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Canadian Journal of Zoology

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Journal canadien de zoologie

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Volume 69 • Number 12 • 1991

Pages 2985–2992

Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*)

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Received January 14, 1991

CRONIN, M. A., AMSTRUP, S. C., GARNER, G. W., and VYSE, E. R. 1991. Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*). Can. J. Zool. **69**: 2985–2992.

We assessed mitochondrial DNA variation in North American black bears (*Ursus americanus*), brown bears (*Ursus arctos*), and polar bears (*Ursus maritimus*). Divergent mitochondrial DNA haplotypes (0.05 base substitutions per nucleotide) were identified in populations of black bears from Montana and Oregon. In contrast, very similar haplotypes occur in black bears across North America. This discordance of haplotype phylogeny and geographic distribution indicates that there has been maintenance of polymorphism and considerable gene flow throughout the history of the species. Intraspecific mitochondrial DNA sequence divergence in brown bears and polar bears is lower than in black bears. The two morphological forms of *U. arctos*, grizzly and coastal brown bears, are not in distinct mtDNA lineages. Interspecific comparisons indicate that brown bears and polar bears share similar mitochondrial DNA (0.023 base substitutions per nucleotide) which is quite divergent (0.078 base substitutions per nucleotide) from that of black bears. High mitochondrial DNA divergence within black bears and paraphyletic relationships of brown and polar bear mitochondrial DNA indicate that intraspecific variation across species' ranges should be considered in phylogenetic analyses of mitochondrial DNA.

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Nous avons évalué la variation de l'ADN des mitochondries chez les trois espèces nord-américaines d'ours, l'Ours noir (*Ursus americanus*), l'Ours brun (*U. arctos*) et l'Ours blanc (*U. maritimus*). Des haplotypes différents d'ADN mitochondrial (0,05 substitutions de base par nucléotide) ont été reconnus chez les populations d'Ours noirs du Montana et de l'Oregon. En revanche, il existe aussi des haplotypes très semblables chez les Ours noirs dans toute l'Amérique du Nord. Cette disparité entre la phylogénie des haplotypes et la répartition géographique indique que le polymorphisme s'est maintenu et qu'il y a eu circulation considérable des gènes durant toute l'histoire de l'espèce. Les différences dans les séquences d'ADN mitochondrial sont beaucoup moins importantes chez l'Ours brun et chez l'Ours blanc que chez l'Ours noir. Les deux formes morphologiques d'*U. arctos*, le grizzli et la forme côtière, ne font pas partie de lignées distinctes d'ADNmt. Des comparaisons interspécifiques indiquent que les Ours bruns et les Ours blancs ont un ADN mitochondrial assez semblable (0,023 substitutions de base par nucléotide), très distinct (0,078 substitutions de base par nucléotide) de celui des Ours noirs. La divergence importante de l'ADNmt chez les Ours noirs et les relations paraphylétiques entre l'ADN mitochondrial des Ours bruns et celui des Ours blancs soulignent l'importance de tenir compte de la variation intraspécifique géographique dans les analyses phylogénétiques de l'ADN mitochondrial.

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Introduction

Analysis of DNA sequence variation allows the phylogenetic relationships of alleles or haplotypes, as well as their distribution among populations, to be inferred (Wilson *et al.* 1985; Avise *et al.* 1987; Avise 1989). Studies of mitochondrial DNA (mtDNA) in natural populations have shown that there is often concordance of phylogenetic relationship and geographic distribution of mtDNA haplotypes as a result of geographic barriers to gene flow (Avise *et al.* 1987). Pocket gophers (*Geomys pinetis*, Avise *et al.* 1979) and deer mice (*Peromyscus maniculatus*, Lansman *et al.* 1983) are examples of small mammals with concordant patterns. In these species, distinct groups of related mtDNA haplotypes occur in different geographic regions. In house mice (*Mus*, Ferris *et al.* 1983) and rats (*Rattus*, Brown and Simpson 1981) the phylogenetic relationships of mtDNA haplotypes do not always correspond to geographic distribution. This may be due to human-mediated gene flow among these commensal rodents.

In some large mammals (white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus hemionus*), moose (*Alces alces*), elk (*Cervus elaphus*), and caribou (*Rangifer tarandus*)), mtDNA haplotype frequencies may vary among geographic areas, but there is no distinct correlation between phylogenetic relationship and geographic distribution of haplotypes (Cronin 1991*a*, 1992). The patterns may be explained by high levels of gene flow among these highly mobile deer, and colonization of recently deglaciated areas from common refugium populations. An exception is the relationship of mule deer and black-tailed deer (*O. h. columbianus*). These morphologically distinct subspecies are separated by the Cascade Mountain range, a well-recognized biogeographic barrier, and have highly divergent mtDNA (base substitutions per nucleotide (p) = 0.07, Carr *et al.* 1986; Cronin *et al.* 1988). The subspecies meet along the crest of the Cascade Mountains, where hybrid populations, with mtDNA characteristic of both subspecies, have been identified (Cronin 1991*a*). Similar patterns of high sequence divergence in contact zones of previously separated populations have been identified in other species (Avise *et al.* 1987).

