Genetic Relationships of Grizzly Bears (*Ursus arctos*) in the Prudhoe Bay Region of Alaska: Inference from Microsatellite DNA, Mitochondrial DNA, and Field Observations

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Grizzly bears are abundant in the region of the Prudhoe Bay oil fields in northern Alaska. We used field observations and molecular genetic data to identify parent-offspring and sibling relationships among bears in this region. We determined genotypes at 14 microsatellite DNA loci and the cytochrome *b* gene of mitochondrial DNA (mtDNA) for 36 bears. We identified 17 possible mother-offspring pairs and 8 possible father-offspring pairs. This includes verification of the relationships of 14 mother-offspring pairs identified from field observations. Three additional mother-offspring pairs and all eight father-offspring pairs were determined from genetic and age data. Relatedness coefficients based on numbers of shared alleles between individuals were as expected: approximately 0.50 for parent-offspring and sibling pairs and approximately 0.75 for a father-offspring pair resulting from a father-daughter mating. The level of genetic variation (mean number of alleles per locus = 6.6, mean heterozygosity = 70%) and allele frequencies in grizzly bears in the Prudhoe Bay region are similar to those in other parts of the species' range.

Genetic issues have become important in the management and conservation of natural populations, particularly in small populations and in species with low reproductive rates (Allendorf and Leary 1986; Ballou and Ralls 1982). Grizzly bears (*Ursus arctos*) have low reproductive rates and population densities and have been the focus of intense management efforts as well as genetic study. Genetic studies of grizzly bears have assessed phylogenetics and population structure (Cronin et al. 1991a; Paetkau et al. 1998a,b; Shields and Kocher 1991; Taberlet and Bouvet 1994; Talbot and Shields 1996a,b; Zhang and Ryder 1993), genetic variation and fitness in small populations (Allendorf et al. 1979; Harris and Allendorf 1989; Paetkau et al. 1998b; Larsen et al. 1983), and practical management applications (i.e., species or sex-identification forensics; Amstrup et al. 1993; Cronin et al. 1991b; Taberlet et al. 1993; Wiig et al. 1998).

Previous genetic studies of grizzly bears in northern Alaska focused on populations in remote, undeveloped areas (Craighead et al. 1995; Talbot and Shields 1996b). A study of reproductive success in a grizzly bear population in the western Brooks Range of northern Alaska indicated that about half the males in this population were effective breeders in a genetically diverse, polygynous, and polyandrous mating system. No male sired more than 11% of the known offspring in the population, and different males sired cubs in the same litter (Craighead et al. 1995). The grizzly bear population in this region has a slow rate of growth because of a short growing season and harsh climate. Previous research has indicated that male bears do not reproduce until they are 9 years old, and female bears do not reproduce until they are 5 years old, with as many as 4 years between litters (Craighead et al. 1995). However, the population dynamics of grizzly bears may be different in the Prudhoe Bay region northeast of the western Brooks Range (Figure 1) where the grizzly bear population has increased considerably over the last 25 years (Shideler and Hechtel, in press). During this time large oil fields in the Prudhoe Bay region have been developed, and more than 35 bears have used ranges that include the oil field areas. Bears sometimes have access to anthropogenic food in garbage and are not hunted in the oil fields, although they are hunted in the surrounding areas. It is possible that the oil fields attract bears and harbor a subpopulation with relatively high fidelity to their natal areas. The bears in the oil field areas may serve as a reservoir subpopulation that provides immigrants to other areas, or as a sink into which bears from other areas are drawn (Knight et al. 1988). A potential wildlife management problem is that some

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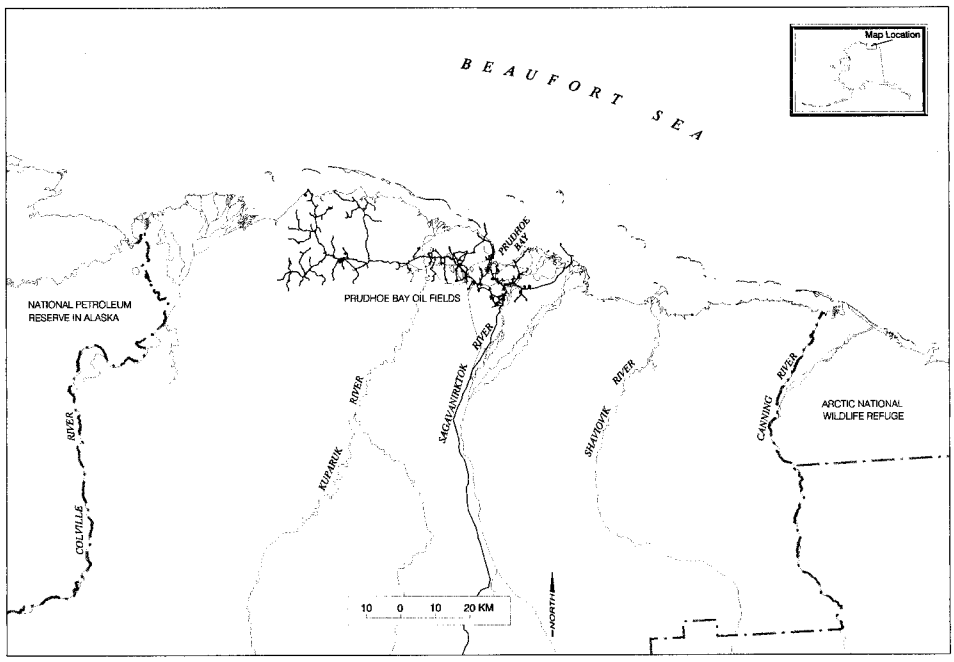

Figure 1. Map of the study area in the Prudhoe Bay region of northern Alaska.

bears become conditioned to human foods in the oil fields and are killed when they move to other areas and become a nuisance and/or a hazard to humans (Shideler and Hechtel, in press).

