POPULATION STRUCTURE AND LANDSCAPE GENETICS OF WESTERN RUFFED GROUSE

ASHLEY MARIE JENSEN
BSc Wildlife Biology, University of Montana (USA), 2009

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ASHLEY MARIE JENSEN

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Dr. T. Burg Thesis Supervisor	Associate Professor	Ph.D.
Dr. A. Iwaniuk Thesis Supervisor	Associate Professor	Ph.D.
Dr. T. Russell Thesis Examination Committee Member	Associate Professor	Ph.D.
Dr. J. Leca Thesis Examination Committee Member	Assistant Professor	Ph.D.
Dr. D. Manzer External Examiner Alberta Conservation Association	Wildlife Program Manager	Ph.D.
Dr. I. Kovalchuk Chair, Thesis Examination Committee	Professor	Ph.D.

DEDICATION

For my mom, Barb, the closest friend anyone could wish for, and the most incredible woman I have ever met.

ABSTRACT

This study was the first to examine the genetic structure of a widespread game bird, the Ruffed Grouse (*Bonasa umbellus*). We uncovered multiple factors acting in concert that are likely responsible for mediating contemporary population connectivity in this species. A combination of autosomal intron, mitochondrial, and high-resolution microsatellite markers revealed many populations of Ruffed Grouse are genetically isolated. Furthermore, the addition of landscape genetic methods not only corroborated genetic structure results, but also uncovered compelling evidence that dispersal resistance created by unsuitable habitat is the most important factor mediating population connectivity among the sampled populations. Our data revealed evidence of high elevation mountains acting as dispersal barriers, as well as two corridors creating limited connectivity among populations that are otherwise isolated by the Rocky Mountains. This research may have implications for both our study species and other inhabitants of the early successional forest habitat required by Ruffed Grouse.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

AB	Alberta
AICc	corrected Akaike's Information Criterion
AK	Alaska
A_R	allelic richness
AUC	area under curve
BIC	Bayesian Information Criterion
bp	base pair
CR	control region
dd	decimal degrees
DEM	digital elevation model
DIC	deviance information criterion
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
ESRI	Environmental Systems Research Institute
FDR	false discovery rate
F_{ST}	Wright's fixation index
F'_{ST}	standardized measure of genetic differentiation
GBIF	Global Biodiversity Information Facility
GIS	Geographical Information System
GPS	Global Positioning System
H_d	haplotype diversity
H_e	expected heterozygosity
H_o	observed heterozygosity
HWE	Hardy-Weinberg equilibrium
IAM	Infinite Alleles Model
IBD	isolation by distance
IBR	isolation by resistance
indel	insertion/deletion
K	number of clusters
km	kilometers
LCC	least cost corridor
LCP	least cost path
LGM	Last Glacial Maximum
LnPr (X|K)	estimated log probability of the data
m	meter
MCMC	Markov chain Monte Carlo
$MgCl_2$	magnesium chloride

mM	millimolar
MU	management unit
n	sample size
Na	number of different alleles occurring at a frequency of ≥ 5%
N(a)	total number of alleles
P	P-value (significance)
PA	private allele
PCo	principal coordinate
PCoA	Principal Coordinate Analysis
PCR	polymerase chain reaction
Q	ancestry coefficient
R^2	Correlation coefficient
s	seconds
SDM	Species Distribution Model
SMM	stepwise mutation model
SNP	single nucleotide polymorphism
SSR	simple sequence repeat
U	units
v	version
VNTR	variable number tandem repeats
µl	microliter
µM	micromolar
π	nucleotide diversity
ΔK	delta K
Ψ	interaction parameter
Φ_{ST}	modified fixation index
˚C	degrees Celsius

Populations

AK	Alaska
AT	Athabasca area, Alberta
BL	Buck Lake area, Alberta
BV	Bonnyville area, Alberta
COA	Cochrane area, Alberta
CP	Crowsnest Pass, Alberta
EA	Edson area, Alberta
FM	Fort McMurray, Alberta
GP	Grande Prairie area, Alberta
LM	Lloydminster area, Alberta

MN	Minnesota
PR	Peace River area, Alberta
WA	Washington
WI	Wisconsin
YT	Yukon

Museums

FMNH	Field Museum of Natural History
RAM	Royal Alberta Museum
UAM	University of Alaska Museum
UWBM	University of Washington Burke Museum

CHAPTER 1: General introduction

1.1 Population genetics

1.1.1 Gene flow

Low genetic diversity due to reduced gene flow is often strongly correlated with decreased population fitness, particularly in small or isolated populations (Reed and Frankham 2003; Frankham 2003). Gene flow is the transfer of genes from one population to another, and in animals usually occurs via individual dispersal (Slatkin 1985). Gene flow aids in maintaining levels of heterozygosity (i.e. genetic diversity) and can prevent fixation of deleterious alleles (Frankham 2003; Frankham et al. 2010). Furthermore, the reduction of gene flow can decrease genetic diversity in a population, which can in turn diminish that population's potential to adapt to changing ecological conditions (i.e. loss of evolutionary potential). The negative effects of decreased genetic diversity can also be compounded by the presence of other ecological pressures, such as habitat loss, edge effects or resource competition (Saccheri et al. 1998; Garza and Williamson 2001; Couvet 2002; Reed and Frankham 2003; Frankham 2005). Small, fragmented populations with increased levels of homozygosity often experience negative biological consequences by way of genetic drift, inbreeding depression, and the reduction of genetic diversity. Increased population connectivity (i.e. successful movement of reproductive individuals between populations) maintains genetic diversity, increases effective population size, and can ameliorate the negative consequences of isolation (particularly in small populations) (Reed and Frankham 2003; Palstra and Ruzzante 2008).

Moreover, measures of gene flow and genetic diversity can aid in identifying Management Units (MU), which are populations that are significantly differentiated with respect to allele frequencies, and may need specialized management strategies (Moritz 1994; Frankham 2003). Assessing gene flow and genetic diversity in wild populations is increasingly important for designing proper management strategies, particularly with the accelerating rate of climate change and anthropogenic landscape alteration (Garza and Williamson 2001; Frankham 2010; Pauls et al. 2013).

1.1.2 Physical barriers

Many factors may affect the genetic structure of a population by influencing gene flow. Geographic barriers are often examined as determinants of genetic differentiation among populations on broad spatial and temporal scales (Frankham 2003; Funk et al. 2005; Frankham et al. 2010). Barriers such as mountains and bodies of water create genetic structuring by reducing movement of individuals between populations (Funk et al. 2005; Holderegger and Wagner 2008). For example, Hapeman et al. (2011) used microsatellite markers to show that two large lakes separating populations of Fishers (*Martes pennanti*) acted as barriers to gene flow by leaving only a narrow corridor between the lakes by which individual dispersal could occur.

Anthropogenic changes in the environment, such as agricultural development and urbanization, can also act as barriers to gene flow because habitat fragmentation leads to limited dispersal ability. Habitat modifications such as timber harvesting and fire suppression are examples of habitat alteration that may not seem to fit the traditional conception of barriers, but still may limit dispersal capabilities in the same fashion as other barriers. Moreover, this type of habitat alteration may be effectively equivalent to

fragmentation because animals may be reluctant or unable to move into unsuitable or marginal habitat (Holderegger and Wagner 2008). In Red Grouse (*Lagopus lagopus scoticus*), microsatellite analyses revealed that both geographic distance and physical barriers affected gene flow, which subsequently led to population structuring (Piertney et al. 1998). Rivers, villages and agricultural lands were all acting as barriers to gene flow by preventing population connectivity (Piertney et al. 1998). Similarly, Fenderson (2014) found urban development was creating severely fragmented habitat for the New England Cottontail (*Sylvilagus transitionalis*), which resulted in genetically differentiated populations. In this species, the loss of connectivity has led to population bottlenecks, and could result in extirpation if management action is not taken (Fenderson et al. 2014).

1.1.3 Population structure and landscape genetics

Population structure is affected by many processes, and the interdisciplinary study of landscape genetics aims to utilize methods from both landscape ecology and population genetics to answer questions about population structuring (Manel et al. 2003). It explores how landscape features reduce or facilitate population connectivity by examining relationships between various landscape data and variation in neutral genetic markers (Holderegger and Wagner 2008). While phylogeography is similarly defined, it differs from landscape genetics in spatiotemporal scale (Manel et al. 2003). Phylogeography has classically used molecular markers such as mitochondrial DNA to investigate the relationship between geography and genetic variation on broad scales, often focusing on historical patterns. Alternatively, landscape genetics focuses on finer spatial and temporal scales, usually through the use of highly variable genetic markers (e.g. microsatellites) (Manel et al. 2003). Before the advent of landscape genetic methods,

the only analysis widely available to examine the relationship between geographic and genetic data was isolation-by-distance (IBD), which measures the correlation between genetic distance and simple geographic distance to determine whether genetic differentiation is a simple function of proximity (Wright 1943; Storfer et al. 2007). While IBD is still utilized, it is now more often being used as one of multiple hypotheses in a landscape genetics approach to investigate genetic structuring. Improvements in the tools available for landscape genetic methods such as Bayesian clustering software, and improvements in GIS have opened up the possibilities for more advanced analyses to explore how genetic divergence is associated with geographic variables (Manel et al. 2003; Manel and Holderegger 2013). A landscape genetics approach can be used to test for the presence of barriers, as well as the ability of the species to cross through heterogeneous landscapes; the latter is termed isolation by resistance (IBR; Holderegger and Wagner 2008; Cushman et al. 2013). IBR incorporates environmental variables to explain genetic differentiation by quantifying an organism's ability to move through different habitats (Cushman et al. 2013). A form of IBR analysis called least cost paths (LCP) is an approach where dispersal routes are calculated as the most likely paths through suitable habitat, as opposed to the straight-line geographic distances used in IBD (Storfer et al. 2007; Manel and Holderegger 2013; Yu et al. 2015). Although LCP shows the single most likely route between population pairs, animals are not realistically expected to always use the single most optimum path for dispersal (Pinto and Keitt 2009). Therefore, it is valuable to not only calculate LCPs, but to also examine resistance values across multiple dispersal routes. This can be achieved using least cost corridors (LCC; Chan et al. 2011) or resistance mapping via circuit theory. In Greater Sage Grouse (*Centrocercus urophasianus*), Row et al. (2015) used circuit theory to test models of

resistance variables (i.e. factors that impede dispersal by varying degree as opposed to an impassable barrier), and found that presence of forested habitat negatively impacts dispersal capabilities of Sage Grouse. From these data they were also able to identify crucial areas of connectivity for this near threatened species. Moreover, an increasing number of studies are discovering the importance of testing multiple models of genetic structure. For example, when comparing models of geographic distance (IBD) and environmental resistance variables (IBR), Fontaine et al. (2007) found that IBD explained most of the genetic structure of Harbour Porpoises (*Phocoena phocoena*), and environmental factors had less of an influence. Conversely, McRae et al. (2007) found by calculating least cost paths and resistance distances between populations of Wolverines (*Gulo gulo*), that IBR rather than IBD best explained the genetic distance between populations.

As methods improve, researchers are also finding a combination of factors are sometimes responsible for genetic patterns. Coulon et al. (2006) used a landscape genetics approach to reveal that a combination of factors, including rivers, highways and high fences explained genetic differentiation of populations of Roe Deer (*Capreolus capreolus*). These factors acted as semi-permeable barriers to gene flow, and due to the additive effect of these factors, the correlation between the landscape and genetic differentiation of Roe Deer was only apparent when all barrier types were considered. The integration of landscape ecology and population genetics has allowed for powerful analyses of contemporary population structure, which allows researchers to uncover meaningful information about genetic connectivity and the evolutionary process (Holderegger and Wagner 2008; Manel and Holderegger 2013).

1.2 Molecular markers

Molecular markers have become an effective way to study animal populations. In the past, census and morphological data were often used, however, these types of data can only provide limited information about overall population trends and in many cases is time-consuming and expensive to obtain (Avise 1994). Molecular methods have become a popular way to study wild populations, and for most population genetics studies, unlinked molecular markers that do not affect phenotype are needed (Parker et al. 1998). Although it is important to note that of late, adaptive genes are being used more often as a means of answering questions about wild populations, neutral genetic markers are most commonly implemented to evaluate genetic diversity and population connectivity because they evolve independently of selective forces (Parker et al. 1998; Holderegger et al. 2006). There are multiple types of markers, which have different rates of evolution. Using multiple marker types can provide better resolution for interpreting genetic patterns as well as provide insights into patterns at multiple spatial and temporal scales (Avise 1994; Wan et al. 2004).

1.2.1 Autosomal nuclear markers

Autosomal nuclear markers typically have a slower rate of evolution than other molecular markers such as mitochondrial DNA or non-coding variable number tandem repeats (VNTR). The introns of genes are sometimes used as population genetic markers because they are effectively neutral in most cases and are not subject to the potential bias that can occur with sex-linked markers (Zhang and Hewitt 2003). Due to the slower rate of evolution in non-repetitive nuclear DNA, longer sequences may be necessary to observe polymorphisms. However, a single nucleotide substitution can provide surprising

statistical power if there are fixed differences among groups (Hare 2001). Palumbi and Baker (1994) used intronic nuclear sequences to evaluate population structure in Humpback Whales (*Megaptera novaeangliae*) and revealed long-term isolation between some populations, and connectivity in the form of male-biased migration between others.

1.2.2 Nuclear Z-linked markers

Loci occurring on sex chromosomes are involved in speciation (e.g., Sætre and Sæther (2010)), and can be useful in identifying the presence of sex-biased dispersal. Li and Merilä (2010) compared the within and among group genetic variation of Z-linked and autosomal markers and found that Siberian Jays (*Perisoreus infaustus*) display sex-biased dispersal, while Cheviron and Brumfield (2009) used a similar approach to show that sex-biased dispersal was not influencing genetic patterns in Rufous-collared Sparrows (*Zonotrichia capensis*). These sex-linked nuclear markers can also be useful in population genetic studies as they have a similar, but slightly higher mutation rate compared to their autosomal counterparts (Prugnolle and de Meeus 2002).

1.2.3 Mitochondrial markers

Mitochondria contain a relatively small, circular genome that is inherited maternally in most animals (exceptions include some mollusks, see Dégletagne et al. 2015). Due to its matrilineal inheritance, the haploid mitochondrial genome does not typically undergo recombination, so the resulting genealogy often shows the patterns of an organism's evolutionary past more clearly (Avise et al. 1987; Galtier et al. 2009). The mutation rate of the mitochondrial genome is also of interest because it is significantly greater than that of the nuclear DNA (approximately 5-10x faster) due to few repair

mechanisms (Wan et al. 2004). This higher mutation rate and lack of recombination makes mitochondrial markers appropriate for phylogeographic studies because it often reveals genetic breaks resulting from prolonged isolation due to long-standing geographic barriers, such as an impassable mountain range or ice sheets creating multiple refugia during the Last Glacial Maximum (LGM; Avise et al. 1987).

Both coding and non-coding regions of the mitochondrial genome are used as genetic markers, and it was long thought that due to the general uniformity of substitution rates among coding mitochondrial loci, that the non-coding regions acted as effectively neutral markers. However, mitochondrial genes are involved in important metabolic functions, and recent studies show that mitochondrial coding regions and nearby loci may be subject to selective sweeps, and do not strictly follow the neutral model of evolution (Ballard and Kreitman 1995; Galtier et al. 2009). However, coding regions are still commonly used as markers in studies of phylogeny, and sometimes population biology (Wan et al. 2004; Galtier et al. 2009). An example is Cytochrome Oxidase I, which is often used to resolve phylogenetic relationships (Wan et al. 2004; Roe and Sperling 2007). For examining intraspecific differences, a highly variable region of the mitochondrial genome, the Control Region (CR) is often employed. This non-coding region of the mitochondrial genome can aid in identifying species, subspecies, or population structure (Parker et al. 1998; Wan et al. 2004).

Despite the apparent advantages of using mitochondrial markers, some considerations should be made. Aside from the possibility of non-neutrality for coding regions, there is the possibility of gender-biased dispersal, as seen with other sex-linked markers (e.g. Z-linked). Another important consideration is rare haplotypes can quickly be lost in smaller populations due to smaller effective population size of mitochondrial

markers compared to that of nuclear markers (Zhang and Hewitt 2003; Wan et al. 2004; Galtier et al. 2009). Uniparentally inherited markers exhibit a lower effective population size compared to biparentally inherited markers because only one copy of the genome is passed down. The smaller effective population size of mitochondrial markers means they are more likely to show evidence of genetic drift in small populations (Wan et al. 2004; Smith et al. 2013). Therefore mitochondrial markers are most useful in population genetics research when used in combination with nuclear markers (Zhang and Hewitt 2003; Wan et al. 2004).

1.2.4 Microsatellites

Variable number tandem repeats (VNTR) are markers occurring in large, typically non-coding areas of the nuclear genome that vary greatly in size due to the number of tandem nucleotide repeats (e.g. $(GATC)_2$ vs. $(GATC)_3$) (Parker et al. 1998). Two main types of VNTRs are used in genetic studies; microsatellites and minisatellites. Minisatellites consist of large repeat blocks (10-100 base pairs) and are seldom used in population genetics due to their hyper-variable nature and issues with allele identification from non-target binding of PCR primers, whereas microsatellites are much better suited for studies of population genetics (Parker et al. 1998; Richard and Pâques 2000). Variation in the number of repeats caused by polymerase slippage during replication characterize microsatellites, also known as simple sequence repeats (SSRs) (Jarne and Lagoda 1996; Parker et al. 1998). The mutation rate of microsatellites is multiple orders of magnitude greater than that of typical single copy nuclear DNA or mitochondrial DNA. This higher mutation rate leads to much faster accumulation of polymorphisms, which can reveal recent divergence and genetic structure on fine spatial and temporal

scales (Parker et al. 1998; Wan et al. 2004). Multiple alleles can be identified for each locus through size identification of PCR products on an acrylamide gel without needing to sequence these products, making it feasible to process large numbers of samples. The high resolution of population trends available through microsatellite analyses due to their high variability and rapid mutation rate makes these markers ideal candidates for any population study, however, some considerations should be made when utilizing these markers (Zhang and Hewitt 2003; Wan et al. 2004). Allelic dropout can be caused by mutations in priming sites of microsatellites or the preferential amplification of one allele over another; this prevents some alleles from being visualized and therefore artificially decreases heterozygosity. Another issue called size homoplasy occurs when two different alleles appear to be the same size, even though they are not identical by descent, which leads to problems inferring the history of the alleles. For example, if one allele gains a repeat ($(AC)_7$ mutates to $(AC)_8$), and another allele loses a repeat ($(AC)_9$ mutates to $(AC)_8$), these two alleles will appear the same, even though they have undergone different mutations to reach their current state (Zhang and Hewitt 2003; Wan et al. 2004). Several models of mutation can be implemented for microsatellites; the two most common are the Stepwise Mutation Model (SMM), where mutations always occur by the addition or subtraction of a single repeat, and the Infinite Alleles Model (IAM), where multiple repeats can be gained or lost (Kimura and Crow 1964; Kimura and Ohta 1978). The SMM is often deemed the most appropriate model for microsatellites, which are frequently subject to mutation via slippage of polymerase during DNA replication (Jarne and Lagoda 1996). It is also important to note that programs now exist to aid in detecting null alleles due to allelic dropout or size homoplasy; an example is MICRO-CHECKER (Van Oosterhout et al. 2004) which is designed to detect the presence of null alleles.

1.3 Study Species

Although population genetics has become an increasingly popular topic of study in recent years, species with very broad distributions are largely understudied. One such species is the Ruffed Grouse (*Bonasa umbellus*). This native game bird occurs across most of Canada and the northern United States (Davis 1970; Gullion 1984). Ruffed Grouse require an early-successional forest habitat, and is closely associated with mixed forest including *Populus* species, particularly quaking aspen (*Populus tremuloides*), as the buds of this tree comprise a large proportion of their diet (Rusch et al. 2000). Not only is the Ruffed Grouse of interest due to its extreme popularity as a game bird (Atwater and Schnell 1989), but it is also an indicator species of the health of early successional forest (USDA Forest Service 2006). This species has experienced significant range reductions over the last century due to fire suppression, maturation of forest stands, land clearing and other anthropogenic activities (Rusch et al. 2000). Habitat degradation has caused declines, particularly in the eastern United States where translocations of birds have been performed in four states over the past 50 years to revive grouse populations (Rusch et al. 2000). Patterns of genetic structure may be valuable in making management decisions for this species, including those involving harvest regulations and translocations. If populations have been sufficiently isolated leading to local adaptation, translocation of individuals between these populations may have negative consequences due to outbreeding depression (Frankham 2010; Frankham et al. 2011). Although translocations can increase a population's evolutionary potential, it is important to ensure that moving individuals from one population to another will not result in outbreeding depression. Moreover, because Ruffed Grouse are heavily hunted throughout most of their range,

population genetic structure may be useful in informing harvest regulations. Overharvesting can decrease genetic variability and therefore reduce a population's evolutionary potential (Allendorf et al. 2008). It is particularly important to identify isolated populations when designing management plans because these populations may require management strategies that are different from those used for populations with high connectivity (Moritz 1994; Frankham 2010).

Ruffed Grouse have been split into as many as 15 subspecies based on morphological data, mainly differing in size, tarsus feathering and plumage colouration (Uttal 1941; Aldrich and Friedmann 1943; Rusch et al. 2000). There are two main colour morphs, grey and red, which occur in different proportions throughout the range (Rusch et al. 2000). For example, the *B. u. castanea* subspecies on the Olympic Peninsula in Washington are predominately red-phased, but the *B. u. umbelloides* subspecies occurring in Alberta and the rest of Canada's interior west consists of mostly grey-phased individuals with feathering midway down the tarsus (Davis 1970; Furtman 2004; Fig. 1.1). The amount of phenotypic variation among populations (or subspecies) of Ruffed Grouse implies genetic variation may be occurring in adaptive genes governing phenotypic characters, and some degree of isolation due to physical or non-physical barriers are likely maintaining this phenotypic variation.

Birds are often thought of as having a high dispersal capability. Tittler et al. (2009) predicts average dispersal distances ranging from 15-95 km for a variety of bird species. However, the Ruffed Grouse has comparatively low dispersal distances (approx. 2-4 km; Yoder 2004). This low dispersal distance combined with a lack of migratory behaviour increases the potential for limited gene flow in Ruffed Grouse, particularly in a heterogeneous landscape. Dispersal success is negatively correlated with the distance

Ruffed Grouse are required to move across unsuitable habitat (Yoder 2004), further reinforcing the possibility that gene flow is restricted in Ruffed Grouse by extrinsic factors.

Although the Ruffed Grouse is a well-studied species in many regards, there is a distinct lack of information on population genetic structure or subspecies genetics (Rusch et al. 2000). The genetic research including Ruffed Grouse is limited to phylogenetic studies of the Tetraoniae family (Ellsworth et al. 1995; Drovetski 2002; Persons et al. 2016). Furthermore, studies of population genetics focusing on any North American Tetraonid species are sparse with the majority focus on species of high conservation concern (Oyler-McCance et al. 2005; Spaulding et al. 2006; Bouzat et al. 2008; Barry and Tallmon 2010).

1.4 Thesis objectives

The overall objective of this thesis is to utilize data from molecular markers to explore contemporary patterns of population connectivity and genetic diversity in the Ruffed Grouse. I used neutral molecular markers with different inheritance patterns and rates of evolution to interpret genetic structure at multiple spatial and temporal scales. To examine potential broad-scale genetic structure, I analyzed non-coding nuclear loci, as well as a mitochondrial marker. The nuclear markers are intron regions, which are predicted to reveal strong genetic breaks resulting from long-term isolation, and the mitochondrial locus is likely to elucidate more recent patterns on a broad spatial scale. While the focus of this study is contemporary patterns, investigating genetic patterns on multiple temporal scales allows us to disentangle factors of contemporary genetic structure from those of a historical nature. To detect finer scale patterns of genetic

structure, I used microsatellite markers, which are more likely to reflect contemporary structure. I used landscape genetic methods to interpret genetic data with respect to environmental variables to allow for more accurate identification of potential barriers to gene flow and to determine the level of permeability of these barriers. My goal was to test three hypotheses:

i. Genetic structuring will be present on both broad and fine spatial scales;

ii. analyzing gene flow will reveal a pattern of isolation by resistance among populations, specifically, areas where physical barriers have narrowed the corridors of habitat that is suitable for individual dispersal will show high resistance, and areas containing contiguous aspen-dominated mixed forest will show low resistance; and

iii. unsuitable habitat will be identified as a barrier to gene flow.

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Figure 1.1. The 13 most agreed upon Ruffed Grouse subspecies classifications. Subspecies distributions created from Aldrich and Friedman (1943), Davis (1970), Furtman (2004), and Ouellet (1990). Of the 15 described races, two of those (*B. u. helmei* and *B. u. obscura*) with small and/or poorly defined ranges are not pictured here.

CHAPTER 2: Landscape effects on the contemporary genetic structure of western Ruffed Grouse populations

2.1 Introduction

Dispersal of organisms across the landscape is ultimately what determines gene flow among populations (Slatkin 1985). The dispersal process is essential in species perseverance, as it maintains the genetic diversity necessary for populations to respond to changing ecological conditions (Reed and Frankham 2003; Frankham 2005). Despite the importance of understanding connectivity, our knowledge of the forces mediating gene flow is limited (Bowne and Bowers 2004). Traditional studies using mark-recapture or telemetry provide little data for the amount of effort required, and the data can be difficult to interpret because the movements of dispersing individuals do not necessarily equate to a successful reproductive event for the individual in a new population. Genetic methods are a more accurate method for this type of study because gene flow can be measured directly (Cushman et al. 2006; Luque et al. 2012).

Many population genetic studies aim to either infer the evolutionary history of species or detect genetic discontinuities created by barriers to gene flow, both past and present (Frankham et al. 2004; Venton 2013). Moreover, researching contemporary population genetic structure can complement studies of phylogeography to tease apart the effects of historical versus contemporary processes (Luque et al. 2012). Historical processes contribute to how species are currently distributed, and in some cases geographic features may restrict gene flow over a long period of time (Irwin et al. 2009). If isolation occurs over a sufficiently large temporal scale, the differentiation will manifest as differences in relatively slowly mutating markers, such as autosomal introns

(Kahn et al. 1999; Cheviron and Brumfield 2009). Although long-term historical isolation may manifest as genetic breaks, contemporary landscape features also influence spatial genetic variation (Tracy and Jamieson 2011; Pilgrim et al. 2012).