Except for secondary contact zones, we are aware of only one example of high intraspecific mtDNA sequence divergence

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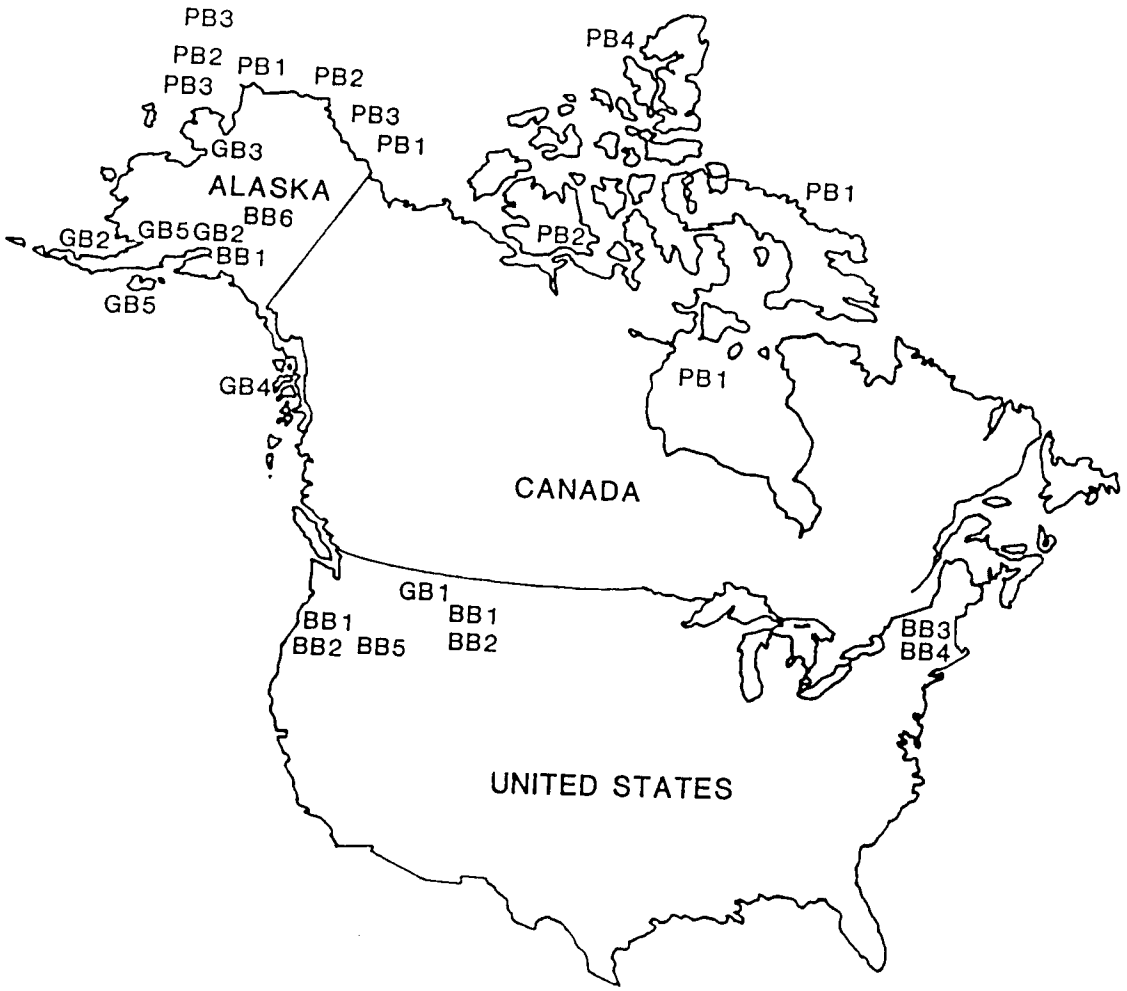

FIG. 1. MtDNA haplotype distribution across North American sampling locations for black bears (BB), brown bears (GB), and polar bears (PB).

within populations. Wayne *et al.* (1990) identified highly divergent mtDNA haplotypes ($p = 0.08$) within a population of black-backed jackals (*Canis mesomelas*). Wayne *et al.* (1990) suggested that the great dispersal capability of carnivores may result in considerable gene flow and co-occurrence of divergent haplotypes within populations.

Our objective was to investigate whether other carnivores exhibit high mtDNA divergence within geographic areas. In this paper, we describe mtDNA sequence divergence within and among three species of North American carnivore: black bears (*Ursus americanus*), brown bears (*Ursus arctos*), and polar bears (*Ursus maritimus*).