In this article we describe the genetic relationships of the bears in the Prudhoe Bay area, including the large oil fields. Field studies have identified many bears that use habitats in the oil fields, including several mother-offspring and sibling groups (Shideler and Hechtel, in press). Our objective was to quantify the genetic relationships, including parent-offspring and family relationships, of the bears in the Prudhoe Bay region to better understand the population structure of this species in northern Alaska. We focused on two types of genetic markers that have previously been used to assess relatedness in grizzly bears: mitochondrial DNA (mtDNA), which is predominantly maternally inherited (Cronin et al. 1991a; Talbot and Shields 1996a,b), and microsatellite DNA, which is biparentally inherited (Craighead et al. 1995; Paetkau et al. 1998a,b).

Materials and Methods

Tissue samples were obtained from 37 bears (21 females, 14 males, 2 unknown sex) that were captured for radio collaring (sample numbers 1–31; see appendix) or killed by hunters (sample numbers 100–105; see appendix) in the Prudhoe Bay region from 1985 to 1994 (Figure 1). Blood was obtained by venipuncture and skin punches were obtained while attaching ear tags to live animals. Muscle tissue was obtained from hunter-killed bears. Mother-offspring and sibling relationships were identified from field observations. The ages of 36 of the 37 bears were determined by counting cementum annuli in excised premolar teeth (Stoneberg and Jonkel 1966) and the age of one bear was unknown.

Genomic DNA was extracted from tissues using QIAamp DNA extraction kits (Qiagen Inc., Chatsworth, CA). The cytochrome *b* gene of mtDNA was amplified from genomic DNA with the polymerase chain reaction (PCR) (Saiki et al. 1988) using the oligonucleotide primers LGL 765, GAAAAACCA(C/T)CGTTGT(T/A)ATTCAACT, and LGL 766, GTTTAATTAGAAT(C/T)T(C/T)AGCTTTGGG. PCR reactions (50 μl) contained 5–50 ng DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM of each of the two primers, and 1.25 units of AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT). Reactions were heated to 95°C for 5 min followed by 32 cycles of amplification. Each cycle consisted of 45 s at 95°C, 40 s at 50°C, and 2.5 min at 70°C. Amplification products were sequenced with the 765 primer on an autosequencer (ABI model 373A; Automated Biosystems Inc., Foster City, CA) using dye-labeled terminators (Carr and Marshall 1991). The DNA sequences were aligned with the SeqEd computer program (Automated Biosystems Inc.) and nucleotide substitutions were used to identify mtDNA haplotypes for each bear. MtDNA haplotypes were compared among bears that were known or suspected to be related, and with other populations (Talbot and Shields 1996b).

Fourteen microsatellite loci were analyzed, including eight (Table 1) which have previously been studied in Alaska grizzly bears (Craighead et al. 1995; Paetkau et al. 1997, 1998a,b). The PCR primers and reaction conditions are described by Paetkau et al. (1998a).

We assessed measures of genetic variation in the Prudhoe Bay bears, including the number of alleles (*A*) and unbiased expected heterozygosity (*H*; Nei and Roychoudhury 1974) for the microsatellite loci. We also used goodness-of-fit chi-square tests (with pooling of rare alleles) to test for Hardy–Weinberg equilibrium for the microsatellite loci using the BIOSYS computer program (Swofford and Selander 1981). We calculated the unbiased probabilities of identity (Paetkau et al. 1998b), probabilities of paternal exclusion (i.e., the probability that an unrelated male can be excluded as a potential father if the mother's genotype is known; Chakravarti and Li 1983), and probabilities of parent-offspring exclusion (i.e., the probability that an unrelated individual can be excluded as a potential parent or offspring if there is no genetic information on relatives, Paetkau et al. 1998a) using the microsatellite allele frequencies. To quantify relationships of all the bears sampled in the Prudhoe Bay population we calculated pairwise relatedness coefficients (r_{xy}; Blouin et al. 1996; Pamilo 1989; Queller and Goodnight 1989) using the Kinship 1.1.2 computer program (Goodnight KF, Rice University, Houston, TX). This coefficient was calculated from the number of microsatellite alleles shared by two bears, weighted by the allele frequencies in the population. This is superior to a simple proportion of shared alleles because it weighs shared rare alleles more than shared common alleles.

We compared the microsatellite genotypes among potential parents and offspring to assess family relationships. We assumed a minimum breeding age of 5 years for males (Craighead et al. 1995), and therefore considered each pair of bears which differed by ≥6 years of age as potential parent-offspring pairs. For example, if a bear born in 1984 conceived an offspring as a 5-year-old in 1989, the offspring's birth year would be 1990, or 6 years from the birth year of the parent.

