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

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Acronyms and Symbols:

ADF&G	Alaska Department of Fish & Game
CMR	Capture-Mark-Recapture analysis
GMU	Game Management Unit
K	Variable depicting the number of genetically distinct clusters
KEFJ	Kenai Fjords National Park
KP	Kenai Peninsula genetic cluster
LISA	Local Indicator of Spatial Association
ML	Mainland genetic cluster
mtDNA	mitochondrial DNA
nDNA	nuclear DNA
PCR	Polymerase Chain Reaction
PWS	Prince William Sound – location and genetic cluster
Q	Variable depicting the proportion of admixture – output by Structure

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GLOSSARY of TERMS

Allele: a variant segment of genetic material; diploid organisms (those with one copy of DNA from each parent) will have two potential alleles for any particular segment of DNA.

Assignment test (Paetkau *et al.* 1995): analysis in which each individual in a sample population is assigned to the group in which its genotype is most likely to occur.

Bayesian assignment test (Pritchard *et al.* 2000): An assignment test in which genetic groups are not predefined; Bayesian algorithms use genotype data to estimate the most likely number of subdivisions in a sample population and assign individuals to appropriate groups.

Continuous-occasion CMR: closed capture abundance estimates in which all (non-invasive genetic) sample collections are combined into a single occasion in which individuals are recorded each time their genotype is observed at different time/trap/location.

Fst (Wright 1965): a measure of genetic differentiation related to statistical analysis of variance; Fst is the proportion of the total genetic variance contained in a subpopulation relative to the total genetic variance. Values range from 0 (complete genetic mixture) to 1 (complete isolation of genetic groups).

Gene flow: movement of genes from one population to another, primarily due to dispersal leading to breeding and passing on of genes outside an individual's natal population.

Genetically distinct group: a term applied in this report to refer to groups of animals in which breeding is unrestricted/random and that differs in genetic composition from all other groups; synonymous with the terms population or subpopulation as used in the genetics literature, or similar to a deme in the population dynamics context.

Genotype: the set of alleles that an organism possesses in a stretch of DNA; in diploid organisms the genotype for each stretch of DNA is composed of one allele from the mother and one from the father.

Haplotype: the particular set of DNA variants in a stretch of DNA sequence; similar to a genotype, but for single-copy DNA, as in DNA inherited from only one parent, such as maternally inherited mtDNA.

Hardy-Weinberg equilibrium (HWE): in a population meeting certain simplifying assumptions (such as no genetic drift or migration, no genetic selection, infinite population size, random mating), equilibrium is reached when the genotype frequencies in the population can be predicted from simply Mendelian expectations: $p^2 + 2pq + q^2$.

Heterozygote: an organism with two different alleles at a given segment of DNA; one from the mother and a different one from the father (heterozygosity = the proportion of DNA segments for which an individual is a heterozygote).

Homozygote: an individual with two copies of the same allele at a given segment of DNA; the same allele from both the mother and father.

Isolation by distance (Wright 1943): results from distance-limited dispersal, leading to decreased mixing (therefore decreased genetic similarity) among individuals separated by increasing geographical distance.

Linkage disequilibrium (LD): the non-random association of alleles at two or more segments of DNA; as with HWE, equilibrium depends on lack of genetic drift or migration, lack of genetic selection, infinite population size, random mating.

Mitochondrial DNA (mtDNA): DNA from the mitochondrion organelle; because organelles exist in the cell body rather than nucleus, they are contained in egg cells, but not sperm, and therefore are inherited only from the mother.

Microsatellite: short DNA sequence that occurs in stings with variable numbers of sequence repeats (e.g. CACACACACACACACA…..); because mutations frequently cause changes in the number of repeats, microsatellites tend to be hyper-variable genetic markers.

Multi-occasion CMR: standard CMR models in which encounter histories are based on multiple discrete trapping occasions in which animal can be observed/recorded once per occasion.

Nuclear DNA (nDNA): DNA from nucleus of the cell; it contains two copies of each DNA segment, one copy from the mother, one from the father (i.e. it is diploid).

Panmixia: condition in which all individuals intermix and interbreed randomly, effectively creating a single large population with no internal subdivision (adj. panmictic).

Polymerase Chain Reaction (PCR): laboratory technique for amplifying targeted segment of DNA so that it can be visualized and analyzed using stains or chemical labels.

Probability of identity (PI): probability of two individuals having the same genotype (identical genetic composition) by chance; PI refers to this probability for two randomly chosen individuals; PIsibs refers to this probability for siblings (which are expected to share some genetic material).

Spatial autocorrelation: when the value of a variable at any one point in space is dependent on values at the surrounding points, i.e. the arrangement of values is not random; positive autocorrelation means that similar values tend to cluster together; negative autocorrelation means that diverse values tend to be near each other.

ABSTRACT

Black bears (*Ursus americanus*) represent a significant component of the fjords ecosystem in south-central Alaska. They occur throughout the coastal portions of Kenai Fjords National Park (KEFJ) and are a focal attraction for park visitors. KEFJ park managers identified resource extraction, land development, landscape fragmentation, and hunting on the Kenai as immediate threats to the coastal ecosystem and resident black bears. In 1998, KEFJ proposed a comprehensive study program on the ecology of black bears and threats to their populations. In this project we used mitochondrial DNA (mtDNA) sequencing and nuclear DNA (nDNA) microsatellite analysis to estimate the abundance of bears using park resources and evaluated phylogeography, population structure, and landscape-genetic relationships of black bears in KEFJ and surrounding areas.

We used non-invasive genetic sampling and DNA-based capture-mark-recapture (CMR) analysis to provide an estimate of black bears using coastal areas within KEFJ. We used models in MARK, and DNA-specific models in Capwire and BayesN. Point estimates based on the most precise estimator (Capwire) were: Aialik 107 (95% CI 63-131); Two Arm 101 (60-154); Nuka 69 (31-132); and Harris 301 (122-750). Based on simulated CMR efforts and trap performance in this study, we recommend making more use of barbwire traps, and increasing the area covered and time period of future trapping efforts in order to achieve better recapture success and more precise abundance estimators. We analyzed mtDNA sequences, and identified 5 unique sequence variants that were closely related evolutionarily. Black bears on the Kenai and mainland showed different distributions of mtDNA lineages indicating a historical distinction between the areas. We used both aspatial and spatial Bayesian assignment tests to cluster individuals into genetically distinct groups. We identified three genetically distinct groups that clustered geographically in the Kenai Peninsula, Alaskan mainland, and Prince William Sound areas. Connectivity among genetic groups was moderate (Fst values ranging from 0.07 to 0.12), indicating that groups were separated by semi-permeable barriers across which some migrants are exchanged each generation. The definition of genetically distinct groups offers a biological basis for defining units for management. If monitored in the future, subdivision of the Kenai genetic group will provide an indicator of novel barriers or population fragmentation. To investigate spatial patterns in genetic variation we used Mantel tests to detect isolation-by distance and a Local Indicator of Spatial Association (LISA) to detect local clusters of genetic similarity. The correlation of genetic and geographic distance was weak suggesting that factors such as physical barriers or evolutionary factors exert a larger influence on genetic structure than distance alone. The LISA analysis identified spatial clusters of genetic similarity within black bears on the Kenai Peninsula. Genetic connectivity was highest within ecological zones (either western lowlands or coastal mountain forests) and lower at the interface of these ecoregions and along the Highway 1 corridor. This showed evidence that dispersing black bears may show fidelity to their natal habitat type and that anthropogenic barriers, such as highways, are likely to impede gene flow. Future monitoring of spatial genetic patterns can be used to assess impacts of novel barriers or the effectiveness of dispersal corridors.

EXECUTIVE SUMMARY

Anthropogenic land-use 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. Habitat loss has been identified as the main threat to black bear populations in North America (Pelton *et al.* 1982) and Alaska in particular (Alaska Dept. Fish and Game 2002). Black bears represent a significant component of the fjords ecosystem in southwest Alaska (National Park Service 2006). They occur throughout the coastal portions of Kenai Fjords National Park (KEFJ) and are a focal attraction for park visitors. KEFJ park managers have identified resource extraction, land development, landscape fragmentation, and hunting on the Kenai as immediate threats to the coastal ecosystem and resident black bears. In response to these threats, in1998, KEFJ proposed a comprehensive study program on the ecology of black bears and threats to their populations. The condensed 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. This study addressed the program objective of evaluating the genetic structure of the KEFJ black bear population in the context of the Kenai Peninsula landscape.

We used genetic data to estimate the abundance of black bears using park resources and to evaluate phylogeography, population structure, and landscape-genetic relationships. Specific objectives and research questions addressed included:

Objective 1: Estimate abundance of black bears in coastal habitats of KEFJ.

- What is the abundance of black bears using coastal food resources in each bay of Kenai Fjords National Park?
- What is the optimal sampling design for future population monitoring using non-invasive genetic sampling and DNA-based Capture-Mark-Recapture estimates?

Objective 2: Describe the phylogeography of black bears on the Kenai Peninsula and south-central Alaska.

- What is the phylogeographic relationship among black bears in south-central Alaska?
- What geographic patterns exist in maternally inherited mtDNA lineages in black bears in south-central Alaska?

Objective 3: Describe population genetic structure and diversity of black bears in south-central Alaska.

- What is the degree of genetic subdivision in the south-central Alaska black bear population?
- What is the degree of differentiation between subdivisions of the population?
- What is the level of genetic diversity in the population or its subdivisions?

Objective 4: Relate population genetic patterns to the landscape of the Kenai Peninsula.

- Is there evidence for increasing genetic isolation with geographic distance?
- What fine scale spatial patterns exist in the genetic variation among black bears in south-central Alaskan?
- Does the landscape of the Kenai Peninsula influence spatial restrictions of black bear gene flow?

Project activities and findings under each objective are described below:

Obtaining Genetic Samples and Data

Within KEFJ we used un-baited, non-invasive genetic sampling techniques to collect samples from many bears in KEFJ, without handling the animals, disturbing their behavior, or impinging on visitors' experiences. We used barbwires strung between trees and break-away hair snares secured to vegetation along bear trails. We timed the trapping sessions with the peak of salmon runs and berry abundance to ensure that baits would be unnecessary and that bear trails would be heavily traveled and easy to identify. In order to provide a broader context in which to analyze the KEFJ genetic data, we supplemented park samples with samples from the rest of the Kenai Peninsula and adjacent mainland areas. The broader study region included the entire Kenai and mainland areas from west of Cook Inlet to north of Prince William Sound. ADF&G provided tissue samples and kill locations from hunted black bears. Only KEFJ samples were used for estimating black bear abundance. Region-wide samples and a representative sub-sample of KEFJ bears were used for the remaining objectives dealing with broader-scale questions of phylogeography, population structure and genetic diversity. DNA was extracted from tissue and hair samples and targeted genetic segments were amplified and analyzed in the University of Idaho Laboratory for Ecological and Conservation Genetics, Moscow, ID.

Objective 1: Estimate abundance of black bears in coastal habitats of KEFJ

An important step in the black bear inventory and monitoring activities was to establish a baseline of bear abundance on which to base future monitoring efforts. In this study we used non-invasive genetic sampling and DNA-based capture-mark-recapture (CMR) analysis to provide an estimate of black bears using coastal areas within KEFJ. We used two traditional closed-capture CMR models in the program MARK (Cooch & White 2006). We also used two novel programs, Capwire (Miller 2005) and BayesN (Petit & Valiere 2006), which offer models specifically designed to use data from multiple captures within non-invasive DNA trapping sessions. There was considerable variation in estimates and precision among models. Data were sparse leading wide confidence intervals, but the most precise estimates were achieved using the programs for non-invasive genetic data, Capwire and BayesN. The most precise point estimates were (from the Capwire model): Aialik 107 (95% CI 63-131); Two Arm 101 (60-154); Nuka 69 (31-132); and Harris 301 (122-750).

We also conducted simulations in CAPTURE (Otis *et al.* 1978) to guide suggestions for achieving more precise abundance estimators in future monitoring efforts. Simulation results indicated that improving capture probabilities would be the most important factor in improving precision of future estimates. Based on trap performance in the current study, we recommend making more use of barbwire (rather than hair snare) traps, and increasing the area covered and time period of future trapping efforts in order to achieve better capture success and more precise abundance estimators.

