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GENETIC VARIATION IN BLACK BEARS IN ARKANSAS AND LOUISIANA USING MICROSATELLITE DNA MARKERS

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In the 1950s and 1960s, translocation projects reintroduced black bears (*Ursus americanus*) from Minnesota and Manitoba to Arkansas and Louisiana. Today, several geographically disconnected populations exist in Arkansas and Louisiana, but their origins are unclear. Some populations may represent a separate subspecies, *U. a. luteolus*, which is federally protected. We characterized 5 microsatellite loci in 5 isolated populations in Arkansas and Louisiana and compared them with genotypes from Minnesota. Our data indicate that bears of the Ozark and Ouachita mountains of Arkansas, an inland area of Louisiana, and those of Minnesota are similar in overall genetic diversity and allele frequencies, consistent with these populations being wholly or mostly descended from bears from the reintroduction programs. In contrast, bears from southeastern Arkansas and the coastal region of Louisiana genetically are more restricted and homogeneous. Because they exhibit a limited set of genotypes found in the other black bear populations, they represent isolated fragments of a single North American black bear population. Furthermore, genetic distance estimates indicate that the bears in southeastern Arkansas are more genetically distinct from bears in Louisiana, which are currently federally protected.

Key words: Arkansas, black bear, DNA, Louisiana, microsatellite, population genetics, *Ursus*

During the 1st half of the 20th century, extirpation of black bear populations progressed at a rapid rate in most of Arkansas and its border states (Smith et al. 1991). By the 1940s, only an estimated 25–50 bears remained in southeastern Arkansas (Dellinger 1942; Holder 1951), mostly in what is now the White River National Wildlife Refuge (NWR). The population was estimated at 160–175 bears in 1990 (Smith and Pelton 1990), and today, they number more than 600 (R. Eastridge, pers. comm.).

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From 1958 to 1967, an estimated 254 bears from northern Minnesota and Manitoba, Canada, were released in the Ozark and Ouachita national forests (Rogers 1973) to repopulate the Interior Highlands of Arkansas. Smith et al. (1991) estimated that there were over 2,500 black bears in the Interior Highlands of Arkansas, Missouri, and Oklahoma. That number has since increased, making it the most successful reintroduction of bears in the world (Smith and Clark 1994). Those populations were assumed to be largely or entirely derived from the translocated bears (Smith and

Clark 1994). From 1964 through 1967, 161 bears from Minnesota were released in northeastern Louisiana (Tensas River) and the Atchafalaya Basin (Lowery 1974). The fate of bears introduced in Louisiana is not well documented (summerized by R. M. Nowak, in litt.). However, some marked bears released in Arkansas remained near release areas for many years (Smith et al. 1991).

Despite conflicting conclusions about the validity and integrity of a subspecies designation (reviewed in Pelton 1991), the Louisana black bear (*Ursus americanus luteolus*) was federally listed as threatened in 1992 (Department of the Interior 1992). All the bears translocated from Minnesota and Manitoba were *U. a. americanus* (Lowery 1974; Sealander and Heidt 1990). However, federal guidelines dictate that all bears in Louisiana, southern Mississippi, and eastern Texas, regardless of origin, are assumed to represent the Louisiana black bear and are therefore protected (Department of the Interior 1992). The federal guidelines further explicitly exclude Arkansas as part of the historic range of the Louisiana black bear, leaving the taxonomic status of the ''native'' population in the White River NWR unresolved. Those bears have been characterized as both *U. a. americanus* (see map in Hall 1981) and *U. a. luteolus* (Sealander and Heidt 1990). Delineation of the 2 subspecies is based on geographic location and morphometrics (Hall 1981), but biochemical comparisons using isozymes and mitochondrial DNA failed to detect differences (Manlove et al. 1980; Pelton 1991). Miller et al. (1998) used a human minisatellite probe for multilocus analysis of black bears and concluded that there were no significant genetic impacts caused by the translocations to Louisiana or Arkansas and that populations in Arkansas are more closely related to those in Louisiana than to those in Minnesota. These conclusions contrast with both historical accounts and those of Smith and Clark (1994) that the Interior Highland populations are descended from translocated stock.

