PHYLOGEOGRAPHY OF THREE HIGH LATITUDE RESIDENT CORVIDS: CLARK'S NUTCRACKER (*NUCIFRAGA COLUMBIANA*), EURASIAN NUTCRACKER (*NUCIFRAGA CARYOCATACTES*), AND GRAY JAY (*PERISOREUS CANADENSIS*)

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DEDICATION

For the owl and the pussy cat.

(And also the centipede.)

GENERAL ABSTRACT

High latitude resident bird species provide an unique opportunity to investigate patterns of postglacial and barrier-mediated dispersal. In this study, multiple genetic markers were used to understand postglacial colonization by and contemporary barriers to gene flow in three corvids. Clark's nutcracker (*Nucifraga columbiana*), Eurasian nutcracker (*N. caryocatactes*), and gray jay (*Perisoreus canadensis*) are year-round resident northern hemisphere passerines with ranges encompassing previously glaciated and unglaciated regions and potential barriers to dispersal (e.g. mountain ranges). Using mitochondrial DNA control region sequences, we found limited geographic genetic structure and one glacial refugium in nutcrackers, contrasting with seven distinct genetic groups and five refugia for gray jays. Nuclear microsatellite markers revealed additional and contrasting patterns of near-panmixia in nutcrackers and multiple hierarchical breaks in gray jays. Genetic patterns are explained by differences in natural history traits, specifically food preferences, for these species.

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> *Science is a way of life. Science is a perspective. Science is the process that takes us from confusion to understanding in a manner that's precise, predictive and reliable - a transformation, for those lucky enough to experience it, that is empowering and emotional.*
>
> Brian Greene

As I write these acknowledgements and reflect upon this project, I could never have predicted how true Greene's words would become. I suggest, though, that the scientist is not the only one that goes through this journey; she also takes a lot of people along for the ride. For all of those who were there for part or all of this process, I want to express my immense gratitude.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

3′	3′-end (tail end of DNA primer sequence)
5′	5′-end (beginning of DNA primer sequence)
A	adenine
ABI	Applied Biosystems
AI	Alberta Innovates
AMOVA	analysis of molecular variance
AMNH	American Museum of Natural History
A_n	number of alleles
ANOVA	analysis of variance
AOU	The American Ornithologists' Union
A_r	allelic richness
ATP	adenosine triphosphate or ATPase 6-8 coding region
AUC	Area Under the Curve
avg	average
bp	base pair
BAPS	Bayesian Analysis of Population Structure (software)
BIOCLIM	bioclimatic layers
C	cytosine
CCAC	Canadian Council on Animal Care
CLNU	Clark's nutcracker (*Nucifraga columbiana*)
CMN	Canadian Museum of Nature
cpDNA	chloroplast deoxyribonucleic acid
CR	mitochondrial control region
CWS	Canadian Wildlife Service
DEM	digital elevation model
DNA	deoxyribonucleic acid
DnaSP	DNA sequence polymorphism (software)
dNTP	deoxyribonucleotide triphosphate
EDTA	ethylenediaminetetraacetic acid
ENM	ecological niche model(ing)
F	forward primer (microsatellite)
FDR	false discovery rate
F_{ST}	Fixation index; measure of population differentiation
g	gram(s)
G	guanine
GBIF	Global Biodiversity Information Facility
GDMG	Geographic Distance Matrix Generator v1.2.3
GenBank	open access online sequence database
GIS	global information system
GRAJ	Gray jay (*Perisoreus canadensis*)
G_{ST}	Fixation index (Nei); measure of genetic differentiation
H	heavy strand

H_d	haplotype diversity
He	expected heterozygosity
HKY	Hasegawa-Kishino-Yano nucleotide substitution model
H_n	number of haplotypes
H_o	observed heterozygosity
HWE	Hardy-Weinberg equilibrium
IBD	Isolation by Distance
IOC	International Ornithological Congress
K	number of clusters/populations
ka	thousand years (geological)
km	kilometre
km^2	square kilometres
kya	thousand years ago
L	light strand
LD	linkage disequilibrium
LGM	last glacial maximum
ln	natural logarithm
LSU	Louisiana State University Museum of Natural Sciences
M	molar
M13	universal primer sequence tag
Ma	million years (geological)
MEGA	Molecular Evolutionary Genetics Analysis (software)
mg	milligram
mg/mL	milligrams per millilitre
$MgCl_2$	magnesium chloride
MJ	median joining (tree algorithm)
min	minutes
MIROC	Model for Interdisciplinary Research on Climate
mM	millimolar
mm	millimetre
MP	maximum parsimony
MSB	Museum of Southwest Biology
mtDNA	mitochondrial deoxyribonucleic acid
mya	million years ago
n	sample size
NaCl	sodium chloride
NBM	New Brunswick Museum
ND2	nicotinamide adenine dinucleotide dehydrogenase 2
nos	numbers
ns	not significant
NSERC	Natural Science and Engineering Research Council
NUCA	Eurasian nutcracker (*Nucifraga caryocatactes*)
P	probability value
PCR	polymerase chain reaction

pH	potential hydrogen
Pr	posterior probabilities
r	weighted calculated sequence mutation rate
R	reverse primer (microsatellite)
R^2	coefficient of determination
RAB	Royal Alberta Museum
RBM	Royal British Columbia Museum
ROM	Royal Ontario Museum
rpm	rotations per minute
RSM	Royal Saskatchewan Museum
R_{ST}	Fixation index (Slatkin); measure of genetic differentiation
s	seconds
SAMOVA	spatial analysis of molecular variance
SAP	shrimp alkaline phosphatase
SDM	species distribution model(ing)
SDS	sodium dodecyl sulfate
SE	standard error
spp	species
SPSS	Statistical Package for the Social Sciences
T	thymine or divergence time
T_1	annealing temperature one
T_2	annealing temperature two
TCS	Templeton Crandall and Sing method (software)
TE	tris-EDTA
TFM	The Field Museum
US	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
USNM	Smithsonian National Museum of Natural History
UWBM	Burke Museum of Natural History and Culture - University of Washington
v	version
Var	variable
ver	version
w/v	weight per volume
WORLDCLIM	global bioclimatic layers
°C	degrees Celsius
°N	degrees north of the equator
°W	degrees west of the meridian
δ	corrected number of nucleotide substitutions per site between populations
ΔK	delta K (Structure Harvester value)
µL	microlitre

μM	micromolar
Φ_{ST}	Phi $_{ST;}$ measure of genetic differentiation
π	nucleotide diversity
+G	discrete Gamma distribution
+I	evolutionarily invariable

C2-4: State and Province Abbreviations

AB	Alberta
BC	British Columbia
CO	Colorado
ID	Idaho
MN	Minnesota
MT	Montana
NB	New Brunswick
NE	Nebraska
NH	New Hampshire
NL	Newfoundland
NM	New Mexico
NS	Nova Scotia
NY	New York
ON	Ontario
PQ	Quebec
SK	Saskatchewan
UT	Utah
VT	Vermont
WA	Washington
WI	Wisconsin
WY	Wyoming

C2-4: Population/Genetic Group Abbreviations

AKA	Alaska Anchorage
AKD	Alaska Denali
AKF	Alaska Fairbanks
AKW	Alaska Wrangell
ANTI	Anticosti Island
BE	Boreal East
BK	Badzhal'skiy Krebet, Russia
CAB	central Alberta
CBC	central British Columbia
ceOR	central Oregon
coWA	coastal Washington
CCA	central California
CO-NM	Colorado-New Mexico
GA	Gorno-Altaysk, Russia

Gasp	Gaspé Peninsula
IMW	Intermountain West
KA	Kamchatka, Russia
KD	Irkutsk Oblast, Russia
Lab	Labrador
MA	Magadanskaya Oblast, Russia

Chapter 1: General introduction

1.1 Phylogeography

Phylogeography investigates the relationship between organisms' genetic lineages and geographic distribution (Avise 2009). The term phylogeography was originally coined by Avise *et al.* in 1987 after a decade of research using mitochondrial DNA has uncovered striking patterns in the relationships between genealogy and geography (Avise 2009) and has rapidly grown in species scope and molecular marker choice. In general, phylogeographers have found that genetic discontinuities are the product of past population isolation induced by historical events, geographic features, life-history characteristics (e.g. migration, behaviour), or a combination of two or more of those factors that hampered exchange of genes among isolated populations of organisms (Avise 2004).

On large geographic scales, there are three commonly cited models regarding genetic diversity in relation to geographic distribution: 1) The latitudinal model is based on climate oscillations and expansion pole-wards after glaciations, predicting a decrease in genetic diversity with increasing latitudes owing to decreased population sizes and shorter time since establishment in populations located in previously glaciated areas (Saitoh et al. 2010). 2) The species diversity model predicts that factors that contribute to species diversity may also contribute to genetic diversity (Vellend and Geber 2005). However, this model is

not mutually exclusive of the latitudinal model as species diversity has long been observed to decrease with increasing latitudes in many groups of organism, though the mechanism behind this pattern is still debated (Buckley et al. 2010). 3) The centre-marginal model predicts that the centre of a species distribution has the highest genetic diversity and the edges have the lowest owing to the centre of the range likely consisting of more prime habitat to experience gene flow and edges being more isolated and more recently colonized (Wisely et al. 2004; Eckert et al. 2008). However, these models are generalizations and can be complicated at smaller scales by barriers to gene flow and species-specific post-colonization events.

1.2 Barriers to gene flow

Barriers to organism's movement often translate into barriers to gene flow between populations. These barriers often leave lasting genetic signatures and can result in isolated and divergent populations. Barriers to gene flow can be broadly classified into two categories: behavioural/natural history and physical/geographic.

1.2.1 Behavioural/natural history

Species' movements across landscapes can be dictated by certain natural history traits, thus limiting or facilitating gene flow between populations. Limited post-natal dispersal and natal philopatry (Steeves et al. 2005), sex-biased dispersal

(Dubey and Shine 2010), sedentary behaviours (Graham and Burg 2012), different migratory and resident populations within species complexes (Salgado-Ortiz et al. 2008), and different migratory routes (Kelly and Hutto 2005) have all been shown to influence gene flow between populations, often contributing to genetically differentiated populations throughout a species' range. In addition, species- and population-specific foraging behaviours (Burg and Croxall 2001) and irruptive dispersal events for foraging purposes (Piertney and Summers 2001; Haring et al. 2007), as well as geographically differentiated song dialects (MacDougall Shackleton and MacDougall Shackleton 2001) and plumage characteristics (Brelsford and Irwin 2009) can promote (in the case of irruptive dispersal) or limit gene flow.

1.2.2 Physical/geographic

Worldwide, natural geographic features such as mountain ranges (Fok et al. 2002; Kelly and Hutto 2005; Fontanella et al. 2008), large water bodies (Soltis et al. 2006) or discontinuities in water flow (e.g. waterfalls, Kano et al. 2012), and river valleys (Bar Yaacov et al. 2012) can influence movement of organisms across landscapes, preventing gene flow and colonization of new habitats. Unsuitable (DeChaine and Martin 2005; Katinas and Crisci 2008) or anthropogenically fragmented (Van Tuinen et al. 2008) habitats can similarly provide barriers to movement, resulting in genetically isolated and divergent populations.

1.2.2.1 North America

Phylogeographic studies in North America have found many common geographic or physical barriers that influence genetic structure in a wide variety of high taxa. Five major mountain chains run north to south through the continent. These ranges, Coastal (Manthey et al. 2011), Cascade (Arbogast 2007), Sierra Nevada (Walstrom et al. 2012), Rocky (Jones et al. 2005), and Appalachian (Fontanella et al. 2008), and the areas between them have been shown to restrict movement between species' populations and gene flow. Large water bodies like the Salish Sea (Burg et al. 2006), Hecate Strait between Haida Gwaii and mainland British Columbia (Graham and Burg 2012), and the Strait of Belle Isle and Gulf of Saint Lawrence (Colbeck et al. 2008; Lait and Burg 2013) are also pointed to as barriers to colonization or gene flow. Genetic breaks have been noted across the Columbia River (Barrowclough et al. 2004), and Mississippi River (Soltis et al. 2006) valleys. Other barriers to gene flow and movement specific to high latitude arboreal species include large expanses of unsuitable habitat like the Snake River (Gugger et al. 2010), Great Basin (Albach et al. 2006; Runck and Cook 2005), and Wyoming Basin (Runck and Cook 2005), and the Okanagon Highlands (Brunsfeld et al. 2001).

1.2.2.2 Eurasia

In Eurasia, phylogeographic studies of widespread species have also revealed a variety of geographical patterns of population structure influenced by current

and historical barriers to movement. East-west splits corresponding with the north-south Volga River or Ural Mountains have been documented for a variety of vertebrates (Flanders et al. 2009; Kryukov et al. 2004; Haring et al. 2007; Zink et al. 2008). For other vertebrate species, multiple splits have been found across other mountain ranges (e.g. the Alps) or large areas of inhospitable habitat (e.g. Tibetan Plateau; Brunhoff et al. 2003; Marmi et al. 2006; Zink et al. 2008), in addition to isolated peninsula (e.g. Iberian Peninsula; Pitra et al. 2000) or island (e.g. Japan; Flanders et al. 2009) populations. In contrast, few barriers to gene flow have been detected in other widespread vertebrate species (Zink et al. 2002; Zink et al. 2008; Zhang et al. 2012). Given the expanse and topographically diverse nature of Eurasia, it would be unreasonable to expect corresponding patterns for all species.

1.3 Pleistocene glaciations

The Pleistocene epoch was a time of worldwide, dramatic climatic fluctuations that began approximately 1.8 million years ago (mya) and ended about 12,000 years ago (Hofreiter and Stewart 2009). Long glacial periods, when temperatures were up to 21°C lower than present day, were interspersed by shorter interglacials, with temperatures occasionally higher than present day (Hofreiter and Stewart 2009). Depending on distance from the equator, continental mass, mountain ranges, and ocean position and currents, these climate oscillations varied in strength of expression (Hewitt 1996). These climate oscillations (glacial-

interglacial/cold-warm) are thought to be the result of the Milankovitch cycle, a combination of variations in the cycles of the Earth's orbit: precession or wobble (19,000 and 23,000 year cycle), axial tilt (42,000 year cycle), and the dominant force of orbital eccentricity (100,000 and 400,000 year cycle; Hays et al. 1976; Webb III and Bartlein 1992; Hofreiter and Stewart 2009). These climate oscillations are thought to have played an important role in shaping species' distribution and diversity throughout a wide range of habitats (Hewitt 2004a). Much of the northern hemisphere was covered by large ice sheets, sea levels dropped substantially, and climate conditions changed considerably during the most recent glaciation, 110 – 12 thousand years ago (kya; Gibbard and Van Kolfschoten 2004). In the northern hemisphere, continental glaciers extended as far as 40°N in North America and 52°N in Europe, (Avise and Walker 1998), though much of northern Eurasia remained ice free, covered instead in thick layers of permafrost (Velichko et al. 1997; Lisitsyna and Romanovskii 1998; Naydenov et al. 2007).

1.3.1 The last glacial maximum

The last glacial maximum (LGM) occurred between 21 and 18 kya and is thought to be the most extensive glaciation in the Pleistocene (Clark et al. 2009). The ice reached its maximum geographic extent and extreme sea level drops uncovered 20% more land than at present in some areas (Pielou 1991; Hewitt 2004a). Distributions of flora and fauna shifted to escape the extremes of climatic

conditions and multiple lines of evidence suggest many mid- and high-latitude organisms spent the LGM in ice-free regions known as glacial refugia (Hofreiter and Stewart 2009).

In North America, the LGM occurred near the end of Wisconsin stage (Gibbard and Van Kolfschoten 2004). During this time, over 17 million km^2 of ice covered the continent (Clark et al. 2009) and two main ice sheets were present (Pielou 1991). The Cordilleran ice sheet in the west extended along the west side of the Rocky Mountains from northern Washington up to Alaska (Pielou 1991). The Laurentide ice sheet covered an area stretching from the Rocky Mountains east to the Atlantic Coast, extending as far south as 40°N in some areas (Pielou 1991). A third ice sheet, the Innuitian, covered Greenland and the Arctic (Dyke et al. 2002).

With much of North America covered in ice and snow, multiple lines of evidence (pollen, fossil, and genetic) suggest that mid- and high-latitude fauna and flora survived in ice-free glacial refugia during the LGM (Pielou 1991; Jaramillo-Correa et al. 2009). Many of these refugia existed in unglaciated areas south of the ice sheets, with six or more locations identified, including the Pacific Coast and south of the Rocky Mountains in the west, and the Appalachian Mountain area in the southeast (Figure 1.1; Pielou 1991; Soltis et al. 2006). Strong evidence also exists for northern refugia, particularly the widely-accepted ice-free corridor in the far northwest between Alaska and the Yukon across to Siberia known as

Beringia (Pielou 1991). Areas of Beringia are known to have been home to a multitude of taxa during the LGM and served as a migration corridor for terrestrial species to travel between North America and Asia (Pielou 1991).

A number of smaller refugia may have existed along the ice sheets' periphery. Along the west coast of Canada, Haida Gwaii (British Columbia), and the Alaska Archipelago are thought to have supported species during the LGM (Soltis et al. 1997). The northern end of Vancouver Island may also have acted as a refugium (Walser et al. 2005). The Atlantic shelf refugium on the east coast of Canada near present-day Newfoundland was exposed for much of the LGM as sea levels drastically dropped, acting as a glacial refugium to multiple taxa (Jaramillo-Correa et al. 2009). Evidence also exists for several other isolated coastal refugia (Pielou 1991), nunataks (unglaciated high-elevation mountain terrain; Pielou 1991), and numerous other southern and northern 'cryptic' refugia (Rull 2010).

1.3.2 Post-glacial colonization

As ice sheets receded after the LGM, species began to move out of refugia and recolonize previously glaciated areas. Each species likely responded individually to climatic change, though phylogeographers have found some common colonization patterns across the landscape (Soltis et al. 2006). Topography also played a role, with comparative phylogeography studies finding common barriers to post-glacial colonization for multiple species (Soltis et al. 2006). Hewitt

(1996) proposed two main patterns of spatial diversity of alleles resulting from post-glacial colonization: 'phalanx' and 'pioneer.' In a phalanx expansion scenario, individuals steadily spread in a diffusive front, resulting in little genetic divergence from the refugial source. If individuals are colonizing from two different refugia, the area where they meet will display a strong differentiation in alleles, creating a 'suture' zone. In pioneer expansion, pockets of populations are established ahead of a main front via long distance migrants or from existing refugia, creating highly differentiated populations (Johansen and Latta 2003).

Patterns of post-glacial colonization world-wide are slowly being unraveled for multiple species through phylogeographic studies. In North America, comparative studies in the Pacific Northwest (Carstens et al. 2005), in the southeast (Soltis et al. 2006), and continent-wide (Jaramillo-Correa et al. 2009) are revealing common colonization patterns for multiple taxa. However, many species responded individually to climate change and barriers to dispersal and molecular marker choice can complicate colonization (see Section 1.2.), making it difficult to diagnose general geographic patterns of post-glacial colonization.

1.4 Avian speciation

Dispersal and survival challenges faced during the climatic oscillations of the Quaternary period (2.6 mya) to present (Webb III and Bartlein 1992) are thought to be a major driving force in speciation for many taxa (Hewitt 2000). Based on

genomic divergence, speciation events for avian taxa may pre-date the Pleistocene glaciations and go back as far as the Pliocene epoch (5.3–2.6 mya; Klicka and Zink 1997; Avise and Walker 1998; Klicka and Zink 1999; Hewitt 2000; Lovette 2005). However, current evidence suggests that these genomic divergences may develop over millions of years, whereas other morphological, physiological, and behavioural changes may act on a much shorter time scale to isolate populations and produce new species or subspecies (Hewitt 2000).

Subspecies have had a long history in zoological taxonomy, originally being designated as "varieties" during the Linnaean period when no distinction was made between individuals and geographical differences (Mayr 1982). Before the advent of modern statistics, sampling, molecular methods, and quantification of phenotypic traits (Haig and Winker 2010), North American bird subspecies were historically designated based on variation in measurements and plumage among prepared museum study skin specimens (often only one specimen per subspecies; James 2010). The median year of description for most current subspecies descriptions is 1908-1909 (Remsen 2010). In 1949, Amadon attempted to formalize "the 75% rule" as the standard for defining subspecies: on the basis of a defining character or set of characters, at least 75% of a population should be distinguishable from greater than 99% of any other population (Amadon 1949). This definition uses effect size rather than statistical significance between population characters (Patten 2010). The American Ornithologists' Union (AOU),

which designates subspecies in North America, presently uses a combination of the biological species concept and a modified Amadon's rule, stating that:

> "Subspecies should represent geographically discrete breeding populations that are diagnosable from other populations on the basis of plumage and/or measurements, but are not yet reproductively isolated. Varying levels of diagnosability have been proposed for subspecies, typically ranging from at least 75% to 95%. Because subspecies represent relatively young points along an evolutionary time scale, genetic differentiation between subspecies may not necessarily parallel phenotypic divergence. Thus, subspecies that are phenotypically but not genetically distinct still warrant recognition if individuals can be assigned to a subspecies with a high degree of certainty. Described subspecies that represent points along a phenotypic continuum (cline) probably would not warrant recognition given further study."

More recent discussions suggest that the most useful definition of a subspecies requires concordance of multiple characters in an isolated population, including morphological, plumage, behavioural, and genetic characteristics (Haig and Winker 2010). The definition of subspecies can have important legal, conservation, and research implications (Waples and Gaggiotti 2006), and the identification and description of demographically isolated populations and subspecies can be very important for monitoring and management decisions (Haig and Winker 2010). However, the current AOU procedure for determining subspecies definitions is lacking without multiple characters for designations, making them a useful tool "of convenience," but not rigorous science at this point (Haig and Winker 2010). With continued work, avian subspecies may one day become well-supported designations based upon multiple characters, though many differences within species fall along a gradient, creating large grey areas for these definitions.

1.5 Study species

The corvid family (crows, magpies, jays, and allied groups) is taxonomically found within the species-rich oscine passerines (Aves: Passeriformes). Corvidae (*sensu* Morony et al. 1975 and Gill and Donsker 2014) is comprised of 130 species, arranged in 25 genera (Cibois and Pasquet 1999; Gill and Donsker 2014). Other accepted taxonomies name Corvidae as much larger group that includes other families and Corvini as the family equivalent (Sibley and Monroe Jr 1990). For the purposes of this thesis, I use the International Ornithological Congress (IOC; Gill and Donsker 2014) definition of Corvidae. Corvidae are thought to have arisen as a lineage during the Oligocene/Miocene period when a sudden explosive radiation of passerine birds occurred (Feduccia 1995) on the supercontinent Gondwana, likely in the Australo-Papua region (Ericson et al. 2002; Ekman and Ericson 2006).

The Corvidae family is principally defined by morphology, specifically the occurrence of a tuft of nasal bristles that extends to the nostril opening on the beak, noted stiffness of primary and tail feathers (particularly in crow species), long scales on the tarsus, and unspotted juvenile plumage (Cibois and Pasquet 1999). Behaviourally, corvids are noted for their cognitive abilities and novel behavioural adaptations, including cooperative breeding (Ekman and Ericson 2006), food storage and retrieval (Olson et al. 1995; Balda and Kamil 2002),

complex social interactions (Bond et al. 2003), social learning (Templeton et al. 1999; Cornell et al. 2012), and use of a sublingual pouch for food transportation (Portenko 1948; Bock et al. 1973). These adaptations have allowed corvids to take advantage of a wide range of habitats worldwide and while contributing to their establishment as specialists in a variety of ecological niches.

1.5.1 Clark's nutcracker *(Nucifraga columbiana)*

The Clark's nutcracker (*Nucifraga columbiana*, Wilson, 1981) is a high latitude resident corvid that inhabits North American coniferous forests. Clark's nutcrackers are most commonly known as essential conifer seed dispersers, particularly *Pinus* species (Tomback 1982; Tomback and Linhart 1990; Lorenz and Sullivan 2010), and for highly developed spatial cognition abilities as demonstrated by extensive food caching behaviours (Tomback 1998). Clark's nutcracker's preferred habitat is higher altitude montane forests dominated by one or more pine species due to the species' specialist diet of pine seeds, which has been shown to influence nutcracker dispersal patterns (Tomback 1998; Lorenz and Sullivan 2009). Clark's nutcrackers have been known to undergo post-fledging, seasonal, altitudinal migration to subalpine areas dominated by *Pinus* species (Tomback 1998; Lorenz and Sullivan 2009). Dispersal may also occur in times of food shortage, and occasionally during the non-breeding season, with large numbers of birds leaving home ranges to travel more than 100 km in search of large pine seed crops (Tomback 1998). No morphological

variation or subspecies have been reported for the species, and it is the only member of the *Nucifraga* genus to occur in North America (Tomback 1998).

1.5.2 Eurasian nutcracker *(Nucifraga caryocatactes)*

The Eurasian nutcracker (*Nucifraga caryocatactes*, Linnaeus, 1758), also known as the spotted nutcracker or simply as "nutcracker", is widespread throughout *Pinus*-dominated high latitude forests of Eurasia and Europe (Haring et al. 2007) and occasionally in other mixed coniferous forests where hazel nut (*Corglus avellana*) are common (Rolando 1996). Similar to their sister species, Clark's nutcracker (Ericson et al. 2005), Eurasian nutcrackers are linked closely to *Pinus* species seed dispersal throughout the continent (Tomback and Linhart 1990; Kajimoto et al. 1998). This specialization influences dispersal patterns (Rolando 1996; Rolando and Carisio 1999), occasionally resulting in long distance dispersal events (Newton 2006). Up to nine subspecies of Eurasian nutcracker have been reported (Gill and Donsker 2014), primarily based on morphological differences (Haring et al. 2007).

1.5.3 Gray jay (*Perisoreus canadensis*)

The gray jay (*Perisoreus canadensis*, Linnaeus, 1766) is found throughout coniferous and mixed coniferous-deciduous forests of northern and western North America, where it is most strongly associated with spruce (*Picea* spp.; Strickland and Ouellet 2011). Similar to their sister species, the Siberian jay

(*Perisoreus infaustus*; Ericson et al. 2005), the gray jay is a relatively sedentary resident species (Li and Merilä 2010; Strickland and Ouellet 2011). Adult gray jays remain in the same territory between breeding seasons and natal dispersal is limited to nearby territories, though occasional irruptive juvenile dispersal has been observed (Strickland and Ouellet 2011). Familiar to recreational users of North American boreal forests as bold "camp robbers", gray jays are known for their generalist diet (Strickland and Ouellet 2011) and as successful forest predators upon other songbirds' nests (Ibarzabal and Desrochers 2004). Gray jays display broad and clinal plumage and morphological trait variation across their range, with up to thirteen described subspecies based on morphological characteristics, though a recent revision suggests nine subspecies (Strickland and Ouellet 2011).

1.6 Molecular markers

The introduction of the polymerase chain reaction (PCR) in the 1980s and other improved laboratory techniques have enabled researchers to answer novel evolutionary questions using only small amounts of genetic material (Arnheim et al. 1990; Avise 2004). This increased ease of data acquisition and lessened expense has led to an explosion in phylogeographic research using molecular markers in recent decades (Avise 2009). The most common markers in phylogeographic studies have historically been mitochondrial DNA (mtDNA), with microsatellite markers and other markers gaining favour as costs associated with these

methods decrease (Avise 2004; Avise 2009). Combining markers with different modes of inheritance and mutation rates for a multi-locus approach has become more common in the past decade, allowing researchers to gain further insight into the influence of pre- and postglacial dynamics on the current population structure of a range of species (Zink and Barrowclough 2008; Wiens et al. 2010; Manthey et al. 2011), particularly in studies with limited sample sizes (Heled and Drummond 2008). Two of the most commonly paired complementary markers used in phylogeographical research are mtDNA and microsatellite (nuclear) markers (Zhang and Hewitt 2003; Avise 2009; Hickerson et al. 2010).

1.6.1 Mitochondrial DNA

In animals, mitochondrial DNA is a maternally transmitted molecule that lacks recombination. MtDNA is highly variable, with an average mutation rate of 2-5% per million years (Ruokonen and Kvist 2002; Avise 2004; Avise 2009) and is present in most cells in large quantities, making it relatively inexpensive and easy to sequence (Zink and Barrowclough 2008). Mutations are generally thought to directly account for the genetic variation seen in populations and many different haplotypes can simultaneously exist within a species (Avise 2004; Avise 2009). The rapid mutation rate of mtDNA is likely due to inefficient DNA repair mechanisms in the mitochondria, the corrosive oxygen-rich environment within the organelle itself, transcription, or translation, and no tightly bound histone proteins around the molecule as seen in nuclear DNA (Avise 2009; Brito and

Edwards 2009). This high mutation rate leads to high nucleotide sequence variation, making mtDNA an excellent marker for phyleographic analyses (Avise 2004; Zink and Barrowclough 2008; Brito and Edwards 2009).

Several coding and non-coding regions exist within mtDNA. In animals, the control region is the major noncoding region of mtDNA and plays a role in replication and transcription (Clayton 1984; Clayton 1992). The control region in vertebrates (including corvids) is subdivided into three domains: domain I, central domain, and domain III (Figure 1.2), which differ in base composition and rate of evolution (Baker and Marshall 1997). The central domain is relatively conserved in contrast to the two domains that flank it, domain I and III, which are typically hypervariable in base substitutions, deletions, and insertions and characterized by a different base composition than that of the central domain (predominantly GC; Wenink et al. 1993; Saunders and Edwards 2000). Combining hypervariable and conserved regions in analyses allows for maximization of sequence variation among individuals and populations in this study.

1.6.2 Microsatellites

Microsatellites are nuclear markers comprised of tandemly repeated sequences with a core motif of one to five base pairs (Jarne and Lagoda 1996; Schlötterer 2000). Repeats generally take three forms: pure (e.g. CTCTCT), compound (e.g.

CTCTAGAG), or interrupted (e.g. CTCTGAACTCTCT; Jarne and Lagoda 1996). Microsatellites are codominant, and inherited biparentally in a standard Mendelian manner. Biparental inheritance means that both male and female histories play a role in the evolutionary patterns elucidated by these markers (Jarne and Lagoda 1996; Avise 2004), potentially complementing information gained from uniparentally-inherited markers, such as maternally inherited mtDNA.

Nuclear mutation rates are generally lower than those in mitochondria, meaning that there will be less variation per sequenced base than found in mtDNA sequences (Brown 1983; Zink and Barrowclough 2008). However, microsatellites still have very high mutation rates (~ 10^{-6}–10^{-2} mutations/ generation; Schlötterer 2000), allowing for a high degree of resolution in studies of gene flow (Hewitt 2004b; Flanders et al. 2009). When multiple microsatellite loci are used, this level of polymorphism is ideal for answering demographic history questions such as coalescence times, gene flow between populations, population growth, and effective population sizes (Hewitt 2004b; Zink and Barrowclough 2008; Avise 2009). Combining nuclear and mitochondrial markers can thus compare and contrast historical and contemporary events that have affected genetic structure, creating a more complete view of a species' phylogeographic history.

1.7 Expanded methods and analyses

The data chapters in this thesis have all been published in or submitted to scientific journals. As such, extensive details of some methods and analyses have not been included in those chapters and are presented here instead.

1.7.1 Sample acquisition

Up to 30 individual birds of each target species were captured at a sampling site using standard mistnetting techniques with call playback. Sampling sites within a population were limited to within 50 km of each other where possible and were not separated by any obvious barriers to dispersal. Population sampling sites were chosen on either side of possible barriers to dispersal (e.g. Rocky Mountains, Great Basin in North America) and from areas that were glaciated and unglaciated during the last glacial maximum (e.g. Central Alberta and New Mexico in North America). Once captured, less than 100 μL of blood from each bird was collected by pricking the brachial vein with a sterile needle and collecting blood in a capillary tube. Each bird was banded with a US Fish & Wildlife Service aluminum band, aged and sexed (if possible), and mass, tarsus, and bill measurements were taken. Additional blood, tissue, and feather samples were obtained from museum collections taken from birds during the breeding season within the past 20 years to ensure all samples were from contemporary populations (Appendix 1, 2, and 3). All birds were handled and samples were

collected according to Animal Care procedures and applicable permits in the geographic area (Appendix 4).

1.7.2 Genetic structure analyses

1.7.2.1 Wright's fixation index (F_{ST}) and analogues

In 1931, Sewall Wright published a landmark paper that quantitatively described the processes that cause genetic variation among populations (Wright 1931). This work gave rise to *F*-statistics as a tool to describe genetic diversity within and among populations (Wright 1951). Wright's fixation index ($F_{ST;}$ Wright 1951) and related statistics (e.g. G_{ST} (Nei 1973); R_{ST} (Slatkin 1995)) are among the most widely used descriptive statistics to measure population genetic differentiation. F_{ST} is directly related to the variance in allele frequency among populations and to the degree of similarity among individuals within populations (Holsinger and Weir 2009). For pairwise comparisons between populations, F_{ST} values may range between 0 (complete panmixia) and 1 (complete differentiation). Values can be cautiously interpreted as: 0 - 0.05 indicates little differentiation, 0.05 - 0.15 indicates moderate differentiation, 0.15 – 0.25 indicates great differentiation, and above 0.25, very great differentiation (Balloux and Lugon-Moulin 2002). However, Wright (1978) noted that values as low as 0.05 can still signify differentiated populations as polymorphism (due to mutation) greatly deflates F_{ST} expectations (Balloux and Lugon-Moulin 2002).

Wright's original F-statistics were developed assuming loci to be biallelic (Wright 1951), but were later adapted for use with multiallelic loci and redefined as a ratio of genetic variances (Cockerham 1973). This led to development of additional F_{ST} analogues, especially for small samples sizes with a limited number of populations (Meirmans and Hedrick 2011). Nei's (1987) G_{ST} and Slatkin's (1995) R_{ST} (specifically for microsatellites) are both based on the ratio of within- and between-population genetic diversity. Excoffier *et al.*'s (1992) Φ_{ST} uses the ratio of within- and between-population genetic diversity (also known as analysis of molecular variance (AMOVA)), but performs this on a matrix of squared Euclidean distances between DNA haplotypes. φ_{ST} allows for mutation rates to differ between different pairs of alleles, making it appropriate for use with haplotype information (Holsinger and Weir 2009), such as that from mitochondrial DNA sequences. However, F_{ST} and its analogues have limitations: F_{ST} and G_{ST} are highly dependent on within-population variation (Hedrick 1999) as is Φ_{ST} (Holsinger and Weir 2009), and R_{ST} is only accurate when loci strictly follow the assumed stepwise mutation model (Meirmans and Hedrick 2011). In addition, for situations with small sample sizes ($n \leq 10$) and limited microsatellite loci ($n \leq 20$), F_{ST} is more accurate than R_{ST} (Gaggiotti et al. 1999). Because a multi-marker approach is used in this thesis, appropriate F-statistics were chosen based on available information outlined above.

1.7.2.2 Additional analyses using F_{ST} and analogues

Once F_{ST} is calculated, subsequent analyses can be performed to further understand genetic patterns and potential barriers to gene flow. These analyses include spatial analysis of molecular variance (SAMOVA; Dupanloup et al. 2002) and Mantel's test for isolation-by-distance (IBD; Slatkin 1993). SAMOVA is based on a simulated annealing approach that incorporates spatial data and aims to maximize the proportion of total genetic variance between populations (Dupanloup et al. 2002). Simulations were run for mitochondrial DNA data using a range of *K* values, from 1 group (no barriers) to the maximum number of populations analysed for differentiation (K_{max}=13 for Clark's nutcracker, K_{max}=28 for gray jay). Unfortunately, SAMOVA is generally used for haploid data only, thus cannot be used to detect barriers to gene flow in microsatellite data. However, IBD can be used for all markers. Under IBD, genetic differences are inversely related to the amount of gene flow (Slatkin 1993). Mantel's test for IBD uses measures of genetic differentiation and spatial data to test for a negative correlation between linearized pairwise F-statistics or analogues (e.g. (F_{ST}/(1 - F_{ST}))) and geographic distances between populations. Geographic distances between populations were calculated using Geographic Distance Matrix Generator v1.2.3 (Ersts 2011).

1.7.2.3 Bayesian clustering analyses

Wright's F-statistics and analogues generally rely on *a priori* grouping

information to characterize genetic differentiation. Bayesian clustering methods avoid this limitation by basing analyses on individual genotypes, not population allele frequencies (Latch et al. 2006). In this thesis, two Bayesian clustering analyses are used: STRUCTURE (Pritchard et al. 2000) and BAPS (Bayesian Analysis of Population Structure; Corander et al. 2008), which both perform very well at detecting clusters, even at low levels of population differentiation (F_{ST} > 0.03; Latch et al. 2006). Both programs attempt to minimize deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) to determine the number of clusters present in the data; deviations from HWE and LD would result if individuals from different, randomly-mating populations were incorrectly grouped into the same population (Latch et al. 2006). STRUCTURE estimates the number of clusters, *K*, using a Markov chain Monte Carlo (MCMC) model to test for likelihood (Falush et al. 2003), whereas BAPS uses a stochastic search algorithm to maximize the posterior likelihood (Corander et al. 2008). To the best of my knowledge, BAPS is the only clustering analysis software that can analyse haploid sequence data; both STRUCTURE and BAPS can use microsatellite loci data. BAPS can also incorporate location data in a spatial model that utilizes coordinate points and plots the spatial pattern of the genetic variation (Corander et al. 2008). BAPS was used for analyses of mitochondrial data and both STRUCTURE and BAPS were used for microsatellite analyses in this thesis.

1.7.3 Species distribution modeling

Species distribution modeling (SDM) estimates the relationship between species' records and environmental and/or spatial characteristics of those sites (Franklin 2009). SDM is alternately known as ecological niche modeling (ENM) in the literature. However, SDM likely more accurately describes the modeling process and resulting model, although niche theory strongly underpins SDM (Franklin 2009). A niche-based model describes habitat suitability in ecological space, but it is typically projected into geographic space, yielding a geographic area of predicted presence for the target species. Modeled areas that satisfy the conditions of a species' fundamental niche represent its potential distribution, whereas the geographic areas it actually inhabits constitutes its realized distribution (Phillips et al. 2006), making SDM a more appropriate term for the models presented here. SDM can also extrapolate distribution data in space and time (Franklin 2009), making it a useful tool for phylogeographers who wish to understand possible species distributions and areas of suitable habitat during the LGM and beyond (Carstens and Richards 2007). These models can be highly complementary to genetic data and provide support to hypotheses formed about LGM refugia based on genetic patterns and structuring.

1.7.3.1 Species occurrence data collection

Geo-referenced locations for all species were obtained from the Global Biodiversity Information Facility (GBIF; http://data.gbif.org). GBIF was

established in 2001 to encourage free and open access to biodiversity information across the world. Data are collected and deposited from a wide range of sources, including museum databases, breeding bird surveys, and vetted citizen science initiatives like eBird (Sullivan et al. 2009). Prior to model-building, duplicate records, and occurrences outside of known species' ranges, without geo-referencing, or recorded before 1950 were excluded from the analyses. Data were further inspected by plotting points using ArcMap 10.1 (ESRI: Redlands, CA). The model was trained and tested using location records from our sample data, from multiple museums, and multiple audio data sources in the GBIF data.

1.7.3.2 Climatic data collection

Current bioclimatic data were extracted from the WORLDCLIM dataset (version 1.4, http://www.worldclim.org/) and LGM bioclimatic data from the Model for Interdisciplinary Research on Climate (MIROC) dataset (Hasumi and Emori 2004) at 2.5 min resolution (~4 x 4 km tiles). The current bioclimatic dataset ranges over a 50 year period (1950 - 2000), hence exclusion of observations prior to 1950. Nineteen bioclimatic variables are included in the WORLDCLIM current and LGM dataset (Table 1.1); Hijmans et al. 2005). ArcGIS 10.1 (ESRI) was used to clip climatic variable layers to include only North America (Clark's nutcracker and gray jay) or Eurasia (Eurasian nutcracker), as using smaller geographic areas can improve predictive power of Maxent models (Anderson and Raza 2010). Some modeling techniques, particularly regressions, can be strongly affected by

correlated predictor variables (artificially increasing the correlation coefficient). Thus, prior to constructing SDM, ENMTools 1.3 (Warren et al. 2010) was used to determine which bioclimatic variables were correlated, using $R > 0.90$ as a cutoff. Nine variables were correlated with at least one other variable and all but one from each set of correlated variables was removed (Table 1.1). Removal was based upon perceived biological relevance of the variable to study species.

1.7.3.3 Maximum entropy modeling of species distributions

Maxent is a machine learning method program that is well-suited for modeling species distributions when presence-only occurrence data are the only available data (Elith et al. 2011). Maxent, similar to other SDM algorithms, associates species occurrences with a specific set of environmental variables (e.g. WORLDCLIM data) and projects these into geographic space to predict species distribution (Figure 1.3). If given current climate data, Maxent will predict a current distribution and can then project through space and time to produce paleodistribution models, if provided with the same climate variables during the desired time frame (i.e. LGM). Typically, the value of the mean area under the receiver operating characteristic curve (AUC) is used as a measure of the suitability of the model for predicting distributions, with values above 0.75 suggesting a "potentially useful" model (Elith 2002). However, AUC values must always be evaluated against the biological suitability of the variables used in the model; a model with a lower AUC value with more biologically relevant

variables may actually represent the most reasonable distribution for the target species (Elith et al. 2011).

For all SDMs created in this thesis, hinge features (variables) and 10-fold cross-validation were used. Hinge features are available for >15 data points and tend to create models with relatively smooth fitted functions (Elith et al. 2011), reducing the chance of overfitting the model to climatic data and generating more reasonable distributions. Cross-validation allows the user to estimate errors around fitted functions by using slightly different training (used to build the model) and testing (held-out from the original model and then used to test predictive performance) data from the whole data set for each model built during the process (Elith et al. 2011), outputting measures of variance in easy-to-interpret geographic formats. Overall, Maxent provides an excellent modeling method to approximate current and paleodistributions (Elith et al. 2006) of corvids in North America and Eurasia.

1.8 Thesis aims

The overall objectives of this thesis are to use genetic markers and species distribution modeling to investigate and compare historical patterns of dispersal and colonization and contemporary geographic genetic structure of three high latitude northern hemisphere corvid species: Clark's and Eurasian nutcrackers and gray jays. Specifically, I test the following hypotheses:

i. Pleistocene glaciations acted as historical barriers to dispersal for high latitude resident corvid species;
ii. Corvid populations historically found in glacial refugia are identifiable using genetic markers;
iii. Historical, contemporary physical, and non-physical barriers to dispersal affect genetic structure in high latitude resident corvid species;
iv. Range-wide morphological variation corresponds to geographic genetic structure.

I predict that:

a. All three study species dispersed into previously glaciated areas from unglaciated areas, and populations will reflect this expansion genetically;
b. Contemporary geographic genetic structure in more vagile nutcracker species will not be as strongly affected by barriers as more sedentary gray jays;
c. Morphological variation, particularly in gray jays, will correspond to patterns of genetic structure.

