Phylogeography and Pleistocene Evolution in the North American Black Bear

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To determine the extent of phylogeographic structuring in North American black bear (*Ursus americanus*) populations, we examined mitochondrial DNA sequences (n = 118) and restriction fragment length polymorphism profiles (n = 258) in individuals from 16 localities. Among the bears examined, 19 lineages falling into two highly divergent clades were identified. The clades differ at 5.0% of nucleotide positions, a distance consistent with an origin 1.8 MYA, and have different but overlapping geographical distributions. Areas of clade cooccurrence show that eastern and western populations are currently mixing, but regional differences in lineage distribution suggest that mixing has begun only recently. The long-term population history of black bears appears to be characterized predominantly by long-term regional isolation followed by recent contact and hybridization. Congruence between the pattern of diversity observed in black bears and patterns of forest refuge formation during the Pleistocene supports earlier speculation that Pleistocene forest fragmentations underlie a common pattern in the phylogeography of North American forest taxa.

Introduction

Among the tools of evolutionary biology, molecular phylogeography has distinguished itself as a means of understanding evolutionary processes within species (Avise 1994). By taking advantage of information contained in the geographical distribution and topological relationships of genetic lineages, which reflects the long term structure and demographic history of populations, phylogeography provides a strategy useful for understanding the historical factors leading to extant patterns of diversity. In a growing number of studies, distinct patterns in the age and geographical distribution of diversity have been identified, implicating specific historical events as the primary source of genetic structuring (e.g., Lamb, Avise, and Whitfield Gibbons 1989; Riddle and Honeycutt 1990; Routman, Wu, and Templeton 1994). In some cases, pervasive regional patterns of diversification have been found, linking patterns of differentiation in many taxa to the same historical events (e.g., Avise 1992). These findings have provided a historical background valuable for interpreting patterns of diversity at various levels of resolution, from population to community, in a number of geographical regions.

The utility of phylogeographic analyses in understanding intraspecific evolutionary processes is not limited to comparisons within localized areas. Across longer distances, species are likely to show patterns of diversification similar to those observed in more confined areas but on a different spatial scale, giving insight into processes inaccessible to studies with a more narrow scope. For example, species can show strong population structure on a continental scale without showing more localized structuring, or populations structured on a large scale may be substructured at a regional level. Efforts to fully understand the phylogeographic history of biota over large areas must include both detailed, regional comparisons and broader, large-scale ones. Nonetheless, large-scale phylogeographic studies are still unusual.

As the location of many ecologically well understood taxa, North America has received special attention in phylogeographic analyses. In several different parts of the continent, regional phylogeographic patterns have been examined in detail, giving information about local evolutionary processes (e.g., Lamb, Avise, and Whitfield Gibbons 1989; Riddle and Honeycutt 1990; Avise 1992; Routman, Wu, and Templeton 1994). However, even where many regional phylogeographies have been examined, only a relatively small number of studies have examined wider areas. Few taxa, for example, have been compared across the North American continent, and most that have are birds (Zink 1995, 1996). Moreover, among the widespread taxa surveyed so far, diverse phylogeographic structures are observed (Zink 1995, 1996). Additional comparisons are essential for defining whether overall patterns of phylogeographic structuring are present on a continental scale in North America.

To determine the character of phylogeographic structuring in a widespread North American carnivore, and to add to the number of taxa examined across the continent, we conducted an analysis of mtDNA polymorphism in the North American black bear (*Ursus americanus*). The black bear is a forest species whose range currently occupies coniferous and broadleaf deciduous woodlands from coast to coast across North America, as far north as Alaska and as far south as Mexico. Fossil evidence indicates that black bears have been present in North America for at least 3 Myr and that the species has maintained a broad distribution through much of its history (Kurtén and Anderson 1980). As a species with a long-standing pan-continental distribution, black bears are likely to provide an informative contrast to widespread species examined previously.

Our analysis focuses on four aspects of genetic diversity. First, we assess the spatial distribution of evolutionary lineages across populations to determine whether distinct patterns of distribution are present. Sec-

