

EVALUATING POPULATION STRUCTURE OF BLACK BEARS ON THE KENAI PENINSULA USING MITOCHONDRIAL AND NUCLEAR DNA ANALYSES

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Increasing human impacts on the Kenai Peninsula, Alaska, have raised questions about potential implications for genetic diversity and population structure of local taxa. Black bears (*Ursus americanus*) occupy most of the Kenai Peninsula and are currently a species of public interest and management focus. In this study, we use 13 nuclear DNA (nDNA) microsatellite loci and sequence data from the mitochondrial DNA (mtDNA) control region to investigate population structure and phylogeographic patterns in black bears on the Kenai and surrounding mainland. We used both aspatial and spatial Bayesian assignment models to evaluate nDNA genetic structure and cluster individuals into genetically distinct groups. Substantial population substructure was detected, indicating restricted gene flow in recent generations as well as signatures of past barriers between the Kenai and mainland. We identified 3 genetically distinct groups that cluster geographically in the Kenai Peninsula, Alaskan mainland, and Prince William Sound areas. Connectivity among genetic groups was moderate, with F_{st} values ranging from 0.07 to 0.12. Five mtDNA haplotypes were detected, 2 of which were primarily restricted to the Kenai. Our results provide important information about current levels of genetic diversity and connectivity among black bears on the Kenai Peninsula and will provide a baseline for future monitoring.

Key words: Bayesian assignment tests, black bear, genetic population structure, Kenai Peninsula, microsatellite, mitochondrial DNA, phylogeography, spatial assignment, *Ursus americanus*

The rugged landscape and distinct ecology of the Kenai Peninsula, Alaska, have led wildlife managers to question whether population substructure exists between black bears (*Ursus americanus*) on the Kenai and the Alaskan mainland. The Kenai Peninsula has been separated from the mainland since the end of the last ice age by a narrow (16-km-wide) isthmus of ice and rock. The coastal climate of the Kenai has fostered unique ecological conditions and biotic communities. Historically, the uniqueness of wildlife on the Kenai has led to the designation of numerous distinct subspecies, such as song sparrows (*Melospiza melodia kenaiensis*—American Ornithologists' Union 1957), American martens (*Martes americana kenaiensis*—Hagmeier 1958), wolverines (*Gulo gulo katschmakensis*—Matschie 1918), wolves (*Canis lupus alces*—Goldman 1944), brown bears (*Ursus arctos gyas*—Merriam 1918), and black bears (*Ursus americanus perniger*—Allen 1910). Although these taxonomic splits have seldom been

upheld by more recent phylogenetic investigations (Paquet and Carbyn 2003; Tomasik and Cook 2005; Waits et al. 1998; Wooding and Ward 1997), they do highlight the biological diversity harbored on the Kenai Peninsula.

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

Historically black bears inhabited most forested areas of North America (Servheen 1990). Their current distribution is restricted to forested areas lacking dense human settlement (Pelton and van Manen 1994). Black bear populations on the Kenai Peninsula have access to relatively continuous habitat and are believed to be

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stable; however, expanding human activity in the area is projected to increase stress on bear populations (Alaska Department of Fish and Game 2002; Schwartz and Franzmann 1992).

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

Genetic analyses offer important insights into the population structure and connectivity among such wide-ranging animals. Genetic data provides information about historic and current levels of gene flow among populations, as well as information about genetic diversity, kinship, and movement patterns within populations (Paetkau et al. 1998; Queller et al. 1993; Schenk et al. 1998; Woods et al. 1999). Nuclear DNA (nDNA) microsatellite analyses coupled with assignment test approaches (used to assign an individual to the population from which its genotype is most likely to have arisen) have proven useful in detecting population structure and gene flow (Funk et al. 2005; McRae et al. 2005; Paetkau et al. 1995; Slatkin 1995). The development of Bayesian assignment tests allows inferences of genetic structure without a priori assumptions about population groupings within a given study area (Corander et al. 2003; Pritchard et al. 2000). Recent innovations also have added the use of geographic coordinates to assign individuals to spatially organized populations (Guillot et al. 2005). Additionally, mitochondrial DNA (mtDNA) sequence data provide important information about maternal gene flow patterns, past isolation events, natural recolonization events, and evolutionary history (Cronin et al. 1991; Onorato et al. 2004; Sunnucks 2000). Such mtDNA data have been widely used to study the phylogeography of bears in North America (Byun et al. 1997; Paetkau and Strobeck 1996; Stone and Cook 2000; Waits et al. 1998; Wooding and Ward 1997).

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

MATERIALS AND METHODS

Study area.—This study was conducted in black bear habitat on the Kenai Peninsula and adjacent mainland game manage-

ment units covering a total of more than 72,000 km². KEFJ occupies a 2,400-km² band of rugged coastline between the Gulf of Alaska and the 32 glaciers of the Harding Icefield. The study area also extended into Alaska Department of Fish and Game game management units 6d, 7, 14a, 14b, 14c 15a, 15b, 15c, 16a, and 16b (Fig. 1). These game management units, although purely political boundaries, provided a useful boundary to the study area and a means for requesting samples from Alaska Department of Fish and Game. The major physiographic landform on the eastern two-thirds of the peninsula is the rugged, heavily glaciated Kenai Mountain Range, which rises to 2,000 m above sea level (Muhs et al. 2001). The Kenai lowlands, a Pleistocene-glaciated plain dotted with lakes, dominates the western one-third of the Kenai (Muhs et al. 2001). The study area was composed of 3 ecoregions: the Pacific Coast Mountain Range ecoregion in high-elevation areas, the Cook Inlet ecoregion covering the Kenai lowlands, and the Coastal Western Hemlock–Sitka Spruce ecoregion along the Gulf of Alaska and Prince William Sound coasts (Gallant et al. 1995).