Microsatellite analyses have been used increasingly to assess relatedness of individuals (Amos et al. 1993) and have been useful for detection of variation in species that have inherently low genetic polymorphisms (Hughes and Queller 1993). Microsatellite loci in the Ursidae have been described, and primers for these loci are conserved sufficiently for use in most bear species (Paetkau and Strobeck 1994; Paetkau et al. 1995). We used several of those microsatellite loci to assess genetic variability in 6 geographically separated black bear populations representing 3 populations in Arkansas, 2 in Louisiana, and 1 in Minnesota. Samples from Minnesota bears were collected near the area where translocated bears were reported to have been originally captured (D. A. Miller, pers. comm.). We compared the genotypic patterns of these 6 populations to assess relatedness. Our goal was to determine whether the populations are distinguishable at the genotypic level, whether the translocated bears were the primary source of any of the populations in Louisiana and Arkansas, and the genetic relationship of these populations to the refugial population of Arkansas.

Materials and Methods

DNA isolation.—DNA was isolated from blood or tissue samples representing the following bear populations (see Fig. 1): Ozarks (n = 13), Ouachitas (n = 6), White River NWR (n = 18), Minnesota (n = 10), Pointe Coupee Parish, an inland region of Louisiana (n = 16), and the southern coastal region of Louisiana (n = 20). Blood samples in the Ozarks and Ouachitas were collected by Arkansas Game and Fish personnel at 2 long-term study sites (Clark and Smith 1994) that are separated by the Arkansas River valley and Interstate Highway 40, which limits gene flow between these allopatric populations. Blood samples from Louisiana were from those populations studied by Pace et al. (2000). Tissue and blood samples from Minnesota and the White River NWR were obtained from researchers at Virginia Polytechnic Insti-

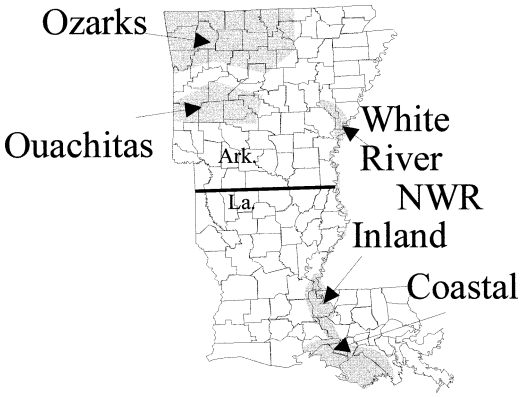

FIG. 1.—Collection sites for samples of black bears from Arkansas and Louisiana. Shaded areas indicate the current distribution of the 5 populations included in this study, and arrows indicate the approximate location of sampling sites. Modified from Pelton and van Manen (1997).

tute and State University; additional blood samples from the White River NWR were obtained from the University of Tennessee. Bear populations will be referred to as Ozark, Ouachita, White River, Minnesota, Inland, and Coastal, respectively.

Some of the blood samples were stored at room temperature as 1:1 mixtures with 2% sodium dodecyl sulfate (SDS, ICN Biomedicals, Aurora, Ohio), 150 mM Tris, 130 mM ethylenediaminetetraacetic acid (EDTA). DNA was isolated by addition of pronase (proteinase E, Sigma Aldrich, St. Louis, Missouri) to 200 μg/ml and incubation at 37°C for 4–12 h. If clots remained after 4 h, additional pronase was added and the incubation continued. The aqueous solution was extracted with phenol : $CHCl_3$, then with $CHCl_3$, and the DNA precipitated with cold ethanol.

