A COMPARISON OF GENETIC DIVERSITY IN NORTH AMERICAN BROWN BEARS

LISETTE WAITS,[1] Laboratoire de Biologie des Populations d'Altitude, CNRS UMR 5553, Universite Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France
DAVID PAETKAU, Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada, email: dpaetkau@gpu.srv.ualberta
CURTIS STROBECK, Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada
RICHARD H. WARD, Institute of Biological Anthropology, University of Oxford, 58 Banbury Road, Oxford, OX2 6QS, England, email: ryk.ward@bioanthropology.oxford.ac.uk

Abstract: To determine if threatened brown bear (*Ursus arctos*) populations of Montana and Wyoming have lower levels of genetic variation than other North American populations, we examined mitochondrial DNA (mtDNA) and nuclear microsatellite DNA diversity in 220 brown bears from 5 areas: Kodiak Island, Alaska; Kluane National Park, Canada; Eastern Slope of the Rockies (East Slope), Canada; Yellowstone ecosystem (YE), Wyoming and Montana; and Northern Continental Divide Ecosystem (NCDE), Montana and British Columbia. Nei's genetic diversity (h) was estimated by analyzing 296 base pairs of control region sequence data from mtDNA and by nuclear microsatellite analysis of 8 independent loci. Genetic diversity was lowest in the Kodiak Island sample. The YE and East Slope samples had intermediate levels of mtDNA diversity and microsatellite diversity. Kluane and NCDE samples had high levels of mtDNA diversity and microsatellite diversity. Genetic diversity in the YE and NCDE samples was lower than in the Kluane sample; however, these differences were statistically signficant ($P < 0.05$) for only 1 microsatellite locus in the YE sample. In contrast, the Kodiak Island sample had significantly less diversity ($P < 0.05$) than the Kluane sample at the mtDNA locus and 6 microsatellite loci. Because genetic diversity has been suggested as critical for the evolutionary fitness of wild populations, the management implications of these results are examined and discussed.

Ursus 10:307–314

Key words: brown bear, genetic variation, inbreeding, microsatellites, mitochondrial DNA, mtDNA, North America, *Ursus arctos*.

Genetic variation within wild populations is believed to be important for maintaining fitness and for adaptating to environmental change (Soulé 1980). Due to human-induced pressures, many brown bears (*Ursus arctos*) are restricted to small, fragmented populations (Servheen 1990). These small, isolated populations are at risk for a loss of genetic diversity and an increase in inbreeding (Wright 1977) due to population bottleneck effects and decreased gene flow. In domestic animals, inbreeding has been recognized as detrimental to fertility, growth rate, survival, disease resistance, and productivity (Wright 1977). In wild populations, the effects of inbreeding are less clear. Population decline of the cheetah (*Acinonyx jubatus*) has been suggested as an example of inbreeding depression (O'Brien et al. 1985); however, several recent studies reject inbreeding as a major factor in the cheetah's decline (Caro and Laurenson 1994, Caughley 1994). In a review of inbreeding in captive and wild populations, Lacy (1993) emphasizes that no wild population is known to have gone extinct due to inbreeding depression, but he suggests that inbreeding depression may impede recovery or accelerate decline in some wild populations.

Brown bear populations in the states of Wyoming and Montana may have suffered a loss of genetic diversity due to historical population bottlenecks. The range of the brown bear in the lower 48 states has been reduced to <1% of its original area, and the estimated population size has plummeted from 100,000 in the early 19th century to <1000 in 1986 (Allendorf and Servheen 1986). In 1975, brown bears in the lower 48 states were declared a threatened species in accordance with the U.S. Endangered Species Act (16 U.S.C. 1531–1544). To determine if endangered brown bear populations of Wyoming and Montana have lower levels of genetic variation than other North American brown bear populations, we examined mtDNA and nuclear microsatellite genetic diversity in 2 endangered brown bear populations and 3 other North American populations.

mtDNA is a double-stranded circular molecule of approximately 16,000 nucleotides (*nt*) which has a uniparental, maternal mode of inheritance in most animals (Birky et al. 1989). The DNA of this molecule has been used extensively to reconstruct phylogenetic histories, to assess population genetic structure and gene flow, and to detect hybridization of closely related species or subspecies (Avise 1994, Moritz 1994). mtDNA has proven exceptionally informative in the study of intraspecific variation of humans and other mammals due to its rapid rate of evolution (Brown et al. 1982). Microsatellite analysis is an appropriate complement to mtDNA sequence analysis because mutation rates (10^{-4}–10^{-5}/generation) are