Objective 2: Describe the phylogeography of Kenai black bear populations

Phylogeographic analyses make use of DNA sequence data to reveal historical relationships between genetic lineages and can be informative regarding recolonization routes and past isolation events (Moritz 1994). We sequenced a 360 base pair region of the mtDNA control region. We analyzed sequences from 110 black bears spanning the region, and identified 5 unique sequence variants, or haplotypes. We constructed a haplotype network illustrating the evolutionary relationships among lineages. A haplotype map displayed the geographical relationship among haplotypes. The five haplotypes were closely related with no evidence of deep evolutionary divergence. The haplotypes found here coincided with haplotypes identified elsewhere in the literature as belonging to the continental black bear clade (Wooding & Ward 1997) – bears that survived the Pleistocene Ice Age (25,000-12,000 years before present) in a refugium in the southeast USA and then expanded to the rest of the North America as the glaciers retreated. The ancestral lineage was widespread across both mainland and Kenai areas. Two of the more recently diverged haplotypes were primarily restricted to the Kenai Peninsula, and the two others to the mainland. This pattern indicated that the entire area was recolonized by bears from the southeast refugium. Then as more ice melted, and sea levels rose, the Kenai became increasingly isolated from the mainland (Pielou 1991) leading to differentiation of black bears on the Kenai. This demonstrates that black bears on the Kenai are an important part of the regional genetic diversity of the species.

Objective 3: Describe population genetic structure and diversity of black bears in south-central Alaska

Population genetic structure refers to the partitioning of individual genetic variation across the landscape. The identification of genetically distinct groups can help to identify biologically meaningful management units, indicate dispersal corridors or barriers and assess the level of connectivity or fragmentation in a population (Deyoung & Honeycutt 2005). We genotyped 13 nuclear DNA microsatellites to investigate population structure of 110 black bears on the Kenai and surrounding mainland. We used both aspatial and spatial Bayesian assignment tests to cluster individuals into genetically distinct groups (Structure, Pritchard *et al.* 2000; BAPS, Corander *et al.* 2006a). We identified three genetically distinct groups that clustered 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. The moderate values found here indicated that groups were separated by semi-permeable barriers across which some migrants were exchanged each generation. The distinction of Kenai black bears from the mainland area further supports the phylogeographical findings that Kenai black bears are an important and distinct segment of the regional black bear population. That the Kenai constituted a single population indicates that dispersal and genetic connectivity are currently high on the peninsula. If monitored in the future, subdivision of this genetic group would be an indicator of novel barriers or habitat degradation leading to population fragmentation.

Objective 4: Relate population genetic patterns to the landscape of the Kenai Peninsula

Describing spatial genetic patterns is essential to identifying the processes affecting gene flow and genetic variation across the landscape. We first used Mantel tests (Mantel 1967) to assess correlation of genetic distance with geographic distance separating individuals. We found only weak correlations, suggesting that factors such as physical barriers or evolutionary factors play a much larger role than distance alone. To investigate other factors, we developed a novel application of local autocorrelation statistics to individual genotypes. We used a Local Indicator of Spatial Association (LISA) (Anselin 1995) to detect local clusters of genetic similarity. These clusters revealed areas where gene flow was locally high. The LISA analysis identified spatial genetic clusters that related to specific ecological divisions within the Kenai Peninsula group. The spatial patterns of genetic similarity helped pinpoint areas of high migration and locally restricted gene flow that were associated with features on the landscape. We found that gene flow was locally highest within ecoregions (the western lowlands versus coastal mountain forests). Connectivity was lower at the interface of these ecoregions and along the Highway 1 corridor. This showed evidence that dispersing black bears may show fidelity to their natal habitat type and that major highways are likely to impede gene flow.

Conclusions and Implications

Kenai Peninsula black bears were genetically distinct from bears in neighboring mainland areas. Although distinct, the genetic groups were not entirely isolated. Migration, low mtDNA divergence, and moderate Fst values indicated only moderate genetic differentiation between genetic groups of black bears in south-central Alaska. Genetic connectivity among black bears on the Kenai Peninsula (KP) is currently high, but not absolute. That bears on the Kenai constituted a single genetic group indicated that migration and interbreeding occurred throughout the area. However, we saw in the spatial analysis that the KP genetic group was not completely intermixed, but exhibited a patchy genetic pattern. Genetic patches were distributed in different ecological regions of the Kenai and separated by anthropogenic features such as major highways. This spatial structuring and relation to roads indicates the potential for black bear populations to become increasingly subdivided if barriers become more severe.

Our results provide a 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 management plans that maintain the current diversity and gene flow by minimizing impacts on bear habitat and linkage zones that connect segments of the population.

INTRODUCTION

Background

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 due 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 land-use changes including increased land clearing, development, transportation, and resource extraction (National Park Service 1999; US Census Bureau 2006). Anthropogenic land-use change and habitat fragmentation are among the most pressing threats to wildlife populations, including bears (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. Habitat loss has been identified as the main threat to black bear (*Ursus americanus*) populations in North America (Pelton *et al.* 1982) and Alaska in particular (Alaska Dept. Fish and Game 2002).

Black bears represent a significant component of the fjords ecosystem in southwest Alaska (National Park Service 2006). They occur throughout the coastal portions of Kenai Fjords National Park (KEFJ) and are a focal attraction for park visitors. KEFJ park managers identified resource extraction, land development, landscape fragmentation, and hunting on the Kenai as immediate threats to the coastal ecosystem and resident wildlife (Hall *et al.* 2007). Such threats have brought to light the need to proactively inventory and monitor natural populations protected by the National Park Service (NPS).

In 1998, KEFJ proposed a comprehensive study program on the ecology of black bears and threats to their populations (Hall *et al.* 2007). The condensed 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). This study addressed the program objective of evaluating the genetic structure of the KEFJ black bear population in the context of the Kenai Peninsula landscape.

Black Bear Biology

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 (Schwartz & Franzmann 1992; Alaska Dept. Fish and Game 2002).

Black bear habitat is characterized by rough terrain and thick understory vegetation; critical components of black bear habitat include food, escape cover, and den sites (Powell *et al.*, 1997). Fecal nutritional analysis within KEFJ found that salmon berries were a key diet item for black bear within the park (Crews, 2002). Wild salmon runs are abundant on the Kenai providing another excellent food source for bears (Robbins *et al.*, 2004). Black bear home ranges vary considerably with quality of habitat and available food resources, though the home ranges of females are consistently a quarter to a third the size of males. Females typically range up to 40 km^2, whereas male home ranges may be upwards of 100km^2 (Powell *et al.*, 1997).

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

Numerous factors may affect dispersal distances and routes. Habitat connectivity and available cover are both influential to black bear dispersal. Interruptions in cover or poor habitat may act as barriers to bear dispersal (Lee & Vaughan 2003). 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 limit black bear dispersal (Peacock 2004). Direct observations (tracking) can reveal the immediate behavioral aspects of dispersal (Schwartz & Franzmann 1992; Lee & Vaughan 2003). Genetic analyses, on the other hand, are useful in discerning the effects of dispersal on population structure over the course of generations (Peacock 2004; Thompson *et al.* 2005; Cushman *et al.* 2006).

Genetic Tools for Population Biology

Genetic analyses offer important insights into the population structure and connectivity among wide-ranging animals such as bears. Genetic data provide information about historic and current levels of gene flow among populations, as well as information about genetic diversity and fitness, relatedness, and movement patterns within populations (Queller *et al.* 1993; Paetkau *et al.* 1998; Schenk *et al.* 1998; Woods *et al.* 1999). Genetic information is essential to estimating population viability and evaluating possible management decisions (Deyoung & Honeycutt 2005).

Two common types of genetic data are mitochondrial DNA (mtDNA) sequences and nuclear DNA (nDNA) microsatellite fragments. MtDNA sequence data provide important information about maternal gene flow patterns, past isolation events, natural recolonization

events, and evolutionary history (Cronin *et al.* 1991; Sunnucks 2000; Onorato *et al.* 2004). Microsatellites are highly variable markers that provide fine genetic resolution for identifying individuals and examining close genetic relationships.

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 (Waits & Paetkau 2005). 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 was critical for the sampling effort within KEFJ 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 by trapping bears. This also helped minimize the impact our research might have on bear behavior or safety, or visitor experience. Rich salmon runs and berry crops in coastal habitats created a natural draw for black bears to these seasonal food resources and the abundance of heavily-used bear trails helped us to identify good areas for hair trapping.

Capture-mark-recapture (CMR) analyses are a well-established tool in wildlife biology (Lukacs and Burnham, 2005b; Otis *et al.*, 1978). With rapid advances in technology and availability of highly variable genetic markers, DNA-based marking has become increasing popular in CMR studies (Kohn *et al.* 1999; Mills *et al.* 2000; Lukacs & Burnham 2005). Traditional CMR methods allow an individual to be observed once per capture occasion, thus requiring multiple, discrete capture occasions (Cooch & White 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, thus blurring the definition of capture occasions (Boulanger *et al.* 2004a; Boulanger *et al.* 2004b; Bellemain *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 (Lukacs 2005; Miller *et al.* 2005; Petit & Valiere 2006). These models, referred to here as continuous-occasion models, treat capture occasions as a single continuous session in which individuals can be observed numerous times in different traps. Petit and Valiere (2006) adapted a Bayesian estimator from Gazey & Staley (1986) to use with a single sampling occasion. Miller *et al.* (2005) developed a maximum likelihood estimator that allows sampling with replacement to estimate abundance from a single sampling occasion. Continuous-occasion 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 and are ideal for situations in which multiple occasions in the field are not logistically feasible (Petit & Valiere 2006).

The study of phylogeography makes use of DNA sequence variation to examine the processes governing the geographic distribution of genetic lineages (Avise & Nelson 2000). Assuming spatial patterns mimic temporal patterns, phylogeographic patterns can be used to deduce routes of historic range expansions and colonizations (Hedrick 2005). Phylogeographic studies make extensive use of mtDNA sequences as they are informative over the historic time scale (Snow & Parker 1998). Because mtDNA is maternally inherited,

patterns in mtDNA lineages are informative regarding female-mediated gene flow as well as historical information (Moritz 1994).

Population genetic techniques are useful in answering myriad ecological and demographic questions. Many applications of genetic data focus on the definition of biological units and the conservation of genetic diversity and evolutionary potential in species or populations (Deyoung & Honeycutt 2005). Genetic diversity has been linked with fitness and long-term viability of wildlife populations. Population genetics provides a finer scale perspective, requiring genetic markers of finer resolution, or greater variability, than phylogeography. Microsatellites have been widespread in wildlife population genetics research (Snow & Parker 1998). 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). Population assignment tests use allele frequencies to detect population structure and determine the natal population of an individual (Paetkau *et al.* 1995). Bayesian assignment tests use probability statistics to detect the number of subdivisions in a sampled population and determine each individual's genetic ancestry in each group.

Accurate description of genetic patterns can help researchers understand the processes that shape gene flow and connectivity within and among populations (Allendorf & Luikart 2007). Genetic patterns, the distribution of alleles across space, may arise from various factors such as limitation on dispersal 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). Spatial analysis using autocorrelation statistics has become a popular tool for describing genetic patterns at the population and individual level (Sokal & Jacquez 1991; Epperson & Li 1996; Arnaud 2003; Epperson 2003; Peakall *et al.* 2003; Scribner *et al.* 2005; Wagner *et al.* 2005). Local autocorrelation of allele frequencies may be attributable to heterogeneous processes acting across the landscape, such as localized environmental selection or local barriers to gene flow (Epperson 2003). Local autocorrelation provides an analytical tool most needed, and offers the greatest insights, for species that tend to be habitat generalist, continuously distributed, and long-range dispersers. Because these species lack obvious population boundaries, or directly observable relationships to the landscape, they exhibit cryptic population structure that may be revealed in spatial genetic patterns.

Objective and Research Questions

Here we attempted 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 study used mitochondrial DNA (mtDNA) sequencing and nuclear DNA (nDNA) microsatellite analysis to estimate the abundance of bears in KEFJ and to evaluate phylogeography, population structure, and landscape-genetic interactions. 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 objectives and research questions addressed in this project included:

Objective 1: Estimate abundance of black bears in coastal habitats of KEFJ.

- What is the abundance of black bears using coastal food resources in each bay of Kenai Fjords National Park?
- What is the optimal sampling design for future population monitoring using non-invasive genetic sampling and DNA-based Capture-Mark-R estimates?

Objective 2: Describe the phylogeography of black bears on the Kenai Peninsula and south-central Alaska.

- What is the phylogeographic relationship among black bears in south-central Alaska?
- Is there evidence of sex-bias dispersal in black bears in south-central Alaska?

Objective 3: Describe population genetic structure and diversity of black bears in south-central Alaska.

- What is the degree of genetic subdivision in the south-central Alaska black bear population?
- What is the degree of differentiation between subdivisions of the population?
- What is the level of genetic diversity in the population or its subdivisions?

Objective 4: Relate population genetic patterns to the landscape of the Kenai Peninsula.