Table 1. Microsatellite allele frequencies, number of alleles (*A*), and heterozygosity (*H*) for grizzly bears from the Prudhoe Bay region, Alaska

Locus	Allele	Frequency	Locus	Allele	Frequency
G10B[a]	140	0.2500	*G10P*[a]	149	0.0139
	148	0.0694		151	0.4028
	150	0.0139		153	0.2222
	152	0.0833		155	0.1250
	158	0.0694		157	0.1250
	160	0.2778		159	0.0694
	164	0.2361		161	0.0417
	A/H	7/80%		A/H	7/76%
G10C[a]	103	0.3194	*G10X*[a]	131	0.1667
	105	0.3472		133	0.0278
	107	0.1667		135	0.0694
	109	0.0278		137	0.5278
	111	0.1389		141	0.2083
	A/H	5/74%		A/H	5/65%
G10L[a]	171	0.0833	*G1A*[a]	180	0.0417
	151	0.0139		184	0.2222
	155	0.4861		186	0.0139
	157	0.3194		190	0.0139
	159	0.0278		192	0.1944
	163	0.0694		194	0.4028
	A/H	6/66%		196	0.0278
				198	0.0694
				200	0.0139
				A/H	9/75%
G10M[a]	206	0.1528	*G1D*[a]	172	0.2778
	208	0.2778		174	0.0556
	210	0.0972		176	0.0278
	212	0.0694		178	0.2500
	214	0.3611		180	0.0278
	218	0.4170		181	0.2083
	A/H	6/76%		182	0.0139
				184	0.0972
				186	0.0417
				A/H	9/81%
CXX20	123	0.0139	*G10O*	182	0.2639
	127	0.0526		192	0.0556
	129	0.4444		198	0.5556
	133	0.0278		200	0.0139
	135	0.0417		204	0.1111
	139	0.0833		A/H	5/61%
	141	0.0417			
	143	0.2917			
	A/H	8/71%			
G10H	221	0.5972	*MU50*	110	0.1111
	229	0.0278		122	0.0556
	231	0.1528		126	0.0139
	233	0.1111		128	0.1389
	237	0.0139		130	0.3056
	252	0.0139		132	0.0139
	254	0.0556		134	0.0556
	257	0.0278		138	0.3056
	A/H	8/61%		A/H	8/79%
G10J	78	0.0278	*MU59*	223	0.0139
	80	0.4861		227	0.7083
	86	0.1667		229	0.0833
	90	0.0694		239	0.0972
	96	0.2500		247	0.0972
	A/H	5/68%		A/H	5/48%

[a] Loci analyzed for other populations by Paetkau et al. (1998b) and Craighead et al. (1995).

Table 2. MtDNA cytochrome *b* nucleotide substitutions and haplotypes

	Nucleotide position					
	14933	14975	15075	15191	Haplotype	Number of Prudhoe Bay bears
Nucleotides	C	A	T	T	GB10*	3
	T	G	T	C	GB14*	7
	T	G	C	C	GB19*	24

Nucleotide positions correspond to those reported by Talbot and Shields (1996b).

The youngest age at which females breed successfully in the western Brooks Range appears to be 5 years old (Craighead et al. 1995). However, field observations in the Prudhoe Bay region suggested that a 5-year-old female had an offspring (conceived when the female was 4). We therefore included all females ≥5 years of age in assessing potential female-offspring pairs. If a pair of bears shared at least one allele at each of the 14 microsatellite loci they were not excluded as a parent-offspring pair. Sharing one allele at each locus by two individuals does not verify a parent-offspring relationship, but indicates it is possible. For the potential female parents, we also compared mtDNA genotypes with those of potential offspring. We combined the microsatellite, mtDNA, and field data to construct pedigrees. We use the term "related bears" to include parent-offspring and sibling pairs and the term "unrelated bears" to include non-parent-offspring and non-sibling pairs.

Results

We obtained 450 nucleotides of cytochrome *b* mtDNA sequence for 34 of the 37 bears sampled. DNA from three of the bears did not amplify in the PCR reactions and was not sequenced (bears 5, 7, 8; see appendix). Our sequences correspond to nucleotide positions 14796–15246 of the 1140 cytochrome *b* nucleotides reported by Talbot and Shields (1996b; GenBank accession numbers U18870–U18899). We detected nucleotide substitutions at four positions that occur in three haplotypes (Table 2). These three haplotypes correspond to haplotypes GB10, GB14, and GB19 which were observed in other northern Alaska grizzly bear populations by Talbot and Shields (1996b). Because our sequences include only 450 nucleotides, we cannot be certain our haplotypes have the same sequences as those of Talbot and Shields for all 1,140 nucleotides of the cytochrome *b* gene. We therefore designated our haplotypes as GB10*, GB14*, and GB19*.

We obtained genotypes at 14 microsatellite loci for 36 of the 37 bears sampled (see appendix). DNA from one bear (no. 8) did not amplify in the PCR reactions. With the exception of one locus (*G1D*), the microsatellite alleles differed in size by two or more nucleotides indicating variable numbers of dinucleotide repeats. Locus *G1D* had alleles that varied by single nucleotides (i.e., 180, 181, and 182 nucleotides; Table 1) as observed by Craighead et al. (1995) and verified with DNA sequencing (Paetkau et al. 1998a). There is abundant variation at the 14 microsatellite loci analyzed, with 5–9 alleles per locus (mean $A = 6.6$), and 48–81% heterozygosity across loci (mean $H = 70.2\%$; Table 1).

