### Mountain Goat Genetic Diversity and Population Connectivity in Washington and Southern British Columbia

By

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Accepted in Partial Completion of the Requirements for the Degree Master of Science

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Leslie C. Parks

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# Mountain Goat Genetic Diversity and Population Connectivity in Washington and Southern British Columbia

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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#### ABSTRACT

Anthropogenic alterations to natural landscapes and the associated habitat fragmentation, habitat degradation, and climatic shifts threaten biodiversity from the local to the global scale. These perturbations disrupt historical patterns of gene flow causing reduced population connectivity, loss of genetic diversity, and increased risk of extinction. A landscape that is permeable to animal movement counteracts local population fluctuations, increases genetic diversity, increases adaptive potential, and provides corridors for range shifts in response to climate change. Maintaining population connectivity is critical for the conservation of small populations isolated by fragmented landscapes. This strategy requires an accurate understanding of the landscape's effect on gene flow and the processes, such as isolation by barrier (IBB), isolation by distance (IBD), or isolation by resistance (IBR), that are driving genetic isolation.

Although the mountain goat (*Oreamnos americanus*) is not globally threatened, mountain goats in Washington have declined by more than 50% since the 1950s. While past unsustainable harvest is likely the cause of this decline, many populations have not recovered, and former historical habitat remains unoccupied despite nearly 20 years of drastically reduced hunting pressure. Mountain goats in Washington exhibit lower genetic diversity than populations from the core of the species' range, raising the possibility that genetic factors are limiting population recovery. Previous research revealed that transportation corridors impede mountain goat gene flow in Washington. In this study, I sought to understand the relationship between the mountain goat population in Washington

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and the much larger and more genetically diverse mountain goat populations in southern British Columbia. Anthropogenic activities in the Fraser lowlands and Okanagan Valley in British Columbia potentially diminish or sever historical linkages between the Washington population and the much larger populations in the Coast Range, Selkirk Mountains, and Purcell Mountains in British Columbia. To this end, I collected 261 genetic samples from scat, tissue, bone, and hair to generate indices of genetic diversity and an accurate model of population connectivity.

In Chapter 1, I used methods based on both discrete and clinal population models to present alternative representations of genetic diversity. Discrete models identified four subpopulations separated by transportation corridors, urbanized areas, and agriculture. Genetic diversity was higher in British Columbia than Washington, illustrating the importance of maintaining gene flow from British Columbia into Washington. Clinal models of population structure found several regions of lower and higher diversity within the subpopulations identified by discrete models, refuting the assumption of IBB and panmixia within subpopulations. In Chapter 2, I examined the relative influence of IBB, IBD, and IBR on genetic isolation. I developed multiple hypotheses of IBR by systematically varying model parameters for four landscape features: distance to escape terrain, roads, landcover type, and elevation. I employed a causal modeling framework to create a multivariate model based on landscape features that met strict criteria for inclusion. This allows for a nonlinear relationship between landscape features and gene flow, accounts for interactions between variables, and minimizes the risk of spurious correlations. The optimized IBR model that I developed was highly correlated with genetic structure and better supported than the

V

alternative models of genetic isolation, IBB and IBD. The best supported model of IBR indicated that urban landcover, agricultural landcover, and freeways present high resistance to mountain goat gene flow, while low elevation valleys resist gene flow to a lesser degree. I used this model of IBR to model gene flow across the study area and identify locations where population connectivity is compromised.

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#### PREFACE

The mountain goat (Oreamnos americanus) is listed as a species of "least concern" (IUCN) 2012) for global extinction because of the species' wide distribution, large population, regulated harvest, and minimal habitat loss and fragmentation. This global security is largely due to the inaccessible nature of the remote alpine environment mountain goats are specialized to inhabit (Festa-Bianchet 2008). Despite the stable global population trend, mountain goats are sensitive to overharvest (Hamel et al. 2006, Rice and Gay 2010) and modern land use changes do threaten some local populations. In particular, the Washington mountain goat population may be at risk due to historical overharvest coupled with anthropogenic development in Washington (Shirk 2009, Shirk et al. 2010). Population connectivity is a key factor affecting a population's viability and resilience when faced with habitat fragmentation, habitat degradation, and climate change. Mountain goats are capable of dispersing long distances, including movements through unsuitable habitat, suggesting that mountain goat conservation should take population connectivity into account (Festa-Bianchet and Côté 2008). Wildlife populations are not defined by political boundaries, and research that transcends regional and national borders sets the framework for conservation of landscape and population connectivity rather than conservation of populations by jurisdiction.

The development of groups that bridge political and agency/organizational boundaries, such as the Washington Wildlife Habitat Working Group (WHCWG), highlights the growing emphasis on research with broader implications for the conservation of ecosystems and biodiversity. Mountain goats were selected as a focal species by the WHCWG as a species with habitat needs representative of wildlife in the subalpine forests and alpine vegetation classes (WHCWG 2010). Modeling of landscape connectivity by the WHCWG for mountain goats was based on an empirical study by Shirk et al. (2010) within the Cascade Range of Washington. Analysis by the WHCWG includes substantial portions of surrounding states and the province of British Columbia. While the WHCWG analysis results in a more holistic model of mountain goat population connectivity, no data outside of Washington were available to explicitly test the predictions of Shirk's model in the extended area.

Though mountain goats are not at risk of global extinction, the loss of this species from the montane ecosystem of Washington would have local consequences. Mountain goats play a role in shaping alpine plant communities (Weisberg and Bugmann 2003) and serve as prey for large predators within the state including mountain lions, grizzly bears, grey wolves, wolverines, and golden eagles (Festa-Bianchet and Côté 2008). The mountain goat also has great cultural significance to Native American Tribes in Washington and is an icon of the rugged Cascade high country for outdoor and wildlife enthusiasts. In addition to the local ecological value of mountain goats, research by Hampe and Petit (2005) found that populations residing at the low-latitude margins of species' distribution ranges, such as the mountain goat population in Washington, may be disproportionately important for the longterm conservation of a species' genetic diversity, phylogenetic history, and evolutionary potential.

With this research I bridge the gap between Shirk's analysis of the mountain goat population in Washington and the landscape connectivity model created by the WHCWG by

collecting genetic samples from southern British Columbia. The expanded genetic sampling will provide the data necessary to identify a model of landscape resistance that accounts for population connectivity beyond the international border between Washington and Canada. In Chapter 1, I evaluated genetic structure based on discrete populations in order to identify potential barriers to gene flow. I also generated population-based and cline-based indices of genetic diversity, providing alternative approaches to quantify genetic diversity. In Chapter 2, I examined the effects of different landscape features on population connectivity relative to alternative mechanisms of genetic isolation. I then modeled gene flow across the study area based on the most supported model of genetic isolation. Collectively, this thesis offers a perspective of mountain goat genetic diversity and population connectivity that is based on empirical data and can be applied by wildlife managers to improve population viability.

#### **CHAPTER 1**

# Mountain Goat Genetic Structure and Molecular Diversity in Washington and Southern British Columbia

#### INTRODUCTION

Anthropogenic activities and the associated loss and fragmentation of natural habitat are key factors driving the range reduction and population extinction observed in many mammalian species worldwide (Lande 1998a, Ceballos and Ehrlich 2002). Small populations isolated by a fragmented landscape are more prone to extinction from stochastic environmental events, Allee effects (Lande 1998a), inbreeding depression (Crnokrak and Roff 1999, Keller and Waller 2002, Mainguy et al. 2009, Dunn et al. 2011), and genetic drift, reducing population viability (Keyghobadi 2007). Inbreeding depression and genetic drift contribute to the loss of allelic diversity, reduce evolutionary potential (Lande 1995, Willi et al. 2006), and increase the risk of random fixation of deleterious alleles (Lynch et al. 1995, Lande 1998b). Reduced fitness resulting from these genetic processes may cause further population decline, increasing vulnerability to stochastic events and the loss of genetic diversity, feeding into a positive feedback loop coined the extinction vortex (Gilpin and Soulé 1986, Fagan and Holmes 2006, Blomqvist et al. 2010, Palomares et al. 2012). Thus, the implications of habitat fragmentation documented by Ceballos and Ehrlich (2002) and Keyghobadi (2007) may serve as a harbinger of species extinction. Conversely, a landscape that supports the movement of organisms among populations moderates local population fluctuations by

facilitating re-occupancy of vacant habitat following local extinctions of small populations caused by stochastic factors and by allowing for gene flow among populations, counteracting the negative effects inbreeding and genetic drift (Crooks and Sanjayan 2006).

An understanding of genetic structure and genetic diversity is necessary to evaluate the extent of population isolation and the subsequent loss of genetic diversity. This analysis requires that the researcher is able to define boundaries around discrete subpopulations, either on the basis of obvious geographic features or by using molecular methods to assign individuals to subpopulations. Indices of genetic diversity can then be calculated for each subpopulation. This approach assumes isolation by barrier (IBB) between subpopulation and panmixia within subpopulations. This scenario fails to account for alternative mechanisms of isolation, such as isolation by distance (IBD) and isolation by resistance (IBR), that are more appropriate for continuously or patchily distributed species that display clinal genetic structure (Cushman et al. 2006). IBD predicts that genetic distance will increase with geographic distance (Wright 1943) and IBR predicts that genetic distance increases at a rate governed by geographic distance and resistance to gene flow from a heterogeneous landscape (Cushman et al. 2006, McRae 2006). Identifying the correct mechanism of genetic isolation is imperative to determine what features in the landscape may be driving genetic isolation. This information can inform decisions made by wildlife managers when assessing the role of genetic factors in population viability.

In this study, I examined the genetic structure and diversity of mountain goat populations from the southern periphery of the species' range in Washington to the southern core of the species' range in British Columbia (Figure 1). In 1961, the Washington

Department of Fish and Wildlife (WDFW) estimated the mountain goat population in the Washington Cascades to be 8,500, excluding Mount Rainier National Park and Yakama Indian Nation lands. More recent surveys by WDFW estimate the population to be around 3,700, including Mount Rainier National Park and Yakama Indian Nation lands (Rice and Gay 2010). Mountain goat populations can be sensitive to overharvest, and this decline is likely the result of overhunting from the 1950s through the 1980s (Hamel et al. 2006, Rice and Gay 2010). Despite drastically reduced hunting pressure since the 1990s, many subpopulations in the Washington Cascades have not recovered and some historical habitat remains unoccupied (C. Rice, WDFW, unpublished).

The lack of recovery for many of these subpopulations may result from the way that the mountain goat population in Washington is structured. Mountain goats occupy the alpine and sub-alpine habitat that is distributed in discrete patches of variable size across the state. This patchy network of suitable habitat may separate the population into subpopulations that comprise a metapopulation, a set of subpopulations linked by migration (Hanksi and Gilpin 1997). Although alpine habitat throughout most of the Washington Cascades is largely intact (approximately 42% of the state is under federal or state ownership), the intervening low elevation habitat has undergone varying degrees of anthropogenic alterations. The development of transportation corridors and the associated recreational areas, ski resorts, agriculture, and residential areas, coupled with timber harvest and the development of forest service roads, all potentially contribute to the isolation of mountain goat subpopulations within Washington and British Columbia.

