HISTORIC AND CONTEMPORARY HYBRIDIZATION OF THREE *SPHYRAPICUS* SAPSUCKER SPECIES

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ABSTRACT

This study examined the rangewide genetic structure of three hybridizing woodpecker species (*Sphyrapicus ruber, S. nuchalis,* and *S. varius*), and hybridization at a *S. nuchalis/S. varius* hybrid zone in central Alberta. Mitochondrial DNA and nuclear Z-linked markers show high intraspecific population connectivity, and limited interspecific connectivity predominantly occurring in hybrid zones. We found evidence of a Haida Gwaii refugium for *S. ruber* and an east-west Pleistocene split for *S. varius*. Genetic data and Ecological Niche Modelling suggest these species diverged, not allopatrically as has long been assumed, but in sympatry with gene flow. Next-generation sequencing methods and traditional SNP analysis showed a well-established *S. nuchalis/S. varius* hybrid zone, with high rates of introgression, particularly in *S. nuchalis*. While hybridization in contact zones is extensive, movement of alleles out of the hybrid zone is limited. As such, plumage, behaviour, or ecology might also impact sapsucker speciation.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

˚C	degrees Celsius
1x	one times
5x	five times
AICc	corrected Akaike's Information Criterion
AMOVA	analysis of molecular variance
AUC	area under curve
bp	base pair
CHD1Z	chromo helicase DNA binding protein 1
COI	cytochrome oxidase I
cpDNA	chloroplast DNA
CR	control region
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
ENM	Ecological Niche Model
Enol	α-enolase
F1	first filial generation of offspring
F2	offspring of F1 generation
FDR	false discovery rate
FST	fixation index
GAPD	glyceraldehyde phosphate dehydrogenase
GBIF	Global Biodiversity Information Facility
GBS	genotyping by sequencing
GSAF	University of Texas Genomic Sequencing and Analysis Facility
h	haplotype diversity
IGD	Cornell University's Institute for Genomic Diversity
ILS	incomplete lineage sorting
indel	insertion/deletion
K	number of clusters
LGM	Last Glacial Maximum
LIG	Last Interglacial
m	meter
MAF	minor allele frequency
MCMC	Markov chain Monte Carlo
$MgCl_2$	magnesium chloride
MIROC	model for interdisciplinary research on climate
mM	millimolar
mtDNA	mitochondrial DNA
MYA	million years ago

n	sample size
NGS	next generation sequencing
PCR	polymerase chain reaction
Q	ancestry coefficient
RADseq	restriction site associated DNA sequencing
s	seconds
SDM	Species Distribution Model
SNP	single nucleotide polymorphism
U	units
v	version
δ	allele-frequency differential
µL	microliter
µM	micromolar
π	nucleotide diversity
χ^2	chi-squared

Populations

AZ	Arizona
CAB	central Alberta
CBC	central British Columbia
CCA	central California
CO	Colorado
ECA	eastern California
FL	Florida
HG	Haida Gwaii
ID	Idaho
IL	Illinois
LA	Louisiana
MI	Michigan
MT	Montana
NBC	northern British Columbia
NC	North Carolina
NCA	northern California
NEOR	northeast Oregon
NEWA	northeast Washington
NJ	New Jersey
NM	New Mexico
NSNB	Nova Scotia/New Brunswick
NWBC	northwest BC

ON	Ontario
SAB	southern Alberta
SCA	southern California
SD	South Dakota
SEAK	southeast Alaska
SEBC	southeast British Columbia
SK	Saskatchewan
SOR	southern Oregon
UT	Utah
VIBC	Vancouver Island, British Columbia
WA	Washington
WY	Wyoming

CHAPTER 1: General introduction

1.1 Evolution

Evolution is by no means a new concept in the field of biology, but our understanding of evolution is constantly undergoing advances and breakthroughs. Since the days of Lamarck and Darwin, biologists have come to understand the forces that drive changes in populations, the hereditary mechanisms that advance these changes, and their expression in organisms' genomes. However, despite centuries of research into how the diversity of life occurred, there are still many advances to be made in the field of evolution. In particular, how do new species arise? How do their unique ecological requirements shape their evolutionary history? What evolutionary forces have affected biota and how? The intent of this thesis is to address these questions to further our knowledge of evolutionary processes.

1.1.1 Mechanisms of evolution

A fundamental requirement for the evolution of a species is to undergo changes in allele frequencies over time (Hamilton, 2009). There are multiple forces that might drive such a change in a population. The most well-known of these is Charles Darwin's and Alfred Russel Wallace's theory of evolution by natural selection, in which better-adapted alleles outcompete other alleles and proliferate in a population (Hamilton, 2009). As conditions change, allele frequencies also change. Over time, the number of changes may accumulate resulting in reproductive isolation and the populations becoming a different species.

1.1.2 Speciation

A detailed understanding of evolutionary theory is not necessary to observe that life is broken into different groups of organisms, which we refer to as species. What is not obvious is how to define a species (Hendry, 2009; Ridley, 2004). Some organisms, such as lazuli and indigo buntings (*Passerina amoena* and *P. cyanea*) appear to be quite different, but interbreed (Baker & Boylan, 1999). Others that are morphologically similar, such as *Niphargus* amphipods, do not (Fiser et al., 2015; Krebes et al., 2010). How do we distinguish between such species? Several species concepts have been developed over the past century in an attempt to create a definition that will allow us to answer these questions.

A widely-used and accepted species concept is the biological species concept developed by Mayr (1942). He defined species as interbreeding groups of organisms. This definition has several limitations, as it does not address asexually reproducing organisms or organisms with the potential to interbreed but are isolated geographically (Hendry, 2009; Ridley, 1996). The ecological species concept on the other hand defines species as a group of organisms exploiting the same ecological niche (Ridley, 2004). With modern advances in molecular methods, biologists may also use the phylogenetic species concept, which states that a species is the smallest group of organisms with shared common ancestry (Cracraft, 1983). Though more than two dozen species concepts have been suggested, we are not any closer to a universal definition (Hendry, 2009).

Despite our tenuous grasp on how to delineate species, it follows that we may ask how do different species arise? Multiple theories have been proposed, including the well-accepted “by-product theory” that populations become separated and the evolution of each group is influenced by its local conditions, and the less popular “reinforcement

theory", whereby natural selection influences group separation (Ridley, 1996). What both theories agree upon is the influence of reproductive isolation, or the inability of the disparate groups to exchange alleles (Dobzhansky, 1970). This isolation may be the result of geographic separation caused by barriers such as mountains, bodies of water, or anthropogenic land use (Apte et al., 2007; Bush et al., 2011; Lait & Burg, 2013). Reproductive isolation may also be caused by behavioural barriers such as mate choice, philopatry, or migratory routes (Baker & Boylan, 1999; Delmore et al., 2016; Johnson & Johnson, 1985). When populations are isolated, they continue to diverge genetically through forces such as genetic drift, random mutation, or adaptation to different environmental conditions (Moore, 1977). Eventually these genetic differences accumulate to the point where we may designate populations as distinct species.

1.1.3 Pleistocene speciation

Events in the Pleistocene era, in particular, are a widely accepted example of the by-product theory, and are credited with impacting the evolutionary history and divergence of species worldwide (Chan et al., 2012; Graham & Burg, 2012; Shafer et al., 2011; Spellman et al., 2007). Extending from approximately 2.58 million years ago to the present, the Quaternary period is divided into two epochs: the Pleistocene and the Holocene, the latter of which began 11,700 years ago and extends to the present day (Dawson, 1992; Gibbard et al., 2010; Walker et al., 2009). The Pleistocene was characterized by generally cooler global temperatures with periodic oscillations of warming and cooling (Davis et al., 2009). Geologists have delineated multiple benchmarks for these climatic trends, and two of the more important points were the Last Interglacial (LIG) and Last Glacial Maximum (LGM). Approximately 130,000 to 116,000

years ago, the LIG saw a period of anomalously warm temperatures and reductions in ice sheets, while the LGM (~ 25,000 – 10,000 years ago) was colder, allowing the ice sheets to reach their greatest extent (Hofreiter et al., 2004; Peltier, 1994).

The changing climate during the Pleistocene affected both terrestrial and aquatic habitats globally. Land masses in the Northern Hemisphere saw extreme habitat change in the form of widespread ice sheets. The northern portion of North America, from present day northern Canada to just south of the Canada/United States border, was covered by two main ice sheets: the Cordilleran from the Pacific Coast to the Rockies, and the Laurentide from the Rockies to the Atlantic Coast (Menounos et al., 2009; Peltier, 1994). These ice sheets were up to 3 km thick and made the land completely uninhabitable for both plants and animals, and as a result life was pushed to the peripheries of these glacial barriers (Lait et al., 2012; Peltier, 1994).

Pleistocene glaciation caused reproductive isolation across taxa, which is supported by evidence of divergence in many contemporary species (Aubry et al., 2009; Crespi et al., 2003; Macfarlane et al., 2016; Walter & Epperson, 2005). While speciation of many sister species actually began before the Pleistocene, this time period played a major role in the divergence and speciation of boreal species (Weir & Schluter, 2004). More specifically, Weir and Schluter (2004) found species inhabiting North America's taiga habitat tend to be basal to their more closely related sister species found in the Rocky Mountains and on the Pacific Coast.

After ice sheets receded at the end of the Pleistocene, many newly differentiated populations experienced range expansion. In some areas, populations that had been isolated for long periods of time experienced secondary contact. Species that were more differentiated would have maintained reproductive isolation after secondary contact, but

those without non-geographic reproductive barriers may have undergone hybridization (Gilman & Behm, 2011; Moore, 1977).

1.1.4 Hybridization

In nature, the forces that contribute to reproductive isolation may be ephemeral, such as the Pleistocene ice sheets. Barriers that once precluded gene exchange between isolated populations may disappear. Most commonly, geographic barriers separate the isolates long enough for them to differentiate into new species, but not long enough for completion of pre- or post-zygotic reproductive isolation mechanisms, resulting in hybridization (Moore, 1977). The hybrid offspring may or not be fertile, and those that are may even backcross with an individual of either parental species, which is known as introgression (Grant & Grant, 1992).

Hybridization is not an uncommon phenomenon, occurring in 25% of plant and 10% of bird species (Grant & Grant, 1992). While hybridization may occur in isolated areas with individuals of one species breeding with vagrants from a closely-related species, it more often occurs as formerly allopatric populations undergo range-expansion and come into contact along the periphery of their ranges (Dobzhansky, 1940). These contact or hybrid zones act as a "natural laboratory" for biologists to study evolution, speciation, and hybridization. Hybrid zones may be broad or narrow, ephemeral or permanent (Grant & Grant, 1992; Moore, 1977; Seneviratne et al., 2016). Many contemporary hybrid zones are the result of post-Pleistocene secondary contact (Moore & Koenig, 1986). These patterns have been seen in a wide variety of biota globally, including kob antelope (*Kob* spp.) in Sub-Saharan Africa, Iberian wall lizards (*Podarcis hispanica*) in Europe, and Brassicaceae (*Arabis* spp.) in North America (Dobeš et al.,

2004; Lorenzen et al., 2007; Renoult et al., 2009). Studying hybridization at contact zones allows us to gain a better understanding of hybrid zones, speciation, and adaptive radiation (Grant et al., 2005).

The long-term existence of hybrid zones was contested by many biologists when they were first proposed, as it was assumed they would either result in speciation or fusion (Dobzhansky, 1940; Moore, 1977). Two models that explain hybrid zone stability include the dynamic-equilibrium (or tension) model, and the bounded hybrid superiority model. In the dynamic-equilibrium model, selection against hybrids is balanced by the migration of parental species into the hybrid zone (Moore, 1977; Seneviratne et al., 2012). The bounded hybrid superiority model suggests that hybrid individuals may be better adapted than either parental species to the environmental conditions within the hybrid zone (Moore, 1977). For example, in gulls within the *Larus occidentalis* and *L. glaucescens* hybrid zone, hybrids had higher fitness because they built nests in habitats with lower predation, and had higher nest success because their diets consisted of more fish than parental species, which provided the chicks with better nutrition (Good et al., 2000).

1.2 Molecular markers

1.2.1 Molecular methods in population genetics

Molecular methods have become integral in the understanding of species' evolutionary history. Biologists are no longer tethered to observable phenotypic data such as morphology or behaviour to determine evolutionary relationships. The advent of PCR and other genetic methods has allowed us the opportunity to examine the genetic makeup of biota, which in turn has opened the possibility to examine evolutionary relationships

and evolutionary histories to a depth that could never be achieved using phenotypic data alone.

A number of different molecular markers are typically used in population genetics, and it is important to select the markers thoughtfully. Molecular markers have different modes of inheritance that influence how they evolve, levels of variation, and how they reflect different ecological or demographic scenarios (Freeland et al., 2012). Because of this, each marker type will have unique advantages and disadvantages, meaning that it is important to use a variety of markers to answer population genetics questions to attain the best, most well-informed results.

1.2.2 Mitochondrial DNA

The primary function of mitochondria in the cell is cellular respiration, and logically, the DNA found inside of them, mitochondrial DNA (mtDNA) codes for these processes (Freeland et al., 2012). MtDNA is commonly used in population genetics studies for a variety of reasons. The mitochondrial genome is 1/10,000 the size of the smallest nuclear genome, which makes it manageable to work with (Brown et al., 1979; Freeland et al., 2012). Because the gene arrangement is highly conserved, universal primers may be designed and used to amplify the same genes in a diverse array of taxa without any *a priori* knowledge about the study species mitochondrial sequence. The mtDNA genome has a high mutation rate (5.7×10^{-8} substitutions/site/year in mammals), about 10 times that of protein-coding nuclear genes, perhaps because of by-products of cellular respiration or less stringent repair mechanisms (Freeland et al., 2012). This high substitution rate allows for high levels of polymorphism and consequently greater potential for detection of distinct lineages among individuals. MtDNA is uniparentally

inherited (in most species) and haploid, so the effective population sizes of mitochondrial markers are theoretically ¼ of the size of nuclear DNA, making mtDNA markers more susceptible to demographic events, such as bottlenecks, which can make these events more discernible (Freeland et al., 2012). The uniparental inheritance of mtDNA greatly reduces recombination, so in the absence of mutation offspring will inherit their mother's mitochondrial haplotype, which facilitates identification of maternal lineages.

Despite being one of the most popular markers used in population genetic studies, mtDNA markers do have disadvantages. Because the effective population size of mtDNA is smaller than nuclear markers, haplotypes are more likely to be eradicated from the population via drift, which can cause researchers to misinterpret the species' evolutionary history. Additionally, because mtDNA only reflects maternal lineages, it does not reflect the genetic histories of males within the population, such as male-biased dispersal, which could cause underestimations of population genetic diversity or migration rates (Freeland et al., 2012).

1.2.3 Z-linked markers

While most nuclear markers are bi-parentally inherited, in many species genes involved in genetic sex determination may be uniparentally inherited (Freeland et al., 2012). In genetic sex determination, there is usually one homogametic and one heterogametic sex. In mammals, females are the homogametic sex with two X chromosomes, and males are heterogametic with one X and one Y (Freeland et al., 2012; Qvarnström et al., 2010). In birds and lepidopterans, however, females are heterogametic (ZW) and males are homogametic (ZZ). The W (or Y) chromosome is usually smaller

than Z (or X) chromosome, and interchromosomal recombination is limited (Qvarnström & Bailey, 2009; Sæther et al., 2007).

Z-linked markers are an excellent choice for studying species barriers, as they contain many important genes directly related to the process of reproductive isolation (Qvarnström & Bailey, 2009; Sæther et al., 2007). Genes on the Z chromosome evolve more rapidly, have low recombination rates, and expose hybrids to incompatible Z-linked recessive alleles (Qvarnström & Bailey, 2009). The male-biased inheritance of Z-linked markers in birds also makes them a good candidate to follow paternal trends over a single generation (Freeland et al., 2012).

1.2.4 Single nucleotide polymorphisms on autosomal nuclear markers

In sexually reproducing species, individuals receive two copies of autosomal markers, one from each parent, and they may undergo recombination. These traits make it more difficult to trace lineages with autosomal nuclear markers than with uniparentally inherited markers, but the larger effective population size and biparental inheritance of autosomal nuclear markers help counterbalance the limitations of mtDNA and sex-linked markers (Freeland et al., 2012).

Single nucleotide polymorphisms (SNPs) are found throughout the genome, and are an excellent choice in studying speciation and population structure (Brumfield et al., 2003). SNPs are often in autosomal regions. They are useful in genetic studies because their low mutation rate translates to few incidences of homoplasy, where a mutation occurs, but mutates back to its original state, confounding mutation rates and genetic distances (Brito & Edwards, 2009; Brumfield et al., 2003). SNPs are limited because the maximum amount of information a single locus can convey is four character states. This

allows for high throughput genotyping, but each locus is less informative therefore studies employing SNPs require the use of multiple loci and the concatenation of these data for analysis (Brumfield et al., 2003). Using multiple unlinked loci, particularly in nuclear autosomal regions, is useful in bypassing many of the pitfalls and biases resulting from use of a single uniparental marker such as mtDNA and sex-linked markers (Brumfield et al., 2003).

1.2.5 Using molecular markers to study speciation and hybridization

To identify hybridization or hybrid individuals, biologists have historically relied upon intermediate phenotypes or breeding domesticated species to demonstrate hybridization (Grant & Grant, 1992). The development of molecular methods has allowed biologists to determine hybridization in an individual or introgression in populations by looking at their genetic makeup. Hybrids or introgressed individuals can be detected as their genomes include alleles from each parental species. By comparing alleles of pure parental species with hybrids or individuals sampled from a hybrid zone, we can identify and quantify hybridization and introgression.

One way that hybridization events are often identified is cytonuclear disequilibrium. Cytonuclear disequilibrium (or discordance) is a phenomenon in which individuals have plastid DNA (mtDNA, cpDNA) characteristic of one species, but nuclear DNA typical of a different species. This is caused by the maternal inheritance and lack of recombination of mtDNA, as explained in section 1.2.2. If hybridization occurs, the offspring will inherit the mtDNA of the mother, but the nuclear DNA of both parents. Cytonuclear disequilibrium has resulted in the detection of hybridization in many species, such as canyon and Arizona tree frogs (*Hyla arenicolor* and *H. wrightorum*), in which a

historic hybridization event caused several canyon tree frogs to have Arizona tree frog mtDNA, yet centuries of backcrossing masked this pattern in nuclear DNA (Klymus et al., 2010). Other examples of species in which cytonuclear disequilibrium revealed a history of hybridization include Cyprinidae fishes, *Podarcis hispanica* lizards, and myrtle/Audubon's warblers (*Septophaga coronata coronata* and *S. c. auduboni*) (Broughton et al., 2011; Renoult et al., 2009; Toews et al., 2011).

Biparentally inherited markers can also be used in identifying hybridization. This is most easily achieved using a series of markers with different fixed alleles in either parental species. In other instances, the two species may be too recently diverged, or hybridization/introgression between the species may be too extensive, in which case fixed alleles may not be identified. Under these circumstances, it is possible for researchers to use Bayesian clustering statistical methods to examine hybridization without any *a priori* knowledge of allele frequencies in parental species (Freeland et al., 2012). Due to the simplicity of working without prior knowledge of parental genotypes, Bayesian clustering has become a commonly used method and has been employed studying a variety of species, from long-tailed tits (*Aegithalos caudatus*) to endangered Cuban crocodiles (*Crocodylus rhombifer*) (Milián-García et al., 2015; Wang et al., 2014).

1.3 Study species

1.3.1 Sapsucker systematics

North America is home to four species of sapsuckers within the *Sphyrapicus* genus: yellow-bellied (*S. varius*), red-naped (*S. nuchalis*), red-breasted (*S. ruber*), and the more distantly-related Williamson's sapsucker (*S. thyroideus*). The taxonomy and systematics of sapsuckers has undergone substantial change in the past century. The

Williamson's sapsucker has a highly divergent plumage, with dark males and heavily barred females, and as such it has always been considered distinct from other sapsuckers (Johnson & Zink, 1983). All other North American sapsuckers were once considered four races of a single "yellow-bellied sapsucker" species, but are now classified as three separate species: yellow-bellied, red-naped, and red-breasted sapsuckers, the latter of which has two subspecies (Howell 1952; Johnson and Zink 1983). Genetic evidence supporting this taxonomy has been demonstrated using allozymes, and next generation sequencing methods, and behavioural research has shown assortative mating in sympatry, which further solidifies these as distinct species (Cicero & Johnson, 1995; Grossen et al., 2016; Johnson & Johnson, 1985; Johnson & Zink, 1983). However, though mtDNA cytochrome b data have confirmed *S. varius* is distinct from *S. ruber/S. nuchalis*, they are not able to differentiate between the latter two species.

Williamson's sapsuckers diverged from the *S. varius* superspecies complex approximately 3.9-2.8 MYA (million years ago) (Cicero & Johnson, 1995). Among the three other species, the yellow-bellied sapsucker lineage is more distant to the red-breasted/red-naped lineage, splitting approximately one million years ago (Cicero & Johnson, 1995). The red-breasted and red-naped sapsuckers are very closely-related, and are estimated to have diverged from each other during or since the end of the Pleistocene era (Johnson & Zink, 1983).

1.3.2 Sapsucker biology

Sapsuckers are named for their singular behaviour of boring holes into the bark of trees from which they drink sap. They have a specialized, bristled tongue adapted for this purpose (Howell, 1952). Sapsuckers drill an elaborate array of sap wells throughout their

territory which they defend, both from other sapsuckers and from the many other species, such as insects, squirrels, and hummingbirds, which drink from the sap wells (Walters et al., 2002b). Sapsuckers are important species within their community because they excavate nesting cavities in trees that are subsequently used by other cavity nesters such as northern flying squirrels (*Glaucomys sabrinus*) and mountain bluebirds (*Sialia currucoides*) (Walters et al., 2002b). While all sapsuckers share similar ecology and life history traits, there are several important characteristics that distinguish each species.

Each species has different plumage (Figure 1.1). The yellow-bellied sapsuckers have the least amount of red, with white on the nape and red patches on the forehead and crown of all birds and on the throat of males. Their chests and bellies are yellower than the other species, and they have white barring on their backs. Red-naped sapsuckers have similar plumage to yellow-bellied, except all birds have red on the forehead, crown, and throat, and males have an additional red patch on the nape. The bellies of red-naped sapsuckers are less yellow, and the white spotting on the back is organized in two vertical stripes. Red-breasted sapsuckers' plumage is unique to the group with increased red plumage covering the head (except for white lores) and extending down the breast. The two subspecies have different plumage as well. *Sphyrapicus r. dagetti* has white bands on the back with a pale belly, while *S. r. ruber* has yellow bands on the back and a yellow upper belly. Another difference from the red-naped and yellow-bellied sapsuckers is the sexually monomorphic plumage of red-breasted sapsuckers.

Each species has a distinct geographic range, associated with different habitats and tree preferences. Yellow-bellied sapsuckers are found across northern North America, mostly in boreal habitats (Figure 1.1). They rely upon early successional forests and prefer riparian habitats dominated by aspen (*Populus* spp.), birch (*Betula*), maple (*Acer*

spp.), or mixed-conifer stands (Walters et al., 2002b). In the winter, yellow-bellied sapsuckers undergo a complete migration, moving to wintering grounds in the southeastern US, central America, and the Caribbean (Walters et al., 2002b).

Red-naped sapsuckers breed in the Rocky Mountains in western North America (Figure 1.1), where conditions are more arid than habitats accessed by yellow-bellied and red-breasted sapsuckers. This species prefers either deciduous or mixed woodlands, particularly aspen groves (Walters et al., 2002a). Their migration is mostly complete, occupying regions from southern Utah, down into central Mexico and Baja California during the winter, but there is overlap between breeding and wintering grounds in the American Southwest.

The breeding range of the red-breasted sapsucker occurs west of the Rocky Mountains, along the Pacific Coast of North America from northwestern British Columbia to northern Baja California (Figure 1.2) (Walters et al., 2002a). This species is associated with moister habitats than either of the other two species (Billerman et al., 2016). The *S. r. ruber* subspecies in the north of the range is a short-distance migrant, usually moving from inland regions to the milder winters of the coast. *S. r. daggetti*, on the other hand migrates very little, with the only migrant individuals moving down to areas of lower elevation within the Sierra Nevadas (Walters et al., 2002a).

1.3.3 Three-way-hybridization complex

Part of what challenged early biologists in classifying these birds is their tendency to hybridize in sympatry (Seneviratne et al., 2012). Sapsuckers are parapatric, so though each species has a distinct range and they are mostly allopatric, there are large zones of sympatry between each species (Figure 1.2). Each sympatric region between two

sapsucker species is a contact zone, or hybrid zone, where there is evidence of interbreeding between species (Billerman et al., 2016; Cicero & Johnson, 1995; Johnson & Johnson, 1985; Johnson & Zink, 1983; Scott et al., 1976; Seneviratne et al., 2012). For this reason, the three species of sapsuckers meet the definition of a single species as delineated by the biological species concept (Mayr, 1942). However, hybrid zone studies show first generation hybrids, are incredibly rare and mating is strongly assortative, leading to a general consensus of dividing these into separate species (Johnson & Johnson, 1985).

Several different hypotheses explain the existence of stable hybrid zones. Multiple sapsucker hybrid zones have been described as tension zones, including a red-naped/red-breasted hybrid zone near the border between Oregon, California, and Nevada, and a red-breasted/yellow-bellied hybrid zone in central British Columbia (Johnson & Johnson, 1985; Seneviratne et al., 2012). In hybrid zones consistent with the tension zone model, selection against hybrids is balanced by migration of parental types into the hybrid zone. Acceptance of this model implies selection against hybrids. Studies have shown that though F1 hybrids are viable, there are fewer F2 individuals in the population than expected, suggesting that F1 individuals have a disadvantage in mate acquisition (Johnson & Johnson, 1985). This could be due to their intermediate plumages being disadvantageous in selection, or there may be partial sterility barriers that reduce their ability to produce offspring (Johnson & Johnson, 1985). Johnson and Johnson (1985) showed females preferentially mate with redder-plumaged males. Within mixed pairings of pure parental phenotypes, almost all males were red-breasted sapsuckers, while females were red-naped (8 of 9), and in 33 of 36 pairs observed, the male was redder than the female (Johnson & Johnson, 1985).

1.4 Thesis aims

The purpose of this study is to expand our knowledge of sapsucker population genetic structure. Therefore, my two primary research objectives were to: 1) determine the range-wide population genetic structure of the three species within the *Sphyrapicus varius* sapsucker superspecies, and 2) quantify introgression of red-naped and yellow-bellied sapsuckers in a central Alberta hybrid zone. To approach these objectives, I used DNA collected from either blood or feathers of museum samples and wild-caught sapsuckers.

1.4.1 Rangewide structure and historic distribution

To address range-wide genetic structure, I used three different markers from individuals across the entire range of each of the three species, and examined patterns of genetic structure. I used Ecological Niche Modelling to model the preferred ecological niche of each species' breeding range and projected these niches to recent historical time periods. With these models, I show the potential historic range overlap of each species to evaluate the degree and history of geographic isolation within these species.

Though these are thoroughly researched species, no study has yet evaluated population genetics of any of these sapsucker species across their range. Each species has a substantial breeding range, so there may be the potential for isolation by distance to impede gene exchange amongst conspecific populations. Furthermore, the yellow-bellied sapsucker has the widest range spanning a variety of habitats and crossing several barriers to movement including unsuitable prairie habitat and the Rocky Mountains. I predict that

eastern and western populations of yellow-bellied sapsuckers will be differentiated as a result of the barriers between the birds on either side of the continent.

Previous genetic studies suggest each sapsucker species is distinct, but these studies were conducted in hybrid zones, where individuals are probably not reflective of the entire species (Cicero & Johnson, 1995; Johnson & Zink, 1983; Seneviratne et al., 2012). Accordingly, I would expect limited gene flow amongst each species. However, each species can hybridize in sympatry to a limited extent, therefore I would expect evidence of some allele exchange in regions of sympatry (Seneviratne et al., 2012, 2016).

1.4.2 S. nuchalis/S. varius Alberta hybrid zone

I examined the hybrid zone between red-naped and yellow-bellied sapsuckers in Alberta, the genetics of which have not been studied. Johnson and Johnson (1985) suggested that red plumage in red-breasted sapsuckers may act as a premating reproductive isolating mechanism. Red-naped and yellow-bellied sapsuckers look the most alike, and because many birds select mates based upon plumage traits (Baker & Boylan, 1999; Seneviratne et al., 2012), more introgression might be expected between these two species. Therefore, I tested a second hypothesis that red-naped and yellow-bellied sapsuckers have high rates of introgression due to their similar plumages.

Though hybridization between red-breasted/red-naped and red-breasted/yellow-bellied sapsuckers has been examined in several genetic studies, hybridization between red-naped and yellow-bellied sapsuckers has not yet been studied from a genetic perspective (Billerman et al., 2016; Cicero & Johnson, 1995; Johnson & Zink, 1983; Seneviratne et al., 2012). Ongoing research based upon the existence of individuals of intermediate plumage within the hybrid zone suggests hybridization occurs (J. Hudon,

personal communication). Plumage has been suggested as an important mechanism in pre-mating reproductive isolation amongst these species (Billerman et al., 2016; Johnson & Johnson, 1985), and red-naped and yellow-bellied sapsuckers have similar plumage. I therefore predict high rates of hybridization and introgression between these species because if plumage does affect mate choice, they have a less powerful reproductive isolating mechanism.

1.5 Thesis organization

This thesis is composed of four chapters. Chapter 1 gives an overview of the biological processes involved in this study, such as evolution, speciation, and hybridization, and describes the use of molecular methods in understanding these topics. Finally, it details three study species that are useful in answering questions of how and why speciation and hybridization occur. Chapter 2 examines the range-wide population genetic structure and how the high likelihood of parapatric historic distributions of yellow-bellied, red-naped, and red-breasted sapsuckers have influenced the genetic patterns. In Chapter 3 I discuss introgression in the contemporary hybrid zone between red-naped and yellow-bellied sapsuckers in central Alberta. The fourth chapter summarizes the results found in Chapters 2 and 3, and discusses what the *Sphyrapicus varius* species complex can tell us about evolutionary processes such as speciation and hybridization.

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a.  b. 

c. 

Figure 1.1. Male breeding plumages of each sapsucker species red-breasted (a), red-naped (b), and yellow-bellied (c).

