# The Cascades Carnivore Connectivity Project: A Landscape Genetic Assessment of Connectivity for Carnivores in Washington's North Cascades Ecosystem

Final Report for the
Seattle City Light Wildlife Research Program

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## Disclaimer

The opinions, findings, and conclusions expressed in this publication are those of the authors and do not necessarily reflect those of Montana State University.

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## Abstract

Washington's North Cascades Ecosystem (NCE) includes one of the most extensive blocks of Federal land in the contiguous United States, and presents a rare opportunity to study and enhance landscape connectivity for wide-ranging carnivores at a very large scale. The region is inhabited by numerous carnivores of conservation concern, including gray wolves, wolverines, Canada lynx, and grizzly bears. Despite its ecological integrity, however, the NCE is fragmented by three major east-west highways: I-90, U.S. Route 2, and State Highway 20. These highways are potential barriers to wildlife movement, and may serve to impede gene flow in certain wildlife populations. The Cascades Carnivore Connectivity Project represents a collaboration of researchers and volunteers working to scientifically evaluate habitat connectivity for carnivores in the NCE.

From 2008–2012, we collected genetic material (i.e., DNA) and occurrence data from American black bears and American martens using noninvasive hair-snagging methods, scat detection dogs, and remotely triggered cameras. All three methods were effective at detecting our target species, although the use of scat detection dogs was discontinued by the project because scats were determined to be an insufficient source of DNA for the genetic tests associated with our objectives. We genotyped 561 unique black bears and 71 unique martens from throughout our study area primarily using DNA from hair captured at hair snagging devices and tissue samples provided by another study. We also detected a number of rare species, including wolves, lynx, wolverines and moose. No grizzly bears were detected during our surveys.

Once our genetic tests were complete we used landscape genetic methods to assess whether landscape features were influencing gene flow in our target species across the NCE. Two genetic clustering approaches identified a region north of Route 2 as a partial barrier to north-south gene flow for bears. This region was characterized by high elevation, rugged topography, and few low-elevation passes connecting forested valleys. We then used individual-based genetic analyses including causal modeling to identify a number of models that described gene flow patterns for black bears. The most supported black bear model was based on a model developed by the Washington Connected Landscapes Project, but also included higher resistance to gene flow across steep and rugged terrain greater than 1500 m in elevation. This outcome is consistent with our genetic clustering results, and suggests that the movement of black bears in the NCE, especially during dispersal, may be mediated by high resistance to movement across higher elevations and rugged topography. Our results highlight the importance of maintaining connectivity among lower elevation, high-quality, forested habitats for black bears.

We did not detect genetic structuring in our marten data set using either genetic clustering or individual-based landscape genetic analyses. It is unclear whether this outcome was due to insufficient sample size and distribution, or whether the marten populations that we examined are not structured by landscape features.

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## Introduction

Landscape fragmentation and reciprocally, habitat connectivity, are vital concepts when planning for the long-term persistence of wildlife, as they affect animal dispersal and mortality and thus the probability of population extirpation or extinction (Lande 1988). Connectivity is particularly important for wide-ranging species such as carnivores, whose large area requirements may exceed contiguous habitat and therefore encompass multiple blocks of suitable habitat. Traveling within and between habitat blocks can also increase the exposure of carnivores to human activities, and thus make them vulnerable to conflicts with people.

The North Cascades Ecosystem (NCE), although extensive and relatively intact, is fragmented by three major east-west highways: I-90, U.S. Route 2, and State Highway 20 (Fig. 1). All three highways completely traverse this ecosystem, potentially creating wildlife movement barriers of varying intensity. At the extreme, the terrain bisected by the I-90 corridor at Snoqualmie Pass is the narrowest north-south band of contiguous public land in the NCE. This area has therefore been identified as a critical connectivity zone for Pacific Northwest wildlife populations (e.g., Thomas et al. 1990, WWHCWG 2010). Singleton and Lehmkuhl (2000) further suggest that this connectivity zone may facilitate the local movement of wildlife, and identify three significant north-south linkage zones—each with its own distinct species assemblages (WSDOT 2006).

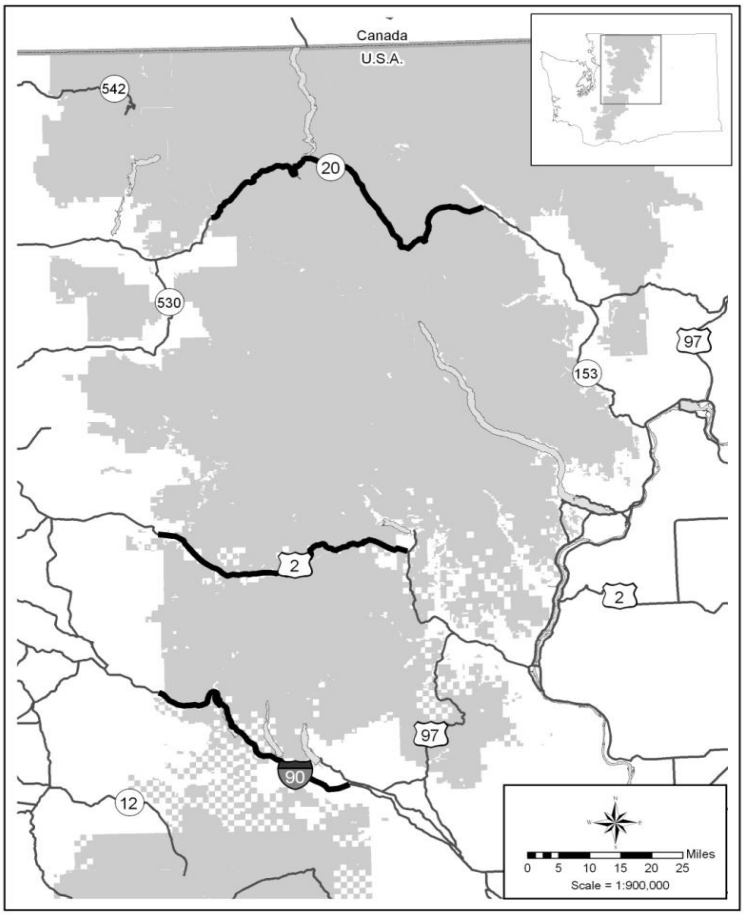



**Figure 1**. Map showing the North Cascades Ecosystem in Washington, including three major east-west highways.

Other landscape features, such as human development, forest successional patterns, lakes and rivers, elevation, and topography, also play a role in determining how carnivores move through the landscape (Cushman et al. 2006). Although major freeways and associated human development might not serve as complete barriers to animal movement, they can affect genetic patterns by hindering or re-directing dispersing individuals, thereby reducing gene flow (McGarigal and Cushman 2005, Cushman et al. 2006).

Studies large enough in scale to evaluate the effects of landscape barriers to animal movement are difficult to design and conduct, particularly for wide-ranging species. Radio-telemetry methods provide vast amounts of information but for relatively few study animals, limiting the potential for inference. In recent years, noninvasive (non-capture-based) survey methods have enhanced our ability to collect occurrence data and biological samples from wide-ranging species across expansive landscapes (Foley et al. 2001, Segelbacher 2002, Waits and Paetkau 2005, Long et al. 2008). For example, hair-snagging mechanisms have been used to collect DNA from a variety of carnivores (see Kendall and McKelvey 2008 for a review), and professionally trained detection dogs have been employed to locate scat samples for genetic and other biological testing (Smith et al. 2003, Wasser et al. 2004, Long et al. 2007, MacKay et al. 2008).

Today's landscape genetic analytical approaches (Manel et al. 2003, Holderegger and Wagner 2008) allow efficient use of the large numbers of genetic samples that can be collected via noninvasive survey methods. These approaches incorporate spatial information associated with individual animals, thus enabling researchers to assess the effects of regional landscape features on gene flow and genetic structuring among populations.

Researchers seeking to assess connectivity for wildlife typically engage experts who are familiar with a given focal species or ecological system to develop expert-based models of habitat connectivity (Murray et al. 2009, Zeller et al. 2012). In effect, such experts are proposing hypotheses about how animals move through landscapes. Genetic data collected from species of interest can be used to evaluate the resulting models by comparing actual patterns of movement—deduced from patterns of gene flow—to the movements predicted by the models. Model parameters can then be revised for greater accuracy. Ultimately, landscape genetic analyses allow the empirical testing of hypotheses about landscape features, whether anthropogenic or natural, that affect animal movement (Cushman et al. 2006, Storfer et al. 2007, Holderegger and Wagner 2008, Spear et al. 2010).

In 2008, we launched the Cascades Carnivore Connectivity Project (CCCP) to harness the potential of noninvasive survey methods and landscape genetic analyses to help advance carnivore conservation in the NCE. More specifically, CCCP was established as a collaboration of researchers from academic institutions, State and Federal agencies, and non-governmental organizations (see Project Collaborators above) interested in evaluating connectivity and barriers for carnivores across the NCE. We identified multiple objectives for this project (see below), and chose the American black bear and the American marten as focal species.

Black bears and martens are both widely distributed in the NCE, but likely respond to habitat fragmentation differently. Black bears are habitat generalists with very large area requirements. Understanding the impacts of roads and other features on the movement of black bears has

significant conservation and management implications. Black bear habitat comprises an interspersion of vegetation types (Rogers and Allen 1987, Schoen 1990), and their seasonal reproductive, denning, and dietary needs (e.g., herbaceous plants, insects, fruits) necessitate extensive animal movement (Schoen 1990, Clark et al. 1993, Lyons et al. 2003, Gaines et al. 2005). Not surprisingly, black bears are one of the principle focal species used in connectivity planning throughout the U.S. (e.g., Dixon et al. 2006, Cushman and Landguth 2012), including Washington (Clevenger et al. 2008, WWHCWG 2010).

In contrast, martens are habitat specialists, preferring mature forest types (Buskirk and Powell 1994, Ruggiero et al. 1994) at moderate to high elevations (Munzing and Gaines 2008). Martens also tend to be sensitive to forest fragmentation (Bissonette et al. 1997, Hargis et al. 1999), making them an excellent focal species for evaluating connectivity among patches of mature forest.

In addition to evaluating habitat connectivity via genetic methods as described above, CCCP also identified the detection of rare carnivores as one of its objectives. In particular, grizzly bears, gray wolves, and Canada lynx are protected under Federal and/or State law, and wolverines are currently a candidate species for listing under the U.S. Endangered Species Act. Wolves have recently recolonized Washington and are rapidly expanding in number and distribution (WDFW 2012). Wolverines, although still very rare, are thought to be expanding their geographic range as well (K. Aubry, USDA Forest Service, unpublished data). Meanwhile, the status of lynx in Washington is poorly understood, with habitat fragmentation considered a primary threat to species persistence (Koehler et al. 2008). Finally, grizzlies are extremely rare and "Endangered" in Washington, with their detection identified as a key objective in the North Cascades Grizzly Bear Ecosystem Recovery Chapter (USFWS 1997). Any data pertaining to animal locations, genetic identification and relatedness, and general proximity to roads and development will contribute to the limited scientific understanding of these species within the NCE.

In summary, CCCP's broad goals were to assess habitat connectivity for carnivores and to advance carnivore conservation in Washington. Our primary objectives were to:

1. evaluate the effects of road characteristics on genetic connectivity for focal carnivores;
2. use landscape genetic methods to identify landscape features that support or inhibit carnivore movement;
3. identify specific fracture zones and potential habitat linkages across them;
4. acquire information about the genetic diversity of surveyed populations;
5. evaluate models developed by the Washington Wildlife Habitat Connectivity Working Group;
6. map occurrences of black bears and martens within the study area;
7. document the presence of grizzlies and other rare carnivores;
8. share study results with relevant agencies, land managers, and conservationists;
9. educate the public about the important ecological role and needs of carnivores.

Below, we report primarily on our findings relating to objectives 1–7. Scientific names for all species mentioned in this report are contained in Appendix 1, and therefore not included in the report body.

## Study Area

The greater NCE includes one of the largest blocks of Federal land in the contiguous U.S., and presents an ideal opportunity for studying landscape connectivity at a very large scale. Our survey sites were distributed throughout the Washington portion of the NCE. Additional black bear samples were acquired from a research effort conducted by the Washington Department of Fish and Wildlife (R. Beausoleil, Department of Fish and Wildlife, unpublished data) during 2005–2009 in the Wenatchee region and the Capitol State Forest at the southern end of Puget Sound (Fig. 1).

The NCE comprises an area of 24,800 km² in Washington, with an additional 10,350 km² extending north into British Columbia (B.C.) (Gaines et al. 2000). In the U.S., 90% of the NCE is managed by the U.S. Forest Service (USFS), the U.S. National Park Service, and the State of Washington, and approximately 41% falls within USFS wilderness and the North Cascades National Park Service Complex (NOCA).

Local vegetation patterns in the NCE are heavily influenced by the region's geological and glacial history. The predominant vegetation zones on the west side of the NCE include western hemlock (*Tsuga heterophylla*), Pacific silver fir (*Abies amabilis*), and mountain hemlock (*Tsuga mertensiana*)(Franklin and Dyrness 1973, Gaines et al. 1994). Subalpine and alpine zones occur throughout the mountainous areas along the crest of the NCE, while east side zones primarily comprise ponderosa pine (*Pinus ponderosa*), grand fir (*Abies grandis*), Douglas-fir (*Pseudotsuga menziesii*), western hemlock, lodgepole pine (*Pinus contorta*), and subalpine fir (*Abies lasiocarpa*)(Franklin and Dyrness 1973, Gaines et al. 1994). The easternmost edge of the NCE largely transitions to shrub-steppe.

Washington's NCE is traversed by three major east-west highways: Interstate 90 (I-90), U.S. Route 2, and State Highway 20 (Fig. 1). I-90 intersects the NCE at Snoqualmie Pass, where growing traffic volumes currently average 27,000 vehicles per day and are increasing by 2.3% per year (WSDOT 2008). Depending on exact location, I-90 in this region is either a divided or joined four-lane highway (i.e., two lanes in each direction) that spans ≥75 m in joined sections and ≥35 m in divided sections. The I-90 corridor has been recognized as a critical link in the north-south movement of wildlife in the NCE (Singleton and Lehmkuhl 2000, Singleton et al. 2002). The highway corridor, which passes through the Okanogan-Wenatchee and Mount Baker-Snoqualmie National Forests, is a National Scenic Byway that also occupies the Upper Yakima River sub-basin east of the Cascade crest and links the dry interior and wet coastal zones of the region.

Given its size and traffic volume, I-90 at Snoqualmie Pass is considered a potential fracture zone for carnivores (Singleton et al. 2002) and ungulates (Shirk et al. 2010). The Washington State Department of Transportation recently began to improve the 15 mile-stretch of I-90 just east of Snoqualmie Pass, with one of its goals being to enhance ecological connectivity via the installation of wildlife crossing structures and fencing (Fig. 2; WSDOT 2008).

**Figure 2.** Visual concept of a wildlife crossing structure on I-90 near Snoqualmie Pass. Photo: WSDOT

Route 2 is a two-lane highway (25–40 m across) and a National Scenic Byway where it passes through the NCE, with an average traffic volume of 3,800 vehicles per day (WSDOT 2009). Ski area development, powerline corridors, and residential development may further affect carnivore movement in the Route 2 corridor. Proposed commercial development will likely lead to an increased residential footprint and pressure to expand the capacity of the highway.

Highway 20 is a two-lane highway (25–40 m across) where it bisects the nearly 900,000 hectares of contiguous wildlands comprising NOCA and the Highway 20 Scenic Byway on the Okanogan-Wenatchee National Forest (Singleton et al. 2002). This remote region provides habitat for some of the most rare and elusive carnivores in the U.S. (e.g., wolverines, grizzly bears), and affords potential transboundary connectivity with carnivores in B.C. When Highway 20 is open, typically April–November, the average traffic volume between Marblemount and Mazama is roughly 1,600 cars/day. In addition, snowmobiles use the road during the winter.

# Methods

The following sub-sections describe our field, genetic, and analytical methods.

## Field Methods

During 2008–2012, we collected genetic material (i.e., DNA) and occurrence data from black bears and martens using a suite of noninvasive hair and scat collection methods. To maximize sampling efficiency, we mapped a tessellation of hexagonal sample units across the study area. Each hexagon comprised 2500 ha, an area slightly smaller than the average home range of a female black bear in this region (Lyons et al. 2003, Koehler and Pierce 2005, Gaines et al. 2005). By focusing a discrete and predefined amount of survey effort on each sample unit and then shifting efforts to a new unit, we were able to efficiently collect DNA samples from as many individuals as possible while minimizing redundant sampling (i.e., capturing hair from the same individual at multiple sites) and associated field and laboratory costs.

A general rule of thumb suggests that DNA be collected from a minimum of 20–30 individuals from each side of a putative barrier to test for barrier effects (D. Paetkau [Wildlife Genetics International] and S. Kalinowski [Montana State University], personal communications). We initially focused our survey efforts on locations adjacent to the three major highways traversing the study area, as the evaluation of highway effects on carnivore connectivity was a primary goal of our research. We ultimately expanded our surveys beyond the highway corridors in an attempt to collect a geographically well-distributed set of samples from across the study area, allowing us to test additional hypotheses about factors that might influence black bear gene flow.

For bears, we deployed two barbed wire corral-type hair snares (Kendall and McKelvey 2008) within each sample unit. Corrals comprised a single strand of barbed wire stretched around four or more trees at a height of 45–50 cm, with one liter of liquid scent lure (i.e., cattle blood and fish oil) poured onto a pile of woody debris in the center of the corral (Fig. 3). Corrals were revisited at 14 days and (1) removed if a sufficient sample was present or (2) rebaited and left for another 14 days if no sample was present. We attempted to locate corrals approximately 2.5–3.0 km apart within a given sample unit.

We collected hair samples from martens with .32 caliber bronze gun cleaning brushes attached to a tree-mounted enclosure baited with chicken and scent lure (Fig. 4). We placed marten devices selectively—usually in groups of two or three spaced at approximately 100 m intervals—in habitat characteristic of martens (i.e., mature forest). After conducting unsuccessful marten surveys during the summers of 2008–2009, we shifted to sampling in winter—when this species appears to be more generally attracted to survey stations (R. Long, unpublished data). During the winter of 2011/12, we enhanced our capacity to conduct marten surveys by enlisting and training roughly two dozen volunteers from Conservation Northwest (I-90 region) and the North Cascades Institute (Highway 20 region) to deploy tree-mounted hair-snagging cubbies.

