LANDSCAPE GENETICS OF BLACK BEARS (*URSUS AMERICANUS*) ON THE KENAI PENINSULA, ALASKA: PHYLOGENETIC, POPULATION GENETIC AND SPATIAL ANALYSES

A Thesis

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ABSTRACT

Anthropogenic landuse change and habitat fragmentation are among the most pressing threats to wildlife populations. Far-ranging species, such as black bears (*Ursus americanus*), may be particularly impacted by habitat loss and impeded dispersal. Wildlife managers on Alaska's Kenai Peninsula recognized the need to proactively inventory and monitor black bear populations on the Kenai leading to a comprehensive black bear study program to evaluate the ecology of Kenai black bears and assess threats to their populations. Molecular ecology research was a key component of this research program.

Investigations of molecular genetics provide important information about wildlife ecology that is not readily observed with direct field methods. This research will use mitochondrial DNA (mtDNA) sequencing and nuclear DNA (nDNA) microsatellite analysis to evaluate population genetic diversity, define biological population units, assess connectivity among populations, assess landscape influences on dispersal and population connectivity, and to estimate the abundance of bears in coastal areas of Kenai Fjords National Park.

Through phylogeographical analysis, I have shown that black bears on the Kenai Peninsula were historically distinct the mainland populations, showing signs that connectivity has been restricted since the end of the Pleistocene Ice Age. Bayesian assignment tests detected population genetic structure suggesting that genetically distinct biological units occupied the Kenai Peninsula, the Alaskan mainland, and Prince William Sound. Genetic diversity was similar among all groups and connectivity moderate between groups. Using spatial statistical analysis, I found that topographic and anthropogenic landscape features influenced gene flow within and among population units. Finally, I used capture-mark-recapture models to provide baseline estimates of black bears in coastal fjord areas and recommendations for future monitoring of bear abundance in these areas. This information will provide an important tool for population monitoring and management. The genetic data have provided insights into landscape patterns of genetic diversity and population structure which will be useful in guiding future investigations and management decisions. Further, novel analytical methods applied here will provide new tools for landscape/geographical genetic research.

VITAE

EDUCATION

Master of Science, Environmental Science, emphasis Wildlife Ecology.
University of Idaho, Moscow. Degree awarded May 2007.
Thesis: Landscape genetics of black bears (*Ursus americanus*) on the Kenai Peninsula, Alaska; phylogenetic, population genetic and spatial analyses.
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Bachelor of Science, Wildlife and Fisheries, Louisiana State University and Agricultural and Mechanical College, Baton Rouge. Degree awarded May 2000.

THESIS-RELATED PUBLICATIONS

In Press: Putting the "Landscape" in Landscape Genetics.
Storfer, Andrew, M. A. Murphy, J. S. Evans, C. S. Goldberg, S. J. Robinson, S. F. Spear, R. J. Dezzani, E. Delmelle, L. Vierling, L. P. Waits.
Heredity, an invited review (advanced online publication 1 November 2006).

Submitted: Evaluating Population Structure of Black Bears on the Kenai Peninsula Using Mitochondrial and Nuclear DNA Analyses.
Robinson, Stacie J., L. P. Waits, I. D. Martin.
Submitted to Journal of Mammalogy (August 2006).

Submitted: Landscape Patterns in Allele Distribution: the Utility of Local Autocorrelation Analyses Within and Between Populations.
Robinson, Stacie J., R. J. Dezzani, L. P. Waits.
Submitted to Molecular Ecology (January 2007).

Submitted: Estimating Black Bear Use of Coastal Food Resources in Kenai Fjords National Park Using Non-Invasive Genetic Techniques.

Robinson, Stacie J., L. P. Waits, I. D. Martin.

Submitted to Journal of Wildlife Management (February 2007).

Submitted: The Impacts of Spatial Sampling Design on Inferences of Population Genetic Structure: A Simulation Study.

Balkenhol, N., S. J. Robinson, R. J. Dezzani, L. A. Vierling, L. P. Waits.

Submitted to Molecular Ecology (March 2007).

Evaluation of Genetic Structure among Black Bears (*Ursus americanus*) in Kenai Fjords National Park (Southwest Alaska Network) and the Kenai Peninsula, Alaska.

Robinson, Stacie J., L. P. Waits, I. D. Martin.

2006. National Park Service Report.

Discovery of Single Nucleotide Polymorphism (SNP) Loci in a Non-Model Organism, the American black bear (*Ursus americanus*).

Robinson, Stacie J., C. L. Anderson, L. P. Waits.

2007. Report on Grant Funds to:

Center for Research on Invasive Species and Small Populations

The American Museum of Natural History

The Wildlife Society.

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TABLE of CONTENTS

LIST of TABLES

Chapter 1

Chapter 2

Chapter 4

LIST of FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 1

Thesis Introduction

BACKGROUND

Motivations

Anthropogenic landuse change and habitat fragmentation are among the most pressing threats to wildlife populations (Smith, Hellmann, 2002). Habitat fragmentation can isolate segments of the population and impede movement between them, decreasing the connectivity necessary to long-term population viability. Far-ranging species, such as black bears (*Ursus americanus*), may be particularly impacted by habitat loss and impeded dispersal (Pelton *et al.*, 1982).

Alaska is valued as one of America's last frontiers, maintaining large tracts of undisturbed wilderness. But, as Alaska becomes a more popular destination for recreation and settlement, even this last stronghold is threatened. The Kenai Peninsula, in south-central Alaska, has been a favored destination thanks to its mild coastal climate, proximity to Anchorage, and recreational opportunities. In the last 25 years the Kenai has received an influx of people and major landuse changes (National Park Service, 1999; US Census Bureau, 2006). Large portions of the peninsula are set aside in protected lands. Kenai Fjords National Park (KEFJ) was established in 1980 to protect the unique coastal fjords ecosystem on the Kenai (National Park Service, 1999). Recent increases in human exploitation of natural resources have brought to light the need to proactively inventory and monitor natural populations protected by the National Park Service (NPS).

NPS initiated the Inventory and Monitoring (I&M) Program in 1992 in an effort to build scientific foundations on which to base sound natural resource management. Among the goals of the I&M program are to integrate research and data management tools in standard park management operations and to integrate scientific findings in park management and planning (NPS, 2006). Park managers and I&M directors identified resource extraction, land development, landscape fragmentation, and hunting on the Kenai as immediate threats to the coastal ecosystem and resident wildlife.

Black bears represent a significant component of the fjords ecosystem. They occur throughout the coastal portions of KEFJ and are a focal attraction for park visitors. In 1998, KEFJ proposed a comprehensive study program on the ecology of black bears and threats to their populations. The goals of the study program were to gather sufficient information on black bear ecology to maintain a natural and healthy population of bears in the park and to develop and implement a coastal bear management plan. As part of the comprehensive black bear study program, a number of studies were launched and have been recently completed to provide insight on black bear habitat selection, identification of critical habitat types, movements and activity patterns (French, 2003), food selection (Crews, 2002), and bear responses to human activities (Smith, Partridge, in prep). My research will address the program objective of evaluating the genetic structure of the KEFJ black bear population in the context of the Kenai Peninsula landscape.

Support

Financial and logistical support for this project comes from Kenai Fjords National Park through the U.S. National Park Service Inventory and Monitoring initiative under cooperative agreement CA9088A0008 with the University of Idaho Department of Environmental Science. Project goals set by KEFJ include:

> "1.Obtain nDNA and mtDNA data from black bears across the Kenai Peninsula.
> 2.Examine female gene flow and phylogeographic structure using mitochondrial DNA sequencing of the control region.
> 3.Examine fine scale population genetic structure of black bears across the Kenai Peninsula using 14 to 16 nuclear DNA microsatellite loci.
> 4.Evaluate barriers to black bear movement across the Kenai Peninsula.
> 5.Describe black bear subpopulations in the Kenai Peninsula.(Martin, 2003)"

Additionally I&M program directors requested an estimate of black bear abundance within KEFJ (Bennet, 2004).

Research Questions

In my thesis research I strive to address the park objectives while expanding the study to provide information on the basic genetic ecology of black bears in south-central Alaska and to provide advancements in the field of molecular ecology. This research will use mitochondrial DNA (mtDNA) sequencing and nuclear DNA (nDNA) microsatellite analysis to evaluate population genetic diversity, population structure, landscape-genetic interactions and to estimate the abundance of bears using park resources. The information gathered from this study in conjunction with information from recently completed studies, will allow KEFJ resource managers to formulate a scientifically-based bear management strategy. Specific research questions addressed in the following chapters will include:

Chapter 2

- What is the phylogeographic relationship between black bears in south-central Alaska?
- What is the degree of genetic subdivision in the south-central Alaska black bear population?

Chapter 3

- What spatial patterns exist in the genetic variation among south-central Alaskan black bears?
- Does the Kenai Peninsula landscape influence spatial restrictions of black bear gene flow?

Chapter 4

- What is the abundance of black bears using concentrated food resources in bay areas of Kenai Fjords National Park?

STUDY LANDSCAPE

Land Management

Public lands cover over half of the Kenai Peninsula (Figure 1.2a) . Kenai Fjords National Park occupies a 2,292 km^2 band of rugged coastline between the Kenai Mountains and the Gulf of Alaska on the eastern coast of the Kenai Peninsula (Figure 1.2b). On the opposite side of the Harding Icefield is the Kenai National Wildlife Refuge (KNWR). The Refuge covers 7,930 km^2 from the Harding Icefield in the east to the north western coast of the Kenai Peninsula at Cook inlet. Kachemak Bay State Park comprises about 1,618 km^2 around the bay on the south-west tip of the Kenai. The 23,000 km^2 (5,179 km^2 on the Kenai) Chugach National Forest encompasses the northern Kenai mountains and all of the islands and coastline in/around Prince William Sound. Hunting is allowed in all of these parcels except KEFJ. All land in Alaska is divided into GMUs for management by Alaska Department of Fish and Game (ADF&G).

Study extent

We used Alaska Department of Fish & Game game management unit (GMU) boundaries to define our study area. While purely political boundaries, the GMU's provided a convenient bound for the study area and means of requesting samples from ADF&G. The study area consisted of GMUs 7 and 15 (a, b, and c), 16b, 14 (a, b, and c) and 6d. The total area was approximately 500km east to west and 300km north to south. Land area was approximately 70,000 km^2 (Figure 1.1). KEFJ formed a core of intense sampling within the large study region.

Legacy of Glaciation

The landscape and ecosystems of North America were shaped by the Pleistocene Ice Age. During the late Wisconsin glacial maximum, about 20,000 years before present (ybp), most

of Canada and the northern portions of the United States were covered by two massive ice sheets, the Cordilleran to the west and the Laurentide to the east (Pielou, 1991). As these bodies of ice advanced and retreated they influenced the climate of the entire continent, influenced sea and lake levels, and carved the landscape (Muhs *et al.*, 2001). Alaska was heavily impacted by past ice coverage (Hall, 2005). The distribution of plant species and wildlife in Alaska are a product of those that survived in refugia either on Beringia or south of the ice mass and then repopulated ice-covered areas after the melt (Pielou, 1991). The Kenai would have received enough warmth from the sea to keep the ice sheet from rising over the highest peaks of the coastal Kenai Mountain range (Pielou, 1991). It is likely that these nunataks received enough sunlight and precipitation to support small plant communities; but conditions would have been too harsh, and areas too small, to support mammalian megafauna (Muhs *et al.*, 2001). Phylogenetic and fossil evidence suggest that black bears survived the ice age in refugia south of the Laurentide ice sheet and in a small coastal area west of the Cordilleran ice sheet (Byun *et al.*, 1997; Stone, Cook, 2000; Wooding, Ward, 1997). Alaska was apparently recolonized from the south while the coastal population was still isolated by ice and high seas (Byun *et al.*, 1997).

The ice sheets started receding from south-central Alaska around 13,000 ybp. The western and southern Kenai were relieved of the Wisconsin glaciations by 11,800 ybp and the western coast (KEFJ) was deglaciated by 10,500 ybp (Wilkes, Calkin, 1994). At this time tremendous amounts of water were still captive in glaciers and smaller ice sheets. Sea levels were lower and the Kenai had not yet become a distinct peninsula (Pielou, 1991). By about 7,000 ybp most of the ice had melted, leaving only smaller glaciations that have survived to present times (Pielou, 1991). The land of south-central Alaska had been scoured and pitted by the ice making its way toward the sea. Between 10,000 ybp and 7,000 ybp these low-lying and channelized lands were flooded as the ice melted and sea level rose (Pielou, 1991). The Kenai became increasingly separated from the mainland; today it is connected to mainland Alaska by a mere 16 km of rock and ice of the Kenai Mountains.

The rugged landscape of south-central Alaska bears the legacy of the glaciers of the past and continues to be characterized by glaciers today. Wilkes and Calkin (1994) used tree ring and radio carbon dating and lichenometrics to record multiple advances and retreats of glaciers on the Kenai during to the Holocene and to date the most recent retreats (Wilkes, Calkin, 1994). They estimate that spruce forests had invaded the Kenai by 8,000 ybp and covered the peninsula by 3,000 ybp (Wilkes, Calkin, 1994). Current glaciers are restricted to high elevations in the Kenai Mountains. Kenai Mountain glaciers advanced during the Medieval Little Ice Age (1200 to 1800 A.D.). Most reached their maxima between 1500 and 1790 and retreated between 1700 and the late 1800's (Wilkes, Calkin, 1994). The glaciers of Nuka and Harris Bays are notable exceptions; they retreated approximately 300 years later than neighboring glaciers (Wilkes, Calkin, 1994) (bays labeled in Figure 1.2). Two Arm Bay was not heavily glaciated during the little ice age, thus ice-free habitat has been stable for 100's of years (Wilkes, Calkin, 1994). Glaciers in Aialik Bay have been stable since little ice age retreat and photo documentation shows these glaciers to be in similar position since the first photos in 1909 (Wilkes, Calkin, 1994). Thus the vegetative communities and potential bear habitat in these bays are considered to be well established. On the other hand, Nuka and Harris Bays have undergone substantial landscape change as their glaciers have rapidly and recently retreated. McCarthy Glacier in Nuka Bay retreated 25 km in the first half of the twentieth century, but ice margins have changed little since photo documentation in 1953 (Wilkes, Calkin, 1994). Northwestern Glacier in Harris Bay retreated 10 km in the last 100 years, much of the retreat in the last 50 years creating the most early successional and dynamic habitat in the KEFJ fjord system.

Present Climate

The Kenai Peninsula and coastal regions of south-central Alaska have among the most moderate climates in the state. Warm ocean currents in the Gulf of Alaska have a moderating influence on regional temperatures, leading to cool summers and relatively mild winters (Gallant *et al.*, 1995). The coastal and mountain regions get large amounts of precipitation averaging 60 inches per year at low elevations (Spencer, Hakala, 1964). Snow

cover is predominate from November to May and may persist through most of the year at high elevations (Spencer, Hakala, 1964). High coastal mountain areas receive substantially more precipitation and snowfall than any other ecotype in the area (Gallant *et al.*, 1995). The Harding Icefield averages 160 inches of precipitation with 400 inches of snowfall annually (Spencer, Hakala, 1964).

Present Ecosystem

The study landscape comprises a diversity of ecolological communities. The level III ecoregions of Baily (1995) provide a useful broad scale description of the area ecosystems. The area is composed primarily of the Cook Inlet region, Pacific Coastal Mountains, and Coastal Western Hemlock – Sitka Spruce Forest (Gallant *et al.*, 1995) (Figure 1.3). Some edges of the study region fall into the Alaska Range. The Cook Inlet region covers the western third of the Kenai. This is a low-lying region scoured by Pleistocene glaciers with rolling topographic relief of only 15 – 100m (Wilkes, Calkin, 1994). This region supports varied plant communities but is dominated by northern boreal forest species, including white spruce (*Picea glauca*), black spruce (*Picea mariana*), black cottonwood (*Populus trichocarpa*), quaking aspen (*Populus tremuloides*), and paper birch (*Betula papyrifera*) (Gallant *et al.*, 1995). The lowlands are peppered with lakes and numerous streams supporting runs of wild salmon. The Pacific Coastal Mountains include the steep and rugged Kenai and Chugach ranges (Gallant *et al.*, 1995). Elevation rises sharply from sea level to over 2,000m. This region was glaciated during the Pleistocene and much of the area above 700m remains glaciated to date (Wilkes, Calkin, 1994). Vegetated zones are dominated by dwarf or low shrub communities (Gallant *et al.*, 1995). The Alaska Range is inland but similar in community structure (Gallant *et al.*, 1995). The Coastal Western Hemlock – Sitka Spruce Forest covers the coastal regions from the southern tip of the Kenai extending beyond Prince William Sound. These deeply fjorded coasts were cut by Pleistocene glaciers flowing to the Gulf of Alaska (Wilkes, Calkin, 1994). Forests are dominated by western hemlock (*Tsuga mertesiana*) and sitka spruce (*Picea sitchensis*) and have substantial shrubby

understory communities (Gallant *et al.*, 1995). Beach grass (*Elymus spp.*) is prevalent in flatter areas immediately adjacent to the water (French, 2003).

Ducks Unlimited (DU) performed extensive fine-scale landcover classification on the Kenai Peninsula. This information was based on two Landsat TM satellite scenes (Path 69, Row 17 and 18 acquired July 10, 1989) and does have some limitations (Ducks Unlimited Inc., 1999). The Landsat path bounds cut off a small strip of land at the extreme northeast coast of the Kenai and do not include the mainland at all. Limited fine-scale landcover data was available for these regions at the time of study. The Ducks Unlimited analysis identified 14 landcover types on the peninsula (and two designations for cloud cover that make up less than 1% of the landcover) (Table 1.1). Forest habitat types included; Closed Needleleaf, Open Needleleaf, Woodland Needleleaf, Closed Deciduous, Open Deciduous, Closed Mixed Needleleaf/Deciduous and Open Mixed Needleleaf/Deciduous (Ducks Unlimited Inc., 1999). Shrub habitat types included Riparian Alder/Willow, Alder, Willow, Other Shrub (Ducks Unlimited Inc., 1999). There was Herbaceous and one Barren/Sparce habitat type (Ducks Unlimited Inc., 1999). There were three water types including; Snow/Ice, Clear Water and Turbid Water (Ducks Unlimited Inc., 1999).

STUDY SPECIES

Physical Description

Black bears are large omnivorous mammals (Figure 1.4). Adults stand 60-90 cm at the shoulder and are 1.5-2 m in total length (Powell *et al.*, 1997). The average weight of adult females is 40 – 70 kg, males 60-140 kg; occasionally males may exceed 250-300kg (Pelton *et al.*, 1982). Bears trapped within KEFJ by French *et al*. (2003) ranged from 37-75 kg for females, and 40 – 117 kg for males. Full growth is reached at 4-5 years for females and 6-7 years for males (Pelton *et al.*, 1982).

History & Status

The family Ursidae underwent rapid evolutionary radiation and recent speciation events, dating back to the mid-Miocene about 20 million years ago (Goldman *et al.*, 1989; Kurten, 1968; Waits, 1999). Eight species exist of which *Ursus americanus* is considered one of the most recently derived (Talbot, Shields, 1996; Yu, Liao, 2000). Its closest taxonomic relative is the Asiatic black bear (*Ursus thibetanus)* (Talbot, Shields, 1996; Yu, Liao, 2000). Black bears have been subdivided into 17 subspecies (Hall, 1981), including a Kenai subspecies, *Ursus americanus perniger* (Allen, 1910). Most of these designations are based on historical type species and morphometric measures. Few are phylogenetically supported (Byun *et al.*, 1997; Wooding, Ward, 1997). Only the Louisiana subspecies (*U.a. luteolus*) and Florida subspecies (*U.a. floridanus*) are recognized with special conservation status (US Fish and Wildlife Service, 2006).

Black bears have inhabited North America for about 500,000 years (Powell *et al.*, 1997). Historically black bears inhabited most forested areas of North America; present distribution is primarily restricted to forested areas without dense human settlement (Pelton *et al.*, 1982). They still inhabit much of their original range in Canada. Habitat fragmentation has been more pronounced in the United States. In Canada black bears are legally hunted big game in

all provinces and are under no major threats given the remoteness of much of the country (Pelton *et al.*, 1982). In the United States black bears are classified as big game species in 33 states and are legally hunted in 28 (Pelton *et al.*, 1982). Seven states classify black bears as rare, threatened, or endangered. A majority of states regard habitat loss and fragmentation as threats to black bear populations (Pelton *et al.*, 1982).

Black bear populations on the Kenai are believed to be stable and have access to relatively continuous habitat, though increased human use of the area could put more stress on bear populations and habitat (Schwartz, Franzmann, 1992). The population is expected to decrease in the next 10 years due to human habitat destruction through development and deforestation, and to declining moose populations (related to forest succession in historic burn areas) (Alaska Dept. Fish and Game, 2002). Hunting is the principal cause of mortality for black bears on the Kenai (Schwartz, Franzmann, 1992). Status of black bears in the study area is primarily measured by hunter takes and occasional flight transects by ADF&G to estimate population densities. Population density in GMU 6 was estimated at 0.54 bears per square kilometer in the western end of PWS and 0.27 bears/km^2 in eastern PWS (current management reports in 2002 are based on 1978 survey data) (Alaska Dept. Fish and Game, 2002). Unit 14 was estimated to contain 530-1080 bears in 17,158 km^2 of habitat leading to a density of 0.03-0.06 bears/km^2 (Alaska Dept. Fish and Game, 2002; Griese, 1999). Unit 16 was estimated to support 0.11 bears/km2 of suitable habitat. Extrapolating this density to the estimated area of suitable habitat yields a total estimate of 2,700 black bears (Alaska Dept. Fish and Game, 2002). Units 7 and 15, on the Kenai were estimated to support 0.21-0.27 bears/km^2 (Schwartz, Franzmann, 1991). Extrapolating this density to the estimated area of suitable habitat on the Kenai yields an estimate of about 3,000 black bears on the Kenai (Alaska Dept. Fish and Game, 2002).

Reproduction

Bears are long lived, mature slowly, and have low reproductive rates and recruitment (Schwartz, Franzmann, 1992). Both males and females reach sexual maturity about 3.5

years. However, 3.5 year old males are not yet full size and may not be able to compete for breeding position for several more years (Powell *et al.*, 1997). Black bears are promiscuous and breed from mid-May to mid-August followed by birth in mid-January to mid-February (Pelton *et al.*, 1982). Natality (breeding interval and litter size) is largely determined by food abundance (Schwartz, Franzmann, 1992). Typical litter size is one to two but three to four are not uncommon when resources are abundant (Schwartz, Franzmann, 1992). Cubs stay with the mother for about 1.5 years becoming independent in their second summer (Schwartz, Franzmann, 1992). Due to the extended period of care female bears typically breed only every two years (Powell *et al.*, 1997).

Habitat & Nutritional Requirements

Black bear habitat is characterized by rough terrain and thick understory vegetation with abundant food sources from tree- and shrub-borne hard and soft mast. Throughout the range of North American black bears the preferred habitats always include some portion of thick (nearly impenetrable) underbrush (Pelton *et al.*, 1982). Critical components of black bear habitat include food, escape cover, and den sites (Powell *et al.*, 1997). It is important for bears to have access to all of the critical habitat components in an unhostile, unfragmented habitat area (Powell *et al.*, 1997). Preliminary data from French *et al*. (2003) suggests that bears in KEFJ select forest cover with substantial understory vegetation. Male bears were more likely to venture into barren areas than were females (French, 2003).

Black bears eat a wide variety of foods. They are highly adaptable and will forage in natural and man-made open areas, but many critical foods and cover sources are available predominantly in mature forests (Powell *et al.*, 1997). They can be active predators on vertebrate prey, particularly elk and moose calves (Schwartz, Franzmann, 1992), though they lack the prey specialization of other carnivores. Black bears rely predominantly on insects and plant foods; mostly fruits, nuts, and young leaves that are easy to digest (Pelton et al., 1982). A fecal nutritional analysis within KEFJ found that salmon berries were the key diet item for black bear within the park (Crews, 2002). Abundant salmon runs also provide a

critical food source to Alaskan populations (Crews, 2002). According to Robins *et al*. (2004) the highly digestible meat proteins may be underrepresented in fecal samples due to much greater digestive efficiency.

Wild salmon runs are abundant on the Kenai providing an excellent protein source for bears (Robbins *et al.*, 2004). While available throughout the Kenai, the lowlands are host to the most substantial salmon runs while the east coast generally supports shorter runs as the rugged topography rapidly becomes too steep for fish to pass. The lowlands are also home to denser brown bear (*Ursus arctos*) populations than the coastal areas (National Park Service, 1999). The abundance of brown bears may limit black bear access to prime fishing territory in lowlands (Fortin *et al.*, 2006). Stable isotope analysis has revealed that marine fish might constitute over 50% of the diet when Alaskan black bears able to access salmon streams without brown bear competition (Robbins *et al.*, 2004). Human use of fisheries is also highest in the lowland areas (Tollefson *et al.*, 2005).

Social Behavior

Black bears are flexible in their social organization and vary in degrees of home range overlap and territoriality. Black bears are typically solitary with the exception of breeding pairs, mother cub groups, and congregations at concentrated food resources (Powell *et al.*, 1997). While they are not highly territorial, dominance hierarchies are maintained between adults and smaller bears (Pelton *et al.*, 1982). Females tolerate their offspring until maturity but may act territorially toward unrelated females (Powell *et al.*, 1997). Female offspring may establish a home range adjacent to the mothers which may improve the survivorship of both through reduced aggressions (Powell *et al.*, 1997). Highly mobile male bears exhibit extensive home range overlap and are thus not considered territorial (Powell *et al.*, 1997).

Space Use & Movement

In different geographic regions, black bear home ranges vary considerably, though the home ranges of females are consistently a quarter to a third the size of males. Females range from 3-40 km^2, while male home ranges may be upwards of 100km^2 (Powell *et al.*, 1997). Within these large home ranges space use tends to be clumped in key areas which may vary seasonally (Horner, Powell, 2006). Extensive home range, even core area, overlap has been documented between all age and sex classes (Horner, Powell, 2006). Genetic methods have shown that overlap is not restricted to related individuals (Schenk *et al.*, 1998). The size and the degree of overlap in home ranges may depend on food abundance and distribution (Rogers, 1987).

Black bears can make complicated selections of habitat (Davis *et al.*, 2006). Important factors in habitat selection appear to be spatial and temportal availability of food resources and freedom from anthropogenic disturbance (Davis *et al.*, 2006). In coastal areas of British Columbia, Davis *et al*. (2006) found that black bears were more likely to select sites with available salmon runs and less likely to select areas with high human disturbance (Davis *et al.*, 2006). Over time, habitat selection affects the distribution of genetic variation (Thompson *et al.*, 2005). Thompson *et al*. (2005) used an allele mapping technique to show that high levels of gene flow aligned with the best available habitat.

