramid Beach 2, Jasper, AB	52.91	-118.10		2710-78274
waviCAB010	MAPS station, Jasper, AB	52.55	-118.65		2340-81929
waviCAB011	MAPS station, Jasper, AB	52.55	-118.65		2340-81933
waviCAB012	Moab Lake, Jasper, AB	52.67	-117.96		2710-78277
waviCAB013	Mushroom Patch, Jasper, AB	53.06	-118.07		2710-78280

waviSEBC048	Field Cemetery, Yoho National Park	51.23	-116.29	2710-78573
waviSAB014	Vermillion Lakes Road, Banff, AB	51.10	-115.35	2710-78576
waviSAB015	Cave and Basin, Banff, AB	51.10	-115.35	2710-78577
waviSAB016	Cave and Basin, Banff, AB	51.10	-115.35	2710-78578
waviSAB017	Cave and Basin, Banff, AB	51.10	-115.35	2710-78579
waviSAB018	Vermillion Lakes 2, Banff, AB	51.10	-115.36	2710-78580
waviSAB019[C]	Vermillion Lakes 3, Banff, AB	51.10	-115.37	2710-78581
waviSAB020	Vermillion Lakes 4, Banff, AB	51.11	-115.35	2710-78582
waviCAB014[C]	Quarry Lake, AB	51.08	-115.37	2221-85084
waviCAB015[C]	Quarry Lake, AB	51.08	-115.37	2221-85085
waviCAB016[C]	Quarry Lake, AB	51.08	-115.37	2221-85086
waviCAB017[C]	Quarry Lake, AB	51.08	-115.37	2221-85087
waviCAB018[C]	Quarry Lake, AB	51.08	-115.37	2221-85088
waviCAB019[C]	Quarry Lake, AB	51.08	-115.37	2221-85089
waviCAB020[C]	Quarry Lake, AB	51.08	-115.37	2221-85090
waviSEBC001	Frisby Rd, Revelstoke, BC	51.35	-118.11	wavi 1
waviSEBC002	Frisby Rd, Revelstoke, BC	51.35	-118.11	wavi 2
waviSEBC003	Frisby Rd, Revelstoke, BC	51.35	-118.11	wavi 3
waviSEBC004	Frisby Ridge Rd, Revelstoke, BC	51.82	-118.12	wavi 4
waviSEBC005	Frisby Ridge Rd, Revelstoke, BC	51.25	-118.13	wavi 5
waviSEBC006	Frisby Ridge Rd, Revelstoke, BC	51.25	-118.13	wavi 6
waviSEBC007	Frisby Ridge Rd, Revelstoke, BC	51.25	-118.13	wavi 7
waviSEBC008	Frisby Ridge Rd, Revelstoke, BC	51.25	-118.13	wavi 8
waviSEBC009	Frisby Ridge Rd, Revelstoke, BC	51.25	-118.13	wavi 9
waviSEBC010	Frisby Ridge Rd, Revelstoke, BC	51.32	-118.13	wavi 10
waviSEBC011	Arrow Lakes Reservoir, BC	50.58	-118.11	wavi 11
waviSEBC012	Revelstoke Dump, Revelstoke, BC	51.12	-118.14	wavi 12
waviSEBC013	Revelstoke Dump, Revelstoke, BC	51.12	-118.14	wavi 13
waviSEBC014	Revelstoke Dump, Revelstoke, BC	51.12	-118.14	wavi 14
waviSEBC018	Revelstoke Industrial Park, Revelstoke, BC	50.59	-118.10	wavi 18
waviSEBC016	Revelstoke Industrial Park, Revelstoke, BC	50.59	-118.10	wavi 16
waviSEBC017	Westside Rd 1, Revelstoke, BC	51.01	-118.13	wavi 17
waviSEBC024	Revelstoke City Park, Revelstoke, BC	50.59	-118.11	wavi 24
waviSEBC035	Frisby Rd, low elevation, Revelstoke BC	51.39	-118.13	2710-78550
waviSEBC036	Frisby Rd, mid elevation, Revelstoke BC	51.39	-118.13	2710-78551
waviSEBC037	Frisby Rd, mid elevation, Revelstoke BC	51.39	-118.13	2710-78552
waviSEBC038	Frisby Rd, mid elevation, Revelstoke BC	51.39	-118.13	2710-78553
waviSEBC039	Frisby Rd, mid elevation, Revelstoke BC	51.39	-118.13	2710-78554
waviSEBC040	Frisby Rd, high elevation, Revelstoke BC	51.39	-118.15	2710-78559
waviSEBC041	Frisby Rd, high elevation, Revelstoke BC	51.39	-118.15	2710-78560
waviSEBC042	Blank Creek Forest Rd, Enderby, BC	50.50	-118.63	2710-78562
waviSEBC043	Blank Creek Forest Rd, Enderby, BC	50.50	-118.63	2710-78563
waviSEBC044	Blank Creek Forest Rd, Enderby, BC	50.50	-118.63	2710-78564
waviSEBC045	Blank Creek Forest Rd, Enderby, BC	50.49	-118.75	2710-78567
waviSEBC046	Blank Creek Forest Rd, Enderby, BC	50.49	-118.75	2710-78568

waviSEBC047	Blank Creek Forest Rd, Enderby, BC	50.49	-118.75	2710-78569
waviSEBC049	Goose Lake Rd, Kamloops, BC	50.59	-120.38	2221-85091
waviSEBC050	Goose Lake Rd, Kamloops, BC	50.59	-120.38	2221-85092
waviSEBC051	Goose Lake Rd, Kamloops, BC	50.59	-120.38	2221-85094
waviSEBC052	Goose Lake Rd, Kamloops, BC	50.59	-120.38	2221-85095
waviSEBC053	Goose Lake Rd, Kamloops, BC	50.59	-120.38	2221-85096
waviSEBC054	McConnel Lake Rd, Kamloops, BC	50.55	-120.44	2221-85097
waviSEBC055	McConnel Lake Rd, Kamloops, BC	50.55	-120.44	2221-85098
waviSEBC058	Silver Star Rd, Vernon, BC	50.33	-119.13	1691-93316
waviSEBC059	Silver Star Rd, Vernon, BC	50.33	-119.13	1691-93317
waviSEBC060	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93318
waviSEBC061	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93319
waviSEBC062	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93320
waviSEBC063	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93321
