Genetic Variation, Relatedness, and Effective Population Size of Polar Bears (*Ursus maritimus*) in the southern Beaufort Sea, Alaska

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Abstract

Polar bears (*Ursus maritimus*) are unique among bears in that they are adapted to the Arctic sea ice environment. Genetic data are useful for understanding their evolution and can contribute to management. We assessed parentage and relatedness of polar bears in the southern Beaufort Sea, Alaska, with genetic data and field observations of age, sex, and mother–offspring and sibling relationships. Genotypes at 14 microsatellite DNA loci for 226 bears indicate that genetic variation is comparable to other populations of polar bears with mean number of alleles per locus of 7.9 and observed and expected heterozygosity of 0.71. The genetic data verified 60 field-identified mother–offspring pairs and identified 10 additional mother–cub pairs and 48 father–offspring pairs. The entire sample of related and unrelated bears had a mean pairwise relatedness index (r_{xy}) of approximately zero, parent–offspring and siblings had r_{xy} of approximately 0.5, and 5.2% of the samples had r_{xy} values within the range expected for parent-offspring. Effective population size ($N_e = 277$) and the ratio of N_e to total population size ($N_e/N = 0.182$) were estimated from the numbers of reproducing males and females. N_e estimates with genetic methods gave variable results. Our results verify and expand field data on reproduction by females and provide new data on reproduction by males and estimates of relatedness and N_e in a polar bear population.

Key words: polar bear, microsatellite DNA, Alaska Beaufort Sea, Ursus maritimus, parentage, relatedness, effective population size-N_e

Polar bears (*Ursus maritimus*) occur in areas of the northern hemisphere that are covered by sea ice for extended periods every year and have interesting characteristics with regard to evolution and genetics. At the interspecific phylogeny level, polar bears have derived morphology and behavior that are apparent adaptations to living on sea ice and preying on marine mammals. Polar bears are thought to have evolved from ancestral brown bears (also called grizzly bears, *U. arctos*) 70,000–1,170,000 years ago (Kurtén 1964, 1988; Stanley 1979; Ingolfsson and Wiig 2008). A close relationship of polar bears and brown bears is reflected in small mitochondrial DNA (mtDNA) sequence divergence and paraphyletic mtDNA phylogeny (Cronin et al. 1991; Shields and Kocher 1991; Taberlet and Bouvet 1992; Talbot and Shields 1996a, 1996b; Waits et al. 1999; Calvignac et al. 2008), small genetic distances derived from protein electrophoresis (Wayne et al. 1991), similar morphology (Kurtén 1988), and the ability to hybridize (Davis 1950; Kowalska 1965). Microsatellite DNA allele frequencies are similar in polar bears and brown bears, although they are not reliable for quantifying relationships of these species (Paetkau et al. 1997).

At the intraspecific population genetic level, polar bears have low protein variation (Allendorf et al. 1979; Larson et al. 1983) and variation comparable to other bears for mtDNA and microsatellite DNA (Cronin et al. 1991; Paetkau et al. 1995, 1997, 1999; Crompton et al. 2008; Zeyl et al. 2009). Analyses of microsatellite DNA have shown little or no genetic subdivision between adjacent polar bear subpopulations and moderate levels of subdivision across broad regions of the Arctic (Paetkau et al. 1999; Cronin et al. 2006; Crompton et al. 2008; Zeyl et al. 2009). This pattern

probably results from high mobility and gene flow of polar bears in a sea ice environment that has few geographic barriers. Genetic data for polar bears in Canada (Lunn et al. 2000) and the Barents Sea (Zeyl et al. 2009) showed that relatedness indices (r_{xy}) derived from microsatellite DNA markers of approximately 0.5 for parent–offspring and full siblings and zero for nonrelatives and instances in which cubs were adopted by females who are not the mother (Lunn et al. 2000) and father–daughter mating (Zeyl et al. 2009).

There is extensive field data on reproduction in polar bears (reviewed by Amstrup 2003). Females are not sexually mature until 4–6 years of age, depending on geographic location, and they can reproduce beyond age 20. Breeding occurs during a prolonged estrus between March and June. Ovulation is induced by copulation, implantation of the embryo is delayed until autumn, and gestation is 195–265 days (including the preimplantation period). Pregnant female polar bears enter snow dens in the autumn and give birth to altricial cubs in mid winter. Litters of 1, 2, or rarely 3 cubs emerge from snow dens between March and May and remain with their mother until weaning at approximately age 2 years 4 months. Females remain anoestrus while lactating and may resume breeding after cubs are weaned or if they die. If a mother successfully raises a litter to weaning at age 2 years 4 months, she can reproduce at most every 3 years, although cubs may occasionally be weaned at age 1 year 4 months and a female could produce cubs every 2 years. However, reproductive intervals ≥ 3 years are more common than shorter intervals.

Studies of spermatogenesis suggest that male polar bears may be fertile by age 3 (Rosing-Asvid et al. 2002). However, observations of male polar bears with estrus females suggest that males may not be effective at breeding until age 5–8, and we have observed male bears in their mid-20s courting estrus females. Males do not accompany offspring so their reproduction cannot be quantified with field observations.

