PATTERNS OF GENETIC DIVERSITY IN A BLACK BEAR POPULATION INDICATE RECENT IMMIGRATION

STEPHEN WOODING,[1] 201 South Biology, University of Utah, Salt Lake City, UT 84112, USA, email: stephen@apollo.med.utah.edu
RYK H. WARD, 2100 EIHG, University of Utah, Salt Lake City, UT 84112, USA, email: ryk@linkers.med.utah.edu

Abstract: To determine whether the recent history of a black bear (*Ursus americanus*) population includes significant immigration, we examined patterns of genetic diversity in mitochondrial DNA (mtDNA) sequences from 37 individuals from the upper North Fork of the Flathead River (NFFH), Montana. Two major evolutionary clades of lineages were identified which differed at an average of 4.4% of nucleotide positions. The divergence found between these clades was much larger than expected and was consistent with the hypothesis that genetic diversity in this population has been supplemented by immigrants from other populations.

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Among managed black bear populations, migration and dispersal patterns are of central importance for maintaining both ecological and genetic integrity. Movement patterns will affect the amount of genetic diversity found in populations, a concern to managers of small populations, and will determine which populations are most closely associated, a concern to managers interested in transplanting animals. Migratory patterns are also important for determining the general habitat requirements of animals, such as range size.

Migratory patterns in black bears have been investigated using both direct observations and inferences from genetic data. The implications of these observations, however, are not always in agreement. Female black bears, for example, have been observed to be relatively nonmigratory and may have small ranges located near their site of birth (Beck 1991), but Cronin et al. (1991:2985) concluded from restriction fragment length polymorphism (RFLP) data that female black bears have experienced "considerable gene flow throughout the history of the species." These contradictory observations are not mutually exclusive: female bears may migrate on a scale imperceptible to humans and have significant gene flow at the same time, or bears may have periods of movement between populations followed by periods that lack immigration.

To investigate the demographic history of a single black bear population, we present an analysis of mtDNA sequence data in a population of black bears from the NFFH in Montana. To test the hypothesis that the pattern of genetic diversity in a black bear population evolved in the absence of immigration, we have adopted an analytical strategy aimed at identifying patterns within a single population rather than using the more conventional approach of comparing 2 putatively interacting populations (Hudson et al. 1992*a,b*). Specifically, we take advantage of the tendency of population genealogies to coalesce, a process that limits the evolutionary divergence found between lineages from the same population. In the analysis, we find levels of divergence greatly exceeding expected values, a pattern consistent with the hypothesis that immigration has been a significant factor in the population's recent history.

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MATERIALS AND METHODS

Sample Collection and Preservation

Blood samples were collected from 37 black bears in a population in the upper NFFH drainage near Glacier National Park, Montana (Fig. 1), as part of management and research efforts by the U.S. Fish and Wildlife Service. Blood specimens were collected in sterile 10 cc vacutainers containing 17.55 mg of EDTA anticlotting agent and kept on ice or in standard freezers until overnight delivery to a laboratory was possible. Upon receipt, samples were immediately placed in a preservative buffer and stored frozen at -20 C.

[1]Present address: 2405 East 2100 South, Salt Lake City, UT 84109, USA, email: stephen.wooding@anthro.utah.edu

Fig. 1. Location of the North Fork of the Flathead River, Montana, population of black bears.

DNA was extracted from blood samples using a chloroform–phenol extraction, and concentrations were quantified by spectrophotometry and standardized to 70 ng/μL for the polymerase chain reaction (PCR). The control region of mtDNA was chosen for sequencing because high levels of variability have been found in other mammals (Wayne et al. 1990, Ward et al. 1991, Baker et al. 1993), indicating that this region would be variable in black bears as well.

Exact protocols for DNA preservation and extraction were similar to those used by Ward et al. (1991).