waviSEBC064	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93322
waviSEBC065	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93323
waviSEBC066	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93324
waviSEBC067	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93325
waviSEBC068	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93326
waviSEBC069	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93327
waviSEBC070	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93328
waviSEBC071	Silver Star Rd, Vernon, BC	50.34	-119.13	1691-93329
waviSEBC072	Rose Valley Regional Park, Kelowna, BC	49.88	-119.56	1691-93330
waviSEBC073	Enterprise Park, Kelowna, BC	49.88	-119.43	1691-93331
waviSEBC074	Westside Rd, Lake Country/Kelowna, BC	50.12	-119.50	1691-93332
waviSEBC075	Westside Rd, Lake Country/Kelowna, BC	50.12	-119.50	1691-93333
waviSEBC076	Westside Rd, Lake Country/Kelowna, BC	50.12	-119.50	1691-93334
waviSEBC077	Westside Rd, Lake Country/Kelowna, BC	50.12	-119.50	1691-93335
waviSEBC086	Max Lake Rd, Penticton, BC	49.50	-119.64	1691-93344
waviSEBC087	Max Lake Rd, Penticton, BC	49.50	-119.64	1691-93345
waviSEBC088	Green Lake Rd, Okanagan Falls/Penticton, BC	49.32	-119.57	1691-93346
waviSEBC089	Green Lake Rd, Okanagan Falls/Penticton, BC	49.32	-119.57	1691-93347
waviSEBC090	Green Lake Rd, Okanagan Falls/Penticton, BC	49.32	-119.57	1691-93348
waviSEBC091	Green Lake Rd, Okanagan Falls/Penticton, BC	49.32	-119.57	1691-93349
waviSEBC031	South Okanagan Grasslands, Osoyoos, BC	49.67	-119.36	2710-78545
waviSEBC032	South Okanagan Grasslands, Osoyoos, BC	49.67	-119.36	2710-78546
waviSEBC033	Robertson House, Osoyoos, BC	49.40	-119.33	2710-78547
waviSEBC034	South Okanagan Grasslands, Osoyoos, BC	49.67	-119.36	2710-78548
waviSEBC082	South Okanagan Grasslands, Osoyoos, BC	49.10	-119.61	1691-93340
waviSEBC083	South Okanagan Grasslands, Osoyoos, BC	49.10	-119.61	1691-93341
waviSEBC084	South Okanagan Grasslands, Osoyoos, BC	49.10	-119.61	1691-93342
waviSEBC085	South Okanagan Grasslands, Osoyoos, BC	49.10	-119.61	1691-93343
waviSEBC092	South Okanagan Grasslands, Osoyoos, BC	49.10	-119.61	1691-93350
waviSEBC093	South Okanagan Grasslands, Osoyoos, BC	49.10	-119.61	1691-93351
waviSEBC094	South Okanagan Grasslands, Osoyoos, BC	49.10	-119.61	1691-93352

waviSEBC078	McKinney Rd, Oliver, BC	49.17	-119.45		1691-93336
waviSEBC079	Oliver Bike Trail, Oliver, BC	49.19	-119.55		1691-93337
waviSEBC080	Oliver Bike Trail, Oliver, BC	49.19	-119.55		1691-93338
waviSEBC081	Oliver Bike Trail, Oliver, BC	49.19	-119.55		1691-93339
waviSWBC001	Lightning Lakes 1, Manning, BC	49.06	-120.84		2710-78281
waviSWBC002	Lightning Lakes 1, Manning, BC	49.06	-120.84		2710-78282
waviSWBC003	PCT Trailhead, Manning, BC	49.06	-120.80		2710-78286
waviSWBC005	Cambie Creek Trailhead, Manning, BC	49.11	-120.85		2710-78288
waviSWBC006	Cambie Creek Trailhead, Manning, BC	49.11	-120.85		2710-78289
waviWA015	Naneum Creek, Kittitas, WA			UWBM	121335
waviWA016	Naneum Creek, Kittitas, WA			UWBM	121383
waviWA017	1801 North B Street, Kittitas, WA			UWBM	89898
waviWA018	Whiskey Dick Creek, Kittitas, WA			UWBM	66875
waviWA019	Whiskey Dick Creek, Kittitas, WA			UWBM	66876
waviWA020	Whiskey Dick Creek, Kittitas, WA			UWBM	66877
waviWA021	Iron Creek, Kittitas, WA			UWBM	120123
waviWA022	Iron Creek, Kittitas, WA			UWBM	118370
waviWA023	South Fork Manastash Creek, Kittitas, WA			UWBM	119559
waviWA024	Whiskey Dick Creek, Kittitas, WA			UWBM	66828
waviWA025	Twisp River, Okanogan, WA			UWBM	118280
waviWA026	Winthrop, Bear Creek, Okanogan, WA	48.48	-120.10	UWBM	113779
waviWA027	Winthrop, Bear Creek, Okanogan, WA	48.48	-120.10	UWBM	113780
waviWA028	Winthrop, Bear Creek, Okanogan, WA	48.48	-120.10	UWBM	113778
waviWA029	Bear Creek, Okanogan, WA			UWBM	113782
waviWA030	Winthrop, Bear Creek, Okanogan, WA	48.48	-120.10	UWBM	113781
waviWA031	Twisp River, Okanogan, WA			UWBM	118297
waviWA032	Benson Creek, Okanogan, WA			UWBM	118332
waviWA033	Little Buck Mountain, Okanogan, WA			UWBM	86319
waviWA034	Okanogan, WA			UWBM	85954
waviNEOR001	Catherine Creek St. park, OR	45.98	-117.44		wavi 1
waviNEOR002	Bird Track Springs Trail, OR	45.18	-118.18		wavi 2