1.9 Thesis overview

This thesis is written in five chapters. Chapter 1 provides a general introduction to phylogeography and the biological processes contributing to population genetic structure in temperate species and methods used to study them. Chapter

2 (Dohms and Burg 2013) examines the effects of Pleistocene glaciations and other barriers to dispersal on Clark's nutcracker geographic genetic structure, using a multilocus (mitochondrial DNA and microsatellite markers) approach coupled with species distribution (SDM) and paleodistribution (PDM) modeling to determine post-glacial colonization scenarios. Chapter 3 (Dohms and Burg 2014) investigates Eurasian nutcracker population genetic structure using a single locus (mitochondrial DNA) approach. Chapter 4 examines barriers to gene flow for gray jays as well as post-glacial movement and subsequent population differentiation using a multilocus (mitochondrial DNA and microsatellite markers) approach complemented by SDM and PDM during the last glacial maximum. Chapter 4 also examines morphological variation across the gray jay range, investigating if this variation corresponds to designated subspecies and genetic groups. The final chapter summarizes results from Chapters 2-4, comparing and contrasting the three corvid species under a lens of natural history traits and biogeographic barriers. Potential future work is suggested.

Table 1.1. Environmental variables available for use in species distribution modeling (SDM). "Var" = variable name; "Code" = abbreviated variable code; "Correlated" = > 90% correlation; "Incl" = included (Y) or removed (N) from (SDM) analyses.

Var	Code	Data description	Correlated	Incl
bio1	1	Annual mean temp	5, 6, 9, 10, 11	Y
bio2	2	Mean of monthly (max T - min T)	None	Y
bio3	3	Isothermality (bio2/bio7) (* 100)	6, 11	Y
bio4	4	Temperature seasonality (std dev *100)	6, 7, 11	Y
bio5	5	Max temp of warmest month	1, 10	N
bio6	6	Min temp of coldest month	1, 3, 4, 9, 11	N
bio7	7	Temperature annual range (bio5-bio6)	4	N
bio8	8	Mean temp of wettest quarter	None	Y
bio9	9	Mean temp of driest quarter	1, 6, 11	N
bio10	10	Mean temp of warmest quarter	1, 5	N
bio11	11	Mean temperature of coldest quarter	1, 3, 4, 6, 9	N
bio12	12	Annual precipitation	13, 16	Y
bio13	13	Precipitation of wettest month	12, 16	N
bio14	14	Precipitation of driest month	17	Y
bio15	15	Precip seasonality (coefficient of variation)	None	Y
bio16	16	Precipitation of wettest quarter	12, 13	N
bio17	17	Precipitation of driest quarter	14	N
bio18	18	Precipitation of warmest quarter	None	Y
bio19	19	Precipitation of coldest quarter	None	Y

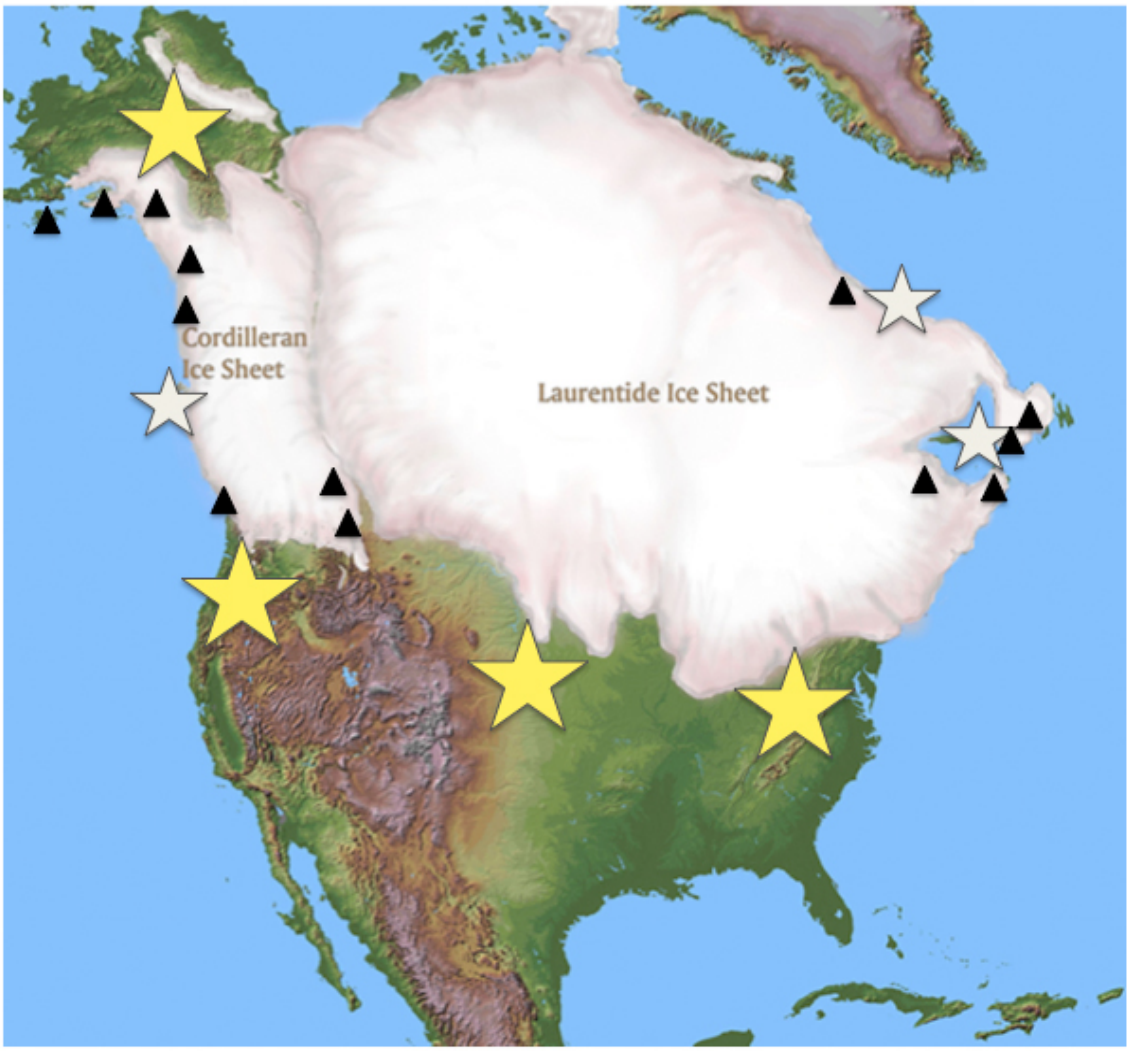

Figure 1.1. North America during the last glacial maximum, 21-18 kya. Much of the continent was covered by the Cordilleran and Laurentide ice sheets, with many species relocating to refugia (signified by stars) or nunataks (small, unglaciated mountain top refugia; signified by solid black triangles). Evidence exists for four putative refugia (yellow stars) and a minimum of four less well-supported refugia (white stars). Figure modified from Pielou (1991) and Cosmographic Research Institute (2009).

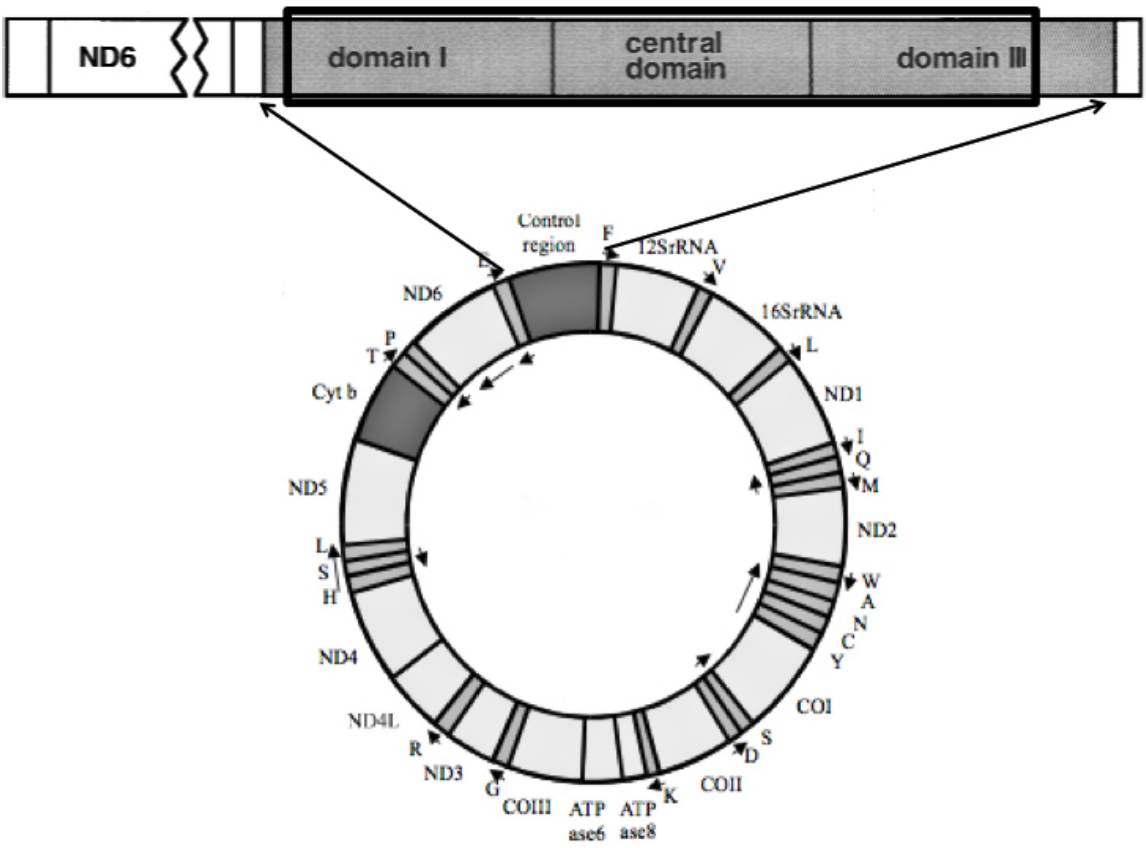

Figure 1.2. Schematic representation of most avian mitochondrial DNA. The expanded area denotes areas of the highly variable control region with the dark lined rectangle approximately encompassing the ~900 bp section sequenced for the corvids in this study. Figure modified from Kvist (2000; complete mtDNA genome) and Saunders & Edwards (2000; expanded section).

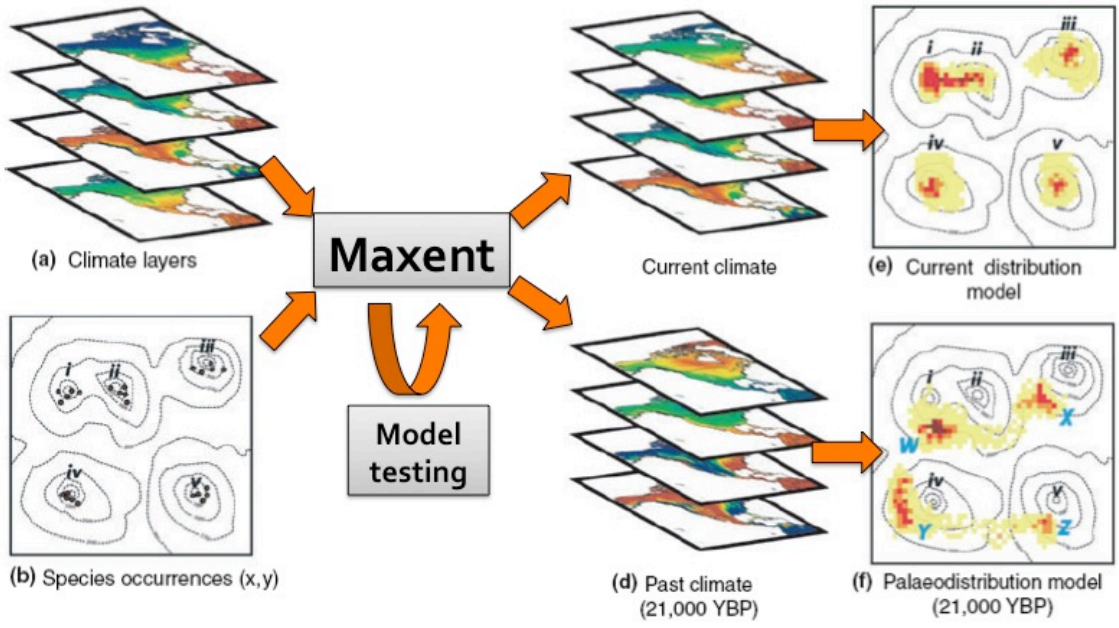

Figure 1.3. Schematic representation of species distribution modeling using Maxent software. Adapted from Richards *et al*. (2007).

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Chapter 2: Molecular markers reveal limited population genetic structure in a North American corvid, Clark's nutcracker (*Nucifraga columbiana*)[1]

2.1 Abstract

The genetic impact of barriers and Pleistocene glaciations on high latitude resident species has not been widely investigated. The Clark's nutcracker is an endemic North American corvid closely associated with *Pinus*-dominated forests. The nutcracker's range encompasses known barriers to dispersal for other species, and glaciated and unglaciated areas. Clark's nutcrackers also irruptively disperse long distances in search of pine seed crops, creating the potential for gene flow among populations. Using the highly variable mitochondrial DNA control region, seven microsatellite loci, and species distribution modeling, we examined the effects of glaciations and dispersal barriers on population genetic patterns and population structure of nutcrackers. We sequenced 900 bp of mitochondrial control region for 169 individuals from 15 populations and analysed seven polymorphic microsatellite loci for 13 populations across the Clark's nutcracker range. We used species distribution modeling and a range of phylogeographic analyses to examine evolutionary history. Clark's nutcracker populations are not highly differentiated throughout their range, suggesting high

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levels of gene flow among populations, though we did find some evidence of isolation by distance and peripheral isolation. Our analyses suggested expansion from a single refugium after the last glacial maximum, but patterns of genetic diversity and paleodistribution modeling of suitable habitat were inconclusive as to the location of this refugium. Potential barriers to dispersal (e.g. mountain ranges) do not appear to restrict gene flow in Clark's nutcracker, and postglacial expansion likely occurred quickly from a single refugium located south of the ice sheets.

2.2 Introduction

Phylogeography is the study of processes underlying spatial and temporal dimensions of genetic variation (Avise 2004). Patterns of genetic variation attained through phylogeographic methods can provide insights into population-level response to disturbance and the processes responsible for historical dispersal and colonization (Milot et al. 2000; Moritz 2002). Examining patterns of genetic variation can help us to evaluate the roles of gene flow, bottlenecks, and historical or geological barriers in explaining contemporary patterns of diversity across geographical regions (Zink 1996). Contemporary genetic patterns are strongly influenced by postglacial expansion from refugia (Weir and Schluter 2004; Williams 2003), historical and contemporary barriers to dispersal (Brunsfeld et al. 2001; Keyghobadi 2007), and dispersal potential (Burg et al. 2003).

The repeated glaciations and climate oscillations of the Pleistocene epoch provide a natural tool to address population responses to large-scale landscape changes. Multiple expansions and contractions of ice sheets created a dynamic landscape that repeatedly fragmented historical populations, alternately creating barriers to dispersal and creating new habitats for colonization (Pielou 1991; Dynesius and Jansson 2000). During the Pleistocene glacial maxima in North America, many populations were confined to refugia (ice-free areas; Pielou 1991). Plant and animal species expanded from several known refugia following the retreat of the ice sheets, including Beringia (present-day Bering Sea and parts of Alaska) and three main areas south of the ice sheets (Pacific Coast, Rockies, and Taiga; Pielou 1991; Hewitt 2004). Additional areas along the periphery of the ice sheets are contested to have been ice-free, such as an Atlantic shelf near present-day Newfoundland (Weir and Schluter 2004; Jaramillo-Correa et al. 2009).

High latitude resident bird species provide a unique opportunity to investigate patterns of postglacial and barrier-mediated dispersal; historical events shaping current population structure should be particularly evident in these species. Non-migratory species have the potential to retain patterns of genetic variation longer due to limited dispersal, allowing researchers to make inferences about past historic events (Burg et al. 2005; Burg et al. 2006). For example, several high latitude tree species show distinct patterns of population genetic structure and have retained information on historic environmental changes (Jaramillo-Correa et

al. 2009; Petit et al. 2005). As the number of studies on resident species increase, similar patterns are emerging in vertebrate taxa (Burg et al. 2006). However, other resident species show limited differentiation despite potential barriers to dispersal (Piertney and Summers 2001; Pulgarin-R and Burg 2012).

Clark's nutcracker (*Nucifraga columbiana*) is a high latitude resident corvid species (Family Corvidae) that inhabits North American coniferous forests. Much of the published work on this species has focused on spatial cognition exhibited by their extensive food caching behaviours (Tomback 1998). Additional research has concentrated on Clark's nutcracker's role as essential seed dispersers for many conifers, particularly *Pinus* species (Tomback 1982). To date, no published phylogeographic work exists for this species, though genetic work has been identified as a priority (Tomback 1998). Clark's nutcracker prefers higher altitude montane forests dominated by one or more *Pinus* species. Their close association with pine-dominated forests is due to a specialised diet of pine seeds, which influences dispersal patterns of both nutcrackers and seeds (Tomback 1998; Richardson et al. 2002). After nestlings fledge, Clark's nutcrackers have been known to undergo seasonal altitudinal movement to nearby *Pinus*-dominated subalpine areas (Tomback 1998). In times of food shortage, and occasionally during the non-breeding season, large numbers of birds have been shown to irruptively disperse, leaving home ranges, and travelling more than 100 km in search of large crops of pine seeds (Tomback 1998; Newton 2006). Range-wide

morphological and plumage variation is well documented for some high latitude resident species (Klicka et al. 2011; Graham and Burg 2012), but there is little evidence of morphological variation in Clark's nutcracker (Tomback 1998). Some suggest that this lack of variation is a reflection of high gene flow between populations due to irruptive dispersal, but this has not been investigated to date (Pyle 1997; Tomback 1998).

To address these gaps in phylogeographic knowledge, our goals for this study were to investigate patterns of dispersal and postglacial colonization in Clark's nutcracker as reflected in genetic structure. We predict that contemporary Clark's nutcracker populations will exhibit low levels of genetic differentiation between populations due to irruptive food-related dispersal patterns. Mountain ranges and unsuitable habitats will not act as strong barriers to gene flow due to this species' preference for high altitude sub-alpine habitats (Tomback 1998) where barriers are limited. Rather, dispersal will be restricted by availability of pine-dominated coniferous forests and seed crops. We expect that postglacial colonization likely occurred from a single refugium south of the ice sheets, similar to patterns of postglacial colonization in several North America *Pinus* species (Jaramillo-Correa et al. 2009).

2.3 Materials and Methods

2.3.1 Ethics statement

All animals captured in the field were handled following animal welfare protocols (#0614 and #1028) approved by the University of Lethbridge Animal Welfare Committee using guidelines set by the Canadian Council on Animal Care (CCAC). Banding in Canada was performed under Canadian Wildlife Service banding permit #10425W (2007) and #10804 (2008 onward) and in the US under US Fish and Wildlife banding permit #23522. All netting and blood sampling was conducted under the appropriate state, provincial, federal, and institutional authorities; detailed permit information is available upon request.

2.3.2 Sample collection and preparation

From 2009-2012, we captured up to 30 individual Clark's nutcrackers at each sampling site (Table 2.1) using standard mistnetting techniques with call playback. For call playback, we used a medley of group interaction vocalizations sourced from multiple tracks found on xeno-canto.org. Sample collection locations for each defined population (i.e. sampling site) were limited to within a 50 km of radius and were not separated by any obvious barriers to dispersal. We chose sites on either side of possible barriers to dispersal (e.g., Rocky Mountains, Great Basin) and from areas that were previously glaciated and unglaciated during the last glacial maximum (e.g., Central Alberta and New Mexico, respectively; Figure 2.1). We collected less than 100 µL of blood from each bird by

pricking the brachial vein with a sterile needle and collecting blood in a capillary tube. Blood was stored in 95% ethanol and archived at -80°C upon return to the University of Lethbridge. Each bird was banded with a USGS aluminum band, aged and sexed (if possible), and mass, tarsus, and other morphological measurements taken. Additional tissue and feather samples were obtained from museum collections taken from birds during the breeding season within the past 20 years to ensure all samples were from contemporary populations (Appendix 1).

2.3.3 Laboratory procedures

2.3.3.1 DNA extraction

DNA was extracted from blood, feather, and tissue samples using a modified Chelex protocol (Burg and Croxall 2001). Once extracted, DNA was stored in low TE buffer at -20°C.

2.3.3.2 Mitochondrial DNA

We amplified a section of the mitochondrial DNA control region (CR) using primers L46 SJ (5'-TTT GGC TAT GTA TTT CTT TGC-3'; Birt & Lemmen, unpublished data) and H1030 JCR 18 (5'-TAA ATG ATT TGG ACA ATC TAG G-3'; Saunders and Edwards 2000) corresponding to positions 46 (Domain I) and 1030 (Domain III) of the corvid mitochondrial control region, respectively. Where the complete fragment would not amplify, we used internal primers designed in-

house, H560 clnuCR (5′-GCA AAG GGA GGA GTA TGC AG-3′) or L530 corvidCR (5′-CGC CTC TGG TTC CTA TTT CA-3′), with L46 SJ or H1030 JCR 18, respectively, to amplify two overlapping fragments.

Polymerase chain reactions (PCR) were performed on a Master gradient thermocycler (Eppendorf: Hauppauge, NY) in a 25 μL volume containing 1x goTaq Flexi buffer (Promega: Madison, WI), 2.5 mM $MgCl_2$, 200 μM dNTP, 0.4 μM of each primer, and 0.5 units goTaq Flexi taq polymerase (Promega) under the following conditions: one cycle of 94°C for 120 s, 52°C for 45 s, and 72°C for 60 s, 37 cycles of 94°C for 30 s, 52°C for 45 s and 72°C for 60 s and one cycle of 72°C for five min. PCR products were run on a 0.8% agarose gel to confirm DNA amplification.

DNA sequencing was performed at McGill University and Génome Québec Innovation Centre on a 3730xl DNA Analyzer (Applied Biosystems: Carlsbad, CA) or at the University of Lethbridge on a 3130 DNA Analyzer (Applied Biosystems). For in-house sequencing we used a shrimp alkaline phosphatase-exonuclease clean up followed by sequencing and sodium acetate precipitation (Graham and Burg 2012) prior to gel electrophoresis.

2.3.3.3 Microsatellite DNA

Three individuals from geographically distant populations (Montana, southern Oregon, and Colorado) were initially screened for 32 microsatellite primer pairs developed for and used in other corvids. Thirteen additional nutcracker-specific microsatellite loci have since been developed by another independent research group (Oyler-McCance et al. 2013). If screened loci were suspected to be polymorphic and/or amplified clearly, two additional individuals from each of the initial test populations plus three individuals from another population potentially separated by dispersal barriers were screened. Eight loci were polymorphic (Table 2.2). All forward primers were modified by adding an M13 sequence (5′ – CAC GAC GTT GTA AAA CGA C – 3′) to the 5′ end, which allowed the integration of a fluorescently labeled primer (700 nm or 800 nm) directly into the PCR product. DNA was amplified in a 10 μL reaction with 1x buffer, 1 mM $MgCl_2$, 200 μM dNTP (Fisher Scientific: Hampton, NH), 1 μM of each primer (forward and reverse), 0.05 μM of the fluorescent primer (Eurofins MWG Operon: Huntsville, AL) and 0.5 units taq polymerase under the following conditions: one cycle of 94°C for 120 s, T_1 for 45 s, and 72°C for 60 s, seven cycles of 94°C for 60 s, T_1 for 30 s and 72°C for 45 s, 31 cycles of 94°C for 30 s, T_2 for 30 s, and 72°C for 45 s, and one final elongation cycle at 72°C for five minutes (Table 2.2).

PCR products were mixed with a stop solution (95% formamide, 20 mM EDTA and bromophenol blue), denatured for 3 min at 94°C, and run on a 6% polyacrylamide gel using a LI-COR 4300 DNA Analyzer (LI-COR Inc.: Lincoln, NE). Alleles were scored via visual inspection, and genotypes were independently confirmed by a second person. Three controls of known allele sizes (pre-screened individuals) plus a size standard were included on each gel to ensure consistent scoring along with a negative control to ensure no contamination was present.

2.3.4 Analyses of genetic structure

2.3.4.1 Mitochondrial DNA

Chromatograms were edited and sequences aligned manually in MEGA v5.0 (Tamura et al. 2011). Shared haplotypes were determined using DnaSP v5.10 (Rozas et al. 2003). We assessed population structure and relationships among haplotypes using a maximum parsimony network constructed using Network v4.611, which is designed to construct the shortest, least complex network (Bandelt et al. 1999). The median-joining (MJ) tree algorithm was used as this allows for multi-state data (e.g., four nucleotide base options at each variable site), with settings as follows: weight = 10 for each site, epsilon = 0, and the MJ squared option turned off. After the tree was constructed, the MP (maximum parsimony) option was used to remove superfluous links and unnecessary

median vectors from the network, which can be produced during the initial network calculations (Polzin and Daneshmand 2003).

Genetic variation within populations was measured by calculating haplotype (H_d) and nucleotide (π) diversity using DnaSP v5.10 (Rozas et al. 2003). We calculated pairwise Φ_{ST} values (an analogue of Wright's fixation index (F_{ST})) using Arlequin v3.5.1.3 (Excoffier and Lischer 2010) to examine population structure and test for genetic differentiation among populations and haplogroups. Significance values were corrected using a Benjamini-Hochberg correction (Benjamini and Hochberg 1995), to control for false discovery rate (FDR), the expected proportion of falsely rejected hypotheses in multiple significance testing situations where multiplicity might occur.

A Mantel's test was performed to examine the correlation between genetic and geographic distances in GenAlEx v6.0 (Peakall and Smouse 2006). We calculated geographic distances using weighted central coordinates for each population (Table 2.1) and the Geographic Distance Matrix Generator v1.2.3 (GDMG; Ersts 2011). Linearised Φ_{ST} values (Φ_{ST} / 1- Φ_{ST}) were used for genetic distances and significance was tested using 9,999 permutations. A mismatch distribution was used to help visualize signatures of demographic expansion, and to test the null hypothesis of historic population growth and expansion (Rogers 1995).

Genetic variation allocated within and among populations was examined using an analysis of molecular variance (AMOVA) in Arlequin v3.5.1.3 (Excoffier and Lischer 2010). We investigated the potential impact of barriers on genetic variation by performing a spatial analysis of molecular variance (SAMOVA; K = 2-12; 100 iterations; Dupanloup et al. 2002); however, the process cannot test for range-wide panmixia as K cannot be set to K = 1. Alternatively, BAPS v6.0 (Cheng et al. 2013), a Bayesian method for analysing population structure, can detect range-wide panmixia. A preliminary population mixture analysis for spatial clustering of individuals in BAPS used no *a priori* population information and K_{max} = 13. A subsequent BAPS run was then conducted using *a priori* population information.

2.3.4.2 Microsatellite DNA

Allele frequencies, deviation from Hardy-Weinberg equilibrium (observed (*Ho*) versus expected (*He*) heterozygosity), and pairwise F_{ST} values (Wright 1978) were calculated with 999 permutations using GenAlEx v6.0 (Peakall and Smouse 2006) and P values were corrected for multiple tests using a Benjamini-Hochberg correction for FDR (Benjamini and Hochberg 1995). Allelic richness was calculated in FSTAT v2.9.3 (Goudet 2001). A Mantel's test was performed to test for isolation by distance using the same procedure as for mitochondrial DNA.

Bayesian clustering analyses were conducted using the programs STRUCTURE v2.3.3 (Pritchard et al. 2000; Falush et al. 2003) and BAPS v6.0 (Corander et al. 2008). STRUCTURE was run with the following settings: K = 1-15, a burn-in of 100,000 followed by 500,000 runs, admixture assumed, correlated allele frequencies and including *a priori* population information. Ten replicates were performed for each value of K. In STRUCTURE, it can be difficult to decide when K captures major structure in the data due to similar lnP(X|K) values, thus two additional analyses were conducted using STRUCTURE results: Bayes factor calculations (Pritchard et al. 2000) and ΔK (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and von Holdt 2012). One limitation with using STRUCTURE HARVESTER is that it cannot detect when K = 1 is the true number of clusters. However, Bayes factors and BAPS are able to detect if K = 1, so the addition of these methods is useful in species where panmixia is a possibility. A preliminary population mixture analysis for spatial clustering of individuals in BAPS used no *a priori* population information and K_{max} = 13. A subsequent BAPS run was then conducted using *a priori* population information.

2.3.5 Species distribution modeling

2.3.5.1 Sample points

In addition to our sample (field and museum) locations, geo-referenced Clark's nutcracker locations were obtained from the Global Biodiversity Information Facility (GBIF; http://data.gbif.org/, accessed on 22 January 2013). We excluded

occurrences outside of North America, without geo-references, or recorded before 1950 from modeling and further inspected accuracy by plotting points using ArcMap v10.1 (ESRI: Redlands, CA). We removed duplicate records prior to model-building.

2.3.5.2 Bioclimatic data

Current bioclimatic data were extracted from the WORLDCLIM dataset (v1.4, http://www.worldclim.org/) and LGM bioclimatic data from the Model for Interdisciplinary Research on Climate (MIROC) database (Hasumi and Emori 2004) at 2.5 min resolution (~4 x 4 km tiles). The current bioclimatic dataset ranges over a 50 year period (1950 - 2000), hence exclusion of observations prior to 1950. Nineteen bioclimatic variables are included in the WORLDCLIM current and LGM dataset (Hijmans et al. 2005). ArcMap v10.1 (ESRI) was used to clip climatic variable layers to include only North America as using smaller geographic areas can improve the predictive power of Maxent models (Anderson and Raza 2010). Prior to constructing SDM, ENMTools v1.3 (Warren et al. 2010) was used to determine which bioclimatic variables were correlated using $R > 0.90$ as a cutoff. Nine variables were correlated with at least one other variable and all but one from each set of correlated variables was removed.

2.3.5.3 Maximum entropy (Maxent) distribution modeling

Maxent v3.3.3 (Phillips et al. 2006) was used to model suitable habitat distributions for Clark's nutcracker at present and during the LGM using hinge features only, regularization multiplier = 1, max number of background points = 10,000, replicate run type of 10 cross-validations, 500 maximum iterations, and 0.00001 convergence threshold. Resulting layers were then exported to ArcMap v10.1, overlayed on a digital elevation map, and processed into images.

2.4 Results

2.4.1 Genetic analyses

2.4.1.1 Mitochondrial DNA

Mitochondrial DNA control region sequences (n = 169; Appendix 1) from 15 populations range-wide revealed 48 variable sites in a 900 base pair (bp) region. In total, 68 haplotypes were recognized in the median-joining analysis (GenBank accession nos. KF687612-KF687679; Figure 2.2; Table 2.3). Eighteen haplotypes accounted for 119 individuals; 50 singleton haplotypes were found (Figure 2.2; Table 2.3). The largest haplotype group contained a total of 50 individuals from 14 of the 15 populations analysed. Most haplotypes were a single mutational step removed from each other. Overall, limited geographic clustering was observed in the maximum parsimony network (Figure 2.2).

Haplotype diversity (H_d) values ranged from 0.583 (southern Alberta (SAB)) to 0.964 (Montana (MT), New Mexico (NM), and central California (CCA)) and nucleotide diversity (π) ranged from 0.00127 (SAB) to 0.00402 (northeastern Oregon) for populations with greater than five samples (Table 2.1). When all samples were combined, overall H_d was 0.854, and overall π was 0.00324 indicating high levels of genetic diversity in Clark's nutcracker. In 78 population pairwise comparisons of genetic differentiation, Φ_{ST} values ranged from -0.050 ($P_{corrected}$ = 0.779) for Utah (UT) and northeast Washington (NEWA) to 0.359 ($P_{corrected}$ = 0.100) for NM and SAB (Table 2.4). Most population pairs (57/78) had Φ_{ST} values less than 0.050, though 14 pairs had Φ_{ST} values between 0.050 and 0.150, four pairs had Φ_{ST} values between 0.150 and 0.250, and three pairs had Φ_{ST} values above 0.250. Only four of those Φ_{ST} values were significant after the FDR correction and all occurred between NM and other populations (UT, Wyoming (WY), southern California (SCA), and NEWA). The mismatch distribution did not deviate significantly from the expected signature, so we cannot reject the null hypothesis of historical population growth and expansion (Rogers 1995). Geographic distance in 78 paired populations ranged from 339 km for WY and UT to 2074 km for NM and central Alberta (CAB). A low, but significant correlation between genetic and geographic distance was detected for mitochondrial DNA (R^2 = 0.082, P = 0.040; Figure 2.3). When the NM population was removed from the analyses, the relationship between genetic and geographic

distance still trended towards isolation by distance, though it was no longer significantly correlated (R^2 = 0.064, P = 0.058; not shown).

Both AMOVA and SAMOVA showed that most genetic variation is contained within rather than among population groups. The highest value of between-group variation was observed when K = 1 for AMOVA analyses (3.4% between groups, 96.6% within groups) and for K = 2 in SAMOVA (17.8% between groups, 80.7% within groups), with one group consisting of NM and the other of all other populations pooled. The preliminary (no *a priori* population information) and secondary (using *a priori* population information) BAPS analyses for spatial clustering of individuals found no significant differentiation between individuals or populations (i.e., K = 1).

2.4.1.2 Microsatellite DNA

A total of seven polymorphic loci (Table 2.5) were used for analyses; an eighth locus (ApCo37) was removed due to limited amplification success and subsequent missing data (~50%). We used the thirteen populations with greater than five samples for microsatellite analyses (Table 2.1; Appendix 1). The total number of alleles detected ranged from four in PnuA106W to 10 in ApCo40 with an average of 6.43. Only 13 of the 91 loci-population comparisons showed significant deviations from Hardy-Weinberg and all showed lower heterozygosity than expected. ApCo41 has a significant heterozygote deficit in

five of the 13 populations sampled and while two of these (NEWA and CAB) had fewer samples ($n \leq 8$), the others had a larger number of samples ($n \geq 14$; Table 2.5).

Limited population differentiation was detected with a global F_{ST} value of 0.070. Paired F_{ST} values ranged from 0.000 (14/78 comparisons) to 0.123 (CCA and central Alberta (CAB); Table 2.6). After Benjamini-Hochberg FDR correction, 21 of 78 paired comparisons were significant (Table 2.6). A weak, but significant, correlation was found between genetic and geographic distance using Mantel's test ($R^2 = 0.078$, $P = 0.029$; Figure 2.4).

STRUCTURE results suggested that the optimal number of groups was $K = 1$ (Figure 2.5A). Further analyses produced peaks at $K = 1$, $K = 4$, and $K = 12$ (Figure 2.5B), though $K = 1$ produced the highest peak and highest posterior probabilities (mean (Pr (K=1)) = -2625.45) and is supported by Bayes factor values (Pr (K=1) = 1) and BAPS results.

2.4.2 Species distribution modeling

A total of 1563 presence records were used for training and 174 for testing the distribution models. After removing correlated bioclimatic layers, 10 layers were used for model construction (bio1-4, 8, 12, 14-15, 18-19). Mean area under the receiver operating curve (AUC) was 0.907 (range = 0.906 - 0.908), suggesting that

the current model was highly suitable for backcasting to the paleodistribution model. Isothermality (mean diurnal temperature range/mean annual temperature range (bio3)), temperature seasonality (bio4), and mean annual temperature (bio1) were the most important variables contributing to the model at 44.3%, 37.7%, and 11.1% respectively.

The current distribution predicted by the Maxent model (Figure 2.6A) closely approximated the known Clark's nutcracker range in North America (Figure 2.1). The model returned a maximum 66% probability of suitable habitat throughout the range. The paleodistribution model returned a maximum 25% probability of predicted suitable habitat, mostly concentrated in the western mountainous regions and along the southeast edge of the ice sheets during the last glacial maximum (Figure 2.6B).

2.5 Discussion

2.5.1 Population genetic structure

Using a combination of measures of population differentiation and clustering analyses, our study suggests high levels of population connectivity between most Clark's nutcracker populations in North America. We found limited differentiation in mitochondrial DNA (Table 2.4), with only the New Mexico population showing significant pairwise differences from four other populations. Microsatellite data indicate higher levels of population differentiation between

Clark's nutcracker populations, though this predominantly occurs between populations at the edges of the species' range (e.g. CAB and SCA; Table 2.6). Geographic distance rather than barriers appear to influence mitochondrial and nuclear gene flow, as there is a weak, but significant effect of isolation by distance on nutcracker populations for both molecular markers (Figures 4 and 5). However, for mitochondrial DNA, this pattern seems to be driven by populations in New Mexico, suggesting limited peripheral isolation for this marker. For microsatellite data, additional peripheral populations (CAB, SAB, and SCA) are significantly differentiated in multiple comparisons (Table 2.6), suggesting peripheral isolation in addition to isolation by distance. Clustering analyses do not support strong population differentiation for either nuclear or mitochondrial markers, with a single population being the best fit for the data in both cases. In the case of microsatellite data, these results should be interpreted with caution as both STRUCTURE and BAPS may struggle with situations where differentiation is low (F_{ST} < 0.030; Latch et al. 2006), though global values (F_{ST} = 0.070) suggest this is likely not the case.

High levels of haplotype, nucleotide, and allelic diversity throughout most nutcracker populations suggest large population sizes and few founder effects or population bottlenecks. The exception to this is the southern Alberta population, which exhibits reduced haplotype and nucleotide diversity for mitochondrial markers relative to the rest of the populations, though microsatellite loci have

high levels of allelic richness and heterozygosity (Table 2.5). This difference between marker genetic diversity may be due to a historical founder effect in previously glaciated southern Alberta (mtDNA), with nuclear gene flow being less limited (microsatellites).

Patterns of potential panmixia and limited population differentiation are not unique to Clark's nutcracker. Many other species that undergo irruptive and seasonal dispersal also exhibit limited population differentiation despite the presence of proposed barriers to dispersal. Studies of *Pinus* seed specialists, such as pygmy nuthatch (*Sitta pygmaea*; Spellman and Klicka 2006), crossbill species (*Loxia* spp.; Piertney and Summers 2001), and Clark's nutcracker's sister species, Eurasian nutcracker (*Nucifraga caryocatactes*; Haring et al. 2007; Dohms and Burg 2014), found no significant genetic differentiation among populations despite potential barriers to dispersal. Limited differentiation has been found in other species with irruptive or seasonal dispersal, such as boreal owl (*Aegolius funereus*; Koopman et al. 2007), and European redpoll species (*Carduelis* spp.; Marthinsen et al. 2008), yet Steller's jay (*Cyanocitta stelleri*; Burg et al. 2005) and white-breasted nuthatch (*Sitta carolinensis*; Spellman and Klicka 2007) exhibit significant differentiation between populations and limited gene flow corresponding with barriers to dispersal. In other taxa, peripheral populations are more likely to be isolated due to reduced gene flow (Burg et al. 2006; Eckert et al. 2008; Lait et al. 2012), while maintaining panmixia in the majority of the range (Stenzler et al.

2009). Overall, Clark's nutcracker population connectivity seems to be primarily limited by distance with some peripheral isolation at the southern and northern edges of the range, which is reasonable for a high elevation species with irruptive and seasonal altitudinal dispersal.

2.5.2 Postglacial colonization

After the LGM, species that rapidly expanded from a single refugium with high levels of gene flow are expected to exhibit three characteristics: 1) a star-like phylogeny with many low frequency single haplotypes separated from high frequency central ancestral haplotypes by few mutational steps (Slatkin and Hudson 1991); 2) low levels of genetic subdivision between and within populations (Zink 1996; Hutchison and Templeton 1999); and 3) a mismatch distribution of pairwise differences among haplotypes indicating a sudden increase in expansion from a single population (Rogers and Harpending 1992). These signals of expansion from a single refugium have been reported in several North American bird species (e.g., multiple species reviewed in Zink 1997, and downy woodpecker (*Picoides pubescens*; Pulgarin-R and Burg 2012) as well as many plant species, including several *Pinus* species that Clark's nutcracker specializes upon (reviewed in Jaramillo-Correa et al. 2009). Clark's nutcracker mitochondrial DNA exhibits a star-like phylogeny with one major shared haplotype, and short connections between this haplotype and others (Figure 2.2), in addition to a modeled paleodistribution that suggests that this species

expanded from a single refugium after the LGM. This pattern is less complex than that found in whitebark pine postglacial expansion: colonization is thought to have occurred from three refugia (Oregon, Idaho, and Colorado; Richardson et al. 2002). Given that Clark's nutcrackers range extends farther south than that of whitebark pine, nutcrackers may have been isolated in a more southern refugium and expanded into whitebark pine refugia from there, subsequently assisting with seed distribution northward.

In addition to the aforementioned characteristics, temperate species that expanded north from a southern refugium have been shown to exhibit genetic differentiation between northern and southern populations corresponding to previously unglaciated and glaciated areas (Hewitt 2004). Southern populations were less impacted by glaciation and should show higher levels of nucleotide diversity and phylogeographic structure compared to northern populations (Milá et al. 2000). This pattern occurs to varying degrees in *Pinus* species on which Clark's nutcracker commonly feed: whitebark pine (*Pinus albicaulis*; Richardson et al. 2002), white pine (*P. monticola*; Kim et al. 2011), limber pine (*P. flexilis*; Mitton et al. 2000; Jørgensen et al. 2002), and Ponderosa pine (*P. ponderosa*; Latta and Mitton 1999; Johansen and Latta 2003). However, this pattern does not distinctly appear in Clark's nutcracker. All southern and northern populations sampled exhibit high levels of heterozygosity, nucleotide and allelic diversity, and limited differentiation from other populations, making it very challenging to

pinpoint a single refugium for this species. In addition, while species distribution models for current suitable nutcracker habitat performed very well (high AUC values), paleodistribution models returned reduced probabilities (< 30%) of suitable LGM habitat throughout unglaciated areas. Taken together, these results point to a single refugium or multiple small, connected refugia, the location of which is not obvious through genetic signatures or paleodistribution modeling.

2.5.3 Conclusions and future research

Throughout its range, Clark's nutcracker exhibits low levels of population differentiation and does not appear to be limited by potential barriers to dispersal, though peripheral populations may be slightly isolated. Future research could integrate additional mitochondrial markers to elucidate the location of a glacial refugium or refugia for this species. Mitochondrial markers with lower rates of mutation in corvids (e.g. cytochrome B; Saunders and Edwards 2000) may retain historic genetic signals longer and thus provide additional historic information to this study. Samples at the northern and isolated southern extremes of the range may also increase our understanding of peripheral isolation and postglacial colonization for this species. Given the importance of Clark's nutcracker to dispersal of *Pinus* species in North America (Tomback 1982) and current conservation and management concerns for many *Pinus* species (McKinney et al. 2011), further understanding of nutcracker dispersal may also assist in future forest management and recovery plans.