[1] Present address: Fish and Wildlife Resources, University of Idaho, Moscow, ID 83844-1136, USA, email:lwaits@uidaho.edu

comparable to mtDNA mutation rates and inheritance at microsatellite loci is biparental (Bruford and Wayne 1993). Microsatellite loci contain short sequence repeats (1–6 base pairs) that vary in the number of times a repeat unit is tandemly reiterated. Microsatellite analysis has been used to study many different aspects of wildlife genetics (Bruford and Wayne 1993), including male reproductive success in brown bears (Craighead et al. 1995), population structure in polar bears (*Ursus maritimus*; Paetkau et al. 1995), and population diversity in the American black bear (*Ursus americanus*; Paetkau and Strobeck 1994).

In this study, we examine genetic variation in 220 brown bears using mtDNA sequence analysis of a 296 base-pair fragment of the control region (D-loop) and nuclear microsatellite DNA analysis of 8 CA repeat loci. At the population level, genetic variation is compared among brown bears sampled from 5 regions of North America: (1) Kodiak Island, Alaska; (2) Kluane National Park, Canada; (3) Eastern Slope of the Rockies (East Slope), Canada; (4) Yellowstone ecosystem (YE) of Montana and Wyoming; and (5) Northern Continental Divide Ecosystem (NCDE) of Montana and southern British Columbia (Fig. 1). Genetic variability data are used to evaluate the following hypotheses: (a) the endangered YE and NCDE populations of the lower 48 states have less genetic diversity than other populations in Alaska and Canada, (b) geographically isolated populations will have lower levels of genetic variation than non-isolated populations, and (c) brown bear populations in regions with limited human impact will have the highest level of genetic diversity.

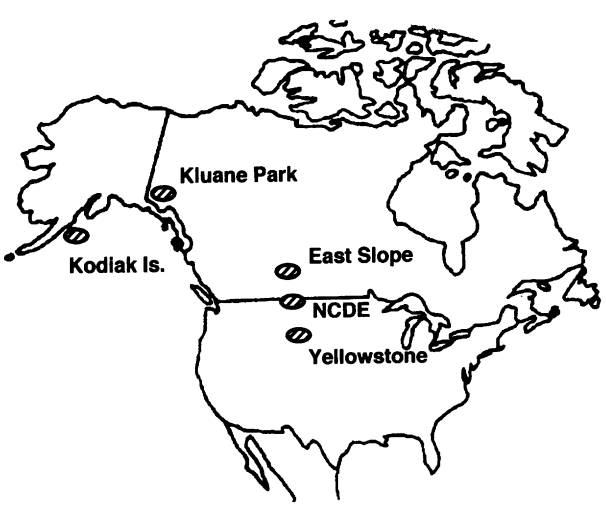

Fig. 1. Geographic locations of the 5 brown bear study populations (NCDE—Northern Continental Divide Ecosystem.)

We thank the wildlife biologists, managers, and agencies who made this study possible by collecting the samples: Kluane Natl Park—R. McCann, Parks Canada; East Slope—M. Gibeau, B. McClymont; East Slope Grizzly bear project—Alberta Fish and Wildlife, Parks Canada; Kodiak Island—V. Barnes, U.S. Geological Survey and R. Smith, Alaska Department of Fish and Game; NCDE—C. Servheen, T. Thier, both U.S. Fish and Wildlife Service, and B. McLellan, British Columbia Ministry of Forests; YE—Interagency Grizzly Bear Study Team, R. Knight; S. French and M. French, Yellowstone Grizzly Foundation. We thank S. Tavare for helpful discussions and computer programming. Funding was provided by Parks Canada, National Science Foundation, and Sigma Xi.