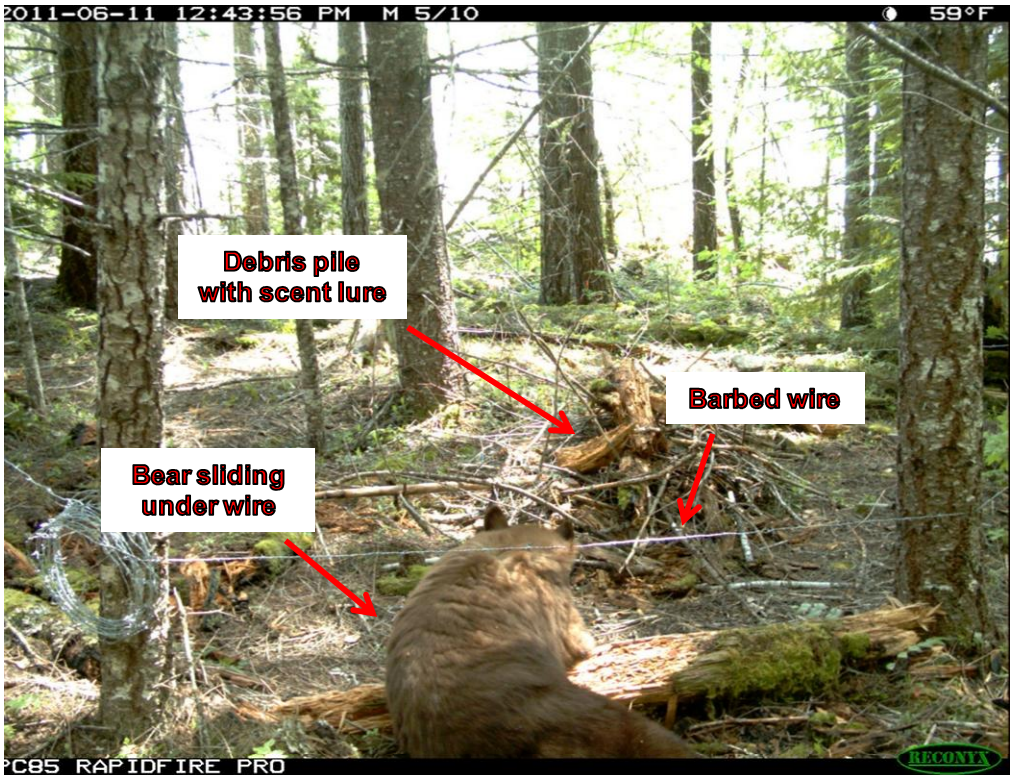



**Figure 3**. Corral-type hair-snagging station for bears, showing barbed wire and debris pile, with black bear sliding under the wire. Photo: WTI

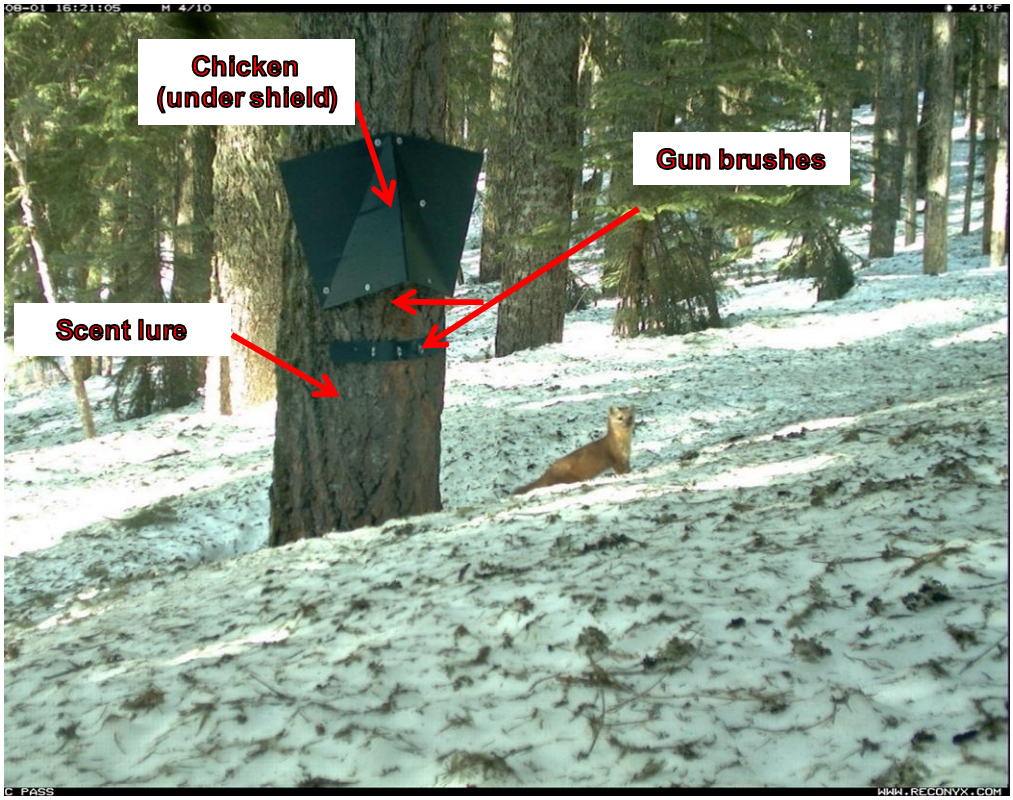



**Figure 4**. Cubby-type hair-snagging station for martens, showing locations for scent lure, bait, and gun brushes, with marten at base of tree. Photo: WTI

We assigned unique sample numbers to hairs collected on a given barb or gun cleaning brush, and then placed them in paper envelopes and stored them with desiccant. In the lab, we subsampled hair samples from each site based on hair quality (e.g., the presence of follicles), the number of hairs in a clump (e.g., single-hair samples were generally discarded unless they represented the only sample at the site), and proximity to other samples snagged by the device (e.g., the best of two adjacent samples was generally selected). We then sent subsamples to Wildlife Genetics International (WGI; Nelson, B.C.) for analysis. Subsampling helped to increase the likelihood that sufficient DNA was obtained from as many samples as possible while at the same time minimizing costs that would have been incurred had all samples been analyzed (Kendall and McKelvey 2008).

During summer/fall 2008, we employed a scat detection dog survey team (Working Dogs for Conservation, Three Forks, MT) to conduct 15 pilot surveys in the region adjacent to I-90 near Snoqualmie Pass. In summer/fall 2009, we employed another detection dog team from the same organization to conduct 20 surveys along Highway 20 in the NCE. Detection dogs have been shown to facilitate the rapid detection of scats from carnivores and other taxa (Long et al. 2007, MacKay et al. 2008). The objective of the pilot effort was to evaluate whether: (1) detection dogs could be an efficient method for collecting DNA samples from the target species in our study area; and (2) DNA extracted from scats would be of sufficient quality and quantity for our landscape genetic analyses. Each survey was carried out by a professional dog handler, an orienteer from our project, and a dog. Dogs were trained to detect scats from target species and other rare carnivores (i.e., wolves, grizzly bears, wolverines, cougars, fishers), and to alert their handler to the specific location of each scat (Fig. 5). We conducted transect-based surveys both on- and off–trail, and dried or froze those scat samples that were collected until they could be analyzed by WGI.

**Figure. 5.** A scat detection dog alerts her handler to a marten scat (foreground) near the I-90 corridor. Photo: P. MacKay/WTI

We deployed digital remote cameras at a subset of survey stations to: (1) detect rare carnivores not detected with our hair-snagging devices; (2) collect additional black bear and marten detection data; (3) gather information about animal behavior and interactions with hair-snagging devices; and (4) attain photos for outreach and educational purposes.

**Genetic Methods**

WGI extracted, amplified, and analyzed DNA with standard procedures (Woods et al. 1999, Paetkau 2003, Roon et al. 2005b). More specifically, DNA was extracted from hair and tissue samples with QIAGEN spin columns and *DNeasy Tissue* kits, and from fecal samples with *DNeasy Stool* kits. WGI analyzed all samples containing 1 or more guard hair follicle(s) or 5 underfur hairs, and up to 10 guard hairs plus underfur when available. Mixed samples (i.e., samples with hair from >1 bear) were reliably identified by evidence of ≥3 alleles at ≥1 locus (Roon et al. 2005a).

To assign individual identification for bears, WGI selected seven microsatellite markers that were known to be highly variable for black bears in northern Idaho. After evaluating genetic variability in the study population, WGI then selected six markers with exceptionally high heterozygosity (G10B, G10H, G10J, G10L, Mu23, Mu59; Paetkau et al. 1995) for use in identifying individuals. WGI did not attempt to assign individual identity to any sample that failed to produce strong, typical, diploid (i.e., not mixed) genotype profiles at all six markers. This strict rejection of all samples whose genotypes featured weak, missing, or suspect data (e.g., unbalanced peak heights) should have dramatically reduced genotyping error by eliminating the most error-prone samples.

WGI attempted to analyze 14 additional loci (G1D, G10C, G1A, G10M, G10P, CXX110, CXX20, MSUT-6, 145P07, CPH9, D123, D1a, G10O, MSUT-2) from each individual bear to enable the assessment of gene flow and barrier effects. Sex was determined by the amelogenin marker, which varies in length by sex (Ennis and Gallagher 1994).

Genotyping errors that result in the creation of “false individuals”, such as allelic dropout and amplification errors, can bias mark–recapture population estimates (Mills et al. 2000, Roon et al. 2005b). WGI used selective reanalysis of similar genotypes to detect and eliminate errors. Reanalysis consisted of replicating genotypes for all: (1) individuals identified in a single sample; (2) pairs of individuals that differed at only 1 or 2 loci (i.e., 1- and 2-mismatch pairs); and (3) pairs of individuals that differed at 3 loci when those differences were consistent with allelic dropout (i.e., homozygous).

WGI took a similar approach for genotyping marten samples, but with a single run (versus the two-step process for bears) of 10 loci (Ma10, MP0085, MP0114, MP0197, Ma7, MP0182, Ma9, MP0055, MP0227, MP0059) analyzed for each sample. Sex determination for martens was accomplished by analyzing an intron in the ZFX and ZFY genes, whose length is sex-specific (D. Paetkau, Wildlife Genetics International, personal communication).

## Landscape Genetic Analysis

Population-Based Genetic Clustering Analyses

We used multiple approaches to detect population-level genetic structure in our target species. More specifically, we employed a Bayesian model-based clustering method (STRUCTURE 2.3; Pritchard et al. 2000) to infer numbers of populations and to quantify admixture (i.e., interbreeding among populations) based only on multi-locus genotype data and without knowledge of sample origin. Additionally, we used a method that employs both genotype data and sample locations to assess population membership (Geneland 3.1, Guillot et al. 2005).

We used STRUCTURE to infer the number of genetically distinct clusters (i.e., subpopulations; K) in our dataset (Falush et al. 2003). We analyzed samples collected in the NCE both separately and together with samples collected from the Capitol Forest region. The unequal distribution of samples across geographic space can obscure analyses of population structure (D. Paetkau, Wildlife Genetics International, personal communication). We therefore pooled the Capitol Forest samples with those from only the I-90 region of the NCE, resulting in a dataset that was more balanced in terms of sample size and distribution relative to potential barriers to gene flow.

STRUCTURE 2.3 (Pritchard et al. 2000) uses Bayesian methods to infer the most likely number of populations sampled, and assigns individuals to populations by minimizing Hardy–Weinberg and linkage disequilibrium within populations. The program further employs Markov chain Monte Carlo (MCMC) simulations to estimate the posterior probability that the data fit the hypothesis of K populations. STRUCTURE allows for some degree of admixture to occur in the analysis, a more realistic scenario when populations do not meet the Hardy-Weinberg assumptions of discrete, panmictic subpopulations and no migration. We ran STRUCTURE with a burn-in of 25,000 iterations and 50,000 MCMC repetitions, assuming admixture and correlated allele frequencies among clusters. We ran 20 repetitions with K varying from 2–5, and combined each set of 20 results using CLUMPP (Jakobsson and Rosenberg 2007). Lastly, we used both a visual assessment of the results and the method of Evanno et al. (2005) to infer the number of genetic clusters (K) in the dataset.

We also assessed population structure using the program Geneland 3.1.3 (Guillot et al. 2005a; Guillot et al. 2005b) as a complement to STRUCTURE. Like STRUCTURE, Geneland performs Bayesian inference with a MCMC simulation and a population model based on minimizing Hardy-Weinberg and linkage disequilibrium. Unlike STRUCTURE however, Geneland allows for parameterizations that do not include admixture, and therefore can provide information about individuals that may have been sampled in locations that differed from their place of origin. We ran 100,000 MCMC repetitions using the uncorrelated allele frequency model, which has been shown to perform better at estimating K than the correlated allele model (Guillot et al. 2005a).

For each genetic population (K) identified by analyses conducted with STRUCTURE and Geneland, we used FSTAT version 2.9.3.2 (Goudet et al. 2002) to calculate observed and expected heterozygosities, mean numbers of alleles, allelic richness, and inbreeding coefficients. We also explored deviations from Hardy–Weinberg and linkage equilibrium using exact tests in the program GENEPOP version 4.2 (Raymond and Rousset 1995). We adjusted results for

multiple comparisons using a sequential Bonferroni correction (Rice 1989).

Individual-Based Analyses

Population-based analyses attempt to detect discrete genetic differences among K populations, but are less effective at detecting continuous population structuring that might be expected when populations are isolated by distance (IBD; Wright 1943) or resistance (McRae 2006). To examine the evidence for continuous population structure, we used an individual rather than population-based approach. We evaluated the correlation between measures of individual pairwise genetic distance (Legendre and Legendre 1998) and landscape distance, given a range of plausible models representing how landscape features (e.g., highways, elevation, habitat type, topographic ruggedness) might resist gene flow. The landscape models exhibiting the strongest correlation with the empirical genetic data are assumed to reflect features that have most influenced gene flow in the study area.

To calculate genetic distances, we used three methods. First, we calculated the proportion of shared alleles (Dps; Bowcock et al. 1994) and Rousset's *a* (Rousset 2000) using SPAGeDi 1.4 (Hardy and Vekemans 2002). We also calculated a principal components analysis (PCA) based distance (Shirk et al. 2010) using a genetic data matrix **G** with *n* rows x *m* columns, where *n* is the number of individuals in the analysis and *m* is the number of alleles present within the population (*m* = 20 for bears, 12 for martens). Each element in the matrix **G**(*i*,*j*) was populated for individual *i* by the number of occurrences for the *j*th allele. We then computed the eigenvectors of **G** in R 2.12 (R Development Core Team 2011). Finally, we used the Ecodist package in R 2.11 (Goslee & Urban 2007) to generate an *n* x *n* pairwise genetic distance matrix (Y) based on the distance between individuals along the first eigenvector (Patterson et al. 2006).

We developed models (i.e., GIS raster layers) of landscape resistance using the Spatial Analyst toolbox in ArcGIS (ESRI 2003). These models represented the various hypotheses pertaining to how landscape features—or combinations of features—might have affected gene flow. The models were created by adding reclassifying raster layers based on resistance parameters, and then adding the layers together into a single surface that represented the cumulative resistance of all input layers.

The 40 models developed for black bears consisted of one or more of the following feature types: roads and highways, human activities, greenness, and topographic complexity (Table 1). Further, as a model-evaluation exercise, we included the Washington Connected Landscapes Project (WA Connected Landscapes Project; WWHCWG 2010) statewide black bear connectivity model (Table 2), and a number of variants of this model, in the set of candidate models (Table 1). Because gene flow tends to decrease with increasing geographic distance even when landscape features are not impeding movement—a process known as isolation-by-distance (IBD; Wright 1943)—we also included an IBD null model that represented landscape distances as simple Euclidian (i.e., straight-line) distances among sample locations. Finally, we included a null model (IBB) representing a north-south barrier to gene flow north of Route 2 based on the results of our genetic clustering analysis. We provide more details in the *Results* section below.

We developed a second set of 24 candidate models for martens, which included many of the same feature types we tested for black bears but with marten-specific resistance values (Table 3). As with bears, we included the WA Connected Landscapes Project (WWHCWG 2010) statewide marten connectivity model (Table 2), variants of this model, and an IBD null model.

To generate pairwise landscape distances, we used the ArcGIS COSTDISTANCE function (ESRI 2003) to calculate least-cost distances between all pairs of bear or marten sample locations based on each model's resistance surface.

To evaluate each model, we used a formal, causal modeling framework (Cushman et al. 2006, 2013). This framework uses rule-based hypothesis testing to identify and select models that capture the most information from an underlying dataset. In our case, we wished to identify those models that best described the underlying genetic structure for each species. The causal modeling framework uses Mantel and partial Mantel's tests (Mantel 1967) implemented in the R software package Ecodist (Goslee & Urban 2007) to calculate the correlation between genetic and landscape distance for each model. This method also permits the testing of one model, while "partialling out" (i.e., controlling for) the effect of one or more other models. In this way, models containing the true drivers of a particular process (e.g., gene flow) can be identified. For example, it is a common practice to partial out the effects of IBD from a given model to evaluate the model after IBD has been controlled for.

We evaluated the relationship between each resistance model and genetic structure by first partialling out the effects of the null models—IBD and IBB—from the resistance models. We considered any resistance models that continued to show significant relationships to the genetic structure candidate models for the next step, which entailed partialling out the effects of each resistance model from the null models. During this step, we expected to observe a non-significant result for supported resistance models; i.e., once the effects of a given resistance model were partialled out from the effects of the null model, a non-significant result would indicate that the null model retained no explanatory power and thus that the resistance model was supported. In contrast, a significant result would indicate that, once the information from the resistance model was partialled out, the null model (versus the resistance model) was the actual factor explaining the observed structure.

We also used an adapted approach (Cushman et al. 2013) designed to address some of the limitations of partial-Mantel tests (see *Criticisms of Mantel-Based Approaches* in *Discussion* below). That is, instead of simply partialling out the null models from each resistance model, and each resistance model in turn from the null models, we also evaluated each resistance model by partialling out its effects from those of other resistance models that still met all the causal requirements (Cushman et al. 2013).