2.6 Chapter acknowledgements

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Table 2.1. Summary table of samples and mitochondrial DNA information for populations included in this study. Population abbreviations are as follows: CAB = central Alberta, SAB = southern Alberta, MT = Montana, WY = Wyoming, UT = Utah, CO = Colorado, NM = New Mexico, SCA = southern California, CCA = central California, NECA = northeast California, SOR = southern Oregon, NEOR = northeast Oregon, WA = central Washington, NEWA = northeast Washington, and SEBC = southeast British Columbia. Column headers are: Lat (^{o}N) = latitude and Long (^{o}W) = longitude of central locations; *n* = number of samples used for mitochondrial DNA analyses; H_n = number of haplotypes; H_d = haplotype diversity; and π = nucleotide diversity multiplied by 100 for ease of interpretation.

Population	**Lat (^{o}N)**	**Long (^{o}W)**	***n***	**H_n**	**H_d**	**π**
CAB	52.687	-118.056	8	6	0.893	0.342
SAB	49.774	-114.397	9	5	0.583	0.127
MT	46.592	-112.158	20	18	0.964	0.281
WY	43.756	-110.581	19	13	0.901	0.295
UT	40.703	-110.612	19	10	0.673	0.226
CO	39.813	-106.195	13	10	0.859	0.221
NM	35.815	-106.852	8	7	0.964	0.396
SCA	34.279	-117.551	14	9	0.912	0.312
CCA	37.771	-118.926	11	9	0.964	0.375
NECA	41.151	-120.205	2	2	1.000	0.456
SOR	42.745	-122.144	12	6	0.849	0.354
NEOR	45.243	-117.524	20	15	0.953	0.402
WA	47.554	-120.958	7	6	0.857	0.329
NEWA	48.845	-119.625	6	4	0.800	0.222
SEBC	49.162	-119.270	1	1	-	-
Overall			**169**		**0.854**	**0.324**

Table 2.2. Microsatellite primer pairs used in this study. Primer names, sequence, focal species, source, and reaction conditions for this study (buffer and taq polymerase, and annealing temperatures, T_1 and T_2 (°C)). Forward primers (shown with an 'F' suffix) were modified to include a short sequence at the 5' end allowing for incorporation of the florescent tag. All loci except PnuA106W are sourced from Florida Scrub Jay (*Aphelocoma coerulescens;* Stenzler and Fitzpatrick 2002). PnuA106W is sourced from Yellow-billed Magpie (*Pica nuttalli*; Ernest et al. 2008).

Primer	Sequence (5' to 3')	Buffer	taq	T_1	T_2
ApCo19F	CAG ACT GCA GTC TTG CTA TAG C	Flexi	crimson	45	48
ApCo19R	GCC TTG GAT GCT TTT ACG				
ApCo30F	GCC CTG ATG CTG TTG ATG GT	Flexi	Flexi	45	48
ApCo30R	CTG GAG CCT GGT TTA GAG TTA TGC				
ApCo37F	TGC CAA ATG CAA CCA AAT CTT	Flexi	Flexi	50	52
ApCo37R	CAT CAC TTG CAG AGA GGG CA				
ApCo41F	CCT ACT CTG GGC ACT GTT ATT ATC	crimson	crimson	50	52
ApCo41R	CCC ATT ATC AGC ATG TCG TAC A				
ApCo46F	GGG AGC CTA GTA TGT TAA GAT GCT	Flexi	crimson	50	52
ApCo46R	TTC CAG GTG AGG TGA TTC AGC				
ApCo91F	GTA GTC CCA ATG GTT TCT CTG TC	crimson	crimson	45	48
ApCo91R	GAT GAA GTA ATG TGA AAC CTG G				
PnuA106WF	GTA TTT TGG GAT GTC TTA GGG TTG	Flexi	Flexi	50	52
PnuA106WR	CAC ACT CGA GCT ACA ATA AAC CTG				

Table 2.3. Geographic distribution of shared haplotypes. Haplotype codes correspond to those found in Figure 2.2. Population abbreviations and locations are found in Table 2.1.

Haplotype	CAB	SAB	MT	WY	UT	CO	NM	SCA	CCA	NECA	SOR	NEOR	WA	NEWA	SEBC	Total
A	3	6	4	5	10	4	1	2	1	1	4	3	3	3	-	**50**
B	-	-	3	4	1	1	-	1	2	-	2	3	-	-	-	**17**
C	-	-	1	1	-	1	-	-	1	-	-	3	-	-	-	**7**
D	-	-	-	-	-	-	-	4	1	-	-	-	-	-	-	**5**
E	-	-	-	-	2	-	-	1	1	-	-	-	-	1	-	**5**
F	-	-	-	1	-	2	1	-	-	-	-	-	-	-	-	**4**
G	-	-	-	-	-	-	1	-	-	-	3	-	-	-	-	**4**
H	-	-	1	1	-	-	-	-	-	-	-	1	1	-	-	**4**
I	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	**3**
J	-	-	1	-	-	-	1	1	-	-	-	-	-	-	-	**3**
K	-	-	-	-	-	-	-	-	2	-	-	1	-	-	-	**3**
L	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	**2**
M	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	**2**
N	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	**2**
O	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	**2**
P	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	**2**
Q	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	**2**
R	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	**2**
Total	**4**	**6**	**13**	**14**	**14**	**9**	**6**	**13**	**9**	**1**	**9**	**12**	**5**	**4**	**0**	**119**
Unique	4	3	7	5	5	4	2	1	2	1	3	8	2	2	1	**50**

Table 2.4. Population pairwise Φ_{ST} values for Clark's nutcracker mitochondrial DNA. Φ_{ST} values are below diagonal, significance values corrected for false discovery rate above diagonal. Values are based on 110 permutations for mitochondrial DNA control region in 166 Clark's nutcrackers from 13 populations (n ≥ 5) in North America. Bolded values indicate significance. See Table 2.1 and Figure 2.1 for sampling locations and abbreviations.

	MT	CO	NM	UT	WY	NEOR	SOR	CCA	SCA	WA	NEWA	SAB	CAB
MT		0.832	0.100	0.509	0.714	0.373	0.316	0.509	0.373	0.825	0.530	0.468	0.574
CO	-0.026		0.270	0.414	0.883	0.546	0.714	0.771	0.414	0.771	0.373	0.373	0.414
NM	0.186	0.147		**<0.001**	**<0.001**	0.234	0.509	0.289	**<0.001**	0.373	**<0.001**	0.100	0.234
UT	0.005	0.017	**0.281**		0.589	0.296	0.289	0.509	0.296	0.714	0.779	0.473	0.270
WY	-0.012	-0.031	**0.156**	0.001		0.462	0.509	0.771	0.373	0.771	0.654	0.479	0.414
NEOR	0.023	0.003	0.099	0.042	0.007		0.383	0.756	0.270	0.756	0.414	0.289	0.414
SOR	0.039	-0.016	0.009	0.072	0.012	0.013		0.779	0.373	0.714	0.373	0.100	0.366
CCA	0.008	-0.025	0.102	0.011	-0.014	-0.015	-0.031		0.654	0.771	0.513	0.270	0.456
SCA	0.022	0.023	**0.202**	0.043	0.034	0.045	0.063	-0.013		0.691	0.499	0.334	0.366
WA	-0.041	-0.035	0.102	-0.019	-0.032	-0.020	-0.025	-0.032	0.003		0.771	0.373	0.714
NEWA	0.005	0.037	**0.282**	-0.050	-0.005	0.031	0.080	0.010	0.023	-0.046		0.876	0.509
SAB	0.017	0.077	0.359	0.017	0.019	0.066	0.153	0.076	0.059	0.032	-0.034		0.289
CAB	0.003	0.028	0.180	0.055	0.028	0.029	0.061	0.021	0.048	-0.018	0.029	0.066	

Table 2.5. Summary table of seven microsatellite loci used to analyze 13 Clark's nutcracker populations. Only populations with greater than five samples were used; *n* = number of samples used in genotyping and analyses; A_n = number of alleles; A_r = allelic richness; H_o = observed and H_e = expected heterozygosity; P = departures from Hardy-Weinberg equilibrium (ns = not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001. See Table 2.1 for population locations.

	ApCo19	ApCo30	ApCo40	ApCo41	ApCo46	ApCo91	PnuA106W
Central Alberta (n=8)							
A_n	3	3	5	2	4	4	2
A_r	2.359	2.361	3.160	1.692	2.967	2.963	1.429
H_o	0.571	0.625	0.750	0.000	0.429	0.625	0.143
H_e	0.500	0.555	0.625	0.245	0.602	0.648	0.133
P	ns	ns	ns	**	ns	ns	ns
Southern Alberta (n=12)							
A_n	3	3	5	2	6	2	2
A_r	2.190	2.274	3.039	2.000	4.018	1.846	1.908
H_o	0.500	0.400	0.583	0.667	0.750	0.143	0.417
H_e	0.497	0.535	0.611	0.444	0.806	0.337	0.413
P	ns	ns	ns	ns	ns	ns	ns
Montana (n=25)							
A_n	3	3	6	4	5	5	3
A_r	2.142	2.566	3.430	1.835	3.446	3.062	1.878
H_o	0.640	0.440	0.840	0.320	0.783	0.750	0.320
H_e	0.486	0.586	0.734	0.282	0.738	0.684	0.351
P	ns	*	ns	ns	ns	ns	ns
Wyoming (n=30)							
A_n	3	4	9	3	5	3	3
A_r	2.022	2.510	3.937	1.620	2.686	2.314	1.800
H_o	0.483	0.357	0.724	0.136	0.550	0.400	0.241
H_e	0.463	0.543	0.793	0.208	0.556	0.460	0.313
P	ns	ns	**	***	*	*	ns
Utah (n=20)							
A_n	2	4	6	3	5	3	3
A_r	1.962	2.558	3.247	1.891	2.926	2.426	1.862
H_o	0.579	0.294	0.650	0.316	0.471	0.571	0.278
H_e	0.478	0.528	0.700	0.342	0.619	0.538	0.323
P	ns	***	ns	ns	*	ns	ns

Colorado (n=13)							
$\mathbf{A_n}$	3	3	6	2	5	4	3
$\mathbf{A_r}$	2.247	2.353	3.509	1.273	3.643	2.632	2.062
$\mathbf{H_o}$	0.385	0.417	0.769	0.091	0.750	0.444	0.385
$\mathbf{H_e}$	0.462	0.517	0.728	0.087	0.760	0.512	0.411
P	ns	ns	ns	ns	ns	ns	ns
New Mexico (n=9)							
$\mathbf{A_n}$	3	3	5	2	4	3	2
$\mathbf{A_r}$	2.241	2.317	3.452	1.867	3.084	2.467	1.975
$\mathbf{H_o}$	0.667	0.778	0.750	0.400	0.429	0.400	0.111
$\mathbf{H_e}$	0.475	0.549	0.719	0.320	0.663	0.460	0.475
P	ns	ns	ns	ns	ns	ns	*
Southern California (n=14)							
$\mathbf{A_n}$	3	3	7	3	5	4	2
$\mathbf{A_r}$	2.111	2.375	3.615	1.646	3.265	2.645	1.976
$\mathbf{H_o}$	0.429	0.571	1.000	0.077	0.571	0.636	0.286
$\mathbf{H_e}$	0.457	0.487	0.737	0.210	0.694	0.566	0.490
P	ns	ns	ns	**	ns	ns	ns
Central California (n=11)							
$\mathbf{A_n}$	3	3	7	2	4	4	3
$\mathbf{A_r}$	1.909	2.231	4.233	1.834	3.173	2.961	2.247
$\mathbf{H_o}$	0.364	0.545	0.636	0.273	0.800	0.636	0.636
$\mathbf{H_e}$	0.310	0.517	0.818	0.351	0.685	0.665	0.533
P	ns	ns	ns	ns	ns	ns	ns
Southern Oregon (n=12)							
$\mathbf{A_n}$	2	5	6	3	4	4	2
$\mathbf{A_r}$	1.940	2.894	3.759	2.202	2.843	2.645	1.908
$\mathbf{H_o}$	0.500	0.750	1.000	0.300	0.750	0.417	0.417
$\mathbf{H_e}$	0.444	0.597	0.760	0.405	0.618	0.549	0.413
P	ns	ns	ns	ns	ns	ns	ns
Northeast Oregon (n=20)							
$\mathbf{A_n}$	2	3	8	3	6	4	2
$\mathbf{A_r}$	1.970	2.681	3.294	1.891	3.798	2.406	1.764
$\mathbf{H_o}$	0.450	0.600	0.700	0.105	0.750	0.500	0.100
$\mathbf{H_e}$	0.489	0.625	0.704	0.342	0.790	0.510	0.320
P	ns	ns	ns	**	ns	ns	**
Washington (n=7)							
$\mathbf{A_n}$	2	3	7	2	3	3	3
$\mathbf{A_r}$	1.992	2.622	4.045	1.500	2.758	2.682	2.359
$\mathbf{H_o}$	0.500	0.571	0.857	0.167	0.500	0.500	0.714
$\mathbf{H_e}$	0.486	0.571	0.765	0.153	0.625	0.569	0.500
P	ns	ns	ns	ns	ns	ns	ns

Northeast Washington (n=6)

A_n	2	3	4	2	5	3	2
A_r	1.998	2.545	2.969	1.773	3.924	2.545	1.992
H_o	0.333	0.667	0.667	0.000	0.833	0.500	0.833
H_e	0.500	0.500	0.625	0.278	0.764	0.500	0.486
P	ns	ns	ns	*	ns	ns	ns
Overall	$A_n = 10$	$A_n = 5$	$A_n = 10$	$A_n = 7$	$A_n = 7$	$A_n = 7$	$A_n = 4$

Table 2.6. Population pairwise F_{ST} values for Clark's nutcracker. F_{ST} values are below diagonal and significance values corrected for false discovery rate are above diagonal. Values are based on 110 permutations using seven polymorphic microsatellite loci in 187 Clark's nutcrackers from 13 populations in North America. See Table 2.2 and Figure 2.1 for population abbreviations.

	MT	**CO**	**NM**	**UT**	**WY**	**NEOR**	**SOR**	**CCA**	**SCA**	**WA**	**NEWA**	**SAB**	**CAB**
MT		0.185	0.454	0.205	0.100	0.454	0.210	**0.032**	**0.028**	0.454	0.311	0.093	0.196
CO	0.016		0.238	0.189	**0.035**	0.189	0.205	0.017	**0.035**	0.454	0.189	0.268	**0.016**
NM	0.001	0.017		0.084	0.189	0.454	0.378	0.196	0.454	0.454	0.454	0.414	0.189
UT	0.009	0.016	0.033		0.454	0.189	0.454	**0.023**	**0.016**	0.454	0.142	**0.017**	0.093
WY	0.012	**0.028**	0.017	0.000		0.081	0.189	**0.016**	**0.016**	0.454	0.072	**0.017**	**0.035**
NEOR	0.000	0.015	0.000	0.012	0.016		0.167	**0.024**	**0.050**	0.454	0.454	0.189	0.210
SOR	0.012	0.017	0.007	0.001	0.013	0.020		0.058	**0.024**	0.467	0.293	**0.031**	**0.032**
CCA	**0.040**	**0.072**	0.021	**0.063**	0.059	**0.054**	0.036		0.234	0.093	0.394	**0.017**	**0.016**
SCA	**0.038**	**0.044**	0.000	**0.071**	**0.066**	**0.039**	**0.062**	0.014		0.196	0.454	0.093	**0.024**
WA	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.043	0.026		0.454	0.358	0.267
NEWA	0.014	0.033	0.000	0.036	0.040	0.000	0.017	0.010	0.000	0.000		0.196	0.072
SAB	0.026	0.011	0.004	**0.045**	**0.046**	0.017	**0.052**	**0.090**	0.034	0.008	0.027		**0.037**
CAB	0.020	**0.075**	0.032	0.034	**0.041**	0.020	**0.066**	**0.123**	**0.096**	0.021	0.071	**0.057**	

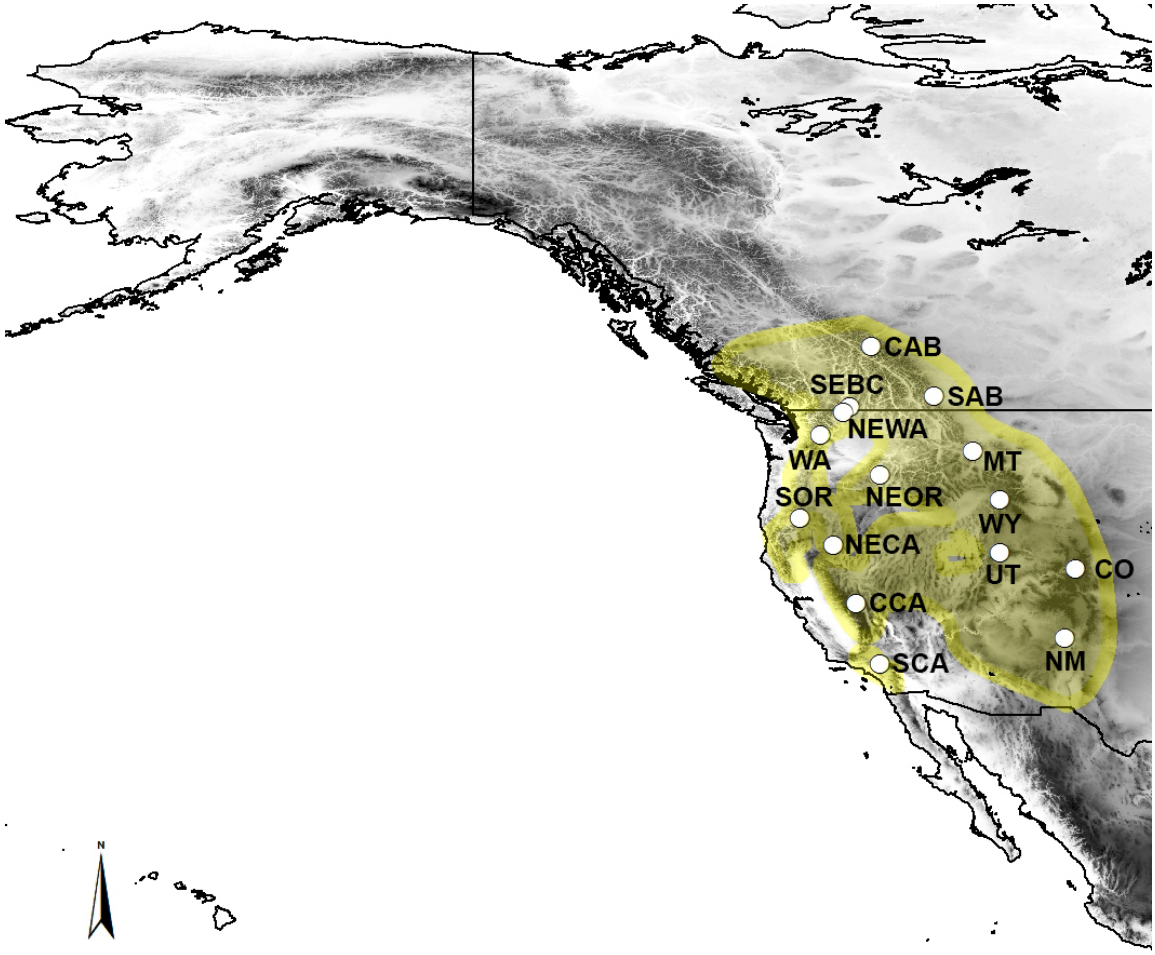

Figure 2.1. Clark's nutcracker population sampling locations. Current range overlaid in yellow (Ridgely et al. 2007). Darker shades imply higher elevation areas. See Table 2.1 for coordinates, sample sizes, and abbreviations per population.

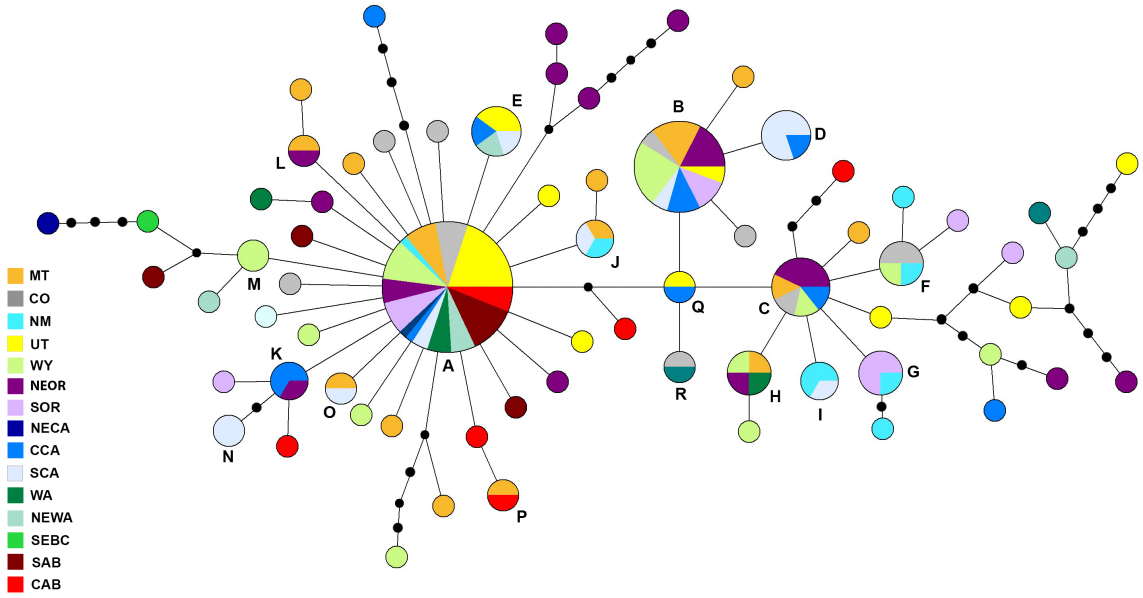

Figure 2.2. Clark's nutcracker mitochondrial DNA haplotype network. Maximum parsimony network constructed using a median joining algorithm and post-processed with a maximum parsimony procedure for a 900 base pair fragment of mitochondrial control region from 169 Clark's nutcrackers across North America. Each colour represents a different population (n = 15). Circle size is proportional to number of individuals with that haplotype. Haplotypes represented as pie charts include individuals from multiple populations with pie segments proportional to the number of individuals from each population. Capital letters are those assigned to shared haplotypes (see Table 2.3). Small black circles represent inferred nodes. See Table 2.1 for population abbreviations.

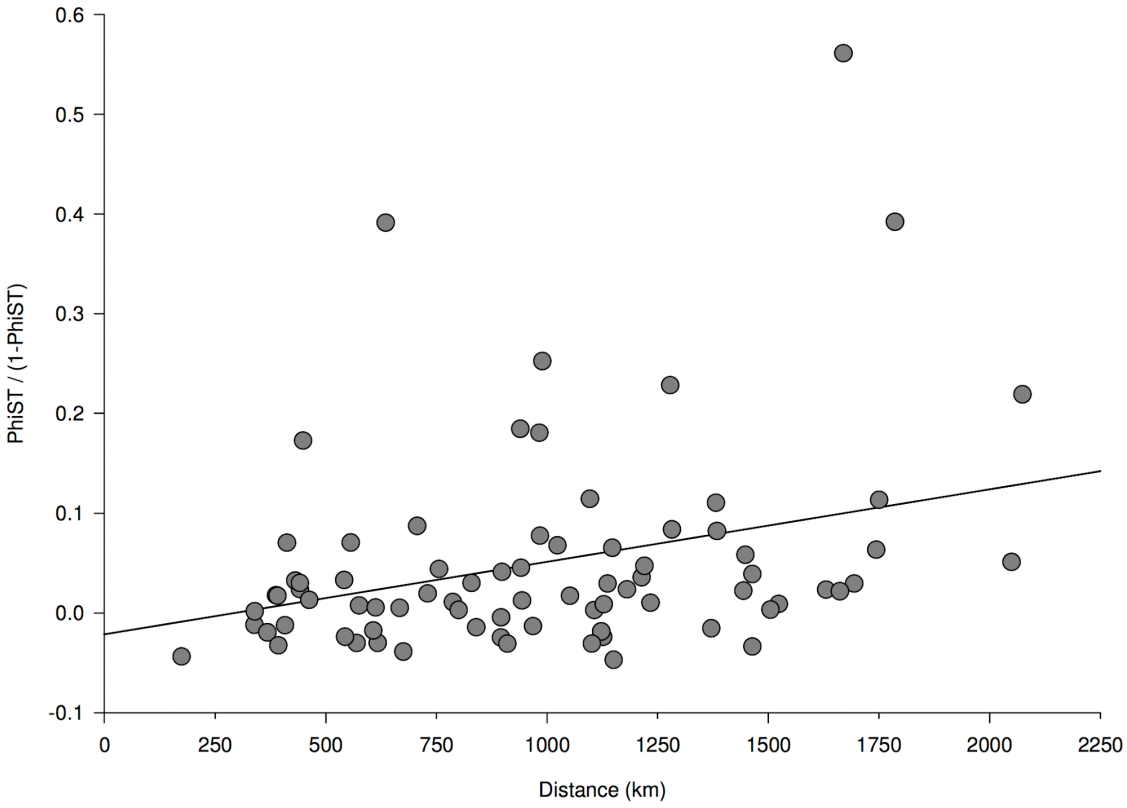

Figure 2.3. Mantel test of isolation by distance for mitochondrial DNA. R^2 = 0.082, P = 0.040. Each point represents one population pairwise Φ_{ST}/ (1- Φ_{ST}) plotted against geographic distance between paired populations.

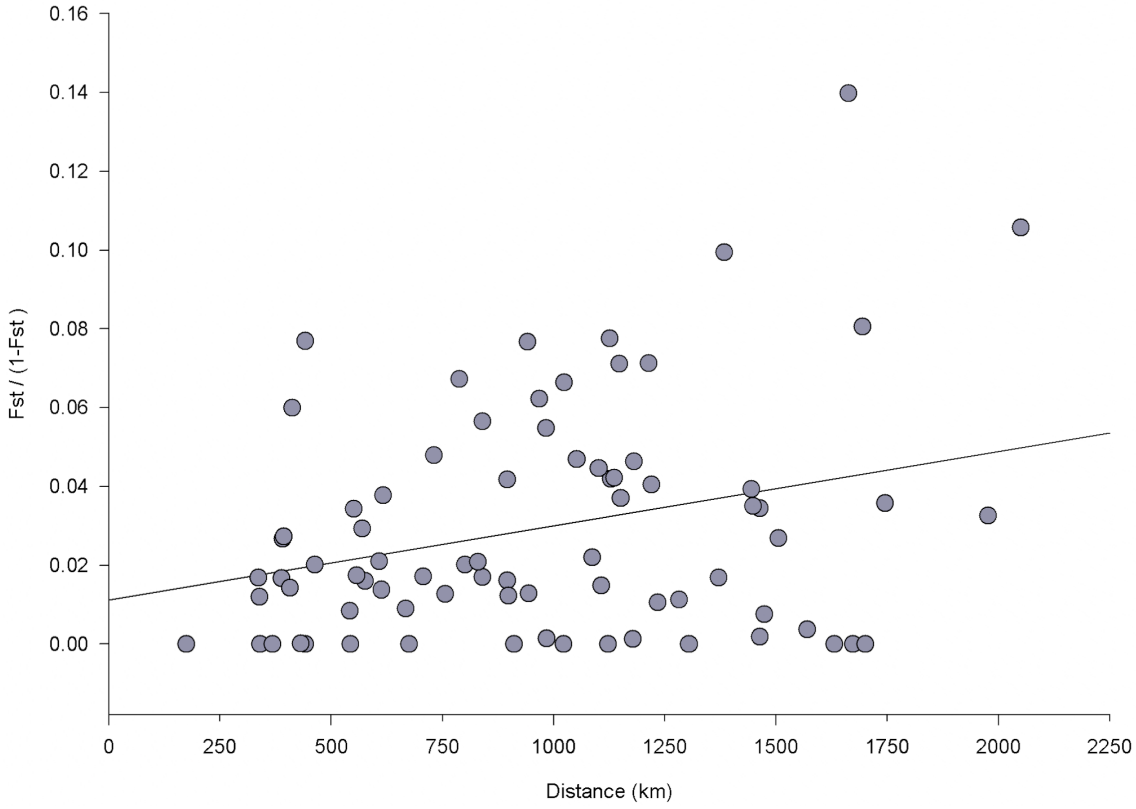

Figure 2.4. Mantel test of isolation by distance for seven microsatellite loci. R^2 = 0.078, P = 0.029. Each point represents one population pairwise F_{ST} / (1- F_{ST}) plotted against straight line geographic distance between paired populations.

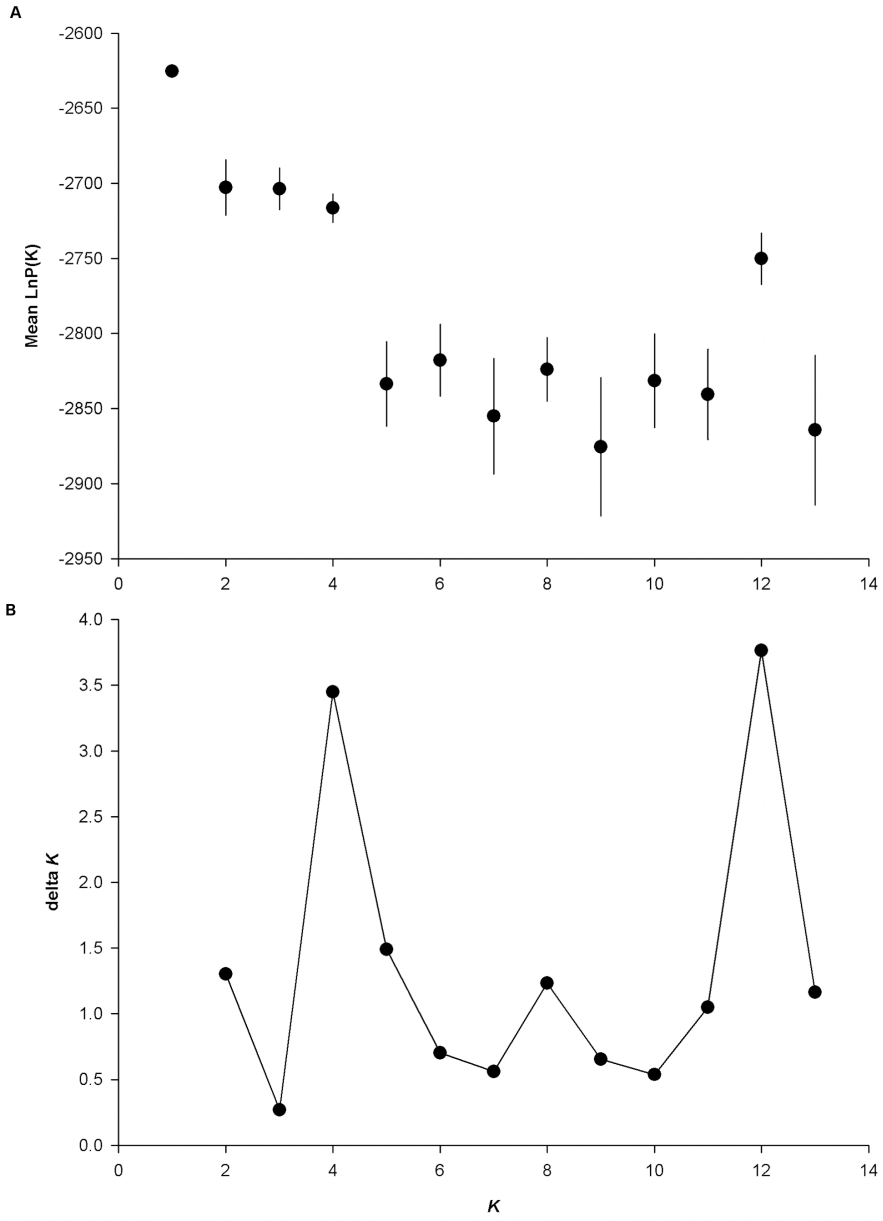

Figure 2.5. Measures of optimal *K* values from STRUCTURE analyses. A) Penalised log likelihood test (mean ± SE) and B) ΔK depicting clusters (K). The penalised log likelihood test takes the maximum ln Pr (X | K) as the correct number of clusters and ΔK infers the number of clusters from the difference between ln Pr (X | K).

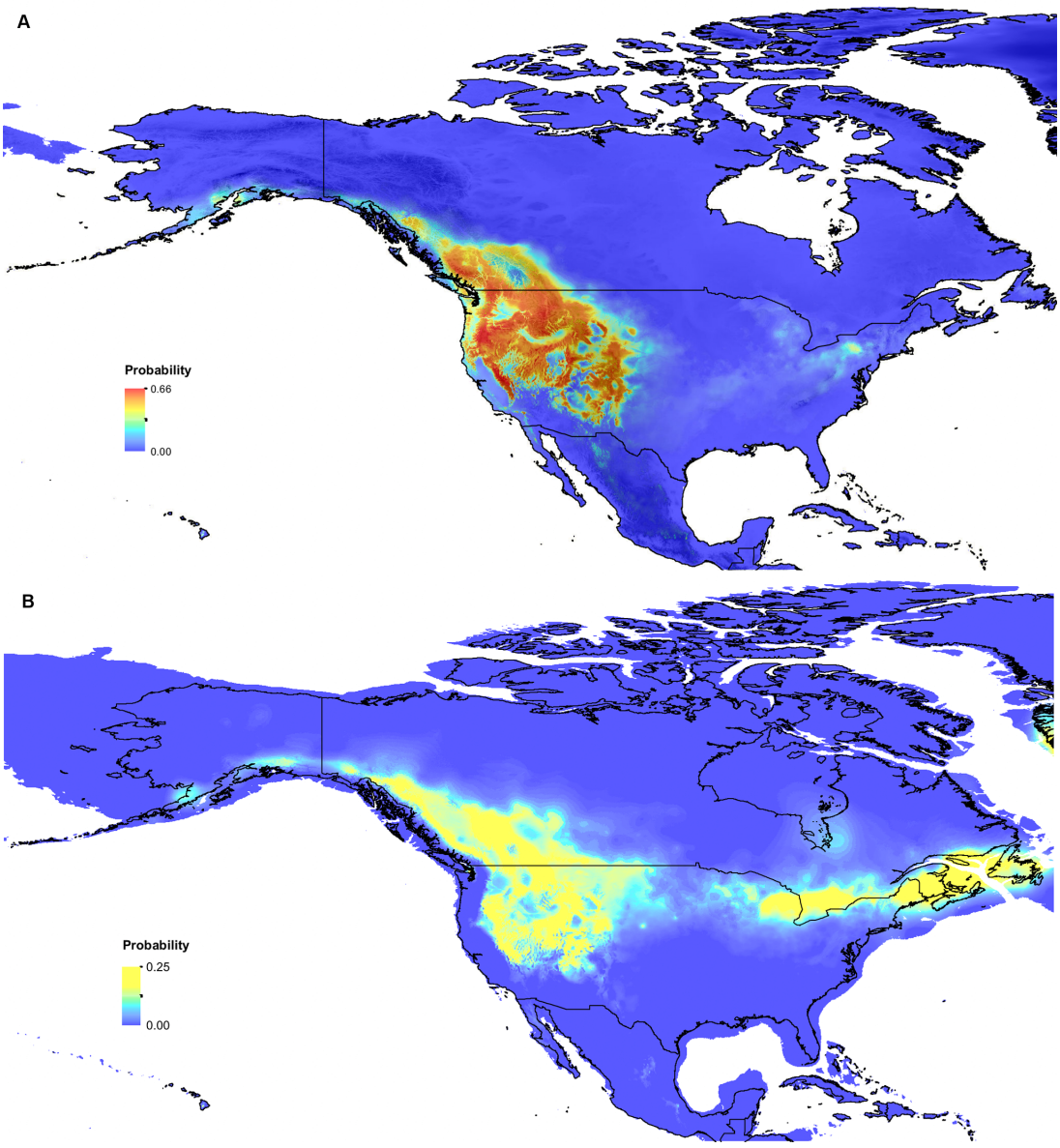

Figure 2.6. Current and paleodistribution models of suitable Clark's nutcracker habitat in North America. Probability of suitable Clark's nutcracker habitat at A) present (overlaid on digital elevation model and B) during last glacial maximum (~21 kya), overlaid on current North American boundaries generated by species distribution modeling. In B), blue areas outside of current coastlines represent historical extent of terrestrial landmass.

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Chapter 3: Limited geographic genetic structure detected in a widespread Palearctic corvid, *Nucifraga caryocatactes* [1]

3.1 Abstract

The Eurasian or spotted nutcracker (*Nucifraga caryocatactes*) is a widespread resident corvid found throughout the Palearctic from Central Europe to Japan. Characterized by periodic bouts of irruptive dispersal in search of *Pinus* seed crops, this species has potential for high levels of gene flow across its range. Previous analysis of 11 individuals did not find significant range-wide population genetic structure. We investigated population structure using 924 base pairs of mitochondrial DNA control region sequence data from 62 individuals from 12 populations distributed throughout the nutcracker's range. We complemented this analysis by incorporating additional genetic data from previously published sequences. High levels of genetic diversity and limited population genetic structure were detected suggesting that potential barriers to dispersal do not restrict gene flow in nutcrackers.

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KM Dohms contribution to publication: study design, data collection and analyses, writing, submission for publication, and revisions.

3.2 Introduction

In Eurasia, phylogeographic studies of many widespread vertebrate species have revealed a variety of geographical patterns of population structure influenced by current and historical landscapes, with little overall consensus among species. Using mitochondrial DNA, east-west splits have been documented for a variety of vertebrates including bats (Flanders et al. 2009), and several avian species (e.g. Eurasian magpie (*Pica pica*; Kryukov et al. 2004), rook (*Corvus frugilegus*; Haring et al. 2007), and red-breasted flycatcher (*Ficedula parva*; Zink et al. 2008)). For other species, multiple splits have occurred (e.g. root vole (*Microtus oeconomus*; Brunhoff et al. 2003) and reed bunting (*Emberiza schoeniclus*; Zink et al. 2008)), or peninsula populations are isolated (e.g. great bustard (*Otis tarda*; Pitra et al. 2000)). In contrast, little population structure has been detected in some widespread species, such as otters (*Lutra lutra*; Ferrando et al. 2004) and several avifauna species (e.g. great spotted woodpecker (*Dendrocopos major*; Zink et al. 2002), common sandpiper (*Actitis hypoleucos*; Zink et al. 2008), and Eurasian magpie (*Pica pica*; Zhang et al. 2012)). Some of the observed phylogeographic patterns have been explained by post-glacial colonization from single or multiple refugia, but may also be influenced by barriers to dispersal, such as mountain ranges (e.g. Ural Mountains), large areas of inhospitable habitat (e.g. Tibetan Plateau), or large bodies of water.

The Eurasian nutcracker (*Nucifraga caryocatactes*, Linnaeus, 1758) is a corvid with a widespread Palearctic distribution. Although generally classified as a resident species of continental coniferous forests, nutcrackers are known to irruptively disperse to take advantage of mast conifer seed crops (Haring et al. 2007), similar to its North American sister species, Clark's nutcracker (*N. columbiana*; Tomback 1998). Strong geographic genetic structure has not been found in Clark's nutcracker, despite numerous potential physical barriers to dispersal and thus gene flow (Dohms and Burg 2013). A previous study by Haring and colleagues (2007) found no population structure in *N. caryocatactes* throughout Eurasia. However, Haring et al. (2007) only used 11 specimens, thus additional data may shed further light on nutcracker population genetic structure.

In this study, we use a highly variable and rapidly evolving mitochondrial DNA marker, the control region, to further investigate population structure of *N. caryocatactes* in Eurasia. Based on the ecology of this species, we predict little range-wide population genetic structure.

3.3 Materials & Methods

3.2.1 Samples

Tissue samples (n = 62) collected throughout the Eurasian nutcracker's range (Figure 3.1) were acquired from the Burke Museum of Natural History and Culture at the University of Washington (Supplemental Table S1). Previously

published control region (CR) sequences (n = 11) were obtained from GenBank (EU070770 and EU070886-EU070895; Haring et al. 2007).

3.2.2 DNA extraction, PCR amplification, and sequencing

DNA from muscle samples stored in ethanol or lysis buffer was extracted using a modified Chelex extraction protocol (Walsh et al. 1991; Burg and Croxall 2001). A 924 bp fragment starting at position 46 of the control region (CR; Saunders and Edwards 2000) was amplified using two primers: L46 SJ (5'-TTT GGC TAT GTA TTT CTT TGC-3'; developed for Steller's jay (*Cyanocitta stelleri*; Birt & Lemmen, unpublished)) and H1030 JCR 18 (5'-TAA ATG ATT TGG ACA ATC TAG G-3'; developed for *Aphelocoma* jays (Saunders and Edwards 2000)). DNA was amplified in a Master gradient thermocycler (Eppendorf) in 25 μL reactions with 1x goTaq Flexi buffer (Promega), 2.5 mM $MgCl_2$, 200 μM dNTP, 0.4 μM of each primer, and 1 unit goTaq Flexi taq polymerase (Promega). DNA sequencing was performed on an ABI 3730xl DNA Analyzer at McGill University and Génome Québec Innovation Centre.

3.2.3 Alignment and analysis

We edited and aligned sequences from chromatograms and an overlapping subset of 305 bp from previously published CR sequences from GenBank (Haring et al. 2007) using MEGA v5.0 (Tamura et al. 2007). Two unrooted statistical parsimony networks (95% probability) were constructed with TCS v1.21

(Clement et al. 2000): one for the samples sequenced as part of this study (924 bp) and a second network that for the 305 bp common fragment (this study; Haring et al. 2007). We calculated the number of haplotypes (H_n), haplotype diversity (H_d), and nucleotide diversity (π) for museum samples using DnaSP v5.10 (Rozas et al. 2003).

3.4 Results

3.4.1 Genetic analyses

We sequenced and aligned the 924 bp control region (CR) sequences for 62 individuals from 12 populations (Table 1; GenBank accession nos. KJ999615–KJ999676). We aligned 11 additional GenBank sequences (Haring et al. 2007) with sequences from our samples and obtained a 305 bp area of overlap. Statistical parsimony networks did not suggest strong geographic structure for the 924 bp sequence (Figure 3.2), nor for the larger dataset using the overlapping 305 bp fragment for all 73 individuals (Figure 3.3). Ncarcar5 could not be connected to the network in the larger dataset, which was also found by Haring et al. (2007).

For the 62 individuals we sequenced, we found 45 unique haplotypes and high levels of genetic diversity in most populations (Table 3.1). We found 57 polymorphic sites within the 924 bp sequence and 22 within the 305 bp sequence. Haplotype diversity for the 924 bp sequence varied from 0.000 (Ola River headwaters (OR)) to 1.000 (five populations), and all but the OR population had

a haplotype diversity equal to or greater than 0.800 (Table 3.1). Nucleotide diversity ranged from 0.00000 (OR) to 0.00993 (PK; Table 3.1). Overall haplotype diversity (H_d) 0.967 and nucleotide diversity (π) was 0.00537.