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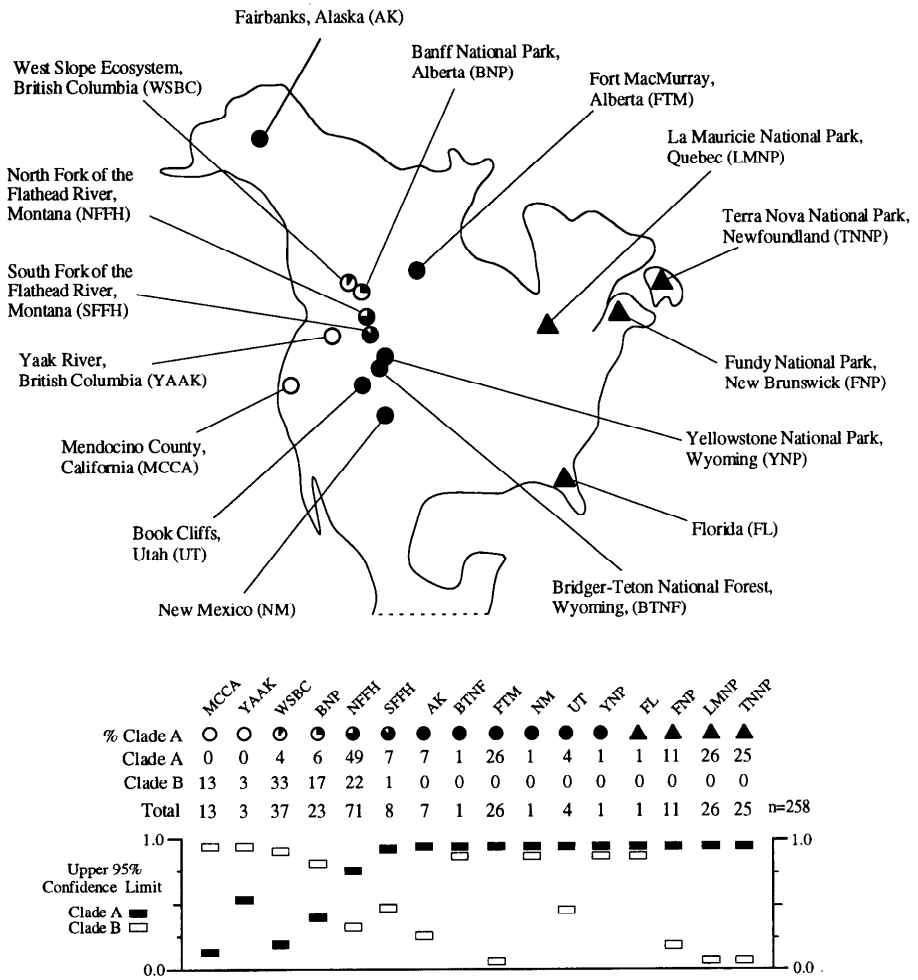

FIG. 1.—Sampled populations and distribution of clades. At each mapped point, color and shape indicate which clades are present. In partially shaded circles, cluster A1 and clade B are both present; black area = % cluster A1 and white area = % clade B. Triangles indicate the presence of cluster A2. Numbered rows represent raw RFLP data sorted by location. The bottom section shows upper 95% confidence limits on the frequencies of clades A and B in each population assuming a binomial distribution.

ond, we analyze lineage ages using a molecular clock to ascertain the time scale over which diversity has evolved. Third, we examine two aspects of long-term demography: geographical distributions of diversity are compared in the context of lineage age to determine the prevalence of migration, and pairwise differences between sequences are compared to determine whether evidence of recent population growth is present. Finally, to place our findings in a meaningful historical context, we discuss the possible relationship between patterns of genetic diversity observed in black bears and geological and habitat changes over the last 2 Myr, during the Pleistocene epoch.

The climatic changes of the Pleistocene are widely regarded as one of the most important factors influencing the current distribution of biological diversity in North America, and patterns of diversity in black bears are consistent in several respects with an influence by Pleistocene events. In particular, the association of black bears with forest habitats, which were divided into refugial islands through the late Pleistocene, suggests that extant patterns of diversity in black bears may be partly due to a long-term fragmentation of forests into eastern and western isolates. The patterns of sequence diversity we observe in black bears clarify earlier conclusions about the origins of several forest songbird species and have a number of implications for the importance of Pleistocene forest divisions in general.

Materials and Methods

Population Samples

Blood and tissue specimens were collected from 258 bears in 16 localities (fig. 1), representing a broad sampling across the range of the species. Most samples were collected as parts of study efforts by U.S. Fish and Game, state Fish and Game departments, and Parks Canada. Sixteen published mtDNA sequences from Paetkau and Strobeck (1995) were also included.

The majority of collected samples were blood specimens. These were collected into vacutainers (Becton Dickinson #6457) containing an anticoagulant (EDTA) and refrigerated at 4°C until delivery to the laboratory was possible. Upon receipt, 10-ml blood specimens were immediately placed in 90 ml of a lysis buffer (0.32 M sucrose; 10 mM Tris-HCl, pH 7.5; 1% Triton X-100).

This solution was centrifuged (15 min, 1,000 × *g*), the supernatant was discarded, and the remaining pellets were resuspended in 90 ml of the same buffer, followed by a second centrifugation. Finally, pellets were suspended in 3 ml of a second lysis buffer (75 mM NaCl, 24 mM EDTA) and kept frozen at −20°C.

Twenty-one muscle tissue specimens were collected into sterile 1.7-ml eppendorf tubes and frozen at −20°C until delivery to the laboratory, where they were stored at −80°C.

DNA Extraction

Blood and tissue samples were treated identically for DNA extraction. To blood cells in lysis buffer, or 5 × 5 mm of frozen tissue, 0.75 ml of a digestive solution (3 mg/ml proteinase K [Boehringer Mannheim], 6.6% SDS) was added. This solution was incubated overnight at 37°C. The solution was then purified first with 1 volume of Tris-equilibrated phenol (pH 7.5), second with 0.5 volumes of equilibrated phenol and 0.5 volumes chloroform/isoamyl alcohol (24:1), and finally with 1 volume of chloroform/isoamyl alcohol (24:1). A precipitation solution (0.1 volume of sodium acetate, 2 volumes of ice-cold isopropanol) was added to the buffer phase, and precipitated DNA was transferred by Pasteur pipet to 1.5 ml TE −4 (10 mM Tris, pH 8.0; 0.1 mM EDTA) and stored frozen at −80°C.