PCR and Sequencing

PCR was carried out in 25 μL volume reactions with 200 ng of template DNA using a Taq polymerase kit (Perkin–Elmer Cetus, Norwalk, Conn.) and primer sets from Ward et al. (1991). The PCR thermal cycle profile consisted of 30 cycles of 1 minute at 94 C, 1 minute at 50 C, and 1 minute at 72 C followed by a 5 minute extension at 72 C. A 5-μL sample of each reaction was subjected to electrophoresis in a 1% agarose gel and visualized with ethidium bromide staining according to the protocols of Sambrook et al. (1989).

Sequencing was done using the reagents and protocols of the Amersham Life Sciences Sequenase 2.0 sequencing kit (American Life Sciences, Arlington Heights, Ill.), and products of sequencing reactions were migrated for 2.5–3.5 hours through a denaturing 8% polyacrylamide vertical gel (Sambrook et al. 1989). Gels were dried, and autoradiograms were obtained by using a 3–4 day exposure on Kodak XAR film (Kodak Inc., Rochester, N.Y.). Finally, sequence data were entered into a database using the MASE software package (Faulkner and Jurka 1988).

Phylogenetic Inference

Phylogenetic trees were constructed using the parsimony algorithms of computer program PAUP (Swofford 1991). PAUP's branch and bound algorithm was used to identify the most parsimonious trees, and 1000 bootstrap replications were performed to determine the robustness of different components of the inferred trees.

RESULTS

In the control region of the 37 individuals sampled, 7 distinct lineages defined by 14 variable nucleotide positions were found (Fig. 2). The lineages were not found with equal frequency in NFFH; some lineages were common, comprising more than 62% of all samples, whereas others were rare and were found in <3% of sampled individuals (Fig. 2).

Parsimony analysis revealed 2 major clades of lineages which were resolved in 100% of bootstrap replications. One clade (A) includes lineages 1 through 5, and a second clade (B) includes lineages 6 and 7 (Fig. 3). The lineages in these clades differ at an average of 4.4% of nucleotide positions. Although the mean pairwise difference between clades A and B is high, the mean pairwise difference within each clade is only 0.5% and 0.3%, respectively.

#	Variable Position	
1	CCTGTAACCGACGG	23
2	.....T.....	2
3	.....T.....	1
4	.....G.....	2
5	T.....	1
6	TTCACG...A.TAA	7
7	TTCACG...AGTAA	1

Fig. 2. Summary of mitochondrial lineages in black bears. Lineage 1 is used as a reference sequence. Dots indicate identity with the bases in the reference sequence and letters represent differences. The number of individuals carrying each lineage is indicated in the right-hand column. Unshaded and shaded regions represent clades A and B, respectively.

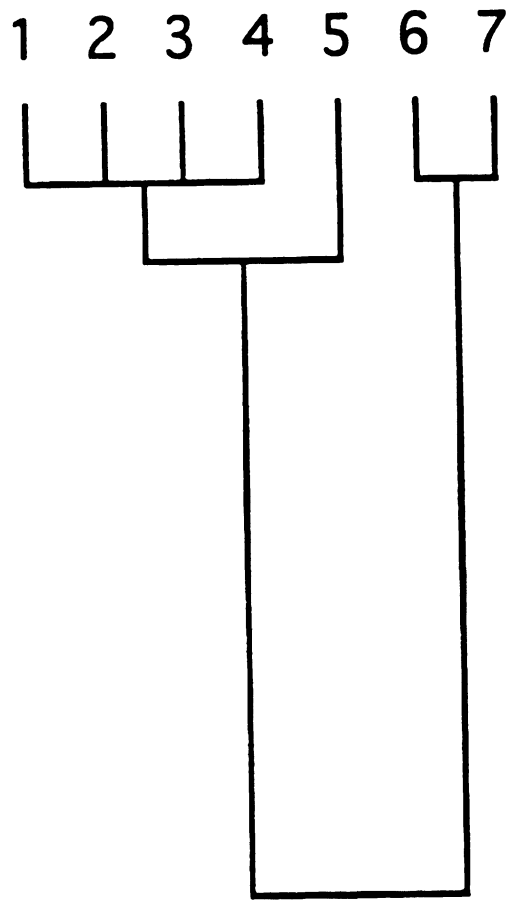

Fig. 3. Phylogenetic tree relating black bear mtDNA lineages. Numbers indicate lineage identifications. Lineages 1–5 are Clade A; 6–7 are Clade B.

