Genetic Relationships of Extant Brown Bears (*Ursus arctos*) and Polar Bears (*Ursus maritimus*)

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Data deposited at Dryad: http://dx.doi.org/10.5061/dryad.q30rt

Polar bears (*Ursus maritimus*) and brown bears (*Ursus arctos*) are closely related species for which extensive mitochondrial and nuclear phylogenetic comparisons have been made. We used previously published genotype data for 8 microsatellite DNA loci from 930 brown bears in 19 populations and 473 polar bears in 16 populations to compare the population genetic relationships of extant populations of the species. Genetic distances (Nei standard distance = 1.157), the proportion of private alleles (52% of alleles are not shared by the species), and Bayesian cluster analysis are consistent with morphological and life-history characteristics that distinguish polar bears and brown bears as different species with little or no gene flow among extant populations.

Key words: *brown bear, genetic distance, microsatellite DNA, polar bear, species*

Polar bears (*Ursus maritimus*) and brown bears (*Ursus arctos*, also called grizzly bears) are related species with Holarctic distributions. Polar bears have derived morphological, physiological, and behavioral characters that differentiate them from brown bears (Kurtén 1964; Amstrup 2003; Stirling 2011). These include cranial and dental characters, body proportions, and hair color. Ranges of the species overlap in Arctic coastal areas, but polar bear range is primarily sea ice, and brown bear range is primarily terrestrial. Polar bears are carnivorous and only pregnant females hibernate, whereas brown bears are omnivorous and both sexes hibernate. The species can hybridize in captivity, and 2 cases of interbreeding in the wild have been observed (Kowalska 1969; Gray 1972; Doupé et al. 2007; Preuß et al. 2009; Stirling 2011). It has been hypothesized that climate change–induced changes to polar bear habitat could increase contact and potential hybridization between the species (Kelly et al. 2010).

Polar bears are believed to have evolved from ancestral brown bears during the Pleistocene, and both species have been the subject of phylogenetic studies (reviewed by Davison et al. 2011; Lindqvist et al. 2010; Edwards et al. 2011; Hailer et al. 2012; Miller et al. 2012). The oldest polar bear fossils are estimated to be 110 000 to 130 000 years old, and the oldest brown bear fossils are about 660 000 years old (Ingólfsson and Wiig 2008; Kurtén 1968). Molecular genetic data for mitochondrial DNA (mtDNA) and nuclear DNA sequences and proteins show polar bears and brown bears are genetically distinct lineages that may have had past introgressive hybridization and molecular clock divergence time estimates of 150 000 to 5 million years ago (Goldman et al. 1989; Wayne et al. 1991; Talbot and Shields 1996a, 1996b; Yu et al. 2004, 2007; Arnason et al. 2007; Bon et al 2008; Krause et al. 2008; Pagès et al. 2008; Lindqvist et al. 2010; Davison et al. 2011; Edwards et al. 2011; Hailer et al. 2012; Miller et al. 2012). A paraphyletic relationship of polar bear and brown bear mtDNA is indicative of their recent common ancestry (Cronin et al. 1991).

Genetic distances (D_s) derived from nuclear microsatellite DNA allele frequencies resulted in inconclusive resolution of the interspecies relationship of brown bears, polar bears, and black bears (*Ursus americanus*, Paetkau et al. 1997). Distances were larger between polar bears and brown bears than between black bears and polar/brown bears, which is inconsistent with the conventional recognition of brown bears and polar bears as sister species and black bears more distantly related. This is believed to be due to microsatellite mutation dynamics that result in the loss of linearity of genetic distance and time (Paetkau et al. 1997).

These studies of brown bear–polar bear relationships focused on the phylogeny and the time of divergence of the species. However, because the species have recent common ancestry and can hybridize, quantification of relationships of extant populations of brown bears and polar bears in a population genetic context is also of interest. By population genetic context, we mean consideration of allele frequencies and distributions without regard for

molecular sequence divergences of alleles. For example, despite mtDNA sequence paraphyly in polar bears and brown bears, different haplotypes occur in each species (Krause et al. 2008; Edwards et al. 2011). From a population genetic perspective, extant populations of each species have different mtDNA gene pools regardless of gene phylogeny and past introgressive hybridization. Interspecies nuclear population genetic relationships have not been characterized for multiple individuals and populations of each species. The most extensive study of nuclear gene sequences in these species included a wide geographic distribution, but sample sizes of <60 individuals of each species (Hailer et al. 2012; Miller et al. 2012). Interspecies comparisons of microsatellites have been restricted to the study noted above, which included only 2 polar bear populations and 8 brown bear populations (Paetkau et al. 1997). Other studies of microsatellites only assessed intraspecies population structure of polar bears and brown bears (e.g., Paetkau et al. 1995, 1998a, 1998b, 1999).