Goodness-of-fit chi-square tests showed that genotypes of 13 of the microsatellite loci in the Prudhoe Bay grizzly bear population did not differ significantly from expected Hardy–Weinberg proportions ($p > .096$). One locus, *G10H,* was not in Hardy–Weinberg equilibrium, and had significantly more heterozygotes than expected ($p = .049$). However, a Bonferroni correction indicates the appropriate level of significance for 14 loci is $p = .0036$ (i.e., 0.05/14), suggesting the deviation at the *G10H* locus may be due to chance. For eight of these loci (Table 1) Craighead et al. (1995) also found no evidence of deviation from Hardy–Weinberg expectation in the western Brooks Range grizzly bear population.

No bears in our sample shared the same genotypes at all 14 loci, and the probability of identity of the Prudhoe Bay bears is high, 4×10^{-13}. This translates to a probability of two unrelated bears sharing the same 14-locus genotype of greater than 1 in 2 trillion. The probability of paternal exclusion is .9999 and the probability of parent/offspring exclusion is .9938. These microsatellite loci are clearly useful for individual identity and pedigree analysis.

Mother-Offspring Relationships

The genetic and field data identified probable family relationships of several bears in the Prudhoe Bay region. Related bears included six multiple litters (five sets of twins and one set of triplets) and five single offspring (Table 3, Figure 2). There are

Table 3. Parent-offspring relationships of grizzly bears determined from 14 microsatellite loci and mtDNA

Sample no.	Breeding age	MtDNA haplotype	Sample no.	Birth year	mtDNA haplotype	Relationship from field observations	r_{xy}
Mother			**Offspring**				
1	4	GB19*	23	1992	GB19*	Suspected	0.5357
2	10	GB19*	1	1987	GB19*	Suspected	0.4612
2	10	GB19*	6	1987	GB19*	Suspected	0.3884
2	13	GB19*	9	1990	GB19*	Known	0.5568
2	16	GB19*	24	1993	GB19*	Suspected	0.4642
2	16	GB19*	25	1993	GB19*	Suspected	0.4170
2	16	GB19*	27	1993	GB19*	Suspected	0.4925
4	5	GB19*	11	1992	GB19*	Known	0.6352
4	5	GB19*	12	1992	GB19*	Known	0.6120
10	7	GB19*	15	1992	GB19*	Known	0.3615
10	7	GB19*	16	1992	GB19*	Known	0.3938
19	12	GB14*	17	1993	GB14*	Suspected	0.4202
19	12	GB14*	18	1993	GB14*	Suspected	0.5241
19	18	GB14*	7	1989	Not Done	Unknown	0.5661
21	9	GB19*	4	1986	GB19*	Unknown	0.4459
21	7	GB19*	10	1984	GB19*	Unknown	0.4989
21	16	GB19*	30	1993	GB19*	Known	0.6379
							$\bar{x}$ = 0.4948
							SD = 0.0869
Father			**Offspring**				
14	10	GB14*	15	1992	GB19*	Unknown	0.4281
14	10	GB14*	16	1992	GB19*	Unknown	0.5065
20	12	GB19*	4	1986	GB19*	Unknown	0.4721
20	13	GB19*	1	1987	GB19*	Unknown	0.5750
20	13	GB19*	6	1987	GB19*	Unknown	0.6128
20	18	GB19*	11	1992	GB19*	Unknown	0.7291
20	18	GB19*	12	1992	GB19*	Unknown	0.7458
31	8	GB10*	30	1993	GB19*	Unknown	0.5085
							$\bar{x}$ = 0.5722
							SD = 0.1168
Hypothetical father			**Offspring**[a]				
Hypothetical	n/a	n/a	7	1989	Not Done	Unknown	0.2277
Hypothetical	n/a	n/a	17	1993	GB14*	Unknown	0.3456
Hypothetical	n/a	n/a	18	1993	GB14*	Unknown	0.3148
Hypothetical	n/a	n/a	23	1992	GB19*	Unknown	0.1298
Hypothetical	n/a	n/a	24	1993	GB19*	Unknown	0.4887
Hypothetical	n/a	n/a	25	1993	GB19*	Unknown	0.4984
Hypothetical	n/a	n/a	27	1993	GB19*	Unknown	0.4928

The parents and offspring listed share at least one allele at each of the 14 loci. Additional pairs not excluded as parent-offspring with genetic data but which field data indicate are not parent-offspring: 2-3, 3-104, 6-23, 1-100, 6-100, 23-100, 27-100.

[a] The hypothetical father was identified from the genotypes for the triplets (bears 24, 25, and 27) and their mother (bear 2). The r_{xy} values were calculated using the inferred genotypes for the hypothetical father (see appendix).

two mtDNA haplotypes in the related bears, including two maternal lineages initiated by bears 2 and 21 (with mtDNA haplotype GB19*), and one maternal lineage initiated by bear 19 (with mtDNA haplotype GB14*). There are 19 bears with mtDNA haplotype GB19* and 4 bears with mtDNA haplotype GB14* in the pedigrees (Table 3, Figure 2). Three bears have the mtDNA GB10* haplotype (Table 2, appendix), but they are not in the maternal lineages of related bears. One of these (bear. 31) is a father in the pedigree, but his (maternally inherited) mtDNA would not be transmitted to offspring.