waviNEOR003	Hilgard junction state park, OR	45.20	-118.14		wavi 3
waviNEOR004	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112650
waviNEOR005	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112649
waviNEOR006	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112648
waviNEOR007	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112647
waviNEOR008	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112646
waviNEOR009	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112645
waviNEOR010	Wallowa-Whitman Nat. Forest, Wallowa, WA			UWBM	112644
waviSEOR001	Steens Mountain Wilderness, OR	42.64	-118.76	DMNS	48097
waviCA007	Emerson Cr., Warner Mountains, Modoc, CA			UWBM	117496
waviCA008	Emerson Cr., Warner Mountains, Modoc, CA			UWBM	117794
waviCA010	Emerson Cr., Warner Mountains, Modoc, CA			UWBM	117448
waviCA011	Emerson Cr., Warner Mountains, Modoc, CA			UWBM	117518
waviCA001	Wyman Creek; White Mountains, Inyo, CA			UWBM	117576

waviCA002	Wyman Creek; White Mountains, Inyo, CA			UWBM	117762
waviCA005	Bishop, White Mountains, Inyo, CA	37.24	-118.14	UWBM	100552
waviNV001	Bonanza Trail, Spring Mountains, Clark, NV	36.22	-115.45	UWBM	109683
waviNV002	Bonanza Trail, Spring Mountains, Clark, NV	36.22	-115.45	UWBM	109684
waviNV003	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111568
waviNV004	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111569
waviNV005	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111584
waviNV006	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111581
waviNV007	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111582
waviNV008	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	106733
waviNV009	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	106734
waviNV010	Bonanza Trail, Spring Mountains, Clark, NV	36.23	-115.45	UWBM	111585
waviNV021	Kingston Canyon, Lander, NV			UWBM	116330
waviNV022	Kingston Canyon, Lander, NV			UWBM	116331
waviNV023	San Juan Creek, Lander, NV			UWBM	116611
waviNV024	Kingston Canyon, Lander, NV			UWBM	116334
waviNV025	San Juan Creek, Lander, NV			UWBM	119129
waviNV026	Kingston Canyon, Lander, NV			UWBM	116326
waviNV027	Toiyabe Range, Lander, NV			UWBM	100362
waviNV028	San Juan Creek, Lander, NV			UWBM	116526
waviNV029	Toiyabe Range, Lander, NV			UWBM	100417
waviNV030	Toiyabe Range, Lander, NV			UWBM	100418
waviNV011	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	112109
waviNV012	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	112107
waviNV013	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	112111
waviNV014	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	107130
waviNV015	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	107126
waviNV016	Jarbidge, 1.5 mi W, Elko, NV	41.53	-115.26	UWBM	107124
waviNV017	Jarbidge, 0.5 mi N, Elko, NV	41.54	-115.25	UWBM	107133
waviNV018	Mountain City, 15 mi ESE, Elko, NV	41.47	-115.42	UWBM	107128
waviNV019	Mountain City, 15 mi ESE, Elko, NV	41.47	-115.42	UWBM	107117
waviNV020	Mountain City, 15 mi ESE, Elko, NV	41.47	-115.42	UWBM	107116
waviID005	Lily Marsh, Ponderosa Park, ID	44.56	-116.43		wavi 3
waviID014	Tyndall Meadows, Valley, ID			UWBM	122085
waviID015	Tyndall Meadows, Valley, ID			UWBM	122088
waviID016	Tyndall Meadows, Valley, ID			UWBM	122052
waviID001	Nature Conservancy Trail, Sandpoint, ID	48.13	-116.33		wavi 1
waviID007	Loop Creek, Shoshone, ID			UWBM	121914
waviID008	Loop Creek, Shoshone, ID			UWBM	121915
waviID009	Loop Creek, Shoshone, ID			UWBM	121998
waviID010	Loop Creek, Shoshone, ID			UWBM	122015
waviID011	Loop Creek, Shoshone, ID			UWBM	122016
waviID012	Loop Creek, Shoshone, ID			UWBM	122022
waviID013	Loop Creek, Shoshone, ID			UWBM	122129
waviWA001	Big Meadow Lake, WA	48.43	-117.33		2540-22996

waviWA002	Big Meadow Lake, WA	48.43	-117.33		2540-22997
waviWA013	Walker Valley ORV Trailhead, WA	48.22	-117.10		2580-49307
waviWA014	Amazon Creek, Steven's County, WA	48.32	-117.36		2580-49324
waviWA003	Amazon Creek, Steven's County, WA	48.32	-117.36		2580-49326
waviWA004	Power Lake, Steven's County, WA	48.15	-117.23		2580-49329
waviWA005	Twin Lakes, Steven's County, WA	48.34	-117.38		2580-49332
waviWA006	Twin Lakes, Steven's County, WA	48.34	-117.38		2580-49333
waviWA007	Twin Lakes, Steven's County, WA	48.34	-117.38		2580-49334
