University of Alberta

On the evolutionary history and population genetic structure of the North American mountain goat (*Oreamnos americanus*)

by

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ABSTRACT

The spatiotemporal scale at which genetic diversity is assessed can provide insights into both broad- and fine-scale patterns in ecology and evolution. I examined the distribution of genetic diversity and the evolutionary history of the North American mountain goat (*Oreamnos americanus*). I first reviewed how the unique physiography and glacial history of northwestern North America shaped the regions' genetic diversity. After reviewing more than 100 published studies, I found that species with high dispersal ability or with large contemporary ranges were the most likely to have resided in multiple refugia. Shifting to mountain goats, I reexamined the phylogenetic affinities of the mountain goat using a total evidence approach and likelihood-based tests of alternative hypotheses. I evaluated all published topologies and found mountain goats to be an independent basal lineage in the Caprinae family. I then examined the phylogeographic and population structure of the mountain goat using a variety of molecular markers. I found evidence of a hitherto unknown northern and coastal refugia, and found no association between immune gene variation and refugial history. The latter finding suggests that the current distribution of immune diversity was not a direct result of the last glacial maximum. Examining the spatial genetic structure of mountain goats, I detected seventeen highly differentiated subpopulations and found that mountains ranges facilitated gene flow. I then examined the fine-scale landscape genetic structure of mountain goats by combining genetic data with mountain goat location data using geographic information systems. I showed that summer habitat used by female mountain goats was the best predictor of gene

flow, and identified a suite of habitat variables important for genetic connectivity. Finally, I found that dispersing mountain goats tended to be less genetically diverse than residents, which supports the fitness associated dispersal hypothesis. These results shed important insight on the evolution and ecology of mountain goats and have implications for conserving the alpine and its inhabitants.

PREFACE

The vast majority of this thesis represents my individual efforts. However, as good science is achieved through mentorship and collaborations, “we” is used throughout the text of the data chapters as a reflection of those involved. All of the data chapters have been published in peer-reviewed journals and follow their respective journals’ referencing format. The appropriate citation is provided at the beginning of each chapter.

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Chapter 1

General Introduction

1.1. Introduction

Both historical and contemporary factors influence the distribution of genetic diversity within a species. Such factors ultimately shape the evolutionary history of a species by influencing population differentiation, local adaptation, and even speciation (Schluter 2000). Evaluating genetic diversity and estimating various population parameters are recognized as important tools for conservation and management of wild populations (Schwartz et al. 2007). Accordingly, the analysis of genetic diversity at a variety of spatial and temporal scales (i.e. phylogeny, phylogeography, and population genetic structure) can provide insights into a suite of ecological, evolutionary, and conservation related questions.

To fully understand the processes shaping species evolution, Avise's (1987) seminal work suggested that we must first begin with its phylogeny. The phylogenetic history of a species can influence its adaptability (Willis et al. 2008), extinction risk (Roy et al. 2009), and population genetic structure (Duminil et al. 2007). Identifying phylogenetically diverse lineages (and their centers of radiation) is also considered important for the preservation of biodiversity (Erwin 1991). Practically speaking, when information is lacking on a study species, researchers can turn to the species' phylogeny to help inform evolutionary and ecological hypotheses (Brooks and Mclennan 1991). Achieving the proper phylogenetic placement of a species is therefore central to understanding its evolution.

Moving down the evolutionary scale (Fig. 1 in Avise 1987), phylogeography bridges the gap between phylogeny and population genetics. In its most basic definition, phylogeography is the study of the geographic distribution of genealogical lineages (Avise 1987). However, since its inception, phylogeographic models and methods have improved tremendously (Knowles 2009), and we can now use them to detect past refugial sites (Hewitt 2000), patterns of demographic expansion (Lessa et al. 2003), and the influence of extrinsic factors such as glaciations and physiography (Soltis et al. 2006). In addition, the identification of evolutionarily significant units has largely been

based on phylogeographic criteria (Moritz 1994). Reconstructing a species' phylogeography is an essential component to tracing its evolutionary history and forms the foundation of determining its population genetic structure.

Among populations, large features such as mountain ranges and ocean currents help shape the spatial distribution of genetic diversity (e.g. Worley et al. 2004, White et al. 2010). However, individual-based analyses are often required to determine the fine scale variables influencing genetic connectivity. As a result, the field of landscape genetics has recently emerged (Manel et al. 2003) and its main goal has been to quantify the influence of heterogenous landscapes on gene flow. In addition to assessing landscape effects, understanding the reasons animals disperse is important as it directly impacts population dynamics and genetic structure (Bowler and Benton 2005). Thus, to achieve a full and broad understanding of a species' evolutionary history we must characterize its phylogeny, phylogeography, and population genetic structure.

1.2. Thesis objectives and data chapters

One of the most enigmatic species in western North America is the mountain goat, *Oreamnos americanus* (Figure 1-1). This charismatic climber inhabits steep, remote alpine terrain and is extremely sensitive to human disturbance (Festa-Bianchet and Côté 2008). The long-term study at Caw Ridge has provided detailed information on the mountain goats' ecology and life-history (Festa-Bianchet and Côté 2008), but our understanding of their phylogenetic relationships, phylogeographic history, and spatial genetic structure (and diversity) is minimal. Addressing such questions in the mountain goat is of value to conservation and management efforts, and provides a framework to test broader evolutionary and biogeographic hypotheses. For my doctoral research, I examined the evolutionary history and population genetic structure of the mountain goat. Although the specific data chapters focus largely on the mountain goat, they address broader biogeographic hypotheses of western North America and test evolutionary hypotheses on the patterns and processes shaping genetic diversity.

The thesis is divided into seven data chapters, the first of which is a review and meta-analysis of the phylogeographic patterns in northwestern North America. Chapters 3 though 8 analyze molecular data generated in mountain goats, with the last two (7 and 8) taking advantage of data on individually marked mountain goats at Caw Ridge, Alberta, and southeast Alaska (Figure 1-1, 1-2).

In **Chapter 2**, I present a review of the phylogeographic history of northwestern North America. I collected data on species' phylogeographic history and looked for co-distributed patterns of refugial sites and colonization routes. I then tested for correlations between refugial history and various ecological characteristics, and discuss the ecological and evolutionary implications of a species' phylogeographic history.

In **Chapter 3**, I re-evaluated the Caprinae phylogeny with the specific goal of resolving the placement of the mountain goat. I collected all available sequence data for members of the Caprinae and constructed a phylogeny using three different tree-building methods. I then quantitatively compared the topological placement of mountain goats that I generated to all those published in the literature.

In **Chapter 4**, I assessed the phylogeographic history and spatial genetic structure of the mountain goat using microsatellite and mitochondrial DNA. I tested various refugial scenarios and evaluated the influence of mountain ranges and refugial history on genetic differentiation. I also compared diversity of populations in the center of the range to those on the periphery to test the central-marginal hypothesis.

In **Chapter 5**, I sequenced a suite of immune genes to get a range-wide estimate of genetic health and diversity. I then compared an individuals' observed immune diversity with their refugial origin to test whether bottlenecks that occurred during the last glaciation shaped the current distribution of immune gene diversity.

In **Chapter 6**, I sequenced a series of Y chromosome genes to clarify the evolutionary history of mountain goats on Baranof Island, Alaska, and to assess whether they conformed to the criteria of an evolutionarily significant unit. I

reviewed the ethnohistory of the region to provide additional support for the purported patterns.

In **Chapter 7**, I evaluated the landscape genetic structure of mountain goats in southeast Alaska. Using GPS radio-collared mountain goats, I constructed landscape resistance surfaces based on the habitat selections patterns of the collared animals. I constructed multiple resistance surfaces according to sex and season, and compared their ability to predict genetic relatedness to two null models of genetic differentiation.

In **Chapter 8**, I calculated the level of genetic heterozygosity of dispersers identified from genetic cross-assignments and individual monitoring at Caw Ridge, Alberta. I tested the fitness associated dispersal hypothesis by comparing levels of heterozygosity seen in dispersers to non-dispersers (residents).

Figure 1-1. A group of mountain goats grazing at Caw Ridge, Alberta. All mountain goats at the Caw Ridge study site are uniquely marked with an ear tag(s) and collar. All three visible mountain goats in this photo are female and partially molted.

Figure 1-2. Mountain goats visiting the traps at Caw Ridge, Alberta. Once captured a variety of morphological measurements are taken and individuals are individually marked. Male mountain goats are fitted with either a VHF or GPS radio-collar.

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Chapter 2

Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North America

A version of this chapter has been published:
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2.1. Introduction

Throughout the Quaternary the distribution of the world's biota has been shaped by climatic fluctuations. During this time, the ebb and flow of glaciers forced populations into several major ice age refugia. These glacial refuges were the location where species survived the ice ages, regardless of geography or size, and contained some degree of suitable habitat (Holderegger & Theil-Egenter 2009). Within these refugia, populations were restricted and often isolated, becoming genetically differentiated over time. When the ice-sheets receded, species colonized newly available terrain (often) in a leading edge expansion, resulting in the reduction of genetic variation in a clinal fashion (Hewitt 2004). However, increased genetic diversity in colonized populations did occur when lineages from separate refugia mixed (Petit *et al*. 2003). Historic subdivision and population isolation within refugia, termed *refugia within refugia*, also helped maintain genetic diversity (Gómez & Lunt 2007). In addition, some species persisted in cryptic glacial refugia (Stewart & Lister 2001; Provan & Bennett 2008) that contained quasi-hospitable climates capable of sustaining at least some temperate flora and fauna. The locations and internal complexity of these major and cryptic ice age refugia, and the subsequent colonization of deglaciated terrain, are partially responsible for shaping the genetic structure and distribution of contemporary biota (Hewitt 1996, 2000, 2004; Widmer & Lexer 2001).

Determining the location of refugia typically requires the knowledge of species distributions prior to glaciation (Waltari *et al.* 2007). Historically, glacial refugia have been inferred from paleoecological data. Fossil and pollen records provide historical snap-shots that are used to reconstruct refugial locations and communities; unfortunately, these methods have considerable shortcomings. Fossil evidence is often sparse or incomplete and pollen analyses have taxonomic and spatial limitations (see Ritchie 1995; McLachlan *et al*. 2005), especially in the Arctic and alpine (Birks & Birks 2000). The lack of sufficient data means that smaller refugia on islands and nunataks may never be discovered because they are either overlooked or leave no discernable evidence (Pielou 1991). For example,

Haida Gwaii has long been hypothesized as a North American coastal refugium (Heusser 1989) and is widely applied in biogeographic models. However, paleoecological findings from the last glacial maximum are extremely limited for this region (i.e. Wigen 2005; Lacourse & Mathewes 2005). This paucity of paleoecological data makes it exceedingly difficult to infer refugial communities and colonization routes from postulated refugia.

Phylogeography provides a valid alternative for inferring refugia and colonization patterns. This approach does not require historical samples, as it is based on the geographic distribution of genealogical lineages that have evolved since the initial separation (Avise *et al*. 1987; Avise 2000). Under this framework, hypotheses regarding how populations responded to geologic and climatic fluctuations can be tested (Knowles 2001). Early phylogeographic models were very simple; for example, the 'southerly refugia model' (Bennett *et al.* 1991) predicted a leading edge expansion from southern refugia to the north following glacial retreat, resulting in a latitudinal gradient of decreased genetic diversity (Hewitt 1996, 2000, 2004). These models have become somewhat obsolete as they do not take into account lineage mixing (Petit *et al*. 2003), additional complexity (e.g. *refugia within refugia*) (Gómez & Lunt 2007), or deeper historical associations (Lovette & Bermingham 1999). To account for such multifaceted phylogeographic histories, analytical methods have expanded to encompass coalescent and ecological-niche modeling (Carstens & Richards 2007; Waltari *et al.* 2007) and phylogeography now pulls from a wide array of disciplines (Knowles & Maddison 2002; Knowles 2009). These advancements coincide with unprecedented growth in the field (Knowles 2009).

In the past decade, the expanding interest in phylogeography has led to the discovery of novel patterns and refugial sites. To better understand these complex patterns, researchers strive to find congruence among co-distributed taxa, and connect the observed patterns to underlying processes (Bermingham & Moritz 1998; Avise 2000). This approach is required to identify refugia and colonization routes in the absence of, and in addition to, paleoecological data. Given the plethora of studies and continued evolution of the field, it has become critical to

periodically review the broader phylogeographic patterns among taxa within regions (e.g. Europe - Taberlet *et al*. 1998; Petit *et al*. 2002; North America - Avise 2000; Soltis *et al*. 2006; Jaramillo-Correa *et al*. 2009). There is now sufficient published data to systematically review the complex biogeographic history of northwestern North America, and thereby identify shared patterns of colonization and identify cryptic refugia.

2.1.1. Reviewing the major patterns of northwestern North America

Ice-sheets covered much of Canada during the Pleistocene, reaching their largest extent some 20,000-years ago (Clark *et al*. 2009). The glacial advances in North America were among the most extensive worldwide (Velichko *et al*. 1997), and followed regular climatic intervals known as the Croll-Milankovich cycle (Imbrie 1985; Muller & MacDonald 1997). This cycle dictated glacial movement throughout the Pleistocene, causing biota to respond by shifting their range or going extinct. The fossil record shows large changes in species distributions that corroborate these cycles (Bennett 1997; Williams *et al*. 1998). For most northwestern biota, two large refugia were available; Beringia and the Pacific Northwest (Fig. 2-1; Hultén 1937; Pielou 1991). Species could have either retreated north, south, or in both directions during glacial advances. This has resulted in the broad classification of colonizing taxa as either Beringian or southern in origin (Youngman 1975; Hoffmann 1981).

While the glaciations are widely recognized as having significant impacts on species distributions across multiple continents (Taberlet *et al.* 1998; Soltis *et al.* 2006), the northwest of North America has a unique complexity due to the physiography of the region. There are essentially two contiguous north-south mountain ranges: the Rocky Mountains, which define the eastern extent of the northwest, and the Coast Mountains, which occur along the Pacific Coast. Moreover, the Cascade/Sierra orogeny was recent enough to have detectable impacts on current species distributions (Graham 1999; Brunsfeld *et al*. 2001). Based on this orogeny, Brunsfeld *et al.* (2001) proposed a historic vicariant pattern between the coast and the northern Rockies.

Adding to the intricacies of this region is the potential existence of cryptic refugia. Soltis *et al*. (1997) suggested that coastal refugia (i.e. Haida Gwaii and the Alexander Archipelago) were important for surviving biota. In areas where glaciation was not complete, species could also have persisted in small within-ice refugia (e.g. nunataks; Pielou 1991). Moreover, the influence of Beringia was largely overlooked in earlier reviews of the northwest. In the decade since Brunsfeld *et al*. (2001), new patterns and processes have been uncovered in this region with many including a role for Beringia and cryptic refugia. Based on this, studies have called for the re-examination of the phylogeographic patterns and a thorough assessment of cryptic refugia in northwestern North America (e.g. Cook *et al*. 2001; Demboski & Cook 2001; Janzen *et al*. 2002; Fedorov *et al*. 2003; Spellman *et al*. 2007).

The aim of this review is to characterize the broad scale phylogeographic patterns in northwestern North America, and to therefore identify refugial locations and colonization routes. We have three major predictions: 1) the phylogeographic history of northwestern North America will be complex, but repeated patterns and substructure (i.e. *refugia within refugia*) will be evident in both plants and animals; 2) major and cryptic refugia will be detected and supported by at least some paleoecological evidence; and 3) certain ecological traits (e.g. habitat specialization) may be associated with refugial history (Table 2-1). Given the complex glacial and physiographic history of this region, we attempted to distinguish between older historical events (e.g. mountain orogeny) and more recent sub-structuring due to multiple glacial refugia. We also discuss the evolutionary and ecological implications of the inferred broad scale phylogeographic patterns in northwestern North America.

2.2. Materials and methods

2.2.1. Study area

For the purpose of the review we are considering northwestern North America to include the following states and province/territories: Alaska, Yukon, Northwest Territories, Alberta, British Columbia, Washington, Oregon, Idaho, Wyoming,

and Montana (Fig. 2-1). The eco-regions within this area include the Pacific coast temperate rain forest, the northwestern-forested mountains, western desert, taiga, and tundra. The physiography of the region is varied, but notably two major mountain ranges run longitudinally: the Coast Mountains along the Pacific Ocean and the Rocky Mountains in the interior (Fig. 2-1).

2.2.2. Literature review

We first searched *Web of Science* using the keywords 'phylogeography' and 'cryptic refugia.' Selected studies had to encompass a portion of northwestern North America. Because biogeographic patterns of the northwest are intertwined with northerly refugia, we included 'Beringia' in our searches. A citation search for all papers referencing the early reviews of Soltis *et al*. (1997) and Brunsfeld *et al*. (2001) was also conducted. Our focus was on literature post-2000 since the reviews by Soltis *et al.* (1997) and Brunsfield *et al.* (2001) summarized much of the earlier work that was done in the region. We included some earlier phylogeographic studies as supportive evidence where applicable. Retrieved papers were qualitatively assessed on: i) sampling, ii) observed pattern, and iii) robustness of pattern. Differing from Brunsfeld *et al*. (2001), we incorporated non-mesic taxa. We sought out additional paleoecological and ecological evidence in the literature to support the purported patterns.

2.2.3. Characteristics of species and refugial type

Following the methodological approach applied by Hickerson & Cunningham (2006), we tested whether certain ecological traits were correlated with the refugial history of vertebrates and plants. We used a dichotomous scale similar to that of Bhagwat & Willis (2008) with each species evaluated for: i) dispersal ability (0 - low, versus 1 - high), ii) habitat specialization (0 - generalist, versus 1- specialist), and iii) size of contemporary range (0 - encompassing a single refugium, or 1 - multiple refugia). The dependent variable was survival during the Pleistocene in single or multiple refugia. The scoring schemata are presented in Appendix I. In an effort to standardize how each ecological variable was scored across species, we utilized three major sources for data collection: the International Union for Conservation of Nature (www.iucn.org), NatureServe

(http://www.natureserve.org/), and the United States Department of Agriculture (http://plants.usda.gov/). The majority of species had range maps and ecological/biological descriptions at these sites. Because we were examining a wide array of taxa, scoring was conservative and standardized when possible. If multiple studies were used to draw inferences on refugial history, species were only entered once in the dataset. Because one of the covariates (i.e. range) appeared highly predictive, Firth's penalized-likelihood logistic regression (Firth 1993; Heinze & Schemper 2002; Heinze 2006) was used to assess the strength and direction of the relationship between the ecological variables and refugial history. This method avoids issues of separation arising from highly predictive covariates and has been shown to provide good estimates of logistic regression coefficients (Heinze & Schemper 2002). Univariate models were conducted on the entire data set as well as on individual groups (mammals, birds, herpetofauna, and plants). The models were evaluated by converting the logistic *b* coefficient to an odds-ratio (OR) using the formula $OR = e^{b}$, with the 95% confidence interval calculated using the formula: $e^{(b \mp 1.96 * \text{standard error})}$.

2.3. Results and discussion

2.3.1. Literature review

Since 2000, over 4800-refereed papers were catalogued under the keyword 'phylogeography.' Of these, 'Beringia' and 'western North America' were listed as keywords 41 and 183 times respectively. An additional 100 studies were listed under the keyword 'cryptic refugia.' All keywords showed a general increase in the number of publications over the past decade. Focusing on species that had a distribution encompassing a part of northwestern North America, 126 relevant phylogeographic studies were retrieved in our literature review (see Table 2-2). Our qualitative assessment of northwestern North America's phylogeographic patterns is included in Table 2-2. Overall, plants and mammals made up the bulk of the phylogeographic studies (60%), but insects, birds, fish, herpetofauna, and parasites contributed to the review. We found that including non-mesic taxa revealed fewer plant studies ($n = 39$) compared to vertebrate studies ($n = 87$). This

contrasts with the review of Brunsfeld *et al*. (2001), but is similar to the review of eastern North America (Soltis *et al*. 2006). Unlike Soltis *et al*. (2006), mammals are not underrepresented in our review (n = 36) and we found no relevant turtle studies. The differences between the composition of our literature review and that of Brunsfeld *et al.* (2001) and Soltis *et al.* (1997) can largely be attributed to our inclusion of non-mesic taxa. The distinctive biotic composition in both northwestern and eastern North America accounts for some of the differences with Soltis *et al*. (2006).

2.3.2. Ecological variables associated with refugial history

We found suitable data on 103 vertebrate and plant species to assess the association between contemporary range, habitat specificity, and dispersal ability with refugial history (Appendix II). Scoring of multiple refugia was based on phylogeographic data and refugial scenarios suggested by the original authors (Table 2-2). All studies had patterns consistent with Pleistocene refugia. Phylogeographic breaks that could not be distinguished from contemporary or pre-Pleistocene influences were not included. We used penalized regression to offset any bias in sample size and because some explanatory variables almost perfectly predicted the dichotomous refugial history (Heinze 2006). Habitat specialists appeared less likely to have persisted in multiple refugia (Table 2-3). On the other hand, we found a positive relationship between multiple refugia and dispersal ability (b = 1.82) and contemporary range (b = 2.86). The OR of dispersal and range were significantly above one, indicating that species with high dispersal ability and large contemporary ranges were more likely to have resided in multiple refugia during glacial advances (Table 2-3). Analyses of individual groups showed the same general pattern but with lower levels of significance (Table 2-3).

After applying a penalty to the log-likelihood, the OR of contemporary range with respect to multiple refugia was the highest across the entire dataset and within groups (Table 2-3). Similar positive associations between northern refugia and range, habitat specialization, and dispersal ability were observed for European plants and vertebrates (Bhagwat & Willis 2008). Svenning & Skov (2004) found

that northern European trees filled most of their potential range (relative to southern species), and attributed the difference to dispersal ability. Efficient dispersal of northern populations would have facilitated efficient range shifts during glacial advances and possibly permitted gene flow between disjunct populations. Moreover, there appears to be a direct link between phylogeography and dispersal ability, such that historical substructure within species can create conditions that promote efficient dispersal (discussed further in section 2.3.10.). Overall these data suggest species with large contemporary ranges and high dispersal ability are significantly more likely to have resided in multiple refugia.

2.3.3. Biotic responses to the changing Pleistocene climate

During glacial advances, most species in western North America retreated to major refugia in either Beringia or the Pacific Northwest (Fig. 2-1: Hultén 1937; Pielou 1991). Some widespread plant species like rockcress, *Boechera* spp. (Dobes *et al*. 2004a) and white spruce, *Picea glauca* (Anderson *et al*. 2006), along with mammals such as the ermine, *Mustela erminea* (Fleming & Cook 2002), caribou, *Rangifer tarandus,* (Flagstad & Roed 2003) and red fox, *Vulpes vulpes*, (Aubry *et al.* 2009) occupied both major refugia. However, it quickly becomes apparent that there is a considerable amount of variation and complexity within species that challenge the view of single northern and southern origins for western North America's biota. In terms of the biotic response, we focused on three major areas: i) south in the Pacific Northwest; ii) north in Beringia; and iii) within areas overlooked or previously undiscovered (i.e. cryptic refugia).

2.3.4. South of the ice-sheets

Refugia in the Pacific Northwest were originally split into two mountainous regions: the Cascade/Coast Range and the northern Rockies (Brunsfeld *et al*. 2001). This phylogeographic split is still identified as a predominant pattern among comparative studies (Carstens *et al.* 2005; Jaramillo-Correa *et al*. 2009). Within vertebrates we see this split in herpatofauna like salamanders *Plethodon vandykei* and *Plethodon idahoensis* (Carstens *et al.* 2004), Pacific tree frog, *Pseudacris regilla* (Ripplinger and Wagner 2004, Recuerco *et al.* 2006), western toad, *Anaxyrus boreas* (Goebel *et al*. 2009), and the spotted

frogs, *Rana luteiventrus* and *Rana pretosia* (Bos & Sites 2001; Funk *et al.* 2008). We also see a similar pattern in the American pika, *Ochotona princeps* (Galbreath *et al*. 2009), long-tailed vole, *Microtus longicaudus* (Conroy & Cook 2000; Spaeth *et al*. 2009), and yellow-pine chipmunk, *Tamias amoenus* (Demboski & Sullivan 2003). Blue grouse, *Dengragapus obscurus*, show this same split (Barrowclough *et al*. 2004) which resulted in recent recognition as distinct species (Banks *et al*. 2006).

In mammals, a larger break of the Coast Range from the continent is commonly observed for species endemic to the northern coniferous forests (Stone *et al*. 2002; Arbogast 2007; Yang & Kenagy 2009) or distributed across North America (Runck & Cook 2005; Latch *et al*. 2009). This overarching phylogeographic split between the Coast Range and northern Rockies/continent can largely be attributed to the orogeny of the Cascade/Sierra chain that occurred 5-2 million years ago (Graham 1999; Brunsfeld *et al*. 2001). The rise of this mountain range created a rain shadow, leading to the xerification of the Columbia River basin and thus a disjunct mesic forest (Daubenmire 1975). Where utilized, molecular dating of clade divergences often corresponds to this event (e.g. Wilke & Duncan 2004; Toews & Irwin 2008). Some other shared phylogeographic splits involving the mountain chains are not specifically from orogeny, but allopatric diversification during historic glacial advances (Johnson & Cicero 2004; Weir & Schluter 2004).

In addition to the east-west split, there is also a north (British Columbia to Oregon) south (Oregon to California) separation within taxa in the Coast/Cascade region that is commonly observed (Soltis *et al.* 1997; Jaramillo-Correa *et al*. 2009). This split has become affectionately known as the "Soltis line" (Fig 2-2; Brunsfeld *et al*. 2007). Examples of this split range from the blue-grey tail-dropper slug, *Prophysaon coeruleum* (Wilke & Duncan 2004), torrent salamander, *Rhyacotriton variegatus* (Miller *et al.* 2006a), to sugar pine, *Pinus lambertiana* (Liston *et al.* 2007). Explanations for the "Soltis line" are less obvious as the break is dynamic (angled dashed line in Fig. 2-2), and occurs in the absence of any obvious physiographic break. Soltis *et al*. (1997) attempted to

reconcile this pattern by proposing different refugial-colonization scenarios resulting from Pleistocene glaciations (Soltis *et al*. 1997). Our accumulated data suggest both multiple Pleistocene refugia in the Pacific Northwest (Table 2-2, Fig. 2-2) and northern colonization (which were Soltis *et al*.'s (1997) original hypotheses) could explain the "Soltis line."

While the large-scale patterns are east-west and north-south separations in the southern refugium, the literature suggests more complex patterns and structure within both the coastal and northern Rocky regions (Fig. 2-2). In plants, Godbout *et al*. (2008) found evidence for three separate refugia of lodgepole pine, *Pinus contorta*, in the Cascades, Columbia River basin, and east of the Rockies in Montana; these refugia are supported by the fossil record (Baker 1976; Mehringer *et al*. 1977; Mack *et al.* 1978; Carrara *et al*. 1986). Similarly, phylogeography of the whitebark pine, *Pinus albicaulis* suggested the coastal mountains, Clearwater basin, and Yellowstone were distinct refugia (Richardson *et al*. 2002), some of which are supported by fossil pollen (Baker 1990). In the Wyoming basin, a phylogeographic split for yellow stonecrop, *Sedum lanceolatum* occurs, suggesting two distinct refugia in this region (DeChaine & Martin 2005). Brunsfeld & Sullivan (2005) and Brunsfeld *et al*. (2007) also found four distinct groups of Constance's bittercress, *Cardamine constancei* in the Clearwater River drainage, as well as a north-south split between the Clearwater and Salmon rivers in the Dusky willow, *Salix melanopsis*. These latter two refugia are also observed in herpetofuana (Nielson *et al*. 2001, 2006; Carstens & Richards 2007). In the long-tailed vole, individuals from Wyoming and Colorado are basal in their phylogeny (Conroy & Cook 2000), suggestive of Pleistocene refugium on the eastern side of the Rockies. In red-tailed chipmunks, *Tamias ruficaudus*, an east-west split occurs in the northern Rockies as well as a subdivision within the Clearwater drainage (Good & Sullivan 2001): all of these examples suggest historical substructure and multiple refugia within the northern Rockies.

We also observed multiple refugial sites in the Cascade/Coast range. For Stellar's jay, *Cyanocitta stelleri* (Burg *et al.* 2005), Canada goose, *Branta canadensis* (Scribner *et al*. 2003), and the spotted owl, *Strix occidentalis*

(Barrowclough *et al*. 1999) the Coast Mountains are implicated as a refugium, but the specific locations could not be determined. More precise refugial locations are inferred from herpatofauna. The Columbia River area was suggested as a refuge for the Oregon salamander, *Batrachoseps wrighti* (Miller *et al.* 2005), and Larch Mountain salamander, *Plethodon larselli* (Wagner *et al.* 2005). The Klamath-Siskiyou Mountains appeared to be a refugium for the tailed frog, *Ascaphus truei* (Nielson *et al.* 2001, 2006), Del Norte salamander, *Plethodon elongatus* (Mahoney 2004), and rough-skinned newt, *Taricha granulosa* (Kutcha & Tan 2005). Some species like the western toad (Goebel *et al.* 2009) and salamanders, *Dicamptodon* spp. (Steele & Storfer 2006, 2007), likely resided in both of these refugia. Additional support for these refugia comes from phylogeographic studies of both foxtail pine, *Pinus balfouriana* (Eckert *et al*. 2008), and rockcress (Kiefer *et al*. 2009) that suggested the Klamath-Siskiyou Mountains was their refugial location. The Blue Mountains in northeast Oregon have also been identified as a regional refuge (Arbogast *et al*. 2001; Thompson & Russell 2005; Nielson *et al*. 2006; Carstens *et al*. 2007; Funk *et al*. 2008; Kiefer *et al*. 2009). Phylogeographic patterns from Dolly Varden, *Salvelinus malma* and bull trout, *Salvelinus confluentus* suggested the Chehalis River in Washington was a coastal refuge (Redenbach & Taylor 2002).

In terms of the physiographic associations, water refuges are clearly important for aquatic taxa, and some of the above phylogeographic substructure can be attributed to geographic barriers like rivers and canyons, elevation, and volcanic events (Wakabayashi & Sawyer 2001; Monsen & Blouin 2003; Miller *et al*. 2006a; Takacs-Vesbach *et al*. 2008; van Tuinen *et al*. 2008; Galbreath *et al*. 2009). Much of the codistributed taxa show the major divisions between the northern Rockies and coastal region, and the "Soltis line" along the Pacific Coast (Soltis *et al*. 1997; Brunsfeld *et al*. 2001). These large breaks are explained by mountain orogeny and Pleistocene glaciations. But the vast majority of phylogeographic structure appears to be related to multiple refugial locales in the Pacific Northwest. There is considerable historic substructure and support for the *refugia within refugia* model in the Pacific Northwest. Similar to refugia on the

Iberian Peninsula (Gómez & Lunt 2007), this historic substructure suggests genetically differentiated populations persisted throughout Pleistocene glaciations in the Pacific Northwest, and have been an important source of genetic variability in colonizing taxa.

2.3.5. Beringia

Phylogeographic studies have shown that Beringia was a refuge for numerous species during the Pleistocene glaciations. Molecular evidence from plants such as mountain avens, *Dryas intergrifolia* (Tremblay & Schoen 1999), purple saxifrage, *Saxifraga oppositifolia* (Abbott *et al*. 2000; Abbott & Comes 2003), locoweeds, *Oxytropis* spp. (Jorgensen *et al*. 2003), white spruce (Anderson *et al*. 2006), and Townsend's daisy, *Townsendia hookeri* (Thompson & Whitton 2006) support Beringia as a refuge. Mammals that show this pattern include lemmings, *Lemmus* and *Dicrostonyx* (Fedorov & Stenseth 2002; Fedorov *et al*. 2003), tundra voles, *Microtus oeconomus* (Brunhoff *et al.* 2003; Galbreath & Cook 2004), thinhorn sheep, *Ovis dalli* (Loehr *et al*. 2006), collared pikas, *Ochotona collaris* (Galbreath *et al*. 2009), Alaska marmot, *Marmota broweri* (Steppan *et al*. 1999), Arctic shrew, *Sorex arcticus* (Fumagalli *et al*. 1999) and brown bears, *Ursus arctos* (Leonard *et al*. 2000; Barnes *et al*. 2002). Many of these mammals have fossil evidence supporting this pattern (e.g. Weber *et al*. 1981; Storer 2004).

Like the Pacific Northwest, additional substructure is observed within Beringia. Abbott & Comes (2003) and Jorgensen *et al*. (2003) found divergent Alaskan clades of saxifrage and locoweeds. In mammals, tundra voles show Beringian splits that appear to have been induced by glaciers (Galbreath & Cook 2004), and thinhorn sheep show evidence for a previously undetected refugium in south-east Beringia (Loehr *et al*. 2006). A distinct haplotype in Keen's mouse, *Peromyscus keeni* from Haines Junction, Yukon (Lucid & Cook 2007) suggests a similar pattern. Ground squirrels (*Spermophilus parryii*) show at least four distinct clades in Alaska where their bifurcations date to glacial events (Eddingsaas *et al*. 2004). In addition, compelling evidence for an inland freshwater refugium south-east of Beringia that centered on the Nahanni river exists for both lake trout, *Salvelinus namaycush* (Wilson & Hebert 1998) and Arctic grayling, *Thymalus*

arcticus (Stamford & Taylor 2004). This refugium was situated at the juncture of the Cordilleran and Laurentide ice-sheets that appeared to contain periglacial freshwater lakes throughout the Pleistocence (Dyke & Prest 1987). The overarching cause of the Beringian splits is still unclear, but Jorgensen *et al*. (2003) attributed the breaks to the coastal ice shield (Fig. 2-2). The Yukon River Delta is also situated between phylogeographic splits (Eddingsaas *et al*. 2004) and likely helps to maintain vicariant signals. Given the ubiquitous nature of glaciations and both the Yukon River and ice shield as potential barriers, they should prove to be a shared phylogeographic break among more of the region's taxa (Riddle 1996). Moreover, these extrinsic factors have served to create and maintain genetic diversity in Beringia throughout multiple Pleistocene glacial cycles.

Beringia also reveals the utility of phylogeography for reconstructing refugial communities when paleoecological data produced different scenarios. Fossil evidence for wolverines, *Gulo gulo* pointed to the possibility of both southern and Beringian refugia (Bryant 1987). However, extensive sampling and phylogeographic analyses found no evidence for a southern refugium (Tomasik & Cook 2005; Cegelski *et al*. 2006). This suggests the southern lineage went extinct (or has had limited success), and Beringia has been the major source for North American wolverines. A slightly different pattern is observed in mountain goats, *Oreamnos americanus*, where fossils only support a southern refugium (Cowan & McCrory 1970; Rideout & Hoffman 1975). The distribution of genetic diversity and haplotypes points to a second northern refugium for mountain goats (Shafer *et al*. 2011a). Thus, instead of the predicted northern decrease in diversity (Hewitt 2004), mountain goats showed discrete hotspots of diversity in both the north and south (Shafer *et al*. 2011a). In both examples, by utilizing both phylogeographic and paleoecological data, a clearer picture of the refugial origin and distribution of diversity was produced.

2.3.6. Cryptic refugia: The Alexander Archipelago and Haida Gwaii

There is considerable evidence in the literature for multiple refugia off the coast of British Columbia and Alaska. Vancouver Island has been suggested as a coastal

refugium (Heusser 1960; Pojar 1980) and some genetic (Walser *et al.* 2005; Godbout *et al*. 2008) and paleoecological (Ward *et al*. 2003) evidence support this. But the vast majority of studies suggest that Haida Gwaii and the Alexander Archipelago were the major coastal refugia. The chestnut-backed chickadee, *Poecile rufescens* (Burg *et al.* 2006), Stellar's jay (Burg *et al.* 2005) and northwestern song sparrow, *Melospiza melodia* (Pruett & Winker 2005) all showed increased genetic diversity on Haida Gwaii - a pattern consistent with refugia (Hewitt 1996). Haida Gwaii and/or the Alexander Archipelago have been suggested as a refuge for the water flea, *Daphnia pulex* complex (Weider *et al.* 1999), and the lichen, *Cuvernularia hultenii* (Printzen *et al.* 2003). It is worth nothing that although garter snakes, *Thamnophis sirtalis*, do not currently reside on Haida Gwaii, Janzen *et al*. (2002) observed a phylogeographic pattern consistent with a Haida Gwaii refuge.

For plant species, phylogeographic evidence of refugia along coastal Alaska and northern British Columbia is accumulating (Gapare & Aitken 2005; Gapare *et al*. 2005; Godbout *et al*. 2008). Fossil plant and pollen records also support refugia in this area (Warner *et al*.1982; Peteet 1991; Hansen & Engstrom 1996). Most contentious is whether mammals utilized these islands as a refuge during the last glacial maximum. Byun *et al*. (1997) postulated that the coastal clade of black bears, *Ursus americanus*, near Haida Gwaii were from an island refuge (but see Demboski *et al*. 1999; Byun *et al.* 1999). Expanded sampling of black bears has failed to fully resolve the debate (Stone & Cook 2000; Peacock *et al*. 2007). However, phylogeographic support is observed in the long-tailed vole (Conroy & Cook 2000) and Keen's mouse, with the latter having fossil evidence predating the Holocene (Lucid & Cook 2004). Mountain goats too may have survived on the Alexander Archipelago (Shafer *et al*. 2011a, 2011b), and fossil evidence does support the presence of an ungulate species around the last glacial maximum (Heaton & Grady 2003). Phylogeography of the ermine and a codistributed nematode, *Soboliphyme baturini,* also support an island refugium (Fleming & Cook 2002; Koehler *et al.* 2009).

Vertebrate fossils are limited on Haida Gwaii (Wigen 2005); but considerable fossil evidence from the Alexander Archipelago supports the persistence of available terrestrial habitat (Heaton *et al*. 1996; Dixon *et al.* 1997; Heaton & Grady 2003). Geological evidence shows portions of this area were ice-free (Scudder & Gessler 1989; Josenhans *et al*. 1995; Carrara *et al*. 2007). Collectively, the accumulation of phylogeographic, fossil, and geological data provide near definitive evidence for a Pleistocene refugium that included mammals. One scenario is an extended coastal refugium including Haida Gwaii, the Alexander Archipelago, and an exposed Queen Charlotte Sound and Hecate Strait. Haida Gwaii was connected to the ice-free coast during the Pleistocene by the Hecate Strait, and both the Hecate Strait and Queen Charlotte Sound were at various times exposed, containing freshwater lakes and terrestrial flora (Barrie *et al.* 1993; Josenhans *et al.* 1995; Hetherington *et al*. 2003, 2004; Lacourse *et al*. 2003, 2005): this appears to be what prompted Byun *et al*. (1997) to suggest Haida Gwaii as a refugium for bears. An exposed Hecate Strait could have facilitated gene flow to the coast and southward, but also potentially north to the Alexander Archipelago. Although a glacier is believed to have separated Haida Gwaii from Alaska (Bornhold & Barrie 1991; Barrie & Conway 1999) open water stretches were considerably shorter (Barrie & Conway 1999). The potential ice or water barrier would therefore have been limited and potentially navigable by terrestrial mammals, or simply bypassed by aerial dispersers. This extended coastal refugium is supported by shared fossil assemblages (Heaton *et al*. 1996; Heaton & Grady 2003; Wigen 2005) and genetic ancestry (Fleming & Cook 2002; Cook *et al*. 2006) of the islands. Such a scenario could be used to explain the widespread coastal haplotype in bears (Byun *et al*. 1997; Demboski *et al*. 1999; Stone & Cook 2000), and essentially doubles the available area making it more plausible that terrestrial vertebrates could have survived the glacial maxima in this region.

2.3.7. Cryptic refugia: The Arctic

In the far north, the Canadian Arctic has been suggested as an important refuge (Macpherson 1965; Pielou 1991). Fossil evidence is limited (Harington 1990) and

those fossils found like caribou (Stewart & England 1986) and muskox, *Ovibos moschatus* (Maher 1968) lack accompanying phylogeographic support. However, a wide variety of taxa show phylogeographic evidence of an Arctic refugium. Arctic hare, *Lepus arcticus* (Waltari & Cook 2005), saxifrage (Abbott *et al*. 2000), and mountain aven (Tremblay & Schoen 1999) populations likely resided in Arctic refugium. Dunlin, *Calidris alpina* (Wennerberg 2001), Canada goose (Scribner *et al*. 2003), and rock ptarmigan, *Lagopus mutus* (Holder *et al*. 1999) appeared to utilize the Arctic throughout the Pleistocene. Some aquatic biota like Arctic charr, *Salvelinus alpinus* (Brunner *et al*. 2001) and water fleas (Weider *et al*. 1999) also persisted in an Arctic refuge.

With the accumulation of data supporting an Arctic refugium (as well any cryptic refugia for that matter), a refinement of biogeographic paradigms begins to arise. Beringia is considered one of the two major sources of biota in the northwest (Hultén 1937; Pielou 1991); however this is not the case for all taxa. In lemmings, their phylogeographic pattern suggests they utilized an Arctic refuge throughout the Pleistocene (Fedorov & Stenseth 2002; but see Fedorov *et al*. (2003) for a possible periglacial refuge). But most interestingly, Fedorov *et al*. (2003) noticed a reduced geographic distribution of Beringian haplotypes in lemmings. This led the authors to suggest that Beringia was only a minor source for postglacial colonization of lemmings, and thus the biogeographic models of Beringia and temperate North America may not be applicable to all northern species (Fedorov *et al*. 2003). It is premature to propose a full shift in biogeographic paradigms for the northwest, but these studies question the Beringian model as the major Pleistocene refuge and biotic source for the Arctic and northwest. Patterns like that observed by Fedorov *et al*. (2003) will undoubtedly be species dependent, but future studies should consider and test the influence of Arctic refugia. Such examples provide hypotheses to be tested in the in statistical phylogeographic framework, and will help refine and rewrite the role of major refugia in shaping contemporary biotic distributions.