Landscape genetics incorporates environmental variables into genetic analyses, which not only allows for detection of barriers, but also identification of environmental factors that are influencing contemporary gene flow (Keyghobadi et al. 1999; Manel et al. 2003; Storfer et al. 2007). Both population and landscape genetics aim to detect extrinsic factors that underlie genetic structuring, such as mountain ranges (Worley et al. 2004; Funk et al. 2005), bodies of water (Díaz-Muñoz 2012), or anthropogenic disturbance (Cegelski et al. 2003; Epps et al. 2005). Unsuitable habitat has long been considered a potential barrier to gene flow, but it may not always act as an impermeable barrier. Instead, habitat often differs in its degree of suitability (Cushman et al. 2006), resulting in a complex matrix of habitat types with varying dispersal costs (or resistance) to individuals moving across the landscape. The difference in landscape resistance can dictate patterns of gene flow, which is termed isolation by resistance (IBR; Ruiz-Gonzalez et al. 2015). Another consideration for landscape genetic studies is the influence of physical distance on genetic variation. While a barrier, such as a mountain range, may create an abrupt genetic break, physical distance between populations can also act as a barrier by creating clinal genetic variation (Ruiz-Gonzalez et al. 2015). These patterns of IBD occur when dispersal routes among populations are straight lines, and for this to occur, the intervening landscape must be relatively homogeneous with respect to the dispersal cost it represents to the individual. When landscape heterogeneity exists between populations, suitable dispersal routes become more complex, and patterns of IBR are more likely to occur (Fontaine et al. 2007; McRae and Beier 2007; Ruiz-Gonzalez et

al. 2015). Sometimes a combination of IBD and IBR best explains genetic structuring (Piertney et al. 1998; Metzger et al. 2015). Moreover, species that are widespread, and relatively continuously distributed are expected to exhibit either panmixia or clinal patterns of genetic structure explained by IBD (Purdue et al. 2000; Alcaide et al. 2009; Ralston and Kirchman 2012). However, a few studies have emerged where widespread, continuously distributed species exhibit unexpected patterns of IBR (Pilot et al. 2006; Pease et al. 2009). Population genetic studies of species with broad geographic ranges are lacking relative to those that focus on species of conservation concern with limited geographic distributions, or species that inhabit fragmented landscapes (Frankham et al. 2010; Basto et al. 2016). It is important to understand how evolutionary processes work, both in species with limited distributions and in broadly ranging species that are not experiencing obvious breaks in population connectivity.

The Ruffed Grouse (*Bonasa umbellus*) is a game bird that is widely distributed across North America (Fig. 1.1). This species is a good model for understanding how landscape features influence gene flow because their broad range encompasses a heterogeneous landscape with many geographic features that may act as barriers to dispersal. The Ruffed Grouse is resident throughout its distribution, and has relatively low dispersal distances for an avian species (approx. 2-4 km; Yoder 2004). Furthermore, they inhabit early successional forest, and are closely tied to the Quaking Aspen (*Populus tremuloides*) because it is an integral part of their diet (Svoboda and Gullion 1972; Rusch et al. 2000; Zimmerman and Gutiérrez 2008). Thus, the presence of suitable mixed forest habitat is important for survival, and likely for successful dispersal events as well. Because of their short dispersal distance and dependence on Quaking Aspen, the Ruffed

Grouse is a species likely to exhibit population genetic structure arising from IBD, IBR or a combination of the two.

Although Ruffed Grouse have been well studied with respect to habitat and behaviour (Gullion 1984; Zimmerman and Gutierrez 2007; Garcia et al. 2012), there is no published literature to date regarding genetics aside from phylogenetic relationships (e.g. Drovetski 2002; Persons et al. 2016). The Ruffed Grouse is one of the most extensively managed game birds due to heavy hunting pressure throughout most of its range (Rusch et al. 2000). Furthermore, this species is of ecological importance; it is considered an indicator species in the management of early successional forest habitats (USDA Forest Service 2006). Therefore, information on how macrogeographic barriers and habitat factors limit gene flow in this species could have important implications for managing not only for Ruffed Grouse but also for other early successional forest species (e.g. American Woodcock (*Scolopax minor*), Mourning Warbler (*Geothlypis philadelphia*), American Redstart (*Setophaga ruticilla*)).

The aims of this study were therefore to quantify population structure by assessing gene flow across a large section of the western extent of the species range, and to identify geographic barriers and other landscape features that may be restricting or facilitating gene flow. We chose to focus on the western extent of the range because this is where macrogeographic barriers are most likely to be influencing population structure, as seen in a range of other species (Pulgarín-R and Burg 2012; Adams and Burg 2015a; Vonhof et al. 2015). Although widespread species with a continuous distribution are expected to show genetic patterns of IBD, we predicted that Ruffed Grouse populations would exhibit patterns of IBR due to the heterogeneous distribution of suitable habitat throughout their range, combined with their low dispersal ability, and early successional forest habitat

preference. We also predicted that Ruffed Grouse populations would show significant population genetic structuring, and of the extrinsic factors that may be affecting gene flow, both mountains and swaths of unsuitable habitat would be the most likely geographic features to act as barriers.

2.2 Methods

2.2.1 Sample acquisition

Fieldwork was conducted from mid-April through May 2016, during the peak activity period of the male Ruffed Grouse's drumming display (Rusch et al. 2000). Birds were located aurally by drumming activity, and the location of each male's drumming log was marked with a handheld GPS unit. Birds were caught with mirror traps (Gullion 1965), which were placed on males' drumming logs adjacent to the drumming stage, a carbon dioxide-powered net gun, or a lift net (Fischer 1974). A suite of morphological measurements (e.g. tarsus, wing and tail lengths, plumage colour) was recorded for each individual, and brachial venipuncture was used to collect a blood sample, which was stored in 99% ethanol. For this study, we collected 75 Ruffed Grouse samples at two Alberta locations (Buck Lake (52.91 N, 115.01 W), and Crowsnest Pass (49.35 N, 114.40 W)). In addition, 159 samples were collected from birds harvested by hunters throughout Alberta in the 2016 hunting season, and 17 were supplied by the Royal Alberta Museum, for a total of 251 samples originating in Alberta (Appendix 1). Outside of Alberta, we obtained 100 samples from various western sites with the goal of sampling populations that are likely to be affected by macrogeographic barriers, such as mountain ranges. This included 13 samples supplied by Yukon Fish and Game, 32 from the University of Washington Burke Museum, and 25 from University of Alaska Museum (Appendix 1).

We also obtained 30 samples in the Great Lakes area from the Field Museum of Natural History to represent a population in the eastern extent of the range.

2.2.2 DNA extraction and amplification

Genomic DNA was isolated from each blood sample with a modified chelex extraction method (Walsh et al. 1991). Samples were screened at two nuclear loci: an intronic region in SLC45a2, a gene in the melanin pigment pathway (Gunnarsson et al. 2007); and intron 6 of Aldolase B, on the Z-chromosome (Cheviron and Brumfield 2009). A portion of the mitochondrial Control Region (domain I and II) was also sequenced to aid in determining the species' evolutionary history. Samples were amplified with polymerase chain reaction (PCR) in a 25 µl reaction containing Green GoTaq® Flexi buffer (Promega), 1.5 mM $MgCl_2$, 0.08 mM dNTP, 0.4 µM of each primer (Appendix 2) and 0.5 U GoTaq® Flexi DNA polymerase (Promega). These reaction mixes were the same for all three loci, except for the Control Region, with 2.0 mM $MgCl_2$. Amplification consisted of one cycle at 95ºC for 120 s, 54ºC for 45 s, and 72ºC for 60 s; 37 cycles of 94ºC for 30 s, 54ºC for 45 s, and 72ºC for 60 s; followed by a final cycle of 72ºC for 300 s, and 4ºC for 20 s. For the Aldolase B primers, the annealing temperature was increased to 62ºC. Successfully amplified samples (SLC45a2 = 80, Aldolase B = 28, and Control Region = 56) were sequenced at Genome Quebec (Montréal, QC, Canada).

The Aldolase B sequences contained a 7 bp indel. The frequency of the indel differed among populations, so a set of three primers was designed to screen for this indel. The forward primer was placed upstream from the indel, while the other two primers were designed to bind to the insertion and deletion regions respectively (see Appendix 2 for primer sequences). An M13 tag was added to the 5' end of the reverse

primer for the insertion to increase the size difference between the fragments. Resulting PCR products were 161 bp for the insertion, and 118 bp for the deletion. All samples were screened on a 3% agarose gel.

Microsatellite loci were chosen from those characterized for species that are closely related to Ruffed Grouse (Appendix 3; Cheng and Crittenden 1994; Segelbacher et al. 2000; Caizergues et al. 2001; Piertney and Hoglund 2001; Burt et al. 2003; Taylor et al. 2003). Extracted DNA was amplified in 10 µl reactions containing Colourless GoTaq® Flexi buffer (Promega), 2.0-2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM of the forward primer, 1.0 µM of the reverse primer, 0.05 µM fluorescent M13 tag, and 0.5 U GoTaq® Flexi DNA polymerase (Promega) (See Appendix 3 for $MgCl_2$ concentrations for each primer set). All forward primers were synthesized with an M13 sequence added to the 5' end of the primer sequence to allow binding of the fluorescent M13 tag, which in turn allows visualization of the PCR products on a LI-COR 4300 DNA Analyzer. To amplify the products, a thermocycling profile with two-step annealing was used: one cycle of 94ºC for 120 s, 45-60ºC for 45 s, and 72ºC for 60 s; 7 cycles of 94ºC for 30 s, 45-60ºC for 30 s, and 72ºC for 45 s; 31 cycles of 94ºC for 30 s, 48-62ºC for 30 s, and 72ºC for 45 s; followed by a final extension step of 72ºC for 300 s (see Appendix 3 for annealing temperatures for each locus). For the 19 successfully amplified loci, a small number of samples (n = 9) were screened for variability; 9 of these loci were monomorphic, while the 10 polymorphic loci (LLSD7, TTD2, TTD6, TUT2, TUT4, SGCA5, BG15, BG18, BG20, and ADL230) were retained. PCR products were visualized on a 6% acrylamide gel using a LI-COR 4300 DNA Analyzer (LI-COR Inc., Lincoln, NE, USA). Three positive controls of known size were chosen from the initial samples amplified at each locus, and these used while scoring samples for each locus. A

second person scored all gels to reduce error in the scoring process. As an additional measure against potential scoring errors, a subset of samples from each population were genotyped a second time at each locus.

2.2.3 Genetic diversity analyses

The chromatograms were aligned and sequences assessed for variation using MEGAv6 (Tamura et al. 2013). Haplotype reconstruction was performed for the autosomal SLC45a2 sequences in PHASE v2.1 (Stephens et al. 2001; Stephens and Scheet 2005). DnaSP v5.1 (Rozas et al. 2003) was used to calculate shared haplotypes, nucleotide diversity (π), and haplotype diversity (H_d) for SLC45a2 and Control Region sequences.

Genetic diversity was measured at the population level using microsatellite loci by calculating observed and expected heterozygosity, the number of alleles per locus, and private alleles in GenAlEx v6.5 (Peakall and Smouse 2012), and FSTAT v2.3.1.0 (Goudet 1995) was used to calculate allelic richness (A_R). Genotypes at the microsatellite loci were checked for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium using GENEPOP v4.2 (Raymond and Rousset 1995) with default parameters. MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004) was used to check for errors in the genotyping data including allelic dropout and null alleles. The resulting significance levels were corrected for multiple tests using a modified False Discovery Rate (Benjamini and Yekutieli 2001). Two loci, ADL230 and TTD2, were removed due to significant deviation from Hardy-Weinberg equilibrium. SGCA5 also had a significant probability of null alleles for several populations, so analyses were performed with and without this marker to determine if the potential presence of null alleles was biasing the data. SGCA5

was kept in the final analyses because its exclusion did not cause noticeable variation in the results; however, SGCA5 had more missing data than the other markers (>25% for some populations), and had to be excluded from fixation index calculations. Of the 351 genotyped samples, 324 were used for analyses after removing samples that amplified at less than six loci. Samples collected in the same area on the same day (i.e. hunter-donated or museum collection samples harvested on the same day) were checked for shared ancestry that would indicate multiple individuals from the same family group; none were found. For analyses that required *a priori* population assignments, sampling sites within 100 km from each other were grouped together as a single 'population'. All sampling sites in Washington, and all sites in Minnesota were grouped together respectively due to low sample sizes at some sites within each state ($n \leq 5$).

2.2.4 Genetic structure

Genetic differentiation between populations was determined by calculating fixation indices in Arlequin v3.5.1.3 (Excoffier et al. 2007; Excoffier and Lischer 2010). A pairwise fixation index, Wright's F-statistic (F_{ST}) (Wright 1951), was used for SLC45a2, and a modified version of the original F-statistic, Φ_{ST} (Wright 1965; Excoffier et al. 1992), for the mitochondrial Control Region sequences. P-values were corrected for multiple tests by a modified False Discovery Rate method (Benjamini and Yekutieli 2001). Haplotypes for both SLC45a2 and Control Region were used to create statistical parsimony networks in PopART v1.7 (Leigh and Bryant 2015).

Individuals were sexed prior to compiling final genotypes for Aldolase B to determine if each individual should be counted as hemizygous (females) or homozygous

(males). The allele frequencies were then tested for significant pairwise population differentiation using Fisher's exact test (Fisher 1922).

Genetic structure was quantified for pairwise comparisons of all populations at microsatellite loci using F'_{ST} calculations in GenAlEx v6.5 (Peakall and Smouse 2012), and p-values were corrected for multiple testing with a modified FDR method (Benjamini and Yekutieli 2001). For better visualization of F'_{ST} across the landscape, a heat map of population divergence was made using the Landscape Genetics Toolbox (Vandergast et al. 2011) in ArcGIS v10.2, which uses spline interpolation to create a colour gradient in geographic space.

2.2.5 Bayesian clustering analyses

Bayesian clustering is widely used in studies of population genetic structure because it remains robust even when only a small number of loci are available. It also identifies genetic clusters without requiring *a priori* population assignments (François et al. 2006). Bayesian analysis applies a Markov chain Monte Carlo (MCMC) simulation method to multilocus genotypes to assign individuals to clusters, and then calculates the posterior probability of the accuracy of the parameters used. The posterior probabilities can then be used to infer the most appropriate number of genetic clusters (K) in the data. Multiple runs were used to accurately assign individuals to respective genetic clusters (Beaumont and Rannala 2004; François et al. 2006). One non-spatial exploratory Bayesian clustering analysis was performed (STRUCTURE v2.3.4), as well as two spatial analyses with different underlying methods of Bayesian analysis (TESS v2.3 and GENLAND v4.0.6). The latter two programs also input geographic coordinates as a parameter for interpreting genetic structure, and the use of multiple Bayesian clustering

analyses (spatial and non-spatial) can help elucidate complex patterns, and aid in validating results if patterns are concordant among different Bayesian clustering methods (Safner et al. 2011).

The data were run through STRUCTURE v2.3.4 (Pritchard et al. 2000) using correlated allele frequencies in the admixture model, and sampling locations as *locpriors*. The *locpriors* option allows sampling location information to be input into the model, but will not create population structuring where there is none. Ten independent runs were performed with 50,000 MCMC repetitions and a 10,000 burn-in period for K values varying from 1-10. After these initial runs, both LnPr(X|K), and delta K (ΔK; Evanno et al. 2005) from runs were averaged in STRUCTURE HARVESTER v0.6.94 (Earl and vonHoldt 2012), and then examined to determine the optimal value of K. Once K was determined, 10 additional runs were performed with K fixed at this value to ensure that the algorithm had converged properly, and that cluster assignment was consistent across runs. For the optimal value of K, any clusters that included more than one population were run through the program independently using the above settings to test for additional substructure.

TESS v2.3 (Chen et al. 2007) was run for K values from 2-10 using 100,000 sweeps and 50,000 burnin, and Ψ (value determining how much geographic coordinates influence clustering) set to 0.6. K was selected based on the runs with the highest posterior probability and highest deviance information criterion (DIC). As with STRUCTURE, once K was determined, any clusters including more than one population were run through the program independently to test for additional substructure. For this hierarchical analysis, K was determined in the same manner as described above.

GENELAND v4.0.6 (Guillot et al. 2005) was first run to evaluate the optimal value of K using a correlated alleles model, 500,000 iterations, thinning of 200, a burnin of 500, and uncertainty of spatial coordinates set to 10 km. Default settings were used for the maximum rate of the Poisson process, and the maximum number of nuclei in the Poisson-Voronoi tessellation. The optimal value of K was determined by examining the posterior probabilities averaged over multiple runs (ten runs allowing K to vary from 1-10), and choosing the K value with the highest average posterior probability. At this fixed K-value, 10 runs were conducted with the same parameters as the original run.

2.2.6 Principal Coordinates Analysis

To examine genetic structure from a multivariate perspective, we ran a principal coordinates analysis (PCoA) using GenAlEx v6.5 (Peakall and Smouse 2012). Multivariate methods can summarize highly variable data, such as multi-locus genotype data, into a few axes that can easily be visualized. Because it does not make any assumptions about the input data (e.g. Hardy-Weinberg Equilibrium), PCoA is well suited for genetic data (Jombart et al. 2009). Furthermore, patterns revealed by multivariate analyses of genetic data are increasingly being used to further validate Bayesian clustering patterns (Basto et al. 2016). The PCoA was run on the matrix of F'_{ST} values for the microsatellite data, and the three axes containing the most variation were retained (Appendix 4). A three-dimensional plot was made in R using the 3D Scatter Plot package (R Core Team 2016) to visualize the first three principal coordinates.

2.2.7 Species distribution modeling

To examine suitable habitat for Ruffed Grouse across the range, we constructed a species distribution model (SDM). The SDM combines data on the current distribution of a species in the form of occurrence data, with environmental data to predict areas where environmental conditions are suitable for a species' habitat requirements. A SDM is more specific than a distribution map because it identifies the suitability of habitat within the range, and can predict suitability of habitat in areas that have not been censused. It is also the first step in creating a resistance surface to model dispersal routes. We obtained 53,145 Ruffed Grouse occurrences from the Global Biodiversity Information Facility (GBIF; http://data.gbif.org/, accessed on 24 January 2017). Observations from non-scientific institutions that were not reviewed or moderated were removed, and we further excluded any occurrences that were recorded before 1980 to ensure accuracy of georeferencing. Environmental data were obtained from the WORLDCLIM dataset (v1.4, http://www.worldclim.org/). We used the BIOCLIM layers (Hijmans et al. 2005) for the current time period, which consist of 19 variables of different measures of climate using precipitation and/or temperature data from 1960-1990. The MODIS-based Global Land Cover Climatology layer (Broxton et al. 2014) was obtained from the USGS Land Cover Institute (https://landcover.usgs.gov/, accessed on 8 February 2017). This layer contains high-resolution data on global land cover types from 2001-2010, which we choose to add to the SDM for more accuracy in predicting suitable habitat for Ruffed Grouse. This layer was clipped to the same extent as the BIOCLIM layers.

Data were prepared for ecological niche modeling using the SDMtoolbox v1.1c (Brown 2014) for ArcGIS. The occurrence data were edited by removing all duplicate records, and then rarified at a distance of 30 km to aid in accounting for sampling bias

towards human settlements and roads; 2,421 occurrences were retained. The BIOCLIM and MODIS layers were clipped to the extent of North America and then projected in World Geodetic System 1984 using ArcMap v10.2 (ESRI®). Due to the similarity of some of the climatic variables used in the layers, we tested for layer autocorrelation at the spatial scale of the North American continent. For pairs of layers that were highly correlated (R>0.90), one of each pair was removed from the model, so as not to bias the SDM. This remaining ten BIOCLIM layers (1, 2, 3, 4, 8, 9, 12, 14, 15, 18; Appendix 5) were used along with the MODIS land cover layer, and rarefied occurrence data to create the SDM. The Gaussian kernel density tool in SDMtoolbox was used to create a bias layer that was added to the model to aid in further accounting for anthropomorphic bias, which is important when modeling very widely distributed species, particularly those that range into areas with relatively low human populations (e.g. near the poles; Phillips et al. 2009).

The environmental layers and occurrence data were imported into MaxEnt v3.3.3 (Phillips et al. 2006) along with the Gaussian kernel density bias file to create the SDM. The most appropriate settings were determined in ENMTools v1.3 (Warren et al. 2010), using Akaike's information criterion (AIC_C), and Bayesian information criterion (BIC) values. Settings used were: hinge features only, regularization multiplier = 1, a replicate run type of 10 cross-validations, maximum number of background points = 10,000, 500 maximum iterations and a 0.00001 convergence threshold. For training the model, 25% of the occurrence points were used and the SDM displayed using the cumulative scale. The most suitable model performed significantly better than expected at random with an area under curve (AUC) of 0.799, where 0.5 is when the fit of the model is no better than random, and values closer to one constitute a better fit. In most cases, it is not possible for

the actual value of AUC to reach one, and in this case the maximum AUC = 0.788. It is important to note that it is not unusual for the actual AUC of the test data to exceed the maximum value slightly (Phillips et al. 2006).

2.2.8 Dispersal route analyses

To evaluate whether the intervening landscape matrix leads to population differentiation by influencing dispersal routes and dispersal costs (i.e. isolation by resistance), we conducted least cost path (LCP) and least cost corridor (LCC) analyses using SDMtoolbox v1.1c (Brown 2014) in ArcGIS v10.2 (ESRI®). The SDM was inverted to create a friction layer, and geographic coordinates for each sampling site were entered in decimal degrees. LCPs and LCCs were calculated between each population pair using the friction values. To calculate LCCs, the LCPs were weighted by resistance values based on the friction layer, then categorized using a 'percentage of LCP' method with cutoffs for inclusion into high-, mid- and low-classes set at 5%, 2%, and 1% of the LCP value respectively. The weighted and categorized LCPs were then summed to create a dispersal network. On the network map, dispersal corridors were heat-mapped using warm colours for high dispersal potential (or low resistance), and cool colours for lower dispersal potential (high resistance).

2.2.9 Isolation by distance

To determine whether genetic structuring between populations is due to physical barriers or simply a product of the geographic distance between them, we tested for isolation by distance in GenAlEx (Peakall and Smouse 2012) by comparing pairwise F'_{ST} with geographic distance. We calculated geographic distance as a straight line between

populations, where lines were kept within the boundaries of the species' geographic distribution.

2.2.10 Isolation by resistance

To explicitly test for isolation by resistance (IBR), we used a similar analysis as described for IBD. Matrices of genetic distance (F'_{ST}) and resistance values were assessed for correlations using a paired Mantel test implemented in GenAlEx v6.5 (Peakall and Smouse 2012). The LCP distance is the distance of the least cost path between each population pair. The resistance distance is the LCP distance weighted with dispersal cost values from the friction layer to account for not only dispersal distance through appropriate habitat, but also the degree of resistance to dispersal depending on the intervening habitat. After calculating the Mantel tests for all population comparisons, the same tests were performed at a regional scale, as well as within each region (if the number of sampling sites permitted).

2.3 Results

2.3.1 Genetic diversity

The autosomal locus, SLC45a2 had a total of eight shared haplotypes and an additional seven that were singletons for sequences from seven populations (Appendix 1). Haplotype diversity (H_d) ranged from 0.000 (AK) to 0.825 (WA) for SLC45a2, and nucleotide diversity (π) from 0.0000 (AK) to 0.00236 (WA). The mitochondrial Control Region sequences from seven populations showed 11 shared haplotypes and 11 singletons, haplotype diversity (H_d) ranging from 0.400 (BL, CP) to 0.970 (MN), and values ranging from 0.00102 (CP) to 0.02300 (MN) for π (Table 2.1).

A total of 351 individuals from 15 sampling sites (Fig. 2.1) were genotyped at 8 variable microsatellite markers. Of those, 324 samples were successfully amplified and scored at six or more of the microsatellite loci, with alleles per locus ranging from five to 28 (Appendix 3). Observed heterozygosity across loci ranged from 0.548 (YT) to 0.663 (CP), and expected heterozygosity ranged from 0.590 (YT) to 0.688 (COA) (Table 2.2). Significant deviations from Hardy-Weinberg equilibrium only occurred at more than one locus in two populations: BL and EA. The BL population had a significant heterozygote deficit at TUT4, SGCA5, and BG18, and the EA population at BG15, SGCA5, LLSD7, TUT2, and BG18. Allelic richness (A_R) ranged from 3.29 (AK) to 4.10 (WI; Table 2.2), and 10 of 15 populations contained private alleles (Table 2.2). Most populations had one to three private alleles, but BL and BV both had five. Also notable was the high frequency (0.14) of a single PA for the PR population.

2.3.2 Genetic structure

Pairwise F_{ST} values for SLC45a2 sequences ranged from the lowest value of -0.013 for BL and EA, to the highest value of 0.348 for YT and WA (Table 2.3). The second highest value was $F_{ST} = 0.303$ for the WA and MN pairwise comparison, and all but one of the significant F_{ST} values were comparisons including the Washington population. The only other significant value (0.182) was for the comparison of Alaska and Crowsnest Pass populations. The mitochondrial Φ_{ST} ranged from -0.057 for the CP:EA comparison to 0.660 for the AK:WA comparison (Table 2.3). Furthermore, all 11 significant Φ_{ST} values included WA or AK.

The statistical parsimony network constructed for SLC45a2 sequences suggests the absence of geographic structure at this marker (Fig. 2.2a). Most haplotypes are only

one mutational step removed from each other, and there are two main haplotypes that include a large proportion (64%) of the total alleles. The two dominant haplotypes among Washington individuals are uncommon in other populations. Aside from Washington, there are no apparent geographic patterns, which is corroborated by the F_{ST} values. The only significant F_{ST} value for SLC45a2 not including the Washington population is between the Crowsnest Pass and Alaska populations.

The statistical parsimony network for the mitochondrial CR exhibits a stronger spatial pattern than the network from the nuclear marker (Fig. 2.3a). The samples from the Alaska population cluster together within the network, as do most of the samples from the Washington population. These two populations are the only two groups that are significantly different from other populations according to Φ_{ST} values. Samples from Alberta loosely cluster together on the network (Fig. 2.3a), but also share haplotypes with other populations (Fig. 2.3b). The Minnesota samples also show a slight geographic pattern with two clusters, but the most noticeable characteristic of this population is the large diversity of haplotypes present (H_d=0.97; Fig. 2.3b).

The Fisher's exact tests performed on the Aldolase B SNP resulted in statistically significant comparisons for all population pairs including AK or CP (Table 2.4; Fig. 2.4). Comparisons between WA and other populations were also statistically significant for all but four pairs (COA, GP, PR, and WI). Of the remaining population comparisons, only three were significant; EA:WI, BV:GP, and BV:WI. Like both the SLC45a2 and Control Region loci, the Aldolase B SNP reveals divergence of the Washington population (Table 2.4; Fig. 2.4).

Pairwise F'_{ST} values of microsatellite loci ranged from -0.083 (BL:PR) and 0.526 (AK:WI; Table 2.5). After FDR corrections, 67 out of 105 comparisons were significant.

Three populations (AK, YT, and WA) were significantly differentiated from all other populations, while CP was significantly differentiated from all but PR. In addition the MN population was significantly differentiated from all but two other populations (WI, and PR), and similarly WI was differentiated from all but three populations (MN, PR, and COA). The population divergence map displaying the interpolated pairwise F'_{ST} values clearly shows the low differentiation among all northern and central Alberta populations, and the AK, YT, and WA populations are the most differentiated (Appendix 6).