In this study, we assessed the genetics of extant populations with 8 nuclear microsatellite loci for 1403 bears including brown bears across their North American range, polar bears across their worldwide range, and areas where the species' ranges are potentially contiguous. Our objective was to assess the interspecies population genetic relationships of brown bears and polar bears and address the question: do extant populations of brown bears and polar bears have different nuclear gene pools? Our approach included quantifying the distribution of private and shared alleles in each species and assessing genetic distances and Bayesian clustering patterns.

Materials and Methods

We obtained individual animal genotypes for 8 microsatellite loci from previous analyses of 19 populations of North American brown bears (Craighead et al. 1995; Paetkau et al. 1997, 1998a, 1998b; Cronin et al. 1999, 2005), 16 worldwide populations of polar bears (Paetkau et al. 1995, 1997, 1999), and 1 population of black bears (*U. americanus*, Paetkau et al. 1997, Table 1, Figure 1). The data were kindly provided by D. Paetkau (Wildlife Genetics International, Nelson, BC, Canada). Data for more than 8 loci exist for some populations of both species, but we limited the analysis to data for 8 microsatellite loci from North American brown bears and polar bears worldwide because genotype data generated in the same laboratory was available for these loci and populations. This avoids the potential problem of different microsatellite allele identification in different laboratories.

The data we used slightly differ from those in the original studies. Four animals were deleted from the Western Brooks Range brown bear population (Craighead et al. 1995; Anonymous 2006), and 6 brown bears were sampled twice, once in each of 2 adjacent populations including 4 bears that were captured in both the East Slope and West Slope Alberta populations and 2 bears that were sampled in both the Anderson Mts. and Paulatuk Northwest Territories

populations (Paetkau D, personal communication). We included these animals in both adjacent populations for our analysis. The data also included combining brown bear samples from Baranof and Chichikof islands in southeast Alaska (Paetkau et al. 1998a) and polar bear samples from the Laptev Sea were included in the Chukchi Sea or Franz Josef L-Novaja Z populations (Paetkau et al. 1999). Populations, sample sizes, and locations are shown in Table 1 and Figure 1.

The laboratory methods for 8 microsatellite loci (G1A, G10B, G10C, G10D, G10L, G10M, G10P, G10X) and analyses of Hardy–Weinberg equilibrium and linkage disequilibrium are described in the original studies (Table 1). We calculated the mean number of alleles per locus (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) with the Microsatellite Toolkit program (Park 2001). Allele frequencies were determined from genotypes and used to calculate allelic richness (AR) and pair-wise F_{st} between populations (Weir and Cockerham 1984) with the F-STAT program (Goudet 1995). We calculated genetic distance (D_s Nei 1972) for each pair of populations from the allele frequencies with the GENDIST program in the PHYLIP 3.62 software package (Felsenstein 2004). Paetkau et al. (1997) previously found that D_s performed well for comparing bear populations with microsatellites.

We calculated D_s and F_{st} between each population pair and then calculated the average of each measure for all interspecies population pairs and intraspecies population pairs. We determined the numbers of alleles restricted to 1 species (i.e., private alleles) and numbers of alleles shared by both species. These analyses were done for the entire data set of 19 brown bear and 16 polar bear populations and for a subset of 7 Arctic brown bear (Western Brooks Range, Prudhoe Bay, Arctic National Wildlife Refuge, Anderson Mts., Richardson Mts., Paulatuk, Coppermine Northwest Territories) and 6 polar bear populations (Chukchi Sea, South Beaufort Sea, North Beaufort Sea, Lancaster Sound, Viscount Melville Sound, M'Clintock Channel) with potentially contiguous ranges (Table 1, Figure 1).

D_s values were used in cluster analysis with the FITCH program (Fitch and Margoliash 1967) in PHYLIP to generate a dendrogram with the black bear population as an out-group and 1000 bootstrap replicates of the allele frequencies with the SEQBOOT program in PHYLIP.