Blood samples collected in Vacutainers (Becton Dickinson, Franklin Lakes, New Jersey) containing EDTA were stored frozen until processed. Cells were lysed with SDS (0.5%) in the presence of EDTA (5 mM), followed by pronase (200 μg/ml) digestion at 37°C for 4 h, extraction with phenol : $CHCl_3$ and then $CHCl_3$, and DNA precipitation with cold ethanol.

Frozen tissue samples (skeletal muscle or ear punches) were initially minced with a razor blade, homogenized in 10 mM Tris–Cl pH 7.5, 5 mM EDTA with a hand-held homogenizer (Virtis, Gardner, New York), and then digested with pronase (200 μg/ml) in the presence of 0.5% SDS at 37°C for 4 h. The digest was then extracted with phenol : $CHCl_3$ and then $CHCl_3$, followed by DNA precipitation with cold ethanol. Purified DNA was redissolved in Te (10 mM Tris–HCl, pH 7.5, 0.1 mM EDTA), quantified using a TKO flourometer (Hoefer Scientific Instruments, San Francisco, California), diluted in Te, and stored at 4°C.

Microsatellite analysis.—Five pairs of microsatellite primers (G1A, G10L, G10B, G10C, and G10P), described previously by Paetkau and Strobeck (1994) and Paetkau et al. (1995), were used to amplify $(CA)_n$ microsatellite loci by polymerase chain reaction (PCR). Genotypes for these 5 loci were determined for 6 bears from the Ouachitas, 13 from the Ozarks, 6 from White River NWR, 10 from Minnesota, 16 from inland Louisiana, and 20 from coastal Louisiana. Genotypes for all loci but G1A were determined for 12 additional bears from the White River NWR. All genotypes were determined at least twice for reproducibility.

One primer from each pair was 5′ labeled with IRD41 for detection on a Licor Model 4000 (Licor Technologies, Lincoln, Nebraska). Unlabeled primers were obtained from Integrated DNA Technologies (Coralville, Iowa). Ten microliters amplified by PCR consisted of 1× PCR buffer (50 mM Tris–Cl pH 8.5, 5 mM $MgCl_2$, 250 mM KCl, 0.5% gelatin, 0.5% Triton-X, 0.5% Ficoll, and 1 mM tartrazine), 1.25 mM $MgCl_2$, 30 ng of genomic DNA, 200 μM diethylnitrophyenyl thiophosphates (dNTPs), 1 M unlabeled primer, 0.2 μM labeled primer (for G10L, G10B, G10C) or 0.4 μM labeled primer (for G10P and G1A), and 0.5 U *Taq* DNA polymerase.

Cycling was completed in sealed capillary tubes in an Idaho Model 1605 thermocycler (Idaho Technology, Salt Lake City, Utah). Samples were heated to 90°C for 30 s, followed by either 30 cycles (for G1A and G10L) or 38 cycles (for G10B, G10C, and G10P) of 15 s at 90°C, 15 s at 60°C, and 60 s at 72°C. Cycling was followed by 3 min at 72°C. After amplification by PCR, the samples were mixed with 6 μl stop buffer (95% formamide, 5% 1× Tris-Borate-EDTA, 3% bromphenol blue–xylene cyanol) and denatured, and 1.5 μl of each sample mixture was loaded on 40-cm 6% denaturing polyacrylamide gels for detection. Sequencing-

reaction ladders were used for size determinations.

Some samples were assayed using either a Cy5™ labeled G10P primer or Cy3™ labeled G10C primer (synthesized by MWG Biotech, High Point, North Carolina). For these analyses, the amplification by PCR consisted of 45 cycles using 1 μM for the labeled primer. Products were resolved on 8% denaturing polyacrylamide gels and detected by scanning with a Typhoon fluorescence scanner (Molecular Dynamics, Amersham Bioscience, Sunnyvale, California). Markers were a rhodamine-labeled 20–base-pair (bp) ladder (Promega Corporation, Madison, Wisconsin). Control DNAs were amplified in all electrophoreses to ensure identical allele size determinations in both the Licor and the Typhoon systems.