Amplification and Sequencing

The control region of mtDNA was chosen for amplification because high levels of variability in other taxa (Ward et al. 1991) suggested that the region would be variable in bears as well. Human primers H16498 and L15997 (Ward et al. 1991) were found to amplify the control region of black bears and were used throughout the experiment. Polymerase chain reaction (PCR) was carried out in 25-μl reactions with 210 ng of template DNA and primer pairs including one biotinylated and one nonbiotinylated member. Purification of PCR products was performed using dynabeads (Dynal) and their accompanying protocols for DNA purification.

Purified single-stranded products were sequenced in 118 individuals using the same primers as for PCR and the reagents and protocols of the Sequenase 2.0 sequencing kit (Amersham Life Sciences). Reaction products were separated by electrophoresis through 8% denaturing polyacrylamide vertical gels, rinsed in 10% glacial acetic acid for 30 min, dried, and exposed to Kodak XAR film for 3–4 days.

Sequences were entered into a database using the MASE software package (Faulkner and Jurka 1988) and aligned by hand. Only minor insertions/deletions were observed, and all sequences were similar, but a nine-nucleotide repeat in the control region showed ambiguities due to sequencing artifacts. The nine ambiguous nucleotides, and three flanking nucleotides on each side, were removed from all analyses.

Restriction Site Polymorphisms

Two distinct clades of lineages were identified during the sequencing phase of the experiment. These were designated clade A and clade B. To allow clades A and B to be distinguished without sequencing, diagnostic restriction fragment length polymorphisms (RFLPs) were identified for each clade by inspection of the obtained sequences. The presence of the two groups was then analyzed in 140 unsequenced individuals by amplifying the control region using primers H16498 and L15997 and digesting the PCR product with the restriction enzymes *Afl* II and *Mbo* I (Amersham Life Sciences), which spanned one and two polymorphic nucleotide positions, respectively. Digests were carried out following the protocols supplied by Amersham Life Sciences, and the products of these reactions were analyzed by electrophoresis in TBE (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) through a 1% agarose gel. As a positive control, all 118 sequenced samples with known clade affiliations were subjected to RFLP analysis. No misleading digestion profiles were found among positive controls. Including both positive controls and unknowns, clade affiliation was determined in a total of 258 individuals.

Phylogenetic Analyses

Phylogenetic trees based on the 314 scored nucleotide positions in the control region were constructed using DNAPARS, packaged with PHYLIP (Felsenstein 1993). Equal weight was given to all substitutions, and trees were rooted using control region sequences from the asiatic black bear (*Ursus thibetanus*) (Waits 1996). Bootstrap replications (250) were used to analyze the robustness of different components of the tree.

In addition to being displayed using conventional diagrams, trees were graphically represented as cladograms using the methods of Templeton, Crandall, and Sing (1992), with ambiguities represented as reticulations and multifurcations.

Divergence Times

To evaluate sequence divergences on a temporal scale, we estimated a nucleotide substitution rate for the control region in bears using the data and methods of Waits (1996). In an evaluation of phylogenetic relationships in the *Ursidae,* Waits (1996) presents a molecular clock based on third-position nucleotide substitutions in four mtDNA-coding regions (Cyt-b, CO-II, ND4, and ND5), and fossil data marking the hypothesized origins of seven species. To calibrate a molecular clock for the control region, we subjected aligned control region data presented by Waits (1996) to an analysis identical to that performed by Waits on coding regions, but including only nucleotide positions analyzed in the present study and excluding positions containing insertions or deletions. This resulted in the calculation of a molecular clock based on the same phylogenetic topology, fossil calibration, and samples used by Waits (1996), but in a different locus—the control region. This procedure resulted in the inference of a control region divergence rate of 2.8% per Myr. Confidence intervals were generated using the method of Hillis, Mable, and Moritz (1996).