In this study, we build on the current knowledge of the reproductive biology of polar bears with assessment of intrapopulation genetics, relatedness, and parentage in the southern Beaufort Sea north of Alaska and western Canada. The bears in the southern Beaufort Sea are considered a subpopulation of the global polar bear population because they have fidelity to home ranges in the region, but there is also movement and gene flow with adjacent subpopulations (Cronin et al. 2006). Genetic data can contribute to a better understanding of polar bear biology, evolution, and management. There are potential changes in population dynamics of polar bears in general and specifically for the southern Beaufort Sea subpopulation because of habitat changes caused by climate change (Regehr et al. 2006, 2007). Field data have provided information on basic reproductive parameters as described above, but there is little known about the numbers and ages of males reproducing, the frequency of multiple paternity and cub adoption, and effective population size

(N_e). N_e is the number of individuals in an idealized population (random mating, discrete generations, no mutation, no migration, no selection; Wright 1931) that would have the same properties observed in the actual population and is often used as an estimate of the number of breeding individuals in a population. N_e is typically smaller than N (the population census size) because not all individuals in a population contribute equally to reproduction. N_e is therefore relevant to management of wild populations, and information on male reproduction can be important for its estimation (Harris and Allendorf 1989).

To obtain such information, we assessed genetic variation and relatedness with 14 microsatellite DNA loci in 226 polar bears including mother–cub pairs observed in the field in which the parent–offspring relationship is known and littermates are likely siblings and males with unknown relationships. Our objectives were to quantify interindividual relatedness, infer parentage and family relationships, and estimate N_e of polar bears in the southern Beaufort Sea. A novel aspect of our study is assessment of reproduction in males because field data have provided information on only female reproduction in the southern Beaufort Sea.

Materials and Methods

Sample Collection

We captured polar bears in the southern Beaufort Sea of Alaska during capture–recapture and movement studies conducted between 1981 and 2004 (Amstrup et al. 2001, 2004; Hunter et al. 2007; Regehr et al. 2007). Bears were captured randomly as encountered in the field (Regehr et al. 2006). All captured polar bears were tattooed on the upper lip for permanent identification. Ages of bears were determined by counting *cementum annuli* in excised vestigial premolar teeth (Calvert and Ramsay 1998). Blood was collected by venipuncture from immobilized animals. During the study period, 490 bears were captured and blood collected. For the genetic analysis, we randomly selected 170 males from this collection. Selection of females was not random, as we selected 56 females identified as mothers with cubs in the field. We sampled more males than females to increase the probability of paternity determination for the cubs with known mothers.

Microsatellite DNA Analysis

Genotypes were determined for the 226 bears for 14 microsatellite DNA loci with polymerase chain reaction (PCR) primers (Supplementary Table 1) developed previously: G1A, G10B, G1D, and G10L (Paetkau and Strobeck 1994); G10C and G10M (Paetkau et al. 1995); G10H, G10J, G10P, and G10X (Paetkau et al. 1997); Mu26, Mu50, and Mu59 (Paetkau et al. 1997; Taberlet et al. 1997); and C203 (Ostrander et al. 1993). PCR amplifications were carried out in 5 multiplex reactions, each in a final volume of

10 μl and contained 2–100 ng genomic DNA, 0.2 mM deoxynucleoside triphosphates, 3.6–4.0 pmol unlabeled primers, 0.06–0.4 pmoles IRD-labeled primer, 1.0 μg bovine serum albumin, 1× PCR buffer (Perkin Elmer Cetus I), and 0.3 units Amplitaq DNA polymerase (PE Biosystems, Forest City, CA). PCRs began with 94 °C for 2 min and continued with 40 cycles each of 94 °C for 15 s, 50 °C for 15 s, and 72 °C for 30 s. A 30 min extension at 72 °C concluded each reaction.

The fluorescently labeled PCR products were electrophoresed on a 48-well 6% polyacrylamide gel on an LI-COR 4200 LR or IR^2 DNA automated sequencer (LI-COR, Lincoln, NE). For allele size standardization for all loci (except C203), genotypes for 6 polar bears were compared with DNA standards of known size provided by D. Paetkau (Wildlife Genetics International, Nelson, British Columbia, Canada). Two individuals that were heterozygous at each of the 13 loci were included in gels as size standards. The sizes of alleles for locus C203 were determined by comparing genotypes for 1 of the 6 bears with an M13 sequence ladder. Using these standards, genotypes for each individual were determined using GeneImagIR 4.05 software (Scanalytics, Inc.).

For quality control to check for genotyping errors, 15% of the samples were extracted, amplified, and genotyped in duplicate. We also did a minimum of 3 replicate analyses of samples in which relationships determined with genetic data were inconsistent with field observations or where multiple paternity or adoption was possible. We used MICRO-CHECKER (Van Oosterhout et al. 2004) to identify genotyping errors. We used sterile technique in the handling of all DNA, and all PCR procedures were done with positive and negative controls to verify amplification without contamination.

