Genetic Connectivity of Boreal Woodland Caribou (*Rangifer tarandus caribou*) in Central Canada

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Abstract

Delineating population units is essential for the conservation and management of a species. Applying a genetic approach to delineate units, this study identifies genetic population structure, and landscape resistance to gene flow, of the nationally threatened boreal woodland caribou (*Rangifer tarandus caribou*) across the ecotypes' southern range in Saskatchewan. Three genetic clusters were delineated across the study area, with moderate genetic connectivity identified with Manitoba. Isolation-by-distance was found to be significant across Saskatchewan, and within each genetic cluster. Gene flow across clusters in Saskatchewan was high (F_{ST} = ~0.01), with genetic connectivity being lowest for the south-central cluster surrounding Prince Albert National Park (F_{ST} = ~0.03). Resistance to gene flow was identified with the following landscape variables: water, forestry, roads, wildfire, and low suitability habitat. Careful consideration of these variables in range planning will help to maintain genetic connectivity of boreal caribou across its southern range in Saskatchewan.

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1.1 Research Problem

Boreal woodland caribou (*Rangifer tarandus caribou*) is an ecotype of woodland caribou that occupies the boreal forest (Environment Canada 2011). It is nationally listed as threatened under the Species At Risk Act (SARA) in Canada (c.29, Schedule 1). In Saskatchewan, the boreal woodland caribou ecotype (from now on referred to as boreal caribou) is not listed provincially; however, population declines have been noted across the Province (Trottier 1988; Godwin and Thorpe 2000; Arsenault 2003). In response to the national Recovery Strategy (Environment Canada 2012), the Saskatchewan Ministry of Environment has developed a Conservation Strategy (2013) that outlines the areas of risk for the ecotype across the Province, as well as range planning objectives. Range plans in Saskatchewan will outline land-use options to maintain suitable habitat and connectivity for the ecotype, and aim to minimize further risk from habitat loss and fragmentation (Saskatchewan Environment 2013).

Currently, areas where boreal caribou reside in Saskatchewan are divided into two Woodland Caribou Conservation Units (WCCU): the Boreal Plains (SK2) and Boreal Shield (SK1) ecozones (Environment Canada 2012; Saskatchewan Environment 2013). These two ecozones have been divided into two separate units for species population assessment purposes; however, more thorough analyses of boreal caribou distribution and connectivity is needed in Saskatchewan (Environment Canada 2012). A main characteristic that separates the two ecozones, and currently WCCUs in Saskatchewan, includes a higher level of anthropogenic disturbance across the Boreal Plains ecozone, including greater road and town development (Environment Canada 2012). This disturbance is expected to create resistance to dispersal for

individual caribou, and consequently landscape resistance to gene flow (Isolation-by-Resistance (IBR); McRae 2006), leading to a greater risk of extirpation for the ecotype (Rettie and Messier 1998; Arsenault and Manseau 2011; Environment Canada 2012). For this reason, attention has been given to WCCU SK2 by the Saskatchewan Ministry of Environment, as more information is needed to aid in the management and recovery of the ecotype across the range where it is most vulnerable. Specifically, more information on genetic population structure and connectivity will allow for the further delineation of these two units into finer scale management units (Saskatchewan Environment 2013). These two current WCCU's should not, however, be treated as distinct areas with independent populations, as the ecotype extends continuously across both ecozones.

Alternatively, Manitoba has recently delineated boreal caribou in its Province into nine Management Units, and at a finer scale, 15 Boreal Caribou Ranges. Connectivity of boreal caribou between provinces has not been outlined in Manitoba's Boreal Woodland Caribou Recovery Strategy; however, inter-jurisdictional cooperation has been addressed in the report as a future opportunity to improve the connectivity of the ecotype across neighbouring provincial boundaries (Manitoba Conservation 2015).

Inter-provincial connectivity of boreal caribou across Saskatchewan and Manitoba has been previously addressed in a study by Ball et al. (2010). The study identified that the boreal caribou sampled within, and north, of Prince Albert National Park (previously known as the Smoothstone-Wapaweka caribou herd in central Saskatchewan), have moderate gene flow with caribou across the provincial boundary in western Manitoba (F_{ST} ranging from 0.03 – 0.08, see paper for more detail). Population structure analysis within central Saskatchewan alone showed evidence of high levels of gene flow across the landscape (Ball et al. 2010), and these results

were replicated in another study evaluating population structure and connectivity across the two provinces (Thompson 2015). The limitation of these two studies includes that sampling was not continuous between the two provinces, leaving spatial gaps that may have had impacts on the population structuring results. Another study by Rettie and Messier (1998), which applied satellite telemetry data to understand the distribution of the ecotype across the Boreal Plains in Saskatchewan, has also described boreal caribou as being continuously distributed without the interfering factors of landscape heterogeneity.

In this study, the genetic population structure of boreal caribou across their southern-most range in Saskatchewan, and genetic connectivity with the neighbouring province of Manitoba, will be identified. This expands on the findings of Ball et al. (2010) and Thompson (2015), with a continuous study area extending from the Boreal Plains in Manitoba to Saskatchewan. From the observed genetic structure, population clusters will be delineated to aid managers in developing finer scale management units for boreal caribou across the study area. The influence of anthropogenic and natural landscape variables, such as roads and wildfires, on population genetic structure will further be determined to aid in range planning for the ecotype across the study area in Saskatchewan.

1.2 General Methods

The study area consists of the eastern to central regions of Saskatchewan and the western region of Manitoba, both within the Boreal Plains ecozone, and extending north into the Boreal Shield ecozone in Manitoba. The following boreal caribou MUs in Manitoba have been included in this study: Naosap, Wabowden, The Bog and Interlake (Manitoba Conservation 2015). By obtaining genetic profiles from the microsatellite DNA of individual boreal caribou fecal pellets collected within the study area, along with GPS locations of sample sites, individual caribou were clustered into genetically defined populations according to population genetic principles set in the software STRUCTURE (Pritchard et al. 2000) and TESS (Chen et al. 2007). These programs inform on the level of gene flow occurring between genetic clusters within the study area, and on the spatial distribution of the ecotype, to identify how many population clusters are present (Pritchard et al. 2000). This information was used to spatially delineate genetic clusters of boreal caribou across the study area. Further, landscape features that are hypothesized to reduce gene flow were assigned resistance values on rasterized maps of the study area in Saskatchewan, and were tested against the genetic distance obtained from the genetic profiles of boreal caribou fecal pellets. This aided in determining if certain landscape features are influencing the population structure patterns observed. Individual boreal caribou sampled in Saskatchewan were also clustered according to their genetic distance to other sampled individuals using the novel program MEMGENE (Galpern et al. 2014). This method determines the spatial distribution of individuals according to spatial autocorrelation patterns, or how genetically similar they are to nearby individuals. This information was further used to determine if certain landscape features are influencing the observed genetic structure of boreal caribou across the study area in Saskatchewan.

1.3 Justification

This study expands on evaluating population structure and genetic connectivity across Saskatchewan and western Manitoba, which will aid in delineating genetically discrete populations with an extended study range and sample collection than in Ball et al. (2010) and Thompson (2015). Delineating population units of boreal caribou across the study area will help to determine if further subdivision of current Conservation Units into Management Units in Saskatchewan, can be validated genetically across the current extent that genetic data is available for within SK2. This information is essential and can assist in range planning for the management and recovery of the ecotype. Additionally, variables on the landscape that are predicted to be influencing genetic connectivity of boreal caribou in Saskatchewan are evaluated. Recommendations for maintaining genetic connectivity of boreal caribou across the landscape are provided in the hope that they will assist in the development of boreal caribou range planning guidelines within the southern range of boreal caribou distribution in the Province, where anthropogenic disturbance is most prevalent.

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2. Chapter Two: Genetic population structure of Boreal woodland caribou (*Rangifer tarandus caribou*), and landscape analysis, across central Canada.

2.1 Introduction

2.1.1 Defining Populations

Identifying populations is essential for effective wildlife management and for applying species conservation plans (Waples and Gaggiotti 2006). However, defining a population, in addition to delineating population boundaries, is a difficult task that will vary across taxa (Putman and Carbone 2014). Different species, or sub-species, have different spatial distributions that influence the level of interaction between individuals and populations on the landscape (Baguette and Van Dyck 2007; Sawyer et al. 2011). Certain species may be divided into many small populations, while others are continuously distributed as one large population (Weir 1996; Frantz et al. 2009). These distribution patterns denote population and genetic structure, and are important parameters to consider when managing a species (Manel et al. 2003). Identifying these patterns is crucial to maintain necessary connectivity across the landscape for conservation practices, but is also often complex, and limited by the tools available in the fields of population biology and population genetics (Manel et al. 2003; Palsbøll et al. 2007). In effect, the recent field of landscape genetics is expanding, and combines landscape ecology and population genetics to answer both genetic and ecological questions about the influence of the landscape on population structure and gene flow for a species (Manel et al. 2003; Manel and Holderegger 2013). These new methods are encouraging and can help to more confidently identify spatial population boundaries that are validated genetically, which are necessary components for the effective conservation and management of species (Waples and Gaggiotti 2006).

2.1.2 Boreal caribou populations

Boreal woodland caribou (*Rangifer tarandus caribou*), from now on referred to as boreal caribou, are non-migratory and typically solitary animals (Stuart-Smith et al. 1997; Callaghan et al. 2010). They are found in low densities compared to the neighbouring barren-ground caribou (*Rangifer tarandus groenlandicus*), which occupies areas just north of their range, and have small, year-round home ranges (Ferguson and Elkie 2004). Dispersal distances are mainly driven by food availability and predator avoidance (Bergerud 1988; Rettie and Messier 2000; Arsenault 2003).

A "local population" of boreal caribou has been defined by Environment Canada (2011; 2012) as "a group of caribou occupying a defined area distinguished spatially from areas occupied by other groups of caribou". Here the term "group", or "population", can be noted as a replacement for the community ecology term "subpopulation". This provides a relevant means for separating spatially isolated animals, with independent demographic parameters, into distinct populations. Difficulties with this approach include relying on an observational method without addressing gene flow patterns, and the connectivity of individuals.

A more useful definition for populations is, therefore, one that includes gene flow across individuals. Dobzhansky (1950) defined a population as "a reproductive community of sexual and cross-fertilizing individuals that share in a common gene pool". This suggests applying a genetic approach for delineating populations, which requires identifying genetic connectivity, and grouping genetically similar individuals. This has proven to be a reliable approach from simulation studies (i.e. Slatkin and Barton 1989); however, measuring gene flow patterns from a sub-set sample of the entire population is not always reliable, as small sample size, and not

sampling from all populations, may bias results (Whitlock and McCauley 1999; Paetkau et al. 2004). These issues are discussed further below. Additionally, interpreting population genetic results from Bayesian Individual-Based-Clustering (IBC) methods alone to delineate population boundaries, has been criticized to have variable success in aiding management decisions (Taylor and Dizon 1999). Improvements to avoid biased results and misleading interpretations may include incorporating analysis of the landscape, and using multiple tools that measure genetic divergence among individuals (Taylor and Dizon 1999; Ball et al. 2010; Safner et al. 2011).

2.1.3 Hardy-Weinberg Equilibrium (HWE) and Linkage Equilibrium (LE)

Once isolated groups of individuals have undergone genetic drift, their genotypes are expected to be in allele frequency equilibrium. This equilibrium can vary across different genetic groups, but is expected to remain constant within a group that has random mating. This theory of allele frequency equilibrium among individuals in a demographically independent group, is termed Hardy-Weinberg Equilibrium (HWE; Hardy 1908; Weinberg 1908).

Deviation from HWE suggests that either allelic mutation, migration, or emigration, or all three, are occurring to cause a shift in allele frequencies, which would be the result of evolution of alleles and continuous gene flow between neighbouring groups of the same sub-species or ecotype (Whitlock and McCauley 1999; Hedrick 2005). Since a population is assumed to be an isolated group with little to no movement of individuals (Waples and Gaggiotti 2006; Environment Canada 2011; 2012), HWE is expected to hold. Therefore, many Bayesian clustering programs (i.e. STRUCTURE and TESS) include this assumption in their algorithms. This, however, is unrealistic in real populations, as the movement of individuals, and the mutation of alleles, is not unlikely. Precautions should therefore be taken when making

interpretations based on these programs (Pritchard et al. 2000; Guillot et al. 2009). Other assumptions of the theory include random mating, no inbreeding, and no sexual or natural selection, which further necessitates that results be taken cautiously (Robertson and Hill 1984). This theory is nonetheless applied in population biology, as it provides a general means to identify a population. Further analysis of gene flow is necessary to identify genetic connectivity between groups of individuals. If connectivity is high, then deviations from HWE can be explained, and the validity of the group as an isolated population can be considered (Guillot et al. 2009).

Similarly, linkage equilibrium (LE) is expected for independent and randomly mating populations, and is based on allele frequency assumptions that adhere to HWE (Hill and Robertson 1968). LE is based on the expectation that all alleles being tested across a chromosome are independent from each other, and is the null hypothesis to testing for linkage disequilibrium (LD) across loci (Weir 1996).

2.1.4 Measuring gene flow

With increasing habitat fragmentation occurring due to anthropogenic disturbances on the landscape in Saskatchewan (Environment Canada 2012) and Manitoba (Manitoba Conservation 2015), historically continuous species may become fragmented and isolated (Gilpin 1991). This highlights the need to use genetics to identify how populations are currently structured, and to identify if gene flow patterns have been altered (Waples and Gaggiotti 2006; Palsbøll et al. 2007; 2010). This information can aid managers in maintaining areas that promote gene flow and connectivity between populations, as well as identify which populations are lacking the genetic diversity required for long-term sustainability (Manel et al. 2003; Palsbøll et al. 2007). Further,

important areas of the landscape that promote gene flow between two genetically dependant populations can be verified, and land-planning decisions can be made accordingly (Palsbøll et al. 2007).

Palsbøll et al. (2007) defined gene flow as "the number of migrants per generation" (N_m). Immigration to a population, or to a group of individuals of one sub-species, can include dispersers that contribute to the next generation of the population, and those that do not contribute to the next generation (due to factors such as sex, age, selection against migrants, etc.; Waples and Gaggiotti 2006; Nosil et al. 2005). The difference between the term "disperser" and "migrant" is that migration rates translate to a measure of gene flow, while dispersal rate refers to simply "the movement of individuals from one genetic population … into another" (Palsbøll et al. 2007). Migration rates can also vary per generation, making capture-mark-recapture (CMR) methods necessary to identify true dispersal patterns over many years (Pradel 1996; Leberg 2005; Putman and Carbone 2014). This, however, may not always be a feasible or available option in the conservation and management of a threatened or endangered species, leaving gene flow to be measured after a single year of sampling in order to make time-sensitive management decisions (Petit and Valiere 2006). Regardless of advances in the field, including the use of genetic data to measure migration rates (i.e. Pritchard et al. 2000; Broquet and Petit 2009; Wang 2005; Luikart et al. 2010), species movement patterns require long-term studies to fully understand. Inferences on genetic structure should therefore be made with precaution, and with the plasticity of animal behaviour and ecology in mind (Baguette and Van Dyck 2007; Yannic et al. 2015). To reduce the problems with short-term sampling, incorporating additional available genetic data, such as mitochondrial haplotypes (Moritz 1994), may be a useful solution. Mitochondrial data may help to answer uncertainties on why certain populations are structured

on the landscape in a particular way, and whether the identified structure may be an artefact of historical interactions and dispersal patterns, or due to recent landscape disturbances (Moritz 1994).

2.1.5 Genetic distance statistics

Groups of individuals that are demographically independent are expected to have different gene, or allele, frequencies after generation(s) of isolation, due to random mating and genetic drift (Hardy 1908; Weinberg 1908). Once alleles become fixed within a group, more individuals are likely to contain homozygous (double) copies of alleles on particular loci, increasing the frequency of that allele. In effect, heterozygosity is expected to decrease, removing certain alleles from within the group (Maudet et al. 2002). Limited interaction between groups of individuals maintains different gene frequencies between groups; however, some levels of gene flow will occur allowing for the sharing of alleles. The fixation index (F_{ST}), first suggested by Wright (1951), is the most common measure of genetic differentiation, also termed genetic distance, to evaluate this frequency of shared alleles between groups (such as sub-populations; Palsbøll et al. 2007). This measure provides either independent or pairwise comparisons between groups of individuals by calculating loss of heterozygosity, or the level of allelic differentiation, for a given group of individuals. Its equation is as follows:

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

In the equation, H_T is the total heterozygosity of both populations being compared (or of all groups in total), and H_S is the average heterozygosity for the population in question (Wright 1951). This measure is equal to the multi-locus genetic distance measure of G_{ST} (Nei 1973).

When alleles become fixed in an isolated group, resulting in high homozygosity, the allelic differentiation between the groups being compared will be at its highest, with F_{ST} being closer to 1. The higher the frequency of shared alleles between the groups being compared, indicating a level of genetic connectivity, the closer F_{ST} will be to 0 (Berg and Hamrick 1997).

High genetic differentiation is important for classifying a population, as it indicates that a group of individuals is independent from the interaction of outside individuals. Extremely high genetic differentiation, however, is not common, as the migration of nearby individuals is expected to lower allelic differentiation (Wright 1978; Whitlock and McCauley 1999). A general F_{ST} threshold has been established to interpret the measure of genetic distance, with a value increasingly greater than 0.20 indicating high allelic diversification, or population structuring (Wright 1978). Recent studies evaluating population structure have suggested that genetic differentiation for most populations is well below this value, with a F_{ST} value of 0.02 being the minimum differentiation for the statistical detection of separate populations (i.e. Latch et al. 2006; Ball et al. 2010). As with HWE assumptions, precaution when relying on F_{ST} measures needs to be taken due to simplifications in the calculation assumptions, such as no allelic mutation (Balloux et al. 2000; Hedrick 1999; 2005), the absence of null alleles, which can increase genetic differentiation (Chapuis and Estoup 2007; Putman and Carbone 2014), and no potential deviation from the infinite-island-model (Meirmans and Hedrick 2011).

R_{ST} can be additionally used with F_{ST} measures to inform on phylogeographic patterns in the data (Slatkin 1995; Hardy et al. 2003). R_{ST} measures genetic differentiation through allele size (repeats between microsatellite alleles created by mutation; Hardy et al. 2003), instead of the number of different alleles present (F_{ST}). Allele size differences will have evolved over time between two isolated groups, or between groups with low levels of gene flow, providing a

measure of historic gene flow events (Hardy et al. 2003). Understanding phylogeographic patterns, in addition to contemporary patterns in gene flow, can help to determine if genetic connectivity between genetic clusters of caribou is historic. Comparing patterns may also provide insight on demographic changes, and when and why they have occurred (Hardy et al. 2003). Limitations of the R_{ST} measure includes that it is sensitive to potential deviation from the stepwise-mutation-model that its calculation assumes (Balloux et al. 2000; Meirmans and Hedrick 2011; Whitlock 2011). F_{ST} has therefore been suggested as a more reliable measure of differentiation when high levels of gene flow between populations is present (Balloux and Goudet 2002). Permutation tests for both F_{ST} and R_{ST} can further help determine the significance of each measure to aid in more accurate inferences of genetic connectivity and differentiation (Hardy et al. 2003).

2.1.6 Lag time and microsatellite data

The effects of landscape changes on genetic differentiation is not always easy to detect, as it may take time to become evident within the genetic data across individuals, especially at the population level. The introduction of landscape resistance to gene flow may cause two groups of individuals to become less genetically connected, yet how well this can be captured in analysis using, for example, microsatellite data, is variable. A study by Landguth et al. (2010) identified that Mantel r, a correlation measure applied in landscape genetic studies, can detect the significance of a landscape variable influencing genetic differentiation across two groups within 1-15 generations. Microsatellite data, although well used in genetic studies, has been criticised to be weak at expressing low levels of gene flow across the landscape (Hedrick 1999). This is because of the long time it takes for genetic distance, which is used to identify gene flow, to reach equilibrium in the microsatellite data of a population (Landguth et al. 2010). For example,

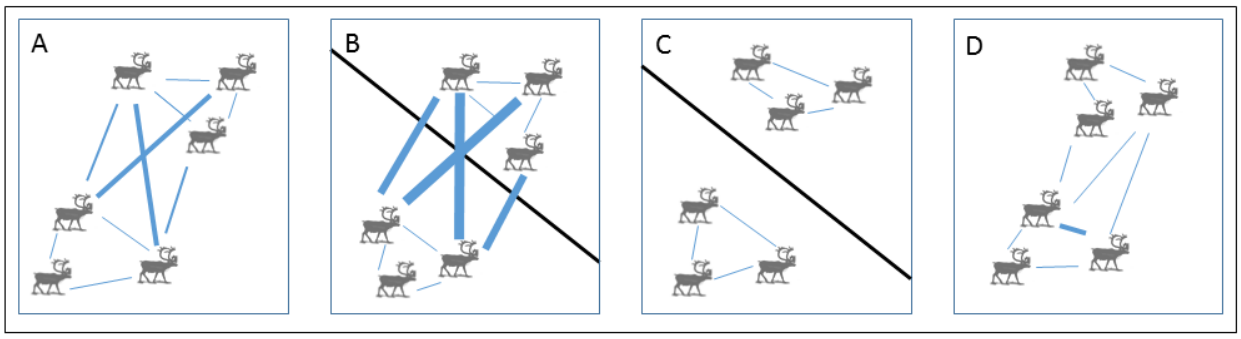
a study by McLoughlin et al. (2004) found that despite high levels of anthropogenic disturbance on the landscape, boreal caribou in western Canada still had low levels of genetic differentiation between groups. Fragmentation and landscape resistance may therefore take many generations to become evident across genetic data, as the effects may be decelerated by even low levels of gene flow. Consideration of this time lag on genetic differentiation needs to be taken when interpreting results based on microsatellite data (Landguth et al. 2010). A further limitation in relying on microsatellite data includes their high rate of mutation, which will influence inferences based on genetic differentiation, such as population structure (Hedrick 2005; Putman and Carbone 2014). This is especially problematic when mutation rates are greater than migration (or dispersal) rates, which may increase measures of genetic differentiation (Meirmans and Hedrick 2011; Whitlock 2011). Missing data in microsatellite genotypes is also an unresolved concern that may bias results, causing issues that vary from identifying unique individuals based on genotypes, to calculating genetic differentiation with measures such as F_{ST} (Putman and Carbone 2014). Incorporating more loci may help reduce some of these limitations, although this option is itself limited by the level of mutation at each locus, and the ability to detect neutral markers (Colonna et al. 2009; Putman and Carbone 2014).

