ON THE EDGE: A SPATIAL AND TEMPORAL ANALYSIS OF GENETIC VARIATION FOR ENDANGERED, PERIPHERAL AMERICAN BADGER POPULATIONS

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Brett Ford

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the degree of   Master of Science   .

Dr. Michael Russello, Department of Biology, University of British Columbia Okanagan

Supervisor

Dr. Karl Larsen, Department of Natural Resource Sciences, Thompson Rivers University

Supervisory Committee Member

Dr. Lael Parrott, Department of Earth, Environmental, and Geographic Sciences, University
of British Columbia Okanagan

Supervisory Committee Member

Dr. Robert Young, Department of Earth, Environmental, and Geographic Sciences,
University of British Columbia Okanagan

University Examiner

Abstract

Peripheral populations are often characterized by small population size and low genetic diversity, with many at risk of extirpation. Threats to these populations may be even more pronounced in human-modified landscapes and areas of recent recolonization, that further reduce resiliency to environmental change. The western American badger (*Taxidea taxus jeffersonii*) is an endangered mammal in Canada inhabiting the sparse grasslands of south-central British Columbia (BC). In addition to being situated at the northwestern edge of the species' range, recent human development and vehicle-induced mortalities have limited population recovery, causing unknown consequences on the genetic variation of these peripheral populations. By collecting mitochondrial haplotypic data and microsatellite genotypic data from roughly 300 samples in BC and neighboring regions, we assess how geographical isolation, anthropogenic disturbance, and glaciation history have shaped the genetic structure of peripheral American badger populations in this region. We discovered that the genetic structure of BC American badger populations epitomizes expectations for peripheral populations, with low levels of genetic diversity, significant differentiation, and population genetic structure all intensifying with an increase in marginality. We find evidence that these patterns, which vastly contrast those observed in central populations, are likely being influenced by geographical and anthropogenic features, that were both significantly correlated with genetic distance between individuals in western BC. Roadways were identified as potential barriers to gene flow across various scales and analyses. We also provide evidence for several glacial refugia impacting population genetic structure across the American badger range, two of which may have existed in British Columbia and the greater Pacific Northwest. Taken together, our study suggests that American badgers in British Columbia exemplify dynamics of peripheral populations, with genetic variation shaped by a unique glacial history and atypical landscape matrix, vastly contrasting central populations. Mitigating the impact of anthropogenic barriers as well as increasing connectivity between populations in BC and with populations in the United States will be essential for conserving the distinct genetic diversity of this endangered species.

Preface

Multiple individuals have contributed to Chapters 2 and 3 of this thesis. Manuscript versions of these chapters will be co-authored when submitted for publication. The co-authors and I share study design and conception of this research. I have been primarily responsible for cataloguing samples, data collection, data analysis, and manuscript preparation.

I collected a large proportion of the samples and all of the data used in the manuscript, except for some ancient DNA sequencing that was collected in the lab of Dr. Jennifer Leonard at the Estación Biológica de Doñana in Seville, Spain. Dr. Michael Russello provided guidance in data collection and analyses, and helped with preparation of the manuscript. Rich Weir collected a large proportion of the samples, aided in study design, and helped in manuscript preparation. Karl Larsen aided in study design, sample collection, and manuscript preparation. Jeff Lewis collected the majority of samples from Washington State. Helen Davis, Adison Fosbery, and Dixon Terbasket assisted in sample collection in British Columbia. The 'we' pronoun is used throughout the thesis to reflect the highly collaborative nature of this work.

The data presented in this thesis were collected from American badgers sampled according to the animal care protocol of the University of British Columbia Research Ethics Board (Animal Care Certificate # A15-0158) and a sampling permit (# SM10-66091) issued by the Ministry of Environment of British Columbia.

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Chapter 1 Introduction

1.1   Defaunation, Conservation, and Genetics

We are undoubtedly in a time of significant biodiversity loss. In the history of Earth there have been five known mass extinctions, and it is argued that we are currently in the sixth (Barnosky *et al.* 2011). While the first five extinctions were attributed to natural disasters, such as a meteor impact and volcanism (Barnosky *et al.* 2011), the sixth extinction is a product of anthropogenic disturbance. More than 322 vertebrate species have gone extinct in the past 600 years and 16 to 33 percent of the remaining vertebrate species are threatened or endangered (IUCN 2013), and even greater deficits are faced by invertebrates, plants and microbes (Dirzo *et al.* 2014). The main drivers of this massive biodiversity loss are habitat destruction, overexploitation, invasive species, and climate disturbance (Hoffmann *et al.* 2010), all associated with humans dominating the landscape. This drastic decrease in biodiversity caused by humans, termed "Anthropocene defaunation" (Dirzo *et al.* 2014), has serious consequences for the proper functioning of ecosystems and has negative feedback loops on human wellbeing. There has never been a more important time to understand the status of and threats to biodiversity.

Conservation biology is a field of science that aims to prevent biodiversity loss and mitigate the cascading effects on ecosystem goods and services. It is a field that integrates a variety of disciplines including ethics, philosophy, economics, population biology, and genetics, to address problems that not only threaten natural species, but humans as well. It was aptly named a crisis-driven discipline (Soulé 1985) upon first recognition, because of the fast decision making that was employed to solve pressing issues of biodiversity loss. Biodiversity is composed of three fundamental levels that conservation biologists strive to preserve: genetic diversity, species diversity, and ecosystem diversity (McNeely *et al.* 1990). Genetic diversity provides a variety of phenotypes, or traits, for natural selection to act upon. Therefore, maintaining a diverse genetic repertoire allows a species to persist in changing environmental conditions. Species diversity, in terms of both richness and evenness, is essential for a proper functioning ecosystem (Dirzo *et al.* 2014). Lastly, maintaining diversity at one of the highest levels of organization, ecosystems, provides the resources needed for the myriad of species inhabiting the globe.

Recent technological and intellectual advances have provided different perspectives on how to address conservation issues. Instead of making decisions in haste, conservation biologists may now provide more conclusive management strategies supported by stronger evidence. One subdiscipline in particular, conservation genetics, has contributed greatly to the field of conservation biology. Conservation genetics applies genetic techniques in an effort to preserve species with the ability to cope with environmental change (Frankham 1995). Broadly, conservation genetics is the study of genetic diversity, the most fundamental level of biodiversity. Genetic diversity is a prerequisite for the variation that natural selection acts upon and it is a crucial component to the fitness of individuals (Reed & Frankham 2003). Low genetic diversity has led to the extirpation of populations (Markert *et al.* 2010), as depicted in a meta-analysis of different bird species, where populations with lower levels of genetic diversity were more likely to be extirpated than populations with higher levels of diversity (Evans & Sheldon 2008). While genetic tools and their continual development have facilitated the conservation of a plethora of species, there are still many species with limited genetic information.

1.2   Conservation Units

One of the most difficult components of measuring genetic diversity of a species is first defining a unit to measure. Almost all species exist as multiple entities, functioning either independently or partially connected to other entities. Due to the uncertainty that often accompanies the definition of a species, it is often difficult for conservationists to determine a unit to conserve. For instance, one of the most widely-used definitions of a species, the biological species concept (BSC), stresses the importance of reproductive isolation and isolation mechanisms (Mayr 1969). However, the problem with this definition is that it does not account for asexual species and faces difficulties when dealing with introgression between distinct organisms. On the contrary, the other main species definition, the phylogenetic species concept (PSC) (Nixon & Wheeler 1990), depends largely on monophyly, where all members of a species share a single common ancestor, or the presence of characters that are fixed and different (diagnosis sensu Cracraft 1983). While this definition better suits the existence of asexual organisms, as with the BSC, hybridization still remains problematic (Frankham *et al.* 2012; but see Russello & Amato 2014).

To avoid prioritization of certain taxonomic groups, which can be hard to delineate and unequal across different species, conservation biologists have shifted their focus to other units for management. One of the first frameworks put forth was evolutionarily significant units (ESUs), defined as a population or group of populations that warrant individual management or conservation due to their distinctiveness (Ryder 1986). The definition of ESUs has been modified and appended following its original designation (Moritz 1994; Waples 1991), but continues to focus on reproductive isolation, adaptive differentiation, and agreement across multiple levels of distinction (genetic, morphological, behavioral). Many legislations, including the Endangered Species Act in the United States and the Species at Risk Act in Canada, adopted the general framework of managing units below the species-level, creating their own units for conservation, including distinct population segments (DPSs), and designatable units (DUs), respectively (Green 2005). While the guidelines for designating such units remains different and challenging to implement for each country, both are largely focused on maintaining populations with ecological and evolutionary distinctiveness.

Defining units for conservation may be simple for species that are naturally patchy and display clearly defined populations, however the designation for species that exist sporadically, in low numbers, is more difficult. For these instances, using genetics can be extremely insightful. A seemingly continuous population of cardinal fish (*Ostorhinchus doederlenini*), for example, was shown to have significant genetic structure, owing to strong and persistent larval homing (Gerlach *et al.* 2007). In addition to behavioral tendencies, other factors such as environmental variables, historical occurrences, and life history strategies can lead to discrete subpopulations in species (Balloux & Lugon-Moulin 2002). Eliciting the "population genetic structure" of species is often how conservation geneticists can define units for prioritization.

One of the most common methods of determining genetic structure is defining the genetic similarity between groups of individuals. Without any *a priori* identification of populations, individuals can be clustered together based on genetic information using Bayesian methods. The most common Bayesian method is implemented in the program STRUCTURE (Pritchard *et al.* 2000), which calculates the likelihood that individuals can be divided into a certain number of clusters (K), given allele frequencies at a maximum of

Hardy-Weinberg equilibrium (Hardy 1908) and linkage equilibrium. Most Bayesian methods are individual-based, which provides the advantage of not needing clearly defined populations for analyses. Furthermore, the program STRUCTURE does not require any *a priori* definition of location relative to other individuals in the data set, so defining clusters is based solely on genetic similarity.

Once units or clusters have been defined one can then measure the levels of genetic diversity and differentiation for each unit. Genetic diversity is affected by a number of evolutionary processes including mutation, migration, genetic drift, natural selection, non-random mating, population size, and gene flow (Hardy 1908). All of these processes work in different ways to either increase or decrease genetic diversity. The null hypothesis in population genetics to test for the effect of these processes is Hardy-Weinberg Equilibrium (Hardy 1908). From this null model, one can calculate departures of allele frequencies from panmictic (i.e. randomly breeding) expectations, and can then ascertain levels of observed genetic diversity.

1.3   Landscape Genetics

Characterizing genetic diversity only provides details about the "status" of a population. However, the ideal goal behind studies measuring genetic diversity is to provide actionable evidence for effective management of endangered populations. Landscape genetics is an emerging field in biology that incorporates spatial statistics, population genetics, and landscape ecology to quantify how the landscape affects evolutionary processes such as genetic variation and gene flow (Manel *et al.* 2003). In other words, by identifying anthropogenic structures that impede connectivity, conservationists may work to mitigate the resistance these structures cause, to increase movement, and therefore increase gene flow, genetic variation, and the subsequent likelihood of survival.

One of the most promising applications of landscape genetics is the identification of barriers to gene flow. A review by Storfer *et al.* (2010) found that 35% of all landscape genetics studies aimed to detect genetic barriers among groups of individuals. From a conservation perspective, identifying barriers is significant because it may determine the cause of population structure and genetic isolation. Barriers may include natural features such as rivers and unsuitable habitat (Côté *et al.* 2012; Rittenhouse & Semlitsch 2006), or

anthropogenic landscape features such as highways and human development (Cushman & Lewis 2010; Frantz *et al.* 2012). Primary approaches used to determine barriers are Bayesian clustering methods, as described above, edge detection methods, and resistance surface modeling. Programs such as Geneland (Guillot *et al.* 2005) and TESS (Durand *et al.* 2009) build on the Bayesian clustering methods implemented in STRUCTURE by incorporating the geographic location of each individual, and then use tessellation to create a landscape of polygons to identify drastic changes in genetic patterns. Resistance surface models are spatial layers where each grid cell represents a value of resistance, or conversely, connectivity, between populations or individuals. Resistance surfaces are parameterized with environmental variables chosen *a priori* to inhibit movement, and then translated into measures of connectivity using least cost path or circuit theory analyses (Spear *et al.* 2010).

1.4   Phylogeography

While studies in landscape genetics have highlighted the impact of contemporary landscape features on population genetic structure, the role of historical factors in shaping genetic patterns must also be accounted for. Glaciation, in particular, has played a significant role in shaping genetic variation of contemporary populations. Since the beginning of the Quaternary period, 2.4 million years ago, Earth has gone through major climactic fluctuations, with a series of major ice ages forming large glaciers that expanded and contracted across the globe. These glaciation cycles led to drastic changes in the distribution and diversity of species. Some species went entirely extinct, others resided in glacial refugia, where environmental conditions were hospitable during glacial maximums (Holderegger & Thiel-Egenter 2009). The drastic change in distribution caused by glaciation, and the recolonization that occurred following glacial retreat, has led to characteristic patterns of genetic variation for contemporary populations, including higher levels of genetic variation in areas where refugia were located, distinct genetic variation for populations that resided in separate refugia, and reduced genetic diversity for populations that recolonized areas once covered by glaciers (Hewitt 2000; Hewitt 1996; Hewitt 2004). To survey the impact of glaciation on genetic variation, many scientists have relied on studies of phylogeography-establishing associations between phylogenetic patterns and the geographic distribution of taxa (Avise 2000). Connecting the evolutionary history of taxonomic lineages with their

current geographical locations provides a better understanding of how populations are related in respect to deep evolutionary history. The most widely used molecular markers to assess phylogeographic history are organelle markers (Beheregaray 2008). Mitochondrial markers have dominated phylogeographic studies due to the ease of working with a much smaller genome size compared to the nuclear genome, relatively high mutation rates, which allow for the detection of genetic lineages between populations, and the lack of recombination, which allows individual lineages to be easily tracked back in time and space (Wan *et al.* 2004). The amount of sequence divergence, or how different two sequences are, provides estimates of how related two populations are. Those populations that have been geographically isolated for longer periods of time are more likely to acquire unique mutations, thus contributing to greater differentiation.

On the surface, the impact of historical factors on genetic variation appears to have little value for informing management. However, characterizing the deep evolutionary relationships between populations can directly inform conservation prioritization. By identifying populations with unique evolutionary histories, conservation resources can be appropriately allocated to conserve the most diverse complement of genetic variation.

1.5 American Badgers in British Columbia

One species that requires a thorough genetic assessment is the American badger of British Columbia (*Taxidea taxus jeffersonii*). American badgers (*Taxidea taxus*) are semifossorial mustelids located throughout much of central and western North America. Currently, there are four recognized subspecies, three of which are found in Canada: *T. t. jacksoni*, of Ontario, *T. t. taxus*, of the Prairie provinces, and *T. t. jeffersonii*, of British Columbia (BC) (Appendix A; Long 1972). The *jeffersonii* badgers in British Columbia lie at the northwestern periphery of the subspecies' range that extends into the United States' Great Basin region (Newhouse & Kinley 2000). While populations in the U.S. seem to be stable, the status of badgers in British Columbia is much more concerning. The subspecies is on the Red List of British Columbia and is currently managed as two designatable units within BC, both of which are designated as "endangered" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2012).

Badgers have strict habitat requirements, mainly residing in grassland or shrubland ecosystems where they can burrow in silty soil and catch their fossorial prey (Apps *et al.* 2002). Given these preferences, badgers largely reside in the dry, southern interior of British Columbia, the same area most preferred for human development. Human residence has drastically increased in this region (Cohen & Neale 2006) causing increased traffic rates and reducing the amount of suitable badger habitat. Both road mortalities and habitat degradation from housing development are cited as the predominant threats to the subspecies (COSEWIC 2012). In addition to direct mortality decreasing population size, human disturbance may have limited the movement of badgers throughout their range, thereby reducing gene flow and leading to unknown consequences for the maintenance and distribution of genetic variation in regional populations.

Recent status assessment of the endangered *jeffersonii* badger suggests very different dynamics within each designatable unit (COSEWIC 2012). While the eastern DU seems stable in population size, possibly due to connection with Montana and the Idaho Panhandle, the status of the western DU is much more concerning, with local-population declines occurring throughout the region (COSEWIC 2012). Coarse genetic evidence also suggests a greater threat to the western DU. Observed heterozygosity in the western unit was only 0.667 compared to the eastern unit's observed heterozygosity of 0.822 (Kyle *et al.* 2004). Furthermore, there were only three mitochondrial haplotypes present in the western DU compared to the five haplotypes present in the eastern DU (Ethier *et al.* 2012).

There are many factors that could account for low levels of genetic variation. One of the distinguishing characteristics of BC badger populations, and many faunal populations of BC, is that populations lie at the periphery of their species' range. Put simply, peripheral populations tend to have lower genetic variation and higher differentiation due to small population size, isolation, and increase genetic drift (Soule 1973). Deep phylogeographic structure remains uncertain for BC badger populations (Ethier *et al.* 2012; Kierepka & Latch 2016b), however given their location relative to the entire species' range, low genetic variation may be a result of post-glacial expansion. On the contrary, low genetic variation could also be a result of recent human disturbance. Human development may be fragmenting badger populations, preventing movement of individuals throughout their range, thereby increasing the likelihood of inbreeding and decreasing genetic diversity. If human

interference is pinpointed as the cause of reduced genetic diversity, strategies may be implemented to promote connectivity and reduce human impact on the badgers at risk.

1.6   Study Objectives

The fine-scale genetic structure, distinctiveness of sampling units, and the factors contributing to the genetic structure of peripheral American badger populations all constitute knowledge gaps hindering effective management. To minimize these knowledge gaps, this thesis seeks to accomplish three main objectives, that will be addressed in the following two chapters:

1. To characterize the extent and distribution of genetic variation in British Columbia and the species' greater northwest distribution, to identify whether populations exhibit genetic patterns consistent with expectations for peripheral populations and how these patterns coincide with current management units.

2. To identify potential barriers to gene flow for American badger populations at the periphery to infer the relative role of contemporary landscape composition on genetic structure.

3. To examine the impact of glacial history on population genetic variation of American badger populations across the species' range.

Chapter 2 Patterns of genetic variation at the northwestern periphery markedly contrast those at the core in the American badger

2.1   Background

Patterns of variation across species' ranges have long been of interest, both for understanding geographical range limits and the conservation of peripheral populations (Eckert *et al.* 2008). Theory predicts that abundance is greatest for species at the center of their range, and gradually decreases towards range edges, where environmental conditions approach physiological limitations (i.e. abundant centre model; Brown 1984). Likewise, population genetic theory predicts reduced genetic diversity and increased differentiation for peripheral populations, owing to smaller effective population size and limited gene flow (i.e. the central-marginal hypothesis; Brussard 1984; Carson 1959; Eckert *et al.* 2008). While evidence for the abundant centre model appears weak (Sagarin *et al.* 2006), the central-marginal theory holds true across many systems; lower expected heterozygosity and greater differentiation for peripheral populations has been found in over 75% of empirical studies (Eckert *et al.* 2008). Nevertheless, there are exceptions to this generalization. Some species exhibit no associations between genetic variation and spatial distribution (Brussard 1984; Garner *et al.* 2004), and some even exhibit higher genetic diversity in peripheral populations (Safriel *et al.* 1994; Zigouris *et al.* 2012).

Many studies have attributed low genetic diversity in peripheral populations to isolation and genetic drift. For instance, a study examining population density and temporal variation in six bird species found that genetic drift could be 2 to 30 times greater in peripheral populations, which was suggested to be a significant factor explaining their low genetic diversity (Vucetich & Waite 2003). However, the ability to disentangle the effects of small population size and drift from historical effects remains challenging (Guo 2012). Founder events following glacial retreat have led to a pattern of decreased genetic diversity towards northern latitudes in many temperate species (Hewitt 2000), a pattern that could be brought about by isolation alone. Connecting pattern to process becomes even more complex when considering the context of the intervening landscape. For instance, Dudaniec *et al.* (2012) found that gene flow in peripheral populations was primarily limited by local

topography, whereas gene flow in central populations was primarily limited by broad-scale variables such as climate.

Nevertheless, characterizing genetic patterns of peripheral populations, and the processes generating such patterns, is critical for assessing conservation priority. Peripheral populations were originally considered to be of low conservation value, because low genetic diversity often limits adaptive capacity, and conserving populations in atypical, suboptimal habitats may be ripe for failure (Carson 1959). However, the points arguing against the conservation of peripheral populations also bolster the argument for their protection. Subject to extreme environmental conditions, peripheral populations are expected to acquire unique genetic and phenotypic variation, owing to strong divergent selection (Lesica & Allendorf 1995). In fact, range margins are suggested to be hotspots of speciation (Carson 1959; Mayr 1954; Simpson 1944), and the loss of genetically distinct populations has been equated to the loss of species (Ehrlich 1988).

Assigning conservation status to peripheral populations becomes particularly difficult when species are abundant across much of their range, but are rare across a political boundary. The Ord's kangaroo rat (*Dipodomys ordii*), for example, is found throughout the Great Plains and Great Basin in the United States (US), but only persists in small, marginal populations in Alberta and Saskatchewan in Canada. Consequently, the species is not listed under the US Endangered Species Act, but is listed as "endangered" under the Species at Risk Act in Canada (COSEWIC 2006). Conserving species that may be labelled as "locally rare," such as the Ord's Kangaroo rat, has been criticized as a parochial practice (Hunter & Hutchinson 1994), and numerous frameworks have been put forth to guide conservation of such populations (Bunnell *et al.* 2004; Lesica & Allendorf 1995). As such, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assigns conservation status to designatable units (DUs; Green 2005), which are subspecies or varieties below the species level that are geographically or genetically distinct (COSEWIC 2015).

