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Black bears in Southeast Alaska: the fate of two ancient lineages in the face of contemporary movement

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Abstract

Biodiversity across a landscape is a product of both historical events and ongoing contemporary forces. The past and present factors that influence black bear *Ursus americanus* diversity on the Alexander Archipelago and mainland of Southeast Alaska were investigated by assessing nuclear genetic variation. The natural fragmentation of the region, the high vagility of black bears and their possible recent post-Pleistocene colonization to Southeast Alaska allowed us to discern between past and present forces characterizing diversity. Two known black bear lineages, estimated previously with mitochondrial DNA to have diverged 1.8 million years ago, remained evident in data from more rapidly evolving nuclear genetic markers. Two nuclear genetic clusters geographically correspond to the lineages, suggesting that contemporary movement since colonization (likely beginning 18 000 cybp) has not been sufficient to eliminate genetic differences between the highly divergent lineages. Concomitantly, the clearest pattern of genetic diversity is related to contemporary geographic patterns; contemporary geography differs from geography immediately after deglaciation due to sea-level change. Narrow saltwater straits, expansive ice fields, narrow beach fringes and saltwater inland bays separate genetically distinct groupings of black bears.

Introduction

Patterns of biodiversity across landscapes provide insight on the colonization and subsequent diversification of species. Although we can only observe a current snapshot of biodiversity, colonization and diversification take place at different temporal scales (Bilodeau, Felder & Neigel, 2005; Durand *et al*., 2005). Teasing apart past and present influences that have shaped biodiversity, such as ancient divergence or species vagility across a modern landscape, requires an instructive model system that can be studied comprehensively. We suggest that the sub-specific and population-level biodiversity of mammals in Southeast Alaska represents such a system for various reasons: colonization after deglaciation to the region was recent and rapid; Southeast Alaska is naturally and highly fragmented, providing an unambiguous geographic structure upon which to study the relationship between biodiversity and the modern landscape; and movement of even large mammals on this relatively pristine landscape is unhindered by human activities. Other biogeographical studies of mammals in Southeast Alaska have highlighted both the contribution of ancient divisions to modern diversity (Talbot & Shields, 1996) and the influence of the modern fragmented landscape on the high levels of diversity (Weckworth *et al*., 2005). Two ancient lineages of black bears *Ursus americanus*, which diverged 1.8 million years ago (Byun, Koop & Reimchen, 1997; Wooding & Ward, 1997; Stone & Cook, 2000), co-occur in Southeast Alaska (Stone & Cook, 2000). Both lineages may have colonized the region during the Holocene (Klein, 1963), or one lineage may have expanded from a refugium within Southeast Alaska, which has been suggested for other mammals (Heaton, Talbot & Shields, 1996). It is currently unknown whether one of the black bear lineages is a paleoendemic taxon or whether one lineage colonized substantially earlier than the other. We investigated the fate of the two black bear lineages in the face of contemporary movement, after colonization or expansion.

It is thought that most mammals colonized Southeast Alaska after the last glaciation, beginning roughly 18 000 cybp (Klein, 1963; Stuiver *et al*., 1998; Conroy, Demboski & Cook, 1999). Some of the sub-specific and population diversity found in mammalian taxa can be attributed to different lineages colonizing from refugia, north and south of the region, which persisted during the last glacial maximum (LGM; Josenhans *et al*., 1995; Byun *et al*., 1997, 1999; Wooding & Ward, 1997; Demboski, Stone & Cook, 1999; Stone, Flynn & Cook, 2002). Alternatively,

some lineages may have expanded from refugia within Southeast Alaska (Heaton *et al*., 1996). Similar patterns of converging, yet spatially segregated lineages are found in several mammalian species in this region: the dusky shrew *Sorex monticolus* (Demboski & Cook, 2001), marten *Martes americana* (Stone *et al*., 2002), brown bear *Ursus arctos* (Talbot & Shields, 1996) and black bears (Stone & Cook, 2000). These parallel patterns of intraspecific variation and high endemism in the region (Cook & MacDonald, 2001) suggest that some lineages may be paleoendemic, and thus expanded from within Southeast Alaska after deglaciation, or colonized much earlier after deglaciation in comparison with other lineages.

In some mammalian species, the distinction between the two lineages has been maintained by a lack of current (here defined as post-deglaciation, after 18 000 cybp) gene flow, whereas in others, continued colonization of local habitats and current movement across the fragmented landscape have begun to dilute differences between lineages. Researchers have described such patterns within and between some of the divergent lineages that co-occur in Southeast Alaska, using genetic markers that mutate at different rates, and thus characterize either historical (mtDNA) or current (nuclear) gene flow (Paetkau, Shields & Strobeck, 1998; Small, Stone, Cook, 2003). Several studies have used microsatellite markers (nuclear) to investigate current movement and highlighted how the combination of species-specific vagility and a fragmented landscape contribute to further differentiation or mixing of ancient lineages. For example, current movement is not observed between the northern flying squirrel sub-species *Glaucomys sabrinus griseifrons* found on the Prince of Wales Island complex and other sub-species in Southeast Alaska (Bidlack & Cook, 2002). This contrasts with contemporary movement among brown bear populations from historically divergent lineages (Paetkau *et al*., 1998). However, mixing within one lineage, between brown bears on Admiralty and Baranof-Chichagof islands, no longer occurs (Paetkau *et al*., 1998), indicating that contemporary movement patterns can contribute to diversification within a historical lineage. A combined knowledge of historical colonization patterns and the highly insular current landscape of Southeast Alaska, paired with genetic techniques spanning a variety of temporal scales, allows us to differentiate the temporal influences that have shaped, and are continuing to characterize, current biodiversity.