2.1.7 Isolation-by-distance (IBD)

Landscape effects on gene flow, or genetic distance, can be described with the following four models pictured in Figure 2-1. Lines connecting individual caribou in the figure represent gene flow, with wider lines representing a higher resistance to gene flow. In the first model, Isolation-by-distance (IBD) is the effect of genetic distance between individuals increasing in response to geographic distance, usually in a linear fashion (Wright 1943; Manel et al. 2003; Figure 2-1.A). Certain variables on the landscape may hinder this effect, causing a resistance to gene flow

between individuals, termed Isolation-by-Resistance (IBR; McRae 2006; Figure 2-1.B). Other variables may act as barriers and completely prevent gene flow between groups of individuals, regardless of distance, causing genetic distance to increase sharply across either side of the barrier. This leads to the model termed Isolation-by-Barrier (IBB; Schwartz and McKelvey 2009; Figure 2-1.C). No effects on genetic distance from either geographical distance, or any landscape variables, is expected to produce panmixia, where genetic distance is arbitrary between individuals, and no genetic structure exists (Figure 2-1.D).

Figure 2-1. The four models of population structure considered across the study area, (A) Isolation-by-Distance, (B) Isolation-by-Resistance, (C) Isolation-by-Barrier, and (D) Panmixia.



When defining an independent population, IBD is expected between genetic groups of individuals across a continuous landscape, while the presence of panmixia suggests that individuals are highly intermixed, and are all part of the same genetic group (Waples and Gagiotti 2006). Across a heterogeneous landscape, a combination of these scenarios can exist. There are no landscape variables known to completely prevent gene flow within the current study area, and therefore only IBD and IBR will be accounted for in this study.

Teasing apart the effects of the landscape from IBD has been proven difficult (i.e. Cushman et al. 2006; van Strien et al. 2015), and the ability to correctly detect genetic patterns to delineate populations remains a challenge in both population and landscape genetics (Frantz et al. 2009; Manel et al. 2003). One study used Bayesian clustering to delineate genetic population structure, and outlined the strong influence that IBD can have on results, causing an overestimation of the optimal number of populations present (type I error; Frantz et al. 2009). A good understanding of the landscape is therefore necessary to correctly interpret and make biologically meaningful conclusions from the results obtained from landscape and population genetic analyses (Pritchard et al. 2000; Schwartz and McKelvey 2009).

2.1.8 Landscape genetics

Landscape genetics is an expanding and relatively new field of study, which combines population genetics and landscape ecology to help address questions of how the landscape influences gene flow, and the spatial distribution, of a species (Taylor et al. 1993; Manel et al. 2003; Storfer et al. 2007; Manel and Holderegger 2013). The main models used to test landscape effects on gene flow include the alternate hypotheses IBD, IBR, and IBB, with the null hypothesis of panmixia. Testing these models can help to validate genetic structure results. This is done by correlating genetic distance with the cost distance of hypothesized landscape variables to determine if a relationship with gene flow patterns exists (Manel and Holderegger 2013). To eliminate confounding effects from IBD when testing landscape models, the approach of causal modelling has been developed by Cushman et al. (2006). This approach is described further on page 35. Alternative spatial genetics tests have also been developed to avoid relying on Mantel tests, which have been criticized for their questionable reliability in correlating matrices (i.e. Raufaste and Rousset 2001). Although not well used or fully advanced in the field, these

methods can further help to evaluate the relationship between spatial and genetic patterns, and be used to validate landscape genetic analyses (i.e. MEMGENE by Galpern et al. (2014) that is described further on page 38).

2.1.9 Boreal caribou and the landscape

Boreal caribou across central Canada have been noted to prefer a continuous landscape of mature and undisturbed high-density coniferous forests (Rettie and Messier 1998; 2000; 2001). Heterogeneity in the landscape due to natural or anthropogenic disturbance, however, is prevalent in the study area of the Boreal Plains eco-zone in Saskatchewan, which is the southern limit of their range in central Canada (Rettie and Messier 1998; Arlt and Manseau 2011), and western Manitoba (Rebizant et al. 2000; Metsaranta et al. 2003). Increasing landscape discontinuity may, in consequence, be influencing the movement behaviour of boreal caribou (Rettie and Messier 1998; 2000; 2001; Arsenault and Manseau 2011). The expansion of anthropogenic disturbances, such as forestry and road development, is also suspected to be a major driver of population declines for the ecotype across central Canada (Trottier 1988; Rettie and Messier 1998; Arlt and Manseau 2011), as boreal caribou are known to be sensitive to landscape changes (Dyer et al. 2001; Vors et al. 2007; Courbin et al. 2009; Moreau et al. 2012).

In the Boreal Plains of central Saskatchewan, boreal caribou prefer mature forested areas with tree species such as black spruce (*Picea mariana*), as well as peat lands with fens and muskeg throughout the Province (Rettie and Messier 1998; Johnson et al. 2001, 2002). Similarly in Manitoba, boreal caribou have been found to prefer continuous stands of mature jack pine (*Pinus banksiana*; Schaefer 1996; O'Brien et al. 2006). A mature conifer forest offers ideal habitat for

boreal caribou, as it provides food such as terrestrial and arboreal lichen (Schaefer 1996; O'Brien et al. 2006), as well as separation from predators (O'Brien et al. 2006; Courbin et al. 2009).

Boreal caribou cross small lakes and rivers within their range (Environment Canada 2012), therefore, these are not complete barriers to gene flow (Galpern 2014). Areas with access to lakes and wetlands are typically preferred by boreal caribou (Ferguson and Elkie 2004, 2005; Fortin et al. 2008), which are a source of water in the summer, and also help support a greater biomass of lichen growth (Bradshaw et al. 1995). In the summer, large lakes with islands are also known to be preferred by calving females as a predator avoidance technique (Bergerud 1985; Stuart-Smith et al. 1997). Larger lakes are also preferred in the winter (between 50-100 ha), and are believed to be, in part, a strategy for predator avoidance, as most predators will hunt more inland for prey (Bergerud 1985; Ferguson and Elkie 2005). Some studies have shown that large bodies of water have a high resistance to gene flow for boreal caribou, and therefore are significant at influencing genetic connectivity across the landscape without being complete barriers to dispersal (Galpern et al. 2012; O'Brien et al. 2006; Koper and Manseau 2009). Examples from previous studies include the Peace River in Alberta, Canada (McLoughlin et al. 2004), the Mackenzie River in the Northwest Territories, Canada (Galpern et al. 2014) and the Interlake region in Manitoba, Canada (Fall et al. 2007; Klütsch et al. 2012).

A frequent natural disturbance in the Boreal Plains ecozone of Saskatchewan is forest fire, and suppression practices are in effect in the Province (Rowe and Scotter 1973). The typical natural wildfire cycle in Saskatchewan in the Boreal Plains ecozone is between 50 -150 years (Weir et al. 2000), and boreal caribou are known to prefer stands that are at least 50 years old (Schaefer and Pruitt 1991; Dalerum et al. 2007). Although suppression of wildfires may be seen as detrimental to forest ecosystems in the long term due to fuel-load buildup, boreal caribou can

benefit from the practise, as they rely on large areas of undisturbed old growth coniferous forests that support an abundance of lichen growth (Arsenault and Manseau 2011). Conversely, wildfire suppression has also been identified to be negatively associated with habitat use for boreal caribou, as it is predicted to prevent the re-establishment of lichen after long periods of fire suppression. This has been observed in, and north, of Prince Albert National Park in Saskatchewan, where wildfire suppression has been occurring since the 1940's, and the avoidance of these old-growth areas by boreal caribou has been detected (Arlt and Manseau 2011).

Anthropogenic disturbances that involve forest clearing, in addition to natural disturbance in the area such as wildfire (Schaefer and Pruitt 1991), typically result in young successional forests that support the increase of other ungulates into traditional boreal caribou habitat ranges (Seip 1992; James and Stuart-Smith 2000; Courbin et al. 2009). This, in turn, is increasing the number of predators in these areas, which is predicted to be having detrimental consequences to boreal caribou in these ranges (Wittmer et al. 2007; Rettie and Messier 1998; Fortin et al. 2008). Predation has been identified as a main cause of mortality for boreal caribou throughout its range, as well as the main limiting factor of recruitment rates (Sorensen et al. 2008), and is predicted to have increased due to expanding human disturbance such as logging activity and associated road development (Trottier 1988). This issue is relevant across central Saskatchewan, where road development has been increasing northward over recent years (Rettie and Messier 1998; Arlt and Manseau 2011).

Roads are also commonly associated with logging practice disturbance, and are avoided by boreal caribou (Courbin et al. 2009; Fortin et al. 2008). High traffic roads will also be avoided by

boreal caribou, especially in the winter (Dyer et al. 2002), with avoidance thresholds documented to be around 0.25 km (Dyer et al. 2001) to 1 km (Courbin et al. 2009).

Forest harvesting is also a major contributor to habitat fragmentation for boreal caribou (Vors et al. 2007), and is reducing boreal caribou habitat ranges (Moreau et al. 2012). Boreal caribou will typically stay around 13 km from clear-cuts for a time range of at least 2 decades before successional growth that can support lichen cover (Vors et al. 2007), and around 1.2 km from smaller fragmented areas. Forested areas with more than 2.5% of recent logging activity have also been found to cause boreal caribou to recede into higher density coniferous forest (Houle et al. 2010; Moreau et al. 2012). With high levels of habitat fragmentation limiting boreal caribou dispersal, genetic connectivity between individuals may become reduced, resulting in a decline in genetic diversity for the ecotype (Galpern et al. 2012). Evidence of this has been found in previous studies where the survival and recruitment rate of boreal caribou in certain areas was negatively influenced by alterations to natural habitat, specifically due to wildfire and anthropogenic disturbance (Wittmer et al. 2007; Environment Canada 2008).

Adaptation to anthropogenic disturbance for boreal caribou has been recorded in some studies, where caribou with high levels of disturbance in their home ranges become less sensitive to the disturbance over time, and reduce their avoidance thresholds (Moreau et al. 2012; Fortin et al. 2008). In general, however, anthropogenic disturbance is typically avoided by boreal caribou, and substantially reduces their habitat ranges (Moreau et al. 2012; Weclaw and Hudson 2004).

2.1.10 Delineating boreal caribou MUs in Saskatchewan

Due to the difficulties discussed above in delineating genetically distinct groups of individuals at a level that can be confidently defined as a distinct population, maintaining Management Units

(MUs) can be a useful approach for conservation and management purposes (Manel et al. 2003; Palsbøll et al. 2007). A MU is defined by Palsbøll et al. (2007) as "populations of conspecific individuals among which the degree of connectivity is sufficiently low so that each population should be monitored and managed separately". Incorporating MUs into management can therefore provide an easier approach for managing a species, with less connotations of complete demographic independence associated with it, which is difficult to define and measure, than the term population (Palsbøll et al. 2007).

In this study, the genetic structure of boreal caribou across the Boreal Plains in Saskatchewan will be assessed to delineate MUs in the Province. This information is critical for the management and recovery planning of the nationally threatened ecotype (Environment Canada 2012; Saskatchewan Environment 2013). Currently, Saskatchewan is divided into two Woodland Caribou Conservation Units (WCCU): the Boreal Plains (SK2) and Boreal Shield (SK1) ecozones (Environment Canada 2012; Saskatchewan Environment 2013), while boreal caribou in the neighbouring province of Manitoba are divided into nine MUs (Manitoba Conservation 2015). More thorough analysis of boreal caribou population structure and genetic connectivity is therefore needed across Saskatchewan to determine if further population sub-structure exists for the ecotype, as in Manitoba, and for more regional MUs to be established in the Province (Environment Canada 2012; Saskatchewan Environment 2013).

Fecal sampling for population genetic analysis of boreal caribou has been occurring across SK2 in Saskatchewan since 2004. Previous studies by Ball et al. (2010) and Thompson (2015) have determined that the northern area of Prince Albert National Park in Saskatchewan (previously known as the Smoothstone-Wapaweka boreal caribou herd range) can be classified as a genetic cluster, with moderate gene flow with neighbouring genetic clusters in Manitoba (F_{ST} ranging

from 0.03 – 0.08; Ball et al. 2010). This study will expand on these two studies, with a study range extending continuously across the Boreal Plains ecozone in Saskatchewan and Manitoba, excluding the western boundary of Saskatchewan where sampling has not yet taken place within the ecozone. With a more complete study area and dataset, genetic population structure, and genetic connectivity with the neighbouring Province of Manitoba, will be determined to delineate MUs across most of the current WCCU SK2 in Saskatchewan where genetic data for the ecotype is available.

Landscape genetics will also be applied across the study area in Saskatchewan to further identify if certain variables on the landscape are influencing the genetic structure observed, and to aid in validating the delineation of cluster boundaries. Thompson (2015) identified that gene flow of boreal caribou across Ontario, Manitoba, and the Smoothstone-Wapaweka region of Saskatchewan, is affected by anthropogenic disturbances, and natural landscape variables, including water bodies and wildfire. Anthropogenic disturbances are increasing across the study area in Saskatchewan and Manitoba, resulting in increasing landscape heterogeneity and fragmentation (Rettie and Messier 1998; Rebizant et al. 2000; Metsaranta et al. 2003; Arlt and Manseau 2011). Understanding how these changes to the landscape may be influencing gene flow, and genetic connectivity, for boreal caribou is critical for the effective conservation and management of this nationally threatened ecotype.

For the purpose of this study, the term “population” will be replaced with the term “cluster” to define a given group of individuals. Within this study, the term cluster will hold the same definition as a Management Unit, defined above, in order to better represent the more genetically admixed demographic that characterizes this boreal caribou ecotype.

2.2 Study Questions and Predictions

2.2.1 Study Questions

1. Are boreal caribou genetically structured into distinct clusters across the Boreal Plains ecozone in Saskatchewan?
2. If structure exists, what is the level of genetic connectivity between observed clusters in Saskatchewan with clusters found in the neighbouring Province of Manitoba?
3. Does the current landscape influence the structure observed through the process of Isolation-by-(landscape) Resistance?

2.2.2 Predictions

According to the findings in Ball et al. (2000) and Thompson (2015) described on pp. 23-24:

1. Boreal caribou are genetically structured into more than one genetic cluster across the study area in Saskatchewan and Manitoba.
2. Genetic connectivity will be moderate to high ($F_{ST} \leq 0.1$) across genetic clusters in Saskatchewan and Manitoba.
3. Gene flow between genetic clusters is present, but is restricted due to the presence of landscape variables. The anthropogenic variables: roads and forestry, natural variables: water bodies and wildfire burns, and general low suitability habitat for the ecotype across the landscape, will restrict gene flow for boreal caribou across the study area.

2.3 Methods

2.3.1 Study area and non-invasive sampling

The study area consists of the eastern to central regions of Saskatchewan, and the western region of Manitoba, both within the Boreal Plains ecozone, and extending north into the Boreal Shield ecozone in Manitoba.

The landscape across the Boreal Plains is made up of mixed deciduous and coniferous forest, with mixed tree stands including trembling aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), tamarack (*Larix laricina*), balsam fir (*Abies balsamea*), black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*; O'Brien et al. 2005). The Boreal Plains landscape is also made up of a moderate number of lakes (Environment Canada 2009) and 25-50% wetlands, including a high number of peat bogs and muskeg (Environment Canada 2009). Lake Winnipeg is the largest lake in Manitoba, being approximately 24,500 km^2 in size, followed by Lake Winnipegosis. In Saskatchewan, the largest lake masses include Montreal Lake, Dore Lake, and Lac La Ronge that is approximately 1400 km^2 in size. Natural wildfires are frequent in the area (Rowe and Scotter 1973), but fire suppression has been enforced in Saskatchewan since the 1940's, when commercial, sport, and recreational activities increased. This lead to an increase in road development, and commercial forest harvests, eventually fragmenting the landscape in Saskatchewan (Arlt and Manseau 2011). The growth and development of new towns has also been increasing across the Boreal Plains in Saskatchewan, leading to further habitat loss and disturbance across the landscape. The Boreal Shield in Manitoba consists of a greater amount of mixed conifer forest, larger lakes, and a greater presence of bedrock (Environment Canada 2009). Similarly, road development and forestry has been increasing in Manitoba in the past

decades, leading to increased habitat loss and fragmentation (Rebizant et al. 2000; Manitoba Conservation 2015).

The main predators for boreal caribou across the boreal study area include wolves (*Canis lupus*), black bears (*Ursus americanus*), coyotes (*Canis latrans*) and lynx (*Lynx Canadensis*). Other ungulate species that may compete for habitat space and resources with boreal caribou include white-tailed deer (*Odocoileus virginianus*), moose (*Alces alces*; O'Brien et al. 2005) and occasionally elk (*Cervus Canadensis*).

Fecal sampling in the east and west ranges of the Boreal Plain ecozone in central Saskatchewan was conducted over the winters of 2004-2005, 2006-2007, 2008-2009, 2011-2012 and 2014 by Parks Canada, and the Saskatchewan Ministry of the Environment, Fish and Wildlife Branch. Fecal pellet sampling in Manitoba has been conducted by Manitoba Conservation, Parks Canada and Manitoba Hydro, over various winter seasons. Aerial surveys were conducted in linear transects across the study area to identify cratering sites (i.e. tracks and foraging) made by boreal caribou in the snow. A second crew would visit each site by helicopter to collect fecal pellets if found on site, and recorded the coordinates of the location where fecal samples were collected. Samples (from individual caribou) were collected separately in the piles that they were found, to prevent cross-contamination of DNA between samples. Samples were then either shipped frozen, or had DNA extracted (by process described in Ball et al. 2007), before being shipped to the Natural Resources DNA Profiling and Forensic Lab at Trent University, Peterborough, Ontario, for analysis. Sample sizes collected varied per year, ranging from 79 – 336 samples in a year. Total number of samples collected and genotyped across the study area was 1,399 samples.

2.3.2 Microsatellite data

To obtain polymorphic microsatellite DNA, the outer mucosal layer of 3-5 fecal pellets per sample were either, swabbed with a quilt-tip, or swished according to a new protocol that involves inserting fecal pellets directly within Qiagen solution in a test tube, and swishing the mucosal layer from the material. The extracted DNA was amplified and then genotyped at 9 microsatellite loci markers (BM848, MAP2C RT24, RT6, RT9, BM888, RT7, RT30, RT5, in addition to a sex-based marker named gender; Moore et al. 1992, Wilson et al. 1997; Bishop et al. 1994; McLoughlin et al. 2004) according to the protocol outlined in Ball et al. (2007). Genotypes were then scored by three different lab personnel to check for errors using GENEMARKER genotyping software (version 1.75). Samples missing more than two allele pairs from their complete genotype at 9 loci (< 7/9 complete loci), were excluded from the analysis. Samples missing one or two pairs of alleles (7/9 or 8/9 loci), were re-amplified to increase genotypes up to 8 or 9 loci. Because individual caribou may have been sampled more than once, duplicate matches of samples were identified using the program ALLELEMATCH (version 2.03; Galpern et al. 2012) in R, and were removed from the dataset so that only unique individuals were included for subsequent analysis.

2.3.3 Individual-based clustering (IBC)

Population genetic structure of boreal caribou across the study area in Saskatchewan and Manitoba was inferred using two individual-based Bayesian clustering programs. Using at least two methods to determine population structure is needed to account for potential spurious results due to the presence of IBD (Frantz et al. 2009; François and Durand 2010; Safner et al. 2011). Chen et al. (2007) recommends using both STRUCTURE (Pritchard et al. 2000) and TESS

(Chen et al. 2007) to supplement results, as the two programs differ slightly in their calculation methods (see next paragraph). Both programs use a Markov Chain Monte Carlo (MCMC) algorithm to group individuals into a specified number of clusters that minimize inbreeding coefficients with HWE and LD assumptions (François et al. 2006). Both programs can also incorporate the assumption that genetic admixture occurs within individuals across clusters. This is a helpful tool for the study species, as boreal caribou are assumed to be continuous with moderate gene flow across the landscape (Ball et al. 2010), and therefore admixture within a genetic cluster would be expected. The proportion of each individual genotype that belongs to a cluster is also reported in decimal form (q) in the admixture models for both programs.

STRUCTURE calculates likelihood values (L(ΔK); Pritchard et al. 2000) or delta K (ΔK; Evanno et al. 2005) for a specified range of clusters (K) in order to determine the optimal number of clusters for the given dataset. TESS, alternatively, calculates Deviance Information Criterion (DIC), which similarly represents model fit to a certain number of clusters (K_{max}; Spiegelhalter et al. 2002). Many studies have compared the two programs with variable results in being able to determine the best model (i.e. Chen et al. 2007; Yoshino et al. 2008; François and Durand 2010). A main difference between the two programs includes that analysis with TESS takes into account the spatial coordinates of each individual, and therefore it incorporates spatial autocorrelation trends when assigning individuals to clusters (François et al. 2006; Chen et al. 2007). This may be beneficial when identifying the admixture across genetic clusters is less important, as individual assignment proportions may become biased under the assumption that nearby individuals are more genetically similar than further individuals (Chen et al. 2007). Chen et al. (2007) have noted that STRUCTURE is better at identifying admixture in individuals than spatial clustering programs, and is better at assigning cluster proportions to individuals when

admixture is high. This provides an advantage in this study, as admixture across individuals is expected to be high, and having accurate admixture proportions may help to more accurately delineate genetic boundaries across the study area. Although results may vary, using both spatial and non-spatial methods can facilitate correct estimation of structured clusters if results are similar, or provide more information on what areas to be cautious of when making inferences, if inconsistencies exist (François and Durand 2010). It is predicted that levels of gene flow among boreal caribou populations would support sufficient admixture to warrant more weighting in STRUCTURE outputs, however, both spatial and non-spatial priors were applied to assess levels of concordance.