We used the Bayesian clustering method with no *a priori* assignment of individuals to populations with the STRUCTURE program (Pritchard et al. 2000; Falush et al. 2003). We ran STRUCTURE for $K = 1$ through $K = 36$ (where $K =$ number of assumed populations) with a 20 000 burn-in period and 300 000 Markov chain Monte Carlo repetitions with the no admixture model. We did 10 replicates of the procedure for each K value. The log probability of data LnP(D) and the statistic ΔK were estimated for each value of K. LnP(D) values are negative, and the value closest to 0 can be used to infer the most probable K, except in cases where population structure results in small differences among LnP(D) values (Pritchard et al. 2010). ΔK quantifies the rate of change of LnP(D) between successive K values, and the

Table I Sampling locations and microsatellite DNA measures of variation for North American brown bears and worldwide polar bears

Location	Population	N	A	AR	H_o	H_e	Original data reference
Brown bears							
Southeast Alaska	Mainland Coast (MC)	15	5.63	5.556	0.6167	0.7569	Paetkau et al. 1998a
	Admiralty Island (AI)	30	4.88	4.395	0.6458	0.6278	
	Baranof & Chichikof Islands[a] (B&C)[a]	35	4.25	3.592	0.4929	0.496	
Yukon Territory	Kluane (KL)	50	7.38	6.1764	0.7875	0.7613	Paetkau et al. 1998a, 1998b
Southwest Alaska	Izembek (IZ)	14	4	4	0.5357	0.5324	Paetkau et al. 1998a
	Kuskokwim Mts. (KK)	55	6.13	4.8971	0.7	0.6819	Paetkau et al. 1998a, 1998b
	Kodiak Island (KI)	34	2.13	1.8918	0.2978	0.265	Paetkau et al. 1997, 1998a, 1998b
Central Alaska	North Alaska Range (NAR)	28	6.63	6.0825	0.7589	0.7794	Paetkau et al. 1998a
Arctic North America	Prudhoe Bay[b] (PB)	78	8.13	6.0753	0.7369	0.754	Cronin et al. 1999, 2005
	Arctic Nat. Wildlife Ref[b] (ANWR)	24	6.63	6.0546	0.776	0.7635	Paetkau et al. 1997
	Western Brooks Range[b] (WBR)	148	7.63	5.8678	0.7745	0.7495	Craighead et al. 1995, Paetkau et al. 1997, 1998a, 1998b
Northwest Territories Canada	Richardson Mts.[b] (RMt)	119	7.5	6.0693	0.7658	0.7554	Paetkau et al. 1997, 1998b
	Anderson Mts.[b] (AC)	23	5.38	4.8071	0.6848	0.6699	Paetkau et al. 1997
	Paulatuk[b] (PK)	58	5.75	4.8831	0.6573	0.6503	Paetkau et al. 1997, 1998b
	Coppermine[b] (CM)	36	5.75	4.16713	0.6181	0.6034	
Southwestern Canada Alberta	East Slope (ES)	45	7	5.6333	0.6444	0.6696	Paetkau et al. 1998b
	West Slope (WS)	41	6.38	5.2275	0.6677	0.6775	
Northern US Rocky Mts.	Yellowstone Nat. Park (YNP)	57	4.38	3.8533	0.5526	0.5545	Paetkau et al. 1998b
	Flathead River (FR)	40	6.5	5.4163	0.6938	0.6943	Paetkau et al. 1997, 1998b
Brown Bears Average		**48.95**	**5.90**	**4.9813**	**0.6530**	**0.6549**	
Polar bears							
Southern Canada	West Hudson Bay (WHB)	33	5.63	4.9369	0.6288	0.6338	Paetkau et al. 1995, 1999
	Davis Strait-Labrador (DS)	30	6.13	5.273	0.5917	0.6008	
	Foxe Basin (FB)	30	5.63	4.9898	0.6292	0.6411	Paetkau et al. 1999
Polar Basin	South Beaufort Sea[b] (SBS)	30	5.75	5.1735	0.6583	0.6364	Paetkau et al. 1995, 1999
	Chukchi Sea[b, c] (CS)	30	5.63	4.9744	0.6083	0.6392	Paetkau et al. 1999
	North Beaufort Sea[b] (NBS)	30	6.38	5.4155	0.6583	0.6405	Paetkau et al. 1995, 1999
	Franz Josef Land-Novaja Z[c] (FN)	32	6.13	4.9925	0.5938	0.5845	Paetkau et al. 1999
	Svalbard (SV)	31	6.5	5.3894	0.6371	0.6265	Paetkau et al. 1999
	East Greenland (EG)	31	6.38	5.3233	0.5927	0.61	Paetkau et al. 1999
Canadian Arctic Archepelago	Lancaster Sound[b] (LS)	30	6.88	5.722	0.7000	0.6888	Paetkau et al. 1999
	M'Clintock Channel[b] (MC)	15	5.25	5.1574	0.6583	0.6661	Paetkau et al. 1999
	Baffin Bay (BB)	31	6.13	5.3128	0.6492	0.6503	Paetkau et al. 1999
	Kane Basin (KB)	30	6.5	5.611	0.675	0.6737	Paetkau et al. 1999
	Gulf of Boothia (GB)	30	6.25	5.6105	0.6667	0.6958	Paetkau et al. 1999
	Viscount-Melville Sound[b] (VM)	30	6	5.4884	0.6083	0.6323	Paetkau et al. 1999
	Norwegian Bay (NW)	30	6.38	5.6024	0.6458	0.6573	Paetkau et al. 1999
Polar Bears Average		**29.56**	**6.10**	**5.3108**	**0.6334**	**0.6423**	
Black bears	La Maurice Nat Park Quebec (LM)	32	8.75	7.4515	0.7891	0.8201	Paetkau et al. 1997