One species that has recently become of conservation concern in Canada, and for which knowledge of peripheral populations is directly applicable, is the American badger (*Taxidea taxus*). The American badger is a semifossorial mustelid typically restricted to grassland-shrubsteppe habitats, where it is well-adapted for burrowing in sandy-loam soils and catching semifossorial prey. Home range size varies across the species' range, but is an

average of 97 km^2 for males and 12 km^2 for females (COSEWIC 2012), with some of the largest ranges recorded at the species' range margins where population densities are low (e.g. 301 km^2; Kinley & Newhouse 2008). Badgers are typically active throughout the warm months of spring and summer, with movement activities greatest in July- when juveniles are dispersing and males are searching for access to multiple females (Weir *et al.* 2004). During this time males may travel up to 64 kilometers in as little as three days (Klafki 2014).

Currently, there are four subspecies recognized for American badgers (Long 1972) that range from the west coast of the United States to as far east as Ohio, and from Oaxaca, Mexico, to its northern limits in British Columbia, the Prairie provinces (Alberta, Saskatchewan, and Manitoba), and southern Ontario (COSEWIC 2012). The American badger is of little concern in the United States ("Not Listed" under the US Endangered Species Act), but has elevated conservation status in Canada, with the *jeffersonii* and *jacksoni* subspecies listed as "endangered," and the *taxus* subspecies listed as "special concern," under the Species at Risk Act (COSEWIC 2012). Population estimates for endangered populations are between 205-405 for *jeffersonii* badgers in British Columbia (British Columbia Badger Recovery Team 2016) and less than 200 *jacksoni* badgers in Ontario (Environment Canada 2013), with primary threats being habitat reduction through human development, and population reduction through vehicle-induced mortalities (COSEWIC 2012).

Previous studies of *Taxidea* populations across the Canadian range found that exemplar, peripheral populations in British Columbia and Ontario were genetically impoverished, but also distinct, compared to other adjacent populations in Canada and northern US (Ethier *et al.* 2012; Rico *et al.* 2016). A broad-scale phylogeographic study of American badgers across the expansive US distribution found contrasting patterns, detecting widespread gene flow and limited structure across much of the species' core range (Kierepka & Latch 2016b). In addition to these range-wide patterns, largely attributed to historical processes of glacial expansion and retreat (Kierepka & Latch 2016b), American badgers are also sensitive to anthropogenic landscape modification, with genetic evidence demonstrating fine-scale structure primarily associated with human agricultural practices (Kierepka & Latch 2016a). Taken together, these results suggest that American badgers represent an excellent mammalian system for explicitly investigating the effects of contemporary processes on

patterns of genetic variation in central and marginal populations, with direct implications for wildlife management at the range periphery.

To this end, we conducted a spatially-explicit, landscape genetic study of American badgers across densely sampled populations at the northwestern periphery in Canada relative to core populations in central and western USA. For this sampling, we collected mitochondrial DNA haplotypic and microsatellite genotypic data to reconstruct the extent and distribution of genetic variation to directly test predictions of the central-marginal hypothesis. Using resistance surface modeling, we infer barriers to gene flow and discuss the conservation and management implications of these results for American badgers and other at-risk species at their range periphery.

2.2   Methods

2.2.1   Sample Site

The range of badgers within BC is separated into two designatable units (DUs). The *T. t. jeffersonii* western designatable unit or WDU, extends from the United States-Canada border near Osoyoos, BC, as far north as Williams Lake, east into the Monashee Mountains and Kettle River drainage, and as far west as the Coast Mountains and Fraser River. The *T. t. jeffersonii* eastern designatable unit or EDU, spans from the United States-Canada border near Roosville, BC, to Golden, BC in the Rocky Mountain trench of British Columbia. Sightings have also occurred at the eastern limits of the province in the Elk Valley (COSEWIC 2012). Within each of these DUs are several elemental occurrences (British Columbia Badger Recovery Team 2016) from which samples were collected, which we here refer to as sampling units, including the Cariboo (CR), Thompson (TH), Nicola (NI), and Okanagan (OK) within the WDU, and the East Kootenay (EK) and Elk Valley within the eastern EDU. We based our BC sampling unit nomenclature on the populations listed in the most recent status report of American badgers in BC (COSEWIC 2012), therefore, East Kootenay and Elk Valley were considered to be a single sampling unit within the EDU. The British Columbia Ministry of Environment is assessing whether the sampling units within both DUs should be designated as distinct management units (British Columbia Badger Recovery Team 2016).

Additional samples were collected from sampling units directly connected to British Columbia in Washington (WA), Alberta (AL), Idaho (ID), and Montana (MT).

2.2.2   Sample Collection

A total of 276 samples were collected from 2001 to 2016, including 195 tissue samples from road mortalities and 81 hair samples from: CR (n=54), TH (n=27), NI (n=11), OK (n=74), EK (n=30), WA (n=68), AL (n=5), ID (n=5), and MT (n=2) (Figure 2.1). Samples from road mortalities consisted of a ~4 cm piece of tissue clipped from the right ear and placed, dry, into a paper envelope with a silica gel packet to avoid moisture collection. Hair samples were collected using non-invasive snaggers placed within the entrance of badger burrows following Klafki (2014). Hair snaggers were checked at least every 7 days, and any captured hair was placed in a dry envelope, and stored at ambient temperature.

To increase our regional sampling distribution in the greater Pacific Northwest (PNW), museum specimens were sampled from five natural history museums (Appendix B). A total of 83 claw powder samples were collected, spanning the years of 1936 to 1988 from: CR (n=1), TH (n=9), NI (n=2), OK (n=21), EK (n=6), WA (n=22), ID (n=8), and MT (n=14) (Figure 2.1, 3 samples had uncertain locality information). Samples were collected following methods described in Casas-Marce *et al.* (2010); briefly, a Dremel® bit tool (1.5-2mm diameter) instrument was used to drill powder from the base of a single foreclaw. Surfaces and tools were cleaned with bleach between collection of each sample. Powder samples were stored, dry, in 2mL centrifuge tubes until DNA extraction.

2.2.3   Data Collection

Genomic DNA was extracted from contemporary samples using the Nucleospin Tissue Kit (Macherey-Nagel) and manufacturers' protocols. Genomic DNA was extracted from historical samples using a MinElute Kit (Qiagen) and manufacturers' protocols in a dedicated facility for handling and processing museum specimens, physically isolated from the main lab where contemporary samples were processed. Extra precautions were carried out to avoid contamination between samples including the use of extraction blanks and the incorporation of multiple negative controls for all PCR reactions.

Haplotypic data were collected from a fragment of the d-loop from the mitochondrial DNA. We first attempted to use previously published primers (Ethier *et al.* 2012), but obtained non-specific amplification (data not shown). Consequently, we designed a new primer set targeting a ~600 basepair fragment at the 5' end of the d-loop (ExtF and ExtR; Appendix B) as well as three internal primer sets to amplify ~200 basepair fragments for the more degraded historical DNA samples (Appendix B). Fragments were amplified using polymerase chain reaction (PCR) in 15 µL volumes containing: 1X PCR Buffer (Applied Biosystems; 150 mM Tris-HCl, pH 8.0, 500 mM KCl), 0.2 mM dNTPs, 0.2 mg/mL bovine serum albumin, 0.67 µM of each primer, and 0.5 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems). Cycling conditions for all fragments included an initial denaturation step at 94°C for ten minutes, followed by 35 cycles at 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 minute, and then a final extension at 72°C for 10 minutes. PCR products were purified using ExoSAP-IT (Affymetrix, Cleveland, OH, USA) and sequenced using BigDye v3.1 Terminator chemistry on an Applied Biosystems 3130XL DNA automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were visualized and edited using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA).

Genotypic data were collected at sixteen microsatellite loci developed for the American badger (Tt-1, Tt-2, Tt-3, Tt-4, Davis & Strobeck 1998; Tt13, Tt15, Tt20, Tt21, Tt27, Rico *et al.* 2014), American marten (Ma-1, Ma-15, Davis & Strobeck 1998), American mink (Mvis072, Fleming *et al.* 1999; Mvis87, O'Connell *et al.* 1996), and wolverine (Gg234, Duffy *et al.* 1998; Gg443, Gg465, Walker *et al.* 2001). All forward primers were 5'-tailed with an M13 sequence [5'-TCCCAGTCACGA-CGT -3'] to facilitate automated genotyping. Specifically, the M13-tailed forward primer was used in combination with a M13 primer of the same sequence that was 5'-labeled with one of four fluorescent dyes (6-FAM, VIC, NED, PET), effectively incorporating the fluorescent label into the resulting PCR amplicon (Schuelke 2000). In addition, reverse primers were modified following Brownstein *et al.* (1996) to improve genotyping. Microsatellite loci were amplified in 12.5 µL PCR reactions containing: 1X PCR Buffer (Applied Biosystems; 150 mM Tris-HCl, pH 8.0, 500 mM KCl), 0.2mM dNTPs, 0.24 mg/mL BSA, 0.4 µM of the M13 fluorescent dye-labeled and reverse primers, 0.04 µM of the forward primer, and 0.5 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems). Cycling conditions used a 'touchdown' protocol that included an

initial denaturation step at 95°C for ten minutes, followed by 35 cycles of 95°C for 30 seconds, annealing for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for ten minutes. The annealing step in the 'touchdown' program decreased by 1°C each cycle from 54°C to 45°C for the first ten cycles, after which it was held at 45°C. Amplified loci were co-loaded and run on an Applied Biosystems 3130XL DNA automated sequencer. Alleles were manually scored using GeneMapper 4.0 (Applied Biosystems, Inc.).

2.2.4   Haplotypic Variation and Population Differentiation

All sequences generated in this study were aligned with previously published mtDNA d-loop sequences from neighboring provinces/states in Alberta (n = 46), Washington (n = 1), Idaho (n = 20), and Montana (n = 67) (Ethier *et al.* 2012; Kierepka & Latch 2016b). As the d-loop fragments did not completely overlap between studies, all sequences were trimmed at the 5' end to align to the range-wide data collected in Kierepkaand Latch (2016b), and 3' terminal gaps were coded as missing data. Initial sequencing efforts discovered a discrete 25 basepair insertion or deletion (indel) in a highly polymorphic region of newly acquired sequences (n=2), as was found previously in Kierepkaand Latch (2016b). Because this indel was the same size across all individuals within which it was found, we perceived this to be a single insertion or deletion event. To retain polymorphic sites in sequences without the indel and to code the indel as a single polymorphism, the 25 basepair region was shortened to only polymorphic sites (five total) for all sequences and sequences with the indel were coded as a single base gap, with the remaining four sites coded as missing data, following Kierepkaand Latch (2016b). Sequences were aligned using default parameters in MUSCLE (Edgar 2004), as implemented in GENEIOUS 10.1.2 (Kearse *et al.* 2012).

To investigate the extent of haplotypic variation in northwestern American badger units, a haplotype network was constructed using statistical parsimony, as implemented in TCS (Clement *et al.* 2000), and visualized using tcsBU (Múrias dos Santos *et al.* 2016).

Estimates of haplotype richness, haplotype diversity (H_d), nucleotide diversity (π), and pairwise difference were calculated for each sampling unit in Arlequin 3.5 (Excoffier *et al.* 2005). To identify whether haplotype diversity decreased as the peripherality of units increased, we used a least-squares linear regression, implemented in the R statistical computing environment (R Core Team 2016). The absolute value of latitude times longitude

was used as a proxy for "peripherality" for each unit, where the most northwestern units were considered the most peripheral, based on the species' entire geographic distribution.

An analysis of molecular variance (AMOVA) was implemented in Arlequin 3.5 (Excoffier *et al.* 2005) for sampling units in British Columbia, using 1000 permutations. Three separate hierarchical groupings were used to determine the configuration that best explained the genetic variation between units: 1) the current management configuration of two designatable units, 2) a configuration of three distinct units, where Cariboo was considered to be its own distinct unit in addition to the current two DUs, and 3) a configuration of five distinct units, where each sampling unit within BC was considered to be its own distinct unit. Measures of population pairwise F_{ST} were conducted in Arlequin 3.5 (Excoffier *et al.* 2005) with significance assessed using 3000 permutations.

2.2.5   Genotypic Variation and Population Differentiation

Genotypic data were examined for evidence of genotyping errors and null alleles using MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004). Deviations from Hardy Weinberg Equilibrium (HWE) and linkage equilibrium were calculated for each locus in each unit using GENEPOP (Rousset 2008), accounting for multiple comparisons using a sequential Bonferroni correction (Rice 1989). Measures of genetic diversity including observed (H_O) and expected heterozygosity (H_E) were calculated in Arlequin 3.5 (Excoffier *et al.* 2005). The program HP-Rare (Kalinowski 2005) was used to measure allelic richness for sampling units and a list of private alleles was obtained using GenAlEx 6 (Peakall & Smouse 2006). To determine whether a relationship existed between genotypic diversity and peripherality, we conducted a least-squares linear regression of allelic diversity on the degree of peripherality, calculated as described above, in the R statistical computing environment (R Core Team 2016). Inbreeding coefficients with 95% confidence intervals were calculated using Genetix 4.05 (Belkhir *et al.* 1996).

Measures of pairwise genetic differentiation (θ) between sampling units were calculated using the program FSTAT (Goudet 1995), with significance assessed after 10,000 permutations. The presence of discrete genetic units was assessed for contemporary badger samples using two approaches. First, we used a spatially explicit method implemented in TESS 2.3 (Durand *et al.* 2009), which incorporates the geographic location of each sample

into a Bayesian admixture model. The analysis was run for 80,000 sweeps, after a burn-in of 10,000, for K=1 to K=12. Each run was repeated for 10 iterations, and variation across iterations was summarized using CLUMPP v. 1.1.2 (Jakobsson & Rosenberg 2007) and visualized using DISTRUCT v. 1.1 (Rosenberg 2004). Optimal population structure was determined using the Deviance Information Criterion (DIC; Spiegelhalter *et al.* 2002). Deviance Information Criterion values were averaged across the 10 iterations, and the optimal model was chosen based on a plateau in ΔDIC values.

In addition, we used an aspatial Bayesian clustering method implemented in STRUCTURE 2.3.4 (Pritchard *et al.* 2000). The program was set to run for 500,000 MCMC iterations, following a burn-in of 200,000 iterations, for K=1 to K= 12, with a standard admixture model, assuming correlated allele frequencies. Each run was repeated for 12 iterations and the programs CLUMPP v. 1.1.2 (Jakobsson & Rosenberg 2007) and DISTRUCT v. 1.1 (Rosenberg 2004) were used to summarize and visualize individual membership coefficients, respectively. Both the ΔK (Evanno *et al.* 2005) and maximum likelihood (Pritchard *et al.* 2000) methods, implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012), were used to determine the optimal value of K.

To test for contemporary genetic structure, we conducted an AMOVA using the genotypic information for all contemporary samples in British Columbia. The AMOVA was implemented in Arlequin 3.5 (Excoffier *et al.* 2005), using 1000 permutations, under the same hierarchical groupings as described for the mitochondrial sequencing analysis.

Measures of bidirectional migration were calculated using a Bayesian inference of recent migration, implemented in BayesAss 1.3 (Wilson & Rannala 2003). Migration rates were tested using the cluster configurations inferred from the TESS analysis, using 10,000,000 MCMC replicates after a burn-in of 1,000,000 replicates, sampling every 100 iterations. Five separate analyses were run and trace files were used to confirm convergence.

2.2.6   Resistance Surface Modeling

To identify potential barriers to gene flow, we examined the relationship between BC landscape resistance and individual-based genetic differentiation estimates (i.e. genetic distance). To do so, we created resistance surfaces using six environmental variables, chosen based on previous studies of American badger connectivity and habitat association (Apps *et*

al. 2002; Kinley *et al.* 2014; Washington Wildlife Habitat Connectivity Working Group 2010) Housing density was not explicitly included, as there are no data available with high enough resolution within British Columbia; however, some of this information was implicitly included in the land use layer (i.e. "human dominated" category). In place of housing density, soil parent material was included, as this variable is a significant factor determining American badger distribution within the province (Hoodicoff *et al.* 2009). American badgers have high dispersal capabilities, with the average dispersal distance being 11 km, and maximum distances being 52 km for females and 110 km for males (COSEWIC 2012). Therefore, to maximize the amount of information retained at the finest scale, all resistance surfaces were set to a resolution of 1000 m^2.

In our analyses, elevation, crown closure, and slope were continuous variables, whereas roadways, land use, and soil parent material were categorical variables (see Appendix B for file sources). Original values were retained for resistance values of continuous variables, then parameterized using methods described below. For the land use layer, initial resistance values were set to those established by expert opinion in Washington Wildlife Habitat Connectivity Working Group (2010). For the roadway layer, each road type was buffered using the values in Appendix B, and then assigned a resistance value out of ten based on the proportion of roadkill samples collected from each roadway type. This reduced the resistance value for some heavily traveled roads in BC (i.e. freeways), but overall, more accurately represented the mortality threat posed by each roadway type. Initial resistance values for soil parent materials were assigned based on a survey of the literature (Apps *et al.* 2002; Kinley *et al.* 2014), using a simple scale from 1 to 6 (Appendix B). This scale minimized subjectivity, and relied on the transformations described below to determine the magnitude between the highest and lowest resistance values.

One of the most difficult aspects of resistance surface modeling is parameterization of resistance surfaces (Spear *et al.* 2010). Inefficiently assigning resistance values can provide inaccurate results and may lead to erroneous conclusions. We used a model optimization approach following the methods described in Epps *et al.* (2013). Briefly, each resistance surface was assigned initial values as described above and then exponentially transformed to change the magnitude of difference between the lowest and highest values of resistance for each surface (e.g. elevation^x; see Appendix B for concept). Each layer was transformed using

the exponent values 0 to 2.0, at intervals of 0.25, and measures of effective distance were calculated between pairs of individuals using least cost path analysis (LCP), implemented in the package landgenreport (Gruber & Adamack 2015) in the R statistical computing environment (R Core Team 2016). Partial Mantel tests (Smouse *et al.* 1986) were used for each of the transformed surfaces to assess the correlation between genetic distance (Bowcock *et al.* 1994) and effective distance (LCP). Partial Mantel tests measure the correlation between two distance matrices (genetic distance and effective distance), while controlling for a third distance matrix (Smouse *et al.* 1986). In this study, Euclidean distance was used as the third matrix to account for a null model of isolation by distance (Wright 1943). Optimal resistance surfaces were chosen based on the highest correlation value (Mantel's r; Mantel 1967). If an optimal surface was not evident in the first round of parameterization, surfaces were continually transformed at values greater than 2.0 until Mantel's r was maximized at a unimodal peak or until we exhausted additional transformations (power transformation= 6.0).

Conducting analyses at multiple spatial scales is highly recommended for resistance surface modeling (Anderson *et al.* 2010) and may identify unique barriers at different spatial scales. Least cost path analyses were conducted for all transformed surfaces at two spatial scales: 1) at a broad scale, between individuals across the entire distribution of badgers in the western DU (n = 119), and 2) at a fine scale, between individuals in the Thompson and Cariboo (n = 75). These two spatial scales were chosen with the direct intent to identify broad-scale barriers across the entire distribution of badgers in western BC, as well as to identify whether the same or different barriers were present between badgers in the two most peripheral sampling units. Resistance surface modeling between British Columbia and Washington was not conducted, due to the different methods used to measure and categorize environmental variables between these regions.

The use of partial Mantel tests for assessing correlations between effective distance and genetic distance has received criticism due to the inflated type I error and presence of spatial autocorrelation (Guillot & Rousset 2013). Therefore, to complement these analyses, we used a multiple regression of distance matrices (Legendre *et al.* 1994), in an information theoretic framework (Burnham & Anderson 2001), to identify the landscape variables that had the greatest impact on gene flow. MRM is a statistical method used to determine which explanatory distance matrices best explain the response distance matrix (e.g. proportion of

shared alleles), and is an improvement to partial Mantel tests by incorporating more than three distance matrices into models. All landscape variables were considered for fitting models regardless of the significance in partial Mantel tests. All distance matrices were standardized to a maximum resistance of 10 and centered around the mean prior to constructing models. The variance inflation factor (VIF) was used to test for collinearity between variables, where values greater than 10 were considered collinear. Those variables that did not show evidence of multicollinearity were used to construct alternative explanatory models. Both a null model (Dps ~ 1) and a model of isolation by distance (Dps ~ Euclidean) were included in model selection. The model with the lowest AICc value and highest model weight (w_i) was considered the best model explaining genetic distance between individuals.

2.3   Results

2.3.1   Mitochondrial DNA Haplotypic Dataset and Analyses

From the initial set of 359 historical and contemporary samples, 301 produced high quality mtDNA d-loop sequences (550 bp). Initial sequence alignments revealed an alignment ambiguity that demonstrated high sequence similarity to a presumed nuclear-mitochondrial DNA segment (NUMT) in the honey badger, *Mellivora capensis* (Rhodes 2006), including a shortened deletion region and variable-length repeats. Consequently, any sequences with variable length in the indel region (i.e. those greater than or less than the 25 bp indel) were considered NUMTs and were removed (n = 87) before conducting downstream analyses. The final haplotypic dataset, after aligning with previously published sequencing data, included 333 mtDNA d-loop sequences from contemporary (n =286) and historical (n=47) samples from the following sampling units: CR (n=26), TH (n=21), NI (n=6), OK (n=40), EK (n=34), WA (n=47), AL (n=51), ID (n=29), and MT (n=79).