Indeed, previous work by Shirk (2009) found that migration across Interstate 90 (I-90) is quite limited. Mountain goat populations at the periphery of the species' range exhibit lower genetic diversity than populations from the core of the species' range (Shafer 2011) and Mainguy et al. (2009) have recently demonstrated that reduced genetic diversity is correlated with lower juvenile survivorship in the Caw Ridge, Alberta mountain goat population. Lower indices of genetic diversity were observed in the two Washington subpopulations (Shirk 2009), and while this may be attributed to the location of these subpopulations at the southern periphery or the species' range, any landscape factors that additionally isolate these subpopulations from core populations in British Columbia may further erode genetic diversity and limit the ability of the Washington population to recover from historical overharvest.

I evaluated the genetic structure of mountain goat populations in Washington and southern British Columbia using both discrete and clinal methods. This allowed me to consider genetic diversity in terms of discrete subpopulations where IBB is the mechanism of isolation and to consider genetic diversity as a gradient dictated by IBD or IBR. These alternative perspectives may provide a better understanding of the relationship between mountain goat populations in Washington and southern British Columbia and may also shed light on the underlying mechanisms of population isolation. This analysis may help identify existing and historical linkages, information that could guide efforts to preserve and improve existing linkages, restore historical linkages, and translocate individuals to artificially facilitate historical gene flow.

#### **Historical translocations**

Mountain goat translocations have been utilized in both the United States and British Columbia to establish non-native populations and augment native populations. In the 1920s, 4 individuals from the Selkirk Range in British Columbia (Hatter and Blower 1996) and 8 individuals from the Chugach Range in Alaska were translocated to the Olympic peninsula prior to the establishment of Olympic National Park (ONP). By the 1980s, the Olympic population had increased to 1,200 individuals and the National Park Service (NPS) launched a program to reduce the size of this non-native mountain goat population in the park. As part of this effort, 130 animals were translocated from ONP to 15 sites within the Cascade Range in an effort to augment declining native populations (Houston et al. 1991). Near some of these release sites in the southern Cascades, Shirk (2009) found genetic evidence that at least some of these translocated animals survived and interbred with native Cascade animals.

Translocations within British Columbia were used to re-establish locally extirpated populations or augment existing populations. Four translocations took place within the study area in British Columbia (Hatter and Blower 1996, Mountain Goat Management Team 2010). Three translocations took place within the Okanagan region: 5 individuals were moved from Penticton Creek to Shorts Creek (~70 km), 3 individuals were moved from Penticton Creek to Tulameen Mountain (~110 km), and 8 individuals were moved from Penticton Creek to Snass Mountain (~100 km). The translocation to Shorts Creek was unsuccessful, low numbers are reported at Tulameen Mountain, and the status of the Snass Mountain translocation is unknown (Mountain Goat Management Team 2010). The fourth translocation was within the Kootenay region where 20 animals were moved from Toby Creek to the

Slocan Valley (~120 km). This translocation was successful (Mountain Goat Management Team 2010). Since source populations were geographically close to the translocation location, the ability to identify descendents of translocated animals in British Columbia is limited. Although these short distance translocations may confound genetic analysis at the local scale, patterns across the entire study area should not be affected.

#### **METHODS**

#### Study area

The study area encompasses 151, 760 km<sup>2</sup> and extends 930 km east to west across southern British Columbia and 760 km north to south from Mount Meager in the Coast Range to Mount Adams in the Cascade Range (Figure 1). The landscape includes four mountain ranges dominated by jagged peaks, serrated ridges, and glaciers that give way to subalpine meadows, montane forests, and glacial valleys. Elevation ranges from sea level to nearly 4,400 m with deep, heavily forested valleys dissecting rugged alpine terrain (Scurlock 2011). The Fraser Valley forms the boundary between the Coast Range to the northwest and the Cascade Range to the south. The expansive Okanagan Valley separates the Selkirk Mountains and Purcell Mountains of southeastern British Columbia from the Coast Range to the west and the Cascade Range to the south. Although much of the study area is remote and inaccessible, the region is not immune from anthropogenic influence. I-90 cuts across the Washington Cascades east -west and the Coquihalla Highway cuts across British Columbia north- south. Several secondary highways and numerous other roads also transect the study area. Developed areas and agricultural lands are present, particularly at lower elevations and along transportation corridors. At higher elevations, ski resorts and residential areas have developed near major passes. Finally, the montane forests surrounding the alpine have been subject to timber harvest.

#### Sample collection

Mountain goats are sensitive to capture and exhibit higher rates of kid abandonment and delayed primiparity with handling (Festa-Bianchet and Côté 2008). In addition, acquiring tissue samples from an alpine specialist requires considerable manpower, supplies, and support. The development of noninvasive genetic sampling (NGS) has enabled the collection of critical genetic data while avoiding the negative effects of capture stress (Waits and Paetkau 2005). Hair is not a reliable source of mountain goat DNA because shed hair produces low genotyping success rates (Gagneux et al. 1997, Poole and Reynolds 2009).

For these reasons, I used a protocol based on the methods developed by Rutledge et al. (2009) to obtain DNA from scat. Fecal samples are prone to genotyping errors because of the low quantity and quality of target DNA obtained (Taberlet et al. 1996). Rutledge et al. (2009) were able to improve amplification success by increasing the amount of high quality target DNA extracted from the sample through swabbing the exterior of fresh scat samples in the field. Fresh pellets on a dry surface were selectively sampled because DNA does degrade quickly when exposed to the environment. Brinkman et al. (2009) found that exposure of deer scat samples to rainfall significantly increases DNA degradation and Poole and Reynolds (2009) found that mountain goat pellets collected on melting snowfields in summer

had lower amplification success, likely due to moisture exposure. In addition, UV exposure degrades DNA (Friedberg 2003).

Pellets were collected in 2010 and 2011 during the months of July, August, and September when mountain goat habitat is most accessible by foot, rainfall events are less frequent, and snow presence is at a minimum. Pellets were swabbed with a cotton-tipped applicator moistened with DET salt solution (20% dimethyl sulfoxide, 0.25 M sodiumethylenediaminetetraacetic acid [EDTA], 100 mM TRIS [tris (hydroxymethyl) aminomethane], pH 7.5, and saturated NaCl; Seutin et al. [1991]). Pellets, or areas of a pellet, that appear to have mucous matter present were assumed to have more contact with the digestive tract and were selectively swabbed. Care was taken to avoid sampling inside the pellet because samples taken from the interior of scat have been found to have lower success rates in other species (Stenglein et al. 2010). The applicator tip was then broken off into a 2 ml vial containing 99% alcohol to preserve the sample.

#### Genotyping

Genotyping was performed by the Washington Department of Fish and Wildlife (WDFW) molecular genetics lab in Olympia, Washington from November 2011 to January 2012. Genomic DNA was extracted from scat material collected on cotton swabs stored in ethanol. Prior to extraction, ethanol was evaporated from the vial and initial extraction steps were conducted in the collection vial to maximize DNA collection. Extraction protocol followed the standard recommendations for the commercial single tube silica-membrane kits DNeasy® blood and tissue DNA isolation kit (Qiagen), except that lysis buffer volumes for scat samples were doubled to cover the entire swab in liquid. DNA was eluted in 180µl and then concentrated to a final volume of 90µl. Polymerase chain reaction (PCR) was used to amplify 19 previously characterized polymorphic microsatellite markers (Mainguy et al. 2005) using six uni- or multiplex reactions (multiplex Oam-A [BM121, BM4107, BM6444, TGLA122], Oam-B [OarCP26, OarHH35, RT27], Oam-C [BM1818, BM4630, RT9, URB038], Oam-D [BM203, BMC1009, HUJ616, McM527], Oam-E [BM1255, BM4513, HEL10] and TGLA10. PCR products were visualized using an ABI 3730 capillary sequencer (Applied Biosystems) and sized using the GeneScan 500-Liz size standard (Applied Biosystems) and GeneMapper 3.7 (Applied Biosystems).

I used GENEPOP 4.1.3 to detect deviations from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium (LE) (Raymond and Rousset 1995). The complete enumeration method was used for loci with fewer than four alleles (Louis and Dempster 1987) and the Markov chain method was used for loci with more than four alleles (Guo and Thompson 1992). Significance values were adjusted with the Bonferroni correction for multiple comparisons (Rice 1989). MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) was used to screen for genotyping errors such as allelic dropout, null alleles, and stuttering that might obscure the presence of heterozygotes. I used GENALEX 6.4 to identify matches and remove samples that were potentially from the same individual.

#### **Population Structure**

Three different methods were used to evaluate multiple mechanisms of genetic isolation. The first two methods evaluated discrete population structure and the third method evaluated

clinal population structure. First, I used STRUCTURE 2.3.3 (Pritchard et al. 2000) without location information to identify ONP and ONP-Cascade admixed individuals without the influence of the spatial location of the sample. Then, I used GENELAND 4.0.2 (Guillot et al. 2005), a program that uses sample location information to inform the population model. Finally, a third method was used in case IBB was not the driving force of genetic isolation. In this case, I assessed clinal population structure with spatial principle component analysis (sPCA) using a method developed by Jombart (2008) that takes genetic variance between samples and spatial autocorrelation into account, allowing for the influence of IBD and IBR.

I used STRUCTURE 2.3.3 (Pritchard et al. 2000) to determine the most likely number of populations present, assign individuals to populations, and identify descendents from ONP mountain goats that were successfully translocated into the Cascades during the 1980s. STRUCTURE uses Bayesian inference to determine the most likely number of populations sampled and assigns individuals to populations by minimizing Hardy-Weinberg disequilibrium and linkage disequilibrium within populations. Markov chain Monte Carlo (MCMC) simulation is then used to estimate the posterior probability that the data fit the hypothesis of K populations P(X/K). I used the admixture model with correlated allele frequencies (100,000 step burn-in followed by 10<sup>6</sup> steps of data collection) to evaluate values of K ranging from 1 to 10. Five independent runs were performed for each value of K. I used the second order rate of change ( $\Delta K$ ) in probability as described by Evanno et al. (2005) to determine the best supported value of K.

The admixture model allows individuals to have mixed ancestry and calculates the fraction of an individual's genome derived from each subpopulation (Q) (Falush et al. 2003).

This enabled me to evaluate an individual's probability of ONP ancestry. While many individuals in the Cascades have some probability of ONP ancestry, samples with a low probability of ONP ancestry are not likely to bias results. In order to retain sufficient samples for analysis, I only classified individuals with *Q* greater than 0.25 for the ONP population to be "ONP-Cascade admixed" individuals. I removed all ONP and ONP-Cascade admixed individuals from the analysis and ran STRUCTURE again using the same parameters listed previously. This reduced the effects of historical translocations on modeled population structure in the study area. I also reran STRUCTURE on subpopulations that I suspected had substructure based on deviation from HWE and LE, the presence of null alleles, and the presence of stuttering within a subpopulation.

I used GENELAND 4.0.2 (Guillot et al. 2005) to introduce location data into the analysis. GENELAND also uses Bayesian inference with MCMC simulation to estimate the number of panmictic groups. GENELAND makes the assumption that some spatial dependence is present among samples and allows spatial coordinates to inform prior distribution. GENELAND identifies genetic discontinuities by minimizing Hardy-Weinberg disequilibrium and linkage disequilibrium. All ONP and ONP-Cascade admixed samples were excluded from this analysis. I used the uncorrelated allele frequency model because it was found to outperform the correlated allele frequency model when estimating the number of populations (K) (Guillot et al. 2005). I evaluated the support for 1 to 10 populations with 10<sup>6</sup> iterations and a burn-in of 1,000. Every 100<sup>th</sup> observation was sampled to reduce sample autocorrelation. After estimating the value of K, I simulated fixed K using the above parameters to determine population membership and generate posterior probability maps.

