Variation in Genetic Diversity across the Range of North American Brown Bears

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Abstract: *Understanding the factors that influence the rate at which natural populations lose genetic diversity is a central aspect of conservation genetics because of the importance of genetic diversity in maintaining evolutionary potential and individual fitness. Concerns about loss of genetic diversity are particularly relevant to large carnivores, such as brown bears (*Ursus arctos*), that are distributed at low densities and are highly susceptible to human-caused population fragmentation. We used eight highly variable nuclear microsatellite markers to study current levels of genetic variation across the North American range of brown bears. The highest levels of within-population genetic diversity (H_e = 0.76) were found in northern populations in the core of the North American distribution. Diversity was significantly lower in populations at the southern fringe of the distribution, in the Northwest Territories, and in southwest Alaska. Diversity was lower still in the Yellowstone Ecosystem population (H_e = 0.55), an isolated remnant of the larger distribution that recently extended south from the Canadian border into Mexico. The insular population on the Kodiak Archipelago had very low genetic diversity (H_e = 0.26). The Yellowstone and Kodiak data suggest that the effective population size for brown bears is much smaller than previously suspected. These results indicate that the levels of diversity in most undisturbed populations can be maintained only through connections to populations on the scale of the current North American distribution. At the same time, the Kodiak data demonstrate that populations well under the size recommended for long-term conservation can persist and thrive for thousands of years, although the probability of such persistence remains unknown.*

Variación en la Diversidad Genética a lo largo del Rango de Distribución del Oso Café de Norteamérica

Resumen: *Entender los factores que influeyen en la tasa a la cual las poblaciones naturales pierden diversidad genética es un aspecto central de la genética de la conservación debido a la importancia de la diversidad genética en el mantenimiento del potencial evolutivo y la condición individual. Las preocupaciones sobre la pérdida de la diversidad genética tiene particular relevancia para los carnivoros mayores, como lo es el oso café (*Ursus arctos*), que se distribuye a bajas densidades y que es altamente susceptible a la fragmentación poblacional causada por humanos. Para estudiar los niveles actuales de variación genética a lo largo del rango de distribución de los osos cafés, usamos ocho marcadores microsatélite nucleares altamente variables. Los niveles mas altos de diversidad genética intrapoblacional (H_e = 0.76) se encontraron en poblaciones del Norte en el centro de la distribución en Norteamérica. La diversidad fue significativamente menor en poblaciones limítrofes sureñas, en los territorios del Noroeste y el Suroeste de Alaska. La diversidad fue mas baja aún en la población de Yellowstone (H_e = 0.55), un remanente de una distribución mayor que recientemente se extendía hacia el sur desde la frontera Canadiense hacia México. La población insular en el archi-*

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pielago de Kodiak tuvo una diversidad genética muy baja (H_e = 0.26). Los datos de Yellowstone y Kodiak sugieren que el tamaño poblacional efectivo para el oso café es mucho mas pequeño que lo previamente estimado. Estos resultados indican que los niveles de diversidad en la mayoría de las poblaciones no perturbadas pueden ser mantenidos mediante conecciones con poblaciones en la escala de la distibución actual en Norteamérica. Al mismo tiempo, los datos de Kodiac demuestran que poblaciones muy por debajo del tamaño recomendado para la conservación a largo plazo pueden persistir por miles de años, aunque su probabilidad de persistencia es aún desconocida.

Introduction

Across the Northern Hemisphere, the number and distribution of brown bears (*Ursus arctos*)—commonly called grizzly bears in parts of North America—have shrunk in the face of habitat alteration and excessive anthropogenic mortality, typically relegating them to isolated northern and mountainous regions (Servheen 1990). In Canada brown bears have been extirpated from the prairies east of the Rocky Mountains and from heavily populated regions of southern British Columbia (Paquet 1995). South of the Canadian border the decline in range and numbers have been on the order of 99%, leaving an isolated population in the Yellowstone Ecosystem and several narrow fingers of distribution penetrating south from Canada along mountain ranges (Fig. 1; Allendorf & Servheen 1986; McLellan, in press). In contrast, the distribution in Alaska and northern Canada remains relatively unaltered by human activity.