For this study, STRUCTURE (version 2.3.4) admixture model was run with 1,000,000 MCMC iterations and a burn in length of 500,000 five times for each K, which ranged from 1-15 for the full study area across both Saskatchewan and Manitoba. This was repeated for samples in just Saskatchewan to delineate hierarchical structure within the Province. Plots of ΔK and L (K) against K were created with STRUCTURE HARVESTER (web version 0.6.94**;** Dent and vanHoldt 2012). TESS (version 2.3.1) no-admixture model was run with 50,000 MCMC iterations and a burn in length of 40,000, 50 times for each K (Thompson 2015), which was set to 1-15 for the full study area. The no admixture model is recommended by Durand et al. (2009) to be run first in TESS to identify the maximal range in the number of parental clusters. The BYM admixture model was then run with the same parameters, but with K ranging from 1-10 once it was determined that DIC, plotted against K, plateaued past this range in the no-admixture model (Durand et al. 2009).

2.3.4 Delineating cluster boundaries

To spatially delineate clusters of individuals in a way that would be useful for management, individuals and their cluster assignments, given as proportions in STRUCTURE and TESS, were visualized on a map of the study area with ArcGIS (ESRI 2010). A cluster assignment threshold of ≥ 60% was used. The rational for applying this threshold is discussed further on page 45. Cluster boundaries were then delineated according to the spatial distribution of individuals and their assignment to genetic clusters. The resulting cluster boundaries were used for subsequent genetic analyses. Cluster boundaries in Manitoba were only used to evaluate connectivity of individuals across the provincial border with Saskatchewan, as previous studies have already delineated finer scale genetic clusters within Manitoba (Ball et al. 2010; Thompson 2015).

2.3.5 Genetic connectivity analysis

In order to validate Bayesian clustering results, gene flow was measured across all proposed genetic clusters identified in Saskatchewan and Manitoba (Guillot et al. 2009). Pairwise F_{ST} was calculated with program SPAGeDi (version 1.5; Hardy and Vekemans 2002) to measure contemporary gene flow (genetic distance). Additionally, pairwise R_{ST} was calculated with SPAGeDi to measure if phylogeographic patterns of gene flow exist across samples, which would be the case if R_{ST} values were found to be higher than F_{ST} (Hardy et al. 2003). Permutation tests for both F_{ST} and R_{ST} were also calculated with the program SPAGeDi to test for the significance of each pairwise genetic distance measure between clusters.

Because IBD is known to influence Bayesian clustering results (Frantz et al. 2009; François and Durand 2010), the influence of geographical distance on gene flow was measured for each genetic cluster using a Mantel test in CRAN R, from the package "ecodist" (version 1.2.9;

Goslee and Urban 2007). Each cluster was further tested for allele frequency deviation from HWE and LE (Guillot et al. 2009) using the probability test in the program GENEPOP (version 4.2; Raymond and Rousset 1995). Allele richness, expected heterozygosity (H_e; Nei 1978), observed heterozygosity (H_o), individual inbreeding coefficient (F_{IS}), and average number of alleles per locus, were further calculated in the Microsoft Excel extension GenAlEx (version 6.501; Peakall and Smouse 2012).

2.3.6 Landscape effects on gene flow

For the landscape genetics analysis in this study, only the extent of the study area in Saskatchewan was included to avoid inconsistencies in the way data had been collected for landscape variables across the two provinces.

The following seven hypothesized landscape variables were tested to identify their influence on genetic distance: roads (main highways and provincial roads), forestry cuts (years 1973-2013), three separate layers for wildfire (years 1953-2013, years 1963-2013, and years 1973-2013), water bodies, and low suitability habitat for boreal caribou that has been classified by the Saskatchewan Ministry of Environment according to the *Field Guide to the Ecosites of Saskatchewan's Provincial Forests* (McLaughlan et al. 2010).

Water bodies, natural wildfire, and major anthropogenic disturbance such as roads and forestry, were included in the analysis as predictors of landscape resistance to gene flow, because they have been identified to be avoided by boreal caribou in previous studies (Dyer et al. 2001; 2002; Fortin et al. 2005; O'Brien et al. 2006; Vors et al. 2007; Wittmer et al. 2007; Artl 2009; Courbin et al. 2009; Koper and Manseau 2009; Galpern et al. 2012). Three ranges of wildfire age were used to test if a difference exists between the landscape resistance of < 60 year old burns, < 50

year old burns, and < 40 year old burns. Wildfire burns that are at least 50 years old have been identified as boreal caribou habitat in previous studies (Schaefer and Pruitt 1991; Dalerum et al. 2007). Testing a range of burn ages in this study will help to identify if burns up to 50 or 60 years old are no longer significant at causing landscape resistance at a level that influences genetic connectivity for boreal caribou.

Low suitability habitat was included in the analysis to determine if this classified habitat type is avoided by caribou, resulting in resistance to gene flow across the landscape. Habitat types that were classified as low suitability habitat for boreal woodland caribou within the Boreal Plains ecozone of Saskatchewan, include: trembling aspen/prickly rose/grass: fresh sand (BP5), trembling aspen/beaked hazel/sarsaparilla: fresh loamy sand (BP6), trembling aspen-white birch/sarsaparilla: fresh loamy sand (BP7), trembling aspen-white birch/mountain maple: fresh sandy clay loam (BP8), white spruce-trembling aspen/feathermoss: fresh sand (BP9), trembling aspen-white spruce/feathermoss: fresh silty loam (BP10), white birch-white spruce-balsam fir: fresh sandy clay loam (BP11), white spruce-balsam fir/feathermoss: fresh sandy clay loam (BP13), balsam poplar-white spruce/feathermoss: very moist silty loam (BP15), balsam poplar-trembling aspen/prickly rose: fresh clay loam (BP16), and Manitoba maple-balsam poplar/ostrich fern: moist silty clay loam (BP17). The classification of this layer is based on habitat potential. Overlap of other landscape variables that exist within the distribution of this habitat type may occur, and this was taken into consideration when interpreting results.

Vector layers of each landscape variable were provided by the Saskatchewan Ministry of Environment, and were each converted into 200m by 200m pixel raster surfaces (Galpern et al. 2012) using ArcMap 10 (ESRI 2010). Each landscape variable was initially tested individually as an independent model of landscape resistance to isolate its effect on gene flow. In order to

measure resistance to gene flow, each pixel that contained the landscape variable being tested was given a value that reflects a predicted landscape resistance. The remaining pixels were given a value of 1. As predicting and interpreting resistance as a numerical value for a landscape variable is difficult, and a main drawback in this analysis (Spear et al. 2010; Segelbacher et al. 2010), three different resistance values: 10, 100 and 1000 were experimentally applied to each variable, resulting in each landscape variable being measured three times. The resistance values 10 and 100 have been determined as equivalent to the resistance of natural features on the landscape to gene flow for boreal caribou (Galpern et al. 2012), and therefore represent low and moderate resistance to gene flow, respectively. A resistance value of 1000 was additionally tested to determine if the landscape variable had a very strong resistance to gene flow across the landscape for boreal caribou.

To measure the cost distance of each landscape variable and its assigned resistance value, raster surfaces of each resistance model were converted into text .asc files using the statistical software R (v. 3.2.1). The following packages were used in the subsequent analysis and downloaded from an R CRAN repository (varied for each package): raster, gdistance, sp, rgdal, igraph, vegan, maptools and ecodist. Each resistance model surface was converted into a transition layer in R, and was set to consider 8 directions (8 neighbour rule) when measuring cost distances. Each transition layer was then geographically corrected for distortion. A spatial points data frame was created using UTM coordinates of each individual caribou sampled across the study area.

Two cost distances reflecting assigned resistance values were computed for each resistance model to test for IBR. This was done in R using the transition layer of each model and the spatial points data frame. Both least-cost pathway, and total landscape resistance distances (also termed circuit-theory; McRae et al. 2008), were calculated. Least cost-pathway analysis considers one

optimal shortest pathway of movement between point locations (individual coordinates) across the resistance surface, which minimizes cost of travel (Dijkstra 1959). This distance was computed using the "costdistance" function in the R package gdistance (van Etten 2012). Total resistance analysis considers the cost of travelling across along all 8 pathways of travel when crossing neighbouring raster cells between sample points, revealing a total resistance to movement across the landscape for any given individual (McRae et al. 2008). This distance was computed using the "commutedistance" function also in the R package gdistance (van Etten 2012).

2.3.7 Model selection: Causal modeling

Following the computation of two cost distances for each of the seven landscape models, a total of three times each to test a gradient of resistance values (10, 100, 1000), 42 partial Mantel's tests were calculated. Partial Mantel tests correlate two distance matrices, allowing for a third distance matrix to be added to "partial" out its effects from the initial matrix. Partialling out the effects of two variables, or models, is an important step in landscape genetics, as many variables are correlated and can lead to spurious results (Cushman et al. 2006; Cushman et al. 2013). A method to address the problem of correlated variables has been developed by Cushman et al. (2006). In this method, termed causal modeling, cost distances for each Isolation-By-Resistance (IBR) model are correlated with genetic distance, with the additional models of Isolation-By-Distance (IBD) or Isolation-By-Barrier (IBB) being partialled out. In this study, IBB is not expected to be significant for any variable across the landscape for boreal caribou, as there are no landscape variables that would completely impede the movement of gene flow, such as a mountain range, across the study area. Therefore, only the IBD model of geographical distance was partialled out from the cost distances of different IBR models:

(Genetic Distance~Cost Distance|Geographic Distance)

Once a resistance model is found significant (p-value < 0.05), indicating that the cost distance is correlated with genetic distance, and is independent of geographic distance, causal modeling continues by switching the independent variables in the partial Mantel test:

(Genetic Distance~Geographic Distance| Cost Distance)

In this second test, geographic distance is correlated with genetic distance, and the cost distance of a given IBR model is partialled out. This test identifies whether IBD alone is the best predictor of genetic distance. If the second test is not found to be significant (p-value ≥ 0.05), then there is sufficient evidence that the relationship between the cost distance of the IBR model and genetic distance is both, significant, and independent of geographic distance (IBD; Cushman et al. 2006; Cushman et al. 2013). IBR models found to be independently correlated with genetic distance were then compared by their Mantel r values to determine the model that has the best fit with genetic distance (Cushman et al. 2013). IBR models that could not be partialled out from IBD with the second test (because the test was significant) were removed from subsequent analyses.

Another approach to model selection has been developed by Wasserman et al. (2010), which similarly applies partial Mantel tests, but tests IBR models together in order to partial out effects. This method is more computationally extensive, as all models compete with each other, instead of first eliminating models that are correlated with IBD as in the causal modeling approach (Cushman et al. 2006; Cushman et al. 2013). Both methods have been identified to have similar success at identifying the correct model when a relationship with genetic distance is present (low type II error), therefore only the Cushman et al. (2006) approach was used in this study, however, partialling out highly correlated models is still a challenge with both approaches (high

type I error; Cushman et al. 2013). To address this concern, variables found significant after causal modeling were correlated with each other to determine if a strong correlation existed. This aided in determining model fit, and in determining which models to further combine and test as new multi-variable models. Models that combined landscape variables were further tested to better represent landscape complexity (Cushman et al. 2006; Spear et al. 2010), as more than a single landscape variable is expected to influence gene flow for boreal caribou with landscape resistance across the study area.

Only the resulting best IBR models were combined into new models, with careful consideration of variable correlations. By combining only significant models of landscape variables into new combined models, the number of models to be tested was reduced, and confounding effects from variables not significantly correlated with genetic distance were avoided (Cushman et al. 2006). Additionally, only the best resistance values (10, 100, 1000) for a landscape variable, determined by Mantel r, were included in the new models, as varying resistances did not greatly change results of model fit. Resistance surfaces and cost distances for models combining landscape variables were created, and calculated using the same methods described above for individual variables. The causal modeling framework was also applied for each combined model, as described above, to partial out the effects of IBD.

Scale of analysis has been identified as crucial in landscape genetic analysis, as certain factors such as IBD can inhibit the detection of weak but significant landscape variables (i.e. Angelone et al. 2011; Galpern et al. 2012; Galpern and Manseau 2013). The study area was therefore further analysed in the same way for each genetic cluster identified for Saskatchewan by the program STRUCTURE, to identify if certain landscape variables were more important predictors of gene flow in certain areas.

The geographic distance matrix was created in R using the distances between coordinates of each individual. All partial Mantel tests were calculated using the R package 'vegan' (version 2.3.1; Oksanen et al. 2015) as a Pearson's correlation test with 1000 permutations.

2.3.8 Model Selection: Eigenvector analysis- MEMGENE

MEMGENE is a package run on R, developed by Galpern et al. (2014) for identifying autocorrelation patterns of gene flow, in relation to the landscape, for a mobile species. It visually determines spatial neighbourhoods from the genetic distances between individuals, without incorporating geographical distance into the analysis. Instead of applying Mantel tests as in landscape genetics analysis, which are highly criticized to accurately correlate distance matrices (i.e. Raufaste and Rousset 2001; Legendre and Fortin 2010; Guillot and Rousset 2013), this program uses a regression to correlate genetic distance with landscape variable predictors generated with Moran's eigenvectors maps (MEM's). These landscape predictors are generated as MEMGENE variables, which represent spatial autocorrelation patterns between each sample, calculated from a Euclidean distance matrix. Each MEMGENE variable, or predictor, represents a genetic distance pattern that is caused by the genetic variation between individuals. Each extracted MEMGENE variable is given a quantitative value that represents how much variation it explains (as a proportion). The spatial pattern of each MEMGENE variable can also be visually expressed on a map by incorporating the spatial coordinates of each sample. This can be used to visually compare genetic distances between individuals, and consequently reveals gene flow patterns across the landscape (Galpern et al. 2014). These results were compared to Bayesian Clustering results, which similarly identify genetic distance patterns to group individuals into separate genetic clusters.

To determine if genetic distance patterns reflect the landscape, and are therefore attributed to the influence of landscape variables on gene flow, MEMGENE was further used as an alternative method to causal modeling, to analyse the influence of specific landscape variables on spatial genetic patterns. Instead of calculating MEMGENE variables from a Euclidean distance matrix as above, a least-cost distance matrix was used, calculated from the resistance surface of each landscape model. All tested landscape models were then compared with each other to determine how much spatial genetic variation can be explained by each landscape variable, in comparison to Euclidean distance alone (Galpern et al. 2014). The full study area in Saskatchewan, and each of the three clusters within the Province, were analysed separately. Only models of uncombined landscape variables found to be both, significant, and partialled out from IBD with causal modeling, were analysed with MEMGENE landscape model analysis. Combined landscape models were not used as all landscape variables are correlated, and only independent variables were of interest to be able to better identify the isolated effects of certain landscape variables on genetic patterns. All MEMGENE analyses were computed using R (v. 3.2.1) following the tutorial by Galpern and Peres-Neto (2014).

2.4 Results

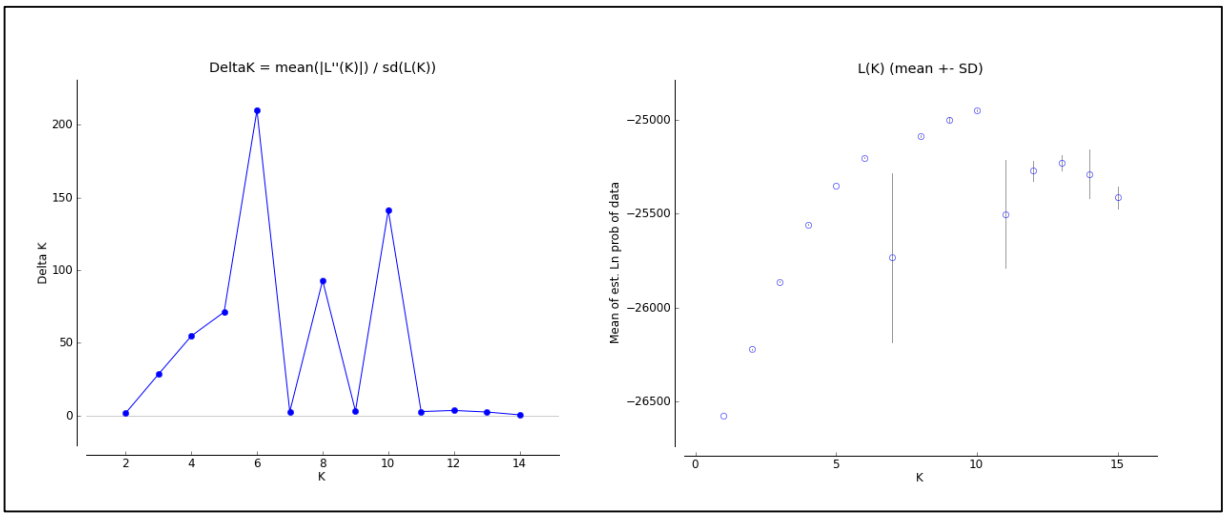
2.4.1 Microsatellite data

Number of unique genotypes across the provinces of Saskatchewan and western Manitoba totaled 945 individuals, with up to one missing set of alleles across all nine loci per individual. In Saskatchewan, 416 unique samples were identified (136 males, 236 females and 44 unknown). In Manitoba, 524 unique samples were identified (124 males, 280 females, 120 unknown).

2.4.2 Individual-based-clustering (IBC)

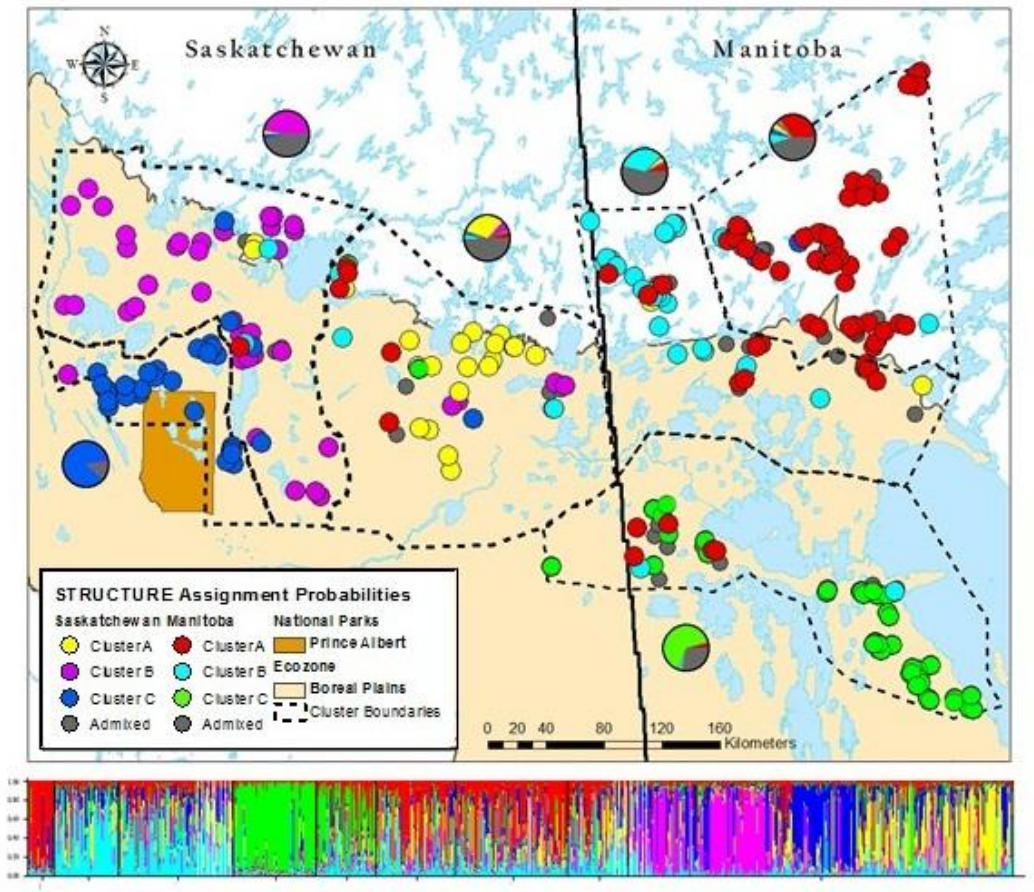
STRUCTURE analysis identified genetic structuring across Saskatchewan and western Manitoba, with an optimal number of K = 6 genetic clusters when the two provinces were pooled (Figure 2-2).

Figure 2-2. STRUCTURE Delta K (ΔK) and log likelihood (L (K)) results for Saskatchewan and Manitoba.



Three genetic clusters were identified across the study area in Saskatchewan, and an additional three genetic clusters were identified across western Manitoba (Figure 2-3). The bar plot included at the bottom of Figure 2-3 corresponds with the coloured dots for each individual on the map, with each vertical line in the bar plot representing an individual sample. Pie charts on the map in Figure 2-3 represent the proportion of animals assigned to a genetic cluster (≥ 60%), and which are found within the boundaries of each delineated cluster (how clusters were delineated is explained in more detail on page 45).

Figure 2-3. The spatial distribution of STRUCTURE cluster assignments across Saskatchewan and Manitoba. The pie charts and bar plot represent cluster assignments for individuals, with colours corresponding with individuals visualized spatially on the map.