Data Analysis

Genetic variation (mean number of alleles per locus [A] observed heterozygosity [H_o] and expected heterozygosity [H_e]) was quantified with the BIOSYS (Swofford and Selander 1981) and Microsatellite Toolkit (Park 2001) computer programs. Exact tests of Hardy–Weinberg equilibrium were calculated using GENEPOP Ver.3.3 (Raymond and Rousset 1995). F_{is}, an inbreeding coefficient and measure of heterozygote deficiency or excess (Weir and Cockerham 1984), was calculated, and analysis of linkage disequilibrium of the 14 microsatellite loci was done with FSTAT (Goudet 1995). These analyses were done with all samples and also with all samples except cubs with known or inferred parents. The cubs were omitted to better represent the entire population by minimizing the potential bias of related individuals.

Pairwise relatedness indices (r_{xy}; Queller and Goodknight 1989) between individual bears were calculated with KINGROUP (Konovalov et al. 2004), and potential parent–offspring relationships were determined with CERVUS (Marshall et al. 1998). Bears that shared at least one allele per locus with potential offspring and were alive and old enough to breed at the time of conception of the offspring were not excluded as parents. Some males were excluded if there was no paternal allele in the potential offspring considering a known mother's genotype. Parent breeding age was calculated as: Cub birth year − parent birth year −1 (i.e., cubs are conceived in the year prior to birth). Previous studies (Rosing-Asvid et al. 2002; Amstrup 2003) and our field observations indicate that female polar bears can begin breeding at 4 years old so we used that as the minimum age of breeding for females. We considered males ≥3 years old at breeding as potential fathers. Male polar bears may be physiologically fertile as young as 3 years old although full fertility and behavioral ability to breed may not occur until 5 or 6 years of age or later (Rosing-Asvid et al. 2002; Amstrup 2003). After we identified parents with genetic data, we calculated the mean age at breeding of females and males. We used one breeding age per litter (single cub or twins) so litters with 2 cubs that represent one breeding year were not counted twice.

To assess the likelihood of parentage, we calculated LOD scores (the sum of log likelihood ratios at each locus) for potential parent–offspring pairs with CERVUS. The potential parent–offspring pair with the highest LOD score includes the most likely parent. We calculated delta scores (the highest LOD score minus the second highest LOD score) and the 0.8 and 0.95 statistical confidence levels for the delta scores from simulations in CERVUS. LOD scores were calculated separately for potential fathers and potential mothers. For calculations involving potential father–offspring pairs with a known mother, we included the mother's genotypes in the analysis. Thus, we identified potential parent–offspring pairs with field observations and genetic data considering nonexclusion and likelihood.

We calculated the probability of exclusion of parentage for the population with CERVUS. This is the probability that 2 unrelated individuals drawn at random from the population would be expected to have alleles in common at every locus (Paetkau and Strobeck 1998). We also used CERVUS to calculate the probability of identity of individuals (the probability that 2 bears shared the same genotypes at all 14 loci; Paetkau et al. 1998) and the probability of identity of siblings which is a conservative estimator of the probability of identity of individuals (Waits et al. 2001).

To estimate the proportion of first-order relatives (i.e., parent–offspring, full siblings) in the sample, we determined the proportion of pairwise r_{xy} values within 2 standard deviations of the mean r_{xy} for the known and inferred parent–offspring pairs as in previous studies of bears (Cronin et al. 2005):

$$\text{Estimated } r_{xy} \text{ of first-order relatives}$$
$$= \text{parent–offspring mean } r_{xy} \pm 2\,\text{SD}.$$

We considered this spread of r_{xy} values likely to include all or most of r_{xy} values of potential parent–offspring and full siblings which have expected $r_{xy} = 0.5$

Table 1. Allele size ranges, numbers of alleles (A), expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{is}) of 226 polar bears from the southern Beaufort Sea for 14 microsatellite loci

Locus	Allele size range	A	H_e	H_o	F_{is}
C203	131-151	9	0.85	0.807	−0.053
G1A	190-200	6	0.735	0.707	−0.039
G10B	142-158	6	0.73	0.753	0.031
G10C	201-215	7	0.372	0.394	0.058
G1D	180-192	7	0.633	0.627	−0.009
G10H	225-249	11	0.841	0.834	−0.009
G10J	186-192	4	0.593	0.581	−0.021
G10L	145-151	4	0.398	0.405	0.017
G10M	200-218	8	0.761	0.775	0.019
G10P	145-161	9	0.704	0.713	0.014
G10X	133-147	8	0.81	0.796	−0.017
Mμ26	188-208	9	0.819	0.812	−0.008
Mμ50	122-138	9	0.814	0.823	0.011
Mμ59	225-253	13	0.85	0.851	0.002
14-Locus totals		7.9	0.706	0.708	−0.003

(Blouin et al. 1996). We emphasize that this is a rough estimate of first-order relatedness because parent–offspring and full siblings could have r_{xy} values outside this range and unrelated pairs could have r_{xy} values within this range.