waviWA008	Starvation Lake, WA	49.29	-117.42		2580-49335
waviWA009	Gillette Lake Road, Steven's County, WA	48.36	-117.33		2580-49336
waviWA010	Gillette Lake Road, Steven's County, WA	48.36	-117.33		2580-49337
waviWA011	Gillette Lake Road, Steven's County, WA	48.36	-117.33		2580-49340
waviWA012	Gillette Lake Road, Steven's County, WA	48.36	-117.33		2580-49343
waviSEBC015	Whitewater Ski Hill Rd, Nelson, BC	49.26	-117.85		wavi 15
waviSEBC096	Whitewater Ski Hill Rd, Nelson, BC	49.44	-117.14		1691-93353
waviSEBC097	Whitewater Ski Hill Rd, Nelson, BC	49.44	-117.14		1691-93354
waviSEBC098	Whitewater Ski Hill Rd, Nelson, BC	49.44	-117.14		1691-93355
waviSEBC099	Whitewater Ski Hill Rd, Nelson, BC	49.44	-117.14		1691-93356
waviSEBC100	Whitewater Ski Hill Rd, Nelson, BC	49.44	-117.14		1691-93357
waviSEBC101	Whitewater Ski Hill Rd, Nelson, BC	49.41	-117.18		1691-93358
waviSEBC102	Whitewater Ski Hill Rd, Nelson, BC	49.41	-117.18		1691-93359
waviSEBC103	Cottonwood Park, Nelson, BC	49.43	-117.25		1691-93360
waviSAB002[C]	West Castle, AB	49.20	-114.25		wavi 2
waviSAB003[C]	West Castle, AB	49.20	-114.25		wavi 3
waviSAB004[C]	West Castle, AB	49.20	-114.25		wavi 4
waviSAB005[C]	West Castle, AB	49.20	-114.25		wavi 5
waviSAB006[C]	West Castle, AB	49.20	-114.25		wavi 6
waviSAB007[C]	West Castle, AB	49.20	-114.25		wavi 7
waviSAB008[C]	West Castle, AB	49.20	-114.25		wavi 8
waviSAB013	River Valley Park, Fort Macleod (811 Bridge)	49.44	-113.24		2430-38995
waviSAB021[C]	West Castle Wetlands, AB	49.22	-114.22		2710-78585
waviSAB022	West Castle Wetlands, AB	49.20	-114.26		2710-78586
waviSAB023	Lynx Creek Road, near Beaver Mines, AB	49.27	-114.22		2710-78587
waviSAB024	Lynx Creek Road, near Beaver Mines, AB	49.27	-114.22		2710-78588
waviSAB025	Beaver Mines Lake Recreation Area, AB	49.22	-114.19		2710-78589
waviSAB026	Beaver Mines Lake Recreation Area, AB	49.22	-114.19		2710-78590
waviSAB027	Beaver Mines Lake Recreation Area, AB	49.22	-114.19		2710-78591
waviSAB028	Beaver Mines Lake Recreation Area, AB	49.24	-114.20		2710-78592
waviSAB029	Beaver Mines Lake Recreation Area, AB	49.24	-114.20		2710-78593
waviSAB035	Castle River, AB	49.31	-114.30	RAM	Z06.4.3
waviSAB009	Hay Barn, Waterton, AB	49.04	-113.51		wavi 9
waviSAB010[C]	Belly River Campground Waterton, AB	49.12	-113.41		A
waviSAB011[C]	Belly River Campground Waterton, AB	49.12	-113.41		B
waviSAB012[C]	Belly River Campground Waterton, AB	49.12	-113.41		C
waviSAB030	Road to Cameron Lake, Waterton Lakes, AB	49.42	-113.59		2710-78594

waviSAB031	Road to Cameron Lake, Waterton Lakes, AB	49.42	-113.59		2710-78595
waviSAB032	Canyon Road, Waterton Lakes, AB	49.54	-113.50		2710-78596
waviSAB033	Canyon Road, Waterton Lakes, AB	49.54	-113.50		2710-78597
waviSAB034	Stables Road, Waterton, AB	49.40	-113.53		2710-78611
waviNWMT001	Flathead National Forest, Whitefish, MT	48.37	-114.53		2591-37601
waviNWMT002	Flathead National Forest, Whitefish, MT	48.37	-114.53		2591-37602
waviNWMT003	Flathead National Forest, Whitefish, MT	48.37	-114.53		2591-37603
waviNWMT004	Tally Lake Campground, Whitefish, MT	48.41	-114.58		2591-37604
waviNWMT005	Flathead National Forest, Whitefish, MT	48.35	-114.50		2591-37605
waviNWMT006	Tally Lake Campground, Whitefish, MT	48.41	-114.58		2591-37606
waviNWMT007	Tally Lake Campground, Whitefish, MT	48.41	-114.58		2591-37607
waviNWMT008	Tally Lake Campground, Whitefish, MT	48.41	-114.58		2591-37608
waviNWMT009	Tally Lake Campground, Whitefish, MT	48.41	-114.58		2591-37609
waviNWMT010	Tally Lake Campground, Whitefish, MT	48.41	-114.58		1881-86711
waviNWMT011	Tally Lake Campground, Whitefish, MT	48.41	-114.58		2591-37610
waviNWMT012	Tally Lake Campground, Whitefish, MT	48.41	-114.58		2591-37611
waviNWMT013	Columbia Mt Trail, Whitefish, MT	48.38	-114.11		2591-37612
waviNWMT014	Columbia Mt Trail, Whitefish, MT	48.38	-114.11		2591-37613
waviNWMT015	Columbia Mt Trail, Whitefish, MT	48.38	-114.11		2591-37614
waviNWMT016	Columbia Mt Trail, Whitefish, MT	48.38	-114.11		2591-37615
waviNWMT017	Columbia Mt Trail, Whitefish, MT	48.38	-114.11		2591-37616
waviNWMT018	Columbia Mt Trail, Whitefish, MT	48.38	-114.11		2591-37617
waviMT038	18 miles northwest of Augusta, MT	47.60	-112.74	DMNS	45392