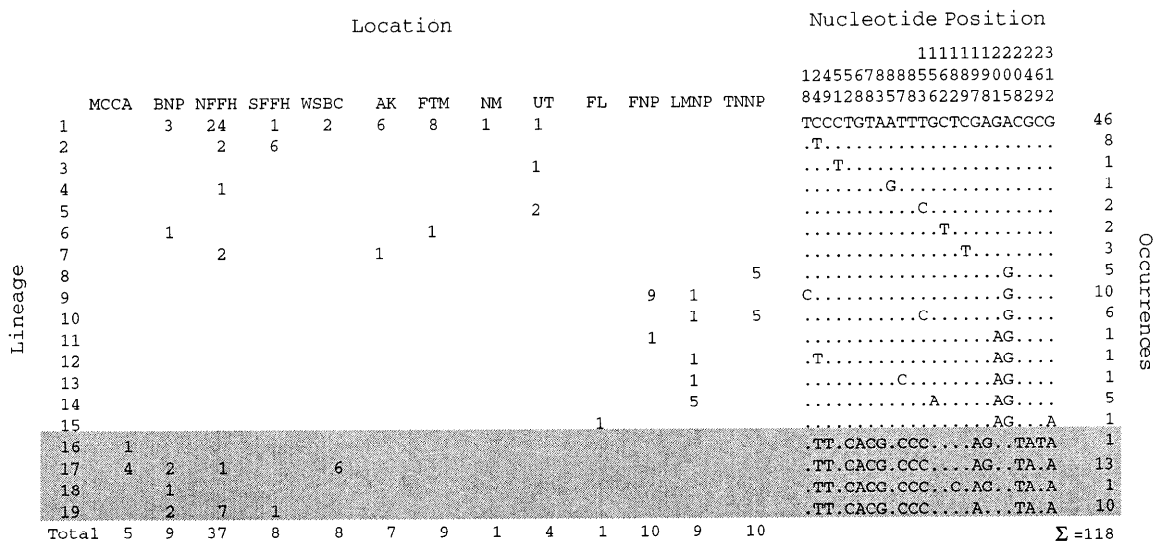

FIG. 2.—Summary of mtDNA lineages and their distributions. In the nucleotide position columns, dots indicate identity with the reference sequence (lineage 1), and letters designate base substitutions. Numbers in location columns represent occurrences and their totals, and the rightmost column of the figure shows total occurrences for each lineage. Unshaded and shaded rows represent clades A and B, respectively.

Population Growth

Mismatch distributions describing the pairwise differences between sequences were analyzed using the three-parameter method of Rogers (1995). This method compares a sample mismatch distribution with the mismatch distributions of simulated populations to test whether the sample evolved in a growing population.

Results

Sequence Variation

In the control region, 19 distinguishable sequences (hereafter referred to as lineages) defined by 24 variable nucleotide positions were observed in 118 individuals from 13 populations (fig. 2). The lineages varied widely in their frequencies, with the most frequent lineage accounting for 39.0% of all samples while other lineages were found in frequencies ranging from 0.8% to 11.0% (fig. 2). The mean number of nucleotide differences per site between sequences (π) was found to be 0.022, with an overall lineage identity probability of 19.0%. All observed substitutions were transitions, and there was no evidence of clustering of variable sites.

Phylogenetic Analyses

Phylogenetic analysis resulted in the discovery of numerous equally parsimonious bifurcating trees; however, these results were confounded by a lack of sites informative in parsimony analyses. Allowing polytomies, only two most-parsimonious trees were found.

Two major clades were identified: clade A, represented by lineages 1 through 15, and clade B, represented by lineages 16 through 19 (fig. 3). These clades are highly divergent, differing at an average of 4.8% of nucleotide positions (a Kimura distance of 5.0%). Although the mean divergence between clades was high, the mean difference between lineages within clades was small: 0.82% and 0.48%, respectively.

The cladogram relating black bear lineages requires 30 mutational events for each of the two alternative trees it represents and fully reflects the topology observed in the parsimony tree, although it graphically displays relationships among lineages in more detail (fig. 3). Unlike many networks previously derived from mtDNA sequence or RFLP data (e.g., Sykes et al. 1995; Nürnberger and Harrison 1995), the cladogram relating black bear lineages contains only minor topological ambiguity: it is as parsimonious to include lineage 2 as an intermediate between lineages 1 and 16 as it is to include lineage 5 as the intermediate (fig. 3).

Geographical Distribution of Variability

Lineages were not equally distributed within or between populations: 12 lineages were found in only one population each, and only three lineages occurred in more than two populations. Lineage 1 was found in eight populations—more than 60% of all locations from which sequences were obtained (fig. 2).

Similarly, the two major clades were not equally distributed. Among the 16 populations examined, clade A was found in 14 locations, while clade B was found in only six. Four populations near the Continental Divide contained both clade A and clade B, and 12 populations contained only one clade (fig. 1). In the four populations containing both clades, clade A was more frequent than clade B in two (NFFH and SFFH), while clade B was more frequent in the other two (BNP and WSBC). Among populations containing both clades, populations located farther east uniformly possessed a higher frequency of clade A. Over all populations, clade B was the more frequent group in 4, while clade A predominated in 12 (fig. 1).

Even within clades, lineages were not equally distributed. In clade A, lineages 1 through 7 (cluster A1) were never found to cooccur with lineages 8 through 15 (cluster A2) (figs. 1 and 3).