For many populations of brown bears, the factors responsible for historic declines are being brought under control, and the public interest in maintaining these populations is growing. In areas such as the 48 conterminous United States or western Europe, however, there is no possibility of reintroducing bears to a large portion of their historic range, and if many current populations are to persist they will do so in physical isolation from larger populations. This situation raises the issue of genetic threats to individual fitness and the evolutionary potential of populations.

The "evil effects of close interbreeding" (Darwin 1882:93) have been recognized for over a century, and there can be little doubt that the detrimental genetic effects associated with rapid declines in population size can threaten the survival of populations (e.g., Lacy 1993; Jiménez et al. 1994; Mills & Smouse 1994; Frankham 1995*a*). Unfortunately, the relative importance of inbreeding in conservation biology remains contentious

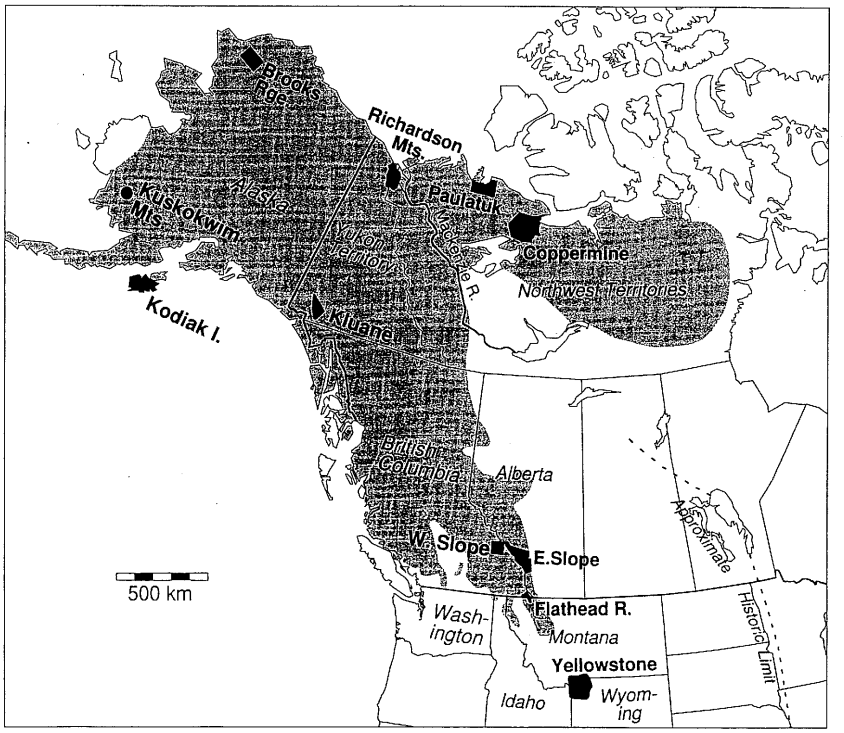

Figure 1. The current distribution of brown bears in North America (shaded; Allendorf & Servheen 1986; Servheen 1990; Gunson 1995; Paquet 1995; McLellan, in press; Ray Case, NWT Renewable Resources, personal communication) and the study areas included in this survey (black). When Europeans arrived in North America, the distribution of brown bears included almost the entire southern half of this map and extended south to Mexico.

because the effects of close breeding are difficult to identify and measure in natural populations and because factors such as the population's history, the rate of decline in population size, and the chance fixation of deleterious alleles can play roles that are important but difficult to quantify. As a starting point it has been suggested that genetic effective population size (N_e) should always be kept above 50 and that targets for long-term conservation should be in the range of 500–5000 (Franklin 1980; Soulé 1980; Lande 1994; Lande 1995). At present it is difficult to apply these numbers to bears because N_e as a proportion of census size (N) is not accurately known—although the implications of a minimum N_e of 500–5000 for brown bear populations are staggering regardless of the ratio of N_e to N because brown bears require very large tracts of relatively undeveloped and uninhabited land.