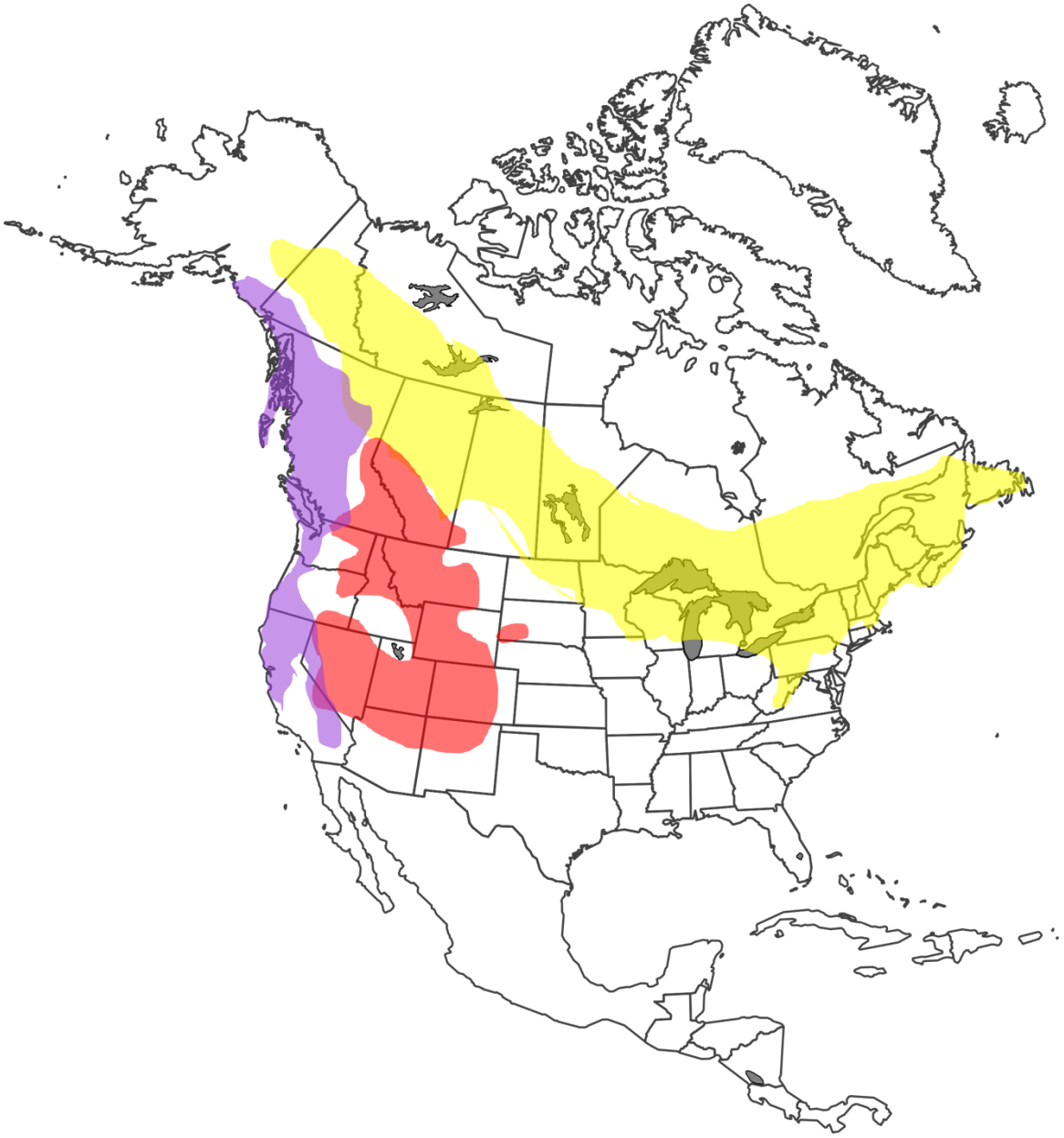

Figure 1.2. Map illustrating breeding ranges of *S. ruber* (vertical lines), *S. nuchalis* (horizontal lines), and *S. varius* (black).

CHAPTER 2

Population genetics and speciation of three woodpecker species (*Sphyrapicus varius*, *S. nuchalis,* and *S. ruber*)

Manuscript submitted for publication

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2.1. Abstract

The root of understanding speciation lies in determining the forces which drive it. In many closely-related species, including *Sphyrapicus varius*, *S. nuchalis*, and *S. ruber*, it is assumed that speciation occurred due to isolation in multiple Pleistocene refugia. We used genetic data from the control region, COI, and CHD1Z to examine rangewide population genetic structure and differentiation amongst these three species across each species' breeding range. In addition, we modelled these species' ecological niches for the Holocene (~6,000 ya), Last Glacial Maximum (~22,000 ya), and Last Interglacial (~120,000-140,000 ya) to determine if Pleistocene glaciations could have contributed to allopatric distributions, therefore allowing these groups to differentiate. Population genetic data show a potential Pleistocene refugium in Haida Gwaii, an east-west split among *S. varius*, and low genetic differentiation within each species. Our control region data show polyphyly, while COI and CHD1Z data show differentiation among species using composite genotypes. Ecological Niche Modelling shows a large amount of niche overlap at each time period suggesting that *S. varius*, *S. nuchalis*, and *S. ruber* did not diverge in allopatry. Speciation in the absence of allopatry is a controversial topic and our data support the growing body of research that suggest mechanisms for reproductive isolation other than vicariance.

Keywords population genetics, Ecological Niche Modeling, Haida Gwaii refugium, hybridization, parapatric speciation

2.2. Introduction

Reproductive isolation is a crucial force in the evolution of species, one which has intrigued biologists since it was suggested by Darwin himself. Isolation is caused by a variety of factors including geographical (vicariance or dispersal), temporal (timing of breeding or migration), and behavioural (differences in song or courtship displays) differences (Baker and Boylan 1999; MacDougall-Shackleton and MacDougall-Shackleton 2001; Weir and Schluter 2004; Billerman et al. 2016; Delmore et al. 2016). Determining what drives two populations to separate can help us better understand evolutionary processes, but it is often difficult to differentiate among the impacts of the different factors.

In studies of recent species divergence, glacial vicariance during the Pleistocene is often cited as a main cause of speciation, as advancing ice sheets separated populations into isolated refugia (Hofreiter et al., 2004; Weir & Schluter, 2004). When ice sheets receded, many newly differentiated populations expanded their geographic ranges, and in some cases, populations that had been isolated experienced secondary contact. At times, the geographical barrier was the only reproductive isolating mechanism between groups and once the ice sheets receded some species experienced hybridization and introgression (Krosby & Rohwer, 2009; Lorenzen et al., 2007).

The *Sphyrapicus* genus is a good example of a group believed to have differentiated during the Pleistocene (Cicero & Johnson, 1995; Weir & Schluter, 2004). These species of *Sphyrapicus* sapsuckers: yellow-bellied (*S. varius*), red-naped (*S. nuchalis*), and red-breasted (*S. ruber*) are included within the superspecies *Sphyrapicus varius*, and are sister to Williamson's sapsuckers (*S. thyroideus*). The taxonomy of the *Sphyrapicus varius* superspecies has undergone substantial changes in the past century, as

what were once considered four races of a single species are now classified as three separate species, with two subspecies of *S. ruber* (Howell, 1952; Johnson & Zink, 1983). This classification is justified by both molecular evidence and assortative mating behaviour (Grossen et al., 2016; Johnson & Johnson, 1985; Seneviratne et al., 2012, 2016). Furthermore, these species exhibit different morphologies, drumming displays, migratory behaviours, and habitat preferences (Johnson and Zink 1983; Walters et al. 2002a; b).

Sphyrapicus ruber and *S. nuchalis* diverged less than 500,000 years ago, while *S. varius* diverged approximately one million years ago (Weir & Schluter, 2004). To date, every genetic study on this group has determined that the three species are highly similar, particularly *S. nuchalis* and *S. ruber* (Cicero & Johnson, 1995; Grossen et al., 2016; Johnson & Johnson, 1985; Johnson & Zink, 1983; Seneviratne et al., 2012). It is believed that these species diverged under allopatry while in separate Pleistocene refugia, and current hybrid zones are the result of secondary contact following range expansion (Cicero & Johnson, 1995; Grossen et al., 2016; Johnson & Zink, 1983; Seneviratne et al., 2012; Weir & Schluter, 2004). However, with the geographic proximity of these species and the dynamic nature of the Pleistocene ice sheets, it is possible that these species were not entirely allopatric in their distribution. If the sapsuckers speciated in limited sympatry, this suggests that other reproductive isolating mechanisms may have been the main driver of divergence within this species complex.

Despite a number of genetic studies on sapsuckers, no study has attempted to determine the rangewide population genetic structure for each species. Using mitochondrial DNA (mtDNA) control region (CR) and cytochrome oxidase I (COI) and z-linked chromo-helicase DNA binding protein (CHD1Z) markers, we have examined

genetic patterns from across each species' breeding range, comparing genetic structure among and within each species. A rangewide sampling approach allows for better evaluation of the historical rangewide genetic structure and a more thorough understanding of the evolutionary processes that influenced the divergence of these species.

To test the hypothesis that *S. ruber*, *S. nuchalis*, and *S. varius* underwent speciation due to geographic isolation in separate refugia during the Pleistocene era, we have projected potential ranges for the mid-Holocene (~6,000 years ago), the last glacial maximum (LGM, ~22,000 years ago), and the last interglacial period (LIG, ~120,000-140,000 years ago) to determine if, when, and the degree to which these species were isolated.

2.3. Methods

2.3.1. Sample acquisition

We collected DNA samples from museum specimens and birds caught with mist nets during the field season. Wild-caught samples were collected from May to July to reduce the number of migrants caught. Birds were called in with playbacks and caught using 12 m mist nets. A small (<50 µL) blood sample was taken from the brachial vein, the birds were banded, and morphometric measurements and photographs were taken. All birds were released on site and blood samples stored in 99% ethanol. A total of 457 samples were collected, comprised of each of the three species from a total of 33 sampling populations.

2.3.2. DNA extraction, amplification, and sequencing

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

An 878 bp (base pair) region of the CR was amplified using primers LThr and HPro (Table 2.1) in 308 sapsuckers. The thermal cycling profile was one cycle of 120 s at 94 ˚C, 45 s at 54 ˚C, 60 s at 72 ˚C; 37 cycles of 30 s at 94 ˚C, 45 s at 54 ˚C, 60 s at 72 ˚C; and one cycle of 300 s at 72 ˚C. The 25 µL PCR reaction contained 5x Green GoTaq® Flexi buffer (Promega), 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.4 µM primers LThr and HPro, 1 U GoTaq® Flexi polymerase, and genomic DNA.

A 749 bp region of COI was sequenced with primers BirdF1 and BirdR1 (Table 2.1) in 61 individuals from all three species using a similar approach to Seneviratne et al. (2012). This COI region was amplified using a similar thermal cycling program to the CR with the exception of a 50 ˚C annealing temperature, and 0.1 mM dNTP, 2.0 mM $MgCl_2$, and 0.5 U GoTaq® Flexi polymerase.

An approximately 250 bp region of the CHD1Z gene on the z-chromosome was sequenced in 81 individuals using primers CHD1Z-F-Sapsucker and CHD1Z-R-Sapsucker (Table 2.1). The PCR conditions were similar to that used in the control region, but with a 45 ˚C annealing temperature, 1 mM dNTP, and 1.5 mM $MgCl_2$. An insertion/deletion (indel) of a single G nucleotide was identified in this sequence.

Successfully amplified samples were sent to NanuQ sequencing service at McGill University, Montreal, Quebec for sequencing. Sequences were checked and aligned using MEGA v. 6 (Tamura et al., 2011). Several samples were sequenced twice to ensure consistency.

2.3.3. SNP screening

The aligned COI and CHD1Z sequences were used to identify SNPs. On COI, we screened for two pairs of SNPs: a CNNT/TNNC SNP at position 303 (hereafter referred to as the CT SNP), and an ANNA/ANNG/GNNG SNP at position 387 (hereafter referred to as the AG SNP). Internal primers BirdFintCT400, BirdFintTC400, and BirdRintCOI530 (Table 2.1) were designed to identify the CT SNP such that the 3' end of each forward primer would only bind to one specific SNP. Two 10 µL PCR reactions were run, one using BirdFintCT400 and the other with BirdFintTC400. The BirdFint reaction was the same as COI with 1 mM $MgCl_2$ (BirdFintCT400) or 1.5 mM $MgCl_2$ (BirdFintTC400). Screening was performed for 367 individuals at the CT SNP site. We designed specific reverse primers for each AG SNP, BirdFintCOI320, BirdRintAA480, BirdRintAG480, and BirdRintGG480 (Table 2.1). Three 10 µL PCR reactions were performed per sample, each containing the primer BirdFintCOI320 and one of the three BirdRint480 primers for 433 birds. The reactions had similar reagent concentrations to the BirdFintCT400 SNP PCR, but 2 mM $MgCl_2$ was used with primers BirdRintAG480 and BirdRintGG480.

Because of imperfect primer binding at the AG and GG SNP, restriction digests were subsequently performed on the amplified DNA for the BirdRintAG480 and BirdRintGG480 PCRs. *AluI* cut the AG allele at a linked SNP 44 bp from the 5' end. The 10 µL digests contained 1x Buffer, 0.04 U *AluI* (New England Biolabs), and PCR product and were incubated at 37 ˚C for a minimum of two hours. Digests were run on a 3% agarose gel and scored as AG (45 and 138 bp) or GG (183 bp) for the 243 samples not identified as AA.

2.3.4. Data analyses

A 753 bp CR alignment was created in MEGA v. 6 (Tamura et al., 2011). DnaSP v 5 (Librado & Rozas, 2009) was used to assign haplotypes and to calculate haplotype (h) and nucleotide (π) diversities.

To determine genetic differentiation among species and among populations, pairwise F_{ST} values measuring population differentiation were calculated in the program Arlequin 3.11 (Excoffier & Lischer, 2010). Populations with fewer than six individuals were removed from this analysis. *p*-values were corrected using the modified False Discovery Rate (FDR) (Benjamini & Hochberg, 1995).

A maximum likelihood tree was created to examine the relationships between haplotypes using the automated model selection function in the program PAUP* (Swofford, 2002). The tree was generated using the K80 + I + G model with 500 bootstrap replicates.

SNP data for CT and AG COI SNPs and the CHD1Z indel were analyzed with descriptive statistics comparing nucleotide composition between both populations and species. Chi-square tests were used to test for significance of SNP variation among species.

2.3.5. Ecological Niche Modelling

Occurrence data were downloaded from the Global Biodiversity Information Facility (GBIF, www.gbif.org). For *S. nuchalis* and *S. ruber*, all entries prior to 1980 were omitted to maximize occurrences with accurate geographic coordinates. Because all sapsuckers were considered *S. varius* until 1985, all occurrences prior to 1986 were

omitted for this species. To restrict occurrences to breeding populations, all *S. nuchalis* and *S. ruber* entries were restricted to those collected from mid-April through August, and *S. varius* from June through August (Walters et al. 2002a, 2002b). All occurrence files were spatially rarefied using the SDM Toolbox (Brown, 2014).

Environmental variables (Appendix 1) were downloaded from WorldClim v 1.4 (Hijmans et al., 2005) and processed in ArcGIS with the SDM Toolbox (Brown, 2014). Variables were checked for autocorrelation with a 0.9 threshold in both SDM Toolbox and ENMTools (Warren et al., 2010), and correlated variables were removed from the analyses. Each species' ecological niche was modelled using Maxent v 3.3 (Phillips et al., 2006). Previous studies have used different studies for this model (Merow et al., 2013; Shcheglovitova & Anderson, 2013; Warren & Seifert, 2011). Therefore, we used a number of regularization multipliers, environmental layers, and feature classes, and tested each model's suitability using the Model Selection tool in ENMTools (Warren et al., 2010). The settings used in Maxent to model the ecological niche of *S. varius* were a 0.1 regularization multiplier with a hinge feature class, and ten bioclim layers: 1, 2, 3, 7, 8, 12, 14, 15, 18, and 19. For *S. nuchalis* and *S. ruber*, we used a 0.5 regularization multiplier and a hinge feature. While the same ten bioclim layers were used for *S. varius* and *S. ruber*, *S. nuchalis* models performed better without layer 2, so only the remaining nine layers were used to model this species' niche. The best fit model for each species was selected by optimal corrected Akaike's Information Criterion (AICc) and area under curve (AUC). These conditions were projected to mid-Holocene and LGM using MIROC-ESM Global Climate Models (Watanabe et al., 2011), and LIG from Otto-Bliesner et al. (2006).

Niche overlap was measured instead of range overlap because even present-day models may include regions of niche overlap but not range overlap, as other factors may impact species ranges, such as competitive exclusion. Because we could not directly measure geographic range overlap in the past time periods, we quantified the projected historical niche overlap for the Holocene and LGM, and compared these with the current niche overlap using ENM Tools (Warren et al., 2010).

2.4. Results

2.4.1. Sequencing

A 753 bp region of CR sequences from 308 individuals was aligned using MEGA v 5 (Tamura et al., 2011) (Table 2.2). DnaSP identified 132 different haplotypes in the 308 individuals, 30 of which were shared among multiple birds and 102 that were found in a single individual (Supplementary Material). Overall haplotype diversity (h) was 0.955 and overall nucleotide diversity was 0.01468 (Table 2.3). Haplotype diversity was highest in *S. varius* (0.969) and lowest in *S. nuchalis* (0.859) (Table 2.3).

Pairwise F_{ST} values for the control region data ranged from 0 (or negative) to 0.803 (*S. varius* SK and *S. ruber* WA) (Table 2.4). A small number of pairwise F_{ST} values between conspecific populations were significant: four comparisons amongst the 10 *S. varius*, two of 21 *S. nuchalis*, and one of 10 *S. ruber* (Table 2.5). In comparison, 25 of 35 comparisons between *S. nuchalis* and *S. ruber* were significant, and all 25 *S. ruber*-*S. varius* comparisons, and 35 *S. nuchalis*-*S. varius* were significant.

The maximum-likelihood tree was polyphyletic with at least three clades that generally, but not definitively, corresponded to species (Figures 2.1a, b). Clade one consisted of two groups that were mostly *S. varius*, and clade two was almost exclusively

S. nuchalis individuals. Almost all *S. ruber* individuals fell within clade three along with remaining *S. nuchalis*. A fourth clade contained four individuals: three *S. ruber* individuals, one from northern British Columbia (NBC) and two from Haida Gwaii (HG), and one *S. varius* from Nova Scotia/New Brunswick (NSNB).

2.4.2. SNP screening

The CT COI SNP differentiated *S. varius* from both *S. nuchalis* and *S. ruber* (χ^2 = 238, $p \leq 0.00001$). In *S. varius,* 1% (2 of 184) of individuals had the CT allele, whereas 86% (72 of 84) of *S. ruber* and 75% (74 of 99) of *S. nuchalis* had the same CT allele (Figure 2.2a). The only two *S. varius* samples that had the CT allele were collected near hybrid zones in CAB and NWBC (Figure 2d). Similar species divisions were found using the AG COI SNP (Figure 2) ($\chi^2 = 518$, $p \leq 0.00001$). All 190 *S. varius* had the AA allele, whereas *S. nuchalis* had 57% (64 of 113) AG and 43% (49 of 113) GG; and *S. ruber* had 15% (19 of 130) AG and 85% (111 of 130) GG. While there was no evidence of geographic clustering of alleles within the *S. nuchalis* populations, 95% (18 of 19) of the *S. ruber* with the AG allele were from the northern Pacific Coast (CBC, HG, VIBC, WA) and 15 of those were from HG (Figure 2.2b).

The CHD1Z sequences showed a G insertion/deletion with significant differences among species ($\chi^2 = 61$, $p \leq 0.00001$). The majority of *S. ruber* (30 of 35 alleles) had the G insertion (Figure 2). This was quite different from *S. nuchalis*, which was nearly fixed for the G deletion, with the exception of one insertion allele collected from a hybrid zone in central Alberta (CAB) (35 of 36). While most *S. varius* alleles (50 of 65) had the G deletion, the majority of G insertions (13 of 15) were found in eastern populations (IL,

ON, and NS) (Figure 2.2) ($\chi^2 = 10$, $p = 0.0014$), and the two western insertion alleles were collected in CAB.

These markers differentiated the species much more effectively in combination. Composite genotypes of each locus were created for all samples screened at both COI loci, as these genotypes allow us to genetically identify each species. The majority of *S. ruber* (107 of 123) had a CTGG genotype, and most of these (88 of 107) fell into clade three. *S. nuchalis* could be separated from *S. ruber* using the deletion on CHD1Z locus, clade two, and the AG COI SNP. *S varius* was quite different from both other species, as the majority of birds had a TCAA genotype (351 of 353). It is interesting to note although the two COI loci are linked, genotypes fall into all possible combinations such that the presence of one SNP does not predict presence of another.

2.4.3. Ecological Niche Modelling

Models for each species were evaluated using AUC: *S. nuchalis* AUC = 0.908 ± 0.003, *S. ruber* AUC = 0.948 ± 0.002, and *S. varius* was 0.848 ± 0.002. Niche overlap was measured using both Schoener's D and the l statistic, which theoretically range from 0 (no overlap) to 1 (complete overlap). The amount of overlap was generally low during the Holocene and high during the present day (Table 2.5). Across each time period, overlap was greatest between *S. nuchalis* and *S. ruber*, and the lowest between *S. varius* and *S. ruber* (Table 2.5).

2.5. Discussion

2.5.1. Effects of Pleistocene biogeography

Within *S. nuchalis*, there does not seem to be much geographic structure determining where certain haplotypes or SNPs are found. However, *S. ruber* shows evidence of population structure in coastal BC, and *S. varius* populations show an east-west split. Both HG and VIBC showed evidence of an evolutionary history separate from other *S. ruber* populations. In each case, both CR and COI show alleles typical of the two other species (Figure 2.1b, Table 2.2). HG in particular contributes the majority of alleles shared with other species in both COI SNPs (Table 2.2). Historical secondary contact between hermit and Townsend's warblers (*Septophaga occidentalis* and *S. townsendi*) is thought to have caused a similar pattern, in which Townsend's warblers on Haida Gwaii had hermit warbler mtDNA haplotypes (Krosby & Rohwer, 2009). Clearly, coastal BC has a unique evolutionary history, possibly as an isolated Pleistocene refugium. Haida Gwaii refugia have been suggested for several North American species, including chestnut-backed chickadees (*Poecile rufescens*) and black bears (*Ursus americanus*) (Burg et al., 2006; Byun et al., 1997). During the Pleistocene when the mainland was covered in ice sheets, small parts of Haida Gwaii and northern Vancouver Island and the adjacent coast were forested regions of inhabitable land for many species (Hetherington et al., 2003). Our ENM data show that this region was within the preferred ecological niche of *S. ruber* during the Pleistocene, and it is possible that an isolated population of sapsuckers persisted in this refugium (Figure 2.3).

Another interesting geographical pattern in our *S. varius* data is the eastern trend for CHD1Z G insertions, whereby the majority of individuals with the insertion were from eastern populations (13 of the 15 insertions), while western populations had a low prevalence (2 of 28 western alleles) of insertions (Table 2.2). This corresponds to the geographical patterns found in western Canada by Seneviratne et al. (2012), but our wider

sampling revealed an unnoticed and unexpected change over the geographic range of *S. varius*. This east west split of CHD1Z alleles corresponds to an abrupt disruption in modeled suitable niche habitat in central Canada in the LIG projection for this species, specifically, bioclim layers 8, 9, 14, and 15 (Figure 2.3). The habitat was associated with an area of low precipitation and temperature during the LIG, which may have isolated eastern from western populations of *S. varius* (Otto-Bliesner et al., 2006). Nuclear Z-linked markers tend to evolve more slowly than the mitochondrial CR or COI markers. This may explain why the remnants of LIG landscape patterns affect CHD1Z, but are absent from the mtDNA data for this species (Renoult et al., 2009).

2.5.2. Systematics and polyphyly

Our data corroborate the findings of previous studies that *S. nuchalis* and *S. ruber* are more closely-related to each other than to *S. varius*. The CR sequence F_{ST} (Table 4) and maximum-likelihood tree data (Figure 2.1) show higher similarity among *S. nuchalis* and *S. ruber* populations than *S. varius* populations. COI SNPs also showed more shared alleles between *S. nuchalis* and *S. ruber.*

On a rangewide scale, mitochondrial markers indicate differentiation between sapsucker species. The maximum-likelihood tree shows three main clades generally corresponding to species, though no species clade is monophyletic. The composite genotype data were able to differentiate between the three species. The near-fixation of the CT COI SNP and complete fixation of the AG COI SNP clearly distinguished *S. varius* from *S. nuchalis/S. ruber* (Figure 2.2), and both the AG SNP and the nuclear CHD1Z marker showed significant differences between the closely related *S. nuchalis* and *S. ruber*.

A number of factors could explain polyphyletic patterns in our data, particularly incomplete lineage sorting (ILS) and introgression, which are often difficult to distinguish (Funk & Omland, 2003). The smaller effective population size of mtDNA causes these markers to sort more quickly than nuclear markers (Renoult et al., 2009). Our pattern is likely the result of hybridization. These species are all known to hybridize in sympatry and hybrid offspring are viable (Johnson & Zink, 1983). The majority of polyphyletic individuals originate near hybrid zones (Figures 2.1, 2.2). Three of the six *S. varius* individuals that fell in the *S. nuchalis/S. ruber* dominated CR clade originated from CAB, a *S. varius*/*S. nuchalis* hybrid zone, as did the only *S. nuchalis* and western *S. varius* individuals with CHD1Z insertion. (Figure 2.1). Furthermore, all five *S. nuchalis* in clade one were from southern Alberta (SAB), just south of the CAB hybrid zone, and one of the three *S. ruber* individuals was from NBC, near a *S. ruber/S. varius* hybrid zone (Seneviratne et al., 2012) (Figure 2.1).

Pairwise F_{ST} values indicate little evidence of structuring among conspecific populations (Table 2.4). However, significant differences exist amongst species and while mixing is not restricted to hybrid zones, a disproportionate number of mixed individuals were found in hybrid zones. In *S. ruber*, eight of the 42 alleles atypical of *S. ruber* were from near hybrid zones (ECA, WA, CBC, or NBC). Similarly, in *S. nuchalis*, 51 of the 80 atypical alleles (from NEOR, WA, NEWA, SAB, and CAB), and seven of the 25 atypical *S. varius* alleles were from near hybrid zones (NWBC and CAB). Because mixing is more evident in hybrid zones, this suggests that hybrid zones may represent sink habitats, or hybrids may be less fit. Sapsuckers are parapatric, and zones of sympatry occur at the peripheries of their range. Range peripheries contain marginal habitats where fitness might be lower, reducing the likelihood that introgressed alleles would move out of these

areas (Micheletti & Storfer, 2016). However, Johnson and Johnson (1985) demonstrated reduced fitness of F1 *S. ruber/S. nuchalis* hybrids, and Seneviratne et al. (2012) suggested selection acts against *S. ruber/S. varius* hybrids, supporting our data of limited movement of these alleles out of hybrid zones.

The ENM data modelled large areas of potential niche overlap for all three species over each time period, which were comparable to present day overlap (Table 2.5, Figures 2.3a-d). During the Holocene, range overlap was likely geographically similar to the present day, particularly in the Canadian Rocky Mountains, where all three species are predicted to have suitable habitat. In the LGM, suitable *S. nuchalis* habitat overlapped with *S. ruber* on the Pacific Coast and with *S. varius* in the southern United States. The two western species, *S. nuchalis* and *S. ruber*, in particular have vast regions of potential range overlap (l statistic > 0.5) during each time period investigated. These projections underscore the high likelihood of at least partial sympatry for the three species at various points of their recent evolutionary history. This is further demonstrated by the fact that the two species showing lowest differentiation, *S. nuchalis* and *S. ruber*, have the largest potential niche overlap. Alves et al. (2003) found similar results of paraphyletic mtDNA clades in European hares (*Lepus timidus*, *L. granatensis*, *L. europaeus*), and came to the conclusion that this resulted from ancient introgression.

As Webb et al. (2011) demonstrated, mtDNA does not necessarily reflect species adaptive divergence, rather mitochondrial patterns may be an artefact of a species' historical isolation. Overwhelming evidence suggests that these three sapsuckers represent distinct species (Cicero & Johnson, 1995; Johnson & Johnson, 1985; Johnson & Zink, 1983). A small number of pairwise comparisons between species were not significant suggesting limited connectivity among species, despite evident speciation

(Grossen et al., 2016; Johnson & Zink, 1983). Assuming that geographic isolation is not completely responsible for reproductive isolation, this raises the question of what additional mechanisms maintain divergence within this species complex.

2.5.3. Reproductive isolating mechanisms

The obvious plumage differences amongst these species would pose an ostensible reproductive barrier. Plumage has been suggested as a barrier in several species, including lazuli and indigo buntings (*Passerina amoena*, *P. cyanea*) and house, Spanish, and Italian sparrows (*Passer domesticus*, *P. hispaniolensis*, and *P. itatliae*) (Bailey et al., 2015; Baker & Boylan, 1999). Furthermore, Toews et al. (2016) demonstrated that the majority of divergence in the genomes of golden-winged and blue-winged warblers (*Vermivora chrysoptera, V. cyanoptera*) is in regions controlling feather colouration and development. Grossen et al. (2016) identified a locus (COG4) using next-generation sequencing that is closely associated with plumage in sapsuckers. Plumage does play a role in mate choice in sapsuckers (Johnson & Johnson, 1985; Seneviratne et al., 2012). Johnson and Johnson (1985) postulated that the highly divergent plumage of *S. ruber* and *S. nuchalis* emerged as a pre-mating isolating mechanism to differentiate the two closely-related species.

Behaviour may also contribute to isolation in these species, particularly their migratory habits. These species exhibit a range of migratory behaviours from the completely migratory *S. varius*, to the partial migrant *S. nuchalis* and nearly resident *S. ruber* (Walters et al., 2002a, 2002b). Differences in migration could lead to population differentiation in multiple ways. Migration may affect the timing of breeding, as resident birds breed earlier (Billerman et al., 2016; Walters et al., 2002a). Furthermore, some birds

may form pair bonds on wintering grounds, in which case resident and migratory birds would be unable to pair bond (Borràs et al., 2011; Davidson et al., 2013; Humphries et al., 2009). Delmore et al. (2016) and Lundberg et al. (2013) have shown reproductive isolation between populations with different migration strategies in both Swainson's thrush (*Catharus ustulatus*) and willow warblers (*Phylloscopus trochilus*).

A third possibility is ecological speciation. Each of the sapsucker species has different breeding habitat preferences: *S. ruber* is found in coniferous forests, *S. nuchalis* breeds in mixed woodlands, especially aspen (*Populus* spp.) and ponderosa pine (*Pinus ponderosa*), and *S. varius* prefers to breed in early successional forests of aspen (*Populus* spp.), birch (*Betula* spp.), and maple (*Acer spp.*) (Walters et al. 2002a, b). Each species may be better adapted to different abiotic factors, such as the high precipitation and mild climate of the Pacific Coast, the arid conditions of the Rocky Mountains, or the cool climate and wetland habitat in the boreal forest. Our Ecological Niche Modelling demonstrates that each species has distinct, albeit slightly overlapping, ecological preferences. Billerman et al. (2016) reported *S. nuchalis* and *S. ruber* ranges in Oregon are shifting in accordance with local climate and habitat change. Grossen et al. (2016) found differentiation among sapsucker species in regions associated with fluid homeostasis, suggesting a genomic underpinning of adaptation to different moisture availability. Species that evolve ecological specialization are more likely to differentiate than generalists, and if these sapsuckers have specialized ecological niches this might contribute to their divergence (Schluter, 2009; Webb et al., 2011).

