Molecular Ecology (1998) **7**, 1283–1292

Gene flow between insular, coastal and interior populations of brown bears in Alaska

DAVID PAETKAU*, GERALD F. SHIELDS† and CURTIS STROBECK*
**Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9, †Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99775, USA*

Abstract

The brown bears of coastal Alaska have been recently regarded as comprising from one to three distinct genetic groups. We sampled brown bears from each of the regions for which hypotheses of genetic uniqueness have been made, including the bears of the Kodiak Archipelago and the bears of Admiralty, Baranof and Chichagof (ABC) Islands in southeast Alaska. These samples were analysed with a suite of nuclear microsatellite markers. The 'big brown bears' of coastal Alaska were found to be part of the continuous continental distribution of brown bears, and not genetically isolated from the physically smaller 'grizzly bears' of the interior. By contrast, Kodiak brown bears appear to have experienced little or no genetic exchange with continental populations in recent generations. The bears of the ABC Islands, which have previously been shown to undergo little or no female-mediated gene flow with mainland populations, were found not to be genetically isolated from mainland bears. The data from the four insular populations indicate that female and male dispersal can be reduced or eliminated by water barriers of 2–4 km and 7 km in width, respectively.

Keywords: Ursus arctos, brown bears, microsatellites, genetic distance, sex-biased dispersal, taxonomy

Received 19 October 1997; revision received 23 March 1998; accepted 23 March 1998

Introduction

Brown bears (*Ursus arctos*, including grizzly bears) are a widely distributed species, occurring throughout large parts of Europe, Asia and North America (Servheen 1990). The habitats in which brown bears can be found include arid regions of countries such as China and Turkey, temperate rain forests, and regions of boreal forest, taiga, and Arctic tundra across the northern hemisphere. Not surprisingly, both body size and population density vary dramatically across this range (e.g. Table 1).

The diversity of brown bear populations has prompted a tremendous effort in systematic description, the legacy of which is one of the most notorious examples of systematic oversplitting (Kurtén & Anderson 1980). In North America extreme synonymy has given way to a general recognition of just two or three subspecies: the large, relatively broad-skulled bears of the Kodiak Archipelago are recognized as *U.a. middendorffi*, but opinions differ as to whether the remaining populations comprise a single subspecies (*U.a. horribilis*; Rausch 1963) or should be broken into *U.a. dalli* (the large bears of coastal Alaska and British Columbia) and *U.a. horribilis* (the smaller 'grizzly bears' of the interior) (Fig. 1; Kurtén 1973).

The understanding of the relationships between North American brown bear populations was recently complicated further when it was found that the morphologically undistinguished brown bears of the ABC Islands of southeast Alaska had a mitochondrial DNA (mtDNA) haplotype that was more similar to haplotypes found in polar bears (*U. maritimus*) than those found in any other brown bears, including brown bears from mainland coastal areas immediately adjacent to the ABC Islands (Talbot & Shields 1996; Cronin *et al.* 1991; Shields & Kocher 1991). These data suggested that ABC brown bears may be reproductively isolated from other brown bear populations and may have been so for an extended period of time (Heaton *et al.* 1996).

Correspondence: D. Paetkau, Department of Zoology, University of Queensland, St Lucia, QLD 4072, Australia. Tel.: +61-7-3365-2966; Fax: +61-7-3365-1655; E-mail: dpaetkau@zoology.uq.edu.au

These mtDNA data and phylogenetic hypotheses make specific suggestions about gene flow in this part of the brown bear distribution. Isolation of the insular groups can be explained by invoking water crossings as barriers to brown bear dispersal. The Kodiak Archipelago is separated from the mainland by more than 35 km at the closest points, and this crossing would have to be made by swimming because these waters do not freeze in winter. The separation of the ABC Islands from the mainland is less than 5 km, and island hopping would allow this distance to be crossed with individual swims of less than 3 km. The genetic isolation between the relatively small bears of the interior (Kurtén's *U.a. horribilis*) and the larger bears of the coastal mainland (Kurtén's *U.a. dalli*) is more difficult to explain and frankly difficult to accept. These putative subspecies have long, common boundaries where no physical barriers to movement exist, so any genetic isolation that could be identified, would have to be explained in terms of barriers to hybridization.

To address the remaining uncertainty surrounding the genetic status of North America's coastal brown bears, we undertook a detailed population genetic survey employing a suite of biparentally inherited (nuclear) genetic markers [$(CA)_n$ microsatellites]; markers which have sufficient variability in brown bears to allow detailed study of population structure (Paetkau *et al.* 1997). Included in this survey were samples from the following: three interior study areas where the physically smaller (Table 1) 'grizzly bears' are found; Kodiak Island; the mainland coasts of southeast and southwest Alaska; each of the ABC Islands (Fig. 1). The distribution of these study areas allowed us to test whether Kodiak bears, coastal brown bears in general, or ABC bears specifically are genetically isolated from interior populations, and to study patterns of gene flow between insular and mainland populations.

Materials and methods

DNA was extracted from blood, skin, hair or meat samples, most of which were obtained during the course of field research projects conducted by others. All individuals were typed at the same eight microsatellite loci used by Paetkau *et al.* (1995) on polar bears. In addition, a subset of 55 animals from the ABC Islands, the Kluane study area and southeast coastal Alaska was typed at nine more loci (Table 2). Three of these additional loci were from a domestic dog library (Ostrander *et al.* 1993), two were from brown bears (Taberlet *et al.* 1997) and the remaining four were isolated from the same black bear library as the eight loci used on all individuals (Paetkau & Strobeck 1994; GenBank Accession numbers UAU 22084–95). The genotypes that we used for Kodiak Island, the Kuskokwim Mountains and Kluane National Park were from an earlier survey of intrapopulation genetic diversity in North American brown bears (Paetkau *et al.* 1998).