The assessments of parentage included 206 pairwise comparisons of 10 females with potential offspring. We identified 17 possible mother-offspring pairs that shared at least one allele at each microsatellite locus and the same mtDNA haplotype (Table 3). This included 6 different females as possible mothers of 17 different offspring (Figure 2). Bear 8, for which there was no genetic data, was known to be an offspring of bear 2 from field observations, and we included him in Figure 2. The r_{xy} values for the mother-offspring pairs were close to 0.5 as expected (mean = 0.4948; Table 3). An additional pair (bears 2 and 3) shared at least one allele per locus and was also identified as a potential mother-offspring pair. However, bear 3 was born in 1988 and bear 2 had twins (bears 1 and 6) in 1987 and probably would not have bred that year. In addition, bears 3 and 5 are siblings (Table 4) and bear 5 shared alleles with bear 2 at only 13 of the 14 loci. Therefore bear 5 is excluded as an offspring of bear 2, and bear 5's sibling, bear 3, is also excluded as an offspring of bear 2 by association. Another bear (bear 23) had two potential mothers (bear 1 or bear 6) identified with genetic data. Field observations suggested that bear 1 was the mother of bear 23 and a sibling of bear 6 (i.e., bear 6 is the aunt of bear 23; Figure 2). Bear 1 was only 4 years old in the conception year (and 5 years old in the birth year) of bear 23, suggesting that bear 1 bred at 4 years of age. The other mothers ranged from 5 to 18 years old when bred (Table 3). There were 6 of 10 (0.60) adult females ≥4 years of age who had offspring in our sample.

The mother-offspring pairs identified with genetic data were consistent with field observations. Of 14 known or suspected mother-offspring pairs identified from field observations, all were confirmed with the microsatellite and mtDNA genotypes (Table 3, Figure 2). As stated previously, the genetic data suggested two potential mothers for bear 23, but field observations allowed identification of the mother (bear 1). This indicates that bears other than parent-offspring pairs (in this case an aunt-niece pair, bears 6 and 23) may share an allele at each locus. Another example is the case of bears 2 and 3 described above. These examples indicate that caution should be exercised when inferring parent-offspring relationships from a limited number of genetic loci without corroborating field and age data. Accordingly, three possible mother-offspring pairs that were identified from genetic data alone can be considered probable (though not verified) parent-offspring pairs: bear 21 as the mother of bears 4 and 10, and bear 19 as the mother of bear 7 (Figure 2; Table 3). In these cases, the probability of identifying a parent-offspring pair without knowledge of the other parent (and without corroborating field evidence) is lower than in cases when one parent is known (i.e., identifying a father when the mother is known). We therefore identified these relationships with dashed lines in the pedigree (Figure 2).

Father-Offspring Relationships

There were no field observations of mating pairs and thus no known or suspected fathers. Our genetic analysis included 100 pairwise comparisons of five males with potential offspring. We identified eight potential father-offspring pairs that shared at least one allele at each microsatellite locus (Table 3, Figure 2). There were three males that were potential fathers: bear 20 who sired two sets of twins (bears 1 and

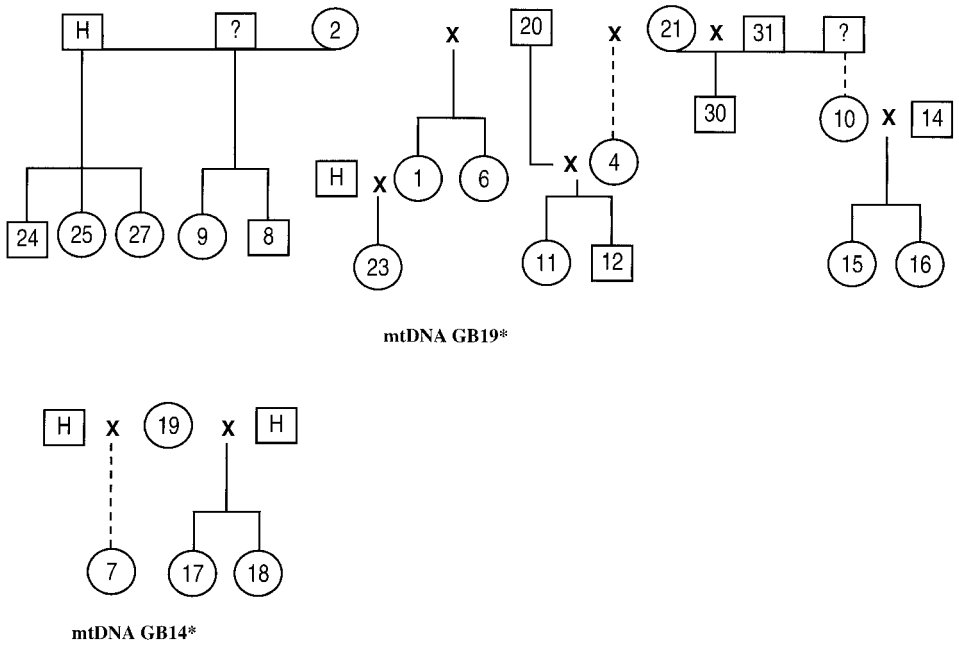

Figure 2. Pedigrees of related grizzly bears from the Prudhoe Bay region of northern Alaska. A hypothetical male (H) represents a possible unsampled father whose genotypes were inferred from the microsatellite data for sampled bears. The bears had the mtDNA haplotypes indicated, except for bear 31 that had mtDNA haplotype GB10* (see text). The dashed lines are for relationships in which the mother is not known or is suspected from field observations and the probability of parentage identity is lower than in the other cases.

6, and bears 11 and 12) and one single offspring (bear 4); bear 14 who sired one set of twins (bears 15 and 16); and bear 31 who sired a single offspring (bear 30). Bear 4 was the only one of these offspring for which the mother was not known or suspected from field observations, so the probability of this parental identity is lower and indicated by a dashed line in the pedigree (Figure 2). The males identified as fathers were between 8 and 18 years old at breeding. It appears that bear 31 bred at 8 years of age to sire bear 30 (Table 3). We found no evidence of littermates with different fathers (i.e., multiple paternity). There were 3 of 5 (0.60) adult males ≥8 years of age who sired offspring in our sample.