Estimation of Effective Population Size N_e

We estimated N_e of the southern Beaufort Sea subpopulation of polar bears considering the numbers of males and females in the subpopulation that reproduced as estimated with our genetic data and field data. We first estimated the proportions of males and females in the population that reproduced during the study period. Because males were sampled randomly, we used our genetic data to estimate the proportion of males reproducing for the study period as: Proportion of males reproducing = (number of fathers identified)/(number of males sampled). We could not estimate the proportion of females reproducing from the genetic data because our sample was biased toward known mother–offspring pairs. However, field data indicate that 0.15–0.22 (mean = 0.183) of the females have cubs over a study period similar to ours (17–22 years; Regehr et al. 2006).

Next we estimated the numbers of males (N_m) and females (N_f) reproducing in the subpopulation. Analyses of comprehensive capture–recapture field data indicate a total subpopulation estimate (N) of 1,526 bears in 2006, which was not statistically different from estimates from the 1980s (Regehr et al. 2006). The sex ratio in the southern Beaufort Sea polar bear subpopulation is 0.54 females and 0.46 males (Regehr et al. 2006), suggesting a total of 702 males (i.e., 1,526 × 0.46) and 824 females (i.e., 1,526 × 0.54) in the subpopulation. We then calculated

$$N_m = \text{Number of males in the subpopulation}$$
$$\times \text{ proportion of males reproducing}$$

$$N_f = \text{Number of females in the subpopulation}$$
$$\times \text{ proportion of females reproducing}$$

We then estimated N_e with the equation:

$$N_e = (4N_m \times N_f)/(N_m + N_f) \text{ (Wright 1931)}.$$

We also used 3 genetic methods to estimate N_e. One method considers linkage disequilibrium, the nonrandom association of alleles at different loci, to estimate N_e (LDNE; Waples and Do 2007). In this analysis, we used only alleles with frequency ≥0.05. We did separate analyses for all our samples and for parent–offspring pairs including only one cub for litters with twins.

Another genetic method (previously applied to brown bears; Harris and Allendorf 1989) considers the change in expected heterozygosity (H_e) between parents and offspring with the equation:

$$H_{et} = H_{e0}[1 - 1/(2N_e)]^t \text{ (Wright 1969)},$$

where H_{e0} = estimated heterozygosity at generation 0 and H_{et} = estimated heterozygosity at generation t. We used the values for parents from our analysis for H_{e0} and values for cubs from our analysis for H_{et}.

A model in which N_e is estimated considering H_e and mutation rate (μ) was also used. This model was used by Paertkau et al. (1998) on brown bears and employs a stepwise mutation model where:

$$H_e = 1 - [1/(1 + 8N_e\mu)^{1/2}] \text{ (Ohta and Kimura 1973)}.$$

We considered a range of $\mu = 0.001$–0.0002 as in previous analyses of microsatellite loci in bears (Paetkau et al. 1998; Miller and Waits 2003).

Results

Genetic Variation

Among the 14 microsatellite loci analyzed in 226 polar bears, there were 4–13 alleles per locus with a mean number of alleles (A) of 7.9 (Table 1; Supplementary Table 2). No bears had identical genotypes at all 14 loci, the probability of identity of individuals was 2.4×10^{-14}, and the probability of identity of siblings was 5.9×10^{-6}. The observed heterozygosity (H_o) ranged from 0.394 to 0.851 across the 14 loci, with mean $H_o = 0.708$ and mean $H_e = 0.706$ (Table 1). All loci had genotypes in Hardy–Weinberg equilibrium for all samples ($P > 0.1363$) and for samples excluding cubs with known and inferred parents ($P > 0.1716$). None of the loci showed significant (adjusted for 92 pairwise locus comparisons, $P = 0.0005$) linkage disequilibrium for all samples ($P > 0.002$) and samples excluding cubs ($P > 0.003$), consistent with previous assessments of these loci (Paetkau et al. 1995, 1997, 1998; Waits et al. 2000). F_{is} values were low for all samples (−0.053 to 0.058, mean = −0.003;

Table 2. Summary data for mean breeding age and mean and range r_{xy} values for polar bear parent-offspring pairs and sibling pairs

Parent type	Number of parent offspring pairs and sibling pairs	Mean (SD) breeding age	Mean (SD) r_{xy} with offspring	Range of r_{xy} with offspring
Known mother-offspring pairs from field data	60	9.44 (3.53)	0.5026 (0.1030)	0.3027-0.7383
Mother-offspring pairs identified with genetic data	10	10.9 (4.91)	0.4614 (0.1524)	0.1930-0.7374
All Mother-offspring pairs	70	9.73 (3.84)	0.4967 (0.1109)	0.1930-0.7383
Fathers-offspring pairs identified with genetic data	48	12.71 (6.33)	0.4616 (0.1115)	0.2066-0.6306
All parent offspring pairs	118	11.11 (5.33)	0.4824 (0.1120)	0.1930-0.7383
Sibling pairs	21	N/A	0.4817 (0.1768)	0.1267-0.8080

Table 1) and samples excluding cubs (F_{is} = −0.073 to 0.052, mean = 0.009). Quality control duplicate samples indicated a scoring error rate of 0.003, and analysis of the genotype data with MICROCHECKER showed no evidence of stuttering, allele dropout, or null alleles.