Microsatellite analysis used ABI's four-colour detection system on a 373A automated sequencer and genotypes were determined using Genotyper software (ABI). The 17 loci used were PCR amplified in eight reactions, and mixing reactions together after amplification allowed all loci from a single individual to be run in two gel lanes (Table 2). PCR reactions contained 50 mM KCl, 0.1% Triton X-100 and 160 μM dNTPs in a volume of 15 μL. The concentrations of $MgCl_2$, *Taq* polymerase and primers were optimized to permit co-amplification (Table 2). Thermal cycling was performed using a Perkin Elmer 9600.

As suggested by Paetkau *et al.* (1997), two genetic distances were calculated between each pair of populations: Nei's standard (D_S; Nei 1972) and the genotype likelihood ratio distance (D_{LR}). D_{LR}, which is based on the ratios of genotype likelihoods in pairs of populations, was chosen because it is calculated in a very different manner

Study area	2*N*	H_O	H_E	CL*	M/F*†	Density*
Admiralty	60	0.646	0.628	361	–/72	399; 440
Baranof	18	0.493	0.496	363	–	–
Chichagof	52			370	–	318
Kluane	100	0.788‡	0.761‡	330	63/43	40
Alaska Rge.	56	0.759	0.779	349§	80/52	15
Kuskokwim	110	0.700‡	0.682‡	–	–	–
Izembek	28	0.536	0.532	404	177/94	191
Kodiak	68	0.298‡	0.265‡	397	142/92	323; 342
Coast (l–z)	30	0.617	0.757	N/A	N/A	N/A

Table 1 Information about study areas. Number of chromosomes sampled (2*N*), mean observed (H_O) and expected (H_E) heterozygosity, mean condylobasal skull length (CL; mm; Rausch 1963), mean weight of adult males and females (M/F; kg; Harting 1987), and density estimate (number of bears per 1000 km^2; Miller *et al.* 1997; Pearson 1975).

* Study areas overlap with, but are not identical to, those used here.

† These values should be compared with caution because of variation in methods between studies.

‡ These data are from Paetkau *et al.* (1998).

§ This value is from the Denali region, west of the study area.

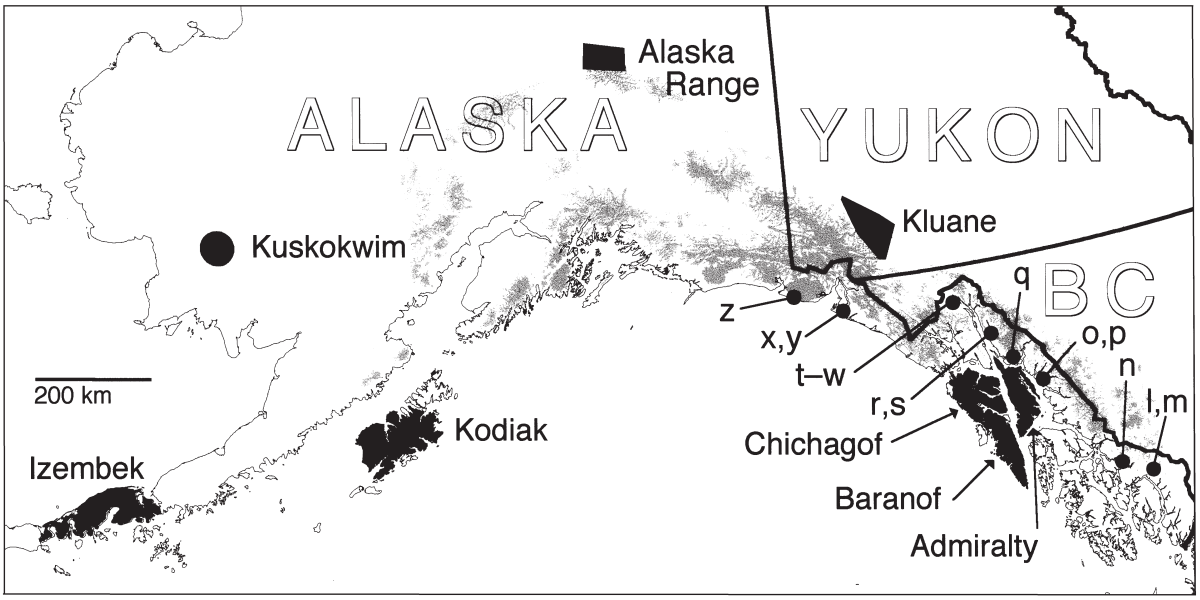

Fig. 1 Study areas (black). Fifteen individual samples were obtained from southeast coastal Alaska (l–z). Glaciers and icefields are shown in grey. According to Kurtén (1973) the Kuskokwim, Alaska Range, and Kluane samples are *Ursus arctos horribilis* whereas the ABC, Izembek and southeast coastal areas fall within the range of *U.a. dalli*.

Table 2 PCR primers and conditions. The 17 loci were amplified in a total of eight reactions (A–H). Four reactions were loaded in each gel lane (I or II). X_F, X_H and X_T refer to FAM, HEX and TET dye groups (ABI), respectively.