DISCUSSION

A variety of phenomena can cause the presence of 2 divergent clades of mtDNA lineages in populations. In many cases, the explanation is a migratory event joining populations after they have been isolated from each other for an extended period of time (Majoram and Donnelly 1994). However, selective forces fostering diversity in DNA sequences, such as frequency dependent selection, can also permit the presence of 2 clades of lineages (Hudson 1990). Two or more separate clades of lineages may even arise in isolated populations in the absence of natural selection because of correlations among mtDNA haplotypes due to coancestry through a particular genealogy, a point explored in detail by Ball et al. (1990). Although 2 clades may evolve under these different conditions, the degree to which the clades can diverge is different in each situation.

In single populations, the divergence among genes unaffected by selection is constrained by the process of genealogical coalescence, which results in the direct relationship of all individuals in a population to a single common ancestor in an amount of time proportional to the population's size. In these populations, the divergence between lineages is limited to levels that can accumulate within the time it takes for the population's genealogy to coalesce (Hudson 1990; Fig. 4). In contrast, diversifying selection can maintain 2 separate lineages indefinitely, even in a relatively small population, and allow them to diverge greatly. Migration can allow high levels of divergence by combining lineages that have evolved completely independently; with migration, divergence is potentially unlimited.

Although populations experiencing immigration or diversifying selection may show the same level of diversity as neutrally evolving populations, they may also sustain much higher levels of divergence than a neutrally evolving population can, and this offers the opportunity to perform tests excluding the possibility that a set of genes has evolved without immigration or selection. A specific test of whether the genetic diversity found in a population is consistent with extended isolation and neutral evolution can be performed by comparing the expected time to coalescence, which can be estimated using population size, with the time to coalescence as estimated using a molecular clock, which can measure the amount of time 2 evolutionary lineages have been diverging.

The model of Ewens (1979) gives the number of generations expected for a population's genealogy to coalesce, assuming no selective forces are present. For a given population size, the time, T, required for a genealogy to coalesce is $T = 2N^eG$, where N^e is female effective population size and G is the generation time in years (Hartl and Clark 1989). We estimate the effective population size of the NFFH population to be approximately 100 females, roughly equal to that found in a nearby drainage (Jonkel and Cowan 1971), so the expected time to coalescence in the NFFH population ($N^e = 100$) is 200 generations or approximately 2000 years. Therefore, if the lineages evolved *in situ* in this population, the time depth of the inferred phylogeny should be approximately 2000 years.

The total length of time lineages have been diverged can be calculated using their degree of divergence and a molecular clock, taking advantage of the fact that mutations seem to accumulate at a steady rate over time. The time that lineages have been diverged is calculated at $T = d/u$, where d is the mean pairwise distance between clades and u is the divergence rate (Li and Graur 1991). The difference between NFFH lineages, $d = 4.4\%$, and the estimated rate of diver-