3.5 Discussion

As predicted, analyses of Eurasian nutcracker mitochondrial DNA control region sequences did not detect significant population genetic structure. All populations except nutcrackers from the Ola River headwaters (OR; n = 2) exhibited high haplotype diversity and relatively high nucleotide diversity. No geographic clustering was observed in statistical parsimony networks, even when integrating samples from the western part of the range. Despite potential barriers to dispersal for this species, such as isolation on an island (e.g. Sakhalin Oblast (SO)) or peninsula (e.g. Kamchatka (KA)), most populations of *N. caryocatactes* do not appear to be geographically differentiated from each other, likely due to gene flow during irruptive dispersal in search of mast pine seed crops. Overall, our work supports that done by Haring et al. (2007) where no significant split was seen between the east and west for nutcrackers and is similar to the pattern found in *N. caryocatactes* sister species, *N. columbiana* (Dohms and Burg 2013).

Compared to Haring et al. (2007), our study found a higher level of haplotype diversity (H_d = 0.967 vs 0.844 and π = 0.00537 vs 0.00279). This may be due to the portion of control region sequenced and the larger sample sizes used in this

study. The sequences obtained from our samples are predominantly composed of domains I and II of the mtDNA control region (Saunders and Edwards 2000), whereas Haring et al. (2007) sequenced primarily central domain II, which is considered less variable (Ruokonen and Kvist 2002).

Haring et al. (2007) state that low genetic diversity may suggest a bottleneck in this species and a single glacial refugium. With high levels of nucleotide and haplotype diversity, our findings do not support a historical bottleneck but rather point toward two possible scenarios: multiple refugia with gene flow or a single refugium with large population size during expansion. Expansion from multiple refugia with gene flow after colonization can produce similar genetic patterns to those in species that expanded slowly from a single refugium with a large population size, retaining high genetic diversity and limited geographic structuring of populations (Hewitt 2004). Given the dispersal potential of nutcrackers, it is possible that a large population expanded out of a single refugium, but it is equally plausible that expansion occurred out of multiple refugia with subsequent gene flow between geographically distinct populations due to irruptive dispersal events. The multiple refugia scenario could have produced the large number of haplotypes, often with high levels of sequence divergence pattern seen here. For example, individuals from Primorsky Kray (PK) are found scattered throughout the parsimony network (Figure 3.2), in some cases with a large number of mutations between PK individuals and other

haplotypes, yet found clustered with geographically distant individuals from Irkutsk Oblast (KD) and KA. This level of divergence is often associated with isolation in and subsequent colonization from multiple refugia (Hewitt 2004). With the high haplotype diversity across all populations, it is not possible to determine which population(s), if any sampled here, may be in the location of the original refugium or refugia. Without additional present day samples from the Alps and Himalaya Mountain ranges, it is difficult to tell using genetic signatures if these areas served as refugia for nutcrackers during the LGM.

Our findings do not support a single refugium in the Altai Mountains of southern Mongolia, as postulated by Haring et al. (2007). Rather, our data show highly divergent haplotypes, which could be the result of prolonged isolation in multiple refugia. Scots pine (*Pinus sylvestris*), an important source of food for nutcrackers, is thought to have survived in refugia near the Alps (Naydenov et al. 2007) and in the east, unglaciated portions of the Himalayas could have served as a refugium for high latitude species (Zhuo et al. 1998; Owen et al. 2002). Alternatively, high levels of plant endemism have been found in the mountains of southern and eastern China, suggestive of long-term suitable habitats (Zhuo et al. 1998). Nutcrackers may have survived in these bands of suitable habitat in the southwest and southeast areas of Eurasia and expanded northward from multiple refugia as glaciers retreated.

3.6 Conclusions

Overall, Eurasian nutcrackers exhibit limited geographic genetic structure throughout their range, as might be expected from a resident bird with irruptive dispersal patterns. Our study found high genetic diversity, which suggests that a population bottleneck has not occurred in this species as previously hypothesized. A more detailed phylogeographical study could include additional genetic sampling from northern and southern parts of *N. caryocatactes'* range to further investigate structure across the range of this species.

3.7 Chapter acknowledgements

We gratefully acknowledge the assistance of Sharon Birks and the Burke Museum of Natural History and Culture – University of Washington; without tissue samples from their collection, this study would not have been possible.

Table 3.1. Diversity of a 924 bp mitochondrial DNA sequence. Haplotype diversity for a 924 bp fragment of mtDNA control region from 12 populations of *Nucifraga caryocatactes* throughout Eurasia.

Population	**Location**	***n***	**H_n**	**H_d**	**π**
BK	Badzhal'skiy Krebet, Russia	3	3	1.000	0.00361
GA	Gorno-Altaysk, Russia	4	4	1.000	0.00328
KA	Kamchatka, Russia	6	4	0.800	0.00369
KD	Irkutsk Oblast, Russia	5	5	1.000	0.00523
MA	Magadanskaya Oblast, Russia	8	8	1.000	0.00397
OK	Kyzyl, Russia	1	1	-	-
OR	Ola River headwaters, Russia	2	1	0.000	0.00000
PK	Primorsky Kray, Russia	11	11	1.000	0.00993
SO	Sakhalinksya Oblast, Russia	5	4	0.900	0.00195
TO	Tyumenskaya Oblast, Russia	7	6	0.952	0.00622
TU	Irkutsk Oblast, Russia	5	4	0.900	0.00326
UL	Ulaanbaatar, Mongolia	5	4	0.900	0.00611
Overall		**62**	**45**	**0.967**	**0.00537**

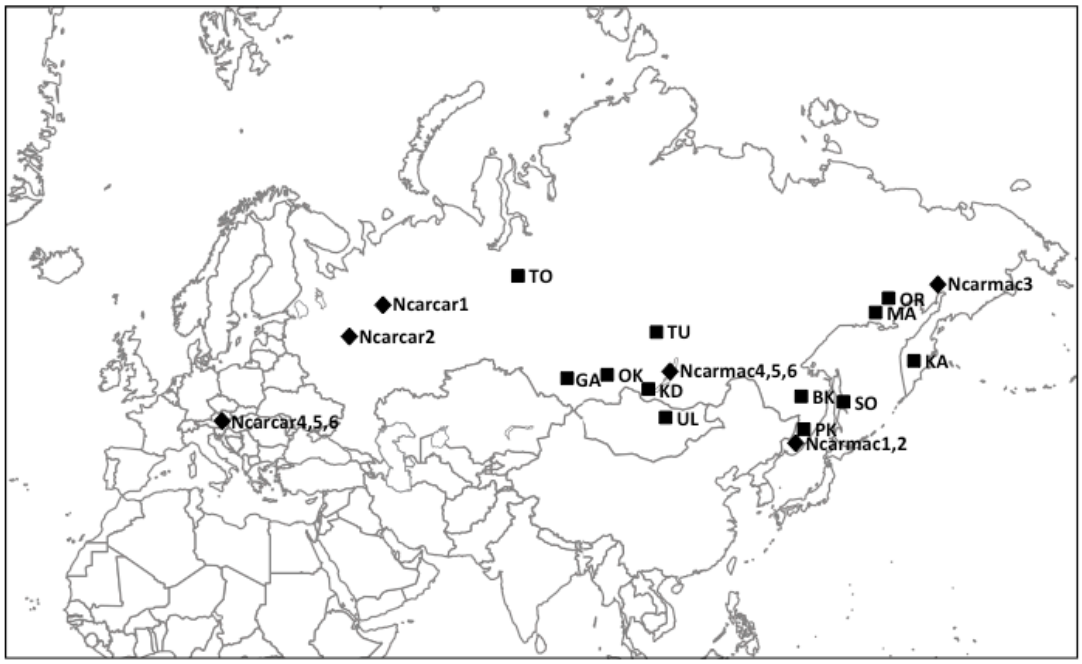

Figure 3.1. Nutcracker tissue sample locations throughout Eurasia. Black squares denote locations; corresponding abbreviations are labelled beside squares. Refer to Table 3.1 and Appendix 2 for further location information. Black diamonds denote locations of previously published partial control region sequences obtained from GenBank with corresponding sample codes from Haring *et al.* (2007).

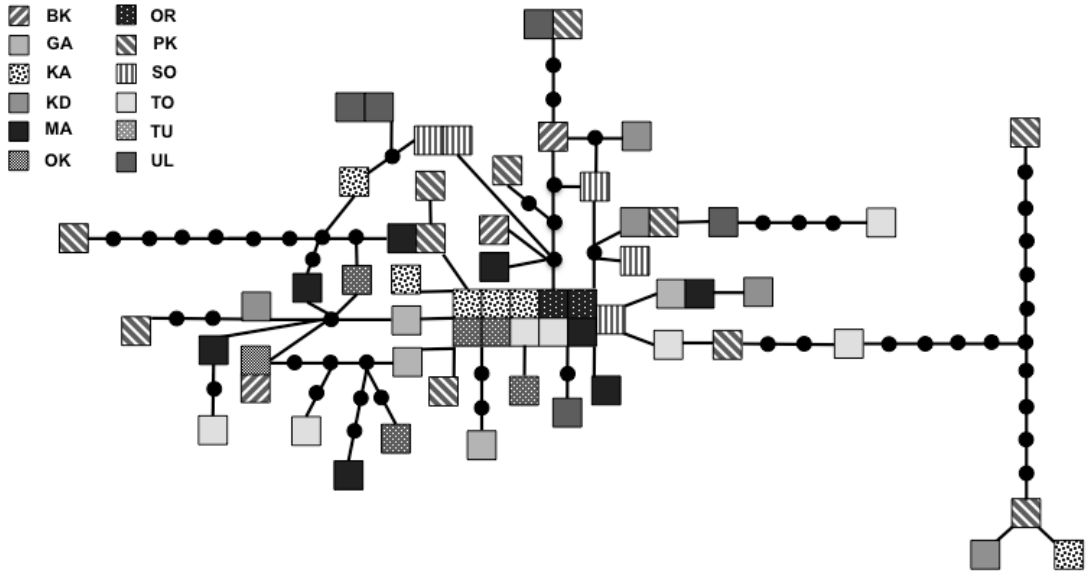

Figure 3.2. Statistical parsimony network for 924 bp mitochondrial DNA sequence. Statistical parsimony network of *Nucifraga caryocatactes* for 924 bp of the mitochondrial DNA control region sequenced from museum samples (n = 62). Each square represents one individual and colours correspond to author-defined populations, as per figure legend. Circles indicate inferred haplotypes. Refer to Table 3.1 for population abbreviations.

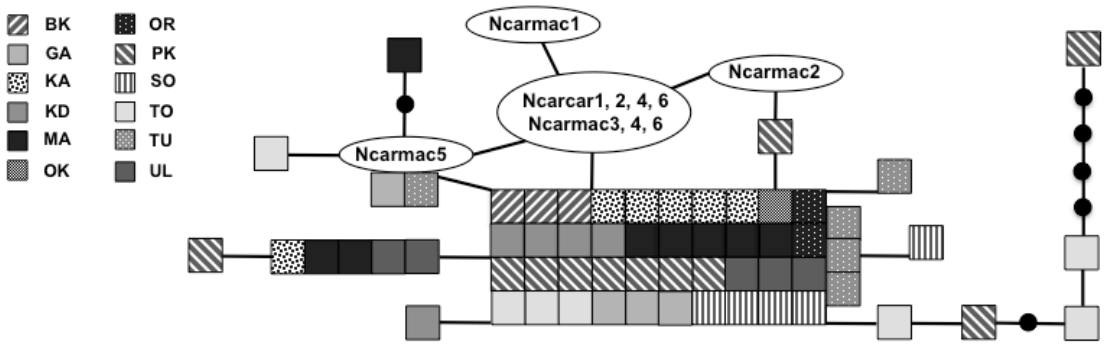

Figure 3.3. Statistical parsimony network of 305 bp mitochondrial DNA sequence. Statistical parsimony network of *Nucifraga caryocatactes* for overlapping sequences of 305 bp of the mitochondrial control region (Domain II) sequenced from museum samples (n = 62) and GenBank sequences (n = 11; (Haring et al. 2007) Each coloured square represents one individual and colours correspond to author-defined populations. Black solid circles indicate inferred haplotypes. Open circles represent haplotypes; text in circles represents GenBank sequences as per Figure 3.1 and Haring *et al.* (2007). Refer to Table 3.1 for population abbreviations found in the legend.

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Chapter 4: Multilocus genetic analyses and spatial modeling reveal complex population structure and history in a widespread resident North American passerine (*Perisoreus canadensis*)

4.1 Abstract

The genetic impact of barriers and Pleistocene glaciations on high latitude resident species has not been widely investigated. The gray jay (*Perisoreus canadensis*) is a high latitude, resident corvid restricted to coniferous and mixed deciduous-coniferous forests. Its range encompasses known barriers to dispersal and much of the land area covered by ice sheets during the Pleistocene. Using the highly variable mitochondrial DNA control region, microsatellite markers, and species distribution modeling, we examined the effects of glaciations and dispersal barriers on the population genetic patterns and population structure of gray jays. We sequenced 914 bp of mitochondrial control region for 375 individuals from 37 populations and screened seven microsatellite loci for 402 individuals from 27 populations across the gray jay range. We used species distribution modeling and a range of phylogeographic analyses (haplotype diversity, Φ_{ST}, SAMOVA, $F_{ST,}$ Bayesian clustering analyses) to examine the evolutionary history and population genetic structure. We found significant amounts of differentiation among populations for both genetic markers, and seven geographically distinct mitochondrial groups with diverse evolutionary histories. We also investigated body size in gray jays and found significant

variation among the seven haplogroups. Paleodistribution models supported at least five potential areas of suitable gray jay habitat during the last glacial maximum. Colonization from and prolonged isolation in multiple refugia is evident in the genetic data. Contemporary gene flow, as assessed by microsatellite markers, is being influenced by barriers such as large bodies of water and large areas of unsuitable habitat. Historical climatic fluctuations, the presence of multiple dispersal barriers, and highly restricted gene flow appear to be responsible for strong genetic diversification of gray jays.

4.2 Introduction

During the last glacial maximum (LGM), large portions of North America were covered by ice sheets (Pielou 1991), fragmenting species' ranges, and restricting surviving individuals and populations to ice-free refugia. Long-term isolation in glacial refugia has been shown to promote genetic diversification in a variety of organisms (Weir and Schluter 2004; Jaramillo-Correa et al. 2009; Shafer et al. 2010). North American plant and animal species expanded from several known refugia following the retreat of the ice sheets, including Beringia (parts of Alaska) and three areas south of the ice sheets (Pacific Coast, Rockies and Taiga), while coastal areas such as Newfoundland are contested to have been ice-free (Pielou 1991; Weir and Schluter 2004; Jaramillo-Correa et al. 2009). Contemporary genetic patterns are strongly influenced by post-glacial expansion from refugia (Weir and Schluter 2004; Williams 2003), historical and contemporary barriers to

dispersal (Brunsfeld et al. 2001; Keyghobadi 2007), and dispersal potential (Burg et al. 2003).

Historical events shaping current population structure should be particularly evident in resident species. Sedentary species retain patterns of genetic variation longer due to limited dispersal, allowing researchers to make inferences about past historic events (Jaramillo-Correa et al. 2009; Burg et al. 2005; Burg et al. 2006; Petit et al. 2005). Indeed, tree species show distinct patterns of population genetic structure and the influence of historical environmental changes (Jaramillo-Correa et al. 2009). Similar patterns are emerging in vertebrate taxa as the number of studies on resident species increases (e.g., Arbogast et al. 2001; Barrowclough et al. 2004; Burg et al. 2005; Graham and Burg 2012; Adams and Burg 2015).

The gray jay (*Perisoreus canadensis*) is ideal for investigating patterns of post-glacial colonization and the impact of dispersal barriers on boreal forest residents for several reasons. Gray jays are a relatively sedentary resident species, like their putative sister species the Siberian jay (*Perisoreus infaustus*; Strickland and Ouellet 2011), which exhibits strong population genetic structure in fragmented habitats (Uimaniemi et al. 2000). Adult gray jays remain in the same territory between breeding seasons and natal dispersal is limited to nearby territories, though some irruptive juvenile dispersal has been observed (Strickland and Ouellet 2011). Gray jays are broadly distributed across northern and western

North America (Figure 4.1) and strongly associated with spruce (*Picea* spp.). The gray jay contemporary range encompasses a number of purported barriers to dispersal (e.g., Salish Sea, Strait of Belle Isle, Columbia Basin), in addition to previously glaciated (e.g., most of Canada) and unglaciated areas (e.g., Alaska, western United States). Gray jays display plumage and morphological trait variation across their range with up to twelve subspecies described based on morphological characteristics, though a recent revision suggests nine subspecies (Strickland and Ouellet 2011). The presence of distinct morphs suggests the potential for reduced gene flow and population structure (Burg et al. 2005), though morphological characteristics have also been shown to vary with temperature and other environmental variables (Diniz-Filho et al. 2009). Plumage and morphological differences are broad and clinal in gray jays, making it difficult to assign individuals to one subspecies based on morphological characteristics alone (Strickland and Ouellet 2011). Physical barriers, including the Cascade and Rocky Mountain ranges and large bodies of water separate mainland subspecies from those found on islands (e.g., *P. canadensis sanfordi* endemic to Newfoundland) delineate some subspecies' ranges, though other boundaries are less well-defined (e.g., *P. c. bicolor* and *P. c. capitalis* overlap in central Idaho; Strickland and Ouellet 2011).

A recent study by van Els *et al.* (2012) using mitochondrial DNA data suggests that gray jays exhibit high levels of genetic diversity and population structure

throughout their range, and resided in multiple refugia during the LGM. While overall samples sizes are relatively large (n=205), many sites had small sample sizes (avg = 3.9 individuals/site). In addition, the study did not incorporate nuclear loci or morphological data. Here, we have examined the effects of Pleistocene glaciations and dispersal barriers on the population genetic structure of gray jays for 37 populations (avg = 10.2 individuals/site) throughout North America, sampling from each of the nine subspecies (Strickland and Ouellet 2011). We used the rapidly evolving mitochondrial DNA control region and seven variable microsatellite markers to investigate population genetic structure, infer locations of glacial refugia, and species distribution modeling to support refugia predicted by genetic patterns in this high latitude resident species. In addition, we investigate relationships between geographic variation in body size and among genetic groups.

4.3 Materials and Methods

4.3.1 Sample collection

From 2007-2012, gray jays were captured at each sampling site (from here on referred to as a population) using standard mistnetting techniques with call playback. We limited mistnetting locations to within a 50 km radius and sites contained no obvious barriers to dispersal. Sampling sites were paired where possible and located on either side of possible barriers to dispersal and from areas that were previously glaciated and unglaciated during the last glacial

maximum. Less than 100 μL of blood was collected from each bird by pricking the brachial vein with a sterile needle and collecting blood in a capillary tube. Blood was stored in 95% ethanol. Each bird was banded with a US Fish & Wildlife Service aluminum band, aged and sexed (if possible), and mass and tarsus measurements were taken. Additional genetic samples were obtained from museum collections taken from birds during the breeding season within the past 20 years (Table 4.1; Appendix 3).

4.3.2 Laboratory procedures

4.3.2.1 DNA extraction

DNA was extracted from blood, tissue, and feather samples from 376 birds using a modified Chelex protocol (Walsh et al. 1991; Burg and Croxall 2001).

4.3.2.2 Mitochondrial DNA

We amplified a section of the mitochondrial DNA control region (CR) using primers L46 SJ (5'-TTT GGC TAT GTA TTT CTT TGC-3'; Birt & Lemmen, unpublished data) and H1030 JCR 18 (5'-TAA ATG ATT TGG ACA ATC TAG G-3'; Saunders and Edwards 2000), corresponding to position 46 (Domain I) to 1030 (Domain III) of the corvid mitochondrial control region. Where the complete fragment would not amplify, we used internal primers designed in-house, H590 grjaCR (5'-GGA GTA TGC ATC CGA CCA CT-3') with L46 SJ or L530 corvidae (5'-CGC CTC TGG TTC CTA TTT CA-3') with H1030 JCR 18, to amplify two overlapping fragments.

PCR reactions were performed on a Master gradient thermocycler (Eppendorf: Hauppauge, NY) in 25 μL reactions with 1x goTaq Flexi buffer (Promega: Madison, WI), 2.5 mM $MgCl_2$, 200 μM dNTP, 0.4 μM of each primer, and 0.5 units goTaq Flexi taq polymerase (Promega) under the following conditions: one cycle of 94°C for 120 s, 52°C for 45 s, and 72°C for 60 s, 37 cycles of 94°C for 30 s, 52°C for 45 s and 72°C for 60 s and one cycle of 72°C for five min. PCR products were run on a 0.8% agarose gel to confirm DNA amplification. One Siberian jay (*Perisoreus infaustus*) was sequenced using the same procedures for use as an outgroup (see Appendix 3).

DNA sequencing was performed at McGill University and Génome Québec Innovation Centre on a 3730xl DNA Analyzer (Applied Biosystems: Carlsbad, CA) or at the University of Lethbridge on a 3130 DNA Analyzer (Applied Biosystems). For in-house sequencing we used a shrimp alkaline phosphatase - exonuclease clean up followed by sequencing and sodium acetate precipitation (Graham and Burg 2012) before electrophoresis.

4.3.2.3 Microsatellite DNA

Initially, three individuals were screened from geographically distant populations (northwest British Columbia, central Alberta, and Colorado) for 30 microsatellite primer pairs developed for and used in other corvids. If loci were

suspected to be polymorphic and/or amplified clearly, two additional individuals from each of the initial test populations plus one individual from each of three other populations, potentially separated by dispersal barriers (Washington, Utah, and southern Alberta), were screened. Seven of the 35 loci were polymorphic (Table 4.2). To allow for integration of a fluorescently labeled primer (700 nm or 800 nm) directly into the PCR product, we modified all forward primers by adding an M13 sequence (5′ – CAC GAC GTT GTA AAA CGA C – 3′) to the 5′ end. DNA was amplified in a 10 μL reaction with 1x buffer, 1 mM $MgCl_2$, 200 μM dNTP (Fisher Scientific), 1 μM of each primer (forward and reverse), 0.05 μM of the fluorescent primer (Eurofins MWG Operon) and 0.5 units taq polymerase under the following conditions: one cycle of 94°C for 120 s, T_1 for 45 s, and 72°C for 60 s, seven cycles of 94°C for 60 s, T_1 for 30 s and 72°C for 45 s, 31 cycles of 94°C for 30 s, T_2 for 30 s, and 72°C for 45 s, and one final elongation cycle at 72°C for five minutes (Table 4.2).

PCR products were mixed with a stop solution (95% formamide, 20 mM EDTA and bromophenol blue), denatured for 3 min at 94°C, then run on a 6% polyacrylamide gel using a LI-COR 4300 DNA Analyzer (LI-COR Inc.: Lincoln, NE). Alleles were scored via visual inspection, and genotypes were independently confirmed by a second person. Three controls of known allele sizes (pre-screened individuals) plus a size standard were included on each load to ensure consistent scoring along with a negative control to ensure no

contamination was present.

4.3.3 Analyses of genetic structure

4.3.3.1 Mitochondrial DNA

We edited and aligned sequences from chromatograms using MEGA v 5.0 (Tamura et al. 2011). To assess population structure and evaluate relationships among haplotypes, we constructed a statistical parsimony network (95% probability) using TCS v 1.21 (Clement et al. 2000). We measured genetic variation within populations and haplogroups by calculating haplotype (H_d) and nucleotide (π) diversity using ARLEQUIN v 3.11 (Excoffier et al. 2005). To examine population structure and assess genetic differentiation among populations and haplogroups, we calculated pairwise Φ_{ST} values (an analogue of Wright's fixation index F_{ST}) using ARLEQUIN v 3.11 (Excoffier et al. 2005). We corrected significance values using a Benjamini-Hochberg correction (Benjamini and Hochberg 1995) to control for false discovery rate (FDR). We examined genetic structure within and among populations by performing an analysis of molecular variance (AMOVA) in ARLEQUIN v 3.11 (Excoffier et al. 2005) and used a spatial analysis of molecular variance (SAMOVA; Dupanloup et al. 2002) approach to assess barriers between gray jay populations.

We calculated divergence times using a standard population genetic technique where $T = \delta/r$ (Wilson et al. 1985), where δ equals the corrected number of

nucleotide substitutions per site between populations or groups (as calculated in ARLEQUIN) and r is the weighted calculated mutation rate of 14.86%/Ma for this sequence (calculated from 303 bp in Domain I at 18%/Ma, 465 bp in Domain II at 9%/Ma, and 146 bp in Domain III at 27%/Ma (mutation rates as per corvid CR mutation rates given in Saunders & Edwards (2000)).

4.3.3.2 Microsatellite DNA

Allelic richness was calculated in FSTAT v2.9.3 (Goudet 2001). Allele frequencies, observed (H_o) and expected (H_e) heterozygosities, and pairwise F_{ST} values Wright 1978 were calculated with 1000 permutations using ARLEQUIN v 3.11 (Excoffier et al. 2005). We corrected *P* values for multiple tests using a Benjamini-Hochberg correction (Benjamini and Hochberg 1995) to control for FDR.

Bayesian clustering analyses were conducted using STRUCTURE v2.3.3 (Pritchard et al. 2000; Falush et al. 2003), run with the following settings: *K* = 1-27, a burn-in of 100,000 followed by 500,000 runs, admixture assumed, correlated allele frequencies and including *a priori* population information. Ten replicates were performed for each value of *K*. In STRUCTURE, it can be difficult to decide when *K* captures major structure in the data due to similar lnP(X|K) values, thus STRUCTURE HARVESTER (Earl and von Holdt 2012) was used to confirm the most parsimonious clustering of groups. Once an overall clustering was determined

for all populations, we re-ran STRUCTURE on populations within the subgroups to elucidate any further structure.

4.3.4 Phylogenetic tree construction using mitochondrial DNA

We used jMODELTEST v 0.1.1 (Posada 2008) and MEGA 5.0 to determine the best model of nucleotide substitution. We then constructed a maximum likelihood tree using 500 bootstrap replicates and rooted the tree with a Siberian jay sequence.

4.3.5 Species distribution and paleodistribution modeling

We used species distribution modeling (SDM) to construct a model of current and LGM (~21 ka) gray jay distributions. Geo-referenced locations were obtained from the Global Biodiversity Information Facility (GBIF; http://data.gbif.org/, accessed on 3 October 2011). Data were inspected and occurrences outside of North America, without geo-referencing, or recorded before 1950 were excluded from the analyses. From the GBIF data, we trained and tested the models using location records from field data, multiple museums, Animal Sound Archive Berlin, Borror Laboratory of Bioacoustics, Macaulay Library Audio Data, USDA Forest Service Lamna Point Count, Point Reyes Bird Observatory Point Counts, Ontario Breeding Bird Atlas 1981-1985 and 2001-2005, and Northwest Territories and Nunavut Bird Checklist. Duplicate records and remaining outliers were removed prior to model-building.

We extracted current bioclimatic data from the WORLDCLIM dataset (v 1.4, http://www.worldclim.org/) and LGM bioclimatic data from the Model for Interdisciplinary Research on Climate (MIROC) dataset at 2.5 min resolution (Hasumi and Emori 2004). The current bioclimatic dataset ranges over a 50-year period (1950-2000), hence we excluded gray jay observations prior to 1950 for consistency. Nineteen bioclimatic variables are included in the WORLDCLIM current and LGM dataset (Hijmans et al. 2005). We used ArcGIS 9.3 (ESRI: Redlands, CA) to clip climatic variable layers to include only North America as using smaller geographic areas can improve predictive power of MAXENT models (Anderson and Raza 2010). Prior to constructing SDM, we used ENMTOOLS (v 1.3; Warren et al. 2010) to determine which bioclimatic variables were correlated, using $R > 0.90$ as a cutoff. Nine variables were correlated with at least one other variable and all but one from each set of correlated variables was removed.

MAXENT (v 3.3.3; Phillips et al. 2006) was used to model current and past gray jay distribution. MAXENT uses a machine-learning algorithm to produce a probability distribution model that uses a set of constraints derived from occurrence data and related environmental variables, which is fit and improved over several iterations (Phillips et al. 2006; Phillips and Dudik 2008). We used the following settings for the MAXENT model: hinge features only, regularization multiplier of 1, 10 000 max number of background points, replicate run type of 10 cross-

validations, 500 maximum iterations, and 0.00001 convergence threshold. We used hinge features only as these are appropriate for samples of greater than 15, improve model performance, and allow for simpler approximations of species response to the environment (Phillips and Dudik 2008). We ran jackknife tests to measure the importance of each bioclimatic variable.

4.3.6 Body size analyses

Using morphological data collected in the field, mean mass and mean tarsus length were calculated for gray jay populations throughout their range. We used spline with barriers (i.e. range boundaries) surface interpolation tool in ArcMap10 to determine how gray jay mass and tarsus vary across their range. After spline analyses were compared to mtDNA and microsatellite groups, we used single-factor analyses of variance (ANOVA) in SPSS 20 (IBM Corp: Armonk, NY) to assess if mass and tarsus varied between the seven mitochondrial genetic groups and the five microsatellite groups.

4.4 Results

4.4.1 Genetic structure

We collected samples from and genotyped mitochondrial DNA of 375 individual gray jays from 37 populations (Table 4.1, Figure 4.1) and seven polymorphic microsatellite loci for 402 individuals from the 27 populations with five or more samples from across the range (Table 4.3).

4.4.1.1 Mitochondrial DNA

We found 261 different haplotypes with overall haplotype diversity (H_d) of 0.982, ranging from 0.707 [north northwest BC (NNWBC)] to 1.000 (11 populations; Table 4.1). Nucleotide diversity (π) ranged from 0.002 [Vancouver Island (VanIsl), coastal Washington (coWA), Newfoundland (NL), and New Mexico (NM)] to 0.014 [northeast Washington (NEWA); Table 4.1; Appendix 5)].

The statistical parsimony network (Figure 4.2) shows at least seven haplogroups throughout North America: Pacific Coast [coWA, western Washington (WA), northwest Washington (NWWA), Washington Olympic Peninsula (WAOP), central Oregon (ceOR) and southern Oregon (SOR)]; VanIsl; Intermountain West [IMW; NEWA, northeast Oregon (NEOR), Idaho (ID), southeast British Columbia (SEBC) and southern Alberta (SAB)]; Colorado-New Mexico [CO-NM; Colorado (CO), southwest CO (SWCO), and (NM)]; Utah (UT); and Boreal East [BE; Alaska Anchorage (AKA), Alaska Wrangell (AKW), Alaska Fairbanks (AKF), Alaska Denali (AKD), northwest BC (NWBC), north northwest BC (NNWBC), central BC (CBC), central AB (CAB), Saskatchewan (SK), Minnesota (MN), northern Ontario (NON), northwest Quebec (NWQC), Nova Scotia-New Brunswick (NSNB), southern Ontario (SON), Gaspé Peninsula (Gasp), north shore (NSH), Anticosti Island (ANTI), Vermont (VT), New Hampshire (NH), Labrador (Lab)]; and NL; Table 4.1). We excluded populations with less than

four birds from further analyses. In pairwise comparisons of the remaining 28 populations, 353 of 378 Φ_{ST} values were significant (B-H corrected $P < 0.047$; Table 4.4, Appendix 6).

A SAMOVA run with K = 7, accounted for the highest amount of variation among groups (79.57%, $F_{CT} = 0.797$, $P < 0.0001$; Table 4.5). SAMOVA population groupings corresponded with those suggested in the statistical parsimony network (Figure 4.2) and the same groups used in the analysis of molecular variance (AMOVA) to explain the most among group variation.

When populations were grouped in to seven haplogroups and analysed using pairwise comparisons for differentiation, all Φ_{ST} values were significant ($P < 0.01$; Table 4.6). When we calculated divergence times using the major haplogroups, we found populations in Pacific Coast group and Vancouver Island split off from other groups first, between ~272-343 ka (Table 4.6). CO-NM split from the BE, UT and IMW ~87-166 ka, BE and IMW 38 ka, NL and IMW 76 ka, BE and UT 41 ka, NL and UT 51 ka, BE and NL 24 ka, and VanIsl from Pacific Coast 26 ka (Table 4.6; Figure 4.3).

jMODELTEST and MEGA 5.0 both suggested a Hasegawa-Kishino-Yano (HKY) model of nucleotide substitution with a discrete Gamma distribution (+G) allowing some sites to be evolutionarily invariable (+I). The maximum likelihood

tree was unable to fully resolve all of the proposed clades due to low bootstrap values, though individuals within each of the seven haplogroups clustered together (Appendix 7).

4.4.1.2 Microsatellite DNA

A total of seven polymorphic microsatellite loci were used for analyses (Table 4.3). Twenty-seven populations with five or more samples were included in general analyses and initial Bayesian analyses of population clustering. One population, NSNB, was excluded from subgroup analyses due to large amounts of missing data and small sample size. Total number of alleles for each locus ranged from six for MJG1 and ApCo41 to 16 in ApCo37 (Table 4.3). Overall allelic richness ranged from 1.857 for MJG1 to 4.444 for ApCo40, ApCo41, ApCo91, and Ck2A5A. Thirty-eight of 189 loci-population comparisons deviated significantly from Hardy-Weinberg equilibrium (Table 4.3).

Significant differentiation was detected in 325 of 351 pairwise population comparisons (Table 4.7), with F_{ST} values ranging from 0.012 (P = 0.616) for NNWBC and AKW to 0.590 for NM and coWA (P < 0.001; Appendix 8). The initial STRUCTURE clustering analysis suggested that the optimal number (K) of gray jay populations was two (Table 4.8; this was supported by STRUCTURE HARVESTER peaks and posterior probabilities (mean (Pr (K=2)) = -5579.66). Due to large amounts of admixture within the two groups, we ran additional STRUCTURE

analyses within each of the two groups. We found three optimal groups within group 1 (mean (Pr (*K*=3)) = -3021.80), comprised of a mixture of BE, CO-NM, UT, and IMW populations, and two optimal groups within group 2 (mean (Pr (*K*=2)) = -2190.09) including the remaining boreal-east populations and all Pacific populations (Table 4.8; Appendix 9).

4.4.2 Species distribution modeling

Using 1447 range-wide presence records for training, 161 records for testing and 10 BIOCLIM environmental layers (bio1-4, 8, 12, 14-15, 18-19), MAXENT modeling predicted a current range similar to that known for gray jays in North America with little variance (Figure 4.4a). Mean Area Under the Curve (AUC) was 0.857 (SD = 0.012; training AUC range 0.859-0.862, test AUC range 0.842-0.870), suggesting that the models were reasonable as AUC values above 0.75 are considered "potentially useful" (Elith 2002). Annual temperature (bio1; 33.2%), precipitation of coldest quarter (bio19; 29.8%), annual precipitation (bio12; 14.5%), and mean diurnal temperature range (bio2; 14.3%) were the largest contributors to the model contributing 91.8%, in addition to having the highest permutation importance (39.7, 25.8, 7.3, and 7.4, respectively) as supported by jackknifing.

When the model used current conditions to predict suitable gray jay habitat during the last glacial maximum (LGM; paleodistribution), five main areas have

a high probability of suitable gray jay habitat (0.5-0.8): most of Alaska and parts of Beringia, two areas in the southern Rockies, the SE US through Tennessee and Virginia, and the Pacific Coast including parts of Vancouver Island, Washington and Oregon (Figure 4.4b). The model also shows suitable gray jay habitat may have existed near Newfoundland.

4.4.3 Body size analyses

Gray jay mass ranged from 59.50 ± 0.76 g in WA to 78.29 ± 2.22 g in NL (Table 4.9). Tarsus length ranged from 32.97 ± 0.32 mm in WA to 36.98 ± 0.57 mm in UT (Table 4.9). Interpolated spline surfaces for mass and tarsus across the gray jay range predict heavier birds throughout the central part of the range and in Newfoundland (Figure 4.5a), but birds with a longer tarsus at the edges of the range, excluding NL (Figure 4.5b). For seven haplotype groups, single-factor ANOVAs suggested that mass (F (6, 134) = 20.53, P < 0.001; Figure 4.6a) and tarsus (F (6, 108) = 9.03, P < 0.001; Figure 4.6b) differ significantly among groups. Single-factor ANOVAs run for microsatellite groups did not detect a significant difference in mass (F (4, 11) = 2.51789, P = 0.102) or tarsus (F (4, 10) = 0.40333, P = 0.802) among groups.

4.5 Discussion

Geographic structuring and population differentiation suggest different evolutionary histories for gray jays in North America. Gray jays are partitioned

into seven geographically distinct mitochondrial groups throughout their range (Figure 4.2, Table 4.5): Vancouver Island; Pacific Coast (western and northwest Washington, central and southern Oregon); Intermountain West (northeast Washington, southeast British Columbia, southern Alberta, Idaho, and northeast Oregon); CO-NM (north-central Colorado, southwest Colorado, and New Mexico); Utah; Newfoundland; and Boreal East (the 20 remaining sampled populations). Only the Newfoundland and Vancouver Island groups correspond directly with a current subspecies (*Perisoreus canadensis sanfordi* and *P. c. obscurus* respectively; Strickland and Ouellet 2011). Microsatellite markers support similar breaks, with significant differentiation (F_{ST}) between most populations and clustering roughly corresponding to larger mitochondrial haplogroups. Exceptions to this include some splits amongst Boreal East populations, inclusion of AKF and CBC with Pacific Coast groups, and several populations that were difficult to consistently assign to a single cluster, suggesting nuclear genetic admixture between some groups. Morphologically, significant differences in mass and tarsus were found among mitochondrial haplogroups, supporting isolation. However, these differences are not as clear when using spline analyses across the range, suggesting broad and clinal differences in gray jay morphology.

4.5.1 LGM refugia and patterns of postglacial colonization

High mitochondrial genetic diversity exists within most groups, suggesting few founder events occurred during gray jay recolonization after deglaciation. Most

areas have haplotype diversity approaching one, some of the highest diversity observed in North American bird species. High haplotype diversity and few shared haplotypes between populations also suggest limited maternal gene flow among groups, as might be expected in a sedentary species (Burg et al. 2005; Burg et al. 2006; Barrowclough et al. 2004; Graham and Burg 2012).

Mitochondrial DNA patterns in the gray jay suggest long-term isolation in multiple refugia and low levels of gene flow following the retreat of the ice sheets. Species distribution modeling (SDM) and fossil data (Wetmore 1962) reinforce the presence of multiple southern refugia and SDM data support a northern refugium. While SDM (Figure 4.4b) shows refugia during the LGM and these maintained isolation of genetically distinct groups (e.g., CO-NM, UT), isolation during earlier glaciations likely created many of the haplogroups seen (Table 4.6). Divergence data for the Pacific Coast and CO-NM groups date to the Illinoian while the Boreal East, Intermountain West and Utah groups split during the Sangamonian and Wisconsin Interglacial (Table 4.6).

High levels of differentiation (Φ_{ST} and AMOVA for mtDNA) suggest the Pacific Coast group (including Vancouver Island) has remained relatively isolated for at least 272 – 343 ka. SDM (Figure 4.4b) shows suitable habitat both on the mainland and Vancouver Island during the LGM. Individuals on Vancouver Island were likely isolated in a different refugium from those on the mainland as

evident from the distinct sets of haplotypes on Vancouver Island. Other North American taxa show evidence of isolation on the mainland (Barrowclough et al. 2004; Graham and Burg 2012; Carstens et al. 2005; Godbout et al. 2008), and a few on Vancouver Island possibly in ice-free portions of the Brooks Peninsula on northern Vancouver Island during the LGM (Godbout et al. 2008; Walser et al. 2005).

Populations in the Intermountain West (IMW), CO-NM, and UT also persisted in separate refugia and show little evidence of post-glacial dispersal. The CO-NM group and UT population each currently exist as disjunct populations from the main gray jay range. Genetic data show the UT population only recently split from the Boreal East and NL groups (41-51 ka). Gray jay populations in the IMW group contain high levels of genetic diversity and are genetically isolated from adjacent populations, a pattern suggestive of long-term isolation. The Clearwater refugium has been suggested as a refugium for other species in the area (Shafer et al. 2010; Godbout et al. 2008), including emerging pollen evidence for *Picea* species (Herring and Gavin 2015). *W*hile our mtDNA data support isolation, the paleodistribution modeling data do not show evidence of suitable gray jay habitat in the area 21 kya. This may be due to modeling limitations or temporal factors. Alternatively, the IMW group may have survived the LGM in a refugium slightly farther south than the Clearwater refugium; paleodistribution models suggest that suitable habitat for gray jays existed in northern Nevada. While the

IMW group is genetically isolated from the Boreal East and other groups, this split occurred ~38 ka, prior to the LGM.

The Boreal East group contains a large number of diverse haplotypes spread over large geographic areas with most populations containing high haplotype and nucleotide diversity. Areas in the SE US and Beringia could have supported populations of gray jays during the LGM, based on suitable habitat models (Figure 4.4b), thus Boreal East populations could have been colonized from birds in the SE US or Beringia or both. Fossil evidence shows gray jays were located in Tennessee and Virginia during the LGM, (Wetmore 1962), though populations are no longer present in those areas. Many other high latitude species survived the LGM in the eastern US (Jaramillo-Correa et al. 2009; Graham and Burg 2012; (Gérardi et al. 2010). Contemporary samples from Alaska, near the Beringia refugium, include haplotypes scattered throughout the statistical parsimony network lending support to a Beringia refugium for gray jays. Alternatively, this could suggest a diverse number of founders from other populations colonizing Beringia after deglaciation. However, given known geographical patterns of deglaciation, genetic evidence from other species (Zink and Dittmann 1993; Shafer et al. 2011), and the diverse nature of haplotypes in Alaska, the former scenario is more likely.

Within the Boreal East, birds from NL form a distinct cluster and pairwise Φ_{ST} values; SAMOVA, AMOVA and SDM raise the possibility of an earlier and separate colonization event. The NL population diverged from other populations in the Boreal East group ~24 ka. The reduced genetic diversity and a clustered set of haplotypes in NL gray jays could be the result of a founder effect or a population bottleneck and no gene flow due to the Strait of Belle Isle acting as a dispersal barrier as it does in other species (Kyle and Strobeck 2003; Lait and Burg 2013). SDM suggests the presence of an Atlantic refugium near Newfoundland and such a refugium is supported by a number of species (Jaramillo-Correa et al. 2009; Lait and Burg 2013; Boulet and Gibbs 2006). Based on lower haplotype and nucleotide diversities, it is possible that the current NL population colonized the area separately from other populations in eastern Canada; however, this is unlikely. The patterns of genetic diversity (e.g., low nucleotide diversity) suggest founder effects and the distribution of haplotypes in the Boreal East support long-term isolation of maternal lineages on NL (Figure 4.4, Table 4.1). Evidence of a founder event, albeit recent, is present in the VT samples. Victory Bog (VT) was recently (1960s) colonized following reestablishment of the forest following extensive logging (W. Barnard, pers. comm.). However, unlike the NL birds, gray jays from VT show lower haplotype diversity, but with highly divergent haplotypes.