Locus	5' primer	3' primer	Primer concentration (nM)	PCR	$MgCl_2$ (nM)	Units of polymerase (*Taq*)‡
CXX20[a]	X_FAGCAACCCCTCCCATTTACT	TTGTCTGAATAGTCCTCTGCG	187	IA	2.1	3.2
CXX110[a]	X_HTGCTTTGGGTTAAATCTAAGCC	CCCCAGAGATGTGGCATC	320	IIB	2.1	3.2
CXX173[a]	X_HATCCAGGTCTGGAATACCCC	TCCTTTGAATTAGCACTTGGC	320	IC	2.1	3.2
G1A[b]*	X_T–ACCCTGCATACTCTCCTCTGATG	GCACTGTCCTTGCGTAGAAGTGAC	227	IID	1.9	2.8
G1D[b]	ACAGATCTGTGGGTTTATAGGTTACA	X_FCTACTCTTCCTACTCTTTAAGAG	320	IIE	1.9	2.8
G10B[b]	X_FGCCTTTTAATGTTCTGTTGAATTTG	GACAAATCACAGAAACCTCCATCC	240	IIE	1.9	2.8
G10C[b]	AAAGCAGAAGGCCTTGATTTCCTG	X_FGGGGACATAAACACCGAGACAGC	160	IIE	1.9	2.8
G10H	CAACAAGAAGACCACTGTAA	X_FAGAGACCACCAAGTAGGATA	227	IIF	1.9	2.0
G10J	X_FGATCAGATATTTTCAGCTTT	AACCCCTCACACTCCACTTC	253	IG	1.9	2.4
G10L[b]	X_TGTACTGATTTAATTCACATTTCCC	GAAGATACAGAAACCTACCCATGC	227	IID	1.9	2.8
G10M[b]†	TTCCCCTCATCGTAGGTTGTA	X_TAATAATTTAAGTGCATCCCAGG	320	IG	1.9	2.4
G10O	TGGTTATGAATCAGGATATTG	X_FCAACAGAACAATCCAAAGATG	320	IH	1.9	2.4
G10P[b]†	ATCATAGTTTTACATAGGAGGAAGAAA	X_HTCATGTGGGGAAATACTCTGAA	207	IC	2.1	3.2
G10U	X_TTGCAGTGTCAGTTGTTACCAA	TATTTCCAATGCCCTAAGTGAT	320	IA	2.1	3.2
G10X[b]†	CCACCTTCTTCCAATTCTC	X_HTCAGTTATCTGTGAAATCAAAA	160	IIB	2.1	3.2
UarMU50[c]†	X_TGGAGGCGTTCTTTCAGTTGGT	TGGAACAAAACTTAACACAAATG	320	IIF	1.9	2.0
UarMU59[c]†	X_TGCTGCTTTGGGACATTGTAA	CAATCAGGCATGGGGAAGAA	320	IID	1.9	2.8

[a]Ostrander *et al.* 1993; [b]Paetkau *et al.* 1995; [c]Taberlet *et al.* 1997.

* The dash indicates a 6-bp restriction site in the 5' primer that was actually used.

† Primers for these loci were altered from those originally published to avoid null alleles (Paetkau & Strobeck 1995), improve the strength of amplification, or to accommodate multiplexing by co-amplification or coloading. Earlier primers (unpublished) for locus G10O also gave null alleles in brown bears.

‡ Concentration approximate; enzyme was isolated using standard methods (Pluthero 1993) and calibrated aginst commercially available *Taq* polymerase (Perkin Elmer).

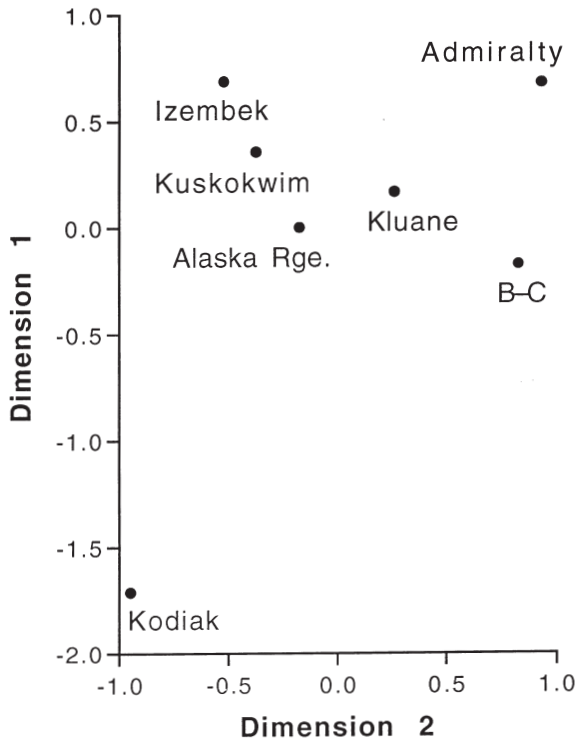

Fig. 2 Cluster analysis of genetic distances (D_{LR}; Table 4) between study areas using multidimensional scaling.

from many traditional genetic distance measures and appears to have low variance (Paetkau *et al.* 1997). Thus, D_{LR} can be used to provide a relatively independent confirmation of the relationships suggested by D_S. A two-dimensional plot of the D_{LR} data (Fig. 2) was created with NTSYS-pc 1.80 using linear nonmetric multidimensional scaling (Kruskal 1964a,b). This was only done for D_{LR} because the higher 'stress' value for the D_S distances (0.26 versus 0.16 for D_{LR}) indicated a relatively poor fit of the actual data to the two-dimensional plot.

For the 55 individuals typed at 17 loci, the distance between each pair of individuals was calculated as one minus the proportion of alleles shared (Bowcock *et al.* 1994). A phenogram (Fig. 3) was constructed from this distance matrix using the FITCH ('global' option on) and DRAWTREE programs in PHYLIP. Branches within the tree were rotated using MacDraw to facilitate comparison to the geographic distribution (Fig. 1).