2.3.3 Bayesian clustering analyses

Plots of delta K (ΔK) and mean log likelihood (LnPr(X|R); Appendix 7) from the initial STRUCTURE runs indicated five groupings as the most appropriate assignment of K. The five clusters identified by STRUCTURE were as follows: Alaska + Yukon, Washington, Crowsnest Pass, the remaining Alberta populations (COA, BL, EA, GP, PR, AT, FM, BV, and LM), and the Great Lakes (MN and WI) (Fig. 2.5). Evidence of potential substructure in the data was indicated by admixture present in the Q values, and the bimodal distribution of ΔK. To investigate this possibility, we ran individuals from each cluster independently. Only the AK-YT cluster was subsequently divided into two populations. None of the other clusters yielded further groupings. For the final groupings, most individuals displayed $Q \geq 0.70$ membership to their respective clusters with the only exceptions in the Alberta cluster (Appendix 8). These exceptions were the GP, PR, and BV populations, and due to the lack of additional substructure, we concluded that the mixed ancestry of these Alberta individuals is true admixture. The BV population has a considerable amount of admixture ($Q = 0.15 – 0.35$) with the WA and Great Lakes clusters, and is the only sampled population that has a substantial amount of admixture

with the Great Lakes cluster. The admixture in the GP and PR clusters, which are in close proximity, varies from Q = 0.15 – 0.40 membership with the AK/YT cluster, and Q = 0.10 – 0.25 membership with the WA cluster. The total number of clusters is therefore six when substructure is included, which may explain why the ΔK plot has a bimodal distribution. Although the ΔK plot showed a second peak at K = 7, we could not clearly identify a seventh cluster in the data. Furthermore, the second highest value in the log likelihood plot corresponds to K = 6, and visual inspection also suggested six clusters, so we took K = 6 to be the true value of K, which is concordant with pairwise F'_{ST} (Table 2.5; Fig. 2.5).

The spatial Bayesian clustering performed in TESS showed K = 4 with potential substructure, as indicated by both DIC and log likelihood values (Appendix 9). The DIC plot was bimodal with a second peak at K = 7; however, when examined, the Q plots for K = 7 showed clear oversplitting of clusters. We therefore concluded as with STRUCTURE, that once hierarchical analysis was performed to reveal substructure, the true number of clusters was K = 6 (Appendix 10; Fig. 2.5).

GENELAND indicated K = 7 at the highest frequency over the MCMC chain, which was in agreement with the highest value for the averaged posterior probabilities of the initial set of runs (Appendix 11). Five of the seven groupings identified by GENELAND corroborated the clusters inferred by STRUCTURE and TESS: AK, YT, WA, CP and Great Lakes. In addition to those five, GENELAND split GP from the remaining Alberta populations (Fig. 2.5; Appendix 12).

2.3.4 Principal Coordinates Analysis

The PCoA using F'_{ST} values showed distinct genetic groupings, with the first and second axes accounting for 35.6% and 19.7% of the variation respectively, and the third axis explaining 16.0% of the variation (Appendix 4). When all three axes are examined together as a three-dimensional plot, it is clear that AK, YT, WA, CP, MN, and WI show separation from all other populations (Fig. 2.6). The majority of the Alberta populations (COA, BL, EA, AT, FM, BV, LM), cluster together as they do in all other analyses, and the GP and PR populations clustered together. Although the GP and PR populations were separated from the main cluster of Alberta populations, they were in much closer proximity to these remaining Alberta populations than to the other sampled populations. The groupings of the PCoA confirm groupings identified by TESS and STRUCTURE, and potentially show evidence of some divergence of the GP population (see above and Fig. 2.5).

2.3.5 Species distribution modeling & dispersal route analyses

The SDM closely matches the species' known distribution (Fig. 2.1), which indicates that the environmental variables used to build the model were sufficient to accurately reflect the species' habitat preferences (Fig. 2.7). The layers that contributed most to the model were land cover, annual mean temperature, and isothermality, at 36.1%, 22.2%, and 21.9% respectively (Appendix 5).

When the dispersal routes are examined across the SDM, it is clear that some populations appear to have direct dispersal routes between them, while others do not (e.g. eastern Alberta to Minnesota versus Washington to Alaska; Fig. 2.8a). The least cost corridors (LCC) revealed high niche connectivity among most of the Alberta populations,

particularly those in the center of the province, and a dispersal route with low resistance stretching across the parkland between eastern Alberta and the Great Lakes area (Fig. 2.8b). The LCC (Fig. 2.8b) implies high elevation mountains may act as barriers to Ruffed Grouse dispersal. There is low niche suitability in much of the high elevation mountains (Fig. 2.7), with one corridor through the intermountain west, and another along the Peace River valley, which is the only river valley to penetrate the entire width of the Rocky Mountains (Fig. 2.8b; Cannings et al. 2011). The corridor through the intermountain west appears to provide connectivity between south-central Alberta and populations west of the Rockies (e.g. Washington). There is potential for moderate dispersal in Washington, and high dispersal through northeastern Washington, and northern Idaho. The dispersal route connecting the Yukon to those populations south of it has moderate to high resistance; it runs between the Rocky and Coast Mountains and then connects with the corridor through the Peace River Valley (Fig. 2.8b). No direct dispersal routes exist among Yukon, Alaska and Washington populations, and the only dispersal route connecting Alaska to the other sampled populations has high resistance.

2.3.6 Isolation by Distance

The Mantel test for isolation by distance using Euclidean distance and pairwise F'_{ST} resulted in a moderate pattern of isolation by distance when all sampled populations were compared ($R^2 = 0.378$; $P = 0.01$; Table 2.6; Appendix 13). A more distinct pattern of IBD was present when only comparing populations east of the Rockies; the Alberta and Great Lakes clusters ($R^2 = 0.567$; $P = 0.02$; Table 2.6). Comparing only western populations (WA, AK, and YT) resulted in a moderate correlation ($R^2 = 0.567$, $P = 0.03$), and IBD was also significant for the comparison of the Alberta, Alaska, and Yukon

populations ($R^2 = 0.806$; $P = 0.002$). The only non-significant comparisons are those comparing CP to the remaining Alberta sampling sites ($R^2 = 0.190$, $P = 0.09$), and the comparison of the other populations within the Alberta (COA, BL, EA, GP, PR, AT, FM, BV, LM; $R^2 = 0.082$, $P = 0.063$).

2.3.7 Isolation by Resistance

When a Mantel test was performed on all populations sampled, the correlation between LCP and genetic distance (F'_{ST}) was considerably higher ($R^2 = 0.649$, $P = 0.01$; Table 2.6; Appendix 13) than the value calculated for IBD ($R^2 = 0.370$, $P = 0.01$), and the correlation between IBR and genetic distance was higher yet ($R^2 = 0.674$, $P = 0.001$). This pattern held for most other comparisons (Table 2.6). When only populations east of the Rockies (COA, BL, EA, GP, PR, AT, FM, BV, LM, MN, and WI) were considered, genetic distance versus resistance values (IBR) performed moderately better than IBD ($R^2 = 0.655$, $P = 0.014$), whereas for populations west of the Rockies (AK, YT, WA, CP; Table 2.6), IBR had a considerably higher correlation value than IBD.

On a finer scale, we compared only populations from Alberta, and the LCP distances alone were not enough to explain the differentiation between CP and the rest of Alberta ($R^2 = 0.111$, $P = 0.050$). Adding the resistance values yielded a significant, but relatively weak correlation ($R^2 = 0.361$, $P = 0.030$). This supports the conjecture that there is a weak signal of IBR explaining only a portion of the divergence of the CP population. Furthermore, the correlation values for regional comparisons including CP all increased noticeably when CP was excluded (Table 2.6). Only the rest of Alberta (i.e., excluding CP) had enough sampling sites to perform a within region comparison, but no significant correlations were detected.

2.4 Discussion

In this study, we used multi-locus genetic data and environmental variables to identify how western populations of Ruffed Grouse are genetically structured. The populations of Ruffed Grouse that were sampled in this study have significant genetic differentiation, and, in some cases, evidence of limited population connectivity. Due to the species' preference for aspen-dominated mixed forest, both macrogeographic barriers and tracts of unsuitable habitat are likely playing important roles in creating genetically structured populations.

2.4.1 Contemporary population genetic structure and macrogeographic barriers

Data from multiple neutral genetic markers show structuring of Ruffed Grouse populations across their range at various spatial scales. Aside from the most highly differentiated populations, AK and WA, there are at least four other distinct genetic groups in our samples: Yukon, southwest Alberta (CP), a large one including most of central/northern Alberta, and one near the Great Lakes (Fig. 2.5). Most pairwise comparisons occurring between these groups are significant with high overall values for F'_{ST}. Although these results confirmed our postulation of genetic differentiation occurring across the range due to the low dispersal capability of this species, the degree of differentiation was somewhat unexpected. The magnitude of fixation indices was high for some populations, with values that are more typical of highly fragmented populations (Barry and Tallmon 2010; Rutkowski et al. 2012).

A number of landscape features correspond with the boundaries of genetic clusters for Ruffed Grouse across western North America. The Columbia River basin and

northern extent of the Great Plains impose sharp limits on the species' range and mountain ranges appear to be a prominent barrier within the western extent of the range. The Alaska, Wrangell, Ray, and Chugach Mountains effectively isolate the Alaska population, as supported by divergence of this population at the microsatellite loci, Z-linked SNP, and Control Region. In addition, the mitochondrial Control Region shows very little haplotype sharing with any of the other sampled populations (Fig. 2.3). The Yukon population is similarly isolated by the same mountain ranges preventing connectivity with the Alaska population, and by the Mackenzie Range restricting connectivity with populations to the east. Mountains also correspond to genetically restricted populations in other parts of the range; a significant genetic break is present between the Washington population and the Alberta populations. These patterns seem to be evidence of the Rocky Mountains acting as a barrier to gene flow. The Washington population is the most genetically distinct, which is evidence that gene flow with other Ruffed Grouse populations has been restricted long-term. This is supported by the microsatellite data (Table 2.5), nuclear marker data (Table 2.3; Table 2.4), and minimal haplotype sharing in the Control Region (Fig. 2.3). The divergence of the WA population also suggests the Rockies may be acting as a barrier. If the Cascade Range was acting as a barrier, substructure should have been detected within the WA cluster through Bayesian analyses because the sampling sites are on both sides of the Cascades. Bayesian methods do not use *a priori* population assignments, so any potential substructure should be apparent in the analyses, regardless of how samples were grouped (Fig. 2.5; Appendix 8, 10). Furthermore, the Cascade Range contains more suitable mixed forest habitat (Pater et al. 1998; Broxton et al. 2014), and generally lower elevation passes than the northern Rockies (Franklin and Dyrness 1973). Similar patterns have been observed in a

widespread generalist passerine, the Black-capped Chickadee (*Poecile atricapillus*), where the habitat composition of a mountain range corresponds to restricted gene flow (Adams and Burg 2015b). The Black-capped Chickadee also shows similar patterns of isolation of populations in Alaska, northwest British Columbia, and on either side of the southern Rockies (Hindley 2013; Adams and Burg 2015b).

The increased number of sampling sites in Alberta allowed us to assess genetic structure on a finer scale for this region. While most of the Alberta populations are not differentiated from one another, the Crowsnest Pass population is consistently isolated from all other populations for most nuclear loci (Fig. 2.4; Fig. 2.5; Fig. 2.6). Because the CP population does not have significant differentiation at the Control Region (Fig. 2.3), it is unlikely that divergence of this population reflects historical population structure. Instead, the differentiation of the CP population likely arose due to contemporary barriers to gene flow. In some species, southwest Alberta populations are divergent from individuals sampled throughout the rest of Alberta, and instead group with either British Columbia populations (Hindley 2013) or with intermountain west (i.e., Montana, Idaho, Wyoming) populations (Pulgarín-R and Burg 2012; Dohms et al. in press), implying that the geography of the Rocky Mountains may affect the genetic structure of species differently depending on their life history. The differentiation of populations located on either side of the Rocky Mountains (central and southern Alberta populations versus Washington populations; Fig. 2.5) is evidence of the Rocky Mountain Range acting as a barrier to gene flow for Ruffed Grouse populations.

The presence of unsuitable habitat may also be restricting gene flow, particularly for the CP population. Only a narrow swath of suitable Ruffed Grouse habitat presently connects southwestern Alberta and the rest of the province; most of the southeast part of

the province is open grassland, which they are reluctant to disperse through (Yoder 2004), and the Rocky Mountains run along the western edge of the province. The habitat in the Rocky Mountains consists mainly of contiguous coniferous forest, with suitable mixed forest habitat occurring mostly on low elevation slopes and valleys (Natural Regions Committee 2006; Broxton et al. 2014). Although Ruffed Grouse are more likely to disperse through coniferous forests than grasslands, their short dispersal distances (approx. 2-4 km; Yoder 2004) suggest that dispersal through vast expanses of coniferous forest are likely to be infrequent. Because the CP population is in close spatial proximity to some of the other populations sampled in Alberta, geographic distance is unlikely to be a causal factor for population differentiation and this is corroborated by IBD analysis (Table 2.6). Therefore, the combination of the Rocky Mountains as a physical barrier, as well as the northwest corner of the Great Plains meeting the Rocky Mountain Front are likely the main factors isolating the CP population. However, this assertion would be strengthened by the addition of more sampling locations west of the Rockies, such as sites in Montana, Idaho, and British Columbia.

All microsatellite analyses were concordant in the assignment of genetic clusters with the notable exceptions of two Alberta populations: GP and PR. The PR population grouped with the main Alberta cluster in all but one analysis (PCoA), where it clustered with GP (Fig. 2.6). However, the GP population either grouped with rest of the Alberta or formed a unique genetic cluster (Table 2.5; Fig. 2.5; Fig. 2.6). GENELAND is more sensitive than other Bayesian clustering programs (Safner et al. 2011), which could be why it identified GP as a differentiated population. Furthermore, this cluster shows a more gradual genetic cline with the populations around it in the GENELAND posterior probability maps, as opposed to the strong genetic breaks evident among some of the

other clusters (Appendix 12). While there is not complete consensus for identification of the GP cluster, there is certainly evidence of differentiation of this population, which could be due to its proximity to the Peace River valley. The Peace River is the only river to cut a continuous valley through the entire width of the Rocky Mountain range (Feinstein 2010). Therefore, it is possible that genotype frequencies at GP are subject to an influx of genes from British Columbia through the Peace River corridor. This is supported by the STRUCTURE results, in which the PR and GP populations show some admixture with the AK/YT cluster (Appendix 8). The Peace River Valley may be a contact zone for Ruffed Grouse populations on either side of the Rocky Mountains. There is evidence that this important corridor facilitates connectivity for multiple species, particularly those reliant on mixed forest or shrubby habitat types, and would have difficulty dispersing through coniferous forest. For example, the Peace Region acts as a contact zone for a number of species (Toews et al. 2011; Seneviratne et al. 2016) that are otherwise geographically isolated by the Rockies, including Mourning (*Geothlypis philadelphia*) and MacGillivray's Warblers (*Geothlypis tolmiei*; Irwin et al. 2009). Furthermore, the permeability of a mountain barrier may range from porous (Vonhof et al. 2015) to impermeable (Irwin et al. 2011) depending on the species. The Bayesian clustering results (Fig. 2.5; Appendix 8) imply the Rocky Mountain Range is a mostly impermeable barrier for Ruffed Grouse, with some porous areas (i.e. Peace River) facilitating limited connectivity.

2.4.2 Landscape genetics: Isolation by distance or resistance?

Aside from mountain ranges, the presence of unsuitable habitat is the most prevalent potential barrier between our sampling sites. Due to the seemingly high degree

of habitat heterogeneity across the landscape, we incorporated environmental variables into our analyses to test their effects on the genetic structuring present, and to help further test the presence of putative geographic barriers discussed previously. LCP/LCC analysis revealed that dispersal cost varies across the landscape, and confirms our earlier hypothesis that mountain ranges are likely to impede dispersal among populations. Mountain ranges in Alaska, as well as the Rockies have markedly high levels of resistance to Ruffed Grouse dispersal, with the exception of two corridors through the mountains: one through the Peace River Valley, and one through the intermountain west, connecting south-central Alberta and northeast Washington (Fig. 2.8b). These corridors are areas with slightly milder climate and a higher proportion of mixed forest than the surrounding mountain slopes based on the MODIS land cover layer (Broxton et al. 2014) and BIOCLIM layers (Hijmans et al. 2005). Furthermore, the LCCs generally seem to correspond with tracts of mesic, mixed forest, which implies that variation in climate and forest type across the landscape may be important in creating population structuring.

Across all sampled populations, IBR explained significantly more of the genetic differentiation among populations than IBD. This pattern held at most spatial scales (Table 2.6); the only exception being the comparison within Alberta. The LCC between Alberta and the Great Lakes had low resistance, stretching across the parkland/boreal forest in a direct path (Fig. 2.8b), which is likely why IBR only moderately outperformed IBD for this comparison. This implies that the genetic distance between Alberta and Great Lakes populations is explained by a combination of physical distance and dispersal cost through intervening habitat at this large spatial scale. In contrast, the genetic differentiation among western populations (AK, YT, WA) was explained more by IBR than by IBD (Table 2.6). This is concordant with the LCP/LCC maps, which do not show

any direct dispersal routes between the AK, YT, and WA populations. Furthermore, the routes that were detected have moderate to high resistance. The patterns among these populations lend support to the idea that the higher heterogeneity of habitat types occurring west of the Rocky Mountains is restricting dispersal in this region.

Due to large sampling gaps among our populations outside of Alberta, it is difficult to be certain whether genetic boundaries between populations are gradual genetic clines or steep genetic breaks. IBD often manifests as smooth, clinal gradients between genetic clusters (see Mims et al. 2016), whereas steep boundaries among genetic clusters are more likely to occur in populations mediated by IBR (Coulon et al. 2006). The high pairwise F'_{ST} values and steep genetic cluster boundaries (Appendix 12) provide further evidence that Ruffed Grouse populations are distinct genetic clusters mediated by IBR. Furthermore, the patterns of IBR among Alberta populations and those west of the Rockies point to the Rocky Mountain Range as a barrier. The conifer-dominated habitat that characterizes high elevation mountains appears to present high dispersal costs to this species, which would explain why high elevation mountain ranges are a dispersal barrier to Ruffed Grouse. This is confirmed by the SDM and LCC, which show high elevation mountains as areas that are highly unsuitable, with the exception of the previously mentioned corridors (Fig. 2.7; Fig. 2.8b). These patterns lend further support to our hypothesis of the Rockies as a semi-permeable barrier.

Although IBR performed consistently better than IBD, neither of these models sufficiently explained the genetic differentiation between CP and adjacent populations. CP might therefore have additional factors affecting connectivity with other Ruffed Grouse populations. The environmental variables used to build the SDM for Ruffed Grouse explained much of the differentiation present among the populations sampled for

this study (Table 2.6), but it is possible that additional, unsampled, environmental factors are contributing to the genetic break at the CP population. In some species, genetic structure may not be evident at a broad spatial scale and is only detected when populations are assessed at a finer scale (Adams and Burg 2015b). Therefore, a smaller scale analysis with more environmental variables may aid in teasing apart the factors underlying the genetic differentiation of the CP population. Furthermore, differences in the vegetation communities of the intermountain west (including southern Alberta) compared to the boreal region (including central-northern Alberta) may be important. The shift in *Populus* species (e.g. *P. trichocarpa*, *P. angustifolia*, *P. balsamifera*) between these regions is an example of how the environmental conditions in these areas differ and may be contributing to genetic structure through local adaptation. These shifts in vegetation communities are also potentially important in other avian species that are genetically differentiated between the intermountain west and central Alberta (Dohms 2015; Adams and Burg 2015b). Although we were able to determine that the distribution of suitable habitat is highly correlated with genetic structure for many Ruffed Grouse populations, positively identifying the environmental factors that are most important in limiting or facilitating population connectivity will likely require explicit tests of competing models of IBR using additional environmental variables (e.g. topographic data, high-resolution data on presence/absence of plant species).

2.5 Conclusions

Our study is a first look into the population genetics of Ruffed Grouse, and we found that contemporary populations in the western extent of the range were highly genetically structured, with the strongest genetic breaks corresponding with high

elevation mountain ranges. Resistance modeling revealed that genetic structure in Ruffed Grouse is primarily influenced by the heterogeneous habitat mosaic of the western North American landscape. Dispersal routes seem to be restricted to areas where suitable mixed forest habitat is present, which lends support to our hypothesis of IBR in Ruffed Grouse, despite the widespread, relatively continuous range of this species. The research presented here is one of relatively few population genetic studies focusing on broadly distributed organisms, and one of even fewer that has revealed patterns of IBR in widespread, fairly continuously distributed organisms that may be expected to exhibit IBD (Pilot et al. 2006; Pease et al. 2009; Ball et al. 2010). This adds to the growing body of work highlighting the importance of evaluating the role of environmental variables in research of population genetic structuring (McRae and Beier 2007; Vergara et al. 2015). It also underscores the need for more landscape genetics studies focusing on broadly distributed taxa because they may be experiencing genetic isolation regardless of their relatively ubiquitous distributions.

2.6 References

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Table 2.1. Number of samples sequenced (n) at each sampling site (ID), number of haplotypes (H_n), haplotype diversity (H_d), and nucleotide diversity for nuclear intron SLC45a2 sequences, and mitochondrial Control Region sequences.

		SLC45a2 sequences				CR sequences			
Population	ID	n	H_n	H_d	π	n	H_n	H_d	π
Alaska	AK	4	1	0.000	0.00000	15	5	0.743	0.00517
Yukon	YT	9	2	0.569	0.00137	-	-	-	-
Washington	WA	8	5	0.825	0.00236	10	3	0.800	0.00609
Crowsnest Pass	CP	18	7	0.687	0.00178	5	2	0.400	0.00102
Buck Lake	BL	25	9	0.706	0.00144	5	2	0.400	0.00203
Edson area	EA	8	7	0.675	0.00166	5	3	0.700	0.00457
Lloydminster area	LM	-	-	-	-	5	3	0.900	0.00609
Minnesota	MN	8	4	0.742	0.00143	12	9	0.970	0.02300

Table 2.2. Sampling site or group of sampling sites used in analyses (Population), sample size per site (n), sampling site abbreviation (ID), number of different alleles occurring at a frequency of ≥ 5% (Na), private alleles (PA), allelic richness, (A_R), observed heterozygosity (Ho), and expected heterozygosity (He). Statistics that may be sensitive to low sample sizes were excluded for populations where N < 10.

Population	ID	n	Na	PA	A_R	Ho	He
Alaska	AK	22	5.25	0	3.29	0.549	0.606
Yukon	YT	13	5.62	2	3.56	0.548	0.590
Washington	WA	23	5.05	2	3.43	0.562	0.635
Crowsnest Pass	CP	36	6.87	2	3.69	0.663	0.674
Cochrane area	COA	12	5.50	0	3.78	0.575	0.688
Buck Lake	BL	29	7.50	5	3.87	0.651	0.682
Edson area	EA	63	9.25	3	3.81	0.586	0.663
Grande Prairie	GP	18	5.87	1	3.85	0.661	0.675
Peace River	PR	8	5.12	-	-	-	-
Athabasca area	AT	29	7.12	2	3.55	0.598	0.620
Fort McMurray	FM	11	5.62	1	3.68	0.589	0.636
Bonnyville area	BV	19	6.62	5	3.77	0.609	0.631
Lloydminster	LM	13	5.62	0	3.64	0.606	0.646
Minnesota	MN	21	6.88	1	4.06	0.590	0.684
Wisconsin	WI	7	5.38	-	-	-	-

Table 2.3. Pairwise F_{ST} values of SLC45a2 sequences for comparisons of seven populations are above the diagonal, and pairwise Φ_{ST} values of Control Region for seven populations are below the diagonal. Population labels for SLC45a2 are on the top and right, and labels for Control Region are on the bottom and left. Warmer colours indicate population pairs that are more highly differentiated. Comparison values that were significantly different after FDR correction are marked with an asterisk.

	AK	YT	WA	CP	BL	EA	MN	
AK	·	0.228	0.302	**0.182***	0.049	0.113	0.145	AK
WA	0.660*	·	**0.348***	0.046	0.051	0.149	0.003	YT
CP	0.656*	0.447*	·	**0.212***	**0.169***	0.025	**0.303***	WA
BL	0.615*	0.442*	0.000	·	0.039	0.060	0.007	CP
EA	0.631*	0.348*	-0.057	0.071	·	-0.013	0.012	BL
LM	0.450*	0.431*	0.222	0.000	0.192	·	0.058	EA
MN	0.280*	0.344*	0.228	0.200	0.212	0.115	·	MN
	AK	WA	CP	BL	EA	LM	MN	

Table 2.4. Significance values of Fisher's exact test (Fisher 1922) for allele frequency pairwise population comparisons of the biallelic Aldolase B SNP. Significant values ($P < 0.05$) are bolded.

	AK	YT	WA	CP	COA	BL	EA	GP	PR	AT	FM	BV	LM	MN	WI
AK	•														
YT	**0.001**	•													
WA	**0.001**	**0.001**	•												
CP	**0.0270**	**0.0245**	**0.001**	•											
COA	**0.001**	0.334	0.060	**0.001**	•										
BL	**0.001**	0.316	**0.003**	**0.001**	0.874	•									
EA	**0.001**	0.605	**0.001**	**0.001**	0.472	0.472	•								
GP	**0.001**	0.072	0.147	**0.001**	0.868	0.389	0.089	•							
PR	**0.001**	0.253	0.331	**0.001**	0.824	0.635	0.361	1.00	•						
AT	**0.001**	0.747	**0.001**	**0.001**	0.566	0.586	1.00	0.130	0.472	•					
FM	**0.001**	0.472	**0.024**	**0.001**	1.00	1.00	0.772	0.472	0.824	0.874	•				
BV	**0.001**	1.00	**0.001**	**0.008**	0.281	0.160	0.472	**0.028**	0.173	0.515	0.403	•			
LM	**0.001**	0.472	**0.024**	**0.001**	1.00	1.00	0.772	0.472	0.824	0.874	1.00	0.403	•		
MN	**0.001**	0.159	**0.046**	**0.001**	1.00	0.635	0.281	0.874	1.00	0.395	0.873	0.100	0.873	•	
WI	**0.001**	0.032	1.00	**0.001**	0.272	0.130	**0.041**	0.472	0.635	0.060	0.158	**0.023**	0.158	0.311	•

Table 2.5. Pairwise F'_{ST} comparisons of data from seven microsatellites for fifteen populations of Ruffed Grouse. Values that were significant values after FDR correction for multiple testing are bolded, and level of significance is indicated by an asterisk.