The r_{xy} values for the father-offspring pairs were close to 0.5 as expected (0.5722; Table 3). Note that bear 20 mated with his daughter (bear 4) to sire twins, bears 11 and 12 (Table 3; Figure 2). The r_{xy} values of bears 20 and 4 (0.47), bears 20 and 11 (0.73), and bears 20 and 12 (0.75) are consistent with values expected for these relationships.

In addition to the possible father-offspring pairs in Table 3 and Figure 2, bear 3 was not excluded as the father of bear 104 with the genetic data. However, bear 3 was only 3 years old when bear 104 was conceived and bear 3 was probably too young to be the father of bear 104. The genetic data also indicated another male (bear 100) was not excluded as the parent or offspring of four bears (bears 1, 6, 23, and 27). However, bear 100's birth year differed by ≤3 years from the birth years of these four bears, so they are not likely parent-offspring pairs. All four of these bears are offspring or grandoffspring of female bear 2 (Figure 2), but bear 100 was excluded as an offspring of bear 2 at one locus (*G10M*; see appendix). Therefore bear 100 is not a parent, offspring, or sibling of these four bears, but he is apparently closely related to their lineage.

The genotypes of the triplets (bears 24, 25, and 27) and their mother (bear 2) allow identification of a "hypothetical male" as the father (Craighead et al. 1995). By identifying the maternal allele in each of the triplets, the alleles contributed by the father can be inferred. Two alleles can be identified for the hypothetical male at six loci, and one allele at eight loci (see appendix). Considering the genotypes at the six loci for which both alleles were identified, this hypothetical male could have sired bears 7, 17, 18, and 23 in addition to the triplets (Table 3; Figure 2). We cannot verify if this hypothetical male represents one unsampled father of these bears, but it is possible.

Fathers and offspring did not share mtDNA haplotypes in three cases. Bear 14 (haplotype GB14*) sired bears 15 and 16 (haplotype GB19*), and bear 31 (haplotype GB10*) sired bear 30 (haplotype GB19*). This result is not unexpected because mtDNA is predominantly maternally inherited. In the pedigrees, fathers of seven bears in the mtDNA GB19* lineage and three bears in the mtDNA GB14* lineage are unidentified (Figure 2).

Table 4. MtDNA haplotypes, number of microsatellite loci with at least one shared allele, known parents, and r_{xy} coefficients for grizzly bear siblings identified in the field

Sibling 1	mtDNA haplotype	Sibling 2	mtDNA haplotype	Number of microsatellite loci with shared allele	Known shared parents[a]	r_{xy}
3	GB19*	5	Not Done	14	Unknown	0.6505
24	GB19*	25	GB19*	11	Mother	0.5201
24	GB19*	27	GB19*	12	Mother	0.4912
25	GB19*	27	GB19*	13	Mother	0.4488
1	GB19*	6	GB19*	14	Mother/Father	0.7238
11	GB19*	12	GB19*	13	Mother/Father	0.6327
15	GB19*	16	GB19*	12	Mother/Father	0.3668
17	GB14*	18	GB14*	13	Mother	0.5349
						$\bar{x}$ = 0.5461
						SD = 0.1167

[a] Shared parents identified from field and/or genetic data.

Sibling and General Relationships

Field observations identified siblings as well as parent-offspring pairs. Siblings may not necessarily share alleles at all loci, as each sibling may receive different alleles for a locus from the same parent. However, siblings share as many alleles, on average, as parent-offspring pairs and inherit the same mtDNA haplotype from their mother. Our genetic data support the sibling relationships determined in the field, as the siblings share the same mtDNA haplotypes and share at least one allele for most or all (11–14 loci) of the microsatellite loci (Table 4). The r_{xy} values for sibling pairs were close to 0.5 (0.5461; Table 4) as expected. We can infer other relationships

from the genetic data (Figure 2) including half siblings (e.g., bears 1 and 11) and grandparent-grandson/granddaughter pairs (e.g., bears 20 and 23).

The relatedness coefficients between related bears (i.e., parent-offspring or sibling pairs) were high (mean r_{xy} = 0.5262) compared to unrelated bears (mean r_{xy} = −0.0376). Among related bears r_{xy} values were similar for mother-offspring pairs (mean r_{xy} = 0.4948), father-offspring pairs (mean r_{xy} = 0.5722), and siblings (mean r_{xy} = 0.5461) (Tables 3 and 4).

Discussion

Our study shows that both field and genetic data should be considered when inferring genetic relationships in natural populations (Pemberton et al. 1992). We identified several bears that were not excluded as parent-offspring pairs with genetic data, but which field and age data indicated were not parent-offspring.

The combination of field and genetic data indicates there are many related individuals and family groups of bears in the Prudhoe Bay region. Although grizzly bears (particularly males) have large home ranges and move long distances (Craighead et al. 1995; Shideler and Hechtel, in press), some bears had fidelity to the Prudhoe Bay region and produced offspring there over several years. Offspring were produced in the region by female bear 2 from 1987 to 1993, by female bear 21 from 1984 to 1993, and by male bear 20 from 1986 to 1992 (Table 3).