We sought to describe black bear population genetic diversity with respect to the two ancient lineages occurring in Southeast Alaska, and assess the comparative influences of modern movement and post-glacial colonization. The two lineages of black bears, termed coastal and continental, likely diverged at the beginning of the Pleistocene (Wooding & Ward, 1997) in two different glacial refugia. These lineages are now spatially segregated, yet converge in the geographic center of our study system (Stone & Cook, 2000). This pattern has allowed us to obtain large sample sizes of animals from the geographic regions where both lineages are found. Specifically, we evaluated whether the deep historic split within black bears, as evidenced by previous mtDNA studies (Stone & Cook, 2000), remains in data collected from rapidly evolving microsatellites. Because of the high vagility of black bears (Hellgren, Onorato & Skiles, 2005), we predicted that the distinction between the two historical lineages would no longer be detected with microsatellite markers, indicating mixing of the lineages since colonization. Consequently, we proposed that modern geographic barriers to movement, such as salt water, glaciers and mountains, would correspond with the observed patterns of nuclear genetic diversity. Lastly, we comment on how our data contribute to the discussion of whether one of the black bear lineages is paleoendemic.

Methods

Study population

We selected 289 black bear tissue samples from individuals killed by sport hunting in Southeast Alaska to assess population genetic structure (Fig. 1). We included samples from the major black bear islands of the Alexander Archipelago: Kuiu (1962 km^2; $n = 39$), Kupreanof (2813 km^2; $n = 35$), Prince of Wales (6675 km^2; $n = 37$), Mitkof (546 km^2; $n = 8$) and Revillagigedo (2965 km^2; $n = 22$). These islands occur in the region where coastal mtDNA lineage has been found (Stone & Cook, 2000). We also incorporated samples from the mainland of Southeast Alaska. The Yakutat sampling region ($n = 19$) is separated from the rest of Southeast Alaska by the Fairweather Range and its associated glaciers. South of the Fairweathers, the Chilkat Peninsula ($n = 34$) is separated from the Skagway ($n = 22$) region by the Chilkat Range at the Davidson Glacier. The Skagway region was bounded to the south at the latitude of Eldred Rock, an area where steep mountains descend immediately into Lynn Canal. We sampled the Juneau region ($n = 30$) from Eldred Rock to the north side of the Taku Inlet, the central mainland (from the Taku Inlet south to the Cleveland Peninsula; $n = 35$), and the southern mainland (the coastal fringe south of the Cleveland Peninsula to Misty Fjords; $n = 8$). All mainland sampling areas occur in the region where the continental mtDNA lineage of black bears was found, except for the southern mainland, which occurs in the geographical region where the coastal lineage occurs (Stone & Cook, 2000). We delineated these sampling regions based on our preconceived notions about barriers (including the idea of isolation-by-distance on the mainland) to black bear movement.

Laboratory methods

We isolated DNA from samples using the Qiagen DNeasy and QiagenDNeasy 96-well extraction kits (www.qiagen.com) according to the manufacturer's protocols, and amplified the DNA extract using polymerase chain reaction (PCR) at 12 microsatellite loci (Ostrander, Sprague & Rine, 1993; Paetkau & Strobeck 1994; Paetkau *et al*., 1995, 1998; Taberlet *et al*., 1997). DNA was amplified at seven of the

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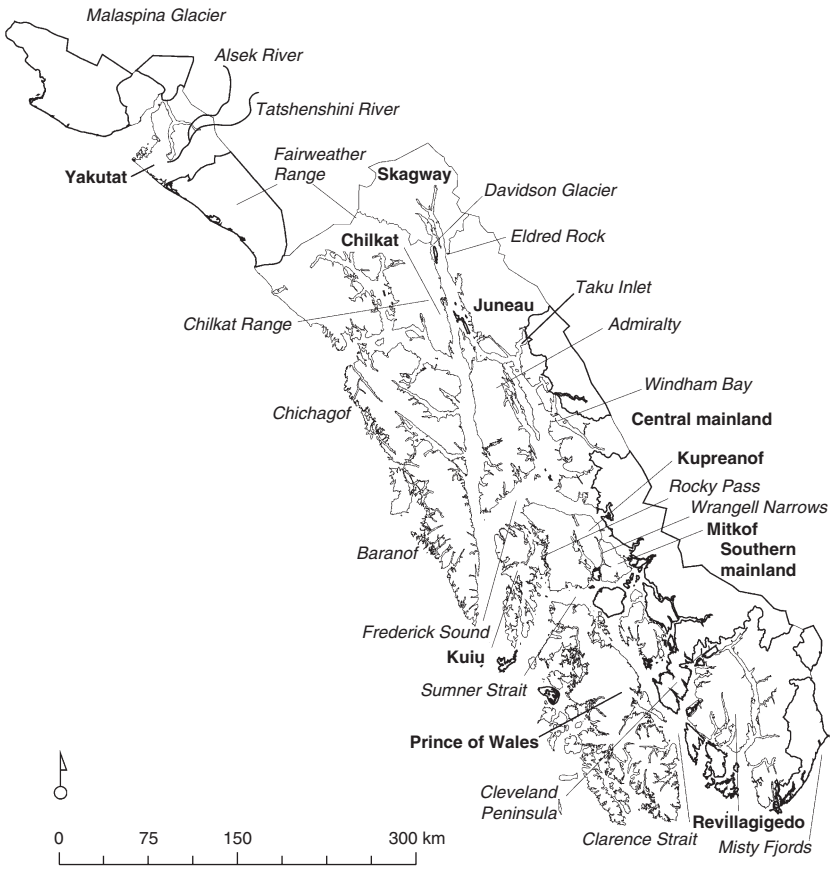

Figure 1 Alexander Archipelago and Southeast Alaska (54°45′N, 132°W). Names of black bear *Ursus americanus* sampling regions are in bold; other place names are in italics.

