

## BIOCHEMICAL VARIATION IN THE BLACK BEAR

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**Abstract:** A total of 35 presumably distinct biochemical loci were analyzed in black bears (*Ursus americanus*) from Alaska, California, Maine, Montana, Tennessee, and Virginia by using starch-gel electrophoresis. Limited spatial subdivision of gene frequency was observed in Tennessee. Overall heterozygosity levels are lower in black bears than would generally be expected for mammalian species. Levels of interpopulation genetic similarity are extremely high for a species with such wide distribution, in contrast to the observed morphological variation.

Recent efforts have been made to evaluate the potential and encourage the use of information on the genetic structure of wildlife populations (Berry 1971, Morgan et al. 1974, Smith et al. 1976). Gene frequencies change through time and space (Krebs et al. 1973, Redfield 1973, Utter et al. 1974, Berry and Peters 1976, Manlove et al. 1976) and these changes may be functionally associated with demographic changes (Smith et al. 1976). Levels of genetic variability need to be assayed to evaluate the potential use of genetic data in the formulation of a comprehensive management program. Our objective is to document levels of biochemical variability in the black bear from various populations throughout its range.

Certain ecological and historical factors affect levels of biochemical variation (Selander 1976, Soulé 1976) and confound the interpretation but not necessarily the application of genetic data. Low levels of biochemical variation could be due to drift or founder effect, but both of these factors imply small population numbers at some time in the past. For example, Bonnell and Selander (1974) have explained low biochemical variability in northern elephant seals (*Mirounga angustirostris*) by suggesting a bottleneck effect of reduced numbers resulting from their interactions with humans. Although this argument is certainly tenable, the ecological effects of low environmental variability on a large animal with high vagility might also explain the reduced variation. Animals with a large body size, great mobility, and a high level of homeostatic control may be expected to exhibit low levels of genetic variability (Selander and Kaufman 1973). For a population experiencing the environment as fine-grained (i.e., apparently homogeneous), the optimum evolutionary strategy will often be a single phenotype adapted to the conditions in the environment that are most frequently encountered (Levins 1968). Valentine (1976) has predicted the occurrence of relatively low genetic variability in large migratory carnivores of the temperate zone since, by perceiving the

environment as relatively fine-grained, they should have alleles whose products function under a variety of conditions.

Studies of biochemical variability in mammal populations have emphasized small mammal species, and few studies exist for large carnivores. Larsen (personal communication) found no evidence of genetic variation in polar bears (*Ursus maritimus*), and Yang (personal communication) found low levels of variation in brown bears (*U. arctos*). Because of the potential use of protein polymorphisms for population studies and the lack of adequate data to verify theoretical generalizations, the assessment of genetic variation in large wildlife species such as black bears is of particular interest.

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### METHODS

The black bears included in this survey were from populations in 6 states and represented diverse habitats across the species range. Blood samples were collected by investigators conducting research on bears in Alaska (Prince William Sound), California (Yosemite National Park), Maine, Montana, Tennessee (Great Smoky Mountains National Park), and Virginia.

Whole blood was collected from bears in the field and separated into red cell and plasma components by centrifugation. Except for the samples from California, these tissues were sent to the Savannah River Ecology Laboratory, where they were frozen and stored at -70 C until analysis. The California collection was comprised of relatively fresh samples including liver and kidney tissue, which were analyzed in the laboratory of S. Yang at the University of California at Berkeley. Plasma and hemolysate were electrophoretically analyzed for bears from California, Montana, and Tennessee; only plasma was analyzed for bears from Alaska, Maine, and Virginia.

Samples were subjected to horizontal starch-gel elec-

trophoresis and protein banding patterns resolved with standard histochemical staining methods as described by Selander et al. (1971) and Manlove et al. (1976). Numbers of enzymatic and general proteins that were successfully resolved varied among populations and among samples within populations, depending on the kind (hemolysate or plasma) and condition of material available. These proteins are listed in Table 1 along with buffer systems used during electrophoresis. Sixteen additional proteins not listed in Table 1 were resolved from the California samples. These include adenosine deaminase, alcohol dehydrogenase, esterase-3, globulin, aspartate amino transferase-1 and -2,  $\alpha$ -glycerophosphate dehydrogenase, gluconate dehyd-

Table 1. Summary of electrophoretic data for proteins assayed from plasma and hemolysate in black bears. The proportion of individuals in a population heterozygous at a given locus is given in parentheses for polymorphic loci. The proportion of loci that are polymorphic and average heterozygosity per individual are given at the end of the table for each population.