Our results are consistent with maternal and biparental modes of inheritance of mtDNA and microsatellites, respectively. All mother-offspring and sibling pairs shared mtDNA genotypes, but three father-offspring pairs did not share mtDNA genotypes. All mother-offspring pairs identified from field data shared at least one microsatellite allele per locus and r_{xy} values were as expected: parent-offspring and sibling pairs had r_{xy} values about 0.5; unrelated bears had r_{xy} values about 0; and the father-offspring pairs resulting from a father-daughter mating had r_{xy} values about 0.75. Blouin et al. (1996) obtained similar results for mice (*Mus musculus*), with r_{xy} values about 0.5 among parent-offspring and siblings and about 0 among unrelated mice.

The genetic patterns of the grizzly bears in the Prudhoe Bay region and the western Brooks Range were similar, although there were some differences. Although Craighead et al. (1995) found evidence of multiple paternity in litters (with a larger sample size) in the western Brooks Range, we found no evidence of this in our study. In addition, it appears that males may reproduce at 8 years old and females at 4 years old in the Prudhoe Bay region. Craighead et al. (1995) reported minimum breeding ages of males as 9 years and females as 5 years in the western Brooks Range.

The extensive genetic and field data in

Appendix. Birth year, sex, and genotypes for 14 microsatellite DNA loci and mtDNA for 36 grizzly bears and one hypothetical male bear from the Prudhoe Bay region of Alaska

Bear number	Birth year/sex	Microsatellite locus														
		G10D	*G10A*	*G10B*	*G10L*	*G10M*	*G10P*	*G10X*	*G10H*	*G10C*	*G10J*	*G10O*	*CX20*	*MU50*	*MU59*	mtDNA
1	1987/female	178/184	192/194	164/164	157/171	208/214	151/151	131/137	221/231	103/107	80/80	182/198	129/143	138/138	227/229	GB19*
2	1976/female	172/178	184/194	148/164	155/157	214/214	151/151	137/137	221/233	103/105	80/96	182/198	129/129	138/138	227/229	GB19*
3	1988/male	172/186	194/194	160/164	155/157	214/214	151/157	131/137	221/231	105/107	80/96	198/198	129/141	122/138	227/229	GB19*
4	1986/female	172/181	184/192	140/152	155/157	206/208	151/151	131/141	221/221	107/111	80/90	198/198	129/143	110/130	227/227	GB19*
5	1988/male	172/186	194/194	160/160	157/157	212/214	151/157	137/137	221/231	105/105	80/96	198/198	129/129	128/138	227/229	ND
6	1987/female	178/181	184/192	164/164	157/171	208/214	151/151	131/137	221/233	103/107	80/80	182/198	129/143	130/138	227/227	GB19*
7	1989/female	172/181	184/194	140/164	157/163	208/214	153/155	131/141	231/254	103/103	80/86	182/198	129/139	128/130	227/239	ND
8	1990/male	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	1990/female	172/181	180/184	148/160	155/155	214/214	151/155	137/137	221/233	103/105	80/80	198/198	129/129	122/138	227/227	GB19*
10	1984/female	176/178	198/200	152/158	155/155	210/212	151/153	137/141	221/221	105/107	86/90	182/198	129/143	130/138	239/247	GB19*
11	1992/female	172/184	184/192	140/152	157/171	208/208	151/151	131/137	221/221	107/111	80/80	198/204	129/143	110/138	227/227	GB19*
12	1992/male	181/184	184/194	140/152	155/157	208/208	151/151	137/141	221/221	107/111	80/80	198/204	143/143	130/130	227/227	GB19*
13	1979/male	178/181	184/194	158/164	151/155	214/214	155/157	135/137	221/233	105/105	80/96	182/192	129/143	130/130	227/239	GB19*
14	1981/male	172/178	194/196	140/160	155/155	214/218	159/161	137/137	221/233	103/111	86/96	182/204	141/143	128/134	227/227	GB14*
15	1992/female	172/176	196/198	140/152	155/155	210/218	153/159	137/141	221/233	105/111	90/96	182/204	143/143	128/138	227/239	GB19*
16	1992/female	178/178	194/198	140/158	155/155	210/214	151/159	137/137	221/221	103/105	86/96	182/204	143/143	130/134	227/239	GB19*
17	1993/female	172/174	194/194	160/164	155/163	206/214	153/155	131/137	221/254	103/103	80/86	198/198	139/139	110/128	227/227	GB14*
18	1993/female	172/174	184/194	160/160	155/163	206/214	155/155	137/141	231/254	103/105	80/86	198/198	129/139	130/130	227/227	GB14*
19	1980/female	172/181	184/194	140/160	163/163	214/214	153/155	137/141	221/254	103/105	80/86	182/198	129/139	110/130	227/239	GB14*
20	1973/male	181/184	192/194	140/164	157/171	208/208	151/151	131/137	221/221	107/107	80/80	198/204	143/143	130/138	227/227	GB19*
21	1976/female	172/178	184/198	152/160	155/155	206/212	151/153	141/141	221/221	105/111	86/90	198/198	129/129	110/130	227/247	GB19*
22	1990/male	178/184	192/194	140/140	155/155	208/208	155/157	133/137	221/229	103/105	80/80	182/200	129/143	132/138	227/227	GB14*
23	1992/female	181/184	192/192	160/164	157/171	208/214	151/153	137/137	221/221	103/103	80/80	182/198	129/143	130/138	227/227	GB19*
24	1993/male	172/174	184/192	148/164	157/157	206/214	151/153	131/137	221/233	103/103	96/96	198/198	129/129	128/138	227/227	GB19*
25	1993/female	178/181	194/194	148/160	155/157	206/214	151/153	131/137	221/233	105/111	96/96	198/198	129/129	128/138	227/227	GB19*
26	1986/male	172/174	192/194	140/158	155/155	210/214	151/153	137/141	221/231	105/105	80/96	182/204	127/127	130/134	223/247	GB19*
27	1993/female	178/181	194/194	148/164	157/157	206/214	151/153	137/137	221/231	103/103	96/96	182/198	129/139	130/138	227/229	GB19*
28	1982/female	180/182	190/194	140/158	157/159	208/214	149/151	137/141	221/231	105/105	80/80	182/198	135/135	110/130	227/227	GB10*
29	1974/female	178/181	184/194	150/160	155/157	208/210	153/157	135/135	221/252	107/111	90/96	192/192	123/143	128/130	227/227	GB14*
30	1993/male	172/178	184/198	160/160	155/155	208/212	153/153	137/141	221/221	105/109	86/86	198/198	129/133	110/122	247/247	GB19*
31	1984/male	172/180	180/184	140/160	155/155	208/210	151/153	137/137	221/229	109/111	80/86	182/198	129/133	110/122	227/247	GB10*
100	1990/male	178/181	192/194	164/164	155/157	206/208	151/153	131/137	221/221	103/103	80/96	182/198	129/129	128/138	227/227	GB19*
101	Unknown/unknown	178/178	186/194	140/160	155/157	206/208	157/157	135/137	221/257	103/103	78/96	182/192	129/143	130/138	227/227	GB14*
102	1978/female	178/184	192/194	160/164	155/155	206/208	155/159	137/141	221/231	105/105	78/80	198/198	129/143	134/138	227/227	GB19*
103	1991/male	172/172	192/194	140/140	155/157	206/210	151/157	131/141	231/257	107/111	80/80	198/198	127/129	126/138	227/247	GB19*
104	1992/female	172/181	180/194	140/164	157/171	212/214	157/159	133/137	221/237	105/107	80/86	198/198	141/143	128/138	227/229	GB19*
105	1990/unknown	181/186	184/192	160/160	155/159	214/218	161/161	135/141	221/231	105/105	80/96	182/204	127/135	130/130	227/239	GB10*
Hypothetical	Male	174/181	192/194	160/	157/	206/	153/	131/137	231/	103/111	96/	198/	129/139	128/130	227/	ND