12 microsatellite loci (G1A, G10B, G10C, G10L, G10 M, G10O and G10X) at the Conservation Genetics Center at the University of Nevada according to conditions described in Peacock (2004). We genotyped PCR products using Applied Biosystems (ABI) 310, 3700 and 3730 automated sequencers and associated GeneScan and GeneMapper software at the Nevada Genomics Center at the University of Nevada – Reno. Wildlife Genetics International amplified and genotyped the samples at the remaining five loci (G10J, G10P, G10L, MU59 and CX110) according to the protocol in Paetkau *et al.* (1998).

Analysis

We assessed allelic richness and Nei's heterozygosity (Nei, 1987) to determine whether loci were sufficiently variable to assess the genetic structure of black bears in Southeast Alaska. To assess independence of loci, we tested for genotypic linkage disequilibrium among loci. We performed all calculations using F-STAT (Goudet, 2001).

We used Pritchard, Stephens & Donnelly's (2000) program STRUCTURE to assess the likelihood of individuals to assign to genetic clusters. These genetic clusters – or genetic types – identify distinct groupings of individuals, which are based on optimizing fit of the genetic data to the equilibrium expectations of Hardy–Weinberg and linkage disequilibrium. We used this Bayesian clustering method to group individuals into these clusters and simultaneously calculate individual assignments to those clusters. The program inferred q, each individual's proportional membership (assignment), to each of K clusters. We determined which value of K was most likely given the data. We suggest this optimal number of clusters represents the clearest pattern of current genetic diversity of black bears in Southeast Alaska. We also observed clustering patterns at non-optimal values of K to infer the pattern of recolonization and diversification that led to the current pattern, and to discover the geographic associations of ancestral groups (Parker *et al.*, 2004). In particular, we assessed the clustering pattern at $K = 2$ to determine whether the deep split associated with the two mtDNA lineages is detectable in the nuclear genetic data. We allowed for population admixture in STRUCTURE's estimation procedure and provided no initial information regarding sampling origin. This method allows us not to define population boundaries, but asks what genetic boundaries arise among black bears.

Results

We used 275 samples that amplified at least seven microsatellite loci to be used in all analyses. The average heterozygosity of black bears in Southeast Alaska ranged from

0.54 in Yakutat to 0.80 in the southern mainland sampling region ($\bar{X} = 0.69 \pm 0.07$, SD), sufficiently variable to address questions regarding genetic structure (Table 1). We found no genotypic disequilibrium between any pair of loci (66 pairwise comparisons) at the Bonferroni-corrected alpha value of 0.0008 after 1320 permutations, indicating that loci were independent.

We determined that the most optimal genetic structure of black bears in Southeast Alaska contained nine clusters or genetic types (Table 2, Figs 2a and 3). The likelihood of the data given nine clusters was 1.0 and was unambiguously highest compared with the likelihood of any other number of clusters; this likelihood was 12 orders of magnitude greater than the next most likely clustering pattern ($K = 10$). The distribution of the probability of the data given K was unimodal.

The genetic clustering pattern at $K = 2$ geographically corresponded with the two mtDNA lineages (Stone & Cook, 2000; Table 3, Figs 4 and 5). Clustering patterns of $K = 4$ and 6 (Fig. 2b and c) also had lower likelihoods, yet provide insight regarding the relative diversification of black bears suggested by the nine-cluster pattern (Figs 2a and 3).

The nine most likely genetic clusters had geographic affinities (Fig. 3). However, individuals within sampling regions were also assigned to various other clusters, and only 55% of the black bears in Southeast Alaska (152 of 275) could be assigned with probability >90% to any cluster; 79% of bears were assigned to a cluster with probability >75% (Table 3). Three pairs of sampling regions grouped together into three single clusters, invalidating these particular a priori assumed sampling regions. The *Northern South-east Cluster* (cluster names are indicated in italics to distinguish them from names of sampling regions) contained black bears from both the Chilkat Peninsula [the average proportional membership (q) of Chilkat bears to the *Northern South-east Cluster* was 0.66] and Skagway ($q = 0.70$). Bears sampled from Revillagigedo Island were associated with the *Southern South-east Cluster* ($q = 0.88$), as were bears from the southern mainland ($q = 0.42$); bears from Mitkof ($q = 0.71$) and Kupreanof ($q = 0.55$) islands grouped together in the *Mitkof–Kupreanof Cluster*. A genetic cluster emerged incorporating black bears sampled from both the Juneau and central mainland sampling regions, near the Taku River (*Taku Cluster*). The average proportional membership of bears sampled in the Juneau region being assigned to the *Taku Cluster* was 0.12, and for those sampled from the central mainland was 0.22. The *Taku Cluster* represented the only grouping that was not a priori assumed to be a distinct group of bears. Black bears from the five remaining sampling regions were assigned to five geographically consistent clusters: *Yakutat* ($q = 0.93$), *Juneau* ($q = 0.76$), *Central South-east* ($q = 0.64$), *Prince of Wales* ($q = 0.65$) and *Kuiu* ($q = 0.90$). Individuals from each sampling region were also assigned to non-geographically consistent clusters with probabilities ranging from <0.01 to 0.30. For example, the average probability of

Table 1 Allelic richness and Nei's heterozygosity of microsatellite data from black bears *Ursus americanus* in sampling regions in Southeast Alaska

Sampling region	Sample size	Allelic richness	Heterozygosity
Yakutat	16	1.58	0.54
Chilkat Peninsula	29	1.73	0.73
Skagway	20	1.72	0.72
Juneau	31	1.74	0.74
Kupreanof Island	34	1.69	0.69
Mitkof Island	8	1.70	0.67
Kuiu Island	39	1.63	0.63
Central mainland	34	1.79	0.79
Prince of Wales Island	37	1.65	0.65
Revillagigedo Island	20	1.68	0.68
Southern mainland	8	1.79	0.80

Table 2 Average proportional membership of black bear *Ursus americanus* individuals (q) from sampling regions to the nine most likely genetic clusters in Southeast Alaska identified by the program STRUCTURE