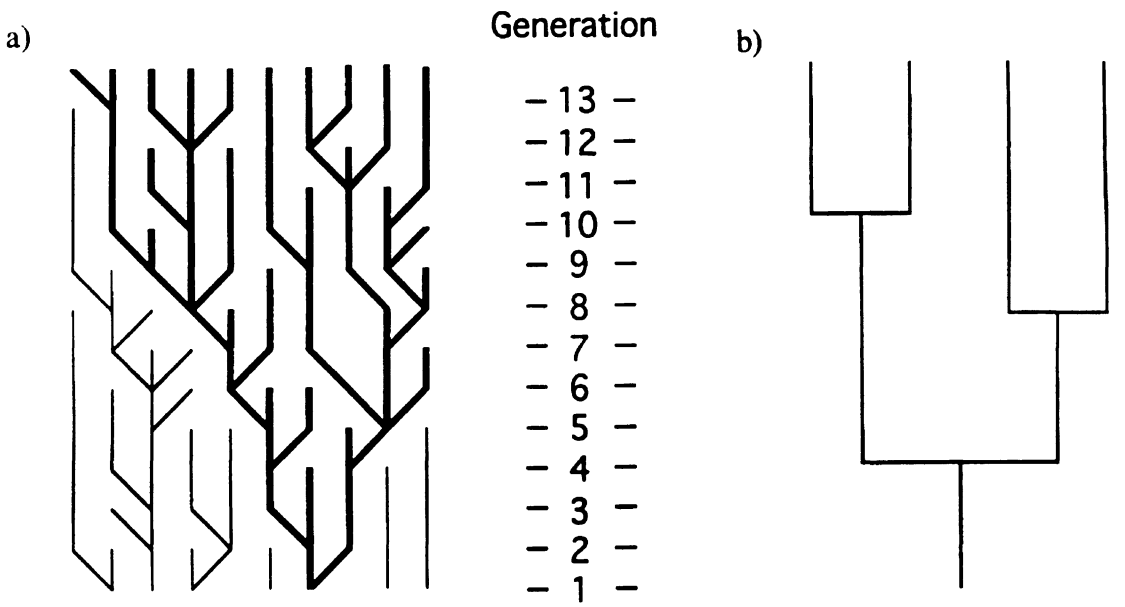

Fig. 4. Coalescent and phylogenetic processes. a) Diagram of the coalescent process in a population with a constant population size of 10. In each generation, some individuals reproduce but others do not. In this example, all members of the population are directly descended from one common ancestor after 13 generations. b) Phylogeny of evolutionary lineages which rose by mutation from the example in a. These lineages share a common ancestor in generation 1, and all other lineages have gone extinct, so the divergence between lineages is limited to the amount that can accumulate in 13 generations.

gence for the control region in bears, 13.8%/million years (Waits 1996), yield a time of divergence of 320,000 years, a value over 150 times as large as the limit defined by Ewens' coalescent model. Although it is difficult to perform a formal statistical comparison of these 2 estimates of the time to the common ancestor, the 2 orders of magnitude separating the estimates are clearly striking.

Three explanations for this result are possible. First, the effective size of the NFFH population might be underestimated. An underestimation of population size would result in an underestimation of coalescence time and, consequently, an underestimation of expected diversity. This possibility is unlikely because it would require an effective female population size of 16,000 to achieve an expected coalescence time of 320,000 years. Second, the presence of diversifying selection could maintain the clades, but the presence of such selective factors seems unlikely since they have not been documented in any mtDNA system. Third, and seemingly most likely, a migratory event may have occurred, bringing distantly related lineages into a new population. This possibility is most plausible since it requires no extraordinary events in the population.

CONCLUSIONS

The distribution of genetic diversity within the NFFH population is consistent with a history of immigration in the population and indicates that other populations having the 2 clades (Cronin et al. 1991) may have similar demographic histories. The immediate cause of this pattern of diversity within the NFFH population seems clear, but the origin of the 2 divergent clades is not. The mixing of the 2 clades in NFFH might represent a local phenomenon, involving the recent interaction of 2 populations with different frequencies of otherwise widespread clades, but the high level of divergence between clades indicates an alternative hypothesis.

Populations containing 2 clades have so far been found only in the vicinity of the Rocky Mountains (Cronin et al. 1991), which are widely recognized as a barrier between eastern and western biogeographic provinces. The NFFH population, located in the Rocky Mountains, might represent a hybrid population composed of migrants from isolated regional groups of populations to the east and west. This hypothesis explains 3 essential features of observed patterns of diversity in black bears: first, it explains why some populations have 2 clades; second, it explains why these populations are localized to the Rocky

Mountain region; third, it explains the high level of divergence between the clades. Recently collected data support this hypothesis. Wooding and Ward (1997) have examined samples from 16 populations, finding the 2 clades have distinctly different but overlapping geographical ranges. This indicates the presence of 2 clades in single populations is not due to local isolations or to natural selection, but population mixing following a long-term division of regional population groups.

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