Under the complete causal modeling framework, therefore, a resistance model would be considered causal if it (1) explained much of the genetic structure (i.e., was significant at $p < 0.05$) after having the effects of IBD and IBB partialled from it; (2) achieved a non-significant result after being partialled *from* the IBD and IBB null models; and (3) remained significant after any competing models had been partialled from it.

**Table 1**. Black bear resistance models (including model type, name, code, and description). Primary models are noted in bold, with variations on primary models falling below them in standard font. WWHCWG refers to a model developed by the Washington Wildlife Habitat Connectivity Working Group. The terms "lower" and "higher" in the description column refer to levels of resistance, where "lower" signifies more permeability to gene flow. "Buffer" is a zone adjacent to roads and highways and is designed to address animal avoidance of roads within a specified distance. "Greenness" is a measure of plant biomass (i.e., primary productivity).

| Type | Model Name | Model Code | Description ("lower" and "higher" refer to levels of resistance) |
|---|---|---|---|
| **WWHCWG** | **WWHCWG_D** | **SW_D** | **WA Habitat Connectivity Working Group black bear model (Default)** |
| WWHCWG | WWHCWG_Alt1 | SW1 | WWHCWG black bear model; No road buffer resistance |
| WWHCWG | WWHCWG_Alt2 | SW2 | WWHCWG black bear model; Lower road buffer resistance |
| WWHCWG | WWHCWG_Alt3 | SW3 | WWHCWG black bear model; Lower road and road buffer resistance |
| WWHCWG | WWHCWG_Alt4 | SW4 | WWHCWG black bear model; Much lower road resistance, lower road buffer resistances |
| WWHCWG | WWHCWG_Alt5 | SW5 | WWHCWG black bear model; Higher road and road buffer resistance |
| WWHCWG | WWHCWG_Alt6 | SW6 | WWHCWG black bear model; Lower I-90 resistance |
| WWHCWG | WWHCWG_Alt7 | SW7 | WWHCWG black bear model; Much lower I-90 resistance |
| WWHCWG | WWHCWG_Alt8 | SW8 | WWHCWG black bear model; Lower non-I-90 resistance |
| WWHCWG | WWHCWG_Alt9 | SW9 | WWHCWG black bear model; Equal wet/dry forest resistance |
| WWHCWG | WWHCWG_Alt10 | SW10 | WWHCWG black bear model; Lower water resistance |
| WWHCWG | WWHCWG_Alt11 | SW11 | WWHCWG black bear model; Higher >1500m resistance |
| WWHCWG | WWHCWG_RND2_Alt2 | RND2_SW2 | WWHCWG black bear model; plus greenness |
| WWHCWG | WWHCWG_RND2_Alt3 | RND2_SW3 | WWHCWG black bear model; plus slope (linear) |
| WWHCWG | WWHCWG_RND2_Alt4 | RND2_SW4 | WWHCWG black bear model; plus slope (linear) & higher >1500m resistance |
| **WWHCWG** | **WWHCWG Landscape Integrity** | **LI** | **WWHCWG Landscape Integrity model (medium)** |
| **Hwy** | **HWYGRAD_D** | **HWG_D** | **Highways gradation (Default)** |
| Hwy | HWYGRAD_Alt1 | HWG1 | Highways gradation; less resistance for all highways |
| Hwy | HWYGRAD_Alt2 | HWG2 | Highways gradation; much less resistance for all highways |
| Hwy | HWYGRAD_Alt3 | HWG3 | Highways gradation; less resistance for all highways, with some road buffer resistance |
| Hwy | HWYGRAD_RND2_Alt2 | RND2_HWG2 | Highways gradation; much less I-90 resistance |
| Hwy | HWYGRAD_RND2_Alt3 | RND2_HWG3 | Highways gradation; less major hwy resistance |
| Hwy | HWYGRAD_RND2_Alt4 | RND2_HWG4 | Highways gradation; much less major hwy resistance |
| Hwy | HWYGRAD_RND2_Alt5 | RND2_HWG5 | Highways gradation; less I-90/major hwy resistance |
| Hwy | HWYGRAD_RND2_Alt6 | RND2_HWG6 | Highways gradation; much less I-90/major hwy resistance |
| Hwy | HWYGRAD_RND2_Alt7 | RND2_HWG7 | Highways gradation; almost no highway resistance |

| | | | |
|---|---|---|---|
| Hwy | HWYGRAD_RND2_Alt8 | RND2_HWG10 | Highways gradation; less resistance, with road buffer resistance; greenness |
| Hwy | HWYGRAD_RND2_Alt9 | RND2_HWG11 | Highways gradation; less resistance, with road buffer resistance; greenness; slope |
| Hwy | HWYGRAD_RND2_Alt10 | RND2_HWG12 | Highways gradation; less resistance, with road buffer resistance; greenness; slope; elevation |
| I-90 | I-90HIGH | I90H | I-90 high resistance; other highways no effect |
| I-91 | I-90MED | I90M | I-90 medium resistance; other highways no effect |
| I-92 | I-90LOW | I90L | I-90 low resistance; other highways no effect |
| I-90 | I-90Breaks | I90B | I-90 medium resistance but with North Bend "bear crossing" location as low resistance |
| **Human** | **Human Activities** | **HA_D** | **Urban/developed high** |
| Human | Human Activities_Alt1 | HA1 | Gradation of road resistance (I-90>Major>Secondary, Local rd. density), urban/developed high |
| Human | Human Activities_Alt2 | HA2 | Gradation of road resistance (Alt1) (I-90>Major>Secondary, Local rd. density), urban/developed high |
| **Landcover** | **Greenness** | **GREEN1** | **Greenness; greener = lower resistance; linear resistance** |
| **Landcover** | **Greenness** | **GREEN2** | **Greenness; greener = lower resistance; power resistance** |
| **Topo** | **Slope** | **SLOPE1** | **Slope; linear resistance** |
| **Topo** | **Slope** | **SLOPE2** | **Slope; power resistance** |

**Table 2**. Resistance values for each layer and attribute of the model developed by the Washington Wildlife Habitat Connectivity Working Group (WWHCWG 2010). This model was used as the default model for many candidate models in our analysis.

| | Species | | | Species | |
|---|---|---|---|---|---|
| **Layer** | **Black Bear** | **Marten** | **Layer** | **Black Bear** | **Marten** |
| Land cover/land-use | | | Housing density (acres per dwelling unit) | | |
| agriculture | 100 | 100 | > 80 | 0 | 0 |
| urban/developed | 200 | 200 | > 40 ≤ 80 | 10 | 5 |
| water | 100 | 100 | > 20 ≤ 40 | 10 | 10 |
| sparsely vegetated | 1 | 50 | > 10 ≤ 20 | 10 | 15 |
| alpine | 0 | 5 | < 10 | 100 | 50 |
| riparian | 0 | 0 | Road type and distance (meters)* | | |
| wetland | 0 | 1 | freeway > 500–1000 buffer | 10 | 1 |
| grass-dominated | 1 | 50 | freeway > 0–500 buffer | 50 | 50 |
| shrub-dominated | 1 | 50 | freeway centerline | 1000 | 1000 |
| dry forest | 1 | 10 | major highway > 500–1000 buffer | 5 | 1 |
| wet forest | 0 | 0 | major highway > 0–500 buffer | 10 | 10 |
| Elevation (meters) | | | major highway centerline | 100 | 100 |
| 0–250 | 5 | 5 | secondary highway > 500–1000 buffer | 4 | 1 |
| > 250–500 | 5 | 5 | secondary highway > 0–500 buffer | 8 | 6 |
| > 500–750 | 4 | 5 | secondary highway centerline | 50 | 50 |
| > 750–1000 | 3 | 5 | local road > 500–1000 buffer | 1 | 1 |
| > 1000–1500 | 2 | 2 | local road > 0–500 buffer | 2 | 1 |
| > 1500–2000 | 1 | 1 | local road centerline | 3 | 1 |
| > 2000–2500 | 0 | 1 | Forest structure (density and height [meters]) | | |
| > 2500–3300 | 1 | 1 | nonforest | 1 | 10 |
| > 3300 meters | 100 | 100 | sparse low (0–40%, ≤ 25) | 0 | 7 |
| Slope (degrees) | | | sparse high (0–40%, > 25) | 0 | 7 |
| 0–20 | 0 | 0 | open low (> 40–70%, ≤ 25) | 0 | 5 |
| > 20–40 | 1 | 1 | open high (> 40–70%, > 25) | 0 | 5 |
| > 40 | 3 | 3 | dense low (> 70–100%, ≤ 25) | 0 | 1 |
| | | | dense high (> 70–100%, > 25) | 0 | 0 |

**Table 3**. Marten resistance models (including model type, name, code, and description). Primary models are noted in bold, with variations on primary models falling below them in standard font. WWHCWG refers to a model developed by the Washington Wildlife Habitat Connectivity Working Group. The terms "lower" and "higher" in the description column refer to levels of resistance, where "lower" signifies more permeability to gene flow. "Buffer" is a zone adjacent to roads and highways and is designed to address animal avoidance of roads within a specified distance. "Greenness" is a measure of plant biomass (i.e., primary productivity).

| Type | ModelName | Model Code | Description ("lower" and "higher" refer to levels of resistance) |
|---|---|---|---|
| **WWHCWG** | **WWHCWG_D** | **SW_D** | **WWHCWG marten model (Default)** |
| WWHCWG | WWHCWG_Alt1 | SW1 | WWHCWG marten model; no road buffer resistance |
| WWHCWG | WWHCWG_Alt3 | SW3 | WWHCWG marten model; lower road buffer resistance |
| WWHCWG | WWHCWG_Alt2 | SW2 | WWHCWG marten model; lower road and road buffer resistance |
| WWHCWG | WWHCWG_Alt4 | SW4 | WWHCWG marten model; lower I-90 resistance |
| WWHCWG | WWHCWG_Alt5 | SW5 | WWHCWG marten model; much lower I-90 resistance |
| WWHCWG | WWHCWG_Alt6 | SW6 | WWHCWG marten model; higher road and road buffer resistance |
| WWHCWG | WWHCWG_Alt7 | SW7 | WWHCWG marten model; lower road resistance, dry forest lower |
| WWHCWG | WWHCWG_Alt8 | SW8 | WWHCWG marten model; lower road resistance; Structure less weight |
| WWHCWG | WWHCWG_Alt9 | SW9 | WWHCWG marten model; lower road resistance, Structure less weight |
| WWHCWG | WWHCWG_Alt10 | SW10 | WWHCWG marten model; lower water resistance |
| WWHCWG | WWHCWG_Alt11 | SW11 | WWHCWG marten model; lower high elevation resistance |
| WWHCWG | WWHCWG_Alt12 | SW12 | WWHCWG marten model; less elevation effect |
| **WWHCWG** | **WWHCWG Landscape Integrity** | **LI** | **WWHCWG Landscape Integrity model (Medium)** |
| Hwy | HWYGRAD_D | HWG_D | Highways gradation (Default) |
| Hwy | HWYGRAD_Alt1 | HWG1 | Highways gradation; less resistance for all highways |
| Hwy | HWYGRAD_Alt2 | HWG2 | Highways gradation; much less resistance for all highways |
| Hwy | HWYGRAD_Alt3 | HWG3 | Highways gradation; less resistance for all highways, with road buffer resistance |
| **Human** | **Human Activities** | **HA_D** | **Urban/developed high** |
| Human | Human Activities_Alt1 | HA1 | Gradation of road resistance; (I-90>Major>Secondary, Local rd. density), urban/developed high |
| Human | Human Activities_Alt2 | HA2 | Gradation of road resistance; (I-90>Major>Secondary, Local rd. density); I-90 higher, urban/developed high |
| **Landcover** | **Landcover_D** | **LC_D** | **Landcover from WWHCWG** |
| Landcover | Landcover_Alt1 | LC1 | Landcover from WWHCWG with lower water resistance |
| **Region** | **Cascade Crest divide** | **CCDIV** | **East/west model** |

# Results

Here, we describe the results of our survey activities and the outcomes of our genetic clustering and individual-based landscape genetic analyses.

**Survey Results**

Black Bears

We deployed 529 corrals throughout the NCE over the course of four field seasons (2008–2011). A total of 2,866 hair samples were collected from these corrals, and 561 individual black bears (268 males, 292 females, 1 sex undetermined) were identified via microsatellite analysis (Table 4, Fig. 6). We detected no grizzly bears, despite our having deployed corrals that were designed to sample both black bears and grizzlies.

**Table 4**. Summary by year of corrals deployed, samples collected and sent for DNA analysis, black bear individuals identified, and sites that included remote cameras. The number of bears identified from tissue samples, collected as part of a separate study, is also reported.

| | 2008 | 2009 | 2010 | 2011 | From Tissue | Total |
|---|---|---|---|---|---|---|
| Corrals deployed | 45 | 98 | 192 | 194 | | 529 |
| Hair samples collected | 184 | 180 | 1,208 | 1,294 | | 2,866 |
| Hair samples sent for analysis | 116 | 153 | 707 | 767 | | 1,743 |
| Black bears identified | 42 | 38 | 216 | 226 | 38 | 561* |
| Corrals with remote cameras | 2 | 12 | 50 | 98 | | 152 |

*This table includes 6 individuals genotyped via scats, and 18 individuals genotyped via hairs collected incidentally or by hair-snagging devices primarily intended for martens.

In sum, genetic testing confirmed black bears at 55% of our corral sites (289 of 529 total sites).

At sites where we individually genotyped bears, we averaged 3.5 genotyping analyses per corral (MIN = 1, MAX = 15) to identify an average of 1.7 unique individuals per corral (MIN = 1, MAX = 6). Only fifty of the 561 individuals (8.9%) were sampled at >1 corral within a given field season.

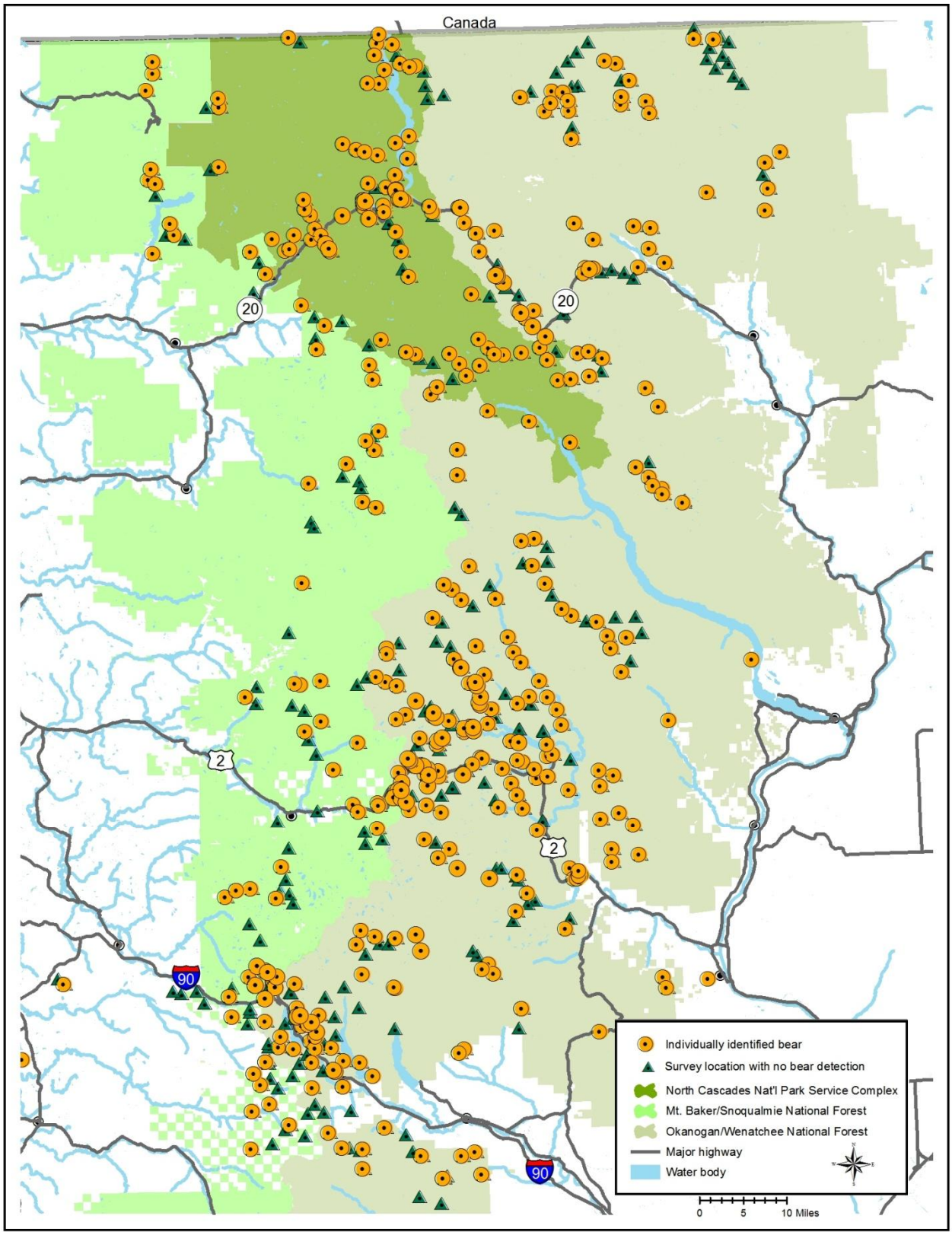



**Figure 6**. Map showing locations of all corral survey sites (dark triangles and orange circles) and individual black bears identified (orange circles) during this study. Note that this map does not include tissue samples collected from the Capitol Forest region.

Martens

We deployed 417 cubbies during the four-year (2008–2011) study period, primarily along the I-90, Route 2, and Highway 20 corridors. Hair samples collected at these cubbies enabled the genetic identification of 71 individual martens (Table 5, Fig. 7). Only one individual—a male—was identified south of I-90. We were also able to genetically identify two martens killed in wildlife-vehicle collisions along Highway 20.