	AK	YT	WA	CP	COA	BL	EA	GP	PR	AT	FM	BV	LM	MN	WI
AK	•														
YT	**0.392*** **	•													
WA	**0.481*** **	**0.324*** **	•												
CP	**0.340*** **	**0.179*** **	**0.210*** **	•											
COA	**0.378*** **	**0.319*** **	**0.198** **	**0.094***	•										
BL	**0.252*** **	**0.271*** **	**0.284*** **	**0.130*** **	0.033	•									
EA	**0.335*** **	**0.251*** **	**0.239*** **	**0.127*** **	0.008	0.005	•								
GP	**0.288*** **	**0.242*** **	**0.193*** **	**0.097** **	-0.016	0.042	**0.0270***	•							
PR	**0.234*** **	**0.196*** **	**0.147*** **	0.017	-0.001	-0.083	-0.044	-0.028	•						
AT	**0.335*** **	**0.266*** **	**0.268*** **	**0.124*** **	0.098	0.024	0.046	**0.081***	-0.014	•					
FM	**0.375*** **	**0.233*** **	**0.296*** **	**0.208*** **	0.045	0.007	0.024	0.096	-0.019	0.022	•				
BV	**0.336*** **	**0.266*** **	**0.200*** **	**0.110** **	0.018	-0.013	0.010	**0.065** **	-0.060	0.051	0.017	•			
LM	**0.288*** **	**0.463*** **	**0.332*** **	**0.175*** **	0.027	0.008	0.058	0.065	-0.016	0.061	0.090	0.016	•		
MN	**0.387*** **	**0.292*** **	**0.186*** **	**0.203*** **	**0.206*** **	**0.225*** **	**0.164*** **	**0.067** **	0.019	**0.284*** **	**0.248*** **	**0.149*** **	**0.247*** **	•	
WI	**0.526*** **	**0.333*** **	**0.405*** **	**0.200** **	0.079	**0.180** **	**0.092** **	**0.042** **	0.046	**0.235** **	**0.174** **	**0.146** **	**0.210** **	0.063	•

* $P < 0.05$, ** $P \leq 0.001$

Table 2.6. Results of Mantel tests for three comparison types; isolation by distance (IBD), isolation by distance using least cost path distance (LCP), and isolation by resistance (IBR). Different combinations of populations were compared at multiple spatial scales for each model. The correlation value for each comparison is reported (R^2), along with the significance level of each test.

Populations compared	IBD	LCP	IBR
Overall	$R^2 = 0.370$	$R^2 = 0.649$	$R^2 = 0.674$
	$p = 0.010$	$p = 0.010$	$p = 0.001$
Alberta and Great Lakes	$R^2 = 0.567$	$R^2 = 0.585$	$R^2 = 0.655$
(COA, BL, EA, GP, PR, AT, FM, BV, LM, MN, WI)	$p = 0.020$	$p = 0.024$	$p = 0.014$
Alaska, Yukon, Washington, and S. Alberta	$R^2 = 0.380$	$R^2 = 0.425$	$R^2 = 0.579$
(AK, YT, WA, CP)	$p = 0.020$	$p = 0.004$	$p = 0.001$
Alaska, Yukon, and Washington	$R^2 = 0.567$	$R^2 = 0.668$	$R^2 = 0.834$
(AK, YT, WA)	$p = 0.030$	$p = 0.042$	$p = 0.019$
Alberta, Alaska, and Yukon	$R^2 = 0.806$	$R^2 = 0.835$	$R^2 = 0.853$
(COA, BL, EA, GP, PR, AT, FM, BV, LM, AK, YT)	$P = 0.002$	$P = 0.001$	$P = 0.004$
Alberta, S. Alberta, and Washington	$R^2 = 0.575$	$R^2 = 0.592$	$R^2 = 0.645$
(COA, BL, EA, GP, PR, AT, FM, BV, LM, CP, WA)	$p = 0.001$	$p = 0.001$	$p = 0.002$
Alberta and Washington	$R^2 = 0.594$	$R^2 = 0.631$	$R^2 = 0.708$
(COA, BL, EA, GP, PR, AT, FM, BV, LM, WA)	$p = 0.010$	$p = 0.010$	$p = 0.020$
Alberta and S. Alberta	$R^2 = 0.190$	$R^2 = 0.111$	$R^2 = 0.361$
(COA, BL, EA, GP, PR, AT, FM, BV, LM, CP)	$p = 0.090$	$p = 0.050$	$p = 0.030$
Alberta (within cluster comparison)	$R^2 = 0.082$	$R^2 = 0.065$	$R^2 = 0.266$
(COA, BL, EA, GP, PR, AT, FM, BV, LM)	$p = 0.063$	$p = 0.065$	$p = 0.267$

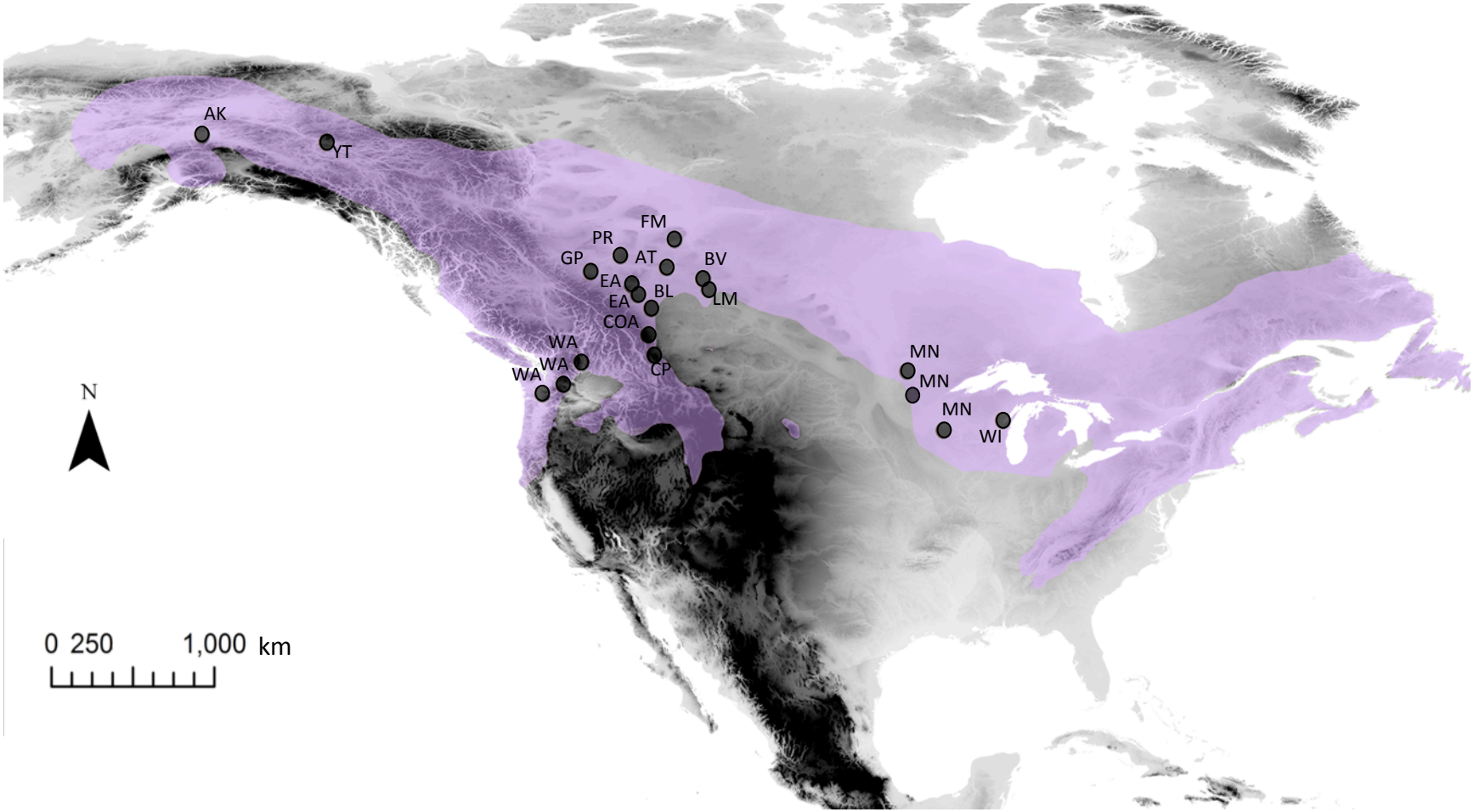
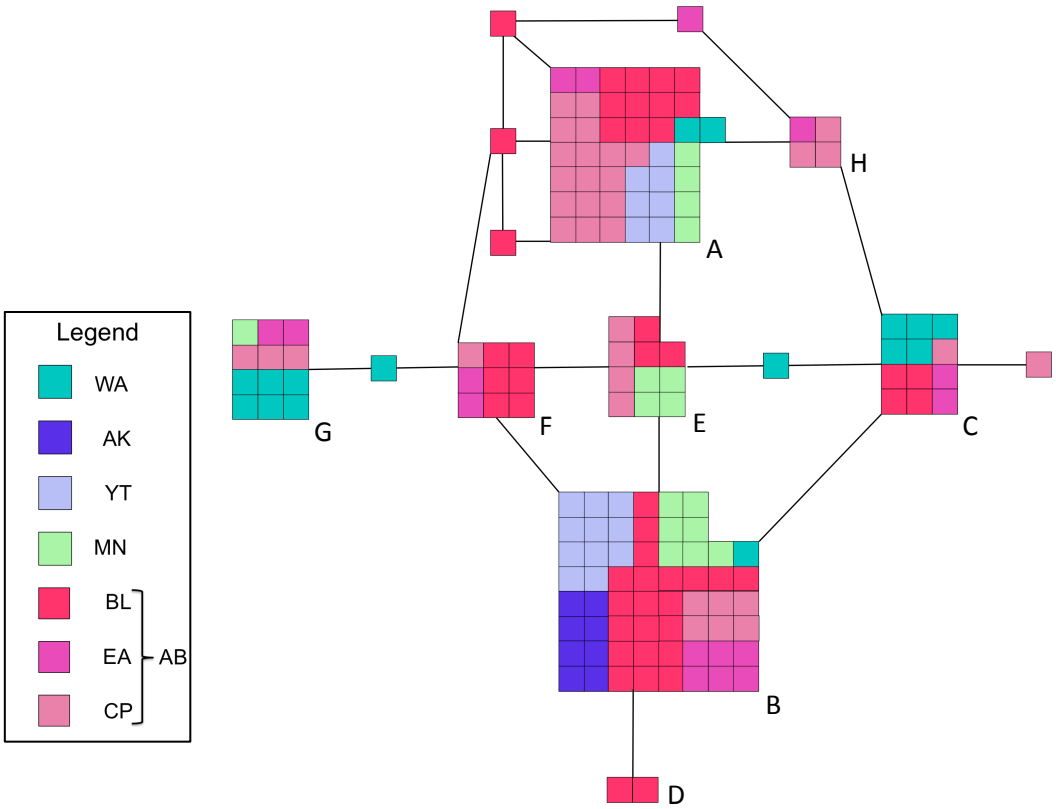


Figure 2.1. Map showing the current range of the Ruffed Grouse (*Bonasa umbellus*), and sampling sites for this study. Sampling sites with the same label were pooled for analyses due to close proximity or lack of sufficient sampling at one or more of these sites. Site abbreviations available in Table 2.3. The data for the range distribution were taken taken from Birds of North America Online, and was projected and overlaid onto a digital elevation map of North America in ArcGIS® v10.2. Digital elevation map courtesy of ESRI®.

a)



b)

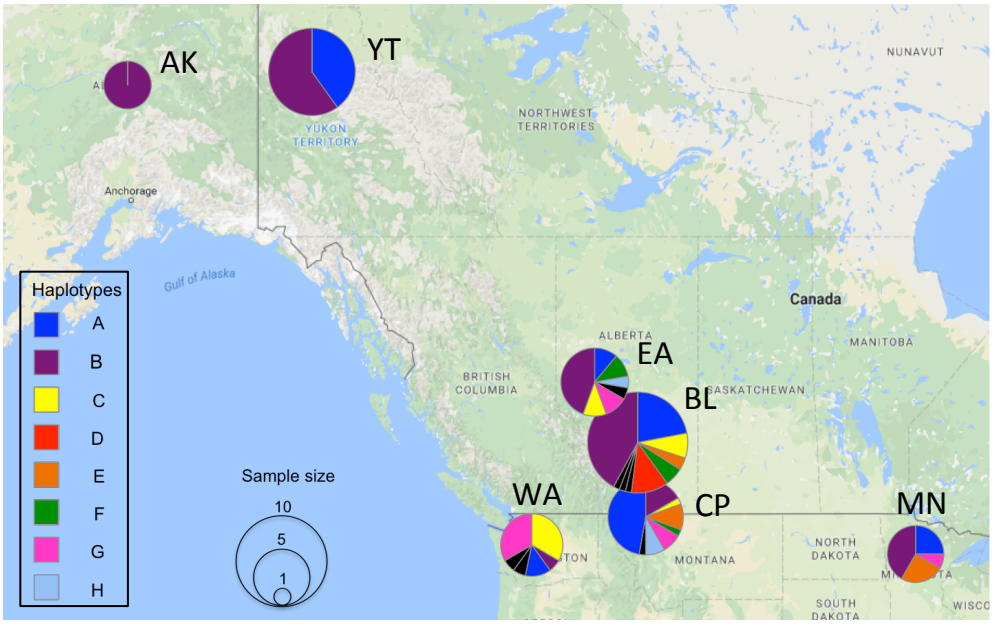


Figure 2.2. The (a) Statistical parsimony network for SLC45a2 sequences from PopART v1.7 (Leigh and Bryant 2015) where each box represents an allele, and groups of boxes share the same haplotype. The lines in the network each represent a single mutation difference. The geographic distribution of the haplotypes can be seen on the (b) haplotype frequency map, where each shared haplotype is represented by a different colour, singletons are denoted in black, and pie charts are sized based on the number of samples (n).

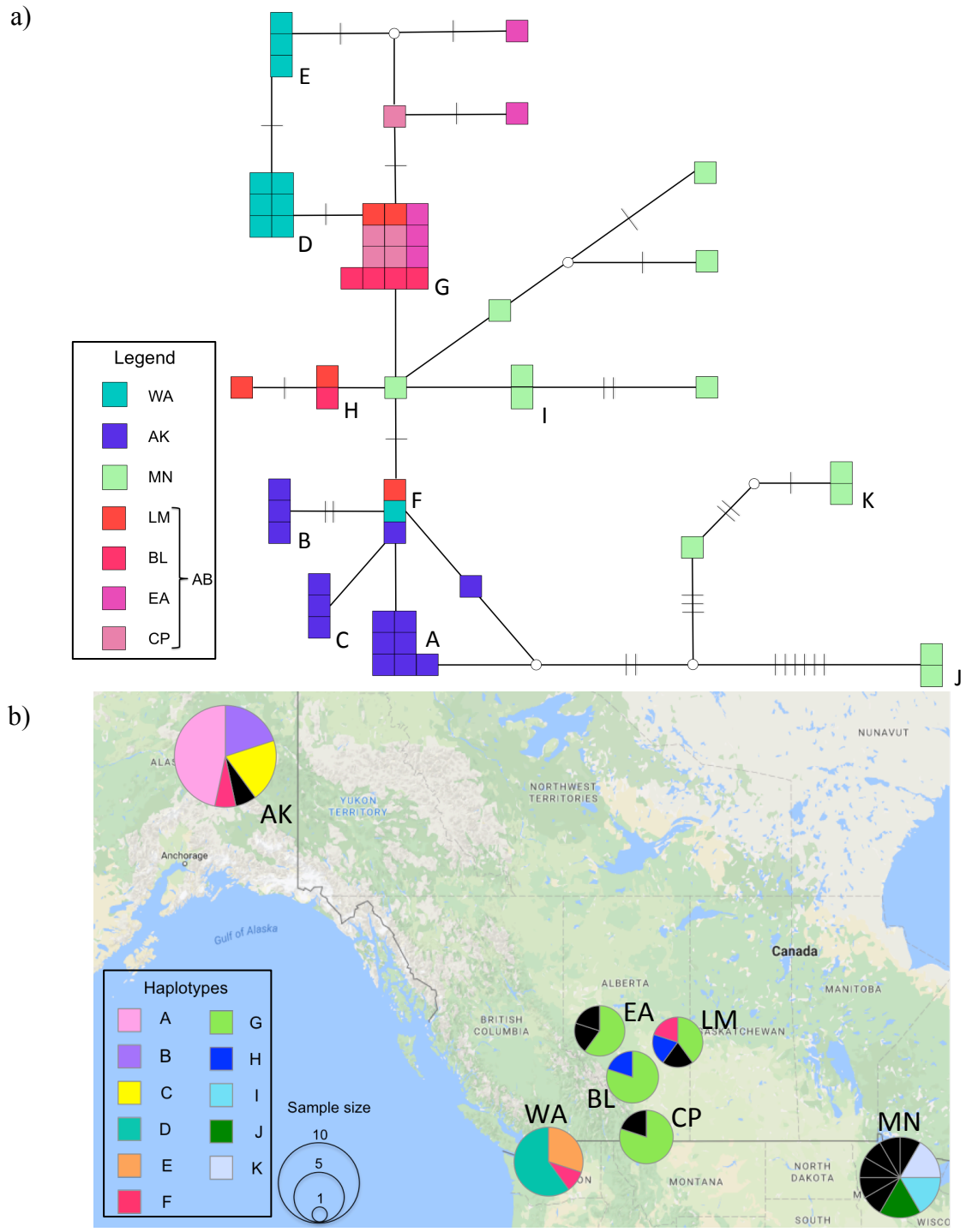


Figure 2.3. The (a) Statistical parsimony network for Control Region sequences from PopART v1.7 (Leigh and Bryant 2015). Each individual is a box, and individuals sharing haplotypes are grouped. The lines in the network each represent a single mutation difference with each hatch-mark across a line representing an additional mutational step, and nodes with inferred haplotypes denoted by open circles. The geographic distribution of shared haplotypes can be seen on the (b) haplotype frequency map for the same sequences. On the map each haplotype is represented by a different colour, singletons are denoted in black, and pie charts are sized based on the number of samples (n).

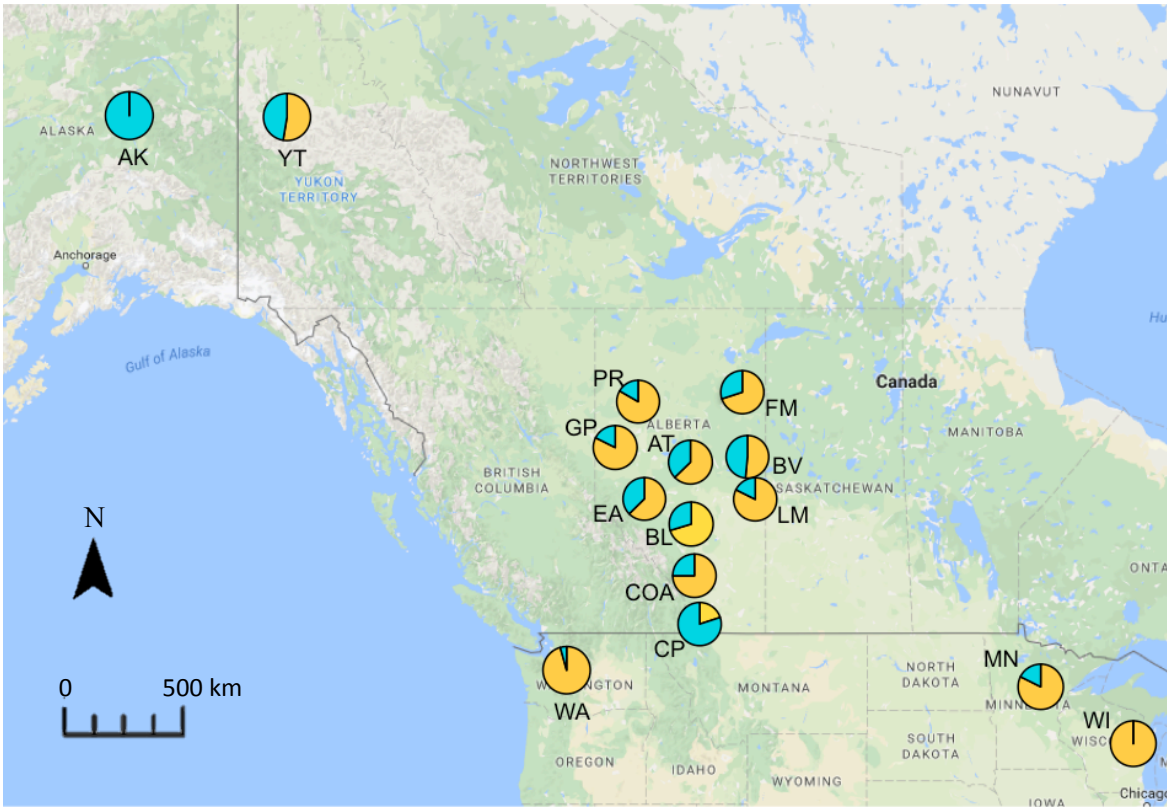


Figure 2.4. Allele frequencies of a SNP on the Z-linked Aldolase B gene for Ruffed Grouse from 15 populations. The pie charts show the proportions of the two possible alleles inferred from the screening data at each population. Pairwise comparisons of these population to test for significant differences among the populations can be seen in Table 2.6.

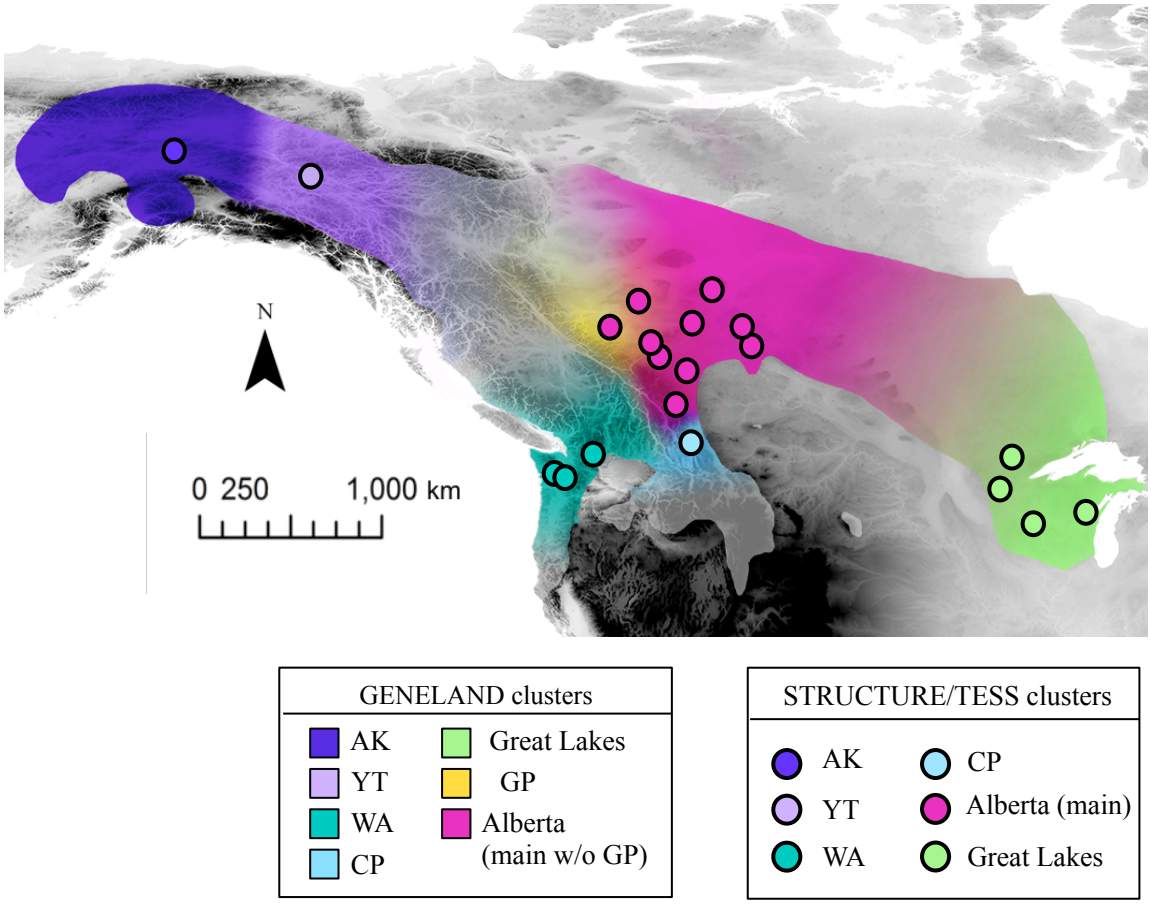


Figure 2.5. Bayesian clustering results from GENELAND v4.0.6, STRUCTURE v2.3.4, and TESS v2.3. The GENELAND clusters from Figure 2.8 have been colour-coded, mapped in geographic space and clipped to limits of the species range. The gradient between colours on the map represents clines created by the contour lines of the posterior probability maps in GENELAND (Appendix 12). The sampling sites are represented by circles and the colour of the circles corresponds to the STRUCTURE and TESS consensus cluster assignments. The colour assignments of the circles match the those used in the Q plots (Appendix 10, 12). The only discordance between any of the programs is the additional cluster assigned by GENELAND for the GP population, which can be seen on the map.

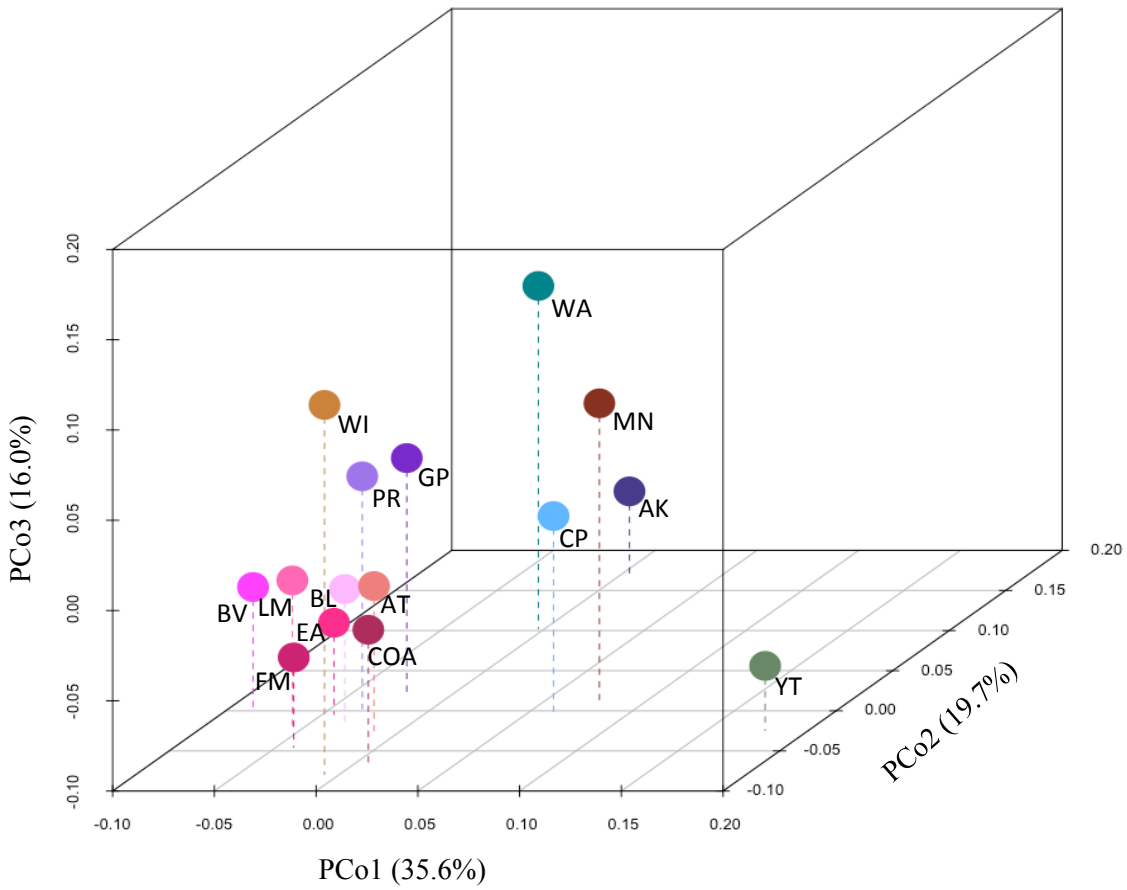


Figure 2.6. A three-dimensional plot of the first three axes of the PCoA as calculated in GenAlEx v6.5 (Peakall and Smouse 2012). Populations are labeled, and principal components are labeled on their respective axes including the amount of variation captured by each. Plot made in R (R Core Team 2016).