- Is there evidence for increasing genetic isolation with geographic distance?
- What fine scale spatial patterns exist in the genetic variation among black bears in south-central Alaskan?
- Does the landscape of the Kenai Peninsula influence spatial restrictions of black bear gene flow?

METHODS and MATERIALS

Study Area

Although KEFJ was the area of focus in this study, the genetic make-up of a given population is often most meaningful in the context of its relation to the regional genetic composition. To provide a broader evolutionary and geographical context for interpreting genetics of the KEFJ black bears, we expanded this study to include the range of black bear habitat on the Kenai Peninsula and adjacent mainland. We used ADF&G game management unit (GMU) boundaries to define our study area. Although purely political boundaries, the GMUs provided a convenient bound for the study area and a means of requesting spatially explicit samples from ADF&G. The study area consisted of GMUs on the Kenai (7 and 15 a, b, and c), mainland adjacent to the peninsula (16b, 14 a, b, and c) and neighboring Prince William Sound area 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). KEFJ formed a core of intense sampling within the study region and was the sole focus of Objective 1. 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 made these rich coastal habitats most vulnerable to human impacts, and makes them an important focus for non-invasive trapping efforts within KEFJ (Figure 2).

The study landscape comprises a diversity of ecological communities. The level III ecoregions of Bailey (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). 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. 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).

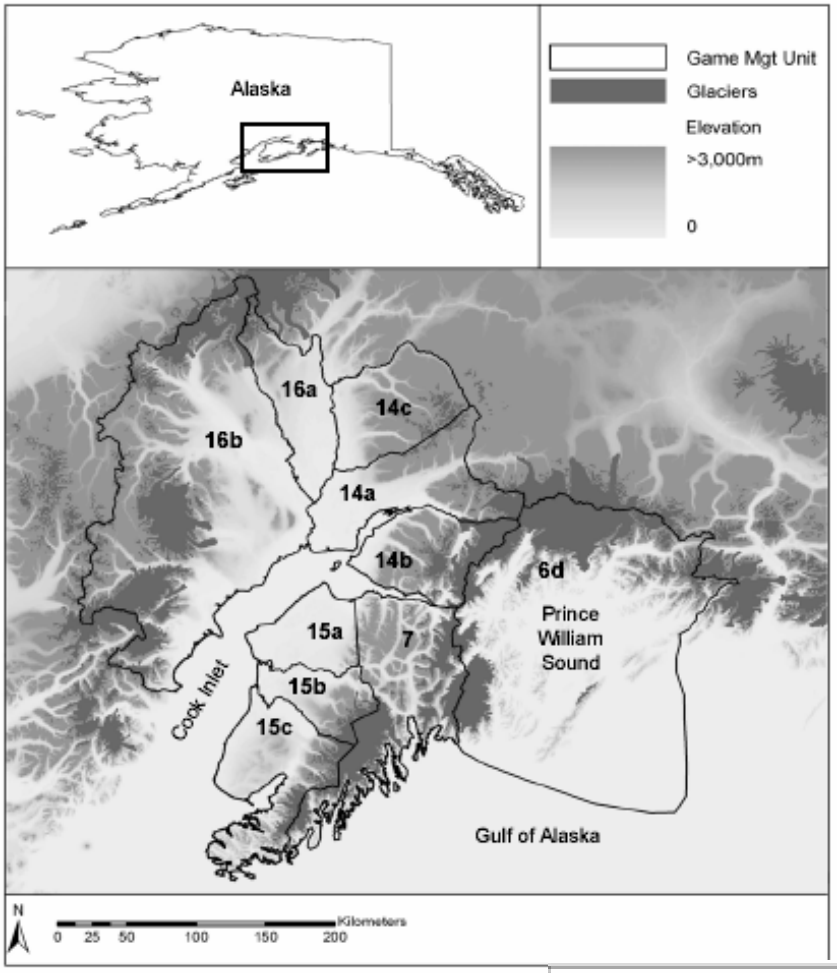
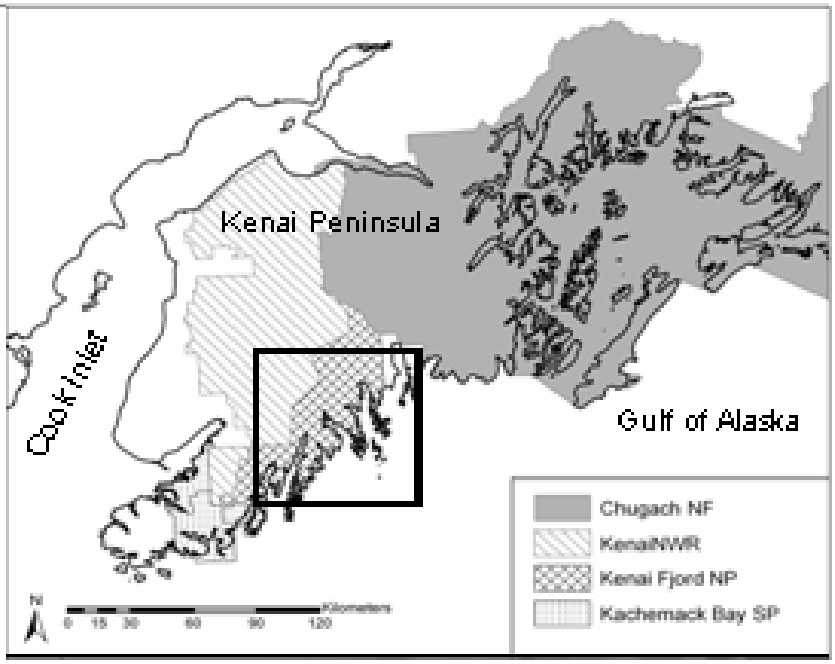


Figure 1: Study region for assessment of phylogeography and population genetic structure of black bears in south-central Alaska.

Figure 2: Focal study area in KEFJ, Alaska (cross-hatched in square), for non-invasive genetic sampling and DNA-based estimate of black bear abundance.



Genetic Sampling

We used non-invasive genetic sampling within KEFJ to collect hairs to provide material for genetic analysis. Non-invasive sampling allowed us to efficiently collect numerous samples without influencing bear behavior through baiting or handling. 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. Black bear samples were collected from the broader study area from hunter-killed bears (covering GMUs 7 and 15 on the Kenai Peninsula, 16b and 14 on the mainland and 6d around Prince William Sound).

Non-invasive Hair Snares and Barbwire Traps - KEFJ

For intensive genetic sampling within KEFJ, 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 (unpublished data, personal communication Farley 2004; Martin 2004; Figure 3A). The 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 tight. The cable was anchored to a secure point; typically a tree trunk or sturdy shrub near the bear trail. The snare was hung over the trail on existing vegetation forming a vertical loop at bear head level so that a bear would walk head-first into the loop. As the bear walked forward, the loop would tighten, catching a few hairs in the barbs, and 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 3B). 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.

Trapping sessions occurred in late July and early August to coincide with the timing of salmon runs and peak berry productivity. Timing was essential to take advantage of natural food draws rather than baiting traps. Each trapping period lasted for approximately 11 days in which traps were set out over days 1-3, checked for the first time from days 5-7, and checked for a second time from days 9-11 (Table 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 salmonberry (*Rubus*) and blueberry (*Vaccinium*) patches. 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. Precise locations of each trap were recorded using a hand-held GPS unit (Trimble, Westminister, CO) (Figure 4).

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.

Table 1: Timing of non-invasive genetic sampling in KEFJ, Alaska (summers 2003 – 2005). The 11-day trapping sessions included approximately 3 days to set traps (from 1st set date) and 3 days to check traps (from Occasion 1, and again from Occasion 2 date).

Bay	Year	First set	Occasion1	Occasion 2	# Traps Set
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

Opportunistic Sampling – Regional

Tissue samples were collected from black bears hunted across the entire study region (GMUs 6d, 7, 14, 15, 16) to provide a broader context for KEFJ samples. 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 DNA 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) (Figure 5). 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).

Figure 3: Break-away hair snares (A) and barbwire hair traps (B) used in non-invasive genetic sampling of black bears in KEFJ, Alaska (summers 2003 – 2005). These unbaited hair traps were set along bear trails in areas of salmon runs or berry thickets.

A)

B)

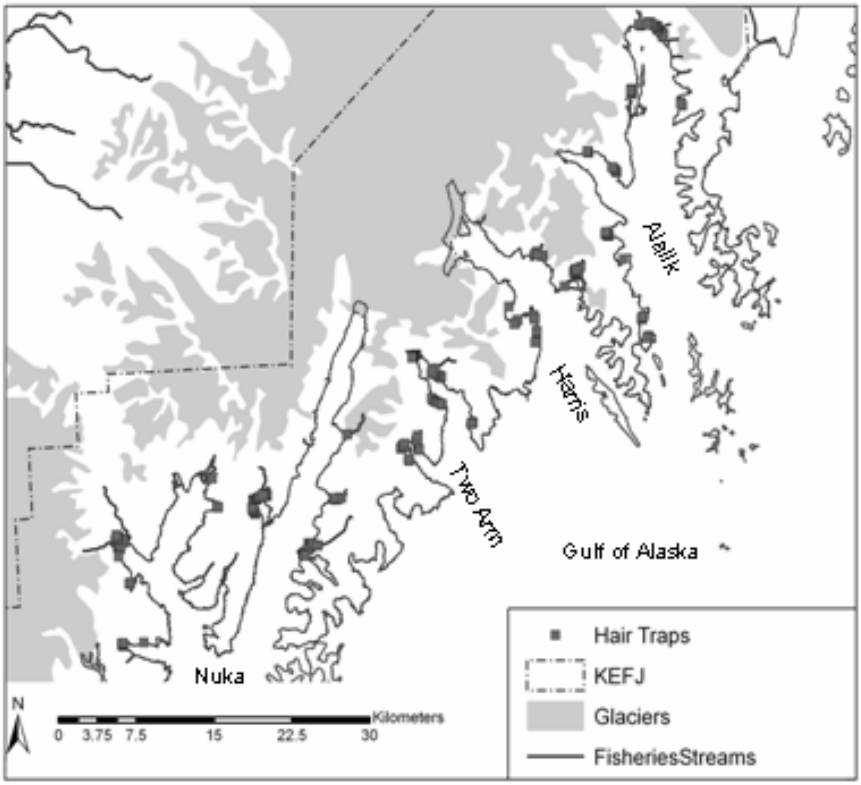


Figure 4: Coverage of 173 black bear hair samples collected using non-invasive genetic trapping in KEFJ, Alaska (summers 2003 – 2005). Samples were used to estimate the abundance of bears in each bay.

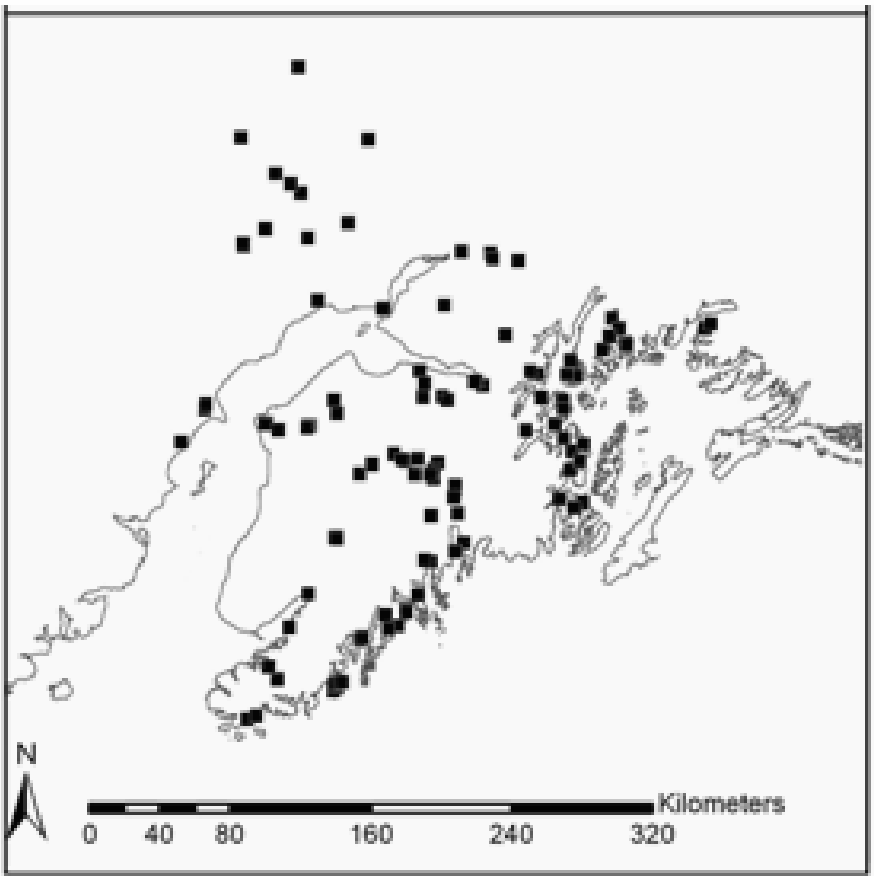


Figure 5: Coverage of 110 black bear tissue samples collected from hunter-killed bears across south-central Alaska (2004 – 2005). Samples were used for phylogeographic, and population structure analyses.