Materials and methods

Tissue samples were collected from across much of the North American ranges of all three species of bears (Fig. 1, Table 1). Numerous subspecies designations have been made for black bears and brown bears, but only one for polar bears (Hall and Kelson 1959). Many of the subspecies designations for black bears and brown bears are questionable because they have been based on analyses of small numbers of specimens and characters, and there may be considerable morphological variation within local populations (Kurtén and Anderson 1980; Stirling and Derocher 1990). Within North American *U. arctos*, however, two forms are commonly considered morphologically distinct, coastal brown bears and grizzly bears (Walker 1964). Both forms are represented in our samples: grizzly bears from Montana and interior Alaska, and coastal brown bears from Kodiak Island, the Alaska Peninsula, and southeastern Alaska. We will refer to these two forms collectively as brown bears. Polar bear samples were collected in the Alaskan Beaufort Sea, the Chukchi Sea, and the Northwest Territories, Canada. Field study suggests some restriction of movements of polar bears between these areas (S. C. Amstrup and G. W. Garner, unpublished data). Black bears were sampled from the eastern (New Hampshire), northern (Alaska), and western (Montana, Oregon) extremes of the species' range.

Blood was collected in tubes containing 15 mg potassium EDTA from 21 immobilized polar bears. All other samples consisted of muscle or brain tissue taken from bears killed by hunters or wildlife biologists. Tissues and blood were frozen at −20°C until processed.

Total genomic DNA was extracted from each sample by the method of Davis *et al.* (1980), with the addition of phenol and chloroform extractions prior to precipitation of nucleic acids. DNA from 29 black bears, 14 brown bears, and 11 polar bears was digested with 11 restriction enzymes with six-base recognition sequences: *EcoRI*, *Hin*dIII, *Bam*HI, *Hpa*I, *Sac*I, *Bcl*I, *Cla*I, *Xba*I, *Eco*RV, *Bgl*I, and *Pst*I. The combination of fragment patterns for these 11 enzymes was used to identify mtDNA haplotypes. DNA from additional bears was analysed with a subset of these 11 enzymes which were diagnostic for various haplotypes (Cronin 1991*a*). This included 17 Montana black bears and 6 Montana grizzly bears analysed with *Eco*RI, *Hin*dIII, *Bam*HI, *Hpa*I, *Sac*I, and *Bcl*I, and 6 Alaska grizzly bears, 33 brown bears, and 29 polar bears analysed with *Hin*dIII, *Cla*I, and *Bcl*I. Digested DNA fragments were separated electrophoretically on 0.7–0.8% agarose gels with Tris–borate–EDTA buffer (Sambrook *et al.* 1989). DNA was transferred to nylon filters by Southern blotting and filters were baked at 80°C for 2 h. Filters were prehybridized at

60–65°C in 5× 0.15 M NaCl plus 0.015 M sodium citrate (SSC), 1% sodium sarkosyl, 1× Denhardt's solution, 0.025 M potassium phosphate, 0.025 mg/mL salmon sperm DNA for 1 h. A P^{32}-labeled mtDNA probe was denatured at 100°C and added to the prehybridization solution. Initially we used a probe consisting of four *Eco*RI fragments constituting the mtDNA of a mule deer which had been cloned into a plasmid vector (PBluescript II, Strategene Inc., La Jolla, CA) (Cronin 1991*b*). When fresh bear tissue became available, we made a probe from polar bear mtDNA, isolated using the methods of Cronin *et al.* (1988) and further purified in low-melting-point agarose gels (Sambrook *et al.* 1989). Hybridization was at 60–65°C for 12–48 h with constant shaking. After hybridization, filters were washed for 30 min at room temperature with 2× SSC, 0.2% SDS, 1× Denhardt's solution, and for 2 h at 37°C with 2× SSC, 0.1% SDS. Filters were air-dried, covered with plastic wrap, and exposed to X-ray film for 3–240 h.

Sizes of digested mtDNA fragments were estimated from comparison with size standards (lambda virus DNA digested with *Hin*dIII). Composite mtDNA haplotypes were identified from variable fragment patterns (Lansman *et al.* 1983; Cronin *et al.* 1988; Lamb *et al.* 1989). Estimates of nucleotide sequence divergence (p) among the haplotypes were made with the fragment method of Nei and Li (1979). Divergence estimates were used to construct a dendrogram using a least squares method (Fitch and Margoliash 1967; Cavalli-Sforza and Edwards 1967) with the Kitsch program of the PHYLIP computer package (Felsenstein 1989). Haplotypes were also subject to phylogenetic analysis using parsimony (PAUP, Swofford 1990), with individual restriction fragments as characters.

Results

Six mtDNA haplotypes were identified in black bears, five in brown bears, and four in polar bears (Figs. 1 and 2, Table 1). The same mtDNA fragment patterns were obtained with both the deer and polar bear mtDNA probes, although the intensity of the patterns on X-ray film was greater with the polar bear probe.