Males are more mobile and tend to disperse much farther than females (Powell *et al.*, 1997). Extreme male dispersals have been recorded over 300 km (Rogers, 1987). This difference in mobility and home range size is typical of polygamous mammals in which the female must secure an area with food resources for herself and her offspring; meanwhile, the male maximizes fitness from maintaining a mating range that gives him access to numerous females (Rogers, 1987).

Family breakup – natal dispersal of cubs – coincides with the onset of the breeding season in the cub's second summer (Schwartz, Franzmann, 1992). Following family breakup male

cubs disperse, while females typically establish a homerange within the maternal range (Lee, Vaughan, 2003; Schwartz, Franzmann, 1992). Dispersal reduced survival of juvenile bears on the Kenai with 50% of dispersing bears dying the same year, and only 11% surviving to maturity (3+ years) (Schwartz, Franzmann, 1992). The only observed female disperser died the same year. Mortality was primarily due to human factors (Schwartz, Franzmann, 1992). Males were 2-3 times less likely to survive to adulthood than females (Schwartz, Franzmann, 1992). Lee and Vaughn (2003) found similar patterns of sex-biased dispersal and survival in a Virginia population. Subadult male bears in search of suitable unoccupied habitat are more likely to be in close proximity to humans and more vulnerable to hunting than non-dispersing subadult females (Schwartz, Franzmann, 1992).

Numerous factors may affect dispersal distances and routes. Habitat connectivity and available cover are both influential to black bear dispersal. Lee and Vaughan (2003) found that in natal dispersals juvenile bears used predominant ridgelines as dispersal cooridors and avoided leaving forest cover. Cushman *et al*. (2006) examined effective dispersal in a landscape genetic framework and found that the landscape, particularly available forest cover, influenced bear movement and genetic structure. Interruptions in cover or poor habitat may act as barriers to bear dispersal. Major roads have been shown to limit black bear (Lee, Vaughan, 2003; Thompson *et al.*, 2005) as well as brown bear (Proctor *et al.*, 2005) movement. Human dominated, agricultural landscapes have also been shown to impede black bear movement (Cushman *et al.*, 2006). Absent human disturbance, large expanses of salt water and/or substantial icefields can also deter black bear dispersal (Peacock, 2004). The factors of reproductive system, habitat selection and space use come together to affect gene flow and the genetic ecology of black bears.

GENERAL METHODOLOGY

Molecular Ecology

Molecular techniques have proven useful in answering a variety of ecological questions, thus giving rise to the discipline of molecular ecology (Burke, 1994). Molecular ecology applications range from considerations of entire taxa over evolutionary time to individuals in the immediate time frame. Many applications of molecular ecology focus on the definition of biological units and the conservation of genetic diversity and evolutionary potential in species or populations threatened with declining population size, loss of habitat or other disruptions (Deyoung, Honeycutt, 2005). Genetics issues have both immediate and long term management implications that are particularly applicable to bears as populations typically have low densities and low effective sizes compared to other mammals (Waits, 1999). Genetic information is essential to estimating population viability and evaluating possible management decisions (Deyoung, Honeycutt, 2005).

Genetic data plays a key role in defining biologically meaningful units. Genetic information has been used extensively in defining/redefining taxonomic relationships (Avise, Ball, 1990); it has even been suggested that taxonomic divisions be reorganized to rely solely on genetic information (Tautz *et al.*, 2003). Evolutionarily significant units (ESUs) have been defined based on deep genetic divergence, requiring reciprocal monophylly (Moritz 1994). Management units (MUs), on the other hand, may lack deep evolutionary divergence, but are demographically and genetically distinct (Moritz, 1994). Such genetic designations may be particularly useful when population units lack distinct habitat associations or geographic boundaries (Deyoung, Honeycutt, 2005).

Key tools of molecular ecology include measures of population connectivity or divergence and similarity or differences in genetic composition. Numerous factors affect genetic variation including selection, mutation, drift, effective population size, bottle necks, founder effects, mating system, population structure, dispersal, gene flow, habitat connectivity and

management actions (DeWoody, 2005). Genetic connectivity, or gene flow, is intimately linked with dispersal and population structure. Gene flow results when individuals disperse beyond their natal population and breed elsewhere thereby spreading their genes to another population. Estimates of gene flow can provide important measures of the cumulative effects of movement and "successful" dispersal over temporal and spatial scales where direct observations would be impractical and imprecise (Scribner *et al.*, 2005). Genetic estimates of dispersal have been found to be significantly correlated with demographic dispersal (Vandewoestijne, Baguette, 1999). Limited gene flow leads to increased genetic differentiation between populations due to independent genetic drift. Fixation indexes are useful in quantifying genetic differentiation between population units (Scribner *et al.*, 2005). Wright (1965) introduced the concept of using correlations of genes to produce fixation indices: Fit, the correlation between gametes among individuals across the total population sampled; Fis, the correlation within individuals relative to each subpopulation sampled; and Fst, the correlation of individuals within subpopulations in relation to the total sample. Because gene flow is heavily influenced by habitat connectivity, levels of gene flow and F statistics are commonly used in studying the effects of landuse change and fragmentation on wildlife populations (DeWoody, 2005).

Measures of similarity or dissimilarity in genetic composition can be used to describe relationships between individuals, populations, or taxonomic units. Relatedness coefficients describe the proportion of shared genetic material between individuals (Queller, Goodnight, 1989). On the other hand, genetic distance measures differences in genotype or allele frequencies between populations or individuals. Several genetic distance measures exist adapted for use over different spatial or temporal scales and different genetic markers (Paetkau *et al.*, 1997).

Phylogenetics

Phylogenetics provides a broad scale perspective to molecular ecology, considering relationships between species or far ranging populations over 1,000´s of generations. The

field of phylogenetics uses DNA sequence analysis to reconstruct evolutionary relationships (Hedrick, 2005). Genetic data are useful in resolving phylogenies that are not clear by the analysis of morphological measurements (Hedrick, 2005). Phenotypic traits may be more directly affected by natural selection, whereas genetic sequences may be more robust to selection and thus more informative (Hedrick, 2005). In fact, much of basic phylogenetics and population genetics relies on neutral genetic markers and is based on the theory that genetic differences are accumulated through mutation and genetic drift and thus genetic differentiation is a product of time since divergence (Hedrick, 2005). Phylogenetic studies make extensive use of mitochondrial DNA sequences as they are informative over the historic time scale (Snow, Parker, 1998).

Phylogeography

The term phylogeography was coined by Avise and Nelson (2000) as the study of "principles and processes governing the geographic distribution of genetic lineages" (Avise, Nelson, 2000). Phylogeography examines the spatial as well as genetic relationship among DNA sequences. Assuming spatial patterns mimic temporal patterns, phylogenetic relationships can be used to deduce historic range expansions and colinizations (Hedrick, 2005). Phylogeographic techniques have also proven useful in detecting hybrid zones (Deyoung, Honeycutt, 2005).

Population Genetics

Population genetics provides a finer scale perspective to molecular ecology, focusing on the individual and/or population as units of study over the period of a few recent generations (Hedrick, 2005). These finer scale investigations require genetic markers of finer resolution, or greater variability. Many population genetic techniques focus on genetic markers that allow the identification of individuals and discrimination between close relationships. Microsatellites have been widespread in wildlife population genetics research (Snow, Parker, 1998).

Population genetic techniques are useful in answering myriad ecological and demographic questions. Genetic diversity has been linked with fitness and long-term viability of populations. The distribution of allele frequencies can be informative regarding definition of genetic populations, the geographic ranges of populations, changes in population size and detection of recent population bottlenecks (Deyoung, Honeycutt, 2005). Individual identification is instrumental in forensic applications (Lewontin, Hartl, 1991) and has also provided a means of estimating abundance (McKelvey, Schwartz, 2004) and migration rates over large geographic areas (Rannala, Mountain, 1997). Parenatage and relatedness studies are useful in determining mating systems, behaviors such as kin selection and kin-cooperation, and population social structure (DeWoody, 2005). See Table 1 in DeWoody (2005) for a valuable summary of applications and genetic markers appropriate to each.

Geographical-Landscape Genetics

Just as phylogeography provides a spatial extension to phylogenetics, the fields of geographical and landscape genetics extend population genetics into the spatial and environmental context. The term "geographical genetics" was coined by Epperson (2003) to describe an extensive body of analysis of spatial genetic patterns. In the same year, Manel *et al*. (2003) coined the term "landscape genetics" to describe the combination of population genetics and landscape ecology. Both fields emphasize fine scale study aimed at discovering the processes affecting the partitioning of genetic variation across the landscape. Geographical genetics more specifically emphasizes the use of spatial statistics, while landscape genetics endeavors more broadly to encourage the joint analysis of spatial/environmental data and genetic data. Both fields have received great attention and inspired a breadth of novel research in recent years (see Scribner *et al.*, 2005; and Storfer *et al.*, 2006 for thorough reviews). It is my feeling that the theory and intent of these two fields are quite similar, the methods substantially overlapping, and their distinction not useful to interdisciplinary collaboration. These two fields will thus be jointly considered and applied throughout this work.

The detection and location of genetic discontinuities is important to managing natural wildlife populations, and is an important first step in more complex analyses of the processes affecting genetic patterns (Manel *et al.*, 2003; Scribner *et al.*, 2005). Genetic patterns are characterized by the distribution of alleles within populations or across space (Epperson, 2003; Manel *et al.*, 2003). These patterns may depart from randomness, panmxia, due to various forms of isolation: geographic distance (Wright, 1943), dispersal barriers (Manni *et al.*, 2004), landscape resistance (Cushman *et al.*, 2006), behavior factors (Deyoung, Honeycutt, 2005), or temporal factors (Vandewoestijne, Baguette, 1999). Identifying genetic patterns and correlating them with influential landscape features can provide ecological information, reveal cryptic population structure and secondary contact between previously isolated populations (Manel *et al.*, 2003). There are many applied examples. Genetic structure has been used to infer metapopulation dynamics (Manier, Arnold, 2005). Differences in spatial genetic structure related to landscape patches has been used to determine effects of anthropogenic landscape change on dispersal and geneflow (Banks *et al.*, 2005). Stock mixture analysis has been applied to aid management of exploited populations (Corander *et al.*, 2006a). Spatial analyses and assignment tests have been useful in identifying sources and tracking the geographic spread of both invasive species (Roman, Palumbi, 2004), and emergent diseases (Blanchong *et al.*, 2006).

The analytical tools available to landscape geneticist are rapidly developing. Some current favorites include: Bayesian assignment tests to delineate genetically distinct units on the landscape (Corander *et al.*, 2006b; Pritchard *et al.*, 2000); barrier detection algorithms to detect zones of maximal genetic change (Barbujani, 1989; Manni *et al.*, 2004); autocorrelation and correlograms to assess degrees of isolation as well as rates and directions of migration (Epperson, Li, 1996; Scribner *et al.*, 2005); matrix correlation tests to examine correlations between genetic and ecological distances (Legendre, Fortin, 1989; Mantel, 1967; Spear *et al.*, 2005); and the interpolation of genetic surfaces to relate to landscape surfaces (Miller, 2005). The advent of spatially-explicit genetic analyses has also forced a reevaluation of sampling methods for population genetics (Scribner *et al.*, 2005). Spatial

sampling design is of increasing importance for inferring population structure or spatial relationships without introducing sampling bias. Manel *et* al. (2003) recommend random sampling of individuals across the study landscape. Others recommend a spatially nested sample so that one can draw inference related to the individual – population – metapopulation – range-wide levels (Scribner *et al.*, 2005; Trapnell *et al.*, 2004).

Genetic Markers

Mitochondrial DNA (mtDNA) sequences are used to examine nucleotide variation between haplotypes. MtDNA is a powerful tool in population analysis due to a relatively rapid rate of base substitution, haploid and maternal inheritance which reduces effective population size (Moritz, 1994). These characters make mtDNA particular sensitive for detecting signals of genetic drift (Avise, 2004; DeWoody, 2005). MtDNA may diverge or show population structure where nuclear DNA sequence does not due to the lower effective number of genes, particularly in cases of male-biased dispersal (Moritz, 1994). MtDNA data applies both to long term phylogenetic studies and short term demographic investigations, providing important information about evolutionary history, historic isolation events, and maternal gene flow patterns (Cronin *et al.*, 1991; Stone, Cook, 2000).

Nuclear DNA microsatellites are simple sequence motifs repeated in various numbers (Ellegren, 2004). Due to the nature of these simple sequence repeats, slippage during DNA replication is common leading to a high mutation rate in microsatellites (up to 0.007 Brinkmann *et al.*, 1998). Microsatellites combine the benefits of high variability and co-dominant inheritance (Frankham *et al.*, 2002). Microsatellite data can be used to detect genetic variation within a population, evaluate gene flow, define genetic structure, identify individuals, and determine paternal and sibling relationships (Parker *et al.*, 1998; Zhang, Hewitt, 2003). These data can also be used to detect migrants and elucidate patterns of dispersal (Schenk *et al.*, 1998). The short length of microsatellite fragments makes them particularly useful in non-invasive sampling (Waits, Paetkau, 2005).

Non-invasive Sampling

Non-invasive genetic sampling has gained popularity in recent years as a means to sample and monitor wild populations with minimal impact on animal behavior or wellbeing. This sampling technique employs means of gathering DNA from sources left behind by the animal (hair, feces, shed skin, feathers) without having to catch, handle, or otherwise disturb the animal (Taberlet *et al.*, 1999). Non-invasive sampling is ideal when dealing with small or endangered populations or in situations where handling the animal may disturb the system (Taberlet *et al.*, 1999). It can even be useful for documenting the presence of rare or hard-to-observe species (Waits, Paetkau, 2005). Limitations do exist related to poor DNA quality or low DNA quantity from noninvasively-collected samples. There is high risk of contamination and most DNA will be degraded into small fragments making it difficult to amplify longer fragments (Frantzen *et al.*, 1998). See Waits and Paetkau (2005) Table 1 for examples of projects applying non-invasive genetics to a variety of DNA source tissues.

Non-invasive sampling was critical for the sampling effort within Kenai Fjords National Park and proved the most efficient means for meeting the NPS goals for this study. Non-invasively collecting hair samples allowed us to collect many more samples than would have been possible trapping bears. This also helped minimize the impacts our research might have on bear behavior or safety, or visitor experience. Using non-baited hair snaring techniques allowed us to discretely collect samples without influencing bear behavior through baiting or handling (details Chapter 4, this document). Samples from the broader study area were collected opportunistically from hunt-monitoring activities (details in Chapter 2, this document).

Laboratory

Whole genomic DNA was extracted from samples. Desired fragments were amplified using polymerase chain reaction (PCR) with specifically designed PCR primers. Primers were

labeled with fluorescent dyes and alleles scored based on the fluorescence detected by an automated sequencer. (Details on lab procedures in Chapter 2, this document).

DNA sequences and fragments were analyzed using a strict protocol to ensure consistent allele scoring and to minimize human error in genotyping. Automated scoring of fragments or sequences was always double-checked and screened for potential errors. A series of cautions was established to eliminate subjectivity in microsatellite genotyping. Allele bins and bin ranges were established to aid automated fragment analysis (Table 1.2). Minimum fluorescent intensities were required for accepting alleles and with particularly stringent thresholds set for accepting homozygote genotypes in size ranges prone to allelic dropout (Table 1.2). Thresholds were also set for discriminating stutter peaks or adenalated peaks from true alleles which can be ambiguous when fluorescent peaks differ by two base pairs (bp). In such cases, if the fluorescent intensity of right most peak was ≤25% that of the peak two bp to the left, the right peak was called the adenylated peak and the left was called the true allele. If the right peak was 26-75% the intensity of the left peak, both were called true alleles. If the right peak was 76-95% the intensity of the left peak, the locus was re-PCRed to confirm the ambiguous genotype. If the right peak was ≥ 96% the intensity of the left peak, the right peak was called the true allele and the left peak was called a stutter.

Recent reviews have pointed out the importance of standardizing data quality-checking protocols and reporting error rates in molecular studies (Bonin *et al.*, 2004; Paetkau, 2003). Genotyping errors have led to misleading inferences when not detected (Wilmer *et al.*, 1999). Allelic dropout (failure of one allele to amplify in a heterozygote) and false alleles (appearance of a nonexistent allele as a result of PCR error) can be particularly problematic in microsatellite data due to PCR slippage in amplifying these simple repeats (Broquet, Petit, 2004; Gerloff *et al.*, 1995; Navidi *et al.*, 1992; Taberlet *et al.*, 1996). We followed the recommendations of Bonin *et al*. (2004) implementing a multi-faceted quality-checking approach described in detail in Chapter 2 (this document).

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Table 1.1: Land cover on the Kenai Peninsula as classified by Ducks Unlimited Inc.
The table gives the class description determined by Ducks Unlimited (1999), representative species, typical areas of occurrence, total acreage and % of Kenai area covered by each landcover class.

Class Name	Class Description	Representative Species	Occurrence	Acres	%
Clear Water	≥80% clear water			1,343,389	21.22
Turbid Water	≥80% turbid water			184,164	2.91
Snow / Ice	≥50% snow and/or ice			805,715	12.73
Barren / Sparsely Vegetated	≥50% barren vegetation sometimes >20%	herbs, graminoids, moss, lichen	riparian gravel bars, rocky slopes, disturbed areas	564,843	8.92
Closed Conifer Forest	≥60% trees >75% of the trees are needleleaf	Primarily Mt Hemlock and Sitka Spruce, then White Spruce	common high elevation	421,819	6.66
Open Conifer Forest	25-59% trees >75% of the trees are needleleaf.	White Spruce, Black Spruce	common low elevation	607,708	9.60
Woodland Conifer Forest	10-24% trees >75% of the trees are needleleaf	varied including Mt Hemlock, Sitka, White and Black Spruce	common throughout the Kenai	301,488	4.76
Closed Deciduous Forest	≥60% trees >75% of the trees are deciduous	Paper Birch, Aspen and Cottonwood	limited in size, primarily riparian	94,608	1.49
Open Deciduous Forest	25-59% trees >75% of the trees are deciduous	Paper Birch, Aspen and Cottonwood	relatively uncommon	16,452	0.26
Closed Mixed Forest	≥60% trees needleleaf nor deciduous >75%	varied	relatively uncommon, primarily riparian	43,693	0.69
Open Mixed Forest	25-59% trees needleleaf nor deciduous >75%	varied	common on slopes and early successional	234,612	3.71
Alder	40-100% shrubs	Alder shrubs makes up at least 80%	common on slopes and unforested flats	612,172	9.67
Alder / Willow Riparian	40-100% shrubs	Alder and/or Willow shrubs make up at least 60%	riparian corridor	25,647	0.41
Willow	40-100% shrubs	Willow shrubs makes up at least 80%	small isolated stands	39,863	0.63
Other Shrub	40-100% shrubs mixed	Dwarf Birch, Willow, Vaccinium, Ledum, dwarf ericaceous, Dryas	ranges from wet muskeg to dry subalpine	345,168	5.45
Herbaceous / Graminoid	≥40% herbaceous species	moss, lichen, forbs and graminoids Carex, Eriophorum, Calamagrostis	ranges from wetlands to alpine	594,478	9.39

Table 1.2: Microsatellite genotyping protocol

Colors in the left column indicate the florescent dye used to label each locus (blue=6fam, green=tet, yellow=hex). Allele sizes are measured in base pairs (bp). For each locus, the table indicates the expected range in allele sizes and the bin ranges set for automated genotyping with Genotyper 2.5 software. Mid point and range are used to describe the center and width of bins set for each allele (example based on first allele in each size range). The appropriate fluorescent peak to call can vary according to the adenylation pattern of each locus, the correct peak is described in the "Peak" column. The "Homo Min" columns give the minimum fluorescent intensity required to accept a homozygous genotype. The intensity must be higher to accept a homozygote at the low end of the size range because larger alleles are more likely to drop out ("Low Homo Min" - varies according to intensity ratio between low and high ends of size range).

	Msat Locus	Size Range	Bin Mid Point	Bin Range	Scoreable Peak	Homo Min	Low Homo Min
6-Fam	B	138-162	138.45	± 0.55	1st pk, not highest	150	250
	C	89-121	89.8	± 0.5	2nd pk aft aden	150	1000
	D	166-190	166.45	± 0.55	2nd pk aft aden	150	500
	M	198-228	199.4	± 0.5	1st pk, ignore smll aden	150	650
	O	174-212	174.2	± 0.4	1st pk, ignore smll aden	150	1000
Tet	A	179-195	174.85	± 0.45	1st pk	150	650
	L	131-169	125.4	± 0.5	1st pk	150	500
	Cxx20	123-153	124	± 0.35	1st pk, ignore smll aden	150	650
	mu15	128-158	128.1	± 0.3	2nd pk aft aden	150	250
Hex	P	151-179	151.95	± 0.45	1st pk	150	250
	J	84-108	85.1	± 0.4	1st pk	150	650
	mu50	110-140	110.1	± 0.5	2nd pk aft aden	150	1000
	mu23	116-146	116.2	± 0.4	1st pk	150	650

Figure 1.1: Study area
This study was conducted in south-central Alaska. Alaska Department of Fish & Game game management units served as the study area boundaries. Much of the area is mountainous and glaciated.

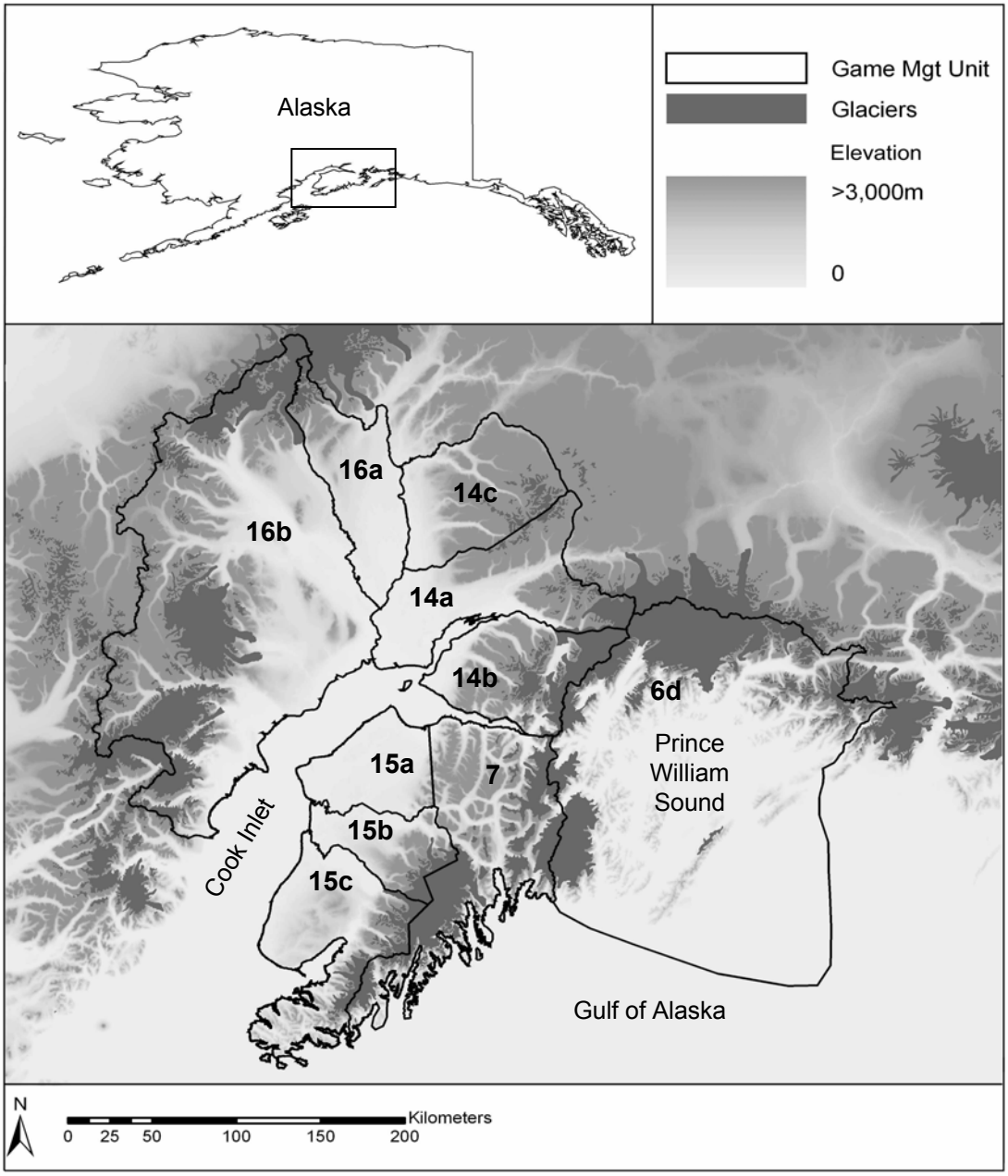

Figure 1.2: Public land parcels on the Kenai Peninsula
A) Shows major public land parcels on the Kenai Peninsula. B) Kenai Fjords National Park was the focal area of this research. Black bears occur in Kenai Fjords primarily in the narrow coastal strip between the glaciers and the Gulf of Alaska. Major bays in Kenai Fjords include Aialik (A), Harris (H), Two Arm (T), and Nuka (N).