We recovered a total of forty-three haplotypes across the entire Pacific Northwest, eight of which were new to our study. Two haplotypes were the most common in the region, with one that was widespread across all sampling units, but found primarily in eastern units of Alberta and Montana (Haplotype 1; Figure 2.2). Haplotype 2 was found only west of the Rocky Mountains, in Idaho, Washington, and western BC, which was the most common haplotype in the WDU (Haplotype 2; Figure 2.2). Only six haplotypes were detected in BC, four of which were previously described in Ethier *et al.* (2012), one previously described in

Kierepkaand Latch (2016b), and one unique to this study. The EDU contained one private haplotype, while the WDU contained two; one was the most common haplotype throughout the region, and the other unique to Thompson. At a finer level within the WDU, Okanagan exhibited the highest haplotypic diversity (0.631), while Cariboo exhibited the lowest (0.00), with one haplotype found across all 26 individuals (Table 2.1). Sampling units outside BC all had a greater number of haplotypes, greater haplotype and nucleotide diversity, and greater pairwise differences (Table 2.1). Values of haplotype diversity significantly decreased with an increase in the degree of peripherality (Figure 2.3).

Significant population structure was detected using an AMOVA, with > 60% of variation distributed among current DUs, and significant variation distributed among sampling units within the WDU (Appendix B). When repeating the AMOVA to consider Cariboo as a separate, distinct unit, the amount of variation explained among sampling units within the WDU reduced to 0 (Appendix B).

Significant pairwise differentiation was common among sampling units (Table 2.2). The comparisons that did not display significant differentiation were between the sampling units in the lower WDU (Thompson, Nicola, and Okanagan SUs), and between sampling units with lower sample sizes, including Nicola and others in the Pacific Northwest, and between Idaho and Montana (Table 2.2).

2.3.2 Microsatellite Genotypic Dataset and Analyses

A total of 219 contemporary samples provided multi-locus genotypes at 16 microsatellites from the following sampling units: CR (n=48), TH (n=27), NI (n=6), OK (n=38), EK (n=28), WA (n=60), AL (n=5), ID (n=5), and MT (n=2). After quality control analyses, loci Ma15 and Mv87 were removed, due to poor genotyping across the dataset and deviations from Hardy Weinberg Equilibrium. After removing these loci, only 13 out of 135 tests significantly deviated from HWE, mostly for loci within Okanagan and Thompson. These deviations almost entirely disappeared after identifying and accounting for population structure (data not shown). Twenty-six out of 945 tests were significant for linkage disequilibrium, however there were no consistent patterns across loci or units. Consequently, all downstream analyses were based on genotypic data at 14 microsatellite loci. The final dataset included 3% missing data.

Moderate to low levels of genotypic variation were observed across all sampling units in the Pacific Northwest, as evidenced by values for both allelic richness and heterozygosity (Table 2.3). Washington and East Kootenay had the highest observed heterozygosity values, while the Thompson and Nicola had the lowest. Values of allelic richness, which are corrected for sample size, were the lowest in the Cariboo, and the highest in Washington and East Kootenay. Similar to patterns for haplotypic variation, values of allelic richness significantly decreased with an increase in the degree of peripherality (Figure 2.4). Inbreeding coefficients were significantly greater than zero for all sampling units, except the Cariboo and Nicola (Table 2.3). Significant variation was distributed among DUs and among sampling units within the WDU (Appendix B). Significant pairwise differentiation was common between sampling units (Table 2.4). The comparisons that did not display significant differentiation were between Nicola and Cariboo and Nicola and Thompson.

The spatially explicit analysis conducted in TESS revealed an optimal number of genetic clusters at K=4 (Appendix B). TESS results display a distinct separation of the Cariboo and eastern sampling units (Figure 2.5), a clustering of lower WDU individuals with northern Washington individuals, and a separate genetic cluster in southern Washington (Figure 2.6).

STRUCTURE results revealed a slightly different configuration with ΔK maximized at K=2 and, to a lesser extent, at K=5, the point at which maximum likelihood also plateaus (Appendix B). At K=2, the western DU largely separates from other badger populations throughout the Pacific Northwest, and at K=5 there is evidence of further structure separating the Cariboo into its own cluster, eastern populations into their own cluster, Washington into its own cluster, and some substructure within the lower western DU (Appendix B). The Nicola and Thompson display some unique clustering relative to the Okanagan, however, the Thompson, Nicola, and Okanagan include individuals from both genetic clusters (Appendix B).

Overall, low migration rates were detected between sampling units ($m<0.1$). Levels suggesting significant migration ($m>0.1$; Hastings 1993) were exclusively north to south in movement, from 1) northern Washington into southern Washington, and from 2) the WDU into northern Washington (Table 2.5).

2.3.3 Resistance Surface Modeling

A total of 72 least cost path analyses were conducted for broad and fine-scale resistance surfaces including: 1) 119 individuals sampled throughout the WDU in BC; and 2) 75 individuals between the Cariboo and Thompson (Table 2.3). For the broad-scale analysis, effective distances from crown closure, elevation, slope, land use, and roadway surfaces were all significantly correlated with genetic distance (Table 2.6). For the fine-scale analysis, only effective distance from roadways was significantly correlated with genetic distance (Table 2.6).

All distance matrices for the broad-scale analysis were highly correlated with each other, with variance inflation factors greater than 10 for all variables. Therefore, multiple regression of distance matrices was not conducted at the broad-scale. Conversely, variation inflation factors were only greater than 10 for slope, land use, and soil for the fine-scale analysis. Therefore, crown closure, elevation, and roadways were used to construct alternative explanatory models using MRM. A total of 16 models were compared for model selection. The top model included elevation, roadways, and Euclidean distance as significant variables explaining genetic distance between individuals in the Thompson and Cariboo (Table 2.7). The next top models were the full model, including crown closure as an additional explanatory variable, and a reduced model with crown closure, roadways, and Euclidean distance as explanatory variables (Table 2.7).

2.4 Discussion

The central-marginal hypothesis is a fundamental concept that has formed the foundation for numerous studies in population genetics, range limits, and species' distributions. While the original theory upon which the central-marginal hypothesis was based (i.e. abundant centre model) has little empirical support (Sagarin *et al.* 2006), the general trend of decreasing genetic diversity and increasing differentiation towards the periphery is commonplace across numerous study systems (Eckert *et al.* 2008). Taken together, these results suggest that the genetic patterns of peripheral populations are generated by more complex processes than population size alone. Other studies have attributed such genetic patterns to founder events following glacial retreat (Hewitt 2000) and niche limitations (Brown 1984).

Very few studies have assessed how contemporary landscape features affect the genetic variation of peripheral populations (although see Sexton et al. 2016 and Micheletti & Storfer 2017). Adverse environmental conditions are expected to increase towards the periphery (Brown 1984), limiting effective gene flow and thereby reducing genetic diversity and adaptive potential for peripheral populations. Identifying genetic barriers at a species' range edge may therefore indicate the factors inhibiting adaptation to novel environments, and thus inhibiting range expansion. For example, Sexton *et al.* (2016) found that patterns of genetic diversity in peripheral populations of monkeyflower (*Mimulus laciniatus*) were best explained by elevation, rather than population size or isolation by distance. Similarly, Michelettiand Storfer (2017) found that a decrease in limestone availability and an increase in growing season were highly correlated with resistance to gene flow in salamander populations, and that both became more common towards the periphery, acting as cryptic barriers to range expansion. Identifying such genetic barriers at the periphery not only provides a better understanding of why range limits exist, but also provides evidence for more effective conservation of peripheral populations.

2.4.1   American Badger Populations and the Central-Marginal Hypothesis

American badger units in the Pacific Northwest exhibit predicted patterns of genetic variation for peripheral populations (Eckert *et al.* 2008). Genetic diversity, both haplotypic and genotypic, decrease linearly as the marginality of populations increases, exemplified by the most peripheral sampling unit (Cariboo) possessing only a single haplotype across all 26 individuals. Our values of haplotype diversity were consistent with complementary studies for American badger populations, ranging from 0.000-0.631 for peripheral populations in BC, similar to estimates for BC DUs (0.378-0.504; Ethier *et al.* 2012), and ranging from 0.703-0.941 for central populations in Montana, Idaho and Washington, similar to estimates for central populations of the *jeffersonii and taxus* subspecies (0.928 and 0.789, respectively; Kierepka & Latch 2016b). Likewise, our genotypic diversity estimates paralleled previous reports, with observed heterozygosity ranging from 0.571 to 0.676 for units in the WDU (0.666; Kyle *et al.* 2004), and ranging from 0.72 to 0.722 for units in Washington and the EDU (0.747; Kierepka & Latch 2016b; 0.822; Kyle *et al.* 2004).

In addition to decreased genetic diversity, we also observed other genetic patterns that coincide with expectations for peripheral populations. TESS and STRUCTURE analyses indicate four or five discrete genetic clusters in the Pacific Northwest. Both programs identified individual clusters for the eastern sampling units and for Cariboo, with some discrepancies for units in the lower WDU and Washington. As spatially explicit methods typically outperform aspatial methods under such conditions (François & Durand 2010), we focus our interpretations on the TESS results. The clustering we observed in the WDU and within Washington show striking similarities with those for mountain goat populations in the same regions (Parks *et al.* 2015). Using individual-based landscape genetics in a causal modeling framework, Parks *et al.* (2015) found that the borders between mountain goat clusters significantly coincided with Highway 5 within BC and Interstate 90 within Washington (Parks *et al.* 2015). While Highway 5 also appears to be the primary feature limiting gene flow in the WDU for our study, the cause of the split within Washington is less clear, potentially due to our sampling distribution in this region. The borders of the two WA genetic clusters roughly coincide with the location of I-90, however they also coincide with the location of major rivers in the Columbia Basin. Indeed, a greater percent of genetic variation is explained among units when badgers are grouped by their location relative to rivers, rather than highways (AMOVA not shown). However, our study was not designed to specifically address genetic barriers in this region. Future modeling efforts with more targeted sampling in Washington and explicitly incorporating highways and waterways, may help to identify the exact genetic barriers in the state.

We found unique haplotypic variation within Thompson and a high frequency of a geographically distinct haplotype in the WDU (Haplotype 2). Furthermore, we found many unique haplotypes in the EDU, falling outside the two major haplotypes in the Pacific Northwest. These findings coincide with previous reports of unique haplotypic variation in the Thompson-Okanagan region (Ethier *et al.* 2012), and parallel reports of unique functional variation for American badgers in BC (Rico *et al.* 2016). We also found significant differentiation between many of the sampling units in our study area, except between the Nicola and other sampling units, although this discrepancy may be affected by small sample size. Significant differentiation in the PNW contradicts the nearly nonexistent differentiation in central and western populations of the United States (Kierepka & Latch 2016b), however

is consistent with previous reports of differentiation at the periphery (Ethier *et al.* 2012; Kyle *et al.* 2004) and consistent with general expectations of the central-marginal hypothesis (Eckert *et al.* 2008). Kierepkaand Latch (2016b) suggested that the limited genetic structure across much of the species' range was due to the high dispersal capabilities of the American badger, which overrides their preference for grassland/shrubland habitats. Badgers in British Columbia also have high dispersal capabilities, if not greater than central populations (maximum dispersal= 52 km for females and 110 km for males; COSEWIC 2012), however, their habitat size is considerably smaller and the environment between sampling units is drastically different compared to central populations. Therefore, the strength of genetic drift from small population size and limited connectivity must exceed effective gene flow for badgers at the periphery, leading to unique and differentiated genetic variation.

While many of the genetic patterns we observed for badgers in the PNW coincided with expectations for peripheral populations, there were some exceptions. Gene flow, if present, is expected to be asymmetrical- from the center to the periphery (Garcia-Ramos & Kirkpatrick 1997). On the contrary, we observed significant migration only occurring in the opposite direction, from northern, peripheral units to southern, central units. Because BayesAss (Wilson & Rannala 2003) estimates recent migration, the significant movement from north to south may represent a contemporary adaptive response by badgers unable to tolerate the unfavorable environmental conditions within British Columbia (Hardie & Hutchings 2010). Examples of southern range shifts are rare (Hickling *et al.* 2006), but most often occur in species that are habitat specialists, such as the American badger, and for species where habitat loss at the northern edge outweighs the threats posed by climate change (Chen *et al.* 2011). The southern interior of British Columbia has seen drastic changes to the landscape over the past century, likely having a strong impact on population persistence and adaptive capacity of American badgers; such impacts may need to be mitigated if ranges are forced to shift north in response to climate change.

Other mesocarnivores show similar, but also more complex patterns of genetic variation that are consistent with our results. For example, Schwartz *et al.* (2003) found that Canada Lynx (*Lynx canadensis*) populations had significantly lower genetic variation at the periphery compared to central populations, but no evidence of significant differentiation, likely due to the animal's high dispersal capabilities. Similarly, Zigouris *et al.* (2012) found

unique genetic variation for peripheral populations of wolverine (*Gulo gulo*) in eastern Canada, although genetic diversity was higher in peripheral populations, potentially due to colonization from multiple glacial refugia. Thus, while some patterns of genetic variation for mammalian peripheral populations are consistent, some are different, likely due to species-specific population history, dispersal capabilities, and habitat specialization.

2.4.2   Peripheral American Badger Units and Landscape Context

At the broad-scale between individuals in the four most peripheral American badger sampling units, we found that crown closure, elevation, slope, land use, and roadways were significantly correlated with genetic distance. However, at the fine-scale, between individuals in the two most peripheral sampling units, only roadways were significantly correlated with genetic distance, and in addition to elevation and Euclidean distance, was one of the variables in the model best explaining the genetic distance between individuals. The low, but significant correlations at the broad-scale suggests that many environmental features may act as barriers in the WDU, but that barriers are likely region-specific, where features that were not barriers to gene flow in certain locations weakened the signal for areas where they were. Furthermore, the significant correlation with roadways at the fine-scale suggests that the gene flow of badgers may be heavily impacted by anthropogenic features at their most peripheral extent. Previous fine-scale landscape genetic studies conducted for American badgers also found that anthropogenic features affect rates of gene flow between badger populations, although in the form of agriculture rather than roadways (Kierepka & Latch 2016a). While agriculture was not a major factor contributing to resistance at the fine-scale in our study, it was a category included our land-use layer, which was a layer significantly correlated with genetic distance at the broad-scale. Notably, the scale at which we conducted our 'broad-scale' analysis was smaller than that conducted for the previous 'fine-scale' analysis (Kierepka & Latch 2016a). Therefore, we too may have found evidence for the impact of agriculture on American badger gene flow. Re-running broad-scale analyses with agriculture separated from the other land use categories (e.g. urban, waterway, wetland) may help to determine its relative impact.

Roadways are clearly a major barrier to connectivity, as this variable was significantly correlated with genetic distance at both scales, is associated with the change in

allele frequencies in western BC, and was a variable in the top model explaining genetic distance. The literature is replete with examples of roadways acting as barriers to gene flow (Balkenhol & Waits 2009), although the effects vary by species (Frantz *et al.* 2012). Roadways in British Columbia do not appear to be absolute barriers, as some individuals assign to different genetic clusters on either side of major roadways. However, the effects of roads clearly have an impact on genetic variation in the province, perhaps by acting as a filter rather than a discrete barrier- where few individuals are able to cross, but those that are successful likely do not reproduce due to additional challenges (e.g. other roadways). Recent evidence suggests that the direct reduction in population size caused by roadway mortalities, rather than the barrier effect, may have a more pronounced impact on genetic structure, by exacerbating the effects of genetic drift through reduced effective population size (Jackson & Fahrig 2011). This may be the case for American badgers in BC, as movement appears to occur regularly across roads (Klafki 2014), but mortality rates are extremely high (Weir *et al.* 2004).

By identifying roadways as major barriers for American badger populations in the PNW, we offer a new perspective about how humans may impact peripheral populations and range limits. The two major highways that transect the WDU may be inhibiting gene flow that is not only essential for the genetically depauperate Cariboo, but also for the southern units in the WDU. Net gene flow from peripheral to central populations has been suggested to contribute towards species' persistence, by supplementing core genetic variation with unique genetic variation from the periphery (Hardie & Hutchings 2010). Therefore, the barrier effect of roadways in the WDU may be preventing the influx of unique adaptive variation from the Cariboo to the more southerly units (Thompson, Okanagan, and Nicola), thus limiting their adaptive capacity. By inhibiting the adaptive capacity of the peripheral units, badgers may be less likely to tolerate stochastic events, thus potentially limiting their potential for range expansion.

2.4.3   Conservation Implications

Examining the genetic variation of peripheral American badger populations can directly inform designation and prioritization of conservation units. Currently, COSEWIC uses designatable units (DUs) for status assessment (COSEWIC 2015) of species at-risk. The two

main criteria for recognizing designatable units, discreteness and significance, may be met by a number of factors, including genetic or geographic distinctiveness and unique ecological or evolutionary significance (see COSEWIC 2015 for more details). At present, the *jeffersonii* subspecies, which is only found in British Columbia in Canada, is currently managed as two designatable units- the EDU and the WDU.

Our results confirm the long-term divergence and evolutionary significance of the two DUs in BC, as evidenced by few shared haplotypes, discrete genetic clustering, and extremely high differentiation values for both molecular markers. However, our results also suggest that EDU badgers are likely not the *jeffersonii* subspecies, as they share a greater amount of genetic variation with eastern populations and are less differentiated from populations in Alberta, which are currently designated as the *taxus* subspecies. However, designating EDU badgers as the *taxus* subspecies also seems inappropriate, as significant differentiation is still evident between Alberta and East Kootenay. Thus, the EDU does not fit neatly into any of the current subspecies designations, at least based on our initial genetic evidence.

The difficulty of using subspecies designations for management has been recognized since the late 1980s (Ryder 1986). Subspecies designations rarely coincide with genetic distinctiveness, as original descriptions are often based on morphology and/or behavior. Indeed, the current designations for American badgers are based off the classification scheme presented by Long (1972), who used skeletal and pelage characteristics to group badgers into four subspecies. A recent range-wide phylogeography study suggests that genetic variation does not coincide with these designations (Kierepka & Latch 2016a). Nevertheless, acknowledging that the EDU shares more genetic variation with eastern units has important management implications, as it suggests that badgers in the WDU are more distinct than previously assumed. A heightened conservation priority should be recognized for units in the WDU to preserve the unique and highly differentiated genetic variation in this region. An extended analysis comparing the EDU to range-wide *taxus* genetic variation is also needed to better understand the relationship between badger populations in the region.

After an extensive survey of genetic variation for BC badger units, the current scheme of the single WDU in western BC also seems inappropriate. Both haplotypic and genotypic variation suggest that the Cariboo is significantly differentiated from badgers in the other

sampling units in the WDU. TESS and STRUCTURE analyses cluster Cariboo badgers into their own discrete genetic cluster, and AMOVA analyses suggest a significant amount of variation explained when the Cariboo is considered its own distinct unit. While the single haplotype that is found in the Cariboo is also found in the Thompson, Nicola, and Okanagan, the extreme fixation of this haplotype across all sequenced individuals suggests long term isolation and limited migration into the region. Distinctiveness of neutral genetic variation is not the only line of evidence to suggest that Cariboo badgers are unique compared to other units in the WDU, as badgers in this region inhabit atypical environments and have different dietary preferences (COSEWIC 2012), thus potentially conferring unique functional variation. We suggest that badgers in the Cariboo region should be recognized as their own DU, and that the unit's population trends and potential threats be reexamined for status assessment.