We investigated the factors affecting levels of within-population genetic diversity in brown bears by using eight highly variable nuclear markers (CA_n microsatellites) to survey 678 brown bears spanning the North American distribution. The study areas (Fig. 1) included relatively pristine northern populations as well as populations at the southern end of the distribution where habitat alteration and human-caused mortality play a central role in the dynamics of brown bear populations. The study areas also spanned a dramatic range of ecosystems ranging from the Rocky Mountains to Arctic tundra. Although the continental distribution of brown bears is relatively continuous, density varies dramatically on both large and fine spatial scales. Density estimates for our study areas ranged from 350 bears per 1000 km^2 in one region of Kodiak Island (Barnes et al. 1995) to 6.3 bears per 1000 km^2 in the Paulatuk study area on the Barren Grounds of the Northwest Territories (P.C., unpublished data).

In addition to sampling a wide range of habitats and population densities, we included two physically isolated populations to study the effect of isolation on genetic diversity. The population in the Yellowstone Ecosystem was once part of a large, continuous distribution extending through the western United States to Mexico, but it has existed in isolation for approximately 100 years (Servheen 1990). In contrast, the population on the Kodiak Archipelago has been separated from the continent by approximately 40 km of open ocean throughout the Holocene. Comparative data from populations of North American black bears (*Ursus americanus*), including an isolated population from insular Newfoundland, were also included. The isolated populations were used to obtain estimates of the relationship between N_e and N in these populations.

Methods

All the brown bear samples were obtained by biologists, typically from drug-immobilized bears, during the course of independent field studies. Samples were either muscle, skin disks from ear punches, blood, or hair. The sample consisted of 678 brown bears, but five animals (four from both the East Slope and West Slope study areas and one from both the Paulatuk and Coppermine study areas) were included in more than one study area, bringing the sample size to 683 (Table 1). Complete eight-locus genotypes were obtained for all individuals.

Microsatellite analysis and sequence analysis of the regions flanking locus G1D used previously described primers and methods (Paetkau & Strobeck 1995; Paetkau et al. 1995). The microsatellite markers were isolated from a North American black bear genomic library (Paetkau & Strobeck 1994). The Brooks Range data and data from insular Newfoundland based on smaller sample sizes and four loci were published previously (Paetkau & Strobeck 1994; Craighead et al. 1995). Allele frequency distributions are given in the Appendix, and individual genotypes are available on request.

Departures from Hardy-Weinberg equilibrium were calculated by means of a Monte Carlo approximation of Fisher's exact test (Guo & Thompson 1992), with results from the eight tests combined for each study area (Sokal & Rohlf 1995). A G test for heterogeneity was used to test for differences in allele frequency distributions between populations, with classes smaller than five observations combined (Sokal & Rohlf 1995).

Unbiased estimates of mean expected heterozygosity (H_e; Nei & Roychoudhury 1974) and total probability of identity [*P(ID)*] were calculated for each study area. The formula for the unbiased estimate of *P(ID)* was based on the previously described formula (Jamieson 1965; Paetkau et al. 1995)—which is simply the sum of squares of the expected frequencies of all possible genotypes and is biased for small sample sizes—and was derived by means of factorial moments (Kendall & Stuart 1977):

$$\frac{n^3(2a_2^2 - a_4) - 2n^2(a_3 + 2a_2) + n(9a_2 + 2) - 6}{(n-1)(n-2)(n-3)},$$

where n is the sample size, a_i equals

$$\sum_j p_j^i,$$

and p_j is the frequency of the jth allele. The values were calculated for each locus and then multiplied across loci to give the overall *P(ID)*.

The significance of differences in genetic diversity between study areas was tested using a paired t test of arcsine-transformed H_e (Archie 1985; Nei 1987).