**Table 5**. Summary by year of cubbies deployed, samples collected and sent for analysis, marten individuals identified, and sites that included remote cameras.

| | **2008–09** | **2009–10** | **2010–11** | **2011–12** | **Total** |
|---|---|---|---|---|---|
| Cubbies deployed | 62 | 100 | 180 | 75 | 417 |
| Hair samples collected | 60 | 252 | 317 | 208 | 837 |
| Hair samples sent for analysis | 46 | 144 | 36 | 71 | 297 |
| Martens identified | 3 | 30 | 23 | 17 | 73 |
| Cubbies with remote cameras | 17 | 6 | 16 | 34 | 73 |

Across all regions, we detected martens at 17% of cubby sites (71 of 417 total sites). Notably, our detection rate south of I-90 was only 0.7% (1 of 134 total sites).

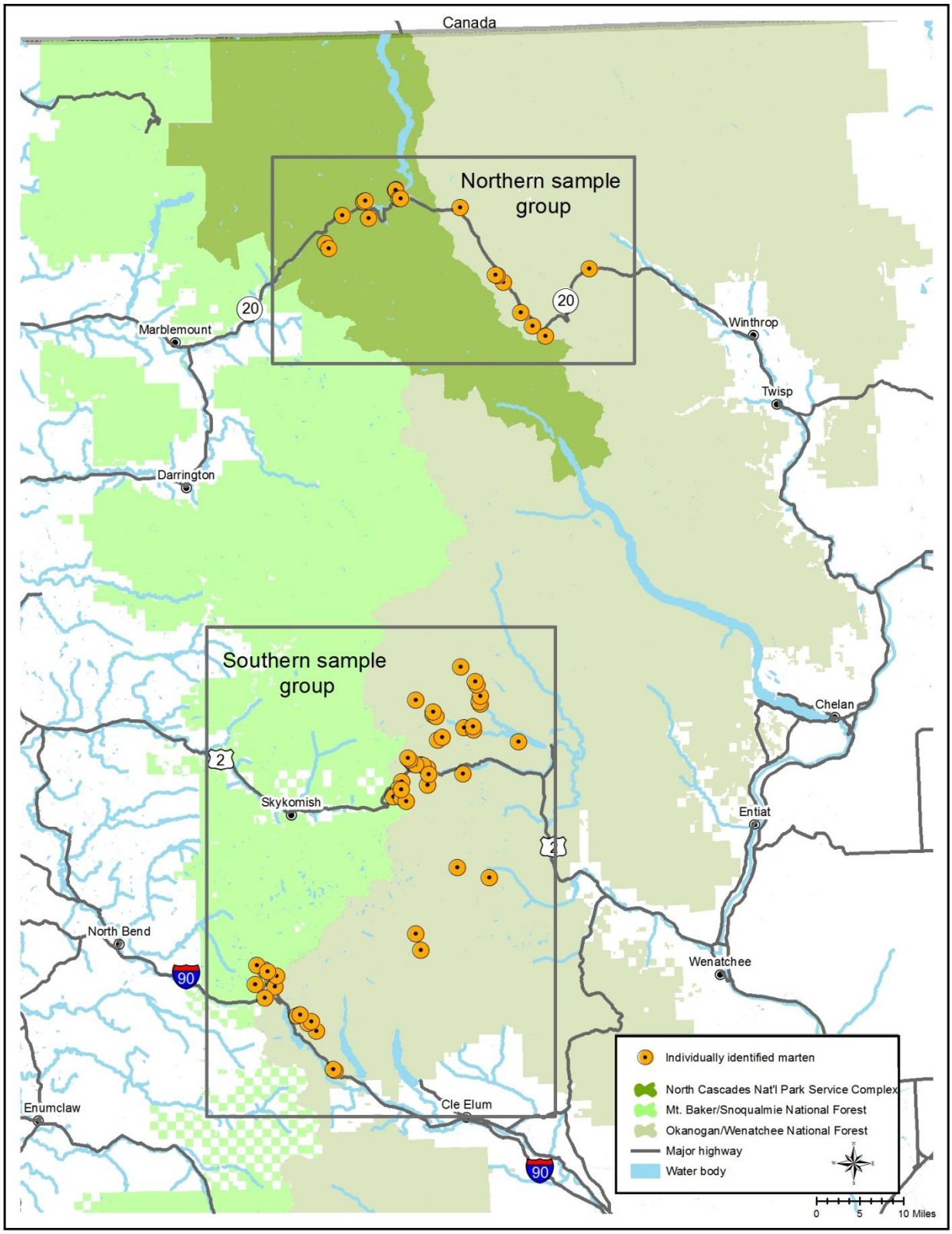



**Figure 7**. Map showing locations of all survey sites (dark triangles and orange circles) and individual martens identified (orange circles) during surveys conducted 2008–2012. Northern and southern sample groups, parsed out for our analysis (below), are also identified.

Scat Detection Dogs

Survey teams equipped with a detection dog were successful at locating black bear scats in the steep, difficult terrain characteristic of the NCE. We collected 37 black bear scats in the I-90 region during 15 pilot surveys conducted in 2008, with 6 of these contributing to the pool of 561 black bear individuals identified for this study. We collected an additional 250 scat samples during 20 additional surveys in the Highway 20 region during summer 2009, but relatively poor success in genetic testing of scats over both years led us to conclude that black bear scats were an inadequate source of DNA for our landscape genetic analyses. This field method was thus discontinued in subsequent years.

In 2008 and 2009, detection dogs contributed to the collection of only three confirmed marten scats. This small number of samples may have reflected the dogs' lack of experience with martens, the challenges associated with detecting small, cryptic scats in our thickly vegetated study area, limited marten presence, or a combination of these factors.

Species Occurrence

We deployed remote cameras at 152 corral sites and 73 cubby sites (Fig. 8) totaling 3,980 and 2,854 camera survey nights at corrals and cubbies, respectively. In addition to black bears and martens (Figs. 9, 10), our hair-snagging devices and remote cameras detected a number of rare species, including gray wolves, wolverines, Canada lynx, and moose (Fig. 11), as well as more common species such as cougars, coyotes, bobcats, deer, and elk (Fig. 12). We detected no grizzly bears at camera sites. In total, 21 species and 2 additional species groups (i.e., birds, small mammals) were detected at our survey sites (Table 6).

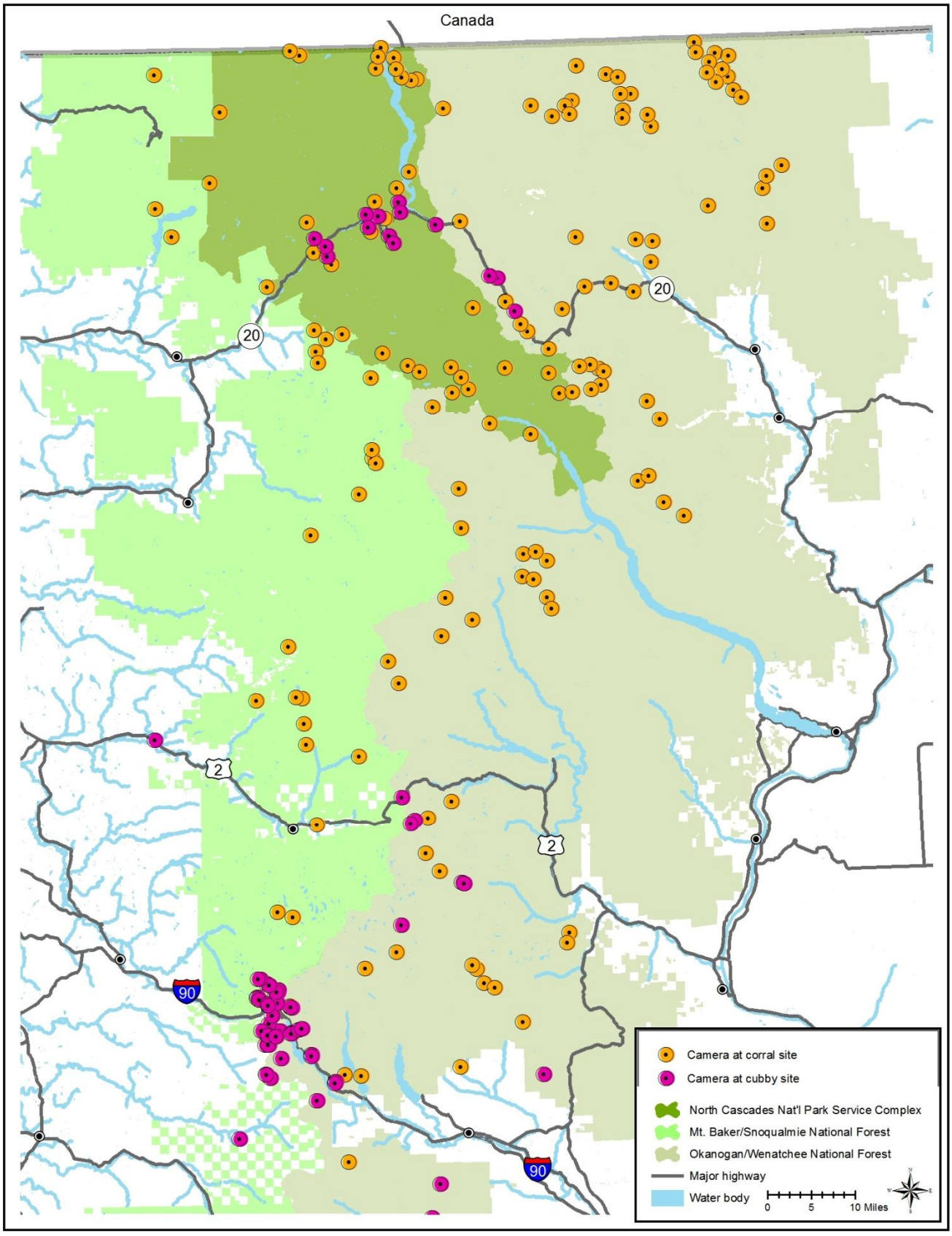
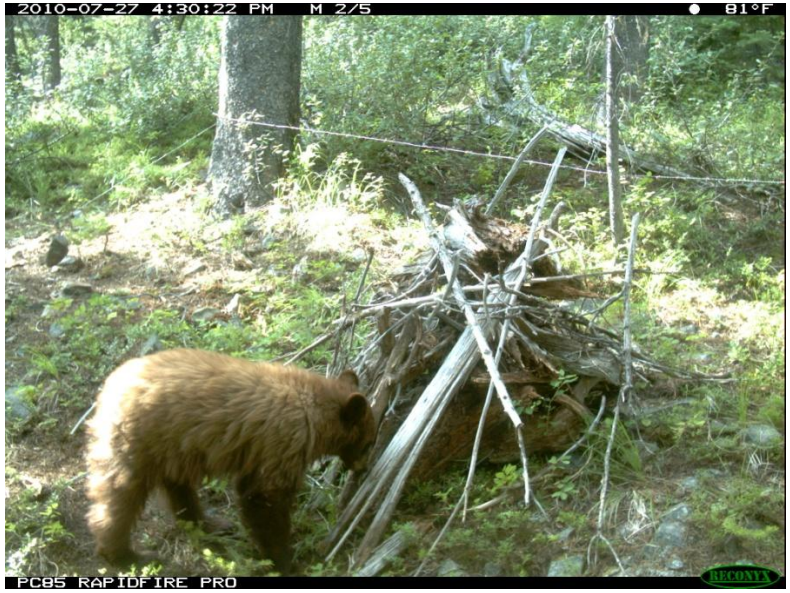



**Figure 8**. Map showing locations of corral and cubby sites where remote cameras were also deployed.

**Table 6**. Species (or species groups) confirmed by remote cameras, DNA extracted from hair or scat, and/or animal tracks.

| Species |
|---|
| American black bear |
| American marten |
| Bird |
| Bobcat |
| Canada lynx |
| Cougar |
| Coyote |
| Deer (mule and black-tailed) |
| Domestic dog |
| Douglas squirrel |
| Elk |
| Gray wolf |
| Hoary marmot |
| Moose |
| Mountain beaver |
| Northern flying squirrel |
| Small mammal (mice, voles, shrews, and chipmunks) |
| Snowshoe hare |
| Short-eared owl |
| Short-tailed weasel |
| Wolverine |

A. 


B. 


C. 


D. 


**Figure 9**. Photos of black bears captured at remote camera/hair-snagging stations.

A. 


B. 

C. 


D. 

**Figure 10**. Photos of martens captured at remote camera/hair-snagging stations.

A. 


B. 


C. 

D. 


**Figure 11.** Photos of rare species captured at remote camera/hair-snagging stations: (A, B) Canada lynx from the Pasayten and Chewuch regions; (C) a gray wolf from the Pasayten region; and (D) a moose near Rainy Pass.

A. 


B. 

C. 


D. 


**Figure 12.** Photos of (A) two cougars; (B) a bobcat; (C) a coyote; and (D) an elk captured at remote camera/hair-snagging stations.

During summer 2012, we collected photos and wolverine hairs from a corral site near the Glacier Peak Wilderness (Fig. 13). These samples were of particular interest to wolverine biologists, as they represented one of the first documented occurrences of this species on the west side of the Cascade crest. Genetic analyses of the hair samples confirmed this individual to be a previously undocumented male wolverine.


**Figure 13**. A wolverine visits a bear corral near the Glacier Peak Wilderness boundary.

## Genetic Clustering Analyses

Black Bears

Both of the genetic clustering programs we employed identified two genetic clusters (i.e., K = 2; Fig. 14) for black bears in the NCE, and plots of cluster probability generally supported this conclusion (Fig. 15). More specifically, output from STRUCTURE identified a shift from one cluster to another just north of Route 2 (Fig. 16). Geneland also identified this region as exhibiting a moderately steep gradation from one cluster to another (Fig. 17), suggesting a reduction in gene flow. While this shift could reflect simple isolation by distance, the rate of the change in probable group assignment from south to north suggests a resistance to gene flow beyond a typical isolation-by-distance effect.

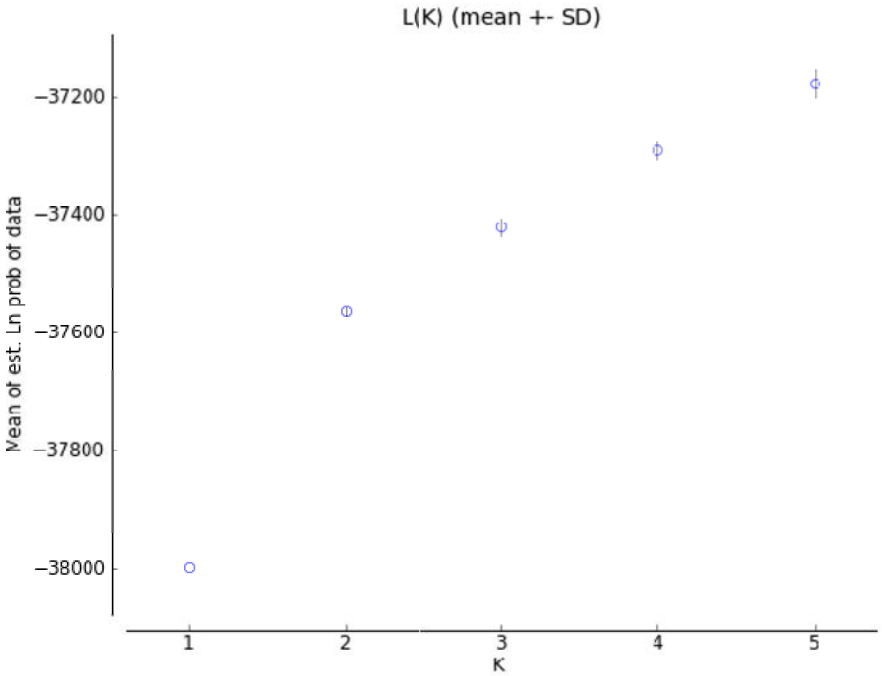



**Figure 14**. Plot of the probability (p; y-axis) that a given value for K was supported by the genetic data for black bears in the NCE. A relatively large jump in p from one K value to the next, followed by smaller increases in p for larger K values, indicates the most supported value for K. In this case, a large increase in p from K = 1 to 2 was followed by smaller increases in p for K = 3, 4, and 5, suggesting that K = 2.

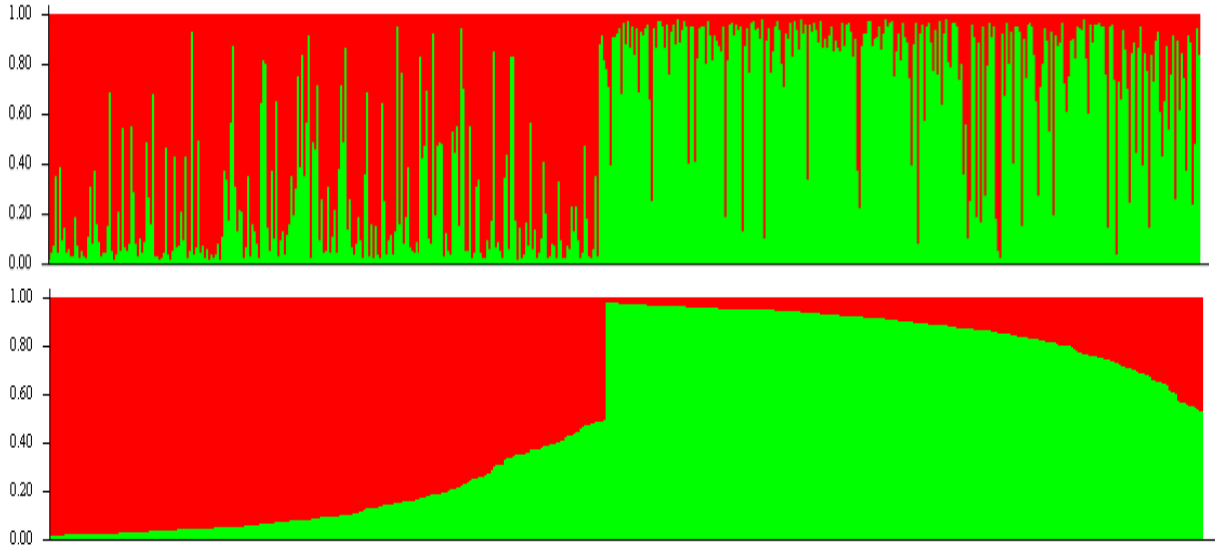



**Figure 15.** Output from program STRUCTURE showing the probability of genetic cluster membership for K= 2 for the NCE sample group of black bears. The top chart shows unsorted probability bars, with each bear represented by a bar, and the proportion of green or red in the bar indicating the probability of a given bear belonging to one or the other genetic cluster. The bottom chart shows the same data, but with probability of membership in one cluster or the other decreasing from left to right. These results suggest some genetic structuring across the sampling extent, but do not indicate a sharp break between the two clusters.