The six black bear haplotypes occurred in two major groups. One group consists of haplotypes BB1, BB3, BB4, and BB6, and the other group consists of haplotypes BB2 and BB5 (Fig. 3). Within each group, mtDNA sequence divergences were low ($p < 0.01$) relative to the divergence between groups ($p = 0.03$–0.057; Table 2). Haplotypes of one group (BB1, BB3, BB4, BB6) were distributed among all locations (Table 1). Haplotypes from both groups occurred together in the same areas of Montana and Oregon. As with jackals (Wayne *et al.* 1990), there are divergent haplotypes within geographic areas. Certain black bear haplotypes were observed in only one location: BB3 and BB4 in New Hampshire, BB5 in Oregon, and BB6 in Alaska, and frequencies vary between Montana and Oregon. Haplotype BB1 occurs in 90% of Montana bears and only 14% of Oregon bears.

Low to moderate divergences were identified among the five mtDNA haplotypes of brown bears ($p = 0.002$–0.03; Table 2). The two morphological forms of *U. arctos*, grizzly and coastal brown bears, do not cluster as distinct mtDNA lineages (Fig. 3). Among grizzly bears, haplotype GB1 occurs in Montana, GB2 and GB5 in south-central interior Alaska, and GB3 on the Seward Peninsula, Alaska. Among coastal brown bears, haplotype GB2 occurs on the Alaska Peninsula, GB5 in all 26 bears from Kodiak Island, and GB4 in all 13 bears from southeastern Alaska. Haplotypes of some coastal brown bears, GB2 and GB5, are more similar to haplotypes of grizzly bears, GB1 and GB3, than to haplotype GB4 of other coastal brown bears. Haplotype GB4 is actually more similar to the polar bear haplotypes than it is to the other brown bear haplotypes (Table 2, Figs. 2 and 3).

Four mtDNA haplotypes with low sequence divergences ($p < 0.01$) were identified in polar bears. PB1 was the most common (73–79%) haplotype in each of the three areas sampled. Haplotype PB2 also occurred in all three areas. Haplotype PB3 occurred only in the two Alaskan locations, and PB4 on Ellesmere Island in the extreme north of the Canadian high arctic. Because of small sample sizes, the geographic distributions of haplotypes in all three species must be considered preliminary.

Average interspecific divergences were $p = 0.023 \pm 0.008$ (SD) for brown bear and polar bear, $p = 0.077 \pm 0.007$ for black bear and polar bear, and $p = 0.079 \pm 0.007$ for black bear and brown bear). The cluster analysis resulted in a dendrogram that reflects these relationships (Fig. 3). Phylogenetic analysis of restriction fragments using parsimony resulted in 14 equally parsimonious cladograms (119 steps long, consistency index 0.731). The topologies of the cladograms were similar to Fig. 3. The only differences between the cladograms and Fig. 3 (and among the 14 cladograms) were the relative placement of BB1, BB3, BB4, and BB6 within the black bear cluster, GB2, GB3, and GB5 within the brown bear cluster, and PB1, PB2, PB3, PB4, and GB4 within the polar bear cluster. In all of the cladograms and Fig. 3, haplotype GB4 clusters with polar bears, separately form the other brown bear haplotypes. This indicates that brown bears are paraphyletic with respect to polar bears in mtDNA phylogeny (Avise *et al.* 1983). That is, some brown bears share a more recent common ancestral mtDNA with polar bears than they do with other brown bears.

Discussion

Intraspecific variation

The level of divergence ($p = 0.046$–0.050) of the black bear haplotypes that occur together in Montana and Oregon (BB1, BB2, BB5) is high, particularly for individuals in the same geographic area (Avise *et al.* 1987; Wayne *et al.* 1990). It is more than twice as high as the average interspecific divergence of brown and polar bears ($p = 0.023$). This indicates that variable mtDNA haplotypes may be maintained in a species for periods of time that are long enough to allow significant divergence to occur. The high divergence among these black bear haplotypes in western North America, and the low divergence among haplotypes BB1, BB3, BB4, BB6 across the continent, indicate that the phylogenetic relationships of haplotypes and their geographic distribution are discordant. Black bears and black-backed jackals (Wayne *et al.* 1990) are two large carnivores with such a pattern.

Wayne *et al.* (1990) suggested that the discordance in distribution and haplotype patterns in jackals may be due to the high dispersal capabilities and large home ranges of carnivores. The same may be true for black bears. Although home range size and dispersal characteristics of black bears may vary with population density, habitat quality, and other factors, the great mobility and dispersal capabilities of black bears have been well documented and may result in the spread of mtDNA haplotypes among areas (Pelton 1982; Wathen *et al.* 1985; Rogers 1987). This may include secondary contact between previously isolated populations (Wayne *et al.* 1990). Black bears probably occurred only south of the North American continental ice sheets during the Pleistocene (Klein 1965; Kurtén and Anderson 1980). The occurrence of very similar black bear haplotypes (BB1, BB3, BB4, and BB6) in Alaska and the other sampling locations may have resulted from postglacial colonization of Alaska from the south.