METHODS

Samples

All samples were obtained from DNA repositories maintained by Ryk Ward, University of Utah, and Curtis Strobeck, University of Alberta. DNA from a total of 220 bears was examined: 34 from Kodiak Island, Alaska; 33, East Slope, Canada; 51, Kluane National Park, Canada; 53, Yellowstone Ecosystem (YE) in Montana and Wyoming; and 49, Northern Continental Divide Ecosystem (NCDE) in Montana and British Columbia. All samples were obtained with the proper documentation according to the regulations of the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

mtDNA Analysis

The mtDNA control region, the most rapidly evolving region of the mitochondrial molecule (Brown et al. 1982), was amplified for 169 brown bears using the polymerase chain reaction (PCR). The PCR primers and conditions were previously reported (Waits 1996). A 296 base pair sequence of the control region was obtained from PCR products using solid-phase sequencing. A hypervariable site with thymine (T) nucleotide stretches ranging from 3 to 16 base pairs was omitted from the analysis because of difficulty in accurately determining the number of repeated T nucleotides. mtDNA sequences were aligned using the multiple aligned sequence editor (MASE; Faulkner 1989), and lineages were identified. A lineage was defined as a unique DNA sequence; it is the haploid equivalent of a nuclear gene allele.

The degree of genetic variation was assessed by comparing the number of mitochondrial lineages and mtDNA diversity (Nei 1987). Diversity was calculated as follows: $h = N(1-X_i^2)/(N-1)$, where X_i denotes frequency of a lineage and N denotes sample size. The diversity statistic is normally termed expected heterozygosity when applied to nuclear genes, but is called diversity when quantifying mtDNA variation because mitochondrial DNA is haploid and individuals are not heterozygous at a mitochondrial locus.

Nuclear DNA Microsatellite Analysis

Microsatellite analysis was performed using 8 independent dinucleotide, CA repeat, loci developed from an American black bear DNA library (Paetkau and Strobeck 1994). Primer sequences and analysis conditions for these loci, named G1A, G10B, G10C, G1D, G10L, G10M, G10P, and G10X, are described in Paetkau et al. (1995). A total of 220 individuals were genotyped at all 8 loci. These individuals included the 169 bears analyzed in the mtDNA analysis plus 51 additional samples. Observed number of alleles and diversity (h) was calculated to estimate genetic variation. Diversity was estimated as described in the mtDNA analysis methods with the exception that N denotes the number of chromosomes ($2N$). The observed number of alleles and diversity was averaged over all 8 loci.

Testing for Significant Differences

An alternative measure of genetic variation is the Ewen's estimation (1979): $\theta = 2N_e m$ for mtDNA and $\theta = 4N_e m$ for microsatellite data, where N_e is effective population size and m is the mutation rate/generation. Assuming random mating and a finite population size, θ can be estimated from the number of alleles (K) and the sample size (N) (Ewens 1979). We used maximum likelihood estimation to obtain estimates of θ at each locus for each population, and the homogeneity of θ estimates was tested at each locus using a likelihood-ratio test as described in Paetkau and Strobeck (1994).

RESULTS

mtDNA Analysis

In a comparison of 296 nucleotides of the mtDNA control region for 169 bears, 25 variable sites were observed and 13 lineages were identified. The maximum number of lineages observed in any population was 5 in the NCDE population and in the Kluane population (Table 1). Three lineages were observed in the YE, 2 in the East Slope population, and 1 in the Kodiak Island population. The distribution of lineages among populations reflects the phylogenetic structure of the brown bear species (Waits et al. 1998). The East Slope, NCDE, and YE populations are in the same mtDNA clade and share lineages 37 or 38 (Table 1). The Kodiak Island and the Kluane lineages are the sole representatives from their respective clades and do not share mtDNA lineages with the other populations. mtDNA diversity within populations ranged from a low of 0% for Kodiak Island to a high of 69% for Kluane (Table 1). The NCDE population had relatively high mtDNA diversity (61%), the YE population had intermediate mtDNA diversity (24%), and the East Slope population had low (7%) mtDNA diversity.

Nuclear DNA Microsatellite Analysis

Microsatellite genotypes were determined for 220 brown bears at 8 loci and are available upon request from Strobeck. An examination of observed and expected genotypes within each population revealed that all loci are in Hardy–Weinburg equilibrium with the exception of the D locus in the Kodiak population ($P < 0.01$). For each population, the number of alleles and genetic diversity was estimated for each locus independently (Table 2). When comparing these values among loci in the same population, the range is highly variable, particularly in populations with low levels of diversity such as Kodiak and YE. To obtain summary statistics for each population, the number of alleles and diversity was averaged over all 8 microsatellite loci (Table 2). Consistent with the mtDNA results, the Kodiak Island sample had the fewest average number of alleles/locus (2.1) and the lowest average diversity (0.27), while the Kluane sample had the highest average diversity (0.76) and the largest average number of alleles (7.4).