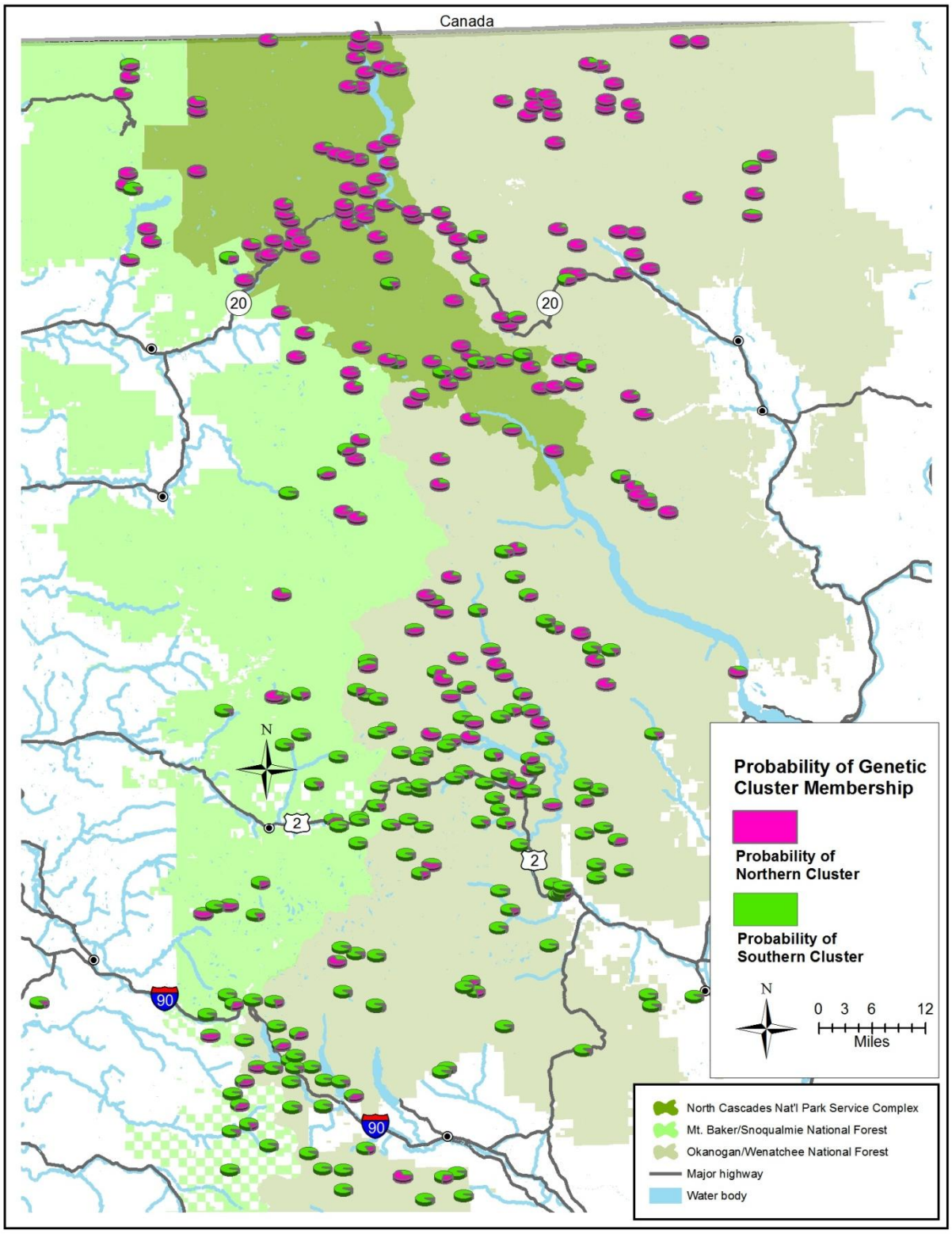



**Figure 16.** Output from STRUCTURE showing probability of genetic cluster membership for each of the NCE bear samples.

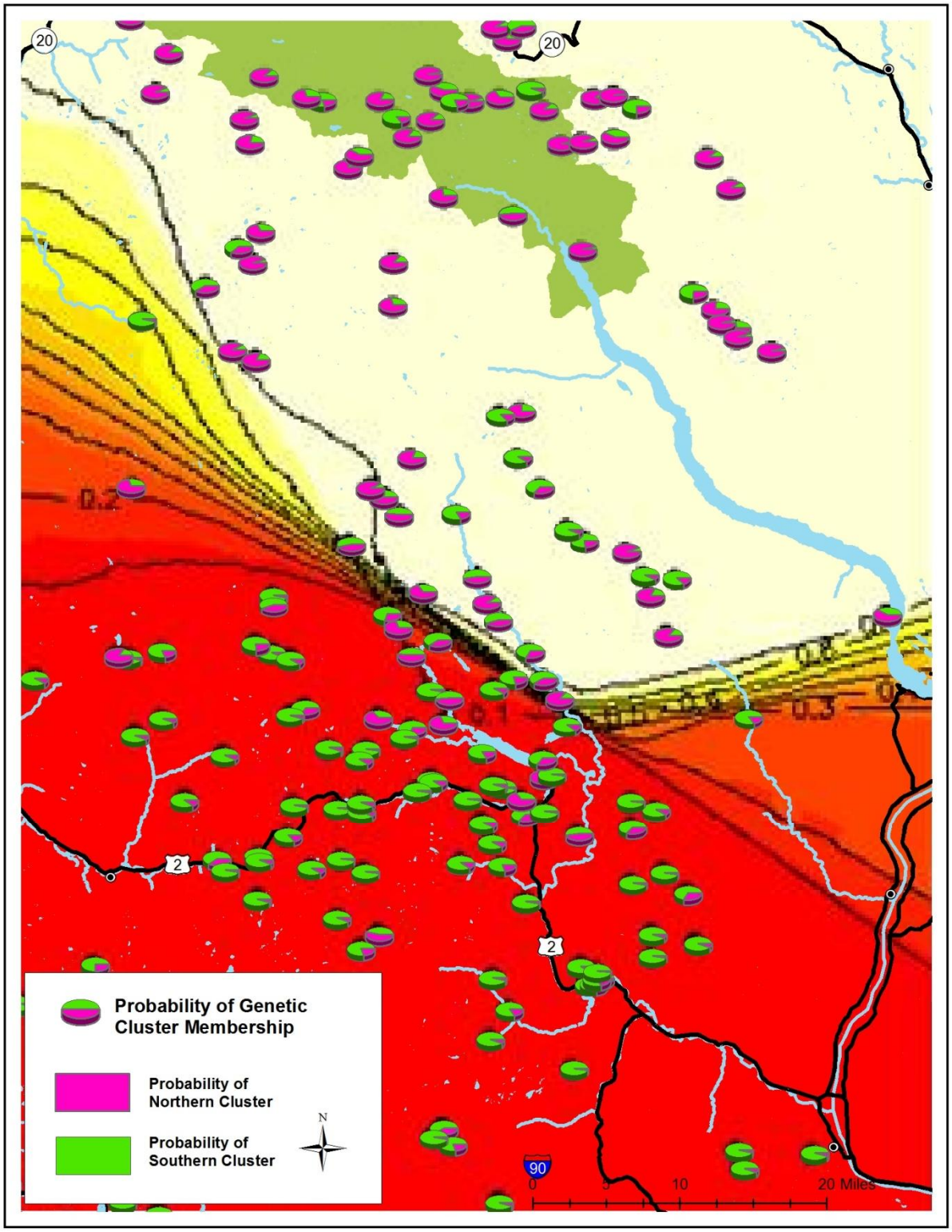



**Figure 17.** STRUCTURE results as shown in Figure 16 but overlaid on a contour map from Geneland, where contours represent relative change in genetic pattern. More closely spaced lines indicate steep gradients in pattern change, suggesting reduced gene flow. This map focuses on the Route 2 region, with Lake Wenatchee located just south of the steepest gradient of genetic pattern change and Lake Chelan in the northwest quadrant of the map.

When samples from the Capitol Forest region (n = 22) were analyzed along with I-90 samples from the NCE (n = 86), STRUCTURE clearly identified two clusters as the most likely value for K (Fig. 18), and plots of cluster probability (Figs. 19) and spatial distribution (Fig. 20) strongly supported this conclusion. Geneland results were identical, with a strong cline identified between the two clusters (Fig. 21). In addition to identifying two clusters, both programs highlighted four individuals sampled in the Capitol Forest region that were identified as having extremely high probability of coming from the NCE genetic cluster. Information from R. Beausoleil (Department of Fish and Wildlife, personal communication) confirmed that two of these individuals were orphaned cubs born in the NCE, reared in captivity, and released in the Capitol Forest region. There was no information available for the other two samples.

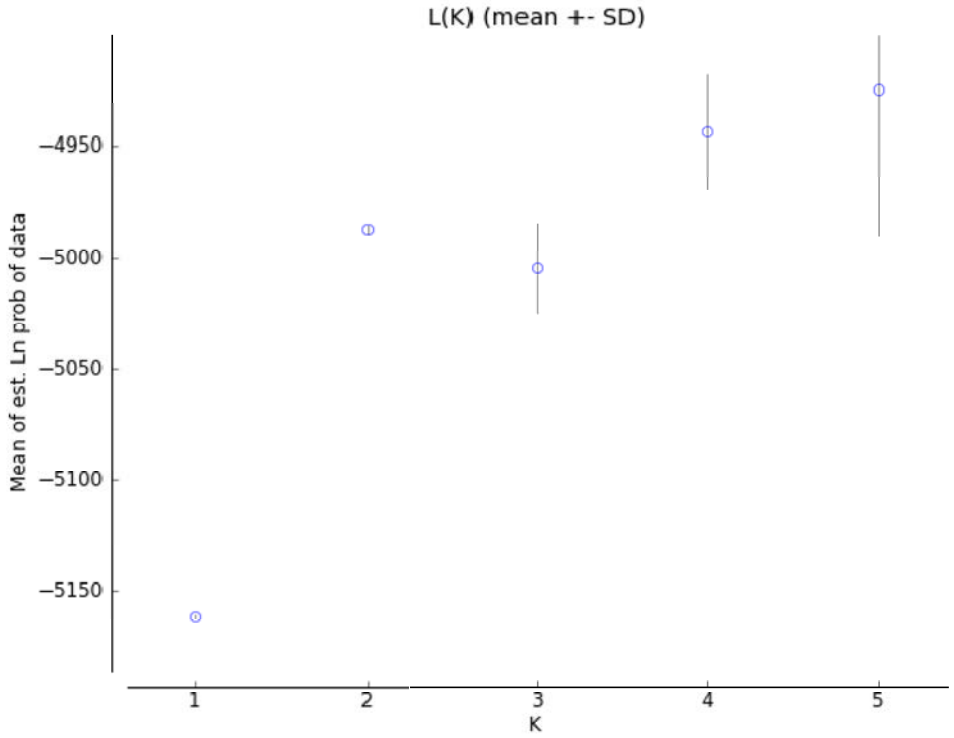



**Figure 18**. Plot of the probability (p; y-axis) that a given value for K was supported by the genetic data for black bears in the Capitol Forest/I-90 regions. A relatively large jump in p from one K value to the next, followed by smaller increases in p for larger K values, indicates the most supported value for K. In this case, a large increase in p from K = 1 to 2 was followed by decreases or smaller increases in p for K = 3, 4, and 5, suggesting that K = 2.

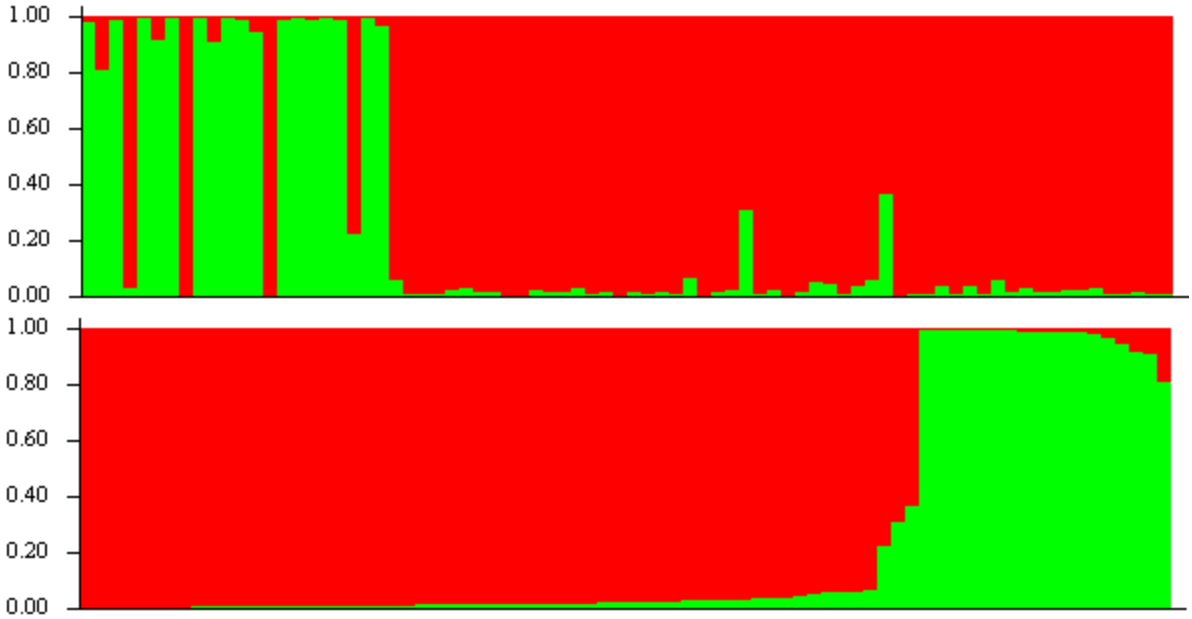



**Figure. 19.** Output from program STRUCTURE showing the probability of genetic cluster membership for K= 2 for the Capitol Forest and I-90 sample group of black bears. The top chart shows unsorted probability bars, with each bear represented by a bar, and the proportion of green or red in the bar indicating the probability of belonging to one or the other genetic cluster. The bottom chart shows the same data, but with probability of membership in one cluster or the other decreasing from left to right. These results suggests strong genetic structuring across the sampling extent, indicating little or no gene flow between the clusters.

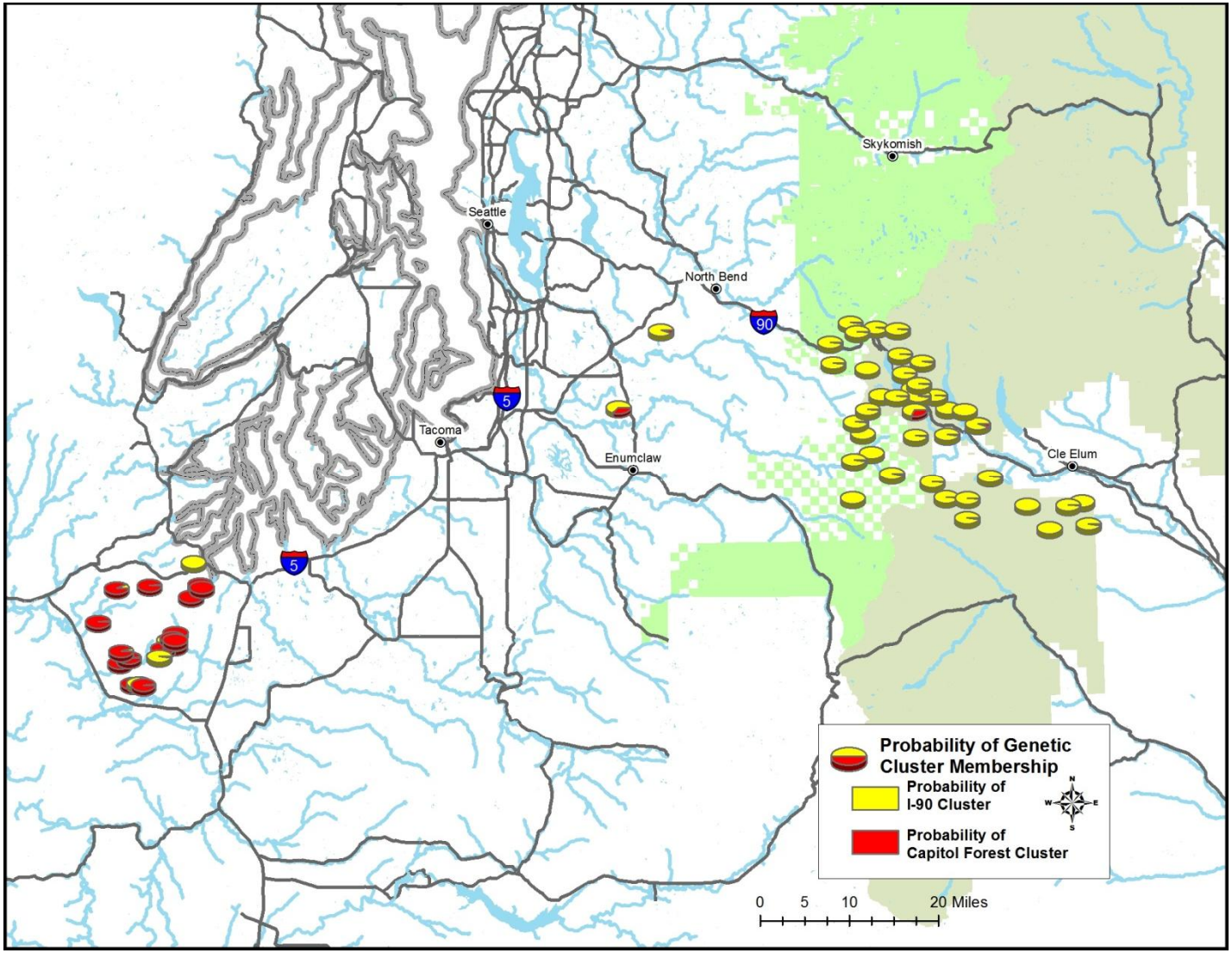



**Figure. 20**. Output from STRUCTURE showing probability of genetic cluster membership for each of the Capitol Forest (on left) and I-90 (on right) bear samples. The samples denoted by almost completely yellow symbols within the Capitol Forest cluster were bears live-captured in the North Cascades Ecosystem and translocated to the Capitol Forest region.