Sample collection.—Black bear samples were collected from hunter-killed bears and supplemented with noninvasive hair snaring on public lands (KEFJ and parts of Kachemak Bay State Park and Kenai National Wildlife Refuge). In 2004 and 2005, Alaska Department of Fish and Game staff collected tissue (hide or muscle tissue) samples from bears processed at Alaska Department of Fish and Game checkpoints as part of regulatory hunt monitoring. Tissue samples were stored in paper envelopes and frozen until the time of extraction. The location of each sample was recorded according to the verbal description of the hunting location on the Alaska Department of Fish and Game 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, California) based on the described locations, and referencing an Alaska place names data layer (Alaska Department of Natural Resources, Land Records Information Section 1967). In the event that more than 1 animal was harvested at a single reported location, we constructed a 500-m buffer around the location point and randomly located the sample points at unique locations within that buffer. This point relocation was used to facilitate visualization of sample points. Further, some spatial models required unique coordinates for each sample point. The error in plotting reported hunt locations was expected to be minimal in comparison to the home range of a black bear, which would extend several kilometers beyond the point of capture (Kernohan et al. 2001).

The area within KEFJ was sampled intensively as part of the National Park Service's Southwest Alaska Network's inventory and monitoring program (National Park Service 2006). Field collections were conducted in KEFJ over 3 summers (July–August 2003–2005) by National Park Service and University of Idaho teams. Sampling within each bay in KEFJ consisted of a 10-day session in which hairs were collected on 2 occasions. Hair samples were collected using barbwire hair traps (Boulanger et al. 2004). Traps were set along presumed bear trails in areas of natural food resource concentration (salmon

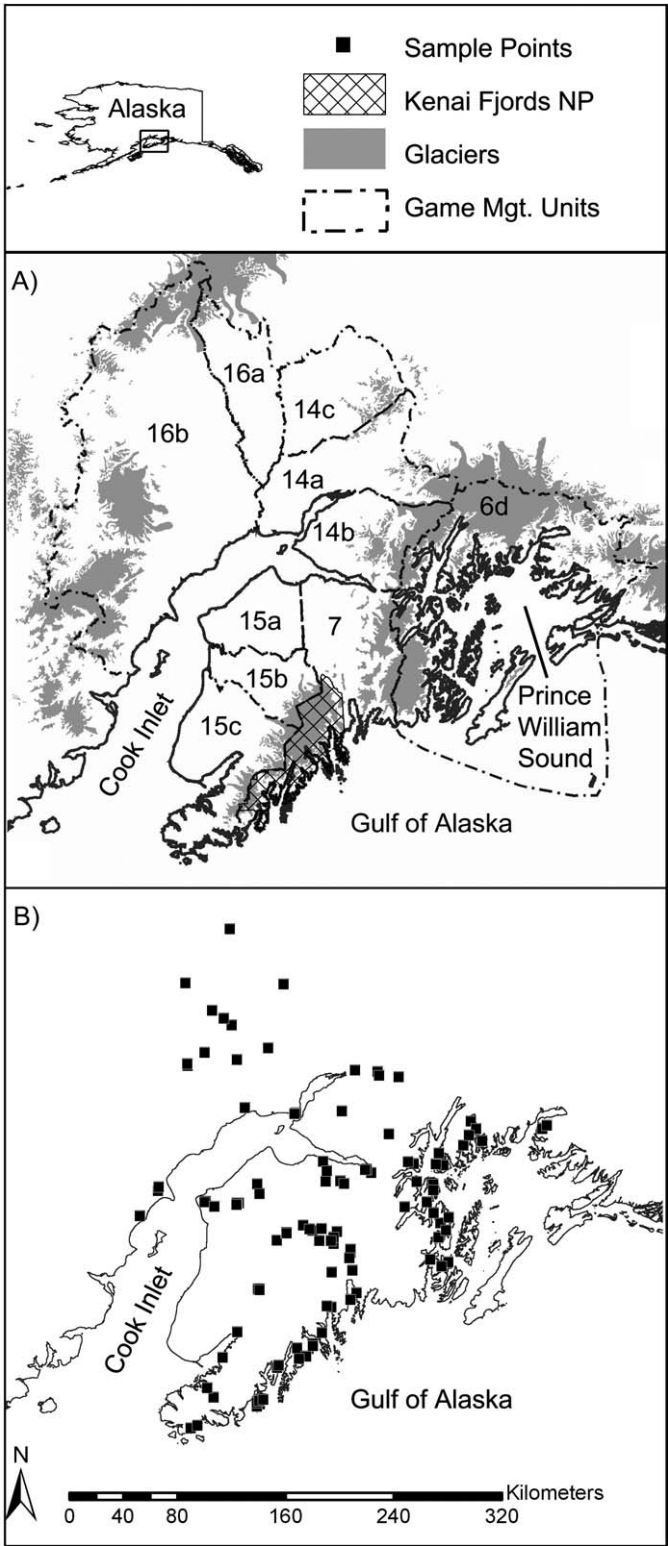


FIG. 1.—A) Study area in south-central Alaska showing geographic features and political boundaries. Game management units (labeled numerically) provided the bounds of the study area and were used to request samples of hunted bears from Alaska Department of Fish and Game. Intensive noninvasive sampling took place in Kenai Fjords National Park. B) Sample coverage for 110 black bear samples collected over the Kenai Peninsula and adjacent mainland.

TABLE 1.—Sample numbers after quality-control measures. Non-invasive samples were collected using hair traps in Kenai Fjords National Park (KEFJ), Kachemak Bay State Park, and Kenai National Wildlife Refuge, Alaska. Tissue samples were collected from bears hunted throughout game management units 6D, 7, 14, 15, and 16. The table includes the number of samples collected using each method; the number discarded because they had no reliable location (No loc.), poor DNA quality (Poor DNA), were recaptures (Recap.), or were thinned from the intensively sampled KEFJ sample set (Thinned); and the final sample size (*n*).