An additional ΔK peak was identified at K = 8 and K = 10 (Appendix A-1, Figure 2-2) suggesting hierarchical structuring (i.e. additional sub-structuring), however, comparing STRUCTURE bar plots revealed that K = 6 gave the least admixed results. Further, the plot of L(K) in Figure 2-2 also revealed that K = 6 was the most likely number of clusters, with points no longer gradually increasing after this value. Samples in the south-east of Saskatchewan were clustered with the Bog samples in Manitoba (Figure 2-3), and therefore these samples were not included in further STRUCTURE analysis of Saskatchewan independent of Manitoba.

Across the Province of Saskatchewan alone, the optimal ΔK remained at K = 3, with a very small additional peak at K = 8 that did not reveal any clear hierarchical structuring (Figure 2-4; Figure 2-5; Appendix A-2). Further levels of sub-structuring could therefore not be inferred for Saskatchewan, but may also not be important, with the high to moderate level of gene flow occurring across the Province.

Figure 2-4. STRUCTURE Delta K (ΔK) and log likelihood (L (K)) results for Saskatchewan.

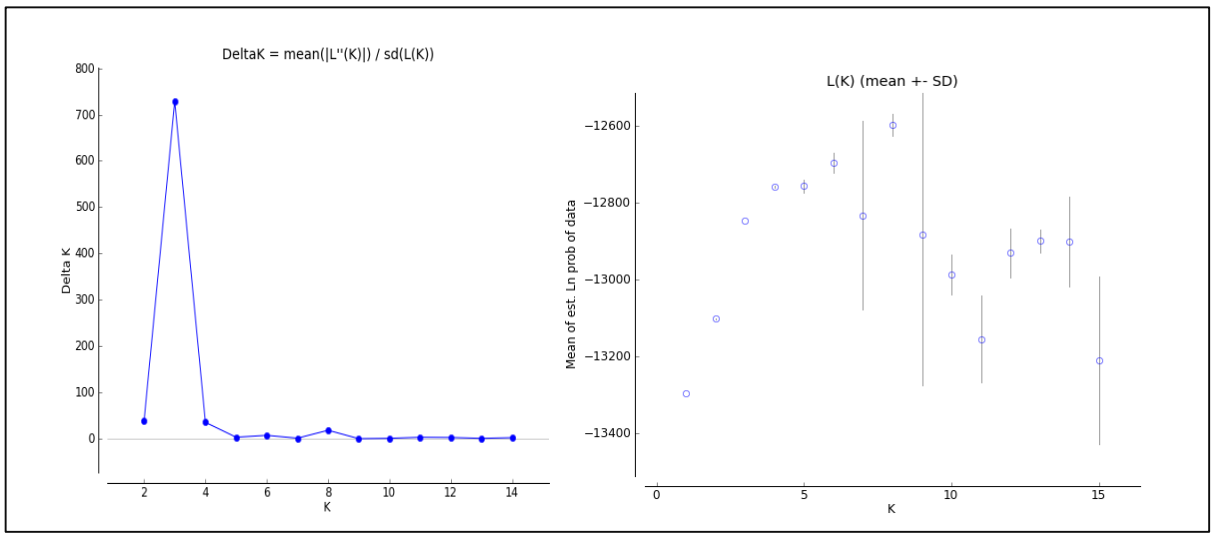
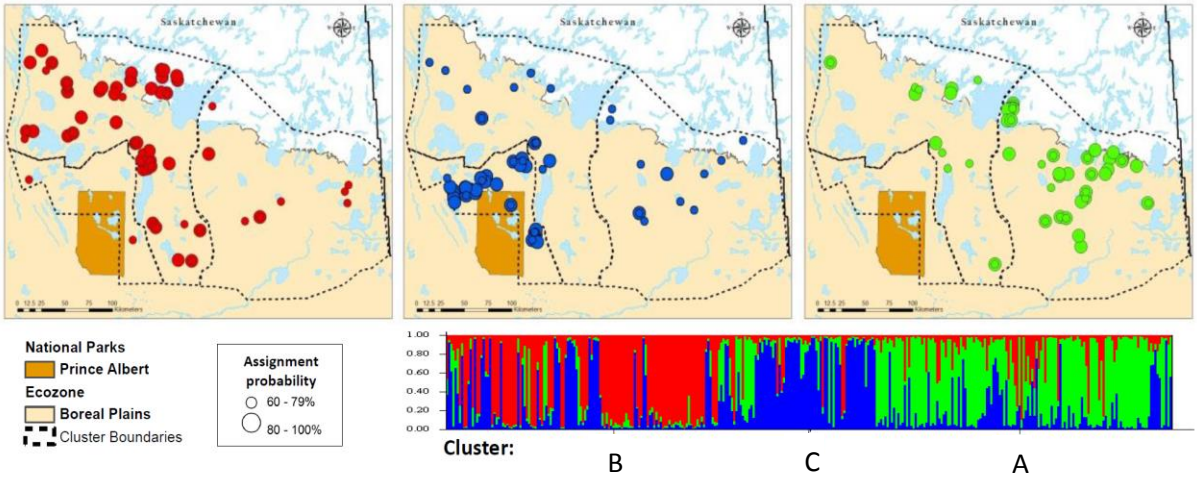
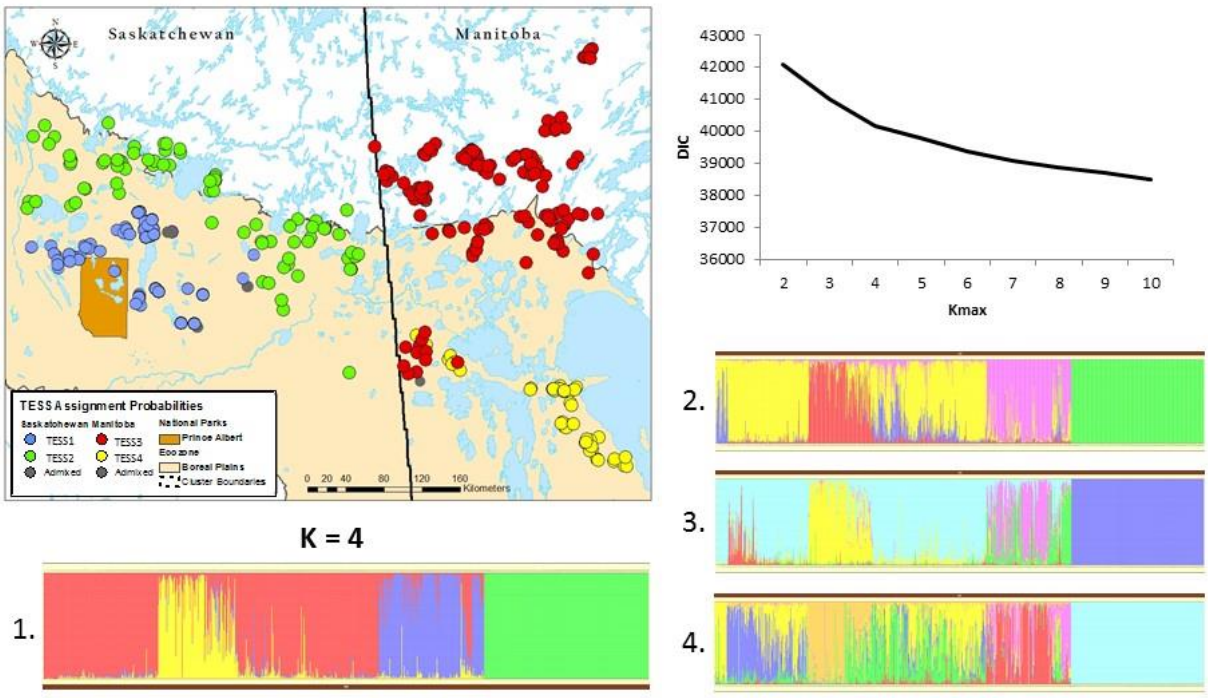


Figure 2-5. The spatial distribution of STRUCTURE cluster assignments for individuals in Saskatchewan. Dot size ranges on each map indicate strength of assignment to a cluster (60-79% and 80-100%). The bar plot in the bottom right corresponds with the colours on the map.



TESS analysis with the no-admixture model identified an optimal $K_{max} = 7$ for Saskatchewan and Manitoba pooled, and the optimal number of parental populations did not increase after $K_{max} = 10$ (Appendix A-3). The optimal K_{max} for the admixture model was difficult to decipher from the second order rate of change DIC values, plotted against K_{max} (top right corner of Figure 2-6); however, comparing bar plots revealed that $K_{max} = 4$ (Figure 2-6.1) provided the clearest division of the two provinces into clusters, with ample sample size within each cluster. Bar plots for $K_{max} = 5$ (Figure 2-6.2), $K_{max} = 6$ (Figure 2-6.3), and $K_{max} = 7$ (Figure 2-6.4) revealed small and biologically insignificant clusters that were likely spurious. The coloured dots in the map of the study area in Figure 2-6 represent 60% assignment to a particular cluster.

Figure 2-6. The spatial distribution of TESS cluster assignments for individuals, including DIC plot against K_{MAX}, and four bar plots (1-4) for K_{max} = 4-7 respectively. The colours on the map correspond with colours in bar plot 1.



The relatively low F_{ST} value between SK A and SK B (F_{ST} = 0.014; Table 2-1) likely influenced partitioning results, and therefore, under low levels of gene flow, STRUCTURE was better than TESS at detecting genetic structure in this study. STRUCTURE was also better at identifying admixture within individuals, and was consistent in detecting an optimal number of clusters at both scales of Saskatchewan and Manitoba, and Saskatchewan alone. For these reasons, analysis at the scale of just Saskatchewan was not repeated with TESS.

2.4.3 Cluster boundaries

As it was determined that STRUCTURE results provided more detailed analysis of admixture across the study area than results obtained with TESS, genetic cluster boundaries were delineated only on maps that visualized STRUCTURE results (Figure 2-3 and Figure 2-5). To delineate cluster boundaries, individuals that fell within a ≥ 80% assignment threshold to a particular cluster were mapped with coloured points that represented their assignment to a particular cluster. For STRUCTURE results of Saskatchewan and western Manitoba pooled, this represented 32% of all individuals in the analysis, as admixture across samples was high (map not shown). The STRUCTURE results of Saskatchewan, independent of Manitoba, was also mapped separately, and with a ≥ 80% threshold, 56% of individuals were mapped across the province (included in Figure 2-5). This threshold allowed for strong assignment to a cluster to be visualized, however, it put many samples into the admixed category making it difficult to draw lines between clusters. In order to remove some of the noise surrounding genetic cluster assignment, the threshold was lowered to ≥ 60% of assignment to a particular cluster. Changing the threshold did not alter the distribution of clusters, but increased the number of individuals that could visually be assigned to a particular cluster, making it easier to draw cluster boundaries around individuals. This increased the number of individuals belonging to a cluster to 60% for Saskatchewan and Manitoba pooled (Figure 2-3), and 81% for Saskatchewan alone (Figure 2-5). The remaining admixed individuals were placed within the boundaries of a cluster based on their spatial location to the cluster, on their level of admixture, and on landscape features that spatially could be used to delineate individuals (i.e. roads and large water bodies; see Appendix A-4). The resulting cluster boundaries are suggestions and it is recognized that interpretation of the results that this delineation was based on can vary.

In Manitoba, two clusters were delineated in the north of the Province. This delineation separated western samples near the Saskatchewan border (Kississing and Naosap-Reed caribou ranges), from samples to the east and north (Harding, Wapisu-Wimapedi, Wabowden and Wheadon caribou ranges) of the study area. The third cluster in Manitoba included the Interlake and Bog ranges in the south-eastern range of the study area (Figure 2-3). The three genetic clusters identified in Manitoba were delineated for genetic connectivity analysis with Saskatchewan, however their purpose is strictly for a general comparison within this study, and their application to management is not being suggested.

Three cluster boundaries were delineated in Saskatchewan. Saskatchewan Cluster A (SK A) extends to the Saskatchewan-Manitoba provincial boundary, with north and south cluster boundaries following collection range polygons (Appendix A-4). Collection ranges do extend further to the south-east in SK A, but this area was clipped to exclude the south eastern area of the Province where samples were identified to be more genetically similar to Manitoba's Bog herd (Figure 2-3). For this reason, these samples were not included in further population structure or landscape genetic analysis of Saskatchewan, and were added to Manitoba Cluster C (MB C). A set of northern samples along the Saskatchewan-Manitoba provincial boundary were also excluded from SK A, as they were more genetically similar to Manitoba Cluster B (MB B; Figure 2-3). The western boundary of SK A was delineated according to genetic partitioning results, extending along the east side of Lac La Ronge (Figure 2-4). Saskatchewan Cluster B (SK B) was similarly delineated following the northern edge of collection range polygons. Further delineation of SK B was based on genetic partitioning of individuals, and following Hwy 2 on the east side of Prince Albert National Park (Figure 2-4; Appendix A-4). Saskatchewan Cluster C

(SK C) was lastly delineated, based on the remaining extent of collection range polygons surrounding Prince Albert National Park (Figure 2-4).

2.4.4 Genetic connectivity analysis

Pairwise genetic distance was calculated for each cluster delineated from STRUCTURE results across Saskatchewan and Manitoba. Clusters were named based on the abbreviation of the Province and an assigned alphabetic letter. Numerical numbering was avoided not to confuse genetic cluster names with current WCCU labels in Saskatchewan. In this study, high differentiation refers to any value ≤ 0.02, and moderate differentiation refers to any value $\geq 0.02 \leq 0.1$. Within provinces, pairwise F_{ST} values were highest between SK C and SK A, and between MB C and MB B, while lowest between SK A and SK B, and between MB A and MB B. Across provinces, SK B and MB C had the highest pairwise F_{ST}, while SK B and MB B had the lowest pairwise F_{ST}. Cluster MB C had the highest genetic differentiation compared with all other clusters, followed by Cluster SK C (Table 2-1).

Table 2-1. Pairwise genetic distances between genetic clusters: F_{ST} (above diagonal) and R_{ST} (below diagonal).

Cluster	SK A	SK B	SK C	MB C	MB B	MB A
SK A	-	0.014	0.035	0.049	0.023	0.024
SK B	0.011	-	0.031	0.05	0.018	0.02
SK C	0.041	0.032	-	0.041	0.043	0.034
MB C	0.031	0.07	0.064	-	0.046	0.037
MB B	*0.006	*<0.001	0.031	0.063	-	0.015
MB A	0.009	0.006	0.017	0.049	*0.003	-

*non-significant (p-value ≥ 0.05)

R_{ST} values reflect the same patterns as F_{ST} results, suggesting that gene flow across the two provinces is high and had not been restricted in the past, with the exception of Cluster C in both Saskatchewan and Manitoba (Table 2-1). Another exception is the relatively high R_{ST} value between SK B and MB C, in comparison to the pairwise F_{ST} value. Three R_{ST} values were also found to be non-significant after applying a permutation test (p-value > 0.05; indicated with asterisks *), therefore, their phylogeographic patterns could not be validated (Table 2-1).

IBD was significant across both Saskatchewan and Manitoba, and all six genetic clusters delineated from STRUCTURE results (Table 2-2). In Table 2-2, p-values indicate significance of the IBD model (p-value ≤ 0.05) and Mantel r values reflect the strength of the correlation at each range. Corresponding sample sizes (n) of provinces and clusters is also provided in Table 2-2. Overall, IBD was weaker across clusters in Saskatchewan than in Manitoba, with the strongest presence of IBD in Saskatchewan being within Cluster SK C. Within Manitoba, IBD was highest across Cluster MB B (Table 2-2).

Table 2-2. Isolation-by-Distance (IBD) for each genetic cluster in Saskatchewan and Manitoba.

Range	Mantel r	P-value	Cluster size (n)
All Saskatchewan	0.081	0.001	413
SK A	0.080	0.002	142
SK B	0.015	0.001	190
SK C	0.155	0.001	81
All Manitoba	0.123	0.001	527
MB C	0.138	0.001	127
MB B	0.191	0.001	170
MB A	0.144	0.001	230

Number of alleles varied from 10 for MAP2C, to 32 for BM888, with size ranges of 79 bp - 387 bp, varying per locus (Table 2-3). Allelic richness (A) varied from 0.806 for BM848, to 16.291 for BM888. Expected heterozygosity (H_e) was always greater than observed heterozygosity (H_o; Table 2-3). Inbreeding coefficient (F_{IS}) ranged from 0.245 for RT30, to 0.037 for RT9 (Table 2-3). HWE analysis for each of the nine microsatellite loci used in this study had similar findings to Ball et al. (2010), with the highest number of deviations from HWE found at the locus RT30, while no deviations occurred at loci RT5 and RT6 (Table 2-3; Appendix A-5). RT30 was not removed from further analysis as it was still in HWE for Saskatchewan Cluster B (Table 2-3; Appendix A-5).

Table 2-3. Descriptive statistics (number of alleles (n), base pairs (bp), allelic richness (A), expected heterozygosity (He), observed heterozygosity (Ho), inbreeding coefficient (F_{IS}), and number of clusters deviating from HWE) per locus across the study area.

Locus	n alleles	Size range (bp)	A	He	Ho	F_{IS}	Clusters deviating from HWE
BM848	12	351 - 387	8.06	0.730	0.670	0.082	1
BM888	32	158 - 214	16.291	0.818	0.802	0.018	1
MAP2C	10	82 - 123	9.484	0.821	0.794	0.032	1
RT24	27	200 - 249	16.95	0.697	0.623	0.106	1
RT30	11	180 - 225	9.882	0.658	0.497	0.245	5
RT5	12	80 - 122	11.006	0.827	0.778	0.060	0
RT6	11	79 - 123	9.23	0.785	0.749	0.046	0
RT7	12	206 - 241	10.08	0.739	0.679	0.081	1
RT9	12	96 - 130	9.006	0.707	0.681	0.037	1

Across populations, the mean number of alleles (n alleles) ranged from 7.67 for SK C, to 11.89 for SK B, and allelic richness (A) ranged from 7.60 for SK C, to 10.56 for SK A (Table 2-4),

showing an increase with cluster size (n; Table 2-2). H_e was always higher than H_o, and F_{IS} values were greatest for MB A, while lowest for SK C (Table 2-4). Number of loci deviating from HWE varied from 1 for SK A and SK C, to 4 for MB A. The number of clusters deviating from LE varied from 0 for SK C, MB B and MB A, to 5 for MB C (Table 2-4). Linkage disequilibrium ranged from 25 associations for MB A, to 36 associations for MB B between all loci pairs across all clusters (Appendix A-6). See Appendix A-5 and A-6 for the full list of HWE and LE p-values, respectively, across clusters and loci.

Table 2-4. Descriptive statistics (number of alleles (n), allelic richness (A), expected heterozygosity (He), observed heterozygosity (Ho), inbreeding coefficient (F_{IS}), and number of loci deviating from HWE and LE) per genetic cluster across the study area.

Cluster	n alleles	A	He	Ho	F_{IS}	Loci deviating from HWE	Loci deviating from LE
SK A	11.56	10.560	0.751	0.724	0.036	1	3
SK B	11.89	10.320	0.758	0.718	0.053	2	3
SK C	7.67	7.600	0.701	0.683	0.026	1	0
MB C	9.00	8.320	0.692	0.662	0.043	2	5
MB B	10.67	9.590	0.749	0.720	0.039	2	0
MB A	11.11	9.670	0.732	0.670	0.085	4	0

Both genetic distance matrices of F_{ST} and R_{ST}, as well as IBD within clusters, was additionally calculated for the four genetic clusters identified with TESS. Genetic differentiation between clusters was found to be reduced by reducing the number of clusters across the study area (Appendix A-7). This further provides support that STRUCTURE was better at delineating genetic clusters across the study area, as genetic differentiation was increased (and likely maximized) by partitioning individuals into three clusters per Province. IBD was also significant

across all four clusters identified with TESS (Appendix A-8), and was comparable with Mantel r values measured across clusters identified with STRUCTURE (Table 2-2).

2.4.5 Landscape effects on gene flow

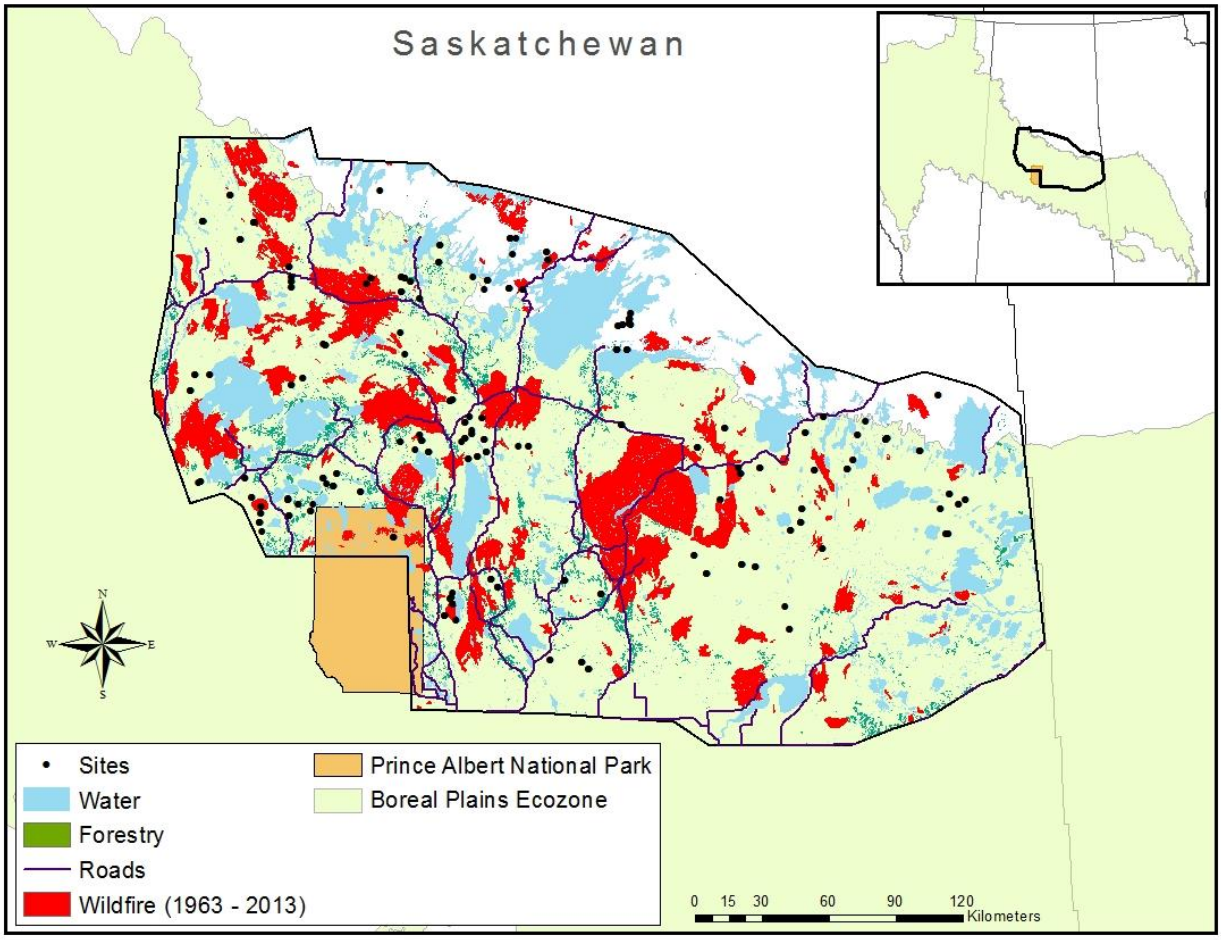
Least-cost pathway analysis was not found to be significant for any variable across the full study area (see Appendix B-1), or within any of the three genetic clusters in Saskatchewan (see Appendix B-2, B-4, and B-6). All following results refer to effective distance, calculated as total landscape resistance based on circuit-theory (McRae et al. 2008).