Intraspecific divergences of brown bear haplotypes are lower than those of black bears, and there is no clear relationship

TABLE 1. Geographic locations, sample size, and mtDNA haplotypes of North American bears

	No. of bears	Haplotype designation	MtDNA description[a]
	Black bears		
U.S.A.			
Montana (south-central part of state)	19	BB1	AAAAAAAAAAA
	2	BB2	BBBABBBAABB
Oregon (statewide)	2	BB1	
	11	BB2	
	1	BB5	BCBABBBAABB
Alaska			
Kenai Peninsula	3	BB1	
Alaska Range	1	BB6	ADAAAAAAAAA
New Hampshire	1	BB3	AAAACAAAAAA
	1	BB4	AAAACAAABAA
	Brown bears[b]		
Montana			
Vicinity of Glacier National Park	6	GB1	CECBDCBBCCA
Vicinity of Yellowstone National Park	5	GB1	
Alaska			
Alaska Peninsula	2	GB2	CECCEDBCDCA
Seward Peninsula	1	GB3	CECCEDBDDCA
Islands of southeastern Alaska	13	GB4	CFDDDEBEDCA
Kodiak Island	26	GB5	CECCEGBDDCA
South-central interior Alaska	3	GB2	
	3	GB5	
	Polar bears		
Alaska			
Beaufort Sea	11	PB1	CFEDDEBEECA
	3	PB2	CGEDDEBEECA
	1	PB3	CFEDDEBFECA
Chukchi Sea	7	PB1	
	2	PB3	
	1	PB2	
Canada			
Northwest Territories	11	PB1	
	3	PB2	
	1	PB4	CFEDDFBGECA

NOTE: Of the bears in the study, 17 Montana black bears and 6 Montana grizzly bears were analysed with only *Eco*RI, *Hin*dIII, *Bam*HI, *Hpa*I, *Sac*I, and *Bcl*I, and 6 Alaskan grizzly bears, 33 brown bears, and 29 polar bears were analysed with only *Hin*dIII, *Cla*I, AND *Bcl*I.

[a]Refers to restriction fragment patterns for the following enzymes (in order from left to right): *Eco*RI, *Hin*dIII, *Bam*HI, *Hpa*I, *Sac*I, *Bcl*I, *Pst*I, *Cla*I, *Eco*RV, *Xba*I, and *Bgl*I. Fragment patterns are available from the senior author.

[b]Brown bears from Montana, south-central interior Alaska, and the Seward Peninsula, Alaska, are grizzly bears, and those from the islands of southeastern Alaska, Kodiak Island, and the Alaska Peninsula are coastal brown bears.

between mtDNA phylogeny and geographic distribution. Haplotypes with low divergence occur in Montana and Alaska (GB1, GB2, GB3, GB5), whereas relatively divergent haplotypes occur within Alaska (GB4 vs. GB5). In addition, the coastal brown bears and grizzly bears are not in distinct mtDNA lineages.

Although the present North American distribution of brown bears is discontinuous, prior to European settlement the distribution of brown bears was nearly continent-wide (Craighead and Mitchell 1982). Brown bears were only found north of the continentnal North American ice sheets during the Pleistocene (Klein 1965; Kurtén and Anderson 1980). The occurrence of similar brown bear haplotypes in Montana and Alaska may have resulted from postglacial colonization from the north of areas south of the ice.

Polar bears are circumpolar in distribution, occurring in the ice-covered seas of the northern hemisphere (Amstrup and DeMaster 1988). The distribution of polar bears differs from those of black bears and brown bears because they are continuously distributed across their range, there being no geographic barriers or disjunct populations. Polar bears are the most mobile of all nonaquatic mammals (Garner *et al.* 1990), and there is the potential for gene flow across the entire range of the species. The