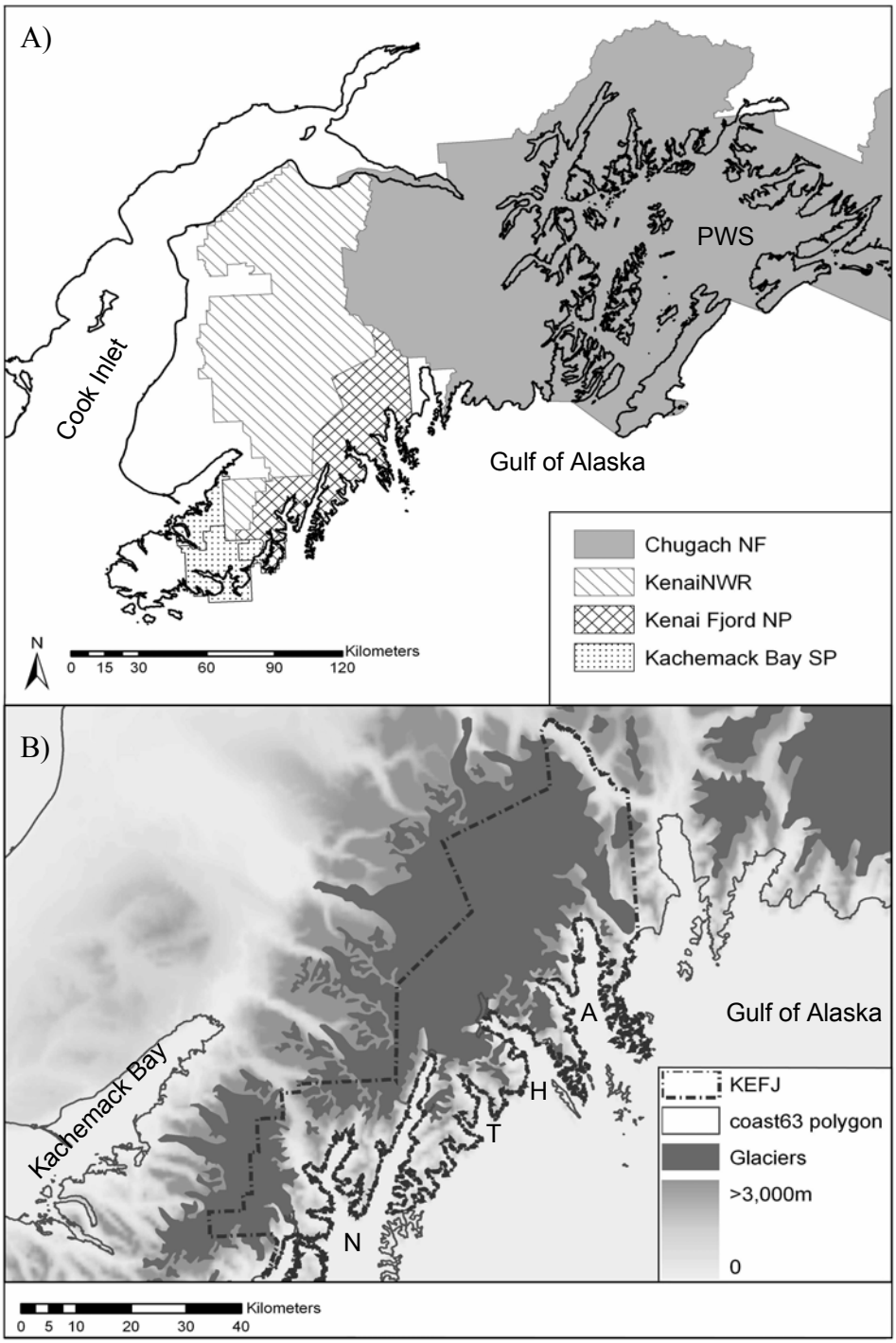

Figure 1.3: Class III ecoregions of south-central Alaska
(Adapted from Galant *et al*. 1995.) A) The Cook Inlet ecoregion is a low-lying plane consisting of boreal forests and wetlands, B) the Pacific Coastal Mountains ecoregion is primarily high-elevation and sparsely vegetated with alpine meadows and low shrubs, C) the Coastal Western Hemlock – Sitka Spruce Forest ecoregion consists of spruce and hemlock forests with varied undergrowth.

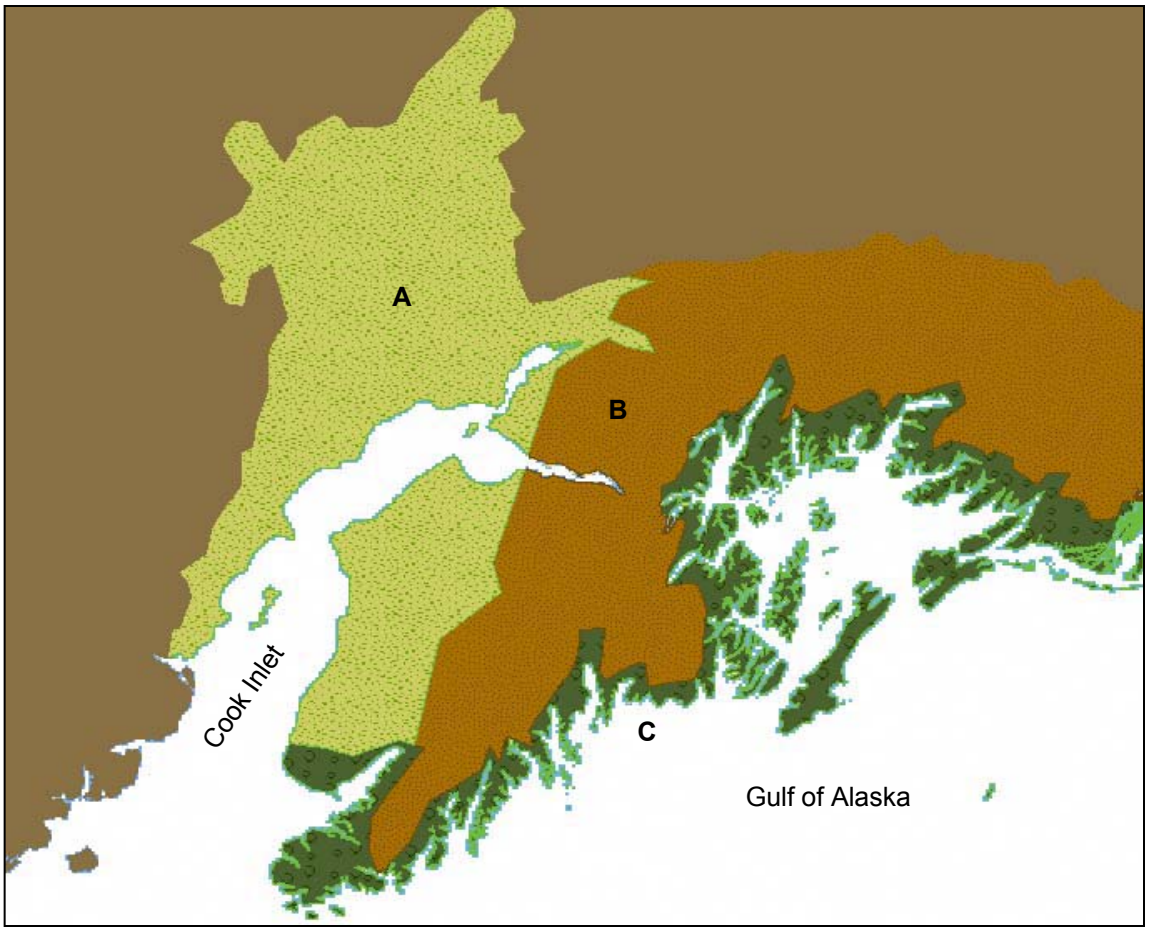

Figure 1.4: American black bear (*Ursus americanus*)
A mother black bear is shown with a cub in forested habitat. Forests are the primary habitat for black bears, providing escape cover, den sites, and vegetative food sources.

Chapter 2

Evaluating Population Structure of Black Bears on the Kenai Peninsula Using Mitochondrial and Nuclear DNA Analyses

ABSTRACT

Increasing human impacts on the Kenai Peninsula, Alaska have raised questions about potential implications for genetic diversity and population structure of local taxa. Black bears (*Ursus americanus*) occupy most of the Kenai Peninsula and are currently a species of public interest and management focus. In this study we use 13 nuclear DNA microsatellite loci and sequence data from the mitochondrial DNA control region to investigate population structure and phylogeographic patterns in black bears on the Kenai and surrounding mainland. We used both aspatial and spatial Bayesian assignment models to evaluate nuclear DNA genetic structure and cluster individuals into genetically distinct groups. Substantial population substructure was detected, indicating restricted gene flow in recent generations as well as signatures of past barriers between the Kenai and mainland. We identified three genetically distinct groups that cluster geographically in the Kenai Peninsula, Alaskan mainland and Prince William Sound areas. Connectivity among genetic groups was moderate with Fst values ranging from 0.07 to 0.12. Five mitochondrial DNA haplotypes were detected, two of which were primarily restricted to the Kenai. Our results provide important information about current levels of genetic diversity and connectivity among black bears on the Kenai Peninsula and will provide a baseline for future monitoring.

KEY WORDS

Bayesian assignment tests, Black bear, Genetic population structure, Kenai Peninsula, Microsatellite, Mitochondrial DNA, Phylogeography, Spatial assignment, *Ursus americanus*.

INTRODUCTION

The rugged landscape and distinct ecology of the Kenai Peninsula, Alaska, USA, have led wildlife managers to question whether population substructure exists between black bears (*Ursus americanus*) on the Kenai and the Alaskan mainland. The Kenai Peninsula has been separated from the mainland since the end of the last ice age by a narrow (16 km) isthmus of ice and rock. The coastal climate of the Kenai has fostered unique ecological conditions and biotic communities. Historically, the uniqueness of wildlife on the Kenai has led to the designation of numerous distinct subspecies such as song sparrows *(Melospiza melodia kenaiensis*, American Ornithologists' Union, 1957), American martens (*Martes americana kenaiensis*, Hagmeier, 1958), wolverines (*Gulo gulo katschemakensis*, Matschie, 1918), wolves (*Canus lupus alces*, Goldman, 1944), brown bears (*Ursus arctos gyas*, Merriam, 1918), and black bears (*Ursus americanus perniger*, Allen, 1910). Although these taxonomic splits have seldom been upheld by more recent phylogenetic investigations (Paquet, Carbyn, 2003; Tomasik, Cook, 2005; Waits *et al.*, 1998; Wooding, Ward, 1997), they do highlight the biological diversity harbored on the Kenai Peninsula.

In recent years wildlife managers at Kenai Fjords National Park (KEFJ) have noted resource extraction, land development, landscape fragmentation, and hunting as potential threats to wildlife populations on the Kenai. KEFJ provides important resources to a number of vertebrate species, including black bears which occur throughout the coastal portions of the park, represent a significant component of the fjords ecosystem, and are a focal attraction for park visitors (National Park Service, 1999). In response to the increasing human impacts on the Kenai, KEFJ launched a comprehensive study program to evaluate the ecology of, and threats to, black bears (National Park Service, 1999). The overall goal of the study program was to gather sufficient information on the status of Kenai black bears so that an appropriate bear management plan could be developed.

Historically black bears inhabited most forested areas of North America (Servheen, 1990). Their current distribution is restricted to forested areas lacking dense human settlement

(Pelton, van Manen, 1994). Black bear populations on the Kenai Peninsula have access to relatively continuous habitat and are believed to be stable; however, expanding human activity in the area is projected to increase stress on bear populations (Alaska Dept. Fish and Game, 2002; Schwartz, Franzmann, 1992).

Black bears are highly vagile and apt to move over great distances. Multiple studies, including work on the Kenai Peninsula have demonstrated that male black bears are more mobile than females and are more likely to disperse from their natal range (Lee, Vaughan, 2003; Schwartz, Franzmann, 1992). Males typically disperse much farther than females (Powell *et al.*, 1997). Dispersal distances of 50 to 100 km are common for male black bears (Texas, Hellgren *et al.*, 2005; Florida, Maehr *et al.*, 1988; Minnesota, Rogers, 1987a; Rogers, 1987b), and dispersals over hundreds of kilometers have been documented (Rogers, 1987a). The effects of such long dispersals can be difficult to document through direct observation.

Genetic analyses offer important insights into the population structure and connectivity among such wide-ranging animals. Genetic data provide information about historic and current levels of gene flow among populations, as well as information about genetic diversity, kinship, and movement patterns within populations (Paetkau *et al.*, 1998; Queller *et al.*, 1993; Schenk *et al.*, 1998; Woods *et al.*, 1999). Nuclear DNA (nDNA) microsatellite analyses coupled with assignment test approaches (used to assign an individual to the population from which its genotype is most likely to have arisen) have proven useful in detecting population structure and gene flow (Funk *et al.*, 2005; McRae *et al.*, 2005; Paetkau *et al.*, 1995; Slatkin, 1995). The development of Bayesian assignment tests allows inferences of genetic structure without *a priori* assumptions about population groupings within a given study area (Corander *et al.*, 2003; Pritchard *et al.*, 2000). Recent innovations have also added the use of geographic coordinates to assign individuals to spatially organized populations (Guillot *et al.*, 2005). Additionally, mitochondrial DNA (mtDNA) sequence data provide important information about maternal gene flow patterns, past isolation events, natural recolonization events, and evolutionary history (Cronin *et al.*, 1991; Onorato *et al.*, 2004; Sunnucks, 2000). MtDNA data have been widely used to study the phylogeography of bears

in North America (Byun *et al.*, 1997; Paetkau, Strobeck, 1996; Stone, Cook, 2000; Waits *et al.*, 1998; Wooding, Ward, 1997).

To assist wildlife managers in developing a scientifically-based bear management strategy, we gathered nDNA microsatellite data and mtDNA sequence data for 110 bears to address the following research questions: 1) What is the level of genetic diversity and population structure of black bears on the Kenai? 2) Are bears in this region panmictic or is there evidence for more than one population unit? 3) Is there evidence for current or historic restriction of gene flow between the Kenai and the mainland? This project will also establish baseline levels of genetic diversity and the degree of population genetic structure critical for defining management goals and monitoring black bear populations on the Kenai Peninsula.

MATERIALS and METHODS

Study Area

This study was conducted in black bear habitat on the Kenai Peninsula and adjacent mainland game management units covering a total of over 72,000 km^2. KEFJ occupies a 2,400 km^2 band of rugged coastline between the Gulf of Alaska and the thirty-two glaciers of the Harding Icefield. The study area also extended into Alaska Department of Fish and Game (ADF&G) game management units (GMUs) 6d, 7, 14a,b,c 15a,b,c and 16a,b (Figure 2.1A). These GMU's, although purely political boundaries, provided a useful bound to the study area and a means for requesting samples from ADF&G. The major physiographic landform on the eastern two-thirds of the peninsula is the rugged, heavily glaciated Kenai Mountain Range, which rises to 2,000 m from sea level (Muhs *et al.*, 2001). The Kenai lowlands, a Pleistocene-glaciated plain dotted with lakes, dominates the western one-third of the Kenai (Muhs *et al.*, 2001). The study area was composed of three ecoregions; the Pacific Coast Mountain Range ecoregion in high elevation areas, the Cook Inlet ecoregion covering the Kenai lowlands, and the Coastal Western Hemlock - Sitka Spruce ecoregion along the Gulf of Alaska and Prince William Sound coasts (Gallant *et al.*, 1995).

Sample Collection

Black bear samples were collected from hunter-killed bears and supplemented with non-invasive hair snaring on public lands (KEFJ and parts of Kachemak Bay State Park and Kenai National Wildlife Refuge). In 2004 and 2005 ADF&G staff collected tissue (hide or muscle tissue) samples from bears processed at ADF&G check points as part of regulatory hunt monitoring. Tissue samples were stored in paper envelopes and frozen until the time of extraction. The location of each sample was recorded according to the verbal description of the hunting location on the ADF&G certificates. Only samples with precise location descriptions using official place names were used in this study. We plotted samples in ArcGIS 9.0 (ESRI, Redlands, CA) based on the described locations, and referencing an

Alaska place names data layer (ADNR LRIS, 1967). In the event that more than one animal was harvested at a single reported location, we constructed a 500 m buffer around the location point and randomly located the sample points at unique locations within that buffer. This point relocation was used to facilitate visualization of sample points. Further, some spatial models required unique coordinates for each sample point. The error in plotting reported hunt locations was expected to be minimal in comparison to the home range of a black bear, which would extend several kilometers beyond the point of capture (Kernohan *et al.*, 2001).

The area within KEFJ was sampled intensively as part of the National Park Service's Southwest Alaska Network's inventory and monitoring program (National Park Service, 2006). Field collections were conducted in KEFJ over three summers (July – August, 2003-2005) by NPS and University of Idaho teams. Sampling within each bay in KEFJ consisted of a 10 day session in which hairs were collected on two occasions. Hair samples were collected using barbwire hair traps (Boulanger *et al.*, 2004). Traps were set along presumed bear trails in areas of natural food resource concentration (salmon streams and berry thickets) so that no baiting was necessary. Hair samples were also collected within Kachemack Bay State Park on the southwest side of the Kenai Peninsula. Samples collected with similar non-invasive methodology were acquired from J. Fortin (Washington State University) (Fortin *et al.*, 2006) from three stream drainages within the Kenai National Wildlife Refuge west of KEFJ. Upon collection, samples were stored in paper envelopes in containers with desiccating silica beads. The location of each sample was recorded as the location of the hair trap using a handheld GPSMAP 76 unit (Garmin International Inc., Olathe, KS). These locations were later plotted in the Alaska Albers projection as a GIS layer using ArcGIS 9.0.

Laboratory Analyses

Whole genomic DNA was extracted using standard protocols for a Qiagen DNeasy tissue extraction kit (Qiagen Ltd., Crawley, West Sussex, UK), using approximately 25mg of tissue or 1-10 follicles clipped from hairs. To avoid contamination, all hair samples were processed

in a separate laboratory that was free of concentrated DNA in any form. We also used one negative control for every 20 samples extracted, and in each amplification reaction.

Not all samples collected were suitable for the current analysis. Some samples did not have sufficient quantity and quality of DNA for microsatellite genotyping. Other samples did not have verifiable geographic locations required for the spatial analyses of the genetic data. The KEFJ area was sampled in particularly high density (125 per 1,000 km^2) and was randomly subsampled to avoid overrepresentation in spatial or genetic analyses. Ten samples were randomly selected from successfully genotyped unique individuals from KEFJ, yielding a sample density similar to other portions of the Kenai Peninsula (averaging four bears per 1,000 square km). The final dataset included 110 black bears genotyped at all microsatellite loci, the sex ID locus, and the mtDNA control region (Table 2.1, see Figure 2.1B for distribution).

Microsatellite analysis was conducted using 13 highly variable independent loci: G1A, G1D, G10B, G10C, G10L, G10M, G10P (Paetkau, Strobeck, 1994), G10J, G10O (Paetkau *et al.*, 1998), Cxx20 (Ostrander *et al.*, 1993), Mu15, Mu23 Mu50 (Taberlet *et al.*, 1997). Sex identification was performed using primers SE47 and SE48 from the amelogin gene (Ennis, Gallagher, 1994). DNA fragments were amplified using Polymerase Chain Reaction (PCR) (reaction conditions in Table 2.2). Hair samples were amplified using a double-amplification step to maximize yields from samples of low DNA concentrations (adapted from Piggott *et al.*, 2004). All nDNA fragments were resolved using an ABI 377 automated sequencer (Applied Biosystems (ABI), Foster City, CA), analyzed using Genescan 3.1.2 (ABI), and alleles scored using Genotyper 2.5 (ABI).

A 360 base pair section of the mitochondrial control region was amplified using primers H16498 and L15997 (Ward *et al.*, 1991) followed by Exosap (USB Corporation, Cleveland, OH), Big Dye 3.1 Sequencing (ABI), and Sephadex (Sigma-Aldrich, St. Louis, MO) steps. Sequences were resolved using an ABI 377 or 3130 automated fluorescent sequencer using Genescan 3.1.2 analysis software. We then edited sequences in Sequencher 4.5 (Gene Codes

Corporation, Inc., Ann Arbor, MI), and then aligned them in ClustalW (Chenna *et al.*, 2003) to identify haplotypes. We included a hyper-variable thymine repeat segment of the control region, which has been excluded from other phylogenetic studies (Waits *et al.*, 1998; Wooding, Ward, 1997), as it is expected to have high homoplasy. Inclusion of this variable site provided additional haploypes for analyses of fine scale variation in gene flow.

Recent reviews have pointed out the importance of standardizing data quality-checking protocols and reporting error rates (Bonin *et al.*, 2004; Paetkau, 2003). Allelic dropout (failure of one allele to amplify in a heterozygote) and false alleles (appearance of a nonexistent allele as a result of PCR error) can be particularly problematic in microsatellite data (Broquet and Petit 2004; Gerloff et al. 1995; Navidi et al. 1992; Taberlet et al. 1996). We followed the recommendations of Bonin *et al*. (2004) implementing a multi-faceted quality-checking approach. All genotypes from non-invasive samples were verified by observing each genotype in at least two instances, either as a capture and a recapture or by repeated genotyping of unique samples. Approximately 1/3 of tissue samples were re-genotyped for verification. Finally, we used the program Validation to identify potentially erroneous genotypes by finding genotypes that differed by two or fewer alleles, which were then reamplified (Roon *et al.*, 2005). Error rates were calculated as the ratio of erroneous alleles (those in disagreement between replicate runs) over the number of allelic comparisons made (Bonin *et al.*, 2004).

Population Structure Analysis

Population genetic structure was assessed using three Bayesian population assignment methods. We used aspatial models in the programs Structure 2.1 (Pritchard *et al.*, 2000) and BAPS 4.0 (Bayesian Analysis of Population Structure, Corander *et al.*, 2006a) as well as a spatial model in BAPS 4.0. These methods are useful in determining the number of genetically distinct groups within a sampled population (Latch *et al.*, 2005). Spatial models are additionally useful for identify geographic boundaries between genetic groups (Corander *et al.*, 2006b; Guillot *et al.*, 2004). While there are numerous definitions of genetic

populations (Waples, Gaggiotti, 2006), herein we refer to genetically distinct groups as groups of individuals in Hardy-Weinberg equilibrium and with significantly divergent allele frequencies from other groups.

Structure (Pritchard *et al.*, 2000) treats the allele frequencies, the number of genetically distinct groups (K) in the sample, and individual ancestry in each group as random variables to be simultaneously determined. The most likely partition of the dataset was selected using 10 replicates with a 100,000 repetition burn-in period and 200,000 Markov Chain Monte Carlo (MCMC) randomizations for each value in the range K=1 to K=10. The optimal K value was chosen according to the maximum log likelihood, L(K), output by Structure and further confirmation using the ΔK statistic developed by Evanno et al. (2005).

BAPS (Corander *et al.*, 2006a) treats the allele frequencies and the number of genetically distinct groups in the sample as random variables. Here individual ancestry in each group is estimated after groups are assigned. The principles of Bayesian inference are similar to those used by the program Structure. However, instead of using MCMC randomizations, BAPS uses stochastic optimization to infer the correct model for the data (Corander *et al.*, 2003). The most likely partition of the dataset was estimated using 10 replicates of a range K = 1 to K = 10. The optimal K value was based on the partition with maximum likelihood and highest probability determined by the program.

BAPS 4.0 also provides a spatially explicit assignment test. The Bayesian algorithms are the same as in the aspatial method with the addition of a spatial prior distribution which favors delineation of groups that are spatially cohesive (Corander *et al.*, 2006a). Parameters for the spatial model were the same as those for the aspatial model with the addition of a geographic coordinate file providing the geographic location of each individual. The optimal K value was based on the partition with maximum likelihood and highest probability determined by the program.

Ancestry of each individual, in each genetic group, was recorded. The q value describes the proportion of an individual's genotypic ancestry that can be attributed to each identified genetic group. Using Structure, individuals were assigned to the group in which their ancestry (q) was highest. Using BAPS, individuals were assigned to genetic groups by the program before calculating q values, so these were used only to judge admixture between groups. When the q value in the assigned group was less than 0.75 the individual was considered to be of mixed ancestry. This arbitrary cut-off was selected to represent the amount of ancestry equivalent to one grandparent from outside the assigned group.

Genetic group assignments were mapped in ArcGIS. Individuals were identified as migrants if they were assigned to a genetic group other than the one in which they were sampled. In the case that genetic groups lacked distinct geographic boundaries, individuals in the range of overlap were not considered migrants. Minimum convex polygons were drawn (using Hawth's Analysis Tools ArcGIS Extension v. 3.25; Beyer, 2004) to encompass all non-migrant points for each detected genetic group. The land area within the polygons was used as a measure of the geographic range of each group.

We tested for linkage disequilibrium (LD) and deviations from Hardy-Weinberg equilibrium (HWE) in the entire dataset and in each identified genetic group using Genepop 3.4 (Raymond, Rousset, 1995). We tested differences in allele frequencies (using Genepop) and calculated pairwise Fst (using Arlequin 3.01, Excoffier *et al.*, 2005) as measures of differentiation between genetic groups. Bonferroni correction was applied to all cases of multiple comparisons. Genetic diversity was measured in terms of expected heterozygosity (Genepop) and allelic richness (AR) (FSTAT 2.9.3.2, Goudet, 1995) in each genetic group.

When using multiple assignment tests, the most likely representation of population structure may differ among methods, making it necessary to develop criteria for selecting among options. In these analyses, we set the following criteria for determining the optimal partition of the dataset: admixture between groups was minimal; LD and HWE deviations were minimal; allele frequencies differed significantly between all groups; Fst values indicated

significant divergence between all groups; and geographic overlap between groups was minimal.

Isolation By Distance Analysis

Limitations to dispersal distance lead to increases in genetic distance with geographic distance, or isolation by distance (IBD) (Wright, 1943). We conducted individual-based Mantel tests (Mantel, 1967) in Genalex 6 following the methods of Smouse and Peakall (1999). The significance of IBD was assessed through 999 randomizations. The Mantel test assumes that a single process is generating the pattern of correlation between variables. This assumption, termed stationarity, may be violated if the sample population is subdivided into distinct units each governed by different processes (Fortin, Dale, 2005). In the population genetic context this means that, if gene flow and genetic distance are governed by different processes in distinct genetic groups, then separate tests within each continuous group may be more appropriate. For this reason, we also tested IBD within each group identified by the assignment tests.

Phylogeographic Analysis

We used mtDNA haplotype data to assess phylogeographic patterns. A haplotype network was drawn by hand according to the number of nucleotide changes between observed haplotypes. The haplotype distributions were mapped using ArcGIS. Minimum convex polygons were drawn (using Hawth's Analysis Tools for ArcGIS) to encompass all samples with each haplotype. As above the polygon area was used to measure the geographic range of each haplotype.

RESULTS

Laboratory Analysis

There was one individual for which the mtDNA locus failed to amplify, thus missing data accounted for 0.9% of the mtDNA dataset. All haplotypes were observed more than once and there was no indication of ambiguity in any of the sequence data.

There were four individuals with data missing at a single microsatellite locus, accounting for 7.7% of those individuals' genotype data and 0.28% of the microsatellite dataset. The total allele-based error rates were 0.4% for hair samples and 1.4% for tissue samples. The discrepancy in hair versus tissue error rates was attributable to the subsampling of only high quality hair samples thus biasing these samples toward a low error rate. This subsampling was not possible for the tissue samples because all samples were needed to maximize geographic coverage. Locus-specific error rates averaged 0.8% (ranging from 0 at loci G10-O, Mu15, Mu50, and Cxx20 to 3.13 % at locus Mu23). Sources of allele-based errors included allelic dropout (30% of errors), false alleles (35% of errors), and allele scoring errors based on unclear adenylation patterns (25% of errors) which occurred solely at locus G10D. In every case of unclear adenylation, the problem was obvious during allele scoring and the sample was rerun for clarification. Closely related individuals or individuals recaptured in hair trapping could be easily distinguished based on a low probability of identity (PI) with 13 microsatellites: PI 6.08×10^{-14} and PI(sibs) 7.56×10^{-6}.