Our extended analysis for units throughout the PNW also afforded the opportunity to look at population dynamics and conservation implications of badgers in Washington State. As expected, levels of genetic variation were much higher in Washington compared to units in British Columbia. However, unexpectedly, we found evidence for two potentially discrete genetic clusters in the state, with potential barriers being Interstate 90 or the major rivers of the Columbia Basin. The admixture coefficients for badgers in Washington show nearly equal amounts of ancestry from both genetic clusters, but it is hard to discern whether this represents recent admixture or recent divergence. We suggest that monitoring of the species should continue in the state, and that future efforts should focus on identifying and mitigating potential barriers to gene flow in this region.
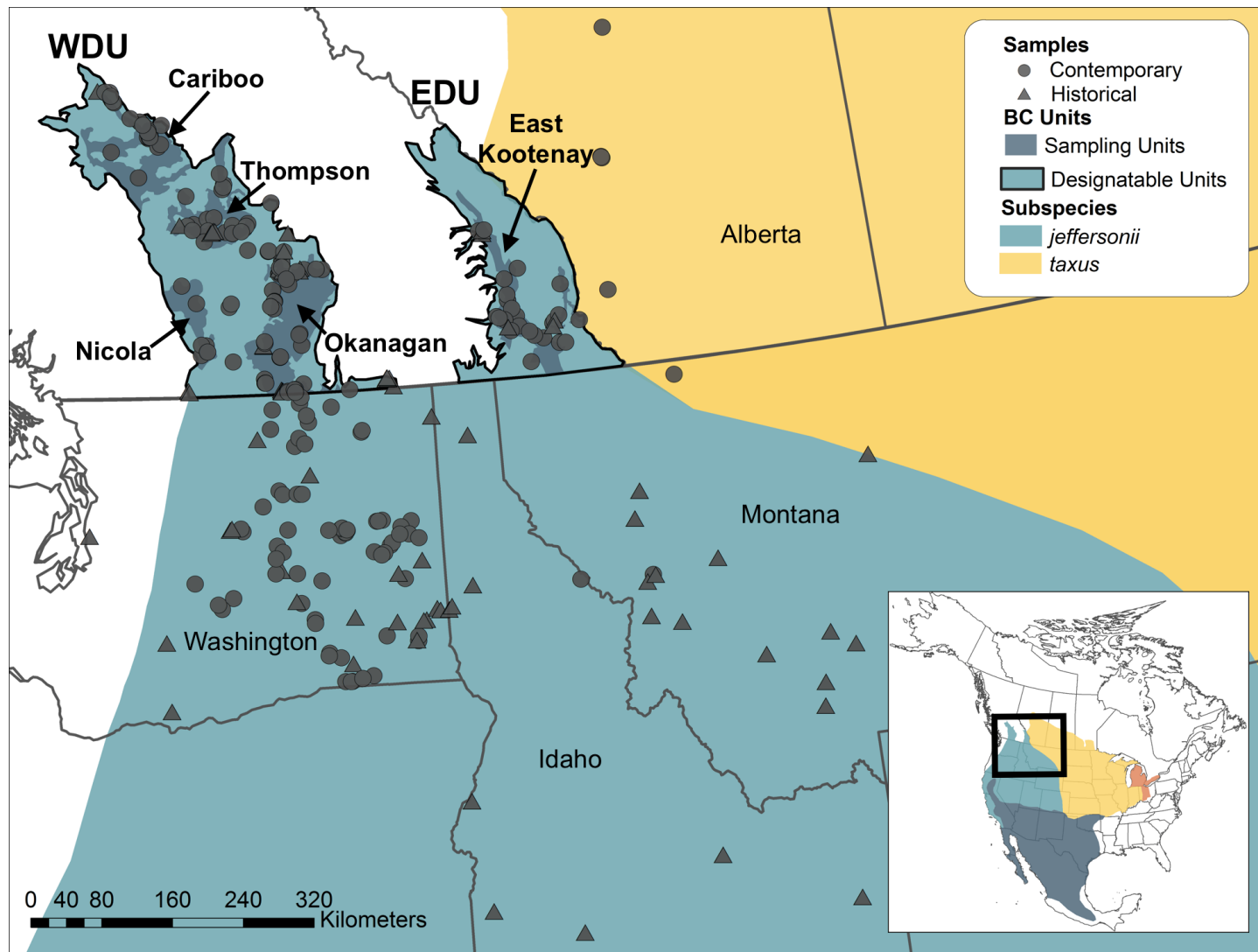
Figure 2.1 Distribution of samples used for haplotypic and genotypic analyses. Inset map displays distribution of the four currently recognized subspecies in North America (*jacksoni,* orange*; taxus,* yellow*; jeffersonii,* green*; berlandieri,* blue).
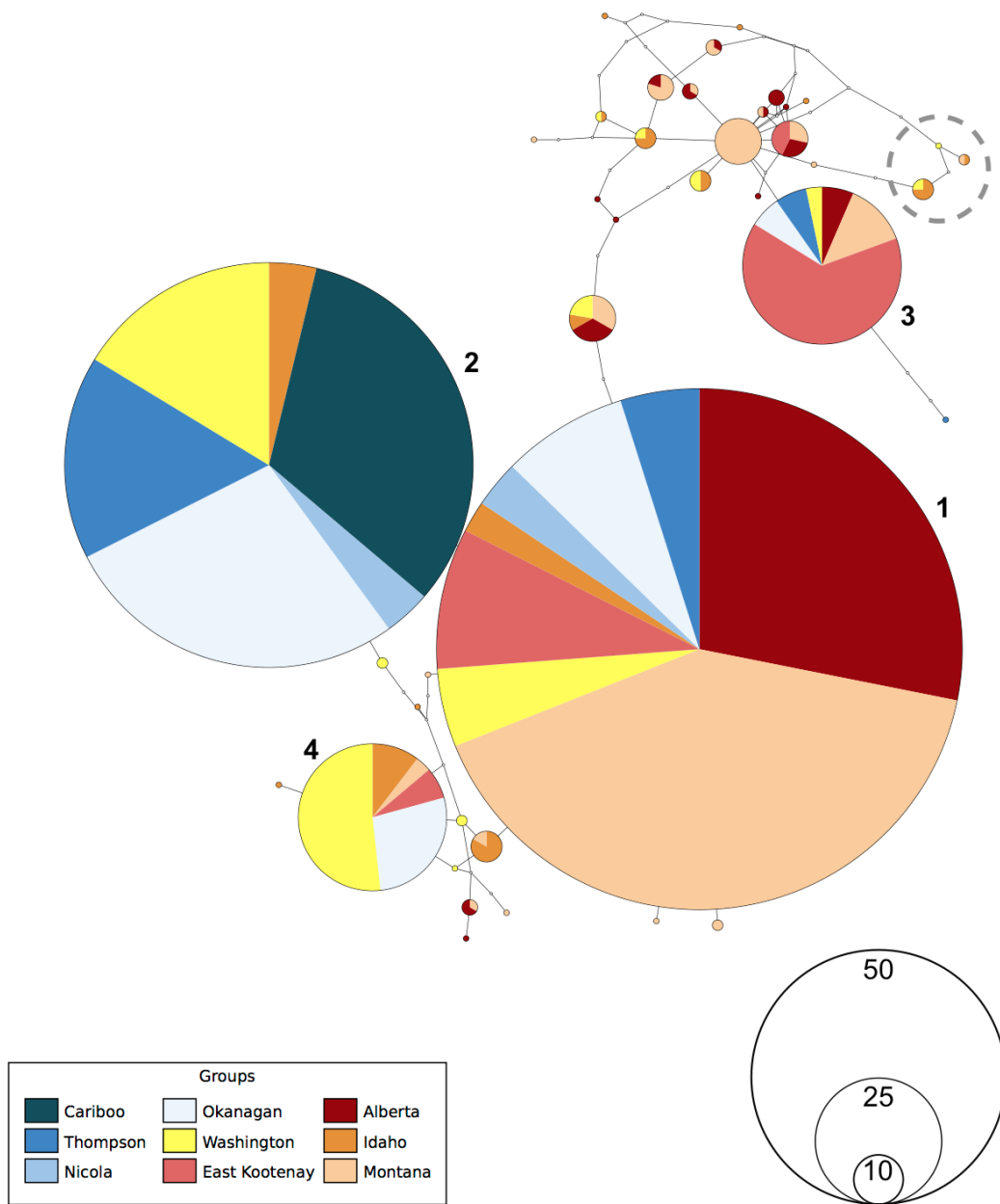
Figure 2.1 Haplotype network based on a fragment of the mtDNA d-loop from American badger sampling units in the Pacific Northwest. Each circle represents a unique haplotype, with size corresponding to the relative number of individuals with each haplotype. Colors designate the sampling units where samples were collected. Open circles represent unsampled transitions between recovered haplotypes. Major and semi-major haplotypes are numbered 1-4. Sequences with a 25 basepair deletion are highlighted by the grey dashed line.

Table 2.1 Haplotype diversity indices based on a fragment of the mtDNA d-loop from American badger sampling units in the Pacific Northwest.

	N	Number of Haplotypes	Haplotype Diversity (Hd ± SE)	Nucleotide Diversity (π ± SE)	Pairwise Difference
Cariboo	26	1	0.00 ± 0.00	0.000 ± 0.000	0.00 ± 0.00
Thompson	21	4	0.558 ± 0.101	0.005 ± 0.003	2.07 ± 1.21
Nicola	6	2	0.60 ± 0.129	0.001 ± 0.002	0.60 ± 0.55
Okanagan	40	4	0.631 ± 0.060	0.003 ± 0.002	1.37 ± 0.87
Washington	47	13	0.818 ± 0.038	0.007 ± 0.004	2.61 ± 1.42
East Kootenay	34	4	0.590 ± 0.071	0.006 ± 0.004	2.70 ± 1.47
Alberta	51	15	0.674 ± 0.074	0.007 ± 0.004	3.01 ± 1.59
Idaho	29	15	0.941 ± 0.022	0.008 ± 0.005	3.15 ± 1.68
Montana	79	19	0.703 ± 0.055	0.007 ± 0.004	3.04 ± 1.60
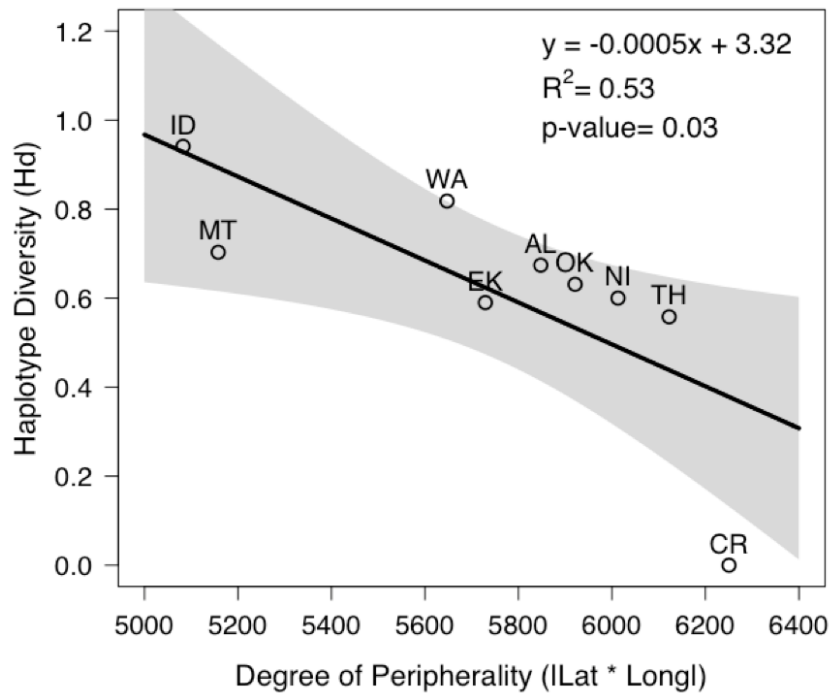
Figure 2.2 Least-squares regression of the degree of peripherality versus haplotype diversity. Grey shading depicts the 95% confidence interval.

Table 2.2 Pairwise differentiation (FST) matrix based on a fragment of the mtDNA d-loop from American badger sampling units in the Pacific Northwest.

	CR	TH	NI	OK	WA	EK	AL	ID	MT
CR		0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000
TH	0.173*		0.517	0.262	0.020	0.000	0.004	0.001	0.001
NI	0.706*	-0.038		0.623	0.271	0.000	0.036	0.120	0.074
OK	0.230*	0.010	-0.037		0.017	0.000	0.000	0.000	0.000
WA	0.204*	0.054*	0.012	0.041*		0.000	0.007	0.014	0.004
EK	0.704*	0.461*	0.529*	0.541*	0.297*		0.000	0.000	0.000
AL	0.399*	0.142*	0.141*	0.171*	0.047*	0.243*		0.002	0.676
ID	0.309*	0.165*	0.112	0.216*	0.074*	0.137*	0.093*		0.005
MT	0.316*	0.124	0.109	0.151*	0.048*	0.180*	-0.007	0.066*	

* significant at P < 0.05

Table 2.3 Genotypic diversity for sampling units within the Pacific Northwest from the analysis of 14-multilocus genotypes. Sampling units with ≤ 5 individuals were excluded from analyses.

Sampling Unit	N	A_R	H_e	H_o	P_A	F_{IS} (95% CI)
Cariboo	48	3.82	0.698	0.676	0	0.006 (-0.056-0.047)
Thompson	27	4.45	0.71	0.571	0	0.150 (0.061-0.202)
Nicola	6	4.29	0.677	0.571	0	0.170 (-0.206-0.223)
Okanagan	38	4.61	0.743	0.613	2	0.150 (0.062-0.209)
Washington	60	5.26	0.794	0.72	6	0.061 (0.010-0.095)
East Kootenay	28	5.28	0.784	0.722	8	0.060 (-0.020-0.102)

N= sample size, A_R= allelic richness, H_e= expected heterozygosity, H_o= observed heterozygosity, P_A= private alleles, F_{IS} (95% CI)= inbreeding coefficient with 95% confidence interval
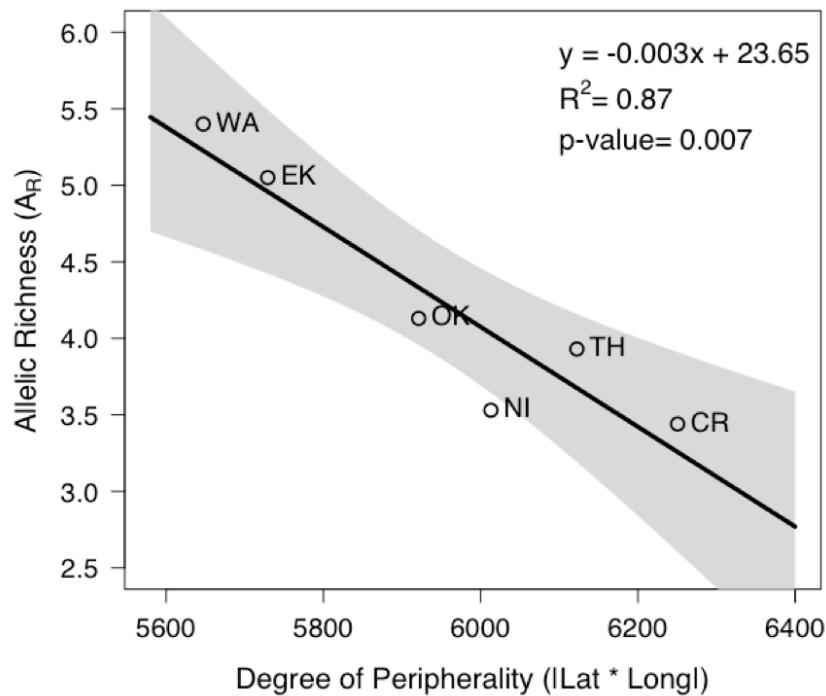
Figure 2.3 Least-squares regression of the degree of peripherality versus allelic richness. Grey shading depicts the 95% confidence interval. Sampling units ≤5 individuals were not included in the analysis.

Table 2.4 Pairwise genetic differentiation (θ) (lower) and associated p-values (upper) based on 14 microsatellite loci from American badger sampling units in the Pacific Northwest. Sampling units with ≤ 5 individuals were excluded from analyses.

	CR	TH	NI	OK	WA	EK
CR		0.001785	0.004000	0.001785	0.001785	0.001785
TH	0.049*		0.071430	0.001785	0.001785	0.001785
NI	0.072	0.027		0.001785	0.001785	0.001785
OK	0.062*	0.03*	0.042*		0.001785	0.001785
WA	0.071*	0.037*	0.042*	0.023*		0.001785
EK	0.116*	0.09*	0.082*	0.072*	0.038*	

* significant after correction for false discovery rate, P < 0.00179
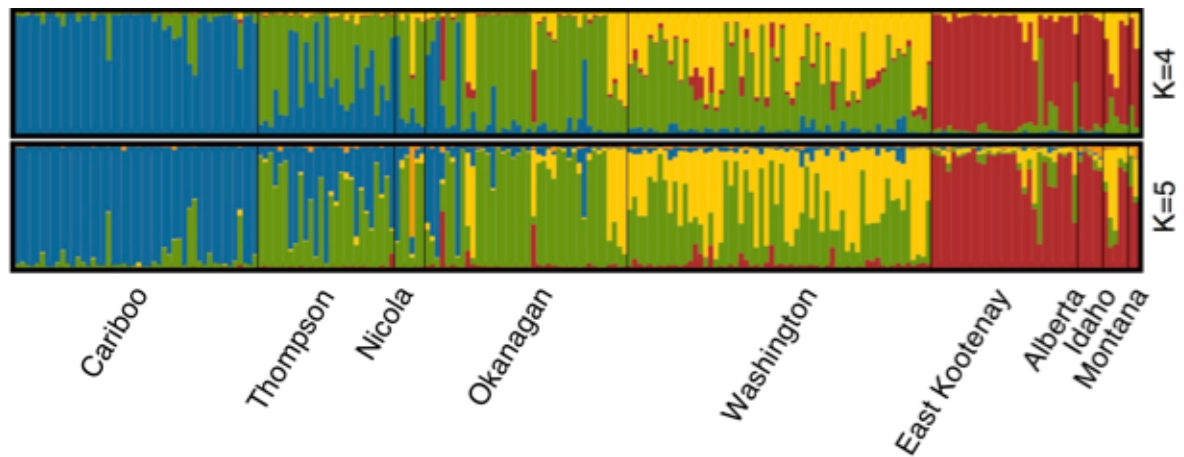
Figure 2.4 TESS barplots for individual American badgers genotyped in the Pacific Northwest, at K=4 and K=5. Color composition of each bar represents the proportion of ancestry from each of the K clusters. Individuals in each sampling unit are sorted from highest to lowest latitude (i.e. North to South).
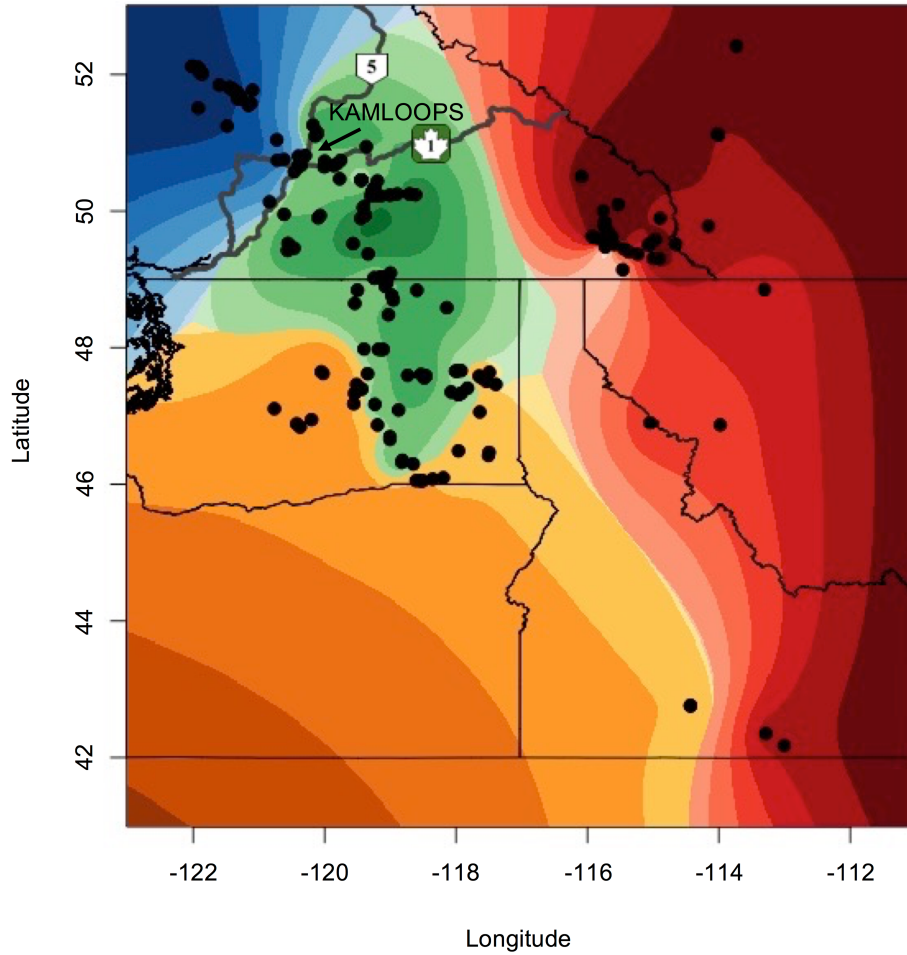
Figure 2.5 Map displaying the interpolation of ancestry coefficients, based on 14 microsatellite loci from American badger sampling units in the Pacific Northwest, from TESS configuration at K=4. Color represents the genetic cluster that individuals are assigned to, with higher proportions of ancestry displayed in darker shading. Major BC highways are depicted in grey.

Table 2.5 Magnitude of migration from units below the diagonal to units above the diagonal, based on TESS units at K=4. For example, *m* from Cariboo to WDU is 0.060. Individuals from the WDU and northern Washington were separated into two units to identify fine-scale migration patterns between these regions. Values in bold represent significant migration (*m*>0.1; Hastings 1993). *WDU= western DU, NWA= Northern Washington, SWA= Southern Washington.*

	Cariboo	WDU	NWA	SWA	Eastern
Cariboo		0.060	0.016	0.011	0.008
WDU	0.022		**0.185**	0.034	0.013
NWA	0.008	0.035		**0.267**	0.033
SWA	0.006	0.005	0.011		0.020
Eastern	0.006	0.005	0.017	0.012	

Table 2.6 Partial mantel tests for genetic distance (proportion of shared alleles, Dps) and landscape variables, when controlling for Euclidean distance. Values display correlations (Mantel's r) for the broad-scale, western DU (upper); and fine-scale, Cariboo-Thompson sampling units (lower), and associated p-values. P-values in bold are significant at $\alpha = 0.05$.

Scale	Power Transformation	Model	Mantel's r	p-value
Western DU				
	0.5	Dps ~ crown cl | Euclidean	0.1382	**0.002**
	0.5	Dps ~ elevation | Euclidean	0.1365	**0.001**
	1	Dps ~ slope | Euclidean	0.081	**0.04**
	0.25	Dps ~ land use | Euclidean	0.0742	**0.025**
	0.5	Dps ~ roads | Euclidean	0.0896	**0.011**
	1.75	Dps ~ soil | Euclidean	-0.021	0.708
Cariboo & Thompson SUs				
	2.75	Dps ~ crown cl | Euclidean	0.0435	0.263
	6	Dps ~ elevation | Euclidean	0.0658	0.075
	0.25	Dps ~ slope | Euclidean	0.0269	0.295
	0.25	Dps ~ land use | Euclidean	-0.1281	0.997
	0.5	Dps ~ roads | Euclidean	0.1198	**0.003**
	0.75	Dps ~ soil | Euclidean	0.0175	0.34

Table 2.7 The top ten models explaining genetic distance (Dps) between individuals in the Thompson and Cariboo sampling units. The best model was selected from the lowest AICc value and the largest Akaike weight (w_i).