An assignment test was performed using the methods of Paetkau *et al.* (1995) except that bias was avoided by subtracting each individual's genotype from the allele distributions in which they were included (instead of adding them to allele distributions in which they were not included). Expected genotype frequencies of zero were avoided by using a frequency of 0.01 for alleles not observed in a particular distribution. Calculators that perform the assignment test as well as calculations of genetic distance and allele sharing can be found at http://www.biology.ualberta.ca/jbrzusto.

Mean observed heterozygosity (H_O; eight loci) and an unbiased estimate of mean expected heterozygosity (H_E; Nei & Roychoudhury 1974) were calculated for each study area. GENEPOP 3.1b was used to test genotype distributions from each study area for conformation to Hardy–Weinberg (HW) expectations using the score (U) test (Rousset & Raymond 1995) with the specific alternative hypothesis of heterozygote deficiency. Global tests across loci and across populations were also made. The same software was used to test the homogeneity of allele distributions at each locus between each pair of populations using the probability test (Raymond & Rousset 1995). Results were combined across loci (Sokal & Rohlf 1995).

The relative effective sizes (N_e values) of insular populations were calculated using the stepwise mutation model [$H_E = 1 - (1/\sqrt{1 + 8N_e\mu})$; μ is mutation rate; Ohta & Kimura 1973]. As only relative sizes were considered, any value of μ could be used with the same result.

Results

The data set consisted of 206 brown bears typed at eight loci plus 55 individuals typed at 17 loci (Table 1). Of 224 single-locus pairwise tests of allele distributions, 203 indicated departures from homogeneity that were significant at the 5% level. When tests were combined across all loci,

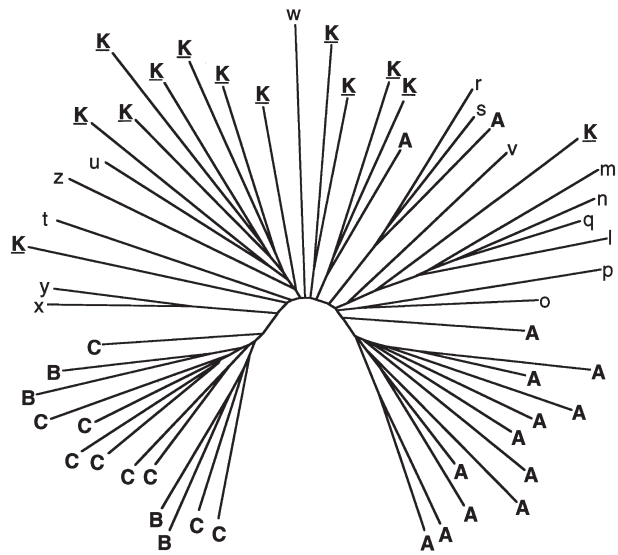

Fig. 3 A phenogram summarizing 17-locus allele-sharing distances between 55 individuals from Admiralty (A), Baranof (B), and Chichagof (C) Islands, Kluane National Park (CK) and coastal Alaska (l–z).

all pairs of study areas had highly significantly different allele distributions ($\chi^2_{16} > 64$, $P << 0.0001$) except Baranof and Chichagof Islands ($\chi^2_{16} = 22.2$, $P > 0.1$). The degree of differentiation seen between these two islands was less than that found between samples collected on two different peninsulas on Kodiak Island ($\chi^2_{10} = 17.4$, $0.01 P > 0.05$) or between two sets of 25 samples from Kluane divided approximately through the centre of that study area ($\chi^2_{16} = 27.7$, $0.05 > P > 0.025$). On this basis we combined the samples from Baranof and Chichagof Islands into a single study area (B-C) for further analysis. Note that this test of population subdivision appears to be so sensitive to population structure that it would be difficult to find study areas large enough to permit reasonable sample sizes of unrelated individuals, yet small enough to have internally homogenous allele distributions.

A total of 95 tests of heterozygote deficiency (≈HW) was performed (three study areas × 17 loci, B-C × 16 loci, three study areas × eight loci, Kodiak Island × four loci; reduced numbers due to nonvariable loci). Two individual tests were significant at the 5% level, but not when the Dunn-Sidák experimentwise error rate (Sokal & Rohlf 1995) was used. It should be noted that small sample sizes compromised the power of the HW test for the nine additional loci used on only 55 individuals.

The data set was checked for nonamplifying (null) alleles (Paetkau & Strobeck 1995; Pemberton *et al.* 1995) through global HW tests of each locus using data from all populations. Locus G1A showed a significant heterozygote deficiency ($P = 0.037$), although not when the number of tests (17 loci) was considered. These results, combined with the fact that complete genotypes were obtained for all individuals, lead us to conclude that most or all alleles were successfully amplified.

Brown bears have finite home ranges and dispersal (Canfield & Harting 1987) so they do not have strict random mating populations. Nonetheless, global tests of HW were also performed across loci for each population to show that the study areas were not large enough to result in a Wahlund (1928) effect. When only the eight loci used on all individuals were considered, the sample of 15 individuals from southeast coastal Alaska departed dramatically from HW proportions ($P < 0.0001$), but when all 17 loci were used the departure was less striking ($P = 0.021$). The large area over which these 15 samples were collected, the significant deficit of heterozygotes, the dramatic difference between H_E and H_O in this sample (Table 1) and the small sample size *per se* all suggested that it would be inappropriate to use this sample as a discrete study area for calculation of genetic distances. By contrast, the combined B-C study area did not differ significantly from HW expectations and had indistinguishable values of H_E and H_O supporting the decision to treat it as a single study area.