Unlike many other species whose current range extends into previously glaciated regions, it appears that most of the current range of the gray jay was colonized by individuals within the Boreal East lineage. A more common pattern for widespread species is movement out of multiple refugia with populations west of the Canadian Rockies being derived from those along the Pacific Coast and populations east of the Rockies from a southeastern Rockies or eastern refugium, or both (Jaramillo-Correa et al. 2009; Burg et al. 2006; Graham and Burg 2012; Godbout et al. 2008; de Lafontaine et al. 2010; Boulet and Gibbs 2006).

4.5.2 Tree refugia

Gray jays are dependent on forested habitat and, in particular, several species of spruce trees (*Picea* spp.). CO-NM, UT, and IMW groups are all closely associated with Engelmann and blue spruce, which are highly fragmented in the southern portion of their range (i.e., UT and CO; Ledig et al. 2006). Populations of Englemann and blue spruce in the IMW and NE UT are genetically distinct (cpDNA) and physically isolated from each other by the Snake River Basin (Ledig et al. 2006), corresponding to the mitochondrial DNA patterns found here.

Support for gray jay colonization throughout the Boreal East from both a Beringia and a southeastern refugium comes from phylogeographical studies of spruce (*Picea* spp; Jaramillo-Correa et al. 2009). The strong association of gray jays with spruce species in these areas (Strickland and Ouellet 2011) means it is

possible that the birds may have followed the colonization of spruce into previously glaciated areas, a pattern seen in other boreal species (Burg et al. 2006; Graham and Burg 2012). The colonization by spruce is suggested to have occurred from multiple refugia north (Beringia) and south (both east and west of the Appalachian Mountains), particularly for white spruce (*Picea glauca*; Jaramillo-Correa et al. 2009; de Lafontaine et al. 2010). Black spruce (*Picea mariana*) has a similar colonization history in the east. However, west of the Rocky Mountains, it is thought to have colonized only from a southern, Pacific refugium (Gérardi et al. 2010), contrary to the pattern of colonization from multiple refugia that we suggest for gray jays in mainland British Columbia.

4.5.3 Physical barriers and peripheral isolation

A number of contemporary dispersal barriers restrict gene flow in gray jays. Barriers include large bodies of water (Strait of Belle Isle and the Salish Sea), large areas of unsuitable habitat (Columbia, Wyoming, and Great Basins) and, in some areas, possibly mountains (Columbia Mountains in southeast BC). With the exception of eight individuals, no haplotypes are shared between the mitochondrial haplogroups (Figure 4.2) suggesting limited female movement. Male movement can be detected when mitochondrial data are compared to microsatellite markers. However, both markers show high levels of structure, suggesting limited male or female movement across landscapes.

Water barriers are evident within both the Pacific Coast and Boreal East haplogroups with the genetic isolation of Vancouver Island and Newfoundland, respectively. Microsatellite markers support this break. While Vancouver Island and Newfoundland populations grouped with their nearest mainland populations in clustering analyses, they were significantly differentiated in all other population differentiation analyses. Birds in both of the island populations possess unique plumage and have morphological variation such that they have historically been considered different species or subspecies of gray jays; Vancouver Island and mainland: *Perisoreus canadensis obscurus* and *Perisoreus canadensis griseus*, respectively; Newfoundland and adjacent mainland: *P. c. sanfordi* and *P. c. canadensis* (Strickland and Ouellet 2011). Similar patterns of genetic isolation for both plant and animal species have been found, though usually with high-resolution nuclear markers and not organellar DNA. The Salish Sea restricts populations on Vancouver Island (e.g., Steller's jay (*Cyanocitta stelleri*; Burg et al. 2005), chestnut-backed chickadee (*Poecile rufescens*; Burg et al. 2005)), and the Strait of Belle Isle isolates populations on Newfoundland (e.g., pine marten (*Martes americana*; Kyle and Strobeck 2003); boreal chickadee (*P. hudsonicus*; Lait and Burg 2013).

Though close in proximity to each other (~530 km apart), the northern Colorado and Utah populations are highly differentiated for both mitochondrial and nuclear DNA, though southern groups cluster together overall in Bayesian

analyses. Two possible reasons are large areas of unsuitable habitat or isolation of peripheral, disjunct populations. The Great Basin to the northwest, Wyoming Basin to the north/northeast and Snake River Basin to the north/northwest all act as barriers to dispersal and gene flow with neighbouring populations. The divergence between Colorado and neighbouring populations in Utah, but not between Colorado and neighbouring populations in New Mexico, has been observed in other taxa (Runck and Cook 2005; Albach et al. 2006). Most notably, congruent patterns of isolation are found in Engelmann and blue spruce (Ledig et al. 2006), which were restricted to higher elevations and isolated on mountains as aridification occurred in the Great and Wyoming Basins. In addition, both the UT and CO populations are currently ~390-700 km, respectively, to the nearest population within the contiguous portion of the gray jay range. Peripheral isolation may also explain the high differentiation and isolation in these disjunct populations. In other taxa, peripheral populations are more likely to be isolated due to reduced gene flow, which is particularly pronounced for disjunct populations (Burg et al. 2006; Eckert et al. 2008).

Peripheral isolation may also explain decreased haplotype and nucleotide diversities in NNWBC birds as this population is at the extreme edge of the gray jay's range. Though this population shows mixed differentiation results when comparing mitochondrial and microsatellite markers from adjacent populations in Alaska, low genetic variation is often characteristic of populations on the

periphery of species' ranges (Eckert et al. 2008). This phenomenon may also explain Labrador gray jays' decreased nucleotide diversity, despite high haplotype diversity.

The Intermountain West (NEWA, SAB, NEOR, SEBC, and ID) group, unlike some of the other isolated populations, occupies a somewhat central portion of the gray jay range, yet they are genetically distinct from surrounding groups for both mitochondrial and nuclear markers. Birds in this area are isolated from adjacent populations by the Columbia Basin/Okanogan Highlands to the west (Pacific populations), Columbia Mountains and Rocky Mountain Trench to the north and Columbia Mountains to the east (Boreal East), and the Snake River Basin to the south (Colorado and Utah). A similar genetic break occurs in mtDNA patterns in Engelmann spruce (Ledig et al. 2006) and Douglas fir (Gugger et al. 2010); both species of trees that gray jays are closely associated with in the Intermountain West area (Strickland and Ouellet 2011).

4.5.4 Morphological variation

Morphological variation in gray jays is very complex as their range covers a large area with high amounts of environmental variation. Body size measurements varied significantly between mitochondrial haplogroups (Figure 4.6, Table 4.9), but not for microsatellite groups. These results should be interpreted with caution given the differences in samples sizes between populations (n = 5-61). In

addition, mass and tarsus splines do not correspond to subspecies descriptions, or directly to genetic groups for either marker, and suggest much variation in size within the Boreal East haplogroup in particular (Figure 4.5). Spline patterns also do not correspond to nuclear DNA groupings determined using microsatellites, suggesting that factors other than geographic genetic variation are likely at play. Variation in gray jay morphology is known to be broad and clinal across the range (Strickland and Ouellet 2011), which corresponds to the patterns seen here.

4.5.5 Marker choice and overall patterns

While some studies question using a highly variable marker like control region versus ND2 or cytochrome b for phylogeographic and phylogenetic studies, previous work has shown that this marker can be used to resolve deep splits in evolutionary history among avian species (Barker et al. 2012) and of corvids in particular (Saunders and Edwards 2000). Within a single species, some loci may not be variable enough to detect differences between populations (e.g. cytochrome b (Steeves et al. 2003) versus control region (Steeves et al. 2005) in masked boobies (*Sula dactylatra*)). Thus, using control region sequences in this study provides a valuable comparison and complement to previous research.

Similar haplogroup patterns and divergence order are found in van Els *et al.* (2012); however, our work differs in several ways. Our results suggest that gray

jay groups diverged more recently from each other (e.g. Pacific group split 3 Ma (van Els et al. 2012) versus 279-369 ka (Table 4.6)). This may be a result of using a more rapidly evolving marker (control region versus ND2), differences in calculation of the timing of splits, or differences in rates of evolution used for calculations. In addition, we suggest that gray jays fall into seven haplogroups across North America compared to four; additional groups are Utah, which is similar to the Boreal group as in van Els *et al.* (2012) but with higher resolution control region data creates a distinct group, and Vancouver Island, with higher diversity in the CO-NM and Pacific Coast groups. While some evidence exists in our paleodistribution model for a Newfoundland LGM refugium, also suggested by van Els *et al.* (2012), genetic data in both studies do not support this refugium and rather suggest a case of long-term isolation, possibly in a nearby refugium. One benefit to using the control region is that it allows us to distinguish additional genetic splits (e.g. NL) that might not be as evident using less variable markers. Adding microsatellite markers to our analyses provided additional support and resolution for geographic patterns. Strong differentiation between most populations is similar to that found with mitochondrial DNA and clustering provides additional insights into patterns throughout the range.

4.5.6 Conclusions and future research

Gray jay populations are highly differentiated, likely a consequence of limited dispersal for both males and females. Historical and contemporary gene flow is

influenced by glaciation, barriers to movement such as large bodies of water and large areas of unsuitable habitat, and peripheral isolation. Additional research could include greater numbers of microsatellite loci or other nuclear markers to further enhance and complete our understanding of gray jay history and contemporary gene flow in North America. Morphological variation does not strongly correspond to range-wide genetic differentiation in this study, though increased sampling could further quantify and advance our understanding of variation throughout the range.

Gray jay geographic genetic patterns are similar to those found in spruce species, the conifer genus most commonly associated with preferred gray jay habitats. Given this parallel, we would recommend future comparative phylogeography research that integrates genetic markers and species distribution modeling for gray jay, spruce, and other codistributed species.

4.6 Chapter acknowledgements

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Table 4.1. Summary table of gray jay samples and mitochondrial DNA information from analyses. Latitude and longitude are central points for population sampling sites. H_d = mitochondrial DNA haplotype and π = nucleotide diversity (multiplied by 100 for ease of viewing). Population and haplogroup abbreviations are: AKA = Alaska Anchorage, AKF = Alaska Fairbanks, AKW = Alaska Wrangell, AKD = Alaska Denali, NWBC = northwest British Columbia (BC), NNWBC = north northwest BC, CBC = central BC, CAB = central Alberta (AB), SK = Saskatchewan, MN = Minnesota, NON = northern Ontario, NWQC = northwest Quebec, SON = southern Ontario, Gasp = Gaspé peninsula, NSH = north shore, ANTI = Anticosti Island, NSNB = Nova Scotia/New Brunswick, VT = Vermont, NH = New Hampshire, Lab = Labrador, NL = Newfoundland (island), UT = Utah, IMW = Intermountain West haplogroup, SAB = southern AB, NEWA = northeast Washington, NEOR = northeast Oregon, ID = Idaho, SEBC = southeast BC, CO-NM = Colorado-New Mexico haplogroup, CO = Colorado, SWCO = southwest Colorado, NM = New Mexico, WA = western Washington, coWA = coastal Washington, NWWA = northwest Washington, WAOP = Washington Olympic Peninsula, ceOR= central Oregon, SOR = southern Oregon, VanIsl = Vancouver Island, Superscript letters indicate museum contributions as follows: [a] Burke Museum of Natural History and Culture – University of Washington, [b] Smithsonian National Museum of Natural History, [c] Louisiana State University Museum of Natural Science, [d] Royal British Columbia Museum, [e] Canadian Museum of Nature, [f] Royal Saskatchewan Museum, [g] The Field Museum, [h] Royal Ontario Museum, [i] New Brunswick Museum, [j] Royal Alberta Museum, and [k] American Museum of Natural History. See Appendix 3 for additional museum collection information including voucher/specimen numbers.

Genetic Group	Pop	Lat (N)	Long (W)	n	H_n	H_d	π
Boreal East				*203*	*163*	*0.998*	*0.008*
	AKA	62.12	-146.57	8	8	1.000	0.012
	AKF	64.95	-146.47	8	7	0.936	0.010
	AKW	61.71	-144.88	17	14	0.969	0.007
	AKD	63.38	-148.47	1	1	-	-
	NWBC	58.45	-130.00	15	11	0.952	0.008
	NNWBC	60.00	-136.87	9	5	0.707	0.004
	CBC	54.77	-127.27	13	10	0.949	0.010
	CAB	53.39	-117.68	20	15	0.968	0.010
	SK	53.97	-106.29	11	9	0.913	0.010
	MN	46.13	-92.87	3	2	0.728	-
	NON	54.56	-84.63	14	9	0.973	0.004
	NWQC	52.24	-78.56	11	11	1.000	0.005
	SON	45.80	-78.56	16	16	1.000	0.005
	Gasp	48.93	-66.40	2	2	1.000	-
	NSH	49.27	-68.09	2	2	1.000	-
	ANTI	49.27	-64.31	11	7	0.728	0.003
	NSNB	46.30	-65.38	6	4	0.800	0.006
	VT	44.55	-71.47	20	13	0.852	0.007
	NH	45.18	-71.15	3	2	0.925	-
	Lab	53.34	-60.41	17	15	0.979	0.005
NL	NL	49.46	-57.76	*12*	*8*	0.897	0.002
UT	UT	40.57	-110.47	*12*	*7*	0.897	0.003
IMW				*40*	*37*	*0.996*	*0.009*
	SAB	49.04	-114.03	13	13	1.000	0.007
	NEWA	48.76	-118.25	11	9	0.913	0.014
	NEOR	45.26	-116.84	10	8	0.955	0.006
	ID	44.95	-116.14	3	3	1.000	-
	SEBC	51.04	-117.87	3	3	1.000	-
CO-NM				*37*	*30*	*0.993*	*0.005*
	CO	40.41	-105.82	20	15	0.949	0.005
	SWCO	37.63	-107.83	12	12	1.000	0.009
	NM	35.81	-105.79	5	5	1.000	0.002
Pacific Coast				*52*	*37*	*0.957*	*0.004*
	WA	46.77	-121.75	33	19	0.938	0.004
	coWA	46.74	-123.80	6	4	0.903	0.002
	NWWA	48.89	-121.90	4	3	0.823	0.003
	WAOP	47.94	-123.07	3	3	1.000	-
	ceOR	43.65	-121.76	5	4	0.900	0.004
	SOR	42.78	-122.08	1	1	-	-
VanIsl	VanIsl	49.74	-124.68	16	10	0.975	0.002
Overall				**375**	**261**	**0.982**	**0.061**

Table 4.2. Microsatellite primer pairs used for nuclear DNA analyses in gray jay. Primer names, sequence, focal species, source, and reaction conditions used in this study (buffer and taq polymerase, and annealing temperatures, T_1 and T_2 (°C)). Forward primers (shown with an 'F' suffix) were modified to include a short sequence (CAC GAC GTT GTA AAA CGA C) at the 5' end allowing for incorporation of a florescent tag. All loci except CK2.A5.A and MJG1 are from Florida Scrub Jay (*Aphelocoma coerulescens;* Stenzler and Fitzpatrick 2002). CK2.A5.A is from Mexican jay (*Aphelocoma ultramarine*; Li et al. 1997) and MJG1 from Mariana crow (*Corvus kubaryi*; Tarr and Fleischer 1998).

Primer	Sequence (5' to 3')	Buffer	taq	T_1	T_2
ApCo30F	GCC CTG ATG CTG TTG ATG GT	crimson	crimson	50	52
ApCo30R	CTG GAG CCT GGT TTA GAG TTA TGC				
ApCo37F	TGC CAA ATG CAA CCA AAT CTT	flexi	flexi	50	52
ApCo37R	CAT CAC TTG CAG AGA GGG CA				
ApCo40F	CTT CTG ACA AGA CAC AGG AGC C	truin	truin	55	57
ApCo40R	GCA CAG ATC TCA GTT GCA TCA CTC				
ApCo41F	CCT ACT CTG GGC ACT GTT ATT ATC	flexi	flexi	50	52
ApCo41R	CCC ATT ATC AGC ATG TCG TAC A				
ApCo91F	GTA GTC CCA ATG GTT TCT CTG TC	flexi	flexi	50	52
ApCo91R	GAT GAA GTA ATG TGA AAC CTG G				
Ck2.A5.AF	TGC TAA GCA CAG TTA GAG AC	flexi	flexi	50	52
Ck2.A5.AR	GAA GAC AGG CAG GAG AGT TG				
MJG1F	CCC GGG AAA GGC TTC GTC TTC	flexi	flexi	50	52
MJG1R	GGA GAT TTT ATA TCG GTG GC				

Table 4.3. Summary table of seven microsatellite loci used to analyze gray jay populations. Only populations with greater than five samples were used; *n* = number of samples used in genotyping and analyses; A_n = number of alleles; A_r = allelic richness; H_o = observed and H_e = expected heterozygosity; P = departures from Hardy-Weinberg equilibrium (-- = not calculated, ns = not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001. See Table 4.1 for population location abbreviations).

	ApCo30	ApCo37	ApCo40	ApCo41	ApCo91	Ck2A5A	MJG1
AKA (n=8)							
A_n	5	5	6	2	3	2	1
A_r	3.47	3.256	4.038	4.038	4.038	4.038	1
H_o	0.857	0.750	0.667	0.625	0.500	0.125	0.000
H_e	0.704	0.664	0.750	0.430	0.403	0.117	0.000
P	ns	ns	ns	ns	ns	ns	--
AKF (n=8)							
A_n	4	6	6	1	5	2	1
A_r	3.119	3.125	4.262	4.262	4.262	4.262	1
H_o	0.571	0.500	0.800	0.000	0.143	0.400	0.000
H_e	0.653	0.578	0.760	0.000	0.724	0.480	0.000
P	ns	ns	ns	--	*	ns	--
AKW (n=18)							
A_n	5	4	5	2	4	1	1
A_r	2.828	2.855	2.965	2.965	2.965	2.965	1
H_o	0.444	0.688	0.308	0.111	0.267	0.000	0.000
H_e	0.559	0.643	0.630	0.105	0.478	0.000	0.000
P	ns	ns	ns	ns	**	--	--
NWBC (n=16)							
A_n	4	9	6	1	4	1	1
A_r	3.293	3.685	3.82	3.82	3.82	3.82	1
H_o	0.786	0.692	1.000	0.000	0.615	0.000	0.000
H_e	0.724	0.710	0.769	0.000	0.642	0.000	0.000
P	*	*	**	--	ns	--	--
NNWBC (n=9)							
A_n	4	6	6	1	3	1	1
A_r	2.632	3.686	4.5	4.5	4.5	4.5	1
H_o	0.444	0.375	0.800	0.000	0.429	0.000	0.000
H_e	0.512	0.727	0.800	0.000	0.357	0.000	0.000
P	ns	*	ns		ns		
CBC (n=13)							
A_n	3	4	5	2	6	3	2
A_r	2.78	2.507	3.718	3.718	3.718	3.718	1.415
H_o	0.500	0.154	0.909	0.077	0.462	0.545	0.154
H_e	0.653	0.485	0.769	0.074	0.675	0.525	0.142
P	ns	***	ns	ns	ns	ns	ns

CAB (n=28)							
A_n	6	5	8	1	5	3	2
A_r	3.328	2.153	3.902	3.902	3.902	3.902	1.809
H_o	0.783	0.381	0.714	0.000	0.500	0.231	0.000
H_e	0.690	0.368	0.794	0.000	0.415	0.208	0.351
P	ns	ns	*		ns	ns	***
SK (n=11)							
A_n	5	4	9	2	3	2	2
A_r	3.569	2.516	4.55	4.55	4.55	4.55	1.481
H_o	0.818	0.727	0.889	0.200	0.375	0.111	0.182
H_e	0.752	0.579	0.833	0.180	0.320	0.105	0.165
P	ns	ns	ns	ns	ns	ns	ns
NON (n=26)							
A_n	7	7	10	3	5	5	3
A_r	3.676	2.895	4.314	4.314	4.314	4.314	1.429
H_o	0.542	0.273	0.850	0.083	0.524	0.333	0.115
H_e	0.763	0.620	0.840	0.081	0.618	0.297	0.144
P	*	***	ns	ns	*	ns	***
NWQC (n=11)							
A_n	6	3	7	3	2	2	2
A_r	3.823	2.231	4.055	4.055	4.055	4.055	1.273
H_o	0.556	0.455	0.600	0.273	0.143	0.091	0.091
H_e	0.765	0.517	0.795	0.417	0.133	0.087	0.087
P	*	ns	ns	ns	ns	ns	ns
SON (n=17)							
A_n	5	5	9	3	1	2	1
A_r	3.494	2.912	4.344	4.344	4.344	4.344	1
H_o	0.429	0.333	0.867	0.250	0.000	0.067	0.000
H_e	0.714	0.640	0.833	0.365	0.000	0.064	0.000
P	ns	ns	*	***		ns	
ANTI (n=12)							
A_n	2	2	5	1	3	2	1
A_r	1.893	1.988	3.484	3.484	3.484	3.484	1
H_o	0.364	0.636	1.000	0.000	0.583	0.083	0.000
H_e	0.397	0.500	0.736	0.000	0.517	0.219	0.000
P	ns	ns	ns		ns	*	
NSNB (n=5)❖							
A_n	3	3	4	1	3	1	2
A_r	2.467	2.2	4	4	4	4	2
H_o	0.600	0.400	1.000	0.000	0.500	0.000	0.333
H_e	0.460	0.340	0.722	0.000	0.531	0.000	0.278
P	ns	ns	ns		ns		ns
VT (n=39)							
A_n	7	6	8	3	4	3	3
A_r	3.324	2.501	3.668	3.668	3.668	3.668	1.451
H_o	0.743	0.462	0.767	0.114	0.324	0.324	0.100
H_e	0.704	0.480	0.768	0.109	0.414	0.314	0.155
P	ns	ns	*	ns	***	ns	ns

Lab (n=18)							
A_n	3	5	9	2	4	6	2
A_r	2.513	2.338	4.578	4.578	4.578	4.578	1.214
H_o	0.235	0.444	0.625	0.188	0.462	0.471	0.071
H_e	0.562	0.446	0.863	0.170	0.521	0.478	0.069
P	**	ns	**	ns	ns	**	ns
NL (n=12)							
A_n	4	3	9	3	3	2	1
A_r	3.399	1.753	4.71	4.71	4.71	4.71	1
H_o	0.417	0.273	0.818	0.200	0.667	0.333	0.000
H_e	0.740	0.244	0.864	0.185	0.486	0.278	0.000
P	*	ns	ns	ns	ns	ns	
UT (n=12)							
A_n	2	6	5	2	4	1	1
A_r	1.273	3.392	3.513	3.513	3.513	3.513	1
H_o	0.091	0.750	0.556	0.167	0.556	0.000	0.000
H_e	0.087	0.701	0.716	0.153	0.691	0.000	0.000
P	ns	ns	*	ns	ns		
SAB (n=13)							
A_n	6	5	6	2	3	1	1
A_r	3.583	2.391	4.667	4.667	4.667	4.667	1
H_o	0.600	0.500	1.000	0.077	0.333	0.000	0.000
H_e	0.720	0.420	0.820	0.074	0.486	0.000	0.000
P	ns	ns	ns	ns	ns		
NEWA (n=12)							
A_n	4	4	10	3	5	2	6
A_r	3.13	2.379	4.681	4.681	4.681	4.681	2.712
H_o	0.500	0.545	0.818	0.182	0.917	0.500	0.667
H_e	0.691	0.479	0.855	0.169	0.681	0.375	0.521
P	ns	ns	*	ns	ns	ns	ns
NEOR (n=11)							
A_n	3	3	7	2	4	2	3
A_r	2.024	2.566	4.225	4.225	4.225	4.225	1.902
H_o	0.455	0.875	0.714	0.200	0.444	0.250	0.333
H_e	0.368	0.570	0.786	0.320	0.377	0.219	0.290
P	ns	ns	ns	ns	ns	ns	ns
CO (n=19)							
A_n	5	4	5	2	6	5	2
A_r	3.385	3.005	3.347	3.347	3.347	3.347	1.535
H_o	0.368	0.500	0.667	0.158	0.706	0.471	0.000
H_e	0.733	0.666	0.720	0.145	0.720	0.441	0.198
P	*	*	ns	ns	ns	ns	***
SWCO (n=12)							
A_n	3	4	6	1	3	1	1
A_r	2.024	2.652	3.497	3.497	3.497	3.497	1
H_o	0.091	0.500	0.750	0.000	0.833	0.000	0.000
H_e	0.368	0.601	0.726	0.000	0.601	0.000	0.000
P	*	*	ns		ns		

NM (n=5)							
A_n	1	2	4	1	1	1	1
A_r	1	1.867	3.429	3.429	3.429	3.429	1
H_o	0.000	0.000	1.000	0.000	0.000	0.000	0.000
H_e	0.000	0.320	0.700	0.000	0.000	0.000	0.000
P		*	ns				
WA (n=38)							
A_n	4	12	11	3	7	3	4
A_r	2.665	3.496	4.418	4.418	4.418	4.418	2.632
H_o	0.469	0.649	0.576	0.105	0.455	0.111	0.261
H_e	0.615	0.713	0.857	0.101	0.739	0.290	0.609
P	ns	***	***	ns	**	***	***
coWA (n=6)							
A_n	2	2	6	1	3	2	1
A_r	1.998	1.995	6	6	6	6	1
H_o	0.667	0.400	1.000	0.000	0.833	0.333	0.000
H_e	0.500	0.480	0.833	0.000	0.569	0.278	0.000
P	ns	ns	ns		ns	ns	
ceOR (n=5)							
A_n	3	2	4	2	2	2	3
A_r	2.75	2	4	4	4	4	2.467
H_o	1.000	0.667	1.000	0.200	1.000	0.400	0.600
H_e	0.594	0.444	0.722	0.180	0.500	0.320	0.460
P	ns	ns	ns	ns	ns	ns	ns
VanIsl (n=18)							
A_n	1	3	7	1	4	2	3
A_r	1	1.909	3.078	3.078	3.078	3.078	2.724
H_o	0.000	0.364	0.467	0.000	0.200	0.111	0.188
H_e	0.000	0.310	0.604	0.000	0.296	0.105	0.643
P	--	ns	ns	--	*	ns	***
Overall (n=402)							
A_n	9	16	15	6	8	10	6

❖ removed from subgroup clustering analyses due to missing data

Table 4.4. Heat map of pairwise Φ_{ST} values of population differentiation. (*) denotes significant values, corrected for false discovery rate ($P < 0.047$). Please see Table 4.1 legend for population abbreviations. See Appendix 6 for Φ_{ST} and p-values.

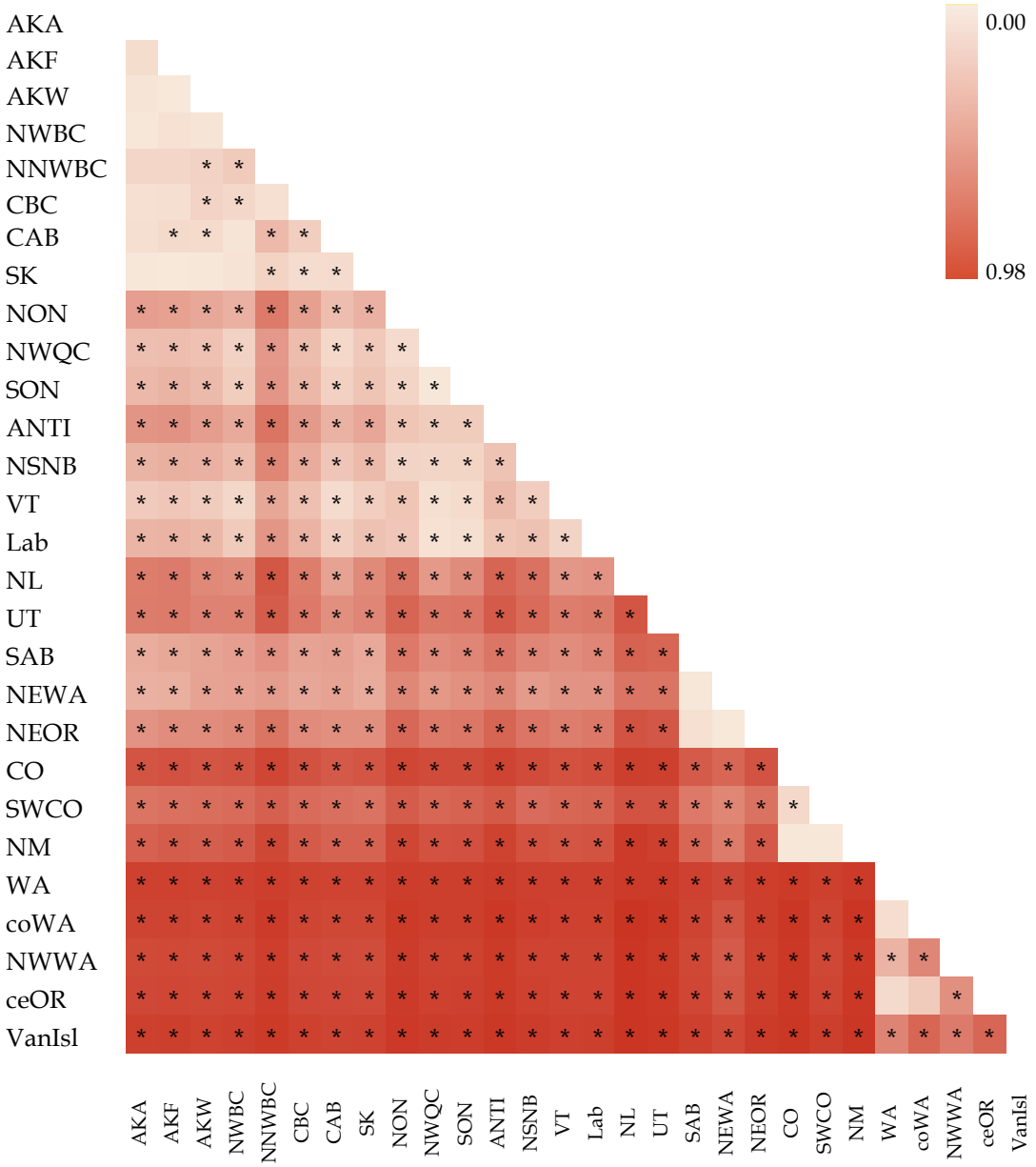

Table 4.5. Spatial analysis of molecular variance (SAMOVA) for gray jay mtDNA control region. The highest amount of between group variation was produced at K=7. SAMOVA software assigned populations to seven groups that were identical to those found in the statistical parsimony network and assigned during AMOVA analysis. **denotes significance tests with $P < 0.001$. Group 1: AKA, AKF, AKW, NNWBC, NWBC, CBC, CAB, SK, NON, NWQC, SON, ANTI, VT, Lab, NSNB. Group 2: NL. Group 3: UT. Group 4: CO, SWCO, NM. Group 5: NEWA, NEOR, SAB. Group 6: WA, NWWA, coWA, ceOR. Group 7: VanIsl. Population abbreviations are explained in Table 4.1.

	d.f.	Variance component	% variation	Fixation index
Among groups	6	11.28	79.57	F_{CT} = 0.797**
Among populations, within groups	21	0.52	3.66	F_{ST} = 0.832**
Within populations	327	2.38	16.78	F_{SC} = 0.179**

Table 4.6. Pairwise Φ st values of population differentiation and estimated divergence times of seven haplotype groups. Φst values (above diagonal; all $P < 0.01$) and estimated divergence times (ka; below diagonal) for gray jay haplogroups (suggested by SAMOVA (Table 4.5) and clustering in the statistical parsimony network (Figure 4.2)). Haplogroup abbreviations are: BE = Boreal East, NL = Newfoundland, UT = Utah, CO-NM = Colorado-New Mexico, IMW = Intermountain West, VanIsl = Vancouver Island. See Figure 4.3 for graphical representation of haplogroup divergence times.

	BE	**NL**	**UT**	**CO-NM**	**IMW**	**Pacific**	**VanIsl**
BE	*	*0.328*	*0.456*	*0.714*	*0.431*	*0.862*	*0.858*
NL	24	*	*0.791*	*0.852*	*0.630*	*0.934*	*0.963*
UT	41	51	*	*0.860*	*0.644*	*0.932*	*0.953*
CO-NM	115	144	166	*	*0.675*	*0.926*	*0.931*
IMW	38	76	85	87	*	*0.879*	*0.870*
Pacific	278	309	323	336	272	*	*0.534*
VanIsl	281	315	328	343	288	26	*

Table 4.7. Heat map of pairwise F_{ST} values of population differentiation for seven microsatellite loci. (*) denotes significant values, corrected for false discovery rate ($P < 0.047$). Please see Table 4.1 legend for population abbreviations. See Appendix 8 for F_{ST} and p-values.

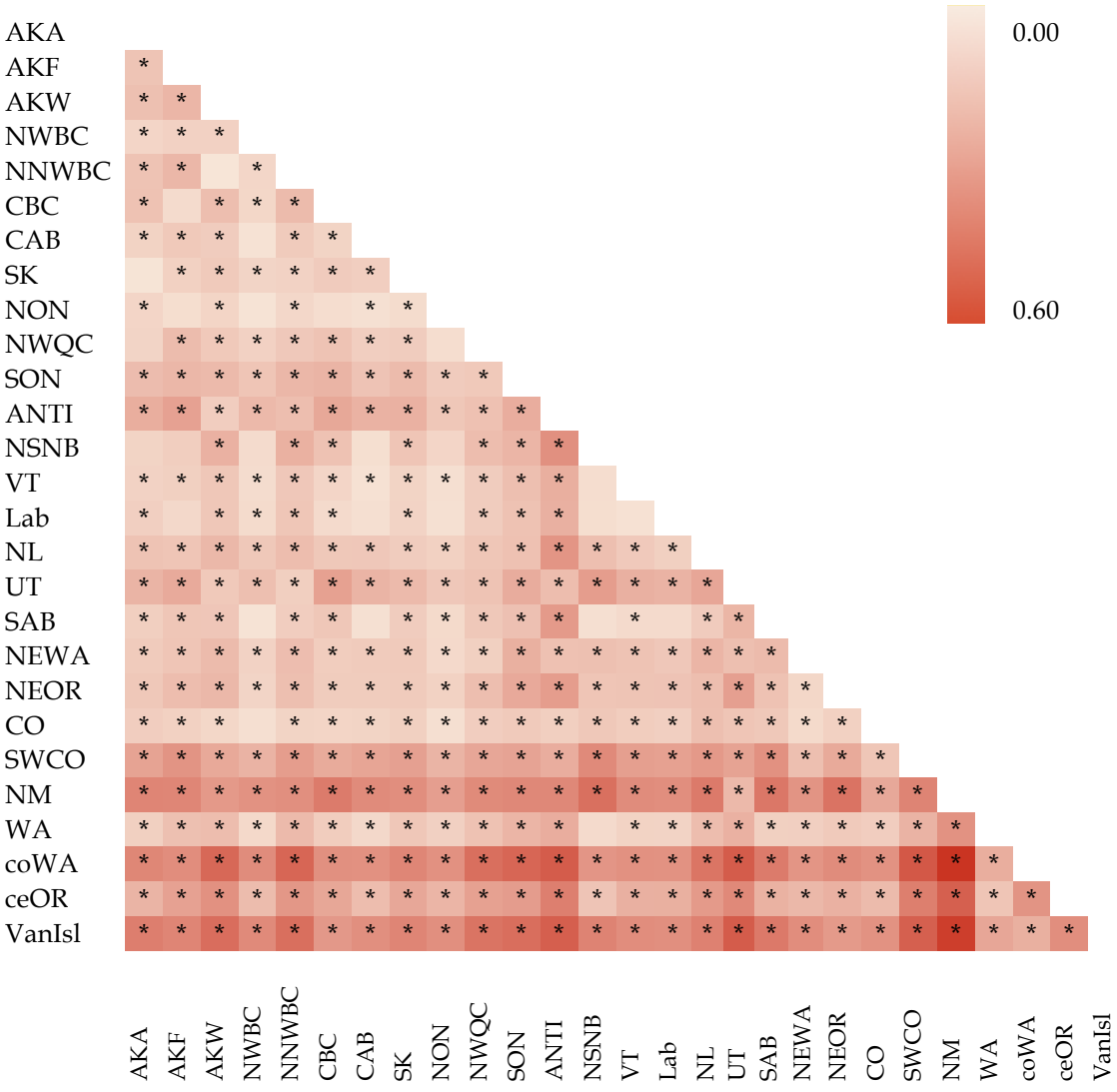

Table 4.8. Groups and subgroups of gray jay populations based on Bayesian clustering analyses of seven microsatellite loci. STRUCTURE and STRUCTURE HARVESTER results both supported two overall groups with three subgroups in Group 1 and two subgroups in Group 2. * denotes populations with high admixture between groups. Colours correspond to haplogroups determined through mitochondrial DNA analyses.

Group 1			Group 2	
I	**II**	**III**	**I**	**II**
AKA	SON	SAB*	AKF	Lab
AKW*	ANTI	NEWA	CBC	NL
NWBC*	UT*	NEOR	coWA	VT
NNWBC	CO		ceOR	
CAB*	SWCO		VanIsl	
SK	NM		WA*	
NWQC				
NON*				

Table 4.9. Gray Jay mean mass and tarsus measurement ± standard error by population and mitochondrial DNA haplogroup. Refer to Table 4.1 for population abbreviations. See Figure 4.6 for graphical representation and ANOVA results.

Haplogroup	Population	Mean Mass (g)	SE	n	Mean Tarsus (mm)	SE	n
Boreal East		*70.41*	*0.75*	*60*	*35.20*	0.21	53
	AKA	72.83	1.38	4	-	-	-
	AKF	73.84	2.28	5	34.70	-	1
	AKW	71.45	1.26	11	35.72	0.49	12
	NWBC	73.11	1.67	9	35.48	0.62	9
	CBC	64.67	3.16	6	34.76	0.01	6
	CAB	66.04	1.24	14	35.87	0.30	14
	SK	72.00	2.52	3	34.83	0.67	3
	NSNB	76.00	-	1	35.20	-	1
	Lab	73.71	2.24	7	33.31	0.38	7
NL	NL	78.29	2.22	7	33.23	0.61	6
UT	UT	78.00	2.70	5	36.98	0.57	5
IMW		68.88	2.19	9	34.66	0.67	7
	SAB	69.00	2.97	6	34.78	0.78	6
	ID	67.50	2.50	2	-	-	-
	SEBC	67.00	-	1	34.00	-	1
CO-NM		72.05	0.98	22	35.33	0.29	22
	CO	73.00	2.31	7	35.34	0.74	7
	SWCO	72.25	1.02	12	35.38	0.29	12
	NM	69.00	2.89	3	35.12	0.77	3
Pacific Coast	WA	59.50	0.76	20	32.97	0.32	20
VanIsl	VanIsl	65.50	0.98	18	33.65	1.45	2
Total				**141**			**115**

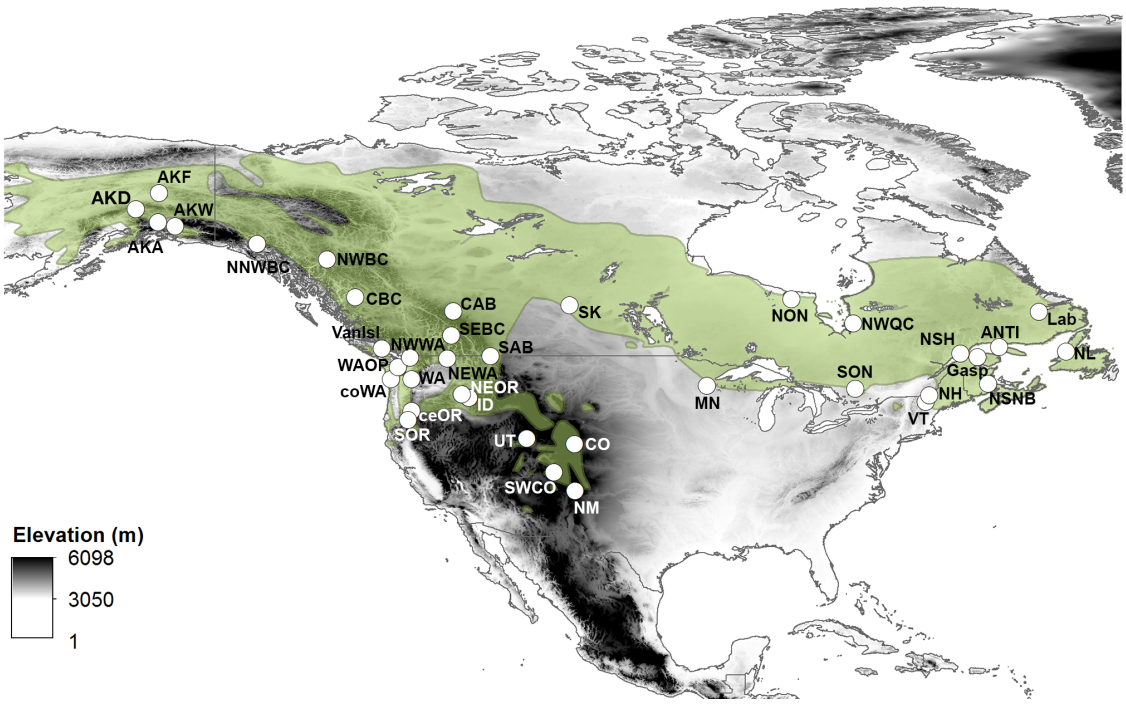

Figure 4.1. Sampled gray jay populations. Gray jay range (light green) in North America and central location of sampled populations (white circles) overlaid on digital elevation model of North America. Population abbreviations and locations are given in Table 4.1.