Model	AIC	AICc	ΔAIC	w_i
Dps ~ elevation + roadways + Euclidean	-4688.22	-4688.21	0.000	0.4966
Dps ~ crown cl + elevation + roadways + Euclidean	-4686.96	-4686.94	1.269	0.2633
Dps ~ crown cl + roadways + Euclidean	-4686.09	-4686.08	2.129	0.1713
Dps ~ roadways + Euclidean	-4684.26	-4684.26	3.950	0.0689
Dps ~ elevation + Euclidean	-4655.65	-4655.64	32.565	0.0000
Dps ~ crown cl + elevation + Euclidean	-4654.13	-4654.12	34.087	0.0000
Dps ~ crown cl + Euclidean	-4649.39	-4649.38	38.821	0.0000
Dps ~ elevation + roadways	-4649.00	-4648.99	39.215	0.0000
Dps ~ crown cl + elevation + roadways	-4647.42	-4647.40	40.802	0.0000
Dps ~ Euclidean	-4646.14	-4646.14	42.065	0.0000

Chapter 3 Spatiotemporal analyses suggest the role of glacial refugia and the ice-free corridor in shaping population genetic variation in American badgers

3.1   Background

Understanding how historical processes shape the genetic variation of natural populations has long been of interest to ecologists and evolutionary biologists. Glaciation, in particular, has been extensively studied in the context of population genetic structure, with numerous studies finding concordance between patterns of genetic variation and the historical locations of ice sheets (Hewitt 2000; Hewitt 1999; Hewitt 2004). Initial studies, based on fossil evidence, suggested that natural populations survived the harsh environments imposed by glaciation by residing in glacial refugia- geographic regions hospitable for flora and fauna during ice ages, primarily south of glacial extents (Petit *et al.* 2003). Indeed, genetic data are consistent with this hypothesis for numerous species, with genetic diversity greatest at lower latitudes and decreasing in a clinal fashion towards northern latitudes, coinciding with the recolonization that occurred when glaciers receded (Hewitt 2004). This 'southerly refugia model' (Bennett *et al.* 1991) has been the longstanding framework for testing the effects of glaciation on contemporary genetic patterns.

Recently, studies of greater breadth and depth, as well as advances in ancient DNA technologies, have depicted more detailed, and oftentimes complex histories of natural populations (Shafer *et al.* 2010; Soltis *et al.* 2006). Cryptic refugia, which were semi-hospitable environments within ice sheets (Provan & Bennett 2008), and a northern refugium in ancient Beringia (Tremblay & Schoen 1999), have both been suggested to account for reconstructed patterns in North America populations (Shafer *et al.* 2010; Soltis *et al.* 2006). Genetic information has supported these alternative refugia hypotheses, with areas of high genetic diversity and discrete genetic variation found at the northern extent of species' ranges (Rowe *et al.* 2004; Shafer *et al.* 2011). However, genetic diversity patterns can be obscured by other complex scenarios, such as admixture between lineages from separate refugia (Petit *et al.* 2003), genetic structure within refugia (i.e. refugia within refugia; Gómez & Lunt 2007), and deeper historical associations (Lovette & Bermingham 1999). Understanding

these alternative scenarios is imperative though, as it can directly inform conservation management, by identifying lineages of distinct ecological and/or evolutionary significance (Bhagwat & Willis 2008; Hampe & Petit 2005).

One interesting species to explore the genetic legacy of glaciation history is the American badger, *Taxidea taxus*. As a semi-fossorial mustelid, *T. taxus* is typically thought to be a habitat specialist, well adapted to dry grassland-shrubsteppe habitats, where it can burrow in silty, sandy-loam soils and catch fossorial prey (COSEWIC 2012). However, habitat associations vary across the species' range, and may drastically differ between local populations. For example, American badgers in British Columbia occupy various habitats from low-elevation grasslands to high-elevation alpine tundras (COSEWIC 2012). Furthermore, the species also shows plasticity in its movement patterns and spatial distribution, with maximum reported dispersal distances at 52 km for females and 110 km for males (Messick & Hornocker 1981), and home ranges between 1.6-34.2 km^2 for females and 2.4-315km^2 for males (British Columbia Badger Recovery Team 2016). Accordingly, the species is found throughout much of central and western North America, with its high dispersal capabilities and ability to traverse through difficult terrain facilitating the discovery of suitable habitat and mates (COSEWIC 2012; Kierepka & Latch 2016b).

Thus, while loose habitat associations suggest that badgers should have resided in one or a few refugia, their dispersal capabilities suggest the potential for multiple refugia (Shafer *et al.* 2010). It has been hypothesized that glaciation displaced badgers into a single, southerly refugium, from which the species then recolonized northern latitudes following glacial retreat, with isolation by major geographic barriers forming the four currently recognized subspecies (*jacksoni*, *taxus*, *berlandieri*, and *jeffersonii*; Kierepka & Latch 2016b; Long 1972). Phylogeographic studies have partially supported this hypothesis, providing evidence for fragmented and isolated units at the northern periphery (Ethier *et al.* 2012), and connected populations with rampant gene flow at the center of the species' range (Kierepka & Latch 2016b). However, a lack of genetic sampling from areas where distinct glacial refugia have been implicated (e.g. the Pacific Northwest and Alaska, where badgers resided historically) has hindered a complete picture of how post-glacial colonization has affected contemporary genetic patterns. Understanding the relative impact of post-glacial colonization

is essential, especially for the management of endangered populations at the periphery (COSEWIC 2012), and a study explicitly addressing their impacts is warranted.

Here, we aimed to gain a broader perspective of the historical influences that shaped the genetic variation of American badger populations across the entire species' range. To do so, we compiled new mitochondrial sequencing data from contemporary, historical, and ancient DNA samples, alongside contemporary mitochondrial sequencing data from previous studies of American badgers (Ethier *et al.* 2012; Kierepka & Latch 2016b), to reconstruct the phylogeographic history of badgers across North America. From preliminary analyses (see Chapter 2), we observed drastically different patterns of gene flow and genetic differentiation for peripheral populations in the Pacific Northwest (PNW), compared to populations in central North America. Therefore, we expected to see distinct and lower overall genetic diversity for peripheral units across the species' range compared to central units, as evidence of post-glacial expansion, along with distinct and higher overall genetic diversity in the PNW compared to other peripheral units. These patterns would reflect a potential glacial refugium in the PNW. We also hypothesized that a glacial refugium may have been present in eastern Beringia, with historical connectivity to populations in western British Columbia, due to its geographic proximity and a distinct genetic signature observed in the northwestern distribution of BC badgers (Chapter 2).

3.2   Methods

3.2.1   Sample Collection

We amassed new DNA sequencing data from contemporary (2000-2017), historical (1900-1999), and ancient (pre-1900) samples throughout the species' range, with focal sampling conducted in the Pacific Northwest (described in Chapter 2). We combined newly acquired DNA sequences with previously published data from American badger studies in Canada (Ethier *et al.* 2012) and central and western North America (Kierepka & Latch 2016b).

Tissue and hair samples (*contemporary* samples) as well as claw powder samples (*historical* samples) were collected from the Pacific Northwest as described in Chapter 2. Ancient subfossils (*ancient* samples) with provenances in Alaska and Yukon, were collected from the American Museum of Natural History and the Canadian Museum of Nature (Table 3.1). All steps before amplification including handling subfossils, DNA extraction, and

preparing polymerase chain reactions were conducted in laboratories dedicated to the processing of ancient DNA at the Estación Biológica de Doñana and the University of British Columbia, Okanagan Campus. Methods to prepare subfossil bones for DNA extraction followed those described in Lippold *et al.* (2011). Briefly, a Dremel tool, fitted with a cut-off wheel, was used to remove surface contaminants from bones. A 0.25 g sample was then removed from each specimen, taking caution not to disturb bone processes used for species identification. Samples were covered with aluminum and pulverized with a mortar and pestle before DNA extraction. All surfaces and equipment were thoroughly cleaned with bleach solutions between each sample.

3.2.2   Data Collection

DNA was extracted from contemporary and historical samples as described in Chapter 2. DNA extractions from ancient samples were conducted using a phenol-chloroform method (Barnett & Larson 2012). All extractions for ancient samples were conducted with small sample sizes and multiple negative controls, carried through all steps, including PCR amplification. A fragment of the mitochondrial DNA (mtDNA) d-loop was amplified from contemporary and historical samples following the methods described in Chapter 2. The mtDNA d-loop fragment was amplified from ancient samples using the same four primer pairs and PCR reaction conditions used for historical samples, with one exception: we used 0.24 mg/mL rabbit album serum (RSA) in place of bovine albumin serum (BSA), due to nonspecific amplification in initial reactions. Ancient DNA samples are subject to degradation over time, that may alter some DNA sequences post-mortem. Therefore, to assure that accurate DNA sequencing information was recovered from ancient samples, each DNA fragment was independently amplified and sequenced at least twice. Additional amplifications were conducted if a mismatch was identified, and the most frequent polymorphism at mismatched sites was retained. PCR products were purified using ExoSAP-IT (Applied Biosystems, Thermo-Fisher Scientific, Inc.) and sequenced using BigDye v3.1 Terminator chemistry on an Applied Biosystems 3130XL DNA automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were visualized and edited using Sequencher 5.0 (Gene Codes Corporation).

3.2.3 Haplotypic Variation and Broad-Scale Geographic Structure

A total of 937 previously published sequences for 4 provinces and 37 states were downloaded from Genbank (Appendix C) to align to newly acquired sequencing information. Samples with uncertain locality information from Kierepkaand Latch (2016b) were excluded from analyses, to facilitate an accurate grouping of badger populations by province or state. All newly acquired sequences from this study and sequences from Ethier *et al.* (2012) were trimmed at the 5' end to align with sequences from Kierepkaand Latch (2016b), and 3' terminal gaps were coded as missing data. We used the same practices as described in Chapter 2 to remove NUMTs and to account for the 25 bp deletion in our dataset. All sequences were aligned using default parameters in MUSCLE (Edgar 2004), as implemented in GENEIOUS 10.1.2 (Kearse *et al.* 2012).

To define populations for downstream analyses, individuals were grouped into sampling units based on their state or province of origin, except for sequences from British Columbia, which were grouped into the five sampling units inferred in Chapter 2 (Cariboo, Thompson, Nicola, Okanagan, and East Kootenay). Specific geographic coordinates for each individual were not provided in Kierepkaand Latch (2016b) or Ethier *et al.* (2012); therefore, the latitude and longitude of the centroid for each state, province, or distribution (i.e. for units at the periphery where badgers are not found throughout the entire state or province) was used for the geographic coordinates of sampling units, following Kierepkaand Latch (2016b). Estimates of haplotype diversity (Hd), nucleotide diversity (π), and pairwise difference were calculated for each sampling unit in Arlequin 3.5 (Excoffier *et al.* 2005). To test for an association between the peripherality of a sampling unit and its genetic diversity, we conducted a least-squares linear regression of haplotype diversity (Hd) on distance from the center (in kilometers), implemented in the R statistical computing environment (R Core Team 2016). The center was calculated by averaging the latitude and longitude of centroids for all states, provinces, and peripheral ranges inhabited by American badgers in North America. The distance from the center was calculated for each sampling unit for which we had adequate haplotype diversity estimates (≥ 5 haplotypes). To test for an association between the latitude of a sampling unit and its genetic diversity, we conducted a least-squares linear regression of haplotype diversity on latitude (in decimal degrees), implemented in the R statistical computing environment (R Core Team 2016).

A range-wide haplotype network was constructed using statistical parsimony, as implemented in TCS (Clement *et al.* 2000). The dissimilarity matrix from the TCS output was used to visualize the haplotype network using Hapstar 0.7 (Teacher & Griffiths 2011).

To better understand populations differentiation across the species' distribution, we conducted a spatial analysis of molecular variance (SAMOVA 1.0; Dupanloup *et al.* 2002). SAMOVA defines groups of populations by identifying the configuration that maximizes the amount of genetic variance explained among differentiated groupings (Dupanloup *et al.* 2002). The optimal configuration of groups was tested from K=2 to K=15, using 100 simulated annealing steps, as suggested in the manual (Dupanloup *et al.* 2002). We chose the optimal configuration based on the grouping that maximized the total amount of genetic variance between groups (ϕ_{CT}).

3.2.4 Fossil Evidence

To corroborate patterns of genetic variation, we also collected information regarding the spatial distribution of American badger fossil specimens throughout North America. Fossil information was acquired from three sources: FossilWorks (<fossilworks.org>), Integrated Digitized Biological Collections (iDigBio; <idigbio.org>), and from the literature describing the sampled subfossil specimens. We used the search term "*Taxidea*" to explore fossil occurrences in the fossilworks.org and iDigBio databases. For each occurrence, we recorded locality information, geographic coordinates, and the estimated median date. When not provided, the estimated median date was assigned to occurrences based on the geological period described, with intervals following those described in the FossilWorks database.

3.3 Results

3.3.1 Sample Collection and Marker Amplification

A total of 211 new mitochondrial sequences were recovered from 164 contemporary and 47 historical samples, after the removal of NUMTs. From the 13 subfossils collected in Alaska, USA, and Yukon, Canada, two yielded the entire d-loop fragment (one from Alaska and one from Yukon), with 5 additional samples recovering partial sequences (Table 3.1). The single Yukon sample that recovered the entire d-loop fragment held the distinguishing 25 bp indel that was found previously across the species' range (Chapter 2; Kierepka & Latch 2016b).

Combined with mitochondrial sequence data from the species' range, our newly acquired sequencing data led to a final dataset of 1207 sequences for downstream analyses.

3.3.2   Haplotypic Variation and Broad-Scale Geographic Structure

A total of 118 haplotypes were recovered across all sampling units; 13 more than the previous large-scale study (Kierepka & Latch 2016b). Levels of haplotype diversity spanned a large range, from 0.000 ± 0.000 in the BC-Cariboo to 1.00 ± 0.096 in Colorado (Table 3.2). Haplotype diversity was significantly associated with distance to center, decreasing as sampling units were closer to the periphery (Figure 3.1). Genetic diversity generally decreased with an increase in latitude, although not significantly so (Figure. 3.2). Sampling units with higher genetic diversity than expected were primarily located in the Pacific Northwest (WA, OR, ID) and the eastern periphery (IL and IN), and sampling units with lower genetic diversity than expected were at the extreme peripheries in BC and Ontario, and to a lesser extent, in the American Midwest (Figure 3.3). Measures of nucleotide diversity were low across all units, ranging from 0.000 in the BC-Cariboo to 0.011 in Oregon (Table 3.2). Haplotypes recovered from Alaska and the Yukon were exact matches to haplotypes in the Canadian Prairie provinces of Saskatchewan and Manitoba, as well as in the American Midwestern states of North Dakota, South Dakota, Nebraska, Wisconsin, and the western state of Wyoming (Figure 3.3).

There were no discrete associations between haplotypes and geographic locations for sampling units across the species' range (Figure 3.4), although many major haplotypes were found in high proportions in certain localities, especially for sampling units at the periphery (Appendix C). One haplotype was the most common across all badger sampling units, found in all four current subspecies designations (Haplotype 1; Figure 3.4). Four other major and semi-major haplotypes were found across the continent (Figure 3.4). There was some evidence of regional geographic structuring, with the *taxus* subspecies sharing haplotypes with most other subspecies, but subspecies at opposite ends of the species' distribution shared very little haplotypic variation (e.g. *jeffersonii* and *jacksoni*; Figure 3.4). Furthermore, haplotypes from the PNW largely fell outside the two major haplogroups (Figure 3.4).

SAMOVA analyses estimated an optimal configuration of populations at K=2, where the BC-Cariboo and BC-Nicola separated from all other sampling units throughout the

species' range (Table 3.3), with 39.26% of variation explained among the two groups. The percent of variation among groups and ϕ_{CT} gradually decreased as the number of groups considered (K) increased. The next configuration that explained the most variation between groups further separated BC-Cariboo and BC-Nicola units into their own groups at K=3 (Table 3.3). Consistent with results from Kierepkaand Latch (2016a), we found that the Lower Peninsula, Ohio, and Ontario samples grouped together at K=5 relative to all other sampling units in North America, besides those identified in BC (Table 3.3).

3.3.3   Fossil Evidence

From our occurrence search, we discovered 230 *Taxidea* fossils, 123 of which had a temporal description. The earliest specimen discovered was from Yepomera, Mexico, dating to the late Miocene, 5.75 million years ago (mya), with the *taxus* species discovered by the Pliocene (3.35 mya). Most of the oldest fossil occurrences, dating before the Pleistocene, were discovered in the American Southwest (Figure 3.5). All subfossil specimens recovered in eastern Beringia were from the late Pleistocene, with the earliest median date being 0.05 mya (Figure 3.5). From our occurrence search, only two specimens had radiocarbon dates, which were both found within the Yukon; one dating back 0.03793 mya and the other 0.01519 mya (Harington 2003).

3.4   Discussion
3.4.1   Broad-Scale Genetic Patterns

The American badger, *Taxidea taxus*, has had a long history in North America, with the earliest fossil specimens dating back to the early Pliocene, ~3.5 million years ago (Long 1972). American badgers are thought to have survived the harsh Pleistocene ice ages by residing in a single refugium, south of glacial extents, from which they recolonized northern latitudes following glacial retreat (Kierepka & Latch 2016b). By expanding on previous genetic studies with newly acquired genetic data, we shed light on more complex patterns across the species' range.

Overall, higher levels of genetic diversity were observed for central sampling units, such as Colorado, Nebraska, and Kansas, compared to units at the periphery, such as British Columbia, Ontario, and Ohio. Moreover, the species-wide haplotype network displayed no

discrete geographic structuring, and a star-like topology, indicative of a largely panmictic glacial refugium and recent population expansion (Slatkin & Hudson 1991). Nevertheless, we did not observe a significant relationship between latitude and genetic diversity of sampling units, which would indicate northern recolonization from a single refugium south of ice sheets. Furthermore, we observed some regional geographic structuring in the haplotype network, with many of the PNW haplotypes falling outside the two major haplotypes across the species' range, and high proportions of major and semi-major haplotypes in specific regions. Levels of haplotype diversity were quite high for sampling units in the PNW, such as Washington and Oregon, coinciding with expectations for areas where glacial refugia once resided (Hewitt 1996). A Pacific Coastal refugium has been suggested for many species (Chavez *et al.* 2014; Cheng *et al.* 2014; Latch *et al.* 2009), and may have also been a refugium for badger populations during the Pleistocene. However, a lack of genetic sampling from other nearby regions cannot exclude other potential possibilities, such as a refugium in California, which has been reported previously for deer and plant species (Latch *et al.* 2009; Soltis *et al.* 1997). The higher than expected genetic diversity for peripheral populations of Illinois and Indiana may also represent a signal of an additional refugium, or alternatively, an admixture zone between a refugium of central North America and a refugium in eastern North America, where badgers resided historically (Figure 3.5; Long 1972). A refugium in the lower peninsula of Michigan, and east of the Appalachian Mountains, have both been suggested in phylogeographic studies of other species (Rowe *et al.* 2004; Soltis *et al.* 2006), and if also a location of an American badger refugium, may explain the high proportion of unique haplotypes in eastern populations. Additional sampling from contemporary southwestern populations and historical eastern populations may expand on this initial evidence of additional refugia.

3.4.2   Population Genetic Variation and the Ice-Free Corridor

Based on previous studies of North American post-glacial colonization (Shafer *et al.* 2010), we expected to see shared or connected haplotypes from ancient populations in eastern Beringia and those from BC. Contrary to these expectations, we discovered that haplotypes recovered from Alaska and the Yukon were identical to haplotypes found in the Prairie provinces, the American Midwest, and the American West. Other species' populations have

displayed this connectivity (Heintzman *et al.* 2016), owing to a hypothesized ice-free corridor between the Cordilleran and Laurentide ice sheets, just east of present-day British Columbia, through which animals migrated during the last glacial maximum (Stalker 1977) . Recent evidence, however, suggests that the Cordilleran and Laurentide glaciers coalesced between 23,000 to 13,000 years ago, completely blocking any migration during this time period (Heintzman *et al.* 2016). Therefore, the shared haplotypes between ancient and contemporary populations suggest one of three possibilities: 1) badgers migrated to eastern Beringia from the grasslands of central North America when the ice-free corridor reopened 13,000 years ago, 2) badgers from central North America migrated north more than 23,000 years ago prior to the closing of the ice-free corridor, or 3) badgers from eastern Beringia colonized central North America after the corridor reopened 13,000 years ago. Unfortunately, we did not have radiocarbon dates for the specimens from which full sequences were recovered, but they are suggested to be late Wisconsin in age, with radiocarbon dates for other species ranging between 12,640 and 40,000 ybp at the Alaska site (Anderson 1977), and between 22,200 and 39,900 ybp at the Yukon site (Harington & Clulow 1973). Furthermore, from our fossil occurrence search, we found two badger specimens, both within the Yukon, radiocarbon dating to 37,930 and 15,190 years ago. These lines of evidence suggest that either scenario 2 or 3 is more plausible, as badgers were present in eastern Beringia before the corridor reopened. In terms of teasing apart the direction of migration, mitochondrial evidence in other studies suggest both directions are plausible. Species such as bison show no evidence of northward expansion into Alaska and the Yukon during the Holocene (Heintzman *et al.* 2016), whereas species such as wolves show complete replacement of northern populations by populations south of the glacial extent, after the glacial period (Leonard *et al.* 2007). Although we cannot unequivocally infer directionality in American badgers, the moderate to high levels of genetic variation, alongside the low frequency of haplotypes with indels in the Midwest (Kierepka & Latch 2016b; also present in the Yukon) suggest a potential recolonization of American badgers into central North America after the ice-free corridor re-opened (scenario 3). A more thorough analysis, incorporating genetic information from Pleistocene fossil specimens south of glacial extents and in the region of the proposed ice-free corridor, with accurate radiocarbon dates, is needed to further test this hypothesis.