	Cluster								
Sampled population	Yakutat	Northern south-east	Juneau	Taku	Central south-east	Kupreanof–Mitkof	Kuiu	Prince of Wales	Southern south-east
Yakutat	**0.933**	0.012	0.007	0.006	0.011	0.007	0.009	0.005	0.011
Chilkat Peninsula	0.096	**0.658**	0.12	0.038	0.037	0.021	0.009	0.013	0.008
Skagway	0.131	**0.702**	0.035	0.016	0.041	0.027	0.016	0.005	0.027
Juneau	0.018	0.056	**0.762**	0.117	0.015	0.008	0.007	0.007	0.011
Central mainland	0.014	0.028	0.028	0.224	**0.639**	0.015	0.017	0.016	0.019
Mitkof Island	0.006	0.016	0.014	0.039	0.052	**0.712**	0.135	0.009	0.017
Kupreanof Island	0.006	0.014	0.008	0.026	0.023	**0.552**	0.304	0.051	0.017
Kuiu Island	0.008	0.006	0.005	0.006	0.01	0.048	**0.900**	0.006	0.01
Prince of Wales Island	0.097	0.027	0.083	0.079	0.01	0.031	0.013	**0.651**	0.01
Revillagigedo Island	0.006	0.006	0.008	0.054	0.009	0.01	0.008	0.016	**0.883**
Southern mainland	0.041	0.059	0.13	0.099	0.113	0.028	0.06	0.05	**0.419**
F_{ST} of cluster	0.29	0.12	0.26	0.10	0.18	0.16	0.34	0.19	0.21

Values in bold show the highest q-values for each sampling region.

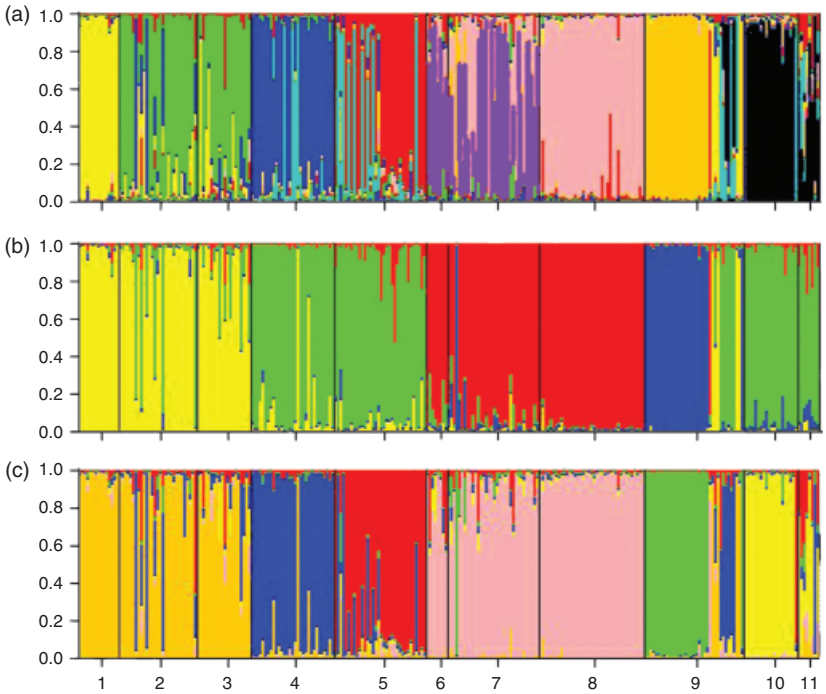

Figure 2 STRUCTURE assignment plots for black bear *Ursus americanus* individuals from sampling regions in Southeast Alaska. Individual samples are organized (each represented by a single vertical line) along the *X*-axis according to sampling region: 1, Yakutat; 2, Chilkat Peninsula; 3, Skagway; 4, Juneau; 5, central mainland; 6, Mitkof; 7, Kupreanof Island; 8, Kuiu Island; 9, Prince of Wales Island; 10, Revillagigedo Island; 11, southern mainland. The *Y*-axis is the probability of an individual assigning to each of the clusters (sum = 1). Plot (a) represents the nine most likely genetic clusters (represented by different colors) of black bears in Southeast Alaska. The colors correspond to the following clusters: yellow, *Yakutat Cluster*; green, *Northern South-east Cluster*; dark blue, *Juneau Cluster*; light blue, *Dispersed Juneau Cluster*; red, *Central South-east Cluster;* purple, *Kuiu–Kupreanof Cluster;* pink, *Kuiu Cluster*; orange, *Prince of Wales Cluster;* black, *Southern South-east Cluster*. Plots (b) and (c) show clustering patterns for four and six clusters, respectively. Note: The colors in (b) and (c) do not correspond to the colors presented for (a), as the clustering patterns are independent.

individuals sampled from the southern mainland region being assigned to the *Central South-east Cluster* was 0.11, whereas the average probability of individuals from Kupreanof Island being assigned to the neighboring *Kuiu Cluster* was 0.30.

The clusters that showed highest divergence were the *Kuiu Cluster*, *Yakutat Cluster* and *Juneau Cluster*, whose F_{ST} values were 0.34, 0.29 and 0.26, respectively. The clusters with least divergence were two mainland clusters: the *Taku Cluster* with an F_{ST} of 0.10 and the *Northern South-east Cluster* with an F_{ST} of 0.12.