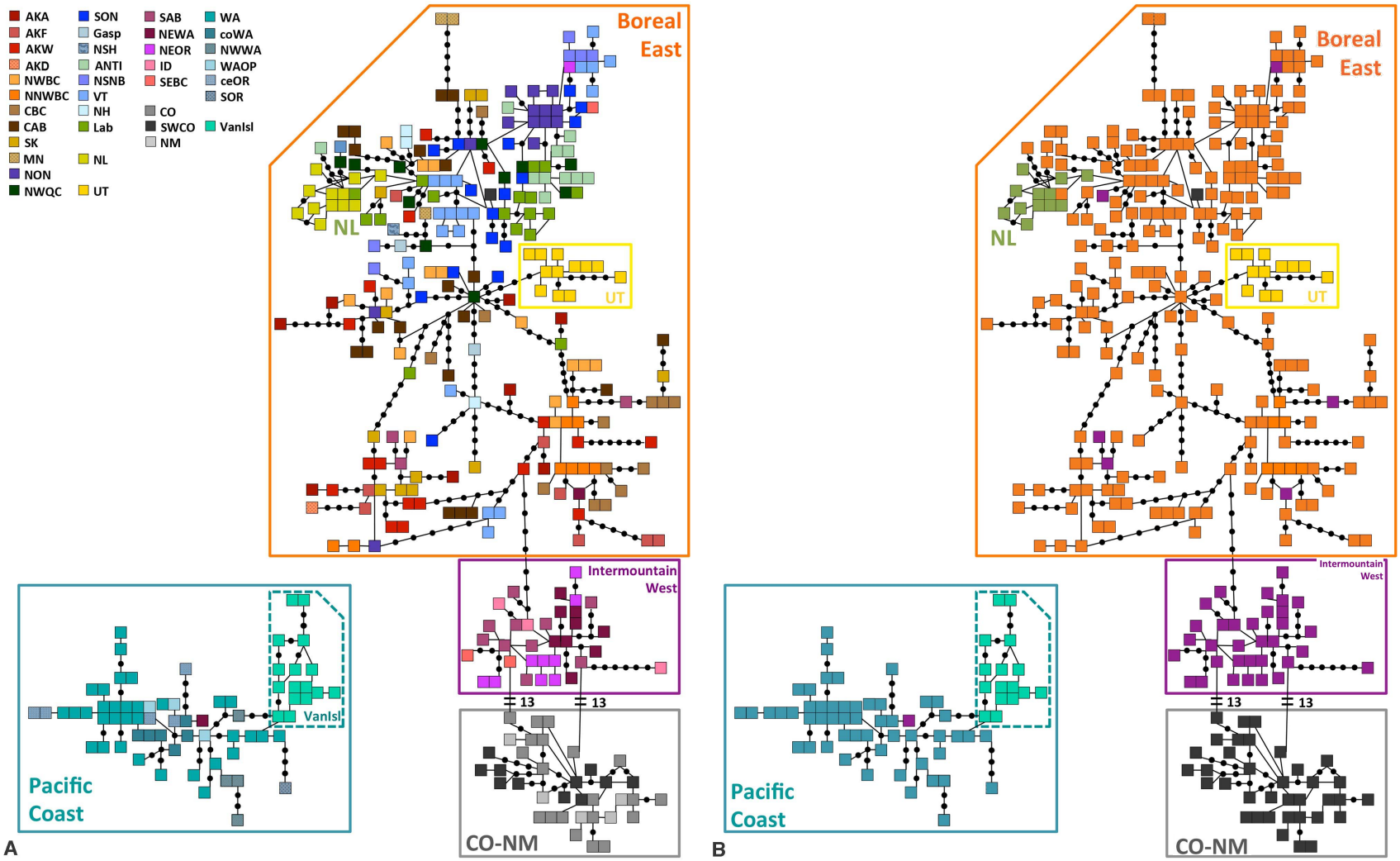
AKA
AKF
AKW
AKD
NWBC
NNWBC
CBC
CAB
SK
MN
NON
NWQC
SON
Gasp
NSH
ANTI
NSNB
VT
NH
Lab
NL
UT
SAB
NEWA
NEOR
ID
SEBC
CO
SWCO
NM
WA
coWA
NWWA
WAOP
ceOR
SOR
VanIsl
Boreal East
NL
UT
Intermountain West
13
13
VanIsl
Pacific Coast
CO-NM
A
Boreal East
NL
UT
Intermountain West
13
13
Pacific Coast
CO-NM
B

Figure 4.2. Statistical parsimony network of mtDNA haplotypes. Statistical parsimony network of 261 gray jay mitochondrial DNA haplotypes for 375 individuals reflecting main haplogroups. Each square represents one individual, individuals with the same haplotype are adjacent, and black dots represent an inferred haplotype. In a) colours correspond to sampled populations (see legend in top left) and b) colours correspond to general haplogroups or population source. Population abbreviations and locations are given in Table 4.1.

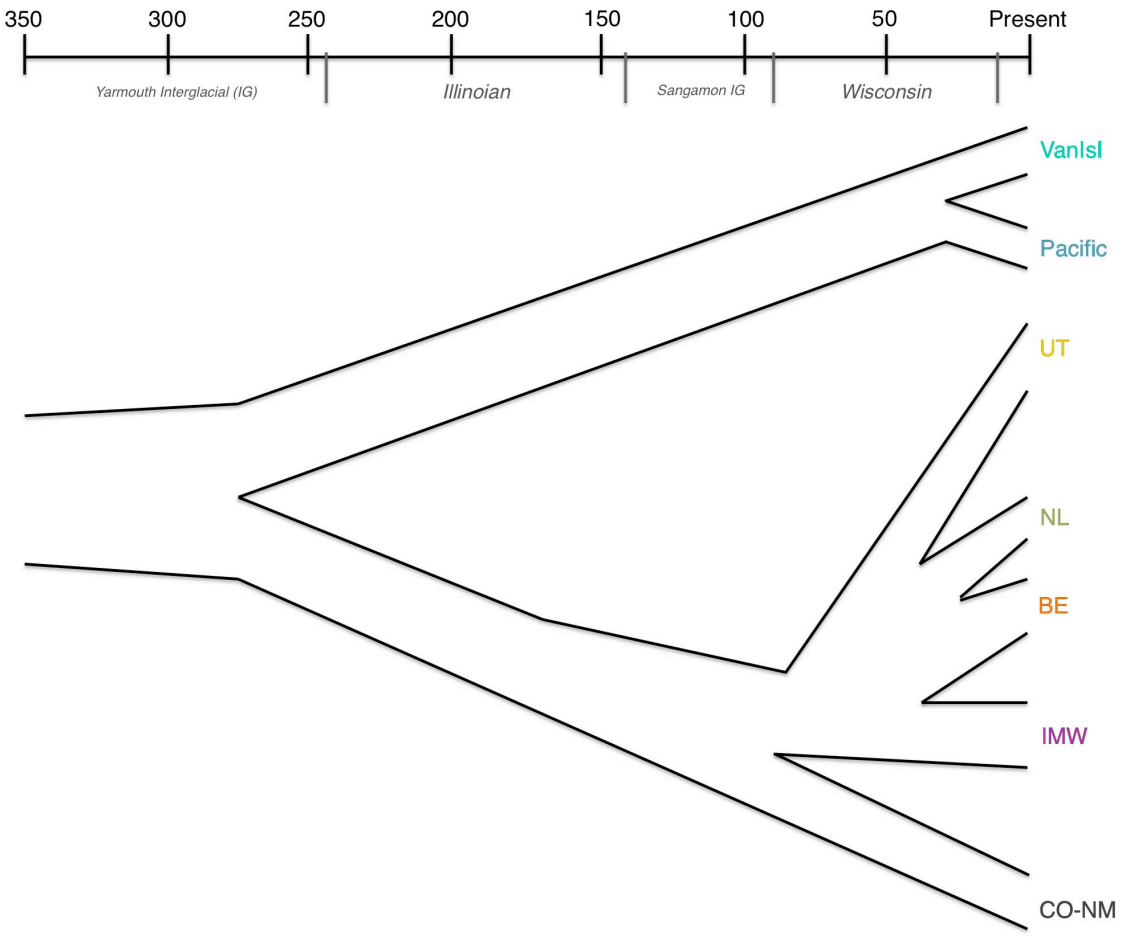

Figure 4.3. Graphical representation of gray jay haplogroup divergence times. Numbers above scale represent thousands of years before present. Words below scale represent glacial and interglacial periods in North America. Names and text colour corresponds to haplogroups in Figure 4.2. See Table 4.1 for haplogroup definitions and Table 4.6 for additional divergence time information.

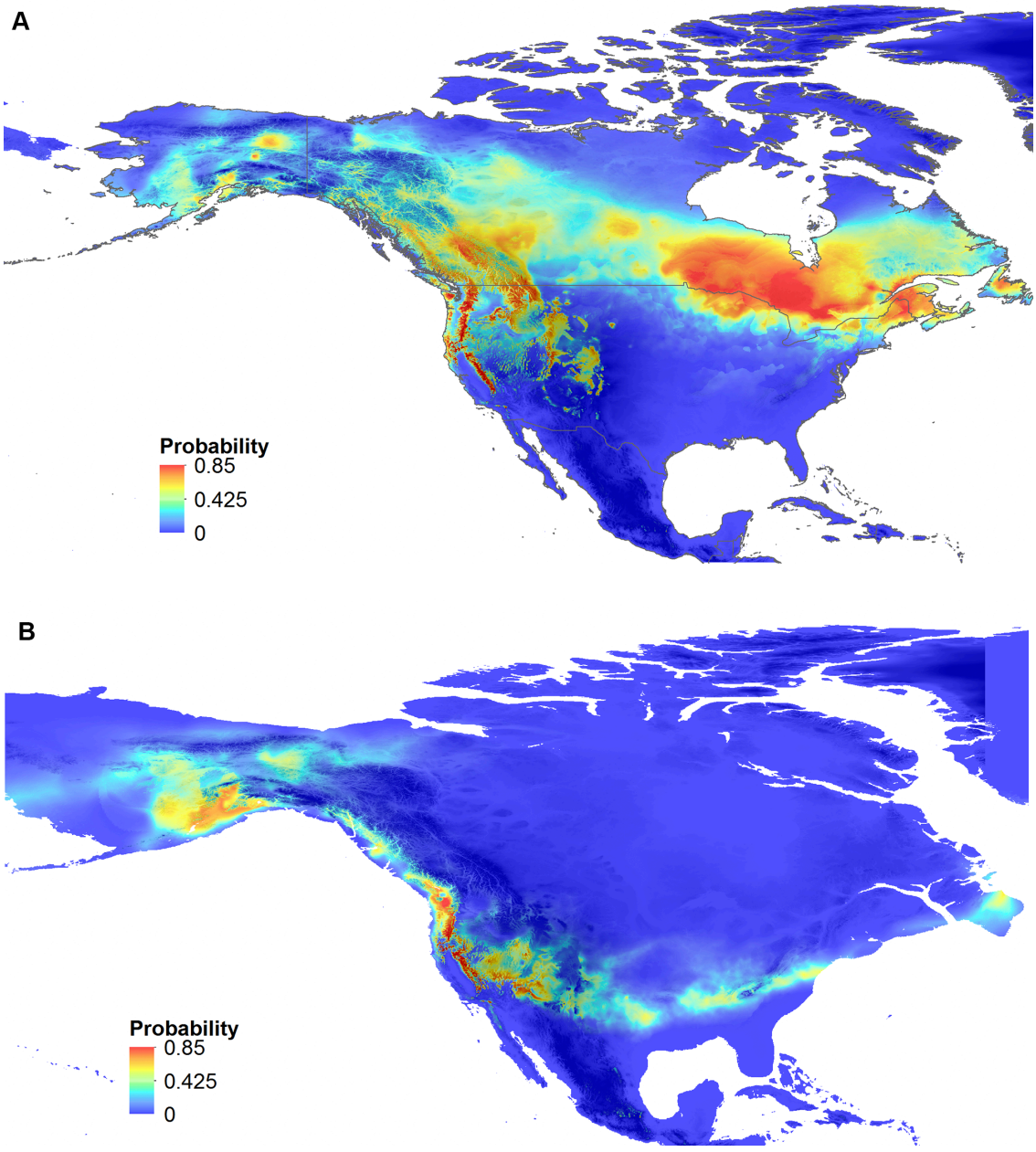

Figure 4.4. Predicted current and paleodistribution of gray jays in North America. a) Current predicted range and b) ~21 ka paleodistribution for gray jay in North America modeled using MAXENT software. Reds and oranges indicate increased probability of species occurrence; probability scale below. Probability maps are layered over digital elevation model (DEM). DEM legend is given in Figure 4.1.

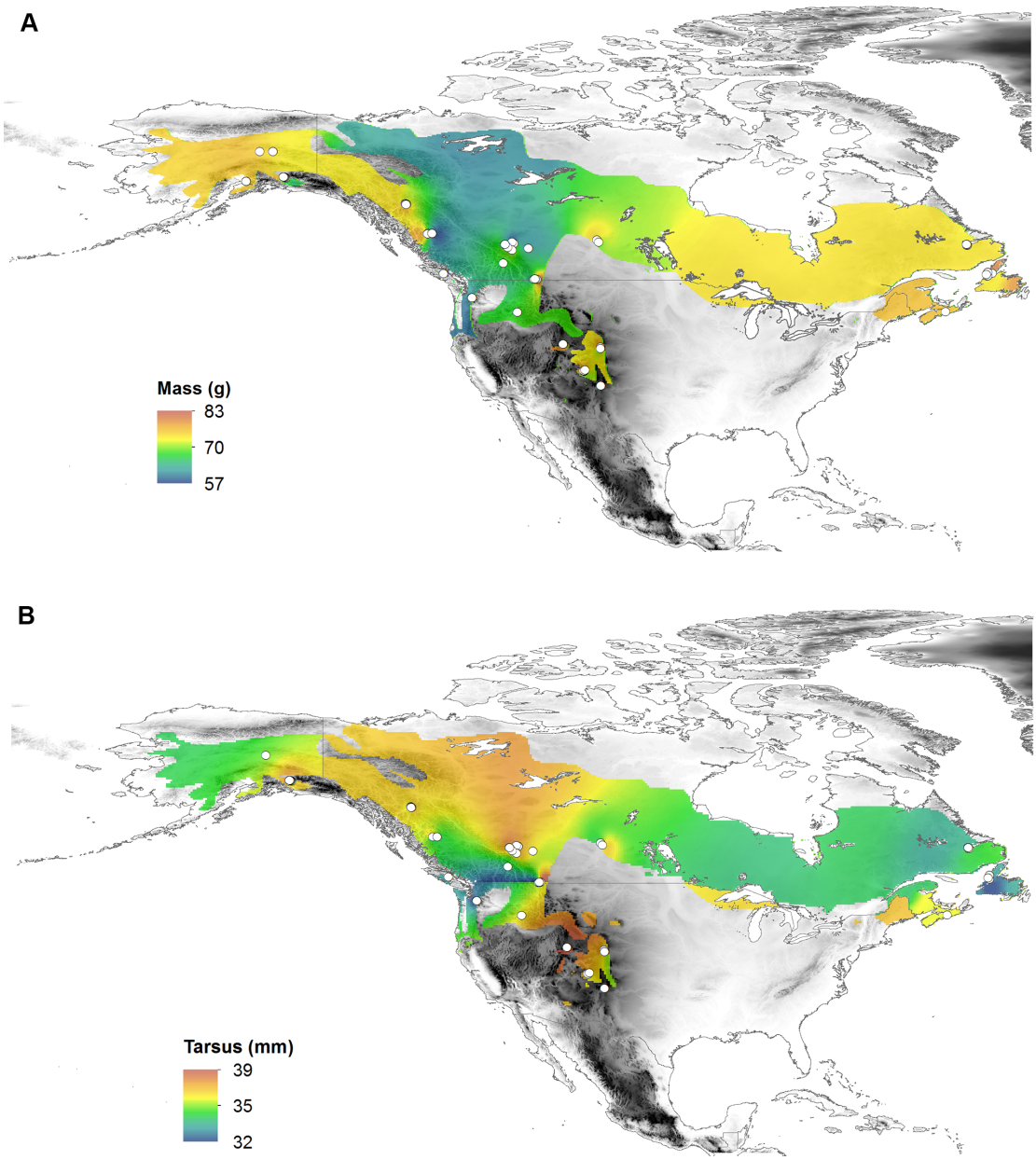

Figure 4.5. Surface interpolation maps of gray jay a) mass and b) tarsus across range. Smoothed spline maps of a) mass (n=141 from 19 populations) and b) tarsus (n=115 from 17 populations) throughout current range created using ArcMap10 Spatial Analyst spline with barriers tool based on measurements taken in the field. Warmer colours indicate larger values. Dots indicate sample locations. See Table 4.5 for populations used in analyses.

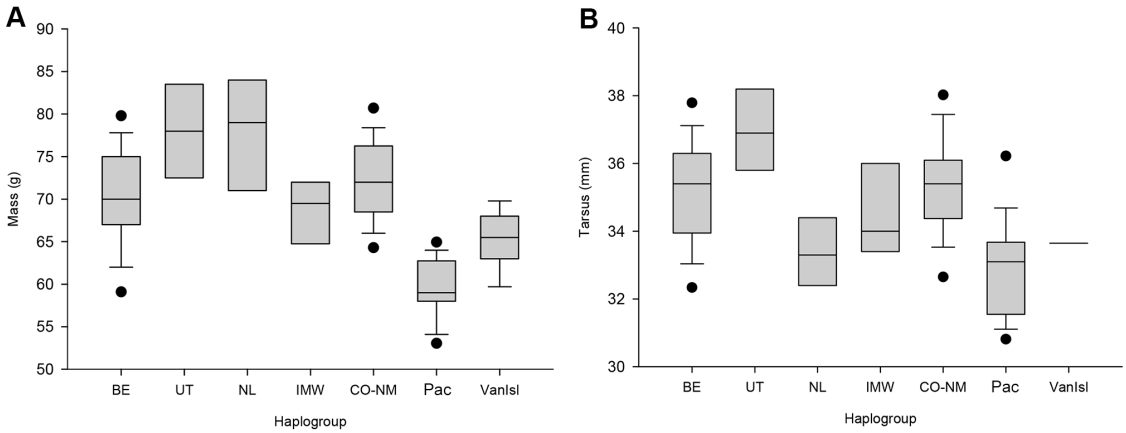

Figure 4.6. Gray Jay a) mass (g) and b) tarsus (mm) compared among mitochondrial haplogroups. 95% confidence intervals; dots are outliers. See Table 4.5 for populations included in analyses.

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Chapter 5: General discussion

5.1 Overall phylogeographic patterns

As predicted, overall phylogeographic patterns based on mitochondrial and nuclear DNA varied by species and location (Table 5.1). All three study species showed high levels of genetic diversity in both mitochondrial DNA control region and, in the North American species, nuclear microsatellite markers. We found evidence that all three species expanded into previously glaciated areas from glacial refugia; however, there were mixed results as to where these refugia were located. We could not find clear evidence of refugial location for nutcrackers, though we suggest they expanded quickly from a single location. This contrasts with gray jays, which likely spent the LGM in at least six different glacial refugia located at the periphery of the ice sheets.

As we predicted, barriers to dispersal do not strongly affect nutcrackers compared to the less vagile gray jay. We found strong breaks across areas of unsuitable habitat, mountain ranges, and large bodies of water for this species, which contrasts strongly to patterns of gene flow across barriers in nutcrackers. Our prediction about morphological variation in gray jays was met with mixed results: geographic breaks for two haplogroups corresponded to significant differences in mass and tarsus between groups, though most variation was clinal and did not show strong breaks across the range.

5.1.1 Glacial refugia and post-glacial colonization

Based on the mtDNA control region marker used in this study, we found that both *Nucifraga* species likely expanded in a phalanx pattern (Hewitt 1996) from a large population in one glacial refugium as ice sheets retreated across the landscape (Table 5.1). All populations sampled exhibit high levels of heterozygosity, nucleotide and allelic diversity, and limited differentiation from other populations, as expected from a species that slowly expanded from a single refugium in a phalanx pattern (Zink 1997; Hewitt 2004). Nutcrackers exhibited star-like mtDNA phylogenies with high levels of genetic diversity (Slatkin and Hudson 1991) and limited genetic subdivision between and within populations (Zink 1996; Hutchison and Templeton 1999). Clark's (*Nucifraga columbiana*) and Eurasian (*N. caryocatactes*) nutcrackers are identified as sister species (Ericson et al. 2005) with no overlap in continental range, though both inhabit *Pinus*-dominated coniferous forests. Both species are known to undergo movement via irruptive dispersal in search of mast crops of *Pinus* seeds (Newton 2006), which likely influenced patterns of genetic diversity and gene flow detected here (Piertney and Summers 2001; Haring et al. 2007). However, no single population stood out genetically as a likely refugial population source, making it very challenging to pinpoint a single (or potentially small, multiple) refugium for these species. Our paleodistribution models were unable to definitively pinpoint refugia for Clark's nutcrackers, modeling was weakly supported and

inconclusive for Eurasian nutcrackers (unpublished data), and fossil records were not available, thus we could not use multiple lines of evidence (Gavin et al. 2014) to locate these areas for either species.

As the main food source for *Nucifraga* spp, one might expect that phylogeography of co-distributed *Pinus* spp could provide clarification for the unresolved number of glacial refugia for nutcrackers. Unfortunately, current studies provide limited or conflicting additional information. For example, studies of *Pinus albicaulis* in North America find ambiguous or single refugial locations (Jaramillo-Correa et al. 2004; Carstens et al. 2005), or multiple north and mid-latitude refugia (Roberts and Hamann 2015). Studies of *Pinus sylvestris* in Eurasia provide some evidence of multiple small refugia on the ice sheets' periphery (Naydenov et al. 2007), but few studies exist of additional *Pinus* spp. in Eurasia to provide more information about refugia for these co-distributed species. Echoing patterns found in nutcrackers, recent studies of western North American trees found that species with few or small refugia and more recent expansion showed limited population differentiation (Roberts and Hamann 2015).

The patterns detected in nutcrackers are in stark contrast to gray jay genetic patterns of substantial genetic differentiation and population subdivision. Similar to trees found in multiple large glacial refugia during the LGM, gray jays isolated

in six to seven refugia followed a pioneer expansion pattern following glacial retreat (Roberts and Hamann 2015). Contrasts in post-glacial expansion and genetic structure are likely the result of differences in natural history traits; in this case dispersal habits driven by diet preferences. Specialization on *Pinus* species seeds by nutcrackers is very different than the generalist diet of the gray jay. Though the dispersal mechanism of *Pinus* seeds out of glacial refugia in North America is debated (e.g. Richardson et al. 2002), nutcrackers have arguably co-evolved in mutualism with *Pinus* spp. and, thus, may have historically and rapidly moved across post-glacial landscapes in search of food sources. This contrasts strongly with gray jay food preferences, which are generalist in nature (Strickland and Ouellet 2011), allowing gray jays to exploit existing food resources in any suitable habitat before dispersing in search of additional food or space. Combined with limited natal dispersal, gray jay movement out of refugia appears to have been slow and resulted in highly differentiated populations and extensive genetic diversity in large portions of their current day range. This dissimilarity in diet preference is likely the driving force for dispersal differences and, consequently, genetic structure in these species.

The specialist-generalist variation hypothesis (SGVH) predicts that specialist species will show lower connectivity and genetic diversity, and high differentiation, relative to generalists (Li et al. 2014; Peled et al. 2016). In a study of nematodes, the majority of species surveyed supported the SGVH (Li et al.

2014). However, this hypothesis comes from assumptions that specialist species exhibit lower levels of dispersal compared to generalists (Santini et al. 2013). In the case of nutcrackers and gray jays, this is reversed. Other studies investigating species with generalist and specialist habitat or diet requirements have found mixed results. A wide-ranging, generalist, avian boreal forest obligate with high dispersal potential exhibited very similar patterns of post-glacial expansion and differentiation to gray jays (Bayard de Volo et al. 2013), yet a generalist, vagile endemic bat species exhibited limited genetic differentiation (Kuo et al. 2014). Comparisons of sedentary specialist and generalist damselfly species in Europe found similar levels of genetic differentiation (Johansson et al. 2013), specialist butterflies have been found to be capable of rapid expansion and gene flow between populations (Betzholtz et al. 2013), and specialist and generalist gerbil species exhibited reversed patterns from those expected (Peled et al. 2016). For many species, as is the case for nutcrackers and gray jays, the SGVH received weak support in the face of complex historical dispersal patterns and potential.

5.1.2 Contemporary barriers and isolation

As originally predicted, mountain ranges did not act as strong barriers to gene flow for either nutcracker species. Both exhibited signs of panmixia and limited population differentiation, which are not unique to species that undergo irruptive dispersal (Cullingham et al. 2012; James et al. 2015). Other northern hemisphere *Pinus* seed specialists characterized by irruptive dispersal

movements (e.g. *Sitta pygmaea* (Spellman and Klicka 2006) and crossbill (*Loxia*) species (Piertney and Summers 2001)) exhibit similar genetic patterns. This irruptive dispersal allows for gene flow to new areas and populations, often leading to limited population differentiation (Haring et al. 2007; James et al. 2015). Mountains may not serve as a barrier to dispersal for high altitude species (Kuo et al. 2014) like nutcrackers, further contributing to limited population differentiation.

When considering North America species only, we used the same mtDNA marker and some overlapping nuclear markers for analyses in both species, allowing for more direct comparison. We found that Clark's nutcracker and gray jay both exhibit peripheral isolation/centre-marginal patterns of current genetic diversity, though isolation by distance plays a larger role in Clark's nutcracker population differentiation. Central-marginal patterns of genetic diversity are not uncommon in other boreal species that do not exhibit long distance seasonal migration (Graham and Burg 2012; Lait and Burg 2013; Roffler et al. 2014; Adams and Burg 2015) and are commonly found in other relatively sedentary taxa (Papadoulou 2009; Degner et al. 2010; Phillipsen and Lytle 2014; Sproul et al. 2014; Micheletti and Storfer 2015). However, mixed support for central-marginal patterns in species with varied dispersal modes has also been found in damselfly species (Johansson et al. 2013), molluscs (Cahill and Levinton 2016), and a variety of other plant and animal (as reviewed in Lira-Noriega and Manthey 2014). This

may be due to finer scale geographic breaks that may not be detected over the range of widely distributed organisms (Lira-Noriega and Manthey 2014; Micheletti and Storfer 2015; Adams and Burg 2016).

We found no notable mtDNA or nuclear breaks in Clark's nutcrackers, but six and two plus additional strong breaks, respectively, in the gray jay. Even in areas in the west where the two species' ranges overlapped, geographical features do not appear to affect nutcrackers and gray jays in the same way. The Okanogan Highlands, Snake River, and Columbia Mountains are found in both species' ranges; for nutcrackers, these do not appear to prevent gene flow between populations as strongly as in the gray jay. This contrast is likely due to dispersal differences driven by diet preferences. Nutcrackers preference for higher altitude habitats where *Pinus* spp. exist, likely reduces the potential of mountain ranges to act as dispersal barriers, as seen in other high altitude species (Kuo et al. 2014). When combined with irruptive dispersal for *Pinus* seeds, this results in gene flow across geographical features that may be barriers to other species, like gray jays. Limited dispersal keeps gray jays more isolated, resulting in these contrasting patterns, even in overlapping areas. Contrasting patterns of genetic differentiation in response to perceived dispersal barriers is not confined to endemic North American corvids. Even in generalist species with high dispersal capabilities, landscape heterogeneity can create complex genetic structure (Balkenhol et al. 2014) and barriers to movement in some species can be

movement corridors for others (Franz et al. 2012; Laurence et al. 2014). Gene flow between populations may depend on features of local topography, such as lower elevation river valleys amongst mountain ranges (Pulgarín-R and Burg 2012), or unexpected overland potential for dispersal (e.g. movement between stream drainages rather than direct connectivity within basins; Philipsen and Lytle 2013). This may create differentially porous barriers (Lait and Burg 2013), which may allow gene flow for species depending on dispersal potential and microgeographic patterns on the landscape.

5.2 Future considerations

Higher resolution climate data for Eurasia and North America could assist in the building of stronger species paleodistribution models and guide additional sampling efforts that might help us to compare perceived historical and more recently established populations. Distribution modeling using data from interglacial periods would allow us to further understand the changes in suitable habitat pre-LGM; this could help us to explain more historical lineage divergences and resolve ambiguous refugial locations. Studies of other widely distributed, high latitude species show a variety of refugial locations and genetic patterns (e.g. Brunhoff et al. 2003; Lait and Burg 2013; Roberts and Hamann 2015). Recently, coalescent and other modeling techniques have been used to complement genetic analyses when investigating finer-scale common refugial histories in co-distributed high latitude alpine mammal species (Eidesen et al.

2013; Lanier et al. 2015), and could provide valuable insight into expansion and contraction history and future distributions for these and other co-distributed species.

Additional microsatellite markers should be developed for all species, and supplementary blood or tissues samples be acquired for these analyses, particularly for populations with less than ideal sample sizes for microsatellites (n=25-30; Hale et al. 2012). Mitochondrial DNA markers are very powerful for measuring inter-population diversity, but nuclear markers may be better suited to measures of intra-population diversity and effective population size fluctuations over time (Hung et al. 2016). Adding more polymorphic microsatellite markers for North American species and increasing sample sizes in Eurasian nutcracker to allow for nuclear marker analyses may also provide an understanding of microgeographic patterns and potential barriers to gene flow (Adams and Burg 2015). Alternatively, whole genome sequencing and other genomic methods may be successful at uncovering fine-scale patterns of divergence and population structure where other genomic methods have missed finer nuances in these species' biogeographical histories (Toews et al. 2016).

Whole genome analyses of existing samples could elucidate more detailed phylogeographic patterns, while allowing us to answer additional questions about morphological differences and subspecies delineation. Phenotypic

differences for isolated small vertebrate populations may be genetically linked (Lomolino 2005). In gray jays, few known morphological differences corresponded to geographic genetic patterns uncovered in this study, though this difference may not exist for all genetic markers. However, recent gray jay morphology research on Anticosti Island does not strongly support this connection (Strickland and Norris 2015). Genomic analyses could also provide a more fulsome understanding of Eurasian nutcracker phylogeography and investigate concordance with subspecies delineations (Toews et al. 2016), which are presently defined by phenotypic differences and geographic associations.

Dispersal potential appears to be one of the main mechanisms behind post-glacial colonization and genetic structure in the endemic corvids in this study. Unfortunately, only limited research exists on nutcracker movement (Rolando 1996; Tomback 1998; Lorenz and Sullivan 2009; Schaming 2016) and most gray jay dispersal information comes from anecdotal accounts (Strickland and Oullet 2011). This makes it particularly difficult to correlate dispersal with gene flow and movement across landscapes, despite the multi-locus genetic data analysed here. Historically, banding recoveries were one of the best (and only tools) available for estimating dispersal distance of birds, but band recoveries, particularly for songbirds, are notoriously low (Thorup et al. 2014) and would significantly limit sample size. Recent research has combined single or multi-locus genetic analyses with various tools for tracking dispersal in a variety of

taxa: stable isotopes with multi-locus genetic markers for long distance migrants (Wilson's warblers (*Wilsonia pusilla*; Rundel et al. 2013)); telemetry combined with microsatellites (radio telemetry in bat-eared foxes (*Otocyon megalotis*; Kamler et al. 2013)) or mitochondrial and nuclear markers (satellite telemetry in ringed seals (*Pusa hispida*; Martinez-Bakker et al. 2013); radio telemetry in two softshell turtle species (Reinertsen et al. 2016)); and geolocators with mitochondrial and microsatellite markers (Alvarado et al. 2014). These combinations have allowed researchers to reach a greater understanding of dispersal and genetic structure and colonization in a variety of species. As with band recoveries, tracking methods that depend on recapture (i.e. geolocators, transmitters via regular monitoring) or transmitters that are small enough to be safely applied to the target study species can be problematic, and also result in small sample sizes relative to the geographic scope of the study. Given that blood or feather samples were taken from many of the individual birds in this thesis, stable isotope analyses are a reasonable option to investigate dispersal distances and combine with the multi-locus genetic analyses in these studies. Based on the time of year samples were collected, differential turnover rates of isotopes in avian tissues (Vander Zanden et al. 2015) could potentially be used to determine dispersal distances for nutcrackers and gray jays. Combining movement information from previous studies, estimates of dispersal from genetic markers, existing band recoveries, and isotopes with our multi-locus genetic analyses would create a

multi-faceted understanding of movement across the landscape and past and present genetic structure in these endemic corvids.

Coordination and collaboration between current researchers would also allow us to optimize our knowledge, reduce redundancies, and contribute in a new way to conservation efforts in the face of climate uncertainty. Recent research has emerged that focuses on using genetic diversity and biodiversity hotspots to advance conservation for co-distributed species assemblages (Wright et al. 2015), particularly for those species that are wide-ranging (Souto et al. 2015). Our understanding of high latitude corvids and other co-distributed species' current geographic genetic patterns combined with models of predicted suitable habitat would allow us to forecast future distributions and proactively respond to potential conservation concerns (Gavin et al. 2014; Malanson et al. 2015; Dalmaris et al. 2015). Combining these methods is highly recommended as a way to most efficiently use funding directed to conservation planning at a landscape level in a rapidly changing world (Brooks et al. 2015; Buerki et al. 2015). Conservation strategies for such co-distributed species may be complex (Ornelas et al. 2013), especially in boreal and subalpine habitats shifting at unexpectedly rapid rates (Bell et al. 2014; Illán et al. 2014). As we address an uncertain future, we must use a more multi-faceted and integrative approach to fully understand the challenges we may face.

Table 5.1 Summary table of results from three corvid phylogeographic studies. IBD = Isolation by Distance.

	Clark's nutcracker *Nucifraga columbiana*	**Eurasian nutcracker** *Nucifraga caryocatactes*	**Gray jay** *Perisoreus canadensis*
Location	Western North America	Eurasia	North America
Habitat	*Pinus*-dominated coniferous forests	Coniferous forests	Spruce-dominated coniferous & mixed forests
Diet type	*Pinus spp* seed specialist	*Pinus spp* seed preference	Generalist
Genetic diversity	High	High	High
Noted mtDNA breaks			
Number	None	None	Six
Locations	-----	-----	Strait of Belle Isle, Okanogan Highlands, Snake River and Great Basins, Columbia Mountains, and Salish Sea
Noted nuclear breaks			
Number	None	-----	Hierarchical: two with three and two subgroups
Glacial refugia			
Number	Likely one	One or many	Six or seven
Locations	Inconclusive	Inconclusive	Beringia, Clearwater, SE US,CO-NM, UT, and Pacific; possibly Brooks Peninsula
Expansion model	Phalanx	Phalanx	Pioneer
Genetic diversity model	IBD; peripheral isolation (centre-marginal)	IBD	Peripheral isolation (centre-marginal)

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Appendices

Appendix 1
Summary table of Clark's nutcracker samples used in analyses.

Appendix 2
Summary table of Eurasian nutcracker samples used in analyses.

Appendix 3
Summary table of gray jay samples used in analyses.

Appendix 4
Protocol and permits information for scientific samples.

Appendix 5
Geographic distribution of shared gray jay haplotypes.

Appendix 6
Mitochondrial DNA pairwise Φ_{ST} values and Benjamini-Hochberg corrected p-values for gray jays.

Appendix 7
Maximum likelihood phylogenetic tree of sampled gray jays rooted with Siberian jay sample.

Appendix 8
F_{ST} values of differentiation and Benjamini-Hochberg corrected p-values for seven microsatellite loci in gray jay.

Appendix 9
Plots of gray jay STRUCTURE clustering analyses.

Appendix 1. Summary table of Clark's Nutcracker samples used in analyses. Sequence (mtDNA): Y = successfully sequenced. N = not sequenced. Genotyped (SSR): Y = successfully genotyped. N = not genotyped. Sources include Burg lab (wild), TS = T. Schaming, The Field Museum (TFM), Burke Museum at the University of Washington (UWBM), Museum of Southwest Biology (MSB), Louisiana State University Museum of Natural Sciences (LSU), American Museum of Natural History (AMNH), Smithsonian National Museum of Natural History (USNM), Royal Saskatchewan Museum (RSM) and Royal Alberta Museum (RAB).

Sample ID	Location	Lat (N)	Long (W)	Source	Band/Museum ID	Sequenced (mtDNA)	Genotyped (SSR)
MT001	Helena, MT	46.542	-112.111	Wild	1783-18001	Y	Y
MT002	Helena, MT	46.542	-112.111	Wild	1783-18002	Y	Y
MT003	Helena, MT	46.566	-112.224	Wild	1783-18003	Y	Y
MT004	Helena, MT	46.566	-112.224	Wild	1783-18004	Y	Y
MT005	Helena, MT	46.566	-112.224	Wild	1783-18005	Y	Y
MT006	Helena, MT	46.566	-112.224	Wild	1783-18006	Y	Y
MT007	Helena, MT	46.566	-112.224	Wild	1783-18007	Y	Y
MT008	Helena, MT	46.566	-112.224	Wild	1783-18008	Y	Y
MT009	Helena, MT	46.566	-112.224	Wild	1783-18009	Y	Y
MT010	Helena, MT	46.566	-112.224	Wild	1783-18010	Y	Y
MT011	Helena, MT	46.566	-112.224	Wild	1783-18011	Y	Y
MT012	Helena, MT	46.566	-112.224	Wild	1783-18012	Y	Y
MT013	Helena, MT	46.566	-112.224	Wild	1783-18013	Y	Y
MT014	Helena, MT	46.566	-112.224	Wild	1783-18015	Y	Y
MT015	Helena, MT	46.566	-112.224	Wild	1783-18016	Y	Y
MT016	Helena, MT	46.566	-112.224	Wild	1783-18017	Y	Y
MT017	Helena, MT	46.566	-112.224	Wild	1783-18018	Y	Y
MT018	Helena, MT	46.566	-112.224	Wild	1783-18019	Y	Y
MT019	Helena, MT	46.566	-112.224	Wild	1783-18020	Y	Y
MT020	Helena, MT	46.566	-112.224	Wild	1783-18021	Y	Y
MT021	Helena, MT	46.566	-112.224	Wild	1783-18022	N	Y

MT022	Helena, MT	46.566	-112.224	Wild	1783-18023	N	Y
MT023	Helena, MT	46.589	-112.129	Wild	1783-18024	N	Y
MT024	Helena, MT	46.589	-112.129	Wild	1783-18025	N	Y
MT025	Helena, MT	46.589	-112.129	Wild	1783-18026	N	Y
CO001	Chambers Lake Campground, CO	40.599	-105.851	Wild	1783-18027	Y	Y
CO002	Chambers Lake Campground, CO	40.599	-105.851	Wild	1783-18028	Y	Y
CO003	Chambers Lake Campground, CO	40.599	-105.851	Wild	1783-18029	Y	Y
CO004	Rocky Mountain National Park, CO	40.394	-105.655	Wild	1783-18031	Y	Y
CO005	Rocky Mountain National Park, CO	40.414	-105.819	Wild	1783-18032	Y	Y
CO006	Rocky Mountain National Park, CO	40.414	-105.819	Wild	1783-18033	Y	Y
CO007	Rocky Mountain National Park, CO	40.414	-105.819	Wild	1783-18034	Y	Y
CO008	Rocky Mountain National Park, CO	40.414	-105.819	Wild	1783-18035	Y	Y
CO009	Rocky Mountain National Park, CO	40.414	-105.819	Wild	1783-18036	Y	Y
CO010	Rocky Mountain National Park, CO	40.414	-105.819	Wild	1783-18037	Y	Y
CO011	2 mi. N and 4 mi. E Grand Mesa, CO	39.041	-107.942	TFM	AMY87-257 334317	Y	Y
CO012	2 mi. N and 4 mi. E Grand Mesa, CO	39.041	-107.942	TFM	AMY87-258 334318	Y	Y
CO013	4.5 mi. E and 2 mi. N Westcreek, CO	39.19	-105.082	TFM	ATP87-266 334319	Y	Y
NM001	La Cueva, Jemez Mountains, NM	35.65	-106.851	MSB	cat #14162	N	Y
NM002	Zuni Mountains, NM	35.059	-108.091	MSB	cat #28605, NK: 169845	Y	Y
NM003	Los Alamos, Barranca Mesa, NM	35.903	-106.286	MSB	cat #14161	Y	Y
NM004	La Cueva, Jemez Mountains, NM	35.862	-106.698	MSB	cat #22798	Y	Y
NM005	Santa Fe, NM	35.687	-105.935	MSB	cat #26349, NK: 165166	Y	Y
NM006	Oso Ridge, Zuni Mountains, NM	35.15	-108.212	MSB	cat #26347, NK: 165118	Y	Y
NM007	Rio Rancho, NM	35.272	-106.69	MSB	cat #26251, NK: 165024	Y	Y
NM008	Oso Ridge, Zuni Mountains, NM	35.15	-108.212	MSB	cat #26348, NK: 165116	Y	Y
NM009	Elk Mountain, NM	35.776	-105.58	MSB	cat #30027	Y	Y
UT001	Lost Creek Campground, UT	40.68	-110.935	Wild	1783-18030	Y	Y
UT002	Vernal, UT	40.716	-109.832	UWBM	#70818 JMB 1693	Y	Y
UT003	Vernal, UT	40.977	-109.092	UWBM	#70850 JMB 1753	Y	Y
UT004	Vernal, UT	40.629	-109.756	UWBM	#70820 JMB 1698	Y	Y

UT005	Vernal, UT	40.717	-109.812	UWBM	#79176 SVD 1248	Y	Y
UT006	Vernal, UT	40.717	-109.812	UWBM	#79175 SVD 1247	Y	Y
UT007	Vernal, UT	40.717	-109.812	UWBM	#79174 SVD 1246	Y	Y
UT008	Uinta Mountains, 12 mi. N and 6 mi. W of Whiterocks, UT	40.719	-110.042	TFM	ATP87-243 334304	Y	Y
UT009	Antelope Canyon, 16 mi. S and 5 mi. E Duchesne, UT	39.321	-111.094	TFM	ATP87-242 334305	Y	Y
UT010	Antelope Canyon, 16 mi. S and 5 mi. E Duchesne, UT	39.321	-111.094	TFM	AMY87-253 334306	Y	Y
UT011	Deseret Peak, Stansbury Mountains, UT	39.321	-111.094	TFM	AMY87-235 334307	Y	Y
UT012	Deseret Peak, Stansbury Mountains, UT	40.46	-112.626	TFM	AMY87-236 334308	Y	Y
UT013	Deseret Peak, Stansbury Mountains, UT	40.46	-112.626	TFM	ATP87-226 334309	Y	Y
UT014	Palos Park, UT	39.321	-111.094	TFM	ATP87-227 334310	N	Y
UT015	Deseret Peak, Stansbury Mountains, UT	40.46	-112.626	TFM	ATP87-228 334311	Y	Y
UT016	Wolf Creek, 11 mi. W and 8 mi. N of Tabiona, UT	40.39	-110.786	TFM	ATP87-239 334312	Y	Y
UT017	Wolf Creek, 11 mi. W and 8 mi. N of Tabiona, UT	40.39	-110.786	TFM	ATP87-240 334313	Y	Y
UT018	Wolf Creek, 11 mi. W and 8 mi. N of Tabiona, UT	40.39	-110.786	TFM	AMY87-250 334314	Y	Y
UT019	Wolf Creek, 11 mi. W and 8 mi. N of Tabiona, UT	40.39	-110.786	TFM	AMY87-251 334315	Y	Y
UT020	Wolf Creek, 11 mi. W and 8 mi. N of Tabiona, UT	40.39	-110.786	TFM	AMY87-252 334316	Y	Y
WY001	Shadow Mountain, Bridger-Teton National Forest, WY	43.703	-110.612	TS	1783-20342	Y	Y
WY002	Shadow Mountain, Bridger-Teton National Forest, WY	43.703	-110.612	TS	1783-20343	Y	Y
WY003	Shadow Mountain, Bridger-Teton National Forest, WY	43.703	-110.612	TS	1783-20344	Y	Y
WY004	Shadow Mountain, Bridger-Teton National Forest, WY	43.703	-110.612	TS	1783-20345	Y	Y
WY005	Shadow Mountain, Bridger-Teton National Forest, WY	43.703	-110.612	TS	1783-20346	Y	Y