Interestingly, we discovered that peripheral populations in northwestern British Columbia are highly differentiated to all other sampling units in North America (Table 3.3). These results suggest that populations in British Columbia either had a long history of isolation relative to all other sampled areas, or, the potential existence of a cryptic glacial refugium within British Columbia. Mounting evidence of complex biogeographic patterns in British Columbia suggests that cryptic refugia may have existed within the Cordilleran ice sheet (Shafer *et al.* 2010). Genetic patterns from both plants (Marr *et al.* 2008) and animals (Loehr *et al.* 2006) have provided support for this hypothesis. Most of the species suggested to exist in cryptic refugia have been either alpine or artic, indicating a predisposition to tolerating cold climates. Badgers are typically restricted to dry grassland-shrubland ecosystems within North America, however, their ability to tolerate unsuitable environmental conditions (COSEWIC 2012), alongside their semifossorial lifestyle, may have permitted badgers to live in refugia within ice sheets. Badgers in the BC-Cariboo show particular hardiness for these environmental conditions, inhabiting atypical alpine and wetland habitats (COSEWIC 2012; Rahme *et al.* 1995) in a region with some of the harshest winter conditions (Symes 2013).

Similar genetic patterns showing a discrete genetic break in the southern interior of British Columbia have been observed for many species (Gayathri Samarasekera *et al.* 2012; Jensen *et al.* 2014; Parks *et al.* 2015; Warren *et al.* 2016). The cause of this break, however, was attributed to various factors, including biological or climatic factors, isolation by distance, anthropogenic landscape features, or unsuitable habitat. However, here, we argue that this genetic pattern, observed across multiple species with different ecologies, may reflect a common glacial refugium within ice sheets. The controversial Haida Gwaii refugium has been suggested to be a source of genetic variation for other natural populations, when southern or northern recolonization patterns were not evident (Burg *et al.* 2006; Janzen *et al.* 2002). Most other species for which the Haida Gwaii refugium has been hypothesized, however, currently reside in coniferous forest environments (Burg *et al.* 2006; Janzen *et al.* 2002), therefore, it seems unlikely that this would be a glacial refugium for badger populations.

3.4.3   Conservation Implications

Reconstructing the phylogeographic history of American badgers in North America offers a broader understanding of contemporary genetic patterns for the species across its range, and provides insights for conservation management. Currently, badger populations within the Prairie provinces are listed as 'special concern' under the Species at Risk Act in Canada (COSEWIC 2012). The potential connectivity and introgression of genetic variation from ancient populations in eastern Beringia into populations in the Prairie provinces suggests that ongoing population monitoring should continue in this region if the unique genetic variation is to be preserved. Furthermore, the distinct genetic variation within the Central Plateau of British Columbia further supports a heightened conservation status for badgers in the western designatable unit of BC. This extended analysis suggests that the genetic signal in this region may not only be unique within British Columbia, but across the entire species' distribution as well. Future work should investigate these patterns with more detailed genomic analyses collected across a denser sampling distribution around the geographic region of drastic genetic change.

Table 3.1 Information for subfossils specimens approved for sampling. Information for successful PCR amplification is still pending for some samples (i.e. CMN 35319).

Museum Identification Number	Museum	Sampled	Locality	Sequence Recovered
F:AM 30840	AMNH	Y	Cripple Creek, AK	N
A.C.C 552	AMNH	Y	Cripple Creek, AK	N
F:AM 30829	AMNH	Y	Ester Creek, AK	P
F:AM 30789	AMNH	Y	Cleary, AK	P
F:AM 117098	AMNH	Y	Goldhill, AK	P
F:AM 30826	AMNH	Y	Cripple Creek, AK	P
F:AM 30831	AMNH	Y	Cripple Creek, AK	N
F:AM 30837-A	AMNH	Y	Ester Creek, AK	N
F:AM 30786	AMNH	Y	Fairbanks, AK	Y
F:AM 30839	AMNH	Y	Fairbanks Creek, AK	N
F:AM 30836	AMNH	Y	Cripple Creek, AK	P
CMN 13486	CMN	Y	Gold Run Creek, YT	Y
CMN 35319	CMN	Y	Dawson Creek, YT	NA

Museum: AMNH= American Museum of Natural History and CMN= Canadian Museum of Nature
Sequence Recovered: N=No, Y= Yes, P= Partial, NA=Not applicable (Specimen not sampled, or data pending)
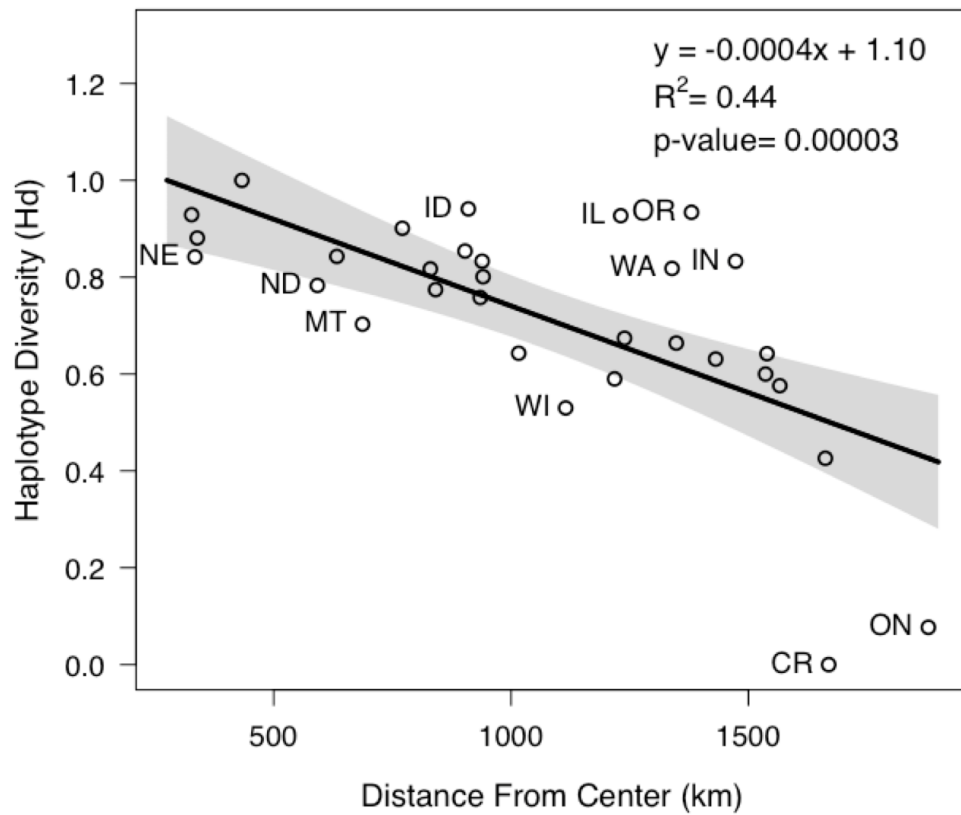
Figure 3.1 Least-squares regression of the distance from center (kilometers) versus haplotype diversity (Hd). Grey shading depicts the 95% confidence interval. Sampling units ≤5 individuals were excluded from analysis. Outlier sampling units, with residuals > 1.0, are labeled with sampling unit abbreviations.
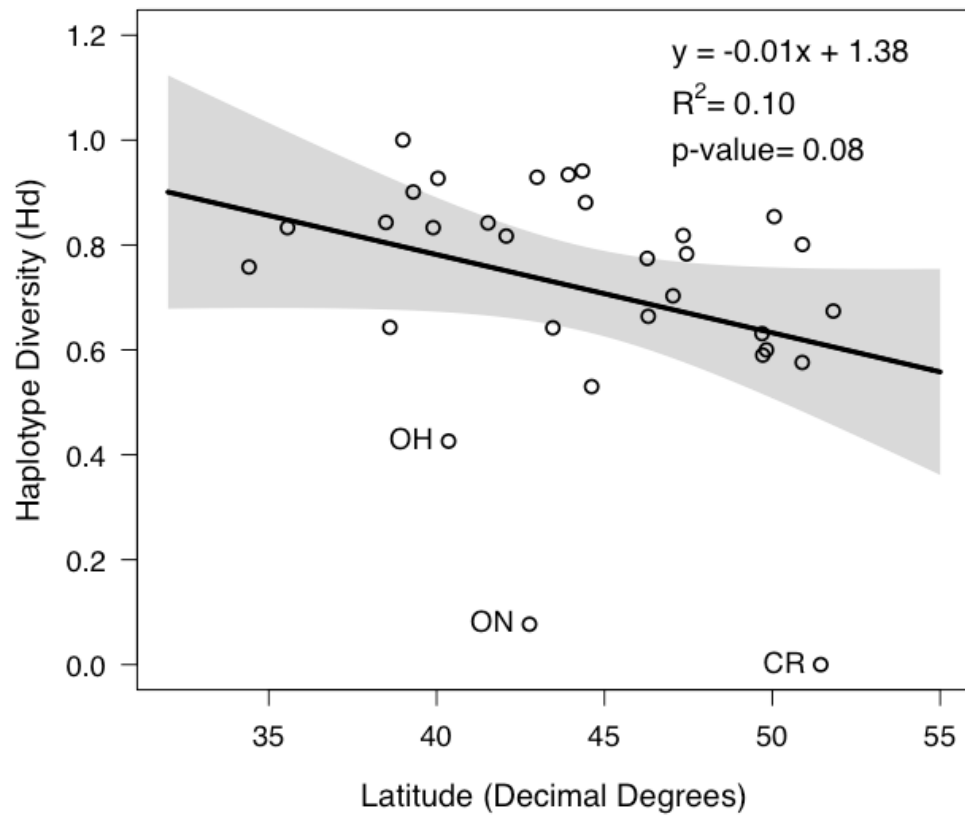
Figure 3.2 Least-squares regression of the latitude (decimal degrees) versus haplotype diversity (Hd). Grey shading depicts the 95% confidence interval. Sampling units ≤5 individuals were excluded from analysis. Outlier sampling units, with residuals > 0.25 are labeled with sampling unit abbreviations.

Figure 3.3 Haplotype diversity across the American badger range and distribution of samples that share haplotypes with ancient specimens in Alaska and Yukon. Sampling units with low sample size (n<4; California, Arizona, Alaska, and Yukon) were excluded from analyses. High diversity estimates are depicted in warm colors and low diversity estimates are depicted in cool colors. Shape of point represents the ancient sample that haplotypes correspond to, and location of points represent sampling units, rather than exact locations of individuals. Sampling units within British Columbia are enlarged for enhanced visibility.

Table 3.2 Haplotypic diversity indices based on a fragment of the mtDNA d-loop in American badger sampling units across the species' range.

| Sampling Unit	N	Number of Haplotypes	Haplotype Diversity (Hd ± SE)	Nucleotide Diversity (π ± SE)	Pairwise Difference
Cariboo	26	1	0.000 ± 0.000	0.000 ± 0.000	0.00 ± 0.00
Thompson	21	4	0.576 ± 0.099	0.005 ± 0.003	2.15 ± 1.24
Nicola	6	2	0.600 ± 0.129	0.002 ± 0.002	0.60 ± 0.55
Okanagan	40	4	0.631 ± 0.060	0.003 ± 0.002	1.38 ± 0.87
Washington	47	13	0.818 ± 0.038	0.007 ± 0.004	2.61 ± 1.42
Oregon	14	9	0.934 ± 0.045	0.011 ± 0.006	4.23 ± 2.23
East Kootenay	34	4	0.590 ± 0.071	0.007 ± 0.004	2.70 ± 1.47
Alberta	51	15	0.674 ± 0.074	0.007 ± 0.004	3.01 ± 1.59
Idaho	29	15	0.941 ± 0.022	0.008 ± 0.005	3.15 ± 1.68
Montana	79	19	0.703 ± 0.055	0.007 ± 0.004	3.04 ± 1.60
Saskatchewan	54	13	0.801 ± 0.037	0.007 ± 0.004	2.86 ± 1.53
Manitoba	60	14	0.854 ± 0.030	0.008 ± 0.005	3.55 ± 1.83
Wyoming	8	6	0.929 ± 0.084	0.007 ± 0.004	3.75 ± 2.11
Utah	44	16	0.901 ± 0.022	0.009 ± 0.005	5.00 ± 2.48
Colorado	6	6	1.00 ± 0.096	0.009 ± 0.006	3.40 ± 2.02
New Mexico	12	6	0.758 ± 0.122	0.009 ± 0.005	3.56 ± 1.95
North Dakota	58	16	0.783 ± 0.044	0.004 ± 0.003	2.44 ± 1.34
South Dakota	78	24	0.881 ± 0.021	0.006 ± 0.004	3.55 ± 1.82
Nebraska	60	23	0.842 ± 0.044	0.005 ± 0.003	2.73 ± 1.47
Oklahoma	12	7	0.833 ± 0.100	0.005 ± 0.003	2.73 ± 1.56
Kansas	31	14	0.843 ± 0.060	0.006 ± 0.003	3.44 ± 1.80
Minnesota	67	17	0.7743 ± 0.045	0.006 ± 0.004	3.69 ± 1.89
Iowa	59	10	0.817 ± 0.034	0.007 ± 0.004	3.83 ± 1.95
Missouri	8	4	0.643 ± 0.184	0.005 ± 0.003	2.89 ± 1.70
Wisconsin	76	9	0.530 ± 0.064	0.004 ± 0.002	2.09 ± 1.18
Illinois	11	8	0.927 ± 0.067	0.007 ± 0.004	3.93 ± 2.13
Indiana	4	3	0.833 ± 0.222	0.004 ± 0.004	2.50 ± 1.69
Ontario	26	2	0.077 ± 0.070	0.000 ± 0.000	0.08 ± 0.15
Upper Peninsula	35	5	0.664 ± 0.048	0.003 ± 0.002	1.32 ± 0.84
Lower Peninsula	118	5	0.642 ± 0.025	0.001 ± 0.001	0.51 ± 0.43
Ohio	28	4	0.426 ± 0.107	0.003 ± 0.002	1.73 ± 1.04
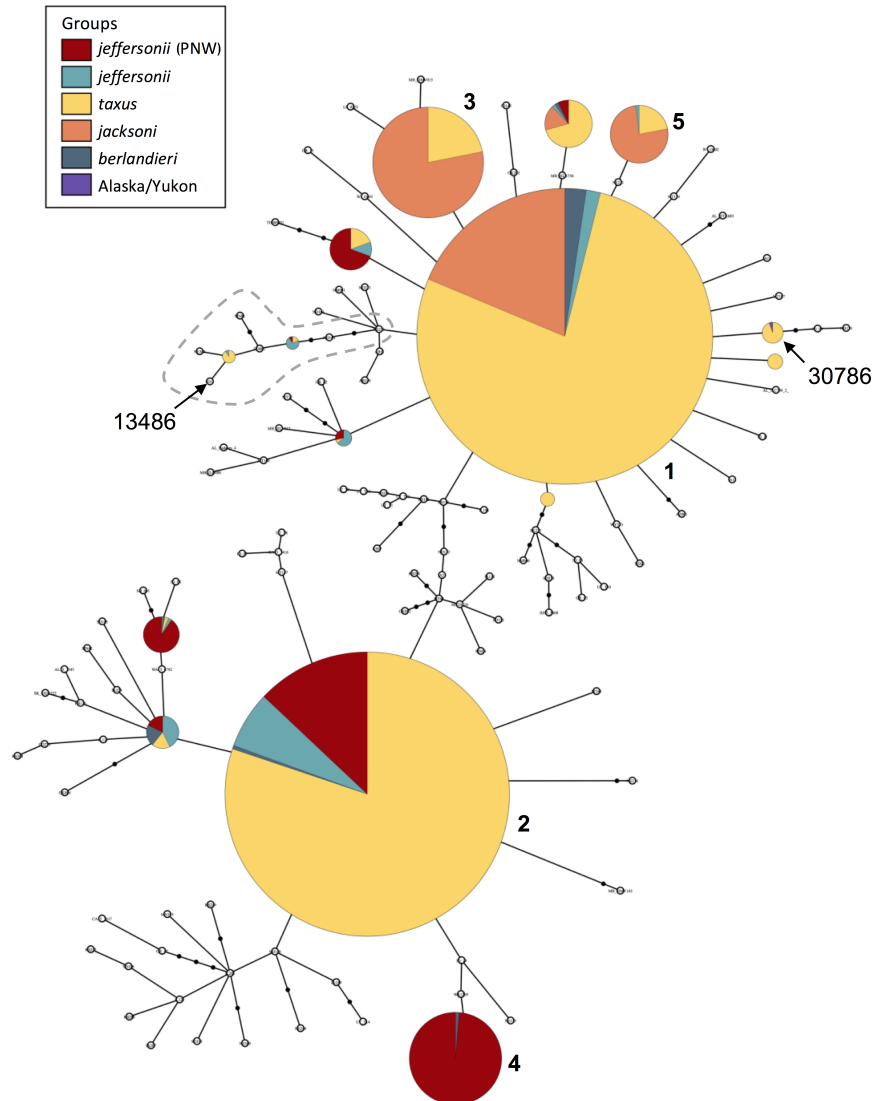
Figure 3.4 Haplotype network based on a fragment of the mtDNA d-loop from American badger sampling units across the species' range. Each circle represents a unique haplotype, with size corresponding to the relative number of individuals with each haplotype. Colors designate the four current subspecies designations in relation to sampling units in the Pacific Northwest [*jeffersonii* (PNW)] and eastern Beringia (Alaska/Yukon). Open circles represent haplotypes with 10 or fewer individuals. Closed, black circles represent unsampled transitions between recovered haplotypes. Major and semi-major haplotypes are numbered 1-5. Haplotypes recovered from ancient specimens are labeled with museum identification numbers. Sequences with a 25 basepair deletion are highlighted by the grey dashed line.

Table 3.3 SAMOVA groupings for K=2 to K=15.

K	Groupings	ϕ_{SC}	ϕ_{ST}	ϕ_{CT}	% var among groups
2	[CR, NI] [TH, OK, WA, OR, EK, AL, ID, MT, SK, MB, WY, UT, CO, NM, ND, SD, NE, OL, KS, MN, IA, MO, WI, IL, IN, ON, UP, LP, OH]	0.252	0.546	0.393	39.26
3	[CR] [NI] [TH, OK, WA, OR, EK, AL, ID, MT, SK, MB, WY, UT, CO, NM, ND, SD, NE, OL, KS, MN, IA, MO, WI, IL, IN, ON, UP, LP, OH]	0.254	0.534	0.376	37.56
4	[CR, TH, OK] [NI] [IN] [WA, OR, EK, AL, ID, MT, SK, MB, WY, UT, CO, NM, ND, SD, NE, OL, KS, MN, IA, MO, WI, IL, ON, UP, LP, OH]	0.217	0.489	0.348	34.76
5	[CR] [TH, OK] [NI] [ON, LP, OH] [WA, OR, EK, AL, ID, MT, SK, MB, WY, UT, CO, NM, ND, SD, NE, OL, KS, MN, IA, MO, WI, IL, IN, UP]	0.134	0.428	0.339	33.91
6	[CR] [TH, OK] [NI] [IN] [ON, LP, OH] [WA, OR, EK, AL, ID, MT, SK, MB, WY, UT, CO, NM, ND, SD, NE, OL, KS, MN, IA, MO, WI, IL, UP]	0.134	0.425	0.336	33.56
7	[CR, TH, OK] [NI] [CO] [MO] [IN] [ON, LP, OH] [WA, OR, EK, AL, ID, MT, SK, MB, WY, UT, NM, ND, SD, NE, OL, KS, MN, IA, WI, IL, UP]	0.133	0.417	0.328	32.76
8	[CR] [TH, OK] [NI] [CO] [NM] [IN] [ON, LP, OH] [WA, OR, EK, AL, ID, MT, SK, MB, WY, UT, ND, SD, NE, OL, KS, MN, IA, MO, WI, IL, UP]	0.134	0.414	0.323	32.28
9	[CR, TH, OK] [NI] [WY] [CO] [MO] [IL] [IN] [ON, LP, OH] [WA, OR, EK, AL, ID, MT, SK, MB, UT, NM, ND, SD, NE, OL, KS, MN, IA, WI, UP]	0.135	0.405	0.313	31.26
10	[CR] [TH, OK] [NI] [WA] [AL, MT, SK] [WY] [CO] [IN] [ON, LP, OH] [OR, EK, ID, MB, UT, NM, ND, SD, NE, OL, KS, MN, IA, MO, WI, IL, UP]	0.064	0.353	0.309	30.86
11	[CR] [TH, OK] [NI] [WA] [EK] [AL, MT, SK, WY] [NM] [IN] [WI, UP] [ON, LP, OH] [OR, ID, MB, UT, CO, ND, SD, NE, OL, KS, MN, IA, MO, IL]	0.030	0.327	0.305	30.54
12	[CR] [TH, OK, NI] [WA] [EK] [AL, MT, SK, WY] [UT] [NM] [IN] [MO] [WI, UP] [ON, LP, OH] [OR, ID, MB, CO, ND, SD, NE, OL, KS, MN, IA, IL]	0.020	0.318	0.304	30.41
13	[CR] [TH, OK, NI] [WA] [OR] [EK] [AL, MT, SK, WY] [UT] [NM] [MO] [WI, IN, UP] [ON] [LP, OH] [ID, MB, CO, ND, SD, NE, OL, KS, MN, IA, IL]	0.017	0.315	0.303	30.31
14	[CR] [TH, OK, NI] [WA] [OR] [EK] [AL, MT, SK, WY] [UT] [CO] [NM] [MO] [IN] [WI, UP] [ON] [LP, OH] [ID, MB, ND, SD, NE, OL, KS, MN, IA, IL]	0.018	0.315	0.302	30.21
15	[CR] [TH, OK, NI] [WA] [OR] [EK] [AL, MT, SK, WY] [UT] [CO] [NM] [MO] [WI, IN, UP] [IL] [ON] [LP, OH] [ID, MB, ND, SD, NE, OL, KS, MN, IA]	0.016	0.312	0.301	30.1
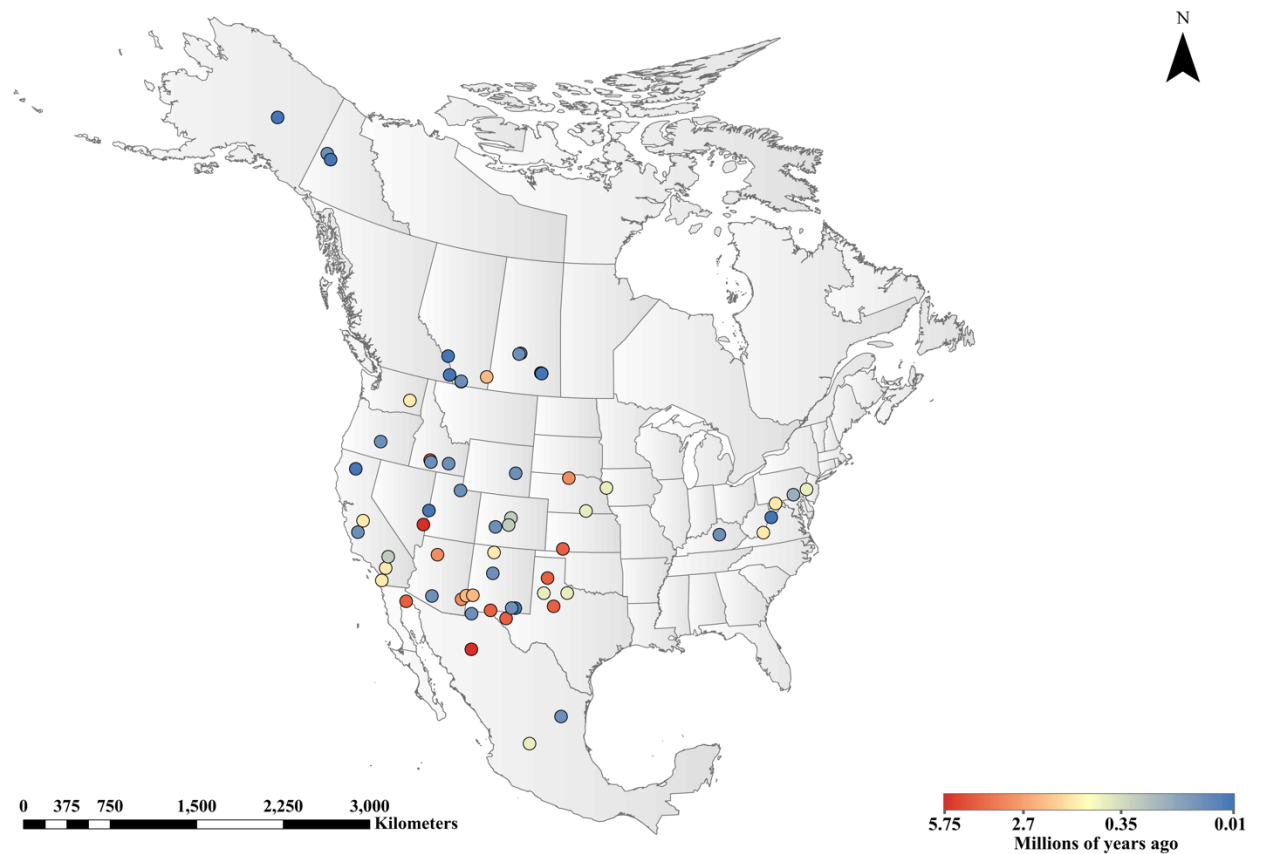
Figure 3.5 Distribution of *Taxidea* fossil specimens in North America, with temporal descriptions aging from the Miocene (5.75 mya) to mid-Holocene (0.005 mya).