Geographical concordance with mtDNA lineages

When we assumed the existence of only two genetic clusters, individuals from sampling regions north of and including the central mainland grouped together in the *Mainland Cluster* ($q = 0.83$–0.97; Table 3, Figs 4 and 5). Individuals sampled from the islands contributed to the *Island Cluster* ($q = 0.72$–0.97). At this broad level of clustering, the two geographic areas that maintained the least distinct bimodal pattern were Prince of Wales Island, in which black bears were assigned to both the *Mainland Cluster* ($q = 0.28$) and *Island Cluster* ($q = 0.72$), and the southern mainland [*Mainland Cluster* ($q = 0.38$) and *Island Cluster* ($q = 0.62$)]. The mean value of F_{ST} of the *Mainland Cluster* was 0.07, whereas F_{ST} of the *Island Cluster* was 0.14. Thirty alleles from 12 loci were found only to occur in either the *Island* (12 alleles) or *Mainland* (18 alleles) cluster. However, 29 of these alleles occurred at a frequency of less than 10% in the other cluster, and thus are not necessarily diagnostic. However, one allele, 154 base pairs in locus G10X, occurred with a frequency of 0.17 in the *Mainland Cluster* ($n = 122$), and never in the *Island Cluster* ($n = 123$).

Discussion

Evidence of the two ancient black bear lineages in Southeast Alaska remains in our more rapidly evolving nuclear genetic data. Two genetic clusters geographically correspond to the mtDNA lineages that have been previously found to co-occur in the region (Stone & Cook, 2000), despite the higher mutation rate of microsatellite markers. This finding is in contrast to our prediction and suggests that black bear movement since the colonization has not been sufficient to eliminate nuclear genetic differences between the two lineages.

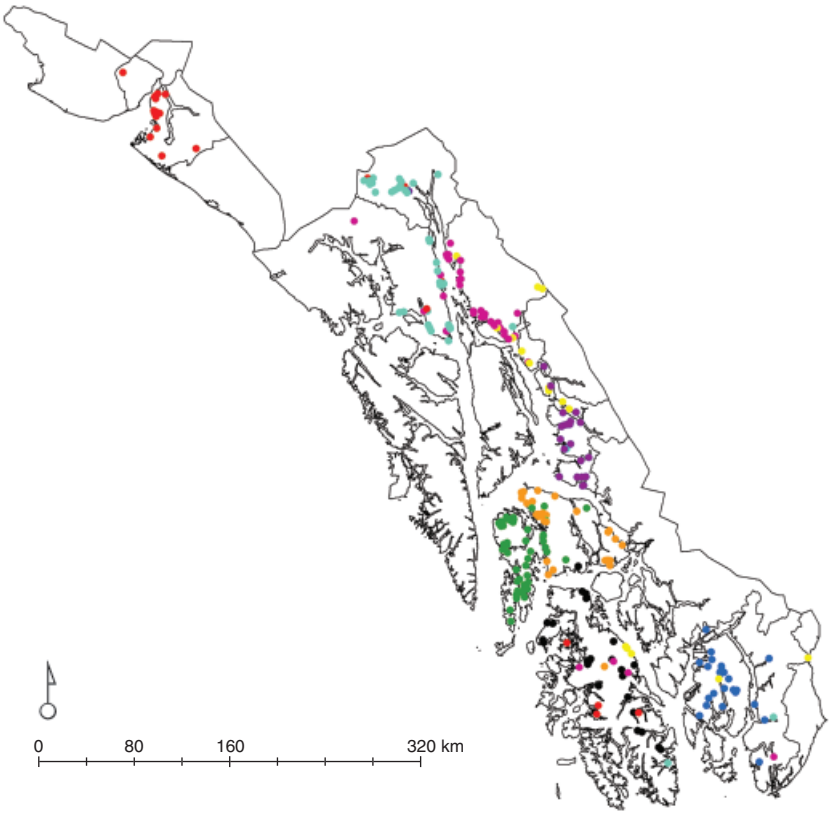

Figure 3 Map of the assignment of sampled black bear *Ursus americanus* individuals to the nine most likely clusters identified by STRUCTURE. The colors of dots represent the same genetic clusters as defined in the caption for Fig. 2a.

Table 3 Average proportional membership of black bear *Ursus americanus* individuals (q) from sampling regions (listed from north to south) to the two genetic clusters in Southeast Alaska identified by the program STRUCTURE; the two genetic clusters geographically correspond to the previously identified mtDNA lineages (Stone & Cook, 2000)

	Cluster	
Sampling region	Island	Mainland
Yakutat	0.019	**0.981**
Chilkat Peninsula	0.040	**0.960**
Skagway	0.041	**0.959**
Juneau	0.033	**0.967**
Central mainland	0.204	**0.796**
Mitkof Island	**0.899**	0.101
Kupreanof Island	**0.953**	0.047
Kuiu Island	**0.974**	0.026
Prince of Wales Island	**0.720**	0.280
Revillagigedo Island	**0.835**	0.165
Southern mainland	**0.624**	0.376

Bold values indicate the most likely cluster to which bears in the sampling regions were assigned.

The likelihood of this bimodal split, however, is much lower than the clearest pattern consisting of nine distinct clusters of black bears in Southeast Alaska. The likelihood of this pattern is 12 orders of magnitude greater than the next most likely clustering pattern, indicating the clarity or significance of the genetic divergence among these nine groups. These clusters may be distinct from one another due to restricted movement, time since contact or non-equilibrium (genetic drift-migration equilibrium) conditions such as genetic bottlenecks. These forces may also act in concert.