Testing for Significant Differences

The significance of interpopulation differences in genetic diversity was examined using a likelihood-ratio test of the maximum-likelihood estimate of $\theta = 4N_e m$ (nuclear loci) and $\theta = 2N_e m$ (mtDNA; see Methods). For a statistical test of our hypotheses, significant decreases in genetic diversity were assessed using a pairwise comparison of $\hat{\theta}$ or the Kluane population with $\hat{\theta}$ for the other populations. When comparing the Kodiak population and the Kluane population, significant differences were detected at 6 out of 8 microsatellite loci (Table 3). When summing over all 8 loci, the difference was highly significant ($\chi_8^2 < 0.00001$). At the mtDNA locus, values $\hat{\theta}$ for the Kluane and Kodiak populations were also significantly different ($\chi_1^2 < 0.01$). Comparison of the Kluane

Table 1. The distribution of mtDNA lineages and genetic diversity (*h*) within 5 North American brown bear populations. *N* is population sample size, NCDE = Northern Continental Divide Ecosystem.

Population	*N*	Lineages (lineage sample size)	Diversity (*h*)
Kodiak Island	34	Lineage 29 (34)	0
Kluane	24	Lineage 21 (2), Lineage 56 (3) Lineage 57 (12), Lineage 58 (6) Lineage 60 (1)	0.689
East Slope	30	Lineage 37 (1), Lineage 38 (29)	0.066
NCDE	34	Lineage 37 (17), Lineage 38 (3) Lineage 39 (3), Lineage 40 (10) Lineage 52 (1)	0.611
Yellowstone	46	Lineage 38 (40), Lineage 51 (4) Lineage 55 (2)	0.240

and YE populations (Table 3) revealed a significant difference at only 1 microsatellite locus ($\chi^2_1 < 0.05$), and the 8 loci comparison was not significantly different ($\chi^2_8 < 0.13$). Additional pairwise comparisons of the Kluane population and the other populations did not reveal significant differences in $\hat{\theta}$ at the microsatellite loci or the mtDNA locus. However, a significant difference in $\hat{\theta}$ was observed between the NCDE population and the YE population at microsatellite locus D ($\chi^2_1 < 0.05$) (data not shown).

DISCUSSION

Population Demographics

The 5 populations surveyed in this study were chosen to represent a range of demographic backgrounds in terms of connectivity to other brown bear populations and the degree of human impact. The Kluane population is in the heart of the continuous distribution of brown bears which extends across Alaska, northern British Columbia, the Yukon, and parts of the Northwest Territories (Servheen 1990), and it has experienced relatively little human impact. The Kluane population was chosen as a population with demographic characteristics that most closely approximate natural populations of brown bears prior to European settlement of North America.

The Kodiak Island population represents a brown bear population with long-term isolation as it is believed to have been separated from populations of continental Alaska since the retreat of the glaciers 12,000 years ago (D. Mann, Univ. Alaska Fairbanks, Geophysical Inst., pers. commun., 1997). Human impact is currently low as two-thirds of Kodiak Island is a protected National Wildlife Refuge (Barnes 1994). However, government-regulated sport hunting is allowed on Kodiak Island, and excessive harvests of brown bears were documented in the mid-1960s (Barnes et al. 1995).

The East Slope, NCDE, and YE populations have been more strongly affected by human development and overhunting than the 2 previously described populations (Allendorf and Servheen 1986). The contiguous North American brown bear population follows the Canadian Rockies and reaches its southern limit at the NCDE. The East Slope population is centralized within the Rocky Mountain distribution of brown bears, but it is at the eastern fringe of the brown bear distribution in Alberta. The NCDE population is currently isolated from other brown bear populations at its western, eastern and southern boundaries. The Yellowstone population is an isolated population at the southeastern extreme of the current North American distribution of brown bears. Researchers be-

Table 2. Summary of the number of alleles (*A*) and genetic diversity (*h*) at 8 microsatellite loci for 5 North American brown bear populations. NCDE and YE are the Northern Continental Divide Ecosystem and the Yellowstone Ecosystem, respectively. *N* denotes sample size.