Statistical methods.—Expected and observed values of heterozygosity testing for Hardy–Weinberg equilibrium values (Guo and Thompson 1992), sample differentiation (Raymond and Rousset 1995), and assignment of test scores to populations were determined using Arlequin 2.00 software (S. Schneider, in litt.). Markov chain lengths were all set to 10,000. Nei's standard genetic distance (Nei 1972) was calculated between all population pairs using Gendist from the PHYLIP software package version 3.5c (J. Felsenstein, in litt.). Unrooted trees were generated using the Fitch program in the same package.

RESULTS

Seven to 10 distinct alleles were observed for each of the 5 loci (Table 1). The average number of alleles per locus was lowest in the White River (1.8) and Coastal populations (4.2), whereas more alleles were detected in the other 4 populations (Ouachita, 4.6; Minnesota, 5.6; Inland, 5.6; and Ozark, 5.8). The range of allele sizes and frequencies is similar to that of the sizes and frequencies reported for western and eastern Canadian black bear populations (Paetkau and Strobeck 1994; Paetkau et al. 1997, 1998). However, the allele frequencies and sizes for the Canadian bears differed slightly from our Minnesota samples. Allele sizes were determined for loci G10L (Fig. 2) and G10P, but our size estimates for alleles for G1A, G10C, and G10P could not be absolutely correlated to the Canadian bears. For example, we estimate band sizes for G10P to be in even numbers of base pairs (Table 1), whereas Paetkau et al. (1998) reported allele sizes in odd numbers of base pairs. Most of our sizes were estimated from comparison with sequence ladders run in parallel, but absolute correlation with other black bear allele frequencies will require standardization of allele sizes.

Of concern is that 3 bear DNA samples (2 from Minnesota and 1 from inland Louisiana) failed to amplify with the primers for G10P despite numerous attempts and despite successful amplifications from these DNA samples for the other 4 loci. Whether this means that these bears are homozygous for a ''null'' allele of G10P is subject to conjecture. Paetkau and Strobeck (1995) described null alleles for G10P in Canadian black bears and Asiatic black bears (*Ursus thibetanus*). If 2 of 10 Minnesota bears are homozygous for a null allele at G10P, then the null allele frequency in the population should be at least 0.447, and we would predict a frequency of null heterozygotes of 0.494 ($2 \times 0.447(1 - 0.447)$). However, only 1 of the other 8 Minnesota bears and only 5 of the 15 inland Louisiana bears were scored as homozygotes. Observed heterozygosities for G10P for these 2 populations (87.5% and 66.7%, respectively) were among the highest (the Ozark population was highest with 92.3% heterozygosity at G10P). Therefore, there does not appear to be a high level of null alleles in our data set. Further examination of those 3 bears for the G10P locus would require examination with the alternative primers described by Paetkau and Strobeck (1995) and was beyond the scope of our study.

Allele frequencies were used to compute expected heterozygosity values for comparison with observed heterozygosity values (Table 2). Expected heterozygosity within populations at individual loci ranged from 0% to 91%, with mean expected heterozygosity for all loci for a population between

28% ± 21 *SD* and 75 ± 9%. There were no sizable differences between expected heterozygosity values and the observed heterozygosities in each of the populations (Table 2). Hardy–Weinberg analysis at each locus failed to show any deviation from expected distributions for any locus over all the populations. However, *P* values for some loci were low for some populations (e.g., G10L for Minnesota and Inland, and G10C for Ozark and Minnesota). Expected heterozygosities for the Minnesota population approximate those reported for Canadian black bears by using some of the same loci (Paetkau et al. 1997). Genetic diversity values for the Ozark, Ouachita, and Inland populations were similarly high, but heterozygosity values for the White River (0.317) and Coastal (0.428) populations were low.