The N_e was estimated from H_e by means of the stepwise mutation model (Ohta & Kimura 1973):

$$H_e = 1 - \left(\frac{1}{\sqrt{1 + 8N_e\mu}}\right),$$

where μ is the mutation rate.

Table 1. Study areas, number of chromosomes sampled ($2n$), mean number of alleles per locus (A), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e), total expected probability of identity [$P(ID)$],[a] and estimated size of population required to maintain the observed H_e (N_t).[b]

Study area (2n)	A *(SE)*	H_o	H_e *(SE)*	*1/P (ID)*	N_t
Brown bears					
Kluane (100)	7.38 (0.56)	0.788	0.761 (0.025)	390,000,000	55,000
Richardson Mts. (238)	7.50 (0.63)	0.766	0.755 (0.030)	360,000,000	52,000
Brooks Rge. (296)	7.63 (0.50)	0.774	0.749 (0.019)	160,000,000	50,000
Flathead R. (80)	6.50 (0.71)	0.694	0.694 (0.027)	15,000,000	n/a
Kuskokwim Mts. (110)	6.13 (0.44)	0.700	0.682 (0.026)	5,300,000	30,000
W. Slope (82)	6.38 (0.56)	0.668	0.678 (0.036)	10,000,000	n/a
E. Slope (90)	7.00 (0.82)	0.644	0.670 (0.062)	20,000,000	n/a
Paulatuk (116)	5.75 (0.88)	0.657	0.650 (0.058)	5,300,000	24,000
Coppermine (72)	5.75 (1.03)	0.611	0.605 (0.073)	1,600,000	18,000
Yellowstone (114)	4.38 (0.60)	0.553	0.554 (0.081)	200,000	n/a
Kodiak I. (68)	2.13 (0.35)	0.298	0.265 (0.098)	101	n/a
Black bears					
W. Slope (232)	9.50 (0.91)	0.800	0.806 (0.017)	6,800,000,000	85,000
Newfoundland I. (66)	3.00 (0.33)	0.427	0.414 (0.055)	1,300	6,400

[a] *The probability that two randomly chosen, unrelated individuals will have identical eight-locus genotypes.*
[b] N_t *was calculated by comparison to the estimated number of animals on the Kodiak Archipelago and assumes that the populations being compared are at equilibrium. Study areas where bears have been highly affected by human activity are unlikely to be at equilibrium, so* N_t *was not calculated for these populations.*

The population size required to maintain the observed H_e in each population (N_t) was calculated by taking the ratio of N_es between other study areas and the Kodiak population and multiplying by 2842, the estimated number of bears on the Kodiak Archipelago (Barnes et al. 1995):

$$N_t = 2842 N_{ex} / N_{eK},$$

where N_{ex}/N_{eK} is the ratio of N_es between Kodiak Island and the population for which calculation is being made.

Inferences regarding the number of generations required for the Kodiak population to reach equilibrium for mutation and genetic drift were based on reiteration of the formula (Hartl & Clark 1989):

$$G_{t+1} = [1/2N_e + (1 - 1/2N_e)G_t](1 - \mu)^2,$$

where G_t and G_t+1 are the homozygosity in generation t and the next generation respectively. This formula is based on the infinite alleles model (Kimura & Crow 1964), which will be similar to the stepwise mutation model in this instance because of the small N_es being considered.

Results and Discussion

Diversity in Brown Bear Populations

The results of the survey are summarized in Table 1. They show that there is a dramatic range of genetic diversity within populations of brown bears across North America (Table 1). The estimated probability of identity ranged from one in hundreds of millions in several northern continental study areas to 1 in 101 for Kodiak Island. In fact, several individuals in the Kodiak Island sample actually had identical genotypes, whereas all other individuals in the survey had unique genotypes. The range of H_e is so great that significant differences ($p < 0.05$) exist between four tiers of study areas: Kodiak Island < Yellowstone < Flathead River < {Brooks Range, Kluane, and Richardson Mountains}.