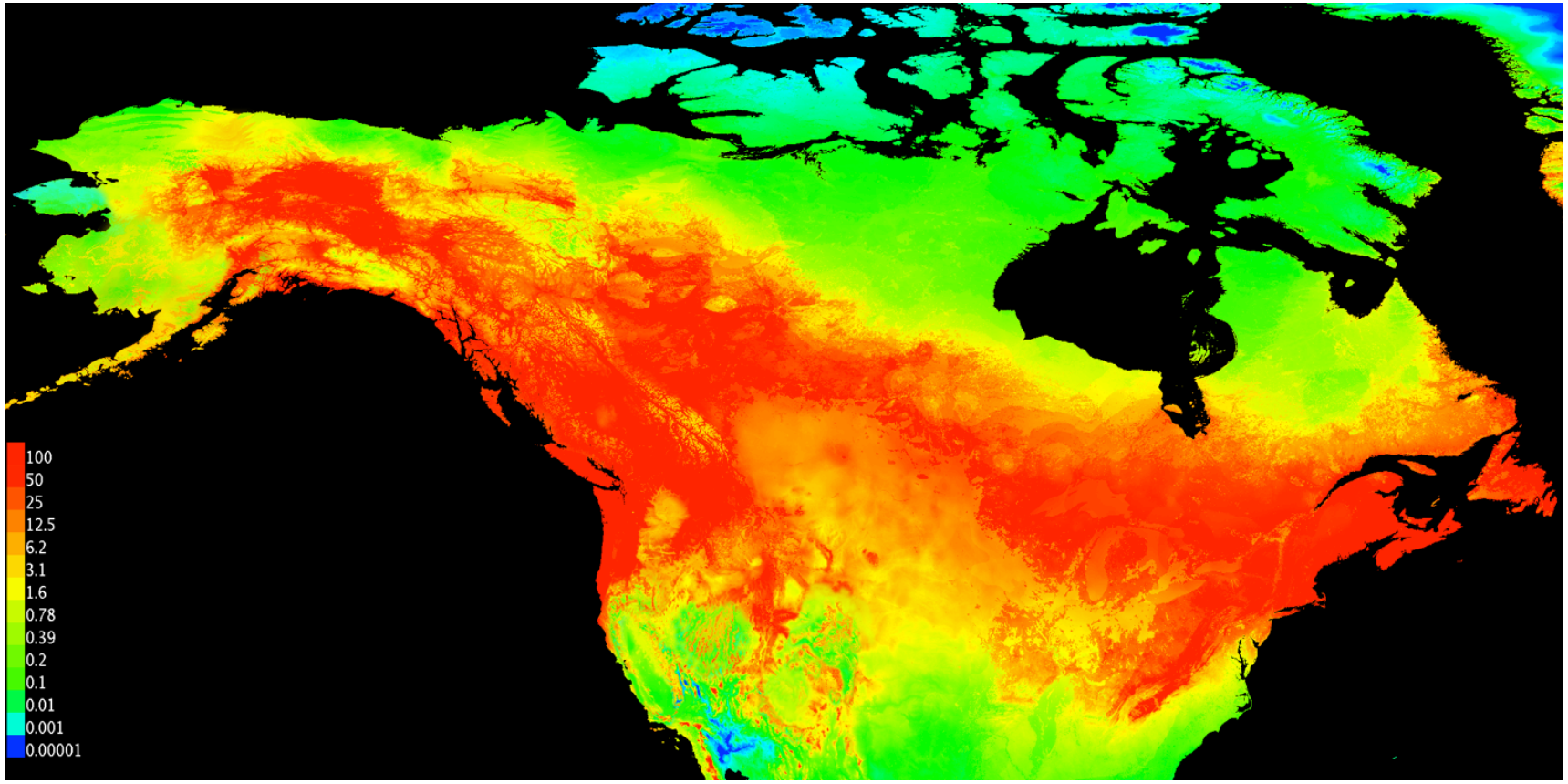


Figure 2.7. Species Distribution model created using occurrences from GBIF and layers including environmental data on vegetation cover across the landscape and multiple climate variables. The model was created using a combination of the SDM toolbox (Brown 2014) implemented in ArcGIS®, and MaxEnt (Phillips et al. 2006). The SDM shows areas of the most suitable habitat (i.e. ecological niche) for Ruffed Grouse, showing areas where environmental conditions are suitable for the species to occur. The scale depicted is cumulative and represents the percent likelihood of habitat suitability for Ruffed Grouse based on the variables input into the model.

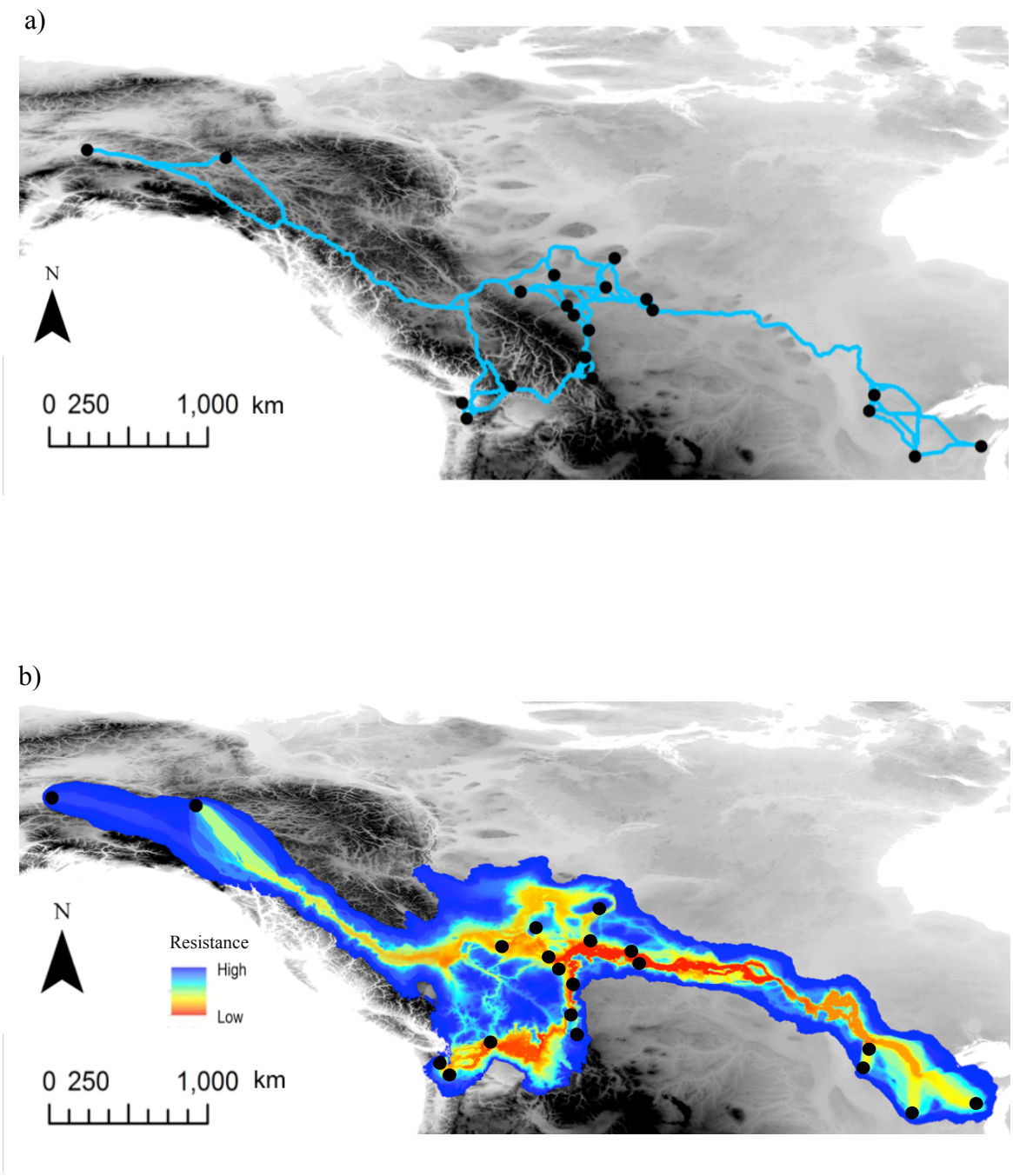


Figure 2.8. The (a) least cost paths (LCP) between the 15 sampled populations of Ruffed Grouse showing the most likely dispersal routes between populations based on the preferred environmental conditions of the species as inferred by the species distribution model (SDM; Fig. 2.8), and the (b) least cost corridors (LCC) among those populations. The LCC shows corridors in place of LCPs, and provides dispersal costs along these corridors coded by colour; red representing areas where there is low resistance (i.e. low dispersal cost), and blue representing areas of high resistance.

CHAPTER 3: General discussion

Understanding population connectivity and the factors influencing it is important for proper species management because it allows for predictions of how continuing environmental change may affect future generations (Frankham et al. 2010). Our research on the population genetic structure of Ruffed Grouse and the environmental factors influencing genetic divergence in this species are important not only in the management of this popular game bird, but for other species as well. Our data revealed Ruffed Grouse populations are highly differentiated, which is likely a consequence of being a relatively sedentary bird, and inhabiting a largely heterogeneous landscape. Of the neutral genetic marker types used, microsatellites showed the highest resolution of contemporary patterns of gene flow. These data revealed multiple distinct genetic groups, and genetic discontinuities coinciding with mountain ranges in western North America. We found that contemporary genetic structure is due to a combination of factors, but isolation by resistance (IBR) is the single best causal factor at all spatial scales examined.

3.1 Management implications

The early successional forest habitat that Ruffed Grouse require is generally at a high risk of decline because it is an ephemeral stage of forest succession, and requires disturbance regimes (e.g. fire cycle) to be maintained (Swanson et al. 2011). Early successional forest habitat supports high biodiversity, and Ruffed Grouse are an indicator species of the quality of this habitat type (USDA Forest Service 2006). This study provides evidence that population connectivity may be restricted by the distribution of suitable habitat, not only in Ruffed Grouse, but potentially in other early successional

forest species. Some species dependent on early successional forest are declining (Hunter et al. 2001), such as the Golden-winged Warbler (*Vermivora chrysoptera*), for which one of the steepest population declines among songbird species in the past century has resulted in a threatened or endangered status across its range (Sauer et al. 2014; Streby et al. 2016). Evaluating population connectivity for early successional species is therefore advocated, as this information may be useful in managing or conserving these taxa.

The Ruffed Grouse is heavily hunted throughout most of its range, and is therefore often the subject of wildlife management strategies to maintain healthy populations. Our findings indicate connectivity between many western populations of Ruffed Grouse is limited. Therefore, it is likely that some of these populations are already experiencing the effects of isolation. When populations become isolated, they may be subject to drift or local adaptation (Reed and Frankham 2003). Many of these isolated or semi-isolated populations should be treated as distinct management units, including Alaska, Yukon, Washington, and southwest Alberta populations. For example, southwestern Alberta (CP) is genetically distinct from the other populations we sampled (Fig. 2.4; Fig. 2.5), and the climate and vegetation communities of southwestern Alberta are more similar to those of the intermountain west than to those of central/northern Alberta (Canadian Parks and Wilderness Society 2011). Therefore, this population is likely to be experiencing different environmental pressures than those in the remainder of Alberta, and should be considered separately from adjacent populations when devising management strategies. Moreover, many of the samples from the southwestern Alberta population were collected in the Castle Special Management Area, which is currently undergoing conversion into a provincial park (Government of Alberta 2017). This diverse and biologically unique area supports species that do not occur elsewhere in Canada, including some that are of

conservation concern such as the Vagrant Shrew (*Sorex vagrans*) and Jones Columbine (*Aquilegia jonesii*; Government of Alberta 2017). Our data showing Ruffed Grouse from this locality are genetically distinct provide further evidence that the Castle area is ecologically unique, and that the area as a whole requires specialized management. Not only does this study imply that further research is warranted in the Castle area, but that perhaps a higher degree of protection should be accorded to this ecologically distinct zone (i.e. conversion into a national park).

A common practice in game bird management is translocating individuals from one population to another, particularly in areas where birds occur in low densities or have been extirpated (Griffith et al. 1989). One issue with translocations occurs when a species range encompasses a large variety of environmental conditions, and populations become adapted to local conditions. If locally adapted individuals are translocated into a new area, they may be less likely to survive and be recruited into the breeding population, or if they do, outbreeding depression may occur (Frankham et al. 2011). Ruffed Grouse have a very wide range, and have been translocated among some populations in the eastern U.S. to restore populations with low numbers (Kelly and Kirkpatrick 1978; Rusch et al. 2000). Although this practice has not yet been deemed necessary in the western extent of the range, this genetic survey of western populations should aid in making appropriate translocation decisions should the need arise in the future.

Another important consideration in species conservation and management is the taxonomic classification of species and subspecies. Delineating subspecies can provide legislative protection, which often affords populations (or groups of populations) with conservation or management attention that would not otherwise be granted (Zink 2004; Frankham et al. 2010). As many as 15 subspecies have been described for Ruffed Grouse

based on varying morphological characteristics such as size, degree of tarsus feathering, and plumage colouration (Uttal 1941; Aldrich and Friedmann 1943; Davis 1970; Hubbard and Banks 1970; Ouellet 1990; Furtman 2004). However, Rusch et al. (2000) have pointed out that some of these subjective subspecies classifications may not be valid, and there is a need to re-assess Ruffed Grouse subspecies descriptions with genetic methods. Although there are no strict criteria for delineating subspecies, the most compelling cases to accept these classifications are when the divergence of behaviour, morphology, and genetics are in agreement (Ball et al. 1992; Mallet 2007; Frankham et al. 2010). Our study is the first to describe intraspecific genetic variation in Ruffed Grouse, and the genetic results for the populations we sampled are mostly congruent with past subspecies classifications based on morphology. The mitochondrial Control Region (CR) is often considered one of the most appropriate markers for resolving subspecies taxonomy due to its high resolution (Mindell 1997; Kahn et al. 1999; Wan et al. 2004), and our CR data from Alaska, Washington, Alberta and Minnesota individuals each form groups respective to their population of origin (Fig. 2.3), although the latter two are not significantly different from each other. The Alaska, Alberta, and Minnesota populations each belong to a single subspecies based on morphological groupings, which is concordant with our genetic results (Fig. 1.1; Fig. 2.3). Based on the locations of samples originating in Washington, these individuals should belong to three different subspecies based on morphological classifications (Fig. 1.1). Conversely, only one grouping was present in the CR data for Washington. Although it is possible this mitochondrial marker did not have enough resolution to reveal subspecies groupings, this scenario is unlikely because even high-resolution microsatellite markers did not reveal any differentiation among Washington individuals. It is therefore possible that subspecies in the Pacific

Northwest have been over split. However, additional data collection will be necessary to confirm this, and to further assess other subspecies descriptions.

3.2 Future directions

We were able to determine the patterns of population connectivity in Ruffed Grouse among multiple populations, mostly focusing on the western extent of the range. However, sampling of additional areas would add a broader perspective of both population connectivity and management units throughout the range. To provide a more complete picture of how mountain barriers and gaps in suitable habitat are restricting gene flow in western Ruffed Grouse populations, sampling at sites in British Columbia focusing on the southern and central areas of the province, and the intermountain west (i.e. western Montana and Idaho panhandle) are required. This additional sampling will not only reveal more information about the functionality of the two corridors identified in this study (Peace River and intermountain west), but will also aid in determining if any population connectivity exists between southern Alberta and other populations (e.g. MT, ID, or BC).

Additionally, sampling at sites throughout eastern North America will allow range-wide comparisons of Ruffed Grouse population structure and also provide insights into how landscape level processes differ across the range. We found that the heterogeneous distribution of habitat across the landscape was the most important overall factor influencing genetic structure of the sampled populations, but the comparisons among populations east of the Rocky Mountains exhibited more evidence of IBD restricting gene flow, while IBR was largely responsible for population differences in the western extent of the range. We speculate that gene flow among populations in the

eastern extent of the range would also show patterns of isolation by resistance. However, the higher degree of habitat fragmentation that has occurred there (Rusch et al. 2000) may lead to larger genetic breaks among these populations. A significant effect of anthropogenic habitat fragmentation on population divergence was apparent in a study of an eastern early successional obligate, the New England Cottontail (*Sylvilagus transitionalis*; Fenderson et al. 2014). To test the hypothesis that fragmentation is similarly mediating population connectivity of Ruffed Grouse in eastern North America, it will be necessary to sample more eastern populations.

Determining the identity of barriers is important in landscape genetics because the goal of this type of research is to determine the key factors mediating gene flow, however, this is not often accomplished because complex models including many variables are required (With et al. 1997; Cushman et al. 2006). In this study, we determined that land cover and climate variables have the greatest effect on population connectivity, and although we are confident of the accuracy of the SDM and resistance modeling, the method we used does not explicitly test which specific environmental variables are significantly influencing genetic structure. For example, when creating the ecological niche model, mean annual temperature, isothermality, and land cover were identified as the variables that contributed most to the model, but it is not clear how they compare to other untested environmental variables (e.g. density of *Populus* species, elevation). It is possible that other environmental factors that were not evaluated in this study might perform slightly better in explaining genetic structuring. The role of specific environmental factors can be determined by testing multiple models containing different combinations of variables (Segelbacher et al. 2010; Row et al. 2015; Adams and Burg 2015). For example, Cushman et al. (2006) found that dense forest cover facilitated gene

flow among populations of Black Bears (*Ursus americanus*) and the effect covaried with elevation, while unforested areas impeded connectivity. Their study was similar to the research presented on Ruffed Grouse in this thesis; both demonstrate how multiple factors can interact to produce observed patterns of genetic structure. However, the study by Cushman et al. (2006) is also an example of how our research could be expanded to evaluate the specific role individual environmental factors might play in genetic differentiation of Ruffed Grouse. Although comparing multiple models consisting of different environmental variables (e.g. fine-scale presence/absence and seral stage data of Quaking Aspen (*Populus tremuloides*) or other associated plant species) is outside the scope of this study, it is certainly a viable option for future research, and may aid in pin-pointing the specific environmental components underlying genetic structure in Ruffed Grouse.

While our study was the first to assess population genetics of Ruffed Grouse, there are still unanswered questions. There is room to expand on the investigation of genetic structure of this species, both in a contemporary and a historical context. Further analyses of genetic data aimed towards uncovering the phylogeography of this species could answer questions about its evolutionary history. For example, patterns in mitochondrial or slowly-evolving autosomal markers can often aid in revealing the glacial refugia that a species inhabited during North America's last glacial maximum, as well how the current range was recolonized after glacial recession (Avise and Walker 1998; Macfarlane et al. 2016). Furthermore, the Ruffed Grouse displays phenotypic variation among populations across the range, particularly with respect to the proportion of two plumage colour morphs in each population (Rusch et al. 2000). It is still unclear how local selection on plumage colouration or other phenotypic characters may be tied to population genetic

structure. In a related study, we attempted to uncover the link between genotype and plumage phenotype by sequencing several genes in the melanin production pathway. However, of the five genetic regions we examined, none of these showed any correlation with phenotypic characters (unpublished data). There are still some coding regions and many regulatory regions related to melanin production that we were not able to examine. Studying patterns of adaptive variation in Ruffed Grouse would benefit from the incorporation of next-generation sequencing or genomic methods that allow the identification of both neutral and non-neutral markers on a larger scale. Identifying the role that both neutral and adaptive loci play in population divergence could reveal the influence environmental factors have on both gene flow and adaptive variation. The phenotypic variation that exists among populations of Ruffed Grouse is evidence that some populations may be experiencing local adaptation due to differential environmental pressures. In other species, such as the Arctic Skua (*Stercorarius parasiticus*), differences in phenotype (in this case plumage colouration) between populations have been linked to polymorphisms on a pigmentation gene as well as local adaptation (Theron et al. 2001; Johnson et al. 2012; Janssen and Mundy 2013). It would therefore be interesting to use next-generation sequencing methods to identify population patterns in adaptive loci to shed light on what environmental pressures or other selective forces are contributing to both phenotypic variation and population structuring in Ruffed Grouse.

3.3 General conclusions

Our research identified patterns of population structure, as well as the most important extrinsic factors influencing population connectivity of an early successional forest specialist, the Ruffed Grouse. Many species that rely on early successional or

mixed forest habitats in North America may similarly be experiencing restricted gene flow due to the heterogeneous distribution of habitat types. The dispersal corridors through the Rocky Mountains identified by our study may also be important for other species, particularly those tied to shrubby, mixed forest, or successional habitats. Not only is there a distinct lack of landscape genetics research on early successional forest obligates, but there are also few studies focusing on wide-ranging species. Studies focusing on these types of organisms may be necessary to identify broad-scale landscape features impacting population connectivity in many taxa (Vergara et al. 2015). This research on Ruffed Grouse not only underscores the importance of genetic studies on wide-ranging species, but also those that incorporate environmental data. Studies such as this may identify important corridors or other landscape patterns affecting a variety of species (Ruiz-Gonzalez et al. 2015). Moreover, understanding species-habitat relationships and accurately assessing population connectivity is becoming increasingly important as environmental change continues to accelerate (Wang et al. 2012).

This study also adds to a growing number of examples (McRae and Beier 2007; Metzger et al. 2015) of why leaving environmental variables out of population structure research may lead to erroneous or incomplete conclusions. Simple patterns of IBD that assume dispersal cost is homogenous across the landscape may not always be sufficient to explain genetic structure, and although IBD was significant in our study, IBR performed better at large scales due to the heterogeneous distribution of suitable habitat across the landscape. Incorrect models of genetic structure can be significant, which creates the risk of spuriously accepting the wrong model if competing models of genetic structure are not tested, as Cushman and Landguth (2010) showed using simulated data. If studies fail to test for the effects of environmental variables on genetic structure, not only may

important factors be overlooked, the importance of other processes (e.g. IBD) may be overestimated. This may be a serious issue for species of conservation concern because incorrectly assessing factors mediating connectivity may lead to poor management practices.

3.4 References

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Appendix 1. Information for all Ruffed Grouse samples used in this study including population abbreviations (Pop), and how samples were obtained (Source); the museum name is listed if obtained from a museum, samples from birds that were wild-caught for this study (wild), or donated by hunters (hunter). If a sample was sequenced for SLC45a2 or CR, the haplotype is listed and unique haplotypes are denoted with an asterisk. For the autosomal SLC45a2 marker, heterozygotes are listed with a backslash between the two different alleles whereas a single letter is used to represent homozygous alleles. If a sample was sequenced, but not included in the microsatellite data set, the sample name is highlighted in grey.