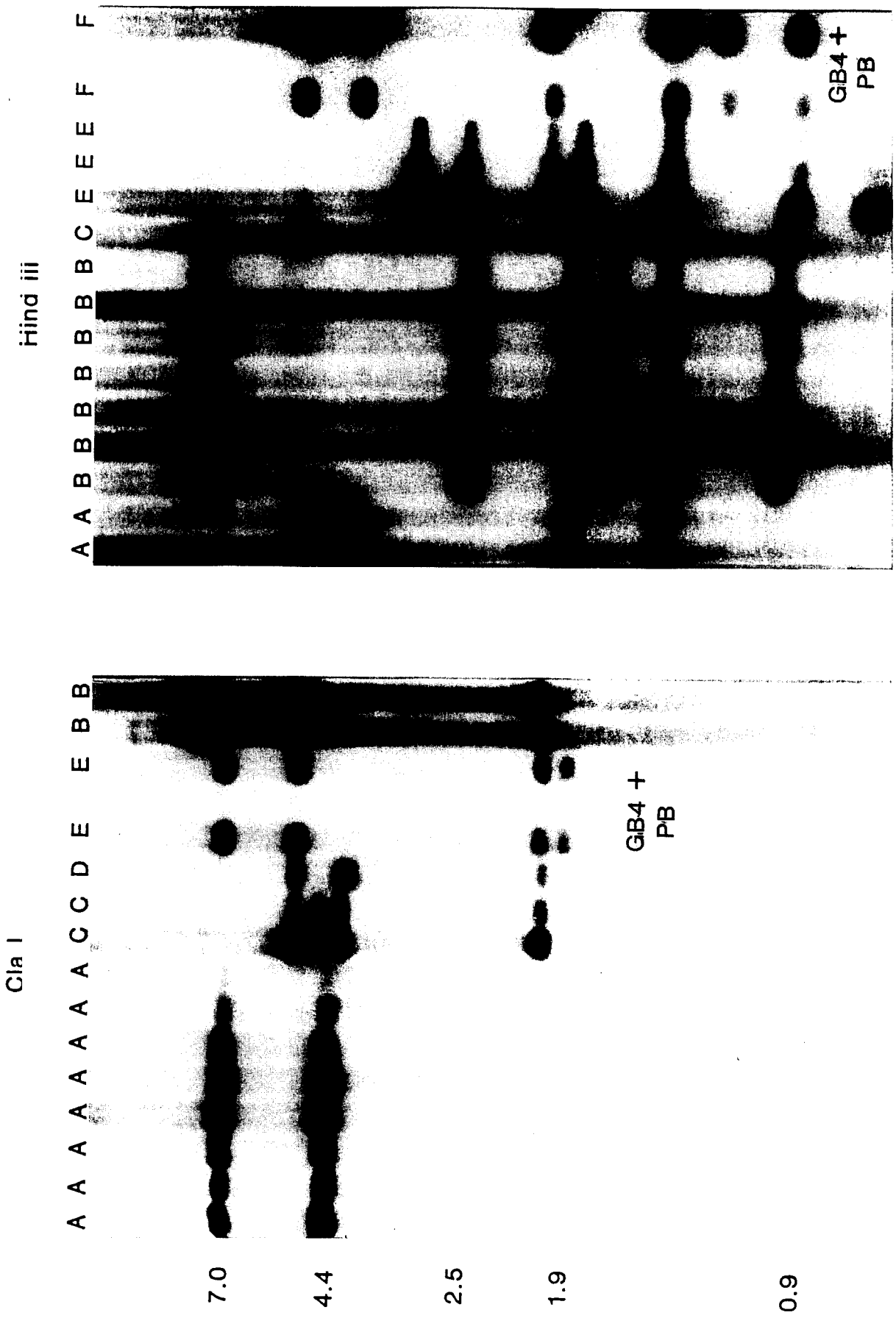

FIG. 2. MtDNA restriction fragment patterns resulting from digestion of DNA with the restriction enzymes *Cla*I and *Hin*dIII, electrophoresis on 0.7% agarose gels, Southern blotting, and hybridization with a polar bear mtDNA probe. Letters refer to fragment patterns (Table 1). Sizes of fragments (in kilobases) are indicated along the left-hand side of the figure. We have marked pattern E for *Cla*I and pattern F for *Hin*dIII, which are shared by polar bears and brown bear haplotype GB4.

TABLE 2. Estimates of sequence divergence (base substitutions per nucleotide) (above the diagonal) and number of shared restriction fragments (below diagonal) for black bear (BB), brown bear (GB), and polar bear (PB) mtDNA haplotypes

	BB1	BB2	BB3	BB4	BB5	BB6	GB1	GB2	GB3	GB4	GB5	PB1	PB2	PB3	PB4
BB1	27	0.046	0.003	0.005	0.050	0.003	0.079	0.074	0.074	0.074	0.079	0.072	0.066	0.078	0.079
BB2	13	30	0.047	0.052	0.001	0.050	0.068	0.070	0.070	0.077	0.083	0.075	0.063	0.082	0.075
BB3	26	13	28	0.002	0.051	0.007	0.089	0.075	0.075	0.075	0.080	0.080	0.074	0.088	0.089
BB4	25	12	27	28	0.057	0.009	0.089	0.075	0.075	0.075	0.080	0.080	0.074	0.088	0.089
BB5	12	29	12	11	29	0.031	0.082	0.084	0.084	0.076	0.100	0.074	0.075	0.080	0.074
BB6	25	12	24	23	16	26	0.087	0.080	0.080	0.073	0.087	0.071	0.072	0.077	0.078
GB1	8	10	7	7	8	7	32	0.016	0.016	0.029	0.016	0.027	0.022	0.023	0.027
GB2	9	10	9	9	8	8	25	34	0.002	0.022	0.005	0.023	0.019	0.022	0.026
GB3	9	10	9	9	8	8	25	33	34	0.022	0.003	0.026	0.022	0.025	0.029
GB4	9	9	9	9	9	9	20	23	23	34	0.029	0.007	0.010	0.010	0.011
GB5	8	8	8	8	6	7	24	30	31	20	32	0.033	0.028	0.032	0.033
PB1	9	9	8	8	9	9	20	22	21	29	18	32	0.003	0.003	0.004
PB2	10	11	9	9	9	9	22	24	23	28	20	31	33	0.006	0.006
PB3	8	8	7	7	8	8	21	22	21	27	18	30	29	31	0.005
PB4	8	9	7	7	9	8	20	21	20	27	18	30	29	29	32