Allele frequencies were used to compute Nei's genetic distance among 6 populations and to perform an exact test of sample differentiation to test for panmixia (Raymond and Rousset 1995). The White River population was the most distinct (Table 3), with genetic distance values ranging from 1.1 to 1.4 compared with the Minnesota, Inland, Ozark, and Ouachita populations. This is supported by *P* values <0.05 for comparisons with all populations except the Ouachita samples ($P = 0.067$). The coastal population yielded intermediate genetic distance values of approximately 0.6 to all other populations and differentiation *P* values above the 0.05 level (except for comparison with White River, 0.000). The smallest genetic distances were found between the Ozark and the Ouachita populations, which in an unrooted tree (Fig. 3), grouped with the inland Louisiana and Minnesota bears. Surprisingly, the White River population appears to be more distantly related to the other 4 populations than does the coastal Louisiana bear population.

The assignment test of Paetkau et al. (1995) uses population allele frequencies to assess whether individual genotypes are more (or less) representative of their source population. The test assigns each bear to the population where its genotype frequency is calculated to be the highest. All but 3 of our 72 bear samples were assigned to their source population. Two Ozark bears gave slightly better assignment scores for the Minnesota population, and 1 Minnesota bear gave a substantially better assignment score for the coastal Louisiana population. The latter misclassification is largely because the bear in question is homozygous for the 193-bp allele of G1A. Coastal bears are all homozygous for allele 193, whereas this allele is only 20% of the G1A alleles in our Minnesota samples (Table 1). No bears from the White River and Coastal populations were misclassified, supporting our conclusions (see below) that these populations are still largely, if not completely, unaltered by the translocation projects. Thus, those 5 microsatellite markers produced sufficient population genotypic differences to discern each of the populations.

Discussion

Based on genetic distances, the Ozark, Ouachita, Inland, and Minnesota populations are most likely representative of the same population (i.e., the Ozark, Ouachita, and Inland populations are derived from the Minnesota population). This conforms to historical accounts that the Ozark, Ouachita, and Inland bear populations resulted largely or exclusively from translocated Minnesota bears. There have been only 2 previous molecular genetic studies published comparing these 6 black bear populations (Miller et al. 1998; Warrilow et al. 2001). Miller et al. (1998) used Southern blots probed with a human minisatellite for multilocus analysis. Genetic comparisons were based on median band-sharing values. They concluded that there is more similarity between and within the populations of Arkansas and those of Louisiana than there is to the population in Minnesota. However, their conclusions were based on small sample sizes, incomplete pairwise comparisons, and pooling choices that may have confused the population comparisons. We had

TABLE 1.—Allele counts and frequencies for 5 microsatellite loci in 6 populations of black bears. Number of individuals (*n*) assayed given for each locus for populations in the Ozarks, Ouchitas, White River National Wildlife Refuge, Minnesota (=Minn.), and inland and coastal Louisiana. Sampling sites are shown in Fig. 1. Allele size is the number of base pairs of the amplified product.

		Allele frequency					
	Allele	Ozarks	Ouachitas	White River	Minn.	Inland	Coastal
GIA							
n		13	6	6	10	16	20
	183				0.100	0.063	
	185					0.156	
	187				0.100	0.063	
	189	0.423	0.667		0.350	0.219	
	191	0.115		0.583	0.150	0.281	
	193	0.192	0.250	0.417	0.200	0.188	1.000
	195	0.269	0.083		0.100	0.031	
G10L							
n		13	6	17	10	16	20
	137	0.192	0.333	0.588	0.100	0.063	0.550
	139	0.462	0.417		0.450	0.334	
	141	0.154			0.050	0.063	
	143	0.077					
	151	0.039			0.100		
	153						0.050
	155	0.039	0.083		0.200	0.031	0.050
	157	0.039			0.100	0.094	0.200
	159		0.167	0.412			0.125
	161					0.406	0.025
G10B							
n		13	6	18	10	16	20
	154					0.030	
	156					0.030	
	158	0.577	0.667	0.056	0.450	0.813	0.900
	160	0.192	0.083		0.250	0.125	0.025
	162	0.192	0.167		0.100		
	164	0.038			0.050		
	166		0.083	0.944	0.150		0.075
G10C							
n		13	6	18	10	16	20
	106	0.077	0.083		0.050	0.219	0.025
	108	0.038					
	110	0.038	0.083		0.100		
	112	0.192	0.250		0.750	0.031	
	114	0.231	0.333	1.000	0.100	0.157	0.800
	116	0.423	0.167			0.063	0.050
	118		0.083			0.031	0.025
	120				0.125		
	122					0.375	0.025
	124						0.075
G10P							
n		13	6	18	8	15	20
	150				0.063	0.433	0.325
	154			0.861	0.188	0.267	0.150