waviMT044	18 miles northwest of Augusta, MT	47.60	-112.74	DMNS	45393
waviMT045	18 miles northwest of Augusta, MT	47.60	-112.74	DMNS	45397
waviMT046	70 miles west of Choteau, MT	47.90	-113.52	DMNS	45407
waviMT048	30 miles northwest of Choteau, MT	48.00	-112.70	DMNS	45401
waviMT049	70 miles northwest of Choteau, MT	47.90	-113.52	DMNS	45414
waviMT050	18 miles northwest of Augusta, MT	47.60	-112.74	DMNS	45394
waviMT051	18 miles northwest of Augusta, MT	47.60	-112.74	DMNS	45396
waviMT052	18 miles northwest of Augusta, MT	47.60	-112.74	DMNS	45398
waviMT058	18 miles northwest of Augusta, MT	47.60	-112.74	DMNS	45395
waviMT001	Helena National Forest, Helena, MT	46.29	-111.51		wavi 1
waviMT002	Orofino, Helena, MT	46.34	-112.40		wavi 2
waviMT003	Road to Park Lake, Helena, MT	46.31	-112.07		wavi 3
waviMT004	Road to Park Lake, Helena, MT	46.31	-112.07		wavi 4
waviMT005	Road to Park Lake, Helena, MT	46.31	-112.07		wavi 5
waviMT009	Canyon off Highway 2, Butte, MT	45.53	-112.28		wavi 9
waviMT010	Cottonwood Creek, Beartooth NWMA, MT	46.56	-111.56		2580-49347
waviMT011	Cottonwood Creek, Beartooth NWMA, MT	46.56	-111.56		2580-49348
waviMT012	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49349
waviMT014	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49351
waviMT016	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49353
waviMT018	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49355
waviMT019	Whitehouse campground, Basin, MT	46.15	-112.28		2580-49356

waviMT020	Whitehouse campground, Basin, MT	46.15	-112.28	2580-49357
waviMT021	Whitehouse campground, Basin, MT	46.15	-112.28	2580-49358
waviMT022	Lowland Creek Rd, Elk Park, MT	46.11	-112.27	2580-49360
waviMT023	Lowland Creek Rd, Elk Park, MT	46.11	-112.27	2580-49361
waviMT024	Lowland Creek Rd, Elk Park, MT	46.11	-112.27	2580-49362
waviMT025	Lowland Creek Rd, Elk Park, MT	46.11	-112.27	2580-49363
waviMT026	Lowland Creek Rd, Elk Park, MT	46.11	-112.27	2580-49365
waviMT027	Lowland Creek Rd, Elk Park, MT	46.11	-112.27	2580-49366
waviMT028	Lowland Creek Rd, Elk Park, MT	46.11	-112.27	2580-49367
waviMT029	Thunderbolt Creek Trailhead, Basin, MT	46.17	-112.28	2580-49368
waviMT030	Thunderbolt Creek Trailhead, Basin, MT	46.17	-112.28	2580-49369
waviMT031	Thunderbolt Creek Trailhead, Basin, MT	46.17	-112.28	2580-49370
waviMT032	Moose Creek Campground, Helena, MT	46.32	-112.15	2580-49372
waviMT033	Moose Creek Campground, Helena, MT	46.32	-112.15	2580-49374
waviMT034	Rimini Rd, Helena, MT	46.32	-112.15	2580-49376
waviMT035	Rimini Rd, Helena, MT	46.32	-112.15	2580-49377
waviMT007	Bridger Woods Rd, Bozeman, MT	45.41	-110.54	wavi 7
waviMT008	Bridger Woods Rd, Bozeman, MT	45.41	-110.54	wavi 8
waviSWMT001	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37618
waviSWMT002	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37619
waviSWMT003	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37620
waviSWMT004	Hood Creek Campground, Bozeman, MT	45.48	-110.97	2591-37621
waviSWMT005	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37622
waviSWMT006	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37623
waviSWMT007	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37624
waviSWMT008	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37625
waviSWMT009	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37626
waviSWMT010	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37627
waviSWMT011	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37628
waviSWMT012	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37629
waviSWMT013	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37630
waviSWMT014	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37631
waviSWMT015	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37632
waviSWMT016	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37633
waviSWMT017	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37634
waviSWMT018	Hood Creek Campground, Bozeman, MT	45.48	-110.97	2591-37635
waviSWMT019	Hood Creek Campground, Bozeman, MT	45.48	-110.97	2591-37636
waviSWMT020	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37637
waviSWMT021	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37638
waviSWMT022	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37639