Genetic Relationships and Reproduction

The probability of exclusion of parentage with neither parent known was 0.9979 and with one parent known it was 0.9999. This means that pairs that are not parent–offspring will share 1 allele/locus <1% of the time. The mean relatedness for the entire sample of 226 bears (25,425 pairwise r_{xy} values) was close to zero (mean r_{xy} = −0.0045, SD = 0.1577). The mean relatedness of 118 known and inferred parent–offspring pairs (mothers and fathers) was r_{xy} = 0.4824 (SD = 0.1120; Table 2). The r_{xy} of first-order relatives was estimated as:

$$\text{Parent–offspring mean } r_{xy} \pm 2\,\text{SD}$$
$$= 0.4824 \pm 0.224 = 0.2584 - 0.7064.$$

Of the 25,425 pairwise r_{xy} values, 1,313 (5.2%) were within this interval and inferred first-order relatives. Of the remaining 94.8%, 5 (0.02%) values exceeded (r_{xy} > 0.7064) and 24,107 (94.8%) values were less than (r_{xy} < 0.2584) the interval of first-order relatives.

Mothers

We identified 32 females as known mothers in 60 mother–cub pairs (including 18 single cubs and 21 pairs of twins) captured in the field (Supplementary Table 3). These mothers were 4–17 years old at breeding (mean 9.44 years old; Table 2). The genetic data showed that all these pairs were not excluded as parent–offspring, with r_{xy} values from 0.303 to 0.738 and a mean r_{xy} = 0.503 (Table 2). Fathers were identified for 14 of these mother–offspring pairs (Table 3). An additional 10 female–cub pairs were not excluded as mother–offspring with genetic data only (Supplementary Table 4). These putative mothers were also between 4 and 17 years of age at breeding and were known mothers of other cubs. The relatedness of these inferred mother–offspring pairs ranged from r_{xy} = 0.193 to 0.737

with a mean r_{xy} = 0.4614. For all 70 known and inferred mother–offspring pairs, the mean r_{xy} = 0.4967 (SD = 0.1109). The mean age of the known and inferred breeding females was 9.73 years (SD = 3.84), and the median age was 9.0 years (Table 2).

Of the 70 known and inferred mother–offspring pairs, 64 had delta values at the 0.95 confidence level, 2 at the 0.80–0.95 confidence level, and 4 < 0.80 confidence level. The LOD scores of the mother–offspring pairs identified were all positive, indicating high likelihood of correct parentage assignment (Supplementary Tables 3, 4). Eight additional female–cub pairs were not excluded as mother–offspring but had lower LOD scores than the known mother. Three female–cub pairs not genetically excluded as mother–offspring pairs were excluded because the mother was too young (<4 years) to breed including two 3-year-old females and one 1-year-old female (Supplementary Table 5).

Of the 56 females sampled, 32 were mothers (0.5714), but this does not reflect the subpopulation as a whole because we preferentially sampled known mothers with cubs. For the 32 mothers, the total numbers of offspring over all years included 7 females with 1 offspring, 17 females with 2 offspring, 4 females with 3 offspring, 3 females with 4 offspring, and 1 female with 5 offspring. Of these 32 females producing litters over the study period, 17 had 1 litter (0.53), 13 had 2 litters (0.40), and 2 had 3 litters (0.06). In all cases in which females had litters in different years and fathers identified, there were different fathers for each litter. The field and genetic data identified 3 females that had cubs ≤2 years after a previous litter (i.e., they bred when the first cubs were 6 months old or 1 year 6 months years old).

Fathers

Thirty-one of the 170 males sampled (0.1824) were not excluded as possible fathers, comprising 48 putative father–offspring pairs. This included 9 males identified in 14 possible father–cub pairs with known mothers, so both parents are identified in these cases (Table 3). These 14 pairs included 4 single cubs and 5 sets of twins. No fathers were identified for 46 cubs with known mothers, and 23 males were identified in 34 possible father–cub pairs without known mothers (Supplementary Table 4). Relatedness

Table 3. Breeding age and relatedness data for polar bear parent-offspring pairs with known mothers and non-excluded fathers. Additional parent-offspring pairs are in the supplementary material