Seven sets of first order relatives were identified in the 110 bear dataset. No related pairs were captured in the same sampling site, thus all appeared to be independent bears with no evidence of capturing undispersed young with parents. The largest group of related bears contained four individuals sampled from different sites within the Prince William Sound (PWS) area. The sex ratio in the dataset was heavily male biased; we identified 81 males and 29 females (2.79 males per female).

Population Structure Analysis

Results from Structure indicated 4 genetic groups in the dataset, showing distinct groups on the mainland (ML), in Prince William Sound (PWS), and two groups on the Kenai Peninsula (KP1, KP2) (Table 2.3, Figure 2.2A). BAPS aspatial indicated 5 groups, the same ML and PWS groups and three groups on the Kenai Peninsula (Table 2.3, Figure 2.2B). Two of these were outliers containing 2 and 3 individuals only. They overlapped completely with the KP group. Allele frequencies failed to differ between outliers and the KP group at 11 of 13 loci. Thus, the outlier groups will be disregarded as suggested by the designers of BAPS (Corander *et al.*, 2006a). Results from BAPS spatial indicated three genetic groups; ML, PWS, and a single Kenai Peninsula group (KP) (Table 2.3, Figure 2.2C).

Taking into account the mixture of ancestry, population parameters and the geographic mapping of group ranges, we concluded that the three groups indicated by BAPS spatial best represented the genetic structure in the study area. HWE deviations were similar, with each partition showing a single locus in disequilibrium in the PWS group. This can be expected given the small sample size representing this genetic group. The BAPS spatial partition minimized linkage disequilibrium (LD) (full dataset: 10 locus pairs in LD, four Structure groups - 5, three BAPS spatial groups - 3). The BAPS spatial partition showed the highest ancestry of assigned individuals and the lowest number of admixed individuals (Table 2.4). Fst values were similar between all comparisons of Kenai groups, ML and PWS groups. Fst values were very low between KP1 and KP2 from Structure (Table 2.5). Maps of the genetic group ranges further supported the designation of three groups suggested by BAPS spatial (Figure 2.2). The designation of KP1 and KP2 by Structure led to an almost complete range overlap, raising questions concerning the distinction between groups.

The KP group had the widest geographic range (27,000 km^2), occupying the entire Kenai and merging onto the mainland. The ML group had a smaller geographic range (15,000 km^2), which was likely related to our sampling boundary rather than a true group boundary. The

PWS group showed a markedly confined geographic range of only 500 km^2, which also may have been influenced by the bounds of the sampling area.

Fst values and inter-group migration indicated moderate levels of gene flow between genetic groups. Divergence was greatest in pairings including PWS (Fst's 0.093-0.120). Divergence was the lowest between the KP and the ML (Fst 0.077). One migrant, from the mainland to the Kenai, was identified in all assignment tests (Figure 2.3). An area of overlap between the ML and KP groups was also consistently identified in all tests. The extent of overlap was estimated most conservatively by BAPS spatial (Figure 2.2).

Genetic diversity (He and AR averaged over 13 loci) was similar between ML and KP groups (Table 2.6). Both allelic richness and expected heterozygosity were lowest in PWS, though they did not differ significantly from other groups (t-test, p value 0.44-AR, 0.32-He). PWS showed heterozygote deficiency at two loci, Mu50 and G10J. Only Mu50 deviated significantly from HWE after Bonferroni correction.

Isolation By Distance Analysis

The global Mantel test, using the full dataset, indicated that genetic distance was significantly, though weakly, correlated with geographic distance (R = 0.231, p value = 0.001). IBD was evident but considerably weaker within the KP group (R=0.112, p value 0.009). IBD was not significant in the PWS group (R = 0.006, p value = 0.527) or in the ML group (R = 0.055, p value = 0.314) when tested alone. It should be noted that sample size of the PWS group was insufficient to consider this IBD test reliable (Legendre, Fortin, 1989).

Phylogeographic Analysis

Five haplotypes were detected in a 360 base pair segment of the mitochondrial control region (Figure 2.4). Haplotypes were based on a single cytosine-thymine substitution and three insertion-deletion (indel) variations in the hyper-variable thymine repeat segment. The

substitution coincided with that identified at nucleotide position 189 by Wooding and Ward (1997) defining lineages 1 and 7 of Clade A, the continental black bear clade. Inclusion of the thymine repeat segment allowed us to further refine these two lineages into five sublineage haplotypes containing 6, 7, or 8 thymine nucleotides in positions 99 – 106. Sublineages defined here are denoted according to the original lineage number and the number of thymine repeats: 1_{t6}, 1_{t7}, 7_{t6}, 7_{t7}, 7_{t8}. The haplotype network in Figure 2.4 illustrates the relationship among haplotypes.

The geographic distribution of haplotypes is depicted in Figure 2.4. Haplotype 1_{t7} was the most common and widespread occurring throughout the study area (n=41, range=40,000 km^2). Haplotype 1_{t6} was also common on the mainland (n=16, range=17,000 km^2) and particularly concentrated in Prince William Sound. Both 7_{t7} (n=28) and 7_{t8} (n=20) were common on the Kenai, but nearly absent on the mainland. Though their ranges overlapped, 7_{t8} appeared to be more concentrated in the east (range=10,000 km^2) while 7_{t7} spanned the northern peninsula and occurred twice on the mainland (range=14,500 km^2). Haplotype 7_{t6} occurred only rarely (n=4) and was confined to the mainland (range=6,000 km^2).

DISCUSSION

Population Structure Analysis

This study has illustrated the importance of using multiple analytical techniques and incorporating geographic context when examining genetic population structure. Slight differences in analytical models can produce differing results and offer different perspectives on the genetic structure of populations (Cegelski *et al.*, 2003; Frantz *et al.*, 2006; Hauser *et al.*, 2006). Here we examined partitions of genetic variation as defined by the program Structure and aspatial and spatial models in the program BAPS.

The population structure defined by BAPS spatial best fit our criteria for the optimal partitioning of the sample population: admixture was minimal, groups showed minimal deviation from HWE, allele frequencies and Fst values indicated significant divergence between all groups and there was little overlap in the geographic ranges of genetic groups. Therefore the delineation of a single genetic group of Kenai black bears was well justified. The inclusion of geographic information in the BAPS spatial model appeared to clarify problems of over-splitting seen in Structure and BAPS aspatial. However, we did detect the potential for *over*-smoothing with BAPS spatial. The spatial smoothing appeared to yield conservative estimates of migration by assigning admixed individuals to the closest group contributing to their ancestry. This illustrates the need to carefully examine the individuals classified as admixed as well as migrants when evaluating gene flow (Figure 2.3).

Structure, perhaps the most commonly used assignment test (Latch *et al.*, 2005), detected two genetic groups on the Kenai Peninsula. Designation of these two groups would entail a substantial range overlap between groups, numerous admixed individuals, and even assignment of first order relatives to different natal groups. This led us to explore reasons for over-splitting. Others have suggested that detection of population structure can be sex-dependent (Tiedemann *et al.*, 2000), or confounded by isolation by distance (Laikre *et al.*, 2005; Pritchard, Wen, 2004). If over-splitting were due to signals of restricted female

dispersal, we would expect population assignments to coincide with mtDNA haplotypes, or for the male portion of the dataset to show less structure. However, the clustering of mtDNA haplotypes did not spatially coincide with the ranges of KP1 or KP2. Further, the same KP1 and KP2 genetic groups were identified when Structure was run with only male individuals. Our analyses showed that IBD was significant among bears within the Kenai. Thus the over-splitting of the Kenai into two groups was most likely an artifact of the model and perhaps attributable to influences of IBD.

BAPS is a newer assignment test software (Corander *et al.*, 2006b). Identification of small outlying groups is a common problem acknowledged in the BAPS aspatial model (Corander, Marttinen, 2006; Latch *et al.*, 2005). It may be tempting to ascribe significance to these outliers, such as representatives of un-sampled populations. However, the prevalence of outlier groups even in simulated datasets make these outliers appear to be artifacts of the model (Latch *et al.*, 2005). Corander et al. (2006b) suggest ignoring these outliers because three or fewer individuals cannot represent a panmictic breeding population (Corander *et al.*, 2006b). However, they do represent a weakness in the model as these individuals could not be assigned to any population or included in estimates of diversity or differentiation.

Isolation By Distance Analysis

In the global Mantel test, geographic distance was significantly correlated with genetic distance between individuals. This correlation was weak, suggesting that, although separation distance may be correlated, it may not be the primary factor affecting genetic distance between individuals. Population substructure was evident, thus violating the assumption of stationarity and potentially confounding the correlation between genetic and geographic distances. It is likely that gene flow barriers isolating bears at the group level were exerting greater influence over genetic distance than separation distance alone. IBD was only significant within the KP group which was sampled across its entire range. This may indicate that the remaining groups were not sufficiently sampled to cover the entire population range and possible distance factors. A population-wise test of IBD over a larger

scale may be more appropriate. For instance Paetkau et al. (1998) showed substantial IBD between populations of brown bears across 1,790 km of northern North America.

Phylogeographic Analysis

Haplotype 1_{t7} was the most widespread in this study as well as others spanning North America (Paetkau, Strobeck, 1996; Roon, 2004; Wooding, Ward, 1997). Lineage 1 was the most prevalent lineage identified by Wooding and Ward (1997), ranging from the American southwest to Alaska, and has been identified in other black bear studies from northeast Alberta, Canada (Paetkau, Strobeck, 1996) and Montana, USA (Roon 2004). Roon (2004) is the only other study to include the thymine repeat segment, and he also found 1_{t7} to be most prevalent in the Greater Glacier Ecosystem, Montana, USA. Lineage 7 was less common in the Wooding and Ward (1997) data and was not found elsewhere in the literature or in sequences published on Genbank (National Center for Biotechnology Information, 2006).

The distribution of mtDNA haplotypes (7_{t7} and 7_{t8}) on the Kenai was more geographically restricted than the groups identified through nDNA microsatellite analysis. MtDNA can diverge or show population structure where nDNA does not due to the lower effective number of genes, particularly in cases of male-biased dispersal (Muhs *et al.*, 2001). Increased geographic restriction of haplotypes on the Kenai suggests greater restriction of (particularly female) black bear movement on the peninsula than on the nearby mainland. Admixture via male dispersal may lead to the weaker spatial structure detected in the nDNA microsatellite data. The haplotype distribution could also reflect signatures of past structure, as mtDNA mutates more slowly than microsatellites. It is likely that the extent and connectivity of black bear habitat has changed dramatically in the past with the advance and recession of glaciers (Wilkes, Calkin, 1994). Future analyses incorporating historic and current landscape features may help to illuminate factors affecting gene flow within the Kenai group.

Biological Interpretation

Our results indicated that black bears on the Kenai Peninsula were genetically distinct from those on the mainland, and further that bears in Prince William Sound appeared to be isolated from other areas. The high male bias in our data set would be expected to over-represent the dispersing sex and make it more difficult to detect population structure. We can thus be confident that our assessment represents the minimum level of differentiation between these populations. Distinctiveness of Kenai populations has been documented in numerous taxa, particularly in carnivores. Kenai populations of Canada lynx (*Lynx canadensis*) and wolverine both show genetic distinction from more interior populations (Schwartz *et al.*, 2003; Tomasik, Cook, 2005). Distinction of gray wolves on the Kenai has been attributed to climatic differences and geographic isolation (Geffen *et al.*, 2004; Weckworth *et al.*, 2005).

The level of differentiation between KP, ML, and PWS groups suggested some restriction of gene flow between segments of the population, though levels of genetic diversity were similar in all groups. This suggests that effective population sizes and migration have been sufficient to maintain diversity within these populations. Genetic diversity levels observed in this study are similar to those observed in nonfragmented populations across the range of black bears (Arakansas and Louisiana, Csiki *et al.*, 2003; British Columbia, Marshall, Ritland, 2002; Quebec and Alberta, Paetkau, Strobeck, 1994).

Fst values ranged from 0.07 between the ML and KP groups separated by a narrow land connection to 0.12 between the KP and PWS groups isolated by ocean water and icefields. This is consistent with population structure detected for black bears in a similarly rugged area of southeast Alaska. Peacock (2004) found Fst values of less than 0.1 between groups separated by large land distances or short water crossings, whereas bear populations separated by more formidable barriers, such as glaciated mountain ranges, or long salt water crossings, showed Fst values as high as 0.12 to 0.29 (Peacock, 2004). Faced with the rugged landscape of south central Alaska, population connectivity may be particularly dependant on important corridors and linkage zones (Clevenger *et al.*, 2002).

We found a high level of genetic connectivity among black bears on the Kenai with more restricted gene flow between the Kenai and mainland. Levels of differentiation between the Kenai and mainland were moderate as reflected in migration levels and Fst values. One male migrant was consistently assigned from the mainland to the Kenai. The geographic distinction between the Kenai and mainland groups did not break cleanly at the isthmus of the peninsula, rather there appeared to be an overlap between the ranges of the Kenai and mainland genetic groups. Further sampling of this area could better indicate the degree of overlap and admixture between these groups.

Black bears of central Prince William Sound appear to be isolated from other regions of the study area to the west and south (but note the small sample coverage of this population). There was no clear indication of migration into or out of the PWS group; though, the presence of two admixed bears indicated migration at least in recent generations. The samples showing the most distinction were from Esther Island and the nearby peninsula which may be particularly isolated within PWS, or simply more connected with poorly sampled regions in the eastern portion of the sound. Four out of 11 bears assigned to this group were closely related ($r > 0.5$), which would affect the distribution of alleles relative to HWE expectations and could, therefore, have influenced the Bayesian assignment methods (Pritchard, Wen, 2004). However it is possible that the bears of central PWS are isolated by salt water crossings and expansive icefields, leading to limited dispersal and high divergence from neighboring groups. Our results are intriguing and additional sampling in this region will be necessary to more thoroughly evaluate the connectivity of PWS bears and their neighbors. Such evaluation will be important as management planning ensues to protect black bears faced with increasing human use of PWS (Lace, Gimblett, 2005).

Climatic history and past glaciations have played a role in shaping the historic levels of separation between black bear groups and continue to affect gene flow in south-central Alaska. Most of south-central Alaska was covered by the Cordilleran Ice Sheet at the last ice age maximum about 25,000 to 13,000 years before present (ybp) (Muhs *et al.*, 2001;

Pielou, 1991). South-central Alaska became deglaciated about 10,000 ybp. At that time ocean levels were such that the Kenai peninsula was largely continuous with the Alaskan mainland (Muhs *et al.*, 2001; Pielou, 1991). The Kenai became distinct and was relatively isolated from the mainland by 7,000 ybp (2003). Haplotype distributions in south-central Alaska suggest that connectivity among black bear ranges was high during the initial recolonization of the area. Haplotype 1_{t7} appears to be the ancestral sublineage spreading from the continental US throughout Alaska. Changing ocean levels and increasing isolation of the Kenai may have fostered the development of unique haplotype distributions on the mainland (7_{t6}) and Kenai (7_{t7} and 7_{t8}).

Our study has indicated that black bears on the Kenai constitute an important component of the genetic diversity of Alaskan black bears. MtDNA data shows that the Kenai population has unique haplotypes, but is not deeply diverged from mainland bears. The distinction of nDNA suggests that the Kenai bears are a distinct management unit as defined by Moritz (Moritz, 1994; Moritz, 2002). At present, population connectivity on the Kenai is high. Corridors such as the Nuka and Resurrection River valleys may be particularly important for maintaining connectivity between coastal regions and inland portions of the peninsula separated by the heavily glaciated Kenai Mountains. Connectivity to the Alaska mainland was much lower; however, functional migration corridors do currently exist between the mainland and the Kenai as shown by our documentation of a migrant from the mainland group to the Kenai group. GPS collar data has also tracked a black bear traveling from the mainland across Turn Again arm and through a Kenai Mountain valley (unpublished pilot study, Farley, 2006)

Our results provide an important measurement of baseline genetic diversity levels and population connectivity of black bears in this region. As human presence on the Kenai increases, it will be critical to develop habitat management plans that maintain the current diversity and structure and minimize impacts to important linkage zones and corridors.

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Table 2.1: Sample numbers after quality control measures
Non-invasive samples were collected using hair traps from Kenai Fjords National Park, Kachemak Bay State Park, and Kenai National Wildlife Refuge. Tissue samples were collected from bears hunted throughout game management units 6D, 7, 14, 15, and 16. The table includes the number of samples collected using each method, the number discarded because they had no reliable location (No Loc.), poor DNA quality (Poor DNA), were recaptures (Recap.), were thinned from the intensively sampled KEFJ sample set (Thin), and the final sample size (N).

Sample Type	# Samples	No Loc.	Poor DNA	Recap.	Thin	N
Non-Invasive	287	0	88	60	119	20
Hunted	160	66	9	0	0	90
TOTAL						110

Table 2.2: Multiplex and singleplex Polymerase Chain Reaction conditions
Conditions are given for reactions used to amplify nDNA microsatellites, sex identification and the mtDNA control region.

Reaction	Primer Set (μM)		Annealing Temp. (C^o)	Amplitaq Gold (units)	$MgCl_2$ (μM)	dNTPs (μM)
A-B-C-D-L	G1A	0.10	57.6	0.5	0.0025	333
	G10B	0.10				
	G10C	0.10				
	G1D	1.33				
	G10L	0.17				
M-P	G10M	0.40	52.0	0.5	0.0025	333
	G10P	0.10				
15-23	Mu15	0.17	52.5	0.5	0.0021	333
	Mu23	0.17				
O-20	Cxx20	0.23	51.0	0.5	0.0025	333
	G10-O	0.15				
J	G10J	0.20	48.0	0.5	0.0025	267
50	Mu50	0.15	52.0	0.5	0.0025	333
Sex ID	SE47/48	0.64	57.0	1.6	0.0019	267
mtDNA	cntrl reg	0.30	50.0	0.5	0.0025	200

Table 2.3: Most likely number of genetic groups determined by assignment tests
Results showing the most likely number of genetically distinct groups within the dataset according to the output of Bayesian assignment tests in Structure 2.1 and BAPS 4.0. For each possible number of distinct groups (K) the log likelihood (L(K)) and the probability (Prob.) are presented. For Structure results we also calculated the ΔK statistic for further verification of the most likely partition. Because BAPS results are based on stochastic optimization, rather than MCMC replicates, not all partitions are equally visited by the model. L(K) was determined based on the 10 best models visited, the probability was determined based on the 30 best models visited. Partitions not included in the best models visited are denoted "nv". The most likely partition produced by each program is indicated in bold text.

	Structure			**BAPS aspatial**		**BAPS spatial**	
K	L(K)	Prob.	ΔK	L(K)	Prob.	L(K)	Prob.
1	-4605	<<0.001	-	nv	nv	nv	nv
2	-4424	<<0.001	41.6	nv	nv	-4714	<<0.001
3	-4377	<<0.001	5.43	nv	0.02	**-4690**	**1.00**
4	**-4308**	**1.00**	**69.87**	-4634	0.36	-4713	<<0.001
5	-4351	<<0.001	6.65	**-4636**	**0.62**	nv	nv
6	-4449	<<0.001	0.52	nv	nv	nv	nv
7	-4530	<<0.001	1.03	nv	nv	nv	nv
8	-4664	<<0.001	1.18	nv	nv	nv	nv
9	-4725	<<0.001	1.38	nv	nv	nv	nv
10	-4996	<<0.001	-	nv	nv	nv	nv

Table 2.4: Individual ancestry and admixture in each genetic group detected
Genetic groups were defined using Structure, BAPS aspatial (BAPSa) and BAPS spatial (BAPSs). The table presents the number of individuals assigned to each group detected in each assignment test program and the average ancestry (q) of individuals assigned to each group. We also show the number of individuals from each group that were admixed, migrants, or inhabiting areas of overlap between group ranges.

	KP	KP1	KP2	ML	PWS	Out1	Out2
Individuals assigned to each group							
Structure	-	36	42	21	11	-	-
BAPSa	73	-	-	23	9	2	3
BAPSs	79	-	-	20	11	-	-
Average q value of assigned individuals							
Structure	-	0.797	0.760	0.910	0.878	-	-
BAPSa	0.861	-	-	0.851	0.926	0.940	9.935
BAPSs	0.914	-	-	0.882	0.933	-	-
Admixed individuals assigned to each group (q<0.75)							
Structure	-	14	18	1	2	-	-
BAPSa	11	-	-	5	0	0	0
BAPSs	4	-	-	2	0	-	-
Migrants detected in each group							
Structure	-	1	0	1	1	-	-
BAPSa	1	-	-	0	0	0	0
BAPSs	1	-	-	0	0	-	-
Individuals in overlapping ranges							
Structure	-	21	23	2	0	-	-
BAPSa	3	-	-	1	0	2	3
BAPSs	3	-	-	1	0	-	-

Abbreviated group names:
KP – Kenai Peninsula, KP1 – Kenai Peninsula 1 of 2, KP2 – Kenai Peninsula 2 of 2, ML – mainland, PWS – Prince William Sound, Out1 – first outlier, Out2 – second outlier.

Table 2.5: Differentiation between genetic groups detected by assignment tests
Genetic groups were defined using Structure, BAPS aspatial (BAPSa) and BAPS spatial (BAPSs). Fst was calculated in Arlequin for each partition of the dataset.

Structure		KP1	KP2	ML
	KP1			
	KP2	0.031		
	ML	0.072	0.096	
	PWS	0.117	0.143	0.091
BAPSa		KP	ML	
	KP			
	ML	0.074		
	PWS	0.129	0.088	
BAPSs		KP	ML	
	KP			
	ML	0.077		
	PWS	0.120	0.093	

Abbreviated group names:
KP – Kenai Peninsula, KP1 – Kenai Peninsula 1 of 2, KP2 – Kenai Peninsula 2 of 2, ML – mainland, PWS – Prince William Sound.

Table 2.6: Heterozygosity and allelic richness in genetic groups defined by BAPS spatial

Measures of genetic diversity are given for each group defined by the BAPS spatial assignment tests. The table shows the number assigned to each group (N), the proportion of heterozygotes expected (He) and observed (Ho) (averaged over 13 microsatellite loci), and the allelic richness adjusted to the smallest sample size (AR_{11}).

Group	N	He	Ho	AR_{11}
ML	20	0.729	0.704	5.173
PWS	11	0.673	0.622	4.846
KP	79	0.710	0.671	5.221

Abbreviated group names:
KP – Kenai Peninsula, KP1 – Kenai Peninsula 1 of 2, KP2 – Kenai Peninsula 2 of 2, ML – mainland, PWS – Prince William Sound.

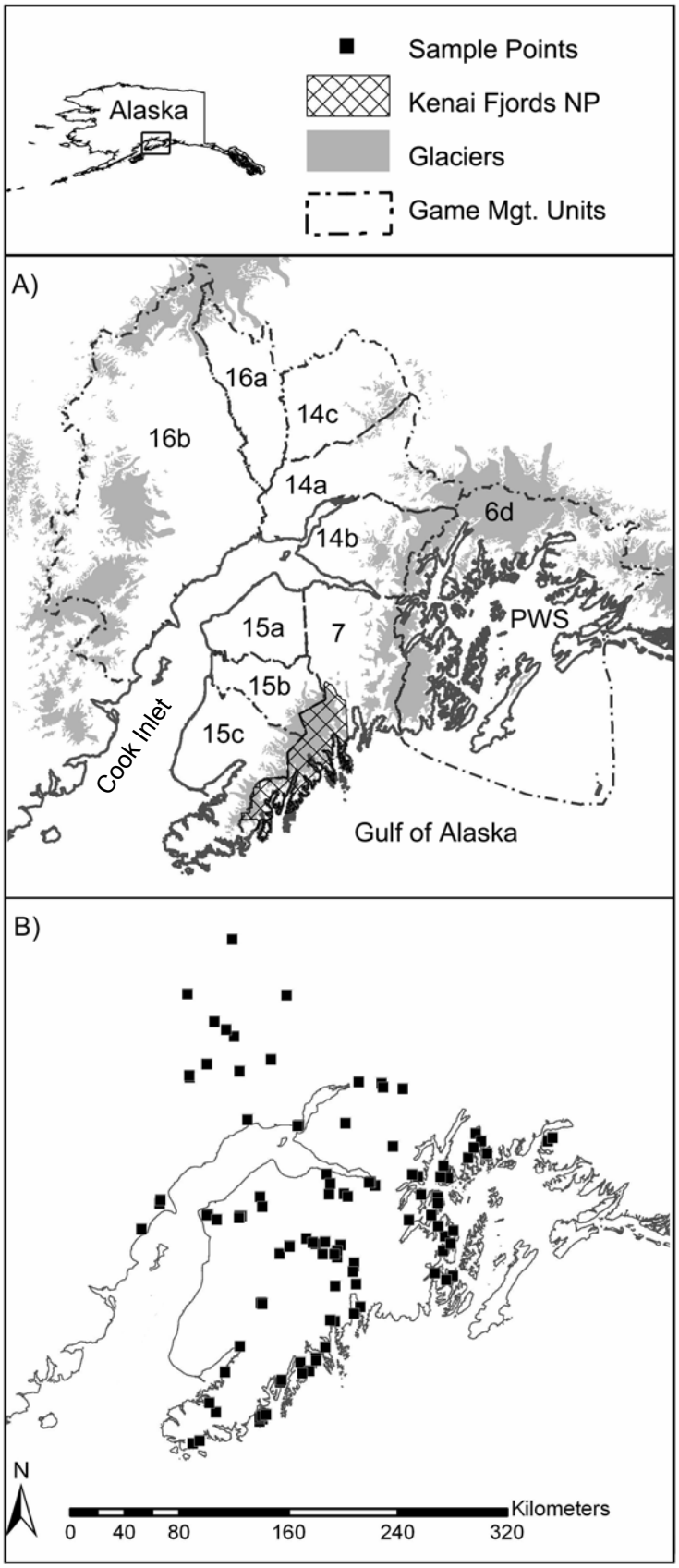


Figure 2.1: Study area and sample coverage
A) Provides a map of the study area in south-central Alaska, showing geographic features and political boundaries. Game Management Units (labeled numerically) provided the bounds of the study area and were used to request samples from hunted bears. Intensive non-invasive sampling took place within Kenai Fjords National Park. B) Shows the coverage of 110 black bear samples collected over the Kenai Peninsula and adjacent mainland.