Proteins or enzymes	Tissue	Buffers <sup>a</sup>	Number of alleles and heterozygosity(h)					
			Alaska N=12	Calif. N=52	Maine N=26	Mont. N=35	Tenn. N=64	Va. N=44
Albumin (ALB)	Plasma	Lithium hydroxide (LiOH)	1	1	1	1	1	1
Esterase-1 (ES-1)	Plasma	LiOH	1	1	-	1	1	1
Esterase-2 (ES-2)	Plasma	LiOH	1	2 (0.273)	-	-	-	1
Esterase-3 (ES-3)	Hemolysate	Tris-maleate (Tm)	-	1	-	1	1	-
Tetrazolium oxidase-1 (TO-1)	Hemolysate	Tris-hydrochloric acid (T-HCl)	-	1	-	-	-	-
Tetrazolium oxidase-2 (TO-2)	Hemolysate	T-HCl	1	2 (0.214)	-	1	1	1
Lactate dehydrogenase-1 (LDH-1)	Hemolysate	T-HCl	-	1	-	1	1	-
Lactate dehydrogenase-2 (LDH-2)	Hemolysate	T-HCl	-	1	-	1	2 (0.206)	-
Malate dehydrogenase-1 (MDH-1)	Hemolysate	Tm	-	1	-	1	1	-
Malate dehydrogenase-2 (MDH-2)	Hemolysate	Tm	-	1	-	1	1	-
Malic enzyme (ME)	Hemolysate	Tm + NADP	-	-	-	1	-	-
6-phosphogluconate dehydrogenase (6-PGD)	Hemolysate	Tm + NADP	-	1	-	1	2 (0.016)	-
Phosphoglucomutase-1 (PGM-1)	Hemolysate	LiOH	-	1	-	-	-	-
Phosphoglucomutase-2 (PGM-2)	Hemolysate	LiOH	-	1	-	1	1	-
Glucose phosphate isomerase (GPI)	Hemolysate	LiOH	-	2 (0.045)	1 (0.156)	2	1	1
Peptidase-1 (PEPT-1) <sup>b</sup>	Hemolysate	Tm	-	1	-	-	1	-
Peptidase-2 (PEPT-2) <sup>b</sup>	Hemolysate	Tm	-	1	-	-	1	-
Plasma protein B (PPB) <sup>c</sup>	Plasma	LiOH	1	-	1	1	1	1
Transferrin (TF)	Plasma	LiOH	1	1	1	1	1	1
Number of loci			6	17 (33) <sup>d</sup>	4	14	15	6
Percentage of polymorphic loci (P)			-	0.176 (0.121) <sup>d</sup>	-	0.077	0.133	-
Mean individual heterozygosity ( $\bar{H}$ )			-	0.031 (0.016) <sup>d</sup>	-	0.013	0.015	-

<sup>a</sup>See Selander et al. (1971) or Manlove et al. (1976) for recipes (except peptidase) and pH values for buffers; NADP= nicotinamide adenine dinucleotide phosphate.  
<sup>b</sup>Stain recipe: 30 mg L-leucyl-L-alanine, 10 mg *Bothrops* snake venom, 20 mg peroxidase, and 10 mg o-dianisidine di-HCl in 50 ml 0.2 M tris-hydrochloric acid buffer (pH=8.0).  
<sup>c</sup>May be identical to PT-1 for California samples listed in the text.  
<sup>d</sup>Includes 16 additional proteins surveyed in California samples as listed in the text.

rogenase, hemoglobin, isocitrate dehydrogenase-1 and -2, leucine amino peptidase-1 and -2, mannose phosphate isomerase, protein-1, and sorbitol dehydrogenase.

The proportion of polymorphic loci ( $P$ ) and average heterozygosity per individual ( $\bar{H}$ ) in a population were calculated for bears from Montana, Tennessee, and California by using the data for proteins listed in Table 1. Comparisons were made of populations from all localities (excluding California) to verify allelic identity across populations. The term *population* as used above refers to all samples from an area or state and is not necessarily meant to be definitive of a biologically functional population. In designating allelic differences for polymorphic loci, superscripts "a" and "b" indicate the relative migration distances of the phenotypes associated with each allele; "a" is the faster-migrating or more electronegative form.

## RESULTS

The proportion of polymorphic loci ( $P$ ) and the average individual heterozygosity ( $\bar{H}$ ) values are given at the end of Table 1. Of the 19 proteins listed in Table 1, 6 were polymorphic, each exhibiting 2 electrophoretic alleles. Although bears from California and Tennessee were polymorphic at more than 1 locus, only 1 locus (GPI) was polymorphic in more than 1 population.

Identical mobilities were observed across populations for all loci fixed for a single allele and when polymorphism occurred, the common allele was the same as that fixed in other populations. For example, LDH-2 had 2 alleles in the Tennessee population (Table 1). The common allele,  $Ldh-2^b$  was 0.18. The  $Ldh-2^a$  allele was fixed in the Montana population.

Gene frequencies for the other 5 polymorphic loci were  $ES-2^a$  and  $-2^b$ , 0.45 and 0.55, respectively (California);  $To-2^a$  and  $-2^b$ , 0.32 and 0.68 (California);  $Gpi^a$  and  $Gpi^b$ , 0.02 and 0.98 (California), 0.11 and 0.89 (Montana);  $6-Pgd^a$  and  $6-Pgd^b$ , 0.01 and 0.99 (Tennessee). Heterozygosity values for these loci are also given in Table 1. Of the additional 16 proteins surveyed in California bears, 1 (protein-1) was polymorphic, having 2 alleles,  $Pt-1^a$  and  $Pt-1^b$ , with frequencies of 0.11 and 0.89, respectively.