Finally, I used sPCA to assess clinal population structure and avoid assigning individuals to discrete subpopulations. This method makes no assumption of HWE or LE (Jombart et al. 2008) and ordinates genotypes by summarizing multivariate genetic data into a few uncorrelated components that maximize variance among genotypes, accounting for both variance between individuals and spatial autocorrelation. This reveals two types of patterns defined as global structure, represented by positive autocorrelation, and local structure, represented by negative autocorrelation. Global structure occurs when individuals are more genetically similar to immediate neighbors than expected in a random spatial distribution, as would be expected in the presence of a genetic cline, and local structure occurs when individuals are more genetically dissimilar to immediate neighbors than expected in random spatial distribution, as would be expected when individuals from the same genetic pool are selected to avoid each other (Jombart et al. 2008). The R package Adegenet was used to implement sPCA (Jombart 2008). Given that few long-distance dispersal events exceed 60 km (Festa-Bianchet and Côté 2008) and to keep methods comparable to previous analysis (Shirk 2009), samples were connected if they were between 0 and 60 km using the neighborhood by distance connection network. Significance of the global and local scores was tested with Monte Carlo tests executed in Adegenet.

#### Genetic diversity and F-statistics

I used GENALEX 6.4 (Peakall and Smouse 2006) to estimate observed heterozygosity  $(H_o)$ , expected heterozygosity  $(H_e)$ , the fixation index  $(F_{IS})$ , and genetic differentiation  $(F_{ST})$  for the discrete subpopulations identified by STRUCTURE and GENELAND. I tested the significance of  $F_{ST}$  values with 9,999 random permutations of the dataset. I used FSTAT 2.9.3.2 (Goudet et al. 2002) to estimate allelic richness ( $A_r$ ), which is adjusted for sample size.

I used the software package sGD (Shirk and Cushman 2011) to detect fine-scale spatial heterogeneity in genetic diversity across the study area. This tool uses microsatellite genotypes and a cost-distance matrix to group individuals into genetic neighborhoods inferred from a correlogram depicting the autocorrelation of genotypes across a range of distance classes. I used a cost-distance matrix based on Euclidean distance between all individuals and a genetic neighborhood diameter defined by the largest cost-weighted distance class that is positively correlated with genetic distance and significant (alpha=0.05). I set the minimum population size to 10 individuals to minimize sampling error. This approach does not assume discretely bound, pannictic subpopulations and is more appropriate for species that form clinal populations (Chambers 1995, Shirk and Cushman 2011).

#### Migration

I examined migration rates between populations with BAYESASS, a software package that employs a Bayesian approach and MCMC techniques to indirectly estimate contemporary migration rates with multilocus genotypes and population genetic models (Wilson and Rannala 2003). This model assumes LE but allows deviations from HWE (Faubet et al. 2007). I used a burn-in period of  $10^6$  iterations followed by  $2x10^6$  iterations and a sample frequency of 1 in 2,000. I set the delta value for allele frequency, migration rate, and inbreeding to 0.22, 0.07, and 0.25 respectively, such that the accepted number of proposed changes was 20-40% of the total number of iterations, the proposed range to optimize mixing of the chain (Wilson and Rannala 2003).

#### RESULTS

#### Sample statistics

In total 250 scat samples were collected for this analysis: 173 scat samples from 2010, 72 scat samples from 2011, 1 scat sample from preliminary work conducted in 2007, and 4 scat samples from field work conducted in 2008. In addition, 2 hair samples with follicles attached, 1 tissue sample, and 1 bone sample were opportunistically collected. I obtained DNA from 24 genetic samples from the Selkirk and Purcell mountains that were acquired by Shafer et al. (2011) as tissue samples from legally permitted hunters from 2005 to 2007. I obtained DNA from 16 genetic samples from Mount Meager in the Coast Rang of British Columbia that were acquired by Poole and Reynolds (2009) in 2009 from scat and hair (Table 1). I used 159 genotypes from genetic samples (100 tissue samples, 58 blood samples and 1 bone sample collected from 2003 to 2008 in collaboration with the NPS and WDFW) obtained by Shirk (2009) from the Cascade Range and ONP in Washington.

Of the 250 scat samples, I removed 101 scat samples collected in 2010 and 26 scat samples collected in 2011 from the analysis because these genotypes were less than 63% complete (amplified at less than 12 of 19 loci). I retained 42% of the scat samples collected in 2010 and 64% of the scat samples collected in 2011. I removed 40 additional samples from the analysis that were potentially from the same individual. In addition to the samples provided by (Shirk 2009), Poole and Reynolds (2009), and Shafer et al. (2011), I retained 83

scat samples, 2 hair samples, 1 tissue sample, and 1 bone sample from the preliminary field work in 2007 and 2008 and the 2010 and 2011 field seasons (Table 1). On average, the 87 samples collected for this study were 92% complete, the 24 genetic samples collected by Shafer et al. (2011) from the Selkirk and Purcell mountains were 92% complete, the 16 genetic samples collected by Poole and Reynolds (2009) from Mount Meager were 98% complete, and the 159 genotypes collected by Shirk (2009) from the Cascades and ONP were 98% complete. I excluded URB038 from the analysis because it was monomorphic, and I excluded McM527 from the analysis because all the samples from the Selkirk and Purcell mountains failed to amplify at this locus. I retained the remaining 17 polymorphic loci for the analysis.

Significant departure from LE was detected in 76 of 136 pairwise comparisons of loci and significant departure from HWE was observed in 16 of 17 loci when all samples were considered a single population. The Wahlund effect (Wahlund 1928) predicts that populations will deviate from HWE and LE when the population is structured. Once I divided the data to the highest level of substructure detected by STRUCTURE (Appendix 1 and 2), I found no significant departure from LE or HWE after Bonferroni correction for multiple comparisons. I did not find any evidence of allelic dropout or stuttering with MICRO-CHECKER, but nine loci (BM203, BM1225, BM1818, BM4107, BM4513, BMC1009, HEL10, OarCP26, and RT9) did show significant homozygote excess, suggesting that null alleles may be present at these loci. Each of these loci only showed significant homozygote excess in one or two of the nine subpopulations detected by STRUCTURE. Since the presence of null alleles was not systematic, I retained all nine of these loci in the analysis.

#### **Population genetic structure**

When considering all samples in the STRUCTURE assignment test, the  $\Delta K$  value peaks at K=6 (Figure 2a). The CR, SC, and ONP all cluster distinctly, with evidence of ONP admixture in the SC (Figure 2c). The SM and PM cluster together with six samples from the northeast region of the OK (Figure 2c). Two clusters occur within the OK and NC region, but there is no clear distinction between the two regions. When the ONP and ONP-Cascade admixed samples are removed from the analysis, the highest peak of support is observed at K=4(Figure 2a) represented by the CR, SM/PM, OK/NC (the latter now only showing evidence for one subpopulation), and the SC. There is evidence for the presence of admixed individuals in the CR, OK/NC, and SC (Figure 3d) but little evidence of admixture in the SM/PM. Two individuals in the OK/NC were identified as SM/PM admixed individuals (Figure 2d), but both were determined to be ONP-Cascade admixed because they were approximately 50 km from ONP translocation sites (the Selkirk Mountains were one of the source populations for the original translocations to ONP) and a considerable distance from the SM/PM (350 km). I removed these two samples from further analysis. I looked for the presence of substructure by evaluating each subpopulation in STRUCTURE and I found evidence of substructure in both the SM/PM and OK/NC subpopulations (Appendix 1).

The GENELAND analysis also supports the presence of four subpopulations when the ONP and ONP-Cascade individuals are removed from analysis because K=4 is the most frequent value along the simulation chain (Figure 2b). GENELAND also detects genetic discontinuities, as suggested by the zones of probability of membership for each subpopulation, that agree with the STRUCTURE subpopulation assignments when K=4 (Figure

4). The sharpest decline in probability of membership occurs between the OK/NC and SC. The delineation between the OK/NC and SM/PM is also fairly abrupt, but the change in probability of membership is more gradual between the CR and OK/NC (Figure 4).

I detected significant global structure across the study area with sPCA analysis (P<0.0001) indicating positive spatial autocorrelation of genotypes. I did not find significant local population structure (P=0.18) indicating negative autocorrelation of genotypes was not present. For visualization purposes, I displayed the first three global axes that contributed significantly to genetic structure (Figure 5a). This revealed a genetic cline from the OK/NC to the SC (represented by the first global score), differentiation of the SM/PM from the three other subpopulations (represented by the second global score), and a genetic cline from the CR to the OK/NC (represented by the third global score) (Figure 5b).

#### **Genetic diversity**

Genetic diversity varied across the discrete populations identified by STRUCTURE and GENELAND (Table 2). The CR exhibited high genetic diversity with the highest  $A_r$  (4.22) and  $H_o$  (0.53) values and the lowest  $F_{IS}$  value (0.07). The SM/PM and OK/NC subpopulations exhibited moderate genetic diversity with high  $A_r$  (3.61 and 3.41) and  $H_o$  (0.38 and 0.38) values. Both of these subpopulations had surprisingly high  $F_{IS}$  values (0.24 and 0.16). The SC and ONP subpopulations exhibited low genetic diversity with the lowest  $A_r$  (2.82 and 2.48) and  $H_o$  (0.35 and 0.36) values and moderate  $F_{IS}$  values (0.10 and 0.11).

The sGD analysis of genetic diversity revealed fine-scale spatial heterogeneity in genetic diversity across the study area. The genetic neighborhood diameter where cost-

weighted distance was no longer positively correlated with genetic distance was 165 km. The CR exhibited high genetic diversity across the region (Figures 6 and 9) and generally low  $F_{IS}$ , with areas of slightly higher  $F_{IS}$  along the southern extent of the region (Figure 8). The SM/PM exhibited moderately high  $A_r$  across the region (Figure 9), but  $H_o$  was variable with moderately high values in the SM and average values in the PM (Figure 6).  $F_{IS}$  was also variable for the SM/PM region with average values in the SM and moderately high values in the PM (Figure 8). The OK region exhibited average to low  $A_r$  (Figure 9), uniformly low  $H_o$ (Figure 6), and uniformly high  $F_{IS}$  (Figure 8). The NC exhibited moderately high  $A_r$  in the center of the region, average  $A_r$  towards the southern extent of the region, and low  $A_r$  around the Mount Baker area (Figure 9).  $H_o$  in the NC was average, except for the Mount Baker area that had moderately low  $H_o$  (Figure 6).  $F_{IS}$  was variable across the NC with lower values in the central and south extent of the study area, trending towards average values to the northeast and low values towards the OK (Figure 8). The SC exhibited increasingly low  $A_r$ towards the southern extent of the region (Figure 9), uniformly low  $H_o$  (Figure 6), and variable  $F_{IS}$ , with average levels in the northern part of the region and lower values to the south (Figure 8).

#### Population differentiation and migration

I found evidence of substantial structure across the study area (Table 3). As expected, the  $F_{ST}$  values between the introduced ONP population and all other subpopulations were the highest calculated. The SM/PM subpopulation had high  $F_{ST}$  values with all other subpopulations, including the adjacent OK/NC subpopulation. The OK/NC had similar  $F_{ST}$  values between

the adjacent CR and SC subpopulations (0.104 and 0.105) and was most differentiated (excluding the ONP population) from the SM/PM (0.194). The CR subpopulation had the lowest  $F_{ST}$  value between the adjacent OK/NC (0.104), followed by the SM/PM and SC subpopulations (0.184 and 0.23). All  $F_{ST}$  calculations were significant (alpha=0.05).