2.3.8. Refugia within the ice-sheets

In addition to refugia flanking the Laurentide and Cordilleran ice-sheets, evidence for refugia within the ice-sheets during the last glacial maximum is mounting. In mountain sorrel, *Oxyria digyna*, a highly cold tolerant species, the haplotype distribution suggests colonization northward to the Arctic from northern British Columbia (Marr *et al*. 2008). This region was mostly covered by ice-sheets, but scattered nunataks (Ryder & Maynard 1991) may have supported persistent populations. A thinhorn sheep refugial population also appears to have persisted in northern British Columbia (Loehr *et al*. 2006). In southwestern Alberta, phylogeographic evidence suggests numerous plants survived within the ice-sheets including groundsel, *Packera* spp. (Golden & Bain 2000), and Easter daisies (Thompson & Whitton 2006) (see also Levsen & Mort 2008). Geological evidence has led to speculation of an ice-free corridor (Jackson 1979; Rutter 1984) or scattered nunataks (Burns 1980; Dyke & Prest 1987; Pielou 1991) that may have sustained disjunct plant populations in this region. Many of the ecological attributes (e.g. cold tolerance) thought to promote survival on nunataks are not restricted to these species (Marr *et al*. 2008), which suggests other northwest taxa may have survived on such refuges. These findings suggest additional colonization routes (Fig. 2-3) and the need to refine the Beringian and southern paradigm for the biota of northwestern North America. Similar to the Arctic refuge, future studies in the northwest should test for the presence of nunatak refugia, especially if the ecological characteristics of the species in question would promote survival in cryptic refugia.

2.3.9. Colonization post-Pleistocene

Dispersal ability was associated with a history of multiple refugia (Table 2-3). However, postulating the colonization routes and dispersal corridors from these refugia proves to be a more challenging task. For highly vagile taxa, colonization appeared to be so efficient that current population structure is a mixture of genetic lineages from different Pleistocene refugia. For example, mule deer, *Odocoileus hemionus* (Latch *et al.* 2009), the common raven, *Corvus corax* (Omland *et al.* 2000), and Canada goose (Scribner *et al.* 2003) all show considerable mixing of

Pleistocene lineages in the northwest, making colonization routes virtually impossible to deduce. On the other hand, taxa that have limited dispersal abilities exhibit population structure that often strongly reflects their Pleistocene distributions. This is true for many herpetofauna, in which limited dispersal ability and habitat specialization acted together to limit their spread post-Pleistocene (e.g. Nielson *et al.* 2001; Carstens *et al.* 2005; Carstens & Richards 2007; Funk *et al.* 2008).

Despite the difficulty, colonization routes have been traced for many plants based on phylogeographic study. These studies come largely from south of the ice-sheets, but northerly routes have also been inferred. The inland dispersal hypothesis and ancient vicariance models (Brunsfeld et al. 2001) still hold for some mesic taxa (Carstens *et al*. 2005; Carstens *et al*. 2007). In contrast, both the dusky willow (Carstens *et al.* 2005; Brunsfeld *et al.* 2007) and whitebark pine (Richardson *et al*. 2002) likely colonized the coast from the northern Rockies. In addition, whitebark pine (Richardson *et al*. 2002) and lodgepole pine (Godbout *et al*. 2008) show a northern colonization route that diverges north of the Columbia River basin and appears to follow mountain ranges. Species surviving within the ice-sheets (e.g. Marr *et al*. 2008) provide novel sources from which colonization would have emanated (Fig. 2-3). In many instances, demographic expansion statistics corroborate colonization from Beringian (Fedorov *et al*. 2003; Galbreath & Cook 2004), southern (Lessa *et al*. 2003; Shafer *et al*. 2011a), and cryptic refugia (Fedorov *et al*. 2003; Lessa *et al*. 2003). There is also evidence that the physiography of mountains helped direct dispersal. Bull trout show a northern colonization pattern that appears to utilize mountain river systems (Redenbach & Taylor 2002). Southerly dispersal along the Coast Mountains has been suggested for garter snakes (Janzen *et al*. 2002), and landscape genetic patterns of northwestern mammals such as thinhorn sheep (Worley *et al.* 2004), wolverines (Schwartz *et al.* 2009), black bears (Cushman *et al.* 2009), and mountain goats (Shafer *et al*. 2011a) all support mountain ranges acting as corridors.

2.3.10. Ecological and evolutionary implications

Two important issues arise from discovering novel refugia. The first is accurate reassessment of migration rates and modelling species-specific responses to climate change. Provan & Bennet (2008) noted the importance of understanding past phylogeographic patterns with respect to predicting future range shifts in species affected by climate change. Phylogeography is important because migration rates that are inferred when there are unrecognized cryptic refugia will be overestimated (McLachlan *et al*. 2005; Svenning & Skov 2007). Thus, predictions of species-specific responses to climate change are likely flawed without correct phylogeographic and refugial inferences. The second issue is identifying units of conservation. Bhagwat & Willis (2008) found evidence that survival in northern refugia was associated with unique biogeographic traits. These localities likely harbour high levels of genetic diversity and should be of highest conservation priority (Bhagwat & Willis 2008). If refugial locations are on the periphery of the range, there is an added urgency (Hampe & Petit 2005). Future studies in northwestern North America should recognize the importance of phylogeography for climate models and identifying conservation units. For example, McLachlan *et al*. (2005) reevaluated migration rates of two tree species in eastern North America based on chloroplast DNA reconstructions; this approach could easily be applied in the northwest. In addition, given the peripheral, endemic, and refugial origin of biota on the Alexander Archipelago and Haida Gwaii, conservation and management plans should recognize the unique phylogeographic status of these islands, especially with anthropogenic pressures mounting (Cook *et al*. 2006).

Some of the patterns we describe have identifiable evolutionary and ecological implications. Similar to our results (Table 2-3), European biota showed an association between dispersal ability and refugial history, such that habitat generalists and efficient dispersers were more likely to have survived in northern refugium (Bhagwat & Willis 2008). Svenning & Skov (2004) also found northerly distributed plants were better dispersers; but it is difficult to tease apart the cause-and-effect relationship between refugial history and dispersal ability. Evidence

suggests the *refugia within refugia* scenario can produce highly efficient dispersers. Admixture between divergent plant populations often produces high-ploidy groups (Abbott & Brochmann 2003). In northern climates, increased ploidy is associated with successful colonization of recently deglaciated terrain (Brochmann *et al*. 2004). Polyploids may be adaptable to a wider array of ecological conditions (Stebbins 1950) and be more likely to maintain genetic variability during long-distance dispersal and bottleneck events (Brochmann *et al.* 2004). This pattern has also been observed in Arctic water fleas (Dufresne & Hebert 1997). Thus a link is formed between refugial history and dispersal, such that (at least in some plants and animals), the *refugia within refugia* scenario may produce efficient dispersers, and can partly explain the success of some northerly distributed taxa.

Speciation and local adaptation are also important evolutionary consequences of multiple refugia. North American mountain sheep provide a textbook example of glacial induced speciation and differentiation during the Pleistocene (Cowan 1940; Pielou 1991; Geist 1999). Thinhorn sheep arose from Beringian refugium while bighorn sheep, *Ovis canadensis*, evolved south of the ice-sheets. During the most recent glacial advance, isolation and differentiation produced detectable morphological and genetic differences between populations of thinhorn sheep (Worley *et al.* 2004). Variation in thinhorn sheep coat colour arose during this time due to hybridization with bighorn sheep in an isolated Plesitocene refuge (Worley *et al*. 2004; Loehr *et al*. 2006) and now plays an important role in dominance hierarchies (Loehr *et al.* 2008). Similarly, glacial induced vicariance also promoted speciation and morphological divergence in northwestern pikas, *Ochotona* spp. (Guthrie 1973; Galbreath *et al*. 2009; Hafner & Smith 2010). Locally adapted variation occurring in part from refugial history has been also observed. One dramatic example comes from lake whitefish, *Coregonus clupeaformis*, where multiple glacial refugia led to divergence among populations in eastern North America (Bernatchez & Dobson 1990, 1991). Secondary contact and sympatric divergence among these lineages produced unique ecotypes (Pigeon *et al*. 1997) that are morphologically and ecologically

distinct. Rogers & Bernatchez (2007) and Renaut *et al*. (2010) found that differential natural selection on adaptive traits likely maintains these ecotypes. With genome-wide scans becoming increasingly accessible, future studies may reveal the full extent to which adaptive variation in populations may stem in part from their phylogeographic history.

2.3.11. Future directions and review of major findings

The accuracy with which phylogeographic patterns are inferred is influenced by a number of interacting factors. One major limitation has been the lack of extensive sampling. Collection of tissues in phylogeographic studies is often opportunistic and may not adequately cover a species distribution. As a result, additional substructure and confidence in phylogenetic topologies may be limited. In our review we found numerous instances where a species was analyzed multiple times in the literature and when more samples were included, additional (or alterations to) refugial locations were inferred (e.g. tailed frog, Nielson *et al.* 2001, 2006; Carstens *et al.* 2005; Columbia spotted frog, Bos & Sites 2001; Funk *et al.* 2008; dusky willow, Carstens *et al.* 2005; Carstens & Richards 2007; Brunsfeld *et al.* 2007). We suggest that more intensive sampling will yield similar substructure and identify additional refugial sites in many species. Although collecting samples from the complete species range would help alleviate this problem, it is clearly not feasible logistically or financially in many cases. One solution is the development and utilization of integrated field inventories and permanent archives. This allows researchers the ability to access information and material for additional study. For example, Kuhn *et al*. (2010) utilized the Parks Canada DNA Repository to augment their ancient DNA with modern samples, enabling them to examine temporal changes in caribou population structure. Online databases also allow for comparisons among laboratories around the world (see Wang *et al*. 2009 for a good example). Such repositories and databases depend upon cooperation and support from parties often with different interests, but are a necessity if we wish to ameliorate the costs and difficulties associated with sample collection.

Another issue is that phylogeographic inference has largely relied on a single locus. The markers of choice, mainly mtDNA and cpDNA, have received

tremendous scrutiny in the literature (e.g. Hurst & Jiggins 2005; Zink & Barrowclough 2008) stemming from their sometimes-inaccurate depiction of population and species histories. For example, in the montane frog, *Rana cascadae*, mtDNA suggested high levels of divergence, almost to the order of separate species, for the Olympic Peninsula population (Monsen & Blouin 2003). When nuclear markers were examined it became clear that the Olympic Peninsula population was not isolated, and gene flow with other Washington populations had been ongoing. In such instances conservation designations and management practices would be misinformed if based solely on mtDNA. These examples of mitochondrial-nuclear discordance are rampant in the literature, which has led to multiple independent nuclear genes being put forth as a potential solution for phylogeography (Hare 2001). However, as evidenced by Table 2-2, nuclear genes have not yet been widely utilized in phylogeographic studies, likely due to a limited number of appropriate markers.

One possible solution may come from the development of genome-wide resources for non-model organisms made possible by the reduced costs of next generation sequencing and single nucleotide polymorphism (SNP) screening (Gilad *et al.* 2009; Pool *et al.* 2010; Thomson *et al.* 2010). SNPs have slow mutation rates and limited homoplasy making them useful markers for inferring population history (Brumfield *et al*. 2003; Brito & Edwards 2009). Genome-wide approaches have recently been used to examine the phylogeography of balsam poplar, *Populus balsamifera* (Keller *et al.* 2010), and *Lycaeides* butterflies (Gompert *et al.* 2010). More importantly, these resources are ripe for the burgeoning field of statistical phylogeography that has shifted away from single gene trees, and towards accounting for genealogical discordance and genetic stochasticity when inferring population histories (Knowles 2009). However, a few limitations still need to be considered with these new genetic markers. Conceptually, genomic resources do not produce a result that is as visually intuitive as gene trees (Brito & Edwards 2009); thus a shift in thought process is required. Unlike what is done with mtDNA, recombination (Nachman 2001) and ascertainment bias (Brumfield *et al*. 2003) should be considered, as they can

impair phylogeographic interpretations. But overall, there is clear promise to these markers and they are being embraced by phylogeographers (Holsinger 2010). As the phylogeographic toolbox expands to include genome-wide data sets, the improved power and resolution will make questions that were inconceivable at one-point, become routine in non-model organisms.

Improved phylogeographic inferences are also being made with the advancement of analytical and statistical methods (Knowles 2009; Nielsen & Beaumont 2009). Already the field has seen considerable growth with the application of coalescent analysis (Knowles & Maddison 2002; Richards *et al.* 2007). Coalescent approaches allow for the testing of specific phylogeographic hypotheses (Carstens & Ritchie 2007, Hickerson *et al.* 2010) providing a quantitative assessment of tree divergence. Although these models are based on certain assumptions that are not likely met in reality (i.e. panmixia, stable population sizes), which could lead to errors in calculating divergence times (Hickerson *et al.* 2006, 2010), software programs now exist (see Excoffier & Heckel 2006; Kuhner 2008) that can untangle complex demographic parameters (e.g. population size and growth), along with estimating the time of divergence. The use of Approximate Bayesian Computational framework could further improve phylogeographic inference (Hickerson *et al.* 2006; Beaumont *et al.* 2010; Bertorelle *et al*. 2010). This approach can incorporate demographic histories that will affect coalescent times (Hickerson *et al.* 2006, 2007), and can be used in a comparative framework that would allow for testing the congruence of divergence times across taxa (Hickerson *et al.* 2006). Ecological-niche modeling (ENM) is another important method being added to the phylogeography toolbox (Peterson 2001; Carstens & Richards 2007). Researchers can use ENM to predict species' distributions pre-Pleistocene and empirically evaluate how predicted historical refugia correspond to the phylogeographic structure (Carstens & Richards 2007; Knowles *et al*. 2007; Waltari *et al*. 2007). Landscape genetics (see Manel *et al*. 2003; Storfer *et al*. 2007) can also be used in conjunction with phylogeography to assess the habitat associations that have helped produce phylogeographic structure. Most importantly, these advances bring phylogeographic analysis

towards a statistically valid comparative approach, rather than purely 'descriptive phylogeography,' which is an important step forward for the field.

In the decade since the review of Brunsfeld *et al*. (2001), empirical studies have revealed a wealth of phylogeographic information from the biota of northwestern North America. The recurrent phylogeographic pattern that emerged was additional complexity, i.e. *refugia within refugia*, in both the Beringia and southern refugia. This substructure is connected to mountain orogeny and common physiographic features like rivers, mountains, and the Alaskan ice shield. There was also near conclusive evidence for multiple cryptic refugia in the Alexander Archipelago and Haida Gwaii, the Canadian Arctic, as well within the ice-sheets. These cryptic refugia force us to refine the classic two-refuge paradigm of Beringian and southern sources for the colonization of northwestern North America. In the next decade as the field transitions from being descriptive to statistical in nature, future phylogeographers should view the purported patterns as hypotheses to test under a more rigorous statistical framework. Because phylogeography is such an integrative field we stress that all available information, especially paleoecological data, should be used in conjunction with new methodology to help formulate and test phylogeographic hypotheses (Cruzan & Templeton 2000). More comprehensive sampling schemes (made available at permanent archives), genomic data, and new analytical methods should be used in future phylogeographic studies. Less conventional approaches like ancient DNA (Krajick 2002), hotspot clusters (Swenson & Howard 2005) and parasites (Criscione *et al*. 2005) can also be used to reconstruct biogeographic scenarios and patterns. With the ever-expanding phylogeographic toolbox, novel patterns and ecological traits associated with refugial history will continue to be discovered, along with our understanding of the ecological and evolutionary consequences of Pleistocene glaciations.

Table 2-1. Predicted phylogeographic patterns in northwestern North America.

I.	Species will have persisted in refugia in either Beringia and/or the continental United States during glacial advances and share recolonization routes
II.	Additional structure, *refugia within refugia*, is likely present in the major refugia
III.	Some species have persisted in cryptic refugia
IV.	Ecological variables will be associated with refugial history
V.	Geological events and barriers may correspond to phylogeographic breaks

Table 2-2. Summary of major phylogeographic studies for northwest North America. Specific refugial sites are noted in the Refugia column. Substructure is in reference to phylogeographic breaks found within a particular refuge. The list of abbreviations is included below the table.

Taxon	Common name	Markers	Observed pattern	Analysis and support[1]	Reference	Refugia			
						Beringia	Southern	Cryptic	Substructure
Review	-	-	N/S split at Cascade/Sierra. HG refuge	Co-distributed patterns	Soltis *et al*. 1997				
Review	-	-	NRM and Coastal clades	Co-distributed patterns	Brunsfeld *et al*. 2001				
Mammals									
Tamiasciurus spp.	Tree squirrels	mtDNA seq, allozymes	Refugia in NRM and Blue Mts	High MP & moderate ML support	Arbogast *et al*. 2001		●		●
Tamias ruficaudus	Red-tail chipmunk	mtDNA seq	E/W divide along Bitterroot Mts, multiple NRM clades	Moderate to high Bayesian, MP, ML & NCA support	Good & Sullivan 2001		●		●
Tamias amoenus	Yellow-pine chipmunk	mtDNA seq	Coastal and continental clades, two refugia in PNW	Moderate to high ML, MP & NCA support	Demboski & Sullivan 2003		●		●
Tamias spp.	Red-tail & yellow-pine chipmunks	mtDNA seq, morphometric	Clearwater refuge, introgression zone	Moderate MP, Bayesian & NCA support, discordant with morphology	Good *et al*. 2003		●		●
Glaucomys sabrinus	Flying squirrel	mtDNA seq	Recent colonization into AA	No ML structure, reduced diversity on island	Bidlack & Cook 2001		●		
Spermophilus paryii	Arctic ground squirrel	mtDNA seq	Multiple clades in Beringia	High ML support, molecular clock	Eddingsaas *et al*. 2004	●			●
Microtus oeconomus	Tundra vole	mtDNA seq	Beringian clade	High NJ & MP support, molecular clock, nucleotide diversity	Brunhoff *et al*. 2003	●			
Microtus oeconomus	Tundra vole	mtDNA seq, nucDNA seq	Multiple clades in Beringia	High Bayesian support, molecular clock, nucleotide diversity	Galbreath & Cook 2004	●			●
Microtus longicaudus	Long-tailed vole	mtDNA seq	Distinct clades along coast, AA & RM	High NJ & MP support, molecular clock	Conroy & Cook 2000 Spaeth *et al*. 2009		●	●	●

Microtus richardsoni	Water vole	mtDNA seq	Coastal and NRM clades with coastal colonization	High ML support but not ENM	Carstens *et al.* 2005; Carstens *et al.* 2007			●		●
Clethrionomys gapperi	Red backed vole	mtDNA seq	Coastal and NRM clades	High MP, NJ & Bayesian support, mismatch & diversity analyses	Runck & Cook 2005			●		●
Peromyscus spp.	Deer and Keen's mouse	mtDNA seq	N & S clades	High NJ support, coalescence based MIGRATE	Zheng *et al.* 2003			●	●	
Peromyscus keeni	Keen's mouse	mtDNA seq	Distinct AA clade	Moderate NJ support, NCA & molecular clock	Lucid & Cook 2004				●	
Peromyscus keeni	Keen's mouse	mtDNA seq	*P. keeni* in Haines Junction distinct, potential Beringian origin	High NJ & MP support, high divergence among conspecifics	Lucid & Cook 2007					
Peromyscus maniculatus	Deer mice	mtDNA seq, μsats	Distinct PNW & California clades mixing in Oregon, recent gene flow	High ML & Bayesian support	Yang & Kenagy 2009			●		●
Phenacomys longicaudus	Red tree vole	mtDNA seq	N/S split in Oregon. E/W division across Willamette Valley	Network analysis, diversity measures	Miller *et al*. 2006b			●		●
Dicrostonyx groenlandicus	Collared lemming	mtDNA seq	Canadian Arctic & Beringian clades	Moderate to high NJ, ML & MP support, molecular clock	Fedorov & Stenseth 2002	●		●		
Lemmus spp.	Lemmings	mtDNA seq	Canadian Arctic & Beringian clades	High NJ & ML support, population expansion models	Fedorov *et al*. 2003	●		●		
Sorex spp.	Shrews	mtDNA seq	Coastal (Oregon to Alaska) & continental clades	High MP & ML support, high divergence	Demboski & Cook 2001			●		●
Ochotona princeps	American pika	mtDNA seq	E/W split & N/S split in RM	High ML support, molecular clock, population expansion models	Galbreath *et al*. 2009			●		●
Lepus arcticus	Arctic hare	mtDNA seq	Canadian Arctic & Beringian clades	High Bayesian & low NJ support, haplotype distribution	Waltari & Cook 2005	●		●		
Mustela erminea	Ermine	mtDNA seq	Beringian, S, continental, AA & HG clades	Moderate to high MP, ML & NCA support	Fleming & Cook 2002	●	●	●		
Martes americana	American marten	mtDNA seq, res sites	Coastal/RM & continental clades	High MP support & network analysis, high divergence	Stone *et al*. 2002			●		
Ursus americanus	Black bear	mtDNA seq	Coastal (HG) & continental clade, Hecate refuge potential HG source	High MP support	Byun *et al*. 1997			●	●	
Ursus americanus	Black bear	mtDNA seq, res sites	Coastal clade to Oregon/California	High MP support	Stone & Cook 2000			●		

Canis lupis	Gray wolf	mtDNA seq	S refuge	Low NJ support, haplotype & diversity distributions	Leonard *et al*. 2005		●		
Canis lupis	Gray wolf	mtDNA seq	BC inland & coastal differentiation	Haplotype distribution, divergence	Munoz-Fuentes *et al*. 2009				●
Gulo gulo	Wolverine	mtDNA seq	Single refuge & rapid colonization	Moderate NJ support, diversity measures	Tomasik & Cook 2005	●			
Gulo gulo	Wolverine	mtDNA seq, µsats	Single Beringian refuge	Star NJ tree, NCA & PCA support	Cegelski *et al*. 2006	●			
Vulpes vulpes	Red fox	mtDNA seq	Beringian refuge, distinct S refuge in PNW	High ML & Bayesian support, haplotype distribution	Aubry *et al*. 2009	●	●		
Odocoileus hemionus	Mule deer	mtDNA seq	Black-tail deer refuge in Oregon/Washington	High MP & NCA support, diversity measures	Latch *et al*. 2009		●		●
Ovis spp.	Bighorn and thinhorn sheep	mtDNA seq	Cryptic refugia in McKenzie Mts & N BC	AMOVA, network analysis, molecular clock	Loehr *et al*. 2006	●	●	●	●
Oreamnos americanus	Mountain goat	mtDNA seq, µsats	N and S clades. Potential refugium in N BC	High ML & Bayesian support, distribution of diversity	Shafer *et al*. 2011a	●	●	●	
Rangifer tarandus	Caribou	mtDNA seq	Beringian & S refugia	Moderate/high Bayesian support, distribution of haplotypes	Flagstad & Roed 2003	●	●		
Herpetofauna									
Ambystoma macrodactylum	Long-toed salamander	mtDNA seq	HG, coastal, Salmon River, Blue Mts, Clearwater & Montana clades	Low ML, ME & MP support, NCA, shallow divergence	Thompson & Russell 2005		●		●
Anaxyrus boreas	Western toad	mtDNA seq, res sites	NW clade & Klamath-Siskiyou Mts refuge, Columbia River refuge	High MP & Bayesian support, shallow divergence	Goebel *et al.* 2009		●		●
Ascaphus truei	Tailed frog	mtDNA seq, allozymes	N & S clades along coast, Klamath-Siskiyou Mts refuge	High MP & ML support, network analysis, molecular clock	Nielson *et al.* 2001, 2006		●		●
Ascaphus montanus	Tailed frog	mtDNA seq, allozymes	Clearwater & Salmon River Mts refugia	High MP & ML support, network analysis, molecular clock	Nielson *et al*. 2006		●		●
Ascaphus spp.	Tailed frog	mtDNA seq	Coastal & NRM clades	ML support, ENM	Carstens *et al.* 2005; Carstens *et al.* 2007		●		●
Batrachoseps wrighti	Oregon salamander	mtDNA seq, RAPD	N/S split in Oregon & N colonization, Columbia River refuge	High NJ & MP support, distribution of diversity	Miller *et al*. 2005		●		●
Plethodon vandykei & *P. idahoensis*	Van Dyke's salamander	mtDNA seq	NRM & coastal clades, Clearwater drainage refuge	High ML & MP support, NCA, molecular clock	Carstens *et al*. 2004		●		●

Plethodon elongatus & P. stormi	Del Norte salamander	mtDNA seq	4 clades, Cascades/Sierra & Klamath Siskiyou refugia	High MP, ML & ME support, high divergence	Mahoney 2004	●	●
Plethodon larselli	Larch Mountain salamander	mtDNA seq, RAPD	Columbia River split & N colonization	Moderate NJ & MP support, NCA, distribution of diversity	Wagner *et al*. 2005	●	●
Plethodon spp.	Plethodontid salamaders	mtDNA seq	Coastal & NRM clades, ancient vicariance	High ML & ENM support	Carstens *et al.* 2005; Carstens *et al.* 2007	●	●
Dicamptodon spp.	Giant salamanders	mtDNA seq	Coastal & NRM clades	High ML support	Carstens *et al.* 2005	●	●
Dicamptodon spp.	Giant salamanders	mtDNA seq	Coastal refuge for *D. aterrimus*	High MP & ML support, Bayesian hypothesis testing	Steele *et al*. 2005	●	
Dicamptodon tenebrosus	Pacific giant salamander	mtDNA seq	N/S clades, Columbia River Valley & Klamath Siskyou Mts refugia	High MP & ML support, Bayesian hypothesis testing	Steele & Storfer 2006	●	●
Dicamptodon copei	Cope's salamander	mtDNA seq	Multiple western clades, Columbia River refuge, N colonization	Moderate MP, ML & Bayesian support, NCA	Steele & Storfer 2007	●	●
Rhyacotriton variegatus	Southern torrent salamander	mtDNA seq	Central Oregon split, Yaquina River barrier, N colonization	Network analysis, AMOVA, spatial autocorrelation	Miller *et al*. 2006a	●	●
Taricha granulosa	Rough-skinned newt	mtDNA seq, allozymes	Oregon split, N colonization from Klamath Siskiyou refuge	Moderate NJ, ML & Bayesian support, distribution of diversity	Kutcha & Tan 2005	●	●
Charina bottae	Rubber boa	mtDNA seq	PNW Clade & N/S split at Sierra Nevada	Moderate MP & ML support	Rodriguez-Robles *et al*. 2001	●	
Crotalis viridis	Western rattlesnake	mtDNA seq	E/W split at RM, effect of NRM & coast orogeny	Moderate MP & ML support, Templeton tests	Pook *et al*. 2000	●	●
Crotalis viridis	Western rattlesnake	mtDNA seq	E/W split at RM, PNW basal for *C. v. oreganus*	Moderate MP & ML support	Ashton & de Queiroz 2001	●	●
Thamnophis sirtalis	Garter snake	mtDNA seq	HG & S clades, N and S colonization	High ML support, hypothesis testing	Janzen *et al*. 2002	●	●
Pseudacris regilla	Pacific tree frog	mtDNA seq	Distinct coast & NRM clades	High MP, ML & ME support, network analysis	Ripplinger & Wagner 2004	●	●
Pseudacris regilla	Pacific tree frog	mtDNA seq	Distinct Coast & NRM clades & a split in Oregon	Moderate MP & Bayesian support, NCA	Recuerco *et al.* 2006	●	●
Rana luteiventrus, R. pretiosia	Columbia spotted frog	mtDNA seq	Separate RM clade, distinct Wyoming and Coast groups	High MP & ML support, NCA	Bos & Sites 2001	●	●
Rana luteiventrus & R. pretiosa	Columbia spotted frog	mtDNA seq	Distinct Coast & NRM clades, unique Blue Mts clade.	High MP, ML & Bayesian support	Funk *et al.* 2008	●	●

Birds

Oporornis tolmiei	MacGillivray's warbler	mtDNA seq	Highest diversity in Oregon, likely N colonization	NJ & MP support, MST, distribution of diversity	Mila *et al*. 2000			●		
Dendroica petechia	Yellow warbler	mtDNA seq	E/W split corresponding to RM	Moderate NJ support, distribution of diversity	Milot *et al*. 2000			●		●
Wilsonia pusilla	Wilson's warbler	mtDNA seq, res sites	Coastal and RM divergence, highest diversity in Alberta & Alaska	Network analysis, distribution of diversity	Kimura *et al*. 2002			●		●
Dendroica coronata	Yellow-rumped warbler	mtDNA seq	Partial differentiation between E & W, Oregon basal	High ML & NJ support, network analysis	Mila *et al*. 2007			●		
Geothylpis trichas	Common yellowthroat	mtDNA seq	E, W & Nevada groups	Network analysis, distribution of diversity	Lovette *et al*. 2004			●		
Poecile rufescen	Chestnut-backed chickadee	µsats	Distinct lineages in HG & Alaska	Bayesian assignment, distribution of diversity, private alleles	Burg *et al*. 2006	●			●	●
Poecile gambeli	Mountain chickadee	mtDNA seq	Coastal & RM clade	High ML support, network analysis, MDIV	Spellman *et al*. 2007			●		●
Melospiza melodia	Northwestern song sparrow	µsats	HG refuge, N colonization	High Bayesian & NJ support, distribution of diversity & alleles	Pruett & Winker 2005			●	●	
Troglodytes troglodytes	Winter wren	mtDNA seq	E/W split potentially from Pleistocene	High ML support, DIVA, distribution of diversity	Drovetski *et al*. 2004			●		
Troglodytes troglodytes	Winter wren	mtDNA seq, AFLP	E/W split at RM, pre-Pleistocene	Bayesian assignment, PCA, molecular clock	Toews & Irwin 2008			●		
Catharus ustulatus	Swainson's thrush	mtDNA seq	Coast & RM split from Pleistocene	Sequence divergence, distribution of diversity, expansion models	Ruegg & Smith 2002			●		●
Cyanocitta stelleri	Stellar's Jay	µsats	HG & S clusters, likely refugia	Bayesian assignment, distribution of diversity & alleles, high F_{ST}	Burg *et al*. 2005			●	●	
Branta canadensis	Canada goose	mtDNA seq, µsats	Multiple clades in Beringia, likely multiple refugia	High ML, ME & MP support	Scribner et al. 2003	●	●	●		
Dendragapus obscurus	Blue grouse	mtDNA seq	Coast & NRM clades, N/S split within NRM	MP support, distribution of diversity, estimates of gene flow	Barrowclough *et al*. 2004			●		●
Lagopus mutus	Rock ptarmigan	mtDNA seq, nuDNA seq	Arctic, Beringia, & Aleutian Island clades	Moderate NJ support, network analysis, distribution of diversity	Holder *et al*. 1999; Holder *et al*. 2000	●			●	●
Grus canadensis	Sandhill crane	mtDNA seq	Arctic clades	High ML & NJ support, coalescent	Rhymer *et al*. 2001				●	●

				molecular clock					
Grus canadensis	Sandhill crane	mtDNA seq, μsats	Beringian clade & secondary contact	Bayesian assignment, PCA, distribution of diversity	Jones *et al*. 2005	●			
Aix sponsa	Wood duck	mtDNA seq	Coast & RM split, Pleistocene divergence	Network analysis, distribution of diversity, molecular clock	Peters *et al*. 2005		●		●
Strix occidentalis	Spotted owl	mtDNA seq	Cascade & Sierra Nevada split, distinct Washington clade	MP support, network analysis, distribution of diversity	Barrowclough *et al*. 1999		●		●
Fish									
Salvelinus alpinus	Arctic charr	mtDNA seq	S, Beringian, & Arctic clades	Moderate, MP, NJ & ML support, haplotype distribution	Brunner *et al*. 2001	●	●	●	
Gobbiesox maeandricus	Northern clingfish	mtDNA seq	N & S coastal clades	Moderate ML support, network analysis	Hickerson & Ross 2001	●	●		
Salvelinus malma & *S. confluentis*	Dolly varden and bull trout	mtDNA seq, nuDNA seq	Beringian & S clades for Dolly varden. S clade for bull trout	Moderate NJ & ML support, distribution of haplotypes	Redenbach & Taylor 2002	●	●		●
Thymallus arcticus	Arctic grayling	μsats, mtDNA seq, res sites	N/S split in Beringia & likely refuge in Nahanni River	Moderate NJ & ML support, distribution of haplotypes	Stamford & Taylor 2004	●		●	●
Salvelinus namaycush	Lake trout	mtDNA res sites	N & S clades & likely a Nahanni River refuge	Moderate ML support, distribution of haplotypes	Wilson & Hebert 1998	●	●	●	
Plants									
Pinus flexilis	Limber pine	mtDNA res sites	Refuge in N Wyoming (E foothills)	Distribution of alleles, degree of differentiation	Mitton *et al*. 2000		●		●
Pinus flexilis	Limber pine	allozymes	E & W refugia for NRM population	High among population G_{ST}, UPGMA support	Jorgenson *et al.* 2002		●		●
Pinus albicaulis	Whitebark pine	mtDNA seq, cpSSR	Yellowstone, Columbia basin, & Oregon clades, N colonization	AMOVA, spatial distribution of haplotypes	Richardson *et al.* 2002		●		●
Pinus lambertiana	Sugar pine	cp seq	N/S split at Cascade-Sierra interface, N refuge in Washington	Moderate MP support, distribution of diversity	Liston *et al*. 2007		●		●
Pinus balfouriana	Foxtail pine	cp, mtDNA, nuDNA seq	Refugia in Klamath Mts & S Sierra Nevada Mts	Bayesian clusters, coalescent-based isolation with migration	Eckert *et al*. 2008		●		●
Pinus contorta	Lodgepole pine	mtDNA, min sat	Montana, Columbia River basin, Cascades, HG/AA & Beringia clades	Bayesian assignment, UPGMA, distribution of diversity	Godbout *et al.* 2008	●	●	●	●

Picea sitchensis	Sitka spruce	cp STS	S refuge & possibly HG	Strong spatial structure, no bottleneck signature	Gapare & Aitken 2005, Gapare *et al.* 2005	●	●	
Picea glauca	White spruce	cp seq	S clade & refuge in Alaska	SAMOVA, K_{ST}, spatial distribution of haplotypes	Anderson *et al*. 2006	●	●	
Salix melanopsis	Dusky willow	cp seq	Clearwater & Salmon River clades, W colonization to the coast	Moderate ML & Bayesian support, divergence values	Brunsfeld et al. 2007	●		●
Salix melanopsis	Dusky willow	cp seq	Coastal & NRM clades	ML support but not ENM	Carstens *et al.* 2005; Carstens *et al.* 2007	●		●
Larix lyallii & *l. occidentalis*	Subalpine and western larch	µsats	E/W split at NRM, likely two distinct refugia for both species	NJ & UPGMA support, distribution of diversity	Khasa *et al.* 2006	●		●
Boechera spp.	Rockcress	cp seq	Montana/Idaho refuge, potential Arctic or Beringian refugia	Moderate MP support, network analysis, distribution of diversity	Dobes *et al.* 2004b	●	●	
Boechera spp.	Rockcress	cp, nucDNA seq, µsats	N/S split in WNA, Cascades basal to NRM	NJ support, distribution of alleles & diversity	Dobes *et al*. 2004a		●	
Boechera spp.	Rockcress	µsats	N/S split in NRM, suggestive of multiple refugia	Bayesian assignment, distribution of diversity	Song *et al*. 2006		●	●
Boechera spp.	Rockcress	cp seq	Possible Klamath–Siskiyou & Blue Mts refugia	Network analysis, distribution of haplotypes & diversity	Keifer *et al*. 2009		●	●
Packera pauciflora	Alpine groundsel	cp res sites	N colonization & refuge in Beringia	Network analysis, AMOVA, distribution of diversity	Bain & Golden 2005	●	●	
Sedum lanceolatum	Yellow stonecrop	cp seq	RM split at Wyoming basin, possible refuge in N. Montana	Distribution of diversity & alleles, high differentiation	DeChaine & Martin 2005		●	●
Townsendia hookeri	Easter daisies	cp seq, cp res sites	Beringia & S refugia, refuge within LGM in SW Alberta	Distribution of diversity, ploidy	Thompson & Whitton 2006	●	●	●
Packera spp.	Ragwort / groundsel	cp res sites	Refugia in SW Alberta on nunataks in *P. pseudaurea* and *P. contermina*	Distribution of haplotypes	Golden & Bain 2000		●	●
Chrysosplenium iowense	Iowa golden saxifrage	ISSR	S refuge, but Alberta population from refuge within ice	PCoA, distribution of diversity, high differentiation	Levsen & Mort 2008		●	●
Cardamine constancei	Constance's bittercress	cp seq	4 NRM refugia (Clearwater)	Moderate ML & Bayesian support, NCA, high divergence	Brunsfeld *et al.* 2005		●	●
Oxyria digyna	Mountain sorrel	cp res sites	Refugia in NBC & western USA, N colonization	NCA, spatial distribution of haplotypes	Marr *et al*. 2008		●	●

Saxifraga oppositifolia	Purple saxifrage	cp res sites	Beringia refuge, suggested Arctic refuge	Moderate MP & NJ support, spatial distribution of haplotypes	Abbott *et al*. 2000		●	●
Saxifraga oppositifolia	Purple saxifrage	cp res sites	Refuge in Beringia, two distinct clusters in Alaska	Distribution of diversity, high differentiation	Abbott & Comes 2003	●		●
Oxytropis spp.	Locoweed	nuDNA seq, RAPD	Refugia in NE Alaska and S Alaska	Moderate ML & UPGMA support, AMOVA	Jorgensen *et al.* 2003	●		●
Dryas integrifolia	Mountain avens	cp res sites	Refugia in Beringia and high Arctic	Moderate NJ support, diversity & differentiation of haplotypes	Tremblay & Schoen 1999	●	●	
Miscellaneous								
Daphnia pulex	Daphnid	mtDNA res sites	Potential refugia in Canadian Arctic	NJ support, distribution of haplotypes & diversity	Weider *et a*l. 1999		●	
Melanoplus spp.	Grasshoppers	mtDNA seq	Multiple NRM refugia in Montana and Idaho	ML, MP & NJ support, coalescent models	Knowles 2001		●	●
Prophysaon coeruleum	Arionid slug	mtDNA seq	Klamath Mts refugia, effect of orogeny	Moderate Bayesian & ML support	Wilke & Duncan 2004		●	
Dendroctonus rufipennis	Spruce beetles	mtDNA seq, µsats	Beringian, S & PNW refugia with deep divergences	Moderate MP & Bayesian support, Bayesian assignment	Maroja *et al*. 2007	●	●	●
Adelges cooleyi	Gall	mtDNA seq, AFLP	Coastal and RM split	Moderate NJ support, Bayesian assignment, NCA	Ahern *et al*. 2009		●	●
Greya politella	Seed parasite	mtDNA seq, AFLP	NRM refugia in Salmon, Bitterroot Rivers & southern Oregon distinct	Moderate MP & Bayesian support, network analysis	Rich *et al.* 2008		●	●
Soboliphyme baturini	Nematode	mtDNA seq	Coastal & interior division, AA refuge	Moderate Bayesian support, distribution of diversity	Koehler *et al.* 2009		●	● ●
Lobaria pulmonaria	Lichen	µsats	Coastal & interior divergence, possible Vancouver Island refuge	UPGMA, AMOVA, distribution of diversity	Walser *et al*. 2005		●	● ●
Cavernularia hultenii	Lichen	nuDNA seq	Beringia & S split in WNA. Possible Pacific Island refuge	Network analysis, diversity distributions (mismatch)	Printzen *et al*. 2003	●	●	●
Armillaria ostoyae	Fungus	nuDNA seq	Coastal & RM split	High NJ, MP & Bayesian support	Hanna *et al.* 2007		●	●
Tricholoma matsutake	Fungus	nuDNA seq, AFLP	RM basal, coastal split corresponds to Sierra/Nevada orogeny	Moderate MP, NJ & ML support, IBD	Chapela & Garbelotto 2004		●	●

[1]Studies showing >80% bootstrap support or posterior probabilities were considered to have high support, with those below 80% considered moderate

Abbreviations for each column listed alphabetically, “Markers”: AFLP – amplified fragment length polymorphism, cp – chloroplast DNA, ISSR – inter-simple sequence repeat, min sat – mini-satellite, mtDNA – mitochondrial DNA, nuDNA – nuclear DNA, res sites – restriction sites, seq – sequence, µsat – microsatellite. “Observed Pattern”: AA – Alexander Archipelago, BC – British Columbia, E – East, HG – Haida Gwaii, LGM – last glacial maxima, Mts – Mountains, N - north, , NRM – Northern Rocky Mountains, PNW - Pacific Northwest, RM – Rocky Mountains, S - south, W – west. “Analysis and Support”: AMOVA - analysis of molecular variance, ENM – ecological niche modeling, IBD – isolation by distance, ME - minimum evolution, ML - maximum likelihood, MP - maximum parsimony, NCA - nested clade analysis, NJ – neighbor joining, PCA - principal component analysis, SAMOVA – spatial analysis of molecular variance, UPGMA – unweighted pair-group method with arithmetic mean.

Table 2-3. Univariate Firth's penalized-likelihood logistic regression results. Data on ecological variables and phylogeographic history were collected from 103 species in northwestern North America (Appendix II).