Chapter 4 Conclusions

4.1   Research Findings and Significance

This, to our knowledge, is the first study to conduct a thorough genetic assessment for American badgers throughout their range in British Columbia and the greater Pacific Northwest. By identifying the contemporary and historical processes generating the patterns of genetic variation for badgers in this region, we provide insight into how humans impact genetic patterns at a species' range edge, but also shed light on the impact of glacial refugia on observed genetic patterns, all with important implications for conservation. Theory suggests that peripheral populations should have lower genetic diversity and greater differentiation (Brussard 1984; Carson 1959), and that gene flow should be asymmetric, from the center to the periphery (Garcia-Ramos & Kirkpatrick 1997). We discovered that the genetic variation of the most northwest American badger populations largely coincide with these expectations, with low genetic diversity, population structure, and significant genetic differentiation vastly contrasting the high levels of genetic diversity and nearly nonexistent differentiation typifying central badger populations (Kierepka & Latch 2016b). On the contrary, instead of centrifugal gene flow to peripheral units, we observed significant gene flow only occurring from peripheral units in the lower WDU into northern Washington, and from northern Washington into southern Washington. Gene flow back into British Columbia appears limited, and gene flow to or from the most isolated Cariboo unit appears nearly nonexistent, with admixture coefficients drastically changing at the intersection of the TransCanda Highway and Highway 5. Concordant with this observation, we identified roadways as a major barrier to gene flow in the WDU, significantly correlated with genetic distance at broad and fine scales and identified as one of the variables in the top model explaining genetic distance between individuals in the Cariboo and Thompson. To date, roadways have been identified as the primary threat limiting badger recovery in the province (COSEWIC 2012), with the highest mortality rates recorded at the major highway intersection in the WDU (British Columbia Badger Recovery Team 2016). By limiting effective gene flow with the most peripheral areas, roadways may be inhibiting gene flow that is essential for badgers to cope with environmental change, and to recover from population decline.

Very few studies have explicitly assessed the impact of human-modified landscapes on peripheral populations, although studying such impacts has been highly encouraged (Guo 2012). Those that have considered landscape features in the context of peripheral populations have primarily identified natural features (e.g. mineral availability, Micheletti & Storfer 2017; and elevation, Sexton *et al.* 2016) as barriers to gene flow. We provide evidence for anthropogenic features acting as barriers to gene flow at a species' range edge and thus present an example of human activity potentially limiting a species' range distribution. These findings are relevant, not only to other peripheral badger populations, such as those in Ontario where land use change and road mortalities also threaten recovery (COSEWIC 2012), but are also relevant to other natural populations at the periphery in British Columbia and across Canada, as 75% of Canadian species at-risk are at their northern range limits (Gibson *et al.* 2009). Mitigating the impact of anthropogenic features will be essential for these species of conservation concern, and should be considered a high priority, especially considering species shifting their ranges towards the north in response to climate change (Gibson *et al.* 2009).

While we highlight the impact of anthropogenic disturbance on contemporary genetic variation, we also emphasize the importance of glacial refugia on broad scale patterns of contemporary genetic variation. Before our study, American badgers were believed to reside in a single refugium, south of the glacial extent, during the Pleistocene (Long 1972). A recent range-wide phylogeography study provided support for this hypothesis, however, the authors noted gaps in their sampling distribution where other potential refugia could reside (Kierepka & Latch 2016b). Indeed, by identifying the paleontological history and genetic patterns across the American badger's historical and contemporary range, we provide evidence for potentially two additional refugia in the Pacific Northwest and Eastern Beringia, and the potential for a cryptic refugium within the Cordilleran ice sheet. The existence of refugia in the Pacific Northwest and Eastern Beringia has been suggested in many other studies (Shafer *et al.* 2010), however, very few have considered the location of these glacial refugia relative to peripheral populations. For species that are found across much of North America, such as the American badger, genetic diversity would be expected to decrease approaching the periphery, in locations such as the Pacific Northwest and Alaska. However, if additional refugia existed towards the periphery, genetic diversity ought to be higher and highly

differentiated in these regions, owing to distinct, long-term population history. Indeed, we found unique genetic variation and high levels of genetic diversity in Washington, Oregon, and Idaho. While we did not recover enough sequencing information from samples in Eastern Beringia to measure levels of genetic diversity relative to the species' range, we found evidence for potential connectivity between Eastern Beringia and the grasslands of central North America, possibly facilitated by the ice-free corridor. Taken together, these results suggest that additional refugia may have existed in the Pacific Northwest and Eastern Beringia, from which American badgers colonized nearby regions. Furthermore, this suggests that genetic variation from badger populations in these regions, or variation from populations historically connected to these regions, may be distinct from the largely panmictic central populations, thus heightening their priority for conservation.

Our study is one of the first to suggest a cryptic glacial refugium in southern BC, primarily based on evidence of a strong genetic signal found from the Cariboo for American badgers, but also for many other species in the region (Gayathri Samarasekera *et al.* 2012; Jensen *et al.* 2014; Parks *et al.* 2015; Warren *et al.* 2016). The genetic patterns of populations in the Northwest are known to be complex, with several cryptic or atypical refugia suggested to exist in locations such as Haida Gwaii, northern BC, or between the Cordilleran and Laurentide ice sheets (Shafer *et al.* 2010). Although consistent with our findings and others from the region, the hypothesis of a southern BC refugium requires further investigation.

4.2 Overarching Conservation Implications

After an extensive genetic assessment of badgers in the Pacific Northwest, we can make several recommendations for management of populations in this region. As outlined in Chapters 2 and 3, there is clear evidence that badgers in the WDU are quite distinct, not only from badgers in the EDU and the Pacific Northwest, but also from populations across the species' range. There is also evidence for a distinct split within the WDU that likely has historical origins, but is exacerbated by recent human activities. Taken together, these findings suggest several points to inform conservation action. First, the distinct variation in BC, and particularly in the WDU, highlights the importance of peripheral American badger populations in the overall scheme of the species' genetic diversity. To conserve such distinct genetic diversity, efforts should first be implemented within British Columbia to alleviate the

impact of vehicle-induced mortality that is indirectly acting as a barrier to gene flow by limiting effective movement across roadways. Both male and female badgers as well as family groups have been shown to use crossing structures when they are available, with 500 mm culverts used the most frequently (Klafki 2014). An obvious starting location for employing such structures would be in Kamloops, where we observed the most distinct change in allele frequencies coinciding with the major highway intersection. This alleviation, alongside habitat restoration in both designatable units, may increase gene flow between units within BC. After such targeted approaches, we also suggest an alleviation of human disturbance between the southern interior of British Columbia and northern Washington. While there appears to be some connectivity present between the WDU and Washington, we also observed evidence of limited gene flow, significant differentiation, and genetic structure between these regions, perhaps not yet reaching equilibrium, thus limiting our ability to detect distinct differences. We didn't explicitly test the association between landscape features and genetic variation in this region, but the habitat associated with badgers between western BC and Washington is connected by a very narrow corridor interspersed with human infrastructure and highway routes. Preserving the connectivity between Washington and BC may promote sufficient levels of gene flow from more central populations to stabilize the debilitating effects of geographic peripherality- such as increased isolation and small population size- in western BC. Lastly, we suggest continual monitoring of badger populations within Washington, to evaluate the impacts of anthropogenic activities on conservation status.

4.3   Limitations and Future Studies

The landscape genetics study we conducted in the second chapter was only an initial assessment of how landscape features impact American badger gene flow. This allowed us to assess what we perceived as the most significant threats to American badger populations, however, it was still limited in breadth and depth. Additional studies working towards a transboundary resistance surface would provide insights into whether patterns of genetic structure are caused by the same genetic barriers, or different genetic barriers, at more localized regions. Initial modeling efforts have made progress towards a transboundary modeling analysis (Washington Wildlife Habitat Connectivity Working Group 2013),

however they have not incorporated genetic information for many species, nor have they addressed connectivity for the entire range of American badgers in BC and Washington. Furthermore, additional landscape features could be included in model construction. We were limited by the environmental layers that were readily available and computational resources at our disposal. A more detailed layer of human development could improve our understanding of how human infrastructure is affecting badger populations in the province. Teasing apart the land use layer into individual resistance surfaces (i.e. rivers, agriculture, human land-use, etc.) may also help to explain the observed genetic patterns. Furthermore, one of the most prominent features that explains badger inhabitance is where prey are located (British Columbia Badger Recovery Team 2016). There is very limited information regarding the distribution, population sizes, and movement patterns of prey species for badgers in British Columbia, and models could be greatly improved by acquiring such information. Identifying barriers could also be greatly improved by using alternative modeling approaches. We attempted to use circuit theory in our initial analyses, but were limited by computational power for the scale and resolution of our analyses. Future studies could expand on our analyses by using circuit-theory at coarser resolutions, or by using individual-based modeling.

Another major limitation to our study was the initial primer design and optimization that was required for amplifying the fragment of the mitochondrial d-loop. We discovered that the published primer sequences we were attempting to use (Ethier *et al.* 2012) were highly divergent from the badger mitochondrial genome, leading to nonspecific amplification of other PCR products. Therefore, we created a new primer pair that would amplify our region of interest. It appears Kierepkaand Latch (2016b) also faced this problem, as they too designed new primers to amplify a fragment of the mitochondrial d-loop. Because both labs independently went about conducting primer design, the fragments chosen were not entirely overlapping. We chose to create new primers at the 5' end, while Kierepkaand Latch (2016b) designed primers towards the 3' end. While much of the fragments overlapped (415 bp), there were still several polymorphisms at the end of Kierepkaand Latch (2016b) sequences that we did not recover using our newly designed primers. This highlights the importance of publishing accurate information, as well as the value of pre-print initiatives. The study by Kierepkaand Latch (2016b) was completed in 2014, however it was not published until 2016.

If we were aware of the data collection strategy of the broad-scale analysis, we would have designed our study to have fully connectible data, which may have provided more detailed insights into the evolutionary history of American badgers.

In addition to marker optimization, our choice of molecular markers inhibited certain aspects of our study. We chose to use microsatellites and a fragment of the mitochondrial control region based on previous studies successfully using such markers to address questions of barriers to gene flow (Kierepka & Latch 2016a), and with the direct intent to connect our sequencing information with exemplar sequences from Canada-wide samples (Ethier *et al.* 2012), to which we did not have access. This connectivity was enhanced even further with the publication of the range-wide study (Kierepka & Latch 2016b). By using such molecular markers, we were able to answer a swath of questions regarding region and species-specific genetic patterns. However, there were instances where we were limited in the conclusions we could make based on our chosen markers. For instance, we did not identify discrete associations between genealogical lineages and geographical regions using the fragment of the mitochondrial d-loop. Perhaps by adding genetic information from other informative markers, such as nuclear genes, more detailed patterns regarding population history would have been found. This poor resolution also hindered the amount of information we recovered from ancient specimens. The use of additional molecular markers may have allowed us to explicitly test whether migration occurred from eastern Beringia to the American Midwest and West. Recent advances in genomic technologies, such as a targeted-capture approaches (Jones & Good 2016), allow researchers to obtain genome-scale information from degraded samples, such as ancient DNA. Using such methods may have provided more information from these unique specimens.

Lastly, there is clearly an interesting genetic pattern occurring between the Cariboo region and the other sampling units in western BC, which may reflect the differences in environment and ecology for badgers between these regions. Because we used neutral molecular markers, we were not able to test for associations between genetic variation and environmental variables to suggest natural selection contributing to differentiation. A study incorporating genome-wide measures of genetic variation, using techniques such as restriction-site associated DNA sequencing (RAD-seq; Baird *et al.* 2008)- a low-cost method for discovering and genotyping thousands of markers across the genome- may indicate the

potential for local adaptation and long-term divergence for badgers between these regions, thereby providing further support for conservation decisions of whether to designate populations in these regions as separate management units.

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Appendices

Appendix A: Chapter 1 Supplementary Material
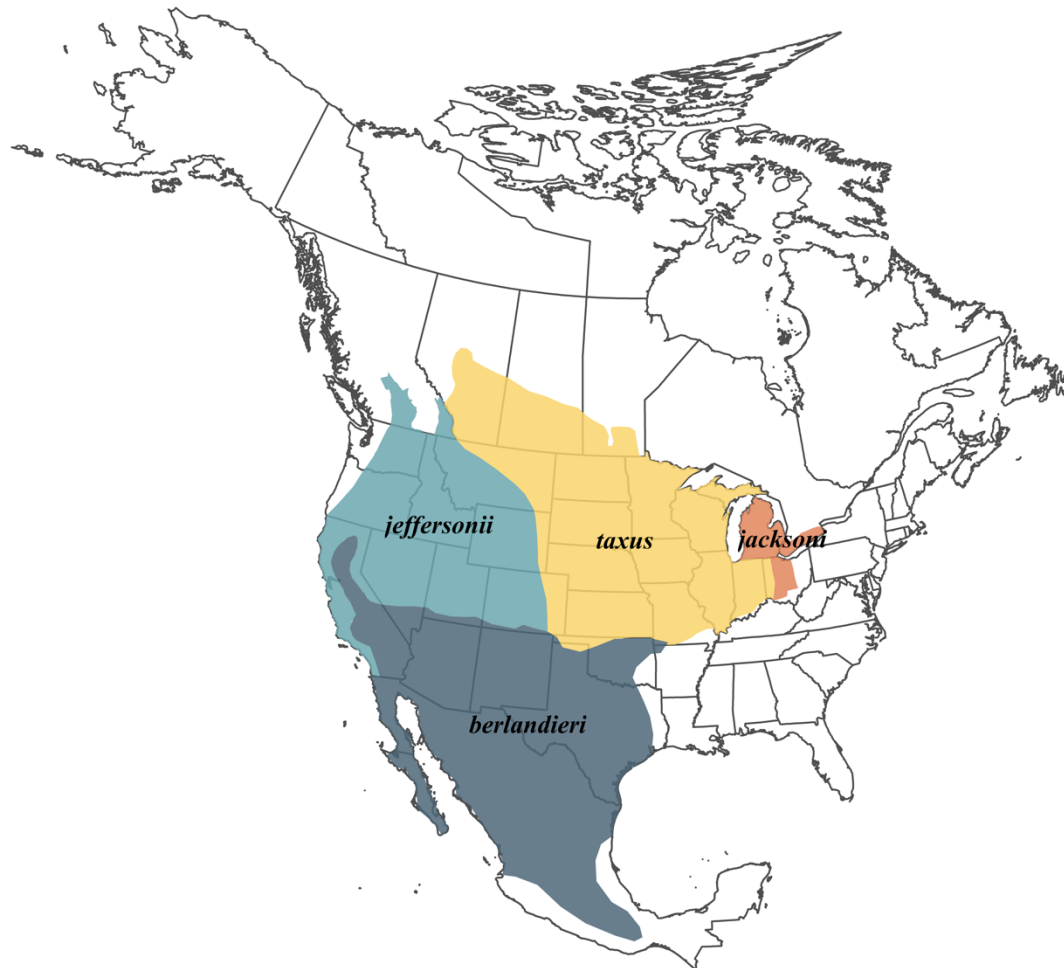
The distribution of the four American badger subspecies across North America.

Appendix B: Chapter 1 Supplementary Material

### Specimen details and sampling dates for historical samples at natural history museums.

Museum	Location	Specimen Localities	# of Samples	Collection Dates	Sampling Date
Royal British Columbia Museum	Victoria, British Columbia	British Columbia	23	1936-1988	8 Dec 2015
Burke Museum of Natural History & Culture	Seattle, Washington	Washington, Idaho	8	1921-2012	15 Apr 2016
Beaty Biodiversity Museum, UBCV	Vancouver, British Columbia	British Columbia	7	1934-1989	18 Apr 2016
Charles R Conner Museum	Pullman, Washington	Washington, Idaho	16	1947-2005	13 Apr 2016
James R Slater Museum of Natural History	Tacoma, Washington	Washington, Idaho	5	1948-1962	14 Apr 2016
Phillip L. Wright Zoological Museum	Missoula, Montana	Montana	13	1943-1971	13 Feb 2017

Primer sequences for the mitochondrial control region of the American badger, *Taxidea taxus*.

Fragment	Primer Sequences, 5' → 3'	T_a(°C)	Amplicon Size
CR1	ExtF: CCAACAATCAGCATTATCGAA R: ATATGAATAGAGTGATATGGTGAGGAA	50	204
CR2	F: TTCTAGCTTAAACTATTCCCTGAT R: AAGCTCGTGATCTAAGTGAAAT	50	200
CR3	F: TTTGCCCCATGCATATAA R: TCTATGGCCCTGAAGTAAGA	50	204
CR4	F: ATACTGAAACTATATCTGACATCTGG ExtR: ATGTGACAAGGCCTTTACGG	50	213

Source information for raster files used in resistance surface modeling analyses.