[a]Samples from Baranof and Chichikof Islands combined.
[b]Potentially contiguous populations of polar and brown bears.
[c]Samples from Laptev Sea included in Chukchi Sea or Franz Josef L-Novaja Z. populations.

highest ΔK is the most likely K in situations when K is not clearly indicated by LnP(D) values (Evanno et al. 2005).

Results

Genotypes of 930 brown bears in 19 populations, 473 polar bears in 16 populations, and 32 black bears in 1 population were used to calculate measures of genetic variation (Table 1), allele frequencies (Supplementary Table 1), and D_s (Table 2, Supplementary Table 2).

Number of alleles per locus among populations ranged from 2.13 to 8.13 (average 5.9) in brown bears and 5.25 to 6.88 (average 6.1) in polar bears. Allelic richness ranged from 1.89 to 6.18 (average 4.98) in brown bears and 4.94 to 5.72 (average 5.31) in polar bears. Observed heterozygosity
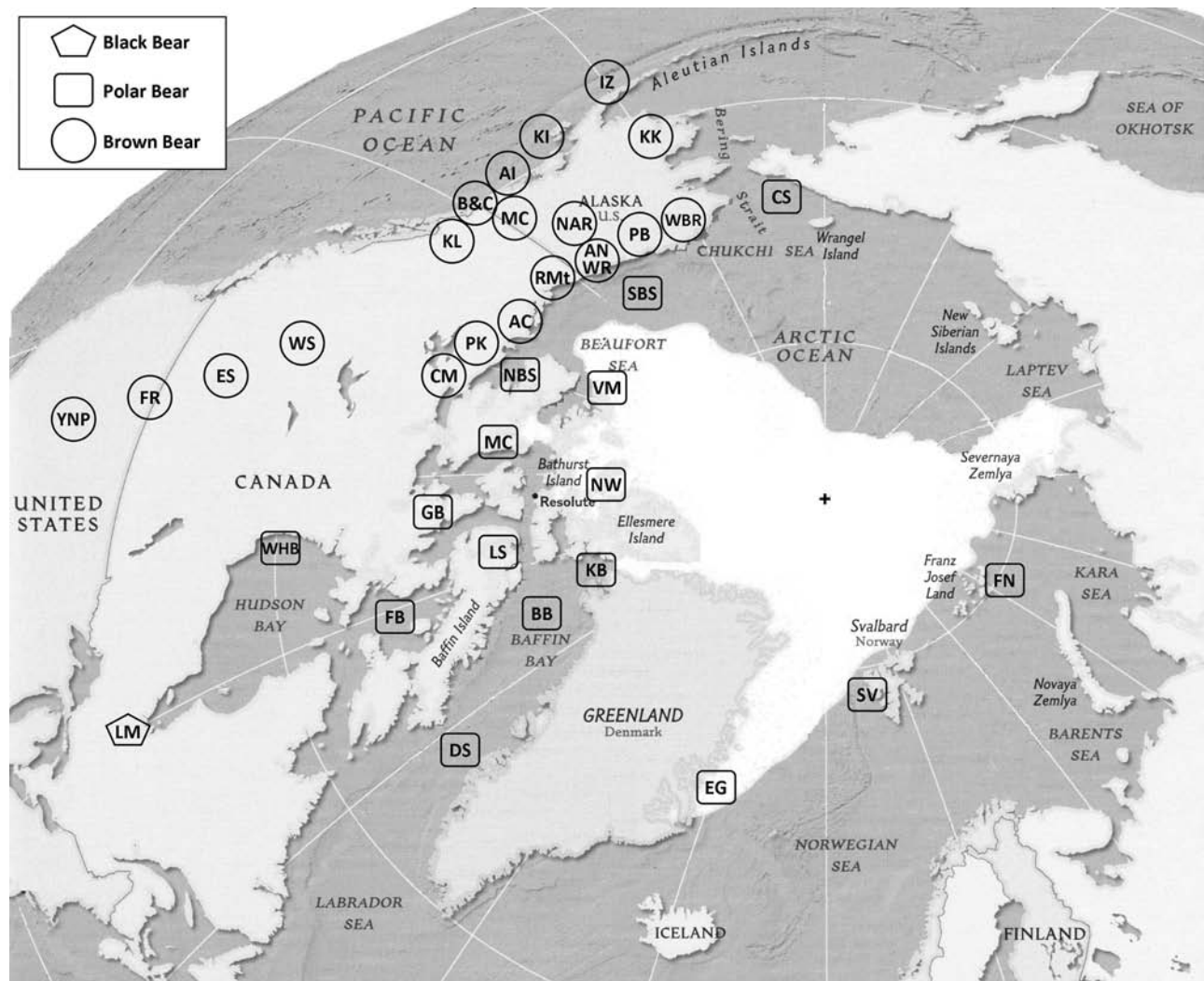
Figure 1. Map of brown bear, polar bear, and black bear sampling locations (population abbreviations described in Table 1).