Genetic diversity within black bears in Southeast Alaska reflects contemporary geography as most of the genetic structure conforms to obvious modern geographic barriers, resulting from the modern sea level, such as narrow strips of beach fringe, glaciated mountain ranges and salt water. Genetic diversity of the wolf *Canis lupus* in the region also reflects fragmentation (Weckworth *et al.*, 2005), but at a larger geographic scale than for black bears, perhaps indicating higher vagility in wolves. The black bears sampled in the Yakutat region were found to be very distinct from black bears in the rest of Southeast Alaska. Of individuals sampled in Yakutat, 80% were assigned at $q = 0.90$. The genetic differentiation of Yakutat suggests that the 3000–4500 m peaks of the Fairweather Range and associated ice fields (~200 km between Yakutat and northern Southeast Alaska) pose a significant barrier to black bear gene flow. Results also suggest that even short saltwater crossing distances (< 100 m) resulted in genetically distinct clusters. The highly distinct *Kuiu Cluster* of bears is separated by the 100-m Rocky Pass from the *Mitkof–Kupreanof*

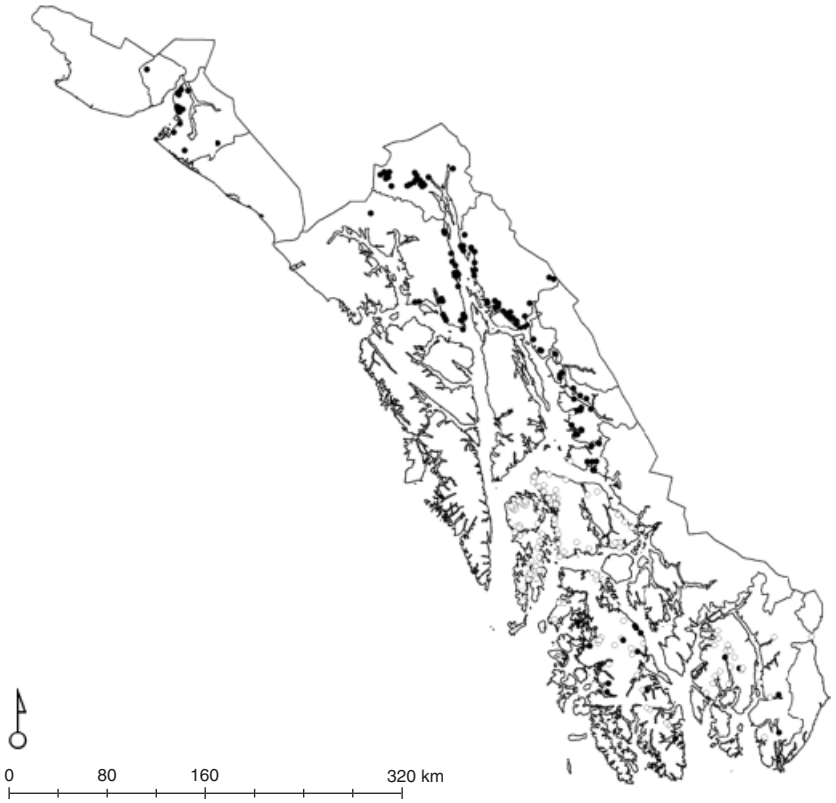

Figure 4 Map of the assignment of sampled black bear *Ursus americanus* individuals to the *Island Cluster* (white dots) and the *Mainland Cluster* (black dots).

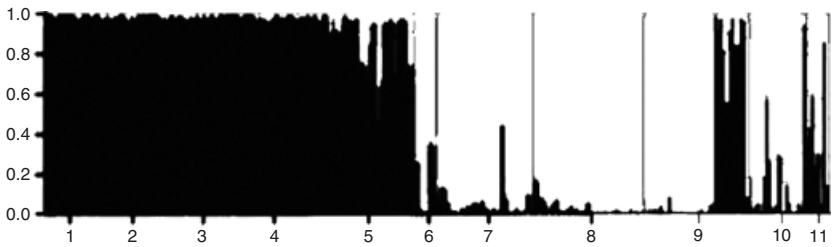

Figure 5 STRUCTURE plot for the two genetic clusters (black represents the *Mainland Cluster*; white represents the *Island Cluster*) that correspond, geographically, to the two mtDNA lineages of black bears *Ursus americanus* in Southeast Alaska. Individual samples are organized (each represented by a single vertical line) on the *X*-axis according to the region in which they were sampled: 1, Yakutat; 2, Chilkat Peninsula; 3, Skagway; 4, Juneau; 5, central mainland; 6, Mitkof; 7, Kupreanof Island; 8, Kuiu Island; 9, Prince of Wales Island; 10, Revillagigedo Island; 11, southern mainland. The *Y*-axis is the probability of an individual assigning to each cluster.

Cluster. This result is surprising for a species that is highly vagile (Hellgren *et al*., 2005). However, Kuiu Island is the only black bear population in Southeast Alaska known to have undergone a significant recent bottleneck (Peacock, 2004), highlighting the potential effect of demography, in addition to barriers to movement, on genetic clustering.

Not all black bear genetic clusters were defined by obvious physical barriers. The bears sampled near the Taku Inlet clustered together in the *Taku Cluster*, although there are no apparent geographic barriers defining the group. In general, the assignment probabilities of black bears to clusters on the Southeast Alaskan mainland were slightly lower than to island clusters. Distinct genetic types on the mainland, however, are apparent, suggesting the influences of even narrow beach fringes and inland bays on black bear movement. For example, while the *Northern South-east* and *Juneau* clusters are adjacent, bears in one region are rarely assigned to the other cluster ($q = 0.04$, 0.06). Again, these data suggest surprisingly little movement for black bears, given that the geographic centers of these adjacent clusters are only 140 km apart.

That genetic pattern and modern geographic structure do not match completely suggests that forces other than the current landscape influence black bear population genetic diversity. The evidence of two clusters that geographically correspond to the extents of the mtDNA lineages (Stone & Cook, 2000) suggest the lasting impact of the original patterns of colonization. Each of the nine most likely

clusters of black bears occurs entirely, with the exception of a few individuals, within either of the two larger clusters. This nested structure suggests that a degree of differentiation remains between the colonizing lineages. We stress, however, that the differentiation between these two clusters is not considerable, due to the high likelihood of the nine-cluster pattern. In comparison, the original colonizing lineage of flying squirrels retains a high degree of isolation, likely due to its much lower vagility (Bidlack & Cook, 2002). This lack of current gene flow among flying squirrels reinforces the distinction between lineages with different colonization histories. In contrast, Paetkau *et al.* (1998) found that modern gene flow provides the potential for homogenization of the two ancient brown bear lineages (Talbot & Shields, 1996). The case of the black bear in Southeast Alaska perhaps represents a case of intermediate vagility. Where the breakdown in clarity of the geographic division between the *Island* and *Mainland* clusters of black bears occurs likely delineates the area of secondary contact of the two mtDNA lineages. We suggest that this contact occurs in the proximity of the Cleveland Peninsula on the Southeast Alaskan mainland and Prince of Wales Island.