When the diversity data are compared to the range map, it appears that the main factor affecting levels of genetic diversity is connectedness to larger populations. At the low end of the diversity spectrum is Kodiak Island, which, along with other islands in the archipelago, has an estimated population size of 2842 brown bears (Barnes et al. 1995). At the other extreme are northern continental populations in the heart of the continuous portion of the distribution.

The amount of open ocean separating the Kodiak Archipelago from the mainland suggests complete isolation since the end of the Wisconsin glaciation. Coastal Alaska, including the Kodiak Archipelago, was glaciated during the Wisconsin (Flint 1971); mitochondrial sequence data suggest that Kodiak brown bears were unlikely to have been isolated in a glacial refugium (Talbot & Shields 1996), implying that this population has been isolated for about 10,000 years.

One possible explanation for the low diversity observed in the Kodiak population is a residual effect from a restricted founder population. The length of time required for a population to reach an equilibrium value of $H_e = 0.265$ (the value observed on Kodiak Island) can be estimated by assuming constant population size and a given mutation rate (μ). Even under the ridiculous as-

sumption that H_e in the founder population was zero, H_e would be 0.210 after only 300 generations (with μ = 0.0007: Weber & Wong 1993; Amos et al. 1996). Thus, with a generation time of 10–15 years (Allendorf & Servheen 1986; Craighead et al. 1995) and a founder population with levels of genetic diversity more consistent with those observed in brown bear populations, a residual founder effect in the Kodiak data seems highly unlikely.

Microsatellite markers have been used to measure genetic diversity in a number of mammalian populations that have undergone sharp declines or are otherwise threatened by low genetic diversity, and comparison to some of the most extreme examples illustrates just how low the genetic diversity in Kodiak brown bears is: a population of Rocky Mountain bighorn sheep (*Ovis canadensis*) founded from 12 individuals (H_e = 0.43; Forbes et al. 1995); the cheetah (*Acinonyx jubatus*), thought to be suffering from reduced fitness due to an ancient genetic bottleneck (H_e = 0.39; Menotti-Raymond & O'Brien 1995); an insular population of Koalas (*Phascolarctos cinereus*) reestablished from 18 adults (H_e = 0.33; Houlden et al. 1996); the northern hairy-nosed wombat (*Lasiorhinus krefftii*), which went through a bottleneck of 20–30 individuals (H_e = 0.27; Taylor et al. 1994); two populations of the critically endangered Ethiopian wolf (*Canis simensis*) (H_e = 0.21, 0.36; Gottelli et al. 1994); a severely bottlenecked population of Asiatic lions (*Panthera leo persica*) (H_e = 0.15; Menotti-Raymond & O'Brien 1995); three captive populations of Mexican gray wolves (*Canis lupus*) founded from two, two, and three individuals (H_e = 0.13, 0.26, and 0.46, respectively; Hedrick et al. 1997). Although most or all of these groups are or have recently been precariously close to extinction, Kodiak brown bears have extremely high density (Barnes et al. 1995), high productivity (Troyer & Hensel 1964), and are famous for their large body size. There is no indication that this is a population in decline.

The significantly reduced diversity in the Coppermine, Kuskokwim Mountains, and Paulatuk study areas relative to the three northern populations with the highest diversity is somewhat puzzling. It is not obvious from the range map (Fig. 1) that these populations are or have recently been more isolated than that of the Brooks Range study area. Part of the explanation may lie in a closer examination of landscape. For example, the area between the Kuskokwim Mountains and the more easterly Alaska Range includes extensive low-elevation wetland. This may cause reduced gene-flow to the east, resulting in a more isolated peninsular distribution than is suggested by the map. Similarly, the population on the Canadian Barren Grounds (the area east of the Mackenzie River) is peninsular, and the Mackenzie River, with its extensive delta, may act to reduce gene flow—although bears are sighted throughout the delta and movements from one side to the other are probably not uncommon.