Laboratory Analysis

KEFJ Sample Set

Only samples from KEFJ were used for abundance estimates in Objective 1. The primary purpose of these samples was individual identification for genetic tagging. To conserve laboratory costs, we limited the number of samples processed from a single collection at a single trap. Because 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 225 samples. We discarded any samples failing to amplify or repeat samples of a single individual from multiple barbs on a single trap. The final sample set for Objective 1 contained 173 samples successfully genotyped for genetic tagging (Table 2).

Table 2: Laboratory processing of non-invasive samples collected from KEFJ, Alaska, for CMR analysis (summers 2003-2005). We extracted up to two samples per hair snare or one sample every five barbs from barbwire traps. Samples were discarded if DNA quality was poor or if more than one sample from an individual was obtained from a single trap on a single date. Note: Captures + Recaptures may not equal Sample Size because some individuals were recaptured more than once.

Bay	Extracted	1ID/Trap	Poor DNA	Sample Size	Captures	Recaptures
Aialik	135	70	9	61	41	10
Harris	50	41	2	39	37	2
Two Arm	95	50	3	47	36	9
Nuka	65	30	4	26	20	3

Regional Sample Set

Analyses of phylogeography, population structure, and spatial genetic patterns required broader scale, so we used samples across the study region and a subset of the KEFJ samples. Some hunted 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 for Objectives 2, 3 and 4 included 110 black bears genotyped at all microsatellite loci, the sex ID locus, and the mtDNA control region (Table 3).

Table 3: Laboratory processing of samples collected throughout south-central Alaska, for phylogeographic and population genetic analyses (2004 – 2005). Samples were discarded if they had no location or if DNA quality was poor. Additionally, non-invasive samples were subsampled for this portion of the study.

Sample Type	Extracted	Location	Poor DNA	Sample Size
Non-Invasive	287	287	88	20
Hunted	160	94	4	90
TOTAL				110

Extraction

Whole genomic DNA was extracted based on 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 (one tube containing only water and no genetic material) for every set of reactions.

Amplification

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). The first seven (G1A, G1D, G10B, G10C, G10L, G10M, G10P) were used for individual identification to screen recaptured individuals (Objective 1). All 13 were used for the population level analyses (Objectives 2, 3 and 4). Sex identification was performed using primers SE47 and SE48 from the amelogin gene (Ennis & Gallagher 1994). A 360 base pair section of the mitochondrial control region was amplified and sequenced using primers H16498 and L15997 (Ward *et al.* 1991). DNA fragments were amplified using Polymerase Chain Reaction (PCR) and visualized on an Applied Biosystems automated sequencer (ABI, Foster City, CA) (see Robinson 2007 for reaction conditions and other details).

Data Quality

Recent reviews have pointed out the importance of standardizing data quality-checking protocols and reporting genotyping error rates (Paetkau 2003; Bonin *et al.* 2004). Quality control is particularly critical in DNA-based CMR studies where misidentification of individuals could bias abundance estimates (Mills *et al.* 2000; Paetkau 2003; McKelvey & Schwartz 2004; Waits & Paetkau 2005). We followed the recommendations of Bonin *et al*. (2004) implementing a multi-faceted quality-checking approach (see Robinson 2006 for details). DNA sequences and fragments were analyzed using a strict protocol to minimize human error in genotyping. 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. 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). Probability of identity (PI and PIsibs) was used to verify that chosen sets of microsatellites contained sufficient variation to confidently identify each unique individual and to provide fine resolution of genetic structure.

Abundance Estimate

We divided KEFJ into four study sites based on the four major bays of the park: Aialik Bay, Harris Bay, Two Arm Bay, and Nuka Bay (north to south in Figure 4 above). Each bay was delineated by topographic features (ridgelines) and supported independent salmon runs. Each area 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.

We used four different CMR models to estimate population abundance in each bay . We first used two traditional closed capture models that required multiple discrete capture occasions; thus dubbed "multi-occasion" models (Otis *et al.* 1978; Huggins 1989). We then applied two models specifically adapted to non-invasive, DNA-based CMR studies wherein individuals may be captured multiple times within a trapping occasion. These models treat sampling as one continuous session in which multiple captures are possible; thus dubbed "continuous-occasion" models (Miller *et al.* 2005; Petit & Valiere 2006).

We first implemented a traditional closed capture analysis in the program MARK. We used both the standard closed capture model, in which abundance (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 simpler models, referred to as the null model (M_o) (Cooch & White 2006). We assumed our strict laboratory protocol ensured accurate genotyping, and that 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 (G. C. White, pers com). We assumed an equal capture probability across time, which is reasonable because we trapped for a short period of time in each bay during which there was negligible 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 expect negligible behavioral response either in avoidance or preference for our traps. We assumed a homogeneous probability of capture because individual capture heterogeneity could not be reliably estimated from our two-occasion dataset (Cooch & White 2006). (But see Lukacs 2005 for potential improvements using all observations within each occasion to estimate individual capture heterogeneity in DNA-based studies.)

We next used a Bayesian closed capture method designed by Petit and Valiere (2006) and implemented in the R routine "BayesN". This routine was based on a Bayesian estimator

that used a non-informative prior distribution of population sizes and individual capture histories to estimate population abundance (Gazey and Staley 1986). Again, in this model, we assumed error-free genetic tagging and capture probabilities that were constant across time and individuals. BayesN does not currently offer models with more complex factors.

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 were two types of individuals with differing capture probabilities (Miller *et al.* 2005). Individual covariates were not required to implement the mixture model; the program assigns individuals to the mixture type and determined the ratio between capture probabilities that maximizes the likelihood of the model (Miller *et al.* 2005). The heterogeneous capture probability was the best justified for our dataset, given that capture distributions were skewed, with few individuals being captured many times. Natural populations seldom exhibit homogeneous capture probabilities (Burnham & Overton 1979), and black bears, in particular, tend to 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 estimators 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 abundance estimators. We simulated populations of 100 individuals. We kept capture probabilities constant across time assuming that future trapping efforts will continue to use 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 by MARK in the KEFJ dataset. 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).

Phylogeography

We used mtDNA sequence data to assess phylogeographic patterns. We identified unique DNA sequences; termed haplotypes because mtDNA contains only a single sequence copy that is inherited from the mother. Thus, haplotypes can be thought of as maternal DNA lineages. We then drew a network connecting haplotypes according to the number of DNA base changes between them. This "haplotype network" illustrated the evolutionary relationship between mtDNA lineages. The haplotype distributions were mapped using ArcGIS. Minimum convex polygons were drawn (using Hawthe's Analysis Tools ArcGIS Extension v. 3.25; Beyer 2004) to encompass all samples with each haplotype. The land area within the polygons was used as a measure of the geographic range of each lineage. Because mtDNA is maternally inherited the distribution of haplotypes also informs us as to the range of female-mediated gene flow. If mtDNA lineages are more geographically restricted than populations indicated by bi-parental microsatellite markers, then male-bias dispersal is evident.

Population Genetic Structure

We used three Bayesian population assignment methods to assess population genetic structure of black bears in the study area. 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 estimating the number of genetically distinct groups within a sampled population (Latch *et al.* 2005). Spatial models are additionally useful for identifying geographic boundaries among genetic groups (Guillot *et al.* 2004; Corander *et al.* 2006b). Although there are numerous definitions of genetic populations (Waples & Gaggiotti 2006), herein we refer to "genetically distinct groups" as groups of individuals in genetic equilibrium and with significantly divergent genetic composition from other groups.

Each of the Bayesian assignment tests estimates the most likely number of genetically distinct groups in the sample according to the genetic data. The dataset is partitioned so that the allele frequencies in each group maximize genetic equilibrium (Hardy-Weinberg and linkage). The partition with the highest likelihood indicates the most likely number of genetic groups (K), and then individuals are assigned to groups in which their genetic ancestry is highest. Structure determines the likelihood of each partition based on Markov Chain Monte Carlo (MCMC) randomizations (we performed 200,000 randomizations for each partition tested). We ran 10 replicate tests for possible values from K=1 through K=10. The K value was chosen according to the maximum log likelihood, L(K) and model probability output by Structure.

Instead of using MCMC randomizations, BAPS uses stochastic optimization to infer the correct model for the data (Corander *et al.* 2003). 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. We ran 10 replicate tests for possible values from K=1 through K=10 for the aspatial and spatial models. The optimal K value from each 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. 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 Hawthe's Tools) 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 subdivision using Genepop 3.4 (Raymond & Rousset 1995). Deviation from equilibrium can imply isolation or inbreeding within a population, or admixture with unsampled populations. We tested differences in allele frequencies (using Genepop) and calculated pair-wise Fst (using Arlequin 3.01, Excoffier *et al.* 2005) as measures of differentiation between genetic groups. Differences in allele frequencies indicate that each group has a distinct genetic composition. The Fst ranges from 0.0 indicating complete mixture among groups to 1.0 indicating complete isolation of groups, thus high Fst values indicate a lack of gene flow between groups. 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. Bonferroni correction was applied to all cases of multiple comparisons.

When using multiple assignment tests, the most likely representation of population structure may differ among models, 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 (mixed ancestry) 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.

Landscape – Genetic Relationships

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. In cases of population subdivision, gene flow and genetic distance may be governed by different processes in distinct genetic groups, making separate tests within each group more appropriate. Thus, we also tested IBD within each group identified by the assignment tests.

Local Spatial Genetic Patterns

Local Indicators of Spatial Association are a class of local spatial autocorrelation statistics (Anselin 1995). Here we applied the Moran's I LISA. Moran's I (Moran 1950) is one of the more popular autocorrelation statistics, and has been widely applied to genetic analyses (Hardy & Vekemans 1999; Epperson 2003). A global Moran's I test provides a summary statistic describing the degree of clustering or dispersion of variable values across space. Unlike the global value, the LISA defines a local neighborhood around each point and the spatial pattern at each point is assessed (Anselin 1995; Fortin & Dale 2005). LISA values for each point can be mapped to identify position, size, shape, and layout of local spatial patterns (Fortin & Dale 2005).

We implemented the Moran's I LISA analysis in the geostatistical program Geoda 0.9.5-i (Anselin 2004). We chose a 100 km threshold to define the local neighborhood. A 100km threshold was well justified because it relates to long dispersal distances typical of male bears (Schwartz & Franzmann 1992; Powell *et al.* 1997; Swenson *et al.* 1998; Lee & Vaughan 2003).

All autocorrelation tests were conducted allele-by-allele using individual-based allele frequencies. For a given allele, an individual had a frequency of 1.0 if homozygous, 0.5 if heterozygous, and 0.0 if lacking that allele. Conversion from genotype to allele frequency data has proven a useful means of adapting autocorrelation measures from population to individual levels of investigation (Heywood 1991; Epperson *et al.* 1999).

For each LISA test Geoda generated an individual Moran's I value (I*i*) and a p value for each point and a cluster map displaying patterns of local autocorrelation (see Figure 6 for an example). Significance of LISA statistics was calculated using 999 randomizations in Geoda to identify spatial clusters. Geoda displayed positive autocorrelation as clusters of either high or low allele frequency values. Clusters indicated local areas where allele frequencies were more similar than expected by chance, signifying a spatially restricted distribution of the allele in question. A cluster of high frequency values indicated an area where the allele was near fixation. A cluster of low frequency values indicated the absence, or extreme rarity, of that allele in the region. Negative autocorrelation indicated that the allele frequency at a

given point was 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 close neighbors. A single outlier point might represent a migrant or admixed individual which differs greatly in genetic 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 6), this indicates that the allele frequencies of neighboring points are no more or less similar than expected under a random distribution of alleles, indicating random mating throughout the area.