ranged from 0.2978 to 0.7875 (average 0.6530) in brown bears and 0.5917 to 0.7000 (average 0.6334) in polar bears. Expected heterozygosity ranged from 0.2650 to 0.7794 (average 0.6549) in brown bears and 0.5845 to 0.6958 (average 0.6423) in polar bears (Table 1). The measures of variation are particularly low for the isolated Kodiak Island brown bear population as reported previously (Paetkau et al. 1997, 1998a, 1998b).

Of 107 total alleles observed for the 8 loci, 56 (0.5234) are not shared by brown bears and polar bears and 51 (0.4766) are shared by both species. Of the alleles not shared by the species (i.e., private alleles), 22 (0.2056) are in polar bears only and 34 (0.3177) are in brown bears only (Supplementary Table 1). Average frequency of the private alleles in polar bears is 0.0849 (standard deviation [SD] = 0.183, range 0.001–0.782), and 14 of 22 (0.636) private alleles are rare (i.e., frequency < 0.05). Average frequency of the private alleles in brown bears is 0.0734 (SD = 0.109, range 0.0005 to 0.413), and 21 of 34 (0.618) private alleles are rare. Average frequency of the alleles shared by both species is

0.120 (SD = 0.142, range 0.001–0.672) in polar bears and 0.108 (SD 0.110, range 0.001–0.501) in brown bears. Some private alleles have limited distribution, including 5 in each species that occur in only 1 population. Other private alleles are widely distributed including 4 that occur in all 16 of the polar bear populations and 3 more that occur in 12–15 of the polar bear populations. Zero private alleles occur in all 19 of the brown bear populations, 2 occur in 18 of the brown bear populations, and 12 occur in 12–16 of the brown bear populations.

The proportion of private alleles are not significantly different in the subsets of 13 potentially contiguous populations and 22 noncontiguous populations of polar bears and brown bears (2 proportion z-test, $P = 0.5751$). The 13 potentially contiguous populations have 92 total alleles, of which 48 (0.5217) are private alleles, 44 (0.4783) are shared by the species, 17 (0.1848) are in polar bear only, and 31 (0.3369) are in brown bear only. The 22 noncontiguous populations have 99 total alleles, of which 53 (0.5354) are private alleles, 46 (0.4646) are shared by the species, 23

(0.2323) are in polar bear only, and 30 (0.3030) are in brown bear only.

The average interspecies D_s between the 16 polar bear populations and the 19 brown bear populations is 1.157. The average interspecies D_s between noncontiguous populations (1.158) is not significantly different from the average interspecies D_s between contiguous populations (1.149) of polar bears and brown bears ($P = 0.800$, 2-tailed z-test of means). The average interspecies D_s between the polar bear populations and the black bear populations is 0.952 and between the brown bear populations and black bear populations is 0.904. When all the polar bear populations are combined into one group and all

Table 2 Pair-wise genetic distance (D_s and F_{st}) summary for 19 populations of brown bears, 16 populations of polar bears, and 1 population of black bears