For the full study area, the best models explaining genetic distance, based on Mantel r, were identified to be the landscape variables: water, forestry, roads, wildfire of 50 years, low suitability habitat, and wildfire of 40 years, all at the resistance value of 10 (Figure 2-7). The only landscape variable not found to be significant was wildfire of 60 years (Appendix B-1).

As wildfire of 50 years was the most significant wildfire variable, wildfire of 60 and 40 years were not included in further analysis of each genetic cluster. All significant variables were further found to be significant when partialled out from the effects of IBD with causal modeling (Table 2-5). All variables were also found to be highly correlated (>0.75), except for wildfire of 50 years and water (0.672).

Combining landscape variables identified to be significant in the previous step, revealed 14 new models that were further tested for landscape resistance across the full study area. The best model was identified to be wildfire of 50 years and water combined, both at a resistance of 10. The following best model combined wildfire of 50 years, roads, forestry, and water (Table 2-5).

Figure 2-7. Significant landscape variables influencing gene flow across Saskatchewan, including water bodies, forestry, roads, and wildfire of 50 years.



Both models were more strongly correlated with genetic distance than the model of water alone (Table 2-5). All landscape variables were correlated at the full scale across Saskatchewan, therefore, although the best model describing gene flow at a coarse scale was one that combined wildfire of 50 years and water (Mantel r = 0.074; Table 2-5), all landscape variables found

significant across the study area, and their effects on gene flow, should be considered in range planning.

Table 2-5. Causal modeling assessing Isolation-by-Resistance (IBR) landscape models and Isolation-by-Distance (IBD) on proportion of shared alleles genetic metric (Gen) across the full study area in Saskatchewan. The landscape variables tested included water, forestry, roads, wildfire of 50 years, low suitability habitat, and wildfire of 40 years. The resistance value tested is indicated in parenthesis.

	Gen~IBR|IBD		Gen~IBD|IBR	
Landscape models (resistance value)	Mantel r	P-value	Mantel r	P-value
water (10)	0.069	0.003	0.002	0.466
forestry (10)	0.057	0.001	0.001	0.465
roads (10)	0.052	0.001	0.001	0.465
wildfire 50 years (10)	0.051	0.033	0.020	0.157
low (10)	0.047	0.024	0.013	0.275
wildfire 40 years (10)	0.045	0.016	0.012	0.256
Combined landscape models (resistance value)				
wildfire 50 years (10) + water (10)	0.074	0.001	-0.011	0.722
wildfire 50 years (10) + roads (10) + forestry (10) + water (10)	0.071	0.001	-0.015	0.836
wildfire 50 (10) + low (10) + water (10)	0.070	0.001	-0.010	0.731
forestry (10) + water (10)	0.070	0.001	-0.009	0.708
roads (10) + forestry (10) + water (10)	0.068	0.001	-0.010	0.749
roads (10) + forestry (10) + water (10) + wildfire 50 years (10) + low (10)	0.068	0.001	-0.013	0.774
roads (10) + water (10)	0.068	0.001	-0.008	0.665
wildfire 40 years (10) + water(10)	0.068	0.001	-0.009	0.703
wildfire 40 years (10) + roads (10) + forestry (10) + water (10)	0.066	0.001	-0.011	0.791
wildfire 40 (10) + low (10) + water (10)	0.064	0.001	-0.007	0.643
low (10) + water (10)	0.064	0.002	-0.001	0.516
roads (10) + forestry (10)	0.057	0.001	-0.003	0.593
wildfire 50 years (10) + low (10)	0.057	0.006	0.003	0.446
wildfire 40 years (10) + low (10)	0.051	0.003	0.003	0.407

Across genetic clusters, Saskatchewan Cluster A had four significant models: wildfire of 50 years and low habitat suitability both a resistance of 1000, water at a resistance of 10, and forestry at a resistance of 1000, in that order (Appendix B-2). All four models were further found to be significant when partialled out from the effects of IBD with causal modeling (Table 2-6). All variables were correlated except for low habitat suitability and water (0.72), and low habitat suitability and wildfire of 40 years (0.649). Based on significant models and correlated variables, five new models combining significant landscape variables were tested. All new models were partialled out from the effects of IBD (Table 2-6).

Table 2-6. Causal modeling assessing Isolation-by-Resistance (IBR) landscape models and Isolation-by-Distance (IBD) on proportion of shared alleles genetic metric (Gen) across Saskatchewan Cluster A (SK A). The landscape variables tested included wildfire of 50 years, low suitability habitat, water, and forestry. The resistance value tested is indicated in parenthesis.

	Gen~IBR|IBD		Gen~IBD|IBR	
Landscape models (resistance value)	Mantel r	P-value	Mantel r	P-value
wildfire50 (1000)	0.094	0.008	-0.009	0.606
low (1000)	0.081	0.024	-0.013	0.620
water (10)	0.051	0.045	0.020	0.274
forestry (1000)	0.042	0.048	0.018	0.294
Combined landscape models (resistance value)				
water (10) + low (1000) + wild50 (1000)	0.094	0.008	-0.010	0.602
wild50 (1000) + low (1000)	0.094	0.008	-0.009	0.630
forestry (1000) + water (10) + wild50 (1000)	0.090	0.011	-0.013	0.677
forestry (1000) + water (10) + wildfire50 (1000) + low (1000)	0.090	0.009	-0.013	0.623
water (10) + low (1000)	0.051	0.042	0.019	0.279

The best two new models for Saskatchewan Cluster A were found to be water and low habitat suitability combined, and water, low habitat suitability and wildfire of 50 years combined; however, they were not better models than wildfire of 50 years alone, based on Mantel r values (Table 2-6). The model combining all four significant landscape variables across SK A still had a high model fit, and therefore all variables found significant within this cluster should be considered in range planning (Appendix B-3).

Saskatchewan Cluster B had four significant models, low habitat suitability at the resistance of 10, roads at the resistance of 1000, and forestry and water at a resistance of 10, in that order (Appendix B-4). Only low habitat suitability and forestry were partialled out from IBD (Table 2-7; Appendix B-5), and both variables were highly correlated (0.968). The model combining them however could not be partialled out from IBD, and therefore low habitat suitability remains the best model for the cluster (Table 2-7).

Table 2-7. Causal modeling assessing Isolation-by-Resistance (IBR) landscape models and Isolation-by-Distance (IBD) on proportion of shared alleles genetic metric (Gen) across Saskatchewan Cluster B (SK B). The landscape variables tested included low suitability habitat, roads, forestry, and water. The resistance value tested is indicated in parenthesis.

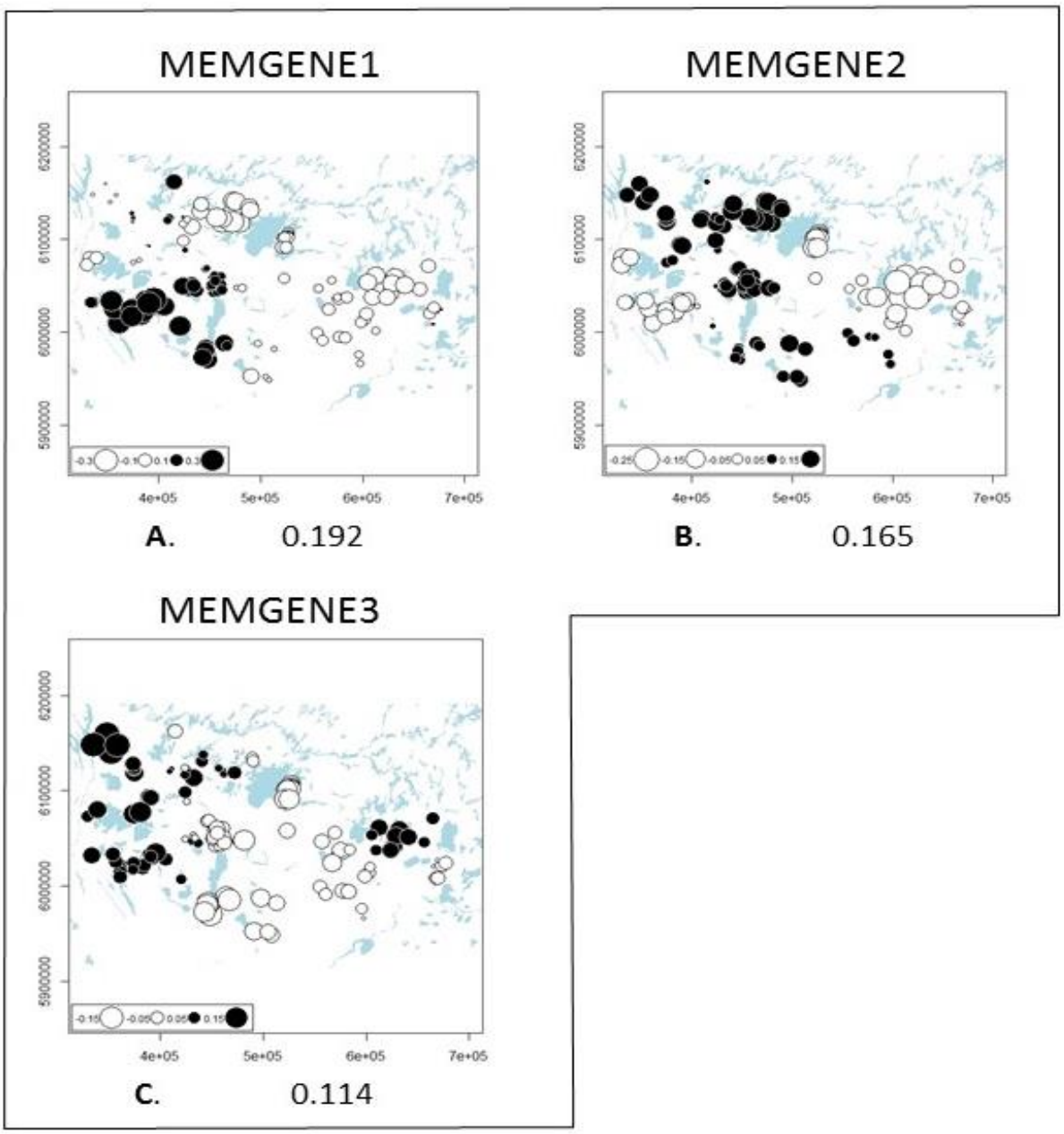
	Gen~IBR|IBD		Gen~IBD|IBR	
Landscape models (resistance value)	Mantel r	P-value	Mantel r	P-value
low (10)	0.078	0.002	0.043	0.066
roads (1000)	0.076	0.003	0.088	0.006
forestry (10)	0.069	0.003	0.042	0.074
water (10)	0.055	0.021	0.057	0.027
Combined landscape models (resistance value)				
forestry (10) + low (10)	0.072	0.016	0.091	0.002

Saskatchewan Cluster C had no significant landscape variables identified to be influencing the gene flow patterns in its area, north and east of Prince Albert National Park (Appendix B-6). Therefore, no combined models were tested for this cluster.

2.4.6 MEMGENE

MEMGENE analysis provided an alternative approach to visualize genetic distance patterns across individuals in the study area in Saskatchewan. Across the full study area in Saskatchewan, the analysis identified 3 main MEMGENE variables that can explain spatial patterns in genetic distance for all individuals across the study area (with proportions greater than 0.1; Figure 2-8). The adjusted R^2 value for the full study area, representing how well genetic distance can be explained by the identified spatial patterns, was 0.123. All MEMGENE maps in Figure 2-8 were superimposed over a raster water layer to better visualize the location of each sample across the landscape. Similar dot size and colour represents similarity in genetic variation between individuals, while greater differences in dot size and colour represents greater genetic distance between individuals. The first predictor, MEMGENE1 (Figure 2-8.A), with the highest contribution to spatial genetic patterns, most clearly separates the south west area (SK C) from the rest of the study area. This supports TESS results that combine SK A and B together, with a separate cluster north and east of Prince Albert National Park. Further, spatial genetic patterns in MEMGENE2 (Figure 2-8.B) and MEMGENE3 (Figure2-8.C) separate the central area of the Province (SK B) from the east (SK A), and west (SK C). This supports STRUCTURE results that partition individuals into three genetic clusters.

Figure 2-8. MEMGENE analysis for the full study area across Saskatchewan revealing discontinuous population structure across the landscape with three main MEMGENE variables. The amount of genetic variation explained by spatial patterns is R^2 =0.123. The number below each figure represents the proportion of R^2 explained by each MEMGENE variable.



Each cluster in Saskatchewan (SK A, SK B and SK C) was further analysed separately with this analysis, providing more detailed spatial autocorrelation insight into each cluster, and can be found in Appendix B-7, B-8, and B-9, respectively. No new information, however, was identified with the finer scale of analysis, and therefore it was not included in the main context of this thesis.

MEMGENE landscape model analysis, which evaluates the influence of different resistance models on the variation in spatial genetic patterns, was done to compare with the causal modelling approach for both the full study area in Saskatchewan and the two genetic clusters SK A and SK B. Cluster SK C was not included in this analysis as no significant landscape variables were found to influence gene flow in this cluster with causal modelling (Appendix B-6), and both analyses were found to provide similar results.

MEMGENE landscape model analysis results are listed below in Table 2-8. From these values, the best model was determined by comparing [abc] values for each model, which represent how well the MEM eigenvectors that are derived from the cost distance of each landscape model explain the variation in spatial genetic patterns. Further, a higher [a] value indicates that spatial genetic variation is best explained by MEMGENE eigenvectors alone, while a higher [c] value indicates that variation is best explained by coordinates (linear pattern of genetic variation) rather than the eigenvectors derived from that model. Any confounded variation that could not be separated from either a linear (coordinates) or non-linear (MEM eigenvectors) pattern is represented in the [b] value. Similar [abc] values indicate that a correlation between models is present (Galpern et al. 2014; Galpern and Peres-Neto 2014).

Table 2-8. MEMGENE landscape model analysis for Saskatchewan's full study area and genetic clusters SK A and SK B. The landscape variables tested included roads, water, forestry, low suitability habitat, wildfire of 50 years, and wildfire of 40 years. Euclidean distance was also tested as a variable. The table describes the proportion of variation in genetic distance that can be explained by- [abc]: spatial predictors (selected MEM eigenvectors), [a]: spatial patterns in the resistance model, [c]: coordinates, [b]: confounded between resistance model and coordinates, and [d]: residual (not spatial), for each resistance model, while P [abc], [a] and [c], represent the significance (p-value) of each calculated proportion (adapted from Galpern et al. 2014; Galpern and Peres-Neto 2014). The resistance value for each model is indicated in parenthesis.

Model	[abc]	P [abc]	[a]	P [a]	[c]	P [c]	[b]	[d]
Saskatchewan full study area								
roads (10)	0.133	0.001	0.100	0.001	< 0.001	0.396	0.033	0.867
water (10)	0.131	0.001	0.098	0.001	< 0.001	0.622	0.034	0.869
forestry (10)	0.130	0.001	0.097	0.001	< 0.001	0.499	0.033	0.870
low (10)	0.127	0.001	0.094	0.001	< 0.001	0.285	0.033	0.870
wildfire 50 years (10)	0.127	0.001	0.094	0.001	< 0.001	0.287	0.033	0.870
wildfire 40 years (10)	0.127	0.001	0.094	0.001	< 0.001	0.43	0.033	0.870
Euclidean	0.123	0.001	0.090	0.001	< 0.001	0.730	0.034	0.877
Saskatchewan Cluster A								
wildfire 50 years (1000)	0.087	0.001	0.064	0.001	0.010	0.015	0.012	0.913
Euclidean	0.080	0.001	0.058	0.001	0.001	0.398	0.021	0.920
forestry (1000)	0.073	0.001	0.051	0.001	0.006	0.073	0.016	0.927
low (1000)	0.073	0.001	0.051	0.001	0.006	0.081	0.016	0.927
water (10)	0.070	0.001	0.047	0.001	0.009	0.016	0.013	0.930
Saskatchewan Cluster B								
Euclidean	0.089	0.001	0.046	0.001	0.002	0.250	0.042	0.911
forestry (10)	0.083	0.001	0.040	0.001	0.009	0.003	0.034	0.917
low (10)	0.083	0.001	0.040	0.001	0.009	0.001	0.034	0.917

MEMGENE landscape model analysis for the full study area in Saskatchewan was done for the six landscape resistance models found significantly correlated with genetic distance and partialled out from IBD with causal modeling (Table 2-5). The best model explaining variation in genetic distance across Saskatchewan was roads, followed by water, then forestry, all at a resistance value of 10 (Table 2-8). These results are similar to causal modeling results, for which the models water, forestry, and roads, were the most strongly correlated with genetic distance across the entire Province (Table 2-5). Low suitability habitat and wildfire of up to 50 and 40 years followed with the same proportions to explain genetic spatial patterns, indicating that they are strongly correlated (Table 2-8). Euclidean distance had the lowest proportion, indicating that IBR from certain landscape variables across the study area in Saskatchewan can better explain genetic distance than IBD alone.

For SK A, significant landscape variables analysed that could be partialled out from IBD with causal modeling included forestry, low suitability habitat, and wildfire of 50 years, all at a resistance value of 1000, and water at a resistance of 10 (Table 2-6). The best model with the highest proportion explaining genetic distance within the cluster was wildfire of 50 years (Table 2-8). This matches results obtained with causal modeling (Table 2-6). Forestry and low suitability habitat shared the same model strength to explain observed genetic patterns [abc], indicating a correlation between the two models. A linear pattern from coordinates was found significant for the variables wildfire of 50 years and water (Table 2-8 $P[c] \leq 0.05$), however the strength of the MEM eigenvectors to describe variation was still greater (Table 2-8 [a]).

For SK B, models analysed that could be partialled out from IBD with causal modeling included forestry and low suitability habitat, both at a resistance value of 10 (Table 2-7). Low suitability habitat and forestry had the same proportion of explaining variation in genetic distance in the

cluster, indicating that they are correlated (Table 2-8 [abc]). While Euclidean distance was the best model, all three landscape models have similar proportions (Table 2-8 [abc]), indicating that they could be correlated (Galpern and Peres-Neto 2014). This supports causal modeling results, which found that the combined model of forestry and low suitability habitat could not be partialled out from IBD (Table 2-7). Coordinates were significant for the models forestry and low suitability habitat (Table 2-8 P[c] ≤ 0.05), however MEM eigenvectors (Table 2-8 [a]) alone better explained spatial variation for each model than the coordinates alone (Table 2-8 [c]), indicating that spatial genetic variation patterns are not linear (Galpern and Peres-Neto 2014).

2.5 Discussion

2.5.1 Delineating boreal caribou MUs in Saskatchewan

Individual- based Bayesian clustering has revealed discontinuous population structure across the study area in Saskatchewan and Manitoba. Genetic connectivity across the provincial boundary was also found to be relatively high (F_{ST} = 0.020-0.050), although enough genetic divergence was identified to separate clusters within the boundaries of each province. While MUs for boreal caribou have already been established in Manitoba (Manitoba Conservation 2015), these results support the further delineation of the southern range of boreal caribou distribution in Saskatchewan into smaller MUs. Establishing MUs is essential for the effective management of a species, and to aid in population recovery, which is critically needed to address the declining populations of boreal caribou across Canada (Environment Canada 2012).

Three clusters were delineated in the study area in Saskatchewan based on STRUCTURE results, revealing a transition of genetic groups for boreal caribou across most of the Boreal Plains in the Province. STRUCTURE results were clear at both the interprovincial scale, and at the provincial scale of Saskatchewan alone, suggesting that three clusters ($\Delta K = 3$; Evanno et al. 2005) is the highest level of structure across the study area in the Province. Further sub-structure may potentially exist across the study area in Saskatchewan, as suggested with the additional peaks of $\Delta K = 8$ and 10 identified with STRUCTURE when the Province was evaluated alone; however, the high to moderate level of gene flow across genetic clusters will confound effects, making it difficult for STRUCTURE to properly assign individuals into a smaller number of clusters (Pritchard et al. 2000). These smaller clusters may also not be biologically significant to aid in the delineation of MUs that support the conservation of the species, and may simply be an

artifact of IBD and the program's sensitivity to genetic distance (Pritchard et al. 2000; Frantz et al. 2009; Meirmans 2012).