Although density estimates for the Kuskokwim Mountains are unavailable, low density probably also explains part of the reduced diversity in the Paulatuk and Coppermine areas. In the Paulatuk study area density was estimated at 6.3 bears/1000 km^2 (P.C., unpublished data)—at the low extreme of published estimates (Harting 1987) and well below estimates for the Brooks Range (29.5 bears/1000 km^2; Reynolds 1992), Richardson Mountains (19 bears/1000 km^2; P. C. unpublished data), and Kluane (37–44 bears/1000 km^2; Pearson 1975) study areas. Although a drop in density could act to reduce the neighborhood size of a population, and therefore its level of genetic diversity, a lowering in density could be offset by increased long-distance movement. Preliminary data on movements of bears in and southeast of the Coppermine study area (Cluff & Case 1995) indicate that females in this area have much larger home ranges than have been reported elsewhere (Canfield & Harting 1987), and that males undertake very large movements—one individual moved 800 km in 3 months.

Explaining the generally lower diversity observed in the southern populations requires consideration of both natural and anthropogenic factors. The area that includes the East Slope, West Slope, and Flathead study areas is characterized by a series of roughly parallel mountain ranges (the Rockies, Purcells, Selkirks, and Monashees) with intervening low valleys. Movement of brown bears through these valleys, although it certainly occurs, may naturally be lower than along individual mountain ranges. In the last century, however, these valleys have also seen a steady growth in human settlement and industrial activity, with the result that some areas are no longer suitable habitat for brown bears. This is illustrated by the fact that bears are found in the Rockies and Selkirks south of the Canadian border but not between them. In addition, the density of bears throughout this region has been affected by habitat alteration and loss, and possibly by the current high rate of human-caused disturbance and mortality (McLellan & Shackleton 1988; Paquet 1995). A better understanding of genetic diversity in this area will require more-detailed local studies that can address issues of gene flow at a landscape level and historic samples that predate the human impacts of the nineteenth and twentieth centuries.

The Yellowstone population is particularly interesting because it has gone from being imbedded in a very large continuous population to being an isolated remnant, separated from other brown bears for nearly a century and with no immediate prospect for renewed connections with more northern populations (Servheen 1990). Given the historical distribution of brown bears and the current reasonably high density of brown bears in the Yellowstone ecosystem (Eberhardt & Knight 1996), the historical levels of genetic diversity in this area were probably at least as high as those currently found in the nearby Flathead River study area, and possibly as high as those

observed in the Yukon and parts of Alaska. This corresponds to a 15–20% drop in H_e in about 100 years. Despite the lack of precise numbers that only historical samples could provide, there can be little doubt that the isolation of the Yellowstone brown bear population has resulted in a significant drop in genetic diversity.

Population Genetic Considerations

One of the motivations for studying genetic diversity in natural populations is to draw inferences about N_e from measurements of H_e. The accuracy of such estimates depends on the use of appropriate mutational models. For example, in a population with H_e of 0.8, the estimate of N_e obtained under the stepwise mutation model (Kimura & Crow 1964) is exactly three times that obtained under the infinite alleles model (Ohta & Kimura 1973; in populations with low diversity, where nearly all mutations give rise to alleles that are not present in the population, the mutational model has relatively little impact.) Although the mutational dynamics of microsatellite loci do not conform exactly to any simple model of mutation, the stepwise model is more appropriate than the infinite alleles model for dinculeotide repeats that have been studied (Shriver et al. 1993; Valdes et al. 1993; Weber & Wong 1993), so calculations of N_e were based on the stepwise model.

One of the most significant ways that microsatellite mutations depart from the stepwise mutational model is through constraints on allele size (Bowcock et al. 1994; Garza et al. 1995; Nauta & Weissing 1996). Such constraints would have the effect of limiting genetic diversity in large populations and would result in underestimation of N_e for these populations. Both the limited range of alleles observed at any one locus in this data set (Appendix) and the fact that diversity is considerably higher for locus G1D—which has both even and odd allele sizes due to a point deletion in the sequence flanking the $(CA)_n$ repeat—than for the other seven loci (Table 2; binomial probability = 0.0078) suggest that the range of alleles, and therefore the amount of genetic diversity, may be constrained in some of the areas we studied.