TABLE 1.—Continued.

		Allele frequency					
	Allele	Ozarks	Ouachitas	White River	Minn.	Inland	Coastal
156	0.077			0.250			
158	0.115	0.167		0.063		0.050	
160	0.077	0.083	0.139	0.188		0.175	
162	0.231	0.167		0.063	0.267	0.300	
164	0.154	0.250		0.125	0.033		
166	0.077	0.167					
168	0.154	0.167		0.063			
172	0.115						

previously attempted to compare some of these 6 populations using randomly amplified polymorphic DNA (RAPD—D. Rhoads, in litt.) and found that the banding patterns were so diverse that the coincidence of band migration confused band-sharing analyses. We suspect that the same was true for the comparisons by Miller et al. (1998), which are based on the human minisatellite probe.

Warrilow et al. (2001) recently reported microsatellite analysis of a large collection of bears from the southeastern United States, including bears from the same regions we examined. Both our data and theirs support a close relationship between the bears of Minnesota and the Interior Highlands (Ozarks and Ouachitas). In contrast to our findings, Warrilow et al. (2001) concluded that the bears of the upper Atchafalaya group with bears from the lower Atchafalaya and the White River NWR. This is surprising because both our analyses and theirs used the same loci. However, we cannot precisely compare their allele sizes with ours. The sizes they report are close to our size determinations in some cases and the same in others. The most notable exception between the 2 data sets is for our Coastal population, which should be analogous to their lower Atchafalaya population. We detected only homozygotes (188 bp) for locus G1A, whereas, Warrilow et al. (2001) reported 6 different alleles with the 186- and 188-bp alleles comprising 27% and 65%, respectively. Because they did not

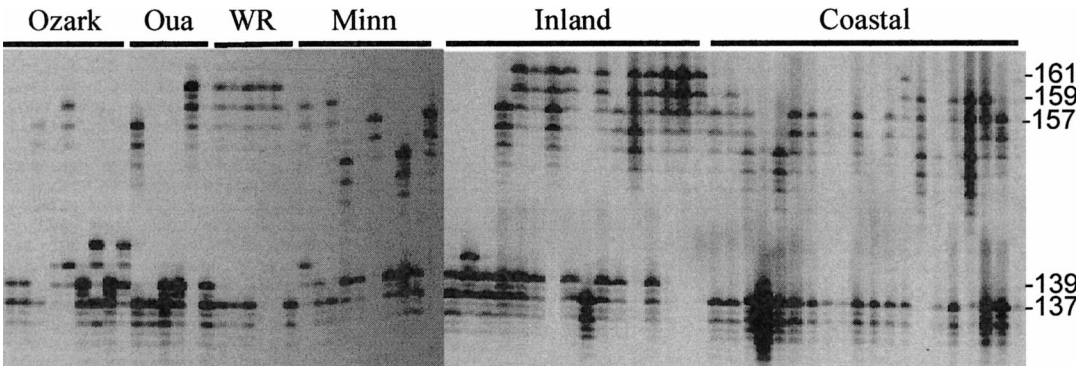

FIG. 2.—Representative gels for microsatellite locus G10L in black bears from populations in Ozarks (=Ozark), Ouachita Mountains (=Oua), and White River National Wildlife Refuge (=WR) in Arkansas, coastal and inland Louisiana (see Fig. 1), and Minnesota (=Minn). Sizes (in number of bases) based on sequence ladders are indicated on the right.