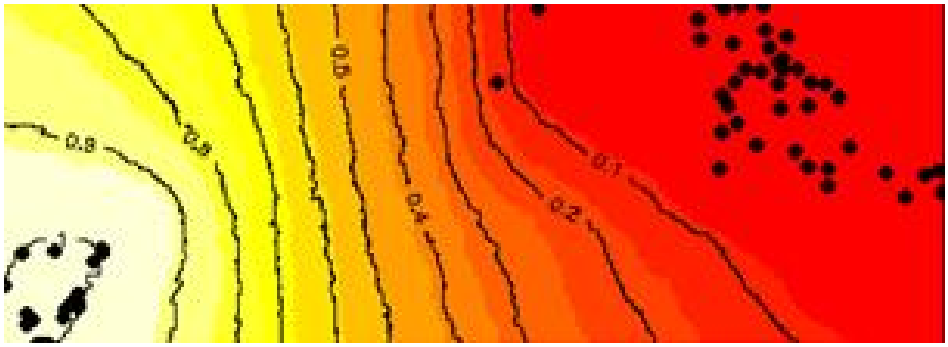



**Fig. 21.** Contour map from Geneland, where contours represent relative change in genetic pattern. More closely spaced lines indicate steeper gradients in genetic pattern change, suggesting reduced gene flow. This map focuses on the area between the Capitol Forest region (on left) and I-90 region (on right), and shows a change in genetic pattern from east–west. Note that this output was generated from a dataset with the outlying translocated bears removed. Further, although this map shows a relatively gradual change in genetic pattern between the clusters, the inter-cluster region includes the Interstate 5 (I-5) corridor. Had samples been collected from within this inter-cluster region, the steepness of the change in contours may have been much greater.

Martens

The geographic gap between marten samples collected in the northern NCE and the southern NCE was large enough to result in genetic differences between them, even if no landscape barriers to movement were present. We therefore analyzed samples from these two regions separately (Fig. 7). We detected no genetic substructuring within the northern or southern groups (Fig. 22, Fig. 23), suggesting that martens within a given group are not genetically isolated from one another.

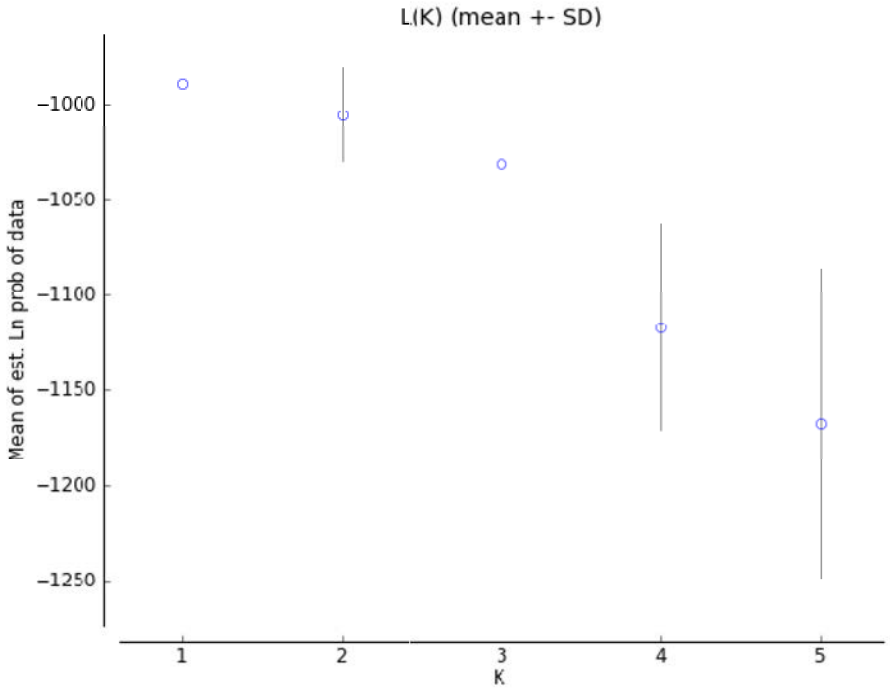



**Figure 22**. Plot of the probability (p; y-axis) that a given value for K was supported by the genetic data for martens in the southern group. A relatively large jump in p from one K value to the next, followed by smaller increases in p for larger K values, indicates the most supported value for K. In this case, the large p associated with K = 1 was followed by decreases in p for K = 2, 3, 4, and 5, suggesting that K = 1.

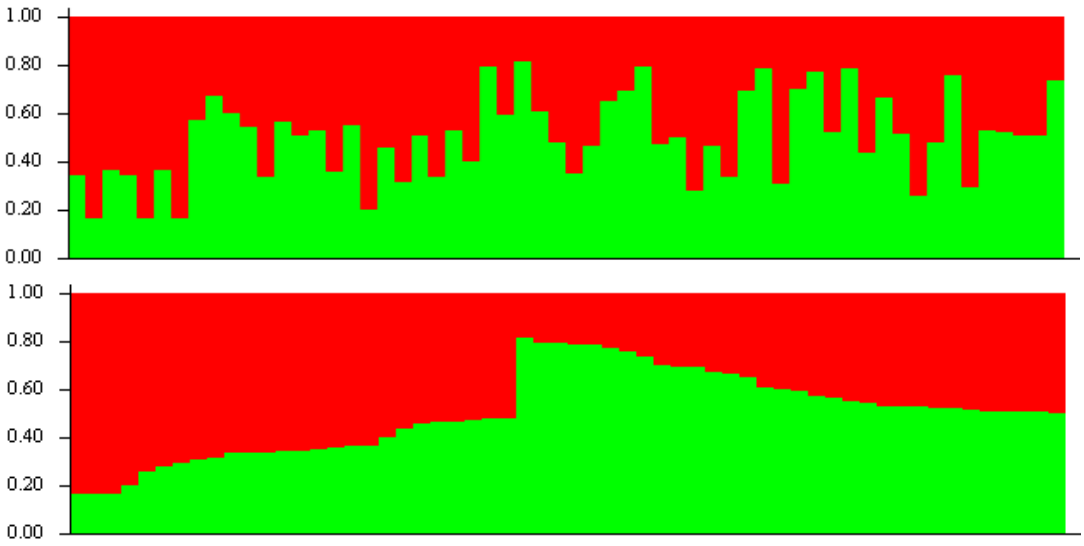



**Figure 23**. Output from program STRUCTURE showing the probability of genetic cluster membership for K= 2 for the southern sample group of martens. The top chart shows unsorted probability bars, with each marten represented by a bar, and the proportion of green or red in the bar indicating the probability of a given marten belonging to one or the other genetic cluster. The bottom chart shows the same data, but with probability of membership in one cluster or the other decreasing from left to right. These results suggest high amounts of admixture if K is set at 2,and therefore given no indication of discrete genetic clusters within this sample group. Patterns for the northern sampling group were similar.

## Genetic Diversity Measures

After separating individual black bear samples by subgroup (Table 7) and adjusting the significance levels for multiple comparisons (Rice 1989), none of the loci within bear subgroups showed significant linkage disequilibrium or deviations from expected Harvey-Weinberg patterns of equilibrium. The lack of linkage disequilibrium suggests no admixture within the subgroups, and the observation of Harvey-Weinberg equilibrium indicates that individuals within each subgroup appeared to be mating randomly. Both of these observations provide additional evidence that little or no genetic substructuring occurred within the subgroups.

**Table 7**. Measures of genetic diversity within the northern and southern bear groups. Genetic variation in black bears was quantified using observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), mean number of alleles (A), allelic richness ($A_r$), and inbreeding coefficients ($F_{IS}$) across 20 loci in FSTAT version 2.9.3.2 (Goudet et al. 2002).

| Sample Group | n | $H_o$ | $H_e$ | A | $A_r$ | $F_{IS}$ |
|---|---|---|---|---|---|---|
| Northern | 321 | 0.767 | 0.778 | 10.55 | 7.89 | 0.014 |
| Southern | 212 | 0.739 | 0.763 | 9.20 | 7.19 | 0.030 |
| Capitol Forest | 22 | 0.682 | 0.676 | 6.05 | 6.05 | -0.006 |

Similarly, after making corrections for multiple tests, we detected neither linkage disequilibrium nor deviations from expected Harvey-Weinberg patterns of equilibrium for any of the marten loci within the two groups (Table 8). As with the bear analysis, the lack of linkage disequilibrium suggests no admixture within the subgroups, and the observation of Harvey-Weinberg equilibrium indicates that individuals within each subgroup appeared to be mating randomly, providing evidence that little or no substructuring occurred within the subgroups. Two loci were fixed and not included in clustering or individual-based analyses.

**Table 8**. Measures of genetic diversity within the northern and southern marten groups. Genetic variation for martens was quantified using observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), mean number of alleles (A), allelic richness ($A_r$), and inbreeding coefficients ($F_{IS}$) across 10 loci in FSTAT version 2.9.3.2 (Goudet et al. 2002).

| Sample Group | n | $H_o$ | $H_e$ | A | $A_r$ | $F_{IS}$ |
|---|---|---|---|---|---|---|
| Northern | 16 | 0.385 | 0.407 | 2.92 | 1.82 | 0.010 |
| Southern | 58 | 0.400 | 0.408 | 3.42 | 2.99 | 0.007 |

## Landscape Genetic Analyses

Black Bears

Because our genetic clustering analyses identified a region north of Route 2 that putatively inhibits gene flow, we added an additional null model—isolation by barrier (IBB)—to the final

set of candidate models for our individual-based analysis. This IBB model was constructed based on our Geneland clustering results, and predicts panmixia (i.e., random interbreeding) within each cluster, with no migration between clusters. We included this as a second null model, along with IBD, against which to test the causal effects of each of the resistance models.

Recall that the formal causal modeling framework calls for evaluating each resistance model by first partialling out the effects of any null models (in our case, IBD and IBB). Any models that continue to show significant relationships to the genetic structure are candidate models for the next step in the causal framework—partialling out the effects of each model from the null models. During this step, we expect to get a non-significant result for models that are still able to explain the genetic data sufficiently. Finally, the effects of each model are partialled from the effects of each of the other models to further elucidate which models are best at explaining the observed genetic structure (Cushman et al. 2013).

After partialling out the effects of both IBD and IBB from the 40 candidate models, we were left with ten resistance models that were significant ($p < 0.05$) in their ability to explain the observed black bear genetic structure (Table 9). Curiously, the three landscape distance metrics we used (Dps, Rousset's *a*, and PCA) provided contrasting results. The Dps and PCA-based metrics identified two and eight resistance models, respectively, but none of these models were the same. Rousset's *a* did not identify any significant resistance models. In addition to the resistance models, the PCA method retained the IBB null model, and the Rousset's *a* method retained the IBD null model, as being consistent with the causal requirements.

Next, we partialled out the effects of the ten remaining models from the IBD and IBB models. Only two resistance models, along with IBD and IBB, continued to meet the causal requirements (Table 10). Models SW11 and RND2_SW4 were both still identified by the Dps method as causal. This indicates that although ten resistance models sufficiently explained the observed genetic structure, most of the explanatory power for eight of the models was the result of either IBD or IBB. Model SW11 was the WWHCWG black bear model with higher resistance values at elevations > 1500 m. Model RND2_SW4 was the WWHCWG black bear model with both high resistance at elevations > 1500 m *and* high resistance in regions with high mean slope values (i.e., high steepness or topographic complexity). Further, IBD and IBB were both still consistent with causal requirements as assessed by the Rousset's *a* and PCA methods, respectively.

When models SW11 and RND2_SW4 were analyzed against one another to partial out the effects of each on the other, only RND2_SW4 was significant in explaining the genetic structure present in the dataset. In addition, the barrier model and the isolation by distance models were also identified by each of the other two genetic distance methods as being consistent with the causal requirements.

**Table 9**. Resistance models that were significant ($p < 0.05$)—for at least one of the three genetic distance methods—in terms of their ability to explain the observed genetic structuring in the black bear dataset after partialling out the effects of both IBD and IBB. WWHCWG refers to a model developed by the Washington Wildlife Habitat Connectivity Working Group.

| Model Code | Description ("lower" and "higher" refer to levels of resistance) |
|---|---|
| SW11 | WWHCWG black bear model; Higher >1500m resistance |
| HWG_D | Highways gradation (Default) |
| HWG1 | Highways gradation; less resistance |
| GREEN1 | Greenness; greener = lower resistance; linear resistance |
| RND2_SW4 | WWHCWG black bear model; plus slope (linear) & higher >1500m resistance |
| RND2_HWG2 | Highways gradation; much less I-90 resistance |
| RND2_HWG5 | Highways gradation; less I-90/major hwy resistance |
| RND2_HWG6 | Highways gradation; much less I-90/major hwy resistance |
| RND2_HWG10 | Highways gradation; less resistance, with road buffer resistance; greenness |
| RND2_HWG11 | Highways gradation; less resistance, with road buffer resistance; greenness; slope |

**Table 10**. Results of causal modeling for each of three genetic distance metrics (Dps, Rousset's *a*, PCA). To meet causal requirements, models must be significant for the first three tests and non-significant for the final two. Models and outcomes that meet the causal modeling requirements across all five tests are in bold. IBR = simple Mantel test of whether the model explains the genetic data; IBR|IBB = partial Mantel test of whether the model explains the data, but with the effects of IBB partialled out; IBR|IBD = partial Mantel test of whether the model explains the data, but with the effects of IBD partialled out; IBB|IBR = partial Mantel test of whether IBB explains the data, but with the effects of the model partialled out; IBD|IBR = partial Mantel test of whether IBD explains the data, but with the effects of the model partialled out.

| | IBR | | | IBR\|IBB | | | IBR\|IBD | | | IBB\|IBR | | | IBD\|IBR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Expect + | | | Expect + | | | Expect + | | | Expect - | | | Expect - | | |
| Model | Dps | Rp | PCA | Dps | Rp | PCA | Dps | Rp | PCA | Dps | Rp | PCA | Dps | Rp | PCA |
| SW11 | + | + | + | + | + | - | + | - | - | - | + | + | - | + | - |
| HWG_D | + | + | + | + | + | + | - | - | + | + | + | + | + | + | + |
| HWG1 | + | + | + | + | + | + | - | - | + | + | - | + | + | + | + |
| GREEN1 | + | + | + | + | + | + | - | - | + | + | - | + | + | - | + |
| RND2_SW4 | + | + | + | + | + | - | + | - | - | - | - | + | - | - | - |
| RND2_HWG2 | + | + | + | + | + | + | - | - | + | + | - | + | + | - | - |
| RND2_HWG5 | + | + | + | + | + | + | - | - | + | + | - | + | + | + | - |
| RND2_HWG6 | + | + | + | + | + | + | - | - | + | + | - | + | + | - | - |
| RND2_HWG10 | + | + | + | + | + | + | - | - | + | + | + | + | + | + | - |
| RND2_HWG11 | + | + | + | + | + | + | - | - | + | + | + | + | + | + | - |

To summarize, after a thorough analysis using Mantel and partial-Mantel tests under a rigorous causal modeling framework, and using three different methods for calculating genetic distance, we identified three different models that appear to explain the observed genetic structure in black bears. One of the models (RND2_SW4) included high resistance at higher elevations and in areas of greater mean slope (i.e., steepness or topographic complexity). The second model (IBB) represents a barrier to north-south gene flow in the region north of Route 2. Finally, the third model (IBD) is a simple isolation-by-distance model.

Martens

After partialling out the effects of both IBD and IBB from the 23 candidate models, no resistance models remained that were significant ($p < 0.05$) in their ability to explain the observed marten genetic structure for the southern sample group. This suggests that any genetic differentiation present in the data was either a consequence of IBD, or that our sample size was too small to have power to detect structuring in this population. Insufficient sample size precluded individual-based modeling of the northern sample group.

# Discussion

## Survey Results

Hair-Snagging vs. Scat Detection Dogs

Our detection dog teams were highly successful at locating black bear scats in a variety of settings, including steep, thick forests. Marten scats, by comparison, were detected only rarely. This disparity was probably due to a combination of factors. Detection dogs are notoriously proficient at detecting bear scats (Long et al. 2007, MacKay et al. 2008), which are relatively large and tend to retain moisture—and therefore odor—for long periods. It's possible that high densities of bear scats may have distracted dogs from detecting the smaller and rarer scats deposited by martens (MacKay et al. 2008). Black bears and bear scats were common along most transects surveyed, and the detection dogs employed in our surveys were highly experienced with this particular species. They were relatively "green" with martens, however, having only recently been trained on marten scats and therefore lacking the field reinforcement necessary to solidify the scent in their scent repertoire. Furthermore, marten densities were likely low in some of the areas we surveyed due to poor habitat quality, providing the dogs with little or no opportunity for detection.

Despite the dogs' effectiveness at finding bear scats, the relatively poor-quality DNA contained in bear scats (when compared with DNA from tissue or hair follicles) could not be sufficiently (or cost-effectively) analyzed by WGI to meet the needs of our landscape genetic analysis. Thus, we elected to discontinue the use of this method after our pilot surveys were completed in 2009. Hair-snagging at scented or baited survey sites, on the other hand, proved an efficient means of collecting genetic samples from both black bears and martens in our survey area (although martens presented challenges, as discussed below), and the samples collected at these sites were highly suitable for DNA analysis. We therefore employed hair-snagging as our sole method for collecting genetic samples in the field after 2009.