WY006	Behind Triangle X, Bridger-Teton National Forest, WY	43.703	-110.612	TS	1783-20347	Y	Y
WY007	Shadow Mountain, Bridger-Teton National Forest, WY	43.76	-110.552	TS	1783-20348	Y	Y
WY008	Shadow Mountain, Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20349	Y	Y
WY009	Behind Triangle X, Bridger-Teton National Forest, WY	43.702	-110.612	TS	1783-20350	Y	Y
WY010	Behind Triangle X, Bridger-Teton National Forest, WY	43.762	-110.553	TS	1783-20351	Y	Y
WY011	Shadow Mountain, Bridger-Teton National Forest, WY	43.762	-110.553	TS	1783-20301	Y	Y
WY012	Shadow Mountain, Bridger-Teton National Forest, WY	43.705	-110.609	TS	1783-20321	Y	Y
WY013	Bridger-Teton National Forest, WY	43.708	-110.613	TS	Unbanded	Y	Y
WY014	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20308	Y	Y
WY015	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20352	Y	Y
WY016	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20353	Y	Y
WY017	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20354	N	Y
WY018	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20355	Y	Y
WY019	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20356	Y	Y
WY020	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20357	Y	Y
WY021	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20358	N	Y
WY022	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20360	N	Y
WY023	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20361	N	Y
WY024	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20632	N	Y
WY025	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20363	N	Y
WY026	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20364	N	Y
WY027	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20365	N	Y
WY028	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20366	N	Y
WY029	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20367	N	Y
WY030	Bridger-Teton National Forest, WY	43.708	-110.613	TS	1783-20368	N	Y

NEOR1	Mt. Howard Summit, OR	45.263	-117.18	Wild	1783-17901	Y	Y
NEOR2	Mt. Howard Summit, OR	45.263	-117.18	Wild	1783-17902	Y	Y
NEOR3	Mt. Howard Summit, OR	45.263	-117.18	Wild	1783-17903	Y	Y
NEOR4	Mt. Howard Summit, OR	45.263	-117.18	Wild	1783-17904	Y	Y
NEOR5	Mt. Howard Summit, OR	45.263	-117.18	Wild	1783-17905	Y	Y
NEOR6	Mt. Howard Summit, OR	45.263	-117.18	Wild	1783-17906	Y	Y
NEOR7	Mt. Howard Summit, OR	45.263	-117.18	Wild	1783-17907	Y	Y
NEOR8	Mt. Howard Summit, OR	45.263	-118.18	Wild	1783-17908	Y	Y
NEOR9	Mt. Howard Summit, OR	45.263	-118.18	Wild	Unbanded	Y	Y
NEOR10	Mt. Howard Summit, OR	45.263	-118.18	Wild	1783-17909	Y	Y
NEOR11	Mt. Howard Summit, OR	45.263	-118.18	Wild	1783-17910	Y	Y
NEOR12	Mt. Howard Summit, OR	45.263	-118.18	Wild	1783-17911	Y	Y
NEOR13	Mt. Howard Summit, OR	45.263	-118.18	Wild	1783-17912	Y	Y
NEOR14	Mt. Howard Summit, OR	45.263	-118.18	Wild	1783-17913	Y	Y
NEOR15	Mt. Howard Summit, OR	45.263	-118.18	Wild	1783-17914	Y	Y
NEOR16	Mt. Howard Summit, OR	45.263	-118.18	Wild	1783-17915	Y	Y
NEOR17	Wallowa, OR	45.22	-117.057	AMNH	DOT-15768	Y	Y
NEOR18	Wallowa, OR	45.22	-117.057	AMNH	DOT-15824	Y	Y
NEOR19	Wallowa, OR	45.22	-117.057	AMNH	DOT-15825	Y	Y
NEOR20	Wallowa, OR	45.22	-117.057	AMNH	DOT-15826	Y	Y
SOR001	Crater Lake Rim Village, OR	42.551	-122.135	Wild	1783-17916	Y	Y
SOR002	Crater Lake Rim Village, OR	42.551	-122.135	Wild	1783-17917	Y	Y
SOR003	Crater Lake Rim Village, OR	42.551	-122.135	Wild	1783-17918	Y	Y
SOR004	Discovery Point, Crater Lake, OR	42.917	-122.152	Wild	1783-17919	Y	Y
SOR005	Discovery Point, Crater Lake, OR	42.917	-122.152	Wild	1783-17920	Y	Y
SOR006	Discovery Point, Crater Lake, OR	42.917	-122.152	Wild	1783-17921	Y	Y
SOR007	Discovery Point, Crater Lake, OR	42.917	-122.152	Wild	1783-17922	Y	Y
SOR008	Discovery Point, Crater Lake, OR	42.917	-122.152	Wild	1783-17923	Y	Y
SOR009	Discovery Point, Crater Lake, OR	42.917	-122.152	Wild	1783-17924	Y	Y
SOR010	Discovery Point, Crater Lake, OR	42.917	-122.152	Wild	1783-17925	Y	Y

SOR011	Discovery Point, Crater Lake, OR	42.917	-122.152	Wild	1783-17926	Y	Y
SOR012	Crater Lake Rim Village, OR	42.551	-122.135	Wild	1783-17927	Y	Y
CCA001	1 mi. NW of Mono Mills on Hwy 120, CA	37.888	-118.958	LSU	LSUMZ #41553	Y	Y
CCA002	Glass Mt. Lookout Rd., 1 mi. E of Hwy. 395, CA	37.775	-118.708	LSU	LSUMZ #21509	Y	Y
CCA003	2km N of Mineret Summit, CA	37.656	-119.06	LSU	LSUMZ #58220	Y	Y
CCA004	Glass Mt. Lookout Rd., 1 mi. E of Hwy. 395, CA	37.775	-118.708	LSU	LSUMZ #21514	Y	Y
CCA005	San Benito Mountain, CA	34.192	-116.709	LSU	LSUMZ #47775	Y	Y
CCA006	2km N of Mineret Summit, CA	37.656	-119.06	LSU	LSUMZ #58222	Y	Y
CCA007	2km N of Mineret Summit, CA	37.656	-119.06	LSU	LSUMZ #62381	Y	Y
CCA008	Glass Mt. Lookout Rd., 1 mi. E of Hwy. 395, CA	37.775	-118.708	LSU	LSUMZ #21512	Y	Y
CCA009	1 mi. NW of Mono Mills on Hwy 120, CA	37.888	-118.958	LSU	LSUMZ #41554	Y	Y
CCA010	2km N of Mineret Summit, CA	37.656	-119.06	LSU	LSUMZ #58221	Y	Y
CCA011	2km N of Mineret Summit, CA	37.656	-119.06	LSU	LSUMZ #58223	Y	Y
SCA001	Cuddy Valley Rd, 1 1/2 mi. ESE Mt. Pinos, CA	34.812	-119.147	LSU	LSUMZ #30275	Y	Y
SCA002	Onyx Peak, San Bernardino Mtns., CA	34.192	-116.709	LSU	LSUMZ #21458	Y	Y
SCA003	Upper end Van Duzen Canyon, San Bernardino Mtns., CA	34.291	-116.884	LSU	LSUMZ #42055	Y	Y
SCA004	W of Wildhorse Meadow, San Bernardino Mtns., CA	34.192	-116.709	LSU	LSUMZ #34559	Y	Y
SCA005	1 mi. NNW of Onyx Peak, San Bernardino Mtns., CA	34.192	-116.709	LSU	LSUMZ #16785	Y	Y
SCA006	Cuddy Valley Rd, 1 1/2 mi. ESE Mt. Pinos, CA	34.812	-119.147	LSU	LSUMZ #30339	Y	Y
SCA007	Jacoby Spring, San Bernardino Mtns., CA	34.318	-116.831	LSU	LSUMZ #42105	Y	Y
SCA008	1 mi. NNW of Onyx Peak, San Bernardino Mtns., CA	34.192	-116.709	LSU	LSUMZ #30430	Y	Y
SCA009	1.5 mi. NW of Onyx Peak, San Bernardino Mtns., CA	34.192	-116.709	LSU	LSUMZ #34416	Y	Y
SCA010	1.5 mi. E of Onyx Peak, San Bernardino Mtns., CA	34.192	-116.709	LSU	LSUMZ #34429	Y	Y

SCA011	1 mi. NNW of Onyx Peak, San Bernardino Mtns., CA	34.192	-116.709	LSU	LSUMZ #30429	Y	Y
SCA012	Heart Bar Campground, San Bernardino NF, CA	34.157	-116.79	Wild	1713-14102	Y	Y
SCA013	Heart Bar Campground, San Bernardino NF, CA	34.157	-116.79	Wild	1713-14103	Y	Y
SCA014	Heart Bar Campground, San Bernardino NF, CA	34.157	-116.79	Wild	1713-14104	Y	Y
WA001	Mt. Rainier Sunrise Visitor Centre, WA	46.901	-121.635	Wild	unbanded	Y	Y
WA002	Mt. Rainier Sunrise Visitor Centre, WA	46.901	-121.635	Wild	1783-17928	Y	Y
WA003	Mt. Rainier Sunrise Visitor Centre, WA	46.901	-121.635	Wild	1783-17929	Y	Y
WA004	Near Jr. Point Campground, Chelan, WA	47.992	-120.388	UWBM	#85352, VGR 681	Y	Y
WA005	Bridgeport, Douglas, WA	48.015	-119.822	UWBM	#72676, CSW 4776	Y	Y
WA006	Chinook Pass, Yakima, WA	46.879	-121.516	UWBM	#46094, CSW 4213	Y	Y
WA007	Naches, Yakima, WA	46.832	-120.973	UWBM	#89883, EVL 1333	Y	Y
NEWA1	Loomis, Okanogan County, WA	48.7	-119.7	UWBM	#74138 NAM 51	Y	Y
NEWA2	Mazama, Okanogan County, WA	48.672	-120.48	UWBM	#66925 SVD 2364	Y	Y
NEWA3	Okanogan County, WA	48.88	-119.691	UWBM	#88835 TNL 018	Y	Y
NEWA4	Okanogan County, WA	48.88	-119.691	UWBM	#88770 KLE 150	Y	Y
NEWA5	Conconully, Okanogan County, WA	48.691	-119.753	UWBM	#85355 VGR 810	Y	Y
NEWA6	Oroville, Okanogan County, WA	48.91	-119.502	UWBM	#67212 NTO 012	Y	Y
SAB001	Porcupine Hills, AB	49.889	-114.011	RAB	acc# Z02.14.3	Y	Y
SAB002	Porcupine Hills, AB	49.884	-114.011	RAB	acc# Z02.14.4	Y	Y
SAB003	Sharples Creek, Imp. District 16, AB	49.9	-114.033	RAB	acc# Z87.20.31	Y	Y
SAB004	12 mi. S & 2 mi. W of Beaver Mines, Impr. Dist 5, AB	49.3	-114.267	RAB	acc# Z86.36.35	Y	Y
SAB005	Plateau Mountain, Imp. District 7, AB	50.2	-114.533	RAB	acc# Z84.35.170	Y	Y
SAB006	Plateau Mountain, Imp. District 6, AB	50.2	-114.567	RAB	acc# Z84.35.143	N	Y
SAB007	Carbondale River, Imp. District 5, AB	49.383	-114.417	RAB	acc# Z84.35.115	Y	Y
SAB008	Wilkinson Creek, Forestry Trunk Rd, AB	50.267	-114.583	RAB	acc# Z79.16.5	Y	Y
SAB009	W slope of Plateau Mtn, Imp. Dist. 6, AB	50.2	-114.533	RAB	acc# Z79.16.4	N	Y
SAB010	Bovin Lake, Imp. District 5, AB	49.217	-114.117	RAB	acc# Z70.57.3	Y	Y

SAB011	11 mi. south of Beauvais Lake, Imp. District 5, AB	49.317	-114.1	RAB	acc# Z92.14.42	N	Y
SAB012	Castle River, Imp. District 5, AB	49.25	-114.25	RAB	acc# Z87.20.69	Y	Y
CAB001	Mt. Edith Cavell Parking Lot, Jasper National Park, AB	52.687	-118.056	Wild	1603-90529	Y	Y
CAB002	Mt. Edith Cavell Parking Lot, Jasper National Park, AB	52.687	-118.056	Wild	1603-90530	Y	Y
CAB003	Mt. Edith Cavell Parking Lot, Jasper National Park, AB	52.687	-118.056	Wild	1603-90531	Y	Y
CAB004	Mt. Edith Cavell Parking Lot, Jasper National Park, AB	52.687	-118.056	Wild	1603-90532	Y	Y
CAB005	Mt. Edith Cavell Parking Lot, Jasper National Park, AB	52.687	-118.056	Wild	1603-90533	Y	Y
CAB006	Mt. Edith Cavell Parking Lot, Jasper National Park, AB	52.687	-118.056	Wild	1603-90534	Y	Y
CAB007	Mt. Edith Cavell Parking Lot, Jasper National Park, AB	52.687	-118.056	Wild	1603-90535	Y	Y
CAB008	Mt. Edith Cavell Parking Lot, Jasper National Park, AB	52.687	-118.056	Wild	1603-90536	Y	Y
SEBC01	Oliver, BC	49.166	-119.254	RSM	catA1168/accn9644	Y	N
NECA01	Mosquito Creek Loop, Lassen, CA	41.151	-120.205	USNM	tissue#B21459	Y	N
NECA02	Mosquito Creek Loop, Lassen, CA	41.151	-120.205	USNM	tissue#B21460	Y	N

Appendix 2. Summary table of Eurasian nutcracker samples used in analyses. Eurasian nutcracker (*Nucifraga caryocatactes*) sample codes, geographic location, voucher number from Burke Museum of Natural History and Culture – University of Washington, and GenBank accession numbers.

Code	Geographic Location	Voucher Number	Genbank Accession #
BK001	Badzhal'skiy Khrebet, Khabarovskiy Kray, Russia	UWBM#47356, SVD 262	KJ999615
BK002	Badzhal'skiy Khrebet, Khabarovskiy Kray, Russia	UWBM#47357, SVD 263	KJ999616
BK003	Badzhal'skiy Khrebet, Khabarovskiy Kray, Russia	UWBM#46908, BKS 961	KJ999617
GA001	Gorno-Altaysk, Altai Republic, Russia	UWBM#46296, CDS 4858	KJ999618
GA002	Gorno-Altaysk, Altai Republic, Russia	UWBM#46293, CDS 4855	KJ999619
GA003	Gorno-Altaysk, Altai Republic, Russia	UWBM#46286, CDS 4848	KJ999620
GA004	Gorno-Altaysk, Altai Republic, Russia	UWBM#46285, CDS 4847	KJ999621
KA001	Koryaki, Kamchatka, Russia	UWBM#44307, JMB 1147	KJ999622
KA002	Milkovo, Kamchatka, Russia	UWBM#44356, JMB 1198	KJ999623
KA003	Milkovo, Kamchatka, Russia	UWBM#44357, JMB 1199	KJ999624
KA004	Milkovo, Kamchatka, Russia	UWBM#44359, JMB 1201	KJ999625
KA005	Milkovo, Kamchatka, Russia	UWBM#44365, JMB 1210	KJ999626
KA006	Milkovo, Kamchatka, Russia	UWBM#44072, CSW 4670	KJ999627
KD001	Khamar-Daban weather station, Slyudyanka, Irkutsk Oblast, Russia	UWBM#51815, SVD 612	KJ999628
KD002	Khamar-Daban weather station, Slyudyanka, Irkutsk Oblast, Russia	UWBM#51817, SVD 614	KJ999629
KD003	Khamar-Daban weather station, Slyudyanka, Irkutsk Oblast, Russia	UWBM#51818, SVD 615	KJ999630
KD004	Khamar-Daban weather station, Slyudyanka, Irkutsk Oblast, Russia	UWBM#51826, SVD 623	KJ999631
KD005	Khamar-Daban weather station, Slyudyanka, Irkutsk Oblast, Russia	UWBM#51841, SVD 638	KJ999632
MA001	Snezhnaya Dolina, Ol'skiy Rayon, Magadanskaya Oblast, Russia	UWBM#51577, SVD 357	KJ999633
MA002	Snezhnaya Dolina, Ol'skiy Rayon, Magadanskaya Oblast, Russia	UWBM#51549, SVD 329	KJ999634
MA003	mouth of Oroholyndja River, Magadan, Magadanskaya Oblast, Russia	UWBM#44100, DAB 41	KJ999635
MA004	mouth of Oroholyndja River, Magadan, Magadanskaya Oblast, Russia	UWBM#44425, SAR 6050	KJ999636
MA005	mouth of Oroholyndja River, Magadan, Magadanskaya Oblast, Russia	UWBM#44400, JMB 987	KJ999637
MA006	mouth of Oroholyndja River, Magadan, Magadanskaya Oblast, Russia	UWBM#43849, CSW 4376	KJ999638
MA007	mouth of Oroholyndja River, Magadan, Magadanskaya Oblast, Russia	UWBM#43817, CSW 4344	KJ999639
MA008	mouth of Oroholyndja River, Magadan, Magadanskaya Oblast, Russia	UWBM#44192, JMB 1016	KJ999640
OK001	Kyzyl, Ovyurskiy Kozhuun, Tuva Republic	UWBM#66384, NAM 213	KJ999641
OR001	headwaters of Ola River, Magadanskaya Oblast, Russia	UWBM#44199, JMB 1024	KJ999642
OR002	headwaters of Ola River, Magadanskaya Oblast, Russia	UWBM#44198, JMB 1023	KJ999643

PK001	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75252, RYA 551	KJ999644
PK002	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75160, RYA 459	KJ999645
PK003	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75159, RYA 458	KJ999646
PK004	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75248, RYA 547	KJ999647
PK005	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75249, RYA 548	KJ999648
PK006	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75250, RYA 549	KJ999649
PK007	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75130, RYA 427	KJ999650
PK008	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75161, RYA 460	KJ999651
PK009	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75200, RYA 499	KJ999652
PK010	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75251, RYA 550	KJ999653
PK011	Khrebet Siniy, Arsen'yev, Anuchinskiy Rayon, Primorskiy Kray, Russia	UWBM#75129, RYA 426	KJ999654
SO001	Sakhalinskaya Oblast, Russia	UWBM#47409, SVD 317	KJ999655
SO002	Sakhalinskaya Oblast, Russia	UWBM#47410, SVD 318	KJ999656
SO003	Sakhalinskaya Oblast, Russia	UWBM#47412, SVD 320	KJ999657
SO004	Sakhalinskaya Oblast, Russia	UWBM#47598, VM 283b	KJ999658
SO005	Sakhalinskaya Oblast, Russia	UWBM#47600, VM 284b	KJ999659
TO001	Vengoyakha river, Noyabr'sk, Yamalo-Nenetskiy Avtonomnyi Okrug, Tyumenskaya Oblast', Russia	UWBM#56723, CSW 5577	KJ999660
TO002	Vengoyakha river, Noyabr'sk, Yamalo-Nenetskiy Avtonomnyi Okrug, Tyumenskaya Oblast', Russia	UWBM#56746, CSW 5601	KJ999661
TO003	Vengoyakha river, Noyabr'sk, Yamalo-Nenetskiy Avtonomnyi Okrug, Tyumenskaya Oblast', Russia	UWBM#56770, CSW 5626	KJ999662
TO004	Vengoyakha river, Noyabr'sk, Yamalo-Nenetskiy Avtonomnyi Okrug, Tyumenskaya Oblast', Russia	UWBM#56792, CSW 5649	KJ999663
TO005	Vengoyakha river, Noyabr'sk, Yamalo-Nenetskiy Avtonomnyi Okrug, Tyumenskaya Oblast', Russia	UWBM#56964, SVD 1198	KJ999664
TO006	Vengoyakha river, Noyabr'sk, Yamalo-Nenetskiy Avtonomnyi Okrug, Tyumenskaya Oblast', Russia	UWBM#56972, SVD 1206	KJ999665
TO007	Vengoyakha river, Noyabr'sk, Yamalo-Nenetskiy Avtonomnyi Okrug, Tyumenskaya Oblast', Russia	UWBM#56782, CSW 5639	KJ999666
TU001	Turuka, Ust-Kut, Ust-Kutskiy Rayon, Irkutsk Oblast, Russia	UWBM#73336, JML 322	KJ999667
TU002	Turuka, Ust-Kut, Ust-Kutskiy Rayon, Irkutsk Oblast, Russia	UWBM#73348, JML 334	KJ999668
TU003	Turuka, Ust-Kut, Ust-Kutskiy Rayon, Irkutsk Oblast, Russia	UWBM#73681, VGR 451	KJ999669
TU004	Turuka, Ust-Kut, Ust-Kutskiy Rayon, Irkutsk Oblast, Russia	UWBM#73478, RCF 2288	KJ999670
TU005	Turuka, Ust-Kut, Ust-Kutskiy Rayon, Irkutsk Oblast, Russia	UWBM#73485, RCF 2295	KJ999671

UL001	Ulaanbaatar, Töv Aymag, Mongolia	UWBM#59995, CSW 5965	KJ999672
UL002	Ulaanbaatar, Töv Aymag, Mongolia	UWBM#60165, DAB 2728	KJ999673
UL003	Ulaanbaatar, Töv Aymag, Mongolia	UWBM#60166, DAB 2729	KJ999674
UL004	Ulaanbaatar, Töv Aymag, Mongolia	UWBM#60167, DAB 2730	KJ999675
UL005	Ulaanbaatar, Töv Aymag	UWBM#60168, DAB 2731	KJ999676

Appendix 3. Summary table of gray jay samples used in analyses. Sources include Burg lab (wild), D Strickland (DS (wild)), DR Norris & D Strickland (DRN/DS (wild)), WH Barnard (WHB (wild)); American Museum of Natural History (AMNH), Burke Museum at the University of Washington (UWBM), Canadian Museum of Nature (CMN), The Field Museum (TFM), Louisiana State University Museum of Natural Sciences (LSU), New Brunswick Museum (NBM), Royal Alberta Museum (RAB), Royal Ontario Museum (ROM), Royal Saskatchewan Museum (RSM) and Smithsonian National Museum of Natural History (USNM).

Sample ID	Location	Source	Band/Museum ID
AKA001	Lake Louise State Recreation Area, Co. AK	UWBM	UWBM#53860 DAB 576
AKA002	Kenai Peninsula Borough Co. AK	UWBM	UWBM# 80319 MNG 035
AKA003	Lake Louise State Recreation Area, Co. AK	UWBM	UWBM#53873 DAB 596
AKA004	Lake Louise State Recreation Area, Co. AK	UWBM	UWBM# 53859 DAB 575
AKA005	Elmendorf Air Force Base, Anchorage, Alaska	USNM	tissue#B13382 voucher#60178
AKA006	Elmendorf Air Force Base, Anchorage, Alaska	USNM	tissue#B13394 voucher#62271
AKA007	Elmendorf Air Force Base, Anchorage, Alaska	USNM	tissue#B13397 voucher#62271
AKA008	Elmendorf Air Force Base, Anchorage, Alaska	USNM	tissue#B13418 voucher#60179
AKE001	11 km E Cantwell in Denali NP, Alaska	LSU	LSUMZ #36851
AKF001	Fort Wainwright Military Reservation, Fairbanks North Star, Alaska	USNM	tissue#B13342 voucer#62267
AKF002	Fort Wainwright Military Reservation, Fairbanks North Star, Alaska	USNM	tissue#B13343 voucher#60171
AKF003	Fort Wainwright Military Reservation, Fairbanks North Star, Alaska	USNM	tissue#B13344 voucher#60171
AKF004	Fort Wainwright Military Reservation, Fairbanks North Star, Alaska	USNM	tossie#B13373 voucher#60171
AKF005	Jones road, AKF	Wild	1713-14083
AKF006	Jones road, AKF	Wild	1713-14084
AKF007	Jones road, AKF	Wild	1713-14085
AKF008	Murphy dome area, AKF	Wild	1713-14086
AKW001	Kenny Lake, AKW	Wild	1713-14066
AKW002	Kenny Lake, AKW	Wild	1713-14067
AKW003	State HWY 10d, AKW	Wild	1713-14068
AKW004	State HWY 10d, AKW	Wild	1713-14069
AKW005	State HWY 10d, AKW	Wild	grja5
AKW006	Old Edgerton HWY, AKW	Wild	1713-14070
AKW007	Old Edgerton HWY, AKW	Wild	1713-14071

AKW008	Old Edgerton HWY, AKW	Wild	1713-14072
AKW009	Old Edgerton HWY, AKW	Wild	1713-14073
AKW010	Old Edgerton HWY, AKW	Wild	1713-14074
AKW011	Old Edgerton HWY, AKW	Wild	1713-14075
AKW012	Old Edgerton HWY, AKW	Wild	1713-14076
AKW013	Old Edgerton HWY, AKW	Wild	1713-14077
AKW014	Old Edgerton HWY, AKW	Wild	1713-14078
AKW015	Richardson HWY x Old Edgerton HWY, AKW	Wild	1713-14079
AKW016	Richardson HWY x Old Edgerton HWY, AKW	Wild	1713-14080
AKW017	Old Edgerton HWY, AKW	Wild	1713-14081
AKW018	Old Edgerton HWY, AKW	Wild	1713-14082
ANTI001	Territory 41, Anticosti Island	DS (Wild)	J-MAS, 1063-03771 (PCA 771)
ANTI002	Territory 41, Anticosti Island	DS (Wild)	B-LAS, 1063-03769 (PCA 769)
ANTI003	Territory 41, Anticosti Island	DS (Wild)	OAS-M, 1063-03770 (PCA 770)
ANTI004	Territory 42, Anticosti Island	DS (Wild)	TAS-V, 1063-03772 (PCA 772)
ANTI005	Territory 42, Anticosti Island	DS (Wild)	NAS-L, 1063-03793 (PCA 793)
ANTI009	Territory 75, Anticosti Island	DS (Wild)	T-RAS, 1063-03765 (PCA 765)
ANTI010	Territory 75, Anticosti Island	DS (Wild)	OAS-B, 1063-03767 (PCA 767)
ANTI011	Territory 75, Anticosti Island	DS (Wild)	JAS-N, 1063-03766 (PCA 766)
ANTI016	Territory 75, Anticosti Island	DS (Wild)	NAS-J,1063-03748 (PCA 748)
ANTI017	Territory 75, Anticosti Island	DS (Wild)	JAS-R, 1063-03752 (PCA 752)
ANTI018	Territory 75, Anticosti Island	DS (Wild)	L-BAS, 1063-03747 (PCA 747)
ANTI021	Anticosti Island	DS (Wild)	PCA 775
CAB001	Buck Lake, AB	Wild	922-97504
CAB002	Buck Lake, AB	Wild	1142-49401
CAB003	Buck Lake, AB	Wild	1142-49402
CAB004	Edson, AB	Wild	1142-49403
CAB005	Edson, AB	Wild	1142-49404
CAB006	Edson, AB	Wild	1142-49405
CAB007	Edson, AB	Wild	991-19823
CAB008	Edson, AB	Wild	1142-44101
CAB009	Edson, AB	Wild	1142-44102
CAB010	Edson, AB	Wild	1142-44103

CAB011	Edson, AB	Wild	1142-49406
CAB012	Hinton, AB	Wild	1142-49407
CAB013	Hinton, AB	Wild	1142-49408
CAB014	Hinton, AB	Wild	1142-49409
CAB015	Hinton, AB	Wild	1142-49410
CAB016	Hinton, AB	Wild	1142-49411
CAB017	Cadomin, AB	Wild	1142-49412
CAB018	Cadomin, AB	Wild	1142-49413
CAB019	Cadomin, AB	Wild	1142-49414
CAB020	Hinton, AB	Wild	1142-49415
CAB021	Hinton, AB	Wild	1142-49416
CAB022	20 miles West, 6 miles North of Sundre, AB	RAM	acc# Z96.23.1, cat#32525
CAB023	3mi. W. of Muskeg River, Improvement District 16, AB	RAM	acc# Z98.10.34, cat#32320
CAB024	3mi. W. of Muskeg River, Improvement District 16, AB	RAM	acc# Z98.10.12, cat#32324
CAB025	3mi. W. of Muskeg River, Improvement District 16, AB	RAM	acc# Z98.10.35, cat#32321
CAB026	3mi. W. of Muskeg River, Improvement District 16, AB	RAM	acc# Z98.10.14, cat#32322
CAB027	3mi. W. of Muskeg River, Improvement District 16, AB	RAM	acc# Z98.10.13, cat#32323
CAB028	10 miles North of Two Lakes, AB	RAM	acc# Z99.10.30, cat#33002
CBC001	Smithers, BC	Wild	1731-05320
CBC002	Smithers, BC	Wild	991-19835
CBC003	Smithers, BC	Wild	1731-05321
CBC004	Smithers, BC	Wild	1731-05322
CBC005	Smithers, BC	Wild	1731-05323
CBC006	Smithers, BC	Wild	991-19836
CBC007	Smithers, BC	Wild	1731-05324
CBC008	Hudson Bay mountain ski hill, Smithers BC	Wild	1142-44122
CBC009	Hudson Bay mountain ski hill, Smithers BC	Wild	1142-44123
CBC010	Hudson Bay mountain ski hill, Smithers BC	Wild	1142-44124
CBC011	Hudson Bay mountain ski hill, Smithers BC	Wild	1142-44125
CBC012	Hudson Bay mountain ski hill, Smithers BC	Wild	1142-44126
CBC013	Hudson Bay mountain ski hill, Smithers BC	Wild	1142-44127
ceOR001	La Pine, Deschutes Co. OR	UWBM	UWBM# 64522 PJG 202
ceOR002	La Pine, Deschutes Co. OR	UWBM	UWBM# 64490 MGS 040

ceOR003	Gilchrist, Klamath Co. OR	UWBM	UWBM# 72662 SMB 52
ceOR004	Gilchrist, Klamath Co. OR	UWBM	UWBM# 72644 EVL 158
ceOR005	Gilchrist, Klamath Co. OR	UWBM	UWBM# 72645 EVL 159
CO001	Chambers Lake campground, CO	Wild	1713-12901
CO002	Chambers Lake Campground, CO	Wild	1713-12902
CO003	RMNP, Lake Irene, CO	Wild	1713-14007
CO004	RMNP, Lake Irene, CO	Wild	1713-14008
CO005	RMNP, Lake Irene, CO	Wild	1713-14009
CO006	RMNP, Lake Irene, CO	Wild	1713-14010
CO007	RMNP, Lake Irene, CO	Wild	1713-14011
CO008	RMNP, Lake Irene, CO	Wild	1713-14012
CO009	2 mi N, 4 mi E Grand Mesa, Mesa Co, CO	TFM	#334295 #AMY87-259
CO010	2 mi N, 4 mi E Grand Mesa, Mesa Co, CO	TFM	#334296 #AMY87-260
CO011	2 mi N, 4 mi E Grand Mesa, Mesa Co, CO	TFM	#334297 #ATP87-251
CO012	2 mi N, 4 mi E Grand Mesa, Mesa Co, CO	TFM	#334298 #ATP87-252
CO013	4.5 mi E, 2 mi N Westcreek, Douglas Co, CO	TFM	#334299 #AMY87-272
CO014	4.5 mi E, 2 mi N Westcreek, Douglas Co, CO	TFM	#334300 #AMY87-273
CO015	Berthoud Pass, Clear Creek, Colorado	UWBM	UWBM#70315 DHB 1997
CO016	Berthoud Pass, Clear Creek, Colorado	UWBM	UWBM#56353 GAV 852
CO017	4 mi west of Cottonwood Pass, Cottonwood Pass Road, Gunnison, CO	UWBM	UWBM#53407 GAV 252
CO018	4 mi west of Cottonwood Pass, Cottonwood Pass Road, Gunnison, CO	UWBM	UWBM#53408 GAV 253
CO019	4 mi west of Cottonwood Pass, Cottonwood Pass Road, Gunnison, CO	UWBM	UWBM#53409 GAV 254
CO020	Frisco, Summit, Colorado	UWBM	UWBM# 70314 DHB 1996
Gasp001	Gaspe Peninsula	DS (Wild)	PCA 681
Gasp002	Gaspe Peninsula	DS (Wild)	PCA 671
Gasp003	Gaspe Peninsula	DS (Wild)	PCA 664
ID001	1 mi. E of USFS Guard Station; Bear Valley, Valley Co., Idaho	LSU	LSUMZ #51588
ID002	Bear Basin, 3 miles NW McCall, ID	Wild	1713-14013
ID003	Bear Basin, 3 miles NW McCall, ID	Wild	1713-14014
Lab001	"Vermouth Road", Building 82, 5-Wing Base, Happy Valley-Goose Bay, Lab	Wild	1603-90509
Lab002	"Vermouth Road", Building 82, 5-Wing Base, Happy Valley-Goose Bay, Lab	Wild	1603-90510
Lab003	30 Montagnais Road, Happy Valley-Goose Bay, Lab	Wild	1603-90511
Lab004	30 Montagnais Road, Happy Valley-Goose Bay, Lab	Wild	1603-90512

Lab005	416 Hamilton River Road, Happy Valley-Goose Bay, Lab	Wild	1603-90513
Lab006	416 Hamilton River Road, Happy Valley-Goose Bay, Lab	Wild	1603-90514
Lab007	Near 'Skidoo Trail' by Main Dock, Rt 520, Happy Valley-Goose Bay, Lab	Wild	D1
Lab008	Near 'Skidoo Trail' by Main Dock, Rt 520, Happy Valley-Goose Bay, Lab	Wild	D2
Lab009	Goose River Lodges Campground, Happy Valley-Goose Bay, Lab	Wild	1603-90515
Lab010	Goose River Lodges Campground, Happy Valley-Goose Bay, Lab	Wild	1603-90516
Lab011	Groves Point Resource Road, Happy Valley-Goose Bay, Lab	Wild	1603-90517
Lab012	Wilburn Bay Campground, Lake Melville, Labrador	Wild	1603-90518
Lab013	Wilburn Bay Campground, Lake Melville, Labrador	Wild	1603-90519
Lab014	Wilburn Bay Rd, by Spruce Meadow Farm, Happy Valley-Goose Bay, Lab	Wild	1603-90520
Lab015	Wilburn Bay Rd, by Spruce Meadow Farm, Happy Valley-Goose Bay, Lab	Wild	1603-90521
Lab016	Wilburn Bay Rd, by Spruce Meadow Farm, Happy Valley-Goose Bay, Lab	Wild	1603-90522
Lab017	Wilburn Bay Rd, by Spruce Meadow Farm, Happy Valley-Goose Bay, Lab	Wild	1603-90523
Lab018	Trans-Labrador Highway, Labrador	Wild	D3
MN001	Greenwood Lake, Cook Co, MN	TFM	#440099 #MDNR-3039
MN002	Hwy 24, near Cook, St Louis Co, MN	TFM	#443519 #MDNR-3253
MN003	Sandstone, Pine, Minnesota	TFM	MDNR-3039 440099
MN004	Sandstone, Pine, Minnesota	Wild	MDNR-3253 443519
NB001	Queens Co., NB	NBM	004531
NB002	Victoria Co., NB	NBM	008135
NB003	Northumberland Co., NB	NBM	009000
NEOR001	Wallowa, OR	AMNH	DOT-15786
NEOR002	Wallowa, OR	AMNH	DOT-15799
NEOR003	Wallowa, OR	AMNH	DOT-15800
NEOR004	Wallowa, OR	AMNH	DOT-15801
NEOR005	Wallowa, OR	AMNH	DOT-15802
NEOR006	Wallowa, OR	AMNH	DOT-15803
NEOR007	Wallowa, OR	AMNH	DOT-15819
NEOR008	Wallowa, OR	AMNH	DOT-15820
NEOR009	Wallowa, OR	AMNH	DOT-15821
NEOR010	Wallowa, OR	AMNH	DOT-15822
NEOR011	Wallowa, OR	AMNH	DOT-15823
NEWA001	Boyds, Ferry Co. WA	UWBM	UWBM# 5331 CSW 3855

NEWA002	Boyds, Ferry Co. WA	UWBM	UWBM# 53318 CSW 3856
NEWA003	Republic, Ferry Co. WA	UWBM	UWBM# 53345 CSW 3884
NEWA004	Republic, Ferry Co. WA	UWBM	UWBM# 53346 CSW 3885
NEWA005	Mazama, Okanogan Co. WA	UWBM	UWBM# 64893 SVD 1263
NEWA006	Mazama, Okanogan Co. WA	UWBM	UWBM# 64894 SVD 1264
NEWA007	Mazama, Okanogan Co. WA	UWBM	UWBM# 64895 SVD 1265
NEWA008	Ione, Pend Oreille Co. WA	UWBM	UWBM# 54054 DAB 492
NEWA009	Cusick, Pend Oreille Co. WA	UWBM	UWBM# 54068 DAB 509
NEWA010	Cusick, Pend Oreille Co. WA	UWBM	UWBM# 54069 DAB 510
NEWA011	Cusick, Pend Oreille Co. WA	UWBM	UWBM# 54070 DAB 511
NEWA012	Tiffany Pass, Conconully, Okanogan, Washington	UWBM	UWBM#58538 PJG 86
NH001	East Inlet Coos Co., NH	WHB (Wild)	912-26714
NH002	East Inlet Coos Co., NH	WHB (Wild)	942-26715
NH003	East Inlet Coos Co., NH	WHB (Wild)	942-26716
NL001	Eagle Mountain (28 km), NL	Wild	grja 1
NL002	Humber Valley Distr., Newfoundland/Labrador	CMN	CNM #77704
NL003	Humber Valley Distr., Newfoundland/Labrador	CMN	CNM #77705
NL004	Humber Valley Distr., Newfoundland/Labrador	CMN	CNM #77706
NL005	The Straits & White Bay Dist., Newfoundland/Labrador	CMN	CNM #77707
NL006	Lomond Campground, Gros Morne NP, NL	Wild	1603-90502
NL007	Lomond Campground, Gros Morne NP, NL	Wild	1603-90503
NL008	Shallow Bay Trail, Gros Morne NP, NL	Wild	1603-90504
NL009	Shallow Bay Trail, Gros Morne NP, NL	Wild	1603-90505
NL010	Shallow Bay Trail, Gros Morne NP, NL	Wild	1603-90506
NL011	Berry Hill Group Camping, Gros Morne NP, NL	Wild	1603-90507
NL012	Berry Hill Group Camping, Gros Morne NP, NL	Wild	1603-90508
NM001	Carson National Forest, ca. 3 mi. by road S Palo Flechado Pass, 2 mi N Hwy. 64 along Forest Service Road 5., Taos Co., NM	LSU	LSUMZ #10197
NM002	Carson National Forest, ca. 3 mi. by road S Palo Flechado Pass, 2 mi N Hwy. 64 along Forest Service Road 5., Taos Co., NM	LSU	LSUMZ #10198
NM003	Pecos Wilderness South Entrance (Trail 254), Santa Fe NF, NM	Wild	1342-17007
NM004	Winsor National Recreational Trail, Santa Fe NF, NM	Wild	1342-17008
NM005	Winsor National Recreational Trail, Santa Fe NF, NM	Wild	1342-17009

NNWBC001	Cassiar Land Distr., British Columbia	CMN	CNM #85902
NNWBC002	Cassiar Land Distr., British Columbia	CMN	CNM #85901
NNWBC003	Cassiar Land Distr., British Columbia	CMN	CNM #85900
NNWBC004	Cassiar Land Distr., British Columbia	CMN	CNM #85903
NNWBC005	Haines Road, BC	RBM	CN #016895
NNWBC006	Haines Road, BC	RBM	CN #016896
NNWBC007	Survey Lake, BC	RBM	CN #016897
NNWBC008	Shini Creek, BC	RBM	CN #017738
NNWBC009	Shini Creek, BC	RBM	CN #017739
NON001	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137215
NON002	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137216
NON003	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137234
NON004	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137217
NON005	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137218
NON006	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137219
NON007	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137220
NON008	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137221
NON009	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137224
NON010	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137225
NON011	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137226
NON012	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137227
NON013	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137228
NON014	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137229
NON015	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137230
NON016	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137231
NON017	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #137232
NON018	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #139839
NON019	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #139929
NON020	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #139930
NON021	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #139956
NON022	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #139957
NON023	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #140015
NON024	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #139847

NON025	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #140131
NON026	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #140132
NON027	near Polar Bear Prov. Park, Kenora District, ON	ROM	cat #140142
NSH001	North Shore (mainland Quebec)	DS (Wild)	PCA 696
NSH002	North Shore (mainland Quebec)	DS (Wild)	PCA 700
NSNB004	Pepper's Property, Musquodoboit Valley, NS	Wild	1603-90524
NSNB005	Three Mile Lake Subdivision, Fox Island, NS	Wild	D1
NSNB006	Ski Bea Eon, East Bay, Cape Breton Co., NS	Wild	D2
NWBC001	Dease Lake, BC	Wild	1142-49417
NWBC002	Dease Lake, BC	Wild	1142-49418
NWBC003	Dease Lake, BC	Wild	1142-49419
NWBC004	Dease Lake, BC	Wild	1142-49420
NWBC005	Dease Lake, BC	Wild	991-19829
NWBC006	Dease Lake, BC	Wild	1731-05314
NWBC007	Dease Lake, BC	Wild	1731-05315
NWBC008	Dease Lake, BC	Wild	991-19830
NWBC009	Dease Lake, BC	Wild	991-19831
NWBC010	Dease Lake, BC	Wild	991-19832
NWBC011	Dease Lake, BC	Wild	991-19833
NWBC012	Dease Lake, BC	Wild	991-19834
NWBC013	Dease Lake, BC	Wild	1731-05316
NWBC014	Dease Lake, BC	Wild	1731-05317
NWBC015	Dease Lake, BC	Wild	1731-05318
NWBC016	Dease Lake, BC	Wild	1731-05319
NWQC001	Territoire de Jamésie, Québec	CMN	CNM #80124
NWQC002	Territoire de Jamésie, Québec	CMN	CNM #80118
NWQC003	Territoire de Jamésie, Québec	CMN	CNM #80119
NWQC004	Territoire de Jamésie, Québec	CMN	CNM #80120
NWQC005	Territoire de Jamésie, Québec	CMN	CNM #80121
NWQC006	Territoire de Jamésie, Québec	CMN	CNM #80122
NWQC007	Territoire de Jamésie, Québec	CMN	CNM #80123
NWQC008	Territoire de Jamésie, Québec	CMN	CNM #80125
NWQC009	Territoire de Jamésie, Québec	CMN	CNM #80126