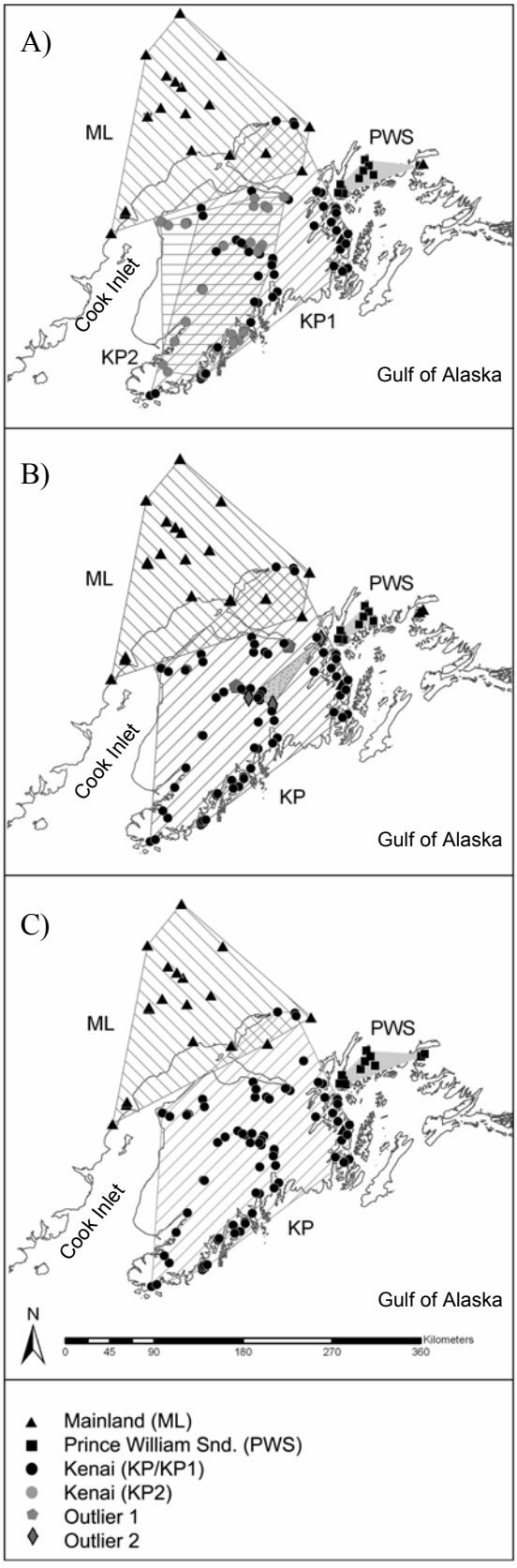


Figure 2.2: Geographic ranges of genetic groups detected by assignment tests
Genetically distinct groups were detected using Bayesian assignment tests: Structure (A), BAPS aspatial (B), BAPS spatial (C). After removing migrants, minimum convex polygons were drawn around the bears assigned to each genetic group.

Figure 2.3: Migration and admixture between genetic groups
Gene flow between genetic groups is depicted by pointing out migrants and admixed individuals. The partition of genetic groups shown is based on assignment test results from BAPS spatial.

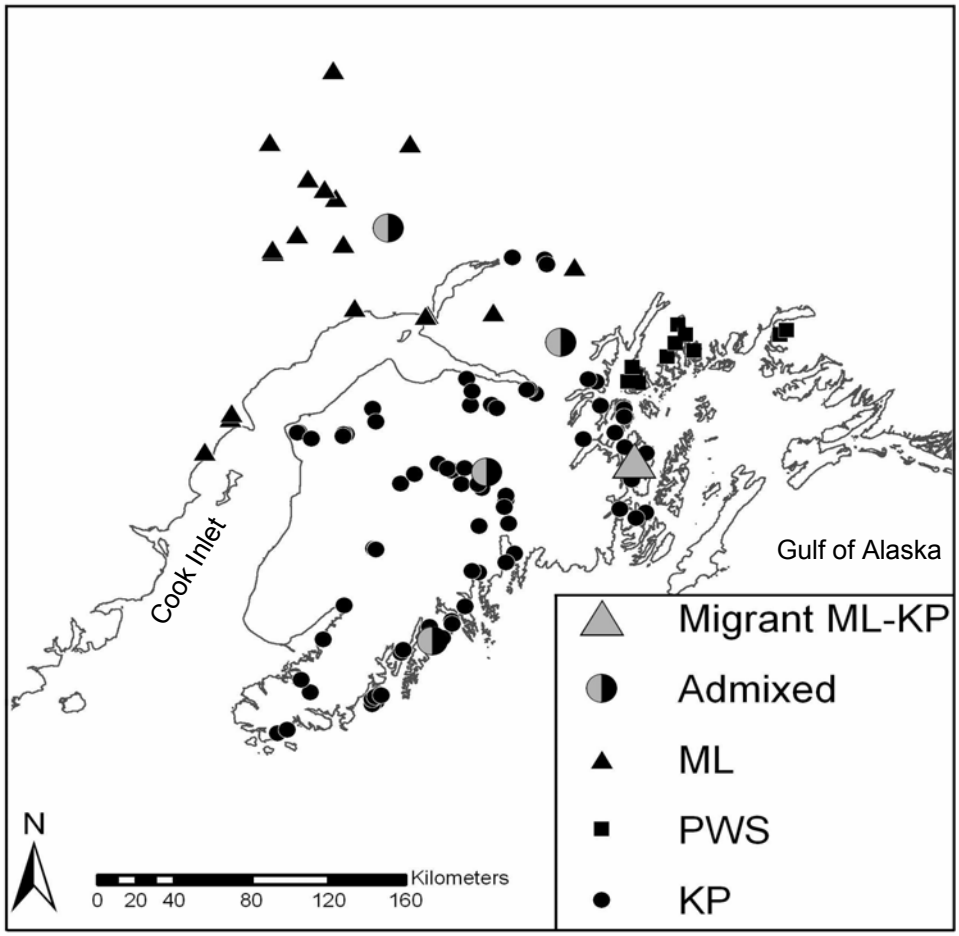
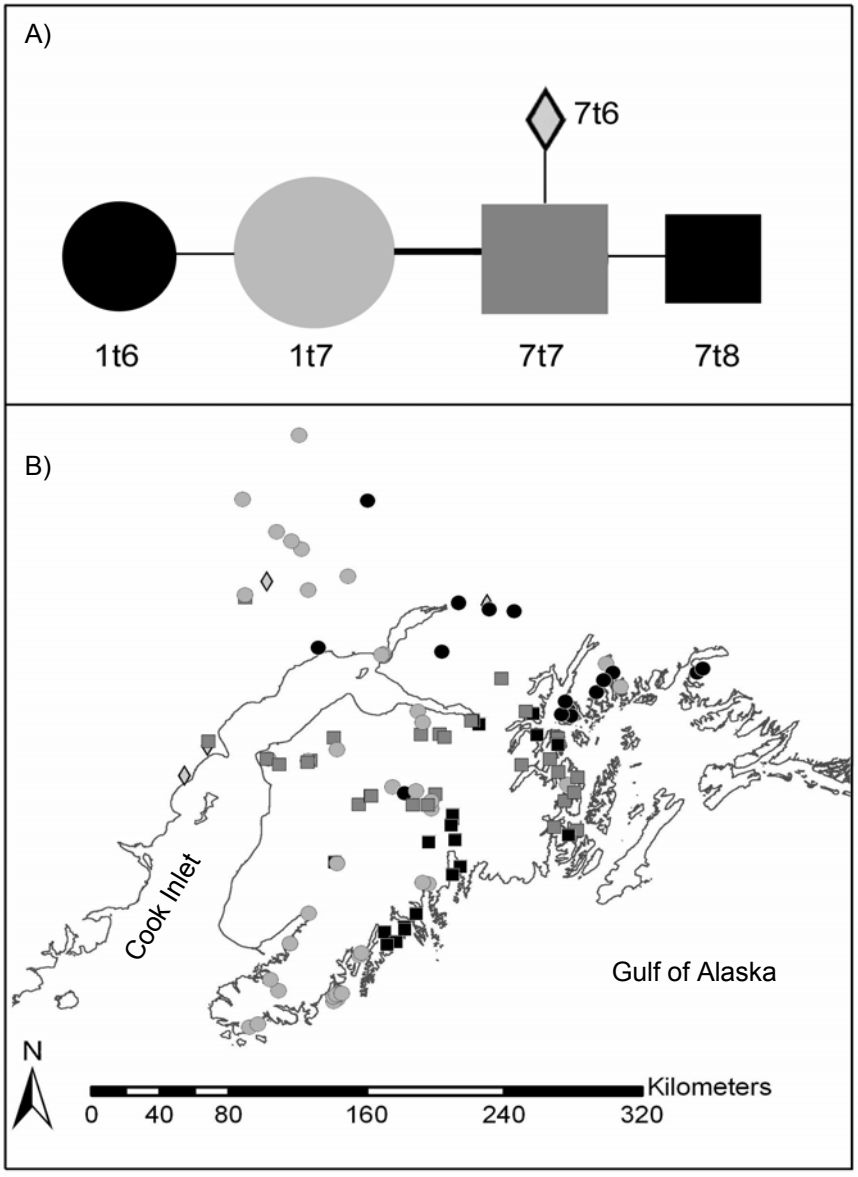


Figure 2.4: mtDNA control region haplotype distribution

A) As a legend of haplotype symbols, we have included a haplotype network in which the C-T substitution is shown as a heavy line and lighter lines depict indels in the hypervariable thymine segment. Symbol size is proportional to haplotype prevalence. B) The map shows the distribution of five MtDNA control region haplotypes identified in south central Alaska.



Chapter 3

Landscape Patterns in Allele Distribution: The Utility of Local Autocorrelation Analyses Within and Between Populations

ABSTRACT

Describing spatial genetic patterns is an essential first step in identifying the processes affecting the partitioning of genetic variation across the landscape. Spatial analysis of genetic data using autocorrelation statistics has become popular; however traditional autocorrelation analyses are limited by their requirement of stationarity and their inability to identify the extent or location of local patterns. We introduce a novel application of local autocorrelation statistics to individual genotypes. We use a Local Indicator of Spatial Association (LISA) to detect local clusters in allele frequencies to describe spatial genetic patterns within and among populations. We apply this technique to two empirical datasets from brown (*Ursus arctos*) and black bears (*Ursus americanus*). The LISA analysis identified biologically meaningful spatial genetic clusters in both datasets. The spatial patterns of allele distribution helped pinpoint areas of high migration and locally restricted gene flow that were related to features on the landscape. The LISA allowed simultaneous analysis of individual data from several distinct populations without assumptions of population structure or the nature of barriers between populations.

KEY WORDS

Black bear, Brown bear, Geographical genetics, Landscape genetics, Local Indicators of Spatial Association, Spatial autocorrelation, *Ursus americanus*, *Ursus arctos*.

INTRODUCTION

Identifying spatial patterns of genetic diversity and structure is of key importance to classical population genetics studies as well as the burgeoning fields of landscape genetics and geographical genetics (Epperson, 2003; Hedrick, 2005; Manel *et al.*, 2003). Detection of spatial patterns is important in the identification of genetically meaningful management units and is a crucial first step in the correlation of genetic patterns and environmental factors (Diniz-Filho, Telles, 2002; Manel *et al.*, 2003). Accurate description of genetic patterns can help researchers understand the processes that shape gene flow and connectivity within and among populations (Allendorf, Luikart, 2007).

Spatial analyses, particularly spatial autocorrelation statistics, have become a popular tool for describing genetic patterns at the population and individual level (Arnaud, 2003; Epperson, 2003; Epperson, Li, 1996; Peakall *et al.*, 2003; Sokal, Jacquez, 1991; Wagner *et al.*, 2005). Spatial autocorrelation explores the correlation of values based on spatial proximity (Haining, 2003). Positive autocorrelation occurs when nearby values of a given variable are more similar than expected under a random spatial distribution; whereas, negative autocorrelation indicates greater dissimilarity between neighboring values than expected under a random spatial distribution (Fortin, Dale, 2005). Positive autocorrelation of allele frequencies is typical of populations subject to spatially restricted dispersal (Hardy, Vekemans, 1999).

Most genetic datasets are analyzed using global spatial statistics to generate a single value summarizing the spatial pattern over the entire study landscape (Boots, 2003). A central issue in the application of global spatial models is the assumption of stationarity. Stationarity implies that a homogeneous process is responsible for generating each of the observed values (Fotheringham *et al.*, 2002), and that differences in observed values may depend on separating distance, but not on their absolute locations. We assume that the study region represents a homogeneous environment and that a single value provides an adequate

summary of the spatial pattern across the entire area (Boots, 2003; Fotheringham *et al.*, 2002). However, in studies designed on the landscape scale, stationarity is likely to be violated by local variations in biological processes (Fortin, Dale, 2005). For example, most landscape genetics studies will sample several biologically distinct population units. It is likely that processes affecting gene flow and genetic drift are different in each population. Further, examining isolation by distance within a continuous population, one can expect isolation over shorter distances where the terrain impedes movement than in open favorable habitat. Such variations at the sampling locations can induce significant differences in observations, thus requiring a different model of the spatial process for each location. The ability to document these differences is essential to understanding and measuring the processes underlying genetic patterns. Global measures may not be appropriate when heterogeneous processes affect the study region (Cressie, 1991), and summary values may be misleading, failing to describe the range in variability or exact localizations of the spatial pattern (Boots, 2003; Fortin, Dale, 2005).

One of the challenges in landscape genetic studies is to sample at a biologically meaningful scale and yet define units of study appropriate for statistical analysis. Manel *et al*. (2003) point out the value of focusing on the individual level, emphasizing the need to sample individuals randomly across a study landscape. Such a sampling scheme is important for examining fine scale landscape genetic processes without relying on researcher assumptions concerning what constitutes a "population" or a "barrier". However, this type of assumption-free, landscape-wide sample presents two major dilemmas for spatial analysis. First, without verifying that one is working within a single continuous population, it would be difficult to justify the assumption of stationarity necessary to global spatial analyses. Second, typical (global) autocorrelation analyses, while useful for describing the overall type and scale of a spatial genetic pattern, fall short of pinpointing genetic clusters or locating discontinuities (Barbujani, 1989; Manel *et al.*, 2003). Thus fine scale analyses of gene flow would greatly benefit from an individual-based statistic for the analysis and visualization of local spatial genetic patterns that is robust to non-stationarity.

One potential solution to both of these dilemmas lies in the analysis of local spatial autocorrelation, (L.S.A.) (Anselin, 1995; Boots, 2003; Ord, Getis, 1995). Because L.S.A. statistics are calculated based on locally defined neighborhoods, they avoid the assumption of stationarity and are, in fact, explicitly designed to detect patterns generated by non-homogeneous processes. Further, because they are individual-based and mappable, L.S.A. statistics are ideal for locating specific areas where spatial genetic patterns are strong or where outliers exist. Local autocorrelation of genotype or allele frequencies may be attributable to non-homogeneous processes acting across the landscape, such as localized environmental selection or local barriers to gene flow (Epperson, 2003). Where gene flow is locally limited we can expect to find greater genetic differentiation in the immediate vicinity (Epperson, 2003). Such influences could lead to abrupt differences in spatial distribution of alleles, to the extent of differentiation among discrete genetic populations (Epperson, Li, 1997), or more subtle differences such as the patchy distribution of alleles within a continuous population (Turner *et al.*, 1982). The ability to identify and locate patches of high genetic connectivity and intervening discontinuities can identify barriers between populations and even cryptic landscape features shaping genetic connectivity within populations.

Despite their utility in geospatial sciences, L.S.A. analyses have rarely been applied by the biological community. One exception is the work of Sokal and colleagues (Sokal *et al.*, 1998; Sokal, Thomson, 2006). An early study employed both extensive simulations and applications to varied empirical datasets to demonstrate the utility of local autocorrelation analysis in identifying localized genetic patterns among populations (Sokal *et al.*, 1998). A recent study revived the use of these statistics examining spatial clustering of genetic and linguist groups in a human population (Sokal, Thomson, 2006). To date, only the "2D LSA" method of Peakall and Smouse (Double *et al.*, 2005; Peakall, Smouse, 2006) has been used to apply local autocorrelation statistics to individual-level analysis using genetic data (ie.pairwise genetic distance rather than allele frequencies). Sokal and Thomson (2006) make note of the growing importance of spatial analysis within populations and state that focusing analysis on individual genotypes in local spatial analysis offers the finest possible resolution for spatial analysis of genetic patterns.

In this paper we evaluate the utility of local spatial autocorrelation analysis for identifying biologically relevant local clustering of alleles, and explore its utility in visualizing spatial genetic patterns without *a priori* information or assumptions regarding population subdivision or presence of barriers. We take a novel approach applying Anselin's (1995) Local Indicators of Spatial Association (LISA) to individual genotype data. This differs from the population-based analysis of Sokal and Thomson (2006) and uses a different statistical method than the "2D LSA" of Peakall and Smouse (2006). Spatial analysis using LISA is applied to two previously published datasets. First, we applied the technique to a well-studied brown bear (*Ursus* arctos) dataset from Scandinavia. This population's history is well-documented (Swenson *et al.*, 1998; Swenson *et al.*, 1995) and numerous genetic investigations (Manel *et al.*, 2004; Taberlet *et al.*, 1995; Waits *et al.*, 2000) offer substantial context for interpreting the performance of this new technique. Second, the same techniques were applied to a dataset of black bears (*Ursus* americanus) from the Kenai Peninsula of Alaska (Robinson *et al.*, submitted) to evaluate spatial genetic patterns within and between populations relative to the landscape.

Using the brown bear dataset we pose the following research questions: 1) Does the LISA statistic detect spatial genetic patterns? 2) Does the pattern detected concur with existing population information? For the black bear dataset we ask: 1) Is there spatial structure within the Kenai Peninsula bear population? 2) Does genetic structure coincide with landscape features on the Kenai?

MATERIALS and METHODS

Scandinavian Brown Bear Data

We used LISA statistics to explore local spatial genetic patterns of 365 bears analyzed by Manel *et al*. (2004). Genetic data included 19 microsatellites genotyped by Waits *et al*. (2000). We removed one bear from the Manel *et al*. (2004) dataset because it was a geographic outlier, greater than 50km from the remaining points, and would have had an insufficient number of local neighbors. In 1930, this population reached a low of about 130 bears distributed in four geographically distinct subpopulations; northern north (NN), southern north (NS), middle (M) and south (S) (Figure 3.1). By 2000 the brown bear population had expanded to about 1,000 individuals. Taberlet *et al*. (1995) showed that the S population belonged to an mtDNA clade divergent from the other groups. Waits *et al*. (2000) characterized microsatellite diversity and gene flow finding substantial differentiation between all but the two northern subpopulations. Manel *et al*. (2004) later reexamined this dataset using neighbor-joining (Satou, Nei, 1987) and Bayesian assignment tests (Structure 2.1, Pritchard *et al.*, 2000). Their results also supported the delineation of three to four subpopulations (Figure 3.1).

Alaskan Black Bear Data

We then applied LISA analysis to 110 black bears genotyped at 13 microsatellite loci by Robinson *et al*. (submitted). In this dataset black bears had been sampled across a continuous area with no *a priori* assumptions of population centers. Robinson *et al*. (submitted) evaluated population structure using Bayesian assignment tests. A spatially informed assignment test (BAPS 4.1, Corander *et al.*, 2006) revealed three genetic subpopulations situated on the Kenai Peninsula (KP), Mainland (ML) and Prince William Sound (PWS) (Figure 3.2) (Robinson *et al.*, submitted).

LISA analysis

Local Indicators of Spatial Association are a class of local spatial autocorrelation statistics defined by Anselin (1995) as having the key properties that: 1) they decompose the global measure to indicate autocorrelation around each point in the dataset, and 2) they sum to a value proportional to the global value. LISA statistics serve two important functions: first, they identify local patterns of autocorrelation; second, they identify points that may be influencing, or deviating from, the overall global pattern (Anselin, 1995). Here we will focus on the Moran's I LISA. Moran's I (Moran, 1950) is one of the most popular autocorrelation statistics, and has been widely applied to genetic analyses (Epperson, 2003; Hardy, Vekemans, 1999). It is a product-moment coefficient of the correlation between values of a given variable within a given distance threshold (Haining, 2003). Similar to the global measure, the Moran LISA computes joint covariation of neighboring localities from the regional average of a variable, in this case an allele frequency. Unlike the global value, the LISA defines a local neighborhood around each point and the spatial pattern at each point is assessed against an expected value generated for each point based on the local neighborhood (Anselin, 1995; Fortin, Dale, 2005). LISA values for each point can be mapped to identify position, size, shape and layout of local spatial structures (Fortin, Dale, 2005).

We implemented the Moran's I LISA analysis in the geostatistical program Geoda 0.9.5-i (Anselin, 2004). The Moran's correlation coefficient for each point, I*i*, is given by the equation:

$$Ii = \left(z_i - \bar{\mathbf{z}}\right)\sum_{\mathbf{j}} \mathbf{w_{ij}}\left(z_{\mathbf{j}} - \bar{\mathbf{z}}\right)$$

where z is the variable of interest (here an allele frequency), z*i* is the frequency of the allele at point i, thus $\left(z_i - \bar{\mathbf{z}}\right)$ is the deviation of the allele frequency at point i from the mean frequency across the study area, $\left(z_{\mathbf{j}} - \bar{\mathbf{z}}\right)$ is the same deviation regarding point j. The summation over j is such that only points in the defined neighborhood are included in the calculation. The local neighborhood is defined through the spatial weighting matrix (w_{ij}),

which can be based either on n^{th} order neighbors, a distance threshold or contiguity (for polygon data).

We calculated the LISA using numerous threshold distances (25 km, 50 km, 100 km, 150 km, 200 km, 250 km). For irregularly distributed point data, we found the distance threshold preferable to the nearest neighbor method in which the distance between neighbors would be highly variable. Based on visual assessments of each dataset we found the clearest pattern using a 100km threshold weighting matrix: points identified as spatially clustered formed spatially cohesive units. The 100km threshold is also well justified because it relates to long dispersal distances typical of male black bears (Lee, Vaughan, 2003; Powell *et al.*, 1997; Schwartz, Franzmann, 1992). Thus, only results based on the 100 km distance weighting scheme are presented.

All autocorrelation tests were conducted allele-by-allele using individual-based allele frequencies. Conversion from genotype to allele frequency data has proven a useful means of adapting autocorrelation measures from population to individual levels of investigation (Epperson *et al.*, 1999; Heywood, 1991). For a given allele an individual was assigned a frequency of 1.0 if homozygous, 0.5 if heterozygous and 0.0 if lacking that allele. When calculated using such frequencies, Moran's I is directly interpretable in terms of Wright's coefficient of relationship (Hardy, Vekemans, 1999); (see Epperson *et al*., 1999 for derivation and statistical properties).

For each LISA test Geoda generates an I*i* value and p value for each point and a cluster map displaying patterns of local autocorrelation (Figure 3.3). Geoda displays positive autocorrelation as clusters of either high or low values. Clusters indicate local areas where allele frequencies are more similar than expected by chance, signifying a spatially restricted distribution of the allele in question. A cluster of high frequency values indicates an area where the allele is near fixation. A cluster of low frequency values indicates the absence, or extreme rarity, of that allele in the region. Negative autocorrelation indicates that the allele frequency at a given point is more dissimilar from neighboring values than expected under a

random distribution. We will refer to these individuals as spatial outliers. These outliers indicate areas of rapid changes in allele frequencies between neighbors. A single outlier point might represent a migrant or admixed individual which differs greatly in allele composition from its neighbors. A zone of numerous outliers might signify a sharp barrier between populations where divergent allele frequencies are in close spatial proximity. If a point lacks local autocorrelation (termed random in Figure 3.3), this indicates that the allele frequencies of neighboring points are no more or less similar than expected under a random distribution of alleles.

We summarized the information from multiple allele-wise tests as suggested by Epperson (2003). Concerns have been raised about the lack of independence between multiple alleles of a single locus (Epperson, 2003). Epperson (2003) provides evidence that if greater than five alleles are present at moderate frequencies (above 0.02), then their distributions and consequent spatial patterns may be treated as independent. We first eliminated any alleles occurring with a frequency less than 0.02 as these would not be present in adequate frequency to reliably test their spatial distribution. We next examined the number and frequencies of alleles remaining at each locus. Loci with fewer than four moderately frequent alleles were removed from the analysis. Then the autocorrelation value of each allele was averaged per locus. Finally, the autocorrelation values were averaged across loci for each individual.

Significance of LISA statistics were calculated using 999 randomizations in Geoda. Significance was used solely to identify allele clusters in each allele-wise test, not to assess significance of the overall LISA pattern. Two problems have been identified concerning the significance of the overall LISA pattern. First, the presence of significant global autocorrelation may bias the p-values in LISA tests (Anselin, 1995; Sokal, Thomson, 2006). To address this, the global Moran's I was calculated to detect the presence of significant global patterns that might cause such bias. A global Moran's I test was performed on each dataset in Geoda using the 100km threshold w*ij* as presented in the LISA analysis. Second, an adjustment is needed for significance values over so many tests; however, it has been

acknowledged that Bonferroni correction over such a large number of tests would be too conservative (Epperson, 2003; Sokal, Thomson, 2006). In the Scandinavian brown bear dataset, for example, correcting for each point-wise test (365) and each allele-wise test (95) would require a Bonferroni correction for 34,675 tests (setting α at an unreasonable 0.0000004).

Due to the influences of global spatial autocorrelation and lack of appropriate p-value corrections for multiple tests we did not rely on significance values for this exploratory technique. Instead, we employed two different methods of summarizing and visualizing the data. We first used a ranking scheme similar to that suggested by Sokal *et al*. (1998 and 2006). The average I values for each individual were ranked from least to greatest. The smallest positive value was ranked 1 and ranks increased with greater positive I*i* values; the least negative value was ranked -1 and ranks became increasingly negative with greater negative I values. Thus, when displayed, the highest negative ranks would indicate the individuals most genetically different from their neighbors and the highest positive ranks would indicate the areas where neighbors were genetically similar. The ranked values were plotted in ArcGIS 9.0 (ESRI, Redland, CA) to visualize local genetic patterns.

Additionally we summarized spatial clustering by calculating the proportion of locally clustered alleles shared by each pair of individuals. A network of lines, or joins, was drawn connecting all possible pairs of individuals. For each pair we counted the number of allele-wise tests in which they appeared in the same cluster of autocorrelated allele frequencies (high or low). We then divided this count by the highest number of clusters including either of the points (the maximum number of clustered alleles potentially shared). This "cluster network" was displayed in ArcGIS 9.0 to visualize areas where points shared similar patterns in spatially clustered alleles. If isolation by distance were the predominant pattern, we would expect to see evidence of a gradient in the cluster network, with substantial joining between any neighboring pairs with no apparent local concentrations or patchiness in allele clustering. On the other hand, if gene flow were restricted by certain landscape features or in certain landscape patches, we would expect a patchy cluster distribution rather than a steady cline.