TABLE 2.—Observed (=Obs.) and expected (=Exp.) heterozygosity values and *P* values for Hardy–Wienberg equilibrium (Guo and Thompson 1992) for the 5 microsatellite loci as tabulated by Arlequin 2.00 software (S. Schneider, in litt.) for the 6 populations of black bears. Minnesota = Minn. Values could not be computed for loci that were monomorphic (=mono).

	Locus											
	G1A			G10L			G10C		G10B			G10P		
Population	Obs.	Exp.	*P*	Obs.	Exp.	*P*	Obs.	Exp.	*P*	Obs.	Exp.	*P*	Obs.	Exp.	*P*
Ozarks	0.846	0.738	0.25	0.615	0.732	0.09	0.462	0.778	0.01	0.769	0.658	1.00	0.923	0.898	0.07
Ouachitas	0.667	0.636	0.21	0.667	0.803	0.64	0.500	0.788	0.10	0.833	0.651	1.00	1.000	0.894	0.71
White River	0.500	0.621	1.00	0.625	0.508	0.61		mono		0.111	0.160	1.00	1.000	0.294	0.27
Minn.	0.700	0.847	0.51	0.500	0.779	0.02	0.100	0.521	0.01	0.800	0.800	0.66	0.778	0.915	0.19
Inland	0.438	0.841	0.00	0.563	0.746	0.06	0.750	0.790	0.19	0.313	0.333	0.28	0.667	0.720	0.12
Coastal		mono		0.800	0.653	0.42	0.250	0.395	0.35	0.200	0.315	1.00	0.650	0.777	0.00

report observed heterozygosities for individual loci, we do not know how many of their samples were homozygous for allele 188. Additionally, for locus G10C we detected a broader range of alleles. Specifically, we detected alleles of 120, 122, and 124 bp in the Inland and Coastal populations, which should correspond to their upper and lower Atchafalaya populations, respectively. Further, comparisons of these 2 data sets are difficult because there are significant differences in the methods used to determine genotypes. Whereas we used the more conventional system of a labeled primer, 40-cm denaturing sequencing gels, and laser detection, Warrilow et al. (2001) used short (18 cm) nondenaturing gels and detection with silver staining of the double-stranded bands. PCR amplification of microsatellite loci yields numerous artifactual bands, and size determination depends on high-resolution electrophoretic systems and accurate band identification. Our analyses are based on high-resolution gels and specific detection of only 1 strand of the amplification products (Fig. 2). To resolve this discrepancy, the samples analyzed by Warrilow et al. (2001) would have to be reanalyzed using a higher-resolution system. Further, it will be important to determine the geographic proximity of their upper and lower Atchafalaya collections to our inland and coastal Louisiana bears.

Heterozygosity values for the White River and Coastal populations were lower than for the others and more comparable with the values others have determined for reproductively isolated bear populations. The isolated black bears of Terra Nova National Park have a mean heterozygosity of 36%, whereas the brown bear (*Ursus arctos middendorffi*) population of Kodiak Island, estimated to have been reproductively isolated for 10,000 years, had an expected heterozygosity of 0.325 (Paetkau et al. 1997). In other areas of southeastern United States, heterozygosity values were 47.4% at Tensas River NWR (Boersen 2001), 39.0% at Chassahowitzka NWR in Florida (*U. a.*

TABLE 3.—Genetic distance and sample differentiation values for 6 populations of black bears. Minnesota = Minn. Upper right half of the matrix contains genetic distance values obtained from Gendist (J. Felsenstein, in litt.), based on allele frequencies at 4 loci (excluding G10P). Lower left are *P* values ± *SE* for sample differentiation (Raymond and Rousset 1995) using Arlequin 2.00 software (S. Schneider, in litt.)