Sample type	No. samples	No loc.	Poor DNA	Recap.	Thinned	<i>n</i>
Noninvasive	287	0	88	60	119	20
Hunted	160	66	9	0	0	90
Total						110

streams and berry thickets) so that no baiting was necessary. Hair samples also were collected within Kachemak Bay State Park on the southwestern side of the Kenai Peninsula. Samples collected with similar noninvasive methodology were acquired from J. Fortin (Washington State University—Fortin et al. 2007) from 3 stream drainages within the Kenai National Wildlife Refuge west of KEFJ. Upon collection, samples were stored in paper envelopes in containers with desiccating silica beads. The location of each sample was recorded as the location of the hair trap using a handheld GPSMAP 76 unit (Garmin International Inc., Olathe, Kansas). These locations were later plotted in the Alaska Albers projection as a global information system layer using ArcGIS 9.0.

Laboratory analyses.—Whole genomic DNA was extracted using standard protocols for a Qiagen DNeasy tissue extraction kit (Qiagen Ltd., Crawley, West Sussex, United Kingdom), using approximately 25 mg 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 1 negative control for every 20 samples extracted, and in each amplification reaction.

Not all samples collected were suitable for the current analysis. Some samples did not have sufficient quantity and quality of DNA for microsatellite genotyping. Other samples did not have verifiable geographic locations required for the spatial analyses of the genetic data. The KEFJ area was sampled in particularly high density (125 bears/1,000 km²) 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 4 bears/1,000 km²). The final data set included 110 black bears genotyped at all microsatellite loci, the sex identification locus, and the mtDNA control region (Table 1; see Fig. 1B for distribution).

nDNA data.—Microsatellite analysis was conducted using 13 highly variable independent loci: G1A, G1D, G10B, G10C, G10L, G10M, and G10P (Paetkau and Strobeck 1994); G10J and G10O (Paetkau et al. 1998); Cxx20 (Ostrander et al. 1993); and Mu15, Mu23, and Mu50 (Taberlet et al. 1997). Sex

TABLE 2.—Multiplex and singleplex polymerase chain reaction conditions for nuclear DNA (nDNA) microsatellites, sex identification, and the mitochondrial DNA (mtDNA) control region.

Reaction	Primer set	Primer amount (μM)	Annealing temperature (°C)	Amplitaq Gold (units)	MgCl ₂ (mM)	dNTPs ^a (nM)
A-B-C-D-L	G1A	0.10	57.6	0.5	2.50	0.33
	G10B	0.10				
	G10C	0.10				
	G1D	1.33				
	G10L	0.17				
M-P	G10M	0.40	52.0	0.5	2.50	0.33
	G10P	0.10				
15-23	Mu15	0.17	52.5	0.5	2.08	0.33
	Mu23	0.17				
O-20	Cxx20	0.23	51.0	0.5	2.50	0.33
	G10-O	0.15				
J	G10J	0.20	48.0	0.5	2.50	0.27
50	Mu50	0.15	52.0	0.5	2.50	0.33
Sex ID	SE47/48	0.64	57.0	1.6	1.87	0.27
	H16498					
mtDNA	L15997	0.30	50.0	0.5	2.50	0.20

^a dNTPs = deoxynucleoside triphosphates.

identification was performed using primers SE47 and SE48 from the amelogenin gene (Ennis and Gallagher 1994). DNA fragments were amplified using polymerase chain reaction (reaction conditions are given in Table 2). Hair samples were amplified using a double-amplification step to maximize yields from samples of low DNA concentrations (adapted from Piggott et al. 2004). All nDNA fragments were resolved using an ABI 377 automated sequencer (Applied Biosystems [ABI], Foster City, California) and analyzed using Genescan 3.1.2 (ABI), and alleles were scored using Genotyper 2.5 (ABI).

mtDNA data.—A 360-base pair section of the mitochondrial control region was amplified using primers H16498 and L15997 (Ward et al. 1991) followed by Exosap (USB Corporation, Cleveland, Ohio), Big Dye 3.1 Sequencing (ABI), and Sephadex (Sigma-Aldrich, St. Louis, Missouri) steps. Sequences were resolved using an ABI 377 or 3130 automated fluorescent sequencer using Genescan 3.1.2 analysis software. We then edited sequences in Sequencher 4.5 (Gene Codes Corporation, Inc., Ann Arbor, Michigan), and then aligned them in ClustalW (Chenna et al. 2003) to identify haplotypes. We included a hypervariable thymine repeat segment of the control region, which has been excluded from other phylogenetic studies (Waits et al. 1998; Wooding and Ward 1997) because it is expected to have high homoplasy. Inclusion of this variable site provided additional haplotypes for analyses of fine-scale variation in gene flow.

Data quality.—Recent reviews have pointed out the importance of standardizing data quality-checking protocols and reporting error rates (Bonin et al. 2004; Paetkau 2003). We followed the recommendations of Bonin et al. (2004) in implementing a multifaceted quality-checking approach. All genotypes from noninvasive samples were verified by observing each genotype in at least 2 instances, either as a capture and a recapture or by repeated genotyping of unique samples. Approximately

one-third of tissue samples were regenotyped for verification. Finally, we used the program Validation (D. Roan, in litt.) to identify potentially erroneous genotypes by finding genotypes that differed by 2 or fewer alleles, which were then reamplified (Roan et al. 2005). Error rates were calculated as the ratio of erroneous alleles (those in disagreement between replicate runs) over the number of allelic comparisons made (Bonin et al. 2004).