Phylogeographic history	Ecological variable	Odds-ratio	95% confidence	*P* value
Multiple refugia	Range overlap			
	All	17.61	5.18 - 59.88	<0.01
	Mammals	9.31	1.26-68.75	0.02
	Herpetofauna	4.71	0.30 - 73.33	0.27
	Birds	3.00	0.34 - 26.60	0.32
	Plants	75.57	3.06 - 1865.60	<0.01
	Habitat specificity			
	All	0.09	0.004 - 1.99	0.13
	Mammals	0.11	0.004 - 2.64	0.17
	Herpetofauna	2.47	0.02 - 277.90	0.71
	Birds	0.94	0.009 - 96.77	0.98
	Plants	0.39	0.004 - 38.81	0.69
	Dispersal ability			
	All	6.19	2.04 - 18.78	<0.01
	Mammals	11.67	0.49 - 277.57	0.13
	Herpetofauna	38.86	0.30 - 4911.40	0.14
	Birds	-	-	-
	Plants	3.18	0.59 - 16.97	0.18

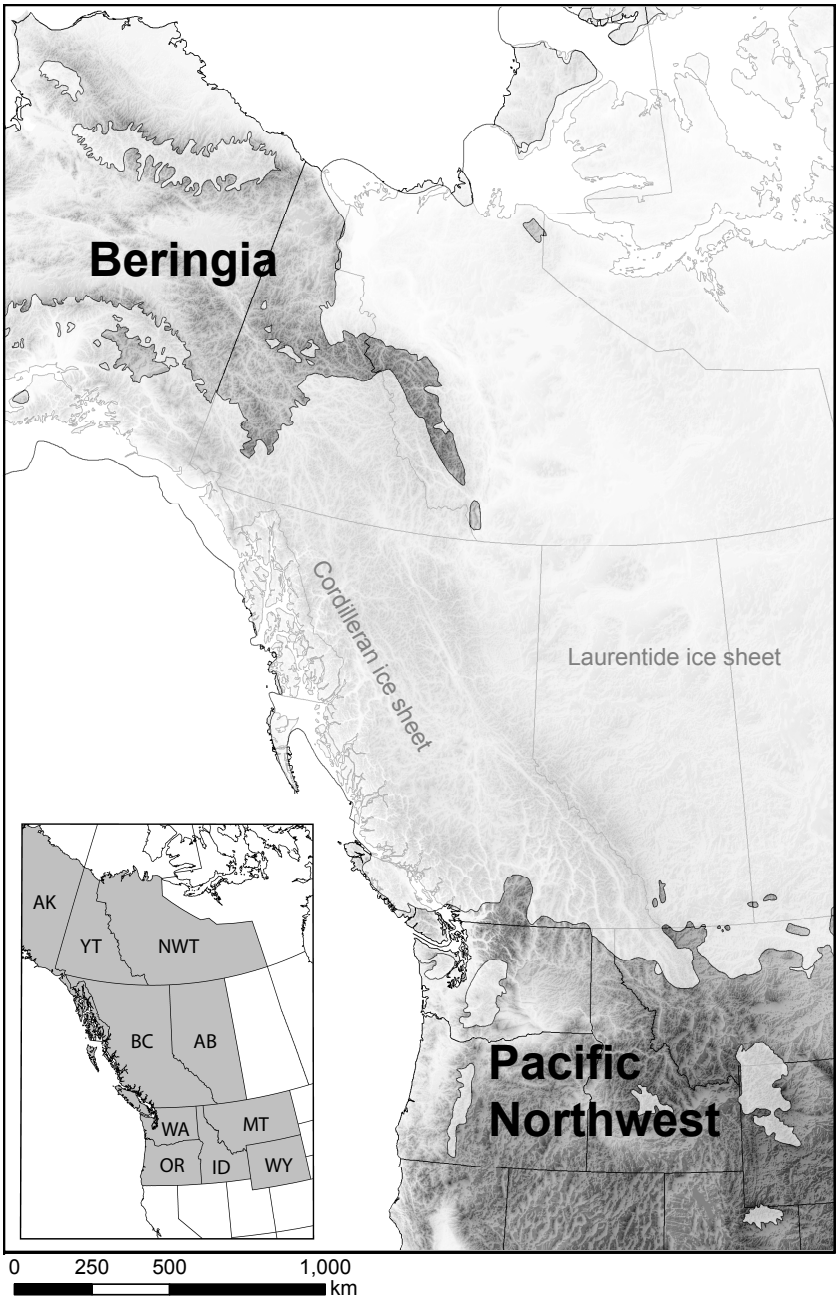

Figure 2-1. Extent of ice during the last glacial maxima in northwestern North America. Ice layer is from Dyke *at al*. (2003) and is partially transparent to show the underlying mountain network. Major refugia and ice-sheets are labeled. Inset map contains the states and provinces of northwestern North America: AK – Alaska, YT – Yukon Territory, NWT – Northwest Territories, BC – British

Columbia, AB – Alberta, WA- Washington, OR – Oregon, ID – Idaho, MT – Montana, WY – Wyoming.

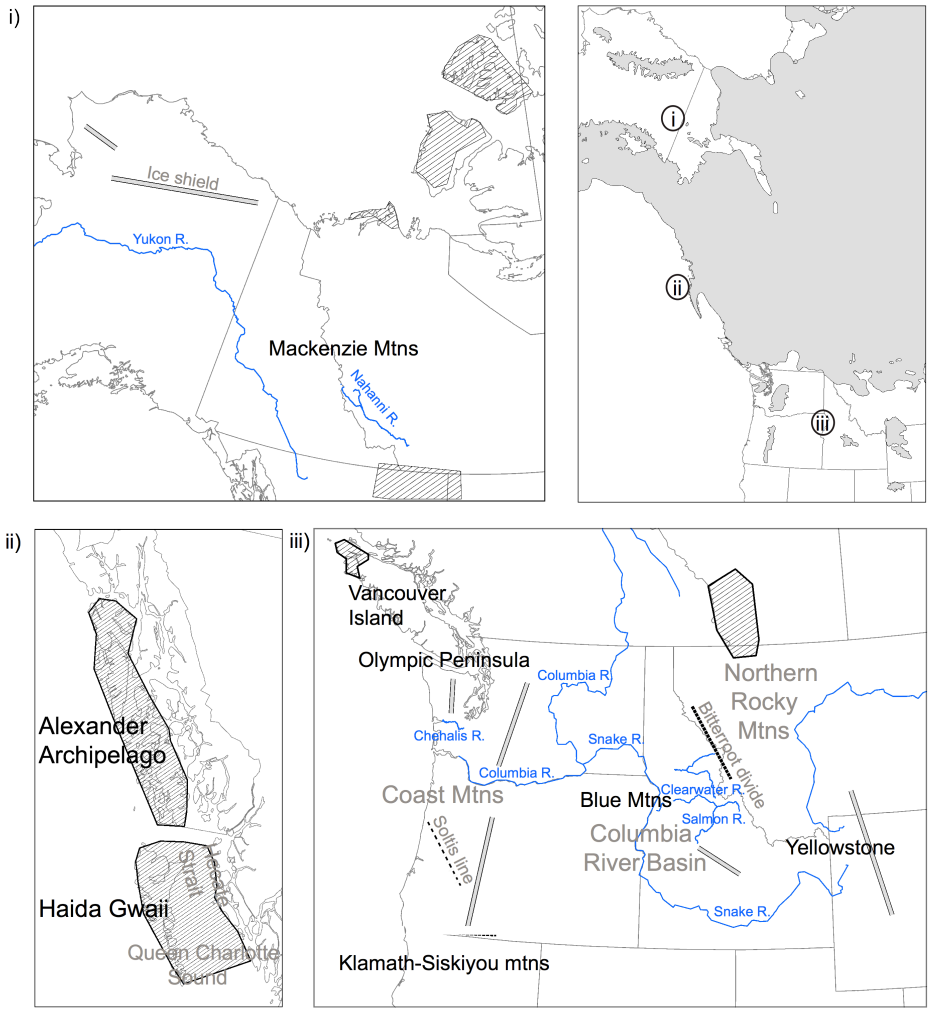

Figure 2-2. Important biogeographic sites within identified refugia. Putative refugial locations are labeled, while important rivers and phylogeographic breaks (dotted line) are shown. In some cases rivers acted as refugial sites. Bordered-dashed areas indicate potential cryptic refugia. Gray lines bordered by black denote separate ice caps inferred from Dyke *at al*. (2003) that may have acted as barriers.

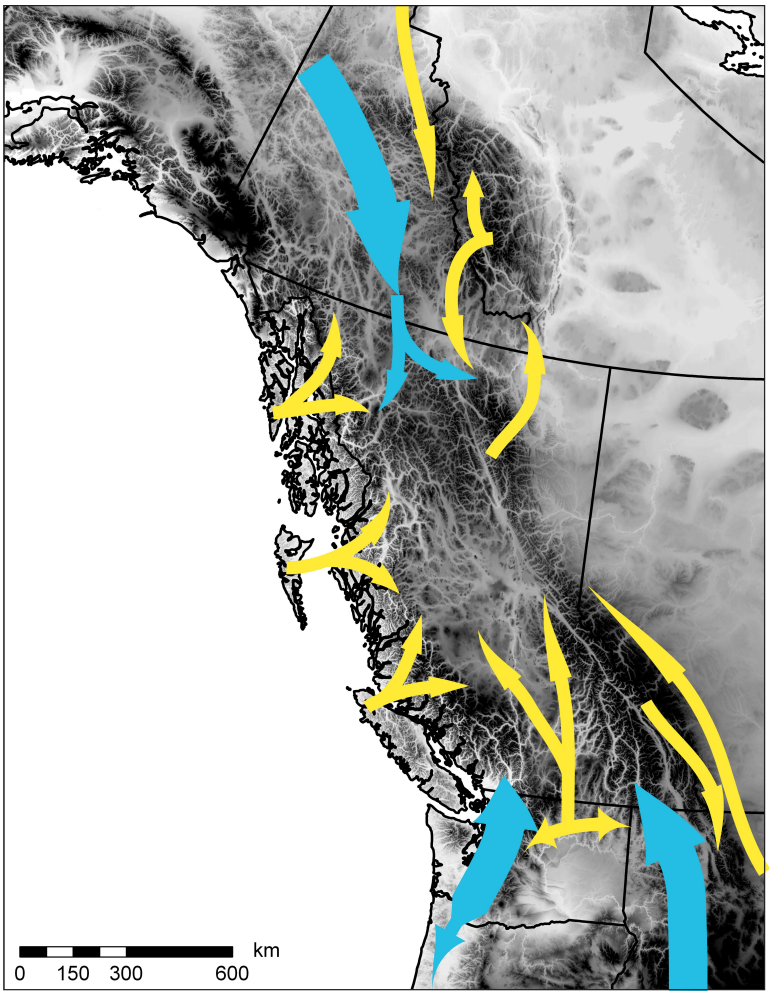

Figure 2-3. Postulated colonization routes in northwestern North America. Large blue arrows indicate major routes, while smaller yellow indicate smaller or postulated routes

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Chapter 3

Placing the mountain goat: a total evidence approach to testing alternative hypotheses

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3.1. Introduction

So-called 'rogue taxa'—defined here as taxa whose phylogenetic position is sensitive to gene sampling or analytical method—present a formidable obstacle to molecular phylogenetics. Their presence is thought to destabilize trees and produce sensitive support values (Sanderson and Shaffer, 2002). Some studies, however, suggest that the removal of 'rogue taxa' does not influence overall support for a particular evolutionary tree (Rokas et al., 2005). Therefore the exact effect of such taxa on phylogenetic hypotheses remains unclear. Traditionally, once a 'rogue taxon' has been identified it is either removed from the analysis altogether, or a series of subtrees and/or consensus trees are constructed with or without the taxon in question (Sumrall et al., 2001; Sanderson and Shaffer, 2002; Rokas et al., 2005). These approaches for addressing problematic taxa are insufficient for two reasons: 1) there is no explicit testing of alternative evolutionary scenarios; or 2) the placement of the taxa in question is never resolved. This latter consequence is particularly unsatisfactory when the taxon is central to the researcher's questions.

To combat problematic groups, some researchers combine all available data (the total evidence approach *sensu* Kluge, 1989). Total evidence has proved useful in resolving many groups (e.g., carnivores: Flynn et al., 2005; wasps: Nylander et al., 2004; spiders: Hedin and Bond, 2006). Empirical work highlights that total evidence is minimally impacted by partition incongruence among data sets and can be superior to congruence-based approaches (e.g., Baker et al., 2001; Flynn and Nedbal, 1998). It has also been suggested that adding more data, not more taxa, increases phylogenetic accuracy (Rokas and Carroll, 2005; but see Hedtke et al., 2006).

When multiple evolutionary scenarios exist, tests that statistically compare topologies can be employed (Huelsenbeck and Crandall, 1997; Huelsenbeck and Rannala, 1997). These tests typically utilize parsimony (e.g., Templeton, 1983) or likelihood-based (e.g., Goldman et al., 2000) approaches to compare hypotheses. However, these tests are commonly restricted to individual data set incongruence

and often examine only a small number of alternative hypotheses. When the literature presents many alternative hypotheses among data sets, the research question is often left in an evolutionary conundrum.

Mountain goats (*Oreamnos americanus*) exemplify the term 'rogue taxa' and are a true evolutionary puzzle. The mountain goat is taxonomically recognized as a member of the tribe Caprini *sensu lato* (*s.l.*), which contains eleven genera (Table 3-1; Hassanin and Douzery, 1999; Ropiquet and Hassanin, 2005a, 2005b). Considerable disagreement surrounds the phylogenetic affinities of the mountain goat. The closest extant relatives of mountain goats are thought to be Asiatic gorals (*Naemorhedus* spp.; Festa-Bianchet and Côté, 2008). However, the mountain goat's phylogenetic position relative to caprin bovids (Caprini *s.l.*) is controversial. Various molecular studies have placed mountain goats as either: 1) sister to *Rupicapra* spp. and caprins (Hartl et al., 1990); 2) sister to *Rupicapra* spp. (Chikuni et al., 1995; Fernandez and Vrba, 2005); 3) sister to muskox (*Ovibos moschatus*: Groves and Shields, 1996); 4) sister to sheep (*Ovis* spp.,: Gatesy et al., 1997; Kuznetsova et al., 2002; Hassanin et al., 2009); 5) sister to *Naemorhedus sumatrensis* and *O. moschatus* (Hassanin et al., 1998; Ropiquet and Hassanin, 2005a); 6) sister to chiru (*Panthalops hodgsoni*: Lalueza-Fox et al., 2002); 7) sister to all Caprini less *N. sumatrenis* and *O. moschatus* (Ropiquet and Hassanin, 2005b); 8) sister to Rupicapra *spp*., *N. sumatrenis* and *O. moschatus* (Ropiquet and Hassanin, 2006); 9) sister to a group consisting of sheep and *Hemitragus jemlahicus* (Gatesy and Swanson, 2007); or 10) unresolved (Hassanin and Douzery, 1999; Ropiquet and Hassanin, 2006). The majority of these studies do not provide convincing support (i.e., >50% bootstrap support [BS] or >0.75 posterior probability [PP]) for their placement of mountain goats within the Caprini. More importantly, none of these studies address phylogenetic uncertainty within their data set or explicitly evaluated alternative hypotheses for the position of mountain goats.

In this paper, we re-analyzed published sequence data from seventeen studies using a total evidence approach to reconstruct the phylogenetic position of mountain goats in the Caprini. This represents the largest combined data set used

to address phylogenetic questions within the subfamily and analyzes members of all genera within the focal group. Because of the aforementioned controversy surrounding the mountain goats phylogenetic affinities, we examined our data set for the ability of different tree building methods to integrate over phylogenetic uncertainty, and statistically evaluated our resulting placement against all published alternatives. In doing so, we have provided the first study in which all available data are used and multiple evolutionary hypotheses are statistically scrutinized.

3.2. Materials and methods

3.2.1. DNA Sequences

DNA-sequence data were obtained from seventeen previous studies that submitted their data to GenBank (Appendix III). Four mitochondrial genes (12S, Cyt b, COII, and ND1) and four nuclear genes (κ Cas, PRKCI, SPTBN1, and TG) were obtained for all recognized tribes (Simpson, 1945) and 11 major species complexes (Table 3-1). Where monophyletic genera contain multiple species (e.g., goral; Hassanin et al., 2009), a single representative was chosen. Three outgroups were used: *Aepyceros melampus* (impala), *Hippotragus niger* (sable antelope), and *Damaliscus dorcas* (blesbok). Sequence data were aligned using CLUSTALW as implemented in BioEdit v.7.0.5.3 (Hall, 1999).

3.2.2. Phylogenetic analyses

The appropriate model(s) of nucleotide substitution for maximum-likelihood (ML) and Bayesian analyses were determined using hierarchical likelihood ratio tests (hLRT) implemented in Modeltest v.3.07 (Posada and Crandall, 1998) and MrModeltest v.2.2 (Nylander, 2004), respectively. Individual data sets were first analyzed in a Bayesian framework separately to identify potential incongruence between data sets. Because Bayesian analysis allows for the implementation of multiple evolutionary models, we then ran a combined analysis under a GTR+I+Γ model with a subsequent separate analysis under the following selected models to cytochrome b, κ-casein, 12S rRNA, COII, ND1, PRKC1, SPTBN1, and TG

respectively: GTR+I+Γ, HKY+Γ, GTR+I+Γ, GTR+I+Γ, GTR+I+Γ, HKY+Γ, K80+Γ, K80+Γ. In addition, stem and loop regions of the 12S rRNA were identified using KNetFold (Bindewald and Shapiro, 2006). Under this nine model partition, the aforementioned models were run along with independent GTR+I+Γ models for the stem and loop regions. In another nine model partition, codon models were selected for the cytochrome b, COII, ND1, and κ-casein data sets, with the remainder using the original models (all other regions were RNA or non-coding). Bayesian analyses were run using MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001) under default priors. Two independent runs of four chains (three heated) were run for 1000000 generations with the first 25% discarded as a burn-in. Under the eight and nine models of evolution, partitions were unlinked to allow each model to optimize its own parameters starting from values specified by MrModeltest (individual models shown in Appendix IV). Chains were considered to have reached convergence if the average standard deviation of split frequencies stabilized at <0.01. For the ML analysis, GTR+I+Γ was the best model of evolution. The ML analysis was run in Garli v.0.95 (Zwickl, 2006) with GTR+I+Γ parameters fixed according to Modeltest specifications. Each run was considered to have reached optimality if 10000 generations occurred with no improvement in topology or a significant increase (0.01) in -ln likelihoods. Confidence in ML topology was evaluated based on 1000 bootstrap replicates.

Maximum parsimony (MP) analyses were conducted in PAUP v.4.0b10 (Swofford, 2002) with gaps treated as missing data and characters unweighted and unordered (Fitch, 1971). Gaps were removed from the analyses because only one representative of each genus was used, and there is considerable intraspecific variation in gaps and repeat numbers within the Caprini (Feng et al., 2008; Zeng et al., 2008). For the MP analysis, we conducted heuristic searches with 1000 random sequence addition replicates and tree-bisection-reconnection with no limit on the number of trees retained per replicate. Branch support was assessed with 1000 bootstrap replicates using the same search strategy.

Due to the many phylogenetic placements of the mountain goat, we hypothesized the MP may have difficulty integrating over phylogenetic

uncertainty within the data set. To evaluate phylogenetic uncertainty, we selected 100 trees at even intervals from the two MCMC chains after burnin. We generated 10 sequence data sets, all with 5987 base pairs, for each selected tree using Mesquite v.2.6 (Maddison and Maddison, 2009) and Modeltest parameters. We then ran MP and ML analyses on the newly generated 1000 data sets using PAUP v.4.0b10. To test for integration over phylogenetic uncertainty, we evaluated each topology with respect to the placement of mountain goats and analytical method used.

3.2.3. Testing alternative phylogenies

To evaluate topologies suggesting alternative phylogenetic positions for mountain goats, we used three different tests. The resulting phylogeny in this study was tested against all published relationships (Table 3-2). If tribe sampling was incomplete in published alternatives (i.e., Gatesy and Swanson 2007), placements were still included as tentative topological hypotheses. We first conducted a pair-wise Templeton test (Templeton, 1983). This is a non-parametric, parsimony-based implementation of the Wilcoxon signed-rank test that determines whether one topology is significantly more parsimonious than an alternative topology for a given set of data. We conducted the test by comparing unconstrained MP trees with alternative MP trees inferred under specific constraints (Table 3-2), using the above search parameters in PAUP v.4.0b10 (Swofford, 2002). Where more than one constrained tree was optimal, all were used in the Templeton test. The calculated *p*-values were halved in order to convert to a one-sided test (Buckley et al., 2001).

We conducted two likelihood-based bootstrap tests: the non-parametric Shimodaira-Hasegawa (SH; 1999) and the parametric Swofford-Olsen-Waddell-Hillis (SOWH) tests (Swofford et al., 1996; Goldman et al., 2000). Maximum likelihood trees were first constructed in PAUP v.4.0b10 (Swofford, 2002) using heuristic searches with as-is sequence addition and tree-bisection-reconnection. Analyses were run under the GTR+I+Γ model of evolution (parameters estimated) with topological constraints according to alternative mountain goat placements (Table 3-2). For the SH test, 10000 bootstrap replicates of each ML tree were resampled using the re-estimated log

likelihoods on ML trees corresponding to each topology and estimated GTR+I+Γ parameters (our ML tree and each one of the nine alternate topologies). All topologies were compared together, with a *p*-value <0.05 in the one-tailed t-test considered incongruent. In the SOWH test, the test statistic (δ) was calculated by subtracting the likelihood of an alternate topology (i.e. H_A) from the maximum likelihood tree (i.e. H_O). We then simulated one hundred replicate data sets in Seq-Gen v.1.5.3 (Rambaut and Grassly, 1997) under the all nine H_A with the GTR+I+Γ model and parameters fixed to be the ML estimates for the alternate ML topology with no polytomies (lcollapse=no). In each simulated data set (n =100), we calculated ML estimates of the H_A and H_O under a GTR+I+Γ model, but with parameters re-estimated (see Goldman et al., 2000). From each of the simulated data ML estimates, we then calculated the $\delta^{(100)}$, which gave a null distribution of the test statistic. One-tailed tests were then conducted to determine whether the observed δ fell below 95% of the ranked list of $\delta^{(100)}$.

3.3. Results

3.3.1. Sequence data

A concatenated data set consisting of 5987 base pairs (bp) was constructed from cytochrome b (1143 bp), κ-casein (408 bp), 12S rRNA (966 bp), COII (582 bp), ND1 (957 bp), PRKC1 (514 bp), SPTBN1 (583 bp), and TG (834 bp). This is the first study to combine all available sequence data, resulting in the most comprehensive dataset yet used to reconstruct the phylogeny of the Caprini. This represents an exhaustive taxonomic sampling and, as such, no additional genera can be added. In the aligned 5987 bp data set, 1577 bp were variable, of which 992 bp were parsimony informative.

3.3.2. Phylogenetic analyses

In the individual analysis (see Appendix IV), mountain goats were either placed (1) in an unresolved polytomy with no support (SPTBN1, PRKC1, Cyt B, COII), (2) had <80% PP support (ND1, k-casein, 12S), or (3) as sister to *Nilgritragus* and *Ovis* with >90% PP (TG). Resulting topologies have no hard incongruence (i.e.,

different placement of mountain goat supported in alternative topologies with greater than 90% PP) and combining data is warranted. Using the combined data set, the strongest support was observed in the ML and Bayesian trees with mountain goats sister to all caprins except *Naemorhedus sumatrensis* and *Ovibos moschatus* with 0.87 PP for eight model, and 0.70 PP for one model analyses (Figures 3-1, 3-2a). The 9 model partition supported this placement, with 0.91 PP for the added RNA stem and loop partitions, but was unresolved for the codon-based models. Thus, four of the five model-based approaches (both simple and complex) support the same placement of mountain goat. In contrast, the MP analysis placed mountain goats in a clade with *N. sumatrensis* and *O. moschatus* at 78% bootstrap support. In the 1000 random data sets used to evaluate parsimony and likelihoods ability to cope with phylogenetic uncertainty, we found parsimony failed to produce a consistent topological placement of mountain goats. Of 1000 trees, MP could only correctly place (i.e. replicate the MP topology) mountain goats on 64 (6.4%) trees. In contrast, ML reproduced its placement in 958 of the 1000 runs (96%).

3.3.3. Testing alternative topologies

Nine alternate topologies were tested against the ML topology based on these data. Using the Templeton test we found our ML topology to differ significantly from four of the nine alternate mountain goat placements (Table 3-2). The likelihood based SH test rejected five of the nine alternatives (Table 3-2). Applying the SOWH test to the reconstructed alternative maximum likelihood trees (Figure 3-2) suggested that the ML and Bayesian placement of mountain goats is significantly more likely than all the proposed alternates (Table 3-2). The calculated δ ranged from 2.65 to 241.89 depending on the alternate trees likelihood score (Fig. 3-2 caption), and in all nine data sets δ had the highest rank in the $\delta^{(100)}$ distribution.

3.4. Discussion

3.4.1. Phylogenetic placement of a 'rogue taxon'

Although various phylogenetic placements within Caprini have been suggested for mountain goats (e.g., Hartl et al., 1990; Chikuni et al., 1995; Groves and Shields 1996; Hassanin et al., 1998; Hassanin & Douzery, 1999; Kuznetsova et al., 2002; Lalueza-Fox et al., 2002; Fernandez and Vrba, 2005; Ropiquet and Hassanin, 2005a, 2005b, 2006), our study provides evidence that mountain goats are an independent lineage, sister to all Caprini members except muskox and goral. In addition, using simulated data we found parsimony an unreliable method for resolving the placement of mountain goats, which is likely a result of the seemingly rapid bifurcations within Caprini (Figure 3-2). Although there are limitations to simulated data, the purpose should be to replicate ideal conditions to test the performance of different tree building methods (Huelsenbeck 1995). Based on this premise, simulations have proven useful in examining inconsistencies within data sets and different phylogenetic methods (e.g., Huelsenbeck 1995; Huelsenbeck and Rannala 1997; Huelsenbeck et al. 2001). If there was no 'uncertainty' within our data, we would predict both likelihood and parsimony to perform equally well in, regardless of the origin and complexity of the data. This is not the case, with likelihood out performing parsimony by 90% in the simulations. This suggests that the caprini topology is complex (i.e. rate heterogeneity, homoplasy), and parsimony is unable to accurately and consistently place the mountain goat. Overall, this study provides increased support for the purported Caprini phylogeny; moreover, we are able to reject some alternative evolutionary hypotheses given all available data.

Our results are congruent with that of Ropiquet and Hassanin (2005b), albeit with stronger levels of support for the purported placement of mountain goats and subtle intra-generic relationships of *Capra* (see Ropiquet and Hassanin, [2005a] for explanation). These similarities between this topology and those of Ropiquet and Hassanin (2005b) are not surprising given that half of the genes used in our analysis were used in that study. Interestingly, most of the alternative topologies that were not rejected share a common theme: a relatively basal

position for mountain goats. Clearly, *Oreamnos* diverged early in the Caprini lineage and may have close affinities to *N. sumatrensis* and *O. moschatus*, evidenced by our MP placement. Our increased character sampling and explicit testing of alternative hypotheses adds important insight to the position of mountain goats within the Caprini phylogeny.

Ruling out alternative evolutionary scenarios of a more derived position for mountain goats sheds light on the historical biogeography of this group. Ropiquet and Hassanin (2005b) suggested that the muskox and goral split off from the main Caprini lineage in the early Pliocene. Our topology suggests that the precursor to *Oreamnos* would have followed shortly thereafter. This finding is in general agreement with the fossil record, as the ancestral mountain goat is believed to have entered North America via the Bering land bridge in the late Pliocene or early Pleistocene (Cowan and McCrory, 1970). Therefore, all three North American Caprini representatives (sheep, mountain goats, muskox) appear to have entered North America separately and independently. Historical vicariance in Eurasia likely precipitated speciation of mountain goats and muskox, rather than glacial dynamics in North America that has been proposed for sheep (Cowan, 1940; Pielou, 1991; Geist, 1999). In mountain goats, their extreme environment, slow growth rate, late age of first reproduction (Festa-Bianchet and Côté, 2008), and limited genetic diversity (Mainguy et al., 2007; Poissant et al., 2009), have likely hindered the rate of evolution (e.g., Martin and Palumbi, 1993; Bleisweiss, 1998). As a result, synapomorphies and lineage sorting have been difficult to detect in any one marker. This is evident in the marker choice, as identical markers have produced different results (Cyt b: Groves and Shields 1996; Hassanin et al., 1998), and in our individual data set analyses, five of the eight markers could not resolve the mountain goats position, with the remaining three all suggesting different placements. Future work should focus on synthesizing all available data, including morphological, to resolve any still controversial taxa, and construct a cohesive story of the Caprini's evolutionary history.

3.4.2. A useful model for placing problematic taxa

Few, if any, phylogenetic analyses do not contain problematic taxa. These include 'rogue taxa,' polytomies, and introgressed markers. From the phylogenetic perspective, these taxa likely resulted from conflicting gene and species trees, missing data, and varying rates of evolution. Total evidence has been an effective means for resolving many difficult phylogenies (e.g., Flynn et al., 2005; Shafer and Stewart 2007), but many systematic relationships remain unclear. With the increased availability, efficiency, and economy of genome-wide scans, total evidence was suggested by Malia et al. (2003) to be the future of systematic studies. However, what happens when using all available data still fails to convincingly place certain taxa? Generally speaking, when such a case arises researchers either display the taxa as a polytomy or select the *best* tree and invoke references suggesting posterior probabilities or bootstrap values are conservative, inflated, or unreliable (e.g., Alfaro et al., 2003; Suzuki et al., 2002; Wilcox et al., 2002; Douady et al., 2003; Alfaro and Holder 2006) to support their placement. None of these responses directly address the problem at hand: placing the problematic taxon.

By utilizing all available data and testing alternative hypotheses, we have developed a convincing argument for the placement of a controversial 'rogue taxon' – the mountain goat. In total evidence, even seemingly small data sets add valuable phylogenetic information (Nylander et al., 2004). When bifurcations appear to be mainly supported by the Bayesian analyses, this framework allows for an objective likelihood-based approach to scrutinize the best tree against all other alternatives. This approach should appeal to those not convinced by Bayesian posterior probabilities. Furthermore, using multiple tests of alternative hypotheses allows the researcher to individually assess the support for each hypothesis. In cases where certain tests are deemed too conservative (Buckley 2002), multiple tests and full disclosure will help diminish lingering concerns. In our placement of mountain goats, the SOWH test rejected all alternatives which some might consider suspect; however, closer examination of the hypotheses not rejected by the SH test revealed that although there were subtle differences in

topological placement, all maintained a common basal placement. As a result of this approach, we have an increased level of confidence in our purported topology.

In conclusion, we have applied a simple approach to placing problematic taxa: 1) acquire all available data; 2) obtain phylogenetic hypotheses (ensuring integration over phylogenetic uncertainty); and 3) use likelihood-based tests of alternative hypotheses to statistically remove possible alternative topologies. This model can also be applied to polytomies and conflicting data sets making it useful for multiple types of problem taxa. At the very least, using total evidence and likelihood based-tests of alternative hypotheses can be used to rule out alternative topologies, which in the case of some phylogenetic discrepancies, is an informative feat in itself.

Table 3-1. Species used in this study and their taxonomic ranking within the tribe Caprini *s.l.* (Ropiquet and Hassanin 2005a, 2005b).

Genus	Subgenus	Specific epithet	Common name
Ammotragus		*lervia*	Aoudad
Budorcas		*taxicolor*	Takin
Capra		*falconeri*	Markhor
		nubiana	Nubian ibex
		ibex	Alpine ibex
		sibirica	Asiatic ibex
Hemitragus		*jemlahicus*	Himalayan tahr
	Aribitragus	*jayakari*	Arabian tahr
	Nilgiritragus	*hylocrius*	Nilgiri tahr
Naemorhedus		*sumatrensis*	Sumatran goral
Oreamnos		*americanus*	Mountain goat
Ovibos		*moschatus*	Muskox
Ovis		*dalli*	Dall's sheep
		aries	Domestic sheep
Panthalops		*hodgsoni*	Chiru
Pseudois		*nayaur*	Bharal
Rupicapra		*rupicapra*	Alpine chamois
		rupicapra	Pyrenean chamois

Table 3-2. Topology tests comparing this study and those published using the Templeton, SH, and SOWH tests. The abbreviation *spp.* is used to denote multiple species belonging to the same genera that are sister taxa.

Topology in this study	Published topologies		Templeton Test	SH test	SOWH test
a) This study	b)	(*O. americanus*, *O. moschatus*)[1]	$p = 0.09$	$p < 0.01$*	$p < 0.01$*
	c)	((*O. americanus*, *P. hodgsoni* (*B. taxicolor* (*Ovis* spp.))(*Hemitragus jemlahicus*, *Capra* spp., *P. nayaur*)(*N. sumatrensis*, *O. moschatus*)(*A. lervia*, *Rupicapra* spp.)))[2]	$p < 0.01$*	$p < 0.01$*	$p < 0.01$*
	d)	(*O. americanus* (*Rupicapra* spp., *Hemitragus jemlahicus* (*A. lervia* (*Capra* spp.))))[3]	$p < 0.01$*	$p < 0.01$*	$p < 0.01$*
	e)	(*O. americanus* (*Rupicapra* spp. (*N. sumatrensis*, *O. moschatus*)))[4]	$p = 0.28$	$p = 0.08$	$p < 0.01$*
	f)	(*O. americanus* (*N. sumatrensis*, *O. moschatus*))[5,6]	$p = 1$	$p = 0.22$	$p < 0.01$*
	g)	(*O. americanus* (*Rupicapra spp.*))[7,8]	$p = 1$	$p = 0.31$	$p < 0.01$*
	h)	(*O. americanus, P. hodgsoni*)[9]	$p = 0.18$	$p < 0.01$*	$p < 0.01$*
	i)	(*O. americanus* (*H. jemlahicus*, *Ovis* spp.))[10]	$p = 0.01$*	$p = 0.01$*	$p < 0.01$*
	j)	(*O. americanus*, *Ovis* spp.)[11, 12]	$p = 0.02$*	$p = 0.01$*	$p < 0.01$*

1. Groves and Shields, 1996. 2. Hassanin & Douzery, 1999. 3. Hartl et al. 1990. 4. Ropiquet and Hassanin, 2006. 5. Hassanin et al. 1998. 6. Ropiquet and Hassanin, 2005a. 7. Fernandez and Vrba, 2005. 8. Chikuni et al. 1995 9. Lalueza-Fox et al. 2002. 10. Gatesy and Swanson 2007. 11. Gatesy et al. 1997. 12. Kuznetsova et al. 2002.

† denotes same placement as Ropiquet and Hassanin 2005b.

* indicates significant *p*-value.

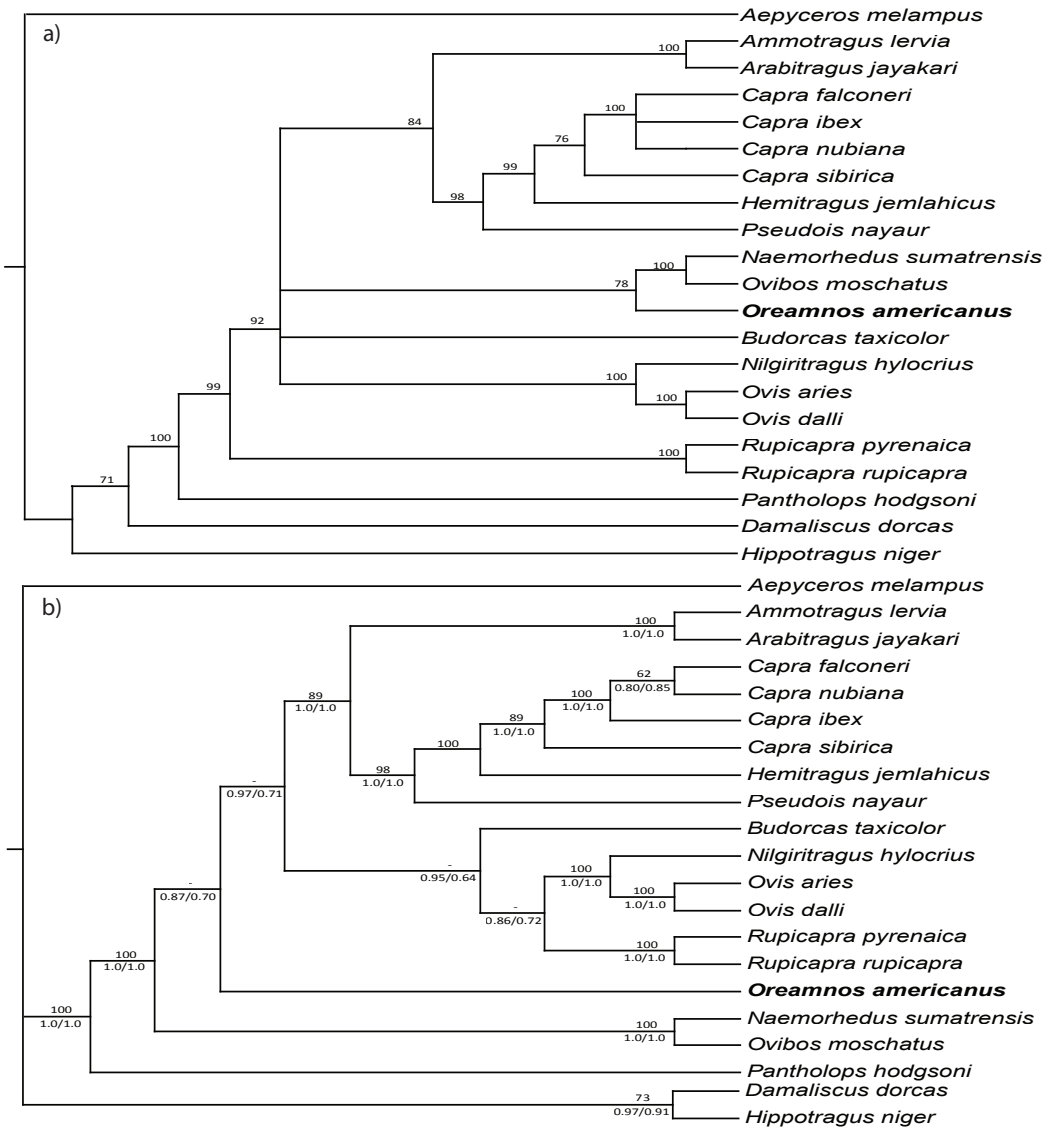

Figure 3-1. Phylogenetic trees of the C0-Iprinae based on eight genes totaling 5987 base pairs (Nuclear: 12S, Cyt b, COII, and ND1; Mitochondrial: κ Cas, PRKCI, SPTBN1, and TG. A. 50% majority rule tree maximum parsimony tree (3840 steps) based on 1000 replicates (bootstrap values shown above branches). B. Bayesian consensus tree. Support values found at the nodes are as follows:

above branch support values (ML) were obtained from 1000 bootstrap replicates; below branch values are Bayesian posterior probabilities (8 models/1 model).

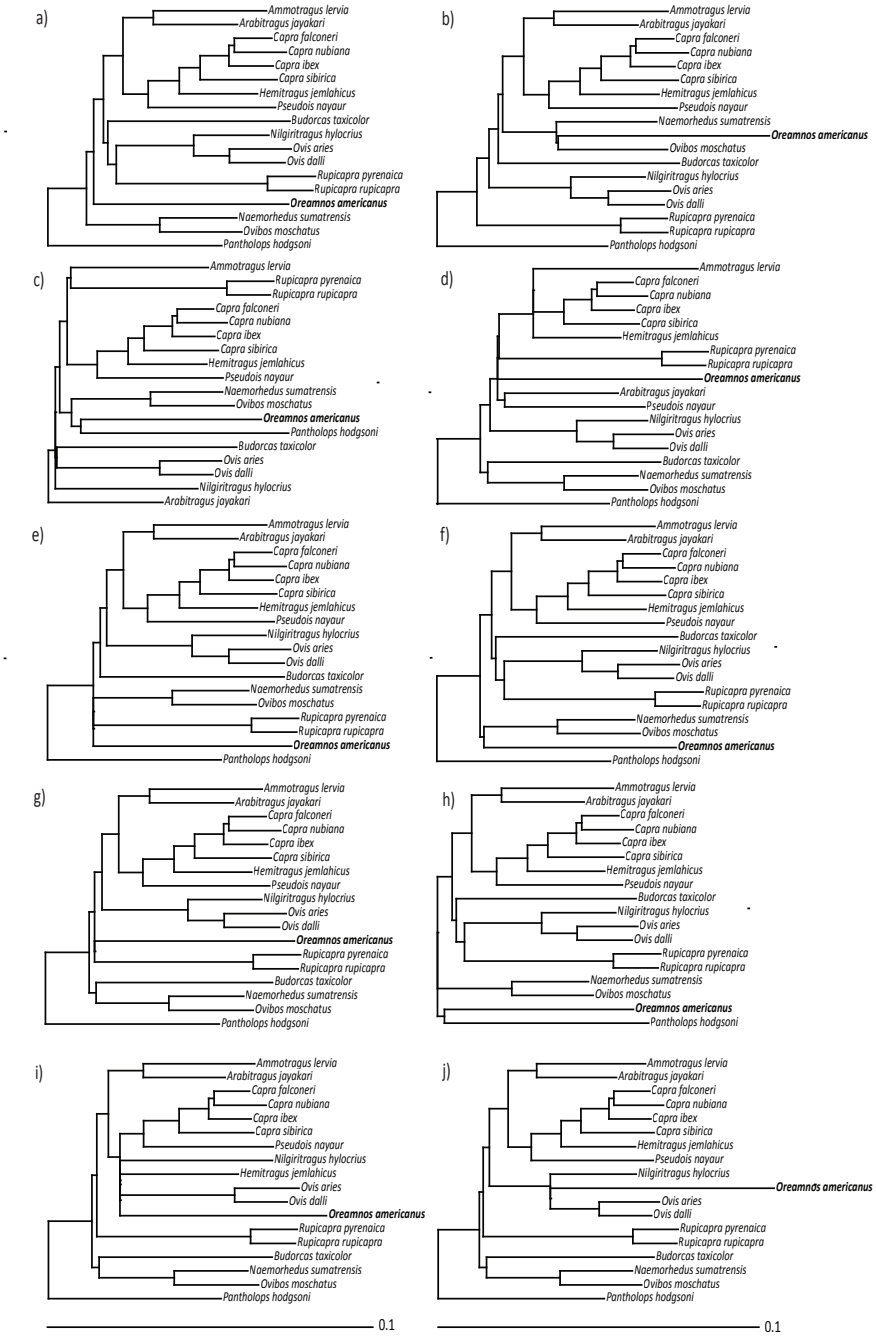

Figure 3-2. Maximum likelihood trees, excluding outgroups, obtained from these data with no topological enforcement (a), and those with a published topological constraint (b – j; see Table 3-1 for references). –ln likelihoods for each tree are: a) -25572.19371, b) -25678.24516, c) -25813.59293, d) - 25707.94395, e) -

25585.05626, f) - 25574.84559, g) - 25577.65514, h) - 25605.18751, i) - 25814.09149, and j) -25669.45709. Mountain goat's taxonomic name is bolded.