Mother[a]	Breeding Age[b]	r_{xy} with offspring	LOD[c]	Delta[c]	Offspring[a]	Birth year	Sex	Father[a]	Breeding Age[b]	r_{xy} with offspring	LOD[c]	Delta[c]
6155	4	0.4843	6.49	6.49	6154	1980	F	6179	15	0.6104	7.91	7.91
6837	6	0.6798	14.1	14.1	6838	1989	F	6250	10	0.4141	10.6	10.6
6700	11	0.5448	11.6	4.63	20122	2001	M	20609	13	0.4235	4.24	4.24
20331	7	0.3027	4.51	4.51	20586	2000	F	9970	21	0.5928	9.98	9.98
20544	12	0.4875	11.3	11.3	20152[d]	2002	F	6681	14	0.4279	9.69	9.69
20544	12	0.4794	9.21	9.21	20153[d]	2002	M	6681	14	0.4245	9.72	9.72
20544	14	0.4644	8.03	8.03	20701[d]	2004	M	20626	Adult[e]	0.5932	14.1	14.1
20544	14	0.5572	8.44	8.44	20702[d]	2004	F	20626	Adult[e]	0.4512	8.91	8.91
20330	9	0.5821	11.1	11.1	20150[d]	2002	M	20636	8	0.4996	7.14	3.36
20330	9	0.4169	7.85	7.85	20151[d]	2002	M	20636	8	0.6108	10.5	10.5
20452	13	0.4552	6.9	6.9	20589[d]	2000	M	20453	17	0.5921	12.4	7.96
20452	13	0.5139	8.44	8.44	20590[d]	2000	M	20453	17	0.5099	9.58	9.58
20472	9	0.5118	7.36	7.36	20505[d]	2000	M	20484	11	0.3951	5.56	5.56
20472	9	0.6146	13.8	13.8	20506[d]	2000	M	20484	11	0.4775	9.76	9.76

[a] Individual bear sample identification number.

[b] Parent breeding age = cub birth year – parent birth year -1 (i.e., cubs are conceived in the year prior to birth).

[c] Different LOD and delta values for a pair indicate there is another potential parent with a lower LOD value.

[d] Same mother and father for sibling pair.

[e] Father's age unknown but was adult.

indices for the 48 potential father–offspring pairs were r_{xy} = 0.2066–0.6306 with a mean r_{xy} = 0.4616 (SD = 0.1115; Table 2). The age of the putative breeding males ranged from 3 to 27 years, with a mean age of 12.71 years (SD = 6.33) and a median age of 12.5 years. This included two 3-year-old and two 4-year-old males identified as fathers. The total numbers of potential offspring for the 31 fathers over all years included 21 males with 1 offspring, 7 males with 2 offspring, 1 male with 3 offspring, and 2 males with 5 offspring. The total numbers of litters for the 31 fathers included 26 males with 1 litter (0.84), 2 males with 2 litters (0.06), 1 male with 3 litters (0.03), and 2 males with 5 litters (0.06).

The LOD scores of 47 of the 48 father–offspring pairs were positive with delta values at the 0.95 confidence level, indicating high likelihood of correct parentage assignment. Forty-seven additional males were identified as potential fathers but excluded because of lower LOD scores than another male, they were dead or <3 years old at the time of conception of the cub, or there was no paternal allele in the offspring considering a known mother's genotypes.

Siblings

Twenty-six pairs of twins in the same litter (i.e., presumed siblings) were identified with their mothers in field observations. We obtained genetic data for 21 of these pairs (Supplementary Table 6). Relatedness of these sibling pairs ranged from r_{xy} = 0.1267 to 0.8080 with a mean r_{xy} = 0.4817 (SD = 0.1768; Table 2). In each case, the mothers were verified with genetic data as the parent of both siblings. Fathers were identified for 5 pairs of siblings (Table 3). We observed 3 pairs of siblings with no father identified and low

r_{xy} values (e.g., r_{xy} < 0.25). These could be half siblings resulting from multiple paternity within a litter, but we cannot verify this as no fathers were identified.

Two bears born the same year were identified as offspring of the same mother and presumed siblings, although only one of them was identified with the mother in the field. The r_{xy} of the cubs was 0.5 as expected for full siblings, and no father was identified for either cub. The genetic identification of siblings without concordant field observations could mean that one of the cubs was present but not captured, the age of one cub was incorrectly determined, or they are not siblings but otherwise related.

Effective Population Size

For the estimation of N_e considering the numbers of reproducing males and females, we calculated the proportion of males reproducing as:

31 fathers identified/170 males sampled = 0.182.

We used this proportion of males reproducing, the proportion of females reproducing (0.183; Regehr et al. 2006), and the number of males (702) and females (824) in the subpopulation (see methods) during the study period to calculate:

$$N_m = 702 \times 0.182 = 128$$

$$N_f = 824 \times 0.183 = 151$$

We estimated N_e as:

$$N_e = 4 \times (128 \times 151)/(128 + 151) = 277.$$

This translates to an N_e/N ratio of 0.182 (277/1,526) or N_e = 18% of the population (Table 4).

Table 4. Effective population size (N_e) and N_e/N estimates for the polar bear subpopulation in the southern Beaufort Sea.