waviSWMT023	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37640
waviSWMT024	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37641
waviSWMT025	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37642
waviSWMT026	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37643
waviSWMT027	Sourdough Trail, Bozeman, MT	45.59	-111.02	2591-37644

waviSWMT028	Sourdough Trail, Bozeman, MT	45.59	-111.02		2591-37645
waviSWMT029	Sourdough Trail, Bozeman, MT	45.59	-111.02		2591-37646
waviWY007	Ferris Mountains, WY	42.29	-107.32	DMNS	46029
waviWY008	18 miles south of Lander, WY	42.51	-108.81	DMNS	45043
waviWY009	18 miles south of Lander, WY	42.51	-108.81	DMNS	45044
waviWY010	18 miles south of Lander, WY	42.57	-108.73	DMNS	45018
waviWY011	18 miles south of Lander, WY	42.57	-108.73	DMNS	44979
waviWY014	18 miles south of Lander, WY	42.57	-108.73	DMNS	44968
waviWY016	18 miles south of Lander, WY	42.57	-108.73	DMNS	44996
waviWY017	19 miles south of Lander, WY	42.57	-108.73	DMNS	45003
waviWY018	20 miles south of Lander, WY	42.57	-108.73	DMNS	45001
waviWY001	Aspen Trailhead, Bridger-Teton NF, WY	43.42	-110.36		wavi 1
waviUT001	W of Woodruff (Hwy 39), UT	41.27	-111.31		wavi 1
waviUT002	W of Woodruff (Hwy 39), UT	41.27	-111.31		wavi 2
waviUT003	Cache National Forest (Forestry Rd.), UT	41.30	-111.30		wavi 3
waviUT004	South Fork campground, UT	41.16	-111.39		wavi 4
waviCO005	Fremont, CO			UWBM	70334
waviCO006	Off highway 9, Fremont, CO			UWBM	113803
waviCO007	Off highway 9, Fremont, CO			UWBM	113802
waviCO008	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112082
waviCO009	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112077
waviCO010	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112076
waviCO011	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112080
waviCO012	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	112078
waviCO013	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	107090
waviCO014	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	107091
waviCO015	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	107093
waviCO016	Cherry Creek Road, La Plata, CO	37.19	-108.08	UWBM	107089
waviCO017	Missionary Ridge Road, La Plata, CO	37.26	-107.45	UWBM	114990
waviWY002	Ferris Mountains, WY	42.29	-107.32	DMNS	46028
waviWY003	Ferris Mountains, WY	42.29	-107.31	DMNS	46026
waviWY004	Green Mountains, WY	42.34	-107.68	DMNS	45973
waviWY005	Ferris Mountains, WY	42.29	-107.32	DMNS	46021
waviWY006	Laramie Mountains near Esterbrook, WY	42.42	-105.34	DMNS	46041
waviWY019	Fremont County - Green Mountains, WY	42.34	-107.68	DMNS	45973
waviSEMT001	Silver Run Trail, CNF, Red Lodge, MT	45.15	-109.34		2591-37647
waviSEMT002	Silver Run Trail, CNF, Red Lodge, MT	45.15	-109.34		2591-37648
waviSEMT003	Silver Run Trail, CNF, Red Lodge, MT	45.15	-109.34		2591-37649
waviSEMT004	Silver Run Trail, CNF, Red Lodge, MT	45.15	-109.34		2591-37650
waviSEMT005	Silver Run Trail, CNF, Red Lodge, MT	45.15	-109.34		2591-37651
waviSEMT006	Silver Run Trail, CNF, Red Lodge, MT	45.15	-109.34		2591-37652
waviSEMT007	Basin Campground, CNF, Red Lodge, MT	45.16	-109.39		2591-37653
waviSEMT008	Basin Campground, CNF, Red Lodge, MT	45.16	-109.39		2591-37654
waviSEMT009	Basin Campground, CNF, Red Lodge, MT	45.16	-109.39		2591-37655
waviSEMT010	CNF, Red Lodge, MT	45.15	-109.36		2591-37656

waviSEMT011	CNF, Red Lodge, MT	45.15	-109.36		2591-37657
waviSEMT012	CNF, Red Lodge, MT	45.15	-109.36		2591-37658
waviSEMT013	Palisades Campground, CNF, Red Lodge, MT	45.17	-109.30		2591-37659
waviSEMT014	Palisades Campground, CNF, Red Lodge, MT	45.17	-109.30		2591-37660
waviSEMT015	Palisades Campground, CNF, Red Lodge, MT	45.17	-109.30		2591-37661
waviSEMT016	CNF, Red Lodge, MT	45.15	-109.31		2591-37662
waviSEMT017	CNF, Red Lodge, MT	45.15	-109.31		2591-37663
waviSEMT018	Basin Campground, CNF, Red Lodge, MT	45.16	-109.39		2591-37664
waviSEMT019	Basin Campground, CNF, Red Lodge, MT	45.16	-109.39		2591-37666
waviSEMT020	CNF, Red Lodge, MT	45.15	-109.37		2591-37667
waviSEMT021	CNF, Red Lodge, MT	45.15	-109.37		2591-37668
waviSEMT022	CNF, Red Lodge, MT	45.15	-109.37		2591-37669
waviSEMT023	CNF, Red Lodge, MT	45.15	-109.37		2591-37670