We summarized the information from multiple allele-wise tests as suggested by Epperson (2003) and averaged the autocorrelation values (I*i*) over alleles and loci at each sample point. Due to the potential influences of global spatial autocorrelation and lack of appropriate p-value corrections for multiple tests we did not rely on significance values for the summarized data. 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*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*i* values. Thus, when displayed, the highest negative ranks indicated the individuals most genetically different from their neighbors and the highest positive ranks indicated the areas where neighbors were most 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. We drew a network of lines connecting all possible pairs of individuals. For each line we calculated the proportion of alleles shared by the two individuals that appeared in the same spatial clusters. This “cluster network” was displayed in ArcGIS 9.0 to visualize areas where points shared similar patterns in spatially clustered alleles. If gene flow were restricted by certain landscape features, we would expect clustering of allele frequencies to appear in certain areas consistently over many allele-wise tests.

RESULTS

Genetic Sampling

We were able to collect over 400 samples in short trapping seasons with non-invasive hair traps (Table 4). Capture success rates (captures/trap*occasion) averaged 0.41 for the break-away hair snares, and 0.58 for barbwire traps (Table 4). Though the success rates were not significantly different (t-test, $P = 0.12$), there was a trend toward higher success with barbwire traps. On average barbwire traps achieved success rates 17% higher than hair snares in the same sampling areas (95% CI = 5% to 29% difference in success rate). Field observations indicated that the hair snares may be bumped or even fully deployed without leaving a hair sample. Barbwires did not exhibit obvious signs of trap encounters so we

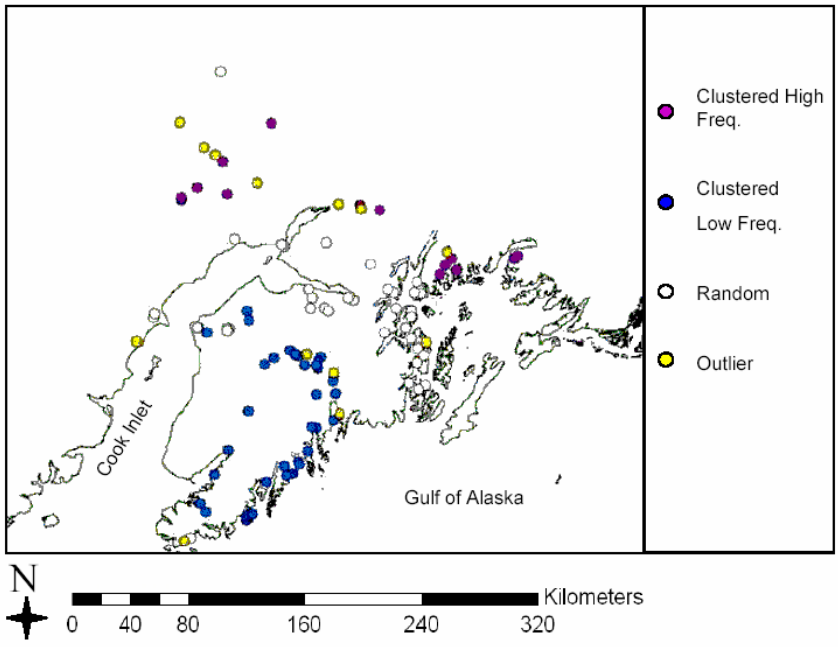


Figure 6: Local clustering of a single allele based on LISA analysis performed in Geoda. Clusters show areas where the allele frequency is highly prevalent; outliers show areas where neighboring individuals differ widely in allele composition.

could not formally quantify differences in trap failure; however, 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.

Table 4: Results of non-invasive genetic sampling using break-away hair snares and barbwire type traps in KEFJ, Alaska (summers 2003-2005).

	Snares		Barbs		Total		
Bay	Number Set	Success Rate	Number Set	Success Rate	# Captures	Success Rate	Samples Obtained
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	

Laboratory Analysis

Data Quality – KEFJ Sample Set

The probability of identity for the full set of seven loci was PI = 1.22×10^{-8}, PIsibs = 0.001. The minimum probability of identity with any five-locus set was PI = 1.41×10^{-6}, PIsibs = 0.007, yielding sufficient power to uniquely identify even closely related individuals. We thus allowed missing data at 1 to 2 loci.

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 0.0025 per genotype. In order for a recaptured genotype to be misidentified as a unique individual after error checking 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.

Data Quality – Regional Samples Set

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.

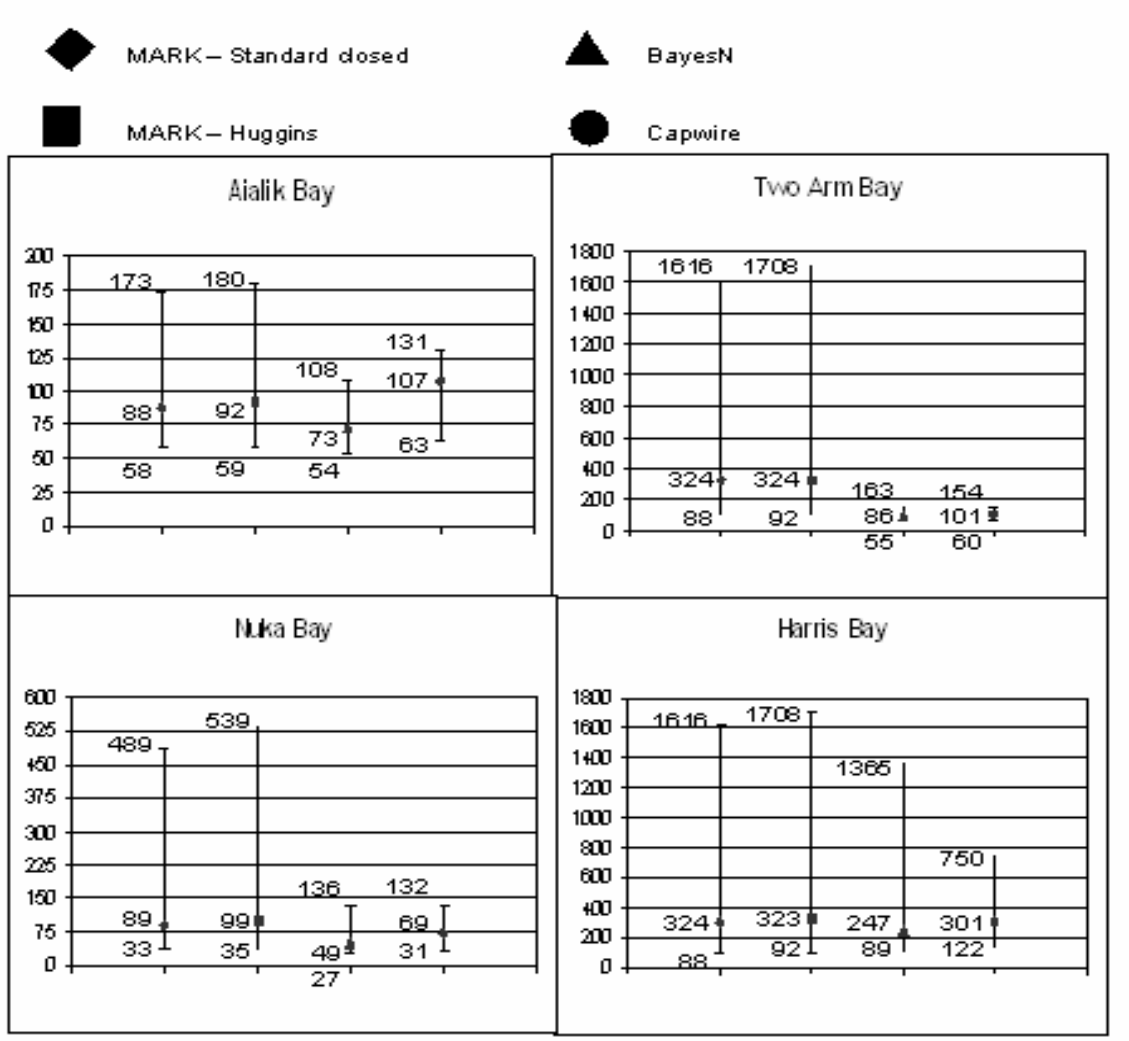
There were four individuals with data missing at a single microsatellite locus, accounting for 7.7% of those individuals' genotype data and 0.28% of the microsatellite dataset. Locus-specific error rates averaged 0.8% (ranging from 0 at loci G10-O, Mu15, Mu50, and Cxx20 to 3.13 % at locus Mu23). The probability of an error occurring in a 13-locus genotype was 0.4% for hair samples and 1.4% for tissue samples. The probability of identity with 13 microsatellites was PI = 6.08×10^{-14} and PI(sibs) = 7.56×10^{-6}, indicating high resolution for discerning close genetic relationships.

Abundance Estimate

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. Many of the recaptures in Aialik Bay occurred during different capture occasions making the encounter histories and results similar between multi-occasion and continuous-occasion models. In other bays several of the recaptures occurred at different traps within a single trapping occasion. This required condensing into a single capture per occasion for the models in MARK, leading to very different encounter histories and limited comparability between the multi-occasion and continuous-occasion models. Probability of capture was estimated by MARK as 0.26 (95% CI = 0.13-0.45) in Aialik Bay, 0.10 (0.09-0.44) in Nuka Bay, 0.05 in Two Arm (0.01-0.29) and Harris Bays (0.01-0.29). The probability of capture parameter was not output by BayesN or Capwire. Capwire, however, provided the number of observations per individual which was 1.49 in Aialik, 1.30 in Two Arm, 1.08 in Harris, and 1.30 in Nuka Bay.

Population estimates were similar from comparable model types and confidence intervals substantially overlapping (Figure 7). Confidence intervals were also similar within model type, with continuous-occasion models always providing the narrowest ones (Figure 7). Capwire tended to estimate the narrowest confidence intervals.

Figure 7: Point estimates and 95% confidence intervals for estimates of black bear abundance in KEFJ, Alaska. Abundance was estimated separately for each bay (chart headings) using four different models (symbols). Note that scale differs among graphs.



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 total captures, similar to our empirical dataset. Simulated CMR estimates showed that, with a capture probability as high as 0.25 (based on the catchability in Aialik Bay), that multi-occasion closed capture models performed well even with few capture occasions (Appendix 1, Figure A). The estimator 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. 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%.

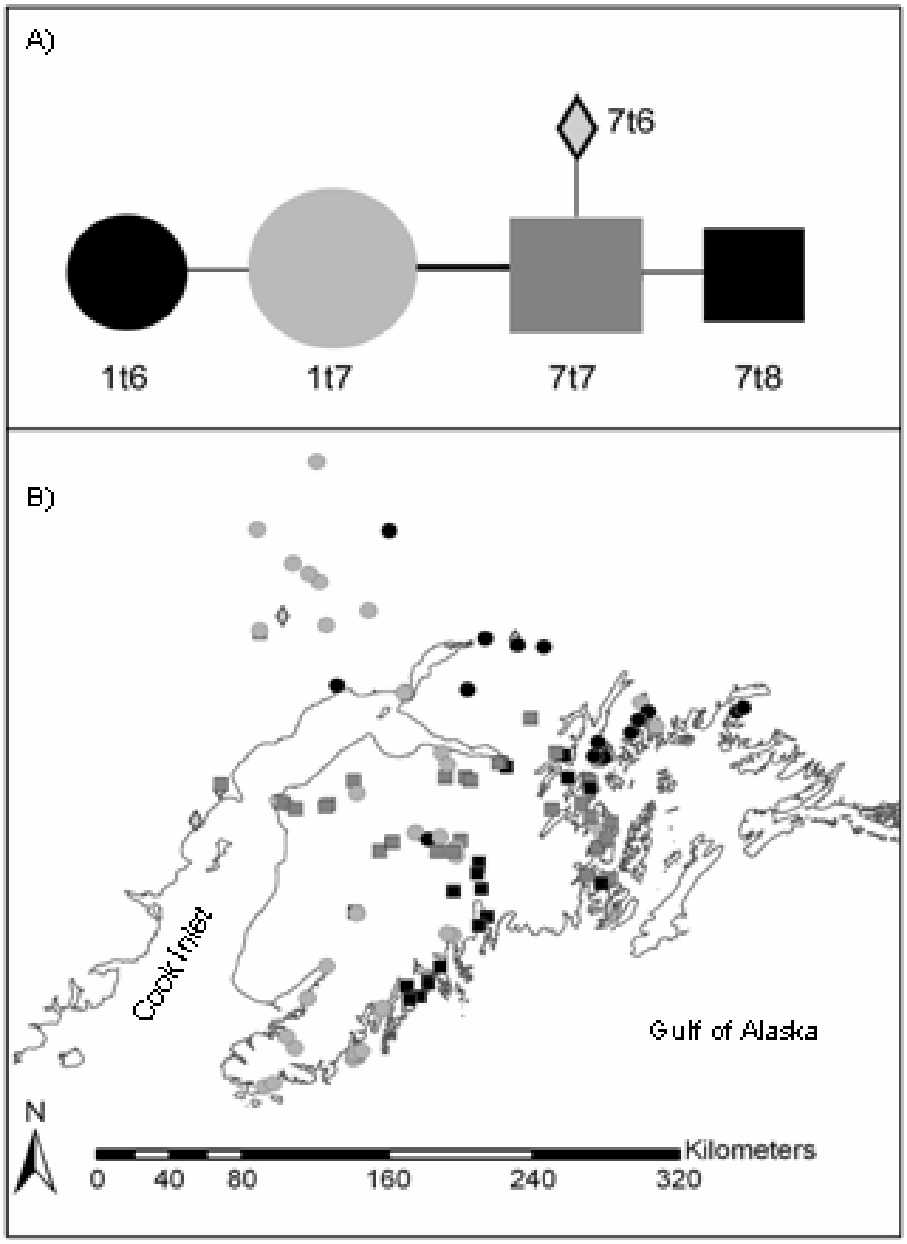
Much greater sampling effort would be required to achieve an unbiased and precise abundance estimator with the lowest capture probability of 0.05 (based on the Harris Bay data) (Appendix 1, Figure B). Bias was high and highly variable across the number of occasions, ranging from -33.75% at 3 occasions 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 of the true population size was high given the wide confidence intervals.