[a] ND = not done.

this study and that of Craighead et al. (1995) identified many related bears, but by no means fully characterized the populations. Fathers for about half of the offspring sampled were not identified in these studies. We identified the fathers of 8 of 18 (0.44) offspring with known mothers and Craighead et al. (1995) identified the fathers of 36 of 57 (0.63) offspring with known mothers in the western Brooks Range. It will be difficult to completely characterize relationships of highly mobile species such as grizzly bears.

There is considerable microsatellite allelic variation and heterozygosity at the microsatellite loci we used in grizzly bears in the Prudhoe Bay region (A = 6.6, H = 70.2%; Table 1), the western Brooks Range (A = 7.6, H = 74.7%, for eight loci; Craighead et al. 1995), and in other mainland North American populations (mean A = 4.4–7.6, H = 55.3–78.8%; Paetkau et al. 1998b). Comparison of the allele frequencies for the eight microsatellite loci analyzed for both the western Brooks Range (Craighead et al. 1995) and the Prudhoe Bay bears (Table 1) indicates there are many shared alleles and a relatively small genetic distance (Nei 1978) of 0.167. The three mtDNA haplotypes we observed, and others defined by additional substitutions within the 450 nucleotides sequenced, were also observed in northern Alaska grizzly bears by Talbot and Shields (1996b). They identified five haplotypes (including GB10, GB14, and GB19) among 19 bears from the western Brooks Range, and four haplotypes (including GB10 and GB19) among 17 bears from the Arctic National Wildlife Refuge east of the Prudhoe Bay region (Figure 1). The combined microsatellite and mtDNA data suggest there is gene flow between bears in the Prudhoe Bay region and adjacent areas.

The overall relatedness of individuals in different populations can also be assessed by comparing r_{xy} values. A relatively high r_{xy} for an entire population could indicate a large proportion of related individuals and the potential for inbreeding to occur. This is not apparent for any of three areas in northern Alaska for which microsatellite data are available. We calculated the mean r_{xy} values for eight microsatellite loci studied in three different areas of arctic Alaska using data from this study and Craighead (1994). Within each area mean r_{xy} values were close to 0: in the Prudhoe Bay region mean r_{xy} (sample size/standard deviation) = −0.0017 (36/0.2434); in the Arctic National Wildlife Refuge mean r_{xy} = −0.0019 (15/0.1825); and in the western Brooks Range mean r_{xy} = −0.0002 (149/0.2020). The mean r_{xy} values of about 0 and the high standard deviations reflect the presence of related and unrelated individuals in these populations, which is consistent with the high mobility of bears.

The grizzly bear population in the Prudhoe Bay region is genetically diverse and apparently has gene flow with neighboring areas. However, our analysis indicates that there are many related bears in the region, and that few adults have contributed a large percentage of the offspring produced in this area. The population genetic structure of bears in the region does not appear to differ appreciably from those in other areas. These results indicate that long-term study of both demography and genetics is necessary to understand the relationships of long-lived species with low reproductive rates, such as grizzly bears.

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