	Populations									
	Kodiak ($2N = 64$)		Kluane ($2N = 102$)		East Slope ($2N = 66$)		NCDE ($2N = 98$)		YE ($2N = 106$)	
	A	*h*	*A*	*h*	*A*	*h*	*A*	*h*	*A*	*h*
Locus A	3	0.606	7	0.733	6	0.727	6	0.709	5	0.661
Locus B	2	0.029	9	0.813	7	0.849	8	0.753	5	0.677
Locus C	3	0.519	6	0.766	5	0.453	5	0.633	4	0.432
Locus D	3	0.492	10	0.848	10	0.820	10	0.831	7	0.789
Locus L	1	0	5	0.616	4	0.643	5	0.604	2	0.405
Locus M	1	0	7	0.821	6	0.776	6	0.704	5	0.658
Locus P	3	0.473	9	0.775	7	0.677	7	0.761	5	0.715
Locus X	1	0	7	0.734	6	0.302	7	0.622	2	0.106
Average	2.1	0.265	7.5	0.763	6.4	0.656	6.8	0.702	4.4	0.554

Table 3. Maximum-likelihood estimates of θ and results of likelihood-ratio tests for significant interpopulational differences. Kl = Kluane, Ko = Kodiak Island, Ye = Yellowstone ecosystem, T = pooled, NS = not significant (*P* > 0.05).

A. Kluane vs. Kodiak Population

Locus	$\hat{\theta}_{Kl}$	$\hat{\theta}_{Ko}$	$\hat{\theta}_{T}$	χ^2_1	*P* value
A	1.53	0.48	1.01	1.96	NS
B	2.20	0.23	1.16	6.38	<0.02
C	1.23	0.48	0.86	1.18	NS
D	2.55	0.48	1.48	4.88	<0.05
L	0.94	0	0.46	5.53	<0.02
M	1.53	0	0.72	8.51	<0.005
P	2.20	0.48	1.32	3.84	<0.05
X	1.53	0	0.72	8.51	<0.005
			χ^2_8 =	40.77	<0.0001

B. Kluane vs. Yellowstone Population

Locus	$\hat{\theta}_{Kl}$	$\hat{\theta}_{Ye}$	$\hat{\theta}_{T}$	χ^2_1	*P* value
A	1.53	0.93	1.22	0.51	NS
B	2.20	0.93	1.52	1.71	NS
C	1.23	0.67	0.94	0.62	NS
D	2.55	1.51	2.01	0.80	NS
L	0.94	0.20	0.55	2.17	NS
M	1.53	0.93	1.22	0.51	NS
P	2.20	0.93	1.52	1.71	NS
X	1.53	0.20	0.80	4.57	<0.05
			χ^2_8 =	12.58	<0.13

lieve that this population has been isolated for 80–100 years and has experienced 2 population bottlenecks in the past 150 years. The first bottleneck occurred from 1850–1920 as the brown bear was exterminated throughout its southern range, and the second bottleneck occurred during the early 1970s due to closure of garbage dumps in Yellowstone National Park (Allendorf and Servheen 1986).

Diversity Comparisons

We cannot reject the possibility that the populational differences in levels of genetic diversity are due to sampling biases. The current methods for collecting DNA samples from brown bears represent opportunistic processes. Samples are generally collected from: (1) bears that are caught in a limited number of trapping locations; (2) problem bears that are influenced by human contact and must be relocated; and (3) dead bears that are discovered in time to collect tissue samples. In order to minimize these biases, we analyzed all collected samples with the exception of individuals that were known to be related, and we obtained samples from different geograpic regions within the ecosystem. However, we recognize the potential biases that may be introduced and are currently working with wildlife managers to develop random sampling methods such as the collection of DNA from scat and hair.