Phylogeography

Five haplotypes were detected in a 360 base pair segment of the mitochondrial control region (Figure 8). 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 defining lineages 1 and 7 of Clade A, the continental black bear clade identified by Wooding and Ward (1997). Inclusion of the thymine repeat segment allowed us to further refine these two lineages into five haplotypes containing 6, 7, or 8 thymine bases. Haplotypes 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 8A illustrates the evolutionary relationship among haplotypes.

The geographic distribution of haplotypes is depicted in Figure 8B. 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).

Figure 8: Evolutionary relationship (A) and geographic distribution (B) of mtDNA haplotypes identified in black bears throughout south-central Alaska. In A, the heavy line represents the C-T base substitution differentiating lineages 1 and 7; the light lines represent variations in the number of thymine repeats. Symbol size is proportional to haplotype prevalence. Symbols in A provide a legend for haplotypes mapped in B.



Population Genetic Structure

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 5, Figure 9A). BAPS aspatial indicated 5 groups, the same ML and PWS groups and three groups on the Kenai Peninsula (Table 5, Figure 9B). Two of these were outliers that contained only 2 and 3 individuals, and 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 5, Figure 9C).

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 population genetic structure in the study area. HWE deviations were similar, with each partition. The BAPS spatial partition minimized linkage disequilibrium (LD). The BAPS spatial partition showed the highest ancestry of assigned individuals and the lowest number of admixed individuals (Table 6). Fst values were similar between all comparisons of Kenai groups, ML and PWS groups, but showed little variation between the KP1 and KP2 split by Structure (Table 7). Maps of the genetic group ranges further supported the designation of three groups suggested by BAPS spatial (Figure 9). 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 10). 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 9).

Genetic diversity (He and AR averaged over 13 loci) was similar between ML and KP groups (Table 8). PWS showed slightly lower genetic diversity, but note the small sample representing this group. Both allelic richness and expected heterozygosity were lowest in PWS, though they did not differ significantly from other groups (t-test, P = 0.44-AR, 0.32-He).

Table 5: Results showing the most likely number of genetically distinct groups of black bears in south-central Alaska, 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. Because BAPS results are based on stochastic optimization, rather than MCMC replicates, not all partitions are equally visited by the model ("nv" denotes those not visited). The most likely partition produced by each program is indicated in bold text.

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

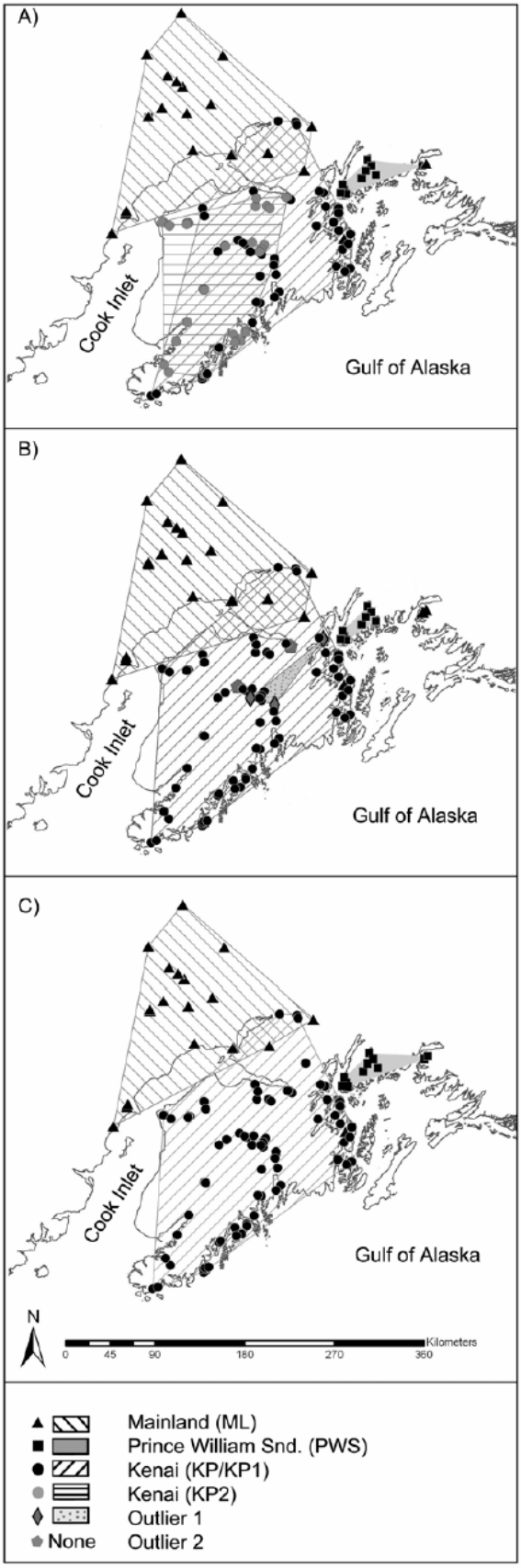


Figure 9: Geographic ranges of genetic groups of black bears in south-central Alaska, detected by Structure (A), BAPS aspatial (B), and BAPS spatial (C). Minimum convex polygons were drawn around all resident bears of each group.

Table 6: Individual ancestry and admixture in each genetic group of Alaskan black bears detected using Structure, BAPS aspatial (BAPSa) and BAPS spatial (BAPSs).

	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 ancestry (q) value of individuals assigned to each group							
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 geographic ranges between groups							
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 7: Pair-wise Fst values showing differentiation between genetic groups of Alaskan black bears (detected using Structure, BAPS aspatial (BAPSa) and BAPS spatial (BAPSs)).

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.

Figure 10: Migration and admixture among genetic groups of black bears in south-central Alaska. (Partitioning of groups based on assignment test from BAPS spatial).

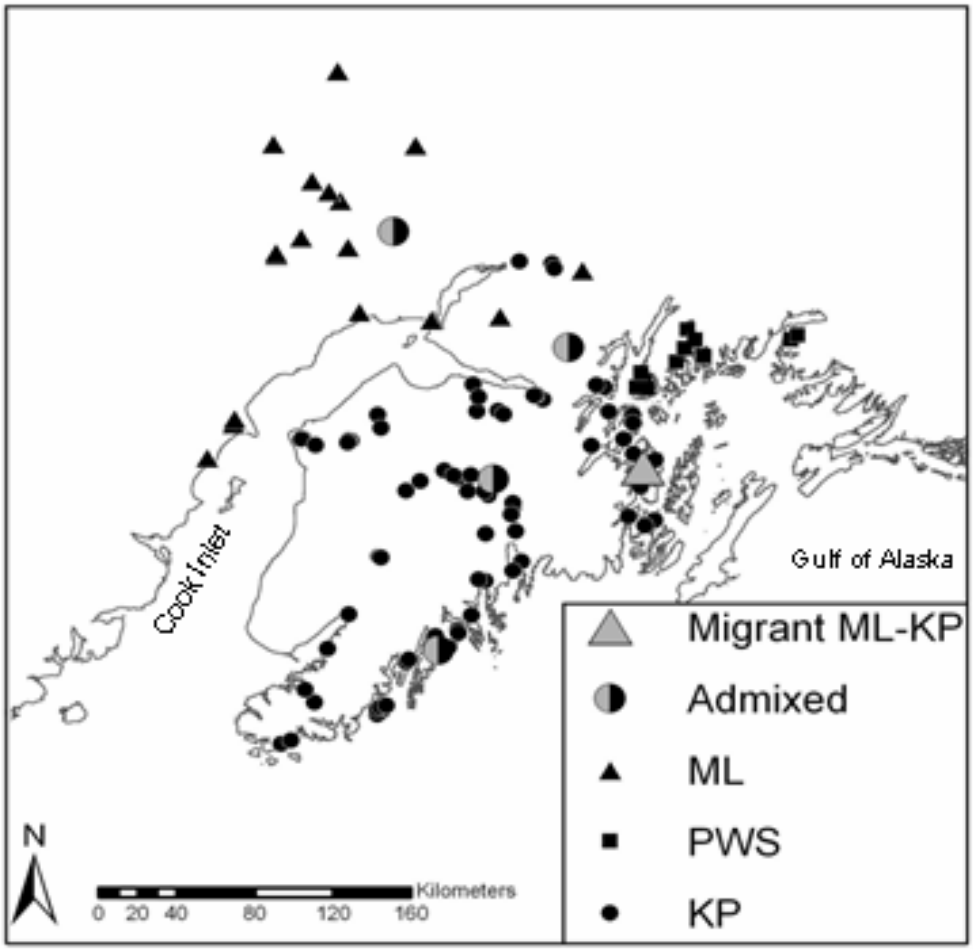


Table 8: Measures of genetic diversity in each genetic group of black bears in south-central Alaska. We show the number assigned to each group (N), the proportion of heterozygotes expected (He) and observed (Ho) (averaged over 13 microsatellite loci), and the allelic richness adjusted to the smallest sample size (AR_{11}). (Partitioning of groups based on assignment test from BAPS spatial).

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.

Landscape – Genetic Relationships

Isolation by Distance

The Mantel test across the full study area indicated that genetic distance was significantly, though weakly, correlated with geographic distance (R = 0.231, (*P*= 0.001). IBD was evident but considerably weaker within the KP group (R=0.112, *P* = 0.009). IBD was not significant in the PWS group (R = 0.006, *P* = 0.527) or in the ML group (R = 0.055, *P* = 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). In all cases the correlation coefficient, R, was low, showing only a weak effect of geographic distance on genetic distance. It is likely that, even in the cases of significant correlation, geographic distance plays only a background role in affecting genetic distance while other ecological or evolutionary factors take the forefront.

Local Spatial Genetic Patterns

Local spatial clustering of allele frequencies was evident in the Geoda cluster maps (individual figures not shown, refer to Figure 6 for an example). High degrees of clustering were evident in points near the core of each of the genetic group areas (ML, KP, and PWS), with the ML and PWS areas showing the highest clustering ranks (Figure 11). Random and outlier points were frequent at the interface between the ML and KP populations. The two highest ranked outliers were points identified as migrants or admixed individuals in the BAPS spatial assignment tests.

In the cluster network the core genetic group areas appeared distinct when viewing lines showing ≥ 0.5 clustered alleles shared (Figure 12). This indicates that, within groups, individuals shared at least half of the locally prevalent alleles. At the 0.5 level there were lines extending between KP and PWS. Examining lines of ≥ 0.6, the KP and PWS groups showed less connectivity. Further, distinct patches of connection took shape within the KP group, showing areas where individuals shared most spatially restricted alleles. Distinct patches of allele clustering centered around the north coastal Kenai Mountains, the southern coastal Kenai Mountains, and a small patch in the western lowlands.

Figure 11: The degree of local autocorrelation in allele frequencies in Alaskan black bears, according to LISA analysis, 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.