2.6. Conclusions

In studying evolution and speciation, evolutionary biologists attempt to identify not only the results of speciation, but also the forces causing it. While these causes may often be assumed based on phylogeographic evidence, the underlying causes for reproductive isolation between different species are undoubtedly complex. Our ENM modelling suggests that *S. varius*, *S. nuchalis*, and *S. ruber* did not speciate under strict allopatry, which calls into question a decades-old assumption and simultaneously reopens the question of what forces have caused the divergence of three species with a well-documented evolutionary history. Parapatric speciation is difficult to prove (Fitzpatrick et al., 2009; Mallet et al., 2009), but given the evidence that three well-supported species were sympatric during the time period molecular evidence says they diverged, it is an explanation that warrants investigation.

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Table 2.1. Primer sets used for the control region (CR) cytochrome oxidase I (COI) and the Z-linked CHD1Z, primer sequences including SNP sites (bold, underlined), and reference.

Region	Primer	Sequence	Source
CR	LThr	CATTGGTCTTGTAARCCAAAG	Graham and Burg 2012
	HPro	AATRCCAGCTTTGGGAGTTGG	This study
COI	BirdF1	TTCTCCAACCACAAAGACATTGGCAC	Hebert et al. 2004
	BirdR1	ACGTGGGAGATAATTCCAAATCCTG	Hebert et al. 2004
	Bird Fint CT400	TGAACTGTCTACCCACCTCT**C**TC**T**	This study
	Bird Fint TC400	AACTGTCTACCCACCTCT**T**TC**C**	This study
	Bird Rint COI530	GGGGGTTTGGTATTGTGARA	This study
	Bird Fint COI320	CCATCATTTCTTCTYCTYCTAGC	This study
	Bird Rint AA480	GCTGTTGTGAATGAAGTTAAT**T**GC**T**	This study
	Bird Rint AG480	GCTGTTGTGGTGAAGTTAAT**C**TC**T**	This study
	Bird Rint GG480	CTGTTGTGGTGAAGTTGAT**C**TC**C**	This study
CHD1Z	CHD1Z-F-Sapsucker	GCAACCTGCTTTAGCTGTCC	Seneviratne et al. 2012
	CHD1Z-R-Sapsucker	GAGCAACTTAAATTCTCAACAGC	Seneviratne et al. 2012

Table 2.2. Sample sizes (n) of each population for each molecular marker. Control region (CR) haplotypes falling within each clade of the maximum-likelihood tree, cytochrome oxidase I (COI) CT and AG SNPs, CHD1Z insertions and deletions, and total number of alleles screened for each marker (n). For CR clades refer to Figure 1, for site locations, refer to Figure 2.

							COI									
		CR clade					CT SNP			AG SNP				CHD1Z		
	Population	1	2	3	4	n	CT	TC	n	AA	AG	GG	n	ins.	del.	n
S. ruber	SCA											3	3			
	ECA			2		2	6		6			7	7			
	CCA						1		1			3	3			
	NCA			5		5	7		7		1	10	11			
	WA			23		23	18	1	19		1	20	21	13	2	15
	VIBC			14		14	12	4	16		1	22	23	6	2	8
	CBC			11		11	6	1	7		1	15	16	4		4
	HG	2		15	2	19	17	6	23		15	18	33	5	1	6
	NBC	1		5	1	7	3		3			9	9			
	SEAK			3		3	2		2			4	4	2		2
	Total	3	0	78	3	84	72	12	84	0	19	111	130	30	5	35
S. nuchalis	AZ						2		2		2		2			
	NM		2	4		6	16		16		15	4	19		7	7
	CO		3	4		7	5	2	7		4	3	7		2	2
	UT		5	2		7	4	1	5		6	1	7		3	3
	SD		1	6		7	4	3	7		1	6	7			
	WY		1	3		4	2	2	4		2	2	4			
	NEOR		10	3		13	1	6	7		5	7	12		4	4
	ID						4		4		7	3	10		11	11
	MT		1	1		2	1	1	2		2		2		1	1
	WA		2	4		6	6		6		2	4	6			
	NEWA		1	2		3	3		3		3		3			

	SAB	5	8	16		29	17	6	23		5	15	20		2	2
	SEBC			4		4	7		7		6	1	7		4	4
	CAB			1		1	2	4	6		4	3	7	1	1	2
	Total	5	34	50	0	89	74	25	99	0	64	49	113	1	35	36
S. varius	SEBC									1			1			
	NWBC	5				5	1	4	5	5			5		8	8
	CAB	20		3		23	1	29	30	33			33	2	4	6
	SK	14				14		14	14	13			13		16	16
	IL	40				40		38	38	40			40	8	9	17
	MI	15				15		17	17	17			17		7	7
	ON	17	1	1		19		44	44	42			42	2	1	3
	NSNB	3		1	1	5		17	17	17			17	3	1	4
	WA			1		1				2			2			
	NM	1				1		3	3	3			3			
	LA	2				2		3	3	3			3			
	NC	9				9		10	10	10			10		2	2
	NJ							2	2	2			2		1	1
	FL	1				1		1	1	2			2		1	1
	Total	127	1	6	1	135	2	182	184	190	0	0	190	15	50	65
	Overall	135	35	134	4	308	148	219	367	190	83	160	433	46	90	136

Table 2.3. Haplotype (h) and nucleotide (π) diversity values, and sample size (n) for CR data.

Species	h	π	n
S. ruber	0.894 ± 0.022	0.00645	84
S. nuchalis	0.859 ± 0.030	0.01111	89
S. varius	0.969 ± 0.009	0.00933	135
All species	0.955 ± 0.007	0.01468	308

Table 2.4. Pairwise F_{ST} values (below diagonal) for populations n ≥ 6 and significance (above diagonal). Values in bold are significantly different with a false discovery rate of 0.05. For site locations see Figure 2.2.

	S. ruber					*S. nuchalis*							*S. varius*				
	WA	VIBC	CBC	HG	NBC	NM	CO	UT	SD	NEOR	WA	SAB	CAB	SK	IL	MI	ON
WA		-	-	*	-	*	*	*	*	*	*	*	*	*	*	*	*
VIBC	-0.024		-	-	-	*	*	*	*	*	-	*	*	*	*	*	*
CBC	-0.027	-0.009		-	-	*	*	*	*	*	-	-	*	*	*	*	*
HG	**0.071**	0.059	0.035		-	-	-	*	*	*	-	-	*	*	*	*	*
NBC	0.140	0.088	0.061	-0.040		*	-	*	*	*	-	-	*	*	*	*	*
NM	**0.302**	**0.262**	**0.223**	0.081	**0.124**		-	-	-	*	-	-	*	*	*	*	*
CO	**0.306**	**0.261**	**0.212**	0.091	0.123	-0.042		-	-	-	-	-	*	*	*	*	*
UT	**0.575**	**0.521**	**0.473**	**0.304**	**0.312**	0.162	0.024		-	-	-	-	*	*	*	*	*
SD	**0.303**	**0.280**	**0.249**	**0.088**	**0.168**	0.011	0.034	0.303		*	-	-	*	*	*	*	*
NEOR	**0.637**	**0.599**	**0.562**	**0.413**	**0.442**	**0.329**	0.202	-0.070	**0.453**		-	-	*	*	*	*	*
WA	**0.217**	0.182	0.127	0.048	0.068	-0.018	-0.085	0.080	0.059	0.221		-	*	*	*	*	*
SAB	**0.152**	**0.136**	0.098	0.050	0.068	0.000	-0.032	0.081	0.029	0.183	-0.053		*	*	*	*	*
CAB	**0.658**	**0.625**	**0.597**	**0.441**	**0.408**	**0.562**	**0.552**	**0.592**	**0.601**	**0.637**	**0.542**	**0.386**		-	-	-	*
SK	**0.803**	**0.781**	**0.761**	**0.573**	**0.568**	**0.723**	**0.711**	**0.734**	**0.768**	**0.762**	**0.713**	**0.499**	0.033		-	-	*
IL	**0.754**	**0.738**	**0.724**	**0.588**	**0.588**	**0.706**	**0.694**	**0.719**	**0.729**	**0.740**	**0.692**	**0.525**	0.017	0.005		-	*
MI	**0.781**	**0.756**	**0.734**	**0.548**	**0.540**	**0.694**	**0.682**	**0.707**	**0.737**	**0.741**	**0.681**	**0.476**	0.015	-0.020	-0.016		*
ON	**0.633**	**0.591**	**0.561**	**0.408**	**0.372**	**0.496**	**0.485**	**0.527**	**0.531**	**0.589**	**0.481**	**0.367**	**0.062**	**0.137**	**0.094**	**0.080**	

Table 2.5. Schoener's D statistic (above diagonal), and l statistic (below diagonal), measuring niche overlap between *S. ruber*, *S. nuchalis*, and *S. varius*.

Present	*S. ruber*	*S. nuchalis*	*S. varius*
S. ruber		0.269	0.071
S. nuchalis	0.557		0.080
S. varius	0.221	0.267	

Holocene	*S. ruber*	*S. nuchalis*	*S. varius*
S. ruber		0.257	0.043
S. nuchalis	0.543		0.050
S. varius	0.140	0.188	

LGM	*S. ruber*	*S. nuchalis*	*S. varius*
S. ruber		0.248	0.054
S. nuchalis	0.502		0.078
S. varius	0.223	0.251	

Figures:

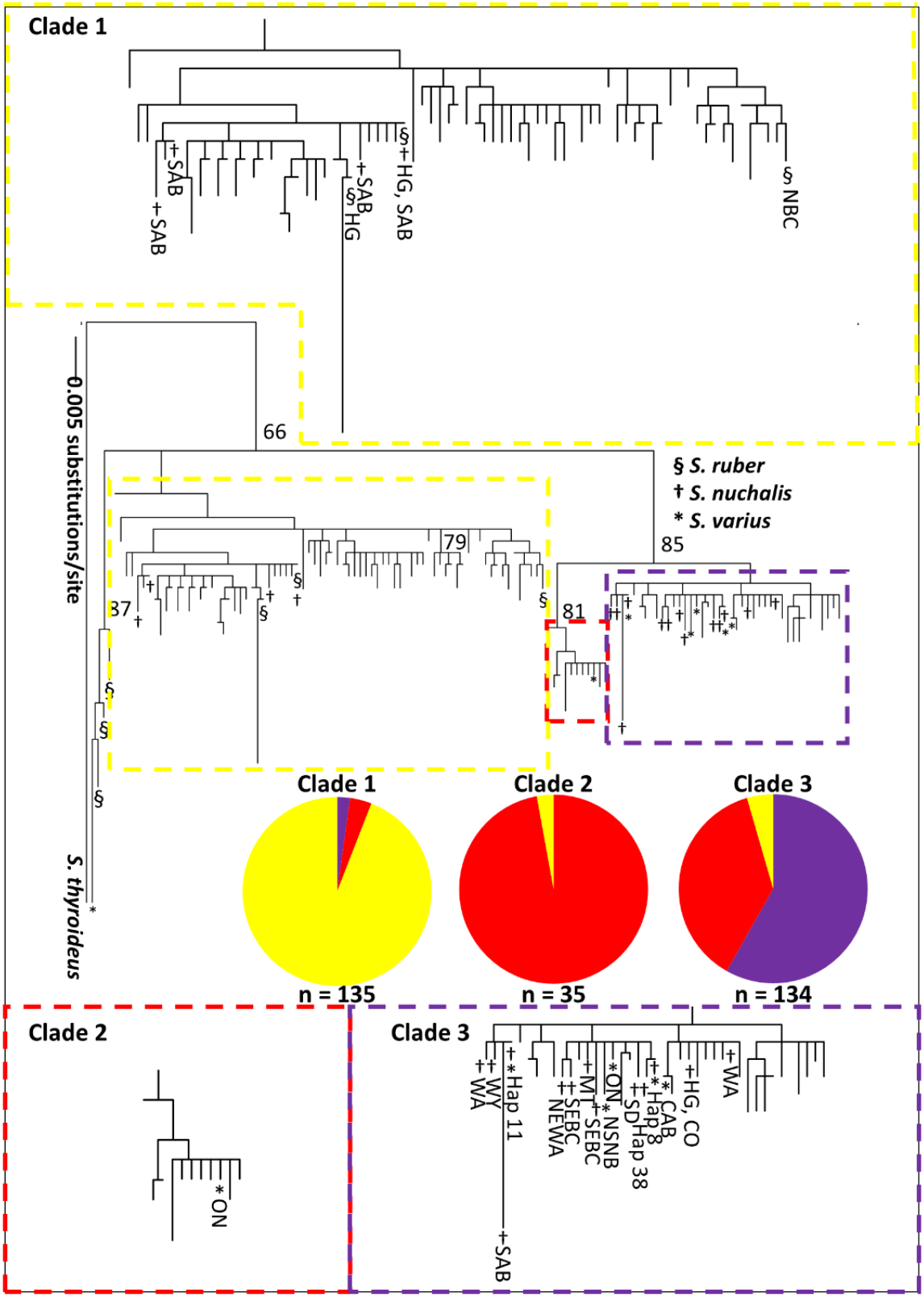
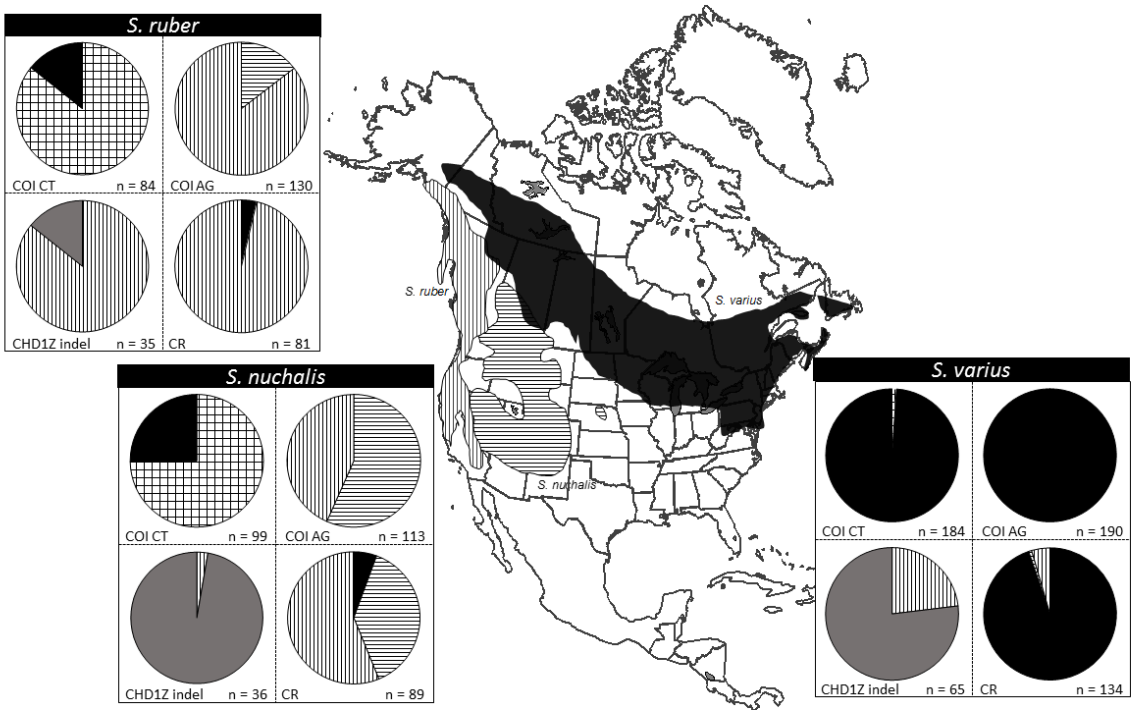
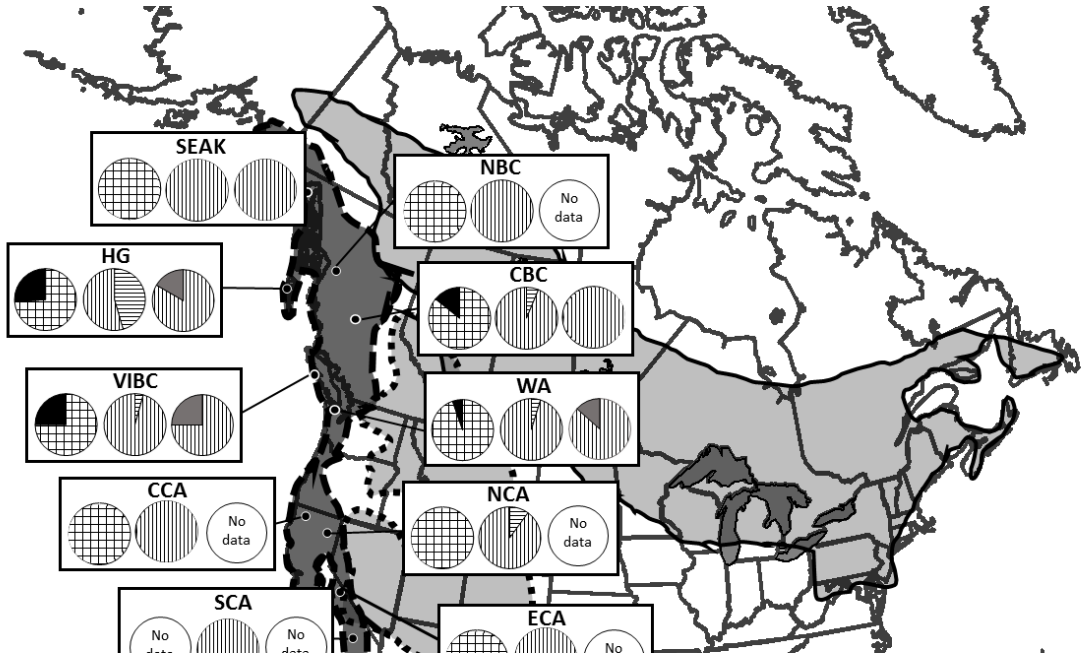

Clade 1
§ S. ruber
† S. nuchalis
* S. varius
0.005 substitutions/site
S. thyroideus
66
79
85
37
81
Clade 1
Clade 2
Clade 3
n = 135
n = 35
n = 134
Clade 2
Clade 3
+SAB
+SAB
+SAB
§+HG, SAB
§+HG
§ NBC
*ON
†*Hap 11
†† WY
WA
†† SEBC
NEWA
†+MT
†* SEBC
*ON NSNB
†† SD
Hap 38
†* CAB
Hap 8
+HG, CO
†WA
+SAB

Figure 2.1. Maximum-likelihood tree of CR haplotypes of three sapsucker species, and *S. thyroideus* outgroup. Bootstrapping values are provided alongside major branches. Haplotypes containing at least one individual of the species not typical of its assigned clade are marked with species specific symbols. In the pie charts, yellow represents the proportion of individuals in each clade that are *S. varius*, red represents *S. nuchalis*, and purple indicates *S. ruber*. Enlarged clades are shown with marked branches showing populations of individuals whose species are not typical of the clade. Shared haplotypes are listed in Table 2.4.

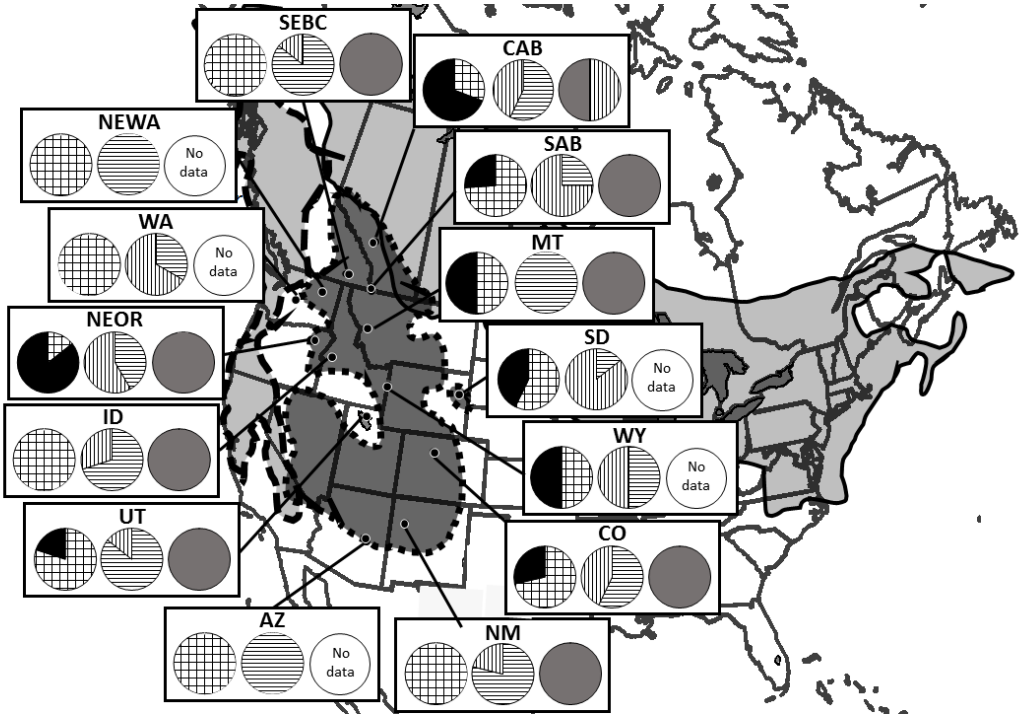
a.


S. ruber
COI CT n = 84
COI AG n = 130
CHD1Z indel n = 35
CR n = 81
S. nuchalis
COI CT n = 99
COI AG n = 113
CHD1Z indel n = 36
CR n = 89
S. ruber
S. varius
S. nuchalis
S. varius
COI CT n = 184
COI AG n = 190
CHD1Z indel n = 65
CR n = 134

b.


SEAK
NBC
No data
HG
CBC
VIBC
WA
CCA
No data
NCA
No data
SCA
No
No
ECA
No

c.


SEBC
CAB
NEWA
No data
SAB
WA
No data
MT
NEOR
SD
No data
ID
WY
No data
UT
CO
AZ
No data
NM

d.

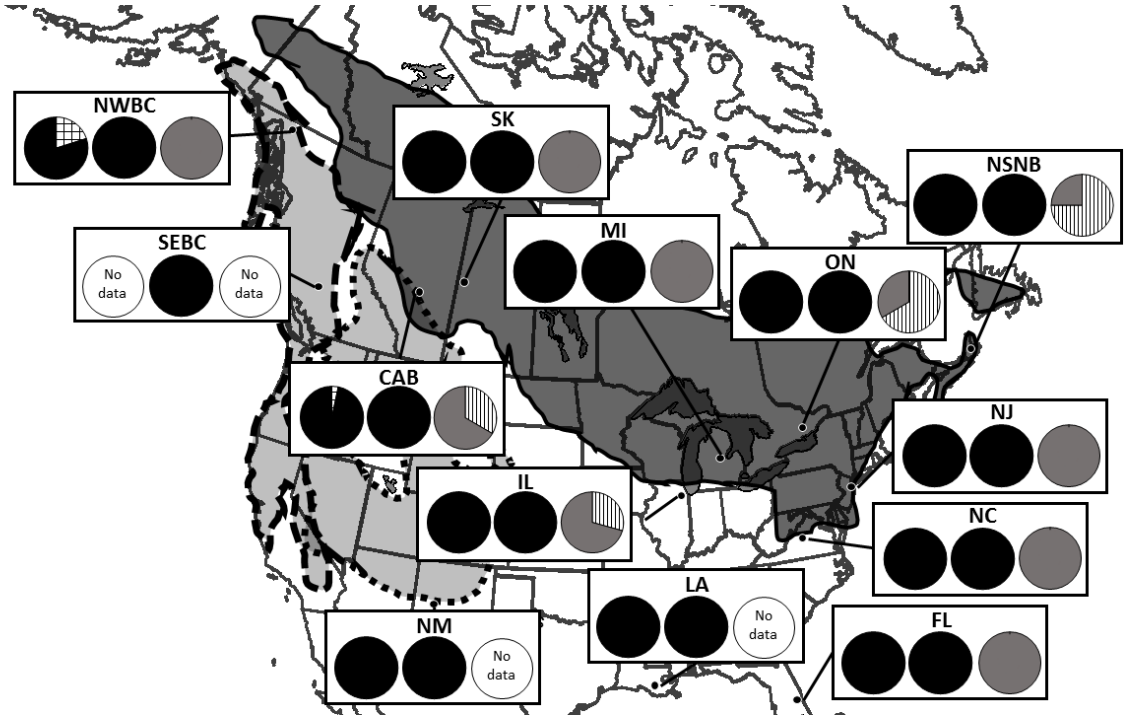

NWBC
SK
NSNB
SEBC
No data
No data
MI
ON
CAB
NJ
IL
NC
LA
No data
NM
No data
FL

Figure 2.2. (a) Proportions of each COI SNP, CHD1Z indel, and CR clade for each sapsucker species. Population COI CT (left) and AG (middle) SNPs and CHD1Z indel (right) proportions from (b) *S. ruber*, (c) *S. nuchalis*, and (d) *S. varius.* Black represents alleles typical of *S. varius* (TC alleles, AA alleles, clade 1), vertical lines depict alleles typical of *S. ruber* (GG, deletion, clade 3), horizontal lines are typical of *S. nuchalis* (AG, clade 2), and grids represent alleles representative of both of these species (CT). Grey represents the CHD1Z insertion indicative of both *S. varius* and *S. nuchalis*. CR pie charts exclude four outliers from CR maximum-likelihood tree.

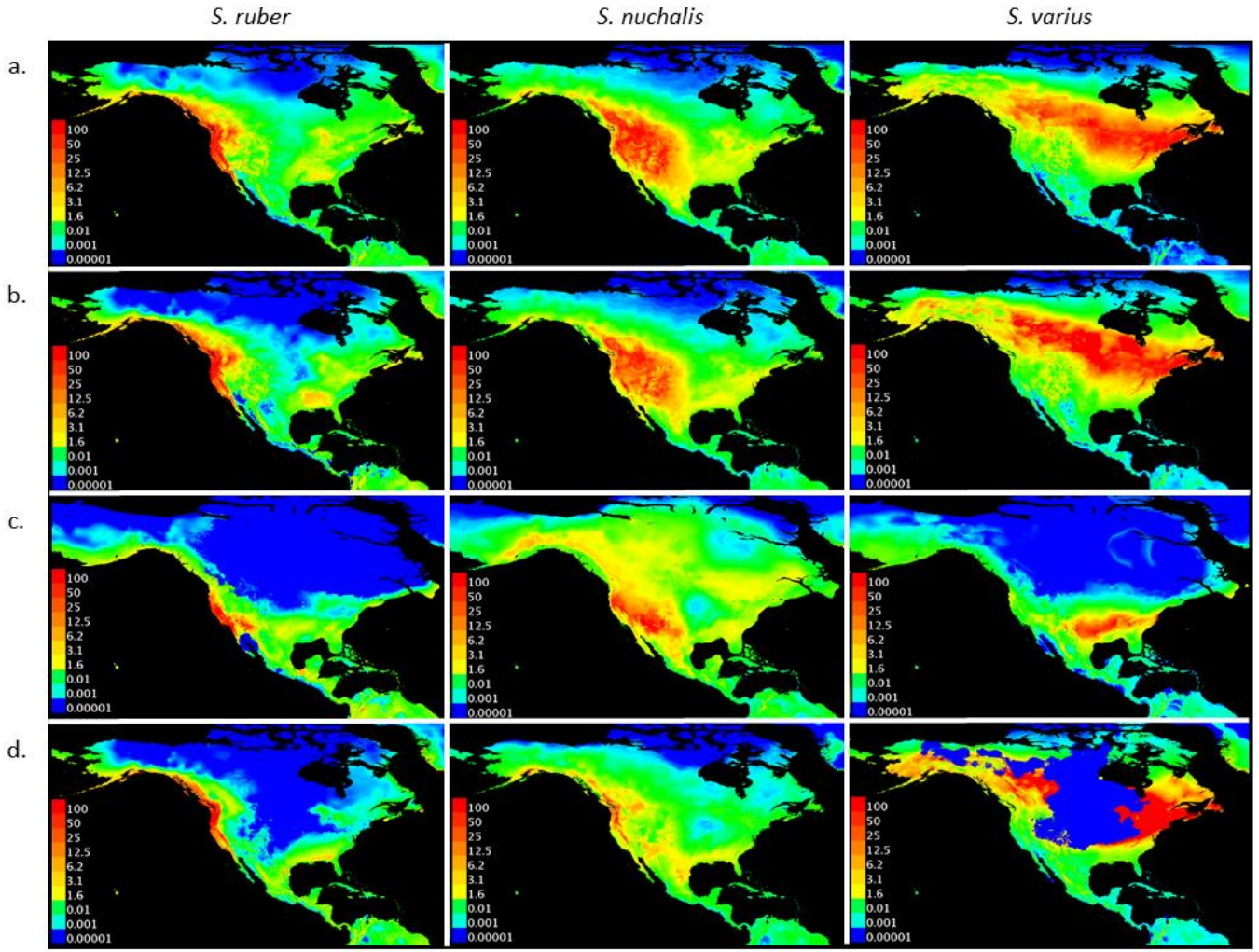


Figure 3. Present day (a), Holocene (b), LGM (c), and LIG projections (d) of ecological niches for *S. varius*, *S. nuchalis*, and *S. ruber*. The logarithmic scale depicts the percent likelihood of climate suitability.

CHAPTER 3

High rates of introgression between *S. nuchalis* and *S. varius* in a central Alberta hybrid zone

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Hybrid zone sapsuckers were collected and identified by phenotype by Jocelyn Hudon. GBS Data for this chapter were processed and analyzed by Ashley Curtis, while traditional genetic markers were processed and analyzed by Libby Natola. Results were synthesized and discussed by Libby Natola.

3.1 Abstract

Hybridization may be the key to unraveling the independent roles of different reproductive isolating mechanisms in closely-related species. In *Sphyrapicus* sapsuckers, hybridization among *S. ruber, S. nuchalis*, and *S. varius* has been studied from several perspectives. Though the genetic aspects of *S. ruber/S. nuchalis* and *S. ruber*/*S. varius* hybrid zones have been studied, no research has yet been undertaken to understand genetic patterns in the *S. nuchalis*/*S. varius* hybrid zone. Using a combination of next-generation genotyping by sequencing (GBS) and traditional genetic methods, we examined patterns of introgression in this hybrid zone in central Alberta. The data show a low incidence of F1 hybrids, but high introgression rates. Patterns of introgression between *S. nuchalis* and *S. varius* are asymmetric with shared alleles extending into *S. nuchalis* populations outside of the hybrid zone, but not into *S. varius* populations. Admixture is higher in in *S. varius* individuals within the hybrid zone, but also high interspecific heterozygosity and Introgression differs along a north-south gradient extending from central to southern Alberta, with more *S. varius* alleles in the north and *S. nuchalis* alleles in the south. We suggest that high rates of introgression occur between these species due to similar plumages, habitat preferences, and migratory behaviours.

3.2 Introduction

The identification of reproductive isolating mechanisms is crucial to understanding both hybridization and speciation. By learning what maintains genetic isolation between groups, we can pinpoint forces that drive divergence, resulting in speciation. Barriers causing reproductive isolation are often geographic, such as mountains, bodies of water, glacial ice, or anthropogenic land use (Apte et al., 2007; Bush et al., 2011; Lait & Burg, 2013). However, they may also be behavioural, such as sexual selection of plumage or song, philopatry, or migratory routes (Baker & Boylan, 1999; Delmore et al., 2016; Johnson & Johnson, 1985). Species are often isolated by multiple barriers.