Migration rates between the four native subpopulations were as expected given STRUCTURE results and  $F_{ST}$  calculations (Table 4). Migration into the CR from the SM/PM and SC subpopulations was negligible (<0.01) and was 0.03 from the OK/NC subpopulation. All migration into the SM/PM from all three other subpopulations was limited, around 0.01. Migration into the OK/NC was negligible from the CR and SM/PM (<0.01) and was 0.02 from the SC. Migration into the OK/NC from the CR is likely lower than migration in the opposite direction due to the higher sample density in the OK/NC, i.e. the fraction of migrants from the CR in the OK/NC is low due to the large difference in sample size. Migration into the SC from all three other subpopulations was negligible (<0.01).

#### DISCUSSION

#### Discrete vs. clinal models of population structure

The STRUCTURE and GENELAND analysis based on discrete subpopulations and the sPCA analysis based on a clinal population offered complementary representations of population structure across the study area. The STRUCTURE assignment tests identified individuals in the CR, OK/NC, and SC with high probability of subpopulation membership from other subpopulations, suggesting the presence of a genetic gradient rather than discrete, pannictic subpopulations bound by impermeable barriers (Figure 3). The presence of a genetic gradient

is also supported by the degree of admixture within these three subpopulations detected in the STRUCTURE Q-plot (Figure 2d). The NC subpopulation had the most evidence of a genetic gradient due to the presence numerous individuals with high probability of membership from the SC and the CR. I also detected substructure within the NC when this subpopulation was evaluated separately (Appendix 1), additional evidence that this subpopulation is not panmictic and that IBD or IBR may be the driving force behind genetic structure. STRUCTURE and GENELEND both had the highest support for the presence of four subpopulations within the study area (Figure 2a and b). The substructure detected in the OK/NC subpopulation did not result in a clear distinction between subpopulations (Appendix 2) and was likely a result of a genetic cline within the subpopulation. Although evaluating substructure in the SM/PM clearly separated the six individuals from the northeast region of the Okanagan (Appendix 1 and 2), this level of substructure was not supported in the GENELAND analysis (Figures 2b and 4). For this reason, I generated all discrete diversity indices based on four subpopulations.

While GENELAND did not identify admixed individuals, both GENELAND and STRUCTURE identified discontinuities between the four subpopulations that correspond with transportation corridors and highly urbanized areas (Figures 3 and 4). The sharp decline in the GENELAND probability of membership between the OK/NC and SC and more gradual change between the OK/NC and CR may be a result of higher sample density adjacent to I-90 rather than a greater barrier effect between those two subpopulations (Figure 4). While no goat mortalities from road traffic have been recorded in the study area (Washington State Department of Transportation, pers. comm. and British Columbia Ministry of Transportation, pers. comm.), mountain goats generally avoid urbanized areas. Reduced movement across
transportation corridors and urbanized areas may be a sign of behavioral avoidance of roads, or simply that mountain goat road kills have not been reported within the study area. Regardless, the development of roads, urban, recreational, and agricultural areas may now infringe upon habitat corridors that historically linked subpopulations.

The sPCA analysis supported clinal population structure across the study area. Evaluating the first three global axes that contributed significantly to global structure revealed the same pattern of genetic structure detected with discrete population analysis. The first global axis corresponds with a south to north gradient of genetic relatedness, the second global axis differentiated the SM/PM subpopulation, and the third global axis detected a genetic gradient from the CR to the OK/NC. Together, these three alternative methods used to evaluate population structure suggest that IBB may not be the driving force of isolation across the study area. I will evaluate the relative support for the alternative mechanisms of genetic isolation, IBD and IBR, with causal modeling and landscape genetics in Chapter 2.

### Patterns of genetic diversity

Evaluating genetic diversity assuming the discretely bound, internally panmictic subpopulations identified by STRUCTURE and GENELAND reveals substantial differences in  $A_r$ ,  $H_0$ , and  $F_{IS}$  between the CR and the three other subpopulations (Table 2). The CR and the SM/PM subpopulations reside in the core of mountain goat habitat, where suitable habitat is present in large, continuous patches and populations are robust. Consequently, both of these subpopulations were expected to have high levels of genetic diversity. The CR had high levels of diversity and low  $F_{IS}$ , but the SM/PM subpopulations had lower  $A_r$ , substantially lower  $H_o$ , and very high  $F_{IS}$  compared to the CR. While this may be a byproduct of the low level of sampling in the SM/PM region,  $A_r$  is corrected for sample size. A recent study by Shafer et al. (2011) of mountain goat genetic diversity across the species' range also reported higher levels of diversity and lower  $F_{IS}$  in the CR compared to the Selkirk and Purcell mountains. In addition,  $F_{IS}$  may be inflated due to substructure and lack of panmixia within the SM/PM subpopulation.

The SC subpopulation had the lowest  $A_r$  and  $H_o$  values of the four subpopulations within the study area (Table 2). This is not surprising given the patchy distribution of suitable habitat across the region, isolation of the subpopulation at the southern extreme of the species' range, and historical overharvest. The low genetic diversity observed within the SC is troubling given that low heterozygosity has been found to be negatively correlated with juvenile survivorship (Mainguy et al. 2009).  $A_r$  and  $H_o$  are moderately higher when the ONP-Cascade admixed animals are included in the SC subpopulation, confirming that the ONP translocations into the Cascades did have a positive impact on genetic diversity. This suggests that additional translocations could be beneficial. The OK/NC had moderate  $A_r$  and  $H_o$  values and unexpectedly high  $F_{IS}$  (Table 2). Once again, the high  $F_{IS}$  observed in the OK/NC subpopulation can likely be attributed to the presence of substructure and lack of panmixia within the subpopulation.  $A_r$  is slightly higher when the ONP-Cascade admixed animals are included in the OK/NC subpopulation, but  $H_o$  does not change. This very modest increase in genetic diversity is not surprising given that I only detected two ONP-Cascade admixed individuals in the OK/NC.

Considering genetic diversity strictly in the terms of discrete subpopulations ignores heterogeneity in diversity within the subpopulations. With the sGD analysis, I was able to detect pockets of higher and lower diversity across all subpopulations (Figures 6, 8, and 9). In particular, the central Cascades within the OK/NC exhibited higher  $A_r$  and  $H_o$  and lower  $F_{IS}$  compared to the Mount Baker area and Okanagan region. The SM had higher  $H_o$  and lower  $F_{IS}$  compared to the PM. Finally,  $A_r$  declined in a north to south gradient across the Cascades. These results support clinal population structure, where a heterogeneous environment structures genetic diversity across the study area according to landscape resistance. Isolation of local populations suffering from historical overharvest and inbreeding depression from more genetically diverse herds will further limit the potential of these local populations to recover. In Chapter 2, I will use sGD (Shirk and Cushman 2011) and an optimized model of landscape resistance to refine these estimates of spatially explicit indices of genetic diversity.

#### **Implications** for conservation

Pockets of low genetic diversity identified by the clinal analysis of diversity may point to a greater risk of local subpopulation extinction than suggested by the population-based analysis of diversity. Low diversity observed in the Mount Baker area, OK, and SC raises the possibility that these local populations have reduced connectivity with the more genetically diverse local populations in the central region of the NC and the highly diverse CR. This implies that the subpopulations identified by discrete methods are not pannictic and that the landscape within discretely bound subpopulations does not uniformly affect mountain goat

movement and subsequent gene flow. Strategies for improving population connectivity should take this information into account. For example, wildlife exclusion fencing along I-90 and wildlife passages across I-90 are currently under construction in an effort to reduce wildlife-vehicle collisions and mitigate the barrier effect of the I-90 corridor to wildlife migration (US Department of Transportation Federal Highway Administration and Washington State Department of Transportation). Assuming that the construction of wildlife exclusion fencing along I-90 and wildlife passages across I-90 will lower the resistance of I-90 to mountain goat movement, these mitigation efforts cannot be expected to uniformly improve genetic diversity in the OK/NC and the SC. Rather, gene flow from the OK/NC into the SC and vice versa will also be affected by the prevailing mechanism of genetic isolation. If the prevailing mechanism is IBD, improvements in genetic diversity will diminish as the distance to a wildlife passage increases. If the mechanism is IBR, gene flow will diminish as the resistance distance to a wildlife passage increases. Only in the case of IBB and entirely panmictic subpopulations would gene flow across I-90 uniformly improve genetic diversity within the OK/NC and SC subpopulations.

The genetic diversity of donor populations should be considered in translocation efforts. These results point to the CR as the premier donor subpopulation for translocation efforts to improve genetic diversity in Washington because this subpopulation has the highest genetic diversity. If feasible, multiple source populations should be utilized as this strategy has the greatest potential to alleviate inbreeding depression. For example, because the ONP population was founded with individuals from Alaska and the Selkirks, translocations utilizing the ONP population may introduce unique alleles into the Cascades even though the

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ONP population has very low genetic diversity. This approach was successful in Oregon, where four different source populations were used to reintroduce mountain goats to the Wallowa Mountains and Elkhorn Mountains (Oregon Department of Fish and Wildlife 2003).

The clinal population structure detected suggests that donor and recipient local populations should be carefully evaluated. Individuals translocated from the CR to the central NC cannot be expected to improve genetic diversity in the Mount Baker herd or the local populations present in the OK. For example, although the OK was included in the OK/NC subpopulation when discrete subpopulations were identified, clinal analysis and sGD analysis suggest that this local population could benefit from the translocation of individuals from the central NC because the OK region exhibits low diversity compared to the central NC. Additionally, any translocated individuals will likely be closely tied to the release site and will not uniformly improve genetic diversity across the region. Indeed, I only detected ONP-Cascade admixed individuals within 50 km of release sites in the SC.

This study has great potential to optimize efforts to improve population connectivity and viability in Washington. Conservation efforts to re-establish historical linkages, preserve existing linkages, and translocate animals cannot be expected to improve genetic diversity uniformly across a subpopulation but rather should be targeted to meet the specific management goals identified for local populations. Source populations need to be carefully selected and, when possible, multiple source populations should be utilized to maximize the potential to improve genetic diversity. Evaluating genetic structure and genetic diversity from alternative perspectives highlights the need to account for the effects of a complex landscape

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on gene flow in conjunction with population dynamics and population demographics when developing and implementing management strategies to improve the population viability of mountain goats in Washington.

# **TABLES**

**Table 1.** Sample sources and sample types retained for analysis after poor quality samples and samples from the same individual were removed.

Sample Source	п
Collected 2007-2011	
Scat	83
Hair	2
Tissue	1
Bone	1
Shafer et al. (2011)	
DNA-tissue	24
Poole and Reynolds (2009)	
DNA-scat and hair	16
Shirk (2009)	
Tissue	100
Blood	58
Bone	1
Total	286

**Table 2.** Sample size (n), allelic richness  $(A_r)$ , observed heterozygosity  $(H_o)$ , expected heterozygosity  $(H_e)$ , and inbreeding coefficient  $(F_{IS})$ .