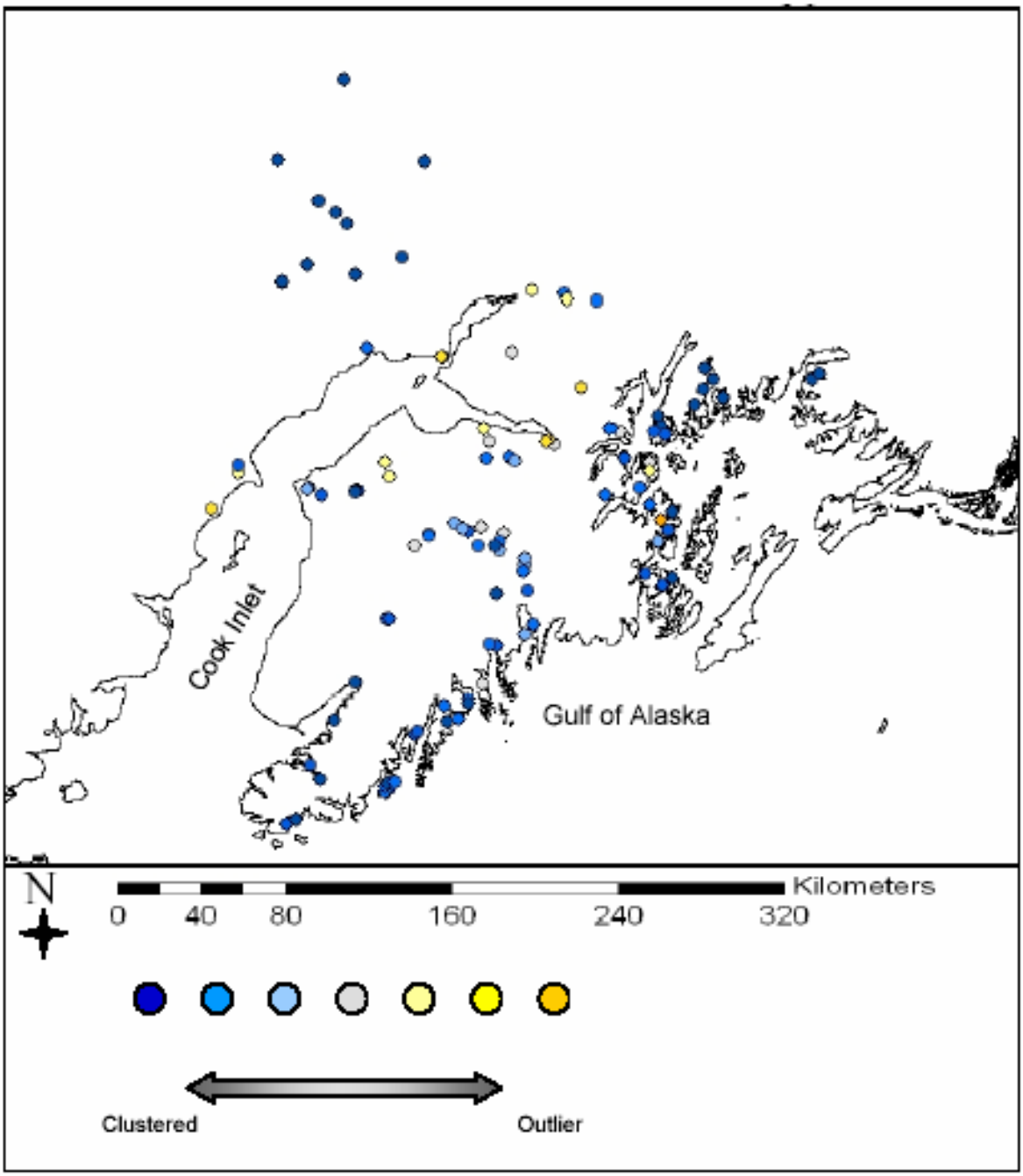


Figure 12: Allele clustering network in which line color indicates the proportion of allele-wise LISA tests in which the joined points appeared in the same spatial cluster of Alaskan black bear allele frequencies. Higher proportions indicate greater similarities in localized genetic information.

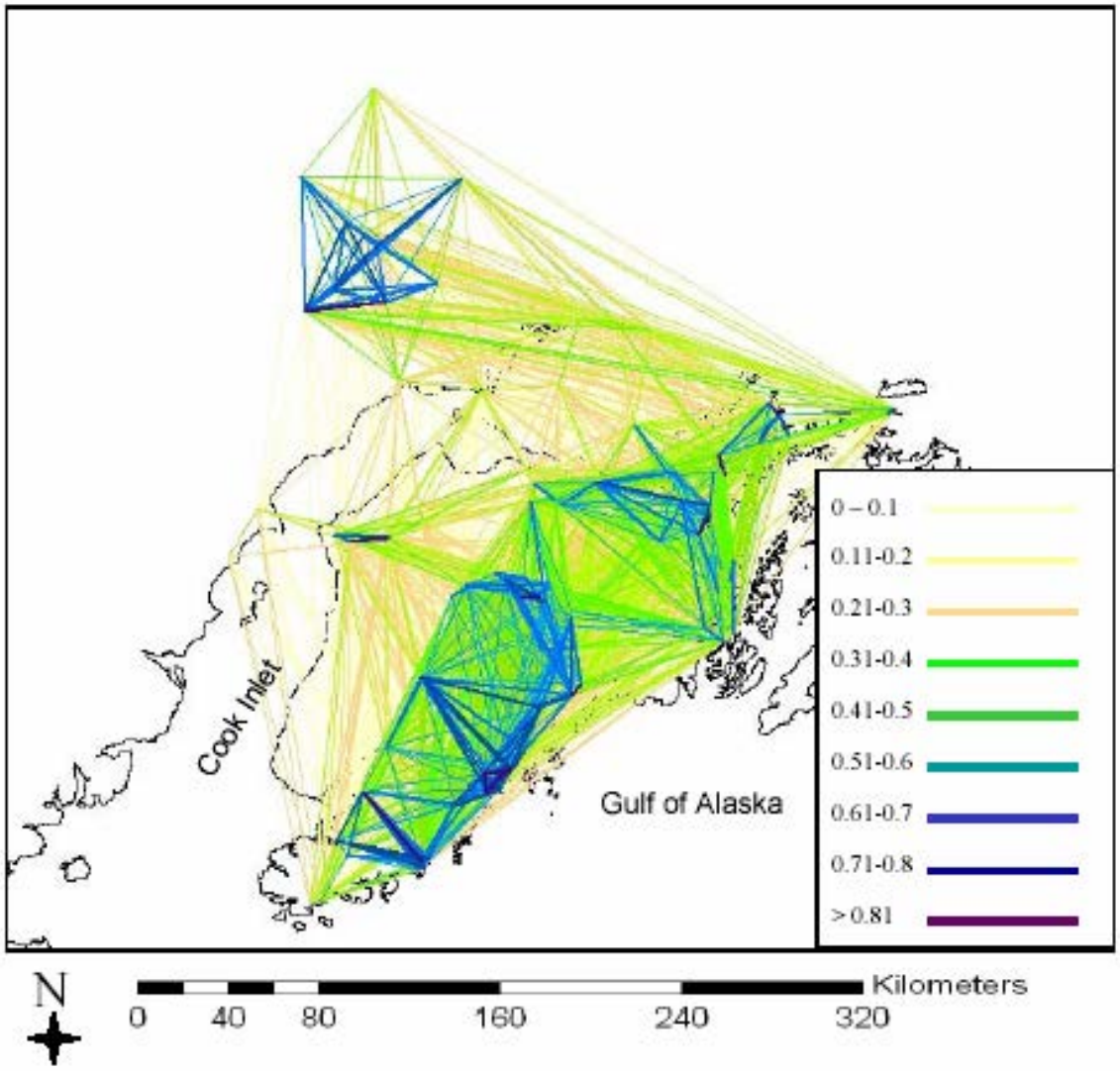
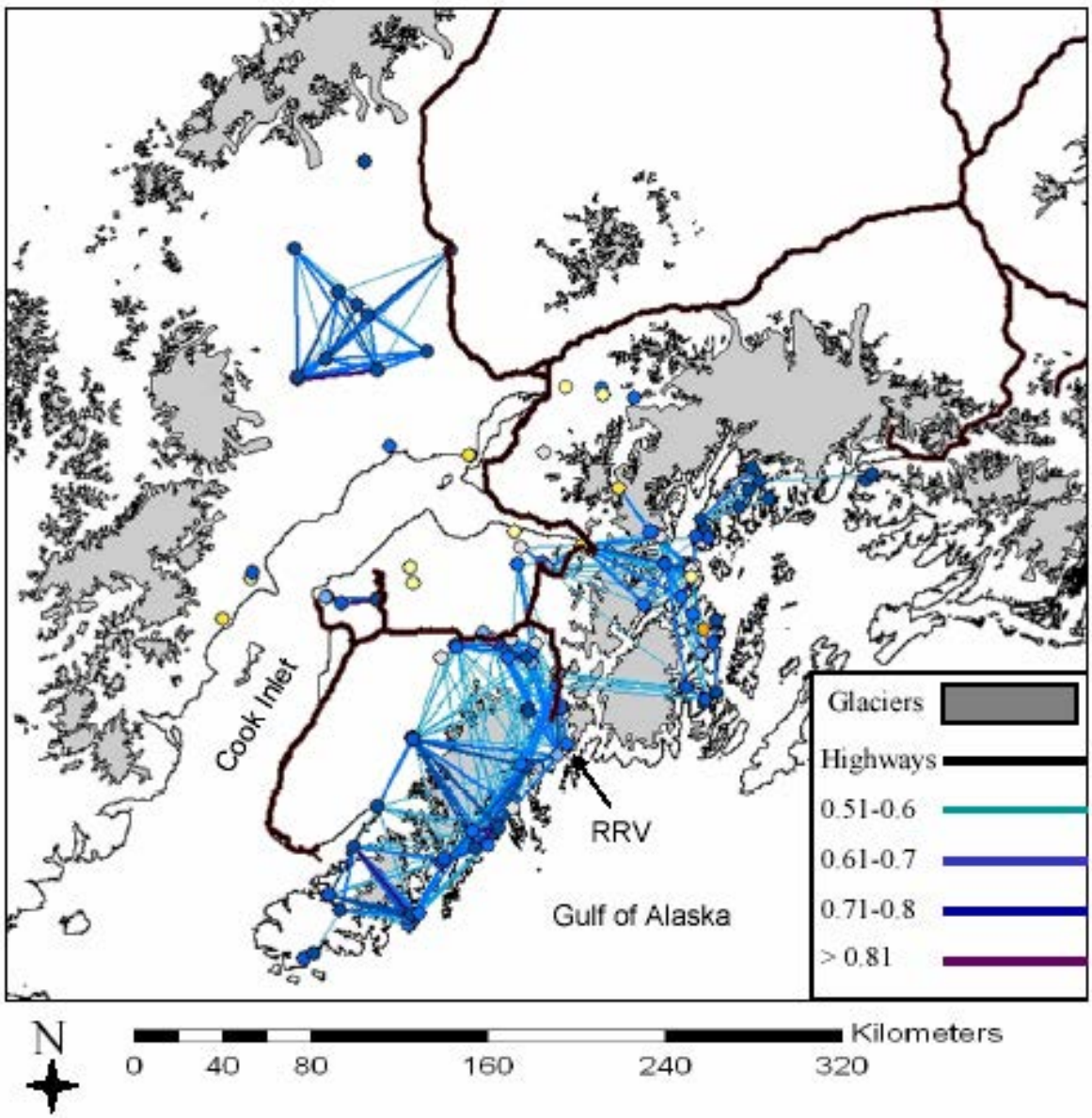


Figure 13: Relationship of geographic features to clusters of genetic similarity in Alaskan black bears. The major road system (heavy black line) and glacial masses (gray) are overlaid on the black bear allele clustering network (only lines ≥0.5 shown). Areas of high local genetic similarity form distinct patches relative to major icefields and/or separated by Alaska Highway 1 or other major roads. The Resurrection River Valley (RRV) is labeled as a potentially important corridor between areas.



DISCUSSION

Abundance Estimate

We achieved 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 tracking trends in black bear abundance. As KEFJ observes increasing visitor use and increasing development in the area, it will be important to monitor black bear use of coastal areas to assess human impacts on these critical habitat resources.

Capture probabilities and recapture rates were especially low in Harris Bay leading to imprecise estimators with confidence intervals so wide as to be uninformative. There were 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 (Wilkes & Calkin 1994; Hall 2005). 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 un-occupied 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 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 get 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 (Otis *et al.* 1978; Huggins 1989; Miller *et al.* 2005; Petit & Valiere 2006).

Models and Assumptions

Continuous-occasion CMR models designed specifically for non-invasive genetic sampling were most informative with our small datasets. The requirement of discrete 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 and led to poor performance. Although models are currently limited for a two-occasion dataset, we should note that Lukacs (2005) has developed methods for using multiple DNA captures per non-invasive trapping occasion to estimate parameters such as capture heterogeneity. Thus future incorporation into publicly available software may provide additional options for maximizing information from multiple captures/occasion within the multi-occasion models.