Reproductive isolating barriers may be ephemeral. For example, several species that were formerly isolated on different continents and have been translocated by humans now hybridize in the absence of geographic isolation (Green & Rothstein, 1998; Rhymer & Simberloff, 1996). Similarly, when the ranges of closely-related species overlap, they may hybridize, allowing biologists the opportunity to study the non-geographic barriers to reproduction in natural populations (Toews et al., 2011). Understanding hybridization is pivotal to gain insight on the process of speciation. Hybridization acts as a source of novel alleles in a population, which may be either adaptive or detrimental, or can sometimes create a hybrid swarm (Rhymer & Simberloff, 1996; Short, 1972).

Sphyrapicus sapsuckers *S. varius*, *S. nuchalis*, and *S. ruber* are three North American woodpecker species that hybridize in sympatry. Hybridization within this species complex has long been of interest to biologists, who have studied the forces maintaining these hybrid zones and changes within them (Howell 1952, Scott et al. 1976, Johnson and Johnson 1985, Seneviratne et al. 2012). *Sphyrapicus ruber*/*S. nuchalis* and *S.*

ruber/S. varius hybrid zones have been studied both behaviourally and genetically (Cicero & Johnson, 1995; Grossen et al., 2016; Johnson & Johnson, 1985; Johnson & Zink, 1983; Seneviratne et al., 2016; Seneviratne et al., 2012). Though these species are known to hybridize, the incidence of F1 hybrids is low, and studies of mate choice show species in these zones tend to mate assortatively (Johnson & Johnson, 1985; Seneviratne et al., 2012, 2016). Mate choice is often dependent upon plumage, and sapsuckers' red plumage has been suggested as an isolating mechanism (Grossen et al., 2016; Johnson & Johnson, 1985). However, it has also been posited that this plumage encourages hybridization, with less red females (*S. nuchalis* and *S. varius*) choosing to mate with redder males (*S. ruber*) (Johnson & Johnson, 1985; Seneviratne et al., 2012). Other reproductive barriers between these species might include migration, breeding phenology, and habitat preferences (Chapter 2).

A *S. nuchalis/S. varius* contact zone exists in central Alberta (CAB), but it has not been studied from a genetic standpoint. Previous research suggests that hybridization does occur within this contact zone (personal communication, Jocelyn Hudon, Royal Alberta Museum), but no studies have quantified the extent of hybridization or introgression between these two species. If red plumage of *S. ruber* is expected to contribute to isolation in *S. ruber/S. nuchalis* and *S. ruber/S. varius* hybrid zones, we expect to see higher rates of introgression in the hybrid zone in which the hybridizing species have similar plumage. Our objective was to determine how the CAB hybrid zone is maintained and to describe the effect of interspecific introgression within the contact zone on the parent populations.

Understanding hybridization has been achieved for decades using traditional genetic methods of studying small, well-understood sections of the genome. Since the

advent of next generation sequencing (NGS) technologies, many researchers have opted to research hybridization and speciation using a genome-wide approach (Hohenlohe et al., 2011; Toews et al., 2016; Twyford & Ennos, 2012). Both methods are used, but they are rarely used in conjunction, and it is unclear how comparable results from the two methods might be. While the higher cost per sample of next generation sequencing limits the number of samples that may be included in analyses, the breadth of data this method yields has the potential for much finer-scale resolution. The resolution of traditional methods may not be as fine-scale, but they allow for wider sampling due to their high-throughput capabilities and low cost. Using both methods allowed us to examine patterns both across the genome and across the geographic range, which provides a broader context to study patterns of hybridization between *S. nuchalis* and *S. varius*.

We used genotyping by sequencing (GBS) and traditional SNP screening of three nuclear markers to observe variation within and outside the hybrid zone. These results were subsequently compared to introgression within other *Sphyrapicus* hybrid zones. We hypothesized that *S. nuchalis* and *S. varius* do hybridize and backcross in central Alberta where their ranges overlap, and predicted higher rates of introgression between these two species compared to *S. ruber/S. nuchalis* or *S. ruber/S. varius*.

3.3 Methods

3.3.1 Sample Acquisition

We collected samples from museum specimens and birds caught with mist nets during the field season. Wild-caught samples were collected from May to July to reduce the number of migrants caught. Birds were called in with playbacks and caught using 12 m mist nets. A small (<50 µL) blood sample was taken from the brachial vein, the birds

were banded, and morphometric measurements and a photograph were taken. All birds were released on site and blood samples stored in 99% ethanol. Museum samples were selected from birds caught within the last 20 years during the breeding season to ensure data reflected contemporary patterns (Appendix 1).

3.3.2 GBS methods

3.3.2.1 GBS DNA extraction, processing

DNA was extracted using a standard phenol-chloroform extraction procedure and 63 samples (34 *S. nuchalis*, 19 *S. varius*, and 10 hybrids) were sent to Cornell University's Institute for Genomic Diversity (IGD) for GBS following Elshire et al. (2011) with the restriction enzyme *PstI*. An additional 84 samples (39 *S. nuchalis*, 25 *S. varius*, and 20 hybrids) were sent to the Genomic Sequencing and Analysis Facility (GSAF) at the University of Texas for double digest RADseq using the restriction enzyme pair *NlaIII* and *MluCI* following Peterson et al. (2012).

3.3.2.2 SNP calling

Due to the lack of a reference genome for *Sphyrapicus*, *de novo* SNP calling was performed. For the Cornell dataset, we used the GBS UNEAK analysis pipeline version 3.0, which is an extension of the JAVA program TASSEL (Bradbury et al., 2007) to filter reads and call SNPs. Quality filtering removed any reads with incorrect, missing, or multiple restriction cut sites or barcodes. Reads were then truncated to 64 bases and aligned into identical sequence tags. A threshold of a minimum of five reads per tag was set for inclusion in the SNP calling process with the error tolerance rate set to 0.03 and a minimum minor allele frequency (MAF) of 0.05 for pairwise alignment identification of

SNPs. A maximum of one SNP per read was kept to aid in minimizing linkage disequilibrium in downstream analyses.

The UTexas data were filtered, demultiplexed, and cleaned using the STACKS v 1.09 process_radtags pipeline (J. Catchen et al., 2013; J. M. Catchen et al., 2011). The denovo_map pipeline in STACKS was then used to identify SNPs *de novo* with both the number of reads required to create a stack and the number of mismatches allowed between loci set to four. UTexas SNPs were then filtered for a MAF of 0.05 (as per the Cornell SNPs) using PLINK v 1.07 (Purcell et al., 2007). Only one SNP per read was kept for downstream genomic analyses.

3.3.2.3 Genomic analysis of hybrid zone individuals

To determine the proportion of ancestry from each species in individuals in the hybrid zone, we used the program ADMIXTURE v 1.2.3 (Alexander et al., 2009). ADMIXTURE was run on the two different datasets (Cornell and UTexas) separately as they both contain different loci and different individuals. The results were then combined after the analyses to view the whole study group together. Each ADMIXTURE analysis was run for populations K = 1-4, using a quasi-Newton algorithm for accelerated convergence (Zhou et al., 2011) and a 5-fold cross-validation. The default block relaxation algorithm was used to perform point estimation with the termination criterion set for the analyses to stop running when the change in the log-likelihood of point estimations between iterations increased by <0.0001. The number of clusters that best fit the data was determined by the K value with the lowest cross-validation error.

3.3.2.4 Genomic structure of hybrid zone

To determine the proportion of F1 and advanced generation hybrids within the CAB hybrid zone, we compared the hybrid index score to the interspecific heterozygosity score for each individual sampled within the CAB contact zone using the R package INTROGRESS v 1.22 (Gompert & Buerkle, 2010; Gompert & Buerkle, 2009; R Development Core Team, 2013). Parental individuals were defined by a hybrid index of either zero (*S. nuchalis*) or one (*S. varius*), with interspecific heterozygosities of close to zero.

We graphed the interspecific heterozygosity against the hybrid index for each individual to distinguish pure individuals from F1 hybrids or advanced generation backcrosses. *Sphyrapicus varius* and *S. nuchalis* individuals from allopatric populations with high assignment of ancestry to their respective species in ADMIXTURE were used as *a priori* parental populations in the INTROGRESS analysis. All individuals located within the CAB hybrid zone (n = 60), regardless of their phenotypic identification as pure or hybrid individuals, were used in the analysis to determine the genetic composition of individuals within the hybrid zone.

3.3.3 Traditional genetic marker methods

3.3.3.1 DNA extraction, amplification, and sequencing

A total of 208 samples were selected from populations outside, near, and within the hybrid zone from each species, in addition to individuals designated as phenotypic hybrids. Fifteen *S. nuchalis* individuals were sampled from New Mexico (NM), along with 10 from Idaho (ID), 31 from southern Alberta (SAB), and 7 from central Alberta (CAB). Sixty-three *S. varius* individuals were sampled from CAB, 14 from Saskatchewan

(SK), 15 from Illinois (IL), and 13 from Nova Scotia/New Brunswick (NSNB). In addition, 40 hybrid individuals were used. Total genomic DNA was extracted from blood samples using a modified Chelex extraction (Walsh et al., 1991). Following extraction, all samples were stored at -20 ˚C.

A 370 bp segment of the α-enolase (Enol) nuclear gene was amplified in 17 individuals from both *S. nuchalis* and *S. varius* using the Enol8L721 and Enol9H912 primers (Table 1). The thermal cycling profile was one cycle of 120 s at 94 ˚C, 45 s at 54 ˚C, 60 s at 72 ˚C; 37 cycles of 30 s at 94 ˚C, 45 s at 54 ˚C, 60 s at 72 ˚C; one cycle of 300 s at 72 ˚C and 20 s at 4 ˚C. The 25 µL PCR reaction contained 5x Green GoTaq® Flexi buffer (Promega), 0.2 mM dNTP, 1 mM $MgCl_2$, 0.4 µM primers Enol8L721 and Enol9H912, 0.5 U GoTaq® Flexi polymerase, and genomic DNA.

A 450 bp region of the glyceraldehyde gene (GAPD) was amplified and sequenced in 11 individuals of both species using primers GAPD11L890 and GAPD12H950 (Table 3.1). The thermal cycling profile was similar to that used to amplify Enol, but with a 60 ˚C annealing temperature, and 0.08 mM $MgCl_2$.

A 760 bp region of an anonymous region was sequenced in 21 individuals from both species using primers TP1-F4 and TP1-R5 (Table 3.1). The thermal cycling profile was similar to the profile used in Enol, but with a 48 ˚C annealing temperature, a 105 s extension time, 0.8 mM dNTP, and 2.5 mM $MgCl_2$.

Successfully amplified samples were sent to NanuQ sequencing service at McGill University, Montreal, Quebec for sequencing. Sequences were aligned using MEGA v. 6 (Tamura et al., 2011).

3.3.3.2 SNP screening

The aligned Enol, GAPD, and anonymous nuclear marker sequences were used to identify SNPs. We detected a C/T SNP 213 bp from the 3' end of the Enol8L721 primer. The GAPD sequences contained a 4 bp insertion/deletion that was associated with a CNC/ANG SNP 118 bp from the 3' end of the GAPD11L890 primer. Sequences from the anonymous nuclear marker revealed a C/T SNP 84 bp from the 3' end of the TP1-F4 primer. New primers were designed to change the sequences of Enol and the anonymous nuclear marker to create cut sites associated with the SNPs to allow screening with restriction enzymes. M13 tags were added to primers either to allow screening to be visualized on an acrylamide gel (Enol), or to increase size differences of digested products on an agarose gel (GAPD, anonymous nuclear marker).

A standard PCR protocol was used with a 10 µL reaction containing 0.1 mM dNTP, 0.4 µM primers, 0.25 U GoTaq® Flexi polymerase, genomic DNA, and varying amounts of $MgCl_2$, 5x GoTaq® Flexi buffer (Promega), and the addition or omission of 0.04 µM fluorescent M13 tag (Table 3.2). Thermal cycling protocols were the same as used in GAPD, with different annealing temperatures (Table 3.2). PCR products were digested in a reaction containing different amounts of restriction enzymes (Table 3.2) and a 1x buffer for a minimum of 3 hours at 37 ˚C.

Digested Enol products were run on a 6% acrylamide gel on the LI-COR 4300 DNA Analyzer. Products were scored as T (187 bp), C (41 bp and 146 bp), or heterozygous (41 bp, 146 bp, and 187 bp) for 188 individuals. GAPD and the anonymous nuclear marker restriction digests were run on a 3% agarose gel. GAPD products were scored as CTC/deletion (469 bp), ATG/insertion (149 bp and 320 bp), or heterozygous (149 bp, 320 bp, and 469 bp). Screening was performed for 174 individuals. The

anonymous nuclear marker products were scored as C (41 bp and 103 bp), T (144 bp), or heterozygous (41 bp, 103 bp, and 144 bp) for 207 individuals.

Fisher's exact tests were used to determine statistical significance of SNP variation between *S. nuchalis* and *S. varius*, between each species and hybrids, and between zones of allopatry and sympatry within each species.

Genotype data at all three loci were run through STRUCTURE v. 2.3.4 (Pritchard et al., 2000) for ancestry assignment. The program was run with a burn in of 10,000 and MCMC length of 150,000 and the loc priors setting. Ten iterations were run for K = 2-4. Optimal K for the data was selected using lowest log-likelihood values, and Q values for each iteration were averaged.

3.4 Results

3.4.1 GBS

After filtering, the Cornell dataset contained 11,311 SNPs shared between *S. varius* and *S. nuchalis* while the UTexas dataset contained 1,638 SNPs. ADMIXTURE identified K = 2 for having the lowest cross-validation error, and therefore the optimal number of clusters for both the UTexas and the Cornell datasets.

The ADMIXTURE plot differentiated the individuals outside of the hybrid zones into two distinct clusters – one for *S. varius* and one for *S. nuchalis* with minimal admixture between these two groups (Figure 3.1a). Individuals identified as phenotypically *S. nuchalis* within the CAB hybrid zone are more admixed than both *S. nuchalis* outside the zone and *S. varius* within the hybrid zone. *S. varius* within the CAB hybrid zone show little or no admixture, with the exception of two individuals with 48% and 32% *S. nuchalis* ancestry (Figure 3.1a).

The hybrid individuals within the contact zone have a mix of ancestry from both species. The amount of admixture changes throughout the hybrid zone, with those individuals located further north having a higher proportion of *S. varius* ancestry and those to the south having a greater proportion of *S. nuchalis* ancestry (Figures 3.1a and 3.2). The proportion of ancestry to either species is indicative of advanced generation hybrids.

The individuals within the hybrid zone exhibited low overall interspecific heterozygosity (0.03-0.31; Figure 3.3). None of the individuals phenotypically identified as *S. varius* (n = 12) or *S. nuchalis* (n = 22) had interspecific heterozygosity of zero, or hybrid indices of 0 or 1, indicating that some of these individuals' genes are from the other species. Similarly, the hybrid index identified no individuals as pure *S. varius* (HINDEX = 1.0) or *S. nuchalis* (HINDEX = 0.0) (Figure 3). The ancestry of the hybrids within the contact zone is not skewed towards one species or the other, but instead ranges across the hybrid index between the two species, similar to the results for hybrids found in ADMIXTURE. There are no F1 generation hybrids (HINDEX = 0.5, interspecific heterozygosity = 1.0) or even any early generation hybrids. Instead the low interspecific heterozygosity shows that the individuals within the hybrid zone are advanced generation hybrids.

To determine if the low interspecific heterozygosity for the hybrid individuals and the higher than expected interspecific heterozygosity for the pure individuals are a result of widespread recombination or incomplete lineage sorting of ancestral traits, we identified a subset of 206 near species-specific loci. These markers (hereafter referred to as nearly diagnostic loci) had an allele-frequency differential (δ) over 0.9 and were compared to all the markers. The diagnostic markers identified three *S. varius* as pure

(HINDEX = 1.0, interspecific heterozygosity = 0); two *S. nuchalis* and one hybrid individual were also identified as pure *S. nuchalis*. Although the interspecific heterozygosity of individuals varied between the loci with $\delta > 0.9$ and all the loci, the over-all patterns were similar (Figure 3.3).

3.4.2 Traditional genetic markers

Each SNP showed significant differences between species. The Enol marker showed nearly diagnostic SNPs, with a T allele typical of *S. nuchalis* (105 of 124 alleles) and C more common in *S. varius* (41 T of 176 alleles) (Table 3.3a, b) ($p < 0.0001$). *Sphyrapicus nuchalis* had a significantly higher proportion of GAPD insertions (106 of 118 alleles) than *S. varius* (92 of 152 alleles) ($p < 0.0001$) (Table 3.3a, b). The anonymous nuclear marker showed a trend of *S. nuchalis* having proportionally fewer T alleles (82 of 126) than *S. varius* (183 of 208 alleles) ($p < 0.0001$) (Table 3.3a, b). All three markers showed significant differences between the two species when considering only parental populations outside central Alberta (Enol, GAPD, anonymous nuclear marker, $p < 0.0001$) (Table 3.3b).

All but nine of the 24 SNP comparisons between parental populations inside or outside the hybrid zone and hybrids were significant (Table 3.3b). The exceptions were in *S. nuchalis* inside versus outside (GAPD and the anonymous nuclear marker), *S. varius* outside versus inside and *S. varius* outside vs. hybrids (GAPD), *S. nuchalis* inside and hybrids (Enol, GAPD) and *S. varius* inside and hybrids (Enol, GAPD, TP1) (Table 3.3b).

STRUCTURE showed optimal K for the admixture plot was K=2 as indicated by log likelihood values. The clusters generally described the two species, with most

individuals in the hybrid zone expressing shared ancestry (Figure 3.1b). In CAB, introgression was more extensive in *S. varius* than in *S. nuchalis* individuals.

The traditional genetic data corroborate the GBS findings that hybrid individuals and many parental individuals in the hybrid zone show admixture, and that admixture of the two species occurs along a north-south gradient. However, GBS data show less admixture of parental species than traditional methods do. Additionally, traditional methods show less admixture in SAB and CAB *S. nuchalis*, and more admixture in CAB *S. varius* than shown in GBS data.

3.5 Discussion

3.5.1 Geographic, genomic, and genetic patterns of introgression

The presence of many advanced generation hybrids with low interspecific heterozygosity and a range of HINDEX scores indicates a well-established hybrid zone with many hybrid individuals backcrossing with individuals from the parental species. The differentiation of populations well outside the hybrid zone from individuals within the hybrid zone and the low differentiation between phenotypic parent species in the hybrid zone and hybrids supports this. Admixture of individuals within and near the contact zone occurs on a north-south gradient. The benefit of our sampling design is that we were able to examine hybridization and introgression along the hybrid zone. Though some studies of sapsucker hybrid zones have examined a transect across the hybrid zone to determine cline width, we sampled along the hybrid zone, allowing us to examine changes in introgression with increasing distance from the range overlap. With this, we have shown that although *S. nuchalis/S. varius* range overlap is almost exclusively

restricted to the CAB population, several hybrids have been identified in SAB, and data show introgression throughout SAB.

The hybrid index showed hybrid individuals group with either parent species. However, there are notable species differences in introgression. Assignment tests of traditional genetic markers show within CAB, *S. varius* individuals are more admixed than *S. nuchalis* individuals (Figure 3.1b). This pattern may be the result of sampling error due to a smaller number of *S. nuchalis* samples in CAB. However, our GBS data also show introgression occurs on a north-south gradient, with fewer *S. nuchalis* alleles admixed into *S. varius* along the contact zone in the north (CAB), and high introgression of *S. varius* alleles into the *S. nuchalis* south of the zone into SAB (Figures 3.2, 3.3). Though *S. varius* shows more introgression when sympatric with *S. nuchalis,* in allopatry introgression of *S. varius* alleles into *S. nuchalis* SAB is high, but there is no evident pattern of introgression of *S. nuchalis* in the nearby SK *S. varius* population. Gene flow from *S. varius* into nearby *S. nuchalis* populations may be the result of habitat features facilitating gene flow between CAB and SAB populations, but not between CAB and SK or larger distance between the contact zone and sampling sites.

3.5.2 Rates of hybridization and introgression

Of the 60 samples analyzed using diagnostic GBS loci, two individuals, a phenotypic *S. nuchalis* and a hybrid, had interspecific heterozygosity scores indicating they were F1 hybrids (Figure 3.4b), although these were not detected using all loci (Figure 3.4a). Research in a *S. ruber/S. nuchalis* hybrid zone along the border of Oregon, California, and Nevada, and a *S. ruber/S. varius* hybrid zone in British Columbia has shown low prevalence of introgression, suggesting selection against F1 individuals

(Johnson & Johnson, 1985; Seneviratne et al., 2016). Our genomic data, however, identified most individuals located within the hybrid zone as hybrids. The abundance of advanced generation hybrids in the SAB and CAB hybrid zone suggests if selection is present, it is weak. Hybrid zones made up of mostly late generation hybrids can be the result of selection on novel hybrid adaptations from an environmental gradient (Hamilton & Aitken, 2013; Milne & Abbott, 2008; Pinheiro et al., 2016). The CAB and SAB hybrid zone is located along an environmentally transitional habitat from the mountains to the foothills, which may represent a mix of the two species' preferred habitats, resulting in selection on intermediate phenotypes of advanced generation hybrids.

3.5.3 Weak isolating barriers may cause high introgression rates

Extensive admixture could result from less stringent reproductive isolating barriers between *S. nuchalis* and *S. varius.* It has been shown that *S. ruber* and *S. nuchalis* tend to mate assortatively in regards to plumage, and it is possible that the higher amount of red plumage of *S. ruber* reduces hybridization in *S. ruber/S. nuchalis* and *S. ruber/S. varius* hybrid zones. Sapsuckers do not sing, therefore they are unable to use this additional behavioural barrier which often divides birds (MacDougall-Shackleton & MacDougall-Shackleton, 2001). Instead, they may rely more heavily upon plumage for mate choice. Baker and Boylan (1999) demonstrated that within lazuli and indigo bunting (*Passerina amoena, P. cyanea*) hybrid zones, females use plumage characteristics to correctly identify conspecific mates. If plumage is a mechanism for assortative mating in sapsuckers, one might expect to see high rates of introgression between *S. nuchalis* and *S. varius*, which have very similar plumages from a human perspective, resulting from misidentification of potential mates. Moreover, intermediate phenotypes would contrast

less with parental phenotypes, which may also facilitate backcrossing of early generation hybrids in comparison to other sapsucker hybrid zones. Our genomic data demonstrate this effect, as an early generation hybrid was identified as a phenotypic parental *S. nuchalis* and a pure *S. nuchalis* individual was identified as a phenotypic hybrid (Figure 3.4).

S. nuchalis and *S. varius* also share more similar habitat preferences than either does to *S. ruber*. Whereas *S. ruber* prefers coniferous forests, *S. nuchalis* and *S. varius* both prefer secondary growth deciduous forests (Walters et al., 2002a, 2002b). While shared habitat preferences may increase the likelihood of parental species occurring in the same habitat, it may also allow for higher fitness of hybrid offspring that are born into a habitat to which both parent species are better adapted.

S. nuchalis and *S. varius* might also experience more extensive introgression because they return to the breeding grounds at similar times. In Oregon, *S. ruber* individuals return to the breeding grounds in hybrid zones up to three weeks before *S. nuchalis*, by which time the majority of *S. ruber* have selected *S. ruber* mates (Walters et al., 2002a). In central Alberta, *S. varius* arrive to their breeding grounds from late March to early April. While arrivals of *S. nuchalis* are poorly understood, local accounts and eBird sightings put the arrival of this species around the same time (Semenchuk, 1992; Sullivan et al., 2009). This might play a role in the higher rates of introgression between *S. nuchalis* and *S. varius* than rates recorded in *S. ruber*/*S. nuchalis* and *S. ruber*/*S. varius* hybrid zones.

3.6 Conclusions

The advantage of a well-studied three species hybridization complex is that it allows biologists to study how each pair of species behaves in dyads. This has allowed us to compare rates of introgression between each species to identify what reproductive isolating mechanisms may be at play between different species and within different habitats. From a rangewide perspective, it is clear that geographic separation is an important isolating mechanism for sapsuckers, however, it appears that other barriers reduce the incidence of hybridization within sapsuckers. These barriers are more influential in hybrid zones involving *S. ruber*.

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Tables.

Table 3.1. Primer sets used for the α-enolase, glyceraldehyde, and anonymous nuclear marker, primer sequences, and source.

Gene	Primer Name	Primer sequence (5'-3')	Source
enol	Enol8 L731	TGGACTTCAAATCCCCCGATGATCCCAGC	(Friesen et al., 1997)
	Enol9 H912	CCAGGCACCCCAGTCTACCTGGTCAAA	(Friesen et al., 1997)
	Enol9 H912M13	CCAGGCACCCCAGTCTACCTGGTCAAA	This study
	Enol SapLM13	GTCCTGTGAATGTTCTTTGAGGCGG	This study
GAPD	GAPD11 L890	ACCTTTAATGCGGGTGCTGGCATTGC	(Friesen et al., 1997)
	GAPD12 H950	CATCAAGTCCACAACACGGTTGCTGTA	(Friesen et al., 1997)
	GAPD11 L890M13	ACCTTTAATGCGGGTGCTGGCATTGC	This study
	GAPD12 H950M13	CATCAAGTCCACAACACGGTTGCTGTA	This study
anonymous marker	TP1F4	CAGCTCTGCTGAACCTGTTG	(Nadeau et al., 2007)
	TP1R5	ATTGGTTTTAGTCACAAGCAAAAA	(Nadeau et al., 2007)
	TP1 SapRM13	GCTGTTGAGTTTTGGCTTACC	This study

Table 3.2. Variations from the standard screening PCR and restriction digest protocols. Bolded, underlined nucleotides in restriction enzyme sequences denote SNP sites used for screening.

	PCR				Restriction Digest				
	5x buffer	$MgCl_2$	M13 tag	Ta	Enzyme	reaction volume	enzyme (U)	PCR product	Gel
enol	clear	2.0 mM	added	60˚	*Fnu4HI (5'-GCNG**C**-3')*	5 µL	0.5 U	1 µL	Acrylamide
GAPD	green	2.0 mM	omitted	60˚	*NlaIII (5'-C**A**T**G**-3')*	10 µL	1 U	6 µL	Agarose
anonymous nuclear marker	green	2.5 mM	omitted	52˚	*MspI* (5'-C**C**GG-3')	10 µL	2 U	6 µL	Agarose

Table 3.3. SNP assignments by locus and population (a). Samples sizes recorded in number of alleles (n). Comparisons among species, hybrids, and populations within and outside central Alberta (b). P-values on the left, bold indicates comparisons are significant ($p < 0.05$), values to the right of the p-values indicate number of alleles (n). Comparison of each species within (inside) and outside hybrid zone.

a.

	enol			GAPD			anonymous nuclear marker		
	T	C	n	insertion	deletion	n	T	C	n
S. nuchalis NM	28	2	30	24	6	30	23	7	30
S. nuchalis ID	20	0	20	19	1	20	13	7	20
S. nuchalis SAB	48	12	60	57	3	60	40	22	62
S. nuchalis CAB	9	5	14	6	2	8	6	8	14
Hybrid	34	42	76	51	27	78	68	12	80
S. varius CAB	34	64	98	51	39	90	105	21	126
S. varius SK	3	25	28	18	10	28	27	1	28
S. varius IL	1	25	26	21	9	30	29	1	30
S. varius NSNB	3	21	24	2	2	4	22	2	24
Total	182	196	376	251	99	348	335	81	414

b.

	enol		GAPD		anonymous nuclear marker	
	p	n	p	n	p	n
S. nuchalis x S. varius	< **0.0001**	300	**0.0001**	270	< **0.0001**	334
S. nuchalis outside x S. varius outside	< **0.0001**	188	**0.0001**	172	< **0.0001**	194
S. nuchalis outside x inside	**0.040**	124	0.188	118	0.079	126
S. nuchalis outside x hybrids	< **0.0001**	186	< **0.0001**	188	**0.011**	192
S. varius outside x inside	< **0.0001**	176	0.311	152	**0.015**	208
S. varius outside x hybrid	< **0.0001**	154	1.000	140	**0.037**	162
S. nuchalis inside x hybrid	0.246	90	0.712	86	**0.002**	94
S. varius inside x hybrid	0.211	174	0.271	168	0.847	206

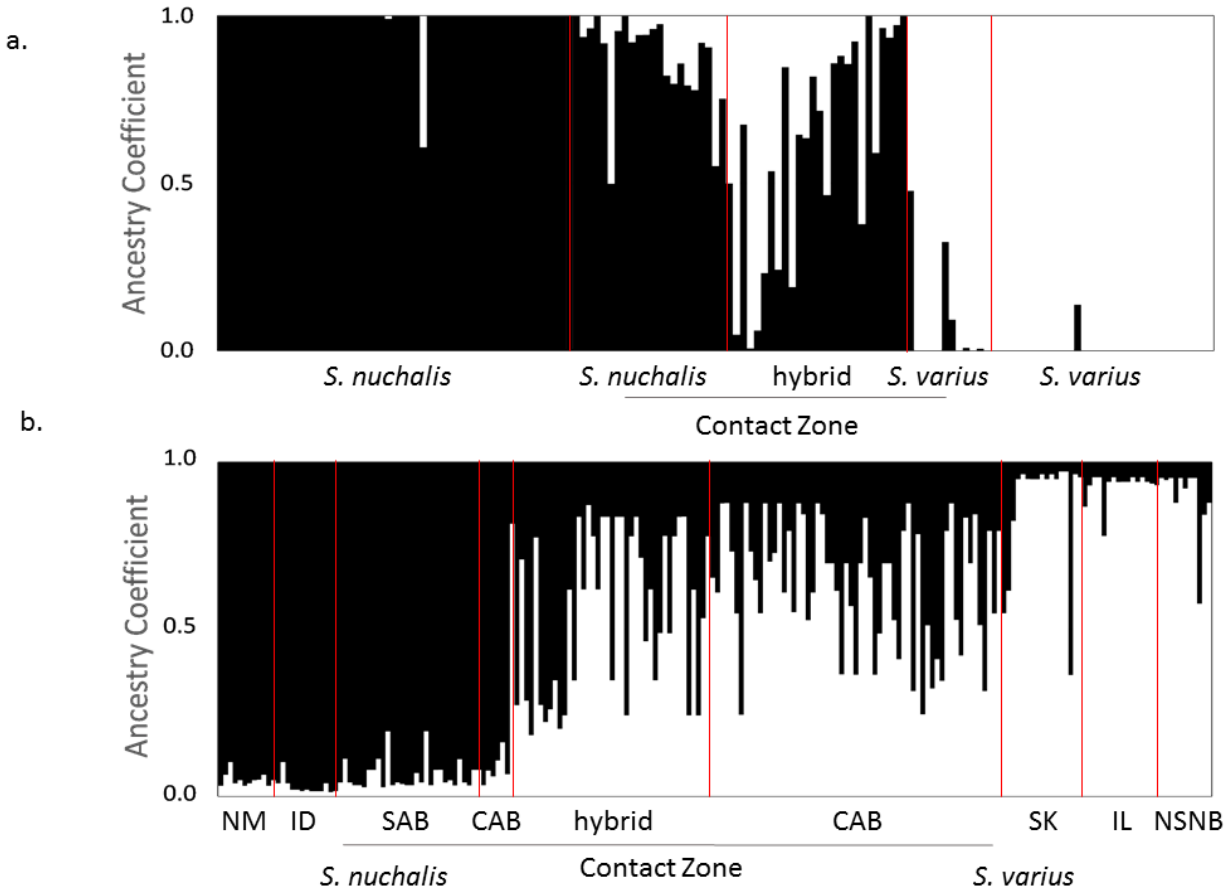

Figure 3.1. ADMIXTURE plot of the sapsuckers using GBS data (a) and STRUCTURE plot using traditional genetic markers (b) showing *S. nuchalis* ancestry (black) and *S. varius* ancestry (white). The individuals within the contact zone are assigned based on the phenotype identity of the bird at capture.