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Chapter 4

Hot spots of genetic diversity descended from multiple Pleistocene refugia in an alpine ungulate

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4.1. Introduction

The spatial genetic structure of a population has a profound effect on evolutionary processes and the maintenance of genetic diversity (Whitlock 2004). The extent to which populations are genetically structured is a consequence of both historical vicariance and contemporary dispersal, in addition to the basic evolutionary processes that regulate genetic variation (e.g., drift, selection). In particular, past refugia and natural habitat fragmentation influence the patterns of genetic differentiation that we observe today (e.g., Poissant et al. 2005; Louy et al. 2007; Paun et al. 2008). These processes create genetic disequilibria, the detection of which can be used to infer the relative influence of both refugia and fragmentation in shaping population genetic structure. Elucidating both historical and spatial effects on genetic structure is considered important for identifying evolutionary significant units (*sensu* Moritz 1994; e.g. Cossíos et al. 2009), understanding the potential for local adaptation (Jorgensen et al. 2006) and speciation (Wagner and McCune 2009), and can be an important predictive tool for modeling the effects of climate change on wildlife (Provan and Bennett 2008).

In western North America, glacial dynamics have had a profound effect on species distributions (Soltis et al. 1997; Brunsfeld et al. 2001; Shafer et al. 2010). For example, glacial induced vicariance is thought to have precipitated speciation in North American wild sheep, *Ovis* spp. (Cowan 1940; Pielou 1991; Geist 1999), and the most recent glacial oscillations have produced detectable morphological and genetic differentiation between populations of thinhorn sheep, *Ovis dalli* (Worley et al. 2004; Loehr et al. 2006). Phylogeographic studies in western North America have also revealed cryptic refuges during the last glaciation (Golden and Bain 2000; Loehr et al. 2006; Marr et al. 2008), and complex recolonization patterns following the recession of ice (Godbout et al. 2008). These historical patterns are generally inferred from unique ice-age signatures, with genetic heterozygosity being highest in areas that acted as a refuge (Hewitt 1996, 2000), or recently colonized areas phylogenetically nested within a refugial base (Brunsfeld et al. 2001; Carstens et al. 2005).

Within many species, genetic differentiation increases with geographic distance resulting from a drift-gene flow equilibrium (Hutchison and Templeton 1999). But in the mountainous regions of western North America, genetic differentiation among alpine populations situated on 'sky islands' is more pronounced among than within contiguous mountain ranges (Worley et al. 2004; Galbreath et al. 2009). This natural habitat fragmentation has important evolutionary consequences, as mammals adapted to alpine environments often have limited dispersal across intervening valleys (Brown 1971; Lomolino and Davis 1997), resulting in isolation and reduced gene flow between mountains. Such spatial heterogeneity may facilitate local adaptation and the maintenance of diversity by producing genetic differentiation (Wegmann et al. 2006), as opposed to highly connected networks where populations remain genetically uniform. Evolutionary hypotheses also predict that populations at the periphery of the range will show less genetic diversity than those in the center (i.e., center-marginal hypothesis: see Eckert et al. 2008). Thus, refugial history and contemporary connectivity influence genetic differentiation and diversity across the range of alpine populations.

Evolutionary and population genetic studies of western North American alpine mammals are limited to a few species (e.g., thinhorn sheep (Sage and Wolff 1986; Worley et al. 2004), arctic ground squirrel *Spermophilus parryii* (Eddingsaas et al. 2004), yellow-bellied marmot *Marmota flaviventris* (Floyd et al. 2005), and American pikas *Ochotona princeps* (Galbreath et al. 2009)). However, the mountain goat (*Oreamnos americanus*) may be the most exemplar alpine species. Mountain goats are endemic to the mainland mountains of western North America, ranging from 44° to 63° latitude North (Cowan and McCrory 1970; Côté and Festa-Bianchet 2003) and are renowned for living in some of the most inhospitable alpine environments (Hornaday 1906). They are thought to have arrived via the Bering land bridge during the Pleistocene (Cowan and McCrory 1970; Rideout and Hoffman 1975), and fossil evidence suggests mountain goats survived in a single refugium south of the ice sheets during the last glacial maximum (Cowan and McCrory 1970). Relatively little is known

about the evolutionary history and population genetic structure of mountain goats. Mainguy et al. (2005, 2007) and Poissant et al. (2009) reported low genetic variability in a small number of individuals sampled from a few locations, and the limited field data available suggests that there is dispersal, but not necessarily gene flow, between herds (Festa-Bianchet and Côté 2008). Mountain goat herds on the range's periphery appear to be small and isolated, often consisting of fewer than 50 individuals (Smith 1988; Hamel et al. 2006). As a result, these peripheral 'sky island' populations may be at risk of becoming genetically impoverished due to the effects of genetic drift and inbreeding (Frankham 1997).

We hypothesized mountain goats would conform to a 'southerly refugia model,' which is characterized by a leading edge expansion, producing decreased genetic heterozygosity from south to north (Hewitt 1996, 2000) and nested haplotypes (Brunsfeld et al. 2001; Carstens et al. 2005). Relative to other large mammals, we anticipated high genetic differentiation between subpopulations (Forbes and Hogg 1999), especially between mountain blocks (Worley et al. 2004). Finally, we expected mountain goats to exhibit reduced genetic diversity at the periphery of their range (Eckert et al. 2008). Thus, examining the mountain goat across its entire range provides the unique opportunity to assess the effects of historical and contemporary vicariance on population genetic structure, and examine the effects of the periphery, distance, mountains, and isolation on gene flow and diversity.

4.2. Materials and methods

4.2.1. Sample collection and DNA extraction

A total of 876 samples were acquired from across the entire native range of mountain goats mostly collected between 2004 and 2009. Sample localities included the Canadian provinces/territories of Alberta and British Columbia, Yukon and Northwest Territories, and the US American states of Alaska, South Dakota, Washington, Idaho, and Montana (Table 4-1). Based on range map and accumulated census data (Festa-Bianchet and Côté 2008), goat populations in the extreme northern (Northwest Territories, Kenai Peninsula Alaska) and southern

(South Dakota, Washington, Idaho, and Montana) periphery of the range appear to be small and isolated (Table 4-1). Most tissue samples were acquired from hunters at compulsory inspection or registration, and subsequently stored in 95% ethanol. A subset of the Alberta, British Columbia, Washington and Alaska samples came from ear punches at field studies. Six hair samples from the Northwest Territories were also acquired. When available, each sample had the age, sex, and location (UTMs) of kill/capture recorded. DNA was extracted using the DNeasy™ Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturer's protocol.

4.2.2. Genotyping

The initial genomic DNA extraction was used as a template in all polymerase chain reactions (PCR). Previously optimized microsatellite markers (Mainguy et al. 2005) were used in duplex and triplex PCRs, totalling 19 markers (Table 4-2). The 10 µl multiplex reactions contained 4.66 to 4.90 µl of double-distilled water, 0.75 to 0.80 µl of $MgCl_2$ (20 mM), 1 µl 10X PCR buffer, 2 µl of dNTPs (0.2 mM each), a 20X primer mix diluted to between 0.24 and 0.34 µM each, 0.08 µl of Taq (0.5 units), and 1 µl of DNA template (25 ng). One primer of the pair was fluorescently labelled (fluorescent tags: 6-FAM, TET, or HEX). The multiplex PCR were hot-started and began with an initial 3-minute denaturation at 95°C, followed by 38 cycles of 30 seconds denaturation at 94°C, 90 seconds annealing at 49°C, and 30 seconds extension at 72°C. The run concluded after 30 minutes at 60°C. The microsatellite amplicons were loaded on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a GS500TAMRA size standard (Applied Biosystems). Microsatellite alleles were detected, scored, and manually verified using GENEMAPPER version 4.0 (Applied Biosystems). To assess the genotyping error rate, we re-extracted DNA and blindly genotyped individuals from the previously genotyped Caw Ridge, Alberta mountain goat herd (Mainguy et al. 2005, 2009a, 2009b).

4.2.3. Microsatellite DNA analysis

Seventeen sampling areas were defined *a priori* according to geographic mountain ranges (Table 4-1; Fig. 4-1a). We quantified genetic diversity as expected (H_E; Nei 1987) and observed heterozygosity (H_O) calculated using GenAlEx 6.2 (Peakall and Smouse 2006). Allelic richness was estimated using the rarefaction method implemented in HP-RARE 1.0 (Kalinowski 2005). We tested for deviation from Hardy-Weinberg equilibrium (HWE) using the exact test (Guo and Thompson 1992) implemented in Genepop 4.0 (Rousset 2008). FSTAT 2.9.3 (Goudet 1995) was used to test for linkage disequilibrium and significance assessed with 1000 permutations.

Microsatellite analyzer (MSA) 4.05 (Dierenger and Schlotterer 2003) was used to calculate Nei's (1972) genetic distance (D_S) between sampling areas. Wright's fixation indices for genetic differentiation (F_{ST}) and inbreeding (F_{IS}) within areas were also estimated using Weir and Cockerham's (1984) unbiased estimators in FSTAT. Significance was tested using 10 000 permutations. Neighbour-joining trees of the sampling areas were constructed with the NEIGHBOR program in PHYLIP 3.69 (Felsenstein 2004). Gene frequencies were bootstrapped over loci 100 times with MSA. Consensus trees were then constructed using the CONSENSE program in PHYLIP and displayed using TreeView (Page 1996).

We used STRUCTURE 2.2 (Pritchard et al. 2000) to assess genetic structure independent of sampling area. STRUCTURE utilizes a Markov chain Monte Carlo algorithm to cluster individuals with multilocus genotypes into populations. We assumed an admixed model with correlated allele frequencies (Falush et al. 2003). The admixed model was selected because male goats are known to move between herds during the rut (Mainguy et al. 2008). Five independent runs from K=1 to K=20 were performed using 1 000 000 iterations with the first 25% removed as a burn in. We used the ΔK method of Evanno et al. (2005) to select the most distinct genetic subdivision in the data. Individuals were then assigned to each genetic cluster based on their highest percentage membership (q) calculated from the five runs using the full search in CLUMPP

1.1.1 (Jakobsson and Rosenberg 2007). We expected a complex and hierarchical pattern of population structure at the scale of our sampling. Therefore, after individuals were assigned to a primary genetic cluster, we repeated the STRUCTURE analyses on each primary cluster using the same methods until there was no longer an increase in likelihood supported by ΔK. Nei's DS and FST quantified population differentiation among hierarchical clusters. Neighbour-joining trees of the final subpopulations were constructed as above.

To identify individuals cross-assigned between subpopulations (i.e., dispersers), polygons were constructed around the core group (*sensu* Bélichon et al. 1996) of individuals assigned to a genetic cluster and belonging to the same mountain range(s) using the HAWTH'S TOOLS (Beyer 2004) extension in ARCGIS 9.0 (ESRI, Redlands, CA, USA). An individual with a q > 0.80 located on a different mountain range not encompassed by its population's polygon was considered cross-assigned. The selection of a 0.80 cut-off is based on the assumption that individuals between 0.20 and 0.80 are admixed (Lecis et al. 2006; Vähä and Primmer 2006; Bergl and Vigilant 2007). Only individuals who moved a sufficient distance (> 100 km) from their clusters were scored as cross-assigned, thus increasing our confidence in identifying true dispersers.

We estimated isolation-by-distance (IBD) between sampling areas (less South Dakota because of small sample size) and STRUCTURE inferred subpopulations. We calculated the Euclidean distance between sampling area and STRUCTURE subpopulations using the mean longitude and latitude of all samples assigned to each group and plotted against pairwise D_S. Genetic distance matrices were obtained from MSA, and a Euclidian distance matrix was constructed using the HAWTH'S TOOLS extension in ARCGIS 9.0. Because many of the sampling areas and subpopulations were nested within a larger mountain range, we examined the effect of mountain range on genetic differentiation by controlling for geographic distance using partial Mantel tests (Mantel 1967). Because the mountain goat's distribution is extensive and may be disjunct between north and south populations (Figure 1.8 in Festa-Bianchet and Côté 2008), we also tested for a second, northern refugium using a partial Mantel

test. Both the mountain range and refugial matrices were binary, consisting of a zero for sampling areas found in the same mountain range or putative refugia, and one for those on different mountain ranges or refugia. Both simple and partial Mantel tests were performed in the program ZT (Bonnet and Van de Peer 2002) using Pearson's correlation coefficient between the matrices. Significance was assessed using 1 000 000 randomizations of the rows, and one column in the matrix.

4.2.4. Mitochondrial DNA sequence

We sequenced the mitochondrial control region of a subset of samples from across the range using the primers L15527 and H00438 (Wu et al. 2003). The control region was amplified via PCR in a 25 µl solution containing 2.5 µl of template DNA (25 ng), 2.5 µl dNTPs (0.2 mM each), 2.5 µl 10X buffer, 0.4 µl each primer (10 µM), 1 µl MgCl (25 mM), 15.5 µl distilled water, and 0.5 µl Taq DNA polymerase (0.5 U). The PCR profile was as follows: hot-start followed by an initial 2-minute denaturation at 94°C, followed by 35 cycles of 45 seconds denaturation at 94°C, 45 seconds annealing at 63.5°C, and 1 minute extension at 72°C. The run concluded after 3 minutes at 72°C.

Double-stranded PCR amplified products were checked by electrophoresis on a 1% agarose gel. Ten µl of PCR product was then treated with 5 µl of ExoSAP (USB Corporation, OH, USA) and incubated at 37°C for 15 minutes followed by heating to 80°C for 15 minutes. A total of 2.5 µL of the ExoSAP treated PCR product was used in a sequencing reaction. Amplicons were directly sequenced in both directions using a Big Dye Terminator Kit (Applied Biosystems, Foster City, CA). Excess of Big Dye Terminator was removed via ethanol precipitation. Sequences were generated on an ABI 3730.

Sequences were aligned using the ClustalW algorithm (Thompson et al. 1994) and edited with Bioedit 7.0.9 (Hall 1999). We calculated haplotype (h) and nucleotide diversity (π) using the software DnaSP 4.0 (Rozas et al. 2003). Three different phylogenetic methods were used to construct evolutionary trees. Neighbour-joining analysis was conducted using the software package MEGA 3 (Kumar et al. 2004). For Bayesian and maximum-likelihood (ML) approaches, the

appropriate model of nucleotide substitution was determined in Modeltest v.3.07 (Posada and Crandall 1998). The ML analysis was run in Garli v.0.95 (Zwickl 2006) with parameters fixed according to Modeltest specifications. MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001) was run under default priors with two independent runs of four chains (three heated) run for 1 000 000 generations, with the first 25% discarded as a burn-in. Confidence in topologies was evaluated based on 1 000 bootstrap replicates or posterior probability.

To test different evolutionary hypotheses, topologies were constrained according to two refugial scenarios (1 versus 2 refugia). The single refugium constraint consisted of northern areas nested within those more southern (a stepping stone model), while the two refugia scenario enforced distinct northern and southern clades. Under a parsimony framework, a heuristic search with tree-bisection-reconnection and maxtrees set to 100 was run with constraints according to refugial scenarios. A pair-wise Templeton test (Templeton 1983), which implements a Wilcoxon signed-rank statistic, was used to determine whether one topology was significantly more parsimonious than the other. We also utilized the likelihood-based SH test (Shimodaira and Hasegawa 1999). Ten thousand bootstrap replicates of each ML tree under different refugial scenarios were resampled using the re-estimated log likelihoods, and significance assessed using a one-tailed *t*-test.

Time of divergence between clades was estimated using MDIV (Nielsen and Wakeley 2001). Under a Markov chain Monte Carlo coalescent-based model, MDIV estimates the parameters θ (where $\theta = 4N_e\mu$) and T (where $T = t / 2N_e$). MDIV was run for 200 000 generations with the first 10% discarded as a burn-in. The migration parameter (M) ranged from 0 to 10, under the assumption of limited historic gene flow between clades. A μ of 24% per locus/per million years (based on sheep control region: Hiendleder et al. 2002; Loehr et al. 2006) was adjusted to account for generation time (gt) and sequence length (l). The following formula was used to calculate time of divergence (t) in years before present (ybp):

$$t = [T \cdot \theta\ ] / [2 \cdot l \cdot \mu \cdot gt]$$

Two approaches were utilized to test for demographic expansion. Fu's F_S (Fu 1997), where negative F_S values represent recent population expansion, was calculated in ARLEQUIN 3.1 (Excoffier et al. 2005) using a coalescent simulation algorithm. Significance of F_S values was evaluated by 1000 permutations. The growth parameter (g) was calculated in LAMARC 2.1.3 (Kuhner 2006). Three runs were conducted using 10 short chains and 2 long chains, with sampling increments of 10 (for 2 000 000 steps) and a burn-in of 1000 samples.

4.3. Results

4.3.1. Microsatellite genetic diversity statistics and population structure

A total of 876 samples (permanently archived at the University of Alberta) were genotyped at 19 loci yielding a >98% complete data set (Dryad Digital Repository, doi:10.5061/dryad.1558). We estimated a genotyping error rate of less than 0.01% by blindly re-genotyping 100 individuals from the Caw Ridge mountain goat herd (Mainguy et al. 2005; 2009a). The total number of alleles per locus ranged from 2 (OarJMP29 and McM64a) to 15 (ILSTS058), and the allelic richness per area ranged from 1.42 to 3.84 (Tables 4-1 and 4-2). Fourteen of the seventeen areas showed positive F_{IS} values (average $F_{IS} = 0.10$, excluding South Dakota), and observed heterozygosities per area ranged from 0.19 to 0.52 (Table 4-1). Linkage disequilibrium (LD) was not detected after Bonferroni correction (Rice 1989).

Significant population subdivision was observed between the sampling areas (Figure 4-1a), with D_S values ranging from 0.03 to 0.66 (Appendix V) and a global D_S, which is a measure of central tendency for the distribution of D_S across loci, of 0.20. Neighbour-joining tree topologies suggested that the southern and northern halves of the range were divergent (Figure 4-2a). We observed two distinct areas of elevated heterozygosity, one in the northern region in southeast Alaska, and one in the south surrounding the Continental mountains (Fig. 4-1b). Peripheral populations had visibly lower heterozygosity (Fig. 4-1b) and less

genetic diversity as measured by H_E (t = -3.4, df = 4.6, P = 0.02) and number of rarified alleles (t = -4.9, df = 5.6, P < 0.01).

Bayesian cluster analysis resolved a primary subdivision at K = 2 based on ΔK, corresponding to north and south clusters (Fig. 4-1c, 4-2b). The north and south clusters were then broken down further into two and three subclusters, respectively, arranged east to west. When these subclusters were broken down as far as possible, we resolved a total of 17 clusters or subpopulations that were in HWE. All clusters were observed in all five replicate STRUCTURE runs and supported by ΔK and CLUMPP with H' (a measure of similarity between runs) of > 0.98. Genetic clusters are presented in Figure 4-1c for individuals assigned to their respective cluster with q > 0.80. Individuals with q < 0.80 were considered admixed.

The degree of differentiation among clusters ranged from D_S 0.04 to 0.61 (Appendix VI) with a global D_S of 0.28. Sixteen of the 17 subpopulations had positive F_{IS} values with an overall average of 0.08 (Table 4-3). A split between north and south clusters was apparent in the neighbour-joining tree topology (Fig. 4-2b). Dispersal between areas was assessed through cross-assignment where we observed 30 (17 males, 9 females, 4 unknown) of 466 individuals (7%) cross-assigned to different populations with a q > 0.80. Most notably, five individuals between the Pacific range (S1.1) and the Rocky and Columbian mountain range populations (S3) were cross-assigned, which represent a distance > 250 km.

A total of 803 samples were sufficiently geo-referenced to test for IBD according to sampling area and STRUCTURE inferred subpopulations. D_S increased significantly with linear distance (Table 4-4; Fig. 4-3), and the fit was better explained by linear distance than by the natural logarithm of distance. When controlling for distance, both mountain ranges and multiple refugia explained additional variance (Table 4-4; Fig. 4-3). All patterns were stronger (i.e., higher r^2) in the STRUCTURE inferred subpopulations

4.3.2. Mitochondrial phylogeographic patterns

A total of 200 samples were sequenced from across the range of mountain goats (Fig. 4-4). All 17 subpopulations were represented and sequences are deposited in

GenBank (HM230898-HM231097). The three phylogenetic methods used all had 100% bootstrap support or posterior probability for the north-south split (Fig. 4-4). Support for phylogenetic structure within the north and south clades were limited, as they were essentially polytomies (Fig. 4-4). Haplotype and nucleotide diversity measures showed that major clades were equally diverse (Table 4-5). The Templeton test found the optimal tree of a north-south split (no. of steps = 562) to be significantly more parsimonious ($Z = -6.46$, $P < 0.01$) than a single refugium model represented by a nested south to north topology (no. of steps = 732). The likelihood-based SH also rejected the single refugium model as a viable phylogeny ($P < 0.01$).

MDIV parameters T and θ both had bell-shaped curves with peaks in likelihood at 0.32 and 109 respectively. Using a generation time of 4 years and sequence length of 811 bp, we estimated the date of divergence between north and south clades to be 224,003 ybp. Both north and south clades showed evidence of recent demographic expansion (North clade: $F_S = -24.03$ ($P < 0.01$), $g = 217$ with 95% CI: 96-358; South clade: $F_S = -23.92$ ($P < 0.01$), $g = 358$ with 95% CI: 172-577).

4.4. Discussion

4.4.1. Historical patterns of genetic differentiation

Our results support the possibility of a second, northerly refugium for mountain goats. In several North American mammals, glacial induced vicariance produced distinct north and south refugia (Fleming and Cook, 2002; Loehr et al. 2006; Aubry et al. 2009); however, fossil evidence suggested only a southern refugium existed for mountain goats during the last glacial maximum (Cowan and McCrory 1970; Rideout and Hoffman 1975). Recent findings have uncovered a now extinct coastal refugium (Nagorsen and Keddie 2000) raising the possibility of additional coastal refugia existing for mountain goats. These data do not support the 'southerly refugia model' for mountain goats, and suggest Beringia, or northern British Columbia (the latter suggested by Loehr et al. 2006), was a major refuge during the last glacial maximum. Given that the current range of mountain goats

falls almost entirely within the proposed extent of ice-sheets during the last glacial maxima (see Dyke et al. 2003), a northern British Columbia refugial site, rather than Beringia proper, appears most likely.

Multiple lines of evidence from these data support a distinct northern refugium. Phylogenetic analyses of both microsatellite and mitochondrial data support a north-south split (Fig. 4-2, Fig. 4-4). The date of this split is ~224,000 ybp, which predates the onset of the last glaciation and is consistent with estimates from mountain sheep from the same area (Loehr et al. 2006). Patterns of genetic differentiation support this hypothesis, as partial Mantel tests that incorporated multiple refugia (Table 4-4) explained an additional 12% of the variance in genetic differentiation. If recolonization had emanated from only a southern refugium following the retreat of the last ice sheet, we would have expected a cline of decreasing genetic diversity from south to north (Hewitt 1996, 2000) or a nested phylogeny (Brunsfeld et al. 2001; Carstens et al. 2005). We do not see this; instead, there are distinct northern and southern hot spots of diversity (Fig. 4-1b) that are separated by a strongly supported bifurcation (Fig. 4-4) and the southern refugium stepping stone model is rejected as a viable scenario. Moreover, these hot spots overlay predicted 'hot spot clusters' (Fig. 4-3 in Swenson and Howard 2005) that reflect Pleistocene glacial refugia and/or expansion and common phylogeographic breaks. In mountain goats, the northern and southern hotspots (Fig. 4-1b) may represent their refugial locations as well where divergent lineages mixed post-glaciation. In addition, the lack of phylogenetic resolution (Fig. 4-4) and signal of demographic expansion (avg. g = 288 and F_S = -24) suggest mountain goats have gone through a recent, rapid expansion from these refugia when the glaciers receded. Similar patterns of post-glacial expansion from refugia have been detected in other northern mammals from the same area (Fedorov et al. 2003; Lessa et al. 2003). Overall, these data suggest that mountain goats were isolated in at least two major refugia during the last glacial maximum, and underwent a rapid demographic expansion following the retreat of the Laurentide and Cordilleran ice-sheets.

A noteworthy anomaly is Alaska's Baranof Island population, which originated from a small number of founders in 1923 (Paul 2009). Two distinct subpopulations were detected on the island: N2.2.1 that was restricted to the island, and N1.1.1 that was primarily found on the mainland (Fig. 4-1c). The island also had a mixture of mitochondrial haplotypes, but was predominantly from the southern clade (Fig. 4-4). Historically, no goats were believed to inhabit Baranof Island, prompting authorities to translocate goats from the adjacent mainland in the early 20th century (Paul 2009). The source population for this translocation is encompassed by the N1.1.1 polygon. Interestingly, N2.2.1 is differentiated from all Alaska populations (minimum $D_s = 0.10$), and is relatively diverse ($H_E = 0.45$). Given the history of the island, it seems unlikely that drift alone could produce two genetically divergent and diverse subpopulations that are not spatially segregated. A possible explanation for this pattern is that N2.2.1 is a glacial relict. There is geological evidence that parts of Baranof Island were ice-free during the last glaciation (Carrara et al. 2007), and Cook et al. (2001, 2006) have compelling phylogeographic data that supports such a refuge. Moreover, Heaton & Grady (2003) discovered a horn core from a hypothesized *Saiga tatarica* dating 32,000 ybp from the closely situated Prince of Wales Island. Given our data, and because *S. tatarica* is currently restricted to central Asia (Sokolov 1974), it is conceivable that this horn core may actually be that of *Oreamnos*. We envision a scenario where at the beginning stages of the glacial advance, mountain goats were split into their major clades (Fig. 4-4) somewhere in southeast Alaska or northern British Columbia, with the Baranof Island population retaining the southern haplotype. During the subsequent isolation, the Baranof Island became population differentiated, yielding the N2.2.1 cluster. The recent translocation from the mainland (Paul 2009) introduced the N1.1.1 genotype, and possibly the northern haplotype. Overall, the detection of a second, northerly refugia and the possibility of a cryptic refugium add additional insight into the broader biogeographic patterns that have shaped species distributions in western North America (Soltis et al. 1997; Brunsfeld et al. 2001).

4.4.2. Gene flow and dispersal

Alpine specialists often show high levels of differentiation over relatively short distances (Perez et al. 2002; Forbes and Hogg 1999; Worley et al. 2004). Mountain goats display such a pattern (Fig. 4-3), and show strong differentiation between subpopulations (Global $D_S = 0.28$; Appendices V and VI). Similar to thinhorn sheep (Worley et al., 2004), mountain ranges also explained a significant portion of variance across the range (Table 4-4). Because mountain ungulates are adapted to naturally patchy alpine terrain (Forbes and Hogg 1999), it is not surprising that mountain ranges facilitate gene flow while valleys would impede it - this is in agreement with Brown's (1971) early observations on boreal mammals. Furthermore, in areas like the Boundary Range of Alaska, subpopulations were visibly separated by fiords and rivers, which goats are unlikely to cross (K. White, unpublished data; but see Klein 1965). Similar breaks are observed in the phylogeography and population structure of numerous Alaska fauna (Cook et al. 2001, 2006). These analogous patterns are largely attributable to shared Pleistocene climatic events that fragmented boreal and alpine terrain.

During the last glacial maximum, global cooling forced species into temporary refugia. Individuals subsequently recolonized available habitat as the glaciers receded. In the alpine, climatic warming permitted the forests to encroach and naturally fragment the terrain creating 'sky islands.' This pattern has been used to explain the patterns of diversity and differentiation in wild sheep (Sage and Wolff 1986; Forbes and Hogg 1999; Worley et al. 2004; Loehr et al. 2006), which occupy similar habitat as mountain goats. Because alpine ungulates typically exhibit a small effective population size, high site fidelity, and limited dispersal (Forbes and Hogg 1999; Worley et al. 2004; Festa-Bianchet and Côté 2008), drift is considered the paramount evolutionary factor affecting genetic diversity in these fragmented populations. However, the distribution of genetic diversity during recolonization is also shaped by long-distance dispersers (Hewitt 1996, 2000).

Long-distance dispersal produces populations with patchily distributed allele frequencies (reviewed by Excoffier et al. 2009). Such a pattern is evident in

mountain goats, as many adjacent subpopulations with a common refugial origin are quite divergent (Fig. 4-3). Moreover, apparent contemporary long-distance dispersal was detected in the data set, most notably across the British Columbia (BC) interior. For BC mountain goats, any east-west movement would involve crossing hundreds of kilometers of sub-optimal habitat. This area is generally considered void of goats - although a handful of observations have been recorded (Mountain Goat Management Team 2010). No recent or direct translocations between these ranges have been conducted (Hatter and Blower 1996), and poaching is unlikely to account for all the cross-assignments. Mountain goats introduced to Oregon moved 71 km from their natal area (Mathews and Heath 2008), and goats in Alberta have been seen 300 kilometers from the nearest known population (Festa-Bianchet and Côté 2008). In addition, mountain goats are known to traverse extensive icefields (Nichols 1985; Hofer 2004) and Mathews and Heath (2008) radio-tracked an individual through agricultural and timber habitat for nearly 250 km. Clearly, long-distance dispersal and movement across sub-optimal habitat has played an important evolutionary role in colonization, and maintenance of gene flow between geographically and temporally distinct subpopulations.

4.4.3. Genetic diversity and effect of geography

Mountain goats can be described as having low to moderate levels of genetic diversity. Relative to other mountain ungulate populations, mountain goats have similar diversity levels to that of the ibex (*Capra ibex*; Maudet et al. 2002) and chamois (*Rupicapra* spp.; Perez et al. 2002) but lower than that of bighorn (*Ovis canadensis*; Forbes et al. 1995; Forbes and Hogg 1999) and thinhorn sheep (Worley et al. 2004). Based on our scale of sampling, there is likely additional, finer scale hierarchies not resolved in our analyses. For example, the cluster S3 encompasses at least 12 discrete herds in Alberta (Hamel et al. 2006) that cannot be discerned without intensive sampling. This unresolved substructure along with the global patterns across loci and populations (Tables 4-1 and 4-2), suggest that the positive F_{IS} values are in part due to the 'Wahlund effect.' That being said, the southern peripheral mountain goat populations in the United States (Montana,

Idaho, and Washington) demonstrate the highest levels of inbreeding in the data set (F_{IS} = 0.15 – 0.26, less South Dakota), which may not entirely be attributed to undetected substructure. Given the sensitive nature of alpine habitat in response to climate change (Sala et al. 2000), populations on the southern end are likely to be impacted disproportionately. In these southern areas, the effects of any future loss of genetic diversity or inbreeding could be exacerbated by climate change and the highly polygynous mating system of mountain goats (Mainguy et al. 2008) where few males obtain most of the paternities (Mainguy et al. 2009b).

Across the range of mountain goats, genetic diversity was significantly diminished in peripheral populations (H_E from 0.18– 0.45), whereas populations located near the center of the species range had higher genetic diversity (H_E from 0.36 – 0.54). This can also be visualized in the individual heterozygosity plots (Figure 4-1b) where peripheral populations are isolated and less diverse. The low levels of genetic diversity in the peripheral range of the mountain goat are in part the result of small population numbers and isolation, as all these sampling areas have less than 4000 mountain goats (Festa-Bianchet and Côté 2008). Empirical support for the central–marginal hypothesis within vertebrates is accumulating (e.g., Beebee and Rowe, 2000; Hutchison 2003; Howes and Lougheed 2008); but none thus far has examined a species as large and mobile across a naturally fragmented environment as the mountain goat. Peripheral populations are thought to occupy ecologically substandard environments, to have suffered from founder effects, genetic drift, and inbreeding, and thus tend to be smaller, isolated, and less reproductively successful (Brussard 1984; Hoffmann and Blows 1994; Lessica and Allendorf 1995; Hutchison 2003). This could explain why southern mountain goat populations went extinct during the hypsithermal (Rideout and Hoffman 1975). Peripheral populations are of particular evolutionary importance, as they may have unique biographical traits (e.g. climate tolerance), have high levels of genetic differentiation, or be locally adapted, and thus important for the maintenance of biodiversity (Eckert et al. 2008; Bhagwat and Willis 2008; Hampe and Petit 2005). Importantly, these populations may require different conservation

practices (see Hampe and Petit (2005) for examples) because of their unique ecological and evolutionary attributes.

Both historical and geographic vicariance has played intricate roles in the distribution and abundance of genetic diversity in species. In western North America, historical climate change and connectivity among mountains have been prominent in shaping genetic differentiation. Across the range of mountain goats, refugial origin and mountain ranges have significantly influenced genetic differentiation, which underscores the need to consider these factors when modeling genetic differentiation in alpine specialists. With alpine ecosystems projected to undergo large changes in biodiversity from small environmental perturbations (Sala et al. 2000), understanding the temporal and geographic effects on the genetic structure of species is required for correctly modeling the effects of climate change (Provan and Bennett 2008), and will be essential for conserving the adaptive and evolutionary potential of both alpine ecosystems and their inhabitants.

Table 4-1. Estimates of genetic variability for individual mountain goats assigned to a sample area belonging to a particular mountain range. Regional population estimates are from Festa-Bianchet & Côté (2008). Sample areas are identified by their abbreviation (Abbr.) throughout the study. Statistics include observed heterozygosity (H_O), expected heterozygosity (H_E), and allelic richness (A) estimated by rarefaction (South Dakota was not included in calculation), and Wright's inbreeding coefficient (F_{IS}). Also reported is the number of individuals assigned to a sample area.

Region	Estimated regional population	Sample Area	Abbr.	Mountain range	N	H_O	H_E	A	F_{IS}
Alaska	24000-33500	Boundary Range*	BouR	Coast mtns	249	0.47	0.54	3.7	0.14
		Kenai peninsula (P)	Ken	Coast mtns	12	0.19	0.18	1.8	-0.03
British Columbia (BC)	39000-67000	Cariboo mtns	Car	Columbian	12	0.50	0.49	3.5	0.03
		Purcell mtns	Pur	Columbian	17	0.47	0.52	3.2	0.12
		Selkirk mtns	Sel	Columbian	33	0.42	0.46	3.2	0.11
		Kitimat & Hazelton mtns	KitH	Coast mtns	71	0.42	0.48	3.5	0.12
		Pacific Range	PacR	Coast mtns	19	0.49	0.51	3.8	0.06
		Omineca	Omi	Interior mtns	16	0.41	0.46	3.2	0.13
		Skeena	Ske	Interior mtns	25	0.50	0.49	3.6	0.00
		Northern Interior	NorI	Interior mtns	43	0.52	0.53	3.7	0.04
		Northern Rockies	NorR	Rocky mtns	14	0.45	0.46	3.4	0.08
Alberta & BC	2750 (Alberta)	Continental mtns	Con	Rocky mtns	221	0.46	0.49	3.3	0.08
Idaho	2700	Salmon River mtns (P)	SalR	Salmon River	20	0.31	0.35	2.4	0.15

Montana	2295-3045	Bitterroot/Absaroka (P)	BitA	Rocky mtns	10	0.31	0.36	2.3	0.22
Northwest Territories & Yukon	1000 1400	McKenzie mtns (P)	MckM	McKenzie mtns	13	0.39	0.45	3.2	0.17
South Dakota	80-100	Black Hills (P)	BlaH	Black Hills	2	0.32	0.18	1.4	-0.46
Washington	2000	Cascades (P)	Cas	Coast mtns	26	0.26	0.34	1.6	0.26

* Boundary Range includes samples from southwest Yukon.

(P) denotes periphery of range

Table 4-2. Number of alleles (A), observed heterozygosity (H_O), and expected heterozygosity (H_E) for each marker used in this study across the entire range of mountain goats. Chromosome locations are based on cow and sheep as described by Mainguy *et al*. (2005).

Locus	A	H_O	H_E	Chromosome location
MAF36a	3	0.20	0.26	22
OARHH35a	9	0.49	0.66	4
OARJMP29a	2	0.31	0.38	24
TGLA122a	13	0.59	0.76	21
AR028a	11	0.42	0.56	2
BM1225a	8	0.47	0.69	20
RT27a	9	0.51	0.66	unassigned
MCM152a	9	0.38	0.47	13
ILSTS058a	15	0.66	0.86	17
TGLA10a	8	0.49	0.57	2
OARHH62a	4	0.22	0.30	16
HUJ1177a	4	0.35	0.45	3
RT9a	7	0.60	0.72	unassigned
OARCP26a	12	0.44	0.52	4
MAF64a	10	0.41	0.56	1
Huj616a	11	0.61	0.78	13
BM1818a	12	0.42	0.50	23
BM6444a	5	0.45	0.57	2
McM64a	2	0.35	0.44	2
Average	8.1	0.44	0.56	
(Std. Error)	(0.89)	(0.03)	(0.04)	

Table 4-3. Number of samples (*N*), Wright's inbreeding coefficient (F_{IS}), and mean percent membership for the 17 subpopulations designated by STRUCTURE 2.2.

Subpopulation	*N*	F_{IS}	Mean percent membership (q)
N111	47	0.06	0.73
N112	53	0.01	0.71
N113	29	0.03	0.77
N12	12	0.03	0.99
N13	71	0.07	0.91
N211	29	0.05	0.78
N212	34	0.17	0.76
N213	48	0.08	0.71
N214	51	0.08	0.70
N221	28	0.08	0.94
N222	55	0.04	0.94
S11	32	0.08	0.92
S12	21	0.17	0.93
S21	35	0.17	0.84
S22	93	0.09	0.73
S23	96	0.06	0.74
S3	142	0.08	0.86

Table 4-4. Results from simple and partial Mantel tests examining the effects of distance, refugia, and shared mountain ranges on population differentiation. Variable following the period is controlled for (i.e. Refugia.Distance). † denotes STRUCTURE 2.2 inferred subpopulations.

Model	r^2	*P* value
Isolation-by-distance		
D_S ~ Distance	0.63	P <0.001
D_S ~ Distance †	0.66	P <0.001
Historical		
D_S ~ Refugia.Distance	0.05	P = 0.09
D_S ~ Refugia.Distance †	0.12	P = 0.04
Contemporary		
D_S ~ Mountains.Distance	0.02	P = 0.06
D_S ~ Mountains.Distance †	0.10	P = 0.04

Table 4-5. Mitochondrial control region diversity statistics for the major mountain goat clades. Included are the number of individuals in each group (*N*), observed number of haplotypes (*Nh*), haplotype diversity (*h*), and nucleotide diversity (π).