Population	п	$A_r$	$H_o$	$H_{e}$	$F_{IS}$
Coast Range (CR)	44	4.22	0.53	0.58	0.07
Selkirk/Purcell Mtns. (SM/PM)	25	3.61	0.38	0.51	0.24
Okanagan/N. Cascades* (OK/NC)	119	3.41	0.38	0.46	0.16
OK/NC + ONP	121	3.44	0.38	0.46	0.16
S. Cascades* (SC)	73	2.82	0.35	0.40	0.10
SC + ONP	84	2.95	0.37	0.42	0.12
Olympic National Park (ONP)	12	2.48	0.36	0.40	0.11
*O)D G 1 1 1 1 1 1 1 1	1 1 1				

\*ONP-Cascade admixed individuals excluded

	CR	SM/PM	OK/NC*	SC*	ONP
CR	-	< 0.001	< 0.001	< 0.001	< 0.001
SM/PM	0.184	-	< 0.001	< 0.001	< 0.001
OK/NC*	0.104	0.194	-	< 0.001	< 0.001
SC*	0.223	0.247	0.105	-	< 0.001
ONP	0.274	0.292	0.317	0.369	-

**Table 3.** Pairwise  $F_{st}$  values for the five mountain goat subpopulations in the study area are given below the diagonal and the associated P values are given above the diagonal.

\*ONP-Cascade admixed individuals are excluded in this table.

**Table 4**. The migration rate into each subpopulation. The subpopulations into which individuals are migrating are listed in the rows, and the origins of the migrants are listed in the columns. Values along the diagonal are the proportions of individuals derived from the source subpopulation each generation.

	CR	SM/PM	OK/NC*	SC*
CR	0.955	0.007	0.030	0.008
SM/PM	0.012	0.966	0.011	0.011
OK/NC*	0.006	0.003	0.970	0.022
SC*	0.004	0.005	0.006	0.986
10177 0 1 1				

\*ONP-Cascade admixed individuals are excluded in this table.

## **FIGURES**



Figure 1. Map of the study area showing location of genetic samples, translocation sites, transportation corridors, and current mountain goat range. CR: Coast Range; OK: Okanagan; SM: Selkirk Mountains; PM: Purcell Mountains; NC: North Cascades; SC: South Cascades; ONP: Olympic National Park. 1: Shorts Creek; 2: Tulameen Mountain; 3: Snass Mountain; 4: Slocan Valley.



**Figure 2. (a)** The second order rate of change in the STRUCTURE assignment probability ( $\Delta K$ ) for 1 to 10 populations. **(b)** Density plot of the number of populations (K) along the GENELAND MCMC chain excluding ONP and ONP-admixed individuals. **(c)** STRUCTURE *Q*-Plot showing the posterior probability of membership (Q) for each sample. Horizontal bars represent individuals grouped by *a priori* populations and ordered from north to south within each *a priori* population. **(d)** STRUCTURE *Q*-Plot showing the posterior probability of membership (Q) excluding ONP and ONP-Cascade admixed individuals. Horizontal bars represent individuals grouped by population and ordered from north to south within each *population*.



**Figure 3**. Samples represented by colored triangles indicate the STRUCTURE subpopulation to which they have the highest probability (>75%) of membership. Samples represented by stars indicate an individual admixed (<75% membership in one subpopulation) between the STRUCTURE subpopulation of the sample's geographic location and the STRUCTURE subpopulation indicated by the color of the star.



Figure 4. The GENELAND posterior probability map of membership for the OK/NC subpopulation.



**Figure 5. (a)** sPCA eigenvalues for each global axis and local axis. **(b)** Individual samples are mapped according to the sample location's geographic coordinates and colored according to the sample's sPCA eigenvalues for the three global axes that contributed significantly to genetic structure. Gradation of red indicates the first global axis, gradation of green indicates the second global axis, and gradation of blue indicates the third global axis.



Figure 6. Observed heterozygosity (*Ho*) calculated using sGD with a genetic neighborhood diameter of 165 km and a minimum population of 10 individuals.



Figure 7. Expected heterozygosity  $(H_e)$  calculated using sGD with a genetic neighborhood diameter of 165 km and a minimum population of 10 individuals.



Figure 8. Inbreeding coefficient ( $F_{IS}$ ) calculated using sGD with a genetic neighborhood diameter of 165 km and a minimum population of 10 individuals.



Figure 9. Allelic richness  $(A_r)$  calculated using sGD with a genetic neighborhood diameter of 165 km and a minimum population of 10 individuals.

### **CHAPTER 2**

### Mountain Goat Population Connectivity in Washington and Southern British Columbia

### **INTRODUCTION**

Anthropogenic modifications to natural landscapes including habitat fragmentation, habitat loss, and climate shifts are contributing to declines in global biodiversity (Thomas et al. 2004, Wiegand et al. 2005, Fischer and Lindenmayer 2007, Butchart et al. 2010). These perturbations reduce population viability by dividing large, well-connected populations into smaller, isolated populations with reduced gene flow and local effective population size,  $N_e$ (Keyghobadi 2007).  $N_e$  is dictated by population size, gene flow, and demographic factors (e.g. sex ratios, mating systems and population structure) and strongly influences the accumulation and loss of genetic diversity (Frankham 1996). The conservation of genetic variation is essential to maintain biodiversity because increased genetic diversity provides populations with the genetic capitol to adapt to environmental change and lower genetic diversity is directly linked to reduced population fitness (Reed and Frankham 2003). Failing to account for genetic factors may lead to an underestimation of extinction risk and the implementation of inadequate recovery plans (Frankham 2005). Maintaining connectivity among patchily distributed habitat is vital for population persistence because it facilitates the movement of individuals and gene flow across the landscape (Crooks and Sanjayan 2006). In addition to habitat loss and fragmentation, climate change is now a serious threat to global biodiversity and poses new conservation challenges (Thomas et al. 2004). Increasing

connectivity is the most common recommendation to reduce the negative impacts of climate change (Heller and Zavaleta 2009) and is the most effective method currently available to conserve biodiversity in the face of climate change (Krosby et al. 2010). For these reasons, it is imperative to consider landscape connectivity when developing conservation strategies for the future (Taylor et al. 1993).

The field of landscape genetics integrates landscape ecology and population genetics to provide powerful methods to evaluate how landscape features influence gene flow across a heterogeneous landscape (Manel et al. 2003). Incorporating landscape ecology into landscape genetics builds on population genetics by accounting for heterogeneity in the matrix that separates patchily distributed habitat (Holderegger and Wagner 2008). This enables researchers to quantify the effects of landscape composition, configuration, and matrix quality on gene flow and spatial genetic variation (Storfer et al. 2007). Spatial genetic patterns are most effectively assessed with genetic data from many individuals with known geographic locations that are sampled across broad landscapes (Manel et al. 2003). Individual-based landscape genetic analyses can be applied to evaluate landscape effects on population connectivity and may lend insight into the genetic health, isolation, and viability of populations.

There are three conceptual models that potentially explain how gene flow occurs across the landscape: isolation by barrier (IBB), isolation by distance (IBD), and isolation by resistance (IBR). IBB predicts that a strong barrier to dispersal exists between subpopulations and results in discrete subpopulations that are internally panmictic. IBD predicts that genetic distance is a function of the Euclidean distance between individuals and the vagility of the species in question, resulting in a genetic gradient (Wright 1943). IBR predicts that genetic distance between individuals is dictated by the resistance of a heterogeneous landscape to gene flow (Cushman et al. 2006, McRae 2006). IBR allows for the assignment of variable resistance costs to landscape features, accounting for heterogeneity in the landscape matrix. Both IBD and IBR accumulate resistance over distance, but IBR builds resistance at a variable rate that is governed by the resistance of landscape features to individual movement. Failing to identify the correct mechanism of genetic isolation may lead to incorrect assumptions regarding the genetic structure of a population and ineffective conservation planning.

In this study, I employed individual-based methods in a causal modeling framework (Cushman et al. 2006, Cushman and Landguth 2010, Shirk et al. 2010) in order to determine the mechanism of genetic isolation for mountain goats in Washington and southern British Columbia (Figure 1). Mountain goats have declined by over 50% in Washington since the 1950s, probably as a result of unsustainable harvest levels (Rice and Gay 2010). Although harvest levels were drastically reduced in the early 1990s, many subpopulations have not recovered and some historical habitat remains unoccupied. In contrast to many ecosystems, the alpine and sub-alpine environments mountain goats inhabit are largely intact within Washington State (approximately 42% of the state is under federal or state ownership). Thus, the lack of recovery of this population may be a result of low genetic diversity and reduced fitness due to inbreeding (Blomqvist et al. 2010, Johnson et al. 2010). Understanding how the landscape matrix may be contributing to genetic isolation is essential for the conservation of this species into the future.

In Chapter 1, I evaluated genetic diversity across the study area using discrete and clinal models of population structure. Both STRUCTURE (Pritchard et al. 2000) and GENELAND (Guillot et al. 2005) identified discontinuities between the four subpopulations that corresponded with transportation corridors, highly urbanized areas, and agriculture, suggesting that IBB may be the dominant mechanism of genetic isolation. In contrast, sPCA analysis (Jombart et al. 2008) found significant global structure (positive autocorrelation) and revealed clinal genetic structure across the study area. The presence of potential migrants and admixed individuals in the STRUCTURE analysis also supports the presence of a genetic cline due to IBD or IBR. Causal modeling provides the framework to evaluate the relative support of IBB, IBD, and IBR and identify the prevailing mechanism of genetic isolation for this population of mountain goats.

Previous research (Shirk et al. 2010) of mountain goat population connectivity within the Cascade Range of Washington identified an optimized multivariate model of IBR that was better supported than IBB or IBD. This model of IBR indicated that an interstate highway, smaller highways, development, low elevation valleys, and water bodies all limit mountain goat gene flow in the Washington Cascades. In Chapter 1, I found that genetic diversity is lower for mountain goats in the Washington Cascades and the Okanagan region than in the Coast Range and Selkirk and Purcell mountain ranges. Together, these results suggest that modern anthropogenic landscape alterations limit gene flow among mountain goat populations in Washington and southern British Columbia.

I expand on Shirk's work by extending the study area into southern British Columbia to gain a better understanding of gene flow between the genetically impoverished population

in Washington and the more robust and genetically diverse populations in British Columbia. Expanding the spatial extent to encompass a multinational study area facilitates an analysis of population connectivity based on the landscape rather than political boundaries. A study area that crosses regional and national borders also encourages the interagency collaboration that will be paramount when planning and implementing efforts to restore and maintain viable populations in the face of climate change and anthropogenic activities that reduce population connectivity (Beier et al. 2011). Simply applying the previously optimized model of IBR to the expanded study area may lead to erroneous conclusions about population connectivity because the ability to detect effects of landscape features on gene flow relies heavily on habitat amount, configuration, and contrast in landscape resistance between habitat and non-habitat (Cushman et al. 2013). For example, even in the case that a species has a globally consistent response to a landscape feature, the effect of that landscape feature will only be detectable when the pattern across the study area is highly variable and limiting to gene flow (Short Bull et al. 2011). For these reasons, replication of landscape genetic research is crucial when identifying species' habitat requirements for gene flow.

In Chapter 2, I evaluate the relative support for IBB, IBD, and multiple hypotheses of IBR that include four landscape features: distance to escape terrain, roads, landcover type and elevation. These features were selected *a priori* as potential factors influencing mountain goat movement based on previous research (Festa-Bianchet and Côté 2008, Shirk 2009, Shirk et al. 2010, Shafer et al. 2012, Wells 2012). I employed individual-based landscape genetic analysis in a causal modeling framework to correlate genetic distance with resistance distance (McRae 2006) and identify a best supported model of genetic isolation. I then used

this optimized model of genetic isolation to parameterize models of gene flow across the study area. I conclude by discussing how this research can facilitate the development of a multiregional, multinational management plan that can effectively improve population viability of mountain goats in Washington.