waviSEMT024	Silver Run Logged Area, CNF Red Lodge, MT	45.15	-109.34		2591-37671
waviSEMT025	Wild Bill Lake, CNF, Red Lodge, MT	45.15	-109.36		2591-37672
waviSEMT026	Wild Bill Lake, CNF, Red Lodge, MT	45.15	-109.36		2591-37673
waviSEMT027	Mountain Bike Trail, CNF, Red Lodge, MT	45.16	-109.31		2591-37674
waviSEMT028	Mountain Bike Trail, CNF, Red Lodge, MT	45.16	-109.31		2591-37675
waviSEMT029	Mountain Bike Trail, CNF, Red Lodge, MT	45.16	-109.31		wavi 29
waviSEMT030	Mountain Bike Trail, CNF, Red Lodge, MT	45.16	-109.31		2591-37676
waviMT041	30 miles south of Lewistown, MT	46.71	-109.33	DMNS	45331
waviMT042	33 miles south of Lewistown, MT	46.71	-109.33	DMNS	45334
waviMT047	30 miles south of Lewistown, MT	46.71	-109.33	DMNS	45335
waviMT053	30 miles south of Lewistown, MT	46.71	-109.33	DMNS	45341
waviMT055	32 miles south of Lewistown, MT	46.71	-109.33	DMNS	45349
waviLETH001[C]	Elizabeth Hall Wetlands, Lethbridge, AB	49.70	-112.86		1691-93389
waviLETH002[C]	Elizabeth Hall Wetlands, Lethbridge, AB	49.70	-112.86		1691-93390
waviLETH003[C]	Elizabeth Hall Wetlands, Lethbridge, AB	49.70	-112.86		1691-93391
waviLETH004[C]	Boterill Bottom Park, Lethbridge, AB	49.68	-112.85		1691-93392
waviLETH005[C]	Helen Schuler Preserve, Lethbridge, AB	49.10	-112.86		1691-93393
waviLETH006[C]	Pavon Park, Lethbridge, AB	49.75	-112.85		1691-93394
waviCAB036[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z06.6.18
waviCAB037[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z06.6.19
waviCAB038[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z06.6.20
waviCAB039[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z07.8.11
waviCAB040[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z07.8.12
waviCAB041[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z07.8.13
waviCAB042[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z07.8.14
waviCAB043[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z07.8.15
waviCAB054[C]	Pine Lake, Red Deer, AB	52.14	-113.48	RAM	Z07.8.26
waviCAB068[C]	Alder Flats, AB	52.92	-115.08	RAM	Z99.11.2
waviCAB069[C]	Alder Flats, AB	52.92	-115.08	RAM	Z99.11.4
waviCAB070[C]	Alder Flats, AB	52.90	-115.09	RAM	Z99.11.5
waviCAB071[C]	Alder Flats, AB	52.90	-115.09	RAM	Z99.11.6
waviCAB072[C]	Alder Flats, AB	52.90	-115.10	RAM	Z99.11.7

waviCAB073^{C}	Alder Flats, AB	52.90	-115.11	RAM	Z99.11.8
waviCAB024^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71910
waviCAB025^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71911
waviCAB026^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71912
waviCAB027^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71913
waviCAB028^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71914
waviCAB029^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71915
waviCAB030^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71916
waviCAB031^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71917
waviCAB032^{C}	Clifford E Lee Preserve, W Edmonton, AB	53.41	-113.79		2091-71918
waviCAB033^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z06.6.13
waviCAB034^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z06.6.14
waviCAB044^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z07.8.16
waviCAB045^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z07.8.17
waviCAB046^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z07.8.18
waviCAB047^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z07.8.19
waviCAB048^{C}	East of Peanut Lake, Barrhead, AB	54.01	-114.33	RAM	Z07.8.20
waviCAB049^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z07.8.21
waviCAB050^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z07.8.22
waviCAB051^{C}	North of Peanut Lake, Barrhead, AB	54.03	-114.33	RAM	Z07.8.23
waviCAB052^{C}	East of Peanut Lake, Barrhead, AB	54.01	-114.33	RAM	Z07.8.24
waviCAB053^{C}	East of Peanut Lake, Barrhead, AB	54.01	-114.33	RAM	Z07.8.25
waviCAB055^{C}	East of Peanut Lake, Barrhead, AB	54.01	-114.33	RAM	Z07.8.27
waviCAB056^{C}	Southeast of Clear Lake, Barrhead, AB	54.22	-114.81	RAM	Z07.8.28
waviCAB057^{C}	Thunder Lake Provincial Park, Barrhead, AB	54.09	-114.71	RAM	Z07.8.29
waviCAB058^{C}	Southeast of Clear Lake, Barrhead, AB	54.22	-114.81	RAM	Z07.8.30
waviCAB059^{C}	Northwest Barrhead, AB	54.23	-114.36	RAM	Z07.8.32