The assignment test was carried out using eight-locus data from all 261 individuals (Table 3). The overall rate of correct assignment was 92%, considerably higher than was observed in four populations of polar bears using the same loci (60%; Paetkau *et al.* 1995). All individuals from the insular Kodiak and B-C study areas were correctly assigned to their own study areas. There was a strong tendency for misassigned individuals to be assigned to the closest neighbouring study areas.

The two measures of genetic distance used here had a correlation coefficient of 0.96 despite the very different ways in which they treat the data. Furthermore, Paetkau *et al.* (1997) used data from the same eight loci to perform a regression of these two distance measures on geographic distance in a series of brown bear populations from North America's northern coast. They found a very strong relationship ($R^2 > 0.87$ in both cases). These data indicate that D_S and D_{LR} can provide meaningful insight into biological relationships, even when as few as eight microsatellite loci are used.

Genetic distance values were calculated between all pairs of study areas using eight-locus data (Table 4; Fig. 2). All the distances from Kodiak Island to other study areas were larger than any distance among those other study areas. Among all the study areas exclusive of Kodiak Island, genetic distances generally increased with the

Table 3 Assignment test results (approximately east to west)

Source population/*N*	Population to which individuals were assigned						
	Adm.	B-C	Klu.	Ala.	Kus.	Ize.	Kod.
Admiralty/30	**29**	1					
B-C/35		**35**					
Kluane/50		1	**45**	4			
Alaska Rge./28	1		1	**24**	1	1	
Kuskokwim/55			1	1	**47**	6	
Izembek/14					2	**12**	
Kodiak/34							**34**
Coast (l–z)/15	1	2	7	5			

Table 4 Genetic distances between study areas ($D_{LR}\backslash D_S$)

	Adm.	B-C	Klu.	Ala.	Kus.	Ize.	Kod.
Admiralty		0.44	0.36	0.54	0.45	0.54	1.40
B-C	5.28		0.22	0.62	0.41	0.45	0.94
Kluane	4.80	3.74		0.31	0.28	0.37	0.91
Alaska Rge.	5.95	7.09	2.66		0.22	0.46	0.66
Kuskokwim	6.95	6.84	3.82	2.75		0.13	0.69
Izembek	8.99	7.32	5.18	4.93	1.78		0.94
Kodiak	16.40	12.60	12.15	10.27	10.88	13.59	

degree of geographic separation between populations. The one exception to this rule was the distance between B-C and Admiralty which was much larger than would be expected for areas less than 8 km apart. Some of the distances between coastal (Kurtén's *U.a. dalli*) study areas and interior study areas were among the smallest distances found.

The allele-sharing tree (Fig. 3) showed a strong clustering of the B-C individuals and of those from Admiralty Island. The overall topology of the tree consisted of the B-C cluster at one end and the Admiralty cluster at the other, with the coastal and Kluane individuals branching off inbetween. The coastal samples from the region east of Admiralty Island (l–s in Fig. 1) grouped towards the Admiralty cluster, and those from northwest of Baranof and Chichagof Islands (x–z) were closer to the B-C cluster. The Kluane samples generally clustered towards the centre of the tree, but not as tightly as the clusters from the insular groups. The relatively weaker clustering of the Kluane samples was expected because there is greater genetic diversity within this study area than within the insular study areas. This caused higher within-population allele sharing distances, and thus poorer clustering.

Discussion

ABC brown bears

The analysis of genetic distance (Table 4, Fig. 2) clearly indicates that ABC brown bears are not genetically distinct from continental brown bears. The genetic distances from B-C to Kluane were among the smallest found among the groups surveyed. The distances between Admiralty and Kluane were larger, but were still smaller than distances between the most widely separated continental study areas (Izembek and Kluane). Interestingly, the Admiralty and B-C study areas were more distinct from each other than either was from the interior Kluane study area.

Although the 15 samples obtained from southeast coastal Alaska could not be used for genetic distance calculations, we wanted to find out how these samples related to those from the ABC Islands. This was done by analysing a subset of samples, including the 15 coastal samples, at 17 loci to allow calculation of genetic distances between individuals (Fig. 3). Consistent with the genetic distance data, the results of this detailed analysis of allele sharing between individuals contradict the hypothesis that ABC bears are a distinct group. If ABC bears were distinct they would be expected to cluster together but, while individuals from Admiralty Island formed one cluster and those from Baranof and Chichagof Islands formed another cluster, these two clusters were closer to individuals from the mainland than they were to each other. The fact that the clustering of the 15 mainland coastal samples reflected their geographical capture location indicates that this analysis can provide a remarkably powerful way to study the impact of landscape features on genetic relationships between individuals.

Another aspect of the data set that argues against the isolation of ABC bears is the level of genetic diversity observed within these study areas. The amount of genetic diversity maintained in a population is a function of its effective population size (N_e) and, while N_e itself is difficult to estimate without accurate knowledge of the mutation rate of the markers used, the ratio of N_e values in different study areas can be estimated under the assumption of equilibrium for genetic drift and mutation and the assumption of a stepwise mutational process (Ohta & Kimura 1973). The Kodiak, B-C and Admiralty study areas currently have similar population densities (Table 1; Miller *et al.* 1997), and the areas of each of the three ABC Islands are approximately half that of Kodiak Island. This would lead to the prediction that the N_e of the combined B-C population would be similar to that of the Kodiak population, and that the N_e of the Admiralty population would be about 50% smaller than either of these. Contrary to this prediction, when H_E was used to estimate N_e, the value obtained for the Kodiak population was 3.5-times smaller than the B-C estimate and 7.3-times smaller than the Admiralty estimate. When these calculations were made under the assumption of an infinite alleles model of mutation (Kimura and Crow 1964), these values were 2.7 and 4.7, respectively. This result could be explained if the densities of these populations have historically been

much different than they are today (i.e. density on the Kodiak Archipelago has been 14 times lower than that of Admiralty Island until very recently), but a more plausible explanation is that the N_e values of the Admiralty and B-C populations are increased by gene flow with populations from the mainland. Note that this argument suggests that there is more gene flow from the mainland into the Admiralty population than from the mainland into the B-C population.