#### METHODS

#### Study area

The study area in Chapter 2 includes the Cascade Range in Washington, the Coast Range in British Columbia, the Selkirk and Purcell mountain ranges in British Columbia, and the Okanagan region in Washington and British Columbia (Figure 1).

### Sample collection and genotyping

See methods section in Chapter 1 for sample collection and genotyping protocol. I identified 13 individuals in the Cascade Range, Washington, that were highly related to the non-native Olympic National Park (ONP) population. These 13 individuals are likely descendents of animals translocated from ONP in the 1980s (Houston et al. 1991) and do not represent natural genetic diversity or gene flow. For this reason, these 13 samples, along with the 12 samples from ONP, were excluded from the landscape genetic analysis in Chapter 2.

#### **GIS** data

I obtained a 30 m resolution digital elevation model (DEM) from the USGS and approximately 20 m resolution Canadian digital elevation data (CDED) from GeoBase. I used ArcGIS 10 (ESRI 2010) to resample the CDED to a 30 m resolution with the nearest neighbor resample technique and combine the CDED with the DEM. Focal statistics were used to fill a small gap in the data along the border between Washington and British Columbia and create a complete DEM of the study area. I used ArcGIS to generate a slope raster from the DEM and the "Euclidean Distance" function to create a distance to escape terrain  $(D_{et})$  raster, with escape terrain defined as slope  $\geq 50^{\circ}$  (Smith 1994). Landcover data and road data at 100 m resolution were both obtained from the Washington Wildlife Habitat Connectivity Working Group (WHCWG 2010). The WHCWG road data were used to reclassify roads as pixels within 500 m from the road centerline for each of the following road categories: freeway, major highway, secondary highway, local road, and no road. Freeways are characterized by multiple lanes in each direction, median or concrete barriers between opposing traffic lanes, and high traffic volumes often exceeding 25,000 vehicles daily. Major highways are nationally and regionally important highways with highly variable traffic volumes between 800 and 20,000 vehicles daily. Secondary highways are hardsurfaced and usually undivided roads with single-lane characteristics and highly variable traffic volumes between 500 and 10,000 vehicles daily. Local roads are characterized by neighborhood streets, short-distance roads connecting small towns, scenic park roads, unimproved or unpaved roads, and industrial roads. The WHCWG landcover data were reclassified into seven categories: alpine/sparsely vegetated, grass- dominated, wet forest/dry forest, shrub-dominated, water/wetland/riparian, agriculture and urban/developed. All GIS data were projected to Albers Equal Area Conic GCS North America Datum of 1983.

### Quantifying genetic distance

Genetic distance between individuals is often estimated by assuming a particular underlying model of microsatellite mutation, such as the infinite alleles model (Kimura and Crow 1964) or the stepwise mutation model (Ohta and Kimura 1973). Neither of these models may be valid for highly polymorphic microsatellite markers (Balloux and Lugon-Moulin 2002). For this reason, I used principle component analysis (PCA) to estimate genetic distance between individuals because PCA makes no biological assumptions regarding the mechanism of mutation. PCA reduces the multidimensional, multilocus microsatellite genotypes into one dimension (1<sup>st</sup> PCA axis) that contains most of the variance within the dataset (Patterson et al. 2006). I created a data frame in R 2.14.2 (The Comprehensive R Archive Network, CRAN) containing all individuals in the dataset in rows and all alleles present in the dataset as columns. Each individual was scored for each allele found in the population as 0 (not present), 1 (heterozygous), or 2 (homozygous). R 2.14.2 was used to calculate the 1<sup>st</sup> PCA axis eigenvalues, and the package Ecodist (Goslee and Urban 2007) was implemented in R 2.14.2 to generate a n x n pairwise genetic distance matrix based on Euclidean distance between individuals on the  $1^{\text{st}}$  axis (n=261).

#### Modeling IBB, IBD, and IBR

I modeled IBB by generating an  $n \ge n$  distance matrix where distance between samples within the same subpopulation (as identified in Chapter 1 Table 2) was zero, distance between samples separated by one hypothetical barrier (Chapter 1 Figure 3) was one and distance between samples separated by two barriers (Chapter 1 Figure 3) was two. To model IBD, I used the Landscape Genetics Arc Toolbox (Etherington 2011) distance matrix tool to generate a  $n \ge n$  matrix of Euclidean distance (in meters) between all individual sample locations. I also tested the correlation of a  $\text{Log}_{10}$  transformed  $n \ge n$  matrix of Euclidean distance between individuals with genetic distance because the logarithm of geographic distance would theoretically have a higher correlation with genetic distance in two-dimensional landscapes (Rousset 1997).

I modeled IBR by generating a  $n \ge n$  matrix of resistance distance for each IBR hypothesis tested (described below). Circuitscape 3.5.8 (McRae and Shah 2009) was used to fill each matrix with pairwise resistance distances between all individual sample locations according to the IBR hypothesis tested. Circuitscape uses graph and circuit theory to account for multiple pathways and calculate resistance distance between points given a landscape resistance surface represented as a GIS raster. I allowed gene flow to the eight nearest cells and calculated resistance between two cells as the average of the resistance value assigned to both cells. I utilized a cell size of 150 m in order to obtain a reasonable computation time. Elevation and  $D_{et}$  were converted to this cell size by aggregating 5 x 5 blocks of 30 m pixels into a single pixel (based on average aggregation technique for the elevation raster and minimum aggregation technique for the  $D_{et}$  raster). The landcover and road rasters were converted from 100 m resolution to 150 m resolution using the nearest resample technique.

#### Evaluating the relative support for IBB, IBD and IBR

I implemented the software package Ecodist (Goslee and Urban 2007) in R 2.14.2 to calculate the Mantel's correlation coefficient between each landscape distance matrix and the

genetic distance matrix in order to assess support for IBB, IBD, and the multiple hypotheses of IBR. I then applied partial Mantel's tests (Smouse 1986) to evaluate the relative support for each model of genetic isolation (IBB, IBD, and the best supported IBR hypothesis) while controlling for the effects of alternative models.

#### Univariate IBR model optimization

I evaluated four factors that were identified by Shirk (2009) as likely to influence mountain goat movement across the landscape: distance to escape terrain ( $D_{el}$ ), roads, landcover type, and elevation. I built multiple hypotheses of resistance for each factor by systematically varying parameters within mathematical functions related to each factor. These mathematical functions were then used to reclassify raster data into alternative hypotheses of IBR for each factor that I evaluated to find the most supported model of IBR.

I reclassified the raster representing Euclidean distance to escape terrain (slope $\geq$  50°) into multiple hypotheses of landscape resistance due to  $D_{et}$  with the following function:

# $R = (D_{et}/Vmax)^{x} * Rmax$

where R is the resistance for that raster cell, x is the response shape exponent, Rmax is the maximum possible resistance value, and Vmax is a constant representing the maximum allowed value of the variable such that as the variable increases to Vmax the resistance increases to Rmax. When x is equal to one, the increase to Rmax is linear, and when x is not equal to one, the increase is nonlinear. I evaluated four different response shape exponents and four different scales of Rmax.

I ranked the five road categories in order of increasing resistance: no road, local road, secondary highway, major highway, and freeway. I then reclassified the road raster into multiple hypotheses of landscape resistance due to roads with the following function:

#### $R = (Rank/Vmax)^{x} * Rmax$

where R is the resistance for that raster cell, x is the response shape exponent, Rmax is the maximum possible resistance value, and Vmax is a constant representing the maximum allowed value of the variable such that as the variable increases to Vmax the resistance increases to Rmax. When x is equal to one, the increase to Rmax is linear, and when x is not equal to one, the increase is nonlinear. I evaluated five different response shape exponents and five different scales of Rmax until I found a unimodal peak of support.

I reclassified the landcover type urban/developed to no data because, given mountain goat avoidance of urbanized areas, no gene flow would likely occur through this landcover type. The remaining six landcover type categories were ranked in order of increasing resistance: alpine/sparsely vegetated, grass-dominated, wet forest/dry forest, shrubdominated, water/wetland/riparian, and agriculture. I then reclassified the landcover raster into multiple hypotheses of landscape resistance due to landcover with the following function:

## $R = (Rank/Vmax)^{x} * Rmax$

where R is the resistance for that raster cell, x is the response shape exponent, Rmax is the maximum possible resistance value, and Vmax is a constant representing the maximum allowed value of the variable such that as the variable increases to Vmax the resistance increases to Rmax. When x is equal to one, the increase to Rmax is linear, and when x is not

equal to one, the increase is nonlinear. I evaluated five different response shape exponents and five different scales of *Rmax* until I found a unimodal peak of support.

Mountain goats are adapted to utilize an optimal elevation range dissected by suboptimal elevations characterized by lowland valleys and glaciated peaks (Festa-Bianchet and Côté 2008, Shirk et al. 2010, Wells 2012). For this reason, I reclassified the elevation raster based on a Gaussian function that defines optimal elevation and standard deviation such that multiple hypotheses of landscape resistance due to elevation were generated by the function:

# $R = Rmax + 1 - Rmax * e^{-(elevation - Eopt)^{2}/(2 * Esd)^{2}}$

where *R* is the resistance for that raster cell, *Rmax* is the maximum possible resistance value, *Eopt* is the optimal elevation, and *Esd* is the standard deviation such that as elevation moves away from *Eopt* resistance increases to *Rmax* at the rate dictated by *Esd*. I evaluated five *Eopt* values, three scales of *Rmax*, and three rates of *Esd* until I found a unimodal peak of support.

#### Multivariate IBR model optimization

After the optimal landscape resistance model for each univariate factor had been identified, I combined univariate factors into a multivariate model. Univariate factors were only included in the multivariate model if the Mantel's correlation reached a unimodal peak of support that had a higher Mantel's correlation value than the IBD null model and the *P*-value was significant (<0.05). I used raster algebra in ArcGIS10 (ESRI 2010) to add the optimized univariate landscape resistance models that met the above requirements into a single

multivariate model. To account for interactions between factors, I optimized the multivariate model by varying parameters for one variable at a time while holding the other variables constant. Factors were optimized in order of decreasing univariate Mantel's correlation. The multivariate model optimization was repeated if any of the parameters changed in the multivariate context.

## Predicting population connectivity

I used Circuitscape 3.5.8 (McRae and Shah 2009) to predict gene flow across the study area using the most highly supported multivariate hypothesis of IBR. Circuitscape calculates the flow of current across a given resistance surface, using current as a proxy for the probability that an individual moving by a "random walk" pattern will pass through a given cell (McRae et al. 2008). This allows for the evaluation of population connectivity and the identification of conservation corridors. I evaluated gene flow across the study area based on the maximum current map created by calculating current between three individuals: one individual located in the Coast Range, one individual located in the south Cascades, and one individual located in the Purcell Mountains, in pairwise mode. In this scenario, current represents the probability that an individual will pass through a given cell as it moves across the study area from the sources to the ground points.

## Spatially explicit genetic diversity

I used the software package sGD (Shirk and Cushman 2011) to estimate genetic diversity indices based on microsatellite genotypes and the pairwise resistance distance matrix

generated from the multivariate optimized IBR model. This approach allows for the grouping of individuals based on spatially explicit genetic neighborhoods that take into account the effects of a heterogeneous landscape on the genetic diversity of clinal populations. I applied a genetic neighborhood diameter defined by the largest cost-weighted resistance distance that is positively correlated with genetic distance and significant (alpha=0.05). I set the minimum population size to 5 individuals.