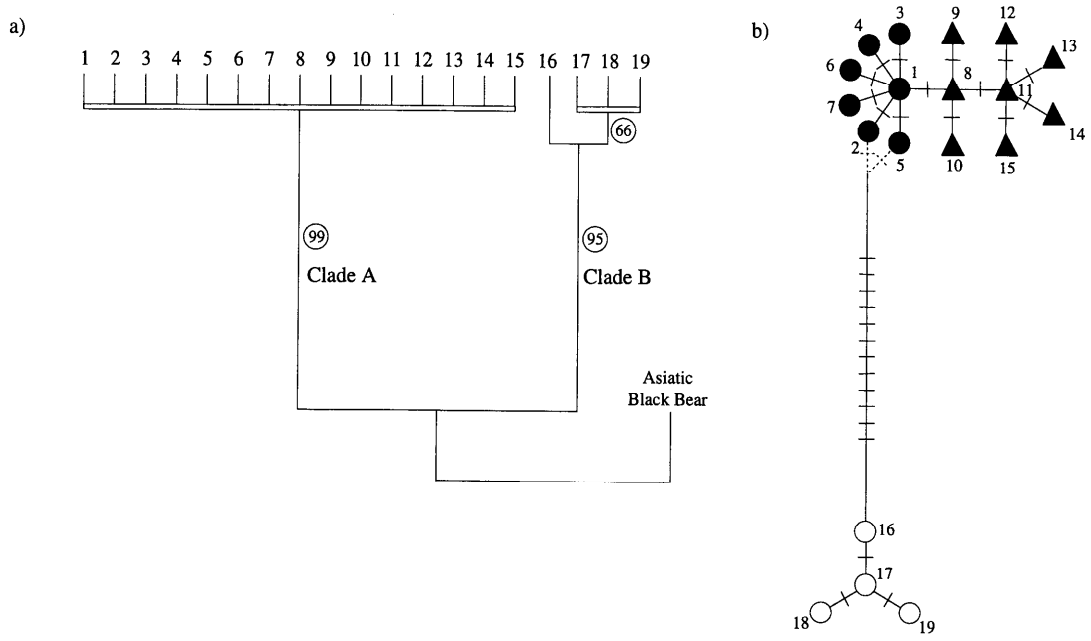

FIG. 3.—Phylogenetic trees relating black bear mtDNA lineages. *a*, Tree topology indicates features defined by parsimony analysis. Circles indicate clusters with bootstrap values greater than 50%. Numbers denote lineage identification. *b*, Maximum-parsimony cladogram. Numbers indicate lineages, crossbars represent single-nucleotide substitutions, and dashed lines indicate alternative topologies. Filled circles represent cluster A1, filled triangles represent cluster A2, and open circles represent clade B.

Discussion

The distribution and topology of mitochondrial diversity in black bears show several distinctive features. Most striking is the presence of two distinct clades. These have been recognized previously in comparisons both within (Waits 1996; Wooding and Ward 1997) and between (Paetkau and Strobeck 1995) black bear populations, and clades apparently corresponding to clades A and B were also recognized by Cronin, Armstrup, and Garner (1991). The most remarkable feature of these clades is their depth of divergence. A difference of 5.0% between mtDNA lineages is unusual within mammalian populations and is suggestive of long-term divergence. However, evolutionary rates can vary considerably among taxa: large sequence divergences do not necessarily reflect large temporal separations.

The molecular clock we estimate for the control region in bears (see *Materials and Methods*) points to an ancient origin of clades. Based on our estimated divergence rate for the control region of 2.8% per Myr, the 5.0% difference between black bear clades supports a divergence 1.8 ± 0.8 MYA. This date is surprisingly early but is corroborated by evidence from two sources. First, in a comparison of coding region third positions in two black bears falling into clades A and B, a Kimura distance of 9.9% was observed by Waits (1996). Based on a reported divergence rate of 6.0% per Myr in coding region third positions in the Ursidae (Waits 1996), a divergence date of 1.7 MYA is implied. Furthermore, the two lineages presented by Waits differ at only 4.2% of control region positions. The 5.0% difference between clades in this study suggests that a comparison of the two lineages sequenced by Waits gives a slight underestimate of divergence time. Second, both Waits' estimated rate of substitution for third positions in coding regions and our estimated rate for the control region are in agreement with rates found in the pinnipeds, a closely related family of carnivores (Árnason et al. 1993, 1996; Slade, Moritz, and Heideman 1994). Although the stochastic nature of the molecular clock makes exact dating impossible, the origin of black bear clades appears to fall on the Pliocene/Pleistocene boundary, dated by several authors as 1.6–2.0 MYA (e.g., Williams et al. 1993).

In addition to showing high levels of sequence divergence, the two clades show spatial clustering, being divided into two primary geographical regions. Whereas clade A occupies a large range encompassing areas north and east of the Rocky Mountains and reaching southeast to Florida, clade B occupies only a limited area south and west of the Rocky Mountains (fig. 1). Furthermore, a zone of contact is present in the Continental Divide region. This distribution conforms to the predicted consequences of secondary contact between previously isolated populations (Marjoram and Donnelly 1994), and is consistent with eastern and western clades having maintained disjunct distributions over a time period sufficient for long-term allopatric divergence to occur. While the separate geographical distributions of the two clades suggest that they evolved in separate areas, the presence of a zone of contact indicates that regional mixing is occurring. However, the limited overlap in clade distributions suggests that regional mixing is recent.

Simulations have shown that lineage age and lineage range are correlated, with older lineages having broader ranges (Neigel and Avise 1993), and in mixing populations, a similar correlation should be present: migrant lineages that are new in a group should be con-

fined relative to migrants that have been present longer. In black bears, the ranges of the two clades overlap, but neither extends far into the primary range of the other. Moreover, a cline in clade frequency occurs across the continental divide (fig. 1). No evidence is present to suggest that competitive interactions or sexual incompatibility has prevented dispersal between regions. On the contrary, in populations where both clades are present, bears assigned to different mtDNA clades are phenotypically indistinguishable. While the presence of populations containing both clades indicates that mixing is occurring at present, the clades' limited overlap and frequency distribution suggests that exchange between regions has been restricted until recently.