| Comparison	Number of population pairs	Average genetic distance, D_s (Nei 1972)	SD	Range	95% Confidence level	F_{st}
Brown versus polar, all populations	304	1.1569	0.2013	0.7360–1.8150	0.0227	0.2688
Brown versus polar, contiguous populations	42	1.1495	0.2046	0.8690–1.661	0.0638	0.2392
Brown versus brown	171	0.4546	0.2552	0.045–1.498	0.0385	0.1590
Polar versus polar	120	0.1329	0.0763	0.0220–0.392	0.0138	0.0497
Brown versus black	19	0.9039	0.2825	0.6220–1.6090	0.1362	0.1759
Polar versus black	16	0.9521	0.1199	0.757–1.138	0.0639	0.1832
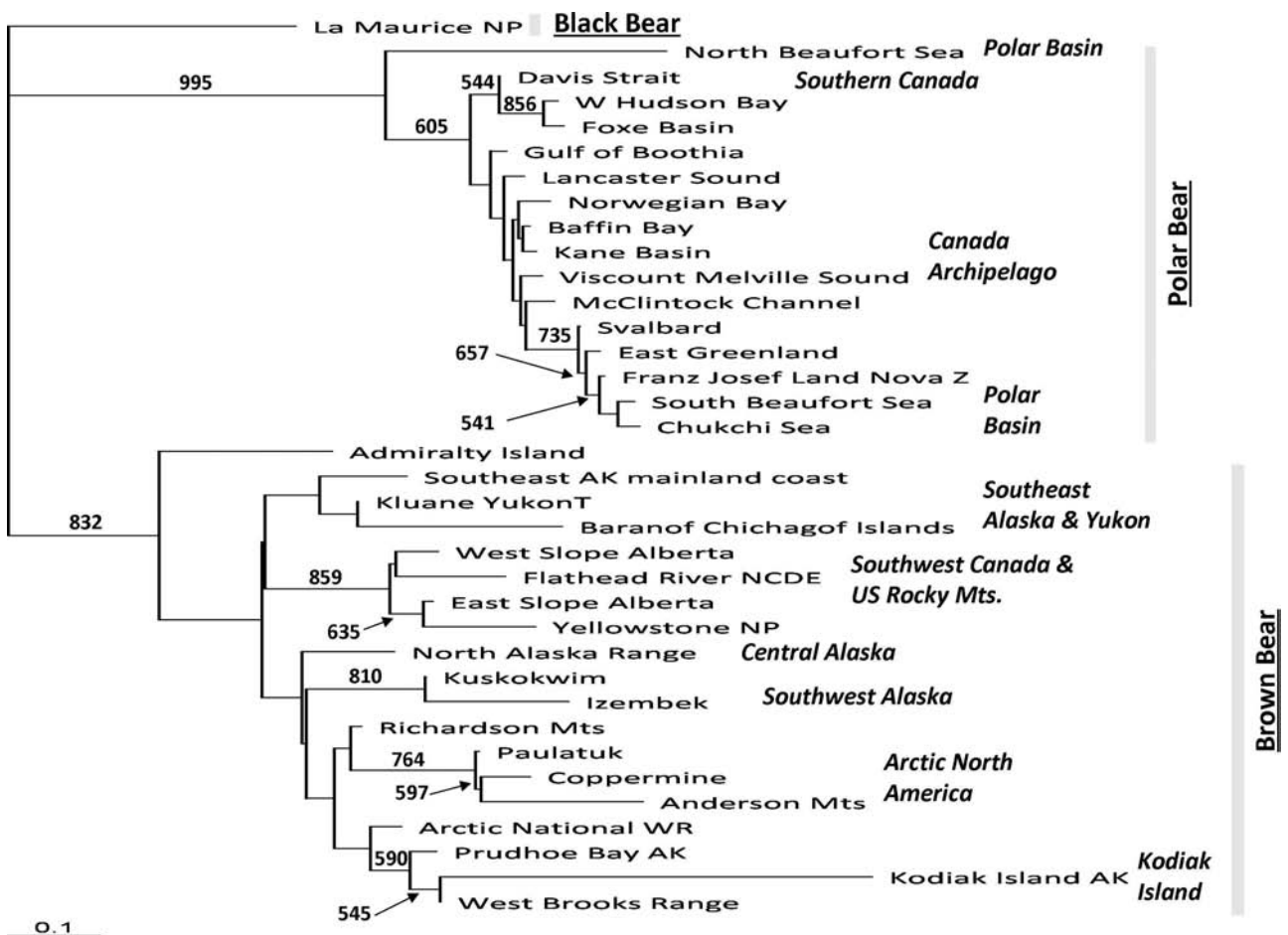
Figure 2. Fitch dendrogram of polar bears, brown bears, and black bears generated with D_s (Nei 1972) genetic distances.

the brown bear populations are combined into another group, the D_s are lower than for the interpopulation averages (polar vs. brown 0.873, polar vs. black 0.886, brown vs. black 0.651).

The D_s between populations and species are shown graphically in the FITCH tree with branch lengths proportional to the D_s (Figure 2). The FITCH tree shows the number of bootstrap replicates that resulted in the topology indicated for nodes with bootstrap values more than 0.50, and black bears as the designated out-group. Brown bears and polar bears occur in separate clusters in both trees. The polar bear cluster has 99% bootstrap support (995 of 1000 replicates), and the brown bear cluster has 83% bootstrap support.