Clade	*N*	*Nh*	*h*	π
North	85	46	0.97	0.013
South	115	54	0.96	0.011
All	200	99	0.98	0.037

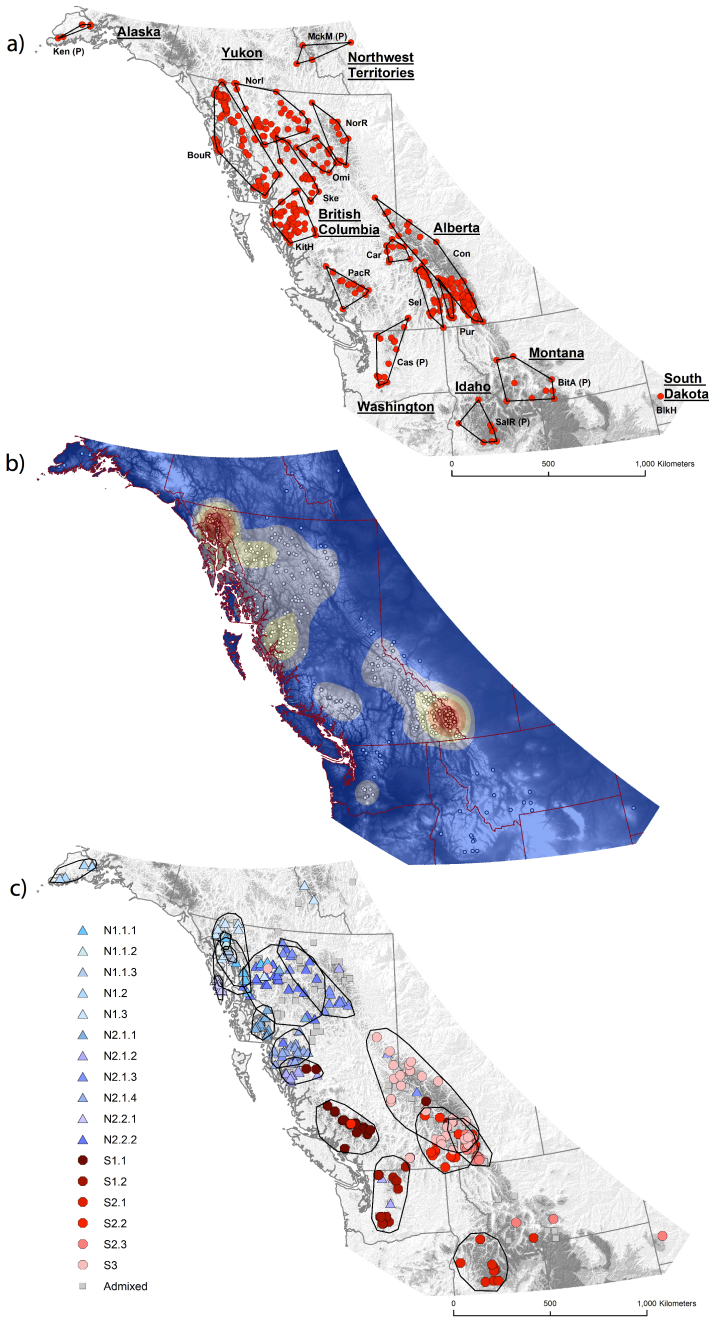

Figure 4-1. Map a) Sample localities for North American mountain goats (*Oreamnos americanus*) across their native range. Mountain ranges are delimitated by polygons and abbreviations are listed in Table 4-1 and peripheral populations are denoted by (P). Map b) Hotspots of individual genetic heterozygosity for mountain goats across their range. Areas with high heterozygosity are warmer (yellow to red) and were detected using spatial analyst

in ARCGIS 9.0. Map c) Locations of seventeen genetic clusters of mountain goats identified by STRUCTURE. Southern (triangles) and northern (circles) samples denote the uppermost hierarchical split supported by STRUCTURE. Individuals assigned to a subpopulation have a $q > 0.80$, while those admixed are left unassigned (gray squares). Because of the scale of sampling, a single point may represent multiple individuals.

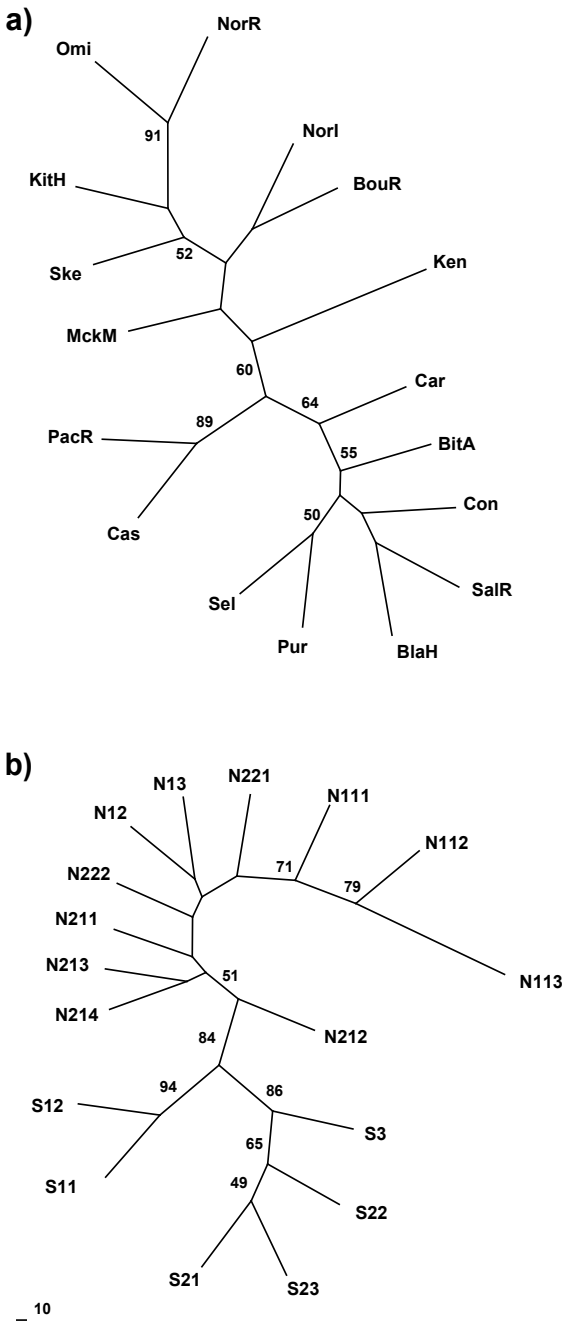

Figure 4-2. Neighbour-joining trees for: a) sampling areas specified *a-priori*, and b) STRUCTURE designated subpopulations. Bootstrapped values are from 100 replicates with those >50% presented. Abbreviations are listed in Table 4-1.

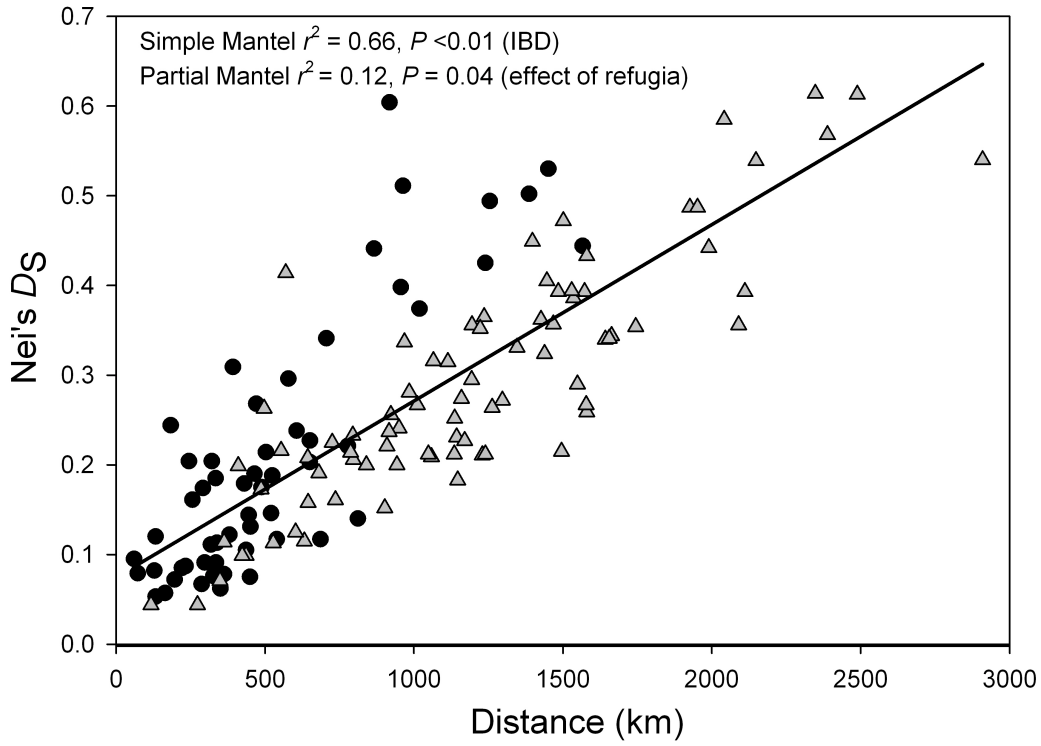

Figure 4-3. Isolation-by-distance relationships between populations of mountain goats defined by subpopulation. Gray triangles indicate population pairs from different refugial origin, and black circles denote a common refugium. The effects of distance and refugia on genetic differentiation are shown.

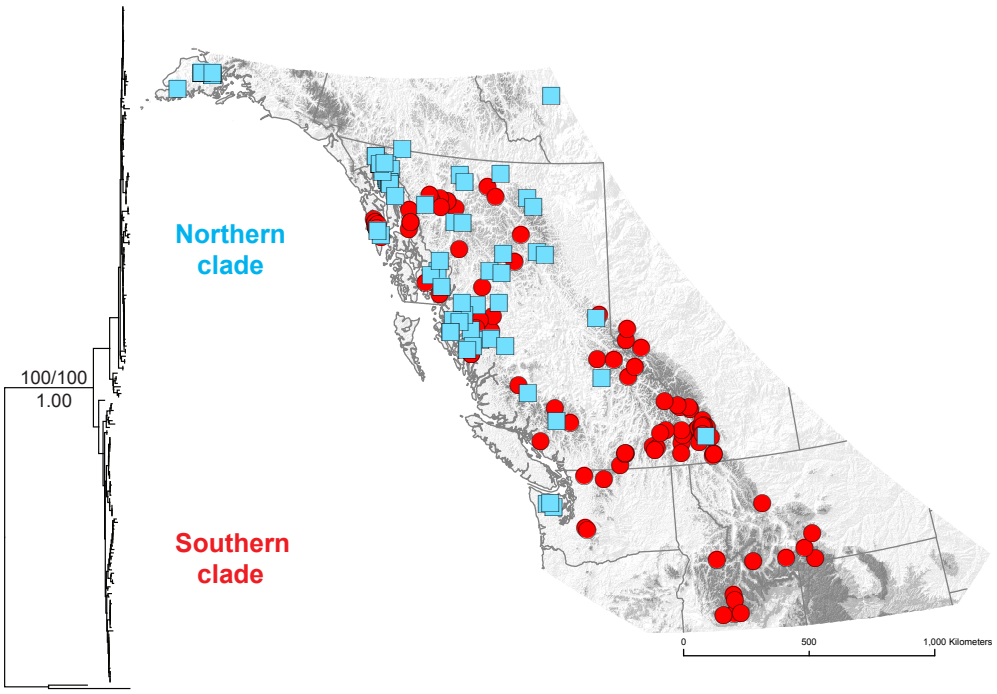

Figure 4-4. Neighbour-joining tree of 200 mountain goat samples with their individual clade designations plotted on the adjacent map. Support values are based on 1000 bootstrapped data sets or posterior probability. Support values on the main branch correspond to neighbour-joining/maximum likelihood above, and Bayesian below.

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Chapter 5

(Lack of) Genetic diversity in immune genes predates glacial isolation in the North American mountain goat (*Oreamnos americanus*)

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5.1. Introduction

The capacity to adapt to environmental perturbations through evolutionary change is dependent upon the presence of adaptive genetic variation (Reed and Frankham 2003). The major histocompatibility complex (MHC) family of genes harbours the greatest number of polymorphic loci in the vertebrate genome (Hedrick 1994), with high variability conferring increased pathogen resistance. Monitoring such diversity is considered an important component to evaluating population-level and species-wide health (Schwartz et al. 2007) and has become an important element in the genetic health assessment of wild populations. Moreover, variation in the MHC of wild populations has been shown to be associated with mate choice (Schwensow et al. 2008), longevity (Huchard et al. 2010), survival (Paterson et al. 1998), parasite load (Oliver et al. 2009), and individual quality (Ditchkoff et al. 2001). For all these reasons, the interest in MHC studies has grown considerably in ecology and evolution over the past two decades (see Milinski 2006; Piertney and Oliver 2006; Sommer 2005).

Part of the increased interest also stems from an increasing number of species becoming 'genome enabled' (Kohn et al. 2006), which permits the transfer and screening of a wider array of molecular markers in wild, and non-model organisms. One recently 'genome enabled' group is the Caprinae, or wild sheep and goats, which have benefited from the genomic analysis of domestic sheep, *Ovis* (e.g., Archibald et al. 2010), and cattle, *Bos* (e.g., Zimin et al. 2009). For example, Poissant et al. (2009) screened over 300 domestic sheep markers in two wild caprids with over 50% success. Miller et al. (2011) genotyped over 45,000 SNP loci in two wild sheep species using an array of markers developed for domestic sheep. Not surprisingly, nearly all MHC and immune gene studies of wild Caprinae have relied on the cow and sheep genome as a resource, often requiring little or no assay modifications between species.

The Caprinae (Order: Artiodactyla) are only found in the northern hemisphere, and primarily inhabit steep, mountainous terrain (Shackleton 1997). As MHC proteins present foreign and self-antigen to immune cells (Klein 1986),

increased sequence diversity should confer greater protection against pathogens. However, in the Caprinae, it has been suggested that their northerly alpine habitats have reduced parasite diversity, which in turn has relaxed the selection pressures that promote MHC variability (Alvarez-Busto et al. 2007; Schaschl et al. 2004). This notion has been used to explain the trend of decreasing MHC diversity with latitude (Mainguy et al. 2007). However, evidence for a latitudinal gradient in terrestrial mammal parasite diversity is equivocal (Bordes et al. 2010), and only a handful of studies have robustly assessed parasite load relative to MHC diversity in wild populations (e.g., Oliver et al. 2009). Alternatively, if alleles are shared between lineages, reduced diversity may be attributed to balancing selection, where pathogen-mediated selection favours only a few alleles (Takahata 1990; Klein et al. 1993; Mona et al. 2008). Yet another possible cause for the low diversity in some Caprinae species could have been the occurrence of historical bottlenecks (Amills et al. 2004; Hedrick et al. 2001; Mainguy et al. 2007; Mikko et al. 1999). Regardless of the mechanism, the limited immunogenetic diversity may be a concern (Radwan et al. 2010), especially under the auspice of climate change (Mainguy et al. 2007). With two-thirds of the Caprinae species considered threatened (Shackleton 1997), species-level assessment of genetic and immunogenetic diversity may prove important to conservation and management (Schwartz et al. 2007; Sommer 2005).

The mountain goat (*Oreamnos americanus*) is one of only four caprids found in North America. Range-wide census data is sporadic, but mountain goats likely number close to 100,000 and are under no immediate threat (Festa-Bianchet and Côté 2008). Initial screening of MHC and neutral genetic markers showed minimal diversity (Mainguy et al. 2005, 2007; Poissant et al. 2009), with the patterns of neutral genetic variation echoed in an extensive range-wide analysis (Shafer et al. 2011). Shafer et al. (2011) also found evidence of two major refugia during the Last Glacial Maximum (LGM); the two refugial clades diverged over 200,000 years ago and are separated latitudinally by more than 1,500 kilometers. The ranges of two other caprids, bighorn sheep (*Ovis canadensis*) and thinhorn sheep (*O. dalli*), cover these refugial sites along with the northern and southern

limits of mountain goats. Interestingly, MHC allelic diversity of bighorn sheep (Gutierrez-Espeleta et al. 2001) and thinhorn sheep (Worley et al. 2006) are among the highest and lowest in wild ungulates, respectively. This is consistent with a clinal pattern (Mainguy et al. 2007 - but see Qutob et al. 2011 for another scenario), as bighorn sheep live south of their thinhorn relatives. Based on this observed latitudinal cline (Mainguy et al. 2007), we hypothesized that immunogenetic diversity would be lower in the mountain goat lineage from the northern refugium than the south. We also screened additional immunogenetic markers to better characterize MHC diversity (Spurgin and Richardson 2010), and to assess whether the paucity of MHC diversity observed by Mainguy et al. (2007) was representative of the species range.

5.2. Materials and methods

5.2.1. Immune gene background and nomenclature

The MHC is an immunologically important gene cluster with the primary function of coding antigen-presenting proteins. The MHC gene structure is conserved among ungulates and is divided into three classes all with different functions (Amills et al. 1998). Class I genes primarily respond to intracellular parasites, viruses, and proteins by presenting antigens to cytotoxic T cells. The Class I protein is a heterodimer consisting of a heavy and light chain, with the a-I and II domains of the heavy chain being the peptide binding region (PBR; Hughes and Yeager 1998). Class II genes code for peptides that present antigens from extracellular parasites and proteins to helper T cells. Similarly, Class II proteins are a heterodimer, but the PBR is found on the a-I and b-I domains (Hughes and Yeager 1998). Class III genes are considered highly conserved and are not well characterized for caprids (Dukkipati et al. 2006; Qin et al. 2008). These genes code for cytokines and proteins involved in the complement cascade (Dukkipati et al. 2006). For the MHC structure, we followed the nomenclature of *Ovis* (Dukkipati et al. 2006; Gao et al. 2010), and used the prefix *Oram* to be consistent with Mainguy et al. (2007). We also analyzed the natural resistance associated macrophage protein (NRAMP), another immunologically relevant gene involved

in responding to intracellular pathogens through controlling cation concentrations (Canonne-Hergaux et al. 1999). The NRAMP protein is located at the endosomal/lysosomal layer of macrophages and is induced by inflammatory agents (Gruenheid et al. 1997).

5.2.2. Tissue sampling and DNA preparation

We obtained tissue samples from across the mountain goat's native range, representing the Canadian provinces of British Columbia and Alberta, Yukon and Northwest Territories, and the American states of Idaho, Alaska, Montana, and South Dakota. Most tissue samples were collected from hunted goats during inspection or registration. Some tissues from Alberta, British Columbia, and Alaska were from ear punches taken during field studies. Handling of live animals was in accordance with animal care guidelines (Gannon et al. 2007). Most samples were stored in 95% ethanol at either -20 or -80^{o}C, but some tissues from Montana were directly frozen at -20^{o}C.

A DNeasy Blood and Tissue kit (QIAGEN) was used to extract DNA from mountain goat tissue samples. We followed the manufacturer's protocol with the following exceptions: 1) An extra one-minute spin at 20,000 x g was performed before elution to ensure the removal of all excess ethanol, and 2) the column was incubated in buffer AE for at least five minutes before centrifugation. Two hundred microlitres of eluent was recovered per sample and stored at -20^{o}C. All samples were quantified using a Nanodrop 2000 (Thermo Scientific).

5.2.3. PCR of Immune Genes

We first screened an array of immune gene primers taken from Bovid and Caprinae species. Those markers that consistently produced a PCR product were analyzed in this study (details on other markers available form corresponding author). We amplified portions of exons 2 and 3 (encoding the a-1 domain) along with intron 2 of the Class I MHC gene using the primers B^{a} (5'-GCT ACG TGG ACG ACA CGC-3') and Br^{a} (5'-AGC GCA GGT CCT CGT TC-3') from Miltiadou et al. (2005). Two Class II genes were amplified: the first domain (exon 2) of the *DRA* gene was amplified using primers DRA-For (5'-CCC CCCT TTC TTG TCT TTT CAG AG-3') and DRA-Rev (5'-CAA TTC CCA AGT CTA GGA

GGA CTG-3') from Sena et al. (2003), and exon 2 of the *DRB* gene using the LA31-K (5'-ATC CTC TCT CTG CAG CAC ATT TCC T-3') and LA32-K (5'-TCA CCT CGC CGC TGC ACA-3') primers modified by Worley *et al.* (2006). The fourth exon and the 3′ UTR of Class III MHC gene, tumour necrosis factor alpha (*TNF-α*), was amplified using primers ovTNF-C1 (5'-CTG CCG GAA TAC CTG GAC TA-3') and ovTNF-C2 (5'-TCC AGT CCT TGG TGA TGG TT-3') from Alvarez-Busto et al. (2004). All PCR ingredients and cycling conditions are provided in appendices VII and VIII. Because Mainguy et al. (2007) only analyzed a small portion of the mountain goats range, we first screened the Class II *Oram-DRB* gene in individuals from across the entire range representing all known subpopulations detected by Shafer et al. (2011).

We amplified exons 5-7 and the intervening introns of the NRAMP gene using two primer sets from Worley et al. (2006): NRAMP-2F (5'-CTC TCC TCT GGC TGA CCA TC-3') and NRAMP-2R (5' CAC GAT GGT GAT GAG GAC AC-3'), and NRAMP-3F (5'-GTG GGA GAT CCA GAC TCC TG-3') and NRAMP-3R (5'-CCG AAG GTC AAA GCC ATT AT-3'). The PCR conditions are in the appendices VII and VIII.

5.2.4. Sequencing and cloning of PCR products

An aliquot of all PCR products was first visualized on an agarose gel (1-2%) to confirm amplification. Ten microlitres of PCR product were treated with 0.25 U each of exonuclease I (Exo; USB) and shrimp alkaline phosphatase (SAP; USB) resulting in a 15 ml reaction. Exo-SAP treated samples were placed in a two-step incubation of 15 minutes at 30^{o}C followed by 15 minutes at 80^{o}C. All samples were then directly sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), ethanol precipitated, and resuspended in 10 ml of water. Two microlitres of the products were mixed in 8 ml Hi-Di (Applied Biosystems) and run on ABI 3730 DNA Analyzer (Applied Biosystems).

The Class I gene amplification produced multiple products, thus requiring cloning before sequencing. For this gene, PCRs were conducted using the high-fidelity Phusion Taq (New England Biolabs). PCR fragments were inserted into a pJET/blunt vector (Fermentas) following the manufacturer's sticky-end protocol.

Vectors were transformed into competent DH5α cells (Invitrogen) and grown on LB agar plates with ampicillin. Colonies were plucked and resuspended in 100 μL of water used for colony PCR (appendices VII and VIII). Sequencing followed the above protocol.

5.2.5. Sequence and statistical analyses

The chromatograms and sequence data were analyzed using Bioedit 7.0.9 (Hall 1999). All sequences were screened on GenBank to find similar sequences and ensure that the appropriate gene was amplified. Sequences were then aligned and converted to amino acids using the software MEGA5 (Tamura et al. 2011). The numbers of SNPs, haplotypes, along with haplotype (*h*) and nucleotide diversity (π) were calculated using the software DnaSP 4.0 (Librado and Rozas 2009). Deviation from Hardy-Weinberg Equilibrium was assessed in GenAlEx 6.2 (Peakall and Smouse 2006) when applicable. We calculated average rate of non-synonymous (dN) and synonymous (dS) substitutions for each coding region according to the Nei–Gojobori method (Nei and Gojobori 1986) implemented in MEGA5. A Z-test of neutrality was conducted between dN and dS using 1,000 bootstrapped replicates. Because of the multiple Class I products, we conducted a phylogenetic analysis on the exonic sequence data to identify gene clusters (*sensu* Miltiadou et al. 2005) and any non-classical genes (*sensu* Zidi et al. 2008). A neighbor-joining tree was constructed from the K2P nucleotide distances in MEGA5. Sequence data from *Ovis*, *Bos, Capra*, and *Sus* were also included. For *Ovis*, we included representatives of all four Class I gene clusters observed in Miltiadou et al. (2005). Confidence in topology was assessed using 1,000 bootstrapped replicates.

We tested for an association between parsimony informative SNPs and amino acid changes with northern and southern refugial origins designated by Shafer et al. (2011). Because of the high levels of differentiation and vicariant refugial history in mountain goats (Shafer et al. 2011), we interpreted a lack of association to mean that the SNPs did not arise from, or post, the LGM. This was done using a binomial logistic regression with bias-reduction (Firth 1993) implemented by the brglm package (Kosmidis 2007) in R2.12.1

(http://www.rproject.org/). Parameter estimates along with the *z* statistic and *P* value are given.

5.3. Results

5.3.1. MHC and NRAMP sequence data

A total of 212 individuals were sequenced at 249 bp of the *Oram-DRB* exon 2. The only SNP was observed was a G→T transversion at position 233 resulting an amino-acid change from glycine to valine. Thirty-two individuals were heterozygous, and only six were homozygous for the T allele. These genotypic frequencies deviated from what is expected under Hardy-Weinberg Equilibrium in the direction of heterozygote deficiency across the range ($P < 0.01$) and in the southern refugium ($P < 0.01$), but not in the northern refugium ($P = 0.08$). Both alleles were previously deposited in GenBank (DQ648492-3) and are most similar to *Bos grunniens* (AY374126) and *Ovibos moschatus* (AF162657).

Due to the limited variability in *Oram-DRB*, we opted to only sequence 31 individuals representing all DRB haplotypes and the entire mountain goat range for remainder immune genes. We sequenced 254 bp of the Class II Oram-DRA exon 2, which was found to be monomorphic (GenBank Accession nos. JN861547-77) and 98% identical to *Capra falconeri* (FM986346) and *Ovis aries* (Z11600). The Class III *Oram-TNF-α* exon 4 and 3' UTR was also monomorphic at the 232 bp sequenced (GenBank Accession nos. JN861578-608), and 98% similar to *Ovis aries* (EF446377). The majority of the *Oram-TNF-α* sequence (189 bp) was the non-coding 3' UTR.

For the Class I gene, we sequenced 298 clones from the 31 individuals. Based on GenBank searches and amino acid conversion, 37 were the Class I *Oram-OLA* gene, 68 were a non-classical Class I gene, one was intermediate, and 192 were pseudogenes (GenBank Accession nos. JN861250-546). On average each individual had only one detectable Class I allele, with the highest number being four. Of the 192 pseudogenes, 73 unique haplotypes were observed. Product sizes of the Class I alleles ranged from 445 to 505 base pairs with the differences in length almost entirely attributable to intronic indels. Since we detected high

variability and could not get genotypes for all individuals we did not test for deviation of HWE. The neighbour-joining tree of exons 2 and 3, with pseudogenes removed, identified four clusters within the *Oram-OLA* clade a *non-classical* clade consisting largely of a monomorphic cluster (Figure 5-1). Both the BLAST searches and phylogenetic placement suggested the *Oram-OLA* and *non-classical* genes were most similar to *Ovis aries*.

We sequenced two NRAMP amplicons in 31 individuals producing a 929 bp fragment spanning exons five, six, and seven, along with introns five and six. We detected three SNPs, two of which were intronic, and one synonymous mutation in exon 7; these were at site 170 (C→G), site 186 (A→C), and site 896 (G→T), respectively (GenBank Accession nos. JN861609-39). The lone exonic SNP was in Hardy-Weinberg Equilibrium ($P = 0.28$), while both intronic SNPs were not ($P < 0.05$). When accounting for refugial origin, all loci were in HWE except for the exonic SNP in the southern refugium. These sequences shared their highest similarity (98%) to *Ovis dalli* (AJ920417). With the exception of the *DRA* gene which used primers from the water buffalo (*Bubalus bubalis*), all sequenced genes used primers from sheep studies.

5.3.2. Distribution of diversity

Diversity statistics for all the genes and refugial lineages are presented in Tables 5-1 and 5-2. For the Class I genes, all pseudogenes and duplicated sequences within individuals were removed from the analyses in Tables 5-1 to 5-3. With the exception of the Class I gene, all genes showed very low levels of diversity and there was no difference between dN and dS. Both Class I genes showed a marked increase in diversity metrics, with the non-classical gene showing evidence of purifying selection (dN < dS). Diversity statistics by refugial origin showed nearly equivalent levels of diversity, except for the *Oram-DRB* that was elevated in the south.

Within the Class II *Oram-DRB* gene, the G haplotype (glycine) was not associated with refugial origin ($ß = -0.63 \pm 0.95$, $z = -0.67$, $df = 211$, $P = 0.50$), but the T haplotype (valine) was found predominantly in individuals from the southern refugium ($ß = -1.02 \pm 0.45$, $z = -2.27$, $df = 211$, $P = 0.02$). Heterozygotes

shared the same association for the southern refugium (ß = -1.00 ± 0.45, z = -2.24, df = 211, P = 0.03). Within the Class I genes, no SNP or amino acid change was associated with refugial origin, nor were any of the NRAMP SNPs (all P's > 0.05). The remaining markers were not suitable for such analysis.

5.4. Discussion

Patterns of immunodiversity in mountain goats from across their range were relatively low with the exception of the Class I *Oram-OLA* gene. No sequence diversity was observed at MHC Class II *Oram-DRA* and Class III *Oram-TNF-α*. Low levels of variation were observed at the Class II *Oram-DRB* and NRAMP genes with one and three SNPs, respectively. The Class I *Oram-OLA* gene showed an increase in diversity (Table 5-1), and appears to offer a novel source of variation in the immune genes of mountain goats. Compared to studies on related ungulates examining the same genes, mountain goats had much lower levels of polymorphism (e.g. Class II *DRA* and *DRB* - Schaschl et al. 2006; Ballingall et al. 2010; Worley et al. 2006, Class III *TNF-α* - Alvarez-Busto et al. 2004, and NRAMP – Worley et al. 2006). With the exception of one *Oram-DRB* allele, we did not find an association or difference between immunodiversity and refugial origin, refuting our hypothesis of a within-species latitudinal cline. Additional screening of non-coding MHC regions will be required to fully substantiate the absence of a cline, but a study by Shafer et al. (2011) showed a similar non-existent clinal pattern in neutral loci. Given the high degree of population differentiation and long-term separation of refugial lineages (Shafer et al. 2011), this lack of an association would suggest that the current distribution of immunodiversity is not a direct result of the LGM, and that the apparent dearth of diversity was present well before the start of the Holocene.

The relative paucity of immunodiversity is somewhat difficult to assess, as few studies of wild organisms have assessed genes other than Class II *DRB* gene. For example, the Class II *DRA* region is generally considered to have relatively low diversity (e.g., Yuhki et al. 2003), but recent studies have shown increased variation in domestic sheep (Ballingall et al. 2010), as well as in wild cetaceans

(Xu et al. 2007, 2008) and caribou, *Rangifer tarandus* (Kennedy et al. 2011). The upregulation of the Class II *DRA* gene is associated with parasite resistance (Diez-Tascón et al. 2005), but the functional relevance of haplotype variation is unknown. The same goes for the Class III *TNF-α* gene, with preliminary studies finding variation in sheep (Alvarez-Busto et al. 2004), with the importance of this variation remaining unclear. In these genes we found complete monomorphism across the range of mountain goats, which may be explained by either an ancient bottleneck (pre-LGM) that wiped out all the diversity or by ongoing purifying selection. Similarly, the NRAMP gene had only three SNPs, all of which were non-informative. Both sheep and cattle have double this number of SNPs (Ables et al. 2002; Worley et al. 2006), and genetic variants have been associated to disease resistance in bovids (Barthel et al. 2001; Borrielo et al. 2006) and mice (Roy and Malo 2002; Vidal et al. 1995). We suggest additional screening within these genes coupled with disease assays may be warranted to confirm the observed low levels of diversity.

The Class II *Oram-DRB* gene is considered among the most diverse in mammals, with evidence of positive selection in wild ungulates (Schaschl et al. 2006). MHC diversity is inversely correlated to latitude in ungulates, with mountain goats containing among the lowest *DRB* allelic variation (Mainguy et al. 2007). Our expanded sampling suggests that the *DRB* paucity is indeed spread across the range of mountain goats. Mainguy et al. (2007) attributed this limited variation to a population bottleneck, which the range-wide analysis would support in the form of two major ice-age refugia (Shafer et al. 2011). However, given the larger sampling scheme presented here, and the significant population structure of mountain goats (Shafer et al. 2011), it seems reasonable to suggest that a bottleneck during the LGM is not the only culprit of low diversity, and perhaps either an ancient pre-LGM bottleneck or some degree of purifying selection is acting upon the Class II *Oram-DRB* locus in mountain goats - the latter conclusion was put forth in great crested newts (*Triturus cristatus*), which like mountain goats had only two *DRB* alleles across a geographically expanse post-glacial range (Babik et al. 2009). Interestingly, in mountain goats the T allele (the

majority found in heterozygous state) is largely restricted to the southern refugial lineage and deviates from Hardy-Weinberg Equilibrium. This suggests there may be a selective pressure favouring the T allele in the southern part of the mountain goat's range, perhaps conferring a heterozygote advantage (e.g., Huchard et al. 2010). Currently, data on pathogen exposure in mountain goats is limited to a handful of historic observations (reviewed by Côté and Festa-Bianchet 2003), but the remote, high altitudinal distribution likely limits pathogen exposure to some extent.

We also identified a largely monomorphic Class I *non-classical* gene that had evidence of purifying selection (Table 5-2), likely due to a functional constraint. Similar *non-classical* genes and phylogenetic structure have been identified in domestic goats (*Capra hircus*) and horses (*Equus caballus*) (Zidi et al. 2008; Tallmadge et al. 2010), and generally have low levels of polymorphism (Tallmadge et al. 2010). We also found one intermediate gene (British Columbia 09 in Figure 5-1) that is nested between non-classical and *Oram-DRA* clades. Similar sequences were detected in horses (Tallmadge et al. 2010) with their exact function unknown. Unlike the other MHC and NRAMP genes that showed minimal sequence variability, the Class I *Oram-OLA* gene showed a high degree of sequence variation. The Class I gene codes for glycoproteins involved in immune surveillance of intracellular pathogens and viruses, and in regulating innate immunity. Our phylogenetic structure is largely in accordance with sheep (Miltiadou et al. 2005) and horses (Tallmadge et al. 2010). Although both Miltiadou et al. (2005) and Tallmadge et al. (2010) examined transcribed sequences, compared to our genomic phylogenetic tree (Figure 5-1), they shared the common structure of four major clades. These clusters may represent differential levels of expression or multiple Class I *OLA* loci (Ballingall et al. 2008; Miltiadou et al. 2005), both of which are unknown at the moment.

We feel there are three plausible explanations for the diversity seen in the mountain goat *Oram* Class I *OLA* gene, none of which are mutually exclusive. The first is that multiple Class I *OLA* loci were sequenced; however, in our screening the majority of individuals had only one detectable functional gene

according to the translated sequence. The second is that mountain goats may be exposed to more intracellular pathogens (e.g., Williams et al. 1979) and viruses (e.g., Samuel et al. 1975) than previously thought; Class I proteins may also present exogenous antigens (Rock 1996), which could maintain this diversity. A possible mechanism comes from the association between killer immunoglobulin-like receptors (KIR) found on natural killer cells and the MHC Class I ligands, where more KIR - Class I combinations confer greater disease resistance in humans (Parham 2005). Indeed, MHC Class I variability has been linked to disease resistance (Bonneaud et al. 2006; Madsen and Ujvari 2006) and longevity (Madsen and Ujvari 2006) in wild populations. Thirdly, the mountain goat MHC Class I gene may be involved in social behavior or mate choice (see Leypold et al. 2002; Leinders-Zufall et al. 2004), which could explain the high individual variation. Overall, the additional screening of immune genes, in particular the Class I *OLA* gene, suggests that the paucity of variation seen by Mainguy et al. (2007) is not entirely reflective of the mountain goat MHC. Identifying specific transcribed Class I loci should be the next step. From such data, we could confirm whether we are amplifying single or multiple loci, verify our initial assessments of diversity, and also examine this variation relative to external factors such as mate choice and parasite exposure; this approach will help disentangle the relative contribution of these hypothesized factors in maintaining the mountain goats' MHC Class I diversity.

In conclusion, by analyzing portions of genes from all the three MHC classes and the NRAMP gene in mountain goats we garnered a better sense of the immunodiversity in this species. With many species becoming genome-enabled, future studies should attempt to screen additional immune genes to better characterize MHC diversity (Spurgin et al. 2010). This would also provide a much-needed database for wild populations, as it is currently limited to the MHC Class II *DRB* gene. Given that in this study there was no strong association between SNPs or diversity and refugial history, it would suggest the limited immunodiversity in mountain goats existed prior to the LGM. Thus, much like the enigmatic cheetah (*Acinonyx jubatus*) (Castro-Prieto et al. 2011), mountain goats

appear to have thrived in the wild despite a paucity of diversity at immune loci. However, this was not entirely true as the Class I *OLA* gene(s) had considerably higher levels of diversity (Table 5-1). Therefore, examining a suite of immune-markers presented a broader picture of diversity and suggests that it is premature to assume genome-wide paucity of immunodiversity in mountain goats. As mountain goats are alpine specialists with limited genetic diversity, concern has been raised over the potential negative impacts of climate change on their survival (Mainguy et al. 2007; Shafer et al. 2011). The identification of a more diverse Class I *Oram-OLA* and the evidence for a long-term paucity of diversity at other immune loci suggest that mountain goats may be better equipped for climatic oscillations and pathogen exposure than we originally thought, as the minimal diversity appears to have persisted through historical glacial cycles

Table 5-1. Genetic diversity statistics for immune genes in mountain goats (*Oreamnos americanus*). n – number of individuals analysed; bp – number of base pairs sequenced; SNPs – total number of single nucleotide polymorphisms; Haplotypes – number of unique haplotypes; *h* – haplotype diversity; π – nucleotide diversity. *NRAMP* data includes introns, with the MHC statistics only in reference to exonic regions. Parentheses in the Class I markers denote the number of analysed sequences as not all individuals had the functional copy amplified.

	Class I – *Oram-OLA*	Class I – *Nonclassical*	Class II – *Oram-DRA*	Class II – *Oram-DRB*	Class III – *Oram-TNF-a*	*NRAMP*
n	31(24)	31(19)	31	212	31	31
bp	476-503	501-504	254	249	232	929
SNPs	91	64	0	1	0	3
Haplotypes	19	5	1	2	1	6
h	0.92	0.31	-	0.18	-	0.65
π	0.08	0.02	-	7.3-E04	-	1.1E-03

Table 5-2. Genetic diversity statistics between north and south glacial refugia for immune genes in mountain goats (*Oreamnos americanus*). *h* – haplotype diversity; π – nucleotide diversity.

	North (h / π)	South (h / π)
Class I – *Oram-OLA*	0.90 / 0.09	0.96 / 0.09
Class II – *Oram-DRB*	0.12 / 0.001	0.23 / 0.001
NRAMP	0.20 / 0.001	0.21 / 0.001

Table 5-3. Non-synonymous (dN) and synonymous substitutions (dS) in immune-related exons of mountain goats (*Oreamnos americanus*) across North America. The Z statistic and corresponding *P* value are reported for the null hypothesis that dN=dS.

	dN	dS	Z	*P*
Class I – *Oram-OLA*	0.08	0.11	-1.30	0.20
Class I – *Nonclassical*	0.02	0.04	-2.56	0.01
Class II – *Oram-DRB*	1.0E-03	0.00	1.12	0.26
NRAMP	0.00	4.0E-03	-1.04	0.30

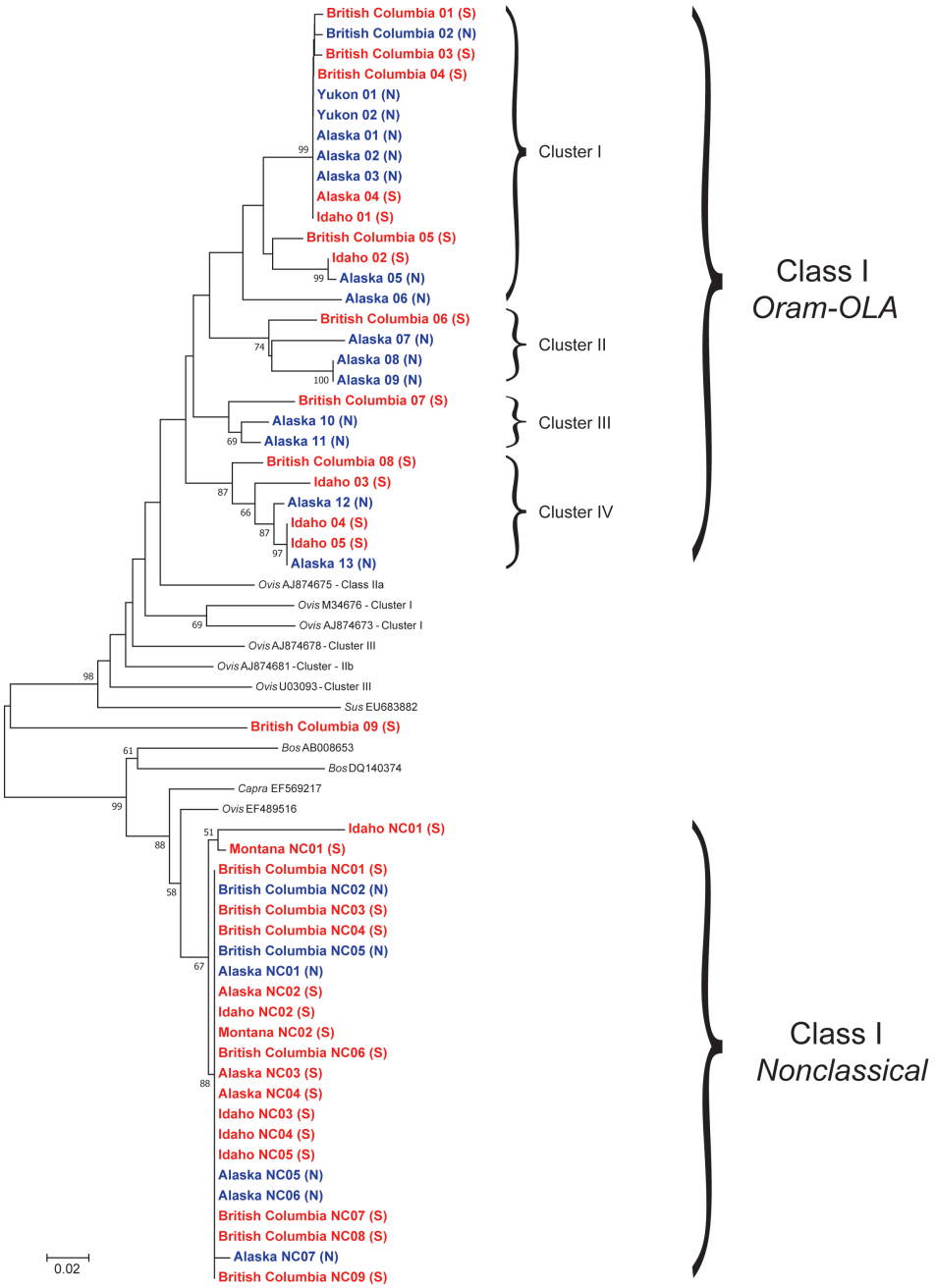

Figure 5-1. Neighbour-joining tree of the mountain goat Class I gene (exons 2 and 3). The non-classical gene is identified, along with four clusters within the *Oram-OLA* gene. Red and blue denote the northern and southern haplotype origin of the individual, respectively, in accordance with Shafer et al. (2011). For *Ovis,* Class I cluster origin is provided from Miltiadou et al. (2005). Support values >50% are shown and are from 1000 bootstrapped replicates.