We would expect clustering of allele frequencies to appear in certain areas consistently over many allele-wise tests.

RESULTS

Genetic Data

After eliminating low frequency alleles and discarding loci with fewer than four alleles, 15 loci remained in the Scandinavian dataset yielding a total of 95 allele-wise tests. The number of alleles in the loci used ranged from 4 to 10 (average 6.3). The average He was 0.739. For the Alaskan dataset, we were able to use all 13 loci which yielded 94 allele-wise tests. The number of alleles in the loci used ranged from 5 to 11 (average 8.6). The average He was 0.736.

LISA - Scandinavian Brown Bears

Global autocorrelation was significant so we relied on the ranking rather than the potentially biased numerical values of significance. Local spatial clustering of allele frequencies was evident in examining the cluster maps output by Geoda (individual figures not shown). The highest degree of clustering across loci was evident in points within each of the historical population areas (NN, NS, M, S) (Figure 3.4A).

In the cluster network, the core population areas appeared as distinct clusters when viewing the joins indicating that greater than 0.5 of clustered alleles were shared between points (Figure 3.5A). The southern population showed the lowest proportion of clustered alleles shared with other populations. No joins above the 0.5 proportion occurred between the S and other areas. Occasional joins >0.5 occurred between the M and neighboring NS population. Many occurred between NN and NS showing substantial similarity in allele frequency distribution between these groups. At the 0.8 proportion, distinction was evident between the western portion and the rest of the NN/NS population.

LISA - Alaskan Black Bears

As in the brown bear dataset, global autocorrelation was again significant in the black bear dataset, so we relied on the ranked LISA scores. Local spatial clustering of allele frequencies was evident in the Geoda cluster maps (individual figures not shown). High degrees of clustering were evident in points near the core of each of the population areas (ML, KP, PWS), with the ML and PWS areas showing the highest clustering ranks (Figure 3.4B). Random and outlier points were frequent at the interface between the ML and KP populations, where the populations were separated by the narrow Cook Inlet or in contact at the peninsula arm. The two highest ranked outliers were points identified as migrants or admixed individuals in previous assignment tests.

In the cluster network the core population areas appeared distinct when viewing joins showing ≥ 0.5 clustered alleles shared (Figure 3.5B). At the 0.5 level there were several joins extending between KP and PWS. Examining joins of ≥ 0.6, the KP and PWS groups showed less connectivity. Further, distinct patches of connection took shape within the KP population. Distinct patches of allele clustering centered around the northeast coast, the southern coast and Kenai Mountains, and a small patch in the western lowlands.

DISCUSSION

Existing literature shows strong support for the utility of L.S.A. analyses, and the under-use of this valuable tool (Double *et al.*, 2005; Peakall *et al.*, 2003; Sokal *et al.*, 1998; Sokal, Thomson, 2006). Sokal and Thomson (2006) provided a thorough explanation of autocorrelation and local statistics in a population genetics context. They used a Moran's I LISA, Geary LISA and Getis-Ord G statistic to explore local clustering of genetic and linguistic patterns among Yanomama populations. They found L.S.A. analysis to be a useful tool in discovering these patterns. Here we build on their techniques by applying a Moran's I LISA analysis at the individual rather than population level. Our results show that the LISA was sensitive and informative regarding spatial structure even at a fine scale within populations. Because the local nature of the LISA relaxes the assumption of stationarity, data from multiple population units could simultaneously be analyzed at the individual level. Peakall and Smouse (2006) developed a multi-locus, distance-based statistic for individual-based L.S.A. analysis. Their "2D LSA" was applied by Double *et al.* (2005) to investigate dispersal and philopatry in a fairy wren population. They found that L.S.A. analysis offered new insights to their demographic and evolutionary questions. The Moran LISA described here differs from the "2D LSA" in its use of individual allele frequencies rather than distance-based measures (Smouse, Peakall, 1999), in the construction of the spatial weighting matrix (Peakall, Smouse, 2006), and in the treatment of significance of the local statistics (Double *et al.*, 2005; Peakall, Smouse, 2006).

Challenges & Assumptions

It is widely acknowledged that LISA statistics are instrumental in exploration of spatial patterns, but should not be counted on for significance testing (Anselin, 1995; Boots, 2003; Fortin, Dale, 2005; Haining, 2003). This is due to two problems: the inflation of LISA significance due to significant global autocorrelation, and the lack of appropriate significance corrections over the numerous tests performed over all sample points and genetic markers. Sokal and colleagues (2006) developed a ranking strategy for interpreting LISA results in the

face of global autocorrelation, and recommend it as yielding reliable and interpretable results. We believe that the ranking scheme employed here accurately conveyed the spatial pattern in the data without introducing bias.

Another issue is the lack of independence between alleles of a single locus. The "2D LSA" (Peakall, Smouse, 2006) escapes this problem by summarizing multi-allelic, multi-locus data in the form of pair-wise genetic distances. Though computationally less convenient, we chose to use multiple allele-based tests for two reasons. First, genotypes converted to individual allele frequencies provide a biologically relevant interpretation of autocorrelation because they relate directly to kinship coefficients (Epperson *et al.*, 1999; Hardy, Vekemans, 1999). Epperson (1999) points out that pair-wise summary measures suffer because they are not directly related to kinship coefficients and what is measured is always relative to the existing sample. Second, we felt that information was best preserved using the multiple allele-wise tests. Sokal *et al.* (1998) point out that by analyzing each allele frequency separately the researcher can examine the contribution of each to the overall LISA. It has been shown that multiple-allele, multiple-locus tests can be averaged to estimate spatial patterns as effectively as multi-locus measures without sacrificing the data contained in each allele-wise test (Epperson, 2003; Sokal, Thomson, 2006). Further, our addition of the cluster network analysis made use of individual allele patterns, greatly enhancing our understanding of spatial patterns in a way that may have been missed had we looked only at the I*i* values. In his recent book Epperson (2003) details simulation studies showing that Moran's I was robust to increasing numbers of alleles and varying allele frequencies. He further illustrates that, despite biological dependence between alleles of a locus, given adequate allele numbers, the alleles act independent in spatial distribution. We took steps (detailed in methods) to ensure that the allele-wise tests could be treated as independent.

Scandinavian Brown Bears

The LISA performed well, yielding clearly interpretable results consistent with the large body of existing literature and clarified the fine scale spatial genetic patterns. The highest

local I*i* values formed clusters that closely coincided with the four historic brown bear population areas (Manel *et al.*, 2004). Further examination of the cluster network showed that, indeed, the bears within each subpopulation shared the greatest proportion of spatially clustered alleles, indicating that spatially restricted gene flow was influenced by historical population isolation (Manel *et al.*, 2004). The level of distinction between S and other groups indicated by the LISA analysis was concordant with that described by previous mtDNA studies (Taberlet *et al.*, 1995; Waits *et al.*, 2000). The two northern subpopulations shared a high proportion of clustered alleles but remained distinct. This was also in agreement with the previous analyses indicating substantial mixing of the NN and NS relic populations (Manel *et al.*, 2004; Waits *et al.*, 2000). Further, the distinct western concentration of clustered allele sharing coincides with potential population split identified by previous assignment tests (Manel *et al*., 2004). When previously detected, Manel *et al*. (2004) dismissed this cluster as it did not coincide with historical population ranges. It is likely that factors other than the historical bottleneck have led to this distinction since the population expansion. In fact, recent work suggests that eastern segments of the NN/NS population exchange migrants with nearby Finnish populations (Swenson, 2006). The distinction in western and eastern allele clustering in the NN/NS population suggests the merit of future investigations of landscape features that might limit the western movement of Finnish migrants. Overall, the LISA offered a powerful spatial analysis over the landscape containing four distinct populations. The cluster network in particular provided a useful visualization of the spatial genetic structure.

Alaskan Black Bears

As observed in the brown bear dataset, the highest local I*i* values formed clusters that closely coincided with previously identified genetic groups (Robinson *et al.*, submitted). The cluster network also showed that the bears within each subpopulation shared the greatest proportion of spatially clustered alleles. The entire KP population was highly connected, most points sharing 0.4 –0.5 of clustered alleles. However, ≥ 0.6 clustered alleles shared, distinct allele clusters were evident within the KP population.

LISA analysis added important details to previous assignment test results. By examining the I*i* values, we were able to judge the strength of the barrier between KP and ML populations. Outliers and non-clustered points occurred primarily at the interface between the KP and ML populations, though the strongest outlier was the one migrant from the ML population on to the Kenai. The prevalence of less extreme outliers at the edge of the Kenai and in the area of range overlap suggests some intermixing at the population boundary. The cluster-sharing patterns within the KP group were particularly informative, showing spatial structure within the population. The patches of allele clustering related to geographically distinct areas within the Kenai Peninsula. The northern patch was located in the Kenai Mountains, north the Resurrection River valley. The southern patch covered the Kenai Mountain range south of the Resurrection River valley. A minor western patch also occurred in the western Kenai lowlands.

Two principle distinctions exist between genetic patches on the Kenai. First the Kenai Mountains (N and S patches) and the Kenai lowlands (W patch) comprise two ecologically divergent areas of the Kenai Peninsula. Topographic differences are extreme; the Kenai Mountains reach elevations over 2000 m with steep and rugged terrain, in contrast the Kenai lowlands have a gentle rolling topography with an elevation range of 10 to 100 m. These areas are further distinguished as different class III ecoregions (Bailey, 1995; Gallant *et al.*, 1995); the eastern regions are in the Coastal Mountain and Hemlock-Sitka Spruce Coastal Forests while the western area is in the Cook Inlet ecoregion. Vegetation communities differ dramatically in these areas. The mountainous regions are primarily composed of sitka spruce and mountain hemlock forests with high elevation alpine zones, while the lowlands are host to a variety of land covers ranging from boreal to mixed forests and substantial riparian and wetland areas (Ducks Unlimited Inc., 1999). I*i* values tended to be lower in the western lowlands indicating that local autocorrelation was highest in the rugged Kenai Mountains where movement might be more restricted. The patchy structure in the cluster network might be influenced by black bear fidelity to the natal ecological zone. The coyote (*Canis latrans*)

is another far-ranging carnivore that shows fidelity to its natal habitat zone, despite its ability to utilize a variety of habitats (Sacks *et al.*, 2005).

Additionally, there are potential barriers between the genetic patches. Alaska Highway 1 is the primary road on the Kenai. In Figure 3.6 we see the highway clearly outlining each of the genetic patches. Other studies have shown major roads to impede dispersal in both black bears (Lee, Vaughan, 2003; Thompson *et al.*, 2005) and brown bears (Proctor *et al.*, 2005). There area also major icefields between the N and S patches, though we note that connectivity is high around both the northern or southern ice masses. It is likely that certain corridors help to maintain connectivity within each of these ice-bound areas. For example, the Nuka and Resurrection River valleys appear to be critical passages around the icefields in the southern Kenai Mountains. There are fewer opportunities to cross between N and S patches without crossing substantial ice, sea cliffs, or Highway 1.

Mapping genetic patches with the LISA cluster network provided a view of landscape features that may facilitate or impede genetic connectivity. Connectivity was highest within similar ecological zones, lowest between areas with intervening highway and icefield crossings. Such features may be key to identifying important movement corridors. Maintaining such corridors will be important in managing the KP population and preserving current gene flow levels that maintain continuity of this population.

LISA Analysis Conclusions

We have presented a novel technique for visualizing biologically relevant spatial patterns in genetic data. This LISA technique performed well producing results concordant with extensive previous research in the Scandinavian brown bear dataset. It also provided important insights into the genetic patterns of the Alaskan black bears. In both of these cases the resultant spatial patterns have provided clues to the role of landscape or habitat features in shaping genetic connectivity across the study landscape.

One benefit of the landscape genetics approach is the treatment of individuals as the unit of investigation and identification of patterns within continuously sampled areas without *a priori* assumptions of population structure (Manel *et al.*, 2003; Storfer *et al.*, 2006). Bayesian assignment tests have provided major improvements to population genetic studies by identifying genetic populations without prior information (Corander *et al.*, 2006; Guillot *et al.*, 2004; Pritchard *et al.*, 2000). To date, most spatial analyses still require the definition of population structure to examine spatial patterns within a continuous population (Epperson, 2003), or the identification of barriers to test for differentiation on either side (Coulon *et al.*, 2004; Proctor *et al.*, 2005). Our application of LISA analysis to allelic data provides a substantial step forward in spatial genetic analysis. LISA can be used to detect spatial genetic patterns without prior information or assumptions of either population structure or specific barriers. Individuals can be sampled across a landscape within one or several genetic "populations". LISA analysis may be usefully applied to detect and map areas of high genetic connectivity or discontinuities whether they occur between or within population units and to point out environmental features to examine in further analyses.

ACKNOWLEDGEMENTS

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Figure 3.1: Population structure of Scandinavian brown bears
(Adapted from Manel et al. 2004.) The map shows the sample coverage of Scandinavian brown bear samples initially analyzed by Manel et al. (2004). Dashed circles indicate areas of population concentrations during a historic population bottleneck in the early 1900's. Solid black circles outline the approximate areas of four genetically distinct groups identified by neighbor-joining methods (Manel et al. 2004). These samples provided the data for our first example.

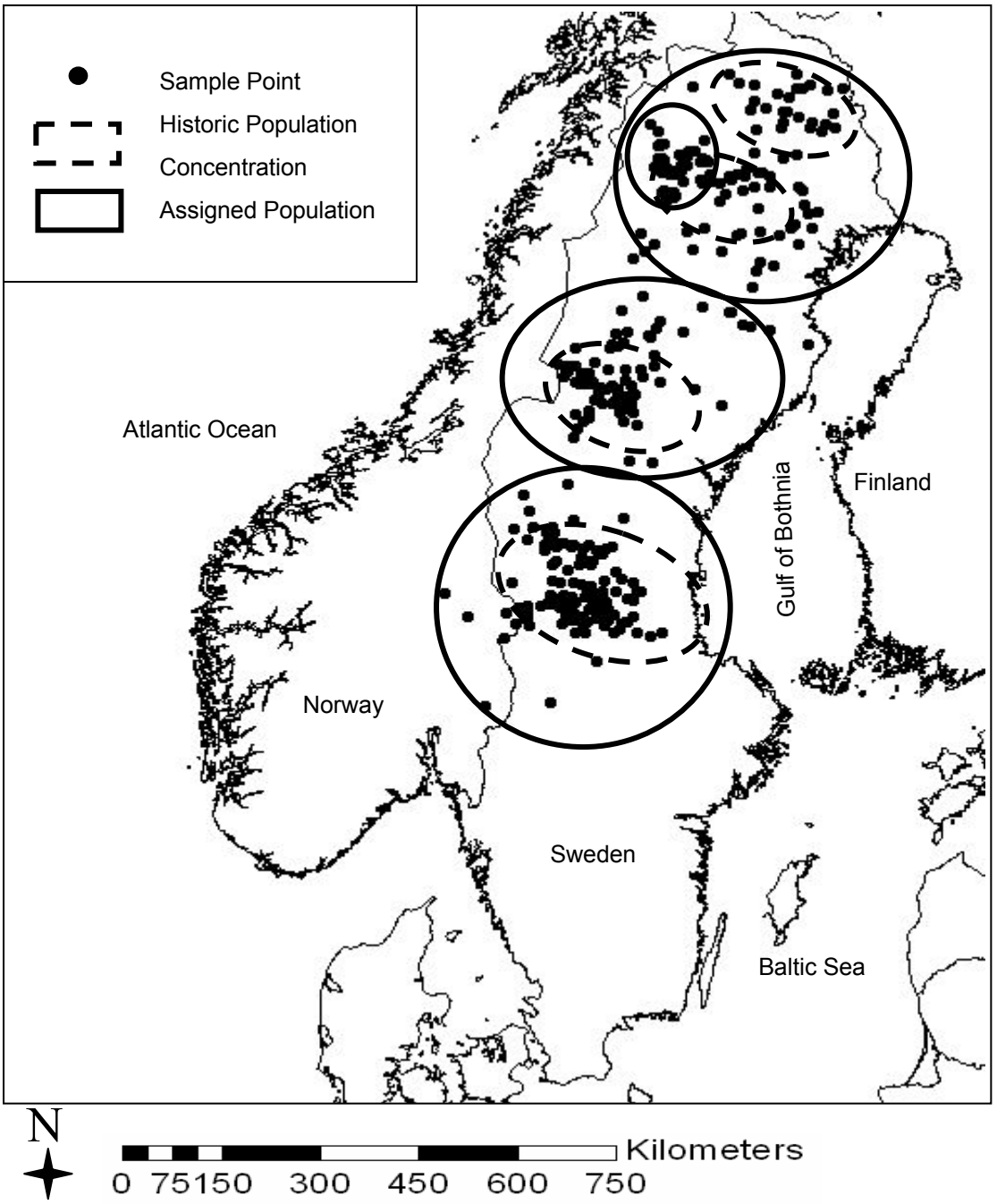


Figure 3.2: Population structure of Alaskan black bears
(Adapted from Robinson et al. 2006.) The map shows sample coverage of Alaskan black bears initially analyzed by Robinson et al. (2006). Different shapes and polygons indicate three genetically distinct groups identified using a Bayesian assignment test (Robinson et al. 2006). These samples provided the data for our second example.

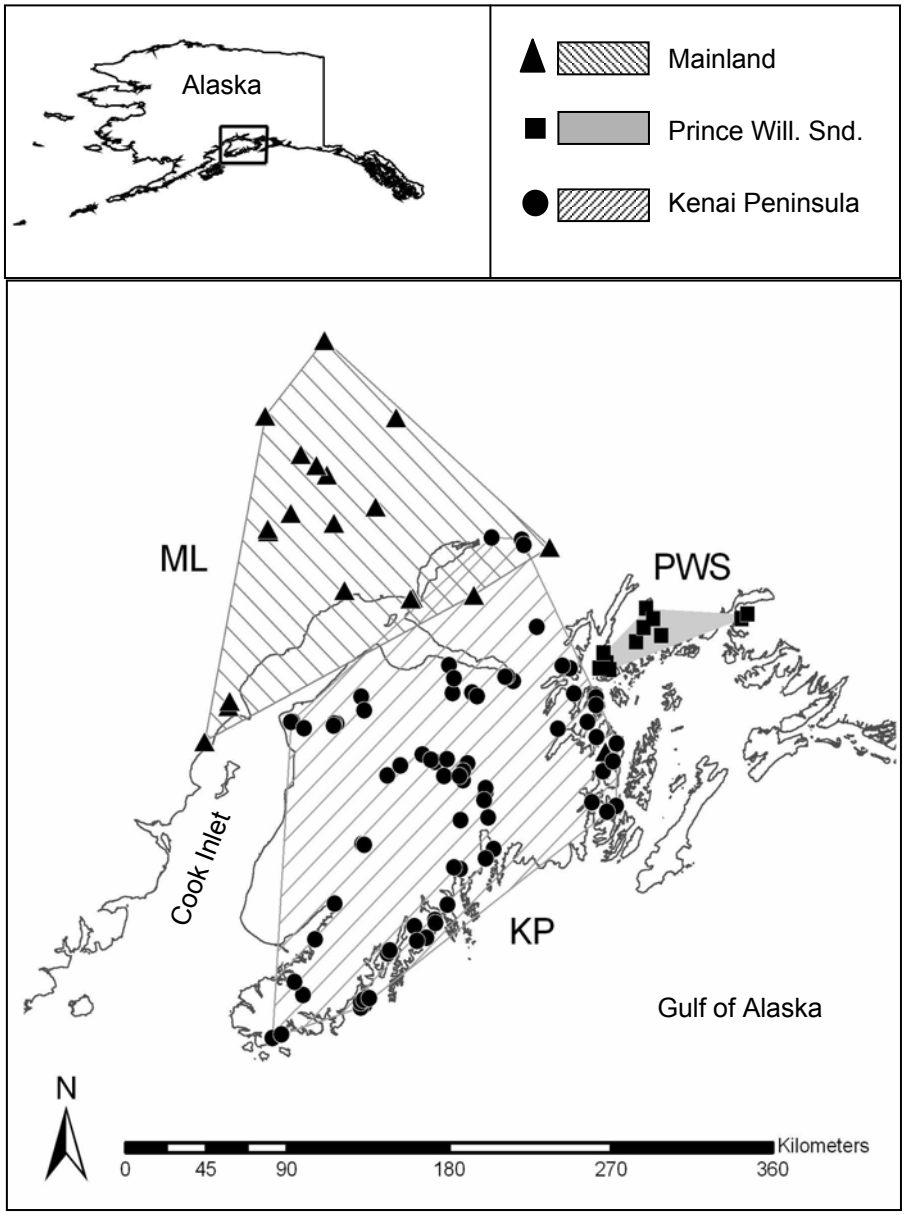


Figure 3.3: Local clustering of a single allele based on LISA analysis in Geoda
This is an example of the cluster map output by Geoda showing the LISA pattern for a single allele. White points show no significant local spatial pattern. Purple points show significant local clustering of high values meaning the allele is highly prevalent in the area. Blue points show clustering of low values, areas where the allele is primarily absent. Yellow points are spatial outliers, points with allele frequencies very different from their neighbors.

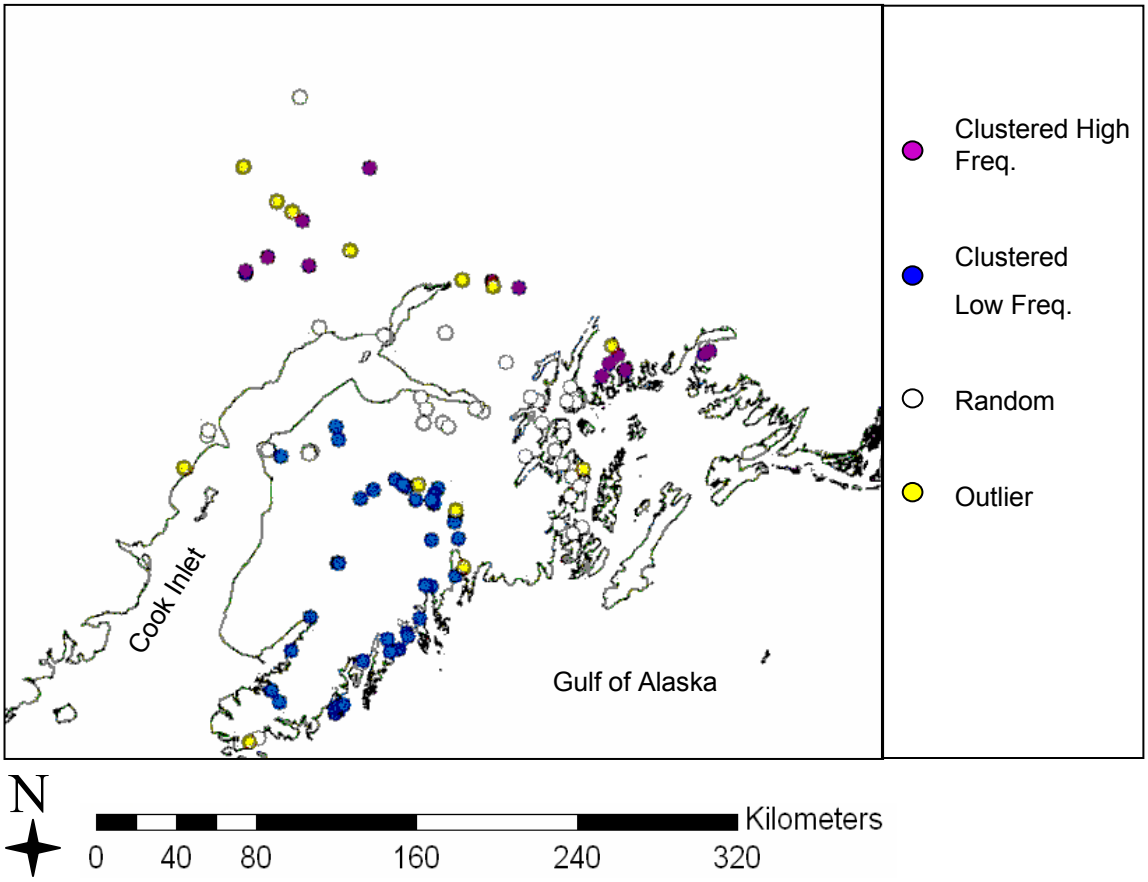


Figure 3.4: Local allele clustering based on LISA analysis
The degree of local autocorrelation is summarized over all allele-wise tests for each sample point. Highly clustered (blue) areas indicate locally patchy genetic connectivity. Outliers (yellow) show sharp genetic differences between neighbors, possibly indicating migrants or genetic discontinuities. A) Scandinavian brown bears. B) Alaskan black bears.