The diversity results were used to test 3 hypotheses: (1) the threatened YE and NCDE populations of the lower 48 states have less genetic diversity than other populations in Alaska and Canada, (2) geographically isolated populations will have lower levels of genetic variation than non-isolated populations, and (3) brown bear populations in regions with limited human impact will have the highest level of genetic diversity. The results of our genetic diversity survey support hypotheses 2 and 3 as the level of genetic variation is correlated with the degree of isolation and the degree of human impact. Both mtDNA and nuclear microsatellite analyses demonstrate that Kluane is the most genetically diverse population. The Kodiak Island population, which has been isolated for approximately 12,000 years (1,200 bear generations) (D. Mann, Univ. Alaska Fairbanks, Geophysical Inst., pers. commun., 1997), has the lowest genetic diversity using both mtDNA and nuclear microsatellite markers. In addition, likelihood-ratio tests comparing $\hat{\theta}$ in Kluane and Kodiak demonstrated that the Kodiak population had significantly lower levels of mtDNA and nuclear microsatellite variation.

Both mtDNA and microsatellite analysis of the NCDE population reject hypothesis 1 and demonstrate that this population has retained relatively high levels of genetic diversity. The YE population had considerably lower mtDNA and microsatellite diversity values than the Kluane population, but likelihood-ratio tests comparing $\hat{\theta}$ detected a significant difference at only 1 microsatellite locus. These results suggest that the period of isolation for brown bears of the YE has resulted in a loss of genetic diversity, but it is not signficant using this statistical test. Additional analyses using more loci, more populations, and different statistical methods are necessary. We did not expect the lower levels of mtDNA and microsatellite diversity in the East Slope population compared to NCDE population because the East Slope population is thought to have suffered less human impact than the NCDE population.

When comparing genetic diversity among populations, the relative ranking was identical for mtDNA and microsatellite diversity with the exception of the YE and East Slope samples. The East Slope population has nuclear microsatellite diversity levels that are approximately 10% higher than the YE population, and the YE population has mtDNA diversity levels that are

approximately 17% higher than the East Slope population. Microsatellite data show moderately reduced diversity in the East Slope and YE populations, and mtDNA sequence variation analyses show dramatically reduced diversity in these populations relative to Kluane and NCDE.

Several factors may cause results to differ between nuclear microsatellite and mtDNA markers. The first factor is the random nature of genetic drift, which can result in different markers giving different results. Diversity levels within the Kodiak population fluctuated by locus from a high of 0.606 at locus A to a low of zero at loci L, M, and X. These fluctuations represent a limitation for mtDNA sequence analysis because the mitochondrial DNA molecule is essentially 1 locus (Birky et al. 1989). In contrast, 8 independent nuclear loci were used to obtain an average estimate for genetic diversity, which should reduce the sample variance. In addition, sample sizes for nuclear diploid loci are 2 times higher than mtDNA (haploid) sample sizes when examining the same number of individuals. Further, effective genetic population size is 4 times smaller for the mitochondrial molecule than for the nuclear loci (Nei 1987). Hence, mtDNA markers are expected to show more rapid and dramatic declines in response to population bottlenecks.

Dispersal differences for male and female brown bears may also be important. Females tend to establish home ranges that overlap with their natal range, whereas males often disperse over relatively large distances (Reynolds and Hechtel 1984). As a consequence, male-mediated migration would be expected to offset the loss of nuclear DNA genetic diversity in fringe populations to a greater degree than maternally inherited mtDNA markers. The differences between mtDNA and nuclear DNA make these genetic markers complementary for studying diversity in natural populations. Mitochondrial markers should be more sensitive to the detection of a loss of genetic variation due to recent bottlenecks and differences in male and female migration rates. Nuclear DNA microsatellite markers provide information about maternal and paternal genetic diversity and can be used to indicate a dramatic loss of genetic diversity, which may lead to inbreeding depression and reduce a population's ability to adapt to future environmental change.