Within regions, no obvious phylogeographic structuring is present. Although clade A is divided into two geographically separated clusters of lineages (figs. 1 and 3), these clusters differ by only a single nucleotide substitution (fig. 3), and their disjunct distributions may be an artifact of inadequate sampling in the central part of the continent. Moreover, the sharing of lineages between populations is common within regions. For example, a single lineage (lineage 1) is found in 75% of the populations containing clade A, and a single lineage (lineage 17) is found in 80% of the populations containing clade B (fig. 2). The prevalence of lineage sharing among populations indicates that at the level of resolution measured by the mtDNA control region, dispersal between populations is probably a regular occurrence.

Cronin, Armstrup, and Garner (1991, p. 2985) concluded from RFLP data that female black bears have experienced "considerable gene flow throughout the history of the species." We come to the opposite conclusion. The age and distribution of genetic diversity in black bears are more consistent with a history of long-term regional division between populations followed only recently by secondary contact. The magnitude of mitochondrial sequence divergence observed between regions, combined with relative homogeneity within regions, represents a previously unrecognized division of *U. americanus* into two markedly different regional variants.

The distribution, age, and topological relationships of lineages in black bears demonstrate that even at an intraspecific level, and even when no major morphological differences are present, distinct phylogeographic patterns can emerge on a continental scale. However, the pattern of diversity found in black bears is not entirely consistent with previous observations of phylogeographic structure in North American species (Zink 1996). The distribution of diversity in black bears resembles distributions in some birds (Bermingham et al. 1992; Gill, Mostrom, and Mack 1993) but is inconsistent with observations in several other widespread taxa (Zink 1996). Black bears add to a growing list of species with diverse phylogeographic structures on a continental scale in North America; among the taxa compared so far, no obvious pervasive trends in differentiation are present (Zink 1996).

A variety of influences might underly the lack of general trends in phylogeographic structure among widespread North American taxa. For example, ecological factors, such as microhabitat use, have been hypothesized as a possible cause of differences among bird species (Zink 1996). Although patterns of diversity observed in black bears do not correspond to larger patterns in North American taxa, they may still be explained by historical events with potentially pervasive effects. In particular, black bears might reflect the result of historical events affecting not all taxa, but only taxa sharing certain ecological attributes. In black bears, phylogeographic patterns may be best understood in the context of historical events in their preferred habitat: coniferous and broadleaf deciduous forests.

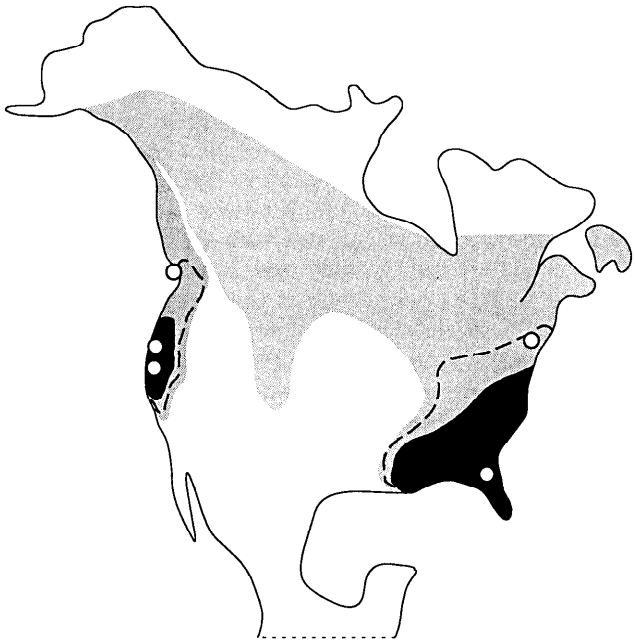

FIG. 4.—Distributions of forest habitats at present, 12,000 years ago, and 18,000 years ago. Lightly shaded areas indicate present forest distributions, dashed outlines indicate forest distributions 12,000 years ago, and dark shaded areas indicate approximate forest distributions 18,000 years ago (Thompson et al. 1993; Webb et al. 1993; Williams et al. 1993). Circles indicate locations of black bear fossils approximately 12,000 years old (Kurtén and Anderson 1980; Heaton and Grady 1993).

Evidence from fossil pollens indicates that although forests currently extend across much of North America, their distribution during the late Pleistocene was restricted to two discrete areas. Xeric habitats in the Rocky Mountains and Great Plains and ice sheets in the far north segregated coniferous and broadleaf forest habitats to the east and west for most of the last 120,000 years, forming a barrier to animal dispersal (fig. 4) (Williams et al. 1993). Following this ice age, eastern and western forests expanded unequally, with eastern forests dispersing rapidly into an area substantially larger than that of western forests (fig. 4). The history of forests during glacial cycles prior to 120,000 years ago is poorly understood but is probably characterized by similar processes (Delcourt and Delcourt 1987).

The potential importance of forest fragmentation as an agent of isolation in North American populations has been recognized for at least 30 years (Mengel 1964). On

the basis of fossil evidence for a long-term separation of forests and the codistribution of morphological variation with the modern vestiges of forest refugia, a number of early studies concluded that eastern and western morphotypes represent the relics of populations isolated by Pleistocene forest fragmentations. Mengel (1970), for example, noticed that several bird taxa are divided into eastern and western groups matching the distributions of eastern and western forests, and argued that regional variants diverged during glacial intervals.