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Chapter 6

Deciphering translocations from relicts in Baranof Island mountain goats: Is an endemic genetic lineage at risk?

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6.1. Introduction

The translocation of animals is a common practice in wildlife management (Griffith et al. 1989; Fischer and Lindenmayer 2000). Anthropogenic movement of wildlife is largely done for aesthetic, conservation, and harvest-related reasons (Kleimen 1989; Hodder & Bullock 1997; Fischer and Lindenmayer 2000). These movements are not, however, without controversy (e.g. Lyman 1998). In addition, translocations have occurred in areas where local populations were thought to be extinct, inadvertently serving to augment the native population (e.g. Vinkey et al. 2006). Such augmentations can have negative effects on local conspecifics by altering the population genetic structure or diminishing adaptive potential (Laikre et al. 2010). Thus, identifying native populations *pre* or *post* translocation can have important conservation implications.

The Alexander Archipelago in southeast Alaska contains one of the largest remaining expanses of temperate old-growth forest in the world. More than 2,000 islands make up the archipelago, and the region is considered a mammalian endemic hotspot and model system for island conservation and biogeography (MacDonald and Cook 1996; Cook and MacDonald 2001; Dawson et al. 2007). The island chains, including Haida Gwaii, have been the source of scientific debate regarding the extent to which they served as a glacial refuge during the last ice-age (e.g. Byun et al. 1997; Demboski et al. 1999; Byun et al. 1999). The archipelago has also been subjected to increased anthropogenic pressures including forestry, human expansions, and introduction of exotics (Cook et al. 2006). Consequently, recognition of island endemics and glacial relicts is considered an important objective for conserving and managing the flora and fauna of the Alexander Archipelago (Cook et al. 2006).

Sufficient data have now accumulated that show a portion of the island network acted as a refugium during the last glacial maximum (Cook et al. 2006; Shafer et al. 2010). However, it is still unclear whether large mammals persisted in this region during the last glacial maximum. Fossil evidence suggested some large mammals utilized the archipelago during glacial advances (Heaton et al.

1996), but phylogeographic support is ambiguous. Both brown bears (*Ursus arctos*) and black bears (*Ursus americanus*) were postulated to have survived the last glacial maximum on island refugia (Heaton et al. 1996; Byun et al. 1997; Waits et al. 1999). However, expanded sampling of individuals and molecular markers failed to provide conclusive evidence in black bears (Demboski et al. 1999; Byun et al. 1999; Stone and Cook 2000; Peacock et al. 2007). In brown bears, the coastal refugium hypothesis has been complicated by the detection of the four major haplotypes (including the island-specific haplotype) on mainland Alaska dating near the glacial maximum (Leonard et al. 2000; Wayne and Morin 2004).

More recently, we found evidence for two distinct subpopulations of mountain goats (*Oreamnos americanus*) on Baranof Island in the Alexander Archipelago (Fig. 6-1; Shafer et al. 2011). In the early 20th century, Baranof Island was considered uninhabited by mountain goats which prompted translocations in 1923 from Tracy Arm, Alaska (Paul 2009). The genetic signature of the source population still is identifiable, but it is accompanied by a distinct subpopulation restricted to Baranof Island. This finding led us to hypothesize that the island subpopulation persisted on a cryptic refugium during the last glacial maximum (Shafer et al. 2011). The primary goal of this study was to utilize additional molecular markers, namely Y chromosome sequence data, to clarify the evolutionary history of the Baranof Island mountain goats. Our second objective was to combine existing mitochondrial sequence and microsatellite genotype data with Y chromosome data to measure the degree of isolation and assess whether the Baranof Island population constitutes an evolutionary significant unit (ESU; *sensu* Moritz 1994).

6.2. Materials and methods

6.2.1. Sample collection and DNA isolation

Mountain goat samples were acquired from across their entire native range between 2004 and 2009. Tissue samples were collected primarily from hunters upon compulsory registration and stored in 95% ethanol, although some were

collected from field studies (e.g. White 2006; Festa-Bianchet and Côté 2008). All samples were sexed in the field and georeferenced. Total genomic DNA was extracted using a QIAGEN DNeasy kit (Qiagen, Valencia, CA). DNA was quantified using a NANODROP 2000 (Thermo Scientific, Wilmingtion, DE) and standardized to a concentration of 10 ng/μL. Additional sample information is available in Shafer et al. (2011).

6.2.2. Amplification and sequencing of Y chromosome markers

We screened five male-specific region (MSY) Y chromosome genes using the primers in Meadows et al. (2004). The initial screening utilized 25 μL PCR reactions that contained: 25 ng of template DNA, 200 μM dNTPs, 1X PCR buffer, 0.75 μM of each primer, $MgCl_2$ (0.8-2.0 mM), 0.3 U Taq DNA polymerase, and distilled water. The initial PCR parameters were run under a gradient annealing temperature (54-64 ºC) for 35 cycles at 45 seconds each step. PCR products were visualized by electrophoresis on a 1% agarose gel. Primer sets that consistently amplified were subsequently optimized. For the sequencing reaction, 10 μL of PCR product was treated with 5 μL of ExoSAP (USB Corporation, OH, USA) and incubated at 37°C for 15 minutes followed by heating to 80°C for 15 minutes. A total of 2.5 μL of the ExoSAP treated PCR product was then used in the sequencing reaction. Fragments were directly sequenced in both directions using a Big Dye Terminator Kit (Applied Biosystems, Foster City, CA). Excess of Big Dye Terminator was removed via ethanol precipitation. Sequences were generated on an ABI 3730 (Applied Biosystems, Foster City, CA).

6.2.3. Analyses of Y chromosome sequence data

DNA sequences were aligned using Bioedit 7.0.9 (Hall 1999). All sequences were compared to GenBank records to ensure the correct gene was sequenced and to identify similar sequences. We calculated haplotype (*h*) and nucleotide diversity (π) using the software DnaSP 4.0 (Rozas et al. 2003). To visualize the spatial distribution of Y chromosome SNPs, individuals were plotted in ARCMAP 9.0 (ESRI, Redlands, CA, USA) and coded according to haplotype. Euclidean distances between individuals and haplogroups were calculated using ARCMAP 9.0.

6.2.4 Testing the criteria of Evolutionary Significant Unit

A strict ESU is defined as being reciprocally monophyletic at mitochondrial DNA (mtDNA) with significant differentiation at nuclear loci (Moritz 1994); however, this definition has been broadened to incorporate both isolation and reduced gene flow (Fraser and Bernatchez 2001). To assess whether Baranof Island mountain goats conforms to ESU criteria, we utilized genotype and mitochondrial data from Shafer et al. (2011) and the newly generated Y chromosome sequence. The Bayesian mtDNA phylogenetic tree and microsatellite-based neighbor-joining tree of subpopulations from Shafer et al. (2011) along with the Y chromosome SNP data were spatially plotted in the software GenGIS v.1.07 (Parks et al. 2009). We used the software MIGRATE 3.2.7 (Beerli et al. 1999, 2001) to examine historical, asymmetrical migration (*M*) between Baranof Island and the mainland, including the putative source population at Tracy Arm inferred from the maximum likelihood estimator (MLE). We employed a continuous Brownian process for the microsatellite data, and a DNA sequence and SNP model for the mitochondrial and Y chromosome data, respectively. Under the likelihood framework, we ran four heated chains for 20,000 genealogies, with sampling every 50 iterations and the first 5,000 discarded as a burn-in.

6.3. Results

Three of the five MSY genes amplified consistently: AMELY intron 4, SRY 5' promoter region, and ZFY intron 5 (Meadows et al. 2004). Genes UTY and DBY could not consistently be amplified and were removed from the data set. The three amplified genes were initially sequenced in ten individuals from across the range. Diversity statistics are presented in Table 6-1. Both ZFY and AMELY were monomorphic and are deposited in GenBank (Accession no. HQ882835-36). These sequences shared 97% identity to *Ovis aries*. The SRY primers 4F/4R amplified a 596 bp gene fragment (Meadows et al. 2004). A single nucleotide polymorphism (SNP), a C/T transition, was observed at position 338 creating two variants henceforth referred to as the C and T haplotypes. The consensus sequence

is deposited in GenBank (Accession no. HQ882834) and shared its highest identity (92%) to the SRY gene of *Capra hircus*.

The SRY gene was subsequently sequenced in 100 males from across the range (Table 6-1). Seventeen individuals contained the C haplotype with the remainder having a T haplotype. Every Baranof Island individual contained the C haplotype (n = 12) with the remainder found to the northeast near Haines, Alaska (Fig. 6-2a). All individuals within 100 km of the Tracy Arm had the T haplotype. Baranof Island was clearly distinct from all other southeast Alaska subpopulations based on the microsatellite neighbor-joining tree (Fig. 6-2a). The mtDNA phylogeny (illustrated with only bifurcations supported by >50 posterior probability) revealed a broad north-south split but essentially a polytomy in southeast Alaska (Fig. 6-2b). All individuals from Haines with the C haplotype contained a northern mtDNA profile, while all but two individuals on Baranof Island had a southern mtDNA haplotype. The Tracy Arm area contained a mixture of southern and northern mtDNA haplotypes. The maximum likelihood estimates of historical migration rates are provided in Table 6-2. All asymmetrical island-mainland scenarios had overlapping 95% confidence intervals, but both mitochondrial and Y chromosome data had the highest MLE migration rate going from the island to the mainland.

6.4. Discussion

The Y chromosome SNP data show that the Baranof Island mountain goat population is genetically distinct. The pattern of Y chromosome SNP variation cannot be attributed to translocation since the C haplotype is restricted to Baranof Island and to two subpopulations in the northeast (Haines and the Lynn Canal). The C haplotype is not found within the presumed Baranof Island source population of Tracy Arm (Fig. 6-2a). In addition, historical migration rates support long-term isolation (i.e. low M values), and for both the mitochondrial and SNP data, MLE migration rates were higher going from the Baranof Island to the mainland, than vice-versa (Table 6-2).

The lack of homoplasy in Y chromosome markers (Underhill et al. 2000) makes them a powerful evolutionary tool; for example, individual Y chromosome SNPs have been shown to segregate according to subspecies and geographic regions in wild sheep (Meadows et al. 2006; Meadows and Kijas 2009). In mountain goats, the distribution of Y-chromosome SNPs and patterns of differentiation at microsatellite loci (Fig. 6-2a; Shafer et al. 2011) support the hypothesis that Baranof Island mountain goats existed on the island prior to the translocation. Although microsatellite-based migration rates showed higher migration going from the mainland to Baranof Island, these values are likely inflated due to some degree of admixture resulting from the translocation, which would influence the microsatellite-based migration rate more than the haploid markers. Therefore, without invoking a scenario of undocumented recent translocations to explain the observed pattern (as there is no anecdotal or recorded translocations from Haines), the principles of parsimony would suggest that prior to 1923, Baranof Island mountain goats were a glacial relict. Thus, the presumed introduction was in fact an accidental augmentation of a native cryptic population.

The human-mediated movement of animals has a long history in North America. Mountain goats are no exception and have been moved extensively across their range (e.g. Hatter and Blower 1996; Paul 2009). In southeast Alaska, regional biologists believed that mountain goats were not indigenous to Baranof Island (e.g. Blee 1989; Whitman 2000). This led to an apparent introduction in 1923 (Paul 2009). However, the ethnohistory of Baranof Island tells a slightly different story. Prior to 1867, Russia's colonial capitol of Sitka was located on Baranof Island and there are many historical documents describing the fur trading industry and natural history of the region. Some of these documents contain peculiar references to "white deer" on Baranof Island (Whitman 2000). Russian speakers on Baranof Island referred to a local ungulate as *iaman*, which is most commonly translated as "wild goat" or "wild sheep" (Blee 1989). In some English translations, *iamen* was interpreted specifically as mountain goat (Tikhmenev 1978) and described as having long white hair (Khlebnikov 1994). Moreover, Tikhmenev (1978) made a distinction between mountain goats and the only other

ungulate on Baranof Island, the Sitka deer (*Odocoileus hemionus sitkensis*). But due to apparent lack of physical evidence, various interpretations of *iamen,* and the assumption that mountain goats were introduced*,* Blee (1989) concluded that the Russian speakers must have been referring to Sitka deer. However, Blee's (1989) interpretation appears questionable in light of ethnographic documents that state local hunters supplied Russian traders with mountain goats found on Baranof Island as early as the 1820's (Andrews 1922; Gibson 1978, 1987). The presence of mountain goat remains at archaeological sites in Sitka further support this conclusion (Petruzelli and Hanson 1998; Grover 2002), but indigenous trade of mountain goat artifacts complicate this interpretation. Nonetheless, examination of paleoecological remains found at other known glacial refugia sites along the northwest coast (i.e. Prince of Wales and Vancouver islands; see also Fig. 6-1) suggest that mountain goats may have existed on a network of outer coast islands during the last glacial maximum (Nagorsen and Keddie 2000; Heaton and Grady 2003). Collectively, these data support the hypothesis that mountain goats were present on Baranof Island prior to the 1923 introduction, but were largely a cryptic population.

Three alternative scenarios could explain the distribution of the SNP data. The first is that mountain goats with the C haplotype dispersed from the Haines area to Baranof Island. Mountain goats have been seen traversing the region's fiords (Klein 1965) and individuals are known to make long-distance movements (Matthews and Heath 2008). If this were to have occurred pre-translocation, males and females would have had to disperse simultaneously to start a founding population; however, this is atypical dispersal behaviour for mountain goats (Festa-Bianchet and Côté 2008). In addition, these two areas are geographically separated (Fig. 6-1), genetically differentiated (Fig. 6-2a), and have different mitochondrial DNA (Fig. 6-2b): these same genetic patterns make a post-translocation dispersal scenario equally implausible. Another possibility is that the C haplotype recently arose because of an extremely successful translocated male (as all Baranof males have the C haplotype). This would explain the elevated microsatellite migration rates from the mainland, but does not account for the

significantly differentiated subpopulation on Baranof, or the presence of the C haplotype in Haines. A third explanation is that the Baranof Island pattern resulted from indigenous translocation of mountain goats earlier in the Holocene. Mountain goat wool was prized by coastal Native Americans, including the Tlingit on Baranof Island, for their use in blankets and ceremonial robes (Samuel 1987). Conceivably, there may have been earlier Holocene translocations of mountain goats to Baranof Island, but the absence of historical evidence and infeasibility of this activity do not support this idea.

Given the limited homoplasy in the Y chromosome (Underhill et al. 2000), it seems unlikely for this mutation to have arisen twice by chance. In our opinion, the distribution of the SNP is best explained by island-to-mainland dispersal during the initial glacial retreat when sea levels were lower (Josenhans et al. 1995) and glaciers bridged islands. Portions of Baranof Island were ice-free during the last glacial maximum (Carrara et al. 2007) and the area near Haines is recognized as a contact zone among biogeographic regions (Swenson and Howard 2005), including mountain goats (Shafer et al. 2011). If the C haplotype arose prior to 1923 in a small, isolated population on the island, drift would have facilitated its fixation. The dispersal of only a few individuals following the retreat of the Cordilleran ice-sheet, and subsequent mating with Haines area mountain goats, would be all that is required to produce the observed pattern. This hypothesis is supported by evidence for historical isolation (Fig. 6-2; Shafer et al. 2011), and asymmetrical mitochondrial migration rates (Table 6-2).

Although Baranof Island mountain goats have a unique evolutionary history, they do not meet Moritz's (1994) strict definition of an ESU. The mtDNA showed very little geographic structuring (Fig 6-2b) and is a complete polytomy on Baranof Island. Alternatively, Fraser and Bernatchez's (2001) definition describes an ESU as an isolated genetic lineage with highly reduced levels of gene flow. This ESU definition applies to Baranof Island mountain goats given the Y chromosome SNP distribution, high genetic differentiation, and non-existent contemporary gene flow. Accompanied by the ethnohistorical evidence, we feel an ESU designation is currently appropriate for Baranof Island mountain goats.

Mountain goat hunting on Baranof Island has been ongoing since 1949 (Whitman 2000) and the population is under no immediate threat (Harper 2008). Local scale habitat disturbances do occur and have the potential to alter genetic structure via fragmentation, but do not likely represent a catastrophic threat to island-wide population persistence. Given our recommended ESU status, the major impetus should be the preservation and identification of this unique genetic lineage. This is obviously confounded by the translocation history of the island, but the relict and introduced lineages are still genetically distinct with limited admixture (Shafer et al. 2011). Future work should attempt to decipher whether these subpopulations are spatially or morphologically segregated in an effort to identify and conserve the unique genetic lineage of Baranof Island mountain goats.

Table 6-1. Male-specific region Y chromosome fragments sequenced. Included are the number of individuals sequenced (*N*), length of fragment (*L*) in base-pairs, number of observed segregating sites (*S*), haplotype diversity (*h*), and nucleotide diversity (π).

Gene	Region	*N*	*L*	S	*h*	π
AMELY	Intron 4	10	786	0	-	-
ZFY	Intron 5	10	706	0	-	-
SRY	5' Promoter	100	596	1	0.30	0.0005

Table 6-2. Historical rates of migration (*M*) between Baranof Island mountain goats and populations on the mainland in southeast Alaska. The maximum likelihood estimate (MLE) of *M*, along with the 95% confidence interval in parentheses, is provided for mitochondrial sequence, nuclear microsatellites, and Y chromosome SNP data. Shaded columns indicate the highest MLE value, which is the average maximum likelihood estimate.

Populations	*M* Island→mainland	*M* Mainland→island
Mitochondrial		
Baranof Island – Tracy Arm	7.1×10^{-10} (5.3×10^{-10}–490)	3.6×10^{-13} (2.7×10^{-13}–505)
Baranof Island – Haines	2.6×10^{-10} (1.94×10^{-10}–310)	3.3×10^{-13} (2.5×10^{-13}–505)
Baranof Island – Lynn Canal	9.5×10^{-14} (5.2×10^{-14}–27)	3.3×10^{-13} (2.5×10^{-13}–505)
Microsatellite		
Baranof Island – Tracy Arm	1.0 (0.7–1.4)	1.5 (1.1–1.9)
Baranof Island – Haines	1.0 (0.7–1.2)	1.3 (1.0–1.8)
Baranof Island – Lynn Canal	1.0 (0.8–1.4)	1.5 (1.1–2.0)
Y chromosome		
Baranof Island – Tracy Arm	3.7×10^{4} (2.8×10^{4}–9.2×10^{7})	7.5×10^{-6} (5.7×10^{-6}–2.0×10^{-2})
Baranof Island – Haines	2.0×10^{4} (1.5×10^{4}–5.0×10^{7})	53.0 (40.0–1.3×10^{5})
Baranof Island – Lynn Canal	3.0×10^{-8} (2.3×10^{-8}–137)	7.5×10^{-6} (5.7×10^{-6}–2.0×10^{-2})

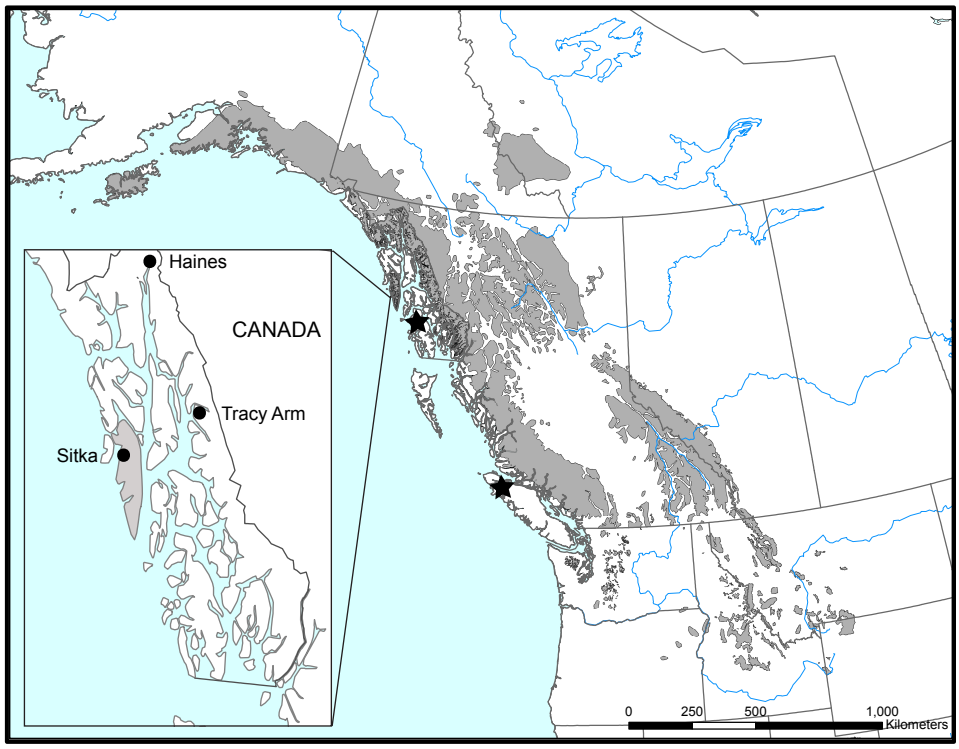

Figure 6-1. Range-wide distribution of mountain goats (in grey) in western North America (modified from Mountain goat management team 2010). Inset map is of the Alexander Archipelago with Baranof Island and the presumed source population of Tracy Arm highlighted. The Russian American capitol of Sitka is shown along with Haines. The two coastal sites where mountain goat and possible mountain goat remains have been discovered are starred (Prince of Wales Island in Alaska, and Vancouver Island off the southern coast of British Columbia).

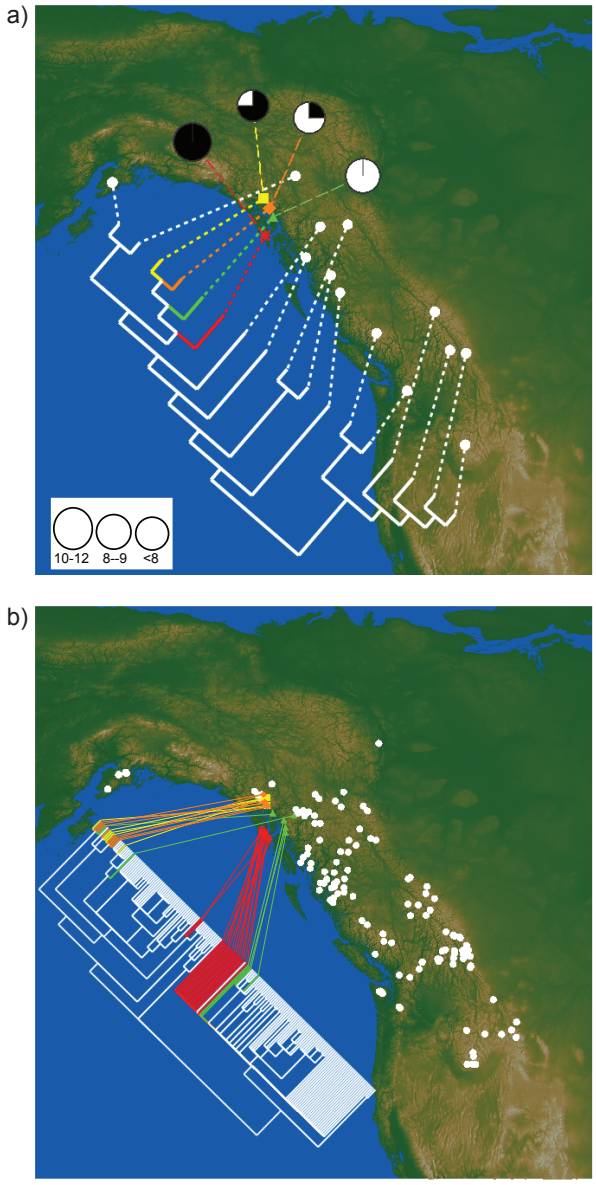

Figure 6-2. a) Neighbour-joining tree of mountain goat subpopulations (based on Shafer et al. 2011), the midpoint of each subpopulation was calculated in ARCMAP. Pie charts represent the Y chromosome SNP composition in each subpopulation and are scaled to the number of individuals sequenced in legend. Grey denotes the C haplotype and white is the T haplotype. b) Bayesian mitochondrial tree based on Shafer et al. (2011). Individuals on Baranof Island are

in yellow triangles and Tracy Arm in red stars. Haines individuals with the C haplotype are shown in blue squares.

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Chapter 7

Habitat selection predicts genetic relatedness in an alpine ungulate

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7.1. Introduction

How organisms interact with the landscape is of fundamental importance to evolutionary ecology. Patterns of habitat selection and landscape-level barriers influence individual movements and alter connectivity, which ultimately affects gene flow. Quantifying gene flow and genetic differentiation are recognized as critical tools in the conservation and management of species (Palsbøll et al. 2007, Schwartz et al. 2007) and should serve as an end goal when identifying the effect of habitat and barriers. To better understand habitat selection and connectivity across the landscape, two distinct approaches have emerged from the fields of landscape ecology and population genetics.

The first method is founded in animal ecology and relies on animal locations, often using global positioning system (GPS) radiotelemetry, to monitor individual movements and habitat interactions (see Cagnacci et al. 2010). The ability to collect large quantities of data using GPS radiotelemetry has allowed researchers to examine the spatial use and relative importance of habitat variables (Aarts et al. 2008, Massé and Côté 2009), identify ecological traps (Nielsen et al. 2006), and design corridors (Chetkiewicz and Boyce 2009). The influence of environmental variables on animal movement has also been assessed using GPS data (Fortin et al. 2005, Coulon et al. 2008, Roever et al. 2010, Leblond et al. 2010). However, field validations of such models have yet to be fully established (Gonzales and Gergel 2007). The second major approach to understanding connectivity has been dubbed landscape genetics (Manel et al. 2003), and is rooted in population genetics but incorporates landscape and spatial processes. Landscape genetics is primarily concerned with quantifying how habitat heterogeneity influences genetic connectivity (see Sork and Waits 2010). Under this framework, studies have identified corridors (Epps et al. 2007) and barriers (Epps et al. 2005, Coulon et al. 2006), and examined the effect of landscape composition (Pavlacky et al. 2009) and time (Shafer et al. 2011a) on genetic differentiation. Traditionally, this approach has relied on expert opinion to select

and penalize habitat variables, which could lead to poor landscape cost schemes (Shirk et al. 2010) and unrepeatable estimates of connectivity.

To date, the above approaches have remained largely disparate, despite common objectives. One area where GPS data could be particularly useful is for identifying key habitat variables and parameterizing landscape-resistance surfaces (Spear et al. 2010). Among models typically used to analyze GPS data, resource selection functions (RSFs; Manly et al. 2002) are commonly used and provide a natural tool for examining landscape resistance. RSFs use known locations of animals to infer preferentially selected habitats, are amenable to geographic comparisons, and have a strong theoretical and empirical backing (Manly et al. 2002, Johnson et al. 2006). Although Spear et al. (2010) identified the potential usefulness of GPS-based resistance surfaces (i.e., Chetkiewicz and Boyce 2009), they have not been directly implemented as resistance surfaces in a landscape genetics framework (but see Cushman and Lewis 2010 for a comparative approach). This merger is particularly attractive because genetic data offer a means to validate spatially explicit connectivity models, while location (GPS) data can be used to select and score habitat variables used in landscape genetic studies. Collectively, this linkage would allow for better inferences on whether habitat selection actually facilitates gene flow (Spear et al. 2010) and what specific habitat variables aid in the maintenance of connectivity.

Here, we explore the landscape genetic structure of the North American mountain goat (*Oreamnos americanus*) in southeast Alaska. We used GPS radiotelemetry data from over 100 individuals to construct landscape resistance surfaces based on habitat selection. Pathways based on least resistance between individuals were then constructed, and correlated to levels of genetic relatedness. Using this approach, we addressed the following questions:

(i) Is habitat selection a good predictor of gene flow?

(ii) Do seasonal and sex-based patterns of habitat selection differ in their ability to predict gene flow?

(iii) How do landscape resistance models compare to null models of genetic differentiation?

Based on mountain goat ecology, we developed the following predictions: 1) summer models will be better predictors than winter models because movement is restricted during winter (Adams et al. 1982) and this is when most juvenile dispersal occurs (Festa-Bianchet and Côté 2008); 2) patterns and predictive ability of models will vary between males and females because of sex-based differences in habitat use (Festa-Bianchet and Côté 2008, White 2006); 3) male habitat selection models will be better than females because gene flow is male-mediated (Festa-Bianchet and Côté 2008, Shafer et al. 2011a, 2011b); 4) models based on locations during the rut will be among the best predictors of genetic relatedness as roving males sire offspring (Mainguy et al. 2009, Ortego et al. 2011); and 5) the habitat selection models will outperform the null models of genetic differentiation.

7.2. Materials and methods

7.2.1. Study area and species

Mountain goats are an alpine ungulate, endemic to the mountainous regions of northwestern North America (Festa-Bianchet and Côté 2008). Our study area was the Lynn Canal region in southeast Alaska. The area is 600 km^2 with elevations ranging from sea level to 1920 m (additional details are provided in White 2006). There are at least three genetic subpopulations of mountain goats in the study area (Shafer et al. 2011a), and they display seasonal migration over short distances (White 2006). Although a handful of studies have examined seasonal movements (White 2006, Rice 2008) and habitat selection (Fox et al. 1989, Lele and Keim 2006, Taylor et al. 2006, Festa-Bianchet and Côté 2008) of native mountain goats, our knowledge of what habitat facilitates gene flow is very broad scale (i.e., Shafer et al. 2011a, Shirk et al. 2010).

7.2.2. Captures, collaring and genetic sampling

Mountain goats were immobilized using helicopter darting techniques (see White 2006 for detailed capture methods) and fitted with Telonics TGW-3590 GPS radiocollars (Telonics Inc., Mesa, AZ), programmed to collect GPS locations every 6 hours. Each individual had a small piece of ear tissue removed during

handling. Tissue samples were placed in 95% ethanol and stored at –80°C. DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following manufacturers protocol and stored at –20°C.

7.2.3. Resource selection functions and resistance maps

A RSF is any function proportional to the probability of selection of a resource unit, where habitat type is an attribute of a resource unit (Manly et al. 2002, Lele et al. 2012). Although RSFs can take many forms, when using GPS location data, typically logistic regression is used to compare the habitat selected by an animal (the used locations) to what is theoretically available on the landscape (available or random locations; see Johnson et al. 2006). The used locations are a subset of what is available to the animal, and when habitat types appear in the used dataset at a higher or lower proportion than in the available dataset the animal is said to be either selecting or avoiding that habitat type (Lele et al. 2012). When estimating resource selection functions we assumed the selection function took the exponential form, and estimated coefficients using logistic regression in a use-available design (Johnson et al. 2006). To represent availability, we drew random points from the 99% composite kernel home range for all animals at a density of 30 points / km^2. As GPS measurement error can influence estimates of coefficients we removed all used locations with positional dilution of precision (PDOP) values greater than 10, where PDOP is a quality metric of GPS data with lower values indicative of higher accuracy (D'eon and Delparte 2005, Lewis et al. 2007).

We fit 10 RSFs representing different combinations of sexes and seasons (Table 7-1), determined from changes in activity patterns (White 2006). GPS radiocollar data often are autocorrelated, which does not influence estimates of model coefficients but can deflate standard errors (Fieberg et al. 2010). Because our interest was in the predictive ability of resistance maps produced from the RSF coefficients, models were evaluated based on their predictive ability. We selected a set of 22 variables (calculated at a pixel size of 30 × 30 m) and biologically feasible interactions between these variables that we hypothesized to influence mountain goat habitat selection and movement (Table 7-2, 7-3).

Because some of these variables were highly correlated ($|r| > 0.7$) we fit univariate models of correlated variables to determine which explained the most variance (lowest log-likelihood; Hosmer and Lemeshow 2000). We then fit a global model of the most explanatory variables, plus all other uncorrelated variables. To obtain our final model we removed variables with $P > 0.10$, and retained variables that were part of interactions with $P < 0.10$ (Hosmer and Lemeshow 2000, Aldridge et al. 2008). Although there are several methods of obtaining models, all have shortcomings (Burnham and Anderson 2002, Guthry et al. 2005, Hastie et al. 2009). Because of the large data set, we selected only variables of biological relevance to mountain goats and assessed models on their individual and global predictive power.

We used the coefficients estimated above to generate maps with the relative probability of habitat selection (RSFs) using the exponential function:

$$w(\mathbf{x}) = \exp(\beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_z x_z).$$

In this equation $w(\mathbf{x})$ is the RSF, and β_1 represents the coefficient for the variable x_1 in a vector, $\mathbf{x}$, of z covariates. For RSFs, coefficients (the values of β) were estimated using logistic regression. Because our primary interest in fitting these models was to obtain predictive maps, to ensure a high level of rigor we used two different validation approaches: RSFs were validated using 5-fold cross validation (Boyce et al. 2002) as well as data from 9 mountain goats withheld from the above analyses (Johnson et al. 2006). To obtain maps representing resistance we calculated the inverse of the RSFs ($[w(\mathbf{x})]^{-1}$) using raster calculator in ArcMap 9.2 (ESRI, Redlands, California).

7.2.3. Genotyping and estimation of pairwise relatedness

Individuals were genotyped at 22 microsatellite loci using previously published PCR parameters (Mainguy et al. 2005, Poissant et al. 2009). Microsatellites were loaded on an ABI 3730 DNA analyzer (Applied Biosystems, Fosters City, CA) with a GS500 size standard (Applied Biosystems). The alleles were visualized and scored using GENEMAPPER 4.0 (Applied Biosystems). We assessed deviation

from Hardy–Weinberg equilibrium and linkage disequilibrium using GENEPOP 4.0 (Rousset 2008). Using the individual as the operational unit is preferred for landscape genetic studies (Manel et al. 2003); however, because the Lynn Canal study area has known spatial genetic structure, individuals were first assigned to a subpopulation using STRUCTURE 2.2 (Pritchard et al. 2000) following the approach of Shafer et al. (2011a). The accuracy of relatedness metrics has been shown to vary between data sets (Van De Casteele et al. 2001), so we estimated relatedness between individuals, referenced according to subpopulation, using three metrics: the Queller and Goodnight (1989) relationship coefficient, Lynch and Ritland's (1999) *r* coefficient, and Moran's *I* statistic (Epperson and Li 1997; Hardy and Vekemans 2002). The three metrics of relatedness vary between –1 and 1 (unrelated to related) and were obtained in a $n \times n$ matrix from the program SPAGeDi 1.2 (Hardy and Vekemans 2002).

7.2.4. Modeling framework

We first constructed two null landscape genetics models. The first, an isolation-by-distance (IBD) model, was created by calculating Euclidean distances between individuals using ArcMap 9.2. The locations of individuals were taken as the centroid of each individual's 100% minimum convex polygon (MCP) home range. The second, an isolation-by-barrier (IBB) model, assessed the effect of the Katzehin River and Berners Bay (a drainage for four glacial rivers), which divide the study area (Figure 7-1a). If a barrier separated the compared individuals they were coded with a 1, with all other individuals given a 0 (King 1987); this was done on each barrier separately, as well as in a single matrix accounting for both barriers. We created a second matrix which accounted for the number of barriers (0, 1, or 2) that separated individuals. We next examined the influence of resistance; landscape resistance models were based on cost surfaces from 30-m cells using the inverse of the RSF maps, described above. Least cost paths were calculated between all individuals using the cost distance, cost backlink, and cost path tools in ArcMap 9.2: this produces a single, pair-wise resistance score between each pair of individuals. We also used Circuitscape 3.0.1 (McCrae 2006), which does not rely on a single pathway, to estimate resistance distances between

all individuals. Pixel size was increased to 50 × 50 m because we had exceeded the maximum number of computable cells (McCrae and Shah 2009). Connections were allowed between all 8 surrounding cells of each pixel. All pair-wise resistances scores, distances, and number of barriers were organized in a $n \times n$ matrix format.

The relationships between matrices were evaluated in R2.9.2 (http://www.r-project.org/) using Mantel tests (Mantel 1967) and multiple regression (Legendre et al. 1994) under 10,000 permutations as implemented by the Ecodist library (Goslee and Urban 2007). Based on the assumption that the best model will retain significant relationships after controlling for competing models (Legendre and Troussellier 1988; Legendre and Fortin 1989), we compared the RSF models to the null IBD and IBB models by controlling for each model using partial Mantel tests under all possible permutations.

7.3. Results

7.3.1. RSF resistance surfaces

Between 2005 and 2009, 111 GPS radiocollars were deployed on 61 male and 50 female goats. We fit RSFs to data from 102 animals (55 males, 47 females; 151,112 total locations), and withheld data from 9 animals (6 males and 3 females) for model validation (Boyce et al. 2002, Johnson et al. 2006). The final RSF models contained between 9 and 15 variables and are shown in Table 7-3. The ratio of GPS fixes to variables in our final models ranged from ~1,350 to 10,800 to one, suggesting overfitting of models was unlikely (Peduzzi et al 1996). In our model validations, most models were highly predictive of withheld data (Appendix VIII). Correlation between overall winter and summer resistance scores was low ($|r| = 0.11$), as were the seasonal sex-based models ($|r|$ 0.01 – 0.55). Within season correlation of sex-based resistance values ranged from 0.53 to 0.90. The rut model was most highly correlated with the overall female model ($r = 0.92$). We observed notable differences between resistance surfaces, particularly between summer and winter (Fig. 7-1b, c; Table 7-3). Of the variables

assessed, heat load was consistently selected, while ice (glaciers), valleys, and areas far from escape terrain were avoided (Table 7-3).

7.3.2. Genotyping and metrics of relatedness

We genotyped 102 individuals at 22 microsatellite loci with a genotyping success rate of 97% (data deposited in the Dryad Repository: doi:10.5061/dryad.6b6k8b83). All markers were in Hardy-Weinberg equilibrium, with only 1 of 231 comparisons showing evidence of linkage disequilibrium after Bonferroni correction (Rice 1989). Diversity statistics for each locus are found in Appendix X. The three relatedness metrics produced the same patterns, but the Queller & Goodnight (QG; 1989) metric explained the most variance and is reported here.

7.3.3. Landscape genetics models

The null IBD and IBB models along with the RSF-based resistance least cost path models are presented in Table 7-4 and Fig. 7-2a (the latter only for IBD). All centroid locations used in the IBD and IBB analyses are deposited with the genotype data in the DRYAD deposition. When we independently compared the two barriers, Berner's Bay (Mantel $r = 0.11$, $p < 0.01$) restricted gene flow more than the Katzehin River (Mantel $r = 0.06$, $p = 0.02$). The IBB model that accounted for the number of barriers separating individuals is reported as it marginally explained more variance than the singular IBB matrix that only considered if individuals were separated by a barrier. Results from the Mantel test from multiple regressions were highly correlated ($r > 0.96$) so only Mantel r values are reported. Circuit theory resistances explained less variance than least cost paths (see Appendix XI). The best predictors of genetic relatedness were resistance models based on summer habitat selection, while winter-based models were the poorest (Table 7-4; Fig. 7-1b, c). Sex-based resistance models were very similar, except for the winter where the male model explained more variance in relatedness than the female model (Table 7-4). After controlling for distance and barriers, the best resistance RSF model (QG ~ Summer; Table 7-4 and Figures 7-1b, 2b) retained a significant negative relationship to relatedness (Table 7-5). The effect of distance on genetic relatedness switched from negative to positive when

the RSF resistance model was controlled for, and the effect of barriers was no longer significant (Table 7-5). The same pattern was observed when both distance and barriers were controlled for in the same model (Table 7-5). When plotted (Fig. 7-2b), the relatedness - summer resistance model showed a distinct break at intermediate resistance values, and there was a positive correlation between resistance scores and the number of barriers separating individuals ($r = 0.82$).

7.4. Discussion

We used measures of habitat selection estimated using RSFs to create landscape resistance maps. We then assessed the relationship between resistance surfaces and genetic relatedness, and with the exception of the winter resistance models, all RSF-based models were better predictors of genetic relatedness than the null IBD and IBB models (Table 7-4). This observation was further supported when we controlled for resistance surface (Table 7-5), which caused the relationship between distance and relatedness to switch (i.e., individuals further apart were more closely related). This finding suggests distance was no longer explanatory when accounting for resistance – a similar pattern was observed for barriers. Based on the expectation that the best model will remain significant after partialling out the effects of competing models (Cushman and Lewis 2010, Shirk et al. 2010), RSF-based resistance models offer a clear improvement over IBD and IBB models for inferring gene flow. Within these analyses, we also found that circuit theory did not explain as much variance as the last cost paths (with the exception of the winter models). We attribute these differences to either the scale of the study being too small for circuit theory to be effective (Anderson et al. 2010, Spear et al. 2010) or that the RSF-based models produced such realistic resistance surfaces that the least cost paths were simply reflective of those actually used by the animals. This latter point highlights perhaps the strongest asset of our approach, which is that the resistance values were based off RSF coefficients inferred from actual animal location data. As a result, they have meaningful biological interpretations and as evidenced by our study, show that habitat selection (or the inverse resistance score) is a good predictor of gene flow.