The number and distribution of samples from the Great Smoky Mountains National Park permitted an analysis of genetic subdivision among these bears. Allele frequencies at the LDH-2 locus in bears from 6 sample areas (3 separate watersheds) were significantly different among areas ( $X^2 = 20.91$ ,  $P < 0.05$ ). Part of this subdivision was due to a high frequency (0.47) of the  $Ldh-2^b$  allele (including 5 homozygotes) in the Rab-

bit Creek area. This allele is relatively rare elsewhere and its possible association with a family unit along Rabbit Creek is supported by data from activity monitoring and field observations.

## DISCUSSION

From the standpoint of using protein polymorphisms as genetic markers on a broad scale to supplement demographic studies of black bear populations, the prospects are not particularly encouraging. There are, however, a few isolated polymorphisms that could be useful for obtaining indices of genetic subdivision and inbreeding and for following patterns of dispersal and gene flow among populations. The localized distribution of the  $Ldh-2^b$  allele among bears in the Smoky Mountains provides a rough index to genetic subdivision of the population(s).

Where allelic variants are spatially concentrated, an opportunity exists to incorporate an index of gene flow into other measures of dispersal. Such information could be very useful for interpreting results from population studies of many wildlife species, including bears. Knowledge of the extent of spatial genetic subdivision among populations may be of value in defining management units (Manlove et al. 1977). Spatial subdivision and short-term genetic changes in time are documented for populations of a variety of species (Smith et al. 1976), and the dynamic nature of the genetic structure of wildlife populations should be an important concern in developing management strategies.

Evaluations of results of  $P$  and  $\bar{H}$  values from Yosemite bears (Table 1) implies that both of these estimates for the Montana and Tennessee populations would be lower if based on more protein systems. However, these values are lower than those generally observed in mainland populations of other mammals.  $\bar{H}$  values for 200 mainland populations of 47 mammal species given by Smith et al. (1978) range from 0 to 0.155 ( $x = 0.042$ ).  $\bar{H}$  values below 0.02 are primarily restricted to species with relatively local distribution or to island populations. Levels of genetic variability in bear populations are toward the lower end of the range for mammals. Since estimates of variability are currently available for only a few large mammals, the significance of low levels of protein polymorphism in black bears remains speculative. Valentine (1976) has suggested that food resource specialists should maintain relatively low levels of genetic variability. As food generalists, bears do not support this speculation. Unpublished data for populations of elk (*Cervus canadensis*) from Montana and caribou (*Rangifer tarandus*)

from Alaska, along with the previously cited studies of bears and elephant seals, conform to Selander and Kaufman's (1973) prediction that large, mobile animals tend to have lower levels of genetic variability than small, less mobile animals. In apparent contrast to this generality, however, are white-tailed deer (*Odocoileus virginianus*), with  $\bar{H}$  values exceeding 12 percent in some populations (Smith et al. 1976), which are among the highest values observed in mammals. Even if body size and mobility affect levels of genetic variation, the relationship is certainly confounded by other environmental and demographic effects. Since fluctuations in number can have a measurable effect on genetic variation (Soulé), an adequate explanation of this measure cannot ignore some account of the population's recent history. It is apparent from laboratory and field studies of small mammals that levels of genetic variability are directly related to absolute densities and reproductive success (Smith et al. 1975). This relationship could have profound implications for managing wildlife populations when genetics data can be systematically incorporated into demographic studies.

Since comparisons on the same gels were not made of Yosemite bears with other populations, their allelic identity to the other populations is not certain. We expect, however, that most if not all loci in the sample would be fixed for alleles common in the other populations across a large geographic area. Electrophoretically detectable genetic uniformity across the black bear's range is greater than that generally found in small mammals. Biochemical similarity among black bear populations also appears to contrast with observed phenotype

variation in morphology across the species range (e.g., variation in body size and coat color). We must recognize the fact that broad-scale generalizations of the causes and consequences of genetic variation are inadequate to explain either the subtle differences in genetic structure among populations or the trends observed in major taxonomic or trophic groups without concurrent knowledge of local environmental, demographic, and historical effects.

This study of black bears should be considered preliminary. With increasing concern for managing optimum bear densities in refuges with limited suitable habitat, some knowledge of the genetic consequences of manipulating habitat quality, population structure, and densities may be important for planning and evaluating the success of a management program. Also, although much data have been accumulated to describe individual movement patterns in populations of many wildlife species, including black bears, we know very little about the extent of effective dispersal (i.e., gene flow) in and among populations. The use of electrophoretic protein variants as genetic markers to complement data on movement and breeding behavior provides an excellent way of beginning to deal with this problem. Future studies of biochemical variation in black bears should attempt to increase the sample size of the populations surveyed and should focus on detectable polymorphisms and genetic indices that will provide data on changes in genetic structure in time and space to supplement concurrent studies of activity patterns, demography, and breeding structure.

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