Layer	Source	Data Layer
Crown Closure	HectaresBC	Crown Closure
Elevation	HectaresBC	Elevation
Slope	HectaresBC	Slope
Land Use	HectaresBC	Baseline Thematic Mapping
Roads	Digital Road Atlas	Road Network
Soil	HectaresBC	Soil Parent Materials
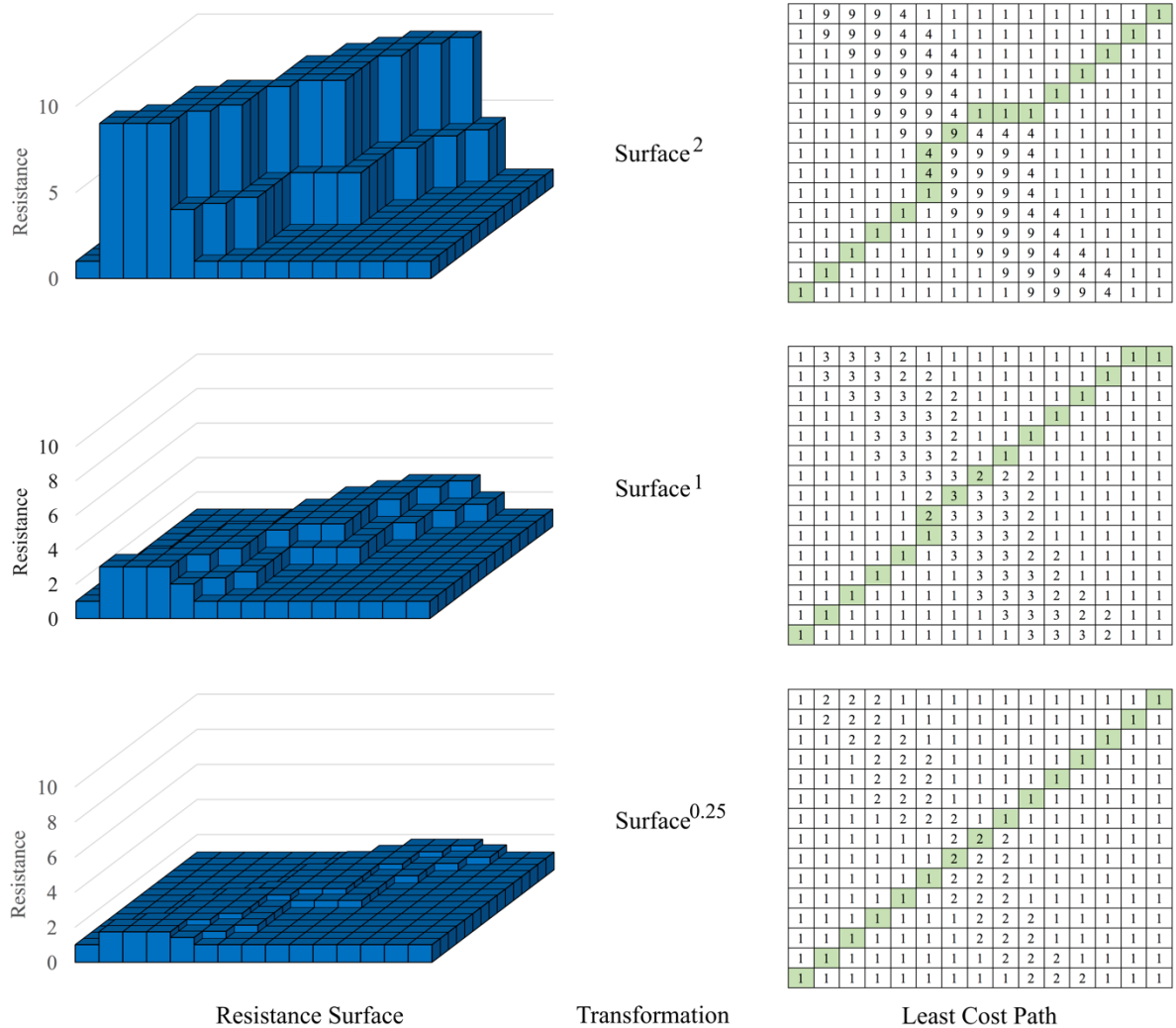
A conceptual outline of resistance surface parameterization. Depicted is the change in magnitude between low and high resistance values for each transformation, and the effect on the least cost path.

Buffer and resistance values for roadways in British Columbia. Road types were based on categories established by the Washington Wildlife Habitat Connectivity Working Group (2010).

BC Digital Road Atlas Class	WHCWG Roadway Type	Buffer (m)	Percent of Samples	Initial Resistance Value
Freeway	Freeway	500	13.4%	2
Highway	Major Highway	400	65.5%	10
Arterials, Collectors	Secondary Highway	300	16.8%	3
All Remaining Roads	Local Road	200	4.2%	1

**Resistance values for soil types in British Columbia, based on literature review.**

Resistance Value	Soil Types
1	Glaciolacustrine
2	Fluvial, Lacustrine
3	Organic, Undifferentiated, Eolian
4	Weathered Rock, Till
5	Anthropogenic, Volcanic, Marine, Glaciomarine, Fresh Water
6	Bedrock, Colluvium

AMOVA results for haplotypic and genotypic variation. Displayed is percent of variation among groups, among sampling units within groups, within groups, and the associated p-values, for three different grouping strategies within British Columbia.

| Hierarchical Level	df	Among Groups	Among Units within Groups	Within Units	p-value
Haplotypic Variation					
2 Designatable Units	1, 3, 123	60.92	2.61	36.37	***
3 Distinct Populations	2, 2, 123	54.07	0	46.03	***
5 Distinct Populations	4, 123	48.34		51.66	***
Genotypic Variation					
2 Designatable Units	1, 145, 147	7.79	10.46	81.75	***
3 Distinct Populations	2, 144, 147	4.36	3.59	92.06	***
5 Distinct Populations	146, 147	13.61		86.39	***

***significant at $p<0.005$
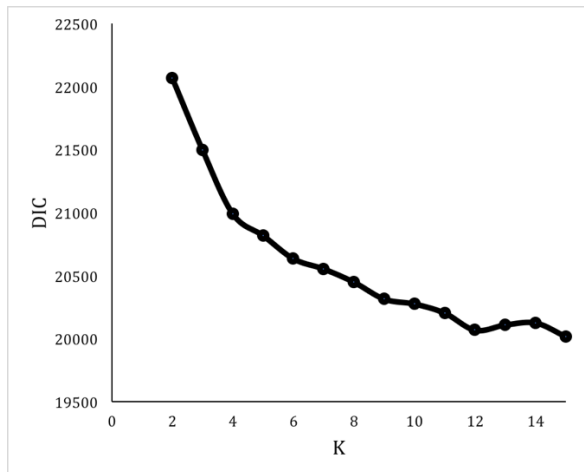
**Plot displaying the change in DIC, from the TESS analysis.**
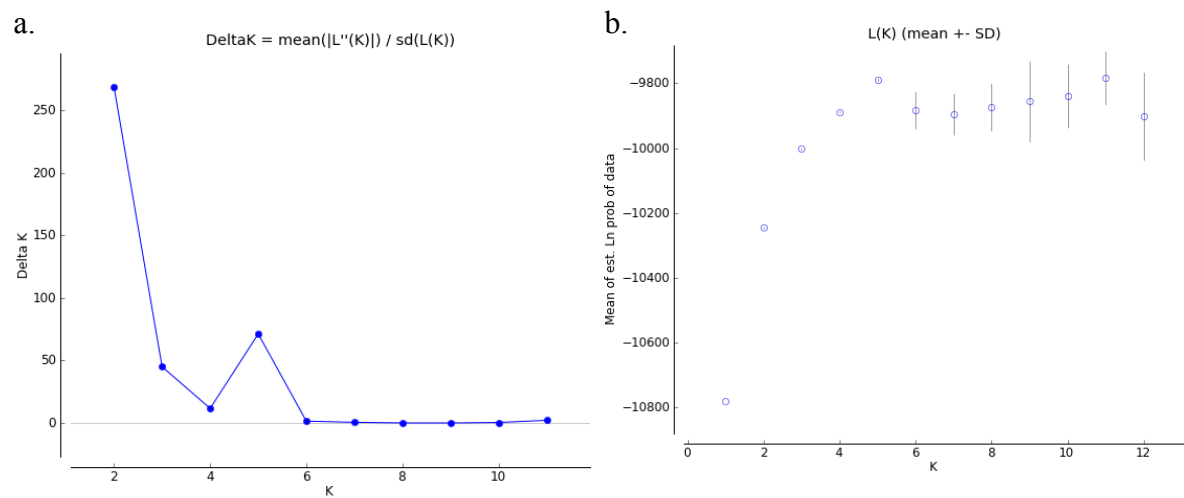


**Plots displaying ΔK (a), and the mean likelihood (b) from STRUCTURE analysis.**

84
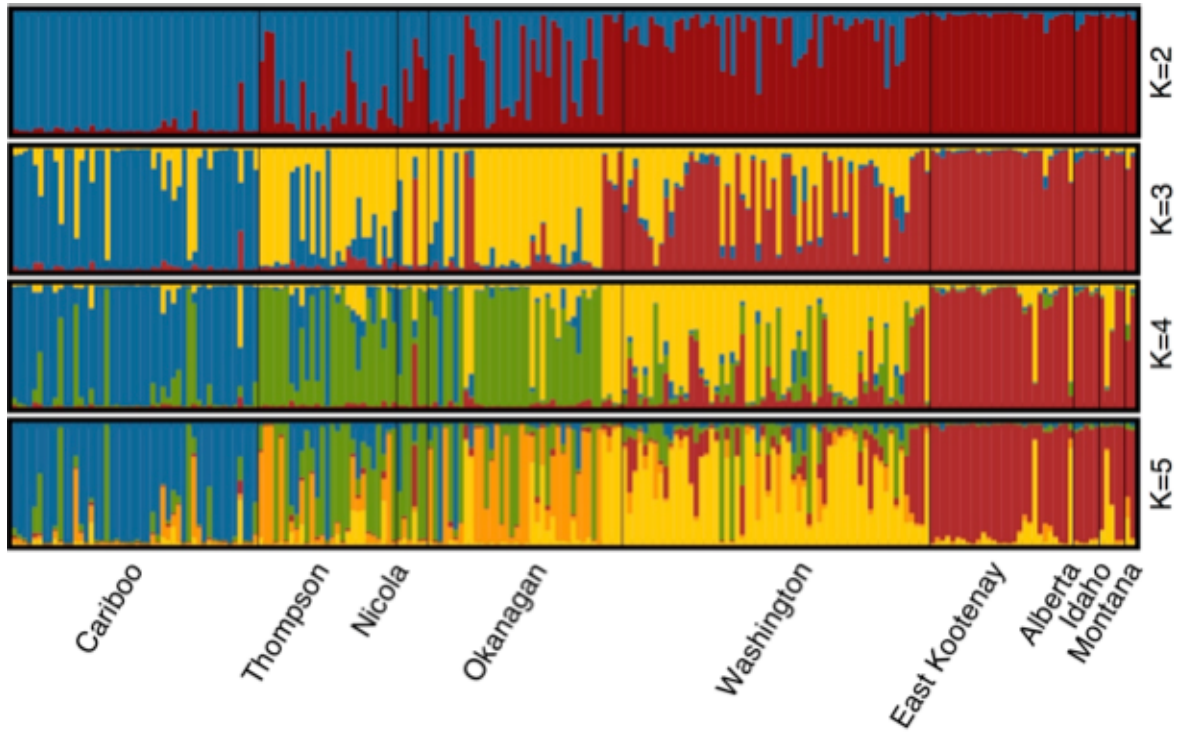
STRUCTURE barplots for individual American badgers genotyped in the Pacific Northwest, at K=2, K=3, K=4, and K=5. Individuals in each sampling units are sorted from highest to lowest latitude (i.e. North to South).
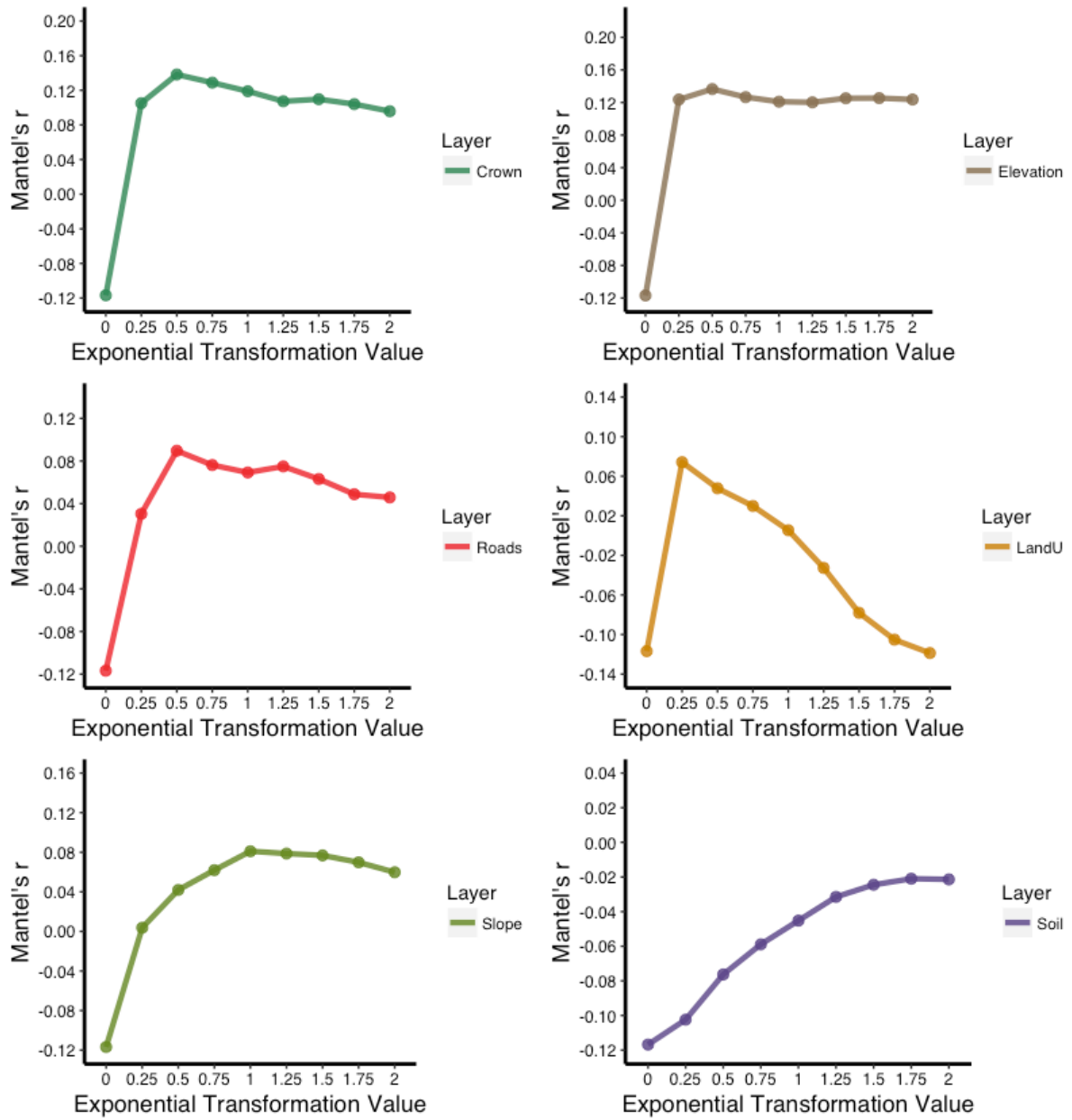
Line plots displaying the parameterization of resistance surfaces for broad-scale resistance surface analyses.
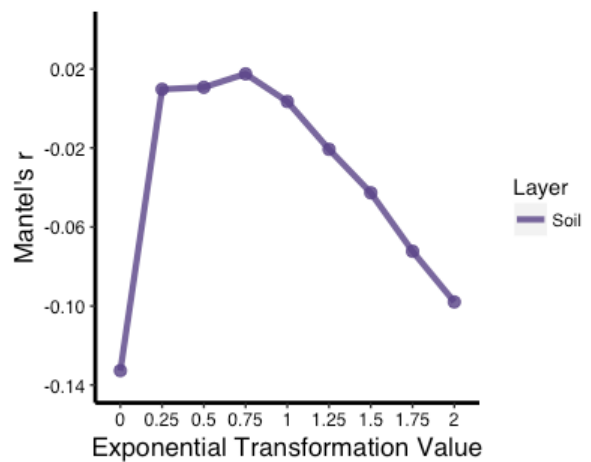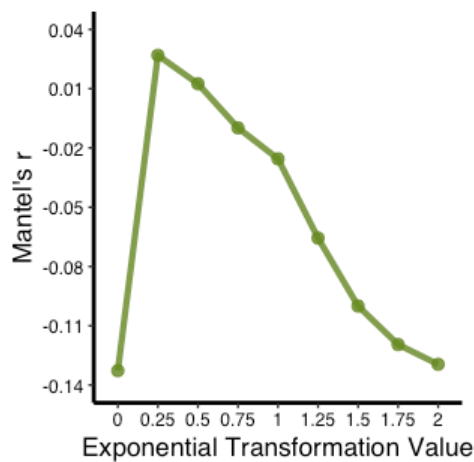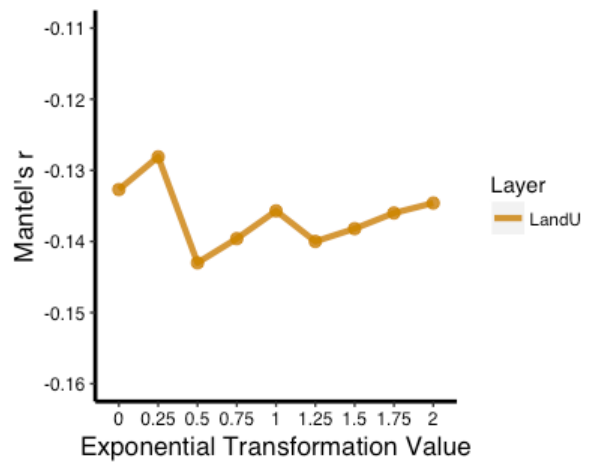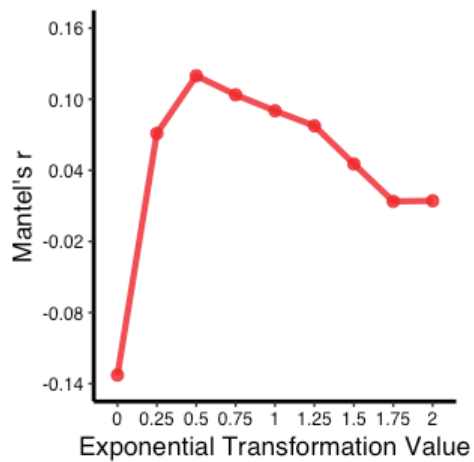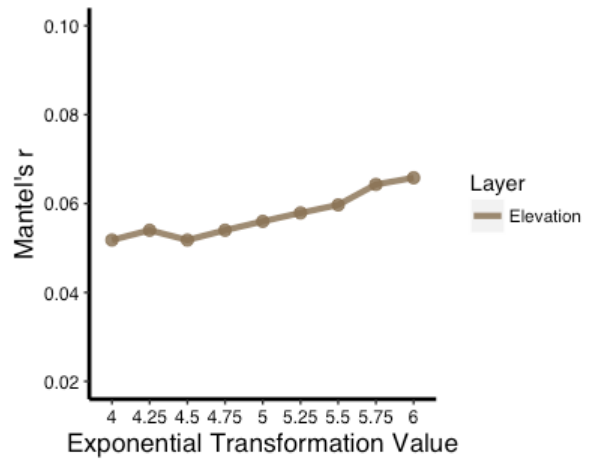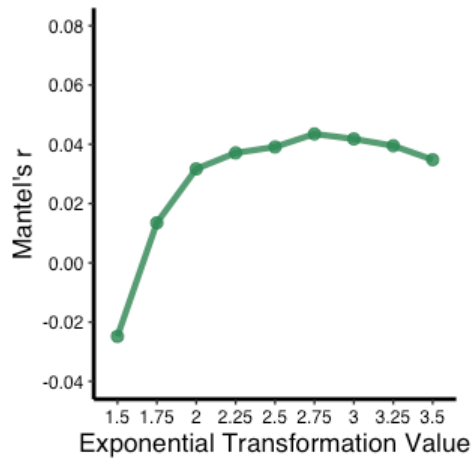
Line plots displaying the parameterization of resistance surfaces for fine-scale resistance surface analyses.

Appendix C: Chapter 3 Supplementary Material

Sampling units and sequence sources used for range-wide phylogeography and temporal analyses (*). K= Kierepka & Latch 2016, E = Ethier et al. 2012, and F = newly acquired sequences. Populations with low sample size (n<4; California, Arizona, Alaska, and Yukon) were excluded.

Sampling Unit	Abbreviation	N	Source
Cariboo*	CR	26	**F**: 26
Thompson*	TH	21	**F**: 21
Nicola*	NI	6	**F**: 6
Okanagan*	OK	40	**F**: 40
Washington*	WA	46	**K**: 1, **F**: 45
Oregon	OR	14	**K**: 13, **F**: 1
East Kootenay*	EK	34	**F**: 34
Alberta*	AL	51	**K**: 5, **E**: 41, **F**: 5
Idaho*	ID	29	**K**: 20, **F**: 9
Montana*	MT	78	**K**: 55, **E**: 12, **F**: 11
California	CA	3	**K: 1 F**: 2
Arizona	AZ	2	**K: 2**
Saskatchewan	SK	54	**K**: 26, **E**: 27, **F**: 1
Manitoba	MB	60	**K**: 4, **E**: 56
Wyoming	WY	8	**K**: 8
Utah	UT	44	**K**: 42, **F**: 2
Colorado	CO	6	**K**: 4, **F**: 2
New Mexico	NM	12	**K**: 11, **F**: 1
North Dakota	ND	58	**K**: 58
South Dakota	SD	78	**K**: 78
Nebraska	NE	60	**K**: 60
Oklahoma	OL	12	**K**: 12
Kansas	KS	31	**K**: 31
Minnesota	MN	67	**K**: 67
Iowa	IA	59	**K**: 59
Missouri	MO	8	**K**: 8
Wisconsin	WI	76	**K**: 73, **F**: 3
Illinois	IL	11	**K**: 11
Indiana	IN	4	**K**: 4
Ontario	ON	26	**E**: 26
Upper Peninsula	UP	35	**K**: 18, **E**: 17
Lower Peninsula	LP	118	**K**: 99, **E**: 19

Ohio	OH	28	**K**: 28
Alaska	AK	1	**F:** 1
Yukon	YT	1	**F:** 1

Distribution of major and semi-major haplotypes across North America. Dots on maps represent sampling units, with size corresponding to the proportion of individuals from the sampling unit with the haplotype.