Table 2. Mean values of H_e and A averaged across 11 brown bear populations.

Locus	H_e	A
G1A	0.649	5.54
G10B	0.700	7.00
G10C	0.557	5.00
G10L	0.547	4.18
G10M	0.627	5.18
G10P	0.708	6.73
G10X	0.552	5.64
G1D	0.798	9.00

Another concern with microsatellite data sets is the presence of nonamplifying (null) alleles (Callen et al. 1993; Paetkau & Strobeck 1995).The conformity of genotypes to expected proportions, the fact that all individuals could be typed at all loci, and the congruence of data from the Brooks Range in known family groups (Craighead et al. 1995) suggest that null alleles are uncommon or absent in the data set.

Most of the study areas in this survey are part of large, continuous populations, and it is important to give some consideration to the size of the study areas because sampling over too large an area would result in exaggerated estimates of genetic diversity (Wahlund 1928). The values of H_o and H_e shown in Table 1 do not differ significantly ($p > 0.1$, signed-rank test; Sokal & Rohlf 1995), and the only two significant departures from Hardy-Weinberg proportions can be explained by the inclusion of relocated animals in one case (East Slope) and by a large deficit of homozygotes ($p < 0.005$) at one locus in the other (Kodiak Island). On the other hand, allele distributions in the adjacent East Slope and West Slope study areas differ dramatically ($G_{36} = 126, p < 0.0001$), and, when data from the two areas are pooled, the resulting genotype distributions depart significantly ($p < 0.05$) from Hardy-Weinberg proportions. This indicates that sampling on a much larger scale would be inappropriate. Note that, although significant departures from Hardy-Weinberg equilibrium were not detected, brown bears normally have finite home ranges that are not consistent with true random mating.

Within populations, particularly those with low levels of diversity, there is a dramatic range in heterozygosity between loci (0–0.606 for Kodiak Island; 0.101–0.805 for Yellowstone). This interlocus variance results in relatively large differences in H_e between populations being insignificant, and it indicates that studies seeking to identify small differences in H_e between populations, or over time within populations, will need to survey more than eight loci. Similarly, precise estimates of genetic distance between populations would be helpful in understanding the observed patterns of within-population genetic diversity, but several distance measures that were tried appeared to be too variable to address such subtle issues. For example, it seems highly unlikely that the Yellowstone population of bears could actually be genetically closer to some northern populations than are bears in the Flathead study area, although this is suggested by values of Nei's (1972) standard distance (Table 3).

Effective Population Size

Estimates of N_e are essential for conservation programs that seek to control loss of genetic diversity because N_e determines the amount of genetic diversity maintained in a population. With selectively neutral markers, N_e can be estimated from H_e if mutation rates are known and

Table 3. Genetic distances between the 11 brown bear study areas.*

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Brooks Rge.		0.145	0.342	0.431	0.212	0.388	0.555	0.479	0.567	0.531	0.429
Richardson Mts.	0.079		0.142	0.237	0.269	0.239	0.349	0.287	0.397	0.291	0.623
Paulatuk	0.289	0.176		0.053	0.322	0.281	0.382	0.400	0.547	0.438	0.903
Coppermine	0.256	0.185	0.074		0.372	0.298	0.360	0.424	0.534	0.486	1.074
Kuskokwim Mts.	0.150	0.162	0.285	0.311		0.276	0.413	0.423	0.444	0.640	0.685
Kluane	0.278	0.172	0.292	0.245	0.259		0.316	0.293	0.318	0.498	0.913
West Slope	0.397	0.313	0.736	0.555	0.489	0.329		0.074	0.164	0.256	1.219
East Slope	0.356	0.267	0.693	0.579	0.440	0.323	0.065		0.172	0.145	1.157
Flathead River	0.284	0.248	0.679	0.546	0.399	0.390	0.164	0.156		0.253	1.498
Yellowstone	0.398	0.328	0.752	0.673	0.534	0.653	0.299	0.215	0.170		1.327
Kodiak Island	0.444	0.523	0.822	0.767	0.603	0.527	0.800	0.715	0.717	0.957	