N_e Method	Reference	N_e	N_e/N
Estimated numbers of reproducing males and females	(Wright 1931)	277	0.1822
Linkage disequilibrium	Waples and Do (2007)		
All samples		183	0.1199
Parents and one offspring		225	0.1474
Change in heterozygosity-parents to offspring	Wright (1969)	111	0.0727
Stepwise mutation model	Ohta and Kimura (1973)		
$\mu = 0.001$		1,321	0.8657
$\mu = 0.0002$		6,608	4.3303

Two of the genetic methods resulted in estimates comparable to the estimate using the numbers of reproducing males and females. The analysis considering linkage disequilibrium LDNE resulted in $N_e = 183$ (95% CI = 150–230; Table 4) for all samples and $N_e = 225$ (95% CI = 177–300) for only parent–offspring.

The analysis considering change in H_e was calculated as

$$0.6922 = 0.6985[1 - (1/2N_e)]^2,$$ resulting in $N_e = 111$.

In contrast, the analysis with the stepwise mutation model resulted in a much higher estimate:

$$0.706 = 1 - [1/(1 + 8N_e\mu)^{1/2}],$$

where $N_e = 1321$ with $\mu = 0.001$ and $N_e = 6608$ with $\mu = 0.0002$ (Table 4).

Our data indicate a generation time of approximately 10 years for polar bears in the southern Beaufort Sea, as the average ages of reproducing females = 9.7 years and reproducing males = 12.7 years (Table 2), so our study period of 23 years comprised about 2 generations.

Discussion

Genetic Variation

Our data indicate that polar bears in the southern Beaufort Sea are a subpopulation with mean relatedness of individuals approximately zero and mean relatedness of first-order relatives approximately 0.5. Values of $r_{xy} = 0.5$ for parent–offspring and full siblings and zero for nonrelatives have also been reported for polar bears in other areas (Lunn et al. 2000; Zeyl et al. 2009). Approximately 5.2% of the polar bears sampled have r_{xy} values within 2 SDs of the mean r_{xy} of parent–offspring. Grizzly bears on the adjacent mainland have a similar proportion (5.3%) of r_{xy} values within 2 SDs of the mean parent–offspring r_{xy} estimated as we did here (Cronin et al. 2005). This may reflect a pattern of relatedness common to bears in relatively large subpopulations that have gene flow with other subpopulations.

Measures of genetic variation ($H_e = 0.706$, A = 7.9) in the southern Beaufort Sea are comparable to those of other subpopulations around the Arctic. Paetkau et al. (1999) reported $H_e = 0.68$ and A = 6.5 for 16 subpopulations of polar bears (mean sample size = 30) with 16 microsatellite loci including 12 of the loci used in this study. The higher A in our study may be due to detection of rare alleles in the southern Beaufort Sea with our larger sample size. Other studies of polar bears with larger sample sizes (N = 377–583) also found measures of genetic variation comparable to ours (A = 7.7–8.0, $H_e = 0.62–0.69$; Crompton et al. 2008; Zeyl et al. 2009).

Genetic Relationships and Reproduction

Our genetic data verified the relationships of 60 mother–offspring pairs and 21 sibling pairs identified in the field. Of the 60 cubs with known mothers, 14 (23%) had fathers identified and 46 (77%) did not have fathers identified. This indicates that our sampling included a limited proportion of the breeding males in the subpopulation, as expected for a nonexhaustive sampling effort. Additional mother–offspring and father–offspring pairs were inferred from genetic data only. It is important to note that some bears not excluded as parent–offspring with genetic data (i.e., shared one allele per locus) were excluded with field data (e.g., age, death, known mothers' genotypes) or likelihood (i.e., lower LOD score). Despite a high probability of exclusion (0.99), we had 58 cases in which a pair sharing one allele per locus was excluded as parent–offspring with field data. These pairs had r_{xy} values between 0.17 and 0.68 and are probably related in some way other than parent–offspring.

The genetic data indicate females in this population can breed at 4 years old and males breed as young as 3 or 4 years old. However, note that 2 females that would have bred as 3-year-olds were not excluded as mothers. One of these female–cub pairs had an r_{xy} (0.36) within the range of known mother–cub pairs and within the range of our estimate for first-order relatives, so it is possible that females may also breed at 3 years. We also noted females that had cubs ≤2 years after a previous litter. Because females usually breed ≥2 years 6 months after giving birth if accompanied by cubs, this may indicate the mother lost the first cubs to mortality, early weaning and dispersal, or adoption by another mother.

We saw no direct evidence of >1 male siring cubs in a litter (i.e., multiple paternity), but we cannot rule out the possibility that twins with relatively low r_{xy} values and no father identified are half-siblings resulting from different fathers of one litter. Multiple paternity was reported in grizzly bears (Craighead et al. 1995) but subsequently found to be undocumented (Anonymous 2006). Other cases of bears with multiple paternity have been reported (Schenk and Kovaks 1995; Onorato et al. 2004; Bellemain et al. 2006). We also did not see evidence of cub adoption, which has been observed in polar bears (Lunn et al. 2000). All the known mother–cub pairs had r_{xy} values close to 0.5 (>0.30) as expected for true parent–offspring.