Intraspecies D_s are considerably smaller than the interspecies D_s, and D_s are larger among brown bear populations than among polar bear populations (Table 2). Note the intraspecies relationships depicted in the dendrogram (Figure 2) have limited reliability as most clusters have bootstrap support less than 0.5. However, the intraspecies brown bear population relationships in the FITCH tree are generally consistent with geographic regions, including clusters in the North American Arctic, southwest Alaska, southern Canada and the northern US Rocky Mountains, and southeast Alaska and the adjacent Yukon Territory. Exceptions are the Admiralty Island population that does not cluster with the other southeast Alaska populations, and the Kodiak Island population clusters with the Arctic populations. Intraspecies clusters of polar bears on the FITCH tree include southern Canada (60% bootstrap support) and polar basin (74% bootstrap support). The Canadian archipelago populations occur in several clusters, and the North Beaufort Sea population occurs outside the other polar basin populations.

F_{st} estimates are of the same relative magnitude as D_s, with the average interspecies polar bear–brown bear value ($F_{st} = 0.2688$) greater than the average intraspecies values (brown bear $F_{st} = 0.1590$, polar bear $F_{st} = 0.0497$). The brown bear–polar bear F_{st} value is also greater than the brown bear–black bear ($F_{st} = 0.1759$) and polar bear–black bear ($F_{st} = 0.1832$) interspecies values (Table 2, Supplementary Table 2).

The STRUCTURE analysis resulted in LnP(D) values ranging from −45428.3 ($K = 1$) to −32873.6 ($K = 32$, Supplementary Table 3). There is little difference in LnP(D) values from $K = 2$–36 and no significantly high value that would indicate a best K value. This is a common pattern in natural populations with varying degrees of structure that may not conform to the STRUCTURE model (Evanno et al. 2005; Pritchard et al. 2010). The ΔK value for $K = 3$ was 57.2, and all other ΔK values were substantially lower (<3.7, Supplementary Table 3), indicating that $K = 3$ is the best-supported number of groups. With $K = 3$, all polar bears have 1.0 probability of being in 1 cluster, and all brown bears and black bears have 0.0 probability of being in this cluster. With $K = 3$, brown bear populations have 0.000–0.999 probability of being in a second cluster and 0.001–1.000 probability of being in a third cluster. Black bears have 0.991 probability of being in the second cluster and 0.009 probability of being in the third cluster. A STRUCTURE analysis with $K = 2$ (with or without black

bears included) indicates that all polar bears have a 1.0 probability of being in 1 cluster, and all brown bears have 1.0 probability of being in a second cluster.

Discussion

Our results show that 52% of the alleles observed are not shared by polar bears and brown bears, and the D_s and the STRUCTURE analysis separate polar bears and brown bears into different clusters. These data are consistent with other data showing the species have separate gene pools including different mtDNA haplotypes, nuclear DNA sequences, morphology, and life history.

Our interspecies D_s are comparable to D_s for other congeneric carnivore species. For example, D_s for 10 microsatellite loci between allopatric wolf (*Canis lupus*) in the Northwest Territories, Canada, and coyote (*Canis latrans*) in California is 0.444, between wolf and golden jackal (*Canis aureus*) in Kenya is 1.031, and between coyote and golden jackal is 1.34 (calculated from allele frequencies in Roy et al. 1994).

Some polar bear and brown bear populations have contiguous ranges, and it has been suggested that climate change–induced changes to polar bear sea ice habitat could increase contact and hybridization between the species (Kelly et al. 2010). Others have questioned the species status of polar bears and brown bears because of potential interspecies hybridization and a paraphyletic mtDNA relationship (Marris 2007, but see Cronin 2007). Our analysis shows genetic distances and the proportions of shared and private alleles between the species are not significantly different for noncontiguous populations and potentially contiguous brown bear and polar bear populations, indicating there is no extensive hybridization in extant populations. DNA sequences for 14 nuclear genes also indicate there is little hybridization in extant populations of polar bears and brown bears (Hailer et al. 2012).