Population structure analysis.—Population genetic structure was assessed using 3 Bayesian population assignment methods. We used aspatial models in the programs STRUCTURE 2.1 (Pritchard et al. 2000) and BAPS 4.0 (Bayesian analysis of population structure—Corander et al. 2006) as well as a spatial model in BAPS 4.0. These methods are useful in determining the number of genetically distinct groups within a sampled population (Latch et al. 2005). Spatial models are additionally useful for identify geographic boundaries between genetic groups (Corander et al., in press; Guillot et al. 2004). Although there are numerous definitions of genetic populations (Waples and Gaggiotti 2006), herein we refer to genetically distinct groups as groups of individuals in Hardy–Weinberg equilibrium and with significantly divergent allele frequencies from other groups.

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

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

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

Ancestry of each individual, in each genetic group, was recorded. The q -value describes the proportion of an individual's

genotypic ancestry that can be attributed to each identified genetic group. Using STRUCTURE, individuals were assigned to the group in which their ancestry (q) was highest. Using BAPS, individuals were assigned to genetic groups by the program before calculating q -values, so these were used only to judge admixture between groups. When the q -value in the assigned group was less than 0.75 the individual was considered to be of mixed ancestry. This arbitrary cutoff was selected to represent the amount of ancestry equivalent to 1 grandparent from outside the assigned group.

Genetic group assignments were mapped in ArcGIS. Individuals were identified as migrants if they were assigned to a genetic group other than the one in which they were sampled. In the case that genetic groups lacked distinct geographic boundaries, individuals in the range of overlap were not considered migrants. Minimum convex polygons were drawn (using Hawth's Analysis Tools ArcGIS Extension version 3.25—Beyer 2004) to encompass all nonmigrant 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 and deviations from Hardy–Weinberg equilibrium in the entire data set and in each identified genetic group using GENEPOP 3.4 (Raymond and Rousset 1995). We tested differences in allele frequencies (using GENEPOP) and calculated pairwise F_{st} (using Arlequin 3.01—Excoffier et al. 2005) as measures of differentiation between genetic groups. Bonferroni correction was applied to all cases of multiple comparisons. Genetic diversity was measured in terms of expected heterozygosity (H_E ; GENEPOP) and allelic richness (AR; FSTAT 2.9.3.2—Goudet 1995) in each genetic group.

When using multiple assignment tests, the most likely representation of population structure may differ among methods, making it necessary to develop criteria for selecting among options. In these analyses, we set the following criteria for determining the optimal partition of the data set: admixture between groups was minimal, linkage disequilibrium and Hardy–Weinberg equilibrium deviations were minimal, allele frequencies differed significantly between all groups, F_{st} values indicated significant divergence between all groups, and geographic overlap between groups was minimal.

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

TABLE 3.—Results showing the most likely number of genetically distinct groups within the data set according to the output of Bayesian assignment tests in STRUCTURE 2.1 and BAPS 4.0 (aspatial and spatial models). For each possible number of distinct groups (K) the log-likelihood ($L(K)$) and the probability (Prob.) are presented. For STRUCTURE results we also calculated the ΔK statistic for further verification of the most likely partition. Because BAPS results are based on stochastic optimization, rather than Markov chain Monte Carlo replicates, not all partitions are equally visited by the model. $L(K)$ was determined by the program based on the 10 best models visited; the probability was determined based on the 30 best models visited. Partitions not included in the best models visited are denoted “NV.” The most likely partition produced by each program is indicated in bold.

K	STRUCTURE			BAPS aspatial		BAPS spatial	
	$L(K)$	Prob.	ΔK	$L(K)$	Prob.	$L(K)$	Prob.
1	−4,605	<<0.001	—	NV	NV	NV	NV
2	−4,424	<<0.001	41.6	NV	NV	−4,714	<<0.001
3	−4,377	<<0.001	5.43	NV	0.02	−4,690	1.00
4	−4,308	1.00	69.87	−4,634	0.36	−4,713	<<0.001
5	−4,351	<<0.001	6.65	−4,636	0.62	NV	NV
6	−4,449	<<0.001	0.52	NV	NV	NV	NV
7	−4,530	<<0.001	1.03	NV	NV	NV	NV
8	−4,664	<<0.001	1.18	NV	NV	NV	NV
9	−4,725	<<0.001	1.38	NV	NV	NV	NV
10	−4,996	<<0.001	—	NV	NV	NV	NV

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

RESULTS

Data quality.—There was 1 individual for which the mtDNA locus failed to amplify, thus missing data accounted for 0.9% of the mtDNA data set. All haplotypes were observed more than once and there was no indication of ambiguity in any of the sequence data.

There were individuals with data missing at a single microsatellite locus, accounting for 7.7% of those individuals' genotype data and 0.28% of the microsatellite data set. The total allele-based error rates were 0.4% for hair samples and 1.4% for tissue samples. Locus-specific error rates averaged 0.8% (ranging from 0% at loci G10-O, Mu15, Mu50, and Cxx20, to 3.13% at locus Mu23). Closely related individuals or individuals recaptured in hair trapping could be easily distinguished based on a low probability of identity (PI) with 13 microsatellites: $PI = 6.08 \times 10^{-14}$ and $PI(sibs) = 7.56 \times 10^{-6}$.