Our approach can be broadly divided into two interrelated components: i) what habitats are mountain goats selecting, and ii) do these patterns of habitat selection predict genetic relatedness. Mountain goats generally selected areas close to escape terrain and avoided valleys, which confirms field and genetic observations (Festa-Bianchet and Côté 2008, Shafer et al. 2011a). Heat load, a metric of incoming sunlight that accounts for aspect, slope, and latitude (McCune and Keon 2002), was selected by both sexes, particularly during winter (Table 7-3). Both males and females display seasonal altitudinal migrations (Rice 2008) that would help explain the selection patterns for higher heat loads, as well as the increased selection of alpine shrubs in the summer. When compared to desert ungulates that utilize areas with reduced heat loads (Cain III et al. 2006), mountain goats in northern climates are probably selecting for areas with increased heat load because they are the most vegetatively productive habitats and have lower snow loads.

One interesting result from these models is the change in habitat selection patterns of males during the rut. We thought this model would be a good predictor of gene flow because males move between female groups during this time and sire offspring (Festa-Bianchet and Côté 2008, Mainguy et al. 2009, Ortego et al. 2011). Fewer variables were important predictors of male habitat selection during this time, and many of the predictive variables had little influence (Table 7-3). Male behavior during the rut changes considerably, with the majority of their time spent standing and interacting socially (Mainguy and Côté 2008). This suggests that during the rut, the importance of habitat selection *per se*, may take a backseat to tending females. As males spend time both tending females and moving between groups of females during the rut (Festa-Bianchet and Côté 2008), they likely select different habitats during these two stages which could be denuding an overall response during the rut. Moreover, this shows that genetic connectivity depends on more than habitat selection across the landscape, and in highly gregarious species like the mountain goat, sociality or behavior are important factors to consider.

Habitat selection models were then evaluated on their ability to predict genetic relatedness. Of the 10 habitat selection models, only the three winter RSF models explained less variance than the null models, with summer RSF models explaining the most (Table 7-4). In addition, visualizing the summer resistance scores (Fig. 7-2b) clearly showed a barrier effect. This demonstrates a major advantage of resistance surfaces; the summer habitat selection model was able to account for variation in genetic relatedness caused by both distance and barriers, while neither the IBD nor IBB models could account for one and the other (Table 7-5). This is visually evident when looking at a subset of comparisons, where we can see the effect of the major barriers and distance on relatedness and resistance scores (Fig. 7-3). Most importantly, these variables, specifically their resistance values, were identified through an objective modeling approach.

The poor predictive power of the winter resistance surface follows our prediction, and is not surprising because mountain goat movement is greatly reduced during winter (Adams et al. 1982) and likely impacted by snow (Smith 1977, Poole and Heard 2003). The only notable difference between sexes was also during the winter, where the male model was a much better predictor of relatedness. The winter difference between sexes may be attributed to sex-based habitat segregation (Festa-Bianchet and Côté 2008), differential sensitivity to weather (Conradt et al. 2000), or reduced vulnerability to predation (White 2006). Clear habitat preferences are known to exist between the sexes (Festa-Bianchet and Côté 2008, this study), but differential sensitivity to weather or predation is unknown. Intersexual habitat segregation may occur during adverse winter weather if males and females differentially select for good foraging and sheltered sites - which has been documented before in ungulates (Conradt et al. 2000, Loe et al. 2006). Although we cannot specifically test the weather or predator sensitivity hypotheses, the large difference in heat load selection during winter (Table 7-3), along with results from White (2006), show that sex-specific habitat selection is occurring, with the underlying mechanism remaining a research avenue to pursue.

7.4.1. Using GPS-telemetry data in landscape genetics

Spear et al. (2010) noted the utility of resistance surfaces based on GPS radiotelemetry, but only a handful studies have used such data in a similar context. Epps et al. (2007) used individual telemetry data to partition slope into different cut-off values according to animal use; the authors then regressed least-cost paths, based on different slopes and penalties, against genetic differentiation and were able to identify potential movement corridors. Alternative approaches by Cushman and Lewis (2010) and Coulon et al. (2008) used GPS data to separately validate important variables inferred from a landscape genetics model (Coulon et al. 2006, Cushman et al. 2006). But none of these models explicitly used the GPS telemetry data to select and score habitat variables for resistance surfaces with the intent of predicting genetic relatedness (see also Spear et al. 2010).

The majority of studies have not used empirical data, instead relying on expert opinion to inform resistance surfaces (Beier et al. 2008, Murray et al. 2009). Such qualitative models can be problematic because they are based on perceived importance and costs by researchers (Beier et al. 2008, Spear et al. 2010) and are not directly repeatable or reflective of optimized costs (Shirk et al. 2010). This can be particularly difficult when attempting to model the intricacies of dispersal (Wang et al. 2008), especially when dispersal routes are seldom validated (Epps et al. 2007). Although an expert could infer variables important for gene flow, as noted, the inferred costs are far from optimal (Shirk et al. 2010): modeling RSFs during times known to be critical to gene flow would yield similar results, but less subjectively. Moreover, resistance surfaces are likely applicable to other species occupying similar habitat niches. At the very least, the selected variables and coefficients (i.e., avoidance or selection) are likely to be good starting points for ecologically similar species.

To reconcile some of the above noted issues, many landscape genetic studies have applied a rigorous model selection framework based on manually adjusting costs until the highest support value is achieved (Shirk et al. 2010, Wasserman et al. 2010); in such cases the selection of variables is based on a mixture of ecological studies and expert opinion. Inherent to such approaches,

however, is the recognition that the initial habitat model is flawed. In contrast, data from randomly selected study animals are used in RSFs to identify the habitat variables and their associated resistance value: this allows less influential variables to be dropped from the model, based on quantitative a priori criteria, and provides an objective and interpretable cost schema. RSFs also examine habitat selection at the home range scale, which is relatively coarse and should provide information on the habitat types that animals prefer over a longer time scale. When animals move through novel areas, they still are likely to respond to the habitat types that they are familiar with in an effort to maximize their success (or survival). This idea is likely even more relevant when discussing animals such as mountain goats, which mediate risk mainly through selection of escape terrain (Hamel and Côté 2007). Indeed distance to escape terrain was among the only two variables found to have an effect in all models (Table 7-3), and exemplifies the interface between gene flow and habitat selection. RSFs are not, however, without potential bias because the modeler must first pick the habitat variables; however this is an unavoidable step in any modeling framework. Screening an array of biologically meaningful variables and using past studies to help with selection, along with removing those that lack information or have a high correlation to another covariate, should alleviate that concern. Overall, GPS telemetry and genetic data form a natural partnership in landscape genetics, both in terms of validating models and habitat parameterization.

7.4.2. Potential applications from linking genetic and GPS data

In this study, we were able to examine the relationship between genetic relatedness and landscape resistance surfaces constructed from over 100 individual mountain goats fitted with GPS radiocollars. However, the question of feasibility still remains (Hebblewhite and Haydon 2010, Spear et al. 2010). Although it is cheaper to genotype an individual than to outfit it with a radiocollar - they provide different, but compatible data with the onus being on using both data sources when available. Researchers routinely collect tissue from immobilized animals being fitted with radiocollars; thus the raw material for genetic and high-resolution location data are simultaneously obtained. Moreover,

the analytical techniques have improved dramatically for landscape genetics (Sork and Waits 2010) and radiotelemetry technology (Cagnacci et al. 2010), along with the anticipated reduced costs of the latter (Cagnacci et al. 2010, Hebblewhite and Haydon 2010). Thus, the time is ripe for a merger of these two approaches. Such a linkage might be particularly useful for understudied species with unclear (or unknown) habitat affinities, or highly panmictic species with limited population structure and for which distance or barriers do not adequately predict genetic relatedness.

We described only one method for fitting a RSF, but several methods exist, including information-theoretic approaches for model selection (Burnham and Anderson 2002) and machine learning (Hastie et al. 2009) that could be used in a similar framework. In addition, step-selection functions (Fortin et al. 2005) have arisen from the RSF framework and specifically examine the habitat variables that facilitate movement. While the most appropriate method will depend on the research question at hand, the relative objectivity of established model selection procedures is a desirable attribute of RSFs. RSF resistance scores could even be incorporated as Bayesian priors and optimized across parameter space (see Choy et al. 2009 for use in expert opinion). Applied to landscape genetics, resistance surfaces and the associated costs-paths would be continually regressed against a metric of genetic differentiation until the optimal resistance surface was found. These approaches can produce surfaces that are easily interpretable (e.g., Fig. 7-1), and when combined with estimates of gene flow (e.g., Table 7-4), could be utilized in a conservation and management context.

As landscape genetic studies are still in their relative infancy, they have yet to reach their full potential. This growth coincides nicely with the fast-evolving field of animal movement ecology (Schick et al. 2008) and GPS-based animal tracking (Cagnacci et al. 2010), where new advances in modeling movement are likely to be applicable to landscape genetics. In this paper, we showed that GPS telemetry data could be used to identify habitat variables and assign costs to a landscape resistance surface through RSF models that can predict gene flow. When compared to the null IBD and IBB models, RSF-based

resistance surfaces offered a better predictor of genetic relatedness. Moreover, resistance scores were able to account for both geographic distance and barriers as shown by the overall model (Table 7-5). With the merger of these different methods, a novel and powerful approach for assessing the influence of landscape on genetic connectivity emerges. Combining GPS telemetry and genetic data should prove to be a promising and fruitful exercise in forthcoming landscape genetic studies.

Table 7-1. Resource Selection Function models broken down by sex and season in Alaskan mountain goats. The number of radio collared individuals and GPS fixes for each model are provided. Nine individuals were withheld to validate each model.

	No. individuals	No. GPS fixes
All individuals		
Year-round	102	151,112
Summer	97	52,566
Winter	94	62,852
Only females		
Year-round	56	77,414
Summer	52	26,592
Winter	52	31,302
Only males		
Year-round	46	73,698
Summer	45	25,974
Winter	42	31,550
Rut	45	13,471

Table 7-2. Variables and descriptions of variables used to estimate resource selection functions for 111 mountain goats collared in the Lynn Canal area, SE Alaska. Distances in parentheses denote the unit distance that the variable is measured.

Variable	Description
d_esc	Distance to escape terrain (100 m), classified as any area with slope greater than 40 degrees[a]
esc	Dummy variable indicating if the location was in escape terrain[a]
elev	Elevation (100 m) determined from ASTER global digital elevation model
heat	Heat load (McCune and Keon 2002)
rad	Incidental radiation (McCune and Keon 2002)
d_lake	Distance to lakes (100 m) obtained from the National Atlas of The United States
vall	Dummy variable indicating if a location was in a valley. A valley was defined as all areas that were not classified as upland or palustrine over 60 m in elevation using a National Wetlands Index layer
ice	Dummy variable indicating if a location was on snow or ice determined from Landfire landcover layer
barr	Dummy variable indicating if a location was barren of vegetation determined from Landfire landcover layer
herb	Dummy variable indicating if a location was herbaceous determined from Landfire landcover layer
alp_shr	Dummy variable indicating if a location was in alpine shrub determined from Landfire landcover layer
wet_shr	Dummy variable indicating if a location was in wetland shrub determined from Landfire landcover layer
shrub	Dummy variable indicating if a location was in any shrub habitat excluding wetland and alpine shrubs determined from Landfire landcover layer
tree	Dummy variable indicating if a location was in treed habitat determined from Landfire landcover layer
d_rds	Distance to roads (100 m) obtained from the USGS National Roads Atlas
d_ice	Distance to *ice* variable (100 m)
d_stm	Distance to streams (100 m) classified as class 1 rivers (Tongass National Forest, 2010)
d_vall	Distance (100 m) to *vall* variable
aspect	Aspect[a]
slope	Slope[a]

[a]Calculated using 15 × 15 m ASTER global digital elevation model (DEM)

Table 7-3. Variables selected and relative selection values (Lele et al. 2012) for the final resource selection function models estimated using data from all individuals, male (♂), and female (♀) mountain goats collared in the Lynn Canal area, SE Alaska. Explanations for each habitat variable are found in Table 7-2.

	Overall	Overall ♀	Overall ♂	Winter	Winter ♀	Winter ♂	Summer	Summer ♀	Summer ♂	Rut ♂
d_esc	0.71	0.65	0.73	0.59	0.48	0.64	0.81	0.82	0.77	0.64
elev	0.95	0.96	0.95	0.84	0.86	0.83	1.08	1.15	1.01	-
heat	2.8	6.04	1.54	4.68	13.13	1.95	1.33	2.49	-	-
rad	-	-	-	-	-	-	-	-	1.97	1.84
d_lake	0.99	0.99	-	0.99	-	0.99	1.00	-	1.00	1.00
d_rds	-	-	-	-	-	-	0.99	0.99	1.01	1.01
vall	0.26	0.35	0.27	-	-	-	-	-	-	0.34
d_ice	-	-	-	-	-	-	0.90	0.82	-	-
d_stm	-	-	-	-	-	-	0.99	1.01	0.98	0.99
d_vall	-	-	-	1.02	1.01	1.02	-	0.96	-	-
ice	0.15	0.16	0.14	0.00	0.01	0.00	-	-	0.30	0.07
barr	0.44	0.54	0.33	0.05	0.07	0.04	3.64	5.41	0.70	0.19
herb	0.81	0.57	-	0.19	0.07	0.26	11.90	11.10	2.63	-
alp_shr	1.2	1.32	1.04	0.24	0.26	0.22	11.79	15.17	2.33	-
wet_shr	0.34	0.19	0.41	0.11	0.04	0.14	9.06	10.07	-	-
shrub	0.82	0.67	-	0.19	0.08	0.24	9.47	10.34	2.25	-
tree	-	-	-	-	-	-	6.11	7.39	-	-
*heat*esc*	1.2	1.32	-	1.23	1.14	1.11	1.13	1.41	-	-
*tree*vall*	1.36	1.67	-	-	-	-	-	-	-	0.24
*tree*d_vall*	-	-	-	0.99	0.97	0.99	-	1.01	-	-
*rad*esc*	-	-	-	-	-	-	-	-	0.88	1.00

Table 7-4. Results from 10 Resource selection function-based landscape resistance models and their least cost paths in Alaskan mountain goats, along with the traditional models of differentiation. When the sex is not denoted the model is based on all of the individuals. Simple Mantel *r* and exact *p* values are provided. The Y intercept and slope are calculated from multiple regressions. QG is the Queller and Goodnight (1989) relationship coefficient. Results are based on 10,000 permutations.

Models	Mantel *r*	*p* value	Intercept	Slope
Traditional				
QG ~ Distance	-0.20	<0.01	0.04	-1.75E-06
QG ~ Barrier	-0.23	<0.01	0.04	-0.07
RSF-based landscape resistance				
QG ~ Year-round	-0.25	<0.01	0.06	-4.4E-07
QG ~ Year-round ♀	-0.25	<0.01	0.06	-2.6E-07
QG ~ Year-round ♂	-0.24	<0.01	0.06	-6.8E-07
QG ~ Summer	-0.27	<0.01	0.05	-4.6E-06
QG ~ Summer ♀	-0.27	<0.01	0.05	-1.9E-06
QG ~ Summer ♂	-0.27	<0.01	0.06	-4.5E-06
QG ~ Winter	-0.08	0.03	0.002	-4.7E-09
QG ~ Winter ♀	-0.08	0.02	0.001	-5.4E-11
QG ~ Winter ♂	-0.19	<0.01	0.003	-2.3E-07
QG ~ Rut ♂	-0.23	<0.01	0.05	-6.1E-07

Table 7-5. Results from the partial Mantel tests on landscape genetic models in Alaskan mountain goats. Partial Mantel *r* and exact *p* values (when >0.01) are in reference to the first variable, with the variables following the vertical bar being controlled for. QG is the Queller and Goodnight (1989) relationship coefficient.

Models	Mantel *r*	*p* value
RSF-based Landscape resistance		
QG ~ Summer | Distance	-0.20	<0.01
QG ~ Distance | Summer	0.03	0.09
QG ~ Summer | Barrier	-0.15	<0.01
QG ~ Barrier | Summer	-0.02	0.25
QG ~ Summer | Barrier + Distance	-0.15	<0.01

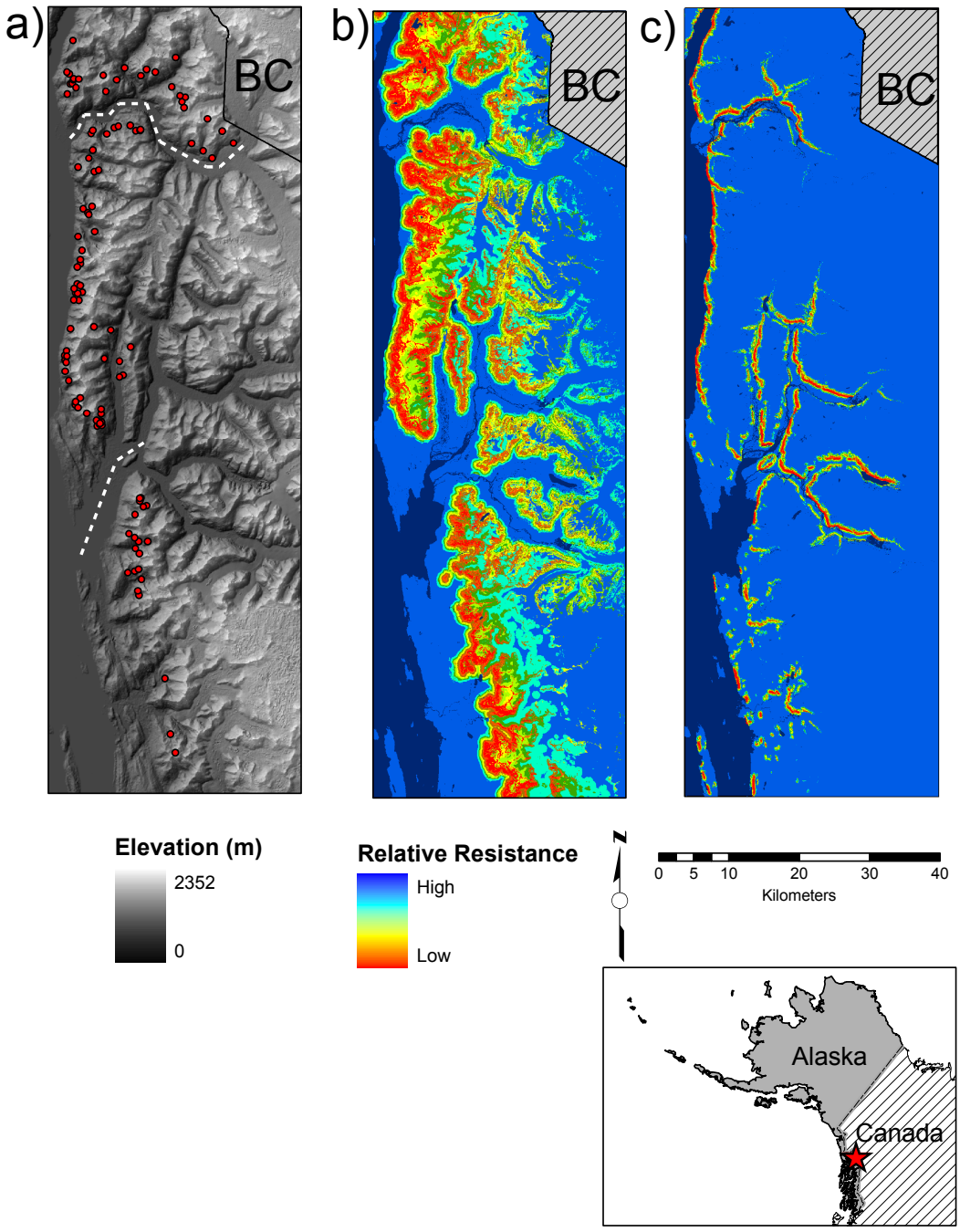

Figure 7-1. Map of sample locations and resistance surfaces of 102 radiocollared mountain goats in southeast Alaska. Map A) shows the topography of the Lynn Canal study region and each individual's home range centroid. Map B) shows the summer resource selection function (RSF)-resistance map, and map C) the winter RSF resistance map. British Columbia (BC) is removed from the northeastern

portion of the maps. The white-dashed lines correspond to the two major barriers in the study area (north - Katzehin River, and south - Berner's Bay).

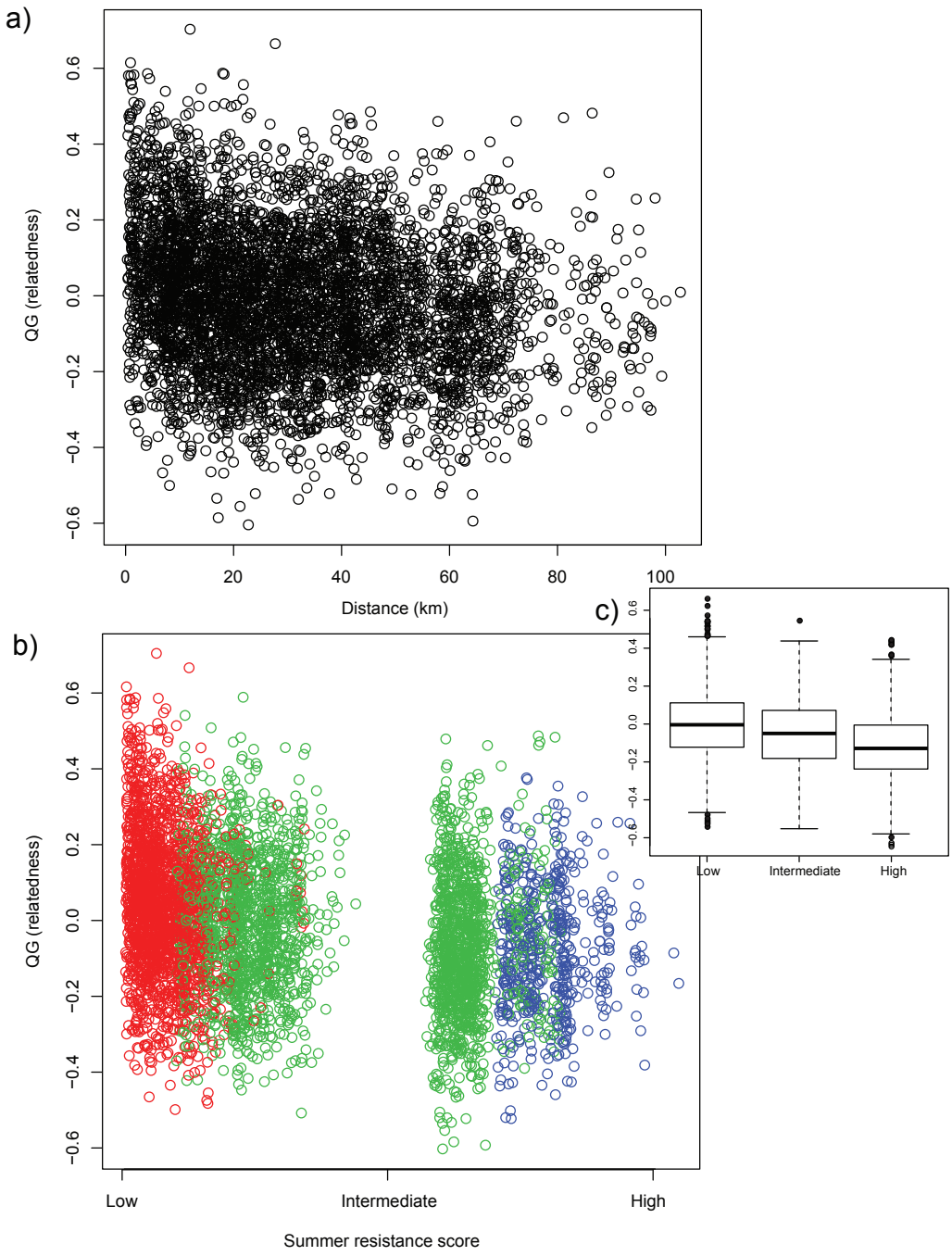

Figure 7-2. Plots of genetic relatedness against a) geographic distance, and b) summer resistance scores inferred from resource selection functions in 102 mountain goats in southeast Alaska. The resistance plot is coloured according to the number of barriers separating individuals with red = 0 barriers, green = 1 barrier, and blue = 2 barriers, and are divided into low, intermediate, and high resistance categories (binned approximately by thirds). In the inset c) a box-and-

whiskers plot provides an alternative view of the relationship between landscape resistance and relatedness. Within each plot the centerline represents the median with the outer box (hinges) the upper and lower quartiles. The vertical lines represent values falling within 1.5 times the box size of the nearest hinge.

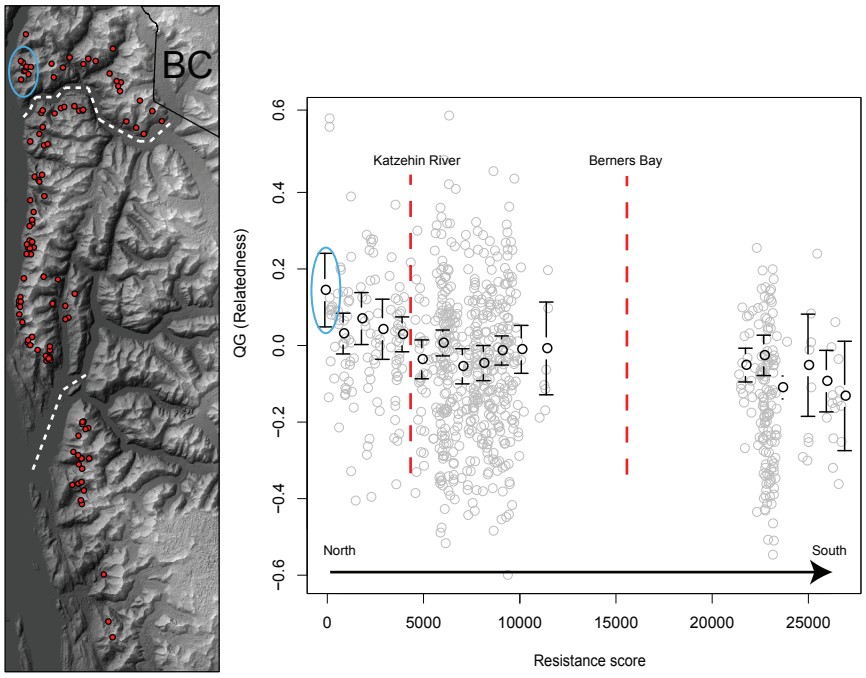

Figure 7-3. A subsampled QG ~ summer resistance plot showing the pattern within, and between animals from Mt. Villard (blue circle) and the rest of the study area. To clearly visualize the effect of landscape resistance on relatedness, comparisons were binned in 1000 resistance unit intervals to obtain mean relatedness and the standard error. Barriers are labeled and a trend of increasing resistance is observed from north to south in the range, showing how the resistance surface accounts for both distance and barriers. The map on the left shows Mt. Villard (blue circle), along with the major barriers in white dashed lines - the Katzehin River (adjacent to Mt. Villard) and Berners Bay at the bottom of the map.

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Chapter 8

Does reduced heterozygosity influence dispersal? A test using spatially structured populations in an alpine ungulate

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8.1. Introduction

The evolution of dispersal is a contentious topic. Ultimate explanations for dispersal have included competition for mates, competition for resources, and inbreeding avoidance (Clobert *et al*. 2001; Bowler & Benton 2005), none of which are mutually exclusive (Dobson & Jones 1985). These ideas lead to the recognition of 'Fitness Associated Dispersal' (Hadany *et al.* 2004 and references therein), which is the preferential dispersal of less successful individuals. Since individual heterozygosity is also positively associated with fitness components in wild populations (Chapman *et al*. 2009), this lead us to hypothesize that dispersed individuals have less heterozygosity than residents.

Here, we assessed whether dispersal was related to heterozygosity in a North American alpine ungulate, the mountain goat (*Oreamnos americanus*). The mountain goat is ideal for testing this hypothesis for two reasons: i) higher individual heterozygosity is associated with increased survival (Mainguy *et al*. 2009); and ii) mountain goat populations are spatially structured (Shafer *et al*. 2011), which allows for dispersal to be detected through genetic assignment tests (Waser & Strobeck 1998). Using data from a range-wide population structure study (Shafer *et al*. 2011) and a long-term study of individually marked goats at Caw Ridge, Alberta, Canada (Festa-Bianchet & Côté 2008), we tested for an association between observed individual multilocus heterozygosity (H_O) and dispersal at two hierarchical levels, subpopulation and herd, and discuss the implications for the evolution of dispersal.

8.2. Materials and methods

Detailed genotyping methods are available in Shafer *et al.* (2011) and Mainguy *et al.* (2009). Briefly, 876 mountain goats were sampled across their native range in western North America (Shafer *et al.* 2011) as well as 311 individuals from the Caw Ridge mountain goat herd in Alberta, Canada (Mainguy *et al.* 2009). All individuals were genotyped at the same 19 microsatellite loci used in Shafer *et al.*

(2011). Although dispersal is male-biased, both sexes were included as they each disperse (Festa-Bianchet & Côté 2008; Shafer *et al.* 2011).

Subpopulations were identified using STRUCTURE 2.2 (Pritchard *et al.* 2000) as described in Shafer *et al*. (2011). Cross-assigned individuals or "dispersers", defined as individuals genetically from one subpopulation but physically found in another (or clearly outside their own), were identified as follows: (i) polygons were constructed around individuals assigned to a subpopulation with a $q > 0.80$ that were confined to specific mountain range(s) using ARCGIS 9.0. These polygons constituted the 'core' populations (*sensu* Bélichon *et al.* 1996) and contained residents - this criteria was based on empirical data showing that mountain ranges help delineate subpopulation boundaries in alpine ungulates (Worley *et al*. 2004; Shafer *et al*. 2011); (ii) individuals with a $q > 0.80$ not found within the polygon and on a separate mountain range were considered cross-assigned; and (iii) individuals with a $q < 0.80$ were considered to have admixed ancestry (Bergl & Vigilant 2007; Lecis *et al*. 2006). This produced three categories: dispersers (*D*), residents (*R*), and admixed (*A*). To ensure the results were not biased by the STRUCTURE algorithm (i.e. failure to detect heterozygous dispersers), we simulated 3 pairs of populations of 25 individuals per popultation with varying degrees of differentiation (F_{ST} of 0.05, 0.15, 0.30) typed at loci with allele frequencies from Shafer *et al*. (2011). For each simulation we measured the covariance between q and H_O. We then randomly designated individuals with $q > 0.80$ as cross-assigned from each simulated population, with the remainder of individuals with $q > 0.80$ classified as residents, and calculated H_O for both categories (Appendix XII contains additional information on the simulations).

Immigrants and emigrants were identified from the long-term Caw Ridge study population where animals aged 1 to 3 disperse (Festa-Bianchet & Côté 2008). This is the only mountain goat herd with such data available. All two-year old males in the herd have been fitted with a radio-collar since 2001 to track long-distance movements. Emigrants from Caw Ridge (D_E) were individuals for whom departure from Caw Ridge was confirmed using live radio signal via helicopter

fly-over or visual means. Individuals that appeared to have emigrated but were found deceased were grouped separately. Immigrants to Caw Ridge (D_I) were easily identified through population monitoring (Figure 8-1) since they were unmarked.

We compared H_O between dispersal categories (D, R, and A) across subpopulations, and at Caw Ridge. We compared the average H_O across categories using a Kruskal-Wallis test. Wilcoxon tests were then conducted between all pairs of categories. H_O of D individuals was also compared to the H_O and expected heterozygosity (H_E) of their subpopulation of origin using a sign-test. For the Caw Ridge herd, we compared D_E and D_I with R using a Wilcoxon test and sign-test. Means are presented ±s.e. and tests were one-tailed. All statistical analyses were done using the freeware R 2.8.0. (http://www.r-project.org) and raw data are provided in the supplementary material.

8.3. Results

A total of 803 animals with sufficient geo-referencing were tested for cross-assignment. Of these, 30 were classified as D, 436 as R, and 309 as A. Assignment of dispersal was unambiguous as D individuals were clearly disjunct (Figure 8-2). H_O differed among the D (0.38±0.02), R (0.43±0.01), and A (0.46±0.01) groups (Kruskal-Wallis test; chi-squared = 10.4, df = 2, $p < 0.01$). H_O was lower in D than R (pair-wise test; W = 5065.5, $p = 0.02$), D than A (W = 3088.5, $p < 0.01$), and R than A (W = 61592.5, $p = 0.02$). Dispersers averaged 6.3% (±2.4 %) less H_O than their subpopulation of origin (s = 10, $p = 0.05$). The same pattern was observed relative to subpopulation H_E (s = 6, $p < 0.01$). In the simulated data, there was no relationship between q and H_O (mean Pearson coefficient 0.0005±0.0007; Appendix XIII) and the H_O of dispersers was not different than residents (W = 730, $p = 0.58$).

Over the past twenty three years at Caw Ridge, 266 animals lived to at least one year of age, of which 15 were classified as D_E, 4 as D_I, and the remainder as R. Mean H_O for D_I, D_E, and R at Caw Ridge were 0.45 (±0.03), 0.55 (±0.03), and 0.50 (±0.01), respectively. A trend of lower H_O in D_I compared to D_E (W = 11, p =

0.03) and R (W = 343, $p = 0.15$) was observed, but R was lower than D_E (W = 1321, $p = 0.03$). There was no difference between pooled D_I and D_E from residents (W = 2669, $p = 0.84$) and between the H_O of D_E and those individuals that died while emigrating (W = 37.5, $p = 0.48$). The four D_I individuals averaged 5.7% (±2.6 %) less H_O than the herd average (s = 0, $p = 0.06$) and were assigned by STRUCTURE to the S3 subpopulation in Shafer *et al*. (2011), which encompasses Caw Ridge.

8.4. Discussion

Dispersing mountain goats appear to be less heterozygous than non-dispersers. Across subpopulations, dispersers had lower H_O than residents, and at the herd level, immigrants to Caw Ridge had the lowest H_O. Simulations indicated that the difference was not due to bias in the assignments. However, the opposite trend was observed in emigrants from Caw Ridge. This latter result suggests alternative mechanisms may be influencing dispersal from Caw Ridge. One scenario we considered was whether Caw Ridge emigrant survival was related to heterozygosity (i.e. less heterozygous individuals were emigrating but had poorer survival); but H_O was not different between D_E and emigrants that were only found deceased. A possible explanation could be related to density. The Caw Ridge herd recently doubled in size (Festa-Bianchet & Côté 2008) and is starting to show signs of density-dependence (Hamel *et al*. 2010). In shrews, Hanski *et al*. (1991) found that as density increased, dispersers tended to be more highly-ranked individuals. Maternal condition was hypothesized to influence dispersal in such instances (Hanski *et al*. 1991; *sensu* Ims 1990). In mountain goats the relationship between heterozygosity, rank, maternal condition, and dispersal, have yet to be examined but may shed additional light on the factors promoting dispersal.

The evolution of dispersal is clearly multifactorial (Dobson & Jones 1985). As a result, models simulating dispersal must take into account a diverse array of costs and processes that often lack realistic assumptions (e.g. condition-dependent strategies; Bowler & Benton 2005). Because multilocus heterozygosity

can be a proxy for inbreeding (Jensen *et al*. 2007), the pattern of lower heterozygosity could be attributed to increased coancestry. Models have shown that inbreeding depression could select for dispersal, resulting in a balance between the costs of coancestry and dispersal (Perrin & Mazalov 1999; Roze & Rousset 2009). In both inbreeding and fitness scenarios, selection could have favoured the dispersal of individuals with reduced genetic heterozygosity because the disperser's fitness would benefit from future heterosis (Morgan 2002; Guillaume & Perrin 2006; Roze & Rousset 2009) and it provides a means of removing deleterious mutations (Hadany *et al.* 2004). Heterosis occurs when individuals from divergent populations reproduce, and is most pronounced in highly differentiated populations with low H_O. In spatially structured species like the mountain goat (Shafer *et al*. 2011), admixture results from matings between dispersers and residents. The distribution of H_O across *D*, *R*, and *A* categories suggests heterosis could be occurring, as the outbred-admixed individuals have the highest H_O. Thus, the cost of philopatry versus dispersal in a fitness- or inbreeding-related context, must consider the potential benefit of heterosis. Future research should also utilize genomic resources (i.e. Poissant *et al*. 2009) to identify chromosomal regions and ultimately the genes responsible for dispersal.

Figure 8-1. All mountain goats at Caw Ridge are uniquely marked, and males are fitted with radio collars allowing us to document both emigration and immigration. Here is a uniquely marked female accompanied by her yet-to be marked kid.

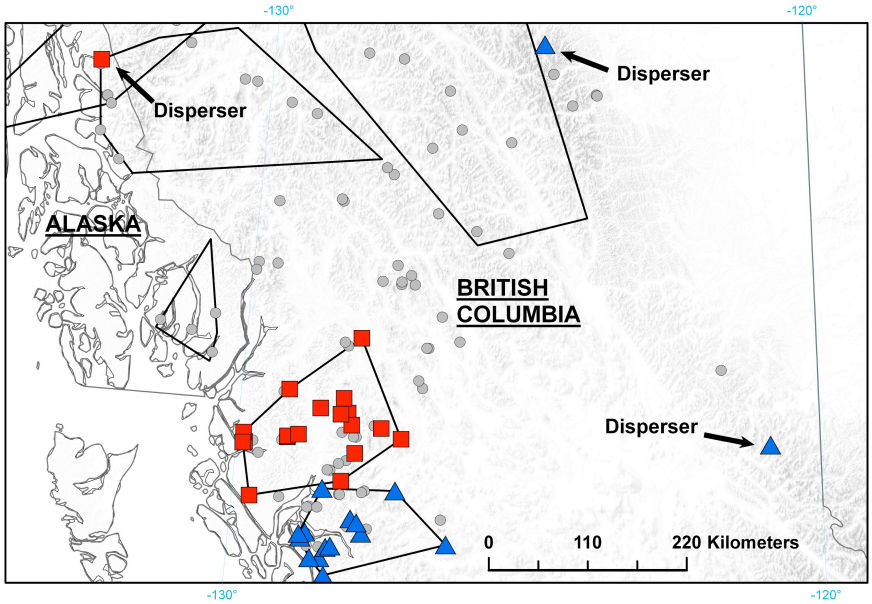

Figure 8-2. Example of cross-assigned mountain goats. Individuals assigned (q > 0.80) to their subpopulations are in coloured squares and triangles, with the dispersers labeled. Grey circles represent admixed individuals. For clarity, all polygons are drawn but only two subpopulations are show.

8.5. Bibliography

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Chapter 9

General Conclusion

9.1. Conclusion

My doctoral thesis focused on understanding and characterizing the evolutionary history and distribution of genetic diversity in mountain goats. First, I reviewed the co-distributed phylogeographic patterns of northwestern North America and tested for correlations between ecological traits and refugial history. I then re-examined the mountain goats' phylogenetic placement in the Caprinae family, and statistically compared the topology I generated to all those proposed in the literature. Using a suite of molecular markers, I examined the phylogeography, spatial genetic structure, and distribution of immune gene diversity in the mountain goat. I also tested the influence of refugia, mountains, and location (core vs. periphery) on genetic diversity and differentiation. Shifting to a more fine scale approach, I examined the landscape genetic structure of mountain goats in southeast Alaska by constructing landscape resistance surfaces based on actual animal movements. I tested for correlations between genetic relatedness and landscape resistance, and compared the results to two null models of genetic differentiation. Finally, I examined the individual genetic characteristics of dispersing mountain goats across the range and at Caw Ridge. I compared levels of heterozygosity between dispersers and residents (or non-dispersers) to test the hypothesis of fitness associated dispersal.

In **Chapter 2**, my review found the phylogeographic history of northwestern North America to be more complex than previously thought (i.e. *refugia within refugia*, cryptic refugia). The overarching co-distributed patterns suggest that we should redefine the classic Beringia-Southern paradigm for biota in northwestern North America. I also found that species with high dispersal ability and large home ranges were most likely to have resided in multiple refugia during glacial advances. Future phylogeographic studies in the region should strive to incorporate genomic data sets and more complex models (coalescent and ecological niche modeling). Public data repositories and the use of non-molecular data (fossils, ethnohistory) should be encouraged and utilized when available.

In **Chapter 3**, I applied a total evidence approach and topological tests to place the mountain goat within the Caprinae phylogeny. I found mountain goats to be an independent basal lineage in the Caprinae lineage. This finding suggests that all North American members of the Caprinae lineage, considering only a single wild sheep ancestor, entered North America independently and separately. This placement would also support mountain goats' early divergence in Pleistocene or late Pliocene. Methodologically, I found that maximum parsimony was unable to integrate over phylogenetic uncertainty. The approach I took will be useful for identifying and dealing with the issue of phylogenetic uncertainty and problematic taxa.

In **Chapter 4**, I found that mountain goats likely survived in two major refugia during the last glacial maximum, and a cryptic refugium on Baranof Island located in the Alexander Archipelago of Alaska. I found that mountain goat populations were structured according to refugial history and mountain blocks, and that animals on the periphery of the range were less genetically diverse than the core of the range. I detected relatively high levels of contemporary dispersal, but paradoxically, populations were still highly differentiated. The overall level of spatial heterogeneity and differentiation may help facilitate and maintain local adaptation in mountain goat populations.