Testing for Significant Differences

To identify statistically significant differences in genetic variation, we used a likelihood-ratio test of maximum likelihood estimates of θ generated according to the Ewens method (1979). This method utilizes 2 underlying models: (1) a fixed model which assumes the populations are independent, and (2) the infinite alleles model which assumes that each new mutation creates a new allele in the population. In the mtDNA analyses, these models are not violated: the data fit the infinite alleles model, and the mtDNA lineages that were compared are not shared between populations and are estimated to have diverged 300,000 to 500,000 years ago (Waits 1996). In the microsatellite analyses, the fit of the data to the underlying models is uncertain. The independence of populations is unclear as microsatellite alleles are shared among populations, and the gene genealogy of these alleles is unknown. The mutational mechanism which creates new microsatellite alleles is also unknown, and some theoretical studies have suggested a step-wise mutation model (Valdes et al. 1993, Di Rienzo et al. 1994) as opposed to an infinite alleles model. We believe that our statistical approach to detecting significant differences among populations is the best method given the current status of theoretical and experimental results. However, it is clear that additional theoretical development is necessary to establish alternative methods for detecting statistical interpopulational differences in microsatellite variability.

MANAGEMENT IMPLICATIONS

These results suggest a connection between the length of population isolation and the loss of genetic diversity in brown bears. Thus, reducing human impacts such as population fragmentation and blocking migration corridors will help curtail additional loss of genetic diversity. The lower levels of mtDNA diversity and nuclear microsatellite diversity in the Yellowstone ecosystem appear to reflect the isolation and recent population bottlenecks of this group. We do not believe genetic augmentation of the Yellowstone population is necessary at this point, but without gene flow from other brown bear populations genetic diversity will continue to decrease. Thus, it is essential to continue genetic monitoring of this population and consider population augmentation to restore gene flow if migration corridors cannot be established.

It is not clear why the NCDE population has retained more genetic diversity than the East Slope population, which has more connectivity to other brown bear populations. The East Slope population has been identified by World Wildlife Fund as a core conservation area for brown bears (Hummel 1990), and thus our observations of lower genetic diversity among East Slope bears raise concern for the genetic health of brown bear populations in southern Canada. Additional examination of demographic differences and differences in human influences

in the NCDE and East Slope populations is warranted. These populations may differ in historical population fluctuations due to hunting, historical and current population densities, and the density of physical barriers to migration such as roads. Examination of these factors and genetic analyses of additional samples from these ecosystems may provide important information for developing strategies to retain genetic diversity.

The extremely low levels of mtDNA and nuclear microsatellite variation among Kodiak Island brown bears supports the hypothesis, based on geological evidence of the last extensive glaciation (D. Mann, Univ. of Alaska Fairbanks, Geophysical Inst., pers. commun., 1997), that Kodiak Island brown bears have been separated from mainland brown bears for up to 12,000 years. The biological and evolutionary implications of such low levels of genetic diversity are much less clear. The Kodiak Island population does not display characteristics of a population suffering from inbreeding depression. Kodiak Island brown bears have one of the highest published density levels for brown bears, presumably due to limited human impact and a highly productive environment (Barnes et al. 1995). In addition, Kodiak Island brown bears have high levels of fertility and survival (V. Barnes, Nat. Biol. Serv., Kodiak, Alas., unpubl. data). However, we cannot exclude the possibility that Kodiak Island brown bears suffered from inbreeding depression in earlier generations. The apparent good health of the Kodiak Island brown bears strongly contrasts the condition of the cheetah, a well-known example of a threatened species with low levels of genetic diversity (O'Brien et al. 1985). Our results demonstrate that the connection between low genetic diversity and fitness in natural populations is a complex interaction that must be studied more extensively.

As the first comprehensive analysis of mtDNA and nuclear DNA microsatellite diversity in brown bears, this study has provided important baseline knowledge of the range of genetic diversity at the population level. Our results demonstrate the importance of testing population genetic theories with empirical data by comparing levels of genetic variability in natural populations with different histories. Genetic variation data from any one population is only informative in the context of genetic variation in other populations. As the anthropogenic stress on brown bear populations escalates, continued cooperation between wildlife managers and population geneticists is essential to monitor levels of genetic variation, interpret results, and plan for the future.

LITERATURE CITED

ALLENDORF, F.W., AND C. SERVHEEN. 1986. Genetics and conservation of grizzly bears. TREE 1:88–89.

AVISE, J.C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, New York, N.Y. 511pp.

BARNES, V.G. 1994. Brown bear–human interactions associated with deer hunting on Kodiak Island. Int. Conf. Bear Res. Manage. 9:63–73.