Population structure analysis.—Results from STRUCTURE indicated 4 genetic groups in the data set, showing distinct groups on the mainland (ML), in Prince William Sound (PWS), and 2 groups on the Kenai Peninsula (KP1 and KP2; Table 3;

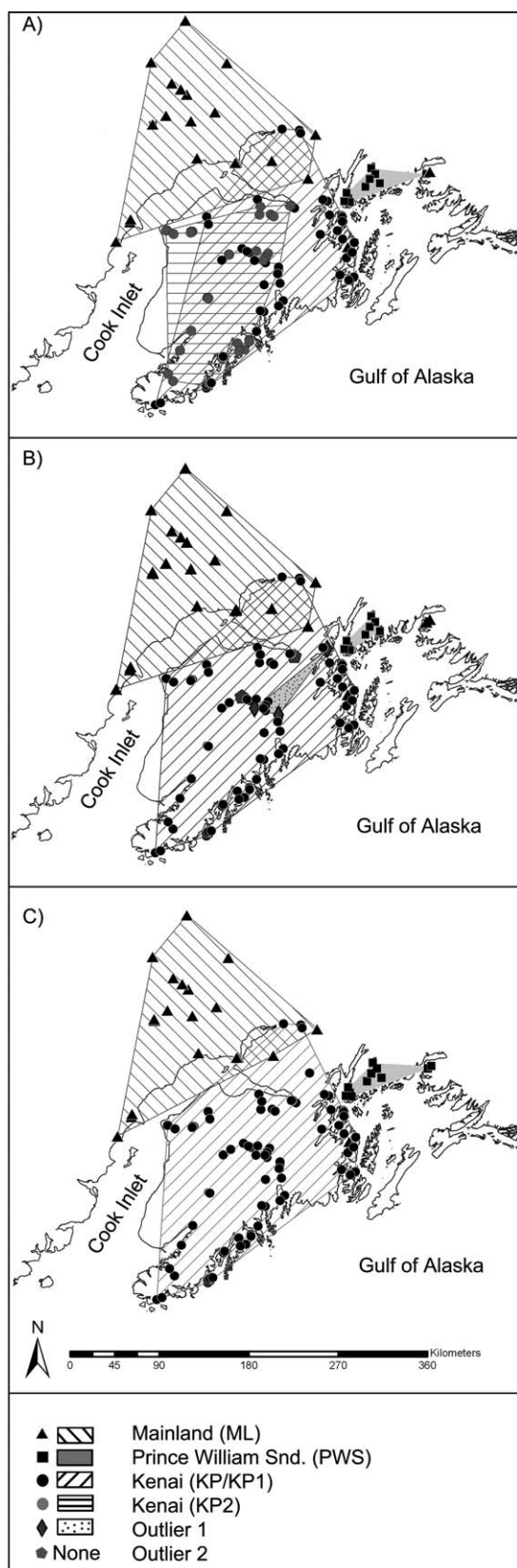


FIG. 2.—Geographic ranges of genetically distinct groups detected using Bayesian assignment tests: A) STRUCTURE, B) BAPS aspatial, and C) BAPS spatial. After removing migrants, minimum convex polygons were drawn to encircle the resident bears assigned to each genetic group. No polygon could be drawn around outlier 2 because it contained only 2 points.

TABLE 4.—Individual ancestry and admixture in each genetic group^a detected. The table presents the number of individuals assigned to each group detected using each assignment test program (STRUCTURE, BAPS aspatial [BAPSa], and BAPS spatial [BAPSs]). As measures of admixture, we show the average ancestry (q) of individuals assigned to each group and the number of individuals from each group that were admixed, migrants, or inhabiting areas of overlap between group ranges.

	KP	KP1	KP2	ML	PWS	Out1	Out2
Individuals assigned to each group							
STRUCTURE	—	36	42	21	11	—	—
BAPSa	73	—	—	23	9	2	3
BAPSs	79	—	—	20	11	—	—
Average q -value of assigned individuals							
STRUCTURE	—	0.797	0.760	0.910	0.878	—	—
BAPSa	0.861	—	—	0.851	0.926	0.940	0.935
BAPSs	0.914	—	—	0.882	0.933	—	—
Admixed individuals assigned to each group ($q < 0.75$)							
STRUCTURE	—	14	18	1	2	—	—
BAPSa	11	—	—	5	0	0	0
BAPSs	4	—	—	2	0	—	—
Migrants detected in each group							
STRUCTURE	—	1	0	1	1	—	—
BAPSa	1	—	—	0	0	0	0
BAPSs	1	—	—	0	0	—	—
Individuals in overlapping ranges							
STRUCTURE	—	21	23	2	0	—	—
BAPSa	3	—	—	1	0	2	3
BAPSs	3	—	—	1	0	—	—

^a KP = Kenai Peninsula; KP1 = Kenai Peninsula 1 of 2; KP2 = Kenai Peninsula 2 of 2; ML = mainland; PWS = Prince William Sound; Out1 = 1st outlier; Out2 = 2nd outlier.

Fig. 2A). BAPS aspatial indicated 5 groups, the same ML and PWS groups and 3 groups on the Kenai Peninsula (Table 3; Fig. 2C). Two of these were outliers containing 2 and 3 individuals only. They overlapped completely with the KP group. Allele frequencies failed to differ between outliers and the KP group at 11 of 13 loci. Thus, the outlier groups will be disregarded as suggested by the designers of BAPS (Corander et al. 2006). Results from BAPS spatial indicated 3 genetic groups; ML, PWS, and a single Kenai Peninsula group (KP; Table 3; Fig. 2B).

Taking into account the mixture of ancestry, population parameters, and the geographic mapping of group ranges, we concluded that the 3 groups indicated by BAPS spatial best represented the genetic structure in the study area. Hardy-Weinberg equilibrium deviations were similar, with each partition showing a single locus in disequilibrium in the PWS group. This can be expected given the small sample size representing this genetic group. The BAPS spatial partition minimized linkage disequilibrium (full data set: 10 locus pairs in linkage disequilibrium, 4 STRUCTURE groups - 5, 3 BAPS spatial groups - 3). The BAPS spatial partition showed the highest ancestry of assigned individuals and the lowest number of admixed individuals (Table 4). F_{st} values were similar between all comparisons of Kenai groups, ML, and PWS groups. F_{st} values were very low between KP1 and KP2 from

TABLE 5.—Differentiation between genetic groups^a detected using STRUCTURE, BAPS aspatial (BAPS_a), and BAPS spatial (BAPS_s). F_{st} was calculated in Arlequin for each partition of the data set.