Patterns of differentiation within the two clusters of black bears also reflect both historical and contemporary factors. The *Island Cluster* shows higher divergence, similar to the coastal clade of martens in Southeast Alaska (*Martes americana caurina*; Small *et al.*, 2003). Small *et al.* (2003) posit that greater diversification within the coastal lineage of martens reflects greater isolation due to fluctuations in sea level earlier in the Holocene. This may also be true for the *Island Cluster* of black bears. There is also evidence for further diversification of brown bears within the paleoendemic mtDNA lineage (Talbot & Shields, 1996) as there is no current gene flow between populations that both belong to this group (Paetkau *et al.*, 1998). When individual black bears are assigned to the two clusters, it is evident that more animals sampled in the southern end of Southeast Alaska assigned to the more northerly *Mainland Cluster* than vice versa. This suggests a current general expansion southward of bears belonging to the *Mainland Cluster*, indicating ongoing differential diversification of the two groups.

We can infer relative timing of diversification from the intermediary clustering patterns (e.g. $K = 2$, 4 and 6) using the convergence of the two mtDNA lineages of black bears in Southeast Alaska as an anchor in time (Stone & Cook, 2000; Parker *et al.*, 2004). These inferences should be viewed as a hypothesis. We hypothesize that after colonization by the two lineages (or expansion of the coastal lineage), bears on the islands and the southern mainland became genetically differentiated from each other (i.e. the *Island Cluster* diversified). Following these events, black bears from Yakutat and northern Southeast Alaska (the Chilkat Peninsula and Skagway area) differentiated from other Southeast Alaskan mainland bears (bears near Juneau, the central and southern mainland), and bears on Prince of Wales Island differentiated from those on Revillagigedo Island. Differentiation of the Kuiu bears from the Kupreanof–Mitkof islands is less, and/or has happened only recently, or is a result of a bottleneck on Kuiu Island (Peacock, 2004). We can speculate on relative timing of diversification events if we assume that time increases genetic differentiation and that populations are at genetic equilibrium between genetic drift and migration. In addition, now submerged land forms may have been previously aerially exposed, as sea levels did not begin to rise significantly until after Southeast Alaska began to be deglaciated. Therefore, there has likely been an interactive effect of time and geographic isolation on the genetic distinctiveness of black bear populations.

Differences in male and female dispersal will influence genetic patterns revealed by mtDNA and nuclear DNA, as mtDNA is solely inherited matrilineally (Paetkau *et al.*, 1998). The bimodal split was still observed in the nuclear DNA despite known male-biased dispersal in black bears, which, if occurring in this region, would be expected to diffuse historical signals of differentiation. The characterization of these lineages with both genetic markers therefore suggests that the mtDNA lineages are not maintained merely by low female dispersal, but constrained by restricted male dispersal as well.

Our data and research questions do not directly address the question of whether the coastal lineage of the black bears is a Southeast Alaskan paleoendemic taxon, and cannot resolve this debate. Our data, however, can contribute to this discussion. Our results do not preclude the possibility of the expansion of the coastal lineage of the black bear from a refugium within Southeast Alaska. Black bear fossils have been found pre- and immediately post-LGM on Prince of Wales Island, supporting the idea of Prince of Wales Island as a refugium or a site of early colonization (Heaton *et al.*, 1996; Talbot & Shields, 1996; Heaton & Grady, 2003). Black bears from Prince of Wales Island in our study were assigned to six of the nine Southeast Alaskan population clusters. The ambiguity of these assignments suggests the island is a zone of population admixture. The maintenance of high black bear genetic diversity on Prince of Wales Island could be due to a combination of the island's large size, high rates of successful current and/or past dispersal. Prince of Wales Island is not currently less geographically isolated than some other studied black bear islands (e.g. Kuiu Island) in the Alexander Archipelago. Thus the current high level of genetic diversity may have resulted from Prince of Wales Island being relatively less isolated from the mainland during periods of lower sea level between 19 000 and 10 000 cypb. As Prince of Wales Island includes the range of black bear genetic variation found in the entirety of Southeast Alaska, the island may have been the site of early colonization post-glaciation, or a source (Cann, Stoneking & Wilson, 1987) of the modern Southeast Alaskan black bears.

Our nuclear genetic data, which reflect contemporary movement, used in concert with existing information on mtDNA genetic diversity have allowed us to discern between current and historical factors that have shaped black bear diversity in Southeast Alaska. Our work on black bears can help decipher the relative timing of glacial events in Southeast Alaska, which have also influenced the diversity of other species. While many studies suggest that black bears

are highly vagile, our work has shown that barriers to movement have curtailed movement to the extent that events occurring 10–16 000 cybp still shape current diversity. Assessing the relative strengths of historical and contemporary forces in shaping biodiversity can provide perspective for studies of biodiversity across the modern landscape.

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References

Bidlack, A.L. & Cook, J.A. (2002). A nuclear perspective on endemism in northern flying squirrels (*Glaucomys sabrinus*) of the Alexander Archipelago, Alaska. *Conserv. Genet.* **3**, 247–259.

Bilodeau, A.L., Felder, D.L. & Neigel, J.E. (2005). Population structure at two geographic scales in the burrowing crustacean *Callichirus islagrande* (Decapoda, Thalassinidea): historical and contemporary barriers to planktonic dispersal. *Evolution* **59**, 2125–2138.