Black Bears

Remote camera and hair-snagging methods were highly effective for collecting both occurrence data and genetic samples from black bears across the study area. Limited backcountry access resulted in some gaps in survey effort in the central Alpine Lakes Wilderness, the Glacier Peak Wilderness, areas of NOCA north of Highway 20, and the Pasayten Wilderness east of Ross Lake. Despite these gaps, sample distribution was relatively even and extensive, and provided a suitable dataset for genetic analyses.

Our survey protocol appears to have successfully minimized the number of bears detected at multiple corrals (i.e., 91% of individuals were detected at only one corral), and therefore also helped minimize excess costs associated with sampling the same individual multiple times. Further, the fact that we sampled male and female bears almost equally suggests that we avoided a sex-related sampling bias.

Martens

We had difficulty attracting martens to survey stations during summer, which has been reported for mustelids elsewhere (Slauson et al. 2012). The probability of detecting a species, given its presence, is a function of both the detection method and the density of the species in the vicinity of the survey site (MacKenzie et al. 2002). Poor marten detection rates during summer have been attributed to various factors, including the consumption of bait by black bears (Slauson et al. 2012); the availability of plentiful prey and other food resources (e.g., berries), resulting in martens being less attracted to bait (Slauson et al. 2009); and females with kits restricting movements and not selecting as much carrion for consumption (Leonard 1986). Recent research suggests that two factors—the absence of persistent bait at survey stations in summer (often as a result of bear visitation) and higher occupancy rates of martens in winter due to larger numbers of individuals following the annual birth pulse, and/or increased home range size—are largely the cause for lower detection rates in summer versus winter (Slauson et al. 2012).

After we shifted our marten survey efforts to winter, in 2010, we experienced increased detection rates north of I-90, where mature forest habitat and larger blocks of contiguous forest were prevalent. We still experienced very low detection rates south of I-90, however, where poorer-quality habitat (e.g., clearcuts, younger forests, smaller forest blocks) may have supported fewer martens. Further research designed to estimate occupancy, distribution, and density would be required to adequately address the causes of low detection rates in this region.

**Genetic Clustering Analyses**

The genetic clustering analyses we employed—STRUCTURE and Geneland—are both designed to detect population structure and to identify distinct populations (Pritchard et al. 2010). As such, they are best applied when fairly strong barriers to gene flow result in clear genetic discontinuity across space. Where population structure is more continuous than discrete, and where significant admixture is present among subpopulations, these types of clustering analyses are less able to identify where subpopulations begin and end, or to differentiate between barriers to gene flow and simple isolation-by-distance. We thus used these analyses as initial screens for distinct genetic barriers, and not necessarily to detect fine-scale resistance to gene flow or more subtle barrier effects.

Black Bears

Our genetic clustering analyses detected a clear genetic discontinuity between bear samples collected in the NCE and those collected in the Capitol Forest region. This is not surprising, as the area between these locations is largely composed of urban and residential development and includes the Interstate 5 (I-5) corridor—and is therefore likely a strong barrier to black bear dispersal and gene flow. More important, however, is the demonstration that the genetic markers and methods we employed were capable of detecting barriers to gene flow where they did occur. Further, our ability to clearly identify a pair of translocated bears (i.e., bears born in the NCE and translocated to the Capitol Forest region) gave us further confidence that we could detect strong barriers to gene flow and subsequent genetic structuring across large regions.

The support for two genetically distinct black bear subpopulations within the NCE, and the identification of a reduction in gene flow between these subpopulations just north of Route 2, was unexpected. This region of reduced gene flow does not appear to reflect any obvious anthropogenic landscape feature (e.g., highway, urban land cover), nor does it correspond to a marked change in land cover or natural community type. It does coincide, however, with an area where the Cascades Range increases dramatically in elevation and ruggedness, and where Glacier Peak and its surrounding ridges and passes are dominant features. Relatively few passes (e.g., Buck Pass, Suiattle Pass, Cloudy Pass) connect lower elevations in this region, much of which is largely snow-covered until late summer. Indeed, previous analyses identified this portion of the Glacier Peak Wilderness as providing limited connectivity for forest carnivores, owing to the rugged topography and naturally fragmented forest patterns resulting from the restriction of forested habitat to a few low-elevation, narrow valley bottoms (Singleton et al. 2002).

Gene flow requires that animals move *and* breed. Black bears typically disperse in early- to mid-summer, at a time when most high-elevation habitat in the NCE remains snow-covered. Snowy and steep terrain may constrain the movement of bears during dispersal. Reciprocally, most of the lower elevation passes in this region are traversed by hiking trails, which have been shown in other ecosystems to displace black bears from adjacent habitats (Kasworm and Manley 1990). Limited human access to higher elevations—again due to persistent spring snow—results in concentrated use of lower elevation trails over a relatively short time period. Such disturbance could further discourage the use of these areas by bears.

Martens

Our failure to detect structuring within the northern or southern groups of martens may reflect a true lack of structure in the dataset. It could also be the result, however, of our relatively small sample size leading to insufficient power to detect structure. Sample sizes from north and south of Highway 20, Route 2, and I-90, respectively, were relatively low (i.e., Highway 20 = 7 N, 9 S; Route 2 = 26 N, 9 S; I-90 = 19 N, 1 S). These numbers fell far below our target sample size of 30 individuals from either side of each highway—the minimum sample size generally recommended for detecting a strong movement barrier (D. Paetkau, Wildlife Genetics International, personal communication). Further, the limited spatial distribution of marten samples likely resulted in a lack of diversity in associated land cover types, and minimal interactions between sampled martens and potential anthropogenic barriers such as developed areas or roads, in our models. Our ability to detect barriers to gene flow from such features was therefore likely limited. Analyses designed to simulate the breeding and movement of martens could help to elucidate the strength of a barrier that would be required to be detected with our methods, or conversely, how large a sample size would be required to detect a barrier of a given strength (see *Next Steps* below for more discussion about this approach).

**Individual-Based Landscape Genetic Analyses**

As stated above, individual-based landscape genetic approaches are more suitable than genetic clustering methods for assessing genetic structure in continuous populations, and when significant admixture is present among subpopulations. Under these conditions, such methods

can often permit the identification of the variables contributing to observed genetic structure.

Black Bears

Our causal modeling approach identified three different models—one for each genetic distance metric used—that sufficiently explained the observed black bear genetic structure. We attempt to reconcile these results below, but first explore each outcome individually.

Model RND2_SW4 was identified by the Dps approach as the top supported model. It was composed of layers and resistance values developed for the WA Connected Landscape Project black bear model (Table 2), but importantly also included higher resistance values for elevations > 1500 m and for areas of high mean slope. The base WA Connected Landscapes Project model included substantial resistance to agriculture, urban and developed landscapes, water, highway centerlines, and highway buffers. Because our study area included few major developed or agricultural areas, these factors likely contributed little to model predictions. Highways were included in the WA Connected Landscapes Project with moderate to substantial resistance (Table 2), and could have contributed to the performance of the RND2_SW4 model. Roads and highways are known to influence habitat use by black bears in the NCE (Gaines et al. 2005), but empirical evidence of their influence on gene flow has not been previously described there. Roads have, however, been identified as important variables influencing black bear gene flow in other ecosystems (e.g., Bull et al. 2011). The clear support for the RND2_SW4 model over the other WA Connected Landscapes Project models (which also contained resistance to highways), however, strongly suggests that elevation and slope were more important factors affecting the selection of the RND2_SW4 model—and therefore gene flow—in our study area.

The resistance layer for the RND2_SW4 model (Fig. 24) shows high resistance values corresponding to increasing elevation and mean slope values just north of Route 2, resulting in a number of almost contiguous east-west bands of very high resistance. This high-resistance region corresponds with the location of the steep gradient between genetic clusters identified with Geneland (Fig. 17) and STRUCTURE (Fig. 16). Although genetic cluster analysis and causal modeling both utilize patterns in the genetic data to infer structure, the former does not take into account landscape features. In other words, the selection of Model RND2_SW4 is consistent with results from the clustering analyses, suggesting that areas characterized by relatively contiguous, high-elevation and rugged terrain inhibit gene flow for black bears.

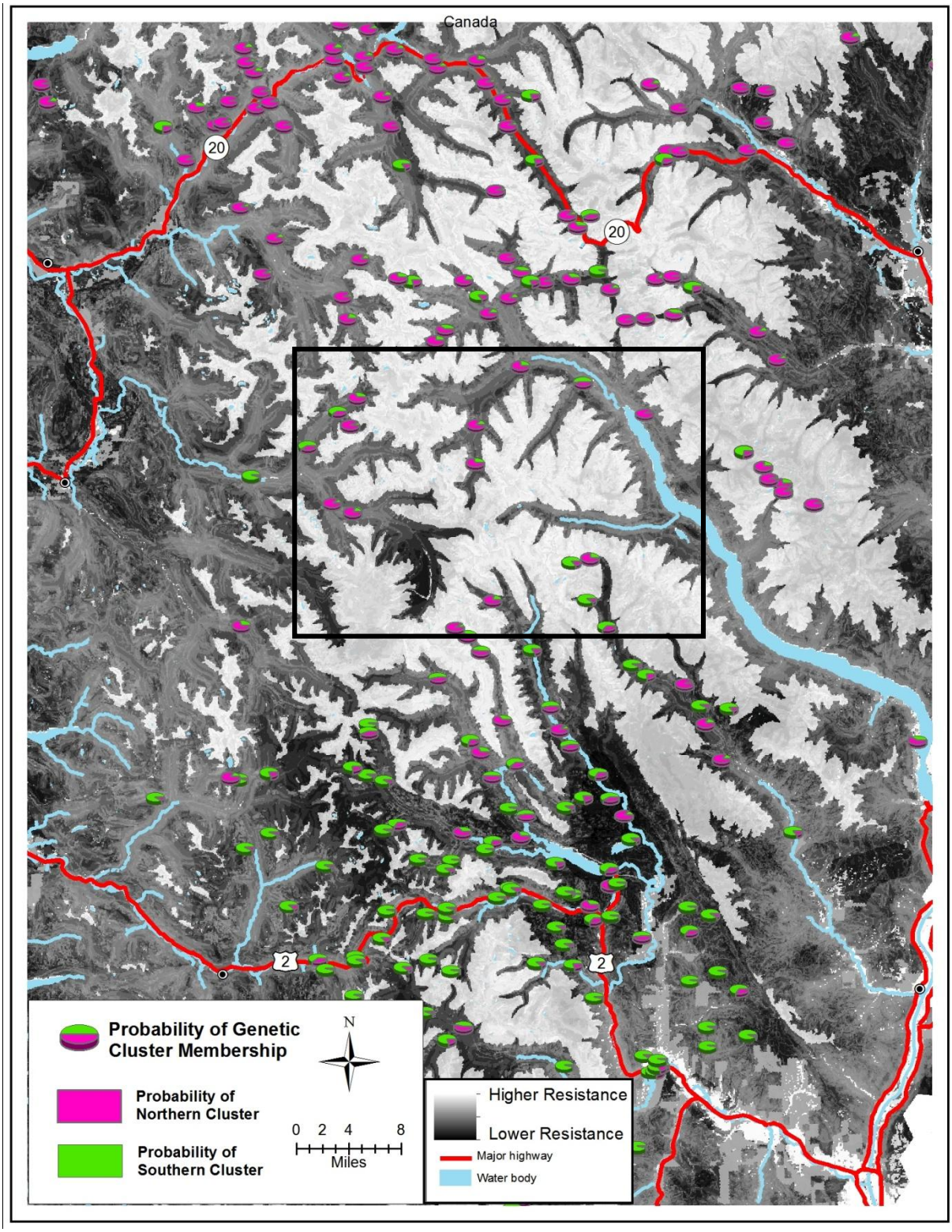



**Figure 24**. Sample locations, including probability of genetic cluster membership plotted over the model RND2_SW4 resistance surface. The rectangular box shows the location of likely reduced gene flow, possibly correlated with high resistance features including areas of elevation > 1500 m and high topographic ruggedness.

The PCA and Rousset's *a* methods for estimating genetic distance each identified a different model as causal than did Dps. PCA identified the IBB model, which was the null model developed to mimic the results from the cluster analysis, as the best model. While this outcome was consistent with the selection of the RND2_SW4 model by the Dps method, the models differed in that the IBB model posits a complete barrier in the region north of Route 2, which is in reality unlikely given the admixture that was detected between the northern and southern bear groups. The RND2_SW4 model, on the other hand, allows for movement based on the parameterization of that model's resistance surface (see Fig. 24). Rousset's *a* identified the IBD model as the best model. As mentioned earlier, IBD is a common phenomenon and would be expected to occur to some extent in most species and populations, especially in the absence of other more significant inhibitors to gene flow.

*Reconciling the Results*

It is somewhat unclear how to interpret the different results provided by the three metrics used to estimate genetic distance, as no quantitative research has been conducted to compare the performance of various genetic distance metrics. Dps has been used routinely for landscape genetic analyses (Johnson et al. 1999, Culver et al. 2000, Warrillow et al. 2001) requires no biological assumptions, and weights alleles based on their rarity. PCA is a relatively new and untested approach for characterizing genetic distance (Shirk et al. 2010), and weights alleles that show the most discrimination among individuals. Finally, Rousset's *a* is based on a lattice model, with individuals distributed evenly and continuously over the landscape. Although our bear dataset was well distributed, it is difficult to know whether the sample distribution was sufficiently even for Rousset's *a* to perform acceptably.

In sum:

- the resistance map of model RND2_SW4 (Fig. 24) appeared to mimic the genetic patterns detected with the genetic clustering algorithm;
- Dps has been used extensively and represents the most basic measure of genetic differentiation among individuals;
- PCA is a new and untested method for characterizing genetic distance; and
- Rousset's *a* may be based on an inappropriate model for our data.

We believe, therefore, that the results of the Dps method (i.e., the selection of model RND2_SW4) are most likely to be valid. Further, the ramifications of model RND2_SW4 for conservation and policy are greater than either the IBD or IBB models, and therefore should be given some weight when discussing potential implications for connectivity planning.

<u>Martens</u>

Given insufficient sample sizes and sample distribution, we were unable to conduct individual-based landscape genetic analyses for martens. We will explore whether alternate analyses, and/or the collection of additional marten samples, can be undertaken in the future to permit further inference (see *Next Steps* below).

## Potential Modeling Limitations and Caveats

Criticisms of Mantel-Based Approaches

Landscape genetics is a relatively new field (Manel et al. 2003, Holderegger & Wagner 2006, Balkenhol et al. 2009), and efforts to explore the relationship between genetic structure and landscape features are still in their infancy. Mantel and partial Mantel tests have been popular methods for attempting to correlate genetic structure with cost-distances reflecting the affect of landscape features on animal movement and gene flow (e.g., Cushman et al. 2006, Storfer et al. 2007, Hapeman et al. 2011, Wasserman et al. 2010). Despite their widespread use, however, a debate has recently emerged focusing on whether these tests are inappropriate under some conditions (Balkenhol et al. 2009, Legendre and Fortin 2010, Guillot and Rousset 2013, Cushman et al. 2013, Graves et al. *in review*). We recognize this debate, and are exploring other alternatives for analyzing our dataset (see *Next Steps* below) as the field of landscape genetics continues to evolve.

Lag Time to Detect Genetic Structuring

Although anthropogenic features did not surface as primary drivers affecting gene flow in our black bear analysis, such features may nonetheless be affecting animal movement. Current microsatellite methods of genetic analysis are capable of detecting strong barriers to gene flow after as few as 1–15 generations (Landguth et al. 2010), but the length of time before structuring can be detected is strongly dependent on the effective population size of the organism under study, as well as the strength of the barrier. Effective population size ($N_e$) can influence genetic patterns, including the rate of loss of rare alleles and heterozygosity via drift (Allendorf and Luikart 2007). A large $N_e$ allows a population to retain its genetic variation, reducing the effects of drift and slowing genetic divergence (Gauffre et al. 2008). Very large effective population sizes can make barriers to movement difficult to detect, as genetic structuring takes longer to manifest. The large black bear population in the NCE may, therefore, make the barrier effects of landscape features that have arisen within the last half-century (e.g., high-volume highways) difficult to detect with current methods.

## Next Steps

As mentioned above, the field of landscape genetics is still young. Each new issue of the major landscape ecology and genetics journals contains articles evaluating the performance and properties of the various methods available to landscape geneticists. Currently, landscape genetics appears to be at a crossroads, where limitations of the methods used consistently until now (e.g., Mantel tests) are being questioned, tested, and, in some cases, shown to be lacking. There are, however, currently few if any good alternatives for relating landscape features to genetic consequences. Our “next steps” may be to re-analyze our models using a different measure of landscape distance—landscape resistance (McRae 2006)—that employs circuit theory instead of least-cost paths, and that can accommodate multiple pathways on the landscape. We will also conduct simulations (e.g., Landguth and Cushman 2010, Landguth et al. 2010) to evaluate whether the models selected in our analyses are able to generate the genetic data that we observed, and to estimate the time required for a strong barrier to create structuring that would be detectable by our methods. And, we will explore new methods for generating

resistance parameters directly from genetic data using more realistic models of animal movement and reproduction (Hanks and Hooten 2013, Graves et al. *in review*).

Finally, we will evaluate whether additional marten samples can be acquired in the region, especially south of I-90. Even if individual-based modeling is not possible given the methodological constraints described above, genetic cluster analysis could help provide insight as to whether I-90 is currently a barrier to gene flow for this species. This information is especially critical if the performance of newly installed wildlife crossing structures (WSDOT 2008) is to be assessed.

**Conclusions and Conservation Implications**

No other study that we are aware of provides empirical evidence that high elevation regions and topographic ruggedness can act as potential mediators of gene flow for black bears. Bears in the NCE commonly occupy alpine habitats, especially during fall when berries are abundant in mountain meadows (Lyons et al. 2003, Gaines et al. 2005). It is in the spring, however, that males typically move long distances from their natal territories to find females for breeding. Since dispersal is a primary factor affecting where and how far genetic material moves within populations, those landscape features that inhibit dispersal are likely to have the most effect on patterns of gene flow.

Black bears are not currently considered rare in the NCE, and forest habitat appears relatively contiguous throughout much of the region. If, however, bears—and especially dispersing bears—avoid moving across higher elevation, snow-covered, rugged terrain, the importance of lower elevation connectivity among forested habitat patches is heightened. The loss of such connections as a result of habitat conversion, climate change, or human development could have ramifications for movement and gene flow for bears.