**Nei's (1972) standard above diagonal and that of Shriver et al. (1995)* D_{SW} *below.*

equilibrium is assumed for migration, mutation, and genetic drift. Gene flow in and out of the Kodiak population has presumably been absent for a period more than sufficient to approach equilibrium, although fluctuations in population size in the last thousand years remain an unknown variable. The best estimates of mutation rates for CA_n microsatellites are in the range of 0.001–0.0002 per generation (Weber & Wong 1993; Amos et al. 1996) and using these numbers for the Kodiak population yields N_e estimates of 106 and 532 respectively. These values correspond to only 3.7% and 18.7% of the N estimated for this population.

Harris and Allendorf (1989) used demographic parameters to arrive at an estimate of N_e/N for brown bears of 0.24 to 0.32, much higher than the estimate obtained from the Kodiak population. But Frankham (1995*b*) recently concluded that demographic estimates of N_e/N tend to be underestimates and found that the average value of N_e/N for comprehensive estimates was 0.11. Our estimate does not seem unreasonable in light of this finding.

The ratio of N_e/N estimated from the Kodiak population is also supported by data from black bears on the island of Newfoundland (Table 1; Paetkau & Strobeck 1994), which have a similar history of isolation. Although estimates are less precise for this population, the same calculation yields N_e estimates of 239–1195, well below the estimated N of 3000 to 10,000 (Payne 1977; Rich 1986).

Another way to evaluate the estimates obtained from the Kodiak Island study area is to compare them to the change in diversity observed in the Yellowstone population. The estimates obtained for N_e/N translate into an N_e of 13–65 for the Yellowstone population ($N = \sim 350$; Eberhardt & Knight 1996), which would result in a drop in H_e of 4% to 0.8% per generation, in the range of the observations.

It is possible that significant fluctuation in N is common in populations of bears, and that the low estimates of N_e/N obtained from the insular Kodiak and Newfoundland populations have been affected by historic population crashes. Because it is the long-term harmonic mean of N_e that is really at issue (Wright 1938), however, these estimates would still be appropriate for conservation purposes, even if the ratio of N_e/N in populations with fixed N is higher.

Another way that H_e can be used to draw inferences about population size is to compare estimated N_es between populations (this procedure is independent of mutation rate because it will cancel out). For example, under the stepwise mutation model the estimated N_e for the Kluane population is 19.4 times that of Kodiak Island, and this is probably an underestimate as discussed above. Because the Kodiak population is estimated to contain 2,842 bears (Barnes et al. 1995), an estimated total population size of 55,000 is required to maintain the diversity observed in the Kluane population. Even if these numbers were high by a factor of two or more, they represent a huge proportion of the 50,000 to 65,000 brown bears estimated to inhabit North America (Servheen 1990).

In short, if the estimates of N_e required for long-term persistence are accurate or if the levels of genetic diversity currently existing in North American brown bear populations are to be maintained, the size of distribution required is on the order of the size of the current North American range. On the other hand, Kodiak bears have apparently persisted and thrived in isolation for a length of time that would satisfy any reasonable conservation plan, despite having an N_e that appears not to satisfy the recommendations for long-term conservation. What the data do not indicate is whether this persistence would be likely in general or whether the Kodiak population survived against all odds after many generations of reduced fitness during which strongly deleterious alleles were being purged.

For the isolated Yellowstone population, loss of genetic diversity probably does not present an immediate threat to survival, but the rate of loss is above the levels considered safe (Franklin 1980; Soulé 1980) and will ultimately lead to a near complete loss of genetic diversity. The long-term survival of this population, as with other

similar populations being left behind as brown bear range contracts throughout the northern hemisphere, will ultimately depend on the reintroduction of gene flow, either through range expansion along the Rocky Mountains or through the regular translocation of bears.

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