———, R.B. SMITH, M.S. UDEVITZ, AND J.R. BELLINGER. 1995. Kodiak brown bears. Pages 349–350 *in* E.T. Laroe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac, eds. Our living resources: a report to the nation on the distribution, abundance, and health of U.S. plants, animals, and ecosytems. U.S. Dep. Int., Nat. Biol. Serv., Washington, D.C.

BIRKY, C.W., P. FUERST, AND T. MARUYAMA. 1989. Organelle gene diversity under migration, mutation and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121:613–627.

BROWN, W.M., E.M. PRAGER, A. WANG, AND A. WILSON. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. J. Molecular Evol.18:225–239.

BRUFORD, M.W., AND R.K. WAYNE. 1993. Microsatellites and their application to population genetic studies. Curr. Opinion Genet. and Dev. 3:939–943.

CARO, T.M., AND M.K. LAURENSON. 1994. Ecological and genetic factors in conservation: a cautionary tale. Science 263:485–486.

CAUGHLEY, G. 1994. Directions in conservation biology. J. Animal Ecol. 63:215–244.

CRAIGHEAD, L., D. PAETKAU, H.V. REYNOLDS, E.R. VYSE, AND C. STROBECK. 1995. Microsatellite analysis of paternity and reproduction in Arctic grizzly bears. J. Hered. 86:255–261.

DI RIENZO A., A.C. PETERSON, J.C. GARZA, A.M. VALDES, M. SLATKIN, AND N.B. FREIMER. 1994. Mutational processes of simple-sequence repeat loci in human populations. Proc. Nat. Acad. Sci., USA 91:3166–3170.

EWENS, W.J. 1979. Mathematical population genetics. Springer-Verlag, New York, N.Y. 221pp.

FAULKNER, D.V. 1989. Multiple aligned sequence editor (MASE). Molecular Biol. Comput. Res. Resour., Boston, Mass. 35pp.

HUMMEL, M. 1990. A conservation strategy for large carnivores in Canada. World Wild. Fund Can., Toronto. 98pp.

LACY, R.C. 1993. Impacts of inbreeding in natural and captive populations of vertebrates: implications for conservation. Perspect. Biol. and Med. 36:480–495.

MORITZ, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecol. 3:401–411.

NEI, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York, N.Y. 512pp.

O'BRIEN, S.J., M.E. ROELKE, L. MARKER, A. NEWMAN, C.A. WINKLER, D. MELTZER, L. COLLY, J.F. EVERMANN, M. BUSH, AND D.E. WILDT. 1985. Genetic basic for species vulnerability in the cheetah. Science 227:1428–1434.

PAETKAU, D., W. CALVERT, I. STIRLING, AND C. STROBECK. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecol. 4:347–354.

———, AND C. STROBECK. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecol. 3:489–495.

———, L.P. WAITS, P.L. CLARKSON, L. CRAIGHEAD, E. VYSE, R.H. WARD, AND C. STROBECK. 1998. Variation in genetic diversity across the range of North American brown bears. Conserv. Biol. 12:418–429.

REYNOLDS, H.V., AND J.L. HECHTEL. 1984. Structure, status, reproductive biology, movement, distribution, and habitat utilization of a grizzly bear population. Fed. Aid Wildl. Restor. Proj. Final Report, Alaska Dep. Fish and Game, Juneau. 29pp.

SERVHEEN, C. 1990. The status and conservation of bears of the world. Int. Conf. Bear Res. and Manage. Monogr. Ser. 2. 32pp.

SOULÉ, M.E. 1980. Conservation biology—an evolutionary perspective. Pages 24–44 *in* M.E. Soulé, and B.A. Wilcox, eds. Conservation biology. Sinauer Assoc., Sunderland, Mass.

VALDES A.M., M. SLATKIN, AND N.B. FREIMER. 1993. Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics. 133:737–749.

WAITS, L.P. 1996. A comprehensive molecular study of the evolution and genetic variation of bears. Ph.D. Diss., Univ. Utah, Salt Lake City. 334pp.

———, S. TALBOT, R.H. WARD, AND G.F. SHIELDS. 1998. Mitocondrial DNA phylogeography of the North American brown bear and implications for conservation. Conserv. Biol. 12:408–417.

WRIGHT, S. 1977. Evolution and the genetics of populations. Vol 3. Univ. Chicago Press, Chicago, Ill. 519pp.