STRUCTURE	KP1	KP2	ML
KP1			
KP2	0.031		
ML	0.072	0.096	
PWS	0.117	0.143	0.091
BAPS _a	KP	ML	
KP			
ML	0.074		
PWS	0.129	0.088	
BAPS _s	KP	ML	
KP			
ML	0.077		
PWS	0.120	0.093	

^a KP = Kenai Peninsula; KP1 = Kenai Peninsula 1 of 2; KP2 = Kenai Peninsula 2 of 2; ML = mainland; PWS = Prince William Sound.

STRUCTURE (Table 5). Maps of the genetic group ranges further supported the designation of 3 groups suggested by BAPS spatial (Fig. 2). The designation of KP1 and KP2 by STRUCTURE led to an almost complete range overlap, raising questions concerning the distinction between groups.

The KP group had the widest geographic range (27,000 km²), occupying the entire Kenai and merging onto the mainland. The ML group had a smaller geographic range (15,000 km²), 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 km², which also may have been influenced by the bounds of the sampling area.

The F_{st} values and intergroup migration indicated moderate levels of gene flow between genetic groups. Divergence was greatest in pairings including PWS (F_{st} = 0.093–0.120). Divergence was the lowest between the KP and the ML (F_{st} = 0.077). One migrant, from the mainland to the Kenai, was identified in all assignment tests (Fig. 3). An area of overlap between the ML and KP groups was also consistently identified in all tests. The extent of overlap was estimated most conservatively by BAPS spatial (Fig. 2).

Genetic diversity (H_E and AR averaged over 13 loci) was similar between ML and KP groups (Table 6). Both allelic richness and expected heterozygosity were lowest in PWS, although they did not differ significantly from other groups (t -test, AR P -value = 0.44, H_E P -value = 0.32). PWS showed heterozygote deficiency at 2 loci, Mu50 and G10J. Only Mu50 deviated significantly from Hardy–Weinberg equilibrium after Bonferroni correction.

Isolation by distance analysis.—The global Mantel test, using the full data set, indicated that genetic distance was significantly, although 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

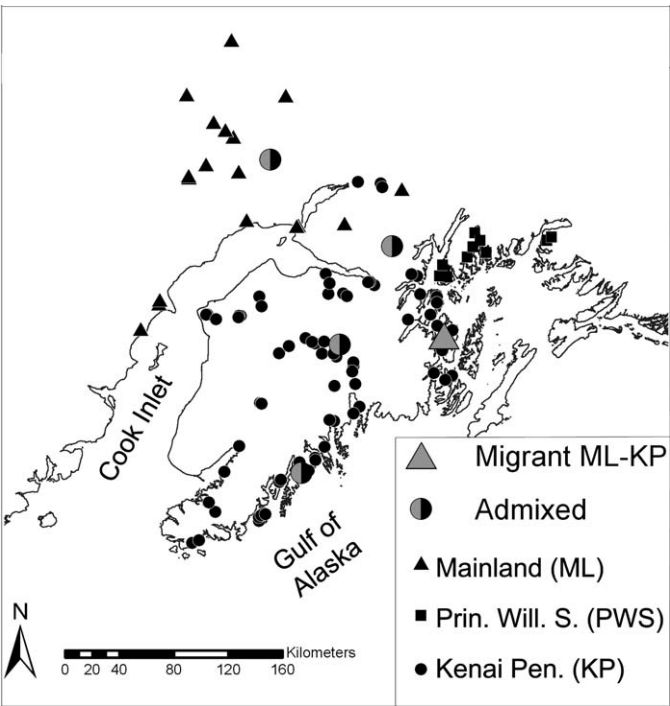


FIG. 3.—Gene flow between genetic groups is depicted showing migrants and admixed individuals. The partition of genetic groups shown is based on assignment test results from BAPS spatial.

group was insufficient to consider this IBD test reliable (Legendre and Fortin 1989).

Phylogeographic analysis.—Five haplotypes were detected in a 360–base pair segment of the mitochondrial control region (Fig. 4). Haplotypes were based on a single cytosine–thymine substitution and 3 insertion–deletion variations in the hyper-variable thymine repeat segment. The substitution coincided with that identified at nucleotide position 189 by Wooding and Ward (1997), defining lineages 1 (GenBank accession AF012305) and 7 (GenBank accession AF012311) of clade A, the continental black bear clade. Inclusion of the thymine repeat segment allowed us to further refine these 2 lineages into 5 sublineage haplotypes containing 6, 7, or 8 thymine nucleotides in positions 99–106. Sublineages defined here are denoted according to the original lineage number and the number of thymine repeats: 1_{t6}, 1_{t7}, 7_{t6}, 7_{t7}, and 7_{t8} (GenBank accessions EF198756–EF198864). The haplotype network in Fig. 4A illustrates the relationship among haplotypes.

TABLE 6.—Heterozygosity and allelic richness (AR) for each group defined by BAPS spatial assignment tests. The table shows the number assigned to each group (n), the proportion of heterozygotes expected (H_E) and observed (H_O), and allelic richness adjusted to the smallest sample size (AR₁₁). Values represent the average over 13 micro-satellite loci.

Group ^a	N	H_E	H_O	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

^a KP = Kenai Peninsula; ML = mainland; PWS = Prince William Sound.