Our data support a generation time of approximately 10 years for polar bears, as estimated by Aars et al. (2006:30) for polar bears and Miller and Waits (2003) for grizzly bears. Other analyses used a generation time of 15 years for polar bears based on age of maturity and length of lifetime reproductive period (Aars et al. 2006:31; Department of the Interior 2007:1070).

Effective Population Size-N_e

Our estimate of N_e using numbers of reproducing males and females (277) and the LDNE and heterozygosity methods (111–225) are comparable in magnitude. Much higher estimates (1321–6608) resulted from the stepwise mutation method. Different methods for calculating N_e can give considerably different results and apply to different timescales (Wang 2005; Waples 2005; Engen et al. 2007), as indicated by our varying estimates for the same subpopulation. The method we applied considering the numbers of reproducing males and females and the model considering change in H_e between parents and offspring reflect N_e over the short timescale of one or a few generations. The linkage disequilibrium model reflects N_e for short to intermediate timescales, and the mutation model reflects long timescales on the order of N_e generations (Wang 2005). The high estimate with the mutation model may reflect gene flow among subpopulations over many generations, which has been reported between the southern Beaufort Sea and other areas (Cronin et al. 1991, 2006; Paetkau et al. 1999; Amstrup et al. 2000, 2004). Migration strongly affects N_e, which will be higher with higher gene flow rates (Nei and Tajima 1981).

A related estimate of N_em (m = migration rate) for polar bears was made considering microsatellite allele frequencies among populations (Paetkau et al. 1999). In this case, N_em was estimated with F_{st} and private allele methods but were considered inaccurate because of discordant results. We do not have good estimates of gene flow rates of polar bears, so calculation of N_e from N_em is problematic and the relationship of F_{st} to N_em is not certain (Whitlock and McCauley 1999). Temporal changes in genetic variation over many generations can also be used to estimate N_e (e.g., Miller and Waits 2003), but we lack such data for polar bears.

N_e estimates depend on factors that are difficult to quantify and vary spatially and temporally in polar bears, such as the proportions of reproducing males and females, census numbers, gene flow rate, and sex ratio (Harris and Allendorf 1989). Other factors (e.g., overlapping generations) also affect estimates of N_e (Waples and Yokota 2007), although the method of Wright (1931) that we used considering numbers of reproducing males and females is valid for populations with overlapping generations and similar demographics of males and females (Engen et al. 2007). This may be the case for polar bears but requires further analysis of demographics of each sex.

Our estimates of N_e/N (other than the mutation model, Table 4) are within the wide range of estimates in natural populations (e.g., 0.11; Frankham 1995; 0.25–0.75 Engen 2007). We can apply the N_e/N ratios derived above to estimate N_e for the entire world population of polar bears. The number of polar bears worldwide is about 20,000–25,000 animals (Aars et al. 2006). Applying our N_e/N ratio for the southern Beaufort Sea of 0.182 gives N_e = 3640–4550 for the entire species. The similar magnitude of this estimate and that derived with the stepwise mutation model (N_e = 1321–6608) for the southern Beaufort Sea subpopulation may reflect considerable gene flow over many generations across the species range. However, these estimates should be used cautiously because they can vary considerably with the method used and over geography and time. For example, N_e/N can be estimated considering only generation times for each sex (eq. 13 of Engen et al 2007). For our data with average ages of breeding females and males of 9.73 and 12.71, respectively (Table 2), this method results in N_e/N = 0.534.

Potential Utility of Genetic Data for Polar Bears

Polar bear population dynamics have been modeled because of predicted habitat changes in sea ice due to climate change (Hunter et al. 2007; Regehr et al. 2007). Our results may provide information useful to such efforts. For example, population models use reproductive rates of different age classes, and field data suggest females begin breeding at 4 or 5 years of age (Hunter et al. 2007; Regehr et al. 2007). Our results suggest that both sexes can reproduce at 4 years, and maybe at 3 years old, and could contribute substantially to production of offspring if cub survival rates are similar for younger and older mothers. For example, of the 49 litters with mothers identified, 6 (12.2%) were produced by 4-year-old females and 3 of these litters were twins. Of the 43 litters with fathers identified, 4 (9.3%) were sired by 3- and 4-year-old males and 7 (16.3%) by 3- to 7-year-old males. Because breeding at younger ages could mean assessments based on previously assumed breeding rates are biased, comparison of reproduction and survival of cubs of younger mothers is a topic for future research. Estimates of the frequency of females breeding at intervals <2 years may contribute to estimates of cub mortality, early weaning, and dispersal. Reproduction by males has not been included in polar bear population models, and our data for male reproduction may provide novel insights for population models and harvest management. Effective population size and relatedness of individuals in a population are also important parameters in population genetics and its application to management and conservation (e.g., Miller and Waits 2003). Our estimates of N_e/N with methods relevant to one or a few generations suggest that about 7–18% of the southern Beaufort Sea subpopulation is breeding each generation. The estimate of 5.2% of the pairwise r_{xy} values within the range expected between parent–offspring for our study period may be useful as polar bear populations are monitored for temporal and spatial genetic patterns.

Supplementary Material

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