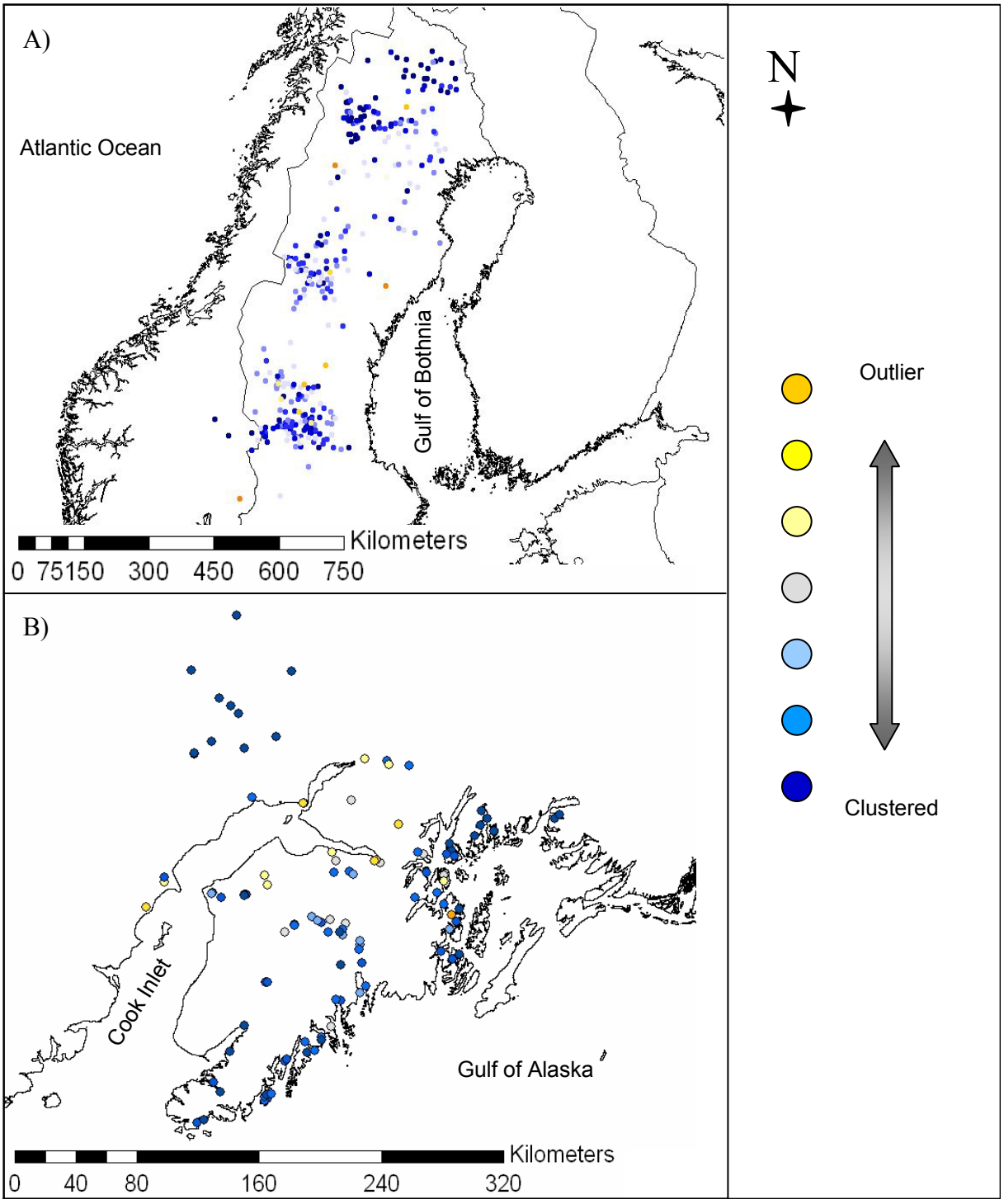


Figure 3.5: Allele clustering network

A network of lines connects all possible pairs of sample points. For each line we calculated the proportion of allele-wise LISA tests in which the joined points appeared in the same positive spatial cluster. Higher proportions indicate greater similarities in localized genetic information. A) Scandinavian brown bears. B) Alaskan black bears.

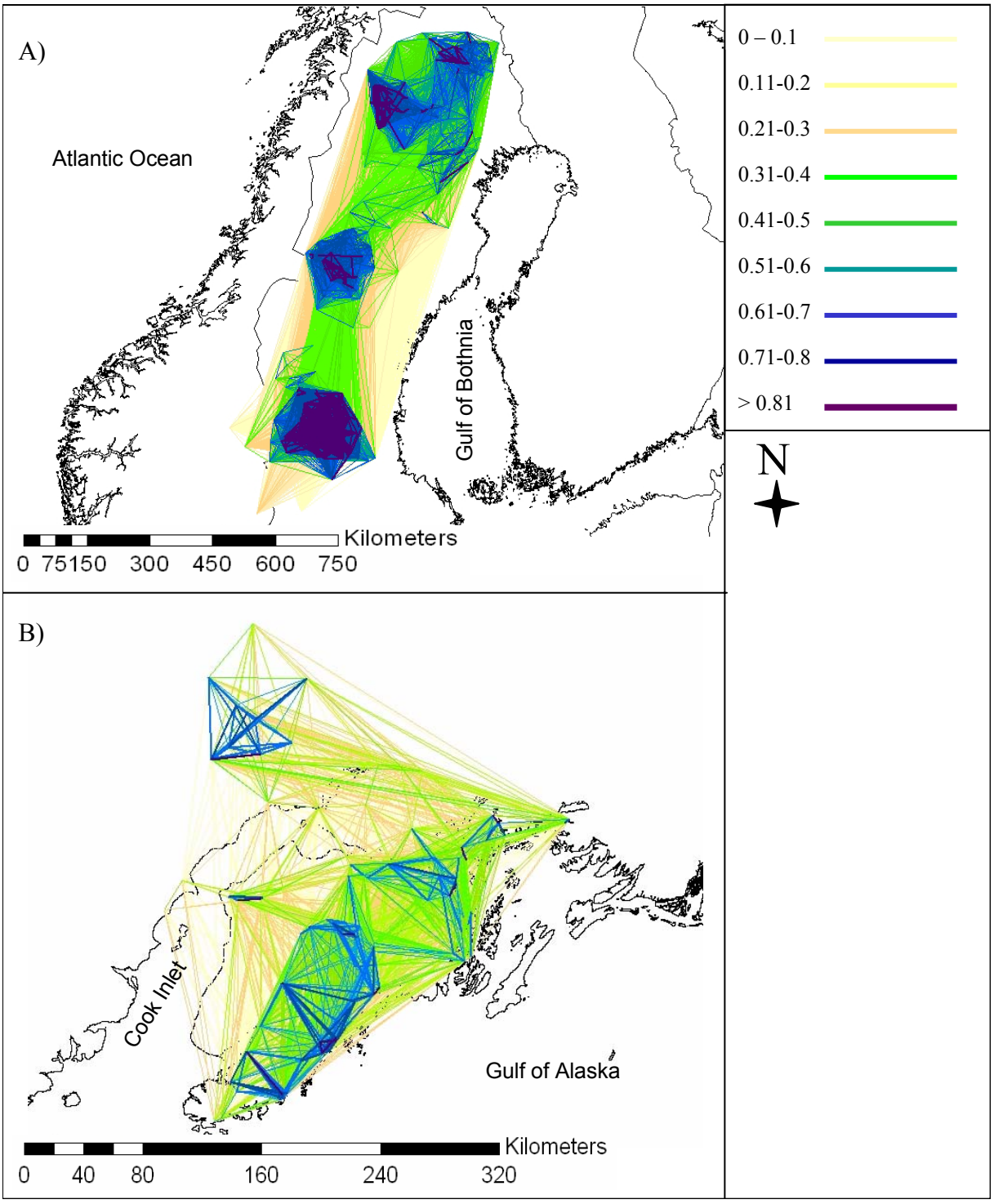
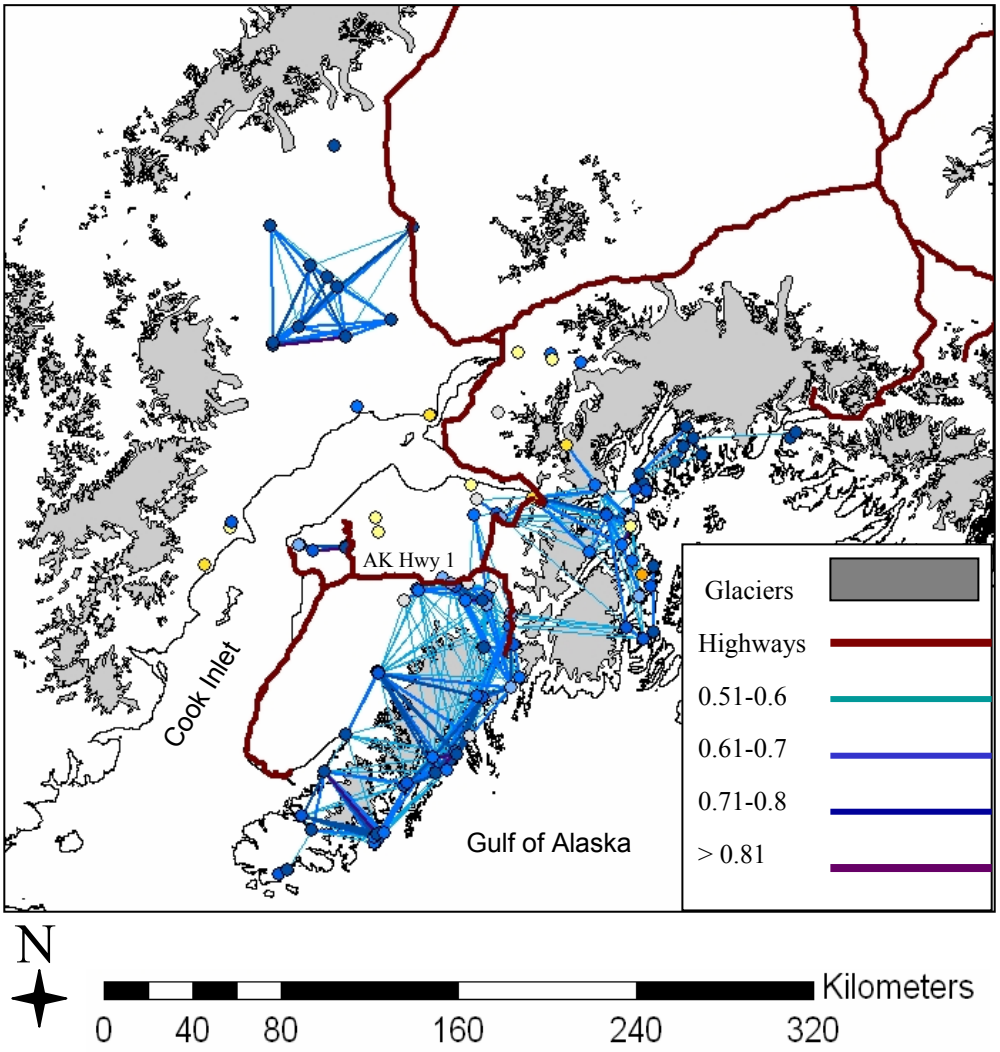


Figure 3.6: Relationship of geographic features to Kenai allele clustering
The major road system (heavy red line) and glacial masses (gray) are overlayed on the black bear allele clustering network. (Only lines ≥0.5 shown). Areas of high local genetic similarity form distinct patches relative to major icefields and/or separated by Alaska Highway 1 or other major roads.



Chapter 4

Estimating Black Bear Use of Coastal Food Resources in Kenai Fjords National Park Using Non-Invasive Genetic Sampling

ABSTRACT

Kenai Fjords National Park protects the unique coastal fjords environment on the eastern coast of Alaska's Kenai Peninsula. Black bears (*Ursus americanus*) are found throughout the park and play a key role in the coastal forest ecosystem. In an effort to establish a scientifically-based bear management plan, the park has undertaken a comprehensive black bear study program. An important first step in this plan is to establish a baseline of bear abundance on which to base future monitoring efforts. In this study we use non-invasive genetic sampling and DNA-based capture-mark-recapture analysis to provide an estimate of black bears utilizing coast food resource areas. We use traditional C-M-R models in the program MARK, as well as novel models specifically designed for genetic data in the programs Capwire and BayesN. Point estimates of bear abundance ranged from 73-324 bears per bay. There was considerable variation between models and estimate precision was low in most models. We conduct simulations in CAPTURE to guide suggestions for achieving more precise abundance estimates in future monitoring efforts.

KEY WORDS

Black bear, Capture-Mark-Recapture, Closed capture models, Continuous session models, Inventory &Monitoring, Kenai Fjords National Park, Kenai Peninsula, Multiple session models, Non-invasive genetic sampling, *Ursus americanus*.

INTRODUCTION

Anthropogenic landscape change and habitat fragmentation are the principle threats to American black bear (*Ursus americanus*) populations (Alaska Dept. Fish and Game, 2002). On the Kenai Peninsula of Alaska, human impacts have brought to light the need to proactively inventory and monitor black bear populations (National Park Service, 1999; National Park Service, 2006). Through the National Park Service (NPS) Inventory and Monitoring program (I&M), Kenai Fjords National Park (KEFJ) has developed a comprehensive black bear study program to gather ecological data to inform science-based black bear management. Black bears occur throughout KEFJ and are a key part of the fjords ecosystem as well as a focal attraction to park visitors (National Park Service, 1999).
An important step in any management program is to establish a baseline estimate of species abundance to guide future monitoring efforts. In this study we present a DNA-based estimate of black bear abundance in important coastal habitats in KEFJ.

Kenai Fjords National Park occupies approximately 2,200 km^2 of coastline between the Harding Icefield and the Gulf of Alaska. The coastline is rugged and deeply fjorded, carved by the glaciers of the Pleistocene Ice Age. Glaciers persist in the Kenai Mountains at elevations above 700 m (Hall, 2005). The mountains rise sharply from the coast to over 2,000 m. The rugged topography of the park restricts human accessibility to low-elevation coast-accessed terrain. These areas receive the majority of on-shore human use in the park including kayak landing, camping and fishing. They are also important areas for bears utilizing beach grass in the early spring and berries and salmon in the late summer to fall. Their importance to both bears and park visitors makes these rich coastal habitats most vulnerable to human impacts, and makes them an important focus for inventory and monitoring efforts (Smith, Partridge, in prep).

Goals of the KEFJ managers include maximizing the wilderness experience of park visitors and minimizing impacts on bear safety or behavior. Non-invasive genetic sampling, using un-baited hair traps, allowed us to efficiently collect samples while working within these

goals. Rich salmon runs and berry crops in coastal habitats create a natural draw for black bears to these seasonal food resources. The abundance of heavily-used bear trails aided tracking and identification of high-traffic areas for hair trapping. The short time window of peak food abundance lends itself to a concentrated trapping effort over short periods in which the population may safely be assumed geographically and demographically closed for analysis with closed population estimation models.

Capture-mark-recapture (CMR) analyses are one of the best established tools available to the wildlife biologist (Lukacs, Burnham, 2005b; Otis *et al.*, 1978). With rapid advances in technology and availability of highly variable genetic markers, DNA-based tagging has become increasing popular in CMR studies (Kohn *et al.*, 1999; Lukacs, Burnham, 2005b; Mills *et al.*, 2000). Closed population models have been particularly popular with non-invasive genetic studies of bears and other wildlife species (Lukacs, Burnham, 2005a; Woods *et al.*, 1999). Closed population CMR models assume a lack of birth or death (demographic closure), or immigration or emigration (geographic closure) from the sampling area during the sampling period (Otis *et al.*, 1978). They further assume that the animal's mark is permanent and correctly read (Otis *et al.*, 1978). DNA genotypes provide an ideal permanent mark. Through careful laboratory controls researchers can ensure that the "mark" is correctly read (Paetkau, 2003). Traditional CMR methods allow an individual to be observed once per capture session (Cooch, White, 2006). Multiple observations of an individual within one capture occasion must be condensed to a single capture per occasion (Bellemain *et al.*, 2005; Boulanger *et al.*, 2003; Mowat, Paetkau, 2002). Disregarding multiple captures could lead to a loss of valuable information (Miller *et al.*, 2005; Petit, Valiere, 2006).

Non-invasive genetic sampling differs from traditional capture methods because the animal is never confined for handling or observation. Individuals move freely over the trapping period allowing deposition of genetic samples (hairs, feathers, feces, etc.) (Bellemain *et al.*, 2005; Boulanger *et al.*, 2004a; Boulanger *et al.*, 2004b). Thus non-invasive genetic sampling resembles random draws from the population allowing replacement (Miller *et al.*, 2005). New models have been developed to take full advantage of the information contained in

multiple observances of genotypes in DNA-based CMR studies (Miller *et al.*, 2005; Petit, Valiere, 2006). Miller *et al.* (2005) developed a maximum likelihood estimator that allows sampling with replacement to estimate abundance from a single continuous sampling session. Petit and Valiere (2006) adapted a Bayesian estimator from Gazey & Staley (1986) to use with a single sampling session. Continuous-session methods may lack the information to estimate demographic parameters such as survival or recruitment, but they are well-suited for use with closed population abundance estimates (Petit, Valiere, 2006).

In this study we endeavor to estimate of black bear use of coastal food resources, and to provide a useful baseline for future monitoring efforts. To best guide the design of future monitoring efforts we also explore ways to make non-invasive genetic sampling most efficient and informative for DNA-based CMR estimates. We address the following research questions: 1) How many bears are utilizing food rich coastal habitats? 2) What is the optimal sampling design for future population monitoring using non-invasive genetic sampling and DNA-based CMR estimates?

MATERIALS and METHODS

Study Area

The mountainous topography and coastal climate bring heavy precipitation to the area, averaging about 120 inches of precipitation per year, with over 400 inches of snow on the Harding Icefield. The wet and mild climate drives the highly productive coastal rain forest ecosystem. KEFJ falls in the Coastal Mountain Hemlock-Sitka Spruce ecoregion. The mature forest is dominated by *Tsuga mertensiana* and *Picea sitchensis* (Bailey, 1995). These rich forests support dense undergrowth of shrubs and berries including (blue berry, *Vaccinium*) and (salmon berry, *Rubus*) (Gallant *et al.*, 1995). Willow (*Salix*) and alder (*Alnus*) form dense thickets along riparian corridors and on some steep hillsides (Ducks Unlimited Inc., 1999). Beach grass dominates the low-lying beach areas (French, 2003). The mature forests and rich berry thickets provide excellent cover and food resources to black bears (Crews, 2002). The park also supports numerous wild salmon runs (*Oncorhynchus tshawytscha, O. keta, O. kisutch, O. gorbuscha, O. nerka*) which provide another important food source to bears.

We divided KEFJ into four study sites based on the four major bays of the park: Aailik Bay, Harris Bay, Two Arm Bay, Nuka Bay (north to south in Figure 4.1). Each bay was delineated by topographic features (ridgelines) and supported independent salmon runs. Each bay was small enough to be sampled within a single trapping period. It was not logistically feasible to trap all bays within a single season. Thus, for abundance estimates, each bay will be considered a separate system, closed during the short trapping period.

Non-Invasive Genetic Sampling

The timing of trapping sessions was critical to maximize the natural food draw. Trapping sessions occurred in late July and early August to coincide with the timing of salmon runs and peak berry productivity. Each trapping period lasted for approximately 11 days in which

traps were set out over days 1-3, checked on the first occasion from days 5-7, and checked on the second occasion from days 9-11 (Table 4.1). This provided us with a sample that could easily be considered as either two distinct occasions or as a single continuous sampling session.

First, areas of high bear concentrations were identified according to salmon spawning streams or dense berry patches (salmon berry and blue berry). Field crews then canvassed these prime habitats to find bear trails. Traps were set on as many different trails as possible using existing vegetation for anchors.

We used two types of hair traps that could easily be set along bear trails. The first, hair snares, consisted of a 3.5 m wire cable with three to four barbs attached (ADF&G, unpublished report, Figure 4.2A). The wire cable was formed into a loop with the barbs facing inward and closed with a loose rubber fastener. This fastener allowed the loop to constrict and then break apart when pulled. The wires were anchored to a secure point; typically a tree trunk or sturdy shrub near the bear trail. The snare was hung over existing vegetation forming a vertical loop at bear head level so that a bear would walk head-first into the loop. As a bear walked into the loop it would tighten the barbs around the bears neck fur, grabbing a few hairs then breaking free without disturbing the passing bear.

The second type, barbwire traps, consisted of a single piece of barbwire strung across a bear trail (Boulanger *et al.*, 2004a) (Figure 4.2B). We used trees on either side of bear trails to provide strong anchors for the barb wire. The wire was pulled tight approximately 50 cm from the ground, such that a black bear would likely rub the wire whether it chose to step over or crawl under the wire.

Any strand or tuft of hair caught on a single barb was considered one sample. Thus, one snare or barbwire trap might capture several samples. Each sample was collected and stored in a separate coin envelope, labeled with the trap location, date of collection, and lettered a-z if multiple samples were collected from a single trap on the same date.

Laboratory Analysis

To conserve laboratory costs, we limited the number of samples processed from a single collection at a single trap. Since snares contained only three to four barbs we would process up to two samples from a snare. Barbwires could contain 10 to 50 barbs. We processed samples at least four barbs apart.

DNA was extracted from samples using the Qiagen DNeasy (Qiagen Inc., Valencia, CA) extraction kit following the manufacturer's protocol with 1-10 hair follicles per extraction. We used seven highly variable microsatellite loci (G1A, G10B, G10C, G1D, G10L, G10M, G10P (Paetkau *et al.*, 1995; Paetkau *et al.*, 1998) to acquire a unique genotype for each individual. We amplified microsatellites using polymerase chain reaction (PCR) as described in Chapter 2 (this document). All hair samples were extracted and amplified in a laboratory free of any form of concentrated DNA. Negative controls were used for every set of 20 and in all PCR reactions extractions to monitor for contamination.

Quality control is critical in non-invasive genotyping, particularly in genetic CMR studies where misidentification of individuals could bias abundance estimates (McKelvey, Schwartz, 2004; Mills *et al.*, 2000; Paetkau, 2003; Waits, Paetkau, 2005). We took a multifaceted approach to ensuring data quality and reliable genotyping (Bonin *et al.*, 2004; Broquet, Petit, 2004). If genotypes are based on too few loci, or on loci that lack variability, a "shadow effect" may occur where multiple individuals share the same genetic tag (Mills *et al.*, 2000). We used the program GenAlEx 6.0 (Peakall, Smouse, 2006) to calculate the probability of identity (PI) and probability of identity sibs(PIsibs) with the given loci to ensure that we had adequate power to distinguish individuals avoiding any "shadow effects". Samples with no results or ambiguous results at three or more loci were discarded in the first screening. In the final screening, we required that each individual genotype be verified in at least two independent amplifications. A genotype could be verified if it occurred in two or more individual samples. If a genotype occurred in only one sample, that sample was re-amplified for verification. Each genotype was amplified until each allele was observed at least twice or

the sample was removed from the dataset. Additionally, we re-genotyped 10% of samples at all loci. From multiple genotypings we were able to assess the rates of allelic dropout and/or false alleles (Bonin *et al.*, 2004). We then used the program Validation (Roon *et al.*, 2005) to identify genotypes that matched at all but 1 or 2 alleles . Close matches were re-amplified to verify the genotype. We report the rate of errors for each locus and the subsequent probability of failing to identify a recapture.

CMR Analysis

We estimated population abundance in each bay using four different CMR models, two traditional multi-session closed capture models (Huggins, 1989; Otis *et al.*, 1978) and two continuous-session models specifically designed for non-invasive genetic sampling (Miller *et al.*, 2005; Petit, Valiere, 2006). We first implemented a traditional closed capture analysis in the program MARK. We used both the full closed capture model in which N is estimated as a model parameter (Otis *et al.*, 1978), and the Huggins closed capture model in which N is a derived parameter (Huggins, 1989). Because the dataset contained only two capture occasions we were limited to the simplest models, referred to as the null model (M*o*) in the notation of program CAPTURE (Cooch, White, 2006). We assumed all genotype identifications were correct. We did not include the misidentification parameter because this parameter is estimated from the distribution of capture frequencies in the dataset and requires at least six capture occasions (White, pers com). Our strict laboratory protocol should ensure accurate genotyping. We assumed an equal capture probability for the first occasion, second occasion, and recaptures. This is a reasonable assumption as we trapped for a short period of time in each bay during which there was minimal change in weather, salmon availability, or other factors that could affect bear movement and capturability. Further, because we used non-baited, non-invasive traps we would not expect a behavioral response to influence either avoidance or preference for our traps. We assumed a homogeneous probability of capture simply because MARK required multiple capture occasions as well as individual covariates to estimate capture heterogeneity (Cooch, White, 2006).

We also used a Bayesian closed capture method designed by Petit and Valiere (2006) and implemented in the R routine "BayesN". This routine is based on a Bayesian estimator which uses a noninformative prior distribution of population sizes and individual capture histories to estimate population abundance (Gazey and Staley 1986). Again in this model we assume error-free genetic tagging and capture probabilities that are constant across time and individuals.

Finally, we used the program Capwire which implements a closed capture model adapted to non-invasive genetic sampling by allowing sampling with replacement (Miller *et al.*, 2005). In this model we again assumed error-free genetic tagging and capture probabilities constant across time. Unlike other models, Capwire did not require the assumption of homogeneous capture probabilities among individuals. Capwire provided a simple mixture model in which there are two types of individuals with differing capture probabilities (Miller *et al.*, 2005). Individual covariates are not required to implement the mixture model; the program assigns individuals to the mixture type and determines the ratio between capture probabilities that maximizes the likelihood of the model (Miller *et al.*, 2005). The heterogenous capture probability is the best justified for our dataset. Capture distributions were skewed, with few individuals being captured multiple times. Natural populations seldom exhibit homogeneous capture probabilities (Burnham, Overton, 1979). Black bears exhibit sex-biased and age-biased capture probabilities (Woods *et al.*, 1999). Further applicable to non-invasive genetic studies, captured individuals may deposit DNA samples at different rates or containing varying amounts of DNA (Miller *et al.*, 2005).

Simulations for Future Sampling Efforts

In this study we used a small dataset to generate a baseline estimate of black bear abundance in coastal habitats of KEFJ. This information will be most useful in the context of future monitoring efforts. More intensive sampling may be desired to achieve more precise estimates for establishing trends of black bear abundance. We used simulation routines available in the program CAPTURE (Otis *et al.*, 1978) to estimate the number of sampling

occasions necessary to improve precision of estimates. We simulated populations of 100 individuals. We kept capture probabilities constant across time assuming that future trapping efforts will continue to utilize the un-baited non-invasive trapping methods developed here. For simplicity, we also kept capture probabilities constant between all individuals. We simulated two different scenarios, one with high, and one with low probability of capture. We set the probabilities of capture based on the highest and lowest capture probabilities estimated in the closed capture models using MARK. Under each scenario we simulated CMR estimates based on 3 – 12 capture occasions (CAPTURE will not simulate data for a simple two occasion study). Each simulation was replicated 1,000 times. We evaluated model performance under the different sampling intensities based on estimate bias, width of the 95% confidence interval (CI), and coverage (inclusion of the true population size in the CI).

RESULTS

Non-Invasive Genetic Sampling

We were able to collect over 100 samples per bay in short trapping seasons with non-invasive hair traps (Table 4.2). Capture success rates (captures/trap*occasion) averaged 0.41 for the break-away hair snares, and 0.58 for barbwire traps (Table 4.2). Though the success rates were not significantly different (t-test, p-value 0.12), there was a trend toward higher success with barbwire traps. Field observations indicated that the hair snares may be bumped or even fully deployed without leaving a hair sample. Barbwires have no such tell-tale signs of trap encounters so we could not quantify differences in trap failure. The barbwire traps were sturdier and contained more barbs than snares, so a successful capture tended to yield more hairs from barbwires than from snares.

Laboratory Analysis

The PI(sibs) for the full set of seven loci was 0.001. The minimum PI(sibs) with any five-locus set was 0.008, yielding sufficient power to uniquely identify unrelated as well as related individuals. We thus allowed missing data at 1 to 2 loci (Table 4.3). After an initial amplification with seven microsatellite loci, we eliminated duplicate samples from an individual bear from the same trap on the same date.

The average number of amplifications per genotype was 2.18. The average error rate was 1.89% per locus (ranged from 0.95% at G10P to 2.88% at G10L). This yielded a probability of 0.132 that at least one locus would be erroneous in each seven-locus genotype. The chance of observing an error in all re-amplifications would be 0.00035 per locus, and 0.0025 per genotype. Identification of close matches with Validation led to the identification of two recaptures that would have been misidentified. In order for a recaptured genotype to be misidentified as a unique individual after error checking and Validation, it would require greater than two errors in the verified consensus genotype. Based on the error rates and

multiple controls used in this study, we would expect this to occur in a negligible number of samples.