#### RESULTS

#### Univariate optimization

The hypothesis of resistance due to *Det* most highly correlated with genetic distance had a shape exponent of 1 with resistance ranging from 1 to 5 (r=0.671, P=0.0001, Figure 2b). Support for this hypothesis did not reach a unimodal peak and the correlation was lower than the IBD null model (r=0.684, P=0.0001). For this reason, I excluded *Det* as a factor in the multivariate model.

The hypothesis of resistance due to roads most highly correlated with genetic distance had an infinite shape exponent with resistance ranging from 1 to 25 (r=0.711, P=0.0001, Figure 3b and 3c) where freeways had a resistance of 25 and all other pixels had a resistance of 1. The actual cost of crossing a freeway was 200 because freeways were generally eight pixels wide. Roads were included in the multivariate model because support for this hypothesis reached a unimodal peak and was more highly correlated with genetic distance than the IBD null model.

The hypothesis of resistance due to landcover most highly correlated with genetic distance had an infinite shape exponent with resistance ranging from 1 to 25 (r=0.713, P=0.0001, Figure 4b and 4c) where agriculture had a resistance of 25 and all other pixels had a resistance of 1. Landcover was included in the multivariate model because support for this hypothesis reached a unimodal peak and was more highly correlated with genetic distance than the IBD null model.

The hypothesis of resistance due to elevation most highly correlated with genetic distance had optimal elevation of 1,600 m with a standard deviation of 1,500 m and resistance ranging from 1 to 5 (r=0.697, P=0.0001, Figure 5b). Elevation was included in the multivariate model because support for this hypothesis reached a unimodal peak and was more highly correlated with genetic distance than the IBD null model.

## Multivariate optimization

I began the multivariate optimization with landcover because this factor had the highest correlation with genetic distance. The optimal shape exponent did not change from the univariate optimal landcover hypothesis; however, the scale increased from 25 to 100 (r=0.715, P=0.0001, Figure 6). I then optimized roads because this factor had the second highest correlation with genetic distance. The optimal shape exponent and scale did not change from the univariate optimal roads hypothesis. Elevation was optimized last because this factor had the lowest correlation with genetic distance. Optimal elevation, standard deviation, and scale did not change from the univariate optimized from the univariate optimal roads hypothesis. I did not perform additional multivariate optimization because only the first landscape feature

considered, landcover, changed in the multivariate context. The resistance costs associated with the final optimized multivariate IBR model are displayed in Table 1.

#### **Relative support for IBB, IBD and IBR**

While the IBB model and IBD model both showed strong correlation with genetic distance (r=0.610, P=0.0001 and r=0.684, P=0.0001), the optimized multivariate IBR model had the highest correlation value (r=0.715, P=0.0001, Table 2). The IBD model was better supported than then IBD<sub>LOG10</sub> model (r=0.684, P=0.0001 and r=0.523, P=0.0001). The IBB model retained a significant relationship with genetic distance when the variance due to IBD was partialled out (r=0.176, P=0.0001) and when the variance due to IBR was partialled out (r=0.105, P=0.0001, Table 2). The IBD model retained a significant relationship with genetic distance when the variance due to IBB was partialled out (r=0.422, P=0.0001) and when the variance due to IBR was partialled out (r=0.080, P=0.0073, Table 2). The IBR model retained a significant relationship with genetic distance when the variance due to IBB was partialled out (r=0.479, P=0.0001) and when the variance due to IBD was partialled out (r=0.297, P=0.0001, Table 2). The IBR model of genetic isolation retained a higher correlation with genetic distance when the variance due to IBB was partialled out than the IBB model of genetic isolation retained when the variance due to IBR was partialled out (r=0.479 and r=0.105, respectively, Table 2). The IBR model of genetic isolation also retained a higher correlation with genetic distance when the variance due to IBD was partialled out than the IBD model of genetic isolation retained when the variance due to IBR was partialled out (r=0.297 and r=0.080, respectively, Table 2). For this reason, the IBR

model of genetic isolation appears to have the strongest relationship with genetic distance among the three models considered.

#### Gene flow

Gene flow from the Coast Range into the north Cascades is restricted most severely by the development along Highway 99 in British Columbia where current is funneled through narrow breaks in the urban and agricultural landcover types (Figure 8). Less severe pinch points are present where gene flow must cross the Coquihalla Highway (Hwy 5) between areas of urban development and agriculture. Gene flow from the Purcell Mountains into the north Cascades is concentrated by agriculture and urban areas as it moves across the Okanagan Valley. As current moves into the north Cascades, it is concentrated by the low elevation and the development associated with the Interstate 5 corridor that runs along the western extent of the study area in Washington, and by the low elevation and agriculture present to the east of the study area in Washington. As current moves south towards Highway 2, urbanization and agriculture begin to force gene flow towards the center of the north Cascades. The most severe channelization of current occurs as gene flow moves across I-90, where current must pass through narrow breaks in urban development and agriculture along I-90. The cost of crossing the Okanagan Valley and I-90 accounts for more than twothirds of the cost of moving all the way from the Purcell Mountains to the south Cascades. Similarly, the cost of moving across Highway 99, the Coquihalla Highway (Hwy 5), and I-90 is very high.
### Spatially explicit genetic diversity

The spatially explicit analysis of genetic diversity revealed variable patterns across the study area. The genetic neighborhood diameter where cost-weighted resistance distance was no longer positively correlated with genetic distance was 4.56. The Coast Range generally exhibited high genetic diversity with high  $H_o$  and  $A_r$  and moderate  $F_{IS}$  (Figure 9, Figure 11, and Figure 12). The Okanagan exhibited the lowest genetic diversity with very low  $H_o$  and  $A_r$ and high  $F_{IS}$ . The south Cascades exhibited very low  $H_o$  at the southern periphery of the study area, with  $H_0$  improving slightly moving north (Figure 9). The south Cascades also exhibited low  $A_r$  (Figure 12).  $F_{IS}$  within the south Cascades was variable and ranged from moderately high to very low (Figure 11). Measures of genetic diversity were also variable within the north Cascades with the central Cascades displaying moderate  $H_o$  (Figure 9) and  $A_r$  (Figure 12).  $H_o$  and  $A_r$  were lower in the north Cascades in the Mount Baker region and to the south (Figure 9 and Figure 12).  $F_{IS}$  within the north Cascades ranged from very low to moderate (Figure 11). Sample density was not high enough in the Purcell and Selkirk mountain ranges to yield any data points. These results should be interpreted with caution because the minimum population applied (5) was lower than the minimum population recommended by the sGD documentation (10, Shirk and Cushman 2011). This could introduce sampling error due to low sample density but was necessary to attain sufficient coverage of the area.

### DISCUSSION

This study expanded upon previous research (Shirk 2009) by extending the extent of the study area to gain insight regarding connectivity between mountain goat populations in Washington that are potentially genetically isolated and mountain goat populations in southern British Columbia that inhabit the core of the species' range. Extending the study area into British Columbia allows for a more realistic analysis of population connectivity because individual movement and metapopulation dynamics are not necessarily bound by regional, state, or international boundaries. In addition, applying a model of landscape resistance created for one area to a different area may lead to incorrect assumptions about species' habitat requirements for gene flow and the implementation of ineffective conservation strategies (Short Bull et al. 2011). I was also able to utilize a smaller cell size (150 m) than previously considered (Shirk 2009), possibly improving the ability to detect an effect of landscape features on gene flow.

The suitability of Mantel testing in landscape genetics has been questioned in the literature (Raufaste and Rousset 2001), but multiple analyses defend the use of this method when used to test hypotheses based on distance matrices (Legendre and Fortin 2010) and implemented within the framework of causal modeling (Cushman and Landguth 2010, Shirk et al. 2010, Cushman et al. 2013). Legendre and Fortin (2010) warn that statistical analyses based on distances, such as Mantel testing, lead to a large loss of statistical power. Standard Mantel tests have also been criticized for having high type 1 error rates when used to assess the relative importance of landscape variables due to high correlation among distance matrices (Balkenhol et al. 2009). Cushman and Landguth (2010) evaluated the power Mantel

testing within a causal model framework in individual-based landscape genetic analysis and found that simple Mantel tests do produce spurious correlations, but partial Mantel tests and causal modeling effectively rejected incorrect explanations and correctly identified the true causal process.

I minimized the risk of spurious correlations complicating model selection by imposing strict criteria for inclusion of univariate landscape features in the multivariate model and by employing partial Mantel tests to evaluate the relative support for IBB, IBD, and IBR. I excluded landscape features that did not have a stronger correlation with genetic distance than IBD or display a unimodal peak of support to ensure only landscape feature that significantly influence gene flow were included in the multivariate model. Systematically varying model parameters allows for nonlinear relationships between landscape features and genetic distance and applies variable scales of resistance to landscape features. Finally, the multivariate optimization process improves the model by accounting for interactions between landscape features.

I applied circuit theory to quantify resistance distance rather than least-cost-path (LCP) analysis because circuit theory takes the width and number of pathways into consideration when calculating resistance distances and does not assume that dispersing individuals have perfect knowledge of the landscape (McRae 2006). The stronger theoretical foundation of isolation by resistance as calculated with circuit theory and the ability of IBR to outperform LCP in both simulated landscapes and empirical analyses supports the use of IBR over LCP (McRae and Beier 2007). Circuit theory can also be used to predict gene flow between points by calculating current, or the net movement probability, in order to identify

important conservation corridors where gene flow is funneled through narrow channels between impermeable landscape features (McRae et al. 2008).

I was able to detect an effect of three landscape features on genetic isolation despite relatively low sample density. Implementing individual-based methods and using PCA to quantify genetic distance may have both improved the power of this analysis. Using the individual as the unit of observation eliminates the requirement to assign individuals to populations, an unrealistic representation of population structure for continuously or patchily distributed species. Individual-based methods also yield a higher number of observations than population-based methods, an important attribute for species that are difficult to sample. Genetic distance quantified as PCA is theoretically more sensitive to genetic dissimilarity than other genetic distance metrics because it reduces multidimensional data into one dimension that contains most of the variance. With this approach, alleles that contain the most genetic variation contribute more to genetic distance than common alleles. This is a benefit over more commonly applied methods, such as Rousset's a (Rousset 2000) or the proportion of shared alleles (DPS; Bowcock et al. 1994), where all alleles contribute equally. Although PCA has not been widely applied to quantify genetic distance in landscape genetics, Shirk et al. (2010) found that causal modeling outcomes were consistent when using genetic distance based on either PCA, proportion of shared alleles, or Rousset's a with PCA yielding the highest correlation values with landscape resistance.

Applying these methods, I was able to identify a model of genetic isolation by landscape resistance that was better supported than IBB or IBD (Table 2). The optimized model of IBR also agreed largely with the results from Chapter 1 and generally agreed with

previous research on mountain goat population connectivity (Shirk 2009). In Chapter 1, I identified genetic discontinuities that correspond with the transportation corridors of I-90 and the Coguihalla Highway (Hwy 5) and development and agriculture in the Okanagan Valley. Evidence of migrants and admixed individuals in Chapter 1 and greater relative support for IBR than IBB in Chapter 2 both suggest that these landscape features are not complete barriers, but do impose high resistance to movement (Figure 7). Assuming that the four subpopulations outlined in Chapter 1 are panmictic would mask considerable heterogeneity in the permeability of the landscape where urban and agriculture landcover types, freeways, and low elevation all resist gene flow within subpopulations. The Mantel's correlation of IBD and genetic distance was only slightly lower than that of IBR and genetic distance, suggesting that IBD is also driving genetic structure in this population. Evaluating the relative support for IBD and IBR reveals that IBR retains a higher Mantel's correlation, supporting the selection of IBR as the strongest driver of genetic isolation. The strong, significant Mantel's correlations of all three conceptual models of genetic isolation emphasize the importance of evaluating the relative support for IBB, IBD, and IBR.