All of the ABC and southeast coastal samples that we analysed have been studied by mtDNA sequencing (Talbot & Shields 1996; G. F. Shields and S. Williamson, unpublished). All ABC brown bear haplotypes differed from those of the coastal mainland by at least 31 fixed nucleotide substitutions in the cytochrome *b* gene alone. These mtDNA data raise very interesting questions about the history of this group, but they appear not to reflect the current genetic position of ABC brown bears as measured here using 17 nuclear genetic markers. A similar but less dramatic situation was seen in brown bears from North America's Arctic coast where a broad boundary between distinct mtDNA lineages, centered around the Yukon–Alaska border (Waits *et al.* 1998), did not correspond to any detectable nuclear genetic discontinuity (Paetkau *et al.* 1997).

The sharp contrast observed between mtDNA and nuclear genetic markers with the ABC brown bears can be explained if dispersal between the islands and the mainland is male-mediated. This explanation is consistent with the known behaviour of brown bears: females have smaller home ranges than males and do not disperse as far from natal ranges (Canfield & Harting 1987). These data emphasize the importance of using multiple biparentally inherited markers for studying the contemporary genetic structure of populations.

Coastal 'big brown bears'

The genetic data also clearly refute the hypothesis that the physically larger coastal brown bears form a genetic group that is isolated from the smaller bears of the interior. A minimal requirement of subspecific recognition for this group would be that the genetic distances between the Izembek study area and the ABC Islands would be smaller than from either of these areas to geographically closer interior populations. This is not the case. The Izembek–Kuskokwim distances ($D_S = 0.13$; $D_{LR} = 1.78$) are actually the smallest observed among any pair of study areas, including pairs of interior study areas, and are smaller than would be predicted by the linear regression of genetic distance on geographic distance in populations of brown bears along the Arctic coast of North America (Paetkau *et al.* 1997).

The allele-sharing data also refute the hypothesis that coastal brown bears are genetically distinct. If this hypothesis were correct one would have expected the ABC and coastal samples to group together, apart from the interior 'grizzly bear' samples from Kluane. In fact, this analysis suggests a simpler situation where the genetic distance between areas is a function of the distance and nature of the intervening landscape (Fig. 3).

Raush (1963) studied condylobasal skull length in an extensive series of skulls from North American brown bears and concluded that there was no basis on which to define a coastal subspecies because variation in skull length was clinal in nature. Strangely, Kurtén (1973) studied Raush's data and argued that, there was a basis for subspecific recognition because the gradient of the cline was so steep between interior populations and coastal populations. From a population genetic perspective, Kurtén's argument is difficult to accept; it suggests that a subspecies with a very long and narrow distribution, and with an extensive common boundary with an adjacent subspecies, can maintain genetic distinctiveness. Given that low levels of gene flow will homogenize populations in the absence of extreme selection against hybrids, it is hard not to suspect that the differences in size have little to do with genetics; the abundant coastal salmon resource is the most obvious single factor that has been cited as accounting for differences in size (Miller *et al.* 1997). The microsatellite data confirm this suspicion and demonstrate that the designation *U.a. horribilis* should be used throughout North America, with the possible exception of bears on the Kodiak Archipelago.

Kodiak brown bears

In an earlier analysis of the Kodiak data presented here, it was concluded that the extreme low genetic diversity observed in this population was best explained by an extended period of severe or complete isolation (Paetkau *et al.* 1998). In the current analysis, all the distances from the Kodiak study area to other study areas were greater than any distance among those other study areas (Table 4; Fig. 2). This supports the view that Kodiak brown bears have had little or no recent genetic exchange with continental populations.

It is tempting to use these data to date the isolation of Kodiak brown bears. However, even among the study areas exclusive of Kodiak Island (i.e. those study areas that do not currently appear to be genetically isolated), the largest distances ($D_S = 0.62$, $D_{LR} = 8.99$) were similar to those seen between populations of brown bears and North American black bears ($D_S = 0.62$, $D_{LR} = 7.50$; Paetkau *et al.* 1997). This indicates that the larger genetic distances in our data set may be at or near a plateau level determined by constraints on allele sizes at microsatellite loci (Feldman *et al.* 1997; Nauta & Weissing 1996), and that it would be unwise to use these data in an attempt to date the isolation of Kodiak brown bears.

Kodiak brown bears are distinguished by relatively broad skulls, not simply greater overall size as is the case for coastal bears, and appear to be isolated at this time. However, Kodiak bears share one of their mtDNA haplotypes with other brown bears from across Alaska. This suggests that the Kodiak Archipelago was colonized relatively recently, probably after the retreat of the Wisconsin ice (Talbot & Shields 1996). The most parsimonious hypothesis that can, therefore, be put forward regarding the history of this group is that the Archipelago was colonized at the end of the Wisconsin, that the founding population may have experienced rapid morphological change due to its small size and isolation, and that this population has been relatively isolated since sea levels approached their present height.