Recent studies have explored the relationship between forest bird taxa and the Pleistocene habitat fragmentation by using molecular genetic markers. Distinct eastern and western clades have been identified in the mtDNA of warblers and chickadees (Bermingham et al. 1992; Gill, Mostrom, and Mack 1993), and these studies have been able to estimate the ages of clades by using a molecular clock calibrated in geese by Shields and Wilson (1987). In a comparison of warblers, Bermingham et al. (1992) identified divergences between eastern and western groups dating to the early and middle Pleistocene, and Gill, Mostrom, and Mack (1993) found evidence for an early Pleistocene divergence in chickadees. The outcomes of these studies show that not only are genetic variants in these species roughly codistributed with the expanded vestiges of forest refugia, but evolutionary distances consistent with divergence on a Pleistocene timescale are present as well. However, the extent to which these patterns represent a generalized motif in the evolution of North American forest species has been unknown. The distribution of diversity in black bears clarifies the pattern.

Black bears show a distribution of diversity consistent in several respects with a division of populations by forest refugia. While the two primary clades are codistributed with the vestiges of forest refugia formed during the Wisconsin, the position and frequency distribution of lineages along the continental divide are consistent with recent contact, possibly coinciding with the expansion of eastern and western habitats following deglaciation (fig. 1 and 4). In addition, fossil evidence supports the argument that black bears were present in the eastern and western forests 12,000 years ago (fig. 4), showing that black bears were present in both eastern and western regions before glacial conditions had fully subsided.

A long-term association between black bear populations and North American forests is also supported by demographic attributes implied by pairwise differences between lineages. If black bear population sizes have remained proportional to forest areas over an extended period, then evidence of population expansion proportional to the magnitude of expansion in forest habitat in each region over the last 18,000 years, the time since the last glacial maximum, should be present. Since the area occupied by western forests has only roughly doubled following the glacial maximum, while eastern forests have expanded to approximately 10 times their original area, population expansions of 2- and 10-fold ($10^{0.3}$- and $10^{1.0}$-fold) are predicted (fig. 4).

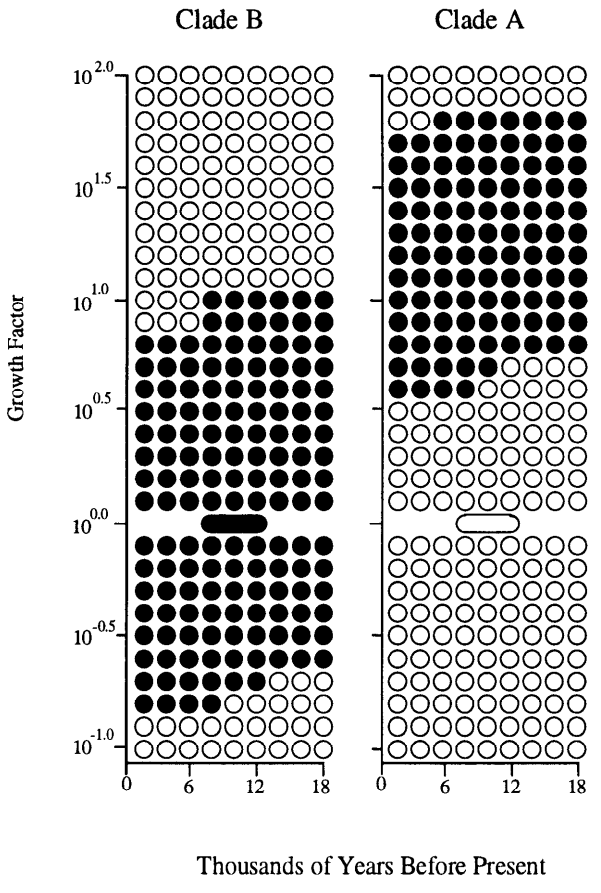

FIG. 5.—Confidence intervals for population growth in clades A and B. Each point on the graph indicates whether the magnitude of growth on the y axis is rejected at the time indicated by the x axis. For example, in clade B, $10^{1.0}$-fold growth is rejected for times more recent than 10,000 years ago, but is not rejected earlier than that. For convenience, Rogers' (1995) scale using τ has been replaced with a time axis. Note that for growth factor = $10^{0.0}$ (i.e., no growth), the timescale is undefined.

The method of Rogers (1995) allows a test of this hypothesis. Under the assumptions that clades A and B represent demographically separate populations and that the female generation time of black bears is 10 years (Jonkel and Cowan 1971), we examined magnitudes of population growth ranging from $10^{-1.0}$ to $10^{2.5}$ and values of τ ($=2\mu t$, where t is the time since population expansion began) ranging from 0.0 to 0.32 (i.e., for times ranging from 0 to 18,000 years ago). Confidence intervals were summarized under the further assumptions that the eastern and western population sizes are at present 600,000 and 100,000, respectively (Brown 1993) and that 20% of individuals contribute to female effective population size (e.g., Hellgren and Vaughan 1989) (fig. 5). In each region, the magnitude of growth predicted from changes in forest area falls well within the 95% confidence interval (fig. 5). Although other magnitudes of increase, or even population reduction, cannot be excluded, pairwise differences within each region are consistent with a connection between black bear population size and forest habitat availability over the last 18,000 years.