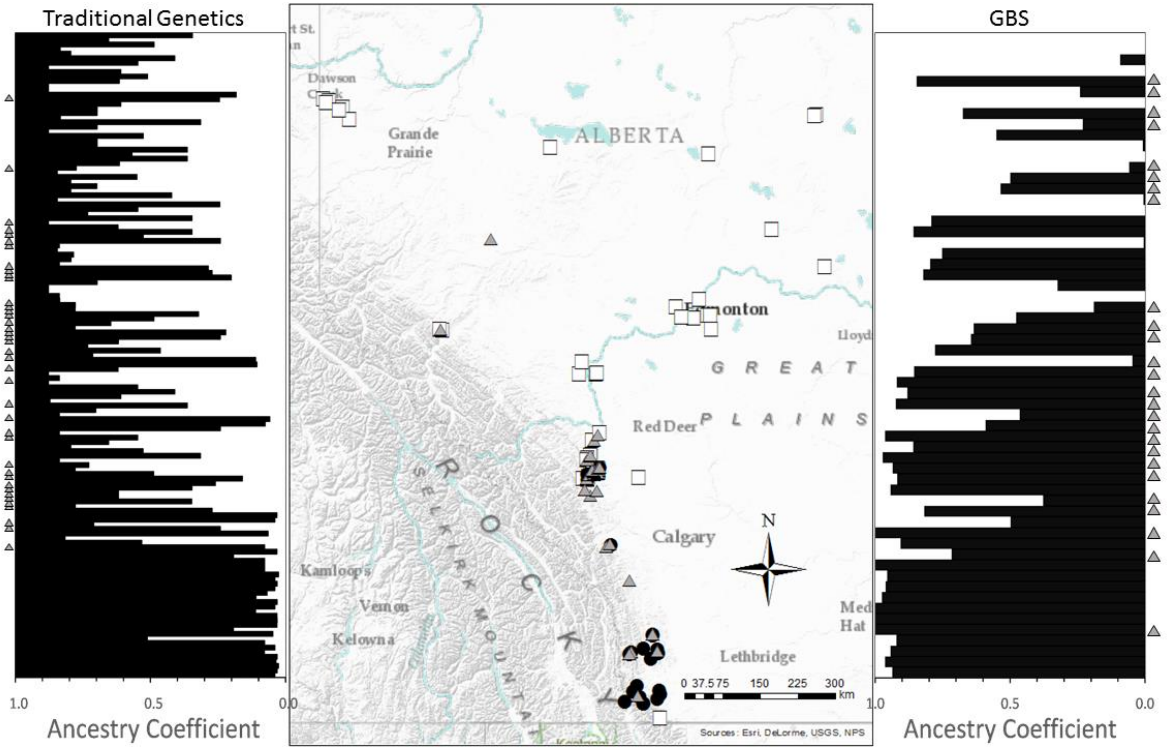

Figure 3.2. Sampling locations of individuals phenotypically identified as *S. nuchalis* (black circles), *S. varius* (white squares), and hybrids (grey triangles) within the central and southern Alberta hybrid zone. The STRUCTURE plot on the left indicates the proportion of the genotype that is *S. varius* ancestry (black) and *S. nuchalis* ancestry (white) using traditional genetic methods, ancestry calculated using GBS is represented in ADMIXTURE plot on the right. Individuals in the plots are arranged by ascending sampling latitude, with the most southern individual at the bottom of the plot and the most northern at the top. Individuals marked with a grey triangle correspond to those hybrid individuals in grey triangles on the map.

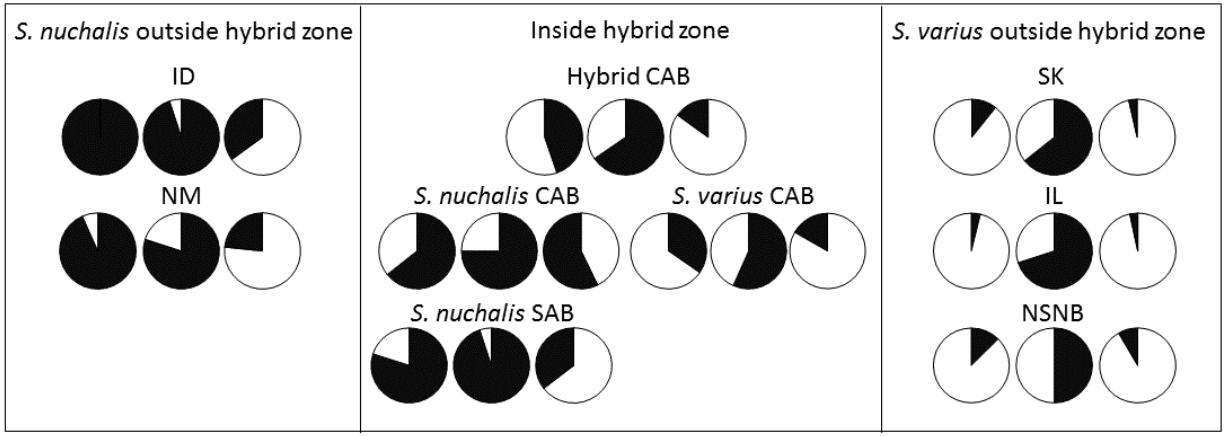

Figure 3.3. The SNP assignments for Enol (left), GAPD (middle), and anonymous nuclear marker (right) for each population sampled. In Enol and anonymous nuclear marker pies black represents C, white T; in GAPD pies black shows insertions, white deletions. Sample sizes in Table 3.3.

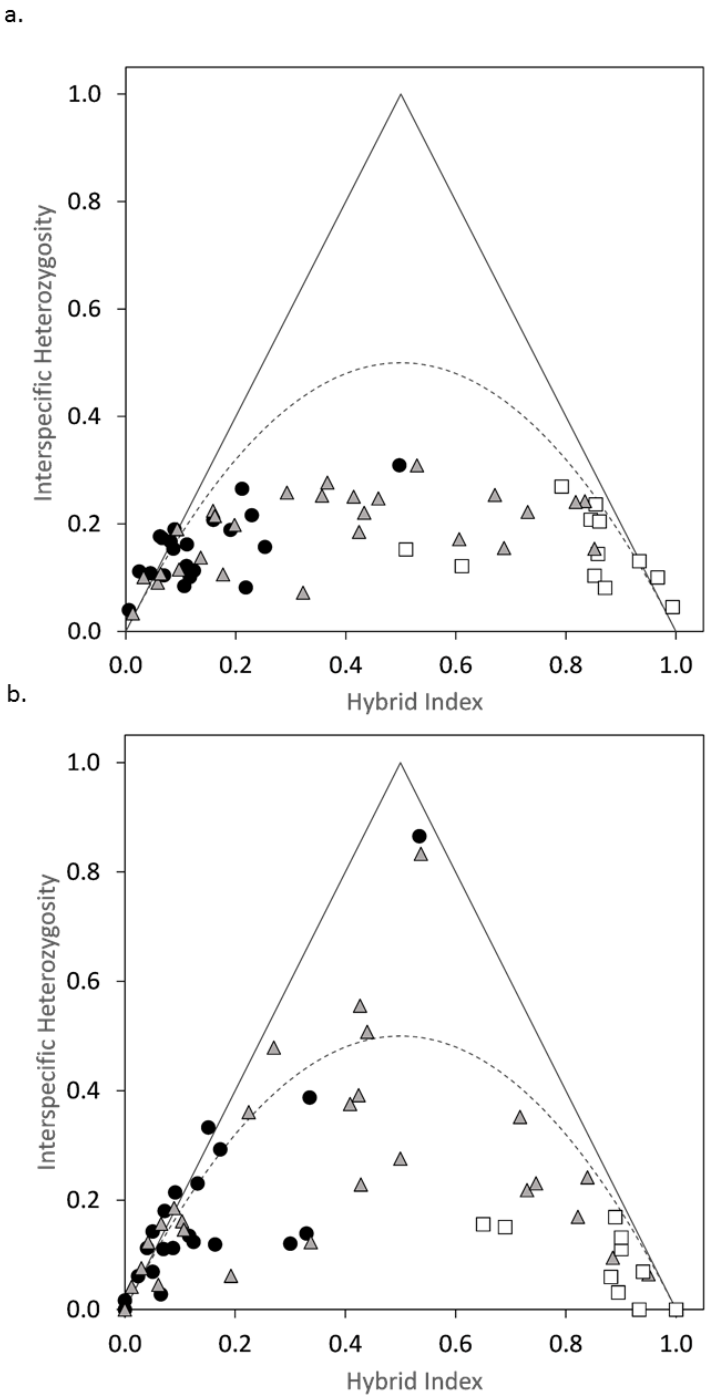

Figure 3.4. Interspecific heterozygosity over hybrid index (HINDEX) scores of each individual in the hybrid zone using (a) all loci and (b) nearly diagnostic loci ($\delta > 0.9$). Black circles are *S. nuchalis*, white squares *S. varius*, and grey triangles are hybrid individuals. A HINDEX score of 0.0 indicates pure *S. nuchalis* genetic ancestry and a score of 1.0 is pure *S. varius*. Individuals located on the solid line are back-crossed individuals. The dotted line indicates the expected distribution for individuals if mating was random throughout the zone.

CHAPTER 4: General Discussion

4.1 General discussion

In many species, including sapsuckers, it is assumed that geographic features historically separated populations rendering them unable to exchange alleles across intervening landscapes and ultimately leading to their divergence, a phenomenon referred to as allopatric speciation (Brelsford et al., 2009; Nosil, 2008). In the case of sapsuckers, Pleistocene glaciation was long thought to be the major driver of species divergence (Cicero & Johnson, 1995; Grossen et al., 2016; Johnson & Johnson, 1985; Seneviratne et al., 2016; Seneviratne et al., 2012). However, there is evidence that non-geographical barriers, such as morphological, ecological, or behavioural barriers, can occur within a landscape and cause the divergence of populations that co-occur within the same range. Data presented in this thesis indicate speciation in *S. ruber*, *S. nuchalis*, and *S. varius* may have occurred with large range overlap, not as a result of allopatric speciation as was previously believed. However, low differentiation between *S. ruber* and *S. nuchalis* suggests these species may be in the early stages of divergence.

Our data suggest sapsuckers diverged in parapatry with regions of range overlap, which falls under the umbrella of sympatric speciation. The concept of sympatric speciation is controversial because divergence is constrained by gene flow, but allele exchange is expected between individuals that share physical space (Fitzpatrick et al., 2009; Mallet et al., 2009). Though the definition and likelihood of sympatric speciation have been argued among evolutionary biologists for decades, a more interesting and relevant question is whether speciation can occur despite the homogenizing effects of

gene flow among diverging populations (Mallet et al., 2009; Nosil, 2008). Biologists have proposed that divergence with gene flow is possible, and modern improvements in detecting gene flow have allowed researchers to identify examples of divergence despite gene flow (Mallet et al., 2009; Nosil, 2008).

Parapatric speciation often occurs with gene flow, and sapsucker speciation likely occurred despite gene flow as well (Martin et al., 2013; Papadopulos et al., 2011). Support for this phenomenon has been shown across taxa, occurring in *Coprosma* spp. and *Howea* palms, *Heliconius* butterflies, and *Coregonus clupeaformis* whitefish, among many others (Gagnaire et al., 2013; Martin et al., 2013; Papadopulos et al., 2011). Differences in phenology of seed and pollen dispersal associated with different altitudes, assortative mating based on divergent morphology, and ecological niche divergence have all been shown to facilitate speciation with gene flow (Gagnaire et al., 2013; Martin et al., 2013; Papadopulos et al., 2011). Several reproductive isolating barriers have been suggested in this thesis which may also have historically constrained gene flow between the diverging species. In sapsuckers, marginal hybrid zone habitats, different ecological adaptations, plumage differences, and migratory behaviours may constrain gene flow, allowing species divergence to occur despite low levels of gene flow.

Our rangewide data show evidence of hybridization or introgression only at or near contact zones for all three species, despite high population connectivity among populations within each species. As outlined in Chapter 2, this could be explained either by low fitness of admixed individuals, or by peripheral hybrid zone habitat acting as population sinks. These same forces may have constrained gene flow during divergence. Each contemporary hybrid zone suggests lower fitness of hybrid individuals (Johnson &

Johnson, 1985; Seneviratne et al., 2012). Additionally, as described in Chapter 3, the *S. nuchalis/S. varius* hybrid zone shows introgression into the nearby SAB population connected to the hybrid zone by a transitional habitat, but not the SK population lacking this transitional habitat connection. This suggests marginal habitats at range peripheries might constrain gene flow between species. However, there is a litany of other forces that may act as barriers that allow divergence despite sympatry and limited gene flow in this system.

4.2 Future directions

The research to date indicates the three sapsuckers are different species, and likely diverged in parapatry with gene flow. Future directions would be well suited to identify reproductive isolation mechanisms between these species to pinpoint what forces allowed them to diverge. We have identified three potential isolating barriers that may be weak in the *S. nuchalis* and *S. varius* hybrid zone to account for its higher introgression than *S. ruber* hybrid zones: 1) plumage, 2) preferred habitat, and 3) migratory behaviour, are potential forces driving speciation in this system (see Chapters 2 and 3). Further investigation of these might provide a clearer picture of sapsucker speciation and evolutionary relationships.

Multiple studies have identified genes related to plumage formation in phenotypically divergent groups of birds (Delmore et al., 2016; Toews et al., 2016). Grossen et al. (2016) found the COG4 locus is closely associated with plumage in sapsuckers. An A/G SNP on the COG4 gene is strongly correlated with colour, with A/G heterozygotes (*S. ruber*) expressing more red than A/A homozygotes (*S. nuchalis* and *S.*

varius), suggesting a genetic basis for plumage differences exists. It might be useful to examine variation at these plumage loci in offspring of interspecific pairs of *S. ruber* and *S. nuchalis/S. varius* to evaluate phenotype and expression of these genes in hybrids. Behavioural studies could be used to determine whether this trait is driving divergence in these species. A study similar to those conducted by Johnson and Johnson (1985) and Seneviratne et al. (2012) could be designed, whereby breeding pairs are tracked in hybrid zones containing each species combination and their plumages scored. The incidence of hybrid or introgressed pairings could be compared among hybrid zones of the different species pairs, and the plumages of those pairs mapped to show whether interspecies mating shows any patterns correlating with plumage.

NGS has led to the discovery that sapsuckers are divergent at a locus that possibly plays a role in cranial skeletal construction, which may be tied to preference for deciduous (hardwood) trees preferred by *S. ruber* versus coniferous (softwood) trees preferred by *S. nuchalis* and *S. varius* (Curtis, 2017). Grossen et al. (2016) also identified enrichment of several loci responsible for fluid homeostasis, which may indicate local adaptation as the sapsuckers have different climate preferences, with *S. nuchalis* occupying arid montane habitats, *S. ruber* occupying moist coastal regions, and *S. varius* occurring in boreal habitats (Chapter 2) (Billerman et al., 2016; Walters et al., 2002a, 2002b). Habitats are clearly important to the divergence of these species. It might be interesting to use whole-genome sequencing to compare genomes of two sapsucker species occupying multiple distinct hybrid zones in different habitats to compare the same species responses to different local adaptations. This could elucidate whether genomic divergence can be attributed to species differences or different environmental

selective pressures. As the only species with multiple, unconnected, hybrid zones in different regions, *S. ruber/S. nuchalis* hybrid zones would be the requisite species pair. By comparing interactions between the same species in different habitats, we can evaluate how environmental factors might affect hybridization and the differentiation of species.

Migration also has the potential to impact speciation among sapsuckers. Because the three species have different migratory behaviours, *S. ruber* may be present on the breeding grounds before *S. nuchalis* and *S. varius* (Walters et al., 2002a, 2002b). This may allow them to begin to form pairs and excavate nests before the arrival of individuals of other species in sympatry, reducing the likelihood of these species interbreeding. The influence of differential breeding phenology could be tested relatively easily by recording arrival of different species and breeding dates of each pair within a hybrid zone. Furthermore, some species have been shown to form pair bonds in wintering grounds, which might potentially affect individual interactions on breeding grounds (Borràs et al., 2011; Davidson et al., 2013; Humphries et al., 2009). Environmental conditions experienced during migration have also been shown to impact plumage coloration, so migration may play a role in sexual signaling and mate choice as well (Sparrow et al., 2017). Migratory routes are poorly understood in *S. nuchalis* and *S. varius*, and it might be beneficial to conduct a study using nanotags or GPS transmitters to determine where sapsuckers overwinter and their migration route.

Understanding what influences the disruption of these species is important in demonstrating how they have evolved, which might allow us to make predictions about the future. Sapsuckers have specific habitat requirements, and changing climates are

already affecting distributions and species interactions (Billerman et al., 2016). Understanding how organisms react to changes in climate or habitat may allow us to better manage species into the future as human activity invariably changes these factors. A more thorough knowledge of species interactions, hybridization, and isolation will allow us to better understand the processes of hybridization and speciation, which we may apply to management of other species of conservational concern, as hybridization threatens many modern species (Garroway et al., 2010; Milián-García et al., 2015; Rhymer & Simberloff, 1996; Toews et al., 2011).

4.3 General conclusions

The population genetic structure of *S. ruber*, *S. nuchalis*, and *S. varius* upholds many of the conclusions determined from earlier genetic and behavioural studies on these sapsucker species: the three groups represent distinct species, *S. ruber* and *S. nuchalis* are more closely related to each other than to *S. varius*, and hybridization and introgression occur among all three species. Using our rangewide approach, we showed paraphyly among *S. ruber* and *S. nuchalis*, and polyphyly among all three species. We were able to identify intraspecific population genetic structure as well. Genetic data and ENM projections suggest a possible separate Pleistocene refugium off the coast of British Columbia in Haida Gwaii, a Pleistocene barrier separating *S. varius* into eastern and western populations, and potential for historical hybridization. Speciation was likely possible because gene flow was constrained by the low likelihood of admixed loci leaving hybrid zone populations. The contemporary *S. nuchalis*/*S. varius* hybrid zone is well established and has a high, asymmetric introgression. We propose that plumage,

habitat preferences, and migratory behaviours, which are similar among *S. nuchalis* and *S. varius*, may reduce hybridization in this system.

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APPENDIX 1: Supplementary Information for Chapter 2

Population genetics and speciation of three woodpecker species

Appendix 1.1. Complete list of layers available from WorldClim. Layers used to model *S. varius* and *S. ruber* (*), and *S. nuchalis* (†).

Layer Number	Bioclim Variable
BIO1*†	Annual Mean Temperature
BIO2*	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3*†	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7* †	Temperature Annual 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 (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18*†	Precipitation of Warmest Quarter
BIO19*†	Precipitation of Coldest Quarter

Appendix 1.2. Haplotype distribution for CR data. Shared haplotype IDs are shown in the left column, with number of individuals from each population with that haplotype recorded across the row. Unique haplotypes are found only in one individual. Shaded areas represent shared haplotypes restricted to a single species. For site locations, refer to Figure 2.2.

	S. ruber								*S. nuchalis*												*S. varius*												
Hap	ECA	NCA	WA	VIBC	CBC	HG	NBC	SEAK	NM	CO	UT	SD	WY	NEOR	MT	WA	NEWA	SAB	SEBC	CAB	NWBC	CAB	SK	IL	MI	ON	NSNB	WA	NM	LA	NC	FL	Total
5	1	1																															2
8			7		3	8		1	3	3	2	1	2	2		2		12	2	1								1					50
9			6	5	2	2	4									1																	20
10			3	1	1																												5
11			2		2	1								1			1	1				2											10
12			1		1																												2
14			1	1		2																											4
28						1												1			1	7		12	2								24
33						1				1																							2
38									1			4						2															7
39																		2															2
40									1	1	3	1		9		1		5								1							22
41																		2															2
42																		1							1								2
43											1							1															2
45										1			1																				2
57																					1			1									2
58																					1	2	1	2	1	1							8
60																						1		1									2
62																						1		1									2
63																						2											2
70																							1	1									2
73																							2		1								3

74																							1	1														2
75																							1									1						2
78																							1	2	1	2	1					1	1					9
79																							2	2	1													5
82																								1		1												2
84																								1		2												3
101																									1								3					4
Unique	1	4	3	7	2	4	3	2	1	1	1	1	1	1	2	2	2	2	2		2	8	5	15	7	12	4			2	5		102					
# Haps	2	5	9	10	7	10	4	3	4	5	4	4	3	4	2	5	3	11	3	1	4	14	12	26	14	17	5	1	1	2	7	1	203					
n	2	5	23	14	11	19	7	3	6	7	7	7	4	13	2	6	3	29	4	1	5	23	14	40	15	19	5	1	1	2	9	1	308					

Appendix 1.3 Sample ID, location, coordinates, source, and band/museum ID for each individual used in Chapter 2, listed by population. Museum samples from the Royal British Columbia Museum (RBCM), University of British Columbia (UNBC), Queen's University (QU), University of Michigan (UMich) Museum of Southwest Biology (MSB), American Museum of Natural History (AMNH), University of Washington Burke Museum (UWBM), Royal Alberta Museum (RABM), Field Museum Natural History (FMNH), New Brunswick Museum (NBM), Canadian Museum of Nature (CMN), Smithsonian (Smith) and Royal Saskatchewan Museum (RSKM).

ID	Location	Lat (˚N)	Long (˚W)	Source	Band/Museum ID
rbsaCBC001	Smithers, BC	54.7833	-127.1500	RBCM	17884
rbsaCBC002	Telkwa, BC	54.7000	-127.0500	RBCM	18393
rbsaCBC003	Hazelton, BC	55.2583	-127.6688	UNBC	13-448
rbsaCBC004	Fort St. James, BC	54.3744	-124.2756	Wild	991-19825
rbsaCBC005	Fort St. James, BC	54.3744	-124.2756	Wild	991-19826
rbsaCBC006	Fort St. James, BC	54.4941	-124.1230	Wild	991-19827
rbsaCBC007	Fort St. James, BC	54.4941	-124.1230	Wild	991-19828
rbsaCBC008	Fort St. James, BC	54.4878	-124.1612	Wild	991-19838
rbsaCBC009	Hazelton, NBC	55.2583	-127.6689	QU	1761-17789
rbsaCBC010	Hazelton, NBC	55.2583	-127.6689	QU	1761-17790
rbsaCBC011	McBride, BC	53.3121	-120.1679	UNBC	08-62
rbsaCBC012	Date Ck Rd - Kispiox	55.3525	-127.6827	QU	1581-43849
rbsaCBC013	Hazelton, NBC	55.2583	-127.6689	QU	1761-17791
rbsaCBC014	Hazelton, NBC	55.2583	-127.6689	QU	1761-17792
rbsaCBC015	Hazelton, NBC	55.2583	-127.6689	QU	1761-17793
rbsaCBC016	Hazelton, NBC	55.2583	-127.6689	QU	1761-17794
rbsaCCA001	Berkeley, Contra Costa Co., CA	37.8704	-122.2808	UMich	236384
rbsaCCA004	Hercules, Contra Costa Co., CA	38.0000	-122.2323	MSB	25303, NK: 142171
rbsaCCA005	El Sobrante, Contra Costa Co., CA	37.9798	-122.2778	MSB	25304, NK: 142175
rbsaECA001	Topaz, S of on Hwy 395, Mono Co., CA	37.9082	-119.0772	UMich	236221

rbsaECA002	June Lake, N of rt 158, Mono Co., CA	37.8000	-158.0667	UMich	236545
rbsaECA003	Upper Poole Power Plant Rd, Inyo National Forest, CA	37.9445	-119.2007	Wild	2331-88114
rbsaECA004	Upper Poole Power Plant Rd, Inyo National Forest, CA	37.9445	-119.2007	Wild	2331-88115
rbsaECA005	Moraine Campground, Inyo National Forest, CA	37.9296	-119.1704	Wild	2331-88116
rbsaECA006	Lower Lundy Creek, Inyo National Forest, CA	38.0378	-119.1625	Wild	2331-88117
rbsaECA007	Meeks Bay Meadow, Lake Tahoe, CA	39.0261	-120.1451	Wild	2331-88120
rbsaECA008	Taylor Creek Rd, Lake Tahoe, CA	33.9241	-120.0593	Wild	2331-88121
rbsaNBC001	Lakeelse, Terrace, BC	54.5179	-128.5630	QU	1761-17780
rbsaNBC002	Lakeelse, Terrace, BC	54.5179	-128.5630	QU	1761-17781
rbsaNBC003	Lakeelse, Terrace, BC	54.5179	-128.5630	QU	1761-17782
rbsaNBC004	Lakeelse, Terrace, BC	54.5179	-128.5630	QU	1761-17783
rbsaNBC005	Diana Lake, Prince Rupert, BC	54.2258	-130.1619	QU	1761-17784
rbsaNBC006	Diana Lake, Prince Rupert, BC	54.2258	-130.1619	QU	1761-17785
rbsaNBC007	Lakeelse, Terrace, BC	54.5179	-128.5630	QU	1761-17786
rbsaNBC008	Lakeelse, Terrace, BC	54.5179	-128.5630	QU	1761-17787
rbsaNBC009	Lakeelse, Terrace, BC	54.5179	-128.5630	QU	1761-17788
rbsaNCA001	Shasta, CA	40.6145	-122.4611	AMNH	DOT-9767 (PRS869)
rbsaNCA002	Shasta, CA	40.6145	-122.4611	AMNH	DOT-9963 (PRS1167)
rbsaNCA003	Trout Creek, Siskiyou Co., CA	41.4333	-122.8833	UMich	226196
rbsaNCA004	Shasta National Forest, Siskiyou Co., CA	41.4333	-122.8833	UMich	227382
rbsaNCA005	Shasta National Forest, Siskiyou Co., CA	41.4333	-122.8833	UMich	227383
rbsaNCA007	Pine Creek Trailhead, Modoc NF, CA	41.3586	-120.2842	Wild	2331-88123
rbsaNCA008	East Creek, Modoc NF, CA	41.1775	-120.1996	Wild	2331-88124
rbsaNCA009	Patterson Guard Station, Modoc NF, CA	41.1980	-120.1886	Wild	2331-88125
rbsaNCA010	Cedar Creek Camping Area, Modoc NF, CA	41.5584	-120.3005	Wild	2331-88126
rbsaNCA011	Garfield St & Townsend Ave, Cedarville, CA	41.5298	-120.1771	Wild	RBSADead1

rbsaQCI001	Sewell Inlet, BC	52.8333	-131.9830	RBCM	018972
rbsaQCI002	Newcombe Inlet, BC	52.8333	-132.0670	RBCM	018973
rbsaQCI003	Newcombe Inlet, BC	52.8333	-132.0670	RBCM	018974
rbsaQCI004	Sewell Inlet, BC	52.8833	-131.9830	RBCM	018977
rbsaQCI005	Sewell Inlet, BC	52.8833	-131.9830	RBCM	018978
rbsaQCI006	Sewell Inlet, BC	52.8833	-131.9830	RBCM	018979
rbsaQCI007	Sewell Inlet, BC	52.8833	-131.9830	RBCM	018980
rbsaQCI008	Sewell Inlet, BC	52.8333	-131.9830	RBCM	018981
rbsaQCI009	Sewell Inlet, BC	52.8833	-131.9830	RBCM	019069
rbsaQCI010	Newcombe Inlet, BC	52.8333	-132.0670	RBCM	019070
rbsaQCI011	Sewell Inlet, BC	52.8333	-131.9830	RBCM	019071
rbsaQCI012	Sewell Inlet, BC	52.8333	-131.9830	RBCM	019072
rbsaQCI013	Sewell Inlet, BC	52.8333	-131.9830	RBCM	019075
rbsaQCI014	Sewell Inlet, BC	52.8333	-131.9830	RBCM	019076
rbsaQCI015	Kaegen Bay Campground, BC	53.2419	-132.1538	Wild	921-16708
rbsaQCI016	Lookout Trail, BC	53.2493	-132.0019	Wild	921-16709
rbsaQCI017	Kaegen Bay Campground, BC	53.2419	-132.1538	Wild	921-16708
rbsaQCI018	Lookout Trail, BC	53.2493	-132.0019	Wild	921-16709
rbsaQCI101	East Limestone, BC	53.2604	-132.0858	QU	8041-49254
rbsaQCI102	East Limestone, BC	53.2604	-132.0858	QU	1581-43721
rbsaQCI103	East Limestone, BC	53.2604	-132.0858	QU	1581-43724
rbsaQCI104	East Limestone, BC	53.2604	-132.0858	QU	1581-43731
rbsaQCI105	East Limestone, BC	53.2604	-132.0858	QU	1581-43737
rbsaQCI106	East Limestone, BC	53.2604	-132.0858	QU	1581-43738
rbsaQCI107	West Skedans, BC	52.9665	-131.6167	QU	1581-43742
rbsaQCI108	East Limestone, BC	53.2604	-132.0858	QU	1581-43743
rbsaQCI109	East Limestone, BC	53.2604	-132.0858	QU	1581-43744
rbsaQCI110	East Limestone, BC	53.2604	-132.0858	QU	1581-43746
rbsaQCI111	East Limestone, BC	53.2604	-132.0858	QU	1581-43725