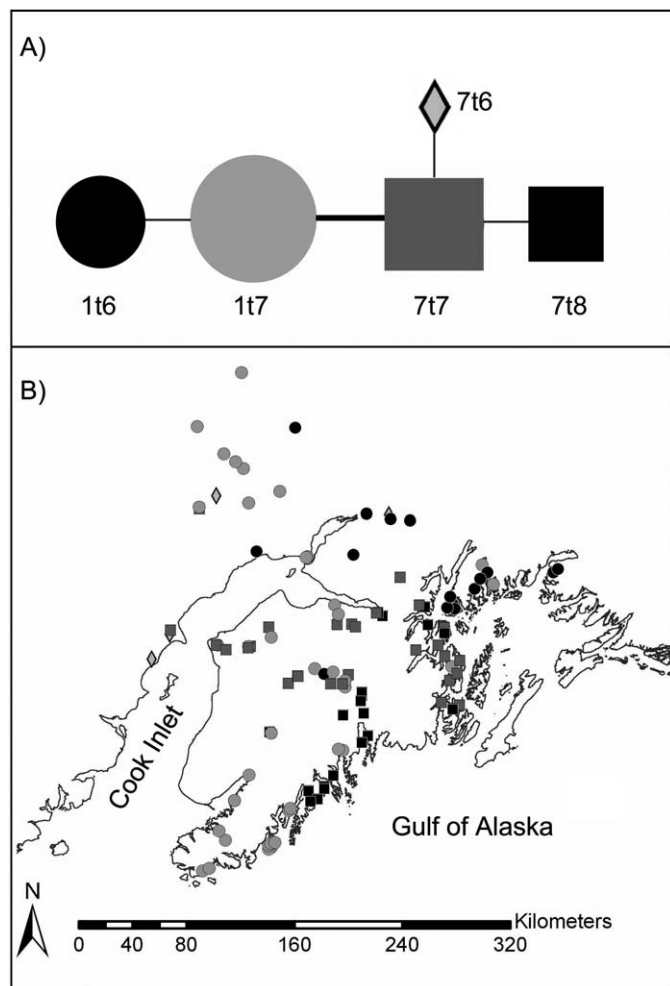


FIG. 4.—Haplotype network and distribution of 5 mitochondrial DNA control region haplotypes identified in black bears in south-central Alaska. A) Haplotype symbols are shown in a haplotype network in which the cytosine–thymine substitution is shown as a heavy line and lighter lines depict insertion–deletions in the hypervariable thymine segment. Symbol size is proportional to haplotype prevalence. B) The map shows the distribution of the 5 haplotypes.

The geographic distribution of haplotypes is depicted in Fig. 4B. Haplotype 1_{t7} was the most common and widespread, occurring throughout the study area ($n = 41$, range = 40,000 km²). Haplotype 1_{t6} was also common on the mainland ($n = 16$, range = 17,000 km²) 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. Although their ranges overlapped, 7_{t8} appeared to be more concentrated in the east (range = 10,000 km²), whereas 7_{t7} spanned the northern peninsula and occurred twice on the mainland (range = 14,500 km²). Haplotype 7_{t6} occurred only rarely ($n = 4$) and was confined to the mainland (range = 6,000 km²).

DISCUSSION

Population 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 deviation from Hardy–Weinberg equilibrium, allele frequencies and F_{ST} 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 oversplitting seen in STRUCTURE and BAPS aspatial. However, we did detect the potential for oversmoothing with BAPS spatial. The spatial smoothing appeared to yield conservative estimates of migration by assigning admixed individuals to the closest group contributing to their ancestry. This illustrates the need to carefully examine the individuals classified as admixed as well as migrants when evaluating gene flow (Fig. 3).

STRUCTURE, perhaps the most commonly used assignment test (Latch et al. 2005), detected 2 genetic groups on the Kenai Peninsula. Designation of these 2 groups would entail a substantial range overlap between groups, numerous admixed individuals, and even assignment of 1st-order relatives to different natal groups. This led us to explore reasons for oversplitting. Others have suggested that detection of population structure can be sex-dependent (Tiedemann et al. 2000), or confounded by isolation by distance (Laikre et al. 2005; Pritchard and Wen 2004). If oversplitting was due to signals of restricted dispersal by females, we would expect population assignments to coincide with mtDNA haplotypes, or for the male portion of the data set to show less structure. However, the clustering of mtDNA haplotypes did not spatially coincide with the ranges of KP1 or KP2. Further, the same KP1 and KP2 genetic groups were identified when STRUCTURE was run with only male individuals. Our analyses showed that IBD was significant among bears within the Kenai. Thus, the oversplitting of the Kenai bears into 2 groups was most likely an artifact of the model and perhaps attributable to influences of IBD.

BAPS is a newer assignment test software (Corander et al., in press). Identification of small outlying groups is a common problem acknowledged in the BAPS aspatial model (Corander and Martinen 2006; Latch et al. 2005). It may be tempting to ascribe significance to these outliers, such as representatives of unsampled populations. However, the prevalence of outlier groups even in simulated data sets makes these outliers appear to be artifacts of the model (Latch et al. 2005). Corander et al. (in press) suggest ignoring these outliers because 3 or fewer individuals cannot represent a panmictic breeding population (Corander et al., in press). However, they do represent a weakness in the model because these individuals could not

be assigned to any population or included in estimates of diversity or differentiation.

Isolation by distance.—In the global Mantel test, geographic distance was significantly correlated with genetic distance between individuals. This correlation was weak, suggesting that, although separation distance may be correlated, it may not be the primary factor affecting genetic distance between individuals. Population substructure was evident, thus violating the assumption of stationarity and potentially confounding the correlation between genetic and geographic distances. It is likely that barriers to gene flow isolating bears at the group level were exerting greater influence over genetic distance than separation distance alone. IBD was only significant within the KP group that was sampled across its entire range. This may indicate that the remaining groups were not sufficiently sampled to cover the entire population range and possible distance factors. A populationwise test of IBD over a larger scale may be more appropriate. For instance, Paetkau et al. (1998) showed substantial IBD between populations of brown bears across 1,790 km of northern North America.