TESS results provided coarser scale structuring estimation across the landscape by dividing the study area in Saskatchewan and Manitoba into four clusters. The results did not differ greatly from STRUCTURE, in that Saskatchewan Cluster C was still identified and separated from the remaining study area, however, Saskatchewan Clusters A and B were combined with TESS into the cluster TESS2. Clusters MB A and MB B in Manitoba were similarly combined into the cluster TESS3, resulting in only two clusters within the study area in each Province. As STRUCTURE was able to identify a higher number of clusters across the study area, the program may therefore, for this dataset, be better than TESS at identifying genetic structure with high level of admixture present. The genetic differentiation between TESS1 and TESS2 was low (F_{ST} = 0.017), compared to SKC and SK A (F_{ST} = 0.035), or SKC and SK B (F_{ST} = 0.031), indicating that genetic differentiation is reduced across the entire study area in Saskatchewan when individuals are grouped into larger clusters. This further indicates that STRUCTURE was more proficient at identifying genetic discontinuity across the study area, and it could group individuals into clusters that maximized genetic differentiation.

Other studies that have used simulations to compare the strength of different programs to identify a pre-defined number of clusters have similarly found that STRUCTURE is more accurate at identifying clusters than TESS (i.e. Latch et al. 2006; Chen et al. 2007), although the opposite has also been found (François and Durand 2010). The benefit of incorporating spatial data may allow TESS to be better at identifying genetic clines created by spatial autocorrelation (Chen et al. 2007; François and Durand 2010); however, this was not found to be the case in this study. A study by Yannic et al. (2015) delineated caribou MUs in eastern Canada (Quebec and Labrador),

and similarly applied STRUCTURE and TESS to determine genetic population structure from microsatellite data. Their results differed for each program, with TESS identifying one more cluster than STRUCTURE, while the opposite occurred in this study. This again shows that different Bayesian programs can differ in their ability to detect sub-structure across different datasets, and that careful consideration of biological relevance for the species in focus needs to be taken when inferring the correct number of clusters (Latch et al. 2006; Chen et al. 2007; Frantz et al. 2009; Yannic et al. 2015). In this study, STRUCTURE was better at detecting admixture, which is an expected characteristic for this ecotype (Rettie and Messier 1998; Ball et al. 2010; Thompson 2015), and therefore cluster boundaries were delineated based on the output of STRUCTURE alone.

The presence of IBD across the study area, and within each cluster, indicates that the boreal caribou ecotype is not panmictic across the study area in Saskatchewan. The low contemporary genetic differentiation (F_{ST}) between SK A and SK B also suggests that movement of individuals between the two clusters does occur, while SK C is the most genetically isolated cluster across the study area in the Province. High admixture in SK A and SK B is further suggested by the high number of loci deviating from LE within the two clusters (3/9; Latch et al. 2011), while no loci deviate from LE within SK C. In Manitoba, five loci deviate from LE for MB C, suggesting the presence of admixture across individuals (Latch et al. 2011), while none deviate for MB A and MB B. Although MB C is the most genetically differentiated cluster in the Province, the inclusion of individuals from the Bog range, between the Saskatchewan and Manitoba provincial boundary, may be contributing to the level of LD in the cluster, as individuals in this area between provinces are highly admixed. While clusters in Manitoba were delineated to compare population structure and genetic connectivity with Saskatchewan only, deviations from HWE

and LE across loci in all MB clusters delineated in this study further confirms that high levels of admixture exist across the current clusters. This indicates that hierarchical sub-structure is present in Manitoba, which supports the findings in previously studies of the Province that have shown that the clusters MB A and MB C, delineated in this study, are further sub-divided into smaller genetic clusters (Ball et al. 2010; Thompson 2015; Hettinga et al. 2012).

Smaller levels of sub-structure could be identified across each of the three main MEMGENE variables derived across the study area in Saskatchewan, expressing variation in genetic spatial patterns. For example, individuals in the north-east corner of Cluster SK C (west of Montreal Lake) were found to differ from individuals in the north-west corner of the Cluster, suggesting further hierarchical structure within the small and genetically isolated Cluster. At a broader scale, the subdivision of each of the three genetic clusters across the study area in Saskatchewan was repeated within the MEMGENE2 pattern of genetic distance (Figure 2-8.B), supporting STRUCTURE results, and indicating that genetic divergence occurs at this level. Conversely, TESS result were more closely repeated within the MEMGENE1 pattern, however, the location of genetic clines between the three clusters proposed with STRUCTURE can still be identified, further supporting STRUCTURE's ability to identify genetic discontinuity.

The repeated partitioning of SK C and MB C with TESS clusters TESS1 and TESS4 respectively, supports the genetic divergence of these two clusters. Manitoba Cluster C, analogous to TESS4, is the most genetically isolated group across the two provinces. This supports results from previous studies (Ball et al. 2010; Hettinga et al. 2012; Thompson 2015) that indicate that the Interlake area in Manitoba is highly isolated due to the presence of Lake Manitoba, Lake Winnipegosis, and Lake Winnipeg. Saskatchewan does not have any lakes of

that size and extent, and therefore, genetic differentiation observed in SK C is likely due to other (combined) variables, which may be mainly anthropogenic.

Unlike Saskatchewan Clusters A and B, a high amount of individuals within SK C have strong assignment to the cluster. The only exception is the north-east corner of the cluster that was delineated following Hwy 2. This delineation separates individuals on either side of the highway, as individuals on the east side of the linear feature had stronger assignment to SK B, according to STRUCTURE results. This delineation was supported by all three MEMGENE spatial predictors (MEMGENE1, MEMGENE2 and MEMGENE3), which show an increase in genetic distance on either side of Hwy 2. Despite the presence of this genetic cline separating the two clusters, which cannot be spatially attributed to IBD, intermixing between SK B and SK C still occurs across the highway. This is reflected in TESS results, which clump individuals on both sides of the highway into SK C that surrounds Prince Albert National Park. This further suggests that the high level of intermixing across this highway makes Hwy 2 a semi-permeable variable to gene flow for boreal caribou in the area.

While there was a low number of caribou found within Prince Albert National Park in SK C, a higher number of animals were found to use the north-west corner of the cluster, which is surrounded by large lakes (i.e. Dore Lake and Smoothstone Lake). This is supported with MEMGENE2, where a clear separation of individuals in the north east of SK B, where Lac La Ronge is located, occurs from the rest of the cluster. Caribou have been documented to prefer to stay near lakes in the winter, as predators are more likely to hunt inland (Bergerud 1985; Ferguson and Elkie 2005). Boreal caribou may therefore be moving to this area of the landscape during the winter season, or they remain there year-round. If habitat conditions are optimal, movement north into Cluster B may be limited due to the preference of staying near these lakes.

This behaviour of habitat selection may explain the low level of gene flow present between these two clusters.

2.5.2 Genetic connectivity of boreal caribou in central Canada

Levels of genetic differentiation were found to be higher between clusters in Manitoba than in Saskatchewan, regardless of how many clusters were delineated with STRUCTURE or TESS. Anthropogenic disturbances are higher in Manitoba, especially along the west side of the Province, with road, city, and hydro line development. These variables may be responsible for the higher level of genetic partitioning observed across Manitoba. While hierarchical structuring of boreal caribou in Manitoba was not analysed in detail in this study, pairwise F_{ST} and R_{ST} values support the findings of previous studies, which identify further subdivision of clusters across the Boreal Plains in Manitoba and into the north-west of the Boreal Shield in the Province (Ball et al. 2010; Thompson et al. 2015).

Ball et al. (2010) evaluated the area north of Prince Albert National Park, and found that at an inter-provincial scale of analysis, boreal caribou in this area were genetically clustered with individuals in western Manitoba. Similar results were found by Thompson (2015). The area evaluated by Ball et al. (2010) included samples that are within Saskatchewan Clusters B and C, delineated in this study. This might suggest why connectivity between provinces was detected by Ball et al. (2010), as Saskatchewan Cluster B and Manitoba Cluster B have the second highest level of gene flow across clusters (F_{ST} = 0.018), suggesting that long-distance dispersal does occur for the ecotype. Genetic connectivity between Saskatchewan Cluster C and Manitoba Cluster B is also lower (F_{ST} = 0.043), which supports the finer scale structuring results in Ball et al. (2010) that divides the two provinces. An explanation for the genetic partitioning between

provinces in the north may be the presence of large town sites near the provincial boundary (Flin Flon, The Pas) that will be avoided by caribou (Dyer et al. 2001), as well as linear features (Dyer et al. 2002) such as Hwy 10 along the west side of Manitoba, and the railway tracks north of the Hwy 10 along the provincial boundary. These variables combined may be restricting gene flow at the level identified between provinces.

To understand if current genetic structure on the landscape is a result of genetic drift (contemporary processes), or inherited from historic patterns such as mutations, F_{ST} and R_{ST} measures of genetic differentiation were compared (Hardy et al. 2003). In this study, F_{ST} and R_{ST} measures reveal similar patterns of genetic differentiation, indicating that historical processes of mutation are not a relevant factor contributing to the genetic differentiation observed between genetic clusters. Results from a study by Klütsch et al. (2012), which analyzed historic lineages from haplotype data of caribou across Canada, indicated that two Woodland Caribou lineages occur in the Boreal Plains ecozone of Saskatchewan (haplogroup A2, which is the most similar with the west of Canada, and haplogroup A3, which is the most similar with the northern regions of central Canada). While the study revealed that the boreal caribou ecotype in Saskatchewan is not intermixed with other caribou sub-species, most of central Canada (Saskatchewan, Manitoba, and Ontario) was identified to be a convergence zone for the different Woodland Caribou haplogroups that occur across the country (Klütsch et al. 2012). The majority of individuals within Saskatchewan, however, were identified to be from the A2 lineage, and therefore genetic structure across the Boreal Plains in Saskatchewan is not a result of the convergence of different lineages of glacial refugia. As both IBD and IBR models were found to influence contemporary gene flow patterns of boreal caribou across the study area, it is most likely that both of these models can describe the genetic structure of the ecotype in the past. This indicates that, in

addition to the effects of IBD, both natural and anthropogenic landscape variables influence the genetic connectivity of this threatened ecotype. Increasing anthropogenic disturbances on the landscape will contribute to further genetic discontinuity of boreal caribou across the landscape, and may lead to additional sub-structure over time.

2.5.3 Landscape effects on gene flow

The measure of total resistance (circuit theory) across the landscape identified significant variables correlated with gene flow, while least-cost pathway analysis did not reveal any significant variables at any scale or resistance. This is likely a factor of the scale of the landscape being analysed. Least-cost pathway identifies one best pathway of travel, while total resistance tests for more than one general pathway of movement, which is more realistic at this scale of analysis, and for this ecotype. Boreal caribou will disperse across their home ranges without necessarily following a single pathway of least resistance (Galpern et al. 2012), and therefore least-cost pathway analysis may only be appropriate at a fine scale of analysis for mobile species with relatively large home ranges (McRae 2006).

Across the study area in Saskatchewan, landscape permeability to gene flow for boreal caribou was found to be reasonably high (a resistance of 10) at the full scale of analysis. IBD results further validate that anthropogenic disturbances, such as road development and forestry, are not the sole cause for the genetic differentiation observed between genetic clusters of boreal caribou across the study area in Saskatchewan. Certain landscape variables, however, were found to be correlated with the genetic structure observed, and therefore could explain some level of the genetic partitioning. Water, a natural feature on the landscape, was found to have the highest correlation with genetic distance across the study area in Saskatchewan. According to previous

studies, it can be assumed that larger lakes, such as Lac La Ronge and Montreal Lake, may contribute strong landscape resistance to gene flow (Ball et al. 2010), while other small water bodies included in the analysis are more permeable.

Forestry (50 years old) was also found significant at a low resistance value, suggesting semipermeable resistance across the landscape for boreal caribou. One study found that while clear cuts were included within boreal caribou home ranges, likely due to their uniform distribution across the landscape, they were unused or avoided year-round with the exception of in the spring (Hins et al. 2009). Older clear cuts may therefore be used to travel between more optimal habitat patches, allowing for gene flow to occur. More recent forestry activity may, however, be causing shifts in habitat use across the landscape, leading to landscape resistance and reduced movement due to its avoidance (Vors et al. 2007; Moreau et al. 2012). Boreal caribou also prefer old growth forests, and are not attracted to new regenerating forest growth that results from forestry practice, unlike moose, white-tailed deer and other ungulate species that feed on new saplings (Seip 1992). If an area is heavily forested, caribou will prefer to leave the area in pursuit of better habitat and resources (Courtois et al. 2007). With increasing habitat fragmentation caused by forestry, this may not always be possible, and therefore forested areas may be forced to be utilized by boreal caribou, and similarly other wildlife (Hins et al. 2009).

Roads were identified to influence gene flow at a low resistance at the scale of the entire study area in Saskatchewan. The low resistance value indicates that roads are semipermeable, as identified in previous studies (Dyer et al. 2002), therefore, occasional road crossings by boreal caribou do occur, permitting some gene flow across the landscape. While the overall resistance of roads was found to be low, road traffic density is still likely to influence how much resistance a specific road is contributing to gene flow across the landscape, with greater traffic density

identified in previous studies to lead to greater avoidance by boreal caribou (Dyer et al. 2002). Human density and road development have been increasing in central Saskatchewan since the late 1960's for forestry and urban expansion (Rettie and Messier 1998; Arlt and Manseau 2011). The influence of these variables on gene flow may also be an indirect effect of predator avoidance by boreal caribou, as an increase in roads has been identified to cause an increase of predators to an area, likely resulting from easier movement across the landscape into less accessible areas that boreal caribou may occupy (Trottier 1988; Rettie and Messier 1998; Wittmer et al. 2007).

Wildfire of 60 years was not found to be significant at any resistance value; however, wildfire of 50 years and 40 years were significantly correlated with genetic distance. This suggests that wildfire burns greater than 50 years old are no longer significant at resisting gene flow for boreal caribou. This supports findings in other studies, which found that optimal habitat types for caribou include forested areas older than 50 years (Schaefer and Pruitt 1991; Dalerum et al. 2007). While wildfire of up to 50 years was found to be significant, it was not identified as one of the most important landscape variables at the scale examined in this study. Previous studies have also identified that boreal caribou will not alter their home ranges after a wildfire, even with over 70% of direct disturbance to their range, suggesting that they are adapted to this natural disturbance on the landscape (Dalerum et al. 2007). While forestry (of up to 40 years) was found to have a greater, yet comparable, effect of restricting gene flow for boreal caribou across the study area in Saskatchewan than wildfire, the two disturbance types vary greatly in their effects on wildlife and ecosystems. For example, forestry activity commonly involves long term noise pollution, trail cuts, and increased road activity in an area, which may all contribute to its avoidance by wildlife for long periods of time, even for dispersal purposes. Conversely, lichen, a

main food source for boreal caribou, requires wildfire disturbance for re-growth and the food source has been found to recover by 40 years following a wildfire (Dunford et al. 2006).

Low suitability habitat was also found to restrict gene flow for boreal caribou across the study area in Saskatchewan. This variable represents areas of the landscape not suitable for boreal caribou, but potentially suitable for other ungulates that do not require large stretches of old growth forest within their habitat ranges. Competition for resources with other ungulate species is still not fully understood for boreal caribou, however, increased predation rates on the ecotype in consequence of the presence of more prey species has been supported (James and Stuart-Smith 2000; Wittmer et al. 2007; Courbin et al. 2009). Boreal caribou may therefore be actively avoiding these areas to avoid interspecies interactions.

Within Saskatchewan Cluster A, forestry was not included in the best model, but was found significantly correlated with gene flow across the cluster, along with wildfire, low suitability habitat, and water. Forestry was also highly correlated with low suitability habitat, and is known to leave behind unsuitable habitat for caribou that prefer ranges with old-growth forest (Seip 1992). This will create an overlap of ranges for the two correlated variables. The results differed slightly for Saskatchewan Cluster B, for which roads and water could not be partialled out from the effects of IBD with causal modeling to validate their independent effects. Their effects on gene flow may still exist, but be weak across the cluster. Lag time of genetic data reflecting landscape effects may also be occurring, and be preventing the identification of any clear effects from roads; however, this will not be the case for water, as it has been on the landscape unchanged for a much longer period of time. The differences in landscape resistance across clusters reflects landscape heterogeneity across the study area, and therefore, variables found important at the finer scale of analysis within clusters should be focused on at a regional scale.

Genetic connectivity between neighbouring clusters SK A and SK B, was found to be higher than any other genetic cluster pair across the study area, suggesting that landscape connectivity between the two clusters is high as well. Dispersal across SK A and SK B may be encouraged by the lower level of development on the east side of Saskatchewan compared with the centre of the Province, and that only a few landscape variables that may cause resistance to gene flow for boreal caribou exist between the two clusters. Roads were identified to cause resistance to gene flow across Saskatchewan, and therefore Hwy 106 may be causing some landscape resistance to dispersal between SK A and SK B, as well as the presence of Narrow Hills Provincial Park and Clarence Steepbank Lakes Provincial Wilderness Park. Saskatchewan Cluster A is also surrounded by a large number of wildfire burns in the west, and an accumulation of low suitability habitat, water, and forestry in the south-east (Appendix B-3). These landscape variables were found significantly correlated with genetic distance within SK A with causal modeling, and, in addition to IBD, are likely contributing factors to the genetic partitioning found between SK A and SK B.

Landscape model analysis with MEMGENE revealed similar results to causal modeling. Both programs use cost distance matrices for each landscape variable to evaluate its relationship with genetic distance (F_{ST}) across samples. MEMGENE landscape model analysis supports causal modeling results, with the same five variables found significant in restricting gene flow across the study area in Saskatchewan. As water bodies are permanent on the landscape, and wildfire burns can be supressed with fire control, but not controlled, considering where the anthropogenic activity of forestry and road development continue to take place within Saskatchewan is important to maintain connectivity for boreal caribou. Forestry and low suitability habitat were also found significant in both SK A and SK B, and should be considered into range planning to

reduce further landscape fragmentation and restrictions to gene flow at a regional level in these areas of the Province.

Saskatchewan Cluster C had no significant landscape variables. This is likely due to the small size of the area, and the low sample size of caribou found within it. Almost no caribou presence had been found, or samples collected, within Prince Albert National Park during the collection periods of 2005 and 2006, suggesting that the Park likely has a strong impact on the way that individuals are distributed and move across the landscape. Small isolated groups of caribou have been documented in this area over the years with low adult survival rates (Arsenault and Manseau 2011). It has been suggested in previous studies that wildfire suppression in this area may be a key factor for this spatial segregation, resulting in the forests of the Park being too over-grown for caribou to utilize, with no new lichen growth (Arlt and Manseau 2011). Further, surrounding forestry activity and (cottage) development near, and in the Park, may be causing other ungulate species, such as white-tailed deer and elk, to utilize the new habitat, driving caribou groups out of the area (Brown et al. 2000; Arlt and Manseau 2011).

A study by Galpern et al. (2012) looked at approximately the same area as Saskatchewan Cluster C, and found that while, similarly, no landscape variables were significantly influencing gene flow, roads could be identified as significant features on the landscape when the analysis was repeated at a larger grain. Instead of using regular raster sizes to measure effective resistance distance, distance was measured between enlarged polygons representing hypothesized suitable habitat patches for boreal caribou. This may present an alternative approach to identify significant landscape variables. Similar to the limitations of the analysis in this study, however, size of analysis (raster or habitat patch), and the value of landscape resistance for each variable,

are hypothesized, and therefore reflect only predictions on how the landscape influences genetic connectivity for the ecotype.

The slight differences in significant landscape variables at the scale of the full study area in Saskatchewan, and within each cluster, provides evidence that scale of analysis is important, and can impact results when evaluating the influence that the landscape has on gene flow (Segelbacher et al. 2010; Short Bull et al. 2011). Attention in range planning should therefore be given to local significant landscape features, such as regional forestry stands within clusters, while large scale effects from variables such as roads should be managed at a provincial scale to minimize loss of genetic connectivity across clusters.

2.5.4 Study limitations

Using genetic data in this study allowed for genetic structure and connectivity to be measured, which aided in the delineation of genetic clusters. Despite its advantages, potential bias may have occurred due to sample collections occurring in the winter season, which may only reflect structure during that time of the year (Latch and Rhodes 2006). This problem, however, is expected to be marginal for this typically sedentary ecotype (Stuart-Smith et al. 1997; Callaghan et al. 2010), as habitat ranges for boreal caribou do not vary greatly throughout the year (Ferguson and Elkie 2004). Additionally, the scale of analysis in this study was coarse enough that short-distance seasonal movements would not have a heavy impact on results.

Actual migration rates could also not be measured across clusters, as samples across the entire study area were collected over a time span of 10 years, without re-sampling of areas. While genetic connectivity and indirect migration rates were assessed in this study, direct migration rates between clusters would provide further information about the movement behaviour of

boreal caribou across the study area in the Boreal Plains (Palsbøll et al. 2010; Robinson and Moyer 2013). Migration rates can best be obtained with repetitive CMR sampling (Gilpin 1991; Palsbøll et al. 2007), which would also provide census data for population size, and effective population size estimates (Kalinowski and Waples 2002). The high admixture observed across the study area and within clusters, in addition to genetic differentiation results, suggests that dispersal (over a variable number of generations) across the Boreal Plains in Saskatchewan is high. Obtaining further direct measurements of migration rates, evaluated for an area over multiple years of sampling (CMR), would aid in validating these results (Palsbøll et al. 2007).