rbsaQCI114	Reef, BC	53.2282	-132.1205	QU	1581-43751
rbsaQCI116	Reef, BC	53.2282	-132.1205	QU	1581-43848
rbsaQCI117	Reef, BC	53.2282	-132.1205	QU	1581-43847
rbsaQCI118	East Limestone, BC	53.2604	-132.0858	QU	museum A66
rbsaQCI119	East Limestone, BC	53.2604	-132.0858	QU	museum A84
rbsaQCI120	Queen Charlotte City, BC	53.2563	-132.0891	QU	feather
rbsaSCA001	San Gorgino Wilderness, San Bernardino NF, CA	34.1449	-116.7900	Wild	2331-88111
rbsaSCA002	Santa Ana River Rd, San Bernardino NF, CA	34.1790	-116.8477	Wild	2331-88112
rbsaSCA003	Heart Bar Group Camp, San Bernardino NF, CA	34.1584	-116.7971	Wild	2331-88113
rbsaSEAK001	Chilkat, Haines AK	59.2294	-135.4524	QU	1761-17795
rbsaSEAK002	Chilkoot, Haines AK	59.2294	-135.4524	QU	1761-17796
rbsaSEAK003	Chilkoot, Haines AK	59.2294	-135.4524	QU	1761-17797
rbsaSEAK004	Lena Peninsula, 17mi N of Juneau, AK	58.3951	-134.7752	UMich	233216
rbsaSOR001	Klamath Falls, Klamath Co., OR	42.2667	-122.1500	UWBM	64453 GKD 78
rbsaVIBC001	Victoria, BC	48.4333	-123.3670	RBCM	018503
rbsaVIBC002	Saanich, BC	48.5333	-123.4000	RBCM	022242
rbsaVIBC003	Miracle Beach, BC	49.8464	-125.0935	Wild	921-16702
rbsaVIBC004	Miracle Beach, BC	49.8464	-125.0935	Wild	921-16703
rbsaVIBC005	Miracle Beach, BC	49.8464	-125.0935	Wild	921-16704
rbsaVIBC006	Miracle Beach, BC	49.8464	-125.0935	Wild	921-16705
rbsaVIBC007	Comox Lake, BC	49.6171	-125.0486	Wild	921-16706
rbsaVIBC008	Comox Lake, BC	49.7424	-124.9665	Wild	921-16707
rbsaVIBC009	Comox Lake, BC	49.6200	-125.0200	QU	1581-43872
rbsaVIBC010	Comox Lake, BC	49.6200	-125.0200	QU	1581-43873
rbsaVIBC011	Comox Lake, BC	49.6200	-125.0200	QU	1581-43874
rbsaVIBC012	Comox Lake, BC	49.6200	-125.0200	QU	1581-43875
rbsaVIBC013	Cumberland, BC	49.6200	-125.0200	QU	1581-43876

rbsaVIBC014	Cumberland, BC	49.6200	-125.0200	QU	1581-43877
rbsaVIBC015	Puntledge Park, BC	49.6800	-124.9800	QU	1581-43878
rbsaVIBC016	Puntledge Park, BC	49.6800	-124.9800	QU	1581-43879
rbsaVIBC017	Puntledge Park, BC	49.6800	-124.9800	QU	1581-43880
rbsaVIBC018	Puntledge Park, BC	49.6800	-124.9800	QU	1581-43881
rbsaVIBC019	Coal Creek, BC	49.6200	-125.0200	QU	1581-43882
rbsaVIBC020	Miracle Beach, BC	49.1900	-125.1100	QU	1581-43883
rbsaVIBC021	Coal Creek, BC	49.6200	-125.0200	QU	1581-43884
rbsaVIBC022	Coal Creek, BC	49.6200	-125.0200	QU	1581-43885
rbsaVIBC023	Saltspring Island, BC	48.7500	-123.4000	RBCM	016433
rbsaVIBC024	Swan Lake, BC	48.4667	-123.3670	RBCM	017882
rbsaWA001	Seattle Animal Acres Park, WA	47.7553	-122.2867	Wild	2431-47306
rbsaWA002	Seattle Animal Acres Park, WA	47.7553	-122.2867	Wild	2431-47307
rbsaWA003	Colonial Creek, WA	48.6930	-121.0990	QU	1581-43886
rbsaWA004	Newhalem, WA	48.6740	-121.2460	QU	1581-43888
rbsaWA005	Newhalem, WA	48.6740	-121.2460	QU	1581-43889
rbsaWA006	Mount Baker summit, Whatcom Co. WA	48.7766	-121.8146	UWBM	57299 JMB 516
rbsaWA007	Mount Baker summit, Whatcom Co. WA	48.7766	-121.8146	UWBM	57300 JMB 517
rbsaWA008	Mount Baker summit, Whatcom Co. WA	48.7766	-121.8146	UWBM	57320 SAR 5893
rbsaWA009	Mount Baker summit, Whatcom Co. WA	48.7766	-121.8146	UWBM	57329 SAR 5894
rbsaWA010	Mount Baker, Whatcom Co. WA	48.8000	-121.9170	UWBM	62614 GAV 906
rbsaWA011	Mount Baker, Whatcom Co. WA	48.8833	-121.9000	UWBM	62615 GAV 907
rbsaWA012	Seattle, King Co. WA	47.6060	-122.3057	UWBM	80436 AMN 21
rbsaWA013	Carnation, King Co. WA	47.6435	-121.9085	UWBM	80431 AMN 04
rbsaWA014	Seattle, King Co. WA	47.6060	-122.3057	UWBM	81654 GHL 005
rbsaWA015	Bellevue, King Co. WA	47.6135	-122.1805	UWBM	85279 JMSE 004
rbsaWA016	Seattle, King Co. WA	47.6060	-122.3057	UWBM	81683 GJY 004
rbsaWA017	Acme, Whatcom Co. WA	48.7117	-122.2086	UWBM	88794 MLD 094
rbsaWA018	Mount Baker, Whatcom Co. WA	48.8000	-121.9167	UWBM	62637 SVD 1213

rbsaWA019	Mount Baker, Whatcom Co. WA	48.7800	-121.9167	UWBM	62638 SVD 1214
rbsaWA020	Mount Baker summit, Whatcom Co. WA	48.8167	-121.9333	UWBM	68268 CSW 6296
rbsaWA021	Mount Baker summit, Whatcom Co. WA	48.8167	-121.9333	UWBM	74557 MLD 049
rbsaWA022	Centralia, Lewis Co., WA	46.7167	-122.9700	UWBM	234154
rbsaWA023	Bellingham, Whatcom Co., WA	48.7161	-122.4606	UWBM	234236
rbsaWA024	Ft. Lewis, Pierce Co., WA	47.1325	-122.5303	UWBM	234237
rnsaAZ001	Chiricahua Mtns., Pine Canyon, AZ	31.9428	-109.3222	MSB	26222, NK: 165046
rnsaAZ002	Chiricahua Mtns., Pine Canyon, AZ	31.9428	-109.3222	MSB	26223, NK: 165042
rnsaCAB001	James River, bridge on 584, AB	51.9000	-115.0000	RABM	Z94.13.9, rnsa 3
rnsaCAB002	James River, Wilson James Campsite, AB	51.8330	-115.2170	RABM	Z94.13.10, rnsa 1
rnsaCAB003	James River, Wilson James Campsite, AB	51.8330	-115.2170	RABM	Z94.13.11, rnsa 2
rnsaCAB004	9 miles west of Bearberry, AB	51.8670	-115.0830	RABM	Z95.11.14, rnsa 4
rnsaCAB005	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.4, rnsa 5
rnsaCAB009	Castle River area	49.3170	-114.3216	RABM	Z12.2.48
rnsaCAB012	Kananaskis Country, AB	51.0484	-114.8026	RABM	Z12.3.4
rnsaCAB013	Kananaskis Country, AB	51.0431	-114.7643	RABM	Z12.3.5
rnsaCAB014	Kananaskis Country, AB	50.9478	-114.7084	RABM	Z12.3.6
rnsaCAB015	Kananaskis Country, AB	50.8114	-114.5989	RABM	Z12.3.7
rnsaCAB016	Kananaskis Country, AB	50.7567	-114.5529	RABM	Z12.3.8
rnsaCAB017	Kananaskis Country, AB	50.8577	-114.6483	RABM	Z12.3.11
rnsaCO001	10 km NE of BlackHawk (Bob Clemans), CO	39.8167	-105.3919	Wild	rnsa 1
rnsaCO002	Pickle Gulch campground, CO	39.8424	-105.5236	Wild	2331-08703
rnsaCO003	Elk Water Campground, CO	40.2558	-105.8413	Wild	2331-08704
rnsaCO004	Gunnison, Gunnison Co. Colorado	38.8412	-106.4783	UWBM	53394 GAV 257
rnsaCO005	Park Co. Colorado	39.4402	-105.7698	UWBM	56359 GAV 858
rnsaCO006	Gunnison, Gunnison Co. Colorado	38.5474	-106.9214	UWBM	56363 GAV 862
rnsaCO007	Sig Creek Campground, San Juan NF, CO	37.6338	-107.8838	Wild	2331-08716
rnsaID001	Day Use Road, Ponderosa State Park, McCall, ID	44.9411	-116.0805	Wild	2291-90482

rnsaID002	Lily Marsh, Ponderosa Park, ID	44.9491	-116.0777	Wild	2291-90483
rnsaID003	W. Mountain Road, McCall, ID	44.8808	-116.1625	Wild	2291-90484
rnsaID004	W. Mountain Road, McCall, ID	44.8808	-116.1625	Wild	2291-90485
rnsaID005	Blue Bunch Road, New Meadows, ID	44.9140	-116.2985	Wild	2291-90486
rnsaID006	Blue Bunch Road, New Meadows, ID	44.9140	-116.2985	Wild	2291-90487
rnsaID007	Aspen Stand by Pedestrian Bridge, McCall	44.8927	-116.1098	Wild	2291-90488
rnsaID008	Lily Marsh, Ponderosa State Park, McCall, ID	44.9491	-116.1128	Wild	2291-90489
rnsaID009	South Beach, McCall, ID	44.9120	-116.1183	Wild	2291-90490
rnsaID010	South Beach, McCall, ID	44.9120	-116.1183	Wild	2291-90491
rnsaMT001	Road to Park Lake, Helena, MT	46.4656	-112.1297	Wld	rnsa 1
rnsaMT002	Unionville (Prospect Rd.), MT	46.5220	-112.1175	Wild	RNSA 2
rnsaNEOR001	Wallowa state park, OR	45.2810	-117.2106	Wild	2431-47308
rnsaNEOR002	Wallowa state park, OR	45.2810	-117.2106	Wild	2431-47309
rnsaNEOR003	Catherine Creek St. park, OR	45.1524	-117.7419	Wild	2431-47310
rnsaNEOR004	Catherine Creek St. park, OR	45.1524	-117.7419	Wild	2431-47311
rnsaNEOR005	Bird Track Springs Trail, OR	45.3030	-118.3084	Wild	2431-47315
rnsaNEOR006	Hilgard junction state park, OR	45.3431	-118.2389	Wild	2431-47316
rnsaNEOR007	Bird Track Springs Trail, OR	45.3030	-118.3084	Wild	2331-88108
rnsaNEOR008	Red Bridge State park, OR	45.2897	-118.3328	Wild	2341-47318
rnsaNEOR009	Hilgard junction state park, OR	45.3431	-118.2389	Wild	2431-47319
rnsaNEOR010	Ukiah Dale State park, OR	45.1246	-118.9726	Wild	2341-47320
rnsaNEOR011	Ukiah Dale State park, OR	45.1246	-118.9726	Wild	2341-47321
rnsaNEOR012	Ukiah Dale State park, OR	45.1246	-118.9726	Wild	2341-47322
rnsaNEOR013	Ukiah Dale State park, OR	45.1246	-118.9726	Wild	2341-47323
rnsaNEWA001	Slide Creek, Boyds, Ferry, Washington	48.7159	-118.1352	UWBM	53324, CSW 3862
rnsaNEWA002	Scatter Creek, Republic, Ferry, Washington	48.5301	-118.8005	UWBM	53350, CSW 3889
rnsaNEWA003	Cusick, Pend Oreille, Washington	48.3384	-117.3060	UWBM	54060, DAB 501
rnsaNM001	Santa Fe, 52 Ojo de la Vacca Tr., NM	35.4431	-105.7763	MSB	25137, NK: 142103
rnsaNM002	between Cowles and Pecos, NM	35.6931	-105.6674	MSB	28720, NK: 169972

rnsaNM003	San Marcial, NM	33.6339	-107.0084	MSB	28952, NK: 170195
rnsaNM004	Albuquerque, NM	35.0874	-106.4897	MSB	20948, NK: 43492
rnsaNM005	Casa Loma Rd, Bernalillo County, NM	35.1101	-106.3802	MSB	25176, NK: 142043
rnsaNM006	Mangas Springs, Grant County, NM	32.8424	-108.5118	MSB	24164, NK: 116335
rnsaNM007	Taos, NM	36.3744	-105.5597	MSB	24656, NK: 130734
rnsaNM008	Black Range, NM	32.9438	-107.7040	MSB	29241, NK: 170616
rnsaNM009	Taos, NM	36.3744	-105.5597	MSB	28620, NK: 169904
rnsaNM010	Hernandez, NM	36.0626	-106.1193	MSB	26720, NK: 165386
rnsaNM011	Sandia Park, NM	35.1678	-106.3673	MSB	26647, NK: 130540
rnsaNM012	Guadalupe Mountains, Dark Canyon, NM	32.1309	-104.7128	MSB	29305, NK: 35770
rnsaNM013	Peloncillo Mtns., Lower Clanton Canyon, NM	31.5213	-108.9805	MSB	26611, NK: 165196
rnsaNM014	Pajarito Village, Pojoaque, NM	35.8785	-106.0032	MSB	23193, NK: 103423
rnsaNM015	Red River, 6 mi S, NM	36.7005	-105.3981	MSB	22316, NK: 37262
rnsaNM016	Pleasanton, NM	33.2723	-108.8728	MSB	18503, NK: 14869
rnsaNM017	Guadalupe Mountains, Dark Canyon, NM	32.1116	-104.7387	MSB	29309, NK: 35775
rnsaNM018	Chimisa Trailhead, Santa Fe NF, NM	35.7286	-105.8664	Wild	2331-88109
rnsaNM019	Chimisa Trailhead, Santa Fe, NM	35.7279	-105.8694	Wild	2331-88110
rnsaSAB001	Belly River Campground, Waterton, AB	49.0219	-113.6873	Wild	rnsa 6
rnsaSAB002	Marquis Hole Picnic Area, Waterton, AB	49.0694	-113.8561	Wild	rnsa 7
rnsaSAB003	Marquis Hole Picnic Area, Waterton, AB	49.0694	-113.8561	Wild	rnsa 8
rnsaSAB004	Marquis Hole, Waterton, AB	49.0683	-113.8823	Wild	rnsa 3
rnsaSAB005	Belly River Campground, Waterton, AB	49.0219	-113.6873	Wild	rnsa 4
rnsaSAB006	Belly River Campground, Waterton, AB	49.0219	-113.6873	Wild	rnsa 5
rnsaSAB007	Field station cabin, AB	49.3491	-114.4108	Wild	991-19821
rnsaSAB008	Field station cabin, AB	49.3491	-114.4108	Wild	991-19822
rnsaSAB009	Red rock canyon rd, AB	49.0944	-113.8866	Wild	991-19839
rnsaSAB010	Waterton lake, AB	49.1030	-113.8519	Wild	991-19840
rnsaSAB011	HWY6, AB	49.0636	-113.7413	Wild	991-19841

rnsaSAB012	HWY6, AB	49.0636	-113.7413	Wild	991-19842
rnsaSAB013	HWY6, AB	49.0489	-113.6941	Wild	991-19843
rnsaSAB014	Castle River, AB	49.3000	-114.2830	RABM	Z96.18.40, rnsa 6
rnsaSAB015	7 miles South East of Beaver Mines Lake, AB	49.4310	-114.3300	RABM	Z99.12.8, rnsa 7
rnsaSAB016	Porcupine Hills, AB	50.0172	-114.0522	RABM	Z02.14.9
rnsaSAB017	Beaver Creek, Porcupine Hills, AB	49.8167	-113.9500	RABM	Z96.18.9
rnsaSAB018	Beaver Creek, Porcupine Hills, AB	49.8500	-113.9667	RABM	Z96.18.18
rnsaSAB019	Beaver Creek, Porcupine Hills, AB	49.8333	-113.9667	RABM	Z96.18.30
rnsaSAB020	Belly River Campground Waterton, AB	49.0227	-113.6874	Wild	921-16716
rnsaSAB021	Hay Barn Picnic Area Waterton, AB	49.0795	-113.8544	Wild	2251-51394
rnsaSAB022	Belly River Campground Waterton, AB	49.0227	-113.6874	Wild	991-19847
rnsaSAB023	Red rock canyon rd, AB	49.0944	-113.8866	Wild	991-19839
rnsaSAB024	Waterton lake, AB	49.1030	-113.8519	Wild	991-19840
rnsaSAB025	HWY6, AB	49.0636	-113.7413	Wild	991-19841
rnsaSAB026	HWY6, AB	49.0636	-113.7413	Wild	991-19842
rnsaSAB027	HWY6, AB	49.0489	-113.6941	Wild	991-19843
rnsaSAB028	HWY6, AB	49.0370	-113.6814	Wild	991-19844
rnsaSAB029	HWY6, AB	49.0370	-113.6814	Wild	991-19845
rnsaSAB030	Stables Road, Waterton, AB	49.0620	-113.8898	Wild	921-16791
rnsaSAB031	Stables Road, Waterton, AB	49.0667	-113.8845	Wild	921-16792
rnsaSAB032	Haybarn, Waterton Lakes, AB	49.0796	-113.8593	Wild	921-16793
rnsaSAB033	Canyon Road, Waterton Lakes, AB	49.0945	-113.8382	Wild	921-16794
rnsaSAB034	Canyon Road, Waterton Lakes, AB	49.0945	-113.8382	Wild	921-16795
rnsaSAB035	Westcastle Wetlands, near Beavermines, AB	49.3766	-114.3784	Wild	921-16796
rnsaSAB036	Allison Creek Road 2 Crowsnest Pass, AB	49.6891	-114.6038	Wild	921-16717
rnsaSAB037	Allison Creek Road 4 Crowsnest Pass, AB	49.6781	-114.5833	Wild	921-16718
rnsaSD001	Custer State Park Office, SD	43.7706	-103.3973	Wild	2331-08707
rnsaSD002	Gate 411, French Creek Road, Custer State Park, SD	43.6995	-103.4342	Wild	2331-08710

rnsaSD003	Sylvan Lake Water Shack, Custer State Park, SD	44.0085	-103.5606	Wild	2331-08712
rnsaSD004	Sylvan Lake Water Shack, Custer State Park, SD	44.0085	-103.5606	Wild	2331-08713
rnsaSD005	Little Devil's Tower Trailhead, Custer State Park, SD	43.8442	-103.5513	Wild	2331-08714
rnsaSD006	Low Powerline Wetland, Custer State Park, SD	43.7757	-103.4525	Wild	2331-08715
rnsaSD007	Sapsucker Enclosure, Custer State Park, SD	43.7591	-103.4836	Wild	2331-08721
rnsaSEBC001	Smokey Bear Campground, Revelstoke, BC	50.9886	-117.7221	Wild	921-16710
rnsaSEBC002	Smokey Bear Campground, Revelstoke, BC	50.9886	-117.7221	Wild	921-16711
rnsaSEBC003	Revelstoke Dump , Revelstoke, BC	51.0223	-117.7665	Wild	921-16712
rnsaSEBC004	Revelstoke Dump , Revelstoke, BC	51.0223	-117.7665	Wild	921-16713
rnsaSEBC005	Revelstoke Forestry Rd, Revelstoke, BC	51.0163	-117.7661	Wild	921-16714
rnsaSEBC006	Revelstoke Forestry Rd, Revelstoke, BC	51.0163	-117.7661	Wild	921-16715
rnsaSEBC007	Smokey Bear Camp, Revelstoke, BC	50.9887	-118.2778	Wild	991-19848
rnsaSEBC008	Smokey Bear Camp, Revelstoke, BC	50.9887	-118.2778	Wild	991-19849
rnsaSEBC009	Mulvehill Creek Wilderness Inn, Revelstoke, BC	50.8526	-118.1149	Wild	921-16797
rnsaSEBC010	Mulvehill Creek Wilderness Inn, Revelstoke, BC	50.8526	-118.1149	Wild	921-16798
rnsaUT001	Cache National Forest (Forestry Rd.), UT	41.5135	-111.5095	Wild	2291-90450
rnsaUT002	Cache National Forest (Forestry Rd.), UT	41.5135	-111.5095	Wild	2291-90451
rnsaUT003	W of Woodruff (Cache Forestry Rd.), UT	41.5321	-111.4575	Wild	2331-08705
rnsaUT004	Boots campground, UT	41.2945	-111.6581	Wild	2291-90453
rnsaUT005	South Fork campground, UT	41.2797	-111.6537	Wild	2291-90454
rnsaUT006	Bridger Lake Cmpgrnd2, UT	40.9691	-110.3872	Wild	2331-08708
rnsaUT007	Bridger Lake Cmpgrnd2, UT	40.9691	-110.3872	Wild	2331-08709
rnsaWA001	Easton, Kittitas Co., WA	47.2418	-121.1843	UWBM	49988 CSW 3959
rnsaWA002	Ellensburg, Kittitas Co., WA	47.1895	-120.6330	UWBM	79033 SVD 2204
rnsaWA003	Kittitas Co., WA	47.1895	-120.6330	UWBM	49001 CSW 4984

rnsaWA004	Kittitas Co., WA	47.1895	-120.6330	UWBM	49002 CSW 4985
rnsaWA005	Cle Elum, Kittitas Co., WA	47.1333	-120.9000	UWBM	63675 MAM 18
rnsaWA006	Kirkland, King Co., WA	47.6719	-122.2035	UWBM	85284 SEZ 045
rnsaWY001	Ditch Creek Road, Bridger-Teton NF, WY	43.7004	-110.5691	Wild	2331-08717
rnsaWY002	Ditch Creek Road, Bridger-Teton NF, WY	43.6955	-110.5743	Wild	2331-08718
rnsaWY003	FR30340A, Shadow Mtn Road, Bridger-Teton NF, WY	43.7002	-110.6196	Wild	2331-08719
rnsaWY004	Turpin Meadows Road, Bridger-Teton NF, WY	43.8558	-110.2981	Wild	2331-08720
ybsaCAB001	Olds, AB	51.7916	-114.2862	Wild	1731-05301
ybsaCAB002	Innisfail, AB	54.0238	-110.9824	Wild	ybsa 2
ybsaCAB003	Buck Lake, AB	54.9721	-115.6046	Wild	ybsa 3
ybsaCAB005	Buck Lake, AB	54.9721	-115.6046	Wild	ybsa 4
ybsaCAB006	9 miles west of Bearberry, Improvement District 10, AB	51.8670	-115.0830	RABM	Z95.11.15, ybsa 6
ybsaCAB007	Brazeau Reservoir, Improvement District 14, AB	52.9170	-115.3670	RABM	Z95.15.18, ybsa 9
ybsaCAB008	Alder Flats, Improvement District 11, AB	52.9170	-115.0670	RABM	Z95.15.22, ybsa 10
ybsaCAB009	Strachan, Improvement District 10, AB	52.2000	-115.1330	RABM	Z95.9.16, ybsa 12
ybsaCAB010	Cow Lake area, Improvement District 10, AB	52.2830	-115.0000	RABM	Z95.9.9, ybsa 14
ybsaCAB011	Smoke Lake, AB	54.3540	-116.9540	RABM	Z99.10.46, ybsa 15
ybsaCAB012	James River area	51.8686	-115.1130	RABM	Z09.8.6
ybsaCAB013	James River area	51.8059	-115.2022	RABM	Z10.3.13
ybsaCAB015	8 Bearberry, Improvement District 10, AB	51.8330	-115.1000	RABM	Z95.11.10, ybsa 2
ybsaCAB016	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.5, ybsa 8
ybsaCAB017	Conklin area	55.6202	-111.1189	RABM	Z11.2.2
ybsaCAB018	Conklin area	55.6137	-111.1470	RABM	Z11.2.3
ybsaCAB019	Brule area	53.3945	-117.8140	RABM	Z11.4.9
ybsaCAB020	Brule area	53.4020	-117.8442	RABM	Z11.4.18
ybsaCAB021	Brule area	53.4005	-117.8685	RABM	Z11.4.30

ybsaCAB022	James River, west of Sundre	51.8900	-115.0475	RABM	Z11.5.2
ybsaCAB023	James River, west of Sundre	51.8941	-115.0138	RABM	Z11.5.4
ybsaCAB024	Clearwater River, AB	52.0250	-115.1547	RABM	Z11.5.8
ybsaCAB025	Clearwater River, AB	52.0390	-115.1604	RABM	Z11.5.9
ybsaCAB026	James River area, Sundre, AB	51.8461	-115.0314	RABM	Z11.5.13
ybsaCAB027	James River area, Sundre, AB	51.8490	-115.0182	RABM	Z11.5.14
ybsaCAB028	Alder Flats, AB	52.9300	-115.0500	RABM	Z02.15.7
ybsaCAB029	St. Albert, Sturgeon Municipal District, AB	53.6330	-113.6330	RABM	Z81.77.1
ybsaCAB030	Fort Saskatchewan, Strathcona County, AB	53.7170	-113.2170	RABM	Z81.146.87
ybsaCAB031	Sherwood Park, Strathcona County, AB	53.5170	-113.3170	RABM	Z87.34.3
ybsaCAB032	Edmonton, Edmonton Municipal District, AB	53.5330	-113.5330	RABM	Z88.17.1
ybsaCAB033	3 miles south of Lodgepole, Parkland County, AB	53.0500	-115.3170	RABM	Z88.19.94
ybsaCAB034	Edmonton, Edmonton Municipal District, AB	53.5330	-113.5330	RABM	Z88.32.6
ybsaCAB035	Edmonton, Edmonton Municipal District, AB	53.5330	-113.5330	RABM	Z88.36.11
ybsaCAB036	Edmonton, Edmonton Municipal District, AB	53.5330	-113.5330	RABM	Z89.72.1
ybsaCAB037	Ardrossan, Strathcona County, AB	53.5500	-113.0670	RABM	Z00.31.1
ybsaCAB038	Kananaskis Country, AB	51.0508	-114.8085	RABM	Z12.3.3
ybsaCAB039	Kananaskis Country, AB	50.64440	-114.4730	RABM	Z12.3.10
ybsaFL001	Cocoa Brevard Community College, FL	28.1654	-80.6691	MSB	25110, NK: 142006
ybsaFL002	Rockledge, Brevard County, FL	28.2910	-80.7587	MSB	25310, NK: 142203
ybsaIL001	Chicago, Madison at LaSalle, Cook Co, IL	41.8819	-87.6325	FMNH	350791
ybsaIL002	Chicago, Ontario and State, Cook Co, IL	41.8932	-87.6284	FMNH	395391
ybsaIL003	Chicago, N side, Cook Co, IL	41.8973	-87.6181	FMNH	434988
ybsaIL004	Chicago, 233 N Michigan Ave, Cook Co, IL	41.8870	-87.6237	FMNH	439018
ybsaIL005	Chicago, Blue Cross Building, Cook Co, IL	41.8846	-87.6204	FMNH	439019
ybsaIL006	Chicago, Illinois Center, Cook Co, IL	41.8879	-87.6242	FMNH	446011
ybsaIL007	Chicago, AON, Cook Co, IL	41.8849	-87.6209	FMNH	446015
ybsaIL008	Chicago, Hyatt Center, Cook Co, IL	41.8881	-87.6227	FMNH	446782

ybsaIL009	Chicago, 311 S Wacker, Cook Co, IL	41.8775	-87.6362	FMNH	446988
ybsaIL010	Naperville, James and George, DuPage Co, IL	41.7718	-88.1273	FMNH	447149
ybsaIL011	Chicago, AON, Cook Co, IL	41.8849	-87.6209	FMNH	452273
ybsaIL012	Chicago, Sears, Cook Co, IL	41.9527	-87.7453	FMNH	452274
ybsaIL013	Chicago, Tribune Tower, Cook Co, IL	41.8903	-87.6238	FMNH	452275
ybsaIL014	Chicago, 111 E Wacker, Cook Co, IL	41.8775	-87.6362	FMNH	452276
ybsaIL015	Chicago, AON, Cook Co, IL	41.8849	-87.6209	FMNH	452277
ybsaIL016	Chicago, AON, Cook Co, IL	41.8849	-87.6209	FMNH	452278
ybsaIL017	Chicago, Wabash and Michigan, Cook Co, IL	41.8965	-87.6268	FMNH	452316
ybsaIL018	Chicago, 150 N Wacker, Cook Co, IL	41.8775	-87.6362	FMNH	453392
ybsaIL019	Chicago, 35 W Wacker, Cook Co, IL	41.8775	-87.6362	FMNH	453926
ybsaIL020	Chicago, 1 N Franklin, Cook Co, IL	41.8822	-87.6353	FMNH	453927
ybsaIL021	Evanston Northwestern University, Cook Co, IL	42.0564	-87.6770	FMNH	454580
ybsaIL022	Chicago, 800 N Wells, Cook Co, IL	41.8967	-87.6346	FMNH	454283
ybsaIL023	Chicago, Union Station, Cook Co, IL	41.8785	-87.6404	FMNH	455101
ybsaIL024	Chicago, Federal Plaza, Cook Co, IL	41.8363	-87.6288	FMNH	456224
ybsaIL025	Chicago, Hyatt, Cook Co, IL	41.8881	-87.6227	FMNH	458063
ybsaIL026	Chicago, Boeing, Cook Co, IL	41.8835	-87.6390	FMNH	458073
ybsaIL027	Chicago, Equitable, Cook Co, IL	41.8895	-87.6228	FMNH	458075
ybsaIL028	Chicago, Swiss Hotel, Cook Co, IL	41.8871	-87.6195	FMNH	458076
ybsaIL029	Chicago, 150 N Wacker, Cook Co, IL	41.8775	-87.6362	FMNH	458078
ybsaIL030	Chicago, R R Donnelly, Cook Co, IL	41.8863	-87.6291	FMNH	458080
ybsaIL031	Chicago, 311 S Wacker, Cook Co, IL	41.8775	-87.6362	FMNH	458923
ybsaIL032	Chicago, 233 Michigan Plaza, Cook Co, IL	41.8864	-87.6244	FMNH	458925
ybsaIL033	Chicago, Prudential 2, Cook Co, IL	41.8854	-87.6230	FMNH	458926
ybsaIL034	Chicago, Sears, Cook Co, IL	41.9527	-87.7453	FMNH	458928
ybsaIL035	Chicago, NBC, Cook Co, IL	41.8837	-87.6310	FMNH	459756
ybsaIL036	Chicago, Sears, Cook Co, IL	41.9527	-87.7453	FMNH	459762