This analysis of a broader study area that incorporated southern British Columbia identified a similar optimized model of landscape resistance to the analysis of the Washington Cascades, but there were key differences between the models. Shirk (2009) conducted his analysis at a coarser scale (450 m), and I hypothesized that reducing the cell size may have given this analysis better sensitivity to detect an effect of distance to escape terrain. Although I conducted this analysis at a finer grain (150 m) than Shirk (2009), I was also unable to detect an effect of distance to escape terrain on genetic distance. This is

surprising because mountain goats depend heavily on escape terrain to avoid predation (Hamel and Côté 2007, Festa-Bianchet and Côté 2008). It is possible that this landscape feature does not influence the movement of dispersing individuals or that escape terrain is not a limiting feature in the landscape. Finally, the higher resolution that I applied may still not be fine enough to detect an effect of distance to escape terrain on genetic distance. Surprisingly, this analysis assigned a drastically lower cost to crossing a freeway (200) than Shirk (10,000) and did not support the inclusion of highways or water in the multivariate model. Low elevation did indirectly apply resistance to the large, low elevation lakes within the study area. Urban and agriculture landcover types along highways in the study area also indirectly add resistance to movement near highways even though I did not find support for inclusion of highways themselves. Low sample density around many of the roads in the Coast Range may have reduced my ability to detect an effect of highways. This low sample density near Highway 99 and Highway 5 in British Columbia is particularly striking in comparison to the much higher sample density near I-90 in Washington (Figure 1). Additionally, due to the expansion of the study area, I used data layers developed for the WHCWG, which covered both British Columbia and Washington, instead of the data layers used by Shirk (2009), which only covered Washington. Ultimately, the model of landscape resistance derived in this analysis, where urban development, agriculture, and freeways are the major resistors to gene flow, resulted in a model of landscape resistance that was qualitatively similar to that identified by Shirk (2009).

Although the model of landscape resistance predicted high gene flow across much of the study area, gene flow is constricted by Highway 99, the Coquihalla Highway (Hwy 5),

the Okanagan Valley, and I-90. Naturally occurring low elevation valleys further restrict movement. This has major implications for the genetic health of the mountain goat population in Washington that relies on connectivity with mountain goat populations in British Columbia as a source of genetic diversity. Within Washington, the south Cascades subpopulation is particularly isolated due to its location at the extreme periphery of the species' range and the high resistance of I-90. The Okanagan region also appears to be fairly isolated with low elevation and high resistance landcover types limiting connectivity with the Coast Range and Selkirk and Purcell mountains. Indeed, spatially explicit analysis of genetic diversity revealed that the south Cascades and Okanagan region had uniformly low indices of diversity (Figure 9 and Figure 12). Genetic isolation increases the risk of inbreeding depression and the fixation of deleterious alleles and also reduced adaptive potential. Ultimately, the genetic consequences of isolated populations with smaller effective local population size lead to reduced population viability.

The results of this analysis are limited to the hypotheses of genetic isolation tested and it is possible that a better model of genetic isolation remained untested. Numerous univariate and multivariate landscape resistance hypotheses were not tested due to computational limitations. In particular, a multimodal response to an environmental variable will be problematic because this could lead to the identification of a local peak of support rather than a global peak of support. The application of complimentary methods could increase confidence in these results. For example, Shirk et al. (2012) used simulation of gene flow under the optimized model of landscape resistance developed by Shirk (2009) to determine if the observed genetic pattern was related to the simulated genetic pattern and effectively validated the optimized landscape resistance model. Genetic algorithms approaches that more efficiently search parameter space to fit landscape resistance surfaces to observed spatial genetic patterns may soon be readily available as computational capabilities increase (Spear et al 2010). Finally, low sample density, particularly in British Columbia, may have limited the ability of this analysis to detect effects of landscape features, such as highways. Despite these limitations, I was able to derive a model of genetic isolation by landscape resistance that exhibited high correlation with genetic structure. This model may be applied to inform efforts to improve population connectivity and optimize the utility of translocation events.

The model of gene flow across the study area identified four priority areas for corridor conservation. Gene flow into the north Cascades from the Coast Range is channeled into several pinch- points along Highway 99 and, to a lesser extent, Highway 5 (Figure 8). Agriculture and urban areas in the Okanagan concentrate modeled movement from the Purcell Mountains into the Cascades. Finally, current is severely restricted to several narrow channels across I-90 as it moves from the north Cascades into the south Cascades. Maintaining and improving connectivity through these pinch-points may increase the landscape's permeability to individual movement and sustain gene flow within the study area. This model of gene flow suggests that the wildlife passages that are currently under construction across I-90 could be quite helpful (US Department of Transportation Federal Highway Administration and Washington State Department of Transportation). Although it is unknown if mountain goats will utilize these crossing structures, strategic placement of wildlife passages in areas with less development may facilitate movement across this highly resistant landscape feature. Results from Chapter 1 suggest that the mountain goat population in Washington was historically better connected to the Coast Range than the Selkirk and Purcell mountains. Given that the Coast Range also has the highest indices of genetic diversity, conservation corridors that allow movement between the Coast Range and the north Cascades should be prioritized to improve the genetic health and resilience to climate change of the mountain goat population in Washington. Finally, translocations may be complimentary to corridor conservation and the construction of wildlife passages to improve genetic diversity in Washington over the short-term. As stated in Chapter 1, the Coast Range is the premier source population because of the evidence of historical linkage with the Washington Cascades and high genetic diversity. I found that the mountain goat populations in the south Cascades are both the most genetically isolated and the least genetically diverse, pointing to these populations as priority recipients of translocated individuals. In the north Cascades the Mount Baker, Okanagan, and Alpine Lakes regions exhibit lower genetic diversity and could also benefit from translocations.

# **TABLES**

Table 1. Resistance cost for each factor that contributes to landscape resistance in the most highly supported IBR model.

Factor	Resistance	
Urban/developed	Barrier	Fixed
Freeway	25	Optimized
Agriculture	100	Optimized
Elevation $< \text{ or } > 1600 \text{ m}$	2-5	Optimized
All other pixels	1	Optimized

Model	Mantel's r	Monte Carlo P value
IBB~G	0.610	0.0001
IBD~G	0.684	0.0001
IBD <sub>LOG10</sub> ~G	0.523	0.0001
IBR~G	0.715	0.0001
IBB~G IBD	0.176	0.0001
IBD~G IBB	0.422	0.0001
IBB~G IBR	0.105	0.0001
IBR~G IBB	0.479	0.0001
IBD~G IBR	0.080	0.0073
IBR~G IBD	0.297	0.0001

**Table 2.** Mantel's correlations and partial Mantel's correlations with genetic distance (G) for the IBB, IBD, and  $IBD_{LOG10}$  null models and the most highly supported IBR model.

# **FIGURES**



Figure 1. The study area showing genetic sample locations (black triangles), freeways (thick grey lines), highways (thin grey lines), elevation represented as a DEM, and study area orientation extent.



0.50

0.45

0.40

0.1

0.3

0.5

Shape Exponent

0.7

0.9

Figure 2. (a) The resistance assigned according to increasing distance from escape terrain (Resistance = (distance/max distance) x \* Rmax, rescaled to range from 1 to the Rmax), given the response shape designated by the exponent (x) in a box along each shape curve. (b) The Mantel's correlation with genetic distance for the 16 hypotheses of resistance due to distance to escape terrain that vary by x and Rmax. The grey area indicates hypotheses with a Mantel's correlation lower than the null model. All hypotheses were significant ( $\alpha$ =0.05).

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**Figure 3. (a)** The resistance assigned according to road type (Resistance = (road rank/max road rank)<sup>x</sup> \* Rmax, rescaled to range from 1 to Rmax), given the response shape designated by the exponent (x) in a box along each shape curve. (b) The Mantel's correlation with genetic distance for the 25 hypotheses of resistance due to roads that vary by x and Rmax. The grey area indicates hypotheses with a Mantel's correlation lower than the null model. All hypotheses were significant ( $\alpha$ =0.05). (c) The Mantel's correlation with genetic distance for the 15 hypotheses of resistance due to roads with the highest Mantel's correlation values.



Figure 4. (a) The resistance assigned according to landcover type (Resistance = (landcover rank/max landcover rank)<sup>x</sup> \* Rmax, rescaled to range from 1 to Rmax), given the response shape designated by the exponent (x) in a box along each shape curve. (b) The Mantel's correlation with genetic distance for the 25 hypotheses of resistance due to landcover that vary by x and Rmax. The grey area indicates hypotheses with a Mantel's correlation lower than the null model. All hypotheses were significant ( $\alpha$ =0.05). (c) The Mantel's correlation with genetic distance for the 14 hypotheses of resistance due to landcover with the highest Mantel's correlation values.



Figure 5. (a) The resistance assigned to various elevations according to a Gaussian function (Resistance = Rmax + 1 – Rmax\* $e^{-(\text{elevation-Eopt})^{2/(2*\text{Esd})^2}}$ , rescaled to range from 1 to Rmax), given the response shape designated by the standard deviation in a box along each shape curve. (b) The Mantel's correlation with genetic distance for the 45 hypotheses of resistance due to elevation that vary by optimal elevation and Rmax/SD. The grey area indicates hypotheses with a Mantel's correlation lower than the null model. All hypotheses were significant ( $\alpha$ =0.05).



Figure 6. The Mantel's correlation with genetic distance for the 12 hypotheses of resistance due to landcover that vary by shape exponent and scale. The resistance for each of these hypotheses was added to the most highly correlated univariate model of road and elevation resistance prior to testing the Mantel's correlation. All hypotheses were significant ( $\alpha$ =0.05).



Figure 7. The best supported model of IBR ranging from lowest resistance (dark grey) to highest resistance (white).



**Figure 8.** Gene glow across the study area represented as current. The color scale represents the amount of current flowing from the Coast Range and Purcell Mountains into the south Cascades. The landscape resistance between pairs of points is also noted.



Figure 9. Observed heterozygosity  $(H_o)$  calculated using sGD with a genetic neighborhood cost distance of 4.56 and a minimum population of 5 individuals.



Figure 10. Expected heterozygosity  $(H_e)$  calculated using sGD with a genetic neighborhood cost distance of 4.56 and a minimum population of 5 individuals.



Figure 11. The inbreeding coefficient  $(F_{IS})$  calculated using sGD with a genetic neighborhood cost distance of 4.56 and a minimum population of 5 individuals.



Figure 12. Allelic richness  $(A_r)$  calculated using sGD with a genetic neighborhood cost distance of 4.56 and a minimum population of 5 individuals.

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## APPENDIX

b)

Appendix 1. The second order rate of change in the STRUCTURE assignment probability ( $\Delta K$ ) for 1 to 5 populations for a) SM/PM subpopulation and b) OK/NC subpopulation.





**Appendix 2.** Samples represented by colored triangles indicate the STRUCTURE subpopulation to which they have the highest probability (>75%) of membership. Samples represented by stars indicate an individual admixed (<75% membership in one subpopulation) between the STRUCTURE subpopulation of the sample's geographic location and the STRUCTURE subpopulation indicated by the color of the star. CR: Coast Range; SM/PM: Selkirk Mountains/Purcell Mountains; OL: Okanagan Lake; OK: Okanagan; MB: Mount Baker; NC: North Cascades; SC: South Cascades.