Additionally, expanding the study area, and incorporating samples from both, north of the Boreal Plains in Saskatchewan, and further west towards, and including, the neighbouring Province of Alberta, would provide further information on how the ecotype is structured across the entire Province of Saskatchewan. This information would confirm that three genetic clusters currently exist across the southern range of boreal caribou distribution in Saskatchewan, as a potential fourth cluster may exist along the provincial boundary with Alberta, and would inform on the potential connectivity with individuals from other ecozones (i.e. the Boreal Shield WCCU SK1). This information would be beneficial for the effective management and conservation of the ecotype. Identifying connectivity of boreal caribou across as large of an area as possible is important, as over-estimating effective number of MUs can potentially be a waste of resources, without benefitting the recovery of a population. Conversely, under-estimating effective number of MUs could be equally as devastating, potentially leading to further population decline due to the lack of proper attention and management to maintaining essential connectivity between individuals of the same genetic group (Palsbøll et al. 2007).

Landscape genetics analyses were beneficial for identifying landscape effects on gene flow in this study, however, variability within the landscape variables tested was ignored, and therefore each variable, and likely its effects, were oversimplified. This is more problematic for certain variables, such as wildfire, where fire severity will influence the level of disturbance caused, as well as roads, where road traffic will influence the level of avoidance by boreal caribou (Dyer et al. 2002). Further limitations include that only a few available landscape variables and models were tested, while in reality the landscape is much more complex (Segelbacher et al. 2010), with many other landscape variables likely influencing gene flow for boreal caribou. For the purpose of this study, a coarse analysis of common natural and anthropogenic variables was enough to identify the presence of landscape resistance to gene flow for the ecotype, and to measure its influence on the genetic structure of boreal caribou across the ecotypes' southern distribution in Saskatchewan. Further analysis can include evaluating a larger extent of landscape variables, and measuring landscape effects at different scales of gene flow, including directly within specific areas of interest for management.

2.6 Conservation and Management Implications

Taking a genetic approach to identify the population structure and distribution of boreal caribou has provided an effective means to delineate genetic population clusters of the ecotype across a range where population distribution was not previously known. Results obtained in this study provide evidence that individuals within the southern range of boreal caribou distribution in Saskatchewan can genetically be partitioned into three smaller MUs. The three cluster boundaries delineated in this study are based on genetic similarities and can be used in future population monitoring to meet management objectives. Since the delineation of genetic boundaries was best explained by natural and anthropogenic landscape features, as determined with landscape genetic analyses, these key areas pointing to reduced gene flow could be incorporated in range planning to ensure, or restore, long term genetic connectivity across the study area.

The effects of IBD were determined to have a strong influence on genetically partitioning individuals across the full study area, indicating that boreal caribou in this range are not panmictic, or randomly distributed, across the landscape. The presence of IBR was also supported across the current landscape, and can therefore explain some level of the genetic partitioning observed. This indicates that gene flow for boreal caribou is influenced by certain aspects of the landscape that create resistance to dispersal, and lower genetic connectivity between clusters.

The high to moderate genetic differentiation identified between boreal caribou clusters in this study suggests that gene flow is active across the landscape, and is a characteristic of the genetic structure of the ecotype, as was previously described by Rettie and Messier (1998). This level of

intermixing between clusters is important to maintain for the conservation of the ecotype. Without at least moderate levels of dispersal and gene flow across the landscape, boreal caribou may become at risk of low genetic diversity and population size, which may increase their risk of extirpation (Hardy 1908; Weinberg 1908; Maudet et al. 2002). Further, maintaining landscape connectivity can help optimize space use by boreal caribou by making better quality habitat more accessible. This is especially important in the Boreal Plains of Saskatchewan, which is the southern limit of boreal caribou in the Province, and where anthropogenic disturbances such as road and town development are increasingly moving northward into their range (Arsenault 2003; Arlt and Manseau 2011; Arsenault and Manseau 2011). Other factors, such as wildfire, may further limit available habitat for the ecotype. If individuals affected by landscape changes are not able to move to new areas due to landscape discontinuity, they may become forced to use less optimal habitat patches that could reduce their rates of survival. This can be a result of both increased predation, and less nutritional food sources, within less optimal habitats (Rettie and Messier 2000; 2001).

Water bodies, and up to 50 year old wildfires, were both relatively strong at explaining landscape resistance to gene flow across the study area, while still being semi-permeable to movement. Similar contemporary and phylogeographic genetic differentiation measures suggests that while these two natural landscape variables are important in shaping the genetic structure of boreal caribou today, they may have potentially also had an IBR effect on the genetic structure of boreal caribou in the past. Forestry and roads were also both identified to have significant effects on gene flow at the scale of the full study area, with forestry further influencing gene flow at a finer scale within genetic clusters. As natural landscape variables cannot be altered, careful consideration of forestry and road development should be incorporated in range planning across

the study area in Saskatchewan. As forestry was identified to influence gene flow at all scales of analysis, attention should be given to this variable to prevent further restrictions to gene flow both across, and within, genetic clusters. Proper planning may help to prevent accumulated disturbance types in one area that may create landscape discontinuity for boreal caribou. Further, because significant landscape variables differed within each of the three clusters identified in Saskatchewan, it is recommended to manage each genetic cluster separately while still maintaining connectivity across clusters. This will help to ensure that suitable land planning decisions are made that minimize further restricting the genetic connectivity of boreal caribou across the entire landscape.

2.6.1 Conclusion

Overall, the methods applied in this study provided valuable information for delineating MUs across the study area. The inclusion of landscape genetics was a useful tool in identifying significant landscape features, such as forestry and wildfire, which are influencing gene flow patterns for boreal caribou. Landscape genetics methods further helped to validate genetic cluster boundaries, and identify areas of concern that may be influencing the genetic structure observed for boreal caribou across the study area. Landscape genetics is an expanding field (Manel et al. 2003; Storfer et al. 2007; Manel and Holderegger 2013), and this study outlines the potential benefits of combining it with population genetics to aid in the delineation of MUs for wildlife. These genetic methods can be similarly applied, and are recommended, for the conservation and management of other threatened species that are sensitive to landscape changes, and for which little previous population distribution and space use information is known.

The findings and recommendations made in this study aim to aid in range planning for boreal caribou across the study area in Saskatchewan, which is the southern limit of boreal caribou distribution in the Province, and to support the recovery of the ecotype across the Province where it is most vulnerable (Environment Canada 2012; Saskatchewan Environment 2013). While anthropogenic disturbance may continue to fragment the landscape across both Saskatchewan and Manitoba, careful range planning and management of genetic clusters may reduce further population decline, and may help to maintain stable populations of boreal caribou across central Canada.

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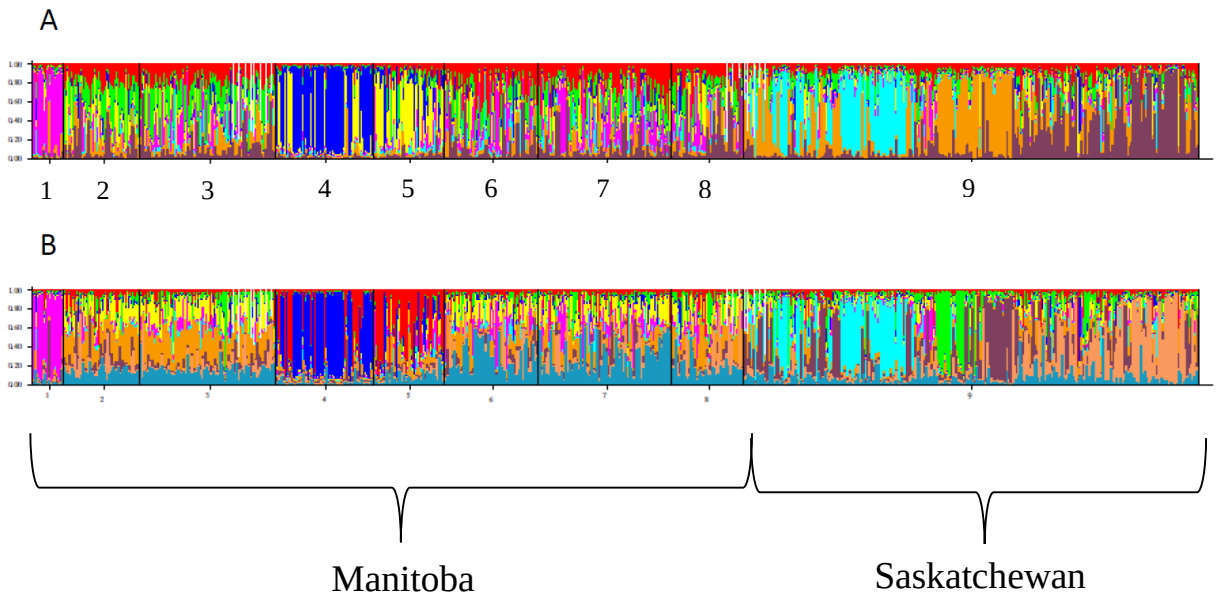
3. Appendices

Appendix A-1. Additional STRUCTURE bar plots for Saskatchewan and Manitoba.

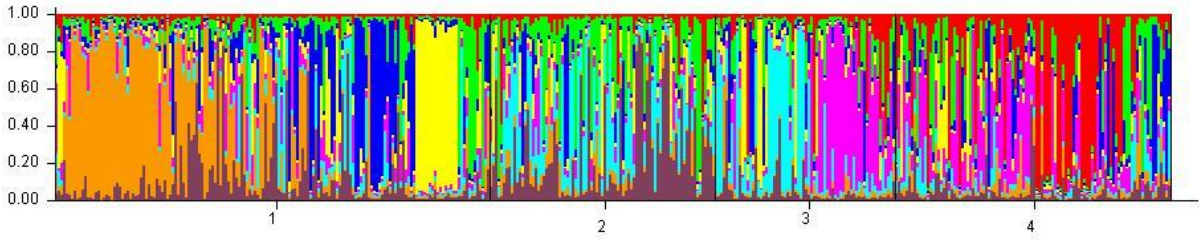
The bar plots for STRUCTURE (A) K = 8 and (B) K = 10 across both provinces Saskatchewan and Manitoba. Structure of more than 6 clusters across both provinces is not well defined and has a high level of admixture for individuals. Each vertical line represents an individual and each colour reflects assignment to a particular cluster. Numbers underneath the bar plots (1-9) correspond to the pre-defined location name of where samples were collected from.



Legend: **1**- Harding Lake, **2**- Kississing, **3**- Naosap-Reed, **4**- North Interlake, **5**- Bog, **6**- Wabowden, **7**- Wapisu-Wimapedi, **8**- Wheadon, **9**- Saskatchewan.

Appendix A-2. Additional STRUCTURE bar plot for Saskatchewan.

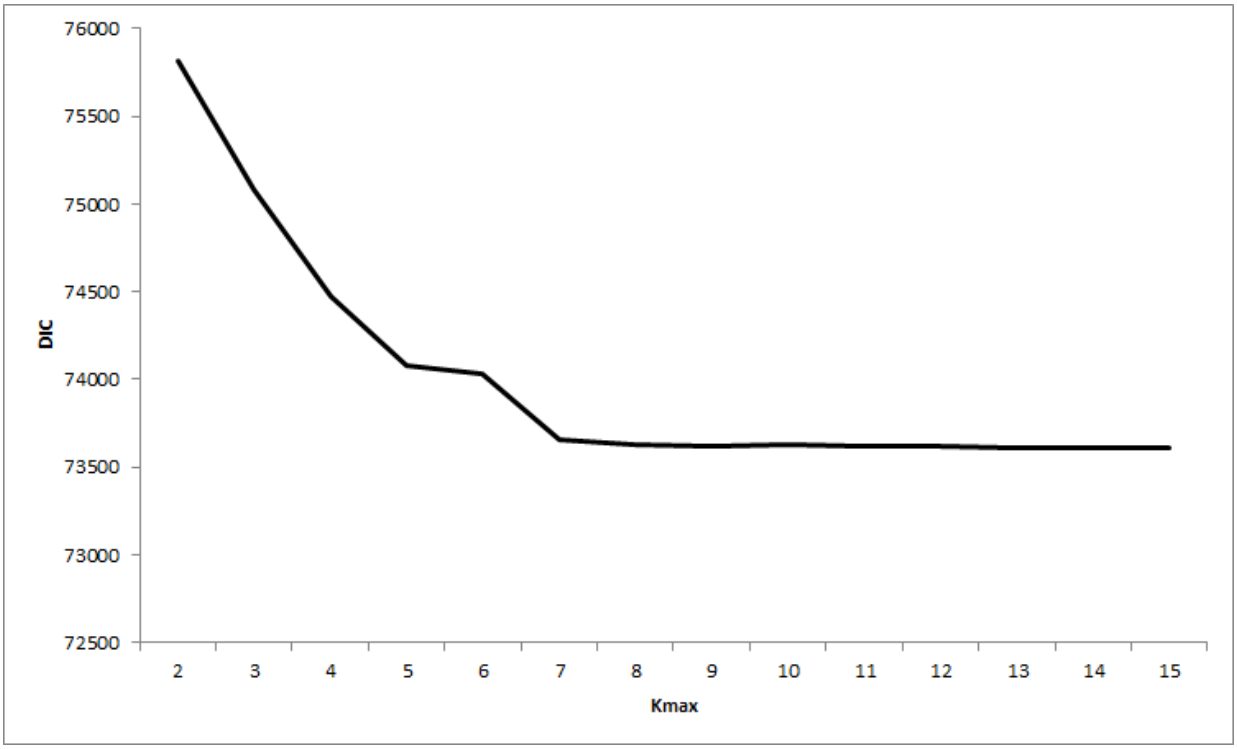
The bar plot for STRUCTURE K = 8 across Saskatchewan. Although ΔK suggests that there is (sub) structure at this level, individual assignment to clusters is not clear and admixture for most individuals is high. Each vertical line represents an individual and each colour reflects assignment to a particular cluster. Numbers underneath the bar plot (1-4) correspond to the pre-defined location name of where samples were collected from.



Legend: 1- Smoothstone, **2, 3-** La Ronge, **4-** Flin Flon.

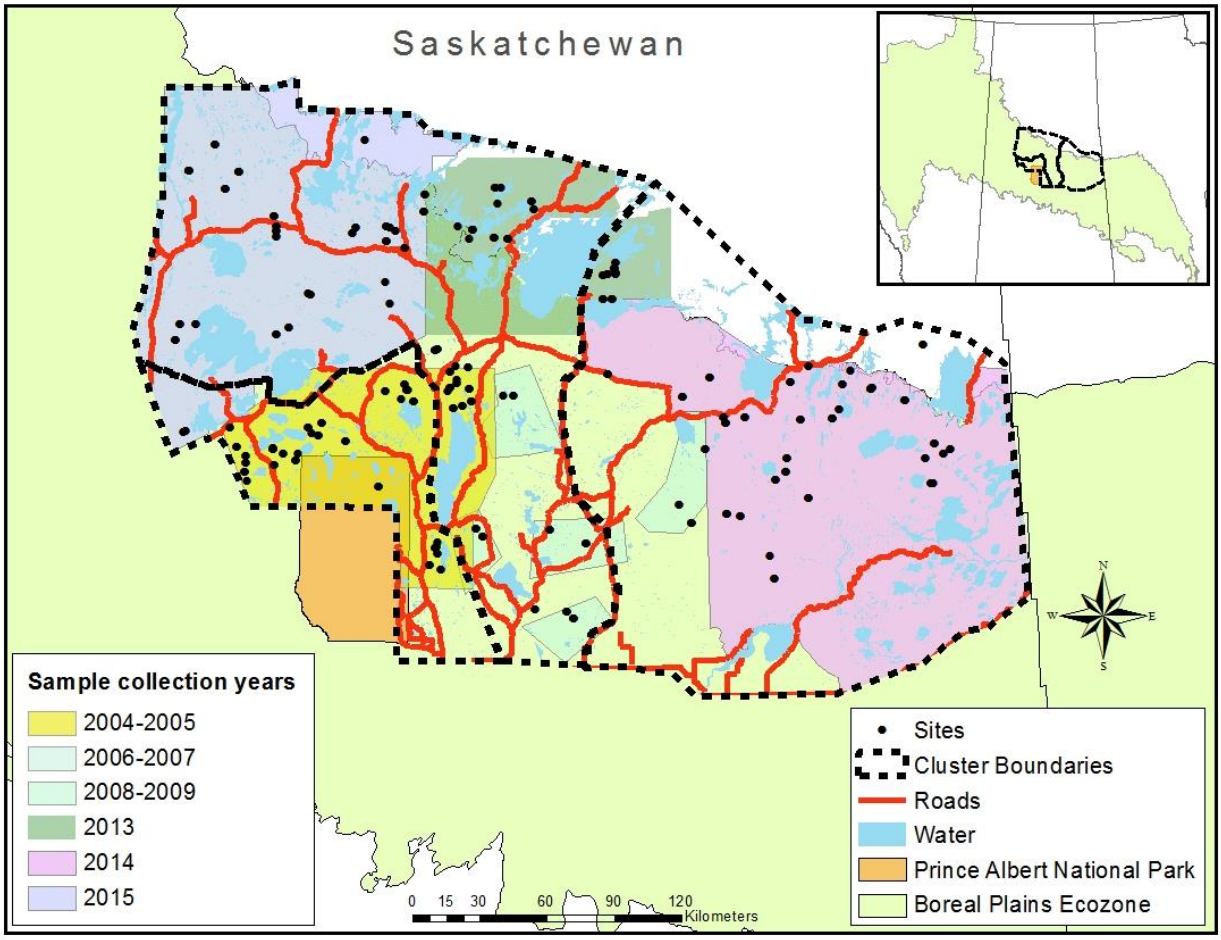
Appendix A-3. TESS K_{max} for the no-admixture model across Saskatchewan and Manitoba.

TESS DIC values plotted against the optimal K_{max} for the no-admixture model across both provinces Saskatchewan and Manitoba. The optimal K_{max}, or optimal number of clusters without admixture, was determined to be between 4 and 7 from this plot (where the plateau begins). These results were used to further test for structure across Saskatchewan and Manitoba using the admixture model, with K_{max} ranging from 2 to 10.



Appendix A-4. Landscape features used in the delineation of cluster boundaries across Saskatchewan.

In addition to STRUCTURE cluster assignments, collection polygons, roads, and water bodies across the study area were used to help delineate the three cluster boundaries in Saskatchewan. Roads and water features were used as they were hypothesized to act as landscape resistors to movement for boreal caribou, and are permanent features on the landscape.



Appendix A-5. Hardy-Weinberg Equilibrium per locus and cluster across Saskatchewan and Manitoba.

Hardy-Weinberg equilibrium p-values for each locus across each cluster. Values indicated with an asterisks (*) indicate significance for non-equilibrium, or deviation from HWE, for each locus after being adjusted for with a Bonferroni correction (Rice 1989).

Locus	SK A	SK B	SK C	MB C	MB B	MB A
BM848	0.0379	0.2939	0.1658	0.0094	0.3981	*0.0005
BM888	0.9336	0.5983	0.7198	0.0366	*0.0055	0.5219
MAP2C	0.0699	0.4733	0.1331	*0.0034	0.9962	0.0291
RT24	0.0597	*0	0.1814	0.0662	0.0196	*0.0033
RT30	*0	0.0095	*0.003	*0	*0	*0
RT5	0.665	0.0352	0.864	0.6897	0.0264	0.7076
RT6	0.2572	0.131	0.2474	0.6326	0.281	0.5085
RT7	0.2985	*0.0019	0.973	0.0551	0.6807	0.0827
RT9	0.038	0.1199	0.7019	0.0496	0.6492	*0.0052

Appendix A-6. Linkage disequilibrium per locus pairs across Saskatchewan and Manitoba.

Locus	Locus	SK A	SK B	SK C	MB C	MB B	MB A
BM848	BM888	*0	0.40462	0.02528	0.34867	0.98901	0.89473
BM848	MAP2C	0.79663	0.46632	0.07549	0.7786	0.32507	0.04856
BM888	MAP2C	0.92976	0.12286	0.63888	*0	0.5204	0.1251
BM848	RT24	0.53527	0.47987	0.42318	0.18556	0.94156	0.01596
BM888	RT24	0.24998	0.02318	0.18095	*0	0.23386	0.6041
MAP2C	RT24	0.68197	0.07106	0.53943	0.05799	0.05937	0.15186
BM848	RT30	0.67239	0.99905	0.57458	0.97094	0.19232	0.03855
BM888	RT30	0.86949	0.05732	0.10736	0.00629	0.61019	0.06792
MAP2C	RT30	0.34605	0.72034	0.97973	0.05799	0.07532	0.16137
RT24	RT30	0.2811	0.07782	0.07609	0.03485	0.37015	0.86732
BM848	RT5	0.07505	0.25756	0.957	0.13664	0.67897	0.23365
BM888	RT5	0.16058	0.08115	0.27761	*0	1	0.49907
MAP2C	RT5	0.01085	0.10558	0.08203	0.00837	0.05243	0.00562
RT24	RT5	0.00582	*0.00425	0.38086	0.23253	0.67158	0.62419
RT30	RT5	0.39172	0.52601	0.83163	*0	0.51787	0.13393
BM848	RT6	0.84148	0.52481	0.0972	0.10221	0.11454	0.12848
BM888	RT6	0.01107	0.90944	0.29108	0.09513	0.07128	0.14524
MAP2C	RT6	0.67886	0.01009	0.29343	0.12191	0.15162	0.22766
RT24	RT6	0.33747	0.05803	0.70464	0.2332	0.82521	0.11469
RT30	RT6	0.4118	0.61097	0.96272	0.10866	0.65215	0.58903
RT5	RT6	0.46909	0.45082	0.03049	0.01881	0.16498	0.61116
BM848	RT7	0.35802	0.64655	0.74379	0.35093	0.98463	0.02526
BM888	RT7	*0	*0	0.02478	*0	0.57636	0.61784
MAP2C	RT7	0.73388	0.008	0.07515	0.03699	0.48178	0.10443
RT24	RT7	0.90282	0.35638	0.79129	0.67996	0.68949	0.53082
RT30	RT7	0.05476	0.66254	0.60183	0.76723	0.08713	0.63992
RT5	RT7	0.94596	0.12408	0.59512	0.08923	0.21921	0.88455
RT6	RT7	0.49345	0.03868	0.93406	0.83437	0.14344	0.14569
BM848	RT9	*0.00117	0.80542	0.3316	0.61359	0.6513	0.80786
BM888	RT9	0.51584	0.37777	0.39247	0.03202	0.23086	0.23555
MAP2C	RT9	0.16851	0.14962	0.26495	0.21384	0.26847	0.84703
RT24	RT9	0.49578	0.85375	0.46258	0.0671	0.26226	0.72857
RT30	RT9	0.25322	0.262	0.95661	0.28783	0.92865	0.73415
RT5	RT9	0.01971	*0.00074	0.59359	0.15791	0.12656	0.0158
RT6	RT9	0.08059	0.41527	0.30079	0.49487	0.77168	0.18188
RT7	RT9	0.20838	0.16158	0.6134	0.6401	0.33785	0.43377
Total deviations after *Bonferroni:		3	3	0	5	0	0

Appendix A-7. Pairwise genetic distances for the four clusters identified with TESS across Saskatchewan and Manitoba: F_{ST} (above diagonal) and R_{ST} (below diagonal).