ybsaIL037	Chicago, Franklin Center, Cook Co, IL	41.8807	-87.6343	FMNH	459777
ybsaIL038	Chicago, Franklin Center, Cook Co, IL	41.8807	-87.6343	FMNH	459778
ybsaIL039	Chicago, IBM, Cook Co, IL	41.8808	-87.6365	FMNH	461075
ybsaIL040	Chicago Westin Hotel, Cook Co, IL	41.8880	-87.6305	FMNH	464630
ybsaLA001	New Orleans, LA	30.0507	-89.9148	MSB	24155, NK: 116324
ybsaLA002	Garner Ridge, Cameron Parish, Louisiana, LA	29.7618	-93.7810	MSB	24230, NK: 116384
ybsaLA003	Johnson Bayou, Cameron Parish, LA	29.7641	-93.7007	MSB	24417, NK: 116380
ybsaMI001	Ann Arbor, Wagner Road, Washtenaw Co., MI	42.2833	-83.7500	UMich	239630
ybsaMI002	Pittsfield, Washtenaw Co., Michigan	42.2058	-83.7234	UMich	227567
ybsaMI003	Univ. Mich. Med. Sci. Complex, Ann Arbor, MI	42.2785	-83.7390	UMich	240776
ybsaMI004	Southeast Michigan	No data	No data	UMich	4721
ybsaMI005	Lodi Twp, Washtenaw Co., Michigan	42.2962	-83.8412	UMich	238463
ybsaMI006	Austin Twp, Mecosta Co., Michigan	43.5767	-85.3667	UMich	238411
ybsaMI007	Georgetown, Wayne Co., Michigan	42.3057	-83.4358	UMich	240790
ybsaMI008	Rapid River, Delta Co., Michigan	45.7045	-86.9362	UMich	239378
ybsaMI009	Summerfield Twp, Clare Co., Michigan	44.1323	-84.9155	UMich	227389
ybsaMI010	Summerfield Twp, Clare Co., Michigan	44.1323	-84.9155	UMich	227390
ybsaMI011	Cross Village, Emmet Co., Michigan	45.6500	-85.0333	UMich	225159
ybsaMI012	Webster Twp, Washtenaw Co., Michigan	42.4000	-83.8000	UMich	225103
ybsaMI013	Wagner Road, ann Arbor., Michigan	42.2833	-83.7500	UMich	225875
ybsaMI014	Mecosta Co., Michigan	43.6485	-85.3652	UMich	235617
ybsaMI015	University of Michigan, Ann Arbor, Michigan	42.2795	-83.7300	UMich	235064
ybsaMI016	Ann Arbor, Washtenaw Co., Michigan	42.2769	-83.7363	UMich	238253
ybsaMI017	Ann Arbor, Washtenaw Co., Michigan	42.2769	-83.7363	UMich	238254
ybsaNC001	Buncombe Co. NC	35.5251	-82.6481	UWBM	87037 NCSM 15281
ybsaNC002	Arden, Buncombe Co. NC	35.4656	-82.5170	UWBM	87061 RBB 772
ybsaNC003	Dillingham, Buncombe Co. NC	35.7537	-82.4070	UWBM	86868 RBB 538

ybsaNC004	Murchison, Buncombe Co. NC	35.8176	-82.2993	UWBM	86869 RBB 539
ybsaNC005	Burnsville, Buncombe Co. NC	35.9111	-82.2927	UWBM	85503 DEA 1196
ybsaNC006	Burnsville, Buncombe Co. NC	35.9111	-82.2927	UWBM	85505 DEA 1198
ybsaNC007	Brevard, Transylvania Co. NC	35.2262	-82.7404	UWBM	85791 NCSM RTB 532
ybsaNC008	Brevard, Transylvania Co. NC	35.2262	-82.7404	UWBM	85577 JAR 025
ybsaNC009	Brevard, Transylvania Co. NC	35.2262	-82.7404	UWBM	85578 JAR 026
ybsaNC010	Transylvania Co. NC	35.1688	-82.8351	UWBM	86710 DEA 1189
ybsaNJ001	Bernardsville, Somerset County, NJ	40.7152	-74.5694	MSB	21159, NK: 37506
ybsaNJ002	Montclair, Essex County, NJ	40.8193	-74.2133	MSB	21158, NK: 37523
ybsaNM001	Edgewood, NM	35.0687	-106.1958	MSB	22209, NK: 103030
ybsaNM002	Guadalupe Canyon (Ranch Headquarters), NM			MSB	20631, NK: 43388
ybsaNM003	Santa Fe County, NM	35.5176	-105.9820	MSB	23862, NK: 116231
ybsaNSNB001	York Co., NB	45.9700	-66.6500	NBM	010432
ybsaNSNB002	Saint John Co., NB	45.2700	-66.0500	NBM	007343
ybsaNSNB003	Victoria Co., New Brunswick	46.8830	-66.9170	CMN	71179
ybsaNSNB004	Victoria Co., New Brunswick	46.8830	-66.9170	CMN	71180
ybsaNSNB005	Restigouche Co., New Brunswick	46.8670	-66.3500	CMN	71181
ybsaNSNB006	Northumberland Co., New Brunswick	47.2330	-66.8670	CMN	71182
ybsaNSNB007	York Co., NB	45.9700	-66.6500	NBM	004985
ybsaNSNB008	Victoria Co., NB	47.2300	-67.1500	NBM	005060
ybsaNSNB009	Victoria Co., NB	47.2300	-67.1500	NBM	005432
ybsaNSNB010	York Co., NB	46.1200	-66.0800	NBM	005484
ybsaNSNB011	York Co., NB	46.1200	-66.8300	NBM	005485
ybsaNSNB012	Victoria Co., NB	47.2300	-67.1500	NBM	008700
ybsaNSNB013	Victoria Co., NB	47.2300	-67.1500	NBM	008288
ybsaNSNB014	Charlotte Co., NB	44.7000	-66.7300	NBM	008585
ybsaNSNB015	Saint John Co., NB	45.2700	-66.0500	NBM	008437

ybsaNSNB016	Victoria Co., NB	47.2300	-67.1500	NBM	009593
ybsaNSNB017	Charlotte Co., NB	44.7700	-66.7500	NBM	010430
ybsaNSNB018	Saint John Co., NB	45.2700	-66.0500	NBM	009680
ybsaNSNB019	Saint John Co., NB	45.2700	-66.0500	NBM	009825
ybsaNWBC001	Dease Lake, BC	58.4350	-129.8940	Wild	ybsa 1
ybsaNWBC002	Dease Lake, BC	58.4350	-129.8940	Wild	ybsa 2
ybsaNWBC003	Dease Lake, BC	58.5069	-130.0231	Wild	ybsa 3
ybsaNWBC004	Dease Lake, BC	58.4303	-129.9868	Wild	ybsa 4
ybsaNWBC005	Dease Lake, BC	58.5069	-130.0231	Wild	ybsa 5
ybsaON001	Lanark Co., Ontario	45.1000	-76.4000	CMN	85199
ybsaON002	Ottawa (metro), Ontario	45.4670	-76.2170	CMN	85200
ybsaON003	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85202
ybsaON004	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85204
ybsaON005	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85205
ybsaON006	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85206
ybsaON007	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85203
ybsaON008	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85207
ybsaON009	Frontenac Co., Ontario	44.2330	-76.5000	CMN	85217
ybsaON010	Ottawa (metro), Ontario	45.2170	-75.6830	CMN	85211
ybsaON011	Ottawa (metro), Ontario	45.2170	-75.6830	CMN	85212
ybsaON012	Ottawa (metro), Ontario	45.2170	-75.6830	CMN	85210
ybsaON013	Frontenac Co., Ontario	44.3000	-76.4670	CMN	85218
ybsaON014	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85215
ybsaON015	Frontenac Co., Ontario	44.2330	-76.5000	CMN	85219
ybsaON016	Lanark Co., Ontario	45.1830	-76.2330	CMN	85221
ybsaON017	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85214
ybsaON018	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85225
ybsaON019	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85224
ybsaON020	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85223

ybsaON021	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85226
ybsaON022	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85227
ybsaON023	Ottawa (metro), Ontario	45.2670	-75.7500	CMN	85228
ybsaON024	Mun. Rég. de Cté des Collines-de-l'Outaouais, Québec	45.6010	-75.6220	CMN	85197
ybsaON025	Hastings Co., Ontario	45.5670	-77.6500	CMN	85222
ybsaON026	Mun. Rég. de Cté de Pontiac, Québec	46.4330	-77.6830	CMN	77964
ybsaON027	Mun. Rég. de Cté de Pontiac, Québec	46.2830	-77.6830	CMN	77967
ybsaON028	Mun. Rég. de Cté de Pontiac, Québec	46.3330	-77.6830	CMN	77968
ybsaON029	Mun. Rég. de Cté de Pontiac, Québec	46.3330	-77.6830	CMN	77969
ybsaON030	Mun. Rég. de Cté de Pontiac, Québec	46.2500	-77.6330	CMN	77970
ybsaON031	Mun. Rég. de Cté de Pontiac, Québec	46.4670	-77.7670	CMN	77971
ybsaON032	Mun. Rég. de Cté de Pontiac, Québec	46.2000	-77.6830	CMN	77972
ybsaON033	Mun. Rég. de Cté de Pontiac, Québec	46.2170	-77.6830	CMN	77973
ybsaON034	Mun. Rég. de Cté de Pontiac, Québec	46.4670	-77.7670	CMN	77974
ybsaON035	Mun. Rég. de Cté de Pontiac, Québec	46.3000	-77.6830	CMN	77975
ybsaON036	Mun. Rég. de Cté de Pontiac, Québec	46.3000	-77.6830	CMN	77976
ybsaON037	Mun. Rég. de Cté de Pontiac, Québec	46.3000	-77.6830	CMN	77977
ybsaON038	Mun. Rég. de Cté de Pontiac, Québec	46.4330	-77.6330	CMN	77978
ybsaON039	Mun. Rég. de Cté de Pontiac, Québec	46.4330	-77.6330	CMN	77979
ybsaON040	Mun. Rég. de Cté de Pontiac, Québec	46.7330	-77.6830	CMN	77980
ybsaON041	Mun. Rég. de Cté de la Vallée-de-la-Gatineau, Québec	46.3000	-76.1000	CMN	85193
ybsaON042	Mun. Rég. de Cté de Pontiac, Québec	45.6000	-76.5000	CMN	85195
ybsaON043	Mun. Rég. de Cté de Pontiac, Québec	45.6830	-76.6170	CMN	85196
ybsaON044	Mun. Rég. de Cté de Pontiac, Québec	45.6000	-76.5000	CMN	85194
ybsaSEBC001	Keremeos, B.C.	49.2014	-119.8285	RSKM	CA652/A9643/E001
ybsaSK001	Treebeard Trail, Prince Albert NP, SK	53.9725	-106.2903	Wild	961-43502
ybsaSK002	Narrows Campground, Prince Albert NP, SK	53.9807	-106.2938	Wild	921-16800

ybsaSK003	Narrows Campground, Prince Albert NP, SK	53.9807	-106.2938	Wild	921-16799
ybsaSK004	Freight Trail, 'D' entrance, Prince Albert NP, SK	53.7067	-106.0536	Wild	961-43503
ybsaSK005	Argyle Street, Regina, SK	50.4500	-104.6167	RSKM	A6548/18264/E1
ybsaSK006	Regina, SK	50.4410	-104.6369	RSKM	A6227/18105/E001
ybsaSK007	Grand Coulee, SK	50.4333	-104.8170	RSKM	A16687/E001
ybsaSK008	Ramada Inn, Regina, SK	50.4500	-104.6170	RSKM	A16698/E002
ybsaSK009	Regina, SK	50.4500	-104.6170	RSKM	A17618/E002
ybsaSK010	Rouleau, SK	50.1833	-104.9330	RSKM	A20030/E
ybsaSK011	Regina, SK	50.4500	-104.6170	RSKM	A20040/E001
ybsaSK012	RSM, Regina, SK	50.4500	-104.6170	RSKM	A18288/E001
ybsaSK013	RSM, Regina, SK	50.4500	-104.6170	RSKM	A16729/E001
ybsaSK014	Regina, SK	50.4500	-104.6170	RSKM	A17134/E002
ybsaWA001	Ellensburg, Kittitas, Washington	46.9941	-120.5561	Smith	V#586086
ybsaWA002	Naches, Yakima, Washington	46.7325	-120.6979	Smith	V#630630

Appendix 1.4. Parsimony informative sites from CR sequences. Letters denote polymorphisms from rbsaNCA02, dots denote consensus with this sequence, ? show missing data. Hap column lists haplotype for each individual.

		1	111111111	1112222222	2222222222	2222222223	3334555556	6666666667	77
		555888990	112466777	7890000122	2234445557	7888899991	2670012380	1237778890	00
	hap	4357235040	496802123	6240179504	6855690171	9138901292	7902459510	4861895834	67
rbsaNCA002	1	TGATCGGATC	CTCCGCGCG	CGATTCAACC	TTCCTGTCGG	TCCTCCTTCG	CTATT?TGTA	CCGCAAGGGC	CG
rbsaNCA003	2	....	........A	....	....	....	.....A....	...T??????	??
rbsaNCA004	3	....	........A	....	....	....	.....A....	...T...C..	..
rbsaNCA011	4	.......G..	........A	....	....	....	.....A....	...T....A.	..
rbsaSOR001	5	....	........A	....	....	....	.....A....	...T......	..
rbsaCCA002	6	........C.	........A	....	....	......C...	.A...A....	...T......	..
rbsaCCA003	5	....	........A	....	....	....	.....A....	...T......	..
rbsaWA001	7	A.........	........A	....	....	C.........	.....A....	...T......	..
rbsaWA002	8	A.......C.	........A	........T.	....	....	.....A....	...T......	..
rbsaWA004	9	?.......C.	........A	....	....	......C...	.....A....	...T......	..
rbsaWA005	9	........C.	........A	....	....	......C...	.....A....	...T......	..
rbsaWA006	8	........C.	........A	........T.	....	....	.....A....	...T......	..
rbsaWA007	10	....	........A	....C.....	....	....	.....A....	...T......	..
rbsaWA008	9	........C.	........A	....	....	......C...	.....A....	...T......	..
rbsaWA009	8	........C.	........A	........T.	....	....	.....A....	...T......	..
rbsaWA010	9	........C.	........A	....	....	......C...	.....A....	...T......	..
rbsaWA011	9	........C.	........A	....	....	......C...	.....A....	...T......	..
rbsaWA012	9	........C.	........A	....	....	......C...	.....A....	...T......	..
rbsaWA013	8	........C.	........A	........T.	....	....	.....A....	...T......	..
rbsaWA014	11	........C.	T.......A	....	....	....	.....A....	...T......	..
rbsaWA015	8	........C.	........A	........T.	....	....	.....A....	...T......	..
rbsaWA016	10	....	........A	....C.....	....	....	.....A....	...T......	..
rbsaWA017	8	........C.	........A	........T.	....	....	.....A....	...T......	..
rbsaWA018	12	........C.	........A	....C.....	....	....	.....AC...	...T......	..
rbsaWA019	8	........C.	........A	........T.	....	....	.....A....	...T......	..
rbsaWA020	13	......A.C.	........A	.A........	....	......C...	.....A....	...T......	..
rbsaWA021	10	....	........A	....C.....	....	....	.....A....	...T......	..
rbsaWA022	14	........C.	........A	...C......	....	......C...	.....A....	...T......	..
rbsaWA023	11	........C.	T.......A	....	....	....	.....A....	...T......	..

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rbsaWA024    15  ........C.  ........A  .........  .........  .........  .....A....  ...T......  ..
rbsaVIBC003   9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaVIBC004   9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaVIBC005  16  ........C.  ........A  .........  .........  .........  .....A....  ...T......  ..
rbsaVIBC006  17  ........C.  ........A  ........T.  ...T......  .........  .....A....  ...T......  ..
rbsaVIBC007  14  ........C.  ........A  ...C......  .........  ......C...  .....A....  ...T......  ..
rbsaVIBC008   9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaVIBC009  18  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaVIBC012   9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaVIBC015  19  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rbsaVIBC016  10  .........  ........A  ....C.....  .........  .........  .....A....  ...T......  ..
rbsaVIBC017  20  ..G.......  ........A  ....C.....  .........  .........  .....A....  ...T......  ..
rbsaVIBC018  21  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rbsaVIBC020  22  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rbsaVIBC021   9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaCBC003    8  A.......C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rbsaCBC004   11  ?.......C.  T.......A  .........  .........  .........  .....A....  ...T......  ..
rbsaCBC005    9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaCBC006   12  ........C.  ........A  ....C.....  .........  .........  .A...AC...  ...T......  ..
rbsaCBC007   11  ?.......C.  T.......A  .........  .........  .........  .....A....  ...T......  ..
rbsaCBC08     8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rbsaCBC009    9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaCBC010   23  ....T.....  T.......A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaCBC011    8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rbsaCBC014   10  .........  ........?  ....C.....  .........  .........  .....A....  ...T......  ..
rbsaCBC016   24  .........  ........?  ....C.....  .........  .........  .....A....  ...T......  ..
rbsaNBC001   25  ......A...  .........  ....C.....  .........  .........  .....A....  ...T......  ..
rbsaNBC002    9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaNBC003    9  ........C.  ........A  .........  .........  ......C...  .A...A....  ...T......  ..
rbsaNBC005   26  .A..TTA.?.  ..TT..??.  ...C.TG.TT  C...?AC..A  ...C...C..  ...?.GC...  ..AT......  ..
rbsaNBC006    9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaNBC007   27  .........T  .CT...A.A  ........TT  C.......A.  .T.C.T.CA.  .C.A.A....  ...T......  ..
rbsaNBC008    9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rbsaQCI006    9  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..  ...A.A....  ...T......  ..
rbsaQCI011   29  .........T  .CT...A.A  T...C..G.T  C.......A.  ..GC...C..  ...A.A....  ...T......  ..
rbsaQCI015   14  ........C.  ........A  ...C......  .........  ......C...  .....A....  ...T......  ..
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rbsaQCI016    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rbsaQCI017   14   ........C.  ........A  ...C......  ..........  ......C...  .....A....  ...T......  ..
rbsaQCI018    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rbsaQCI105    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rbsaQCI106   28   ........C.  ........A  ..........  ..........  ......C...  .....A....  ...T......  ..
rbsaQCI107    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rbsaQCI108    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rbsaQCI109    8   ........C.  ........A  ........T.  ..........  ..........  .?...A....  ...T......  ..
rbsaQCI110   14   ........C.  ........A  ...C......  ..........  ......C...  .?...A....  ...T......  ..
rbsaQCI111    8   ........C.  ........A  ........T.  ..........  ..........  .A...A....  ...T....?.  ..
rbsaQCI114    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rbsaQCI116   30   ....T.A...  T.TT..AT.  ...C.TG.TT  A....AC.AA  ...C...C..  ...A.ACA.?  ..?T....A?  ?A
rbsaQCI117   31   ......A...  T.TT..AT.  ...C.TG.TT  A..TCAC.A?  ...C...C..  ...AAAC...  .??T?.????  ??
rbsaSrQ101   32   ........C.  ........A  ........T.  ...G......  ..........  .....G....  ...TG.....  ..
rbsaSrQ102    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rbsaSrQ103   33   ........C.  ........A  ........T.  ..........  ......C...  .....A....  ...T......  ..
rbsaSrQ104   11   ........C.  T.......A  ..........  ..........  ..........  .....A....  ...T......  ..
rbsaSrQ120    9   ........C.  ........A  ..........  ..........  ......C...  .....A....  ...T......  ..
rbsaSEAK01    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rbsaSEAK02   34   ........C.  ........A  ..........  ........A.  ......C...  .....A....  ...T......  ..
rbsaSEAK03   35   ........C.  ........A  ..........  ..........  ......C...  .....A....  ...T......  ..
rnsaMT001    36   ..G.....C.  T.T.A.A.A  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaMT002    37   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB001   38   A.......C.  ........A  ........T.  ..........  ..........  .....A..G.  ...T......  ..
rnsaSAB002   39   A.G.....C.  T.T.A.A.A  ..........  ........A.  CT......T.  .....A..G.  ...T......  ..
rnsaSAB003   39   A.G.....C.  T.T.A.A.A  ..........  ........A.  CT......T.  .....A..G.  ...T......  ..
rnsaSAB004    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB005    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB006    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB007   40   ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaSAB008    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB009   41   ..G.....C.  T...A.A.A  ........T.  ........A.  CT......T.  .....A..G.  ...T......  ..
rnsaSAB010   40   ..G.....C.  T.T.A.A.A  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaSAB011   40   ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaSAB012   40   ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaSAB013    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
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rnsaSAB014    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB015    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB016   42   .........T  .CT...A.A  ....C....T  C.......A.  ..GC...C..  ...A.A....  ...T......  ..
rnsaSAB017   43   .........T  T.T...A.A  ....C....T  C.......A.  .TGC...C..  ...A.A....  ...T......  ..
rnsaSAB018    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB019   38   ........C.  ........A  ........T.  ..........  ..........  .....A..G.  ...T......  ..
rnsaSAB020    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB022    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB023   41   ..G.....C.  T...A.A.A  ........T.  ........A.  CT......T.  .....A..G.  ...T......  ..
rnsaSAB024   40   ..G.....C.  T.T.A.A.A  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaSAB025   40   ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaSAB026   40   ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaSAB027    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB028    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSAB029    8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSD001     8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaSD002    38   ........C.  ........A  ........T.  ..........  ..........  .....A..G.  ...T......  ..
rnsaSD003    40   ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaSD004    38   ........C.  ........A  ........T.  ..........  ..........  .....A..G.  ...T......  ..
rnsaSD005    38   ........C.  ........A  ........T.  ..........  ..........  .....A..G.  ...T......  ..
rnsaSD006    38   ........C.  ........A  ........T.  ..........  ..........  .....A..G.  ...T......  ..
rnsaSD007    44   ..G.....C.  ........A  ........T.  ..........  ..........  .....A..G.  ...T......  ..
rnsaWY001    45   ..G.....C.  T...A.A.A  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaWY002    46   ........C.  T.......A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaWY003     8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaWY004     8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaCO001     8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaCO002    47   ........C.  T...A.A.A  ........T.  ........A.  CT......T.  .?...A..G.  ...T......  ..
rnsaCO003    33   ........C.  ......A.A  ........T.  ..........  ......C...  .?...A....  ...T......  ..
rnsaCO004     8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaCO005    45   ..G.....C.  T...A.A.A  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaCO006    40   ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaCO007     8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaUT001    40   ..G.....C.  T.T.A.A.A  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaUT002    41   ..G.....C.  T...A.A.A  ........T.  ........A.  CT......T.  .....A..G.  ...T......  ..
rnsaUT003     8   ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
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rnsaUT004     40  ..G.....C.  T.T.A.ATA  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaUT005     40  ..G.....C.  T.T.A.ATA  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaUT006      8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rnsaUT007     48  ..G.....C.  T.T.A.A.A  .........  ........A.  C.......T.  ..G..A..G.  ...T......  ..
rnsaNM003     40  ..G.....C.  T.T.A.ATA  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNM004      8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T...C..  ..
rnsaNM008     38  ........C.  ........A  ........T.  .........  .........  .....A..G.  ...T...CA.  ..
rnsaNM012      8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T..????  ??
rnsaNM015     49  ..G.....C.  ....A.A.A  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNM016      8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T...C..  ..
rnsaSEBC001    8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rnsaSEBC003   50  ........C.  ........A  ........T.  .........  .........  .....A....  ...T...C..  ..
rnsaNEWA001   51  ........C.  ........A  ....C...T.  .........  .........  .....A....  ...T...C?.  ..
rnsaNEWA002   52  ..G.....C.  T.T.A.ATA  .........  ........A.  C.......T.  .....A....  ...T...CA.  ..
rnsaNEWA003   11  ........C.  T.......A  .........  .........  .........  .....A....  ...T...C..  ..
rnsaWA001      8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rnsaWA002     53  ........C.  T.T.....A  .........  .........  .........  .....A....  ...T......  ..
rnsaWA003     54  ..G.....C.  T.T.A.A.A  .........  ........A.  C.........  .....A..G.  ...T......  ..
rnsaWA004      9  ........C.  ........A  .........  .........  ......C...  .....A....  ...T......  ..
rnsaWA005      8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rnsaWA006     40  ..G.....C.  T.T.A.A.A  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR001   55  ?.G.....C.  T.T.A.A.A  .....T....  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR002   11  ........C.  T.....A.A  .........  .........  .........  .....A....  ...T......  ..
rnsaNEOR003   40  ..G.....C.  T.T.A.A.A  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR004   40  ..G.....C.  T.T.A.ATA  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR005   40  ..G.......  T.T.A.ATA  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR006    8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rnsaNEOR007   40  ..G.....C.  T.T.A.A.A  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR008   40  ..G.....C.  T.T.A.ATA  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR009   40  ..G.....C.  T.T.A.A.A  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR010   40  ..G.....C.  T.T.A.A.A  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR011    8  ........C.  ........A  ........T.  .........  .........  .....A....  ...T......  ..
rnsaNEOR012   40  ..G.....C.  T.T.A.A.A  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaNEOR013   40  ..G.....C.  T.T.A.A.A  .........  ........A.  C.......T.  .....A..G.  ...T......  ..
ybsaNWBC001   56  .........T  .CT...A.A  ........TT  C.......A.  .TGC...C..  ...A.A....  ...T..A...  ..
ybsaNWBC002   28  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..  ...A.A....  ...T......  ..
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ybsaNWBC003   57  .........  .CT...A.A  ........TT  C....A..A.  .TGC.T.CA.   ...A.A....   ...T..A...   ..
ybsaNWBC004   58  ...C.....T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..   .C.A.A....   ...T......   ..
ybsaNWBC005   59  .........  .CT...A.A  ........TT  C.......A.  .TGC.T.CA.   ...A.A....   ...T.GA...   ..
ybsaCAB001    28  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB002    60  ...C.....T  .CT...A.A  ....C....T  C....A..A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB003    61  .........T  .CT...A.A  ........TT  C.......A.  ..GC...C..   T..A.A....   ...T..A...   ..
ybsaCAB005    28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB006    28  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T.....?   ??
ybsaCAB007    62  ...C.....T  .CT...A.A  ....C...TT  C.......AA  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB008    58  ...C.....T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB009    58  ...C.....T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB010    63  .........  .CT...A.A  ........TT  C.......A.  .TGC.T.CA.   .?.A.A....   ...T..A...   ..
ybsaCAB011    43  .........T  T.T...A.A  ....C....T  C.......A.  .TGC...C..   .?.A.A....   ...T......   ..
ybsaCAB012    28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   .?.A.A....   ...T......   ..
ybsaCAB013    11  ........C.  T.......A  ..........  ..........  ..........   .....A....   ...T......   ..
ybsaCAB015    28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB016    64  ....T.....  ........A  ..........  ..........  ......C...   .....A....   ...T......   ..
ybsaCAB017    65  ...C.....T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..   ..GA.A....   ...T??????   ??
ybsaCAB018    66  .........T  .CT...A.A  ....C..G.T  C.......A.  ..GCT..C..   ...A.A....   ...T...?..   ..
ybsaCAB019    67  .........  .CT...A.A  T.......TT  C......TA.  .TGC...CA.   ...A.A....   ...T..A.A.   ..
ybsaCAB020    68  .........  .CT...A.A  ........TT  C.......AA  .TGC.T.CA.   ...A.A....   ...T..A...   ..
ybsaCAB021    28  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB022    63  .........  .CT...A.A  ........TT  C.......A.  .TGC.T.CA.   ...A.A....   ...T..A...   ..
ybsaCAB023    69  .........T  .CT...A.A  ........TT  C.......A.  .TGC...C..   ...A.A....   ...T..A...   ..
ybsaCAB024    70  .........  .CT...A.A  ........TT  C.......A.  ..GC...CA.   ...A.A....   ...T..A...   ..
ybsaCAB025    28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaCAB026    71  .........T  .CT...A.A  ........TT  C.......A.  .TGC...C..   T..A.A....   ...T..A...   ..
ybsaCAB027    11  ........C.  T.......A  ..........  ..........  ..........   .....A....   ...T......   ..
ybsaSK001     72  .........T  .CT...A.A  T...C..G.T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaSK002     73  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A   ...A.A....   ...T..A...   ..
ybsaSK003     74  ...C.....T  .CT...A.A  ....C..G.T  CC......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaSK004     75  ...C.....T  .CT..TA.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaSK005     69  .........T  .CT...A.A  ........TT  C.......A.  .TGC...C..   ...A.A....   ...T..A...   ..
ybsaSK006     76  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.AC...   ...T......   ..
ybsaSK007     77  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGCT..C..   ...A.A....   ...T...C..   ..
ybsaSK008     75  ...C.....T  .CT..TA.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
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ybsaSK009     76  ...C.....T  .CT...A.A  ....C....T  C.....C.A.  .TGC...C..   ...A.AC...   ...T......   ..
ybsaSK010     78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A   ...A.A....   ...T..A...   ..
ybsaSK011     79  ...C.....T  .CT...A.A  ....C.....  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaSK012     80  .........T  .CT...A.A  ....C....T  C.......A.  ..GC...C..   ...A.A....   ...T......   ..
ybsaSK013     58  .........T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaSK014     79  ...C.....T  .CT...A.A  ....C.....  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaWA002      8  ........C.  ........A  ........T.  ..........  ..........   .....A....   ...T...AA.   ..
ybsaNM001     76  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.AC...   ...T......   ..
ybsaIL001     81  ...C.....T  .CT..TA.A  ....C.....  C.......A.  .TGC......   ...A.A....   ...T......   ..
ybsaIL002     57  ..........  .CT...A.A  ........TT  C....A..A.  .TGC.T.CA.   ...A.A....   ...T..A...   ..
ybsaIL003     82  ..........  .CT...A.A  T.......TT  C.......A.  ..GC...CA.   ...A.A....   ...T..A...   ..
ybsaIL004     83  .........T  .CT...A.A  .A.......T  C.......A.  .TGC...C.A   ...A.A....   ...T..A...   ..
ybsaIL005     84  .........T  .CT...A.A  ........T.  C.......A.  .TGC...C..   ...A.A....   ..AT......   ..
ybsaCAB028    85  .........T  .CT...A.A  ........TT  C.......A.  .TGC...C..   ...A.A....   ..AT..A...   ..
ybsaIL006     28  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL007     86  .........T  .CT...A.A  .A......TT  C.......A.  ..GC...C.A   T..A.A....   ...T..A...   ..
ybsaIL008     78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A   ...A.A....   ...T..A...   ..
ybsaIL009     87  ..G......T  .CT...A.A  ........TT  C....A..A.  ..GC...C..   ...A.A....   ...T.GA...   ..
ybsaIL010     88  .........T  .CT...A.A  ........TT  C.......A.  .TGC.T.CA.   ...A.A....   ...T..A...   ..
ybsaIL011     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL012     89  .........T  .CT...A.A  ....C...TT  C.......A.  ..GC...C..   ...A.A....   ...T......   ..
ybsaIL013     72  .........T  .CT...A.A  T...C..G.T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL014     69  .........T  .CT...A.A  ........TT  C.......A.  .TGC...C..   ...A.A....   ...T..A...   ..
ybsaIL015     28  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL016     60  .........T  .CT...A.A  ....C....T  C....A..A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL017     58  ...C.....T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL018     90  ....T....T  .CT.....A  ..C.....TT  C.......A.  .TGC...C..   ...A.A....   ...T..A...   ..
ybsaIL019     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL020     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL021     91  ..G......T  .CT...A.A  .........T  C......TAA  .TGC...C..   ...A.A....   ...T..A...   ..
ybsaIL022     92  .........T  .CT...A.A  ........T.  C.......AA  ..GC...C..   ...A.A....   ...T..A...   ..
ybsaIL023     93  ..........  .CT...A.A  ........TT  C.......AA  .TGC...CA.   ...A.A....   ...T..A...   ..
ybsaIL024     28  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL025     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL026     94  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A   ...A.A....   G..T..A...   ..
ybsaIL027     79  ...C.....T  .CT...A.A  ....C.....  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
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ybsaIL028     58  .........T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL029     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL030     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL031     95  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL032     78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A   ...A.A....   ...T..A...   ..
ybsaIL033     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL034     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL035     96  .........T  .C....A.A  ........TT  C.......A.  .TGC...C..   ...A.A....   ...T..A...   ..
ybsaIL036     97  ...C.....T  ..T...A.A  ....C.....  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL037     62  ...C.....T  .CT...A.A  ....C...TT  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL038     28  ...C.....T  .CT...A.A  ....C....T  C.......AA  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL039     98  ...C.A...T  .CT...A.A  ....C....T  CCT.....A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaIL040     79  ...C.....T  .CT...A.A  ....C.....  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI001     43  .........T  T.T...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI002     99  ...C.....T  .CT...A.A  ....C....T  CCT.....A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI003     28  ...?.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI004    100  .........T  .CT..TA.A  ........TT  C.......A.  ..GC...C..   ...A.A....   ...T..A...   ..
ybsaMI006    101  ........CT  .CT.....A  ..C.....TT  C.......A.  .TGC...C..   ...A.A....   ...T..A...   ..
ybsaMI007    102  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI008    103  ...C.....T  .CT...A.A  ....C....T  C......TA.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI009    104  ..G......T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A   ...A.A....   ...T......   ..
ybsaMI010    105  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI012     78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A   ...A.A....   ...T..A...   ..
ybsaMI013     28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI014    106  .........T  .CT.....A  ..C.....TT  C.......A.  .TGC...C..   ...AAA....   ...T..A...   ..
ybsaMI015     75  ...C.....T  .CT..TA.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI016     58  .........T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaMI017     79  ...C.....T  .CT...A.A  ....C.....  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaON001     84  .........T  .CT...A.A  ........T.  C.......A.  .TGC...C..   ...A.A....   ..AT......   ..
ybsaON002     82  ..........  .CT...A.A  T.......TT  C.......A.  ..GC...CA.   ...A.A....   ...T......   ..
ybsaON006     78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A   ...A.A....   ...T..A...   ..
ybsaON018    107  .......G.T  .CT...A.A  .A......TT  C....A..A.  .TGC...C.A   ...A.A....   ...T..A...   ..
ybsaON020    108  .........T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
ybsaON021    109  .........T  .CT...A.A  ........TT  C.........  .TGC.T.CA.   ...A.A....   ...T......   ..
ybsaON023     40  ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.   .....A..G.   ...T......   ..
ybsaON025    110  .........T  TCT...A.A  .A......TT  C....A..A.  .TGC...C.A   ...A.A....   ...T..A...   ..
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ybsaON026     58  .........T  .CT...A.A  ....C..G.T  C.......A.  .TGC...C..  ...A.A....  ...T......  ..
ybsaON029     78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A  ...A.A....  ...T..A...  ..
ybsaON030    111  .........T  .CT.....A  ..C.....TT  CC......A.  .TGC...C..  ...A.A....  ...T..A...  ..
ybsaON031    112  ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
ybsaON032    113  .........T  .GT...A.A  .A......TT  C.......A.  .TGC...C.A  ...A.A....  ...T..A...  ..
ybsaON033    114  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A  ...A.A....  ...T..A...  ..
ybsaON035    115  ....T...CT  .CT.....A  ..C.....TT  C.......A.  .TGC...C..  ...A.A....  ...T..A...  ..
ybsaON036     84  .........T  .CT...A.A  ........T.  C.......A.  .TGC...C..  ...A.A....  ..AT......  ..
ybsaON039    116  .........T  .CT...A.A  T...C..G.T  C.......A.  ..GC...C..  ...AAGCA.C  .AA.G.A..T  TA
ybsaON040    117  .........T  .CT...A.A  ........TT  C.......A.  .TG....C.A  ...A.A....  ...T..A...  ..
ybsaON042    118  .........T  .CT...A.A  ........TT  C.......A.  ..GC...C..  ...A.A....  ...T..A...  ..
ybsaNSNB008  119  ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
ybsaNSNB014  120  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A  ...A.A....  ...T.GA...  ..
ybsaNSNB016  121  .A..TTA...  T.TT..AT.  ...C.TG.TT  A...CAC.AA  ..GC...C..  ...AAGCA.C  .AA.G.A..T  TA
ybsaNSNB017  122  ...C.....T  .CT...A.A  ........TT  C.......A.  .TGC...C.A  .?.A.A....  ...T..A...  ..
ybsaNSNB018   78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A  ...A.A....  ...T......  ..
ybsaNC001     78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A  ...A.A....  ...T..A...  ..
ybsaNC002    123  ..........  .CT...A.A  T.......TT  C.......AA  .TGC...CA.  ...A.A....  ...T..A...  ..
ybsaNC003    124  ......A.CT  .CT.....A  ..C.....TT  C.......A.  .TGC...C..  ...A.A....  ...T..A...  ..
ybsaNC004    125  .........T  .CT...A.A  .A......TT  C.......A.  .TG....C.A  ...A.A....  ...T..A...  ..
ybsaNC005    101  ........CT  .CT.....A  ..C.....TT  C.......A.  .TGC...C..  ...A.A....  ...T..A...  ..
ybsaNC006    101  ........CT  .CT.....A  ..C.....TT  C.......AA  .TGC...C..  ...A.A....  ...T..A...  ..
ybsaNC007    126  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A  .A.A.A....  ...T..?...  ..
ybsaNC009    127  ..........  .CT...A.A  .A......TT  C.......A.  .TGC...C.A  ...A.A....  ...T.GA...  ..
ybsaNC010    101  ........CT  .CT.....A  ..C.....TT  C.......A.  .TGC...C..  ...A.A....  ...T..A...  ..
ybsaFL002     78  .........T  .CT...A.A  .A......TT  C.......A.  .TGC...C.A  .?.A.A....  ...T..A...  ..
ybsaLA001    128  ...C.....T  .CT...A.A  ....C..G.T  C.......A.  ..GC...C..  ...A.A....  ...T.G....  ..
ybsaLA002    129  ...C.....T  .CT...A.A  ....C....T  C......TA.  .TGC...C..  ...A.A....  ...T......  ..
rnsaCAB006     8  ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaCAB008     8  ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaCAB009    40  ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaCAB013     8  ........C.  ........A  ........T.  ......?...  ..........  .....A....  ...T......  ..
rnsaCAB014     8  ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
rnsaCAB016    40  ..G.....C.  T.T.A.ATA  ..........  ........A.  C.......T.  .....A..G.  ...T......  ..
rnsaCAB017     8  ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
ybsaCAB039   130  ........C.  ........A  ........T.  ..........  ..........  .....A....  ...T......  ..
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rnsaSAB030    42  .........T  .CT...A.A  ....C....T  C.......A.  ..GC...C..   ...A.A....   ...T......   ..
rnsaSAB031   131  .........T  .CT...A.A  ....C....T  C.......A.  ..GC...C..   ...A.A....   G..T......   ..
rnsaSAB032    11  ........C.  T.......A  ..........  ..........  ..........   .....A....   ...T......   ..
rnsaSAB033    28  ...C.....T  .CT...A.A  ....C....T  C.......A.  .TGC...C..   ...A.A....   ...T......   ..
rnsaSAB034    40  ..G.....C.  T.T.A.A.A  ..........  ........A.  C.......T.   .....A..G.   ...T......   ..
rnsaSAB035   132  ........C.  T.......A  ..........  ..........  ..........   .....A....   ...T...A..   ..
rnsaSEBC009  133  ........C.  ........A  ........T.  ..........  ..........   .....A....   ...T......   ..
rnsaSEBC010    8  ........C.  ........A  ........T.  ..........  ..........   .....A....   ...T......   ..
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Appendix 1.5. Composite genotypes of all individuals with complete COI genotypes used in Chapter 2, CR clade