Rapid genetic change in small isolated populations is probably a common theme in evolution (it has been suggested, for example, that this is the explanation for the rapid divergence of polar bears from brown bears (Stanley 1979)), but the growing consensus among molecular biologists that taxonomic status should reflect only the length of time that two groups have been isolated (as measured by DNA sequences that accumulate mutations in a pseudoclockwise fashion) does not allow for such mechanisms. This probably makes it impossible to provide a suggestion for the subspecific status of Kodiak bears that will satisfy all people. However, it is the evolutionary history of Kodiak bears *per se* that is of primary interest, not their formal taxonomic description, and the genetic data collected to date have certainly enhanced our understanding of this history.

Gene flow in coastal populations

Among the continental regions included in this survey, the Izembek study area and the sample of fifteen southeast coastal bears stand out as having low H_O (0.54 and 0.62, respectively; Table 1). Because heterozygosity is a function of population size at equilibrium, these data suggest a high degree of genetic isolation in these regions relative to other continental populations. Southeast coastal Alaska is characterized by a thin strip of land backed by, and often interrupted by, huge icefields (Fig. 1). The reduced diversity observed in the southeast coastal samples probably results from the fragmented nature of the habitat in this region. Similarly, the Izembek data can be explained by the fact that this sample was obtained at the tip of the long, narrow Alaska Peninsula, and is thus relatively isolated compared to most continental populations. This effect may be exaggerated by moderately lower diversity in bears at the base of the Alaska Peninsula, as suggested by the Kuskokwim data (H_O = 0.70 versus 0.76 and 0.79 for Kluane and Alaska Range, respectively).

Dispersal over water barriers

By comparing the microsatellite data from southeast Alaska to a detailed map of the region (Fig. 4), it is possible to draw inference about the long-term dispersal habits of brown bears. First, Baranof and Chichagof Islands are approximately 600 m apart at their closest point. The amount of genetic differentiation (as measured by the allele distribution homogeneity test and the H–W test) between animals from these two islands was not measurably greater than that observed over similar distances on land (within Kluane or Kodiak Island). It appears that this water barrier is of little significance, and that bears on these two islands can be treated as a single population for genetic purposes.

Next, movement between the mainland and either Chichagof or Admiralty Islands requires two water crossings of around 2 km each, or a single larger swim. In this case there is strong mtDNA evidence that females rarely, if ever, undertake these movements. By contrast, it seems clear that male-mediated gene flow occurs at a rate sufficient to prevent these populations from becoming genetically isolated from continental populations.

Finally, despite the geographic proximity of the B-C and Admiralty populations, the allele-sharing tree and the genetic distances indicate that these populations are less genetically similar to each other than either is to the Kluane study area. This indicates that gene flow directly across the intervening Chatham Strait, which is never less than 7 km wide, is very limited if not absent. Considering the genetic differentiation of the B-C and Admiralty populations, and given that a direct crossing of over 35 km is required to

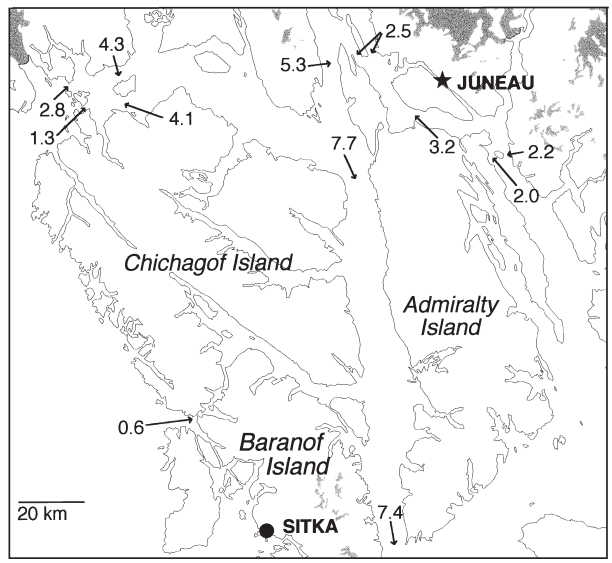

Fig. 4 Enlarged view of the ABC Islands showing the distances (km) between land areas.

move from the mainland to the Kodiak Archipelago, it is not surprising that the genetic evidence indicates little or no gene flow between Kodiak and the mainland.

It would clearly be desirable to compare these genetic data to field data on brown bear movements over water, and many bears on Admiralty and Chichagof Islands have been fitted with radio collars. Unfortunately, there is a strong bias towards fitting collars on females, and movements are only monitored within discrete study areas that do not include the adjacent mainland. This means that the inability to detect a radio collar could be due to instrument failure, movement outside the study area, or dispersal to the mainland. For these reasons no cases of movement between the ABC Islands and the mainland have been documented (K. Titus, Alaska Department of Fish and Game, personal communication).

Conclusions

The bears of the ABC Islands are not currently genetically distinct from adjacent mainland populations, although female-mediated dispersal from the islands to the mainland has apparently been limited or absent for some time. The bears of Baranof and Chichagof Islands can be considered as a single genetic population, and those on Admiralty Island as a second discrete, but not isolated, population. The 7-km wide straight separating these populations has apparently reduced or eliminated dispersal by either sex.

The brown bears of coastal Alaska are not genetically distinct from interior populations, and the designation *U.a. dalli* should be dropped in favour of *U.a. horribilis*, the designation used throughout most of North America. This suggestion was made more than 30 years ago (Rausch 1963), but now has a much stronger basis of support. Differences in body size between these groups can probably best be explained by ecological rather than genetic factors.

Kodiak brown bears are genetically isolated at the present time, but while the history of this group is now better understood, a final decision about its taxonomic status must await broader consensus on subspecies definitions.