Pop	Location	Lab Sample ID	Source	Museum/Hunter/ Field ID	Latitude (dd)	Longitude (dd)	Slc45a2 Haplotype	CR Haplotype
AK	Fairbanks, Alaska	RUGR AK 001	museum (UAM)	UAMX4692	64.46382	-148.72298	B	C
AK	Fairbanks, Alaska	RUGR AK 002	museum (UAM)	UAMX4506	64.85700	-147.80430	B	C
AK	Fairbanks, Alaska	RUGR AK 003	museum (UAM)	UAMX4505	64.52478	-148.11338	B	B
AK	Fairbanks, Alaska	RUGR AK 004	museum (UAM)	UAMX4488	64.49340	-147.60000	B	B
AK	Fairbanks, Alaska	RUGR AK 005	museum (UAM)	UAMX5312	64.332291	-149.92010		A
AK	Fairbanks, Alaska	RUGR AK 006	museum (UAM)	UAMX1324	64.49584	-147.19568		C
AK	Fairbanks, Alaska	RUGR AK 007	museum (UAM)	UAMX2318	64.28225	-146.58261		F
AK	Fairbanks, Alaska	RUGR AK 008	museum (UAM)	UAMX2321	65.54900	-147.79600		*
AK	Fairbanks, Alaska	RUGR AK 009	museum (UAM)	UAMX2324	64.82100	-147.37030		A
AK	Fairbanks, Alaska	RUGR AK 010	museum (UAM)	UAMX2325	64.45600	-147.02300		A
AK	Fairbanks, Alaska	RUGR AK 011	museum (UAM)	UAMX2392	64.51230	-148.12090		A
AK	Fairbanks, Alaska	RUGR AK 012	museum (UAM)	JJW681	64.55132	-148.85916		B
AK	Fairbanks, Alaska	RUGR AK 013	museum (UAM)	UAMX5482	64.54250	-148.13196		A

AK	Fairbanks, Alaska	RUGR AK 013	museum (UAM)	UAMX5482	64.54250	-148.13196		A
AK	Fairbanks, Alaska	RUGR AK 014	museum (UAM)	UAMX5471	64.54310	-148.12981		A
AK	Fairbanks, Alaska	RUGR AK 015	museum (UAM)	UAMX5693	64.52227	-147.35210		A
AK	Fairbanks, Alaska	RUGR AK 016	museum (UAM)	UAMX5709	64.97210	-147.35021		
AK	Fairbanks, Alaska	RUGR AK 017	museum (UAM)	UAMX5757	64.84600	-147.81023		
AK	Fairbanks, Alaska	RUGR AK 018	museum (UAM)	UAMX5845	64.90120	-147.91280		
AK	Fairbanks, Alaska	RUGR AK 019	museum (UAM)	UAMX5593	65.02350	-147.80430		
AK	Fairbanks, Alaska	RUGR AK 020	museum (UAM)	JJW1901	64.84380	-148.02130		
AK	Fairbanks, Alaska	RUGR AK 021	museum (UAM)	JJW1445	64.56275	-147.56462		
AK	Fairbanks, Alaska	RUGR AK 022	museum (UAM)	AF7188	64.48339	-148.13423		
AK	Fairbanks, Alaska	RUGR AK 023	museum (UAM)	AF14367	64.83333	-147.83333		
YT	Dawson City, Yukon Territory	RUGR YT 001	Environment Yukon	YT1	64.03937	-138.88134	A	
YT	Dawson City, Yukon Territory	RUGR YT 002	Environment Yukon	YT2	64.05709	-139.05286	B	
YT	Dawson City, Yukon Territory	RUGR YT 003	Environment Yukon	YT3	64.00740	-138.60300	A	
YT	Dawson City, Yukon Territory	RUGR YT 004	Environment Yukon	YT4	64.04860	-138.88772		
YT	Dawson City, Yukon Territory	RUGR YT 005	Environment Yukon	YT5	64.04899	-138.87911	A	
YT	Dawson City, Yukon Territory	RUGR YT 006	Environment Yukon	YT6	64.04799	-138.89088		
YT	Dawson City, Yukon Territory	RUGR YT 007	Environment Yukon	YT7	64.02990	-138.93000		

YT	Dawson City, Yukon Territory	RUGR YT 008	Environment Yukon	YT8	64.30404	-140.17851	B	
YT	Dawson City, Yukon Territory	RUGR YT 009	Environment Yukon	YT9	64.06800	-139.33050	B	
YT	Dawson City, Yukon Territory	RUGR YT 010	Environment Yukon	YT10	64.07017	-139.36971	B	
YT	Dawson City, Yukon Territory	RUGR YT 011	Environment Yukon	YT11	64.07090	-139.37043	B	
YT	Dawson City, Yukon Territory	RUGR YT 012	Environment Yukon	YT12	64.02436	-139.44776	A/B	
YT	Dawson City, Yukon Territory	RUGR YT 013	Environment Yukon	YT13	64.03231	-138.81221		
WA	Puget Sound, Washington	RUGR WA 001	museum (UWBM)	UWBM# 19506	47.55223	-122.25189		D
WA	Snohomish, Washington	RUGR WA 011	museum (UWBM)	UWBM# 81695	47.49566	-121.78678	*	D
WA	Snohomish, Washington	RUGR WA 012	museum (UWBM)	UWBM# 80184	47.85838	-122.19111		E
WA	Snohomish, Washington	RUGR WA 013	museum (UWBM)	UWBM# 91559	47.89686	-121.82817	C	
WA	Olympic Penninsula, Washington	RUGR WA 014	museum (UWBM)	UWBM# 63809	47.97638	-122.91885		
WA	Olympic Penninsula, Washington	RUGR WA 015	museum (UWBM)	UWBM# 63808	47.98978	-122.90624	G	D
WA	Olympic Penninsula, Washington	RUGR WA 016	museum (UWBM)	UWBM# 79195	47.92537	-122.68349	G	D
WA	Olympic Penninsula, Washington	RUGR WA 017	museum (UWBM)	UWBM# 80267	47.58496	-122.93141		
WA	Puget Sound, Washington	RUGR WA 018	museum (UWBM)	UWBM# 50954	46.53205	-122.62595		D
WA	Puget Sound, Washington	RUGR WA 019	museum (UWBM)	UWBM# 87204	46.31953	-122.76271		D
WA	Puget Sound, Washington	RUGR WA 021	museum (UWBM)	UWBM# 81667	47.78087	-122.12078	G	
WA	Puget Sound, Washington	RUGR WA 022	museum (UWBM)	UWBM# 81632	47.53010	-122.03262	A	
WA	Kittitas County, Washington	RUGR WA 023	museum (UWBM)	UWBM# 63839	47.15638	-120.91726	B/C	E

WA	Kittitas County, Washington	RUGR WA 024	museum (UWBM)	UWBM# 57085	47.14043	-120.98132	C	E
WA	Kittitas County, Washington	RUGR WA 025	museum (UWBM)	UWBM# 58114	47.01823	-120.75943		
WA	Kittitas County, Washington	RUGR WA 026	museum (UWBM)	UWBM# 90007	46.94619	-120.53522		
WA	Kittitas County, Washington	RUGR WA 027	museum (UWBM)	UWBM# 87148	47.19499	-121.01774		
WA	Okanogan County, Washington	RUGR WA 028	museum (UWBM)	UWBM# 65251	48.71833	-119.67899		
WA	Okanogan County, Washington	RUGR WA 029	museum (UWBM)	UWBM# 72629	48.70793	-119.68248		F
WA	Okanogan County, Washington	RUGR WA 030	museum (UWBM)	UWBM# 81814	48.52502	-120.31714		
WA	Okanogan County, Washington	RUGR WA 031	museum (UWBM)	UWBM# 67218	48.91011	-119.50152		
WA	Okanogan County, Washington	RUGR WA 032	museum (UWBM)	UWBM# 72617	48.90287	-119.54546		
WA	Okanogan County, Washington	RUGR WA 033	museum (UWBM)	UWBM# 86232	48.93335	-119.78287		
WA	Okanogan County, Washington	RUGR WA 034	museum (UWBM)	UWBM# 80299	48.83479	-119.77708		
CP	Crowsnest Pass, Alberta	RUGR CP 001	wild	091110-2 (014)	49.42853	-114.39605	F/*	
CP	Crowsnest Pass, Alberta	RUGR CP 002	wild	232 (059)	49.40842	-114.36446	A	G
CP	Crowsnest Pass, Alberta	RUGR CP 003	wild	2009-01	49.48003	-114.32108		
CP	Crowsnest Pass, Alberta	RUGR CP 004	wild	440	49.39897	-114.36288	A/B	
CP	Crowsnest Pass, Alberta	RUGR CP 005	wild	445	49.52562	-114.39233	B	*
CP	Crowsnest Pass, Alberta	RUGR CP 006	wild	231	49.39905	-114.36408	A/G	G
CP	Crowsnest Pass, Alberta	RUGR CP 007	wild	27	49.38288	-114.34945	A	G
CP	Crowsnest Pass, Alberta	RUGR CP 008	wild	091110-1	49.40530	-114.37766	A	G

CP	Crowsnest Pass, Alberta	RUGR CP 009	wild	233	49.43228	-114.33032	A
CP	Crowsnest Pass, Alberta	RUGR CP 010	wild	2009-02	49.44798	-114.37986	
CP	Crowsnest Pass, Alberta	RUGR CP 011	wild	RG 09	49.43228	-114.32850	A
CP	Crowsnest Pass, Alberta	RUGR CP 012	wild	JK 2015 #1	49.38288	-114.35500	B
CP	Crowsnest Pass, Alberta	RUGR CP 013	wild	RUGR529	49.79046	-114.21836	
CP	Crowsnest Pass, Alberta	RUGR CP 014	wild	638	49.35616	-114.40036	
CP	Crowsnest Pass, Alberta	RUGR CP 015	wild	605	49.36553	-114.31428	
CP	Crowsnest Pass, Alberta	RUGR CP 016	wild	602	49.69260	-114.58333	A/G
CP	Crowsnest Pass, Alberta	RUGR CP 017	wild	604	49.36933	-114.31538	E/H
CP	Crowsnest Pass, Alberta	RUGR CP 018	wild	611	49.40586	-114.34593	
CP	Crowsnest Pass, Alberta	RUGR CP 019	wild	627	49.40965	-114.33890	A/E
CP	Crowsnest Pass, Alberta	RUGR CP 020	wild	601	49.37870	-114.37618	B/C
CP	Crowsnest Pass, Alberta	RUGR CP 021	wild	617	49.37751	-114.37020	E/H
CP	Crowsnest Pass, Alberta	RUGR CP 022	wild	616	49.36413	-114.31350	
CP	Crowsnest Pass, Alberta	RUGR CP 023	wild	610	49.40523	-114.34733	
CP	Crowsnest Pass, Alberta	RUGR CP 024	wild	608	49.40351	-114.34608	
CP	Crowsnest Pass, Alberta	RUGR CP 025	wild	612	49.39033	-114.36651	
CP	Crowsnest Pass, Alberta	RUGR CP 026	wild	632	49.40386	-114.33285	
CP	Crowsnest Pass, Alberta	RUGR CP 027	wild	634	49.40486	-114.33531	

CP	Crowsnest Pass, Alberta	RUGR CP 028	wild	657	49.41345	-114.33947	
CP	Crowsnest Pass, Alberta	RUGR CP 029	wild	630	49.37482	-114.38817	
CP	Crowsnest Pass, Alberta	RUGR CP 030	wild	614	49.38085	-114.35867	A/G
CP	Crowsnest Pass, Alberta	RUGR CP 031	wild	658	49.35636	-114.40760	
CP	Crowsnest Pass, Alberta	RUGR CP 032	wild	656	49.36447	-114.41300	E/H
CP	Crowsnest Pass, Alberta	RUGR CP 033	wild	623	49.42098	-114.33568	
CP	Crowsnest Pass, Alberta	RUGR CP 034	wild	606	49.40680	-114.34308	
CP	Crowsnest Pass, Alberta	RUGR CP 035	wild	662	49.47482	-114.39078	A
CP	Crowsnest Pass, Alberta	RUGR CP 036	wild	664	49.53288	-114.37295	
COA	Cochrane, Alberta	RUGR COA 001	hunter	COA1	50.64402	-114.65545	
COA	Cochrane, Alberta	RUGR COA 002	hunter	COA2	50.64901	-114.66022	
COA	Cochrane, Alberta	RUGR COA 003	hunter	COA3	50.85554	-114.53864	
COA	Cochrane, Alberta	RUGR COA 004	hunter	COA4	51.13127	-114.84900	
COA	Cochrane, Alberta	RUGR COA 005	hunter	COA5	51.13977	-114.85226	
COA	Cochrane, Alberta	RUGR COA 006	hunter	COA6	50.70665	-114.46241	
COA	Cochrane, Alberta	RUGR COA 007	hunter	COA7	50.70665	-114.46241	
COA	Ghost River area, Alberta	RUGR COA 008	hunter	COA bird 2	51.43801	-115.21209	
COA	Ghost River area, Alberta	RUGR COA 009	hunter	COA bird 3	51.28631	-114.93345	
COA	Ghost River area, Alberta	RUGR COA 010	hunter	COA bird 4	51.28012	-114.92899	

COA	Ghost River area, Alberta	RUGR COA 011	hunter	COA bird 5	51.38022	-114.93989		
COA	Cochrane, Alberta	RUGR COA 012	hunter	COA12	51.14527	-114.84569		
BL	Buck Lake, Alberta	RUGR BL 001	wild	225	52.81538	-114.87312	D	
BL	Buck Lake, Alberta	RUGR BL 002	wild	522	52.77200	-114.76113	E	
BL	Buck Lake, Alberta	RUGR BL 003	wild	203	52.93247	-115.05622	A/B	
BL	Buck Lake, Alberta	RUGR BL 004	wild	206	52.94245	-115.09055	A/B	G
BL	Buck Lake, Alberta	RUGR BL 005	wild	182	52.78682	-114.81638	B	
BL	Buck Lake, Alberta	RUGR BL 006	wild	215	52.90668	-115.12867	B/*	G
BL	Buck Lake, Alberta	RUGR BL 007	wild	344	52.78683	-114.81398	B	G
BL	Buck Lake, Alberta	RUGR BL 008	wild	345	52.78317	-114.79998	C	G
BL	Buck Lake, Alberta	RUGR BL 009	wild	346	52.78667	-114.81500	B/C	H
BL	Buck Lake, Alberta	RUGR BL 010	wild	340	52.81460	-114.85273	A	
BL	Buck Lake, Alberta	RUGR BL 011	wild	205	52.76335	-114.72930	A	
BL	Buck Lake, Alberta	RUGR BL 012	wild	45	52.77898	-114.80662	A/F	
BL	Buck Lake, Alberta	RUGR BL 013	wild	144	52.76870	-114.72903	A/F	
BL	Buck Lake, Alberta	RUGR BL 014	wild	343	52.81460	-114.85273	A/F	
BL	Buck Lake, Alberta	RUGR BL 015	wild	342	52.81460	-114.85273	B/*	
BL	Buck Lake, Alberta	RUGR BL 016	wild	95	52.80365	-114.83955	B	
BL	Buck Lake, Alberta	RUGR BL 017	wild	121	52.81367	-114.86368	B	

BL	Buck Lake, Alberta	RUGR BL 019	wild	208	52.80433	-114.84115	F	
BL	Buck Lake, Alberta	RUGR BL 021	wild	341	52.77788	-114.76406		
BL	Buck Lake, Alberta	RUGR BL 022	wild	226	52.81538	-114.87311	B	
BL	Buck Lake, Alberta	RUGR BL 023	wild	88	53.06333	-114.74550	B	
BL	Buck Lake, Alberta	RUGR BL 024	wild	130	52.78616	-114.78033	A/B	
BL	Buck Lake, Alberta	RUGR BL 025	wild	160	53.02956	-114.80793	A/*	
BL	Buck Lake, Alberta	RUGR BL 026	wild	230	52.81465	-114.84868	E/F	
BL	Buck Lake, Alberta	RUGR BL 027	wild	?X	53.03038	-114.79126	B	
BL	Buck Lake, Alberta	RUGR BL 028	wild	OctBL 2014	52.02145	-115.63900	B/C	
BL	Buck Lake, Alberta	RUGR BL 029	wild	BLR0001	52.02038	-115.37166		
BL	Buck Lake, Alberta	RUGR BL 030	wild	BLR0002	52.88685	-115.21633		
BL	Buck Lake, Alberta	RUGR BL 031	wild	BLB0001	53.19705	-114.84745		
BL	Buck Lake, Alberta	RUGR BL 032	wild	OctBL24 2015	52.02383	-115.00205		
BL	Buck Lake, Alberta	RUGR BL 033	wild	643	52.85371	-115.37166		
BL	Buck Lake, Alberta	RUGR BL 034	wild	647	52.88685	-115.54966		
BL	Buck Lake, Alberta	RUGR BL 035	wild	651	52.80600	-114.84148		
BL	Buck Lake, Alberta	RUGR BL 037	wild	RUGR 661	52.03349	-115.64012		
EA	Edson, Alberta	RUGR EA 001	hunter	SCLR0001	53.45392	-115.44379		G
EA	Edson, Alberta	RUGR EA 002	hunter	SCLR0002	53.45992	-115.46379		

EA	Edson, Alberta	RUGR EA 003	hunter	SCLR0003	53.43992	-115.46379		
EA	Edson, Alberta	RUGR EA 004	hunter	SCLR0004	53.45992	-115.45359		
EA	Edson, Alberta	RUGR EA 005	hunter	SCLR0005	53.40992	-115.43290		
EA	Whitecourt, Alberta	RUGR EA 006	hunter	Price1	54.09849	-115.89082	A	G
EA	Whitecourt, Alberta	RUGR EA 007	hunter	Price2	54.10848	-115.85080	B	*
EA	Whitecourt, Alberta	RUGR EA 008	hunter	Price3	54.13850	-115.89089	B/F	*
EA	Whitecourt, Alberta	RUGR EA 009	hunter	Price4	54.10829	-115.90082	B/F	G
EA	Whitecourt, Alberta	RUGR EA 010	hunter	Price5	54.09849	-115.91001		
EA	Whitecourt, Alberta	RUGR EA 011	hunter	Price6	54.09049	-115.84087		
EA	Whitecourt, Alberta	RUGR EA 012	hunter	Price7	54.09849	-115.89008		
EA	Whitecourt, Alberta	RUGR EA 013	hunter	Price8	54.09949	-115.89183		
EA	Whitecourt, Alberta	RUGR EA 014	hunter	Price9	54.09749	-115.89086		
EA	Whitecourt, Alberta	RUGR EA 015	hunter	Price10	54.09849	-115.90090		
EA	Whitecourt, Alberta	RUGR EA 016	hunter	Price11	54.09849	-115.89171		
EA	Whitecourt, Alberta	RUGR EA 017	hunter	Price12	54.09749	-115.89083		
EA	Whitecourt, Alberta	RUGR EA 018	hunter	Price13	54.09919	-115.89090		
EA	Whitecourt, Alberta	RUGR EA 019	hunter	Price14	54.09849	-115.89184		
EA	Whitecourt, Alberta	RUGR EA 020	hunter	Price15	54.09849	-115.89083		
EA	Edson, Alberta	RUGR EA 021	hunter	EA21	53.53433	-116.57675	H/*	

EA	Edson, Alberta	RUGR EA 022	hunter	EA22	53.53903	-116.58016	G
EA	Edson, Alberta	RUGR EA 025	hunter	EA25	53.53127	-116.56115	C
EA	Edson, Alberta	RUGR EA 026	hunter	Niton1	53.52635	-116.55904	B
EA	Edson, Alberta	RUGR EA 027	hunter	Niton2	53.53672	-116.55903	
EA	Edson, Alberta	RUGR EA 028	hunter	Niton3	53.52634	-116.57936	
EA	Edson, Alberta	RUGR EA 029	hunter	1(EA29)	53.52175	-116.59836	
EA	Edson, Alberta	RUGR EA 030	hunter	2(EA30)	53.53837	-116.59364	
EA	Edson, Alberta	RUGR EA 031	hunter	3(EA31)	53.63117	-116.76060	
EA	Edson, Alberta	RUGR EA 032	hunter	4(EA32)	53.63836	-116.76934	
EA	Edson, Alberta	RUGR EA 033	hunter	5(EA33)	53.61732	-116.76973	
EA	Edson, Alberta	RUGR EA 035	hunter	7(EA35)	53.65227	-116.75335	
EA	Edson, Alberta	RUGR EA 036	hunter	8(EA36)	53.62240	-116.76187	
EA	Edson, Alberta	RUGR EA 037	hunter	9(EA37)	53.61945	-116.74972	
EA	Edson, Alberta	RUGR EA 038	hunter	10(EA38)	53.63936	-116.77055	
EA	Edson, Alberta	RUGR EA 039	hunter	11(EA39)	53.87711	-116.33901	
EA	Edson, Alberta	RUGR EA 040	hunter	12(EA40)	53.54617	-116.24898	
EA	Edson, Alberta	RUGR EA 041	hunter	13(EA41)	53.58530	-116.27833	
EA	Edson, Alberta	RUGR EA 042	hunter	14(EA42)	53.58492	-116.28022	
EA	Edson, Alberta	RUGR EA 043	hunter	15(EA43)	53.58679	-116.26110	

EA	Hinton, Alberta	RUGR EA 044	hunter	Mike1	53.35636	-117.63734
EA	Hinton, Alberta	RUGR EA 045	hunter	Terry2	53.29347	-117.67095
EA	Hinton, Alberta	RUGR EA 046	hunter	Terry1	53.30912	-117.66723
EA	Hinton, Alberta	RUGR EA 047	hunter	Terry 3	53.29023	-117.68123
EA	Edson, Alberta	RUGR EA 048	hunter	16(EA48)	53.65187	-115.67756
EA	Edson, Alberta	RUGR EA 049	hunter	17(EA49)	53.65904	-115.67934
EA	Edson, Alberta	RUGR EA 050	hunter	18(EA50)	53.64872	-115.66755
EA	Edson, Alberta	RUGR EA 051	hunter	19(EA51)	53.65236	-115.67934
EA	Edson, Alberta	RUGR EA 052	hunter	20(EA52)	53.68178	-115.69899
EA	Edson, Alberta	RUGR EA 053	hunter	21(EA53)	53.67711	-115.70691
EA	Edson, Alberta	RUGR EA 054	hunter	EA54	53.40298	-115.42904
EA	Edson, Alberta	RUGR EA 055	hunter	EA55	53.41179	-115.43449
EA	Edson, Alberta	RUGR EA 056	hunter	EA56	53.39023	-115.42088
EA	Edson, Alberta	RUGR EA 057	hunter	EA57	53.41273	-115.43449
EA	Edson, Alberta	RUGR EA 058	hunter	EA58	53.37752	-115.42629
EA	Edson, Alberta	RUGR EA 059	hunter	EA59	53.37022	-115.42101
EA	Edson, Alberta	RUGR EA 060	hunter	EA60	53.36930	-115.43958
EA	Edson, Alberta	RUGR EA 061	hunter	EA61	53.37022	-115.44589
EA	Edson, Alberta	RUGR EA 062	hunter	EA62	53.37225	-115.44599

EA	Edson, Alberta	RUGR EA 063	hunter	EA63	53.35990	-115.43011
EA	Edson, Alberta	RUGR EA 065	hunter	EA65	53.39023	-115.36990
EA	Edson, Alberta	RUGR EA 066	hunter	EA66	53.38923	-115.37883
EA	Edson, Alberta	RUGR EA 067	hunter	EA67	53.38002	-115.35331
GP	Valleyview, Alberta	RUGR GP 003	hunter	GP3	55.31650	-117.00285
GP	Valleyview, Alberta	RUGR GP 004	museum (RAM)	B1	55.41303	-117.73250
GP	Spirit River, Alberta	RUGR GP 005	museum (RAM)	B2	55.61303	-119.25670
GP	Spirit River, Alberta	RUGR GP 007	museum (RAM)	B4	55.59303	-119.19690
GP	Spirit River, Alberta	RUGR GP 008	museum (RAM)	B5	55.58303	-119.28700
GP	Spirit River, Alberta	RUGR GP 009	museum (RAM)	B6	55.61903	-119.30710
GP	Spirit River, Alberta	RUGR GP 010	museum (RAM)	B7	55.58303	-119.32720
GP	Spirit River, Alberta	RUGR GP 011	museum (RAM)	B8	55.65303	-119.20730
GP	Spirit River, Alberta	RUGR GP 012	hunter	GP12	55.72377	-119.12255
GP	Spirit River, Alberta	RUGR GP 013	hunter	GP13	55.69411	-119.12963
GP	Spirit River, Alberta	RUGR GP 014	hunter	GP14	55.65673	-119.13091
GP	Spirit River, Alberta	RUGR GP 015	hunter	GP15	55.63489	-119.12860
GP	Spirit River, Alberta	RUGR GP 016	hunter	GP16	55.62206	-119.73996
GP	Spirit River, Alberta	RUGR GP 017	hunter	GP17	55.63092	-119.74568
GP	Spirit River, Alberta	RUGR GP 018	hunter	GP18	55.61993	-119.72884

GP	Spirit River, Alberta	RUGR GP 019	hunter	GP19	55.66473	-119.84270
GP	Spirit River, Alberta	RUGR GP 020	hunter	GP20	55.67088	-119.85190
GP	Spirit River, Alberta	RUGR GP 021	hunter	GP21	55.66348	-119.84991
PR	Manning, Alberta	RUGR PR 001	museum (UAM)	UAM 18791	56.78772	-118.02235
PR	Manning, Alberta	RUGR PR 002	museum (UAM)	UAM 18796	56.79002	-118.02199
PR	Peace River, Alberta	RUGR PR 003	museum (RAM)	G1	56.25288	-116.73551
PR	Peace River, Alberta	RUGR PR 004	museum (RAM)	G2	56.26112	-116.74771
PR	Peace River, Alberta	RUGR PR 005	museum (RAM)	G3	56.39636	-116.70706
PR	Manning, Alberta	RUGR PR 006	hunter	PR6	56.61396	-118.21697
PR	Peace River, Alberta	RUGR PR 007	hunter	PR7	56.15284	-117.41062
PR	Peace River, Alberta	RUGR PR 008	hunter	PR8	56.15308	-116.75352
AT	Athabasca area, Alberta	RUGR AT 001	hunter	ATC1	55.32884	-113.28963
AT	Athabasca area, Alberta	RUGR AT 002	hunter	ATC2	55.29882	-113.29187
AT	Athabasca area, Alberta	RUGR AT 003	hunter	ATC3	55.33891	-113.28961
AT	Athabasca area, Alberta	RUGR AT 004	hunter	ATC4	55.32846	-113.28855
AT	Athabasca area, Alberta	RUGR AT 005	hunter	ATC5	55.32795	-113.30024
AT	Athabasca area, Alberta	RUGR AT 006	hunter	ATC6	55.33872	-113.19792
AT	Athabasca area, Alberta	RUGR AT 007	hunter	ATC7	55.14175	-113.20818
AT	Athabasca area, Alberta	RUGR AT 008	hunter	ATC8	55.15173	-113.21287

AT	Athabasca area, Alberta	RUGR AT 009	hunter	ATC9	55.17177	-113.23790
AT	Athabasca area, Alberta	RUGR AT 010	hunter	ATC10	55.15175	-113.22193
AT	Athabasca area, Alberta	RUGR AT 011	hunter	ATC11	55.14938	-113.21760
AT	Athabasca area, Alberta	RUGR AT 013	hunter	ATC13	55.25991	-113.47126
AT	Athabasca area, Alberta	RUGR AT 014	hunter	ATC14	55.26197	-113.48221
AT	Athabasca area, Alberta	RUGR AT 015	hunter	ATC15	55.26091	-113.49128
AT	Athabasca area, Alberta	RUGR AT 016	hunter	ATL16	54.62234	-112.63670
AT	Athabasca area, Alberta	RUGR AT 017	hunter	ATL17	54.61071	-112.61648
AT	Athabasca area, Alberta	RUGR AT 018	hunter	ATL18	54.60945	-112.62354
AT	Athabasca area, Alberta	RUGR AT 019	hunter	ATL19	54.61985	-112.61982
AT	Athabasca area, Alberta	RUGR AT 021	hunter	ATL21	54.60040	-112.61873
AT	Athabasca area, Alberta	RUGR AT 022	hunter	ATL22	54.65095	-112.64754
AT	Athabasca area, Alberta	RUGR AT 023	hunter	ATL23	54.65822	-112.641026
AT	Athabasca area, Alberta	RUGR AT 024	hunter	ATL24	54.65298	-112.63491
AT	Athabasca area, Alberta	RUGR AT 025	hunter	ATL25	54.65302	-112.65283
AT	Athabasca area, Alberta	RUGR AT 026	hunter	ATL26	54.68195	-112.61170
AT	Athabasca area, Alberta	RUGR AT 027	hunter	ATL27	54.69022	-112.62345
AT	Athabasca area, Alberta	RUGR AT 028	hunter	ATL28	54.67899	-112.61903
AT	Athabasca area, Alberta	RUGR AT 029	hunter	ATL29	54.69236	-112.62349

AT	Athabasca area, Alberta	RUGR AT 030	hunter	ATL30	54.66546	-112.57374
AT	Athabasca area, Alberta	RUGR AT 031	hunter	ATL31	54.66238	-112.58293
FM	Fort McMurray, Alberta	RUGR FM 001	hunter	FM#1	57.28159	-112.69066
FM	Fort McMurray, Alberta	RUGR FM 002	hunter	FM#2	57.28200	-112.69122
FM	Fort McMurray, Alberta	RUGR FM 003	hunter	FM#3	57.28399	-112.69066
FM	Fort McMurray, Alberta	RUGR FM 004	hunter	FM#4	57.28299	-112.70001
FM	Fort McMurray, Alberta	RUGR FM 005	hunter	FM#5	57.56012	-111.58226
FM	Fort McMurray, Alberta	RUGR FM 006	hunter	FM6	57.56178	-111.57011
FM	Fort McMurray, Alberta	RUGR FM 007	hunter	FM7	56.39102	-111.12834
FM	Fort McMurray, Alberta	RUGR FM 008	hunter	FM8	56.39207	-111.13129
FM	Fort McMurray, Alberta	RUGR FM 009	hunter	FM9	56.37110	-111.18526
FM	Fort McMurray, Alberta	RUGR FM 010	hunter	FM10	56.33331	-111.38198
FM	Fort McMurray, Alberta	RUGR FM 011	hunter	FM11	56.25603	-111.55044
BV	Bonnyville, Alberta	RUGR BV 001	hunter	BV1	54.52043	-110.54210
BV	Bonnyville, Alberta	RUGR BV 002	hunter	BV2	54.54377	-110.60104
BV	Bonnyville, Alberta	RUGR BV 003	hunter	O'TooleBV3	54.53943	-110.53208
BV	Bonnyville, Alberta	RUGR BV 004	hunter	O'TooleBV4	54.53543	-110.54910
BV	Bonnyville, Alberta	RUGR BV 006	hunter	O'TooleBV5	54.53943	-110.54810
BV	Bonnyville, Alberta	RUGR BV 007	hunter	LM20	54.54491	-111.26049