The continuous-occasion 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-occasion CMR models have been well-tested through simulation studies (see Miller *et al.* 2005 and Petit & Valiere 2006 for details on simulations). In previous research with similar sample coverage (50 simulated samples), the continuous-occasion Bayesian estimator showed lower error, similar bias, and lower variance as compared to the null, M*o*, model of Otis (1978) with multiple occasions (Petit & Valiere 2006). In simulations Capwire performed well (in situations of homogeneous and heterogeneous capture probabilities) concerning coverage, confidence interval, and bias relative to multi-occasion CMR models (Miller *et al.* 2005). All of the tested models tended toward a small 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 applied here. Correcting genotyping error is imperative for meeting this assumption in DNA-based CMR studies (Taberlet *et al.* 1999; Paetkau 2003). 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 CIs in this study. By detecting the imbalance of one-time captures, the misidentification parameter in MARK does not adequately capture the likelihood of genotyping error. 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 rates may provide a more realistic and more flexible reflection of genotyping error in DNA-based CMR studies.

Recommendations for Future Sampling 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 set. 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 catchability.

The best strategy may be to follow the big law of CMR studies and work toward increasing capture probability. Miller *et al.* (2005) suggest that, in their continuous-occasion model, 2.0 to 2.5 observations per individual would be necessary to achieve estimates within 10% to 15% of the true value. In this study we saw 1.08 to 1.49 observations per individual and much lower precision. We saw from the simulations that scenarios with higher capture probabilities led to less biased and more precise population estimates even with few capture occasions.

The 4 to 5 day capture occasions used here were relatively short. Longer trapping occasions may help improve capture probabilities without necessitating baits or lures. Other non-

invasive genetic studies have used trap occasions up to 14 days long (Boulanger *et al.*, 2004b). A wider spread of traps may also help in recapturing bears moving between salmon runs and other parts of the bay. An alternative to longer capture occasions would be to use more short occasions. Regardless of capturability, more than two capture occasions would be preferable if using traditional CMR models. With additional capture occasion we could apply more advanced models incorporating variables such as covariates related to environmental factors or capture heterogeneity.

Trap performance may be another important factor in improving capture probabilities. Barbwire traps tended 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 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 DNA-based black bear abundance estimates. The balance of trap selection and arrangement, and length and number of trapping occasions should offer a variety of options from which to determine the most economical sampling strategy. 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 collaboration with other projects incorporating salmon abundance or 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 black bear population.

Phylogeography

Haplotype 1_{t7} was the most widespread in this study as well as others spanning North America (Paetkau & Strobeck 1996; Wooding & Ward 1997; Roon 2004). 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 haplotype 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 lineages (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

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) (Pielou 1991; Muhs *et al.* 2001). 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 (Pielou 1991; Muhs *et al.* 2001). The Kenai became distinct as a result of continued ice melt and sea level rise, and was relatively isolated from the mainland by 7,000 ybp (Pielou 1991). 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 lineage spreading from the continental US throughout Alaska. Increasing isolation of the Kenai Peninsula may have fostered the development of unique haplotype distributions on the mainland (1_{t6} 7_{t6}) and Kenai (7_{t7} and 7_{t8}).

Population Genetic Structure

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 LD or 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 (see Robinson 2006 for model details).

Biological Interpretation

Our results indicated that black bears on the Kenai Peninsula were genetically distinct from those on the mainland and the Prince William Sound areas. 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). 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, thus probably not constituting an evolutionary significant unit (ESU as defined by Moritz 1994; Moritz 2002). The distinction of nDNA suggests that the Kenai bears are a distinct management unit (MU) (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).

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 unfragmented populations across the range of black bears (Quebec and Alberta, Paetkau & Strobeck 1994; British Columbia, Marshall & Ritland 2002; Arkansas and Louisiana, Csiki *et al.* 2003).

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. These values are considered moderate in the Fst range observed in black bear populations. The values indicate genetic distinction, but occasional migration connecting groups. This would be expected of groups separated by semi-permeable barriers. 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 long over-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 that permeate the boundaries between genetic groups (Clevenger *et al.* 2002).

Landscape – Genetic Patterns

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, potentially confounding the correlation between genetic and geographic distances. It is likely that gene flow barriers isolating bears at the group level exerted 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.

Local Spatial Genetic Patterns

We have presented a novel technique for visualizing biologically relevant spatial patterns in genetic data. 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. This technique can be applied to management decisions, corridor design, and studies of environmental selection. Similar methods of spatial analysis have been well tested in other studies (Sokal *et al.* 1998; Double *et al.* 2005; Sokal & Thomson 2006). (See Robinson 2006 for further analyses and greater technical detail on spatial analyses.)

Biological Interpretation

The highest local autocorrelation values formed clusters that closely coincided with genetic groups identified in assignment tests. The cluster network also showed that the bears within each group 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, beyond 0.6 shared allele clusters, distinct genetic clusters were evident within the KP population.

LISA analysis added important details to the assignment test results. By examining the local autocorrelation values, we were able to judge the strength of the barrier between KP and ML populations. Non-clustered points and low-ranked outliers occurred primarily at the interface between the KP and ML populations. 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 this genetic group. The patches of allele clustering related to

geographically distinct regions within the Kenai Peninsula (Figure 13). The northern patch was located in the Kenai Mountains north of the Resurrection River valley. The southern patch covered the Kenai Mountain range south of the Resurrection River valley, including KEFJ. A minor western patch also occurred in the western Kenai lowlands.

Two principal distinctions exist between genetic patches on the Kenai. First the Kenai Mountains and the Kenai lowlands 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 span 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). Local autocorrelation values tended to be lower in the western lowlands and higher in the Kenai Mountains. This local difference in spatial autocorrelation indicated that alleles were more spatially restricted in the Kenai Mountains where movement might be more limited by rugged topography. The patchy structure in the cluster network might also be influenced by black bear fidelity to their natal ecological zone. The coyote (*Canis latrans*) is another far-ranging carnivore that shows fidelity to its natal habitat zone, despite its ability to use a variety of habitats (Sacks *et al.* 2005).

Additionally, there were potential barriers between the genetic patches. Alaska Highway 1 is the primary road on the Kenai. In Figure 15 we see the highway clearly outlining each of the genetic patches. A partial Mantel test confirmed the significance of the highway barrier (data not shown). 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 were also major icefields between the northeastern and southeastern patches, though we note that connectivity is high around either the northern or southern ice masses. It is likely that specific corridors help to maintain connectivity within each of these ice-bound areas. For example, river valleys appear to provide critical passages around the icefields in the southern Kenai Mountains. There are fewer opportunities to cross between northeast and southeast patches without crossing substantial ice, sea cliffs, or Highway 1. The local spatial analysis developed here can be useful in monitoring changes in spatial pattern to detect effects of emerging barriers or isolation due to landscape change. The same methods could also be used to evaluate the effectiveness of wildlife corridors established in future efforts.

CONCLUSIONS

Black bear populations in south-central Alaska appear to be abundant and show high genetic diversity and connectivity. Kenai Peninsula black bears are genetically distinct from bears in neighboring mainland areas. It is likely that this distinction is a product of isolation of the Kenai from the mainland since the end of the Pleistocene Ice Age. Although distinct, the populations are not entirely isolated. Migration, low mtDNA divergence, and moderate Fst values indicate only moderate genetic differentiation between genetic groups of black bears in south-central Alaska. Genetic diversity is similar among all genetic groups, and in the range expected for relatively large and stable populations. There was no evidence of genetic subdivision of black bears within KEFJ, or pronounced isolation of KEFJ bears from the remainder of the KP group. The definition of genetically distinct groups provides a biological basis for defining management units. Maintaining connectivity within the KP genetic group may require the collaboration of agencies managing various lands on the Kenai Peninsula.

Genetic connectivity among black bears on the Kenai Peninsula is currently high, but not absolute. That bears on the Kenai constituted a single genetically distinct group indicating that migration and interbreeding occur throughout the area. However, we saw in the spatial analysis that the KP genetic group is not completely intermixed (panmictic), but exhibits a patchy genetic pattern. Genetic patches were distributed in different ecological regions of the Kenai and separated by anthropogenic features such as major roads. This spatial structuring and relation to roads indicates the potential for black bear populations to become increasingly subdivided if barriers become more severe.

Our results provide an important measurement of baseline genetic diversity levels and population connectivity of black bears in this region. Mapping genetic patches with the LISA cluster network provided a view of landscape features that may facilitate or impede genetic connectivity on the Kenai Peninsula. Connectivity was highest within similar ecological zones, lowest between areas across topographic divides or anthropogenic barriers. Such features may be key to identifying important movement corridors. As human presence on the Kenai increases, it will be critical to develop habitat management plans that maintain the current diversity and gene flow and minimize impacts to important linkage zones and corridors.

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APPENDIX 1
Results of Simulated CMR Efforts

Figure A: Results of simulated C-M-R efforts with high capture probability, based on the highest capture probability observed in black bears in the KEFJ (Aialik Bay, p=0.25). 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.

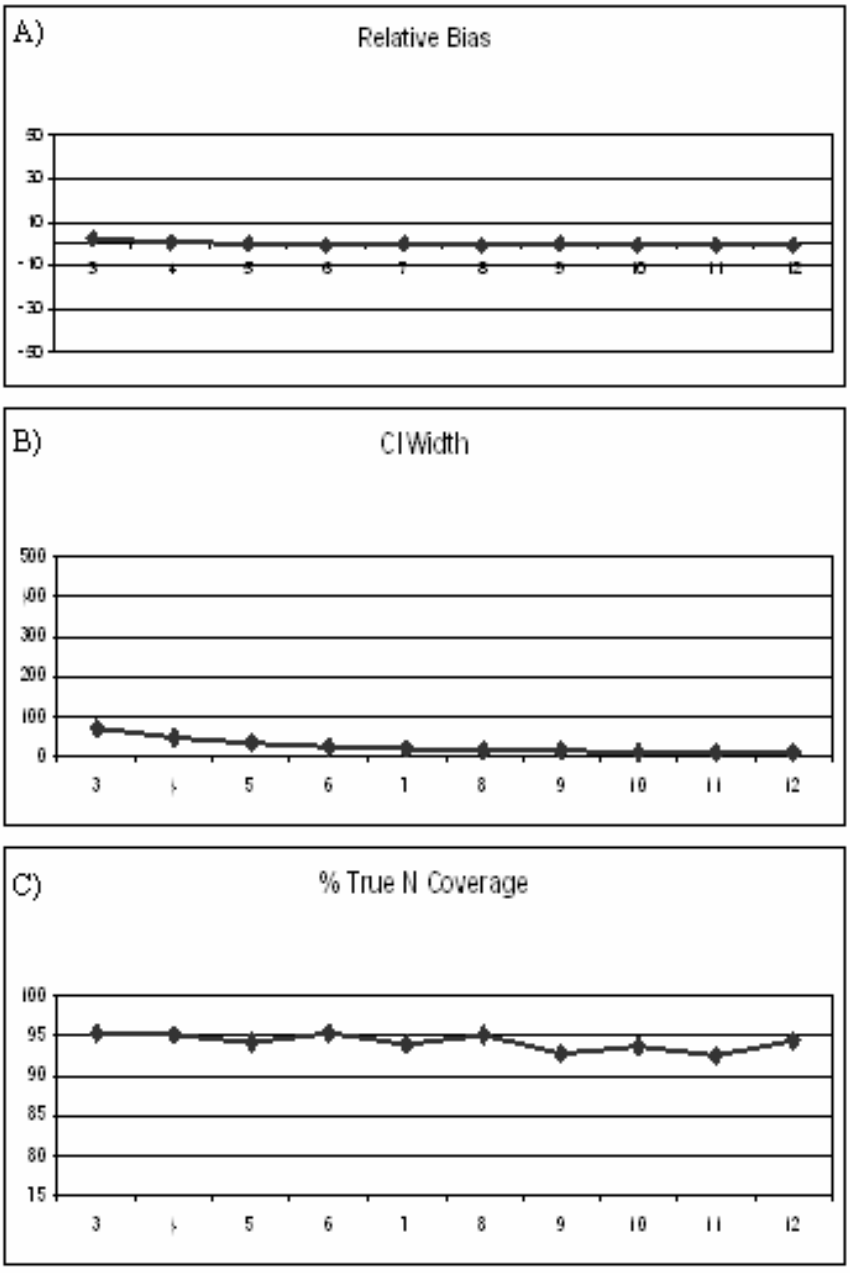

Figure B: Results of simulated C-M-R efforts with low capture probability, based on the lowest capture probability observed in black bears in the KEFJ (Harris Bay, p=0.05). Simulations were based on populations of 100 individuals. The performance can be judged by the accuracy of the estimate (A), the 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.