

# USE OF MICROSATELLITE DNA ANALYSES TO INFER BREEDING BEHAVIOR AND DEMOGRAPHIC PROCESSES IN AN ARCTIC GRIZZLY BEAR POPULATION

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**Abstract:** Analyses of microsatellite DNA, combined with behavioral observations, indicated that female grizzly bears (*Ursus arctos*) in the Arctic have a large male gene pool from which to choose. Males from a large surrounding area bred successfully with the females in our study area and competed with males who centered most of their activities in the study area. Observations of breeding activity did not reliably indicate paternity, particularly under conditions where constant monitoring was not possible. Since females tend to be strongly philopatric, male behavior (influenced to some degree by female choice) is thus the primary mechanism for maintaining genetic diversity in brown or grizzly bear populations. In isolated populations with no influx of male genes from neighboring areas, genetic diversity should be correspondingly lower.

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A grizzly bear population in the Western Brooks Range of Alaska (Fig. 1) was studied for 17 years using conventional ecological and demographic techniques. Observations of breeding behavior and animal movements raised questions of paternity, relatedness, and genetic diversity that we sought to answer using DNA fingerprinting techniques. Microsatellites are short, tandem repeat units (1–5 base pairs) of genomic DNA (Beckman and Weber 1992) that can be amplified as single loci using polymerase chain reaction (PCR) techniques to reveal genotypes of an individual. Microsatellite fingerprinting techniques can provide definitive answers to these questions and illuminate other aspects of breeding behavior and effective population size that were previously unclear.

The variation observed at these noncoding microsatellite repeat loci should be selectively neutral. Assumption of neutral gene theory is necessary to allow standard population genetic analyses to be made with the population. If alleles are selectively neutral, variations in allele frequency between populations or between generations should be caused only by the breeding structure of the species, which in turn is related to migration rates and local population sizes (Lewontin and Krakauer 1973, Slatkin 1982). Breeding structure should affect all alleles and loci in the same way.

## METHODS

Paetkau and Strobeck (1994) and Paetkau et al. (1995) identified tandem repeat microsatellite sequences from

genomic DNA of black bears (*Ursus americanus*). They developed single-locus primer sets which also amplified polar bear (*Ursus maritimus*) and brown or grizzly bear DNA loci. For this study, 8 loci, termed G1A, G1D, G10B, G10C, G10L, G10M, G10P, and G10X (Paetkau et al. 1995), were amplified from 152 grizzly bears in an Alaska Department of Fish and Game study population in the Western Brooks Range (WBR). Primer sets were labeled with FAM or HEX fluorescent dye. Genomic DNA was amplified using a Perkin–Elmer 9600 thermal cycler (Perkin–Elmer Corporation, 761 Main Avenue, Norwalk, CT 06859-0001). PCR products were analyzed (Craighead et al. 1995) using an Applied BioSystems 373A automated sequencer (Perkin–Elmer, Applied BioSystems, 850 Lincoln Center Drive, Foster City, CA 94404). Data were collected and analyzed on Apple Macintosh computers using GeneScan software (Perkin–Elmer, Applied BioSystems, 850 Lincoln Center Drive, Foster City, CA 94404).

Within the Western Brooks Range population, expected heterozygosity ( $\hat{H}$ ) was calculated for each genotype as  $1 - p_i^2$  at each locus, where  $p_i$  equals the frequency of the  $i$ th allele at that locus. Average  $\hat{H}$  was calculated as  $1 - \sum p_i^2/8$  because 8 loci were studied. Observed heterozygosity ( $H$ ) was calculated by enumerating all heterozygotes and homozygotes at each locus from the database. Chi-square values ( $\sum (H - \hat{H})^2/\hat{H}$ ) were calculated to determine if there was a deficiency of heterozygotes (or excess of homozygotes, i.e., a Wahlund effect) in the population as evi-