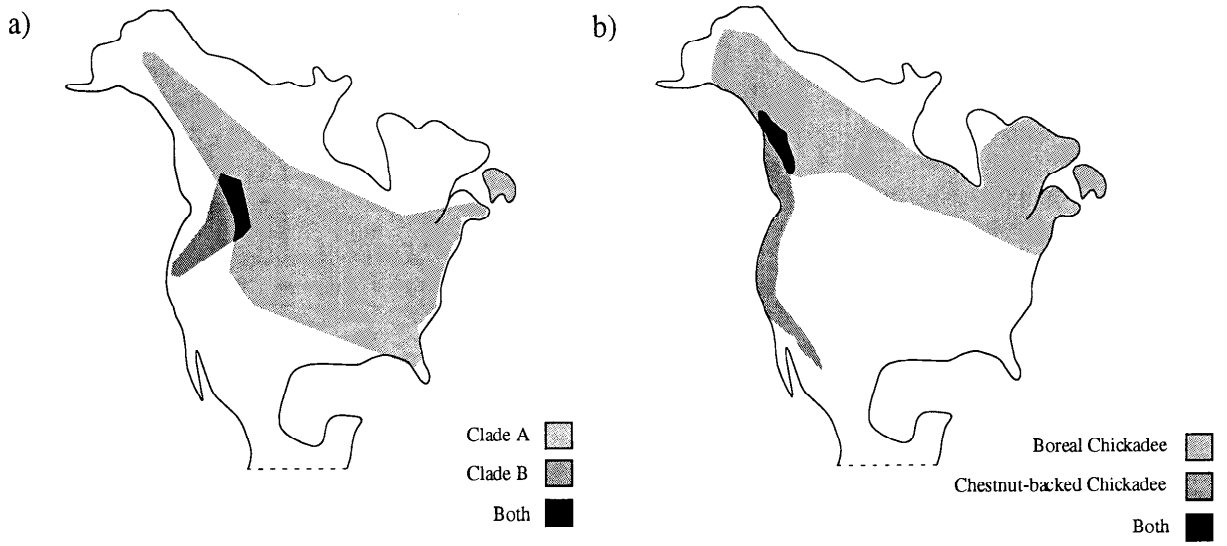

FIG. 6.—Distributions of eastern and western taxa. *a*, Distributions of clades A and B in black bears. *b*, Distributions of the boreal chickadee (*Parus hudsonicus*) and chestnut-backed chickadee (*Parus rufescens*).

The ages of black bear clades, their distributions, and their demographic properties are all consistent the hypothesis that black bears have historically been divided into separate forest refugia. It seems most likely that black bears became partitioned into groups corresponding to eastern and western forest fragments formed by ice age climate changes approximately 2 MYA and then diverged in isolation to form distinct clades. Most recently, with the recession of the Wisconsin ice sheets, bear populations have expanded with changes in their forest habitat and, although impeded by the continental divide, are currently mixing. Throughout the Pleistocene, the role of interglacial warm periods as a source of regional mixing seems to have been inconsequential relative to that of the isolation imposed during glacial intervals. Although occasional between-region dispersal cannot be ruled out, eastern and western black bear populations appear to have been largely isolated over the last 1.8 Myr.

A history of population division in black bears not only explains the distribution and age of clades, but also has implications for the interpretation of observations at higher levels of resolution. Patterns of diversity within regions and within populations might be strongly affected by both regional mixing and population growth. The zone of contact observed near the continental divide, for example, may be an important consideration in comparisons of allele number and heterozygosity among populations, and overall levels of diversity in each region may best be interpreted with regard to their historically different effective population sizes and growth patterns. The finding that the BNP and LMNP populations show no significant difference in heterozygosity (Paetkau and Strobeck 1994), for instance, is interesting in light of the location of the BNP population in the zone of mtDNA clade mixing observed in this study.

The patterns of diversity we have identified in mtDNA do not necessarily reflect patterns of diversity at all loci. mtDNA is maternally inherited and, in the strictest sense, reflects only the demographic history of females. However, male black bears are known to disperse farther than females and could, in principle, foster extensive regional mixing among autosomal loci, which are transmitted by both parents. Contrasts of diversity in nuclear genes with expectations based on our findings in mtDNA may be especially informative in understanding the full effects of Pleistocene population divisions. Although we anticipate that male and female population structures will be largely similar with respect to the regional division found in mtDNA, nuclear and mitochondrial loci could show different patterns of phylogeographic structuring, especially at local levels.

In a more general context, the diversity we observe in black bears supports earlier speculation that forest divisions have imposed a significant influence on their resident taxa. The presence of similar east/west divisions in birds and the distinctness of the pattern in bears confirm that not only are patterns of differentiation consistent with forest divisions present (fig. 6), but these patterns are found in highly divergent taxonomic groups. Although the effect of forest divisions cannot be fully defined until both the vegetational fossil record is explored in more detail and more taxa are compared, patterns of diversity in black bears point to a strong and pervasive effect of forest refuge formation in North American forest taxa.

GenBank Accession

The sequences referred to in this paper have been deposited in the GenBank sequence database under accession numbers AF012305–AF012323.

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