NWQC010	Territoire de Jamésie, Québec	CMN	CNM #80127
NWQC011	Territoire de Jamésie, Québec	CMN	CNM #80128
SAB001	Syncline Ski Area, AB	Wild	grja1
SAB002	Far picnic area on Akamina Parkway, Waterton, S AB	Wild	grja2
SAB003	9 mi S and 6 mi W of Beaver Mines, Improvement District 5, AB	RAM	acc# Z86.36.27, cat#22741
SAB004	Porcupine Hills, Improvement District 6, AB	RAM	acc# Z89.67.33, cat#26163
SAB005	Castle River, Improvement District 5, AB	RAM	acc# Z91.14.45, cat#29254
SAB006	Lake Cameron, SAB	Wild	1783-03101
SAB007	Lake Cameron, SAB	Wild	1783-03102
SAB008	HWY6, SAB	Wild	1783-03103
SAB009	HWY6, SAB	Wild	1783-03104
SAB010	Cameron Lake Ski Trail, SAB	Wild	1142-44130
SAB011	Little Prairie Picnic Area (Cameron Lake Ski Trail Parking Lot), SAB	Wild	1142-44131
SAB012	McNeally's Picnic Area, Waterton Lakes NP, SAB	Wild	1142-44132
SAB013	McNeally's Picnic Area, Waterton Lakes NP, SAB	Wild	1142-44133
SEBC001	Falkland, B.C.	RSM	catA1152/accn9722 exn 001
SEBC002	Mt Revelstoke, Revelstoke, BC	Wild	1142-44128
SEBC003	Mt Revelstoke, Revelstoke, BC	Wild	1142-44129
SK001	10km N of LaRonge, SK	RSM	catA6302/accn17623 exn 001
SK002	Duck Mountain Provincial Park, SK	RSM	accn15199 exn 007
SK003	Duck Mountain Provincial Park, SK	RSM	accn15199 exn 008
SK004	2 mi SW of Reserve, SK	RSM	accn17749 exn 003
SK005	Treebeard Trail, Prince Albert NP, SK	Wild	1603-90525
SK006	Treebeard Trail, Prince Albert NP, SK	Wild	1603-90526
SK007	Treebeard Trail, Prince Albert NP, SK	Wild	1603-90527
SK008	Narrows Campground, Campsite 82, Prince Albert NP, SK	Wild	1513-25699
SK009	Narrows Campground, Campsite 82, Prince Albert NP, SK	Wild	1513-25700
SK010	Freight Trail, 'D' entrance, Prince Albert NP, SK	Wild	1603-90528
SK011	Crean Lake Road, Prince Albert NP, SK	Wild	1513-25698
SON001	Mile 36 - Algonquin Provincial Park, ON	DRN/DS (Wild)	OOKLBOSR
SON004	Aligator - Algonquin Provincial Park, ON	DRN/DS (Wild)	WOSLTOBR
SON008	Cam Lk Rd - Algonquin Provincial Park, ON	DRN/DS (Wild)	LOBLGOSR
SON011	Sim's Pit - Algonquin Provincial Park, ON	DRN/DS (Wild)	YOYLBOSR

SON012	Davies Bog - Algonquin Provincial Park, ON	DRN/DS (Wild)	BOLLWOSR
SON016	Bat Lake - Algonquin Provincial Park, ON	DRN/DS (Wild)	YOSLYOTR
SON019	Crossroads - Algonquin Provincial Park, ON	DRN/DS (Wild)	BOSLPOTR
SON020	Crossroads - Algonquin Provincial Park, ON	DRN/DS (Wild)	GOSLBOLR
SON022	Arohowa East (N) - Algonquin Provincial Park, ON	DRN/DS (Wild)	POLLWOSR
SON025	Big Pines (N) - Algonquin Provincial Park, ON	DRN/DS (Wild)	KOLLTOSR
SON027	Opeongo Br - Algonquin Provincial Park, ON	DRN/DS (Wild)	BOPLLOSR
SON030	TDS North - Algonquin Provincial Park, ON	DRN/DS (Wild)	KOSLBOTR
SON036	Rock Yard (N) - Algonquin Provincial Park, ON	DRN/DS (Wild)	TOSLYOPR
SON040	Hermit Creek (N) - Algonquin Provincial Park, ON	DRN/DS (Wild)	YOLLTOSR
SON044	Wolf Howl (N) - Algonquin Provincial Park, ON	DRN/DS (Wild)	POWLKOSR
SON047	Sunday Creek (N) - Algonquin Provincial Park, ON	DRN/DS (Wild)	KOPLOOSR
SON049	North Bog (N) - Algonquin Provincial Park, ON	DRN/DS (Wild)	LOSLBOPR 1513-19054
SOR001	Chiloquin, Klamath Co. OR	UWBM	UWBM# 64584 EVL 239
SWCO001	"Feeling Corner", Durango Mtn Resort, San Juan NF, CO	Wild	1272-31664
SWCO002	"Feeling Corner", Durango Mtn Resort, San Juan NF, CO	Wild	1272-31665
SWCO003	Mile 4 Dispersed Campsite, San Juan NF, CO	Wild	1272-31666
SWCO004	Mile 4 Dispersed Campsite, San Juan NF, CO	Wild	1272-31667
SWCO005	FR578/581 Junction, San Juan NF, CO	Wild	1272-31668
SWCO006	FR578/581 Junction, San Juan NF, CO	Wild	1272-31669
SWCO007	FR578/581 Junction, San Juan NF, CO	Wild	1272-31670
SWCO008	Trailhead, Improved/Unimproved Transition, FR 579, San Juan NF, CO	Wild	1272-31671
SWCO009	"Top of the World", FR550, San Juan NF, CO	Wild	1272-31672
SWCO010	"Top of the World", FR550, San Juan NF, CO	Wild	1272-31673
SWCO011	Seventh Heaven Picnic Tables, Durango Mtn Resort, San Juan NF, CO	Wild	1272-31674
SWCO012	Mile 4 Dispersed Campsite, San Juan NF, CO	Wild	1272-31675
UT001	Bridger Lake Cmpgrnd, UT	Wild	1713-14001
UT002	Bridger Lake Cmpgrnd, UT	Wild	1713-14002
UT003	Bridger Lake Cmpgrnd, UT	Wild	1713-14003
UT004	Bridger Lake Cmpgrnd, UT	Wild	1713-14004
UT005	Bridger Lake Cmpgrnd, UT	Wild	1713-14005
UT006	Bridger Lake Cmpgrnd, UT	Wild	1713-14006
UT007	Vernal, Uintah Co. UT	UWBM	UWBM# 70817 JMB 1692

UT008	Vernal, Uintah Co. UT	UWBM	UWBM# 70816 JMB 1691
UT009	Vernal, Uintah Co. UT	UWBM	UWBM# 79117 SVD 1249
UT010	Uinta Mts., 14 mi N, 6 mi W Whiterocks, Duchesne Co, UT	TFM	#334301 #AMY87-254
UT011	Uinta Mts., 14 mi N, 6 mi W Whiterocks, Duchesne Co, UT	TFM	#334302 #ATP87-245
UT012	Uinta Mts., 14 mi N, 6 mi W Whiterocks, Duchesne Co, UT	TFM	#334303 #ATP87-246
VI001	Raven Lodge, BC	Wild	1142-44104
VI002	Raven Lodge, BC	Wild	1142-44105
VI003	Raven Lodge, BC	Wild	1142-44106
VI004	Raven Lodge, BC	Wild	1142-44107
VI005	Raven Lodge, BC	Wild	1142-44108
VI006	Raven Lodge, BC	Wild	1142-44109
VI007	Raven Lodge, BC	Wild	1142-44110
VI008	Raven Lodge, BC	Wild	1142-44111
VI009	Mt Washington Ski Hill, BC	Wild	1142-44112
VI010	Mt Washington Ski Hill, BC	Wild	1142-44113
VI011	Mt Washington Ski Hill, BC	Wild	1142-44114
VI012	Mt Washington Ski Hill, BC	Wild	1142-44115
VI013	Mt Washington Ski Hill, BC	Wild	1142-44116
VI014	Mt Washington Ski Hill, BC	Wild	1142-44117
VI015	Mt Washington Ski Hill, BC	Wild	1142-44118
VI016	Mt Washington Ski Hill, BC	Wild	1142-44119
VI017	Mt Washington Ski Hill, BC	Wild	1142-44120
VI018	Mt Washington Ski Hill, BC	Wild	1142-44121
VT001	Victory Bo, Essex Co., VT	WHB (Wild)	1453-96909
VT002	Victory Bo, Essex Co., VT	WHB (Wild)	942-26089
VT003	Victory Bo, Essex Co., VT	WHB (Wild)	942-26099
VT004	Victory Bo, Essex Co., VT	WHB (Wild)	942-26111
VT005	Victory Bo, Essex Co., VT	WHB (Wild)	942-26113
VT006	Victory Bo, Essex Co., VT	WHB (Wild)	942-26129
VT007	Victory Bo, Essex Co., VT	WHB (Wild)	942-26135
VT008	Victory Bo, Essex Co., VT	WHB (Wild)	942-26153
VT009	Victory Bo, Essex Co., VT	WHB (Wild)	942-26161
VT010	Victory Bo, Essex Co., VT	WHB (Wild)	942-26175

VT011	Victory Bo, Essex Co., VT	WHB (Wild)	942-26212
VT012	Victory Bo, Essex Co., VT	WHB (Wild)	942-26213
VT013	Victory Bo, Essex Co., VT	WHB (Wild)	942-26214
VT014	Victory Bo, Essex Co., VT	WHB (Wild)	942-26215
VT015	Victory Bo, Essex Co., VT	WHB (Wild)	942-26216
VT016	Victory Bo, Essex Co., VT	WHB (Wild)	942-26217
VT017	Victory Bo, Essex Co., VT	WHB (Wild)	942-26218
VT018	Victory Bo, Essex Co., VT	WHB (Wild)	942-26219
VT019	Victory Bo, Essex Co., VT	WHB (Wild)	942-26221
VT020	Victory Bo, Essex Co., VT	WHB (Wild)	942-26222
VT021	Victory Bo, Essex Co., VT	WHB (Wild)	942-26223
VT022	Victory Bo, Essex Co., VT	WHB (Wild)	942-26224
VT023	Victory Bo, Essex Co., VT	WHB (Wild)	942-26225
VT024	Victory Bo, Essex Co., VT	WHB (Wild)	942-26226
VT025	Victory Bo, Essex Co., VT	WHB (Wild)	942-26227
VT026	Victory Bo, Essex Co., VT	WHB (Wild)	942-26228
VT027	Victory Bo, Essex Co., VT	WHB (Wild)	942-26229
VT028	Victory Bo, Essex Co., VT	WHB (Wild)	942-26230
VT029	Victory Bo, Essex Co., VT	WHB (Wild)	942-26232
VT030	Victory Bo, Essex Co., VT	WHB (Wild)	942-26710
VT031	Victory Bo, Essex Co., VT	WHB (Wild)	942-26711
VT032	Victory Bo, Essex Co., VT	WHB (Wild)	942-26712
VT033	Victory Bo, Essex Co., VT	WHB (Wild)	942-26713
VT034	Victory Bo, Essex Co., VT	WHB (Wild)	942-26717
VT035	Victory Bo, Essex Co., VT	WHB (Wild)	942-26718
VT036	Victory Bo, Essex Co., VT	WHB (Wild)	942-26720
VT037	Victory Bo, Essex Co., VT	WHB (Wild)	942-26721
VT038	Ferd. Bog Essex Co., VT	WHB (Wild)	942-26234
VT039	Ferd. Bog Essex Co., VT	WHB (Wild)	942-26235
VT040	Moose bog Essex Co., VT	WHB (Wild)	942-26220
VT041	Moose bog Essex Co., VT	WHB (Wild)	942-26238
WA001	MT. Rainier Paradise, WA	Wild	1713-14026
WA002	Mt. Rainier Narada Falls, WA	Wild	1713-14027

WA003	Mt. Rainier Narada Falls, WA	Wild	1713-14028
WA004	Mt. Rainier Narada Falls, WA	Wild	1713-14029
WA005	Mt. Rainier Narada Falls, WA	Wild	1713-14030
WA006	Mt. Rainier Narada Falls, WA	Wild	1713-14031
WA007	Mt. Rainier Paradise, WA	Wild	1713-14032
WA008	Mt. Rainier Paradise, WA	Wild	1713-14033
WA009	Mt. Rainier Paradise, WA	Wild	1713-14034
WA010	Cougar Rock Campground, WA	Wild	852-44053
WA011	Mt. Rainier Paradise, WA	Wild	1713-14035
WA012	Mt. Rainier Paradise, WA	Wild	1713-14036
WA013	Mt Rainier Narada falls	Wild	1713-14037
WA014	Mt Rainier Narada falls	Wild	1713-14038
WA015	Mt Rainier Narada falls	Wild	1713-14039
WA016	Mt Rainier Narada falls	Wild	1713-14040
WA017	Mt. Rainier Stevens Canyon Road, WA	Wild	1713-14041
WA018	Mt. Rainier Stevens Canyon Road, WA	Wild	1713-14042
WA019	Mt. Rainier Stevens Canyon Road, WA	Wild	1713-14043
WA020	Mt. Rainier Paradise, WA	Wild	1713-14044
WA021	Mt Rainier Paradise, WA	Wild	1713-14045
WA022	Mt Rainier Paradise, WA	Wild	1713-14046
WA023	Port Angeles, Clallam, Washington	UWBM	UWBM#79173 PLG 68
WA024	Uncas, Clallam, Washington	UWBM	UWBM#72761 PLG 63
WA025	Hurricane Ridge Road, Clallam, Washington	UWBM	UWBM#85991 DRF 387
WA026	T19N R15E Sec. 31,Cle Elum,Kittitas,Washington	UWBM	UWBM#79592 EVL 699
WA027	South Fork Taneum Creek; T19N R15E Sec. 31, Cle Elum, Kittitas, WA	UWBM	UWBM#79811 GKD 279
WA028	Experimental mammal trapping grid near town, Cle Elum, Kittitas, WA	UWBM	UWBM#79824 JMF024
WA029	Experimental mammal trapping grid near town, Cle Elum, Kittitas, WA	UWBM	UWBM#79858 WCW 019
WA030	Experimental mammal trapping grid near town, Cle Elum, Kittitas, WA	UWBM	UWBM#79828 JMG 021
WA031	Experimental mammal trapping grid near town, Cle Elum, Kittitas, WA	UWBM	UWBM#79864 WLM 018
WA032	Snoqualmie Pass, Kittitas, Washington	UWBM	UWBM#44800 JMB 1233
WA033	Snoqualmie Pass, Kittitas, Washington	UWBM	UWBM#44801 JMB 1234
WA034	Snoqualmie Pass, Kittitas, Washington	UWBM	UWBM#44802 JMB 1235
WA035	Snoqualmie Pass, Kittitas, Washington	UWBM	UWBM#44803 JMB 1236

WA036	Snoqualmie Pass, Kittitas, Washington	UWBM	UWBM#44804 JMB 1237
WA037	Snoqualmie Pass, Kittitas, Washington	UWBM	UWBM#44805 JMB 1238
WA038	Snoqualmie Pass, Kittitas, Washington	UWBM	UWBM#44806 JMB 1239
WA039	Raymond, Pacific, Washington	UWBM	UWBM#69675 PSM 20322
WA040	Raymond, Pacific, Washington	UWBM	UWBM#69676 PSM 20323
WA041	Raymond, Pacific, Washington	UWBM	UWBM#69677 PSM 20324
WA042	Raymond, Pacific, Washington	UWBM	UWBM#69678 PSM 20325
WA043	Raymond, Pacific, Washington	UWBM	UWBM#69679 PSM 20327
WA044	Raymond, Pacific, Washington	UWBM	UWBM#69680 PSM 20328
WA045	Mount Baker, Whatcom, Washington	UWBM	UWBM#64889 SVD 1220
WA046	Mount Baker, Whatcom, Washington	UWBM	UWBM#64890 SVD 1221
WA047	Mount Baker, Whatcom, Washington	UWBM	UWBM#64891 SVD 1222
WA048	Mount Baker summit, Whatcom, Washington	UWBM	UWBM#74553 EVL 392

Appendix 4. Protocol and permit information for scientific samples.

All animals captured in the field were handled following animal welfare protocols (#0614 and #1028) approved by the University of Lethbridge Animal Welfare Committee using guidelines set by the Canadian Council on Animal Care (CCAC). Banding in Canada was performed under Canadian Wildlife Service (CWS) banding permit #10425W (2007) and #10804 (2008 onward) and in the United States under US Fish and Wildlife Service banding permit #23522. Blood, feather, and tissue samples were imported from the US to Canada under US Export Permit MB072258-0 and MB072258-1.

In Canada, blood sampling was conducted under the following permits: CWS Permit SC2529 and SC2699 (Atlantic Canada regional), 09-SK-SC006, 11-AB-SCO010, CWS07-A003, and CWS008-S007 (Prairie and Northern regional), and 59-08-0228, 09-0249, BC-10-0011, and BC-12-0021 (Pacific regional); Parks Canada Permits FNP-2010-5033 (Gros Morne National Park), MRGNP-2008-1500 (Mount Revelstoke, Prince Albert, and Waterton Lakes National Parks), and JNP-2011-7993 (Mount Revelstoke, Jasper, and Waterton Lakes National Parks); Newfoundland Labrador Provincial Scientific Research Permit IW2009-24a; Newfoundland Labrador Parks and National Areas Division Scientific Research Permit (no permit number provided); Nova Scotia Wildlife Division Provincial Scientific Permit (no permit number provided); New Brunswick Fish and Wildlife Scientific and Export Permit SP10-001; Saskatchewan Ministry of Environment Scientific Research Permit 10FW041; Alberta Fish and Wildlife Research Permits 27953, 29198, 34437, and 35275 and Collection Permits 27929, 28052CN, 35338, and 35270; and British Columbia Provincial Wildlife Permits VI09-51090 and VI12-76938.

In the US, blood sampling was conducted under the following permits: US Department of Agriculture Forest Service Permits in San Bernardino National Forest (special letter of permission; no permit number), San Juan National Forest (COL261), and Santa Fe National Forest (ESP210901); US Department of the Interior National Park Service Research and Collecting Permits in Crater Lake (CRLA-2009-SCI-001) and Mount Rainier (MORA-2009-SCI-004) National Parks and Wrangler-St. Elias National Park and Preserve (WRST-2010-SCI-004); California State Department of Fish and Game Scientific Collection Permit D-0003961061-6; Colorado Department of Natural Resources Scientific Collection Licenses 09TRb1019 and 11TRb2037; Idaho Wildlife Collection/ Banding/ Possession Permit 100216; Idaho forestry permit (no permit number); Montana Department of Fish, Wildlife and Parks Scientific Collector's Permit/Bird Banding Permits 2008-011 and 2009-030; New Mexico Department of Wildlife Scientific Collection Permit 3525; Oregon State Parks Permit 004-09; Oregon State Scientific Taking Permit 057-09); Utah State Collection Permit (no permit number); and Washington State Collection Permit 09-077.

All banded birds were annually reported to the Environment Canada Bird Banding Office and US Geological Survey Bird Banding Laboratory and field reports filed with the respective agencies outlined above.

Appendix 5. Geographic distribution of shared gray jay haplotypes.

	Boreal																							IMW					CO-NM		Pacific								
Hap	AKA	AKF	AKW	AKD	NWBC	NNWBC	CBC	CAB	SK	MN	NON	NWQC	SON	Gasp	NSH	ANTI	NSNB	VT	NH	Lab	**NL**	**UT**	SAB	NEWA	NEOR	ID	SEBC	CO	SWCO	NM	WA	coWA	NWWA	WAOP	ceOR	SOR	**VanIsl**
A	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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SS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
TT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
VV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
WW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-
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ZZ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-

AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	1	1	-	-
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AD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
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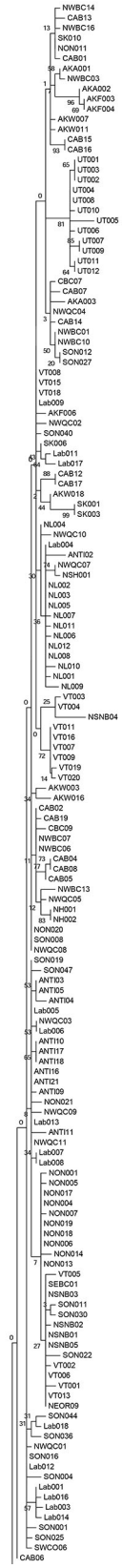
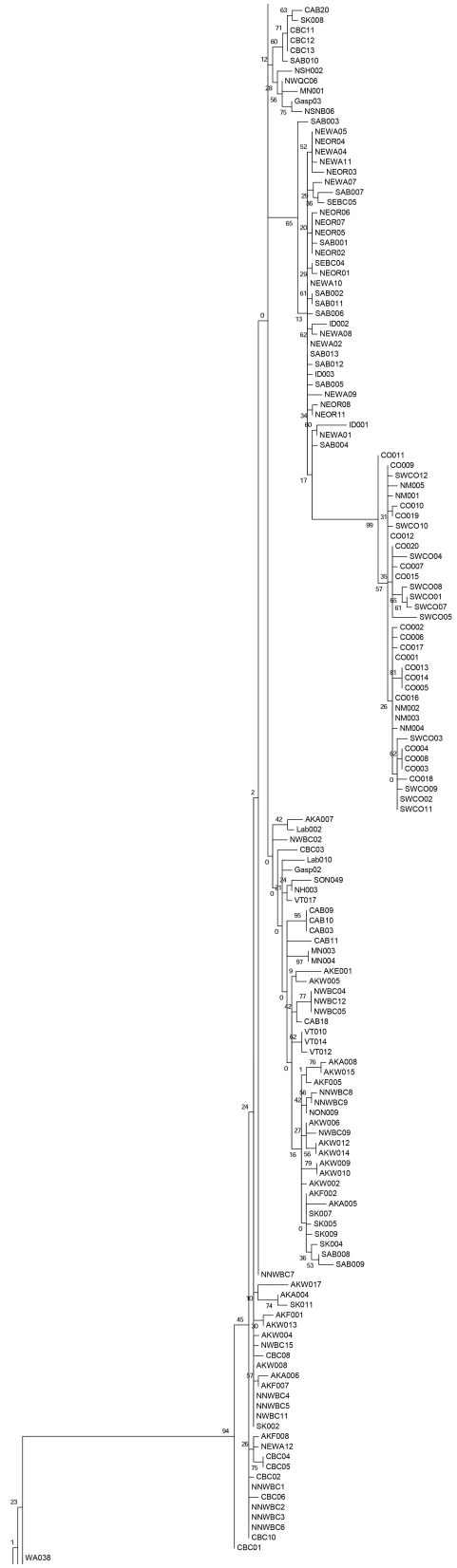
Unique	8	5	10	1	7	3	7	10	5	1	6	8	11	2	2	4	3	9	1	9	7	3	12	7	3	2	2	8	11	3	11	2	2	1	1	1	7
Total	8	8	17	1	15	9	13	20	11	3	14	11	16	2	2	11	6	20	3	17	12	12	13	11	10	3	3	20	12	5	33	6	4	3	5	1	16

Appendix 6. Mitochondrial DNA pairwise Φ_{ST} values and Benjamini-Hochberg corrected p-values for gray jays. Φ_{ST} values are below diagonal, corrected p-values above.

	AKA	AKF	AKW	NWBC	NNWBC	CBC	CAB	SK	NON	NWQC	SON	ANTI	NSNB	VT	Lab	NL	UT	SAB	NEWA	NEOR	CO	SWCO	NM	WA	coWA	NWWA	ceOR	VanIsl
AKA	*	0.941	0.714	0.313	0.050	0.106	0.073	0.493	0.001	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.001	0.000
AKF	-0.064	*	0.420	0.156	0.090	0.108	0.022	0.442	0.000	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.003	0.003	0.000
AKW	-0.026	-0.003	*	0.156	0.012	0.000	0.014	0.542	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NWBC	0.010	0.041	0.025	*	0.003	0.012	0.160	0.184	0.000	0.001	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NNWBC	0.101	0.100	0.121	0.164	*	0.138	0.000	0.035	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.002	0.000
CBC	0.048	0.052	0.115	0.091	0.047	*	0.000	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CAB	0.052	0.080	0.074	0.023	0.255	0.143	*	0.020	0.000	0.011	0.000	0.000	0.002	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SK	-0.008	-0.001	-0.010	0.029	0.116	0.068	0.074	*	0.000	0.003	0.000	0.000	0.004	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NON	0.400	0.386	0.347	0.298	0.592	0.393	0.227	0.299	*	0.045	0.013	0.000	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
NWQC	0.225	0.229	0.214	0.120	0.432	0.241	0.081	0.168	0.071	*	0.581	0.003	0.045	0.083	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
SON	0.262	0.281	0.250	0.148	0.443	0.269	0.125	0.196	0.103	-0.010	*	0.000	0.046	0.018	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ANTI	0.451	0.469	0.401	0.339	0.630	0.424	0.294	0.356	0.191	0.153	0.155	*	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.000
NSNB	0.286	0.311	0.298	0.247	0.534	0.330	0.189	0.250	0.110	0.104	0.107	0.201	*	0.042	0.001	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.003	0.000	0.000	0.006	0.000	0.000
VT	0.162	0.183	0.152	0.093	0.354	0.219	0.070	0.145	0.192	0.049	0.075	0.252	0.148	*	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
LAB	0.278	0.291	0.264	0.164	0.445	0.290	0.140	0.208	0.186	0.040	0.049	0.191	0.206	0.111	*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NL	0.581	0.590	0.514	0.492	0.786	0.582	0.376	0.510	0.626	0.428	0.498	0.711	0.638	0.438	0.460	*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000
UT	0.585	0.591	0.547	0.555	0.756	0.604	0.477	0.530	0.708	0.610	0.615	0.765	0.676	0.580	0.598	0.791	*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.000
SAB	0.321	0.350	0.368	0.400	0.470	0.381	0.389	0.342	0.601	0.506	0.530	0.617	0.531	0.493	0.529	0.719	0.704	*	0.423	0.135	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEWA	0.297	0.312	0.374	0.385	0.413	0.352	0.378	0.330	0.522	0.436	0.472	0.526	0.419	0.455	0.469	0.627	0.630	-0.006	*	0.314	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000
NEOR	0.460	0.495	0.492	0.518	0.628	0.503	0.478	0.477	0.694	0.602	0.614	0.711	0.622	0.579	0.609	0.804	0.785	0.048	0.001	*	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000
CO	0.803	0.817	0.791	0.809	0.878	0.812	0.772	0.791	0.878	0.847	0.845	0.894	0.852	0.819	0.836	0.915	0.908	0.762	0.702	0.806	*	0.006	0.471	0.000	0.000	0.000	0.000	0.000
SWCO	0.630	0.647	0.663	0.677	0.739	0.676	0.651	0.635	0.755	0.704	0.724	0.771	0.675	0.704	0.715	0.806	0.808	0.600	0.533	0.639	0.085	*	0.363	0.000	0.000	0.002	0.000	0.000
NM	0.733	0.756	0.741	0.763	0.871	0.761	0.718	0.721	0.876	0.817	0.821	0.898	0.818	0.789	0.807	0.937	0.909	0.704	0.592	0.772	-0.008	0.008	*	0.000	0.003	0.008	0.008	0.000
WA	0.905	0.904	0.896	0.902	0.921	0.898	0.891	0.895	0.925	0.915	0.915	0.929	0.913	0.907	0.907	0.939	0.934	0.902	0.864	0.919	0.941	0.911	0.937	*	0.072	0.005	0.065	0.000
coWA	0.885	0.890	0.877	0.887	0.939	0.880	0.866	0.867	0.943	0.915	0.915	0.952	0.920	0.901	0.903	0.971	0.953	0.885	0.802	0.920	0.956	0.889	0.962	0.056	*	0.005	0.042	0.000
NWWA	0.856	0.862	0.857	0.868	0.922	0.858	0.848	0.840	0.932	0.897	0.901	0.940	0.894	0.887	0.887	0.964	0.943	0.866	0.770	0.904	0.950	0.871	0.948	0.290	0.525	*	0.012	0.000
CEOR	0.870	0.874	0.868	0.878	0.928	0.869	0.858	0.854	0.936	0.905	0.908	0.943	0.903	0.894	0.894	0.964	0.946	0.875	0.787	0.910	0.952	0.880	0.951	0.077	0.165	0.484	*	0.000
VanIsl	0.912	0.915	0.898	0.907	0.942	0.903	0.889	0.897	0.944	0.928	0.925	0.950	0.930	0.912	0.916	0.963	0.953	0.910	0.860	0.934	0.956	0.914	0.959	0.541	0.703	0.590	0.706	*
	AKA	AKF	AKW	NWBC	NNWBC	CBC	CAB	SK	NON	NWQC	SON	ANTI	NSNB	VT	Lab	NL	UT	SAB	NEWA	NEOR	CO	SWCO	NM	WA	coWA	NWWA	ceOR	VanIsl

Appendix 7. Maximum likelihood phylogenetic tree of sampled gray jays rooted with Siberian jay sample.

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NEWA11
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NEWA07
SAB007
SEBC05
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NEOR07
NEOR05
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CAB11
NN003
MN004
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CBC10
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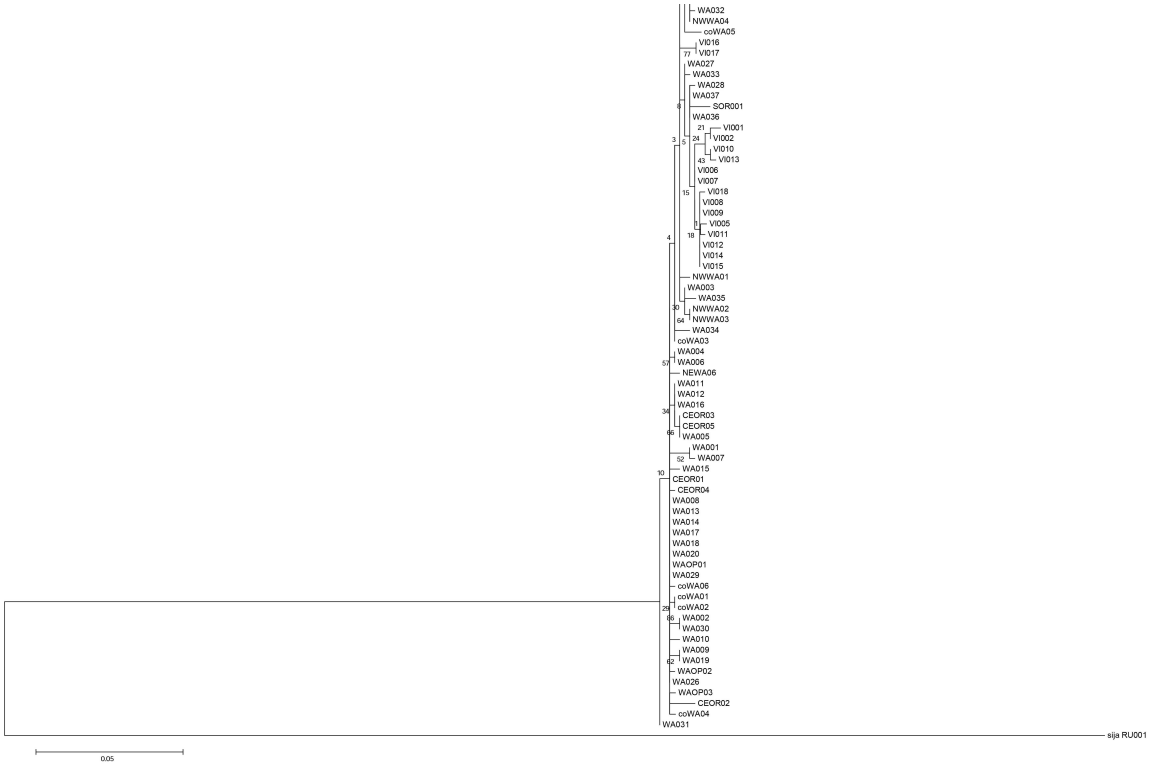
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VI017
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24
VI002
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VI006
VI007
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VI018
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VI009
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coWA02
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WA030
WA010
WA009
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WA019
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WA026
WAOP03
CEOR02
coWA04
WA031
sija RU001
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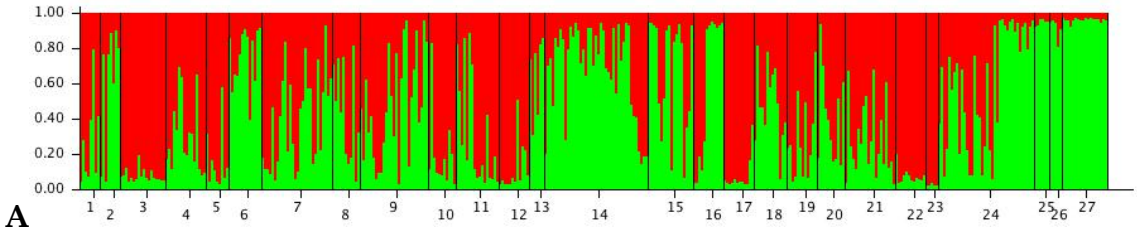
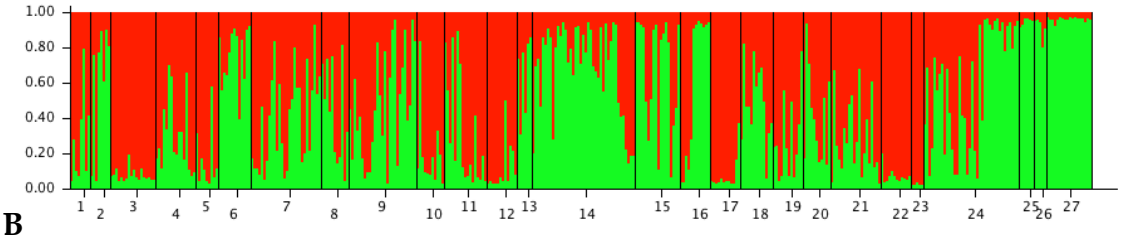
Appendix 8. F_{ST} values of differentiation and Benjamini-Hochberg corrected p-values for seven microsatellite loci in gray jay. F_{ST} values are below diagonal, corrected p-values above.

	AKA	AKF	AKW	NWBC	NNWBC	CBC	CAB	SK	NON	NWQC	SON	ANTI	NSNB	VT	Lab	NL	UT	SAB	NEWA	NEOR	CO	SWCO	NM	WA	coWA	ceOR	VanIsl
AKA	*	0.011	0.000	0.030	0.005	0.001	0.017	0.442	0.016	0.063	0.000	0.000	0.152	0.004	0.006	0.000	0.000	0.028	0.000	0.001	0.002	0.000	0.001	0.005	0.000	0.000	0.000
AKF	**0.121**	*	0.000	0.029	0.001	0.166	0.001	0.023	0.231	0.000	0.000	0.000	0.146	0.007	0.097	0.002	0.001	0.008	0.001	0.001	0.019	0.000	0.001	0.000	0.000	0.003	0.000
AKW	**0.132**	**0.166**	*	0.004	0.616	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
NWBC	**0.063**	**0.076**	**0.076**	*	0.030	0.013	0.201	0.004	0.252	0.003	0.000	0.000	0.243	0.008	0.023	0.000	0.000	0.404	0.000	0.012	0.097	0.000	0.000	0.001	0.000	0.000	0.000
NNWBC	**0.122**	**0.163**	0.012	**0.060**	*	0.001	0.001	0.017	0.028	0.015	0.001	0.000	0.000	0.000	0.002	0.000	0.024	0.042	0.000	0.000	0.015	0.000	0.001	0.000	0.000	0.000	0.000
CBC	**0.125**	0.043	**0.140**	**0.059**	**0.148**	*	0.003	0.001	0.053	0.000	0.000	0.000	0.006	0.001	0.037	0.000	0.000	0.003	0.000	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000
CAB	**0.072**	**0.105**	**0.093**	0.020	**0.097**	**0.068**	*	0.002	0.041	0.001	0.000	0.000	0.326	0.035	0.048	0.000	0.000	0.207	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.001	0.000
SK	0.013	**0.078**	**0.101**	**0.066**	**0.073**	**0.096**	**0.086**	*	0.039	0.005	0.000	0.000	0.014	0.003	0.003	0.001	0.000	0.006	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
NON	**0.063**	0.035	**0.065**	0.017	**0.056**	0.037	**0.028**	**0.042**	*	0.086	0.000	0.001	0.085	0.006	0.061	0.002	0.000	0.037	0.017	0.003	0.038	0.000	0.000	0.000	0.000	0.000	0.000
NWQC	0.066	**0.146**	**0.104**	**0.076**	**0.105**	**0.125**	**0.086**	**0.092**	0.037	*	0.004	0.000	0.003	0.001	0.002	0.001	0.001	0.010	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000
SON	**0.144**	**0.161**	**0.152**	**0.118**	**0.162**	**0.171**	**0.121**	**0.148**	**0.096**	**0.104**	*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.000
ANTI	**0.193**	**0.234**	**0.089**	**0.156**	**0.140**	**0.213**	**0.178**	**0.181**	**0.111**	**0.129**	**0.195**	*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NSNB	0.068	0.089	**0.183**	0.045	**0.184**	**0.126**	0.032	**0.116**	0.063	**0.144**	**0.168**	**0.289**	*	0.176	0.327	0.005	0.001	0.514	0.002	0.021	0.010	0.000	0.004	0.150	0.002	0.020	0.000
VT	**0.074**	**0.080**	**0.111**	**0.042**	**0.109**	**0.063**	**0.024**	**0.068**	**0.031**	**0.093**	**0.136**	**0.189**	0.038	*	0.079	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lab	**0.083**	0.054	**0.111**	**0.045**	**0.113**	**0.046**	0.032	**0.069**	0.028	**0.093**	**0.126**	**0.186**	0.036	0.023	*	0.000	0.000	0.072	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NL	**0.122**	**0.118**	**0.160**	**0.107**	**0.144**	**0.107**	**0.109**	**0.092**	**0.077**	**0.115**	**0.130**	**0.274**	**0.136**	**0.103**	**0.081**	*	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
UT	**0.172**	**0.204**	**0.103**	**0.137**	**0.084**	**0.234**	**0.172**	**0.151**	**0.109**	**0.122**	**0.198**	**0.143**	**0.248**	**0.184**	**0.174**	**0.217**	*	0.002	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.001	0.000
SAB	**0.084**	**0.113**	**0.112**	0.018	**0.089**	**0.110**	0.029	**0.092**	**0.049**	**0.108**	**0.131**	**0.257**	0.030	**0.047**	0.048	**0.095**	**0.167**	*	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.000
NEWA	**0.098**	**0.116**	**0.150**	**0.073**	**0.141**	**0.090**	**0.097**	**0.102**	**0.050**	**0.082**	**0.187**	**0.129**	**0.132**	**0.122**	**0.109**	**0.170**	**0.136**	**0.149**	*	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.000
NEOR	**0.106**	**0.143**	**0.160**	**0.067**	**0.137**	**0.093**	**0.094**	**0.097**	**0.074**	**0.140**	**0.207**	**0.249**	**0.118**	**0.121**	**0.121**	**0.138**	**0.239**	**0.126**	**0.059**	*	0.003	0.000	0.000	0.000	0.001	0.000	0.000
CO	**0.089**	**0.077**	**0.059**	0.030	**0.068**	**0.066**	**0.069**	**0.082**	**0.032**	**0.089**	**0.100**	**0.085**	**0.106**	**0.091**	**0.082**	**0.135**	**0.117**	**0.106**	**0.049**	**0.078**	*	0.001	0.000	0.000	0.000	0.001	0.000
SWCO	**0.229**	**0.277**	**0.210**	**0.174**	**0.248**	**0.203**	**0.221**	**0.237**	**0.171**	**0.218**	**0.227**	**0.194**	**0.310**	**0.241**	**0.234**	**0.262**	**0.228**	**0.286**	**0.136**	**0.206**	**0.114**	*	0.000	0.000	0.000	0.000	0.000
NM	**0.325**	**0.322**	**0.260**	**0.284**	**0.293**	**0.358**	**0.307**	**0.297**	**0.244**	**0.308**	**0.315**	**0.316**	**0.395**	**0.302**	**0.298**	**0.361**	**0.156**	**0.372**	**0.278**	**0.384**	**0.212**	**0.328**	*	0.000	0.001	0.005	0.000
WA	**0.081**	**0.133**	**0.141**	**0.052**	**0.153**	**0.091**	**0.053**	**0.111**	**0.079**	**0.125**	**0.168**	**0.195**	0.047	**0.072**	**0.070**	**0.144**	**0.181**	**0.080**	**0.083**	**0.100**	**0.089**	**0.175**	**0.283**	*	0.000	0.000	0.000
coWA	**0.321**	**0.299**	**0.421**	**0.302**	**0.428**	**0.290**	**0.287**	**0.324**	**0.286**	**0.398**	**0.428**	**0.460**	**0.272**	**0.286**	**0.284**	**0.380**	**0.461**	**0.357**	**0.274**	**0.302**	**0.283**	**0.473**	**0.590**	**0.193**	*	0.004	0.005
ceOR	**0.172**	**0.234**	**0.288**	**0.144**	**0.267**	**0.215**	**0.142**	**0.215**	**0.169**	**0.234**	**0.250**	**0.346**	**0.118**	**0.184**	**0.187**	**0.257**	**0.314**	**0.180**	**0.160**	**0.182**	**0.149**	**0.347**	**0.447**	**0.118**	**0.278**	*	0.001
VanIsl	**0.348**	**0.325**	**0.404**	**0.310**	**0.399**	**0.262**	**0.294**	**0.327**	**0.293**	**0.383**	**0.403**	**0.450**	**0.333**	**0.301**	**0.292**	**0.338**	**0.459**	**0.365**	**0.299**	**0.258**	**0.284**	**0.448**	**0.560**	**0.218**	**0.187**	**0.297**	*

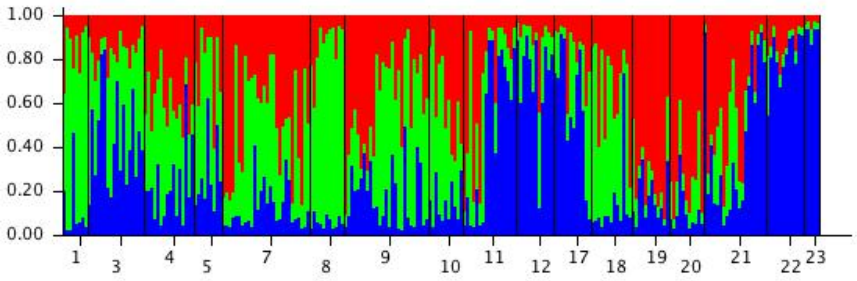
Appendix 9. Plots of gray jay STRUCTURE clustering analyses.



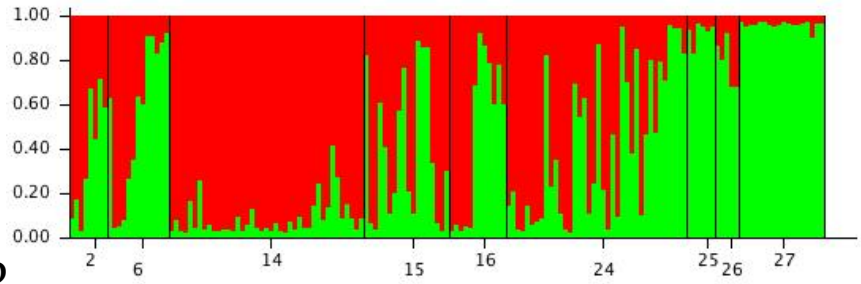
A



B



C



D

Figure a) and b) Two different iterations of overall clustering results for 27 gray jay populations for K=2. Note population admixture as referenced in Table 4.8.
Figure c) Example plot of clustering results for 18 populations identified as best suited to Group 1 for K=3.
Figure d) Example plot of clustering results for 9 populations identified as best suited to Group 2 for K=2.