Byun, A.S., Koop, B.F. & Reimchen, T.E. (1997). North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. *Evolution*. **51**, 1647–1653.

Byun, A.S., Koop, B. & Reimchen, T.E. (1999). Coastal refugia and postglacial recolonization routes: a reply to Demboski, Stone, and Cook. *Evolution* **53**, 2013–2015.

Cann, R.L., Stoneking, M. & Wilson, A.C. (1987). Mitochondrial DNA and human evolution. *Nature* **325**, 32–36.

Conroy, C.J., Demboski, J.R. & Cook, J.A. (1999). Mammalian biogeography of the Alexander Archipelago of Alaska: a north temperate nested fauna. *J. Biogeogr.* **26**, 343–352.

Cook, J.A. & MacDonald, S.O. (2001). Should endemism be a focus of conservation efforts along the North Pacific Coast of North America? *Biol. Conserv.* **97**, 207–213.

Demboski, J.R. & Cook, J.A. (2001). Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America. *Mol. Ecol.* **10**, 1227–1240.

Demboski, J.R., Stone, K.D. & Cook, J.A. (1999). Further perspectives on the Haida Gwaii glacial refugium. *Evolution* **53**, 2008–2012.

Durand, J.D., Tine, M., Panfili, J., Thiaw, O.T. & Lae, R. (2005). Impact of glaciations and geographic distance on the genetic structure of a tropical estuarine fish, *Ethmalosa fimbriata* (Clupeidae, S. Bowdich, 1825). *Mol. Phylogenet. Evol.* **36**, 277–287.

Goudet, J. (2001). *F-STAT, a program to estimate and test gene diversities and fixation indices, 2.9.3.2*. Lausanne, Switzerland: Institut d'Ecologie, Universite de Lausanne.

Heaton, T.H. & Grady, F. (2003). The late Wisconsin vertebrate history of Prince of Wales Island, Southeast Alaska. In *Ice age cave faunas of North America*: 17–53. Schubert, B.W., Mead, J.I. & Graham, R.W. (Eds). Indiana University Press, Indianapolis.

Heaton, T.H., Talbot, S.L. & Shields, G.F. (1996). An Ice Age refugium for large mammals in the Alexander Archipelago, southeastern Alaska. *Quat. Res.* **46**, 186–192.

Hellgren, E.C., Onorato, D.P. & Skiles, J.R. (2005). Dynamics of a black bear population within a desert metapopulation. *Biol. Conserv.* **122**, 131–140.

Josenhans, H., Fedje, D.W., Conway, K.W. & Barrie, J.V. (1995). Post glacial sea levels on the western Canadian continental shelf: evidence for rapid change, extensive subaerial exposure and early human habitation. *Mar. Geol.* **125**, 73–94.

Klein, D.R. (1963). Postglacial distribution patterns of mammals in the southern coastal regions of Alaska. *Fourteenth Alaskan science conference*, Anchorage, Alaska.

Nei, M. (1987). *Molecular evolutionary genetics*. New York: Columbia University Press.

Ostrander, E.A., Sprague, G.F. & Rine, J. (1993). Identification and characterization of dinucleotide repeat (Ca)N markers for genetic-mapping in dog. *Genomics* **16**, 207–213.

Paetkau, D., Calvert, W., Stirling, I. & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.* **4**, 347–354.

Paetkau, D., Shields, G.F. & Strobeck, C. (1998). Gene flow between insular, coastal and interior populations of brown bears in Alaska. *Mol. Ecol.* **7**, 1283–1292.

Paetkau, D. & Strobeck, C. (1994). Microsatellite analysis of genetic-variation in black bear populations. *Mol. Ecol.* **3**, 489–495.

Parker, H.G., Kim, L.V., Sutter, N.B., Carlson, S.C., Lorentzen, T.D., Malek, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E.A. & Kruglyak, L. (2004). Genetic structure of the purebred domestic dog. *Science* **304**, 1160–1164.

Peacock, E. (2004). *Population, behavioral and genetic studies of black bears (Ursus americanus) in Southeast Alaska*. PhD dissertation, University of Nevada – Reno. Reno, NV, USA.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 645–959.

Small, M.P., Stone, K.D. & Cook, J.A. (2003). American marten (*Martes americana*) in the Pacific Northwest:

population differentiation across a landscape fragmented in time and space. *Mol. Ecol.* **12**, 89–103.

Stone, K.D. & Cook, J.A. (2000). Phylogeography of black bears (*Ursus americanus*) of the Pacific Northwest. *Can. J. Zool.* **78**, 1218–1223.

Stone, K.D., Flynn, R.W. & Cook, J.A. (2002). Post-glacial colonization of northwestern North America by the forest-associated American marten (*Martes americana*, Mammalia: Carnivora: Mustelidae). *Mol. Ecol.* **11**, 2049–2063.

Stuiver, M., Reimer, P.J., Bard, E., Beck, J.W., Burr, G.S., Hughen, K.A., Kromer, B., McCormac, G., Van der Plicht, J. & Spurk, M. (1998). INTCAL98 radiocarbon age calibration, 24,000–0 cal BP. *Radiocarbon* **40**, 1041–1083.

Taberlet, P., Camarra, J.J., Griffin, S., Uhres, E., Hanotte, O., Waits, L.P., DuboisPaganon, C., Burke, T. & Bouvet, J. (1997). Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Mol. Ecol.* **6**, 869–876.

Talbot, S.L. & Shields, G.F. (1996). Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Mol. Phylogenet. Evol.* **5**, 477–494.

Weckworth, B.V., Talbot, S., Sage, G.K., Person, D.K. & Cook, J. (2005). A signal for independent coastal and continental histories among North American wolves. *Mol. Ecol.* **14**, 917–931.

Wooding, S. & Ward, R. (1997). Phylogeography and Pleistocene evolution in the North American black bear. *Mol. Biol. Evol.* **14**, 1096–1105.

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