Cluster	TESS1	TESS2	TESS3	TESS4
TESS1	-	0.017	0.019	0.04
TESS2	0.016	-	0.016	0.046
TESS3	*0.006	*0.002	-	0.037
TESS4	0.068	0.048	0.059	-

*non-significant (p-value ≥ 0.05)

Appendix A-8. IBD for the four clusters identified with TESS across Saskatchewan (TESS 1 and TESS2) and Manitoba (TESS3 and TESS4).

Cluster	Mantel r	P-value	Cluster size (n)
TESS1	0.158	0.001	157
TESS2	0.079	0.001	267
TESS3	0.043	0.002	397
TESS4	0.156	0.001	124

*non-significant (p-value ≥ 0.05)

Appendix B-1. Landscape models for Saskatchewan.

Models of the 10 landscape variables tested for the full study area of Saskatchewan at three resistance values (10, 100, 1000) with both least-cost pathway analysis and circuit-theory resistance analysis.

	Least-cost		Resistance	
10	Mantel's r	P value(0.05)	Mantel's r	P value(0.05)
roads	-0.037	0.210	0.052	*0.001
forestry	-0.035	0.164	0.057	*0.001
wildfire 60 years	0.023	0.393	0.037	0.109
wildfire 50 years	0.017	0.239	0.051	*0.033
wildfire 40 years	0.015	0.298	0.045	*0.016
water	-0.015	0.552	0.069	*0.003
low	0.020	0.215	0.047	*0.024
100				
roads	-0.039	0.203	0.031	0.053
forestry	-0.033	0.230	0.048	*0.013
wildfire 60 years	0.025	0.372	0.031	0.329
wildfire 50 years	0.019	0.234	0.044	0.051
wildfire 40 years	0.014	0.307	0.038	0.069
water	-0.012	0.629	0.064	*0.011
low	0.039	0.103	0.027	0.190
1000				
roads	-0.039	0.202	0.013	0.299
forestry	-0.033	0.218	0.043	*0.038
wildfire 60 years	0.025	0.369	0.029	0.346
wildfire 50 years	0.019	0.253	0.042	0.084
wildfire 40 years	0.013	0.317	0.036	0.075
water	-0.011	0.630	0.064	*0.024
low	0.040	0.108	0.022	0.233

Significant (p-value ≤ 0.05) models are indicated with an asterisks(*)

Appendix B-2. Landscape models for Saskatchewan Cluster A (SK A).

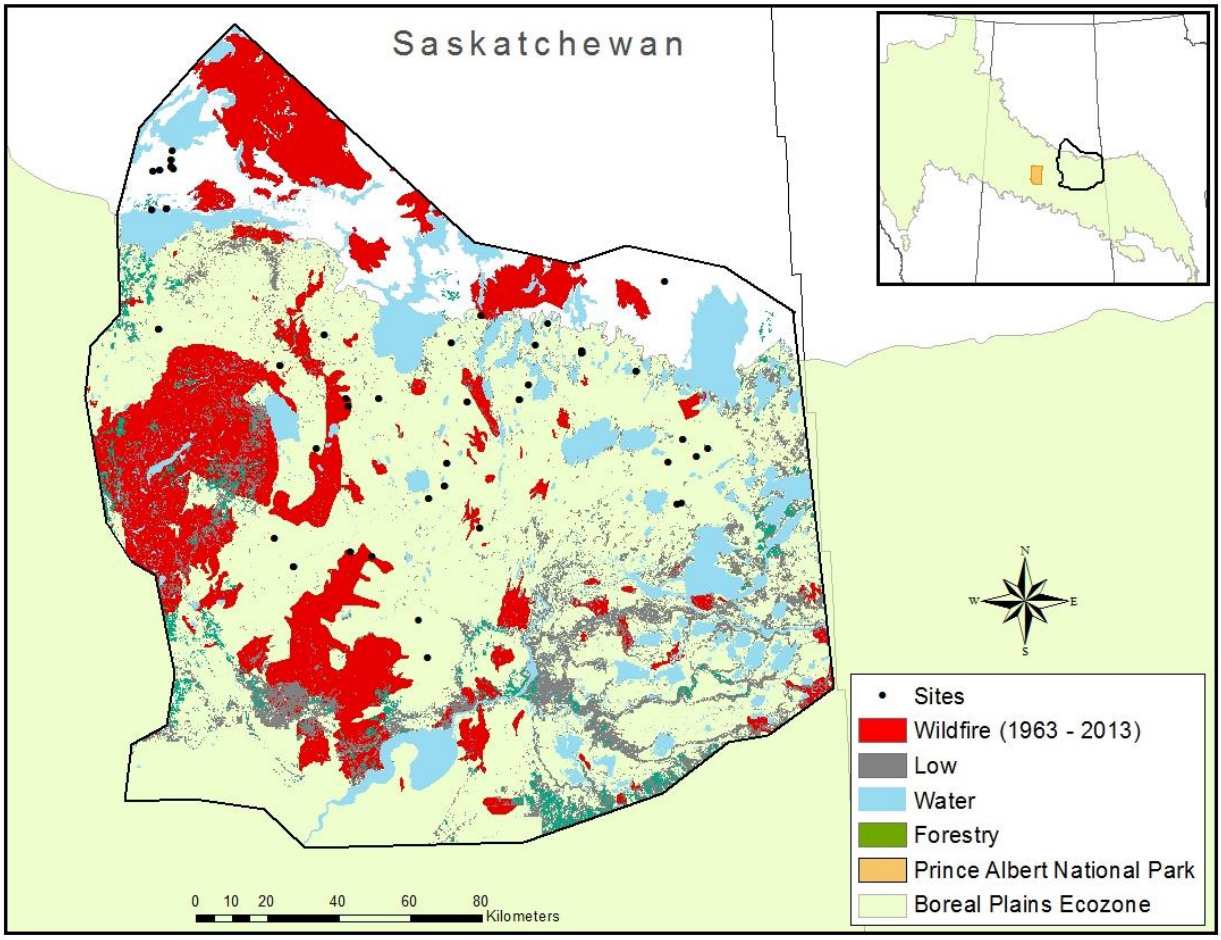
Models of the 6 landscape variables tested for Saskatchewan Cluster A at three resistance values (10, 100, 1000) with both least-cost pathway analysis and circuit-theory resistance analysis.

	Least-cost		Resistance	
10	Mantel's r	P value(0.05)	Mantel's r	P value(0.05)
roads	-0.020	0.654	0.032	0.128
forestry	-0.011	0.748	0.042	*0.036
wildfire 50 years	0.015	0.356	0.089	*0.004
water	0.010	0.832	0.051	*0.045
low	0.027	0.225	0.011	0.370
100				
roads	-0.013	0.777	0.015	0.649
forestry	0.001	0.959	0.042	*0.030
wildfire 50 years	0.018	0.334	0.094	*0.007
water	0.006	0.887	0.046	0.219
low	-0.016	0.632	0.078	*0.016
1000				
roads	-0.012	0.822	0.003	0.942
forestry	0.002	0.946	0.042	*0.048
wildfire 50 years	0.019	0.321	0.094	*0.008
water	0.006	0.905	0.043	0.256
low	-0.020	0.677	0.081	*0.024

Significant (p-value ≤ 0.05) models are indicated with an asterisks (*).

Appendix B-3. The four landscape variables found to significantly influence gene flow within Saskatchewan Cluster A (SK A), along with sample sites.

All four landscape variables mapped here were found to significantly influence gene flow with causal modeling, and should be considered in range planning. Wildfire of 50 years had the highest Mantel r, suggesting that it has the strongest influence on genetic distance across the cluster.



Appendix B-4. Landscape models for Saskatchewan Cluster B (SK B).

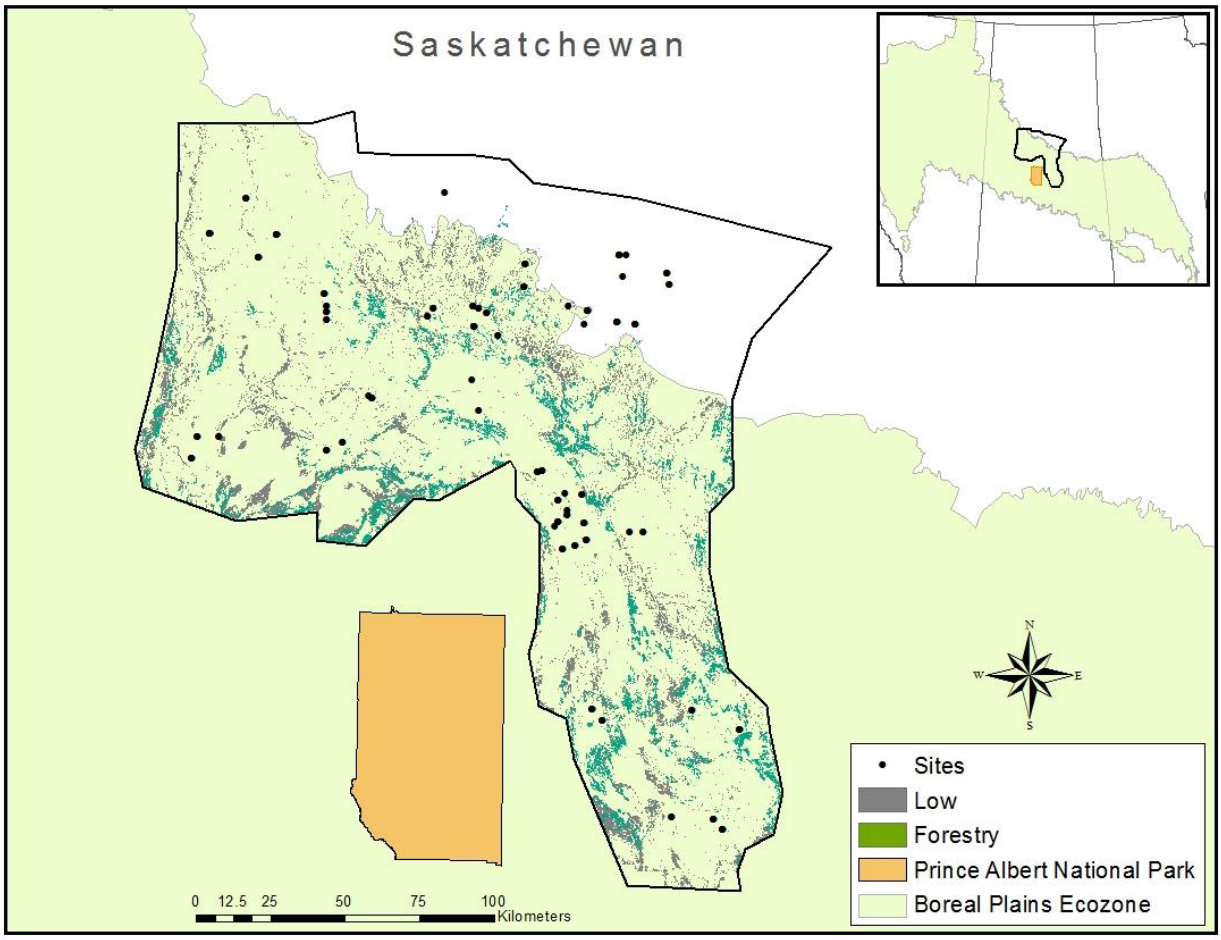
Models of the 6 landscape variables tested for Saskatchewan Cluster B at three resistance values (10, 100, 1000) with both least-cost pathway analysis and circuit-theory resistance analysis.

	Least-cost		Resistance	
10	Mantel's r	P value(0.05)	Mantel's r	P value(0.05)
roads	0.045	0.271	0.061	*0.001
forestry	0.062	0.070	0.069	*0.003
wildfire 50 years	-0.013	0.666	0.041	0.088
water	-0.020	0.585	0.055	*0.021
low	0.035	0.194	0.078	*0.002
100				
roads	0.050	0.229	0.073	*0.002
forestry	0.054	0.136	0.063	*0.016
wildfire 50 years	-0.009	0.600	0.032	0.175
water	-0.022	0.522	0.055	0.059
low	0.025	0.249	0.075	*0.009
1000				
roads	0.049	0.259	0.076	*0.003
forestry	0.054	0.140	0.062	*0.017
wildfire 50 years	-0.009	0.588	0.030	0.199
water	-0.023	0.478	0.050	0.083
low	0.023	0.276	0.071	*0.021

Significant (p-value ≤ 0.05) models are indicated with an asterisks (*).

Appendix B-5. The two landscape variables found to significantly influence gene flow within Saskatchewan Cluster B (SK B), along with sample sites.

Both low suitability habitat and forestry could be partialled out from IBD within the cluster, however, the two landscape variables were highly correlated (0.968), making their effects difficult to tease apart for independent analysis.



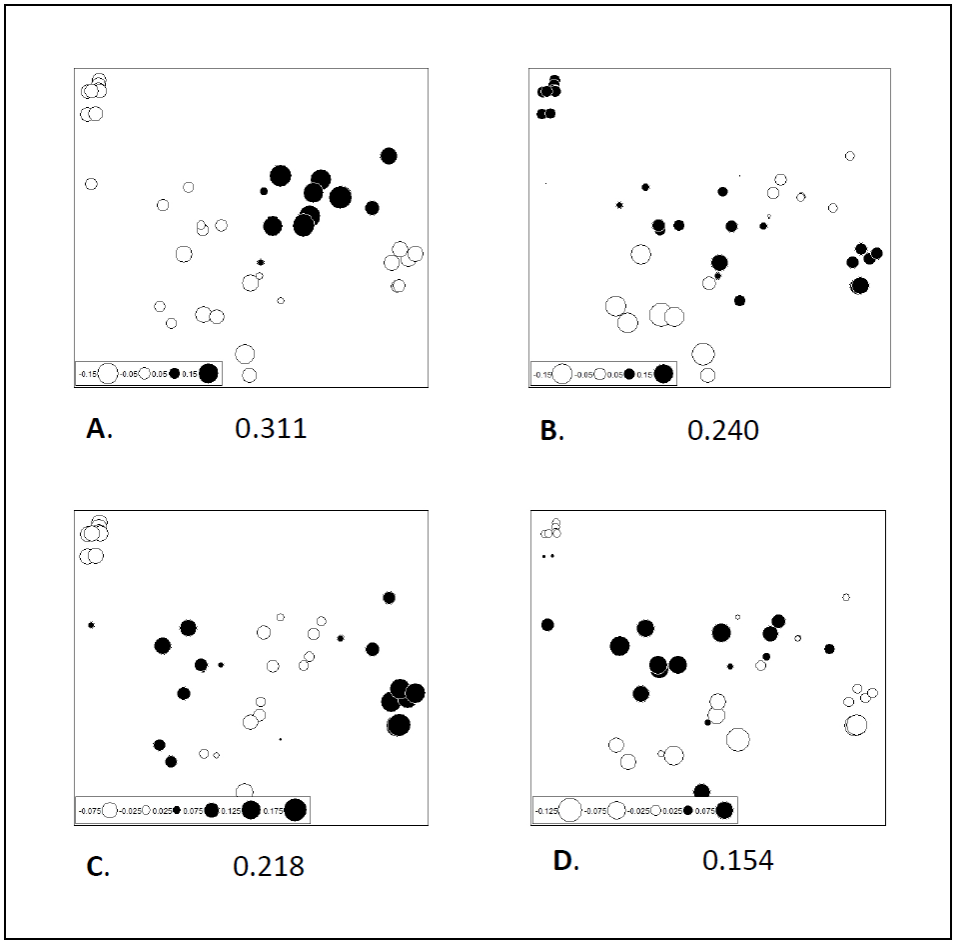
Appendix B-6. Landscape models for Saskatchewan Cluster C (SK C).

Models of the 6 landscape variables tested for Saskatchewan Cluster C, at three resistance values (10, 100, 1000), with both least-cost pathway analysis and circuit-theory resistance analysis. No model was found to be significant (p-value ≤ 0.05).

	Least-cost		Resistance	
10	Mantel's r	P value(0.05)	Mantel's r	P value(0.05)
roads	-0.034	0.562	-0.035	0.893
forestry	-0.034	0.568	-0.006	0.560
wildfire 50 years	0.002	0.494	-0.009	0.573
water	-0.006	0.915	-0.011	0.613
low	-0.041	0.771	-0.028	0.762
100				
roads	-0.020	0.748	-0.033	0.853
forestry	-0.029	0.636	0.005	0.484
wildfire 50 years	0.000	0.505	-0.012	0.586
water	-0.002	0.973	-0.006	0.527
low	-0.033	0.700	-0.024	0.677
1000				
roads	-0.019	0.736	-0.030	0.807
forestry	-0.031	0.633	0.007	0.463
wildfire 50 years	0.000	0.494	-0.013	0.580
water	-0.001	0.979	-0.005	0.537
low	-0.030	0.716	-0.023	0.658

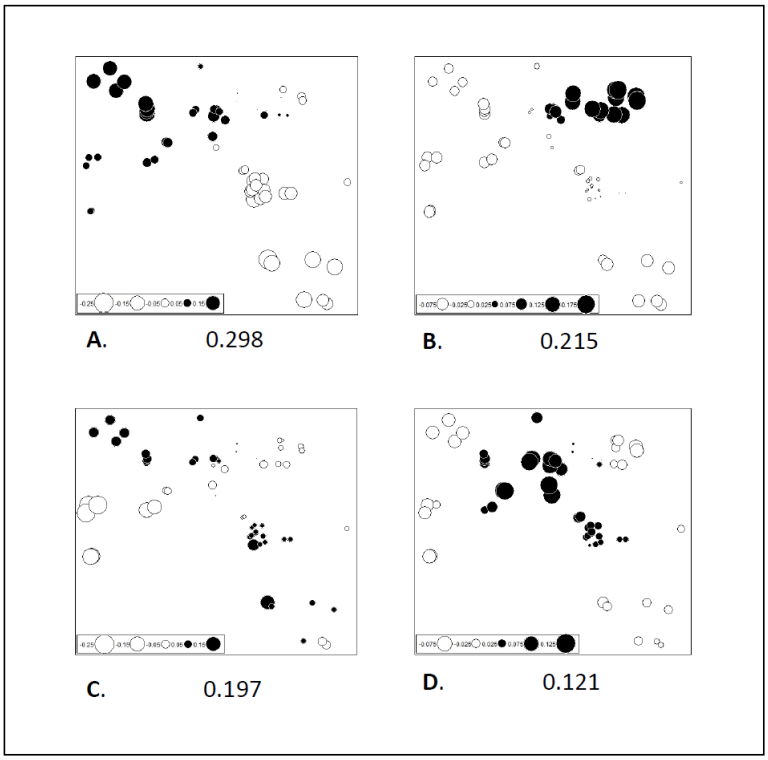
Appendix B-7. MEMGENE for Saskatchewan Cluster A (SK A).

Within Saskatchewan Cluster A, four main MEMGENE variables were identified with proportions contributing to spatial patterns in the genetic distance across individuals in the cluster greater than 0.1. The adjusted R^2 value for SK A was 0.079. Genetic spatial patterns varied within this cluster but MEMGENE consistently separated individuals in the north-west group corner of the cluster.



Appendix B-8. MEMGENE for Saskatchewan Cluster B (SK B).

Within Saskatchewan Cluster B, four main MEMGENE variables were identified that represent patterns in the genetic distance with proportions contributing to spatial patterns in the genetic distance across individuals in the cluster greater than 0.1. The adjusted R^2 value for SK B was 0.084. Within this genetic cluster, MEMGENE1 most clearly separates the north-west of the cluster that has the least amount of intermixing with other clusters (A). This suggests that high levels of intermixing can strongly confound spatial genetic (distance) patterns. MEMGENE2 further most clearly separates individuals in the north east where Lac La Ronge is located, from the rest of the cluster (B). MEMGENE3 (C) and MEMGENE4 (D) identify that the central area of the cluster is the most genetically separated from the rest of the cluster, with further separation of the north and east in the central area of the cluster. This central area in SK B is the most admixed with SK C.



Appendix B-9. MEMGENE for Saskatchewan Cluster C (SK C).

Within Saskatchewan Cluster C only two MEMGENE variables were identified with proportions contributing to spatial patterns in the genetic distance across individuals in the cluster greater than 0.1. The adjusted R^2 value for SK C was 0.040. MEMGENE analysis for this cluster further suggests that individuals in the north-west and south east of the cluster are also genetically separated within the cluster boundaries. The individuals in the north east are also genetically separated, falling along the east side of Hwy 2, along with individuals in the south-east.