The difference in the D_s between polar bears and brown bears (i.e., 1.157 for the interpopulation–interspecies average and 0.873 for combined populations of each species) is primarily because the interpopulation average gives equal weight to each interpopulation distance regardless of the number of samples in them, whereas the combined population gives average weight contributions from each population in proportion to the number of samples in each. For example, there are 2 brown bear populations with disproportionately large sample sizes (Western Brooks Range, $N = 148$, and Richardson Mts., $N = 119$, Table 1) that have relatively low interpopulation D_s (average 0.983 and 0.969) with polar bear populations. This serves to increase the number of brown bear samples with allele frequencies more similar to those of polar bears in the combined sample pool, resulting in a lower D_s. These brown bear populations are contiguous with polar bear range, and the relatively low D_s could suggest hybridization. However, 2 points argue against this. First, these populations, and the 2 polar bear populations contiguous to them (South Beaufort Sea, Chukchi Sea) do not have significantly different proportions of shared alleles than do all of the contiguous populations (2 proportion z-test, $P = 0.5514$). Second,

some noncontiguous brown bear and polar bear populations also have relatively low D_s (Supplementary Table 2).

Our analysis detected similar levels of genetic variation in polar bears and brown bears (Table 1) and a higher level of intraspecies population subdivision of brown bears than polar bears. Previous studies of intraspecies microsatellite variation using more than 8 loci have better resolution than our analysis and have shown that brown bears occur in geographically separate populations with limited gene flow among some areas (Paetkau et al. 1997, 1998a, 1998b; Cronin et al. 1999, 2005; Miller and Waits 2003; Jackson et al. 2008), whereas polar bear populations are relatively homogeneous over geography (Paetkau et al. 1995, 1997, 1999; Cronin et al. 2006; Zeyl et al. 2009) although there may be localized structure (Crompton et al. 2008). MtDNA variation also shows considerable geographic structure of brown bear populations (Talbot and Shields 1996b; Waits et al. 1998; Shields et al. 2000; Davison et al. 2011). These patterns are probably due to the lack of geographic barriers to gene flow across the polar bear range of Arctic sea ice, whereas the brown bear range has geographic barriers (e.g., mountain ranges, water bodies, human settlements) that restrict gene flow.

Two characteristics of genetic data have complicated assessing the relationships of polar bears and brown bears. First, the species have a paraphyletic mtDNA phylogeny, indicating some brown bears have mtDNA related to that of polar bears. However, different mtDNA haplotypes occur in each species (Krause et al. 2008; Edwards et al. 2011), and extant populations of each species have different mtDNA gene pools.

Second, microsatellite D_s between polar bears and brown bears are higher than those between either of them and black bears. This is inconsistent with the recognition of polar bears and brown bears as sister species and black bears are more distantly related. Paetkau et al. (1997) showed that genetic distances (including D_s) plateau at a maximum level after short evolutionary time periods (3000–30 000 generations) due to constraints on allele size and a rapid mutation rate. This suggests that microsatellite alleles are shared by the 3 species because of homoplasy, not identity by descent. Similarly, Wayne et al. (1991) found with 2-dimensional protein electorphoresis data for carnivores (including brown bears and polar bears) that there is less genetic change per unit time with increasing D_s. This indicates that estimation of interspecies phylogeny and divergence times with rapidly evolving markers such as microsatellites may be inappropriate, although the average square distance (ASD) has been demonstrated to be linear with time to about 2 million years ago (Sun et al. 2009). Regardless, our data for only 8 loci are too limited to reliably assess the species phylogeny and divergence times with ASD. Other studies of molecular phylogeny show a wide range of divergence times (150 000–5 million years ago) of polar bears and brown bears (Talbot and Shields 1996b; Waits et al. 1999; Yu et al. 2004, 2007; Lindqvist et al. 2010; Davison et al. 2011; Edwards et al. 2011; Hailer et al. 2012; Miller et al. 2012).

The uncertainty of the polar bear–brown bear–black bear phylogeny and divergence times does not prevent recognition that extant polar bears and brown bears have different nuclear gene pools as indicated by our analysis. Likewise, paraphyletic mtDNA phylogeny does not detract from the fact that there are different mtDNA haplotypes and distinct nuclear DNA lineages in each species. These genetic data combined with derived morphological, physiological, behavioral, and life-history characters in polar bears, and very rare interbreeding in nature supports recognition of polar bears and brown bears as different species under the biological species (Mayr 1963), genetic species (Baker and Bradley 2006), and phylogenetic species (Eldredge and Cracraft 1980) concepts.

Future assessment of the relationship of polar bears and brown bears will be aided by many new genetic markers from genome-sequencing projects (BGI 2011; Medrano JF, University of California Davis, personal communication; Miller et al. 2012). Single nucleotide polymorphisms and nuclear DNA sequences in general may be particularly useful as they may exhibit less homoplasy than microsatellite loci (Coates et al. 2009).

Supplementary Material

Supplementary Tables 1–4 can be found at http://www.jhered.oxfordjournals.org/. The genotype data reported in this paper has been deposited in the Dryad data archive.

Funding

Acknowledgments

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