waviCAB060^{C}	Northwest Barrhead, AB	54.23	-114.36	RAM	Z07.8.33
waviCAB062^{C}	Northwest Barrhead, AB	54.23	-114.36	RAM	Z07.8.35
waviCAB077^{C}	Northwest Barrhead, AB	54.23	-114.36	RAM	Z07.8.37
waviCAB061^{C}	Southeast Barrhead, AB	54.95	-114.17	RAM	Z07.8.34
waviCAB063^{C}	Southeast Barrhead, AB	54.95	-114.17	RAM	Z07.8.36
waviCAB064^{C}	Southeast Barrhead, AB	54.95	-114.17	RAM	Z07.8.38
waviCAB065^{C}	Southeast Barrhead, AB	54.95	-114.17	RAM	Z07.8.39
waviCAB066^{C}	Southeast Barrhead, AB	54.95	-114.17	RAM	Z07.8.40
waviNAB006^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z06.3.13
waviNAB008^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z06.3.5
waviNAB009^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z06.3.6
waviCAB074^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z06.3.12
waviCAB075^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z07.8.02
waviCAB076^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z07.8.03
waviCAB080^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z07.8.07
waviCAB081^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z07.8.08
waviCAB082^{C}	Swan Hills, AB	55.02	-115.48	RAM	Z07.8.09
waviNAB001^{C}	Slave Lake, AB	55.49	-113.98	UMMZ	244743

waviNAB002^{C}	Slave Lake, AB	55.71	-115.13	UMMZ	244769
waviNAB004^{C}	Slave Lake, AB	55.50	-114.03	UMMZ	244728
waviNAB005^{C}	Slave Lake, AB	55.50	-114.14	UMMZ	244746
waviNAB010^{C}	Chinchaga River, AB	57.37	-119.13	RAM	Z93.10.30
waviNAB011^{C}	Chinchaga River, AB	57.37	-119.13	RAM	Z93.10.40
waviNAB003^{C}	Cold Lake, AB	54.60	-110.20	UMMZ	244792
waviCAB021^{C}	Teleford Lake, Leduc, AB	53.26	-113.52		2091-71907
waviCAB022^{C}	Teleford Lake, Leduc, AB	53.26	-113.52		2091-71908
waviCAB023^{C}	Teleford Lake, Leduc, AB	53.26	-113.52		2091-71909
waviCAB035^{C}	Camrose, AB	53.13	-113.35	RAM	Z06.6.17
waviCAB067^{C}	Camrose, AB	53.47	-112.79	RAM	Z99.11.10
waviSEAB001^{C}	Police Point Park, Medicine Hat, AB	50.04	-110.64		1691-93363
waviSEAB002^{C}	Police Point Park, Medicine Hat, AB	50.04	-110.64		1691-93364
waviSEAB003^{C}	Strathcona Island Park, Medicine Hat, AB	50.04	-110.65		1691-93384
waviSEAB004^{C}	Strathcona Island Park, Medicine Hat, AB	50.04	-110.65		1691-93385
waviSEAB005^{C}	Strathcona Island Park, Medicine Hat, AB	50.04	-110.65		1691-93386
waviSEAB006^{C}	Police Point Park, Medicine Hat, AB	50.04	-110.65		1691-93387
waviSEAB007^{C}	Police Point Park, Medicine Hat, AB	50.04	-110.65		1691-93388
waviCYHILLS001^{C}	Cypress Hills, AB	49.66	-110.08		1691-93365
waviCYHILLS002^{C}	Cypress Hills, AB	49.66	-110.08		1691-93366
waviCYHILLS003^{C}	Cypress Hills, AB	49.66	-110.04		1691-93367
waviCYHILLS004^{C}	Cypress Hills, AB	49.64	-110.03		1691-93368
waviCYHILLS005^{C}	Cypress Hills, AB	49.64	-110.03		1691-93369
waviCYHILLS006^{C}	Cypress Hills, AB	49.64	-110.03		1691-93370
waviCYHILLS007^{C}	Cypress Hills, AB	49.64	-110.03		1691-93371
waviCYHILLS008^{C}	Cypress Hills, AB	49.64	-110.03		1691-93372
waviCYHILLS009^{C}	Cypress Hills, AB	49.64	-110.03		1691-93373
waviCYHILLS010^{C}	Cypress Hills, AB	49.66	-110.05		1691-93374
waviCYHILLS011^{C}	Cypress Hills, AB	49.66	-110.05		1691-93375
waviCYHILLS012^{C}	Cypress Hills, AB	49.66	-110.10		1691-93376
waviCYHILLS013^{C}	Cypress Hills, AB	49.66	-110.10		1691-93377
waviCYHILLS016^{C}	Cypress Hills, AB	49.66	-110.10		1691-93380
waviCYHILLS017^{C}	Cypress Hills, AB	49.66	-110.10		1691-93381
waviCYHILLS018^{C}	Cypress Hills, AB	49.66	-110.10		1691-93382
waviCYHILLS019^{C}	Cypress Hills, AB	49.66	-110.10		1691-93383
waviSK002^{C}	Wascana Park, Regina, SK	50.43	-104.61		1691-93396
waviSK003^{C}	Wascana Park, Regina, SK	50.43	-104.61		1691-93397
waviSK004^{C}	Wascana Park, Regina, SK	50.43	-104.61		1691-93398
waviSK005^{C}	Wascana Park, Regina, SK	50.43	-104.61		1691-93399
waviSK006^{C}	Wascana Lake Display Pond, Regina, SK	50.43	-104.59		1691-93400
waviSK008^{C}	North Wascana Lake, Regina, SK	50.43	-104.59		2091-71902
waviSK009^{C}	North Wascana Lake, Regina, SK	50.43	-104.59		2091-71903
waviSK010^{C}	North Wascana Lake, Regina, SK	50.43	-104.59		2091-71904
waviSK011^{C}	North Wascana Lake, Regina, SK	50.43	-104.59		2091-71905
waviSK012^{C}	Les Sherman Park, Regina, SK	50.49	-104.64		2091-71906