		clade 3	no clade info	clades 1+4	all RBSA
rbsa	CTGG	88	16	3	107
	TCAG	2	1	1	4
	TCGG	2	6		8
	CTAG		4		4

		clade 2	clade 3	no clade info	clade 1	all RNSA
rnsa	CTGG	12	9	34	2	57
	TCAG	5	20	8		33
	TCGG	3	5	8	1	17
	CTAG	2	20	2		24

ybsa		clade 1	no clade info	clades 2-4	all YBSA
	CTGG				0
	TCAG	1			1
	TCGG				0
	CTAG				0
	CTAA	2			2
	TCAA	238	110	4	352

APPENDIX 2: Supplementary Information for Chapter 3

High rates of introgression between *S. nuchalis* and *S. varius* in central Alberta hybrid zone

Appendix 2.1 Sample ID, location, coordinates, source, and band/museum ID for each individual used in Chapter 3, listed by population. Museum samples from the Museum of Southwest Biology (MSB), University of Washington Burke Museum (UWBM), Royal Alberta Museum (RABM), Field Museum Natural History (FMNH), New Brunswick Museum (NBM), Canadian Museum of Nature (CMN), and Royal Saskatchewan Museum (RSKM).

ID	Location	Lat (˚N)	Long (˚W)	Source	Band/Museum ID
GBS Samples					
rnsaCAB001	James River, bridge on 584, AB	51.9000	-115.0000	RABM	Z94.13.9
rnsaCAB002	James River, Wilson James Campsite, AB	51.8330	-115.2170	RABM	Z94.13.10
rnsaCAB003	James River, Wilson James Campsite, AB	51.8330	-115.2170	RABM	Z94.13.11
rnsaCAB003	James River, Wilson James Campsite, AB	51.8330	-115.2170	RABM	Z94.13.11
rnsaCAB004	9 miles west of Bearberry, Improvement District 10, AB	51.8670	-115.0830	RABM	Z95.11.14
rnsaCAB004	9 miles west of Bearberry, Improvement District 10, AB	51.8670	-115.0830	RABM	Z95.11.14
rnsaCAB005	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.4
rnsaCO004	Gunnison, Gunnison Co. Colorado	38.8412	-106.4783	RABM	53394 GAV 257
rnsaCO005	Park Co. Colorado	39.4402	-105.7698	RABM	56359 GAV 858
rnsaCO006	Gunnison, Gunnison Co. Colorado	38.5474	-106.9214	RABM	56363 GAV 862
rnsaNM003	San Marcial, NM	33.6339	-107.0084	RABM	28952, NK 170195
rnsaNM008	Black Range, NM	32.9438	-107.7040	RABM	29241, NK 170616
rnsaNM017	Guadalupe Mountains, Dark Canyon, NM	32.1116	-104.7387	RABM	29309, NK 35775
rnsaSAB016	Porcupine Hills, AB	50.0172	-114.0522	RABM	Z02.14.9
rnsaSAB017	Beaver Creek, Porcupine Hills, AB	49.8167	-113.9500	RABM	Z96.18.9
rnsaWA001	Easton, Kittitas Co. WA	47.2418	-121.1843	UWBM	49988 CSW 3959
rnsaWA003	Kittitas Co. WA	47.1895	-120.6329	UWBM	49001 CSW 4984
rnsaWA004	Kittitas Co. WA	47.1895	-120.6329	UWBM	49002 CSW 4985
rnsaWA004	Kittitas Co. WA	47.1895	-120.6329	UWBM	49002 CSW 4985
rnsaWA005	Cle Elum, Kittitas Co. WA	47.1333	-120.9000	RABM	63675 MAM 18

rnsaWA006	Kirkland, King Co. WA	47.6719	-122.2035	UWBM	85284 SEZ 045
rnybCAB001	James River area, AB	51.8685	-115.0437	RABM	Z09.8.1
rnybCAB002	James River area, AB	51.6554	-115.2607	RABM	Z09.8.2
rnybCAB005	James River area, AB	52.0256	-115.1573	RABM	Z09.8.5
rnybCAB009	James River area, AB	51.8678	-115.0068	RABM	Z10.3.4
rnybCAB011	James River area, AB	51.8685	-115.0156	RABM	Z10.3.6
rnybCAB014	Clearwater River area, AB	51.9890	-115.2330	RABM	Z10.3.9
rnybCAB018	James River area, AB	51.8681	-115.1149	RABM	Z10.3.14
rnybCAB023	1.5 miles south Strachan, Improvement District 10, AB	52.2000	-115.1000	RABM	Z95.9.1
rnybCAB024	Cow Lake area, Improvement District 10, AB	52.2670	-115.0330	RABM	Z95.9.7
rnybCAB025	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.7
rnybCAB026	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.6
rnybCAB030	James River area, west of Sundre, AB	51.7774	-115.2274	RABM	Z11.5.10
rnybCAB033	Vicary Creek Area, AB	49.8215	-114.4246	UWBM	Z12.2.7
rnybCAB034	Vicary Creek Area, AB	49.7995	-114.4678	UWBM	Z12.2.20
rnybCAB035	Porcupine Hills, AB	50.0243	-114.0447	RSKM	Z12.2.27
rnybCAB036	Porcupine Hills, AB	50.0235	-114.0457	RSKM	Z12.2.30
rnybCAB037	Kananaskis Country, AB	51.0265	-114.8734	RSKM	Z12.3.1
rnybCAB038	Kananaskis Country, AB	51.0526	-114.8117	RSKM	Z12.3.2
rnybCAB039	Kananaskis Country, AB	50.6437	-114.4661	RSKM	Z12.3.9
ybsaCAB013	James River area, AB	51.8059	-115.2022	RSKM	Z10.3.13
ybsaCAB021	Brule area, AB	53.4005	-117.8685	RSKM	Z11.4.30
ybsaCAB022	James River area, west of Sundre, AB	51.8900	-115.0475	RSKM	Z11.5.2
ybsaCAB025	Clearwater River area, AB	52.0390	-115.1604	UWBM	Z11.5.9
ybsaCAB027	James River area, west of Sundre, AB	51.8490	-115.0182	UWBM	Z11.5.14
ybsaNC003	Dillingham, Buncombe Co. NC	35.7537	-82.4070	UWBM	86868 RBB 538
ybsaNC004	Murchison, Buncombe Co. NC	35.8176	-82.2993	UWBM	86869 RBB 539
ybsaSK007	Grand Coulee, SK	50.4333	-104.8167	UWBM	A16687/E001

ybsaSK008	Ramada Inn, Regina, SK	50.4500	-104.6167	MSB	A16698/E002
ybsaSK009	Regina, SK	50.4500	-104.6167	MSB	A17618/E002
ybsaSK010	Rouleau, SK	50.1833	-104.9333	MSB	A20030/E
ybsaSK011	Regina, SK	50.4500	-104.6167	UWBM	A20040/E001
ybsaSK012	RSM, Regina, SK	50.4500	-104.6167	UWBM	A18288/E001
ybsaSK013	RSM, Regina, SK	50.4500	-104.6167	UWBM	A16729/E001
ybsaSK014	Regina, SK	50.4500	-104.6167	UWBM	A17134/E002
rnsaSAB029	HWY6, AB	49.0370	-113.6814	Wild	991-19845
rnsaSAB030	Stables Road, Waterton, AB	49.0620	-113.8898	Wild	921-16791
rnsaSAB031	Stables Road, Waterton, AB	49.0667	-113.8845	Wild	921-16792
rnsaSAB032	Haybarn, Waterton Lakes, AB	49.0796	-113.8593	Wild	921-16793
rnsaSAB033	Canyon Road, Waterton Lakes, AB	49.0945	-113.8382	Wild	921-16794
rnsaSAB034	Canyon Road, Waterton Lakes, AB	49.0945	-113.8382	Wild	921-16795
rnsaSAB035	Westcastle Wetlands, near Beavermines, AB	49.3766	-114.3784	Wild	921-16796
rnsaSAB036	Allison Creek Road 2 Crowsnest Pass, AB	49.6891	-114.6038	Wild	921-16717
rnsaSAB037	Allison Creek Road 4 Crowsnest Pass, AB	49.6781	-114.5833	Wild	921-16718
Traditional Methods Samples					
rnsaCAB002	James River, Wilson James Campsite, AB	51.8330	-115.2170	RABM	Z94.13.10, rnsa 1
rnsaCAB003	James River, Wilson James Campsite, AB	51.8330	-115.2170	RABM	Z94.13.11, rnsa 2
rnsaCAB004	Bearberry, Improvement District 10, AB	51.8670	-115.0830	RABM	Z95.11.14, rnsa 4
rnsaCAB005	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.4, rnsa 5
rnsaCAB006	Vicary Creek Area, AB	49.8046	-114.4531	RABM	Z12.2.9
rnsaCAB007	Vicary Creek Area, AB	49.8008	-114.4712	RABM	Z12.2.13
rnsaCAB008	Porcupine Hills	50.0237	-114.0459	RABM	Z12.2.28
rnsaSAB019	Beaver Creek, Porcupine Hills	49.8333	-113.9667	RABM	Z96.18.30
rnsaCAB001	James River, bridge on 584, AB	51.9000	-115.0000	RABM	Z94.13.9
ybsaCAB011	Smoke Lake, AB	54.3540	-116.9540	RABM	Z99.10.46
ybsaCAB020	Brule area	53.4020	-117.8442	RABM	Z11.4.18
ybsaCAB023	James River area, west of Sundre	51.8941	-115.0138	RABM	Z11.5.4

rnybCAB001	James River area	51.8685	-115.0437	RABM	Z09.8.1
rnybCAB002	James River area	51.6554	-115.2607	RABM	Z09.8.2
rnybCAB003	Red Deer River area	51.5918	-115.1682	RABM	Z09.8.3
rnybCAB004	Burnt Timber Creek	51.8912	-115.0333	RABM	Z09.8.4
rnybCAB005	James River area	52.0256	-115.1573	RABM	Z09.8.5
rnybCAB006	Clearwater River area	51.6437	-115.0417	RABM	Z09.8.7
rnybCAB007	Red Deer River area	51.6554	-115.2607	RABM	Z10.3.2
rnybCAB008	Red Deer River area	51.8483	-115.1166	RABM	Z10.3.3
rnybCAB009	James River area	51.8678	-115.0068	RABM	Z10.3.4
rnybCAB010	James River area	51.8460	-115.0315	RABM	Z10.3.5
rnybCAB011	James River area	51.8685	-115.0156	RABM	Z10.3.6
rnybCAB012	James River area	51.8503	-115.1040	RABM	Z10.3.7
rnybCAB013	James River area	51.8722	-115.1365	RABM	Z10.3.8
rnybCAB014	Clearwater River area	51.9890	-115.2330	RABM	Z10.3.9
rnybCAB015	Clearwater River area	52.0394	-115.1595	RABM	Z10.3.10
rnybCAB016	Clearwater River area	52.0255	-115.1575	RABM	Z10.3.11
rnybCAB017	Clearwater River area	51.8202	-115.1865	RABM	Z10.3.12
rnybCAB018	James River area	51.8681	-115.1149	RABM	Z10.3.14
rnybCAB019	James River area	51.8714	-115.1376	RABM	Z10.3.15
rnybCAB020	Bearberry, Improvement District 10, AB	51.8330	-115.1000	RABM	Z95.11.11
rnybCAB021	Bearberry, Improvement District 10, AB	51.8670	-115.1000	RABM	Z95.11.13
rnybCAB022	Bearberry, Improvement District 10, AB	51.8670	-115.1000	RABM	Z95.11.12
rnybCAB023	Strachan, Improvement District 10, AB	52.2000	-115.1000	RABM	Z95.9.1
rnybCAB024	Cow Lake area, Improvement District 10, AB	52.2670	-115.0330	RABM	Z95.9.7
rnybCAB025	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.7
rnybCAB026	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.6
rnybCAB027	James River area, west of Sundre	51.8690	-115.1134	RABM	Z11.5.1
rnybCAB028	James River area, west of Sundre	51.8718	-115.1060	RABM	Z11.5.5
rnybCAB029	James River area, west of Sundre	51.8730	-115.1371	RABM	Z11.5.6

rnybCAB030	James River area, west of Sundre	51.7774	-115.2274	RABM	Z11.5.10
rnybCAB031	James River area, west of Sundre	51.8210	-115.1858	RABM	Z11.5.11
rnybCAB032	Red Deer River area	51.6441	-115.0413	RABM	Z11.5.12
rnybCAB033	Vicary Creek Area	49.8215	-114.4246	RABM	Z12.2.7
rnybCAB034	Vicary Creek Area	49.7995	-114.4678	RABM	Z12.2.20
rnybCAB035	Porcupine Hills	50.0243	-114.0447	RABM	Z12.2.27
ybsaCAB001	Olds, AB	51.7916	-114.2862	Wild	1731-05301
ybsaCAB002	Innisfail, AB	54.0238	-110.9824	Wild	ybsa 2
ybsaCAB003	Buck Lake, AB	54.9721	-115.6046	Wild	ybsa 3
ybsaCAB005	Buck Lake, AB	54.9721	-115.6046	Wild	ybsa 4
ybsaCAB006	Bearberry, Improvement District 10, AB	51.8670	-115.0830	RABM	Z95.11.15, ybsa 6
ybsaCAB007	Brazeau Reservoir, Improvement District 14, AB	52.9170	-115.3670	RABM	Z95.15.18, ybsa 9
ybsaCAB008	Alder Flats, Improvement District 11, AB	52.9170	-115.0670	RABM	Z95.15.22, ybsa 10
ybsaCAB009	Strachan, Improvement District 10, AB	52.2000	-115.1330	RABM	Z95.9.16, ybsa 12
ybsaCAB010	Cow Lake area, Improvement District 10, AB	52.2830	-115.0000	RABM	Z95.9.9, ybsa 14
ybsaCAB012	Smoke Lake, AB	54.3540	-116.9540	RABM	Z99.10.46, ybsa 15
ybsaCAB013	James River area	51.8686	-115.1130	RABM	Z09.8.6
ybsaCAB014	James River area	51.8059	-115.2022	RABM	Z10.3.13
ybsaCAB015	Bearberry, Improvement District 10, AB	51.8330	-115.1000	RABM	Z95.11.10, ybsa 2
ybsaCAB016	James River, Improvement District 10, AB	51.7670	-115.2170	RABM	Z95.12.5, ybsa 8
ybsaCAB017	Conklin area	55.6202	-111.1189	RABM	Z11.2.2
ybsaCAB018	Conklin area	55.6137	-111.1470	RABM	Z11.2.3
ybsaCAB019	Brule area	53.3945	-117.8140	RABM	Z11.4.9
ybsaCAB021	Brule area	53.4005	-117.8685	RABM	Z11.4.30
ybsaCAB022	James River area, west of Sundre	51.8900	-115.0475	RABM	Z11.5.2
ybsaCAB024	Clearwater River area	52.0250	-115.1547	RABM	Z11.5.8
ybsaCAB025	Clearwater River area	52.0390	-115.1604	RABM	Z11.5.9
ybsaCAB026	James River area, west of Sundre	51.8461	-115.0314	RABM	Z11.5.13
ybsaCAB027	James River area, west of Sundre	51.8490	-115.0182	RABM	Z11.5.14

ybsaCAB028	Alder Flats, AB	52.9300	-115.0500	RABM	Z02.15.7
ybsaCAB029	St. Albert, Sturgeon Municipal District, AB	53.6330	-113.6330	RABM	Z81.77.1
ybsaCAB030	Fort Saskatchewan, Strathcona County, AB	53.7170	-113.2170	RABM	Z81.146.87
ybsaCAB031	Sherwood Park, Strathcona County, AB	53.5170	-113.3170	RABM	Z87.34.3
ybsaCAB032	Edmonton, Edmonton Municipal District, AB	53.5330	-113.5330	RABM	Z88.17.1
ybsaCAB033	Lodgepole, Parkland County, AB	53.0500	-115.3170	RABM	Z88.19.94
ybsaCAB034	Edmonton, Edmonton Municipal District, AB	53.5330	-113.5330	RABM	Z88.32.6
ybsaCAB035	Edmonton, Edmonton Municipal District, AB	53.5330	-113.5330	RABM	Z88.36.11
ybsaCAB036	Edmonton, Edmonton Municipal District, AB	53.5330	-113.5330	RABM	Z89.72.1
ybsaCAB037	Ardrossan, Strathcona County, AB	53.5500	-113.0670	RABM	Z00.31.1
ybsaCAB041	Saddle Hills area, AB	55.7800	-119.9476	RABM	Z13.4.41
ybsaCAB042	James River area, AB	51.8361	-115.1563	RABM	Z15.4.12
ybsaCAB043	Saddle Hills area, AB	55.7686	-119.8388	RABM	Z13.4.52
ybsaCAB044	James River area, AB	51.8903	-115.0313	RABM	Z16.5.12
ybsaCAB045	Ardrossan, AB	53.5477	-113.0236	RABM	Z16.1.35
ybsaCAB046	Ardrossan, AB	53.5477	-113.0236	RABM	Z16.1.32
ybsaCAB047	James River area, AB	51.8474	-115.1135	RABM	Z15.4.15
ybsaCAB048	Saddle Hills area, AB	55.6941	-119.5916	RABM	Z13.4.60
ybsaCAB049	Ardrossan, AB	53.5477	-113.0236	RABM	Z16.1.31
ybsaCAB050	James River area, AB	51.7792	-115.2800	RABM	Z15.4.11
ybsaCAB051	James River area, AB	52.0038	-115.2091	RABM	Z16.5.11
ybsaCAB052	St. Paul, AB	54.0567	-110.9710	RABM	Z16.1.27
ybsaCAB053	Saddle Hills area, AB	55.5746	-119.4777	RABM	Z13.4.13
ybsaCAB054	James River area, AB	51.8706	-115.1084	RABM	Z16.5.6
ybsaCAB055	Saddle Hills area, AB	55.6701	-119.6615	RABM	Z13.4.25
ybsaCAB056	Fort Smith, AB	59.9779	-111.8291	RABM	Z13.5.7
ybsaCAB057	James River area, AB	51.9892	-115.2318	RABM	Z16.5.10
ybsaCAB058	James River area, AB	51.8475	-115.1131	RABM	Z16.5.13
ybsaCAB059	James River area, AB	51.7792	-115.2800	RABM	Z15.4.10

ybsaCAB060	Alder Flats, Improvement District 11, AB	52.9300	-115.0500	RABM	Z93.15.2
ybsaCAB061	Saddle Hills area, AB	55.7489	-119.8904	RABM	Z13.4.46
ybsaCAB062	Ardrossan, AB	53.5477	-113.0236	RABM	Z14.3.19
ybsaCAB063	Ministik, AB	53.4010	-113.0006	RABM	Z16.1.33
ybsaCAB064	Waterton Lakes Townsite, AB	49.0512	-113.9115	RABM	Z16.1.29
ybsaCAB065	Ardrossan, AB	53.5477	-113.0236	RABM	Z16.1.34
ybsaCAB066	James River area, AB	51.7831	-115.2926	RABM	Z16.5.14
ybsaCAB067	Saddle Hills area, AB	55.6659	-119.6615	RABM	Z13.4.24
ybsaCAB068	Alder flats area, AB	52.9316	-115.0483	RABM	Z15.10.3
ybsaCAB069	James River area, AB	51.8474	-115.1135	RABM	Z15.4.14
ybsaCAB070	Calling Lake area, AB	55.2250	-113.0524	RABM	Z16.4.3
ybsaSK001	Treebeard Trail, Prince Albert NP, SK	53.9725	-106.2903	Wild	961-43502
ybsaSK002	Narrows Campground, Campsite 82, Prince Albert NP, SK	53.9807	-106.2938	Wild	921-16800
ybsaSK003	Narrows Campground, Campsite 82, Prince Albert NP, SK	53.9807	-106.2938	Wild	921-16799
ybsaSK004	Freight Trail, 'D' entrance, Prince Albert NP, SK	53.7067	-106.0536	Wild	961-43503
ybsaSK005	Argyle Street, Regina, SK	50.4500	-104.6167	RSKM	A6548/18264/E1
ybsaSK006	Regina, SK	50.4410	-104.6369	RSKM	A6227/18105/E001
ybsaSK007	Grand Coulee, SK	50.4333	-104.8167	RSKM	A16687/E001
ybsaSK008	Ramada Inn, Regina, SK	50.4500	-104.6167	RSKM	A16698/E002
ybsaSK009	Regina, SK	50.4500	-104.6167	RSKM	A17618/E002
ybsaSK010	Rouleau, SK	50.1833	-104.9333	RSKM	A20030/E
ybsaSK011	Regina, SK	50.4500	-104.6167	RSKM	A20040/E001
ybsaSK012	RSM, Regina, SK	50.4500	-104.6167	RSKM	A18288/E001
ybsaSK013	RSM, Regina, SK	50.4500	-104.6167	RSKM	A16729/E001
ybsaSK014	Regina, SK	50.4500	-104.6167	RSKM	A17134/E002
ybsaIL001	Chicago, Madison at LaSalle, Cook Co, IL	41.8819	-87.6325	FMNH	350791
ybsaIL002	Chicago, Ontario and State, Cook Co, IL	41.8932	-87.6284	FMNH	395391

ybsaIL003	Chicago, N side, Cook Co, IL	41.8973	-87.6181	FMNH	434988
ybsaIL004	Chicago, 233 N Michigan Ave, Cook Co, IL	41.8870	-87.6237	FMNH	439018
ybsaIL005	Chicago, Blue Cross Building, Cook Co, IL	41.8846	-87.6204	FMNH	439019
ybsaIL006	Chicago, Illinois Center, Cook Co, IL	41.8879	-87.6242	FMNH	446011
ybsaIL007	Chicago, AON, Cook Co, IL	41.8849	-87.6209	FMNH	446015
ybsaIL008	Chicago, Hyatt Center, Cook Co, IL	41.8881	-87.6227	FMNH	446782
ybsaIL009	Chicago, 311 S Wacker, Cook Co, IL	41.8775	-87.6362	FMNH	446988
ybsaIL010	Naperville, James and George, DuPage Co, IL	41.7718	-88.1273	FMNH	447149
ybsaIL011	Chicago, AON, Cook Co, IL	41.8849	-87.6209	FMNH	452273
ybsaIL012	Chicago, Sears, Cook Co, IL	41.9527	-87.7453	FMNH	452274
ybsaIL022	Chicago, 800 N Wells, Cook Co, IL	41.8967	-87.6346	FMNH	454283
ybsaIL013	Chicago, Tribune Tower, Cook Co, IL	41.8903	-87.6238	FMNH	452275
ybsaIL014	Chicago, 111 E Wacker, Cook Co, IL	41.8775	-87.6362	FMNH	452276
ybsaNSNB001	York Co., NB	45.9700	-66.6500	NBM	010432
ybsaNSNB002	Saint John Co., NB	45.2700	-66.0500	NBM	007343
ybsaNSNB004	Victoria Co., NB	46.8830	-66.9170	CMN	71180
ybsaNSNB005	Restigouche Co., NB	46.8670	-66.3500	CMN	71181
ybsaNSNB006	Northumberland Co., NB	47.2330	-66.8670	CMN	71182
ybsaNSNB007	York Co., NB	45.9700	-66.6500	NBM	004985
ybsaNSNB008	Victoria Co., NB	47.2300	-67.1500	NBM	005060
ybsaNSNB009	Victoria Co., NB	47.2300	-67.1500	NBM	005432
ybsaNSNB010	York Co., NB	46.1200	-66.0800	NBM	005484
ybsaNSNB011	York Co., NB	46.1200	-66.8300	NBM	005485
ybsaNSNB012	Victoria Co., NB	47.2300	-67.1500	NBM	008700
ybsaNSNB013	Victoria Co., NB	47.2300	-67.1500	NBM	008288
ybsaNSNB014	Charlotte Co., NB	44.7000	-66.7300	NBM	008585