NOTE: Numbers on the diagonal are total number of fragments for each haplotype. Estimates of base substitutions per nucleotide site were made using the fragment method of Nei and Li (1979).

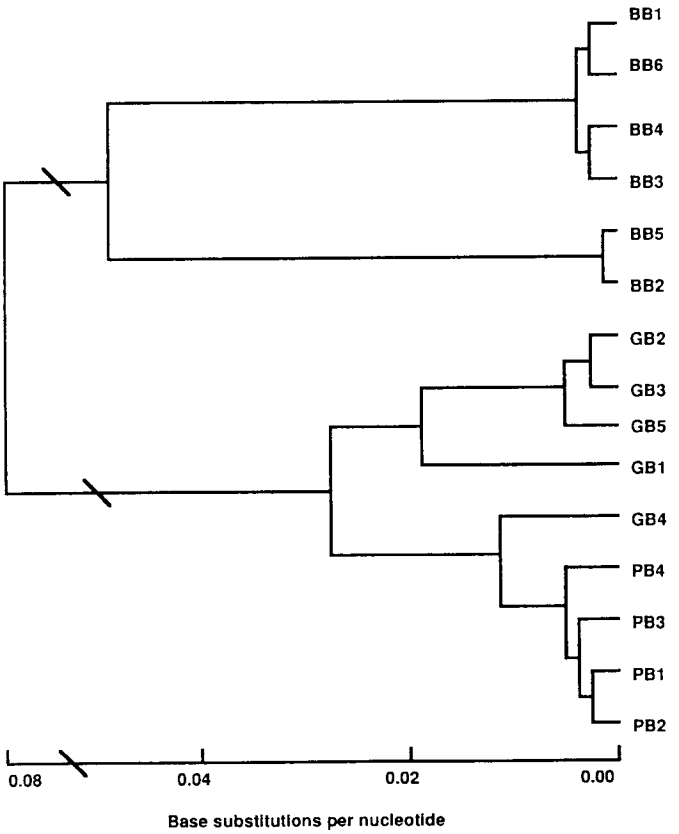

FIG. 3. Dendrogram constructed with the Kitsch computer analysis of mtDNA divergence estimates (Table 2) of bear mtDNA haplotypes (Table 1). The scale at the bottom reflects the total branch lengths between two taxa.

occurrence of the same haplotypes across the sampling locations may reflect this.

Our data suggest that there has been considerable movement of mtDNA haplotypes of all three species among areas in the recent past. However, bear populations may be structured into subpopulations on a large or a small geographic scale, with limited interchange of individuals (Manlove *et al.* 1980; Wathen *et al.* 1985). As in other species, bear populations may undergo episodes of dispersal and gene flow (e.g., postglacial colonization) interspersed with periods of stable population structure with restricted gene flow (Avise *et al.* 1984; Slatkin 1987, 1989; Wayne *et al.* 1990). The occurrence of different haplotypes among areas (Table 1) may reflect such a population structure, but larger sample sizes are needed for proper assessment of this.

Black bears and polar bears are characterized by relatively low levels of protein variation (Allendorf *et al.* 1979; Manlove *et al.* 1980; Larsen *et al.* 1983). Gene flow, population bottlenecks during recent speciation, and selection pressure in the harsh arctic habitat of the polar bear have been suggested as possible causes for low protein variation. In contrast, there is considerable intraspecific mtDNA variation (as indicated by numbers of haplotypes, Table 1) in bears compared with some other large mammals. For example, in an extensive survey of several subspecies and geographic areas across North America, Cronin (1992) found only one mtDNA haplotype in moose and two in elk. Conclusions about the causes of these relative levels of variation would be speculative until a more intensive assessment of nuclear and mitochondrial DNA variation in bears is done.

The relative levels of intraspecific variation may also be compared in terms of the magnitude of sequence divergences between haplotypes. Our results show that the oldest species (Kurtén 1976), the black bear, has the highest intraspecific mtDNA divergences among haplotypes, and the youngest species, the polar bear, has the lowest level. Brown bears are intermediate in age and mtDNA divergence.