BV	Bonnyville, Alberta	RUGR BV 008	hunter	LM21	54.53494	-111.25912	
BV	Bonnyville, Alberta	RUGR BV 009	hunter	LM22	54.52495	-111.26191	
BV	Bonnyville, Alberta	RUGR BV 010	hunter	LM23	54.08599	-110.66360	
BV	Bonnyville, Alberta	RUGR BV 011	hunter	Bonny1	54.08603	-110.64362	
BV	Bonnyville, Alberta	RUGR BV 012	hunter	Bonny2	54.08999	-110.70231	
BV	Bonnyville, Alberta	RUGR BV 013	museum (RAM)	A1	54.08599	-110.66360	
BV	Bonnyville, Alberta	RUGR BV 014	museum (RAM)	A2	54.14500	-110.99010	
BV	Bonnyville, Alberta	RUGR BV 015	museum (RAM)	A3	54.16326	-111.06286	
BV	Bonnyville, Alberta	RUGR BV 016	museum (RAM)	A4	54.16399	-111.06593	
BV	Bonnyville, Alberta	RUGR BV 017	museum (RAM)	A5	54.17399	-111.06493	
BV	Bonnyville, Alberta	RUGR BV 019	museum (RAM)	C2	54.06833	-110.93948	
BV	Bonnyville, Alberta	RUGR BV 021	museum (RAM)	D1	54.61533	-110.67041	
BV	Bonnyville, Alberta	RUGR BV 022	hunter	BV22	54.02934	-110.96014	
LM	Lloydminster, Alberta	RUGR LM 001	hunter	443	53.87565	-110.11316	F
LM	Lloydminster, Alberta	RUGR LM 002	hunter	441	53.87027	-110.13228	G
LM	Lloydminster, Alberta	RUGR LM 003	hunter	442	53.80496	-110.13876	*
LM	Wainwright, Alberta	RUGR LM 004	hunter	LM1	52.83544	-111.06307	G
LM	Wainwright, Alberta	RUGR LM 005	hunter	LM2	52.64053	-110.93542	H
LM	Wainwright, Alberta	RUGR LM 006	hunter	LM6	52.80886	-110.50530	

LM	Wainwright, Alberta	RUGR LM 007	hunter	LM7	52.80686	-110.51530		
LM	Wainwright, Alberta	RUGR LM 008	hunter	LM8	52.80799	-110.49990		
LM	Wainwright, Alberta	RUGR LM 009	hunter	LM9	52.71885	-111.18210		
LM	Wainwright, Alberta	RUGR LM 010	hunter	LM10	52.71800	-111.18202		
LM	Wainwright, Alberta	RUGR LM 011	hunter	LM11	52.71885	-111.18193		
LM	Wainwright, Alberta	RUGR LM 012	hunter	LM12	52.64814	-111.04908		
LM	Wainwright, Alberta	RUGR LM 013	hunter	LM13	52.64808	-111.04893		
MN	Cook County, Minnesota	RUGR MN(n) 001	museum (FMNH)	FMNH 435429	47.93817	-90.32744	E	*
MN	Cook County, Minnesota	RUGR MN(n) 002	museum (FMNH)	FMNH 441492	48.02695	-90.39707		*
MN	Cook County, Minnesota	RUGR MN(n) 004	museum (FMNH)	FMNH 441493	48.02064	-90.12137		I
MN	St. Louis County, Minnesota	RUGR MN(n) 005	museum (FMNH)	FMNH 464461	47.79039	-92.14999	A	*
MN	Itasca County, Minnesota	RUGR MN(n) 006	museum (FMNH)	FMNH 464464	47.84929	-93.36724	A	
MN	Beltrami County, Minnesota	RUGR MN(n) 007	museum (FMNH)	FMNH 464462	48.10898	-95.25744	E	
MN	Crow Wing County, Minnesota	RUGR MN(c) 008	museum (FMNH)	FMNH 490452	47.05069	-95.59133	B/G	*
MN	Crow Wing County, Minnesota	RUGR MN(c) 009	museum (FMNH)	FMNH 430505	47.07991	-95.60022	B	I
MN	Aitkin County, Minnesota	RUGR MN(c) 010	museum (FMNH)	FMNH 395697	46.50340	-93.31019	B	*
MN	Crow Wing County, Minnesota	RUGR MN(c) 011	museum (FMNH)	FMNH 486735	46.67039	-93.91705		K
MN	Crow Wing County, Minnesota	RUGR MN(c) 012	museum (FMNH)	FMNH 434414	46.67935	-93.92335	B	
MN	Crow Wing County, Minnesota	RUGR MN(c) 013	museum (FMNH)	FMNH 396978	46.66834	-93.90944		

MN	Crow Wing County, Minnesota	RUGR MN(c) 014	museum (FMNH)	FMNH 429054	46.68133	-93.91277	
MN	Crow Wing County, Minnesota	RUGR MN(c) 015	museum (FMNH)	FMNH 454406	46.68923	-93.91934	
MN	Crow Wing County, Minnesota	RUGR MN(c) 016	museum (FMNH)	FMNH 454405	46.66812	-93.90934	
MN	Crow Wing County, Minnesota	RUGR MN(c) 017	museum (FMNH)	FMNH 396977	46.51033	-93.94142	
MN	Chisago County, Minnesota	RUGR MN(c) 018	museum (FMNH)	FMNH 465217	45.52176	-92.74326	
MN	Winona County, Minnesota	RUGR MN(s) 019	museum (FMNH)	FMNH 437493	44.06695	-91.66336	J
MN	Olmsted County, Minnesota	RUGR MN(s) 020	museum (FMNH)	FMNH 396979	43.97632	-92.51214	K
MN	Olmsted County, Minnesota	RUGR MN(s) 021	museum (FMNH)	FMNH 387962	43.98857	-92.52676	
MN	Olmsted County, Minnesota	RUGR MN(s) 022	museum (FMNH)	FMNH 429053	43.98782	-92.53588	J
MN	Olmsted County, Minnesota	RUGR MN(s) 023	museum (FMNH)	FMNH 438320	43.90602	-92.32113	*
WI	Brown County, Wisconsin	RUGR WI 001	museum (FMNH)	FMNH 431072	44.66641	-88.07266	
WI	Brown County, Wisconsin	RUGR WI 002	museum (FMNH)	FMNH 348440	44.66821	-88.07220	
WI	Brown County, Wisconsin	RUGR WI 003	museum (FMNH)	FMNH 348438	44.66791	-88.07290	
WI	Brown County, Wisconsin	RUGR WI 004	museum (FMNH)	FMNH 348442	44.67283	-88.09585	
WI	Brown County, Wisconsin	RUGR WI 005	museum (FMNH)	FMNH 441623	44.67911	-88.09103	
WI	Brown County, Wisconsin	RUGR WI 006	museum (FMNH)	FMNH 467698	44.67879	-87.66217	
WI	Door County, Wisconsin	RUGR WI 007	museum (FMNH)	FMNH 490305	44.69020	-87.66263	

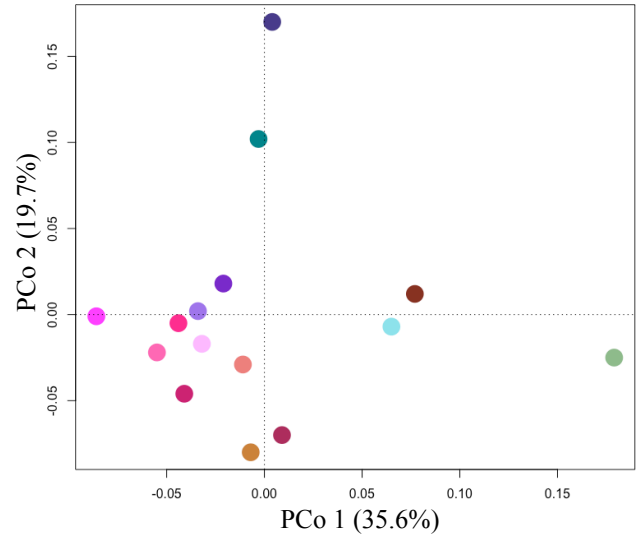
Appendix 2. Information for nuclear intron and mitochondrial primers used in this study, including the annealing temperature (T_a), magnesium chloride concentration, and the reference for primers used (if not designed for this study).

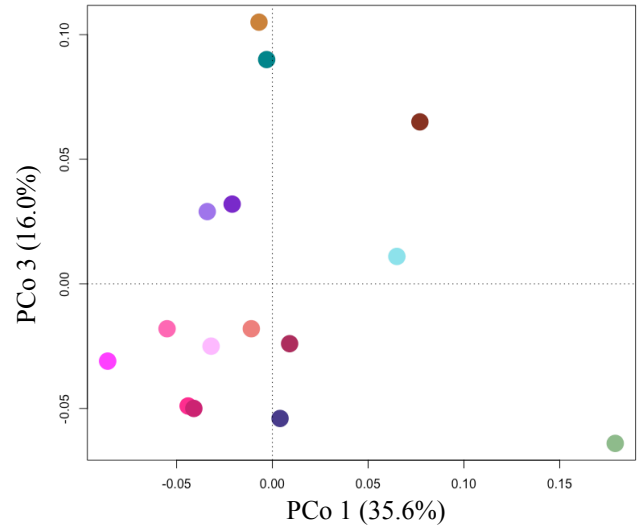
Locus	Oligo sequence	Product Size (bp)	T_a (ºC)	$MgCl_2$ (mM)	Reference
SLC45a2 (intron 4)	SlcEx5F: 5' - AGGCTCAAAGGCGGATGA - 3'	865	54	1.5	this study
	SlcEx5R: 5' - ATAGRCTGAGGATGCAATCG - 3'				this study
Aldolase B (intron 6)	AldoB6F: 5' – AAGATCACCAGCACAACAC CCTCT - 3'	550	62	1.5	Cheviron and Brumfield 2009
	AldoB6R: 5' – AGGCTGCTGTGGAAAGACA GCTTA - 3'				Cheviron and Brumfield 2009
Control Region	H505: 5' - GAAAGAATGGTCCTGAAGC -3'	394	54	2.0	Burg and Croxall 2001
	tRNA Glu L16758: 5' – GGYTTGAAAAGCYGTY GTTG -3'				Sorenson et al. 1999
Aldolase B (indel)	AldoBscrnF: 5' – AGTTCCTTCAAAACTACAC CACAT - 3'	-	54	2.0	this study (designed to screen for indel)
	AldoBdelR: 5'- TTTCTGAGGTCAAGAATGTTTATG -3'	118			this study (designed to screen for indel)
	AldoBinsR M13: 5' –ggataacaatttcacacaggTGAGGTC AAGAATGTTTATATTGAGAG -3'	161			this study (designed to screen for indel)

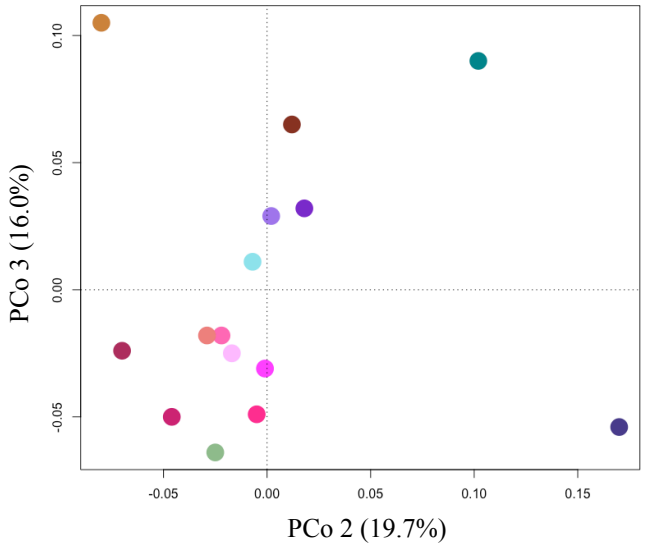
Appendix 3. Information for microsatellite primers used in this study, including the temperatures for the two-step annealing PCR program: ($T_{A(1)}$) annealing temperature for the first 8 cycles, and ($T_{A(2)}$) for the last 31 cycles, magnesium chloride concentration, total number of alleles (N(a)), and reference. Loci completely removed from analyses due to significant deviation from HWE are denoted with an asterisk. An M13 tag sequence (M13) was added to the 5' end of forward primers.
M13 sequence: 5' – CACGACGTTGTAAAACGAC - 3'

Locus	Repeat type	Oligo sequence	Size Range (bp)	$T_{A(1)}$ (ºC)	$T_{A(2)}$ (ºC)	$MgCl_2$ (mM)	N(a)	Reference
LLSD7	CA	5' - M13 - CATGCTTCCAGTACCTGTGC - 3' 5' - GTTAGCCATCACAGTGTCACC - 3'	162-172	60	62	2.0	6	Piertney and Dallas 1997
TTD6	CA	5' - M13 - GGACTGCTTGTGATACTTGCT -3' 5' - CATGCAGATGACTTTCAGCA - 3'	127-155	52	54	2.3	12	Caizergues et al. 2001
TUT4	TATC	5' - M13 - GAGCATCTCCCAGAGTCAGC - 3' 5' - TGTGAACCAGCAATCTGAGC - 3'	170-234	48	50	2.3	17	Segelbacher et al. 2000
SGCA5	CA	5' - M13 - CACTATTAATTAACCTGAGA -3' 5' - GTCAGAATCTACAAATGAG - 3'	264-272	55	57	2.0	5	Taylor et al. 2003
BG15	CTAT	5' - M13 - AAATATGTTTGCTAGGGCTTAC - 3' 5' - TACATTTTTCATTGTGGACTTC - 3'	140-160	52	54	2.5	6	Piertney and Hoglund 2001
BG20	GATA	5' - M13 - AACACTTACAATGGTGAGGAC - 3' 5' - TATGTTTTCCTTTTCAGTGGTATG - 3'	123-171	52	54	2.5	9	Piertney and Hoglund 2001
BG18	CTAT	5' - M13 - CCATAACTTAACTTGCACTTTC - 3' 5' - CTGATACAAAGATGCCTACAA - 3'	160-268	48	50	2.5	28	Piertney and Hoglund 2001
TUT2	GATA	5' - M13 - CCGTGTCAAGTTCTCCAAAC - 3' 5' - TTCAAAGCTGTGTTTCATTAGTTG - 3'	144-184	52	54	2.5	11	Segelbacher et al. 2000
ADL230*	AC	5' - M13 - CAGCCAATAGGGATAAAAGC - 3' 5' - CAGCCAATAGGGATAAAAGC - 3'	123-129	55	57	2.5	4	Cheng 1994
TTD2*	CA	5' - M13 - AACAGCCTGAAATACTGAACTT - 3' 5' - ATGTGGTTTTGAAGTAAGTTGAC - 3'	166-180	45	48	2.5	8	Caizergues et al. 2001

Appendix 4. Two-dimensional plots of the first three principal coordinates plotted against each other.

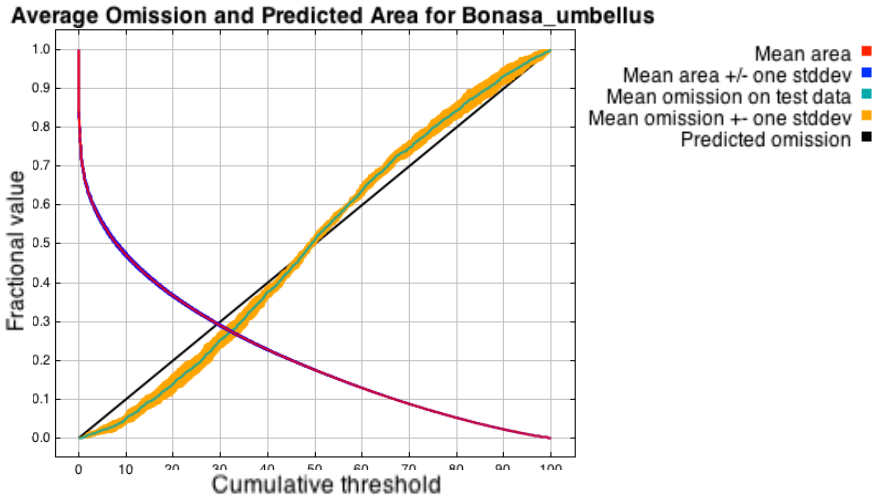


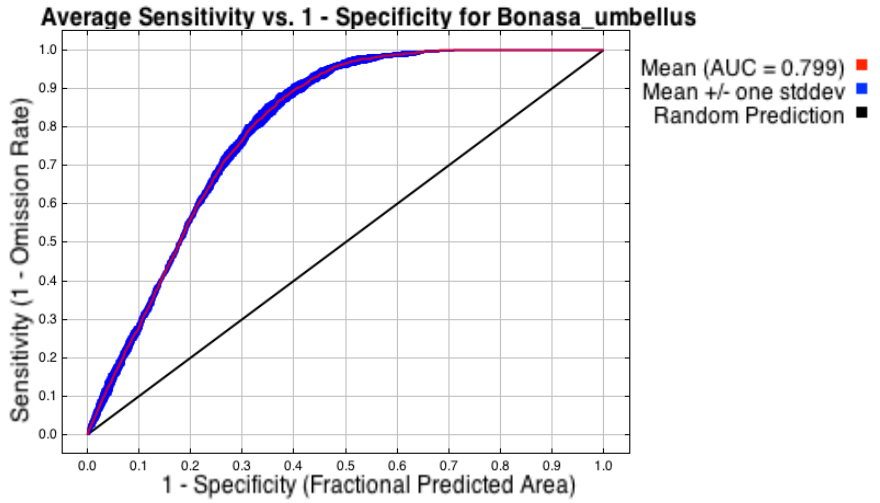


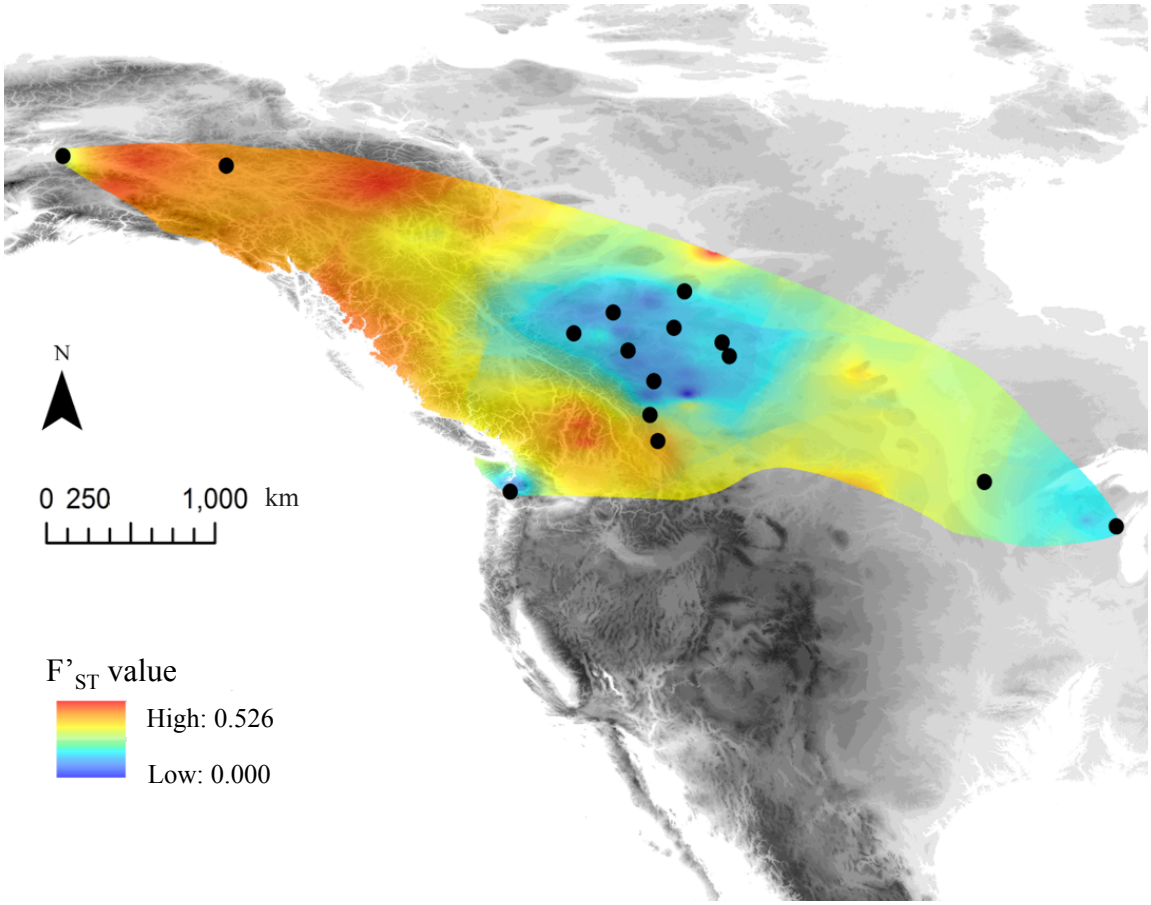


Appendix 5. Contribution of layers used in SDM, with BIOCLIM layers labeled by number. Average/predicted omission and AUC curves are also included.

Layer	description	% contribution to model	permutation importance
LC	MODIS land cover layer	36.1	16
1	Annual mean temperature	22.2	38.5
3	Isothermality (mean diurnal temp range/annual temp range (* 100))	21.9	19.6
8	Mean temperature of wettest quarter	7.1	0.2
9	Mean temperature of driest quarter	6.1	13.4
18	Precipitation of warmest quarter	2.6	0.3
2	Mean diurnal range (mean of monthly (max - min temp))	2	7.3
4	Temperature seasonality (standard deviation *100)	0.7	3.4
14	Precipitation of driest month	0.7	0.5
15	Precipitation seasonality (coefficient of variation)	0.3	0.7
12	Annual precipitation	0.2	0

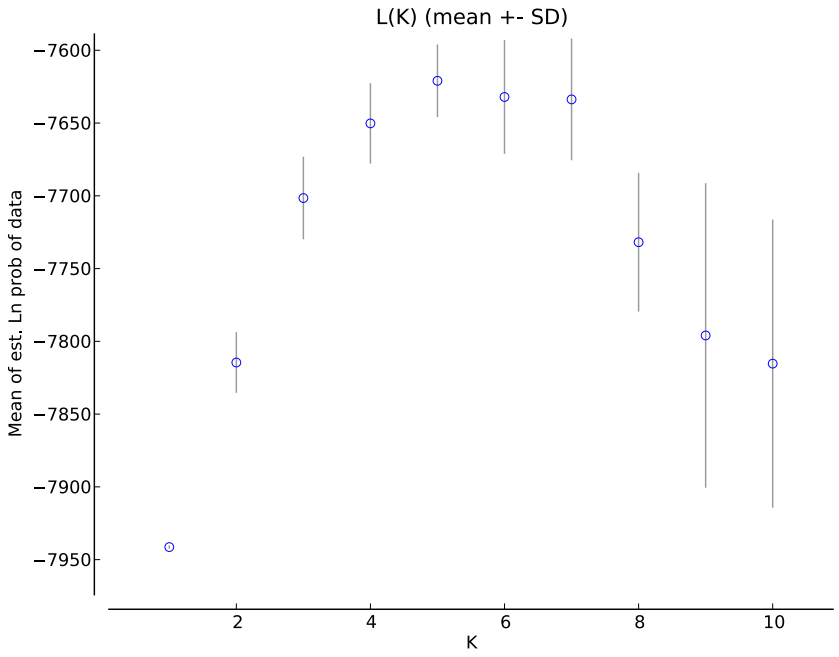




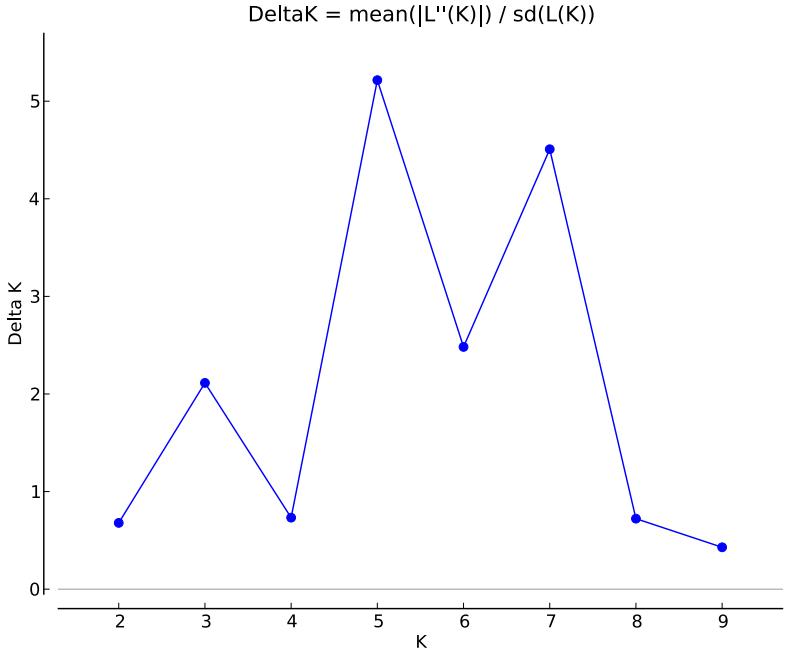


Appendix 6. The species divergence map made using the Landscape Genetics toolbox (Vandergast et al. 2011) in ArcGIS®. Pairwise F'_{ST} values (Table 2.5) were colour-coded and interpolated across a geographic map of the sampling sites. Colours that are green or warmer are equivalent to statistically significant F'_{ST} values.