Although the results give a dramatic example of how mtDNA data can provide an inaccurate measure of contemporary population structure, they also emphasize the special utility of mtDNA data for studying differential dispersal between the sexes (Avise 1995) and for studying historical relationships for which evidence has been lost from nuclear markers through male-mediated gene flow.

Acknowledgements

This project was made possible through help from L. Beier, J. Brzustowski, L. Craighead, H. Reynolds, P. Taberlet, S. Talbot, K. Titus, E. Vyse and S. Williamson, and support from Parks Canada, NSERC, the Southeast Regional Office of the Alaska Department of Fish and Game, as well as the Department of Biology and Wildlife, the Institute of Arctic Biology and the Center for Global Change at the University of Alaska Fairbanks.

References

Avise JC (1995) Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. *Conservation Biology*, **9**, 686–690.

Bowcock AM, Ruiz-Linares A, Tomfohrde J *et al.* (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368**, 455–457.

Canfield J, Harting AL (1987) Home range and movements. In: *Grizzly Bear Compendium* (eds LeFranc MN, Moss MB, Patnode KA, Sugg WC), pp. 27–35. National Wildlife Federation, Washington, DC.

Cronin MA, Amstrup SC, Garner GW, Vyse ER (1991) Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*). *Canadian Journal of Zoology*, **69**, 2985–2992.

Feldman MW, Bergman A, Pollock DD, Goldstein DB (1997) Microsatellite genetic distances with range constraints: analytic description and problems of estimation. *Genetics*, **145**, 207-216.

Harting AL (1987) Size. In: *Grizzly Bear Compendium* (eds LeFranc MN, Moss MB, Patnode KA, Sugg WC), pp. 13–15. National Wildlife Federation, Washington, D.C.

Heaton TH, Talbot SL, Shields GF (1996) An Ice Age refugium for large mammals in the Alexander Archipelago, southeastern Alaska. *Quaternary Research*, **46**, 186–192.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics*, **49**, 725–738.

Kruskal JB (1964a) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, **29**, 1–27.

Kruskal JB (1964b) Nonmetric multidimensional scaling: a numerical method. *Psychometrika*, **29**, 28–42.

Kurtén B (1973) Transberingian relationships of *Ursus arctos* Linné (brown and grizzly bears). *Commentationes Biologicae*, **65**, 1–10.

Kurtén B, Anderson E (1980) *Pleistocene Mammals of North America*. Columbia University Press, New York.

Miller SD, White GC, Sellers RA *et al.* (1997) Brown and black bear density estimation in Alaska using radiotelemetry and replicated mark-resight techniques. *Wildlife Monographs*, **133**, 1–55.

Nei M (1972) Genetic distance between populations. *The American Naturalist*, **106**, 283–292.

Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. *Genetics*, **76**, 379–390.

Nauta MJ, Weissing FJ (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. *Genetics*, **143**, 1021-1032.

Ohta T, Kimura M (1973) A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research*, **22**, 201–204.

Ostrander EA, Sprague GF, 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. *Molecular Ecology*, **4**, 347–354.

Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, **3**, 489–495.

Paetkau D, Strobeck C (1995) The molecular basis and evolutionary history of a microsatellite null allele in bears. *Molecular Ecology*, **4**, 519–520.

Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**, 1943–1957.

Paetkau D, Waits LP, Clarkson PL *et al.* (1998) Variation in genetic diversity across the range of North American brown bears. *Conservation Biology*, **12**, 418–429.

Pearson AM (1975) The northern interior grizzly bear *Ursus arctos* L. *Canadian Wildlife Service Report Series*, No. 34, 1–86.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, **4**, 249–252.

Pluthero FG (1993) Rapid purification of high-activity *Taq* DNA polymerase. *Nucleic Acids Research*, **21**, 4850–4851.

Rausch RL (1963) Geographic variation in size in North American brown bears, *Ursus arctos* L., as indicated by condylobasal length. *Canadian Journal of Zoology*, **41**, 33–45.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution*, **49**, 1280-1283.

Rousset F, Raymond M (1995) Testing heterozygote excess and deficiency. *Genetics*, **140**, 1413-1419.

Servheen C (1990) The Status and Conservation of the Bears of the World. *International Conference on Bear Research and Managment Monograph Series*, No. 2, 1–32.

Shields GF, Kocher TD (1991) Phylogenetic relationships of North American ursids based on analysis of mitochondrial DNA. *Evolution*, **45**, 218–219.

Sokal RR, Rohlf FJ (1995) *Biometry*, 3rd edn. Freeman, New York.

Stanley SM (1979) *Macroevolution: Pattern and Process*. Freeman, San Francisco.

Taberlet P, Camarra J-J, Griffin S *et al.* (1997) Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Molecular Ecology*, **6**, 869–876.

Talbot SL, Shields GF (1996) Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Molecular Phylogenetics and Evolution*, **5**, 477–494.

Wahlund S (1928) Zusammensetzung von populationen und korrelationserscheinungen vom standpunkt der vererbungslehre aus betrachtet. *Hereditas*, **11**, 65–106.

Waits LP, Talbot SL, Ward RH, Shields GF (1998) Mitochondrial DNA phylogeography of the North American brown bear and implications for conservation. *Conservation Biology*, **12**, 408–417.

Gerald Shields, who has been working on evolutionary and phylogeographic problems in wildlife species, and his student Sandra Talbot became interested in ABC brown bears when they obtained polar bear-like mtDNA from these bears. David Paetkau had been working on a PhD in brown bear population genetics in Curtis Strobeck's laboratory, where numerous projects on mammalian population genetics are currently underway, and had a special interest in studying the impact of landscape features on gene flow. The two groups came together at a bear conference and this project resulted.