	Ozarks	Ouachitas	White River	Minn.	Inland	Coastal
Ozarks	—	0.116	1.454	0.323	0.460	0.617
Ouachitas	1.00 ± 0.00	—	1.111	0.313	0.495	0.485
White River	0.003 ± 0.002	0.07 ± 0.01	—	1.248	1.271	0.591
Minn.	1.00 ± 0.00	1.00 ± 0.00	0.007 ± 0.004	—	0.605	0.893
Inland	1.00 ± 0.00	1.00 ± 0.00	0.007 ± 0.005	1.00 ± 0.00	—	0.509
Coastal	0.16 ± 0.03	0.16 ± 0.03	0.00 ± 0.00	0.12 ± 0.01	0.09 ± 0.02	—

floridanus—D. S. Maehr, in litt.), and 31.6% at Mobile River Basin in Alabama (*U. a. floridanus*—Edwards 2002). Therefore, genetic diversities in the White River and Coastal black bear populations are consistent with these being reproductively isolated populations.

Differences in the conclusions drawn between our study and the study by Warrilow et al. (2001) are of critical importance to management of bear populations in Arkansas and Louisiana. Our data and conclusions do not support the current policies of the U.S. Fish and Wildlife Service to protect all black bears of Louisiana as *U. a. luteolus*. Our data indicate that some of the federally protected black bear populations of Louisiana are largely derived from translocated bears. Conversely, the White River and Coastal bear populations appear unaltered by the translocation projects. They show low levels of heterozygosity and few alleles, and none of the bears were misclassified in the assignment tests. Therefore, these 2 populations appear to have remained reproductively isolated from the more diverse populations.

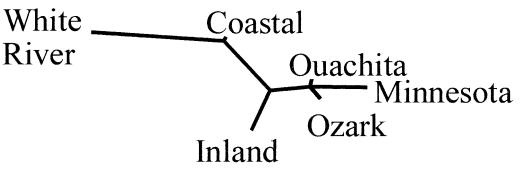

FIG. 3.—Phylogenetic tree for the 6 populations of black bears examined in this study. Nei's (1972) genetic distances (Table 3) were used to construct an unrooted tree based on all 5 loci measured. See ''Materials and Methods'' for details.

What is the genetic status of the White River population and that of the Coastal population representing *U. a. luteolus*, relative to the other black bear populations? The microsatellite alleles we detected in these 2 populations are not unique to either population. Rather, these populations contain a subset of alleles identified in the other populations. Therefore, based on these genetic analyses, we conclude that neither the White River bears nor the Coastal bears are distinguishable from being restricted subsets of a greater North American black bear population. Although these isolated populations differ in terms of their level of genetic diversity, they might not be sufficiently different to be considered different subspecies. Morphometric data argue for subspecific status for the coastal Louisiana bear (Pelton 1991; but see Kennedy et al. 2002). However, our data suggest that the bears of the White River NWR are even more distinct at the genotypic level. Therefore, the morphological variation seen in the Louisiana black bear may be more the result of factors such as genetic bottleneck and founder effects or possibly environmental plasticity (Pelton 1991) rather than true genetic differences. Alternatively, if the coast-

al Louisiana bears are to be given subspecies status, the more genetically distinct Arkansas native bears of the White River NWR also should be considered for separate subspecies status. However, inclusion of the large population of bears at White River NWR as *U. a. luteolus* would warrant reconsideration of the threatened status of the subspecies, given that current legislation explicitly excludes Arkansas for the range of *U. a. luteolus*. Finally, consideration should be given as to whether some of the inland Louisiana black bear populations (specifically those of Pointe Coupee Parish) should be included for protection as *U. a. luteolus*.

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