The negative influence of roads and highways on black bear habitat connectivity has been demonstrated elsewhere. The inclusion of these variables in our most supported model suggests that road effects on black bears in the NCE warrant further exploration.

One of the objectives of our research was to evaluate the expert-based habitat connectivity models used in the WA Connected Landscapes Project state-wide assessment (WWHCWG 2010). The WA Connected Landscapes Project black bear model formed the basis for our most supported black bear model (RND2_SW4), and therefore its core parameterization appears to relate to gene flow and bear movement in the NCE. Our results suggest, however, that some modifications to the WA Connected Landscapes Project statewide black bear model may be warranted to more accurately reflect the influences of high elevations (>1500 m) and steep slopes.

Finally, although black bears and grizzly bears have different life requisites, both species require large, contiguous or well-connected blocks of suitable habitat and must disperse long distances during the spring and early summer to mate and secure new territories. The results of our analyses may be helpful for future assessments of suitable grizzly habitat in the NCE, and for informing grizzly bear recovery in this region.

## Literature Cited

Allendorf, F.W. and G. Luikart. 2007. *Conservation and the Genetics of Populations*. Blackwell, Malden, Massachusetts.

Balkenhol, N., F. Gugerli, S.A. Cushman, L.P. Waits, A. Coulon, J.W. Arntzen, R. Holderegger, H.H. Wagner, P. Arens, P. Campagne, V.H. Dale, A.G. Nicieza, M.J.M Smulders, E. Tedesco, H. Wang, and T.N. Wasserman. 2009. Identifying future research needs in landscape genetics: where to from here? Landscape Ecology 24:455–463.

Bissonette, J.A., D.J. Harrison, C.D. Hargis, and T.G. Chapin. 1997. The influence of spatial scale and scale-sensitive properties on habitat selection by American marten. Pages 368–385 *in* J.A. Bissonette, editor. *Wildlife and landscape ecology*. Springer-Verlag, New York.

Bowcock, A.M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J.R. Kidd, and L.L. Cavalli-Sforza. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368:455–457.

Buskirk , S.W., and R.A. Powell. 1994. Habitat ecology of fishers and American martens. Pages 283–296 *in* S.W. Buskirk, A.S. Harestad, M.G. Raphael, and R. A. Powell, editors. *Martens, sables and fishers: biology and conservation*. Cornell University Press, Ithaca, New York.

Clark, J.D., J.E. Dunn, and K.G. Smith. 1993. A multivariate model of female black bear habitat use for a geographic information system. Journal of Wildlife Management 57:519–526

Clevenger, A.P., R.A. Long, and R. Ament. 2008. I-90 Snoqualmie Pass East wildlife monitoring plan. Final report prepared for the Washington Department of Transportation.

Culver, M., W.E. Johnson, J. Pecon-Slattery, and S.J. O'Brien. 2000. Genomic ancestry of the American puma (*Puma concolor*). Journal of Heredity 91:186–197.

Cushman, S.A., and E.L. Landguth. 2012. Multi-taxa population connectivity in the Northern Rocky Mountains. Ecological Modelling 231:101–112.

Cushman, S. A., K. S. McKelvey, J. Hayden, and M. K. Schwartz. 2006. Gene flow in complex landscapes: Testing multiple hypotheses with causal modeling. The American Naturalist 168:486–499.

Cushman, S., T. Wasserman, E. Landguth, and A. Shirk. 2013. Re-evaluating causal modeling with Mantel tests in Landscape Genetics. Diversity 5:51–72.

Dixon, J.D., M.K. Oli, M.C. Wooten, T.H. Eason, J.W. McCown, and D. Paetkau. 2006. Effectiveness of a regional corridor in connecting two Florida black bear populations. Conservation Biology 20:155–162.

Ennis, S., and T.F. Gallagher. 1994. PCR-based sex determination assay in cattle based on the bovine Amelogenin locus. Animal Genetics 25:425–427.

ESRI. 2003. ArcGIS. Environmental Systems Research Incorporated, Redlands, CA.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14:2611–2620.

Falush D., M. Stephens, and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Foley, C. A.H., S. Papageorge, and S.K. Wasser. 2001. Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Conservation Biology 15:1134–1142.

Franklin, J.F., and C.T. Dyrness. 1973. Natural vegetation of Oregon and Washington. USDA Forest Service, Gen. Tech. Rep. PNW-GTR-8. 417pp.

Gaines, W.L., A.L. Lyons, J.F. Lehmkuhl, and K.J. Raedeke. 2005. Landscape evaluation of female black bear habitat effectiveness and capability in the North Cascades, Washington. Biological Conservation. 125:411–425.

Gaines, W.L., R.H. Naney, P.H. Morrison, J.R. Eby, G.F. Wooten, and J.A. Almack. 1994. Use of LANDSAT multispectral scanner imagery and geographic information systems to map vegetation in the North Cascades Grizzly Bear Ecosystem. International Conference on Bear Research and Management 9:533–547.

Gaines, W.L., P. Singleton, and A.L. Gold. 2000. Conservation of rare carnivores in the North Cascades Ecosystem, western North America. Natural Areas Journal 20: 366–375.

Gauffre, B., A. Estoup, B. Bretagnolle, and J. Cosson. 2008. Spatial genetic structure of a small rodent in a heterogeneous landscape. Molecular Ecology 17:4619–4629.

Goslee, S., and D. Urban. 2010. Ecodist: Dissimilarity-based analysis functions including ordination and Mantel test functions, intended for use with spatial and community data. R Foundation for Statistical Computing.

Goudet, J. 2002. FSTAT 2.9.3.2, a program to estimate and test gene diversities and fixation indices. Available from http://www.unil.ch/izea/softwares/fstat.html.

Graves, T.A., P. Beier, and A.J. Royle. In review. Current approaches using genetic distances produce poor estimates of landscape resistance to inter-individual dispersal. Molecular Ecology.

Guillot, G., A. Estoup, F. Mortier, and J.F. Cosson. 2005a. A spatial statistical model for landscape genetics. Genetics 170:1261–80.

Guillot, G., F. Mortier, and A. Estoup. 2005b. Geneland: a program for landscape genetics. Mol Ecological Notes 5:712–715.

Guillot, G., and F. Rousset. 2013. Dismantling the Mantel tests. Methods in Ecology and Evolution: OnlineEarly.

Hapeman, P., E.K. Latch, J.A. Fike, O.E. Rhodes, and C.W. Kilpatrick. 2011. Landscape genetics of fishers (*Martes pennanti*) in the Northeast: dispersal barriers and historical influences. The Journal of Heredity 102:251–9.

Hardy, O.J., and X. Vekemans. 2002. spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620.

Hanks, E.M., and M.B. Hooten. In press. Circuit theory and model-based inference for landscape connectivity. Journal of the American Statistical Association.

Hargis, C.D., J.A. Bissonette, and D.L. Turner. 1999. The influence of forest fragmentation and landscape pattern on American martens. Journal of Applied Ecology 36:157–172.

Holderegger, R., and H.H. Wagner. 2008. Landscape genetics. BioScience 58:199–207.

Jakobsson, M., and N.A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806.

Johnson, W.E., J.P. Slattery, E. Eizirik, J. Kim, M.M. Raymond, Cristian Bonacic, R. Cambre, P. Crawshaw, A. Nunes, H.N. Seuánez, M.A.M. Moreira, K.L. Seymour, F. Simon, W. Swanson, and S.J. O'Brien. 1999. Disparate phylogeographic patterns of molecular genetic variation in four closely related South American small cat species. Molecular Ecology 8:S79–S94.

Kasworm, W.F., and T. Manley. 1990. Road and trail influences on grizzly bears and black bears in northwest Montana. International Conference on Bear Research and Management. 8: 79–84.

Kendall, K.C., and K.S. McKelvey. 2008. Hair collection. Pages 141–182 in Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray, editors. *Noninvasive survey methods for carnivores*. Island Press, Washington, D.C.

Koehler, G.M. and D.J. Pierce. 2005. Survival, cause-specific mortality, sex, and ages of American black bears in Washington state, USA. Ursus 16:157–166.

Koehler, G.M., B.T. Maletzke, J.A. von Kienast, K.B. Aubry, R.B. Wielgus, and R.H. Naney. 2008. Habitat fragmentation and the persistence of lynx populations in Washington State. Journal of Wildlife Management 72:1518–1524.

Lande, R. 1988. Genetics and demography in biological conservation. Science 241:1455–1460.

Landguth E.L, and S.A. Cushman. 2010. CDPOP: a spatially explicit cost distance population genetics program. Molecular Ecology Resources 10:156–161.

Landguth, E.L., S.A. Cushman, M.K. Schwartz, K.S. McKelvey, M. Murphy, and G. Luikart. 2010. Quantifying the lag time to detect barriers in landscape genetics. Molecular Ecology 19:4179–4191.

Legendre, P., and L. Legendre. 1998. Numerical ecology. 2nd English edition. Elsevier, Amsterdam.

Legendre, P., and M.J. Fortin. 2010. Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. Molecular ecology resources 10:831–44.

Leonard, R.D. 1986. Aspects of the reproduction of the fisher, *Martes pennanti*, in Manitoba. Canadian Field-Naturalist 100:32-44.

Long, R.A., T.M. Donovan, P. MacKay, W.J. Zielinski, and J.S. Buzas. 2007. Effectiveness of scat detection dogs for detecting forest carnivores. Journal of Wildlife Management 71:2007–2017.

Long, R.A., P. MacKay, W.J. Zielinski, and J.C. Ray, editors. 2008. *Noninvasive survey methods for carnivores*. Island Press, Washington D.C.

Lyons, A.L., W.L. Gaines, and C. Servheen. 2003. Black bear resource selection in the northeast Cascades, Washington. Biological Conservation 113:55–62.

MacKay, P., D.A. Smith, R.A. Long, and M. Parker. 2008. Scat detection dogs. Pages 183–222 *in* R. A. Long, P. MacKay, W. J. Zielinski, and J. C. Ray, editors. *Noninvasive survey methods for carnivores*. Island Press, Washington, D.C.

MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83:2248–2255.

Manel, S., M.K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology & Evolution 18:189–197.

Mantel, N. 1967. Detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

McGarigal, K., and S.A. Cushman. 2005. The gradient concept of landscape structure. Pages 112–119 *in* J. Wiens and M. Moss, eds. Issues and perspectives in landscape ecology. Cambridge University Press, Cambridge.

McRae, B.H. 2006. Isolation by resistance. Evolution 60:1551–1561.

Mills, L.S., J.J. Citta, K.P. Lair, M.K. Schwartz, and D.A. Tallmon.2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. Ecological Applications 10:283–294.

Munzing, D., and W.L. Gaines. 2008. Monitoring American marten on the east-side of the North Cascades of Washington. Northwestern Naturalist. 89: 67–75.

Murray J.V., A.W. Goldizen, R.A. O'Leary, C.A. McAlpine, H.P. Possingham, and S.L. Choy. 2009. How useful is expert opinion for predicting the distribution of a species within and beyond the region of expertise? A case study using brush-tailed rock-wallabies *Petrogale penicillata.* Journal of Applied Ecology 46:842–851.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology 12:1375–1387.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4:347–354.

Patterson, N., A.L. Price, and D. Reich. 2006. Population structure and eigenanalysis. PLoS Genet 2:190.

Pritchard J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 4.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Rogers, L.L., and A.W. Allen. 1987. Habitat suitability index models: black bear, Upper Great Lakes Region. USDI, Fish and Wildlife Service, Biological Report No. 82.

Roon, D. A., M.E. Thomas, K.C. Kendall, and L.P. Waits. 2005a. Evaluating mixed samples as a source of error in non-invasive genetic studies using microsatellites. Molecular Ecology 14:195–201.

Roon, D.A., L.P. Waits, and K.C. Kendall. 2005b. A simulation test of the effectiveness of several methods for error-checking non-invasive genetic data. Animal Conservation 8:203–215.

Rousset, F. 2000. Genetic differentiation between individuals. Journal of Evolutionary Biology 13:58–62.

Ruggiero, L.F., W.J. Zielinski, K.B. Aubry, S.W. Buskirk, and L.J. Lyon. 1994. A conservation assessment framework for forest carnivores. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO. Pages 1–6.

Schoen, J.W. 1993. Bear habitat management: a review and future perspective. International Conference on Bear Research and Management, 8:143–154.

Segelbacher, G. 2002. Noninvasive genetic analysis in birds: testing reliability of feather samples. Molecular Ecology Notes 2:367-369.

Shirk, A.J., D.O. Wallin, S.A. Cushman, C.G. Rice, and K.I. Warheit. 2010. Inferring landscape effects on gene flow: a new model selection framework. Molecular ecology 19:3603–3619.

Bull, R.A.S., S.A. Cushman, R. Mace, T. Chilton, K.C. Kendall, E.L. Landguth, M.K. Schwartz, K. McKelvey, F.W. Allendorf, and G. Luikart. 2011. Why replication is important in landscape genetics: American black bear in the Rocky Mountains. Molecular Ecology 20:1092–107.

Singleton, P.H., W.L. Gaines, and J.F. Lehmkuhl. 2002. Landscape permeability for large carnivores in Washington: a geographic information system weighted-distance and least-cost corridor assessment. USDA Forest Service, Pacific Northwest Research Station. PNW-GTR.549.

Singleton, P.H., and J.F. Lehmkuhl. 2000. I-90 Snoqualmie Pass Wildlife Habitat Linkage Assessment. Final Report to the Washington State Department of Transportation GCA1177. USDA Forest Service Pacific Northwest Research Station. PNW-98-0513-CC.

Slauson, K.M., J.A. Baldwin, and W.J. Zielinski. 2012. Occupancy estimation and modeling in Martes research and monitoring. Pages 343–368 *in* K.B. Aubry, W.J. Zielinski, M.G. Raphael, G. Proulx and S.W. Buskirk, editors. Martens, sables, and fishers: a new synthesis. Cornell University Press, Ithaca, New York.

Slauson, K.M., J.A. Baldwin, W.J. Zielinski, and M.K. Schwartz. 2009. Estimating detection probabilities for fishers using non-invasive methods and implications for survey protocols. Final report prepared for U.S. Fish and Wildlife Service, Yreka Field Office and the Fisher Science Team. U.S.D.A. Forest Service, 1 Pacific Southwest Research Station, Redwood Sciences Laboratory, Arcata, California. 68pp.

Smith, D.A., K. Ralls, A. Hurt, B. Adams, M. Parker, B. Davenport, M.C. Smith, and J.E. Maldonado. 2003. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Animal Conservation 6:339–346.

Spear, S.F., N. Balkenhol, M.-J. Fortin, B.H. McRae, and K. Scribner. 2010. Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis. Molecular Ecology 19:3576–3591.

Storfer, A., M.A. Murphy, J.S. Evans, C.S. Goldberg, S. Robinson, S.F. Spear, R. Dezzani, E. Delmelle, L. Vierling, and L.P. Waits. 2007. Putting the 'landscape' in landscape genetics. Heredity 98:128–142.

Thomas, J.W., E.D. Forsman, J.B. Lint, E.C. Meslow, B.R. Noon, and J. Verner. 1990. A conservation strategy for the northern spotted owl. Report of the Interagency Scientific Committee to Address the Conservation of the Northern Spotted Owl. Portland, OR.

USFWS. 1997. Grizzly Bear Recovery Plan Supplement: North Cascades Ecosystem Recovery Plan Chapter. USFWS Forestry Sciences Laboratory, Missoula, Montana.

Wasser, S.K., B. Davenport, E.R. Ramage, K.E. Hunt, M.Parker, C. Clarke, and G. Stenhouse. 2004. Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead ecosystem, Alberta, Canada. Canadian Journal of Zoology 82:475–492.

Wasserman, T.N., S.A. Cushman, M.K. Schwartz, and D.O. Wallin. 2010. Spatial scaling and multi-model inference in landscape genetics: *Martes americana* in northern Idaho. Landscape Ecology 25:1601–1612.

Warrillow, J., M. Culver, E. Hallerman, and M. Vaughan. 2001. Subspecific affinity of black bears in the White River National Wildlife Refuge. Journal of Heredity 92:226–233.

Waits, L. P., and D. Paetkau. 2005. Noninvasive sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 64:1419–1433.

Wiles, G.J., H.L. Allen, and G.E. Hayes. 2011. Wolf conservation and management plan for Washington. Washington Department of Fish and Wildlife, Olympia, Washington. 297 pp.

Woods, J.G., D. Paetkau, D. Lewis, B.N. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin 27:616–627.

Wright, S. 1943. Isolation by distance. Heredity 28.114–138.

WSDOT. 2006. Recommendation package: Interstate 90 Snoqualmie Pass East mitigation development team. Washington State Department of Transportation, Yakima, WA. July 2006.

WSDOT. 2008. I-90 Snoqualmie Pass East Final Environmental Impact Statement and Section 4(f) Evaluation.

WWHCWG:Washington Wildlife Habitat Connectivity Working Group. 2010. Washington Connected Landscapes Project: Statewide Analysis. Washington Departments of Fish and Wildlife, and Transportation, Olympia, WA.

Zeller, K.A, K. McGarigal, and A.R. Whiteley. 2012. Estimating landscape resistance to movement: a review. Landscape Ecology 27:777–797.

# Appendices

**Appendix 1**. Common and scientific names of species mentioned in this report.

Names are listed in alphabetical order and were taken from the Smithsonian National Museum of Natural History North American Mammals website (http://www.mnh.si.edu/mna/main.cfm).

**Mammals**
American black bear (*Ursus americanus*)
American marten (*Martes americana*)
Bobcat (*Lynx rufus*)
Canada lynx (*Lynx canadensis*)
Cougar (*Puma concolor*)
Coyote (*Canis latrans*)
Elk (*Cervus elaphus*)
Gray wolf (*Canis lupus*)
Grizzly bear (*Ursus arctos*)
Long-tailed weasel (*Mustela frenata*)
Mountain beaver (*Aplodontia rufa*)
Mule deer (*Odocoileus hemionus*)
Northern flying squirrel (*Glaucomys sabrinus*)
Short-tailed weasel (ermine; *Mustela erminea*)
Snowshoe hare (*Lepus americanus*)
Wolverine (*Gulo gulo*)