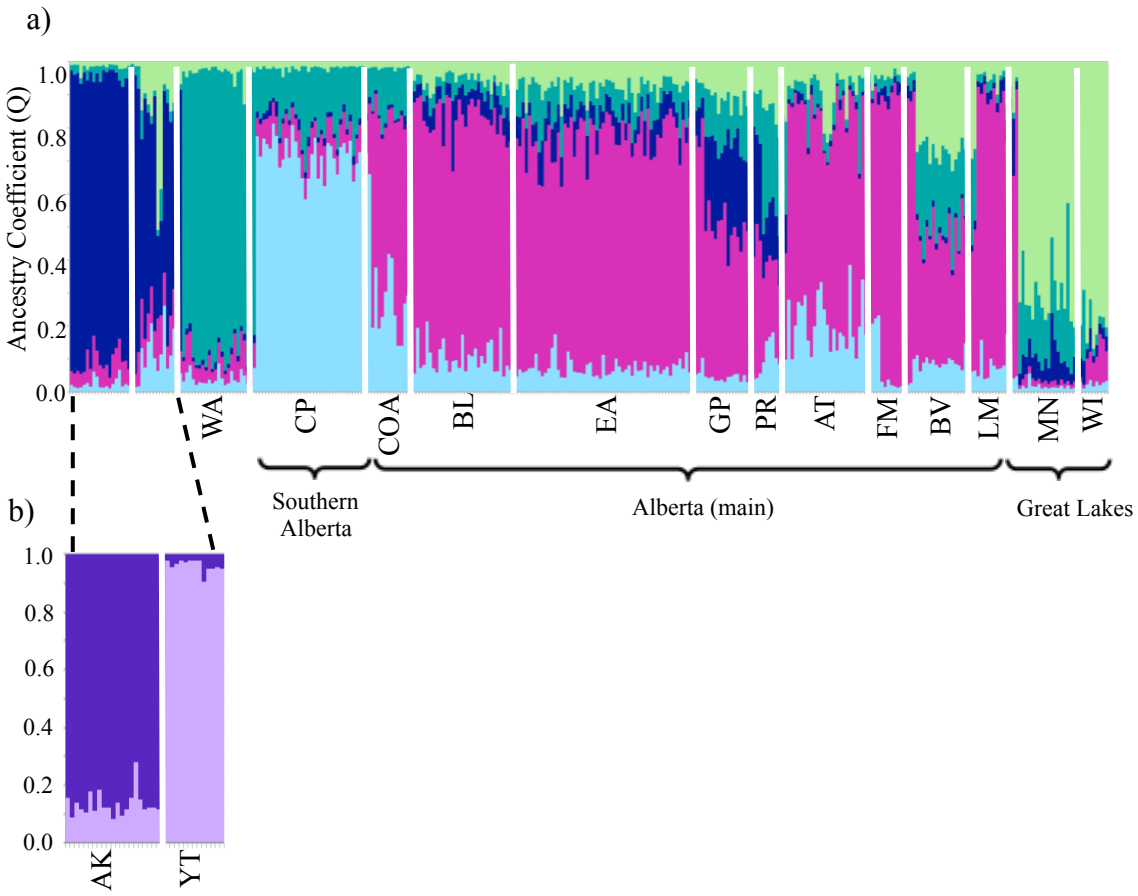
a)



b)

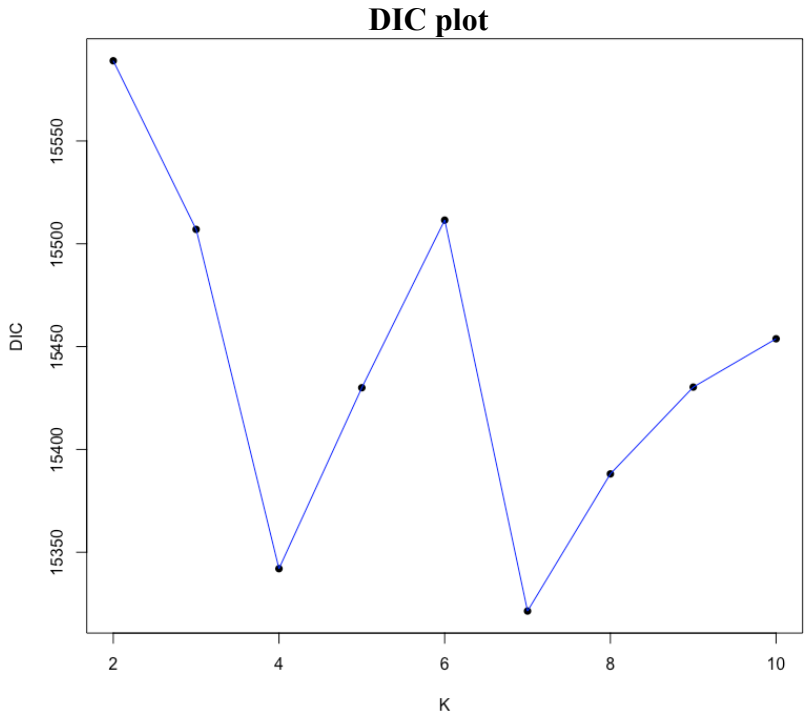


Appendix 7. Plots of (a) log likelihood (LnPr(X|K)) and (b) delta K (ΔK) over 10 replicates of STRUCTURE run for each value of K from 1-10. Plots were created using STRUCTURE HARVESTER v0.6.94 (Earl and vonHoldt 2012), and were used to evaluate all 15 populations run together; subsequent runs involving only two populations to investigate substructure can not be plotted. The most likely number of populations (K) is determined by the highest estimated log probability of the data and highest delta K.

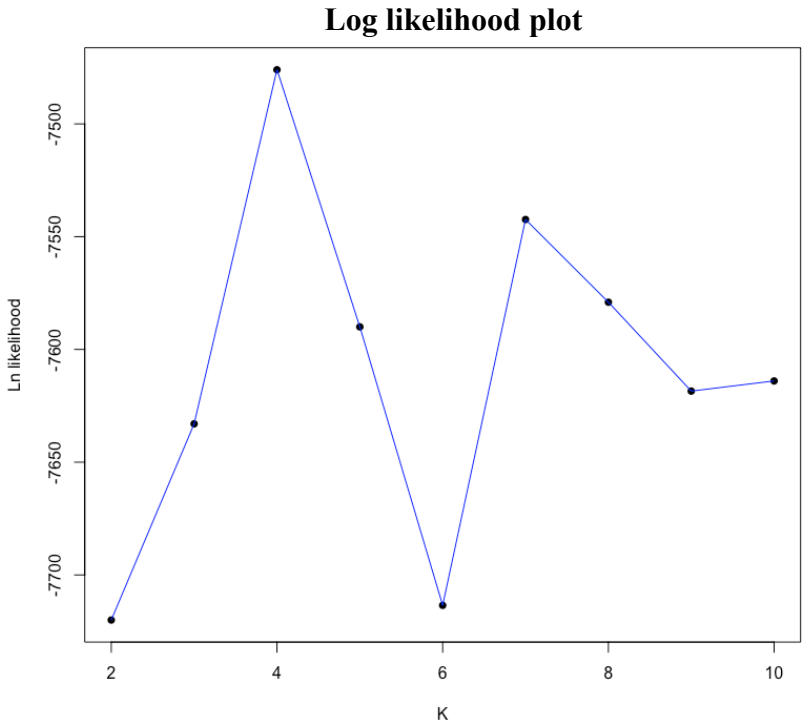


Appendix 8. Ruffed Grouse population structure as inferred by STRUCTURE v2.3.4 (Pritchard et al. 2000) from eight microsatellite loci . The histogram plots show ancestry coefficient (Q) on the y-axis, and individual samples on the x-axis. The number of genetic cluster and inferred membership to these clusters for each individual for all 324 individuals at 15 sampling sites is shown at (a) K = 5. The five clusters formed were one for the Great Lakes populations (MN, WI), one for northern and central Alberta (COA, BL, EA, GP, PR, AT, FM, BV, LM), one for southern Alberta (CP), one for Washington (WA), and one for Alaska and Yukon (AK, YT) with further substructure. All groups aside from Alaska and Yukon were removed, which resulted in (b) K = 2, which was supported by higher values of log likelihood (LnPr(X|K)) than for K = 1. No further substructure was found when the remaining clusters were run independently.

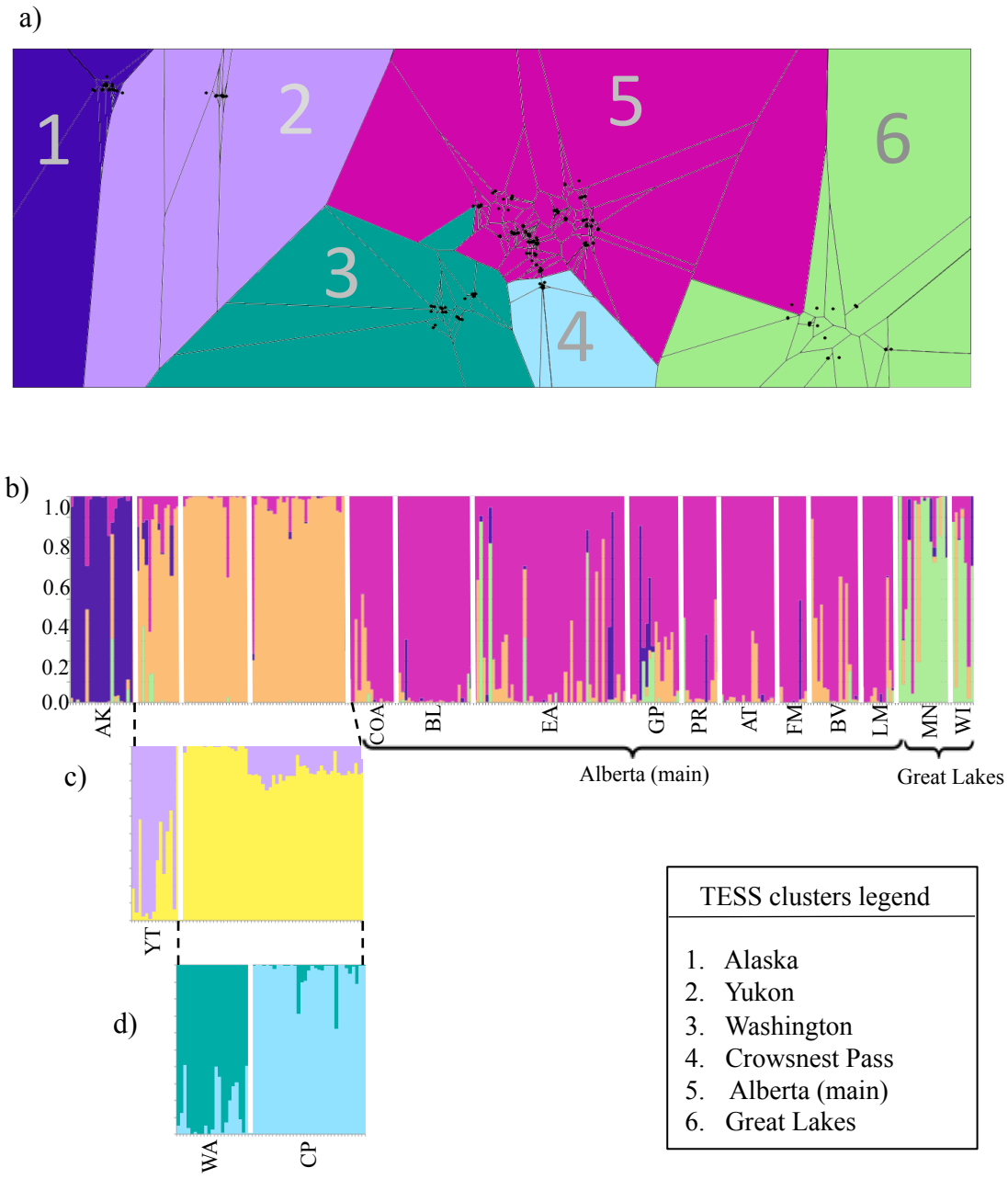
a)



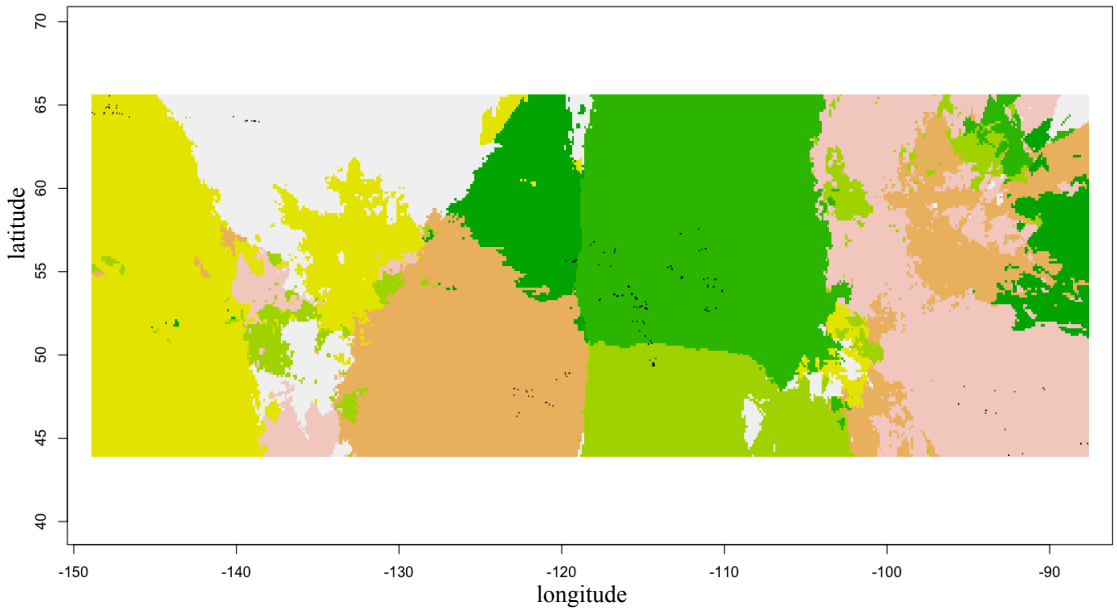
b)



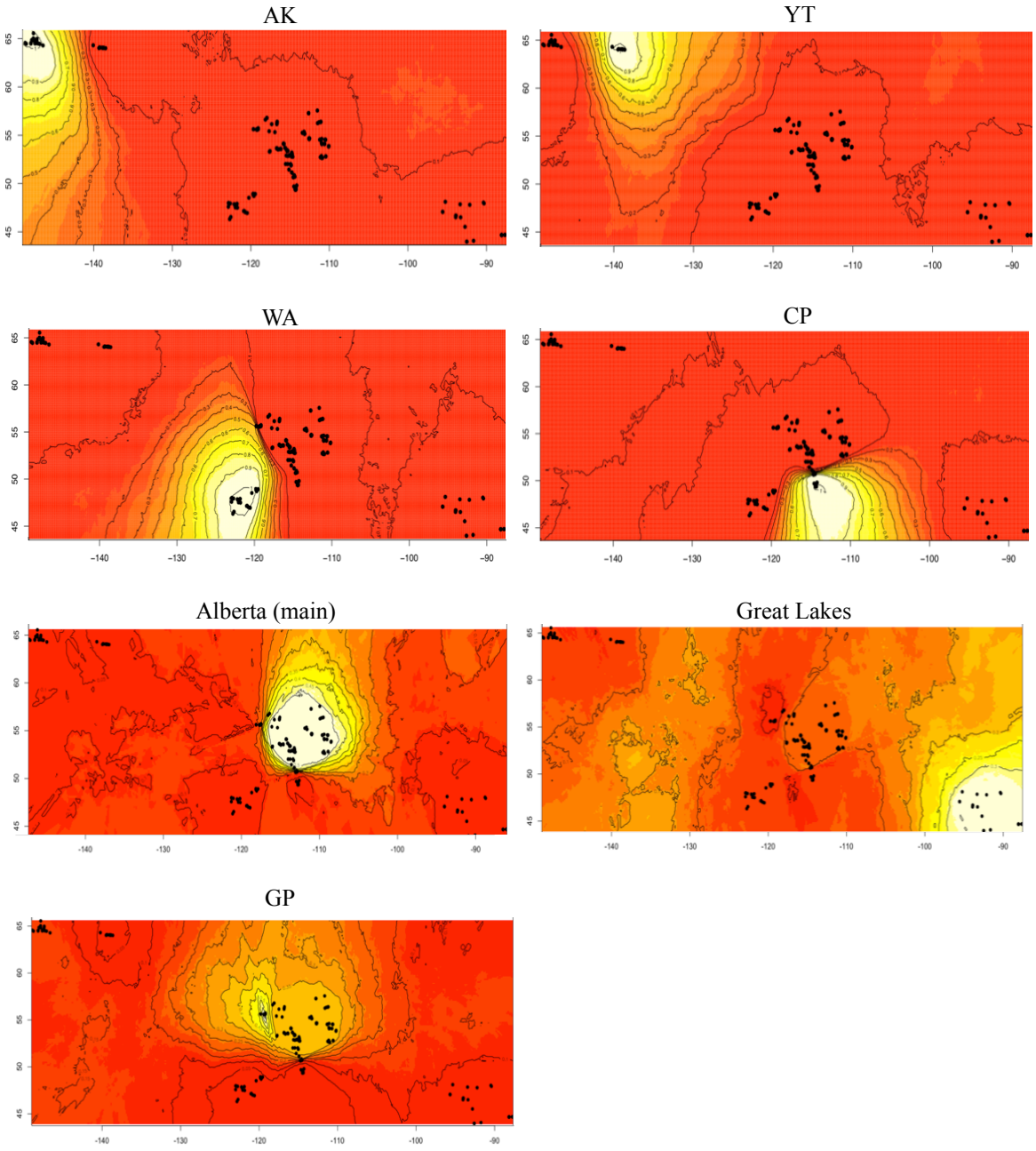
Appendix 9. Plots of (a) DIC and (b) log likelihood averaged over 10 runs in TESS v2.3 from K = 2 – 10. The lowest value of DIC suggests the best value of K, whereas the highest value of log likelihood (LnPr(X|K)) indicates the most appropriate value of K.



Appendix 10. Ruffed Grouse population structure as inferred by TESS v2.3 (Chen et al. 2007) from eight microsatellite loci. The (a) Voronoi tesselation shows the membership of each of the cluster inferred by TESS v2.3 plotted in geographic space. The histogram plots show ancestry coefficient (Q) on the y-axis, and individual samples on the x-axis. The number of genetic cluster and inferred membership to these clusters for (b) K = 4. Further substructure was identified by running the cluster containing YT, WA, and CP independently at (c) K = 2, and after removing YT, populations WA and CP were run at (d) K = 2. No further substructure was found when the remaining clusters were run independently.

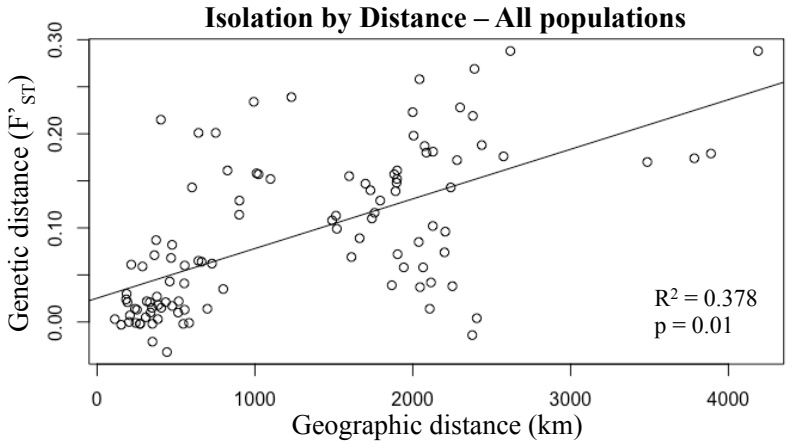


Appendix 11. GENELAND population membership map. Each colour represents a different genetic cluster, and black dots are individual samples. Coloured clusters with no black dots within them should be be considered with caution because these areas are lacking genetic data, and have been assigned based on a "best guess" by the program. The map is in geographic space with latitude on the y-axis and longitude on the x-axis.

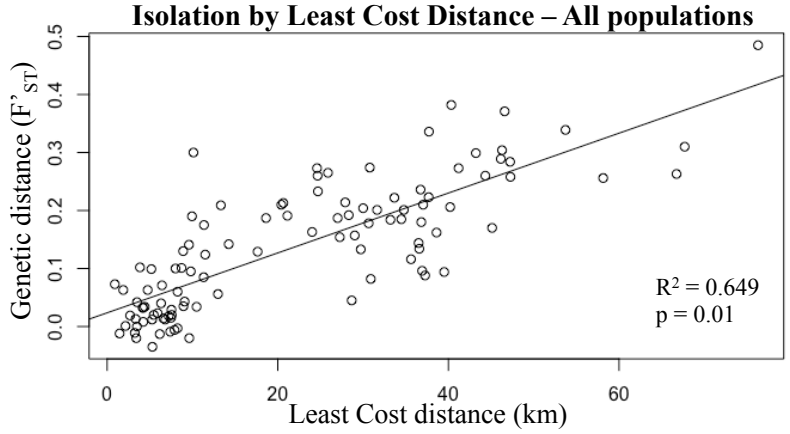


Appendix 12. Ruffed Grouse population structure as inferred by GENELAND v4.0.6 (Guillot et al. 2005) from eight microsatellite loci. The posterior probability maps of cluster membership are shown here. The gradient lines (i.e. contour lines) connect points of the same degree of differentiation to show genetic clines, and the clusters are plotted in geographic space with latitude and longitude in decimal degrees on the y- and x-axes respectively.

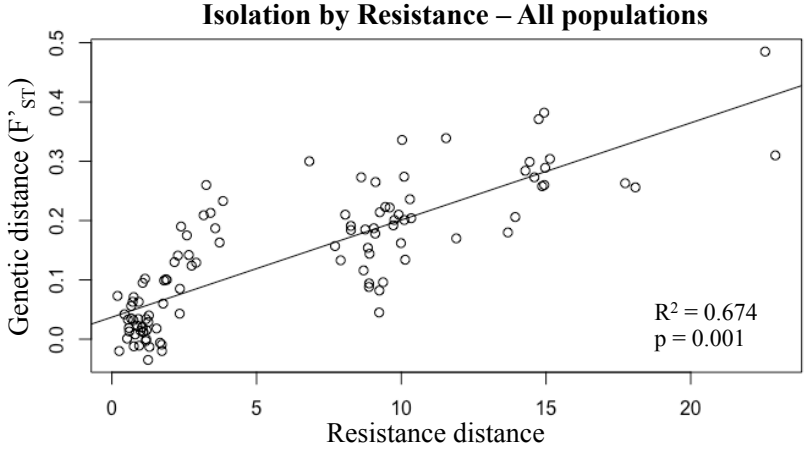
a)



b)



c)



Appendix 13. Plots showing Mantel test of (a) isolation by distance (IBD) comparing genetic distance as measured by F'_{ST} and geographic distance between populations, (b) isolation by distance using least cost path distance in place of geographic distance, and (c) isolation by resistance which compares genetic distance to resistance distances (least cost paths weighted with dispersal cost values). Correlation values (R^2) and p-values of each Mantel test are given for each respective plot as calculated in GenAlEx v6.5 (Peakall and Smouse 2012). Plots shown are comparisons including all 15 sampled populations.

Appendix 14. Variable sites and geographic distribution of haplotypes for SLC45a2 sequences. Each variable site is labeled vertically by its nucleotide position. Sites labeled with (.) are identical to the top sequence, and (-) indicates a deletion. The deletion sites are also highlighted in orange. Shared haplotypes are denoted by letter and singletons are labeled with the corresponding sample name.

	SLC45a2 Variable Sites																SLC45a2 Geographic Distribution of Haplotypes						
	224	241	242	243	244	245	246	247	248	249	250	299	541	633	674	744							
Haplotype/ Sample ID																	Alaska (AK)	Yukon (YT)	Washington (WA)	S. AB (CP)	Cent. AB (BL)	Cent. AB (EA)	Minnesota (MN)
A	T	T	G	G	T	T	T	T	T	C	T	T	T	G	G	C		7	2	17	11	2	4
B	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	8	11	1	6	21	6	7
C	C	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.			5	1	4	2	
D	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					2		
E	C	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.				4	3		4
F	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.				1	6	2	
G	C	-	-	-	-	-	-	-	-	-	-	.	C	.	.	.				3		2	1
H	.	-	-	-	-	-	-	-	-	-	-	.	C	.	.	.				3		1	
RUGR BL025 allele b	.	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.					1		
RUGR CP001 allele a	C	.	.	.	.	.	.	.	.	.	.	C	C	.	C	.				1			
RUGR CP016 allele b	.	-	-	-	-	-	-	-	-	-	-	.	C	.	.	T				1			
RUGR CP017 allele a	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.				1			
RUGR WAp011 allele a	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.			1				
RUGR WAp011 allele b	C	-	-	-	-	-	-	-	-	-	-	.	C	.	.	T			1				
RUGR EA021 allele b	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.						1	

Appendix 15. Variable sites and geographic distribution of haplotypes for Control Region sequences. Each variable site is labeled vertically by its nucleotide position. Sites labeled with (.) are identical to the top sequence. Shared haplotypes are denoted by letter and singletons are labeled with the corresponding sample name.

	Control Region Variable Sites																													Control Region Geographic Distribution of Haplotypes						
	7	8	8	9	1 0	0	0	1 1 0 2	1 3	1 3	1 4	1 4	1 5	1 5	1 5	1 6	1 6	1 6	1 7	1 7	1 8	1 9	1 9	1 9	2 1	2 1	2 4	3 0							
	9	0	2	1	0	1	2	7	5	6	0	1	2	5	6	0	1	2	0	8	0	1	3	4	2	4	6	5							
Haplotype/ Sample ID																													Alaska (AK)	Washington (WA)	S. AB (CP)	Cent. AB (BL)	Cent. AB (EA)	E. Cent. AB (LM)	Minnesota (MN)
A	G	A	A	G	A	C	A	G	G	G	T	T	T	G	G	G	G	C	A	G	A	A	G	G	G	C	C		7						
B	.	.	G	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.		3						
C	.	G	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		3						
D	.	.	.	A	.	.	.	.	.	A	.	.	.	.	.	.	A	.	.	.	.	G	.	.	.	.	T	T			6					
E	.	.	.	A	.	.	.	.	.	A	.	.	.	.	.	.	A	.	.	A	.	G	.	.	.	.	T	T			3					
F	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1	1				1	
G	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	T	T				4	4	3	2	
H	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	T	T					1		1	
I	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T								2
J	A	G	.	.	G	T	G	.	.	A	.	.	.	.	A	.	A	T	.	.	.	.	A	A	.	.	.	.								2
K	.	G	.	.	G	T	.	A	A	A	.	.	.	A	A	.	.	.	A	G	.	.	A	.	A	.	.									2
RUGR AK008	.	.	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1						
RUGR CP005	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	T	T				1					
RUGR EA007	.	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	T	T						1			
RUGR EA008	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	A	.	.	A	.	G	.	.	.	.	T	T						1		
RUGR LM003	.	.	.	.	.	.	.	.	.	A	.	.	.	A	A	.	.	.	.	.	.	.	.	.	.	.	T	T							1	
RUGR MNn001	.	G	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T								1
RUGR MNn002	.	G	.	.	G	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T								1
RUGR MNn005	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T								1
RUGR MNc008	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T								1
RUGR MNc010	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	T	T								1
RUGR MNs023	.	G	.	.	G	T	.	A	A	A	.	.	.	.	.	A	.	.	.	A	G	.	.	A	.	.	.	.								1