CMR Analysis

There was considerable variation in the estimates of bear populations in each bay. Probability of capture and recapture rates were highest in Aialik Bay leading to the most consistent estimates (Table 4.4). Many of the recaptures in Aialik Bay occurred during different capture occasions making the encounter histories and results comparable between multi-session and continuous-session models. In other bays several of the recaptures occurred at different traps within a single occasion leading to very different encounter histories and limited comparability between the multi-session and continuous-session models (Table 4.4). Probability of capture was estimated by MARK as 0.51 in Aialik Bay, 0.25 in Two Arm Bay, 0.05 in Nuka and Harris Bays. The single-session models did not provide the probability of capture in output. However, Capwire output the ratio of heterogeneous capture types. Ratios ranged from 4.7 in Aialik Bay to 12.3 in Harris Bay, indicating that some individuals were much more likely to be captured than others.

Population estimates were similar from comparable model types. Of the multi-session models, the Huggins model typically gave estimates higher than the Otis model. Of the continuous-session models, Capwire gave higher estimates than BayesN. Confidence intervals were also similar within model type, with continuous-session models always providing narrower CI's (Figure 4.3). Capwire estimated the narrowest confidence interval for the challenging Harris Bay dataset.

Simulations for Future Sampling Efforts

Our simulated population size of 100 bears was a realistic representation of a typical KEFJ bay population. Sample sizes in simulated datasets ranged from 21 to 58, similar to our empirical dataset. Simulated CMR estimates showed that, with a capture probability as high

as 0.25 (based on the capturability in Aialik Bay), that multi-session closed capture models performed well even with few capture occasions (Figure 4.4). The estimate bias was low and coverage of the true population size high under all occasion scenarios. Confidence intervals were widest with three occasions (72.06), meaning that even with few capture occasions the population size could be estimated ± 36%. CI width rapidly tightened with increasing capture occasions. With five occasions, one could estimate the populations with a certainty of ± 16%. Seven occasions would be required to achieve a certainty within 10% of the estimated value. Precision continued to increase with additional occasions with the CI widths approaching zero at the highest sampling intensities. Note that the increasing precision led to a decrease in coverage of the true value, though it remained above 90%.

Much greater sampling effort would be required to achieve an accurate and precise abundance estimate with the lowest capture probability of 0.05 (based on the Harris Bay data) (Figure 4.5). Bias was high and highly variable across the number of occasions, ranging from -33.75% at 3 occasions to +19.86% at seven occasions. The bias was minimized at four occasions where the curve crossed from negative to positive bias. However the precision was still quite poor with a CI width of 365.17. The CI did narrow with increasing capture occasions, however even at twelve occasions the CI was still ± 51% of the estimate. Coverage was high given the wide confidence intervals.

DISCUSSION

Bear Abundance in Coastal Habitats

The continuous-session models provided informative estimates for Aialik, Two Arm and Nuka Bays. Estimates varied between models and confidence intervals were wide, but should provide a range informative for management. Monitoring trends in bear use will be important in managing human impacts to these critical coastal habitat areas.

Capture probabilities and recapture rates were low in Harris Bay leading to imprecise estimates with confidence intervals so wide as to be uninformative. There are several possible reasons for this. First, we must acknowledge the lower number of traps set in Harris Bay. However, Harris Bay had the highest success rate per trapping session for both trap types and we acquired a sample size comparable to the other sample areas. The high success rates of Harris Bay trapping suggests that it was not a failure to trap to bears, but a failure to recapture bears that led to poor estimates. Because of recent deglaciation, Harris Bay contains less mature forest habitat and hosts some newly established salmon runs (Hall, 2005; Wilkes, Calkin, 1994). It is possible that the opening of new and productive habitat has led to expansion and growth of the bear population in Harris Bay. It is also possible that feeding areas invite transient bears from Aialik and Two Arm Bays, leading to closure violations in the Harris Bay sampling area. Given the low bound of the CI, we cannot be certain that this bay has a much higher bear abundance than others. It seems most likely that severe closure violations occurred as bears moved in and out of salmon fishing areas. Closure violations would result in a positive bias in the estimate as new individuals might migrate in and be captured and marked individuals could leave the area preventing recapture (Boulanger, McLellan, 2001). Movement in and out of one of the study sites would violate the closure assumption of all of the models used (Huggins, 1989; Miller *et al.*, 2005; Otis *et al.*, 1978; Petit, Valiere, 2006).

There were potential violations of geographic closure in each of the study sites. Our estimates can best be described as representing the "superpopulation" of bears in the area surrounding the sampled food concentrations (Kendal *et* al. 1999). Our resource-focused trapping scheme was invaluable for achieving high sampling success without disrupting bear behavior. However this sampling design limits inferences to an index of bears utilizing coastal food resources rather than a precise point estimate or density estimate.

Continuous vs Multi-Session Models

Our sampling design and dataset best fit the continuous session models designed for non-invasive genetic sampling. Compression into two discrete sampling occasions required a substantial loss of information and led to poor performance by the closed capture models in MARK. Though MARK is recommended for its flexibility and options of constructing complex and elegant CMR models (Lukacs, Burnham, 2005b), models in the program are poorly conditioned for use with only two capture occasions (White pers com). Numerous capture occasions would be needed to estimate varying capture probabilities, capture heterogeneity, or misidentification (Cooch, White, 2006).

Continuous session CMR models designed specifically for non-invasive genetic sampling were most informative with our small datasets. The requirement of distinct capture occasions in MARK models required us to eliminate captures at multiple traps during a single session. This limited the number of recaptures in encounter histories entered into MARK models. The lack of information led to an extreme lack of precision in MARK estimates (Figure 4.3). The continuous session models performed well in all but the Harris Bay dataset in which capture probabilities and recapture success were low regardless of demarcation of occasion.

Continuous-session CMR models performed well in this study and have been well-tested through simulation studies. In previous research with similar sample coverage (50 simulated samples), the single session Bayesian estimator showed lower error, similar bias, and lower variance as compared to the null, M*o*, model of Otis (1978) with multiple sessions (Petit,

Valiere, 2006). In simulations, Capwire performed well concerning coverage, confidence interval, and bias relative to multi-session CMR models (Miller *et al.*, 2005). All of the tested models tended toward a positive estimate bias with sample sizes similar to ours (n=50, Petit, Valiere, 2006; or n=25% of the population, Miller *et al.*, 2005).

We assumed error-free genotyping in all the CMR models used. Correcting genotyping error is imperative for meeting this assumption in DNA-based CMR studies (Paetkau, 2003; Taberlet *et al.*, 1999). Our laboratory protocol was designed to ensure that errors leading to misidentification would be extremely unlikely. Petit and Valiere (2006) showed that, with small datasets, error rates as high as 6% only introduced 1.5 and 2% positive bias into their population estimates. Such minimal bias would be negligible compared to the wide CI's in this study. By detecting the imbalance of one-time captures, the misidentification parameter in MARK fails to adequately capture the likelihood of genotyping error and cannot replace good laboratory error checking. It fails to account for multiple observances of a genotype in the laboratory before the consensus genotype enters the CMR analysis. A misidentification parameter based on lab-based error rate estimates may provide a more realistic and more flexible reflection of genotyping error in DNA-based CMR studies.

Recommendations for Future CMR Efforts

Trapping success was good with the unbaited, non-invasive hair traps used in this study. We acquired hair samples from about 30 – 65% of the traps put out. However the high trap success did not directly translate to high success in recapturing individuals. Low capture probability (particularly in Harris Bay) made it nearly impossible to estimate abundance with any confidence. Simulations showed that even much increased sampling efforts would not yield reliable population estimates with such a low capturability.

Regardless of capturability, more than two capture occasions would be preferable if using traditional CMR models. With six or more capture occasion advanced models could be used to incorporate a number variables to make the estimate most realistic, including covariates

related to environmental factors or individual capture heterogeneity. Miller *et al.* (2005) suggest that, in their continuous session model, 2.0 to 2.5 observations per individual would be necessary to achieve estimates within 10% to 15% of the true value. Though six sessions may not be logistically feasible, even a small increase in occasions will provide more information whether using multi- or continuous-session models.

The best strategy may be to work toward increasing capture probability. We saw from the simulations that scenarios with higher capture probabilities led to more accurate and precise population estimates even with low numbers of capture occasions. Improved capture probability will be necessary for adequate performance of any CMR model. The 4 to 5 day capture occasions used here were relatively short. Longer trapping occasions may help improve capture probabilities. Other non-invasive genetic studies have used trap occasions of up to 14 days long (Boulanger *et al.*, 2004b). Higher trap density may help to capture and recapture more individuals using or passing through an area. A wider spread of traps may also help in recapturing bears moving between salmon runs and other parts of parts of the bay.

Barbwire traps tend to collect more hairs than break-away hair snares. Barbwires can also be better adapted to trails of various widths. Hair snares require a relatively restricted trail to channel the bear through the loop opening. Barbwires may particularly outperform hair snares in mature forests where bear trails often wind between large trees and in sparsely vegetated, rocky beaches or riparian zones where long barbwires may be strung between the odd willow or alder tree. Hair snares may be best used as supplemental traps, particularly in areas where trails are narrow and channeled through substantial underbrush such as some berry thickets. In these areas there are ample overhanging branches on which to fasten snare loops. Also snares may be anchored to bunches of small shrub stems that would be unable to support a barbwire.

By maximizing trap performance and increasing the area and period of hair trapping, managers can increase capturability and improve black bear abundance estimates. Others

have combined DNA-based CMR estimates with information on salmon availability to provide important information on the relationship of bear abundance to food resources (Boulanger *et al.*, 2004a). Future research might benefit most from incorporating salmon abundance and human use data with black bear trend data. This would allow park managers to connect trends in black bear abundance with the state of resources and possible disturbances affecting the population.

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Table 4.1: Season of non-invasive genetic sampling in KEFJ bays
Each bay in KEFJ was trapped for approximate 11 days during the peak of salmon runs and berry availability. The 11 day trapping sessions included approximately 3 days to set traps (from 1st Set date), 3 days to check traps (from Occassion 1, and again from Occasion 2 date). We show the total number of traps set in each bay.

Bay	Year	1st Set	Occassion1	Occassion 2	# Traps
Aialik	2004	5-Aug	9-Aug	12-Aug	95
Harris	2005	24-Jul	28-Jul	1-Aug	47
Two Arm	2004	20-Jul	24-Jul	28-Jul	108
Nuka	2003	16-Jul	20-Jul	24-Jul	81

Table 4.2: Non-invasive genetic sampling in KEFJ bays
Non-invasive genetic sampling was conducted using break-away hair snares and barbwire type traps. The trapping success for each is shown as the ratio of captures per number of traps set over two trapping occasions. The table also shows the total success rate and number of samples collected from all captures (samples may exceed captures because more than one barb might contain a hair sample from each successful trap).

	Snares		Barbs		Total		
Bay	Number	Success Rt	Number	Success Rt	# Captures	Success Rt	Samples
Aialik	60	0.45	35	0.6	93	0.57	211
Harris	26	0.52	21	0.75	62	0.66	203
Two Arm	70	0.33	28	0.34	49	0.29	94
Nuka	62	0.32	19	0.61	63	0.39	113
Avg Rate		0.41		0.58		0.48	

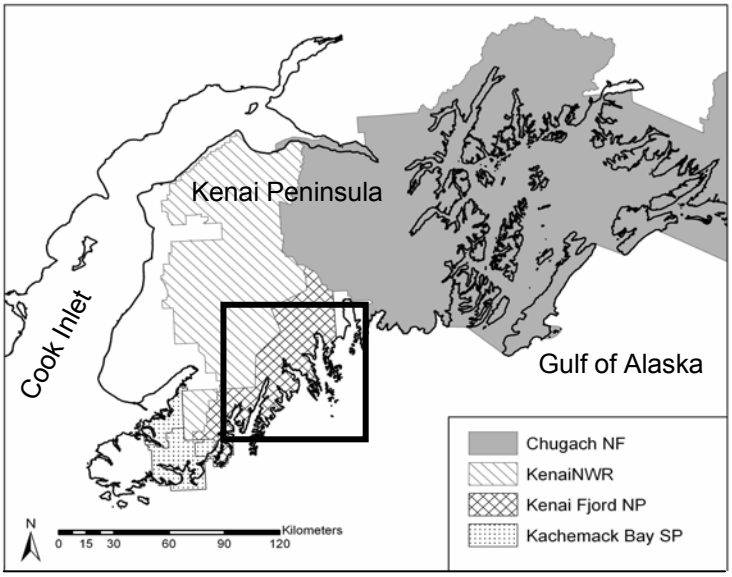
Table 4.3: Laboratory processing of non-invasive samples from KEFJ bays
Because successful hair traps often contained numerous samples, we did not process every sample collected. We extracted up to two samples from break-away hair snares, and up to one sample/5 barbs from barbwire traps. We culled any samples failing to amplify at 2 or more of seven microsatellite loci. After genotyping seven loci we kept only one sample per unique individual collected at one trap within one occasion (1ID/Trap). The sample size shows the number of individual samples used for C-M-R analysis.

Bay	# Extracted	Culled	1ID/Trap	Sample Size
Aialik	135	9	70	61
Harris	50	2	41	39
Two Arm	95	3	50	47
Nuka	65	4	30	26

Table 4.4: CMR estimates of black bears using coastal habitats in KEFJ bays
We used four models to estimate abundance of black bears utilizing coastal food resource areas in KEFJ bays. Each bay was estimated separately to maintain closure within each system. From each model we provide the point estimate (N) and lower (min) and upper (max) bound of the 95% confidence interval.

	Aialik			Two Arm			Nuka			Harris		
Model	min	N	max	min	N	max	min	N	max	min	N	max
MARK - Full	58	88	173	88	324	1616	33	89	489	88	305	1616
MARK - Huggins	59	92	180	92	323	1708	35	99	539	92	323	1708
BayesN	54	73	108	55	86	163	27	49	136	89	247	1365
CapWire	63	107	131	60	101	154	31	69	132	122	301	750

Figure 4.1: Area of non-invasive genetic sampling within Kenai Fjords N. P.
The map shows Kenai Fjords National Park situated on the east coast of the Kenai Peninsula in south-central Alaska. We set hair snares and barbwire hair traps along bear trails in each bay of the park. Trapping in each bay took place in 11 day sessions timed with peak food abundance to avoid use of any baits or lures.



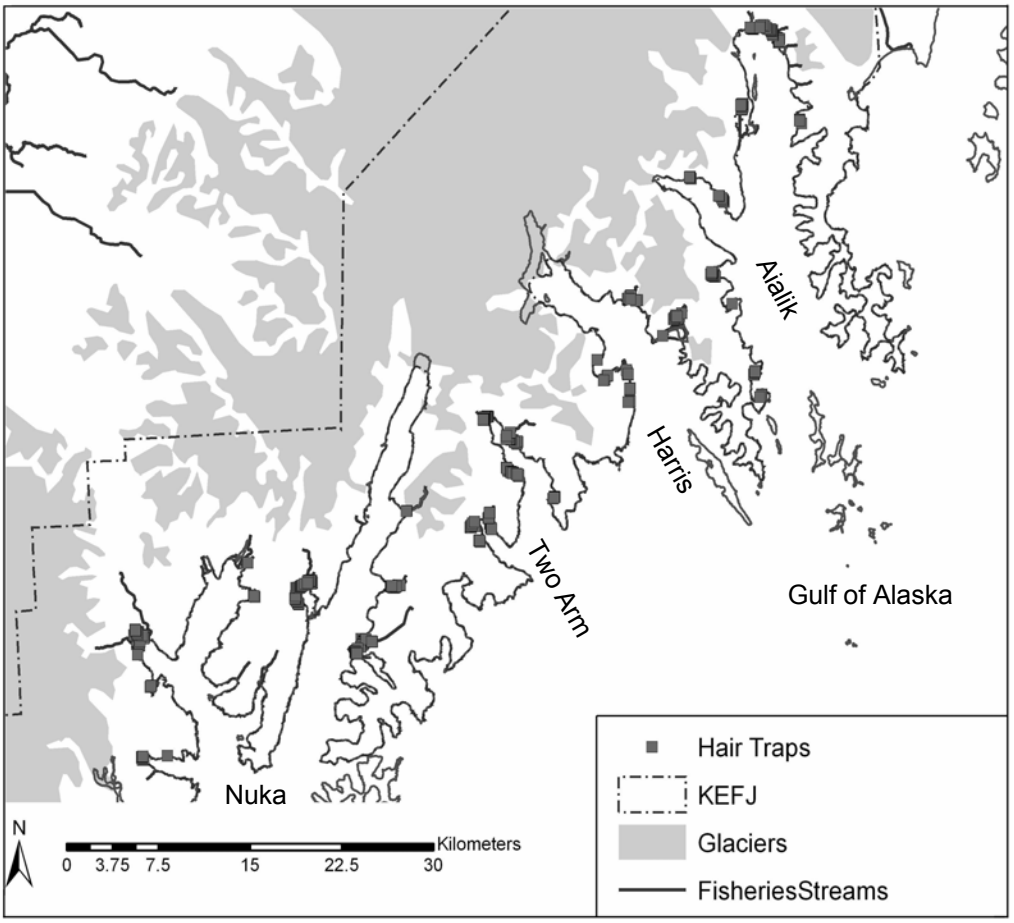


Figure 4.2: Non-invasive hair trapping methods

Non-invasive hair traps were used to collect samples for genetic analysis. Un-baited traps were set along presumed bear trails in areas of food resource concentrations. A) A black bear approaches a break-away hair snare mounted on vegetation near a bear trail (photo from NPS). B) A tuft of bear hair is left on a barbed wire strung between trees along a bear trail.

A)

B)

Figure 4.3: Abundances of black bears in KEFJ bays, estimated by four models

The graphs illustrate the point estimate and width of confidence interval derived from each of four models; the full closed capture model in program MARK, the Huggins closed capture model in MARK, the Bayesian estimator in BayesN, and the maximum likelihood estimator with unequal capture probabilities in Capwire. Abundance was estimated separately for each bay in KEFJ (A-D), note that the scale differs for each graph.

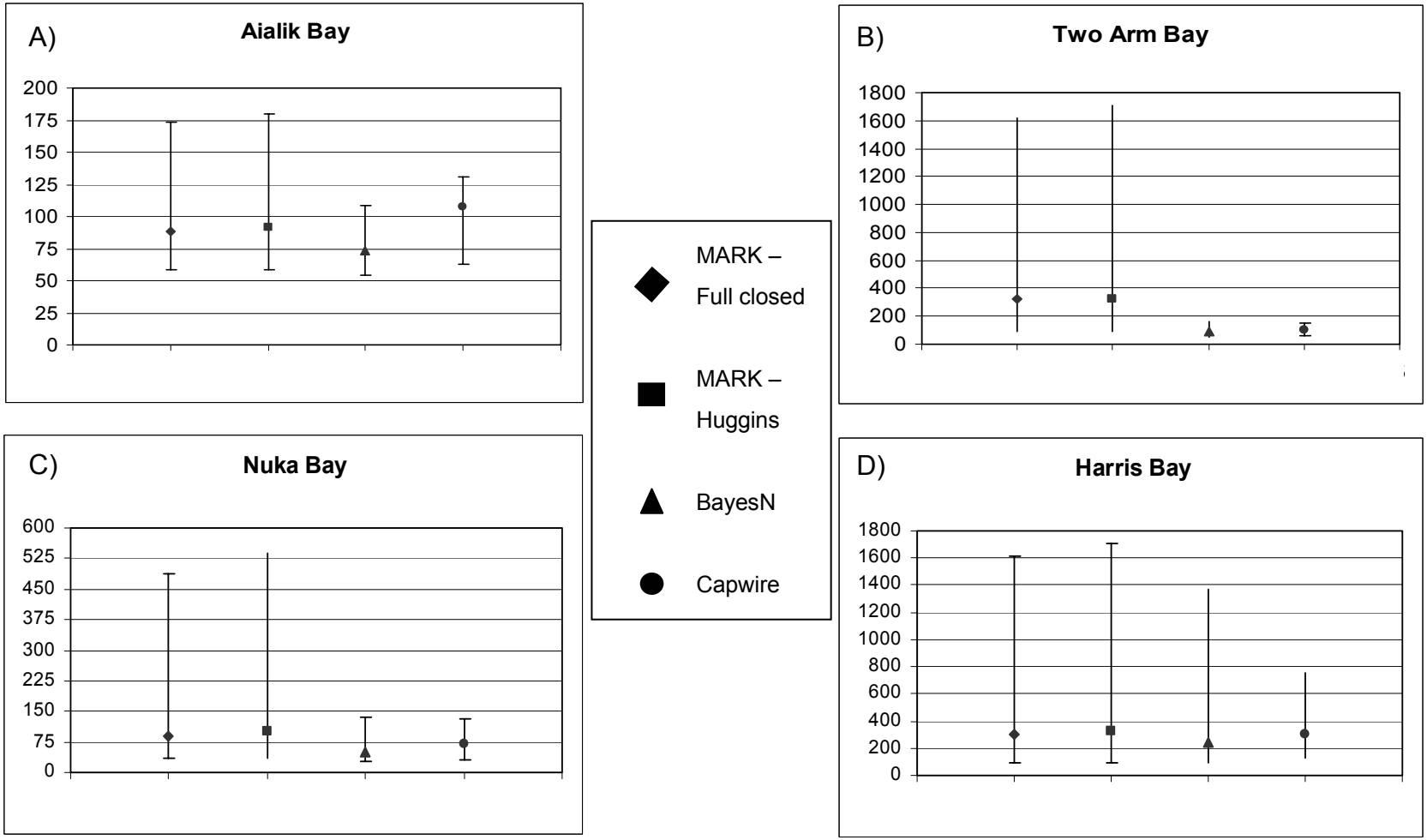


Figure 4.4: Results of simulated C-M-R efforts with high capture probability
We show the trend in closed capture estimator performance using varying numbers of capture occasions. Simulations were based on populations of 100 individuals. The performance can be judged by the accuracy of the estimate (A), the width of the associated confidence interval (B), and the coverage (C) - % of time that the true population size of 100 appears within the confidence interval. This simulation was based on the highest capture probability observed in black bears in the KEFJ (Aialik Bay, p=0.25).

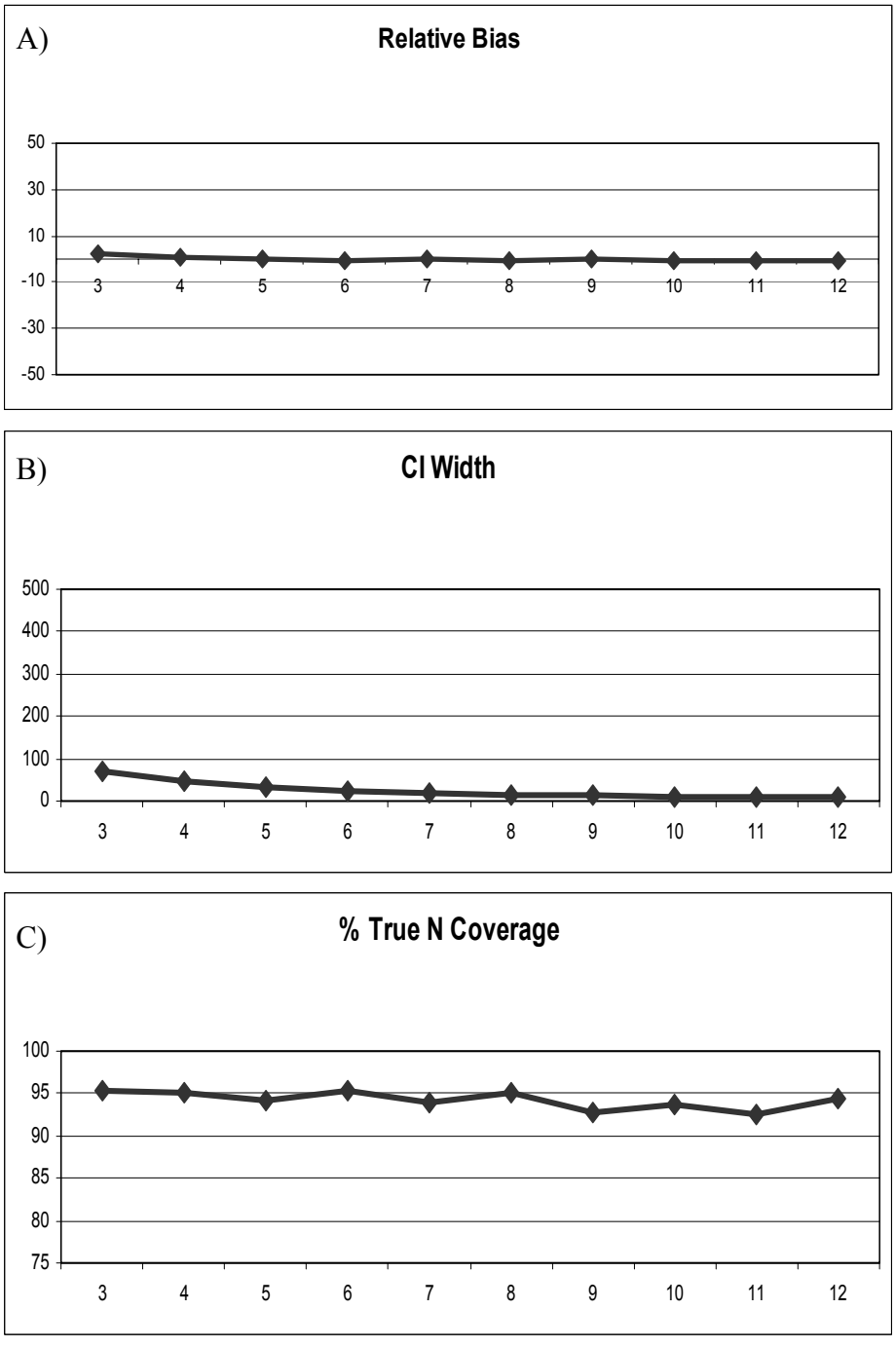


Figure 4.5: Results of simulated C-M-R efforts with low capture probability
We show the trend in closed capture estimator performance using varying numbers of capture occasions. Simulations were based on populations of 100 individuals. The performance can be judged by the accuracy of the estimate (A), the width of the associated confidence interval (B), and the coverage (C) - % of time that the true population size of 100 appears within the confidence interval. This simulation was based on the lowest capture probability observed in black bears in the KEFJ (Harris Bay, p=0.05).