In **Chapter 5**, I found mountain goats to have low levels of diversity at 4 of 5 immune loci. The Class I MHC immune gene, however, was nearly 30 fold more diverse than the rest of the immune genes and represents a source of increased immunogenetic variation. I found that the polymorphisms observed within each locus had no association with refugial history, suggesting that they did not arise in the Holocene. Thus, mountain goats may be better equipped for climate change and anthropogenic disturbance than previously thought because the paucity of diversity has persisted since before the last glacial maximum, and the there is increased diversity in the Class I gene.

In **Chapter 6**, I revisited the possibility that Baranof Island was a cryptic refugium for mountain goats. Using Y chromosome sequence data, I found a single polymorphic site that was restricted to Baranof Island and an area to the

northeast. The polymorphism was not found in the presumed source population of Tracy Arm. Reviewing historical Russian documents of the region, I found multiple references to mountain goats on Baranof Island stemming from the early 1800s. Collectively, these data support the hypothesis that Baranof Island acted as a refugium during the last glaciation, and I recommend the population be recognized as an evolutionarily significant unit.

In **Chapter 7**, I examined the landscape genetic structure of mountain goats in southeast Alaska. I first constructed landscape resistance surfaces based on animal location data and habitat selection. Multiple landscape resistance models were constructed according to sex and season along with two null models of genetic differentiation (isolation by distance and isolation by barrier). Each surface and null model was evaluated on its ability to predict genetic relatedness in a causative framework. Summer resistance surfaces were the best overall models, whereas winter was the worst. When all permutations were considered in the partial Mantel tests, the summer resistance surface significantly outperformed the barrier and distance model. This analysis provides an objective modeling approach for selecting and scoring habitat variables in landscape genetic studies, and identified important habitat variables that facilitate genetic connectivity among mountain goats.

In **Chapter 8**, I found that dispersal in mountain goats was associated with reduced heterozygosity. This finding provides empirical support to the fitness associated dispersal hypothesis. However, the pattern was not seen in emigrants from Caw Ridge, which I suggested might be due to density dependence. Overall, associations between lower heterozygosity and dispersal may be advantageous because it will remove deleterious alleles and the individual should benefit from heterosis.

When I began this doctoral research five years ago, very little was known about the evolutionary history of mountain goats. Although we had excellent data on mountain goat ecology and life-history (Festa-Bianchet and Côté 2008), we were missing much of the larger evolutionary picture. My dissertation research provides a comprehensive overview of the evolutionary history and distribution of

diversity in the mountain goat. Using molecular markers I identified important features that influenced genetic connectivity among populations, I found a source of increased immunogenetic diversity, and I made the case for Baranof Island mountain goats being an evolutionarily significant unit – all of which are important to the conservation and management of this species. In a broader context, I built on Brunsfeld et al.'s (2001) early review of northwestern North America and I suggested that it is time we revisit northwestern North America's biotic paradigm. I added empirical evidence for the central-marginal hypothesis (Eckert et al. 2008) and the fitness-associated dispersal model (Hadany et al. 2005), and I provided methodological advancements in phylogenetics and landscape genetics. Overall, this thesis has filled in numerous gaps regarding mountain goat evolution and added numerous insights into evolutionary and ecological hypotheses.

I think it is worth ending by acknowledging that none of this dissertation would have been possible without long-term monitoring and local hunter/stakeholder participation. Long-term monitoring of wild populations has high ecological and evolutionary value (Clutton-Brock and Sheldon 2010); however, there are only a handful of long-term studies of natural ungulate populations (Festa-Bianchet and Côté 2008). It goes without saying that if we lose these projects, we lose an irreplaceable source of ecological and evolutionary data. Without local participation, range-wide data sets such as the one I have accumulated in Figure 2-1 would not exist, and conservation recommendations like those made in Chapter 6 would be meaningless. Mountain goats are among the few North American ungulates that have been extirpated from large parts of their range through sport hunting (Glasgow et al. 2003) making stakeholder participation vital to conservation. The continued dissemination of information from scientists, combined with local stakeholder participation, will help ensure the long-term preservation of mountain goats and their alpine home.

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APPENDICES

Appendix I. Scoring schemata for refugia-ecology association.

The major data sources are included in Table 2-2. In cases where the main source provided insufficient data, primary literature was sought out. Refugial history was scored 1 if phylogeographic data supported more than one refugium (Table 2-2), which included Beringia, Southern, Coastal, and within the Ice sheets. Range was scored 1 if species range map covered more than one major refugia (southern or Beringia) or cryptic refugia (Arctic, Pacific Islands, or within the ice-sheets). Species were considered habitat specialists (1) if a specific habitat feature is required for species existence (e.g. talus) and mentioned in species descriptions and ecology. Dispersal ability was qualitatively measured on a variety of characteristics. In plants, seed spread rate, growth rate, and growth requirements were considered important for dispersal. For vertebrates, aerial transport or high mobility was considered important, while slowly reproducing species with specific habitat requirement for were considered less efficient. High dispersal ability was scored 1.

Appendix II. Species with their refugial history and the ecological characteristics utilized in the logistic regression. Refugia locations are: south (s), Beringia (n), and cryptic (c). Appendix I discusses the scoring system. Source acronyms are the International Union for Conservation of Nature (IUCN) and the United States Department of Agriculture (USDA).

Common name	Refugia locale	Refugia code	Range	Habitat	Dispersal	Source
Douglas squirrel	s	0	0	1	0	IUCN
Red squirrel	s	0	1	1	0	IUCN
Tundra vole	n	0	0	0	1	IUCN
Black bears	s c	1	1	0	1	IUCN
Flying squirrel	s	0	1	0	0	IUCN
Long-tailed vole	s c	1	1	0	1	IUCN
Deer mice	s	0	1	0	1	IUCN
Keens mouse	s c	1	1	0	1	IUCN
Red-tree vole	s	0	0	1	0	IUCN
Collared lemming	n	0	0	1	1	IUCN
Brown lemming	n	0	0	0	1	IUCN
Wandering shrew	s	0	0	0	1	IUCN
Yellow-pine chipmunk	s	0	0	0	1	IUCN
Ermine	n s c	1	1	0	1	IUCN
Red-tail chipmunk	s	0	0	0	0	IUCN
Mule deer	s	0	1	0	1	IUCN
American marten	s	0	1	0	1	IUCN
Red-backed vole	s	0	1	0	1	IUCN
Thinhorn sheep	n c	1	1	0	1	IUCN
Bighorn sheep	s c	1	1	0	1	IUCN
Mountain goat	n s	1	1	0	1	IUCN
Gray wolf	s	0	1	0	1	IUCN
Red fox	n s	1	1	0	1	IUCN
Wolverine	n	0	1	0	1	IUCN
Arctic hare	n c	1	1	0	1	IUCN

Caribou	n s	1	0	0	1	IUCN
Arctic ground squirrel	n	0	0	0	1	IUCN
Grizzly bear	n	0	0	0	1	IUCN
Water vole	s	0	0	1	0	IUCN
American pika	s	0	0	1	0	IUCN
Lodgepole pine	n s c	1	1	0	1	USDA
Mountain sorrel	s c	1	1	0	0	USDA
Purple saxifrage	n c	1	1	0	1	USDA
White spruce	s c	1	1	0	0	USDA
Whitebark pine	s	0	0	0	0	USDA
Foxtail pine	s	0	0	0	0	USDA
Dusky willow	s	0	0	0	1	USDA
Western red cedar	s	0	0	0	1	USDA
Western hemlock	s	0	1	0	1	USDA
Noble fir	s	0	0	0	0	USDA
Subalpine larch	s	0	0	0	0	USDA
Western larch	s	0	0	0	1	USDA
Englemann spruce	s	0	0	0	0	USDA
Limber pine	s	0	0	0	1	USDA
Rockcress	n s	1	1	0	1	USDA
Alpine groundsel	n s	1	1	0	1	USDA
Stonecrop	s	0	1	0	0	USDA
Sitka spruce	s c	1	1	0	1	USDA
Easter daisies	n s c	1	1	0	1	USDA
Ragwort	s c	1	1	0	1	USDA
Iowa golden saxifrage	s c	1	1	0	0	USDA
Constances bittercress	s	0	0	1	1	USDA
Sugar pine	s	0	0	0	0	USDA
Locoweeds	n	0	1	0	0	USDA
Mountain avens	n c	1	1	0	1	USDA
Spotted owl	s	0	0	0	1	NatureServe
MacGillivray's warbler	s	0	0	0	1	NatureServe
Yellow warbler	s	0	1	0	1	NatureServe
Common raven	s	0	1	0	1	NatureServe
Blue grouse	s	0	1	1	1	BNA
Canada goose	s c	1	1	0	1	NatureServe

Wilson's warbler	s	0	1	0	1	NatureServe
Chestnut-backed chickadee	n c	1	1	0	1	NatureServe
Mountain chickadee	s	0	0	0	1	NatureServe
Yellow-breasted chat	s	0	0	0	1	NatureServe
Common yellowthroat	s	0	1	0	1	NatureServe
Nashville warbler	s	0	0	0	1	NatureServe
Stellar's Jay	s c	1	1	0	1	NatureServe
Song sparrow	s c	1	1	0	1	NatureServe
Sandhill crane	s	0	1	0	1	NatureServe
Yellow-rumped warbler	s	0	1	0	1	NatureServe
American redstart	s	0	0	0	1	NatureServe
Swainson's thrush	s	0	1	0	1	NatureServe
Winter wren	s	0	0	0	1	NatureServe
Wood duck	s	0	0	0	1	NatureServe
Rock ptarmigan	n c	1	0	0	1	NatureServe
Long-toed salamander	s c	1	1	0	0	IUCN
Western toad	s	0	0	0	0	IUCN
Tailed frog	s	0	0	0	0	IUCN
Oregon salamander	s	0	0	0	0	IUCN
Rubber boa	s	0	1	0	0	IUCN
painted turtle	s	0	0	0	0	NatureServe
Western rattlesnake	s	0	0	0	0	IUCN
Ringneck snake	s	0	0	0	0	IUCN
Van Dyke's salamander	s	0	0	0	0	IUCN
Cope's giant salamander	s	0	0	0	0	IUCN
Giant salamanders	s	0	0	0	0	IUCN
Del Norte salamander	s	0	0	0	0	IUCN
Larch Mountain salamander	s	0	0	1	0	IUCN
Pacific tree frog	s	0	0	0	0	IUCN
Red-legged frog	s	0	0	0	0	IUCN
Columbia spotted frog	s	0	1	0	0	IUCN
Northern leopard frog	s	0	0	0	0	IUCN
Wood frog	s	0	1	0	0	IUCN
Southern torrent	s	0	0	0	0	IUCN

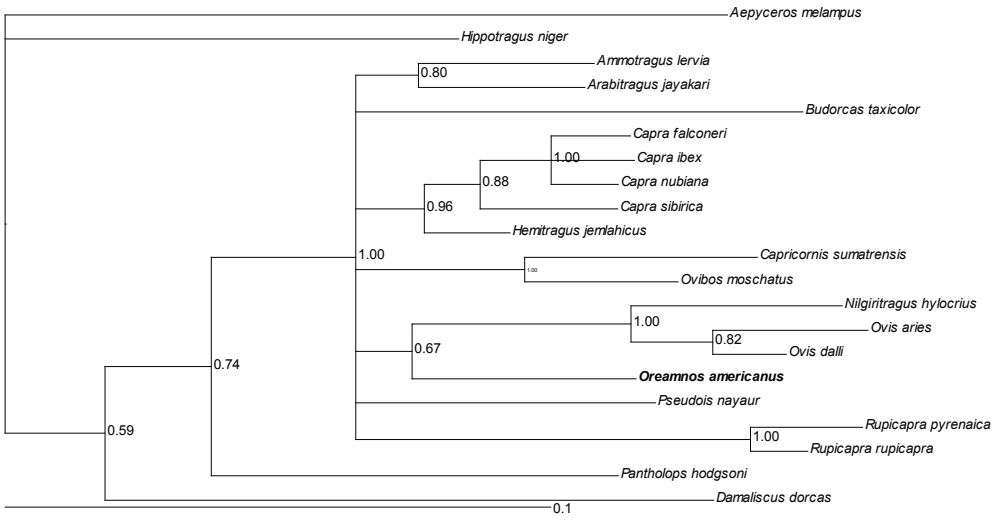
salamander						
Rough-skinned newt	s	0	0	0	0	IUCN
Garter snake	s c	1	0	0	1	IUCN
Arctic charr	n s c	1	1	0	1	NatureServe
Northern clingfish	n s	1	1	0	1	Hickerson & Ross
Dolly varden	n s	1	1	0	1	NatureServe
Bull trout	s	0	1	0	1	NatureServe
Arctic grayling	n c	1	1	0	1	NatureServe
Lake trout	n s c	1	1	0	1	NatureServe

Appendix III. Genes used in Chapter 3 with their GenBank accession number.

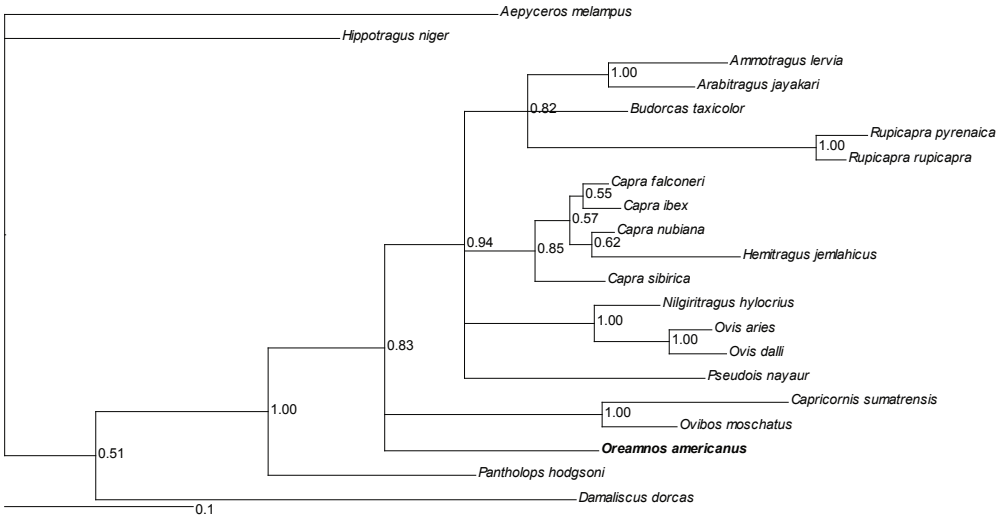
Species	12S	CO2	Cyb	ND1	κ-cas	SPTBN1	PRKCI	TG
Aepyceros melampus	M86496	AY689194	AF036289	DQ236320	AY121998	AF165782	AF165781	AF165786
Damaliscus pygargus	M86499	AY689195	AF036287	DQ236321	AY122002	DQ236280	AY846794	AF165778
Hippotragus niger	AY670653	AY846771	AF036285	DQ236322	AY122001	DQ236281	AY846795	AF165746
Ammotragus lervia	AY670654	AY846772	AF034731	DQ236323	AY670670	DQ236282	AY846803	DQ236302
Buborcas taxicolor	AY670655	AY846773	AY669320	DQ236324	AY670671	DQ236283	AY846811	DQ236303
Capra falconeri	AY670656	AY846774	AF034736	DQ236325	AY670672	DQ236284	AY846797	DQ236304
Capra ibex	AY846815	AY846775	AF034735	DQ236326	AF525023	DQ236285	AY846798	DQ236305
Capra nubiana	AY670657	AY846776	AF034740	DQ236327	AY670673	DQ236286	AY846799	DQ236306
Capra sibirica	AY670658	AY846777	AF034734	DQ236328	AY670674	DQ236287	AY846800	DQ236307
Hemitragus jemlahicus	AY670659	AY846780	AF034733	DQ236329	AY670675	DQ236288	AY846801	DQ236308
Arabitragus jayakari	AY846816	AY846779	AY846791	DQ236330	DQ236300	DQ236289	AY846804	DQ236309
Nilgiritragus hylocrius	AY846817	AY846778	AY846792	DQ236331	DQ236301	DQ236290	AY846808	DQ236310
Naemorhedus sumatraensis	AY670660	AY846781	AY669321	DQ236332	AY670676	DQ236291	AY846812	DQ236311
Oreamnos americanus	AY670661	AY846782	AF190632	DQ236333	AY670677	DQ236292	AY846814	DQ236312
Ovibos moschatus	AY670662	AY846783	AY669322	DQ236334	AY670678	DQ236293	AY846813	DQ236313
Ovis aries	AY670663	AY846785	AF034730	DQ236335	AY670679	DQ236294	AY846806	DQ236314
Ovis dalli	AY670664	AY846786	AF034728	DQ236336	AY670680	DQ236295	AY846807	DQ236315
Pantholops hodgsonii	AF400659	AY846787	AF034724	DQ236340	AY670681	DQ236299	AY846796	DQ236319
Pseudois nayaur	AY670665	AY846788	AF034732	DQ236337	AY670682	DQ236296	AY846802	DQ236316
Rupicapra pyrenaica	AY846818	AY846789	AF034726	DQ236338	DQ236341	DQ236297	AY846810	DQ236318
Rupicapra rupicapra	AY670666	AY846790	AF034725	DQ236339	D32182	DQ236298	AY846809	DQ236317

Appendix IV. Eight trees from individual gene phylogenic analysis. Above each tree in bold is the name of the gene followed by the model of evolution.

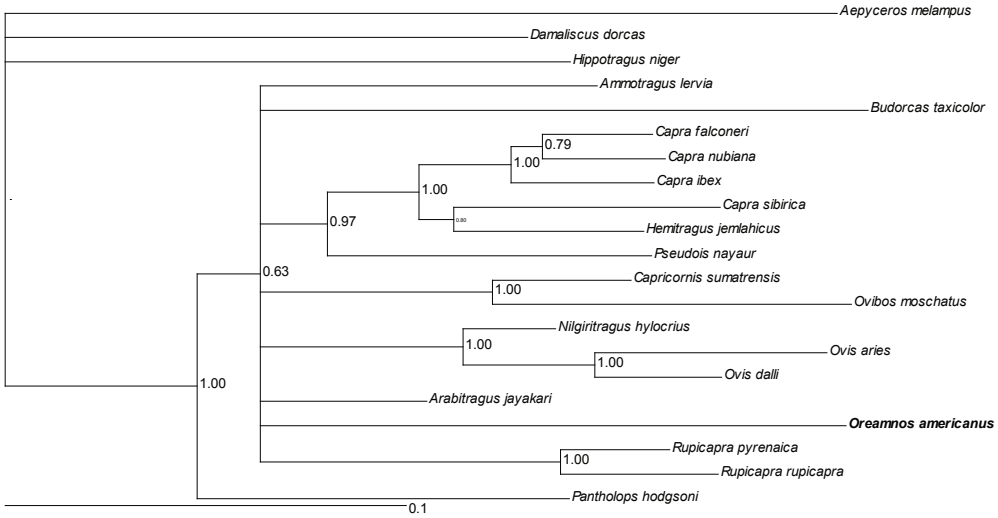
12S rRNA - GTR+I+G



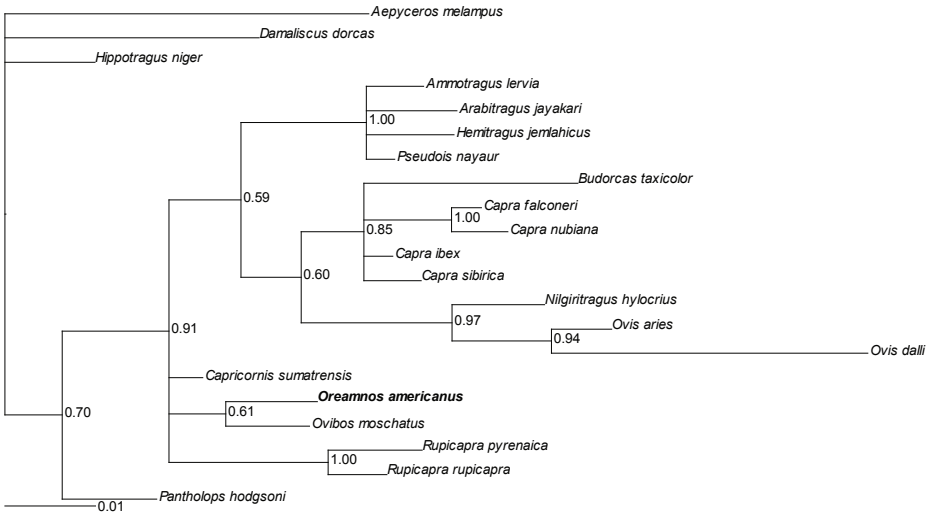
COII- GTR+I+G



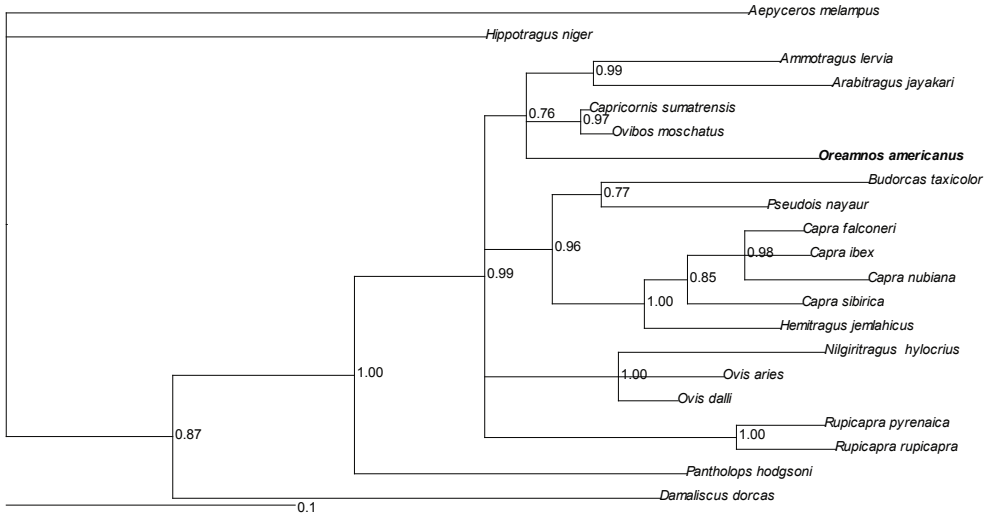
Cyt b - GTR+I+G



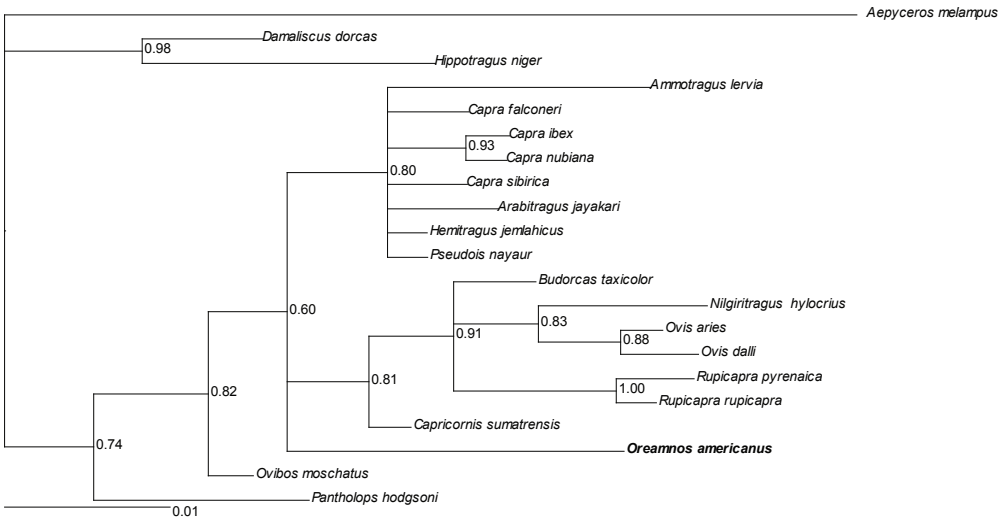
k-casein - HKY+G



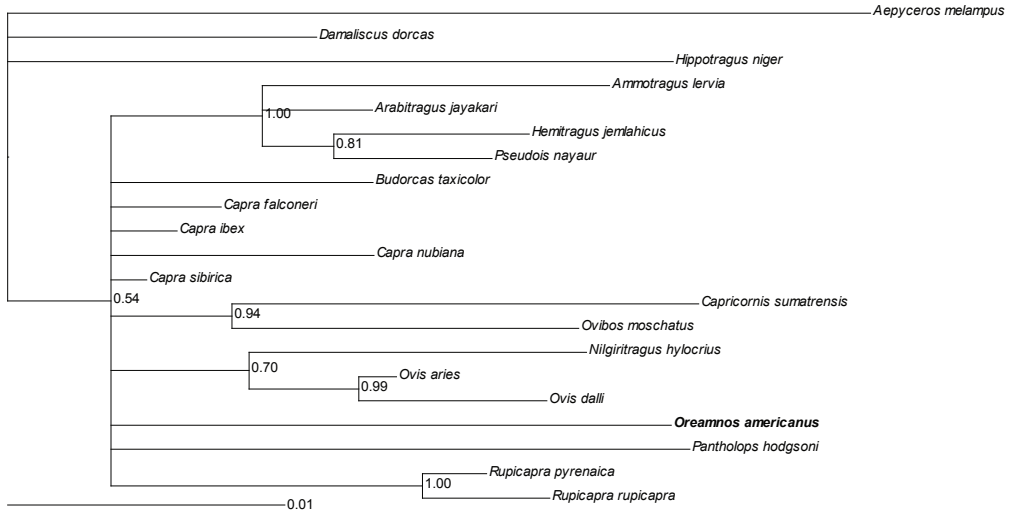
ND1 - GTR+I+G



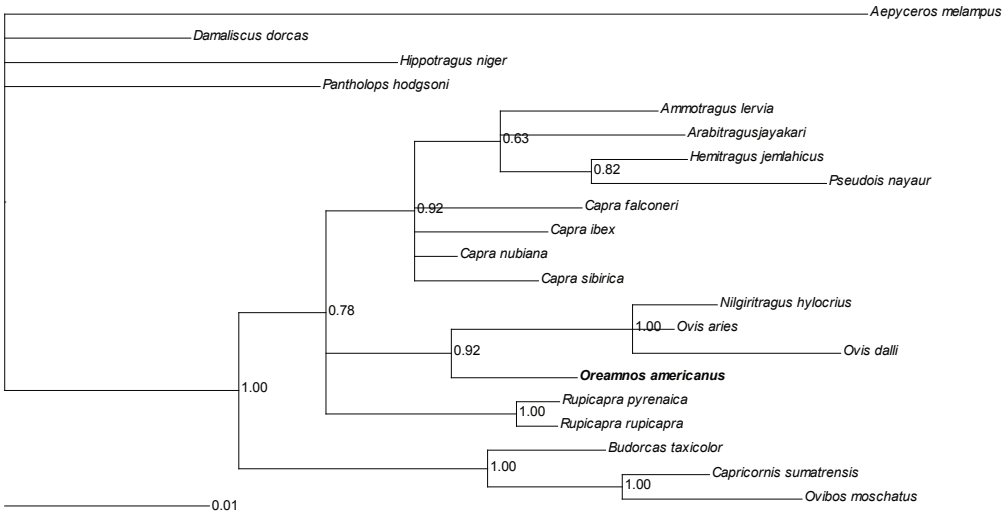
PRKC1 - K80+G



SPTBN1 - K80+G



TG - HKY+G



Appendix V. Pairwise D_S and F_{ST} and for designated areas (Table 4-1: Figure 4-1a).

D_S

	SalR	BitA	BlaH	Cas	Sel	Con	Pur	PacR	Car	KitH	BouR	Ske	Omi	NorR	NorI	MckM
SalR	-															
BitA	0.18	-														
BlaH	0.28	0.26	-													
Cas	0.34	0.28	0.32	-												
Sel	0.15	0.10	0.24	0.19	-											
Con	0.14	0.10	0.15	0.16	0.04	-										
Pur	0.11	0.08	0.22	0.19	0.00	0.04	-									
PacR	0.26	0.22	0.28	0.07	0.12	0.10	0.12	-								
Car	0.21	0.10	0.25	0.17	0.06	0.04	0.05	0.09	-							
KitH	0.29	0.22	0.28	0.18	0.22	0.16	0.16	0.11	0.17	-						
BouR	0.33	0.24	0.39	0.25	0.23	0.21	0.19	0.16	0.18	0.10	-					
Ske	0.25	0.21	0.31	0.22	0.21	0.15	0.15	0.13	0.16	0.03	0.06	-				
Omi	0.30	0.24	0.33	0.24	0.25	0.21	0.19	0.16	0.23	0.05	0.08	0.03	-			
NorR	0.32	0.22	0.34	0.20	0.25	0.20	0.20	0.14	0.21	0.04	0.09	0.05	0.01	-		
NorI	0.29	0.22	0.32	0.21	0.21	0.17	0.16	0.14	0.16	0.05	0.02	0.02	0.03	0.04	-	
MckM	0.37	0.28	0.42	0.16	0.22	0.19	0.20	0.11	0.19	0.12	0.08	0.10	0.09	0.09	0.06	-
Ken	0.43	0.42	0.66	0.43	0.42	0.43	0.38	0.39	0.34	0.37	0.32	0.34	0.40	0.41	0.34	0.33

F_{ST}

	SalR	BitA	BlaH	Cas	Sel	Con	Pur	PacR	Car	KitH	BouR	Ske	Omi	NorR	NorI	MckM
SalR	-															
BitA	0.22	-														
BlaH	0.34	0.30	-													
Cas	0.38	0.33	0.36	-												
Sel	0.17	0.11	0.22	0.21	-											
Con	0.15	0.10	0.12	0.17	0.04	-										
Pur	0.13	0.08	0.17	0.21	0.00	0.04	-									
PacR	0.25	0.20	0.21	0.09	0.11	0.09	0.10	-								
Car	0.23	0.10	0.21	0.20	0.06	0.04	0.04	0.08	-							
KitH	0.27	0.21	0.23	0.19	0.19	0.14	0.14	0.10	0.15	-						
BouR	0.26	0.19	0.26	0.21	0.18	0.16	0.14	0.12	0.13	0.08	-					
Ske	0.25	0.20	0.26	0.23	0.19	0.13	0.12	0.11	0.14	0.03	0.05	-				
Omi	0.30	0.23	0.28	0.26	0.22	0.18	0.16	0.13	0.19	0.05	0.07	0.03	-			
NorR	0.32	0.21	0.28	0.23	0.21	0.17	0.16	0.12	0.18	0.03	0.08	0.05	0.00	-		
NorI	0.25	0.18	0.23	0.21	0.17	0.14	0.12	0.11	0.12	0.05	0.02	0.02	0.03	0.04	-	
MckM	0.35	0.26	0.33	0.20	0.20	0.17	0.16	0.10	0.16	0.11	0.06	0.10	0.09	0.08	0.06	-
Ken	0.52	0.52	0.73	0.51	0.43	0.37	0.40	0.40	0.40	0.36	0.29	0.37	0.44	0.45	0.33	0.41

Appendix VI. Pairwise D_S, and F_{ST} for STRUCTURE assigned populations (Figure 4-1c).

D_S

	N111	N112	N113	N12	N13	N211	N212	N213	N214	N221	N222	S11	S12	S21	S22	S23
N111	-															
N112	0.04	-														
N113	0.08	0.06	-													
N12	0.32	0.35	0.45	-												
N13	0.08	0.08	0.14	0.31	-											
N211	0.16	0.16	0.26	0.38	0.10	-										
N212	0.19	0.19	0.28	0.35	0.12	0.09	-									
N213	0.12	0.13	0.22	0.39	0.09	0.08	0.06	-								
N214	0.16	0.18	0.25	0.41	0.10	0.06	0.07	0.05	-							
N221	0.10	0.17	0.20	0.39	0.15	0.15	0.20	0.18	0.15	-						
N222	0.05	0.11	0.17	0.34	0.07	0.07	0.11	0.05	0.06	0.10	-					
S11	0.22	0.21	0.26	0.38	0.16	0.16	0.09	0.14	0.15	0.28	0.18	-				
S12	0.33	0.32	0.37	0.45	0.22	0.26	0.18	0.23	0.22	0.35	0.29	0.08	-			
S21	0.35	0.32	0.44	0.41	0.29	0.28	0.27	0.28	0.29	0.38	0.29	0.23	0.33	-		
S22	0.27	0.25	0.32	0.43	0.19	0.18	0.17	0.18	0.18	0.30	0.20	0.10	0.17	0.10	-	
S23	0.31	0.28	0.34	0.45	0.23	0.18	0.21	0.20	0.19	0.32	0.23	0.14	0.23	0.11	0.04	-
S3	0.25	0.23	0.30	0.41	0.19	0.19	0.18	0.20	0.20	0.29	0.21	0.09	0.19	0.14	0.04	0.06

F_{ST}

	N111	N112	N113	N12	N13	N211	N212	N213	N214	N221	N222	S11	S12	S21	S22	S23
N111	-															
N112	0.04	-														
N113	0.07	0.07	-													
N12	0.32	0.36	0.43	-												
N13	0.07	0.07	0.12	0.31	-											
N211	0.16	0.18	0.25	0.45	0.11	-										
N212	0.16	0.17	0.22	0.37	0.11	0.11	-									
N213	0.11	0.12	0.19	0.38	0.08	0.09	0.06	-								
N214	0.14	0.17	0.21	0.40	0.10	0.07	0.07	0.05	-							
N221	0.10	0.17	0.19	0.42	0.14	0.17	0.18	0.17	0.15	-						
N222	0.04	0.10	0.14	0.32	0.06	0.08	0.09	0.04	0.06	0.09	-					
S11	0.17	0.18	0.21	0.38	0.13	0.17	0.09	0.12	0.14	0.23	0.14	-				
S12	0.30	0.33	0.36	0.59	0.23	0.34	0.22	0.25	0.25	0.38	0.27	0.12	-			
S21	0.29	0.29	0.36	0.47	0.26	0.31	0.26	0.26	0.28	0.35	0.24	0.23	0.39	-		
S22	0.21	0.21	0.25	0.38	0.16	0.18	0.15	0.16	0.16	0.24	0.16	0.09	0.20	0.11	-	
S23	0.25	0.25	0.28	0.42	0.20	0.19	0.19	0.18	0.18	0.28	0.19	0.13	0.25	0.13	0.04	-
S3	0.20	0.20	0.23	0.37	0.16	0.18	0.16	0.17	0.17	0.24	0.16	0.08	0.20	0.14	0.04	0.07

Appendix VII. Immune gene PCR reagent concentrations.

	Class I – *Oram-OLA*	Class II – *Oram-DRA*	Class II – *Oram-DRB*	Class III – *Oram-TNFA*	NRAMP - 2	NRAMP - 3	Colony PCR
Buffer	1X*	1X	1X	1X	1X	1X	0.8X
dNTPs	0.2 mM	0.2 mM	0.2 mM	0.2 mM	0.2 mM	0.2 mM	0.16 µM
$MgCl_2$	-*	1.3 mM	1.6 mM	1.5 mM	1.3 mM	1.2 mM	1.2 mM
Forward Primer	0.4 µM	0.5 µM	0.5 µM	0.5 µM	0.5 µM	0.5 µM	0.16 µM
Reverse Primer	0.4 µM	0.5 µM	0.5 µM	0.5 µM	0.5 µM	0.5 µM	0.16 µM
Water	to 20 µl	to 25 µl	to 25 µl	to 25 µl	to 25 µl	to 25 µl	to 25 µl
Taq	0.1 U	0.5 U	0.5 U	0.5 U	0.5 U	0.5 U	0.25 U
DNA	~25 ng	~80 ng	~40 ng	~40 ng	~40 ng	~40 ng	5 µl

*5X Phusion Taq buffer contains 7.5 mM $MgCl_2$ for a final concentration of 1.5 mM $MgCl_2$.
**The Class I marker was amplified using the Phusion high fidelity taq (New England Biolabs).

Appendix VIII. Immune gene PCR thermocycler parameters for the immuno-related genes analyzed in this study.

	Class I – *Oram-OLA*	Class II – *Oram-DRA*	Class II – *Oram-DRB*	Class III – *Oram-TNFA*	NRAMP - 2	NRAMP - 3	Colony PCR
Pre-Cycle	98°C / 30 s	94°C / 4 min	94°C / 3 min	94°C / 4 min	94°C / 3 min	94°C / 3 min	94°C / 3 min
Denature	98°C / 15 s	94°C / 30 s	94°C / 30 s	94°C / 30 s	94°C / 30 s	94°C / 30 s	94°C / 30 s
Annealing	55.2°C / 30 s	60.5°C / 30 s	61.5°C / 30 s	55.2°C / 30 s	61.3°C / 30 s	57°C/30 s	60°C / 30 s
Extension	72°C / 4 s	72°C / 30 s	72°C / 30 s	72°C / 30 s	72°C / 45 s	72°C /45 s	72°C / 1 min
No. Cycles	30	35	35	30	35	40	25
Post-cycle	72°C / 7 min	72°C / 7 min	72°C / 5 min	72°C / 7 min	72°C / 5 min	72°C / 5 min	72°C / 2 min

Appendix IX. Results of two separate validation methods. Spearman rank correlation coefficients for 5-fold cross validations and r^2 values, slopes and indications for if intercepts were significantly greater than 0 and if X^2 goodness of fit tests were significant for validations conducted using withheld data.

	r_s	r^2	Slope	Intercept	X^2
All individuals					
Year-round	1.00	0.99	1.03	N	N
Summer	1.00	0.99	1.05	N	N
Winter	0.99	0.94	1.12	N	N
Only females					
Year-round	1.00	0.79	0.44	Y	N
Summer	1.00	0.74	0.15	N	N
Winter	0.99	0.90	0.74	N	N
Only males					
Year-round	1	0.91	1.46	N	N
Summer	0.99	0.59	0.34	Y	N
Winter	0.99	0.98	1.40	Y	N
Rut	1.00	0.15	0.27	N	N

Appendix X. Microsatellite markers used in this study on mountain goats from Alaska and diversity statistics. Observed heterozygosity (H_O), expected heterozygosity (H_O), and Wright's inbreeding coefficient (F_{IS}) are reported.

Locus	H_O	H_O	F_{IS}
MAF36	0.46	0.46	0.00
OARHH35	0.60	0.70	0.14
OARJMP29	0.01	0.01	0.00
TGLA122	0.66	0.80	0.17
AR028	0.51	0.53	0.04
RT27	0.51	0.53	0.03
MCM152	0.64	0.60	-0.07
ILSTS058	0.70	0.75	0.07
TGLA10	0.49	0.47	-0.03
OARHH62	0.03	0.03	-0.01
HUJ1177	0.40	0.39	-0.04
RT9	0.68	0.74	0.07
OARCP26	0.24	0.26	0.05
MAF64	0.56	0.64	0.13
Huj616	0.81	0.79	-0.02
BM1818	0.56	0.56	-0.01
BM6444	0.49	0.50	0.01
McM64	0.41	0.44	0.07
UWCA4	0.59	0.63	0.05
BMC5221	0.73	0.77	0.04
BMS1341	0.48	0.52	0.07
BL1080	0.44	0.46	0.04

Appendix XI. Results from 10 Resource Selection Function-based landscape resistance models using circuit theory on Alaskan mountain goats. When sex is not denoted the model is based on all of the individuals. Simple Mantel *r* and exact *p* values >0.01 are provided. The Y intercept and slope are calculated from multiple regressions. Results are based on 10,000 permutations.

Models	Mantel *r*	*p* value	Intercept	Slope
RSF-based landscape resistance				
QG ~ Year-round ♀ (Circuit theory)	-0.19	<0.01	1.02E-01	-1.94E-02
QG ~ Year-round ♂ (Circuit theory)	-0.19	<0.01	9.99E-02	-2.44E-02
QG ~ Rut ♂ (Circuit theory)	-0.19	<0.01	1.11E-01	-2.08E-02
QG ~ Year-round (Circuit theory)	-0.19	<0.01	9.76E-02	-2.12E-02
QG ~ Summer (Circuit theory)	-0.23	<0.01	1.24E-01	-2.71E-02
QG ~ Summer ♀ (Circuit theory)	-0.27	<0.01	5.57E-02	-1.23E-02
QG ~ Summer ♂ (Circuit theory)	-0.21	<0.01	1.23E-01	-2.40E-02
QG ~ Winter (Circuit theory)	-0.19	<0.01	1.03E-01	-1.63E-02
QG ~ Winter ♀ (Circuit theory)	-0.21	<0.01	1.59E-01	-1.59E-02
QG ~ Winter ♂ (Circuit theory)	-0.19	<0.01	1.01E-01	-1.67E-02

Appendix XII. Additional information on dispersal simulations.

There was no relationship between q and H_O (mean Pearson coefficient 0.0005±0.0007), which can be visualized in Appendix XII. In the simulated populations we had only one individual naturally cross-assign. This suggests false cross-assignments are rare (<0.01%). This individual did not have a significantly lower H_O than residents (W = 32.5, p =0.18) and was removed from the reported analyses where we randomly assigned individuals as cross-assigned. When we only examined individuals with a q > 0.80, we were left with 143 simulated individuals. Based on Shafer *et al*. (2011) in which 7% of individuals with q > 0.80 appeared to have dispersed, we randomly selected 10 of the 143 individuals as cross-assigned. There was not relationship between H_O and cross-assignment which is reported in the chapter.

Appendix XIII. Simulated relationship between q (assignment to a subpopulation) and heterozygosity (h).