Phylogeography.—Haplotype 1₁₇ was the most widespread in this study as well as others spanning North America (Paetkau and Strobeck 1996; Roon 2004; Wooding and Ward 1997). Lineage 1 was the most prevalent lineage identified by Wooding and Ward (1997), ranging from the American southwest to Alaska, and has been identified in other black bear studies from northeastern Alberta, Canada (Paetkau and Strobeck 1996), and Montana (Roon 2004). The work by Roon (2004) is the only other study to include the thymine repeat segment, and he also found 1₁₇ to be most prevalent in the Greater Glacier Ecosystem of Montana. Lineage 7 was less common in the data of Wooding and Ward (1997) and was not found elsewhere in the literature or in sequences published on GenBank (National Center for Biotechnology Information 2006).

The distribution of mtDNA haplotypes (7₁₇ and 7₁₈) on the Kenai was more geographically restricted than the groups identified through nDNA microsatellite analysis. The mtDNA can diverge or show population structure where nDNA does not because of 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 movement by black bears (particularly by females) on the peninsula than on the nearby mainland. Admixture via dispersal of males may lead to the weaker spatial structure detected in the nDNA microsatellite data. The haplotype distribution also could reflect signatures of past structure, because 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 and Calkin 1994). Future analyses incorporating historic and current landscape features may help to illuminate factors affecting gene flow within the Kenai group.

Biological interpretation.—Our results indicated that black bears on the Kenai Peninsula were genetically distinct from those on the mainland, and further that bears in Prince William Sound appeared to be isolated from other areas. The high male

bias in our data set would be expected to overrepresent the dispersing sex and make it more difficult to detect population structure. We can thus be confident that our assessment represents the minimum level of differentiation between these populations. Distinctiveness of Kenai populations has been documented in numerous taxa, particularly in carnivores. Kenai populations of Canada lynx (*Lynx canadensis*) and wolverine both show genetic distinction from more interior populations (Schwartz et al. 2003; Tomasik and Cook 2005). Distinction of gray wolves on the Kenai has been attributed to climatic differences and geographic isolation (Geffen et al. 2004; Weckworth et al. 2005).

The level of differentiation between KP, ML, and PWS groups suggested some restriction of gene flow between segments of the population, although 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 non-fragmented populations across the range of black bears (Arkansas and Louisiana—Csiki et al. 2003; British Columbia, Canada—Marshall and Ritland 2002; Quebec and Alberta, Canada—Paetkau and Strobeck 1994).

The F_{st} 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 ice fields. This is consistent with population structure detected for black bears in a similarly rugged area of southeastern Alaska. Peacock (2004) found F_{st} values of less than 0.1 between groups separated by large land distances or short water crossings, whereas bear populations separated by more formidable barriers, such as glaciated mountain ranges, or long saltwater crossings, showed F_{st} values as high as 0.12–0.29 (Peacock 2004). Faced with the rugged landscape of south-central Alaska, population connectivity may be particularly dependant on important corridors and linkage zones (Clevenger et al. 2002; Graves et al. 2007).

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

Black bears of central Prince William Sound appear to be isolated from other regions of the study area to the west and south (but note the small sample coverage of this population). There was no clear indication of migration into or out of the PWS group, although the presence of 2 admixed bears indicated migration at least in recent generations. The samples showing the most distinction were from Esther Island and the nearby peninsula, which may be particularly isolated within

PWS, or simply more connected with poorly sampled regions in the eastern portion of the sound. Four of 11 bears assigned to this group were closely related ($r > 0.5$), which would affect the distribution of alleles relative to Hardy–Weinberg equilibrium expectations and could, therefore, have influenced the Bayesian assignment methods (Pritchard and Wen 2004). However, it is possible that the bears of central PWS are isolated by saltwater crossings and expansive ice fields, leading to limited dispersal and high divergence from neighboring groups. Our results are intriguing and additional sampling in this region will be necessary to more thoroughly evaluate the connectivity of PWS bears and their neighbors. Such evaluation will be important as management planning ensues to protect black bears faced with increasing human use of PWS (Gimblett and Lace 2005).

Climatic history and past glaciations have played a role in shaping the historic levels of separation between black bear groups and continue to affect gene flow in south-central Alaska. Most of south-central Alaska was covered by the Cordilleran Ice Sheet at the last ice age maximum about 25,000 to 13,000 years ago (Muhs et al. 2001; Pielou 1991). South-central Alaska became deglaciated about 10,000 years ago. At that time ocean levels were such that the Kenai peninsula was largely continuous with the Alaskan mainland (Muhs et al. 2001; Pielou 1991). The Kenai became distinct and was relatively isolated from the mainland by 7,000 years ago. Haplotype distributions in south-central Alaska suggest that connectivity among black bear ranges was high during the initial recolonization of the area. Haplotype 1₇ appears to be the ancestral sublineage spreading from the continental United States throughout Alaska. Changing ocean levels and increasing isolation of the Kenai may have fostered the development of unique haplotype distributions on the mainland (7₁₆) and Kenai (7₁₇ and 7₁₈).

Our study has indicated that black bears on the Kenai constitute an important component of the genetic diversity of Alaskan black bears. Examination of mtDNA data shows that the Kenai population has unique haplotypes, but is not deeply diverged from mainland bears. The distinction of nDNA suggests that the Kenai bears are a distinct management unit as defined by Moritz (Moritz 1994, 2002). At present, population connectivity on the Kenai is high. Corridors such as the Nuka River 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. Use of a global positioning system collar also has tracked a black bear traveling from the mainland across Turn Again arm and through a Kenai Mountain valley (S. Farley, in litt.).

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

maintain the current diversity and structure and minimize impacts to important linkage zones and corridors.

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