Interspecific relationships

Our results are consistent with other analyses of mtDNA (Shields and Kocher 1991), morphology (Kurtén 1964), and proteins (Goldman *et al.* 1989; Wayne *et al.* 1991), which indicate a close phylogenetic relationship of brown bears and polar bears relative to black bears (Fig. 3). Shields and Kocher (1991) reported a divergence estimate derived from restriction fragment analysis ($p = 0.011$) between brown bears and polar bears considerably lower than our average ($p = 0.023$). However, Shields and Kocher's brown bears were from southeastern Alaska (G. F. Shields, personal communication), and the average divergence between our brown bears from southeastern Alaska

(GB4) and polar bears is similar to theirs ($p = 0.01$). This suggests that their bears had haplotypes similar to our GB4. Our higher interspecific divergence estimate and the identification of the paraphyletic relationship of brown bear and polar bear mtDNA reflect intraspecific variation across much of the North American range of these species.

The clustering of haplotype GB4 with polar bears (Fig. 3) suggests that brown bears are paraphyletic with respect to polar bears in mtDNA (and thus matriarchal) phylogeny. Paraphyletic mtDNA relationships among closely related species are common and may result from introgressive hybridization or random sorting of ancestral haplotypes during speciation (Avise 1986; Neigel and Avise 1986). Paraphyletic patterns have been described in mice (*P. maniculatus* and *Peromyscus polionotus*; Avise *et al.* 1983), ducks (*Anas platyrhynchos* and *Anas rubripes*; Avise *et al.* 1990), and deer (*O. virginianus* and *O. hemionus*; Cronin *et al.* 1988; Cronin 1991*b*). The GB4 and PB1–PB4 haplotypes probably represent the descendents of one mtDNA variant that occurred in ancestral populations, and GB1–GB3 and GB5 represent a different ancestral haplotype. Although the mtDNA phylogeny of brown and polar bears is paraphyletic, polar bears have many derived morphological characters (Stanley 1979) that identify them as a monophyletic group. This relationship between brown bears and polar bears is an example of an mtDNA phylogenetic tree that is not the same as a species tree (Pamilo and Nei 1988).

The similarity of brown bear and polar bear mtDNA is not surprising, considering that these species can produce fertile hybrid offspring (Kowalska 1965) and are thought to have been derived recently from a common brown bear ancestor (Kurtén 1964). Much of the morphological divergence of polar bears and brown bears may have occurred rapidly, within the last 20 000–40 000 years, and has been cited as an example of quantum speciation (Stanley 1979). The clustering of black bears separately from brown and polar bears reflects the older separation of these groups (Goldman *et al.* 1989; Kurtén 1976; Wayne *et al.* 1991). The fossil record suggests that the black bear and brown bear – polar bear lineages diverged 1.5–2.5 million years ago, and polar bears diverged from the brown bear lineage less than 300 000 years ago. Black bear fossils date from the Early Pleistocene, more than 3 million years ago (Kurtén 1964, 1968, 1976; Kurtén and Anderson 1980). These are rough estimates of divergence times because the fossil record of bears is not complete.

MtDNA sequence divergence has been used widely to date divergence times of taxa, assuming a rate of about 0.02 base substitutions per nucleotide per million years (Brown *et al.* 1979; Wilson *et al.* 1985). However, this rate was initially calibrated for primates and should not be uncritically applied to other groups (Moritz *et al.* 1987). This is particularly true for comparisons of closely related species in which intraspecific variation is great and there are paraphyletic relationships. In addition, Wayne *et al.* (1990) reported substantial variation in the rate of mtDNA sequence evolution in jackals. We feel that applying the primate rate to date speciations of carnivores (e.g., bears) is unwarranted.

In summary, carnivores provide evidence that the genetics of populations are dynamic and need to be interpreted in light of historical as well as current levels of gene flow (Slatkin 1987, 1989; Avise 1989). MtDNA polymorphism can be maintained for long periods of time, and gene flow can result in discordance of phylogeny and geographic or taxonomic distribution of haplotypes (Slatkin 1989). It is important to assess intraspecific variation and distinguish gene phylogenies and species phylogenies when using mtDNA as a phylogenetic tool (Pamilo and Nei 1988).

Acknowledgements

Samples were provided by D. A. Palmisciano and K. Ohn, Montana Department of Fish, Wildlife, and Parks; E. H. Orff, the New Hampshire Fish and Game Department; C. E. Trainer, Oregon Department of Fish and Wildlife; J. Wolfe, Alaska State Crime Laboratory; L. Aumiller, S. D. Miller, R. B. Smith, B. P. Minn, and K. Koenen, Alaska Department of Fish and Game; V. G. Barnes, Jr., U.S. Fish and Wildlife Service; and the Northwest Territories Division of Wildlife. Laboratory assistance was provided by J. R. Powell and R. DeSalle, Department of Biology, Yale University, K. Cease, and S. Kavanaugh. R. L. Wilmot, W. J. Spearman, and K. L. Denning provided technical assistance, and R. Wayne and two anonymous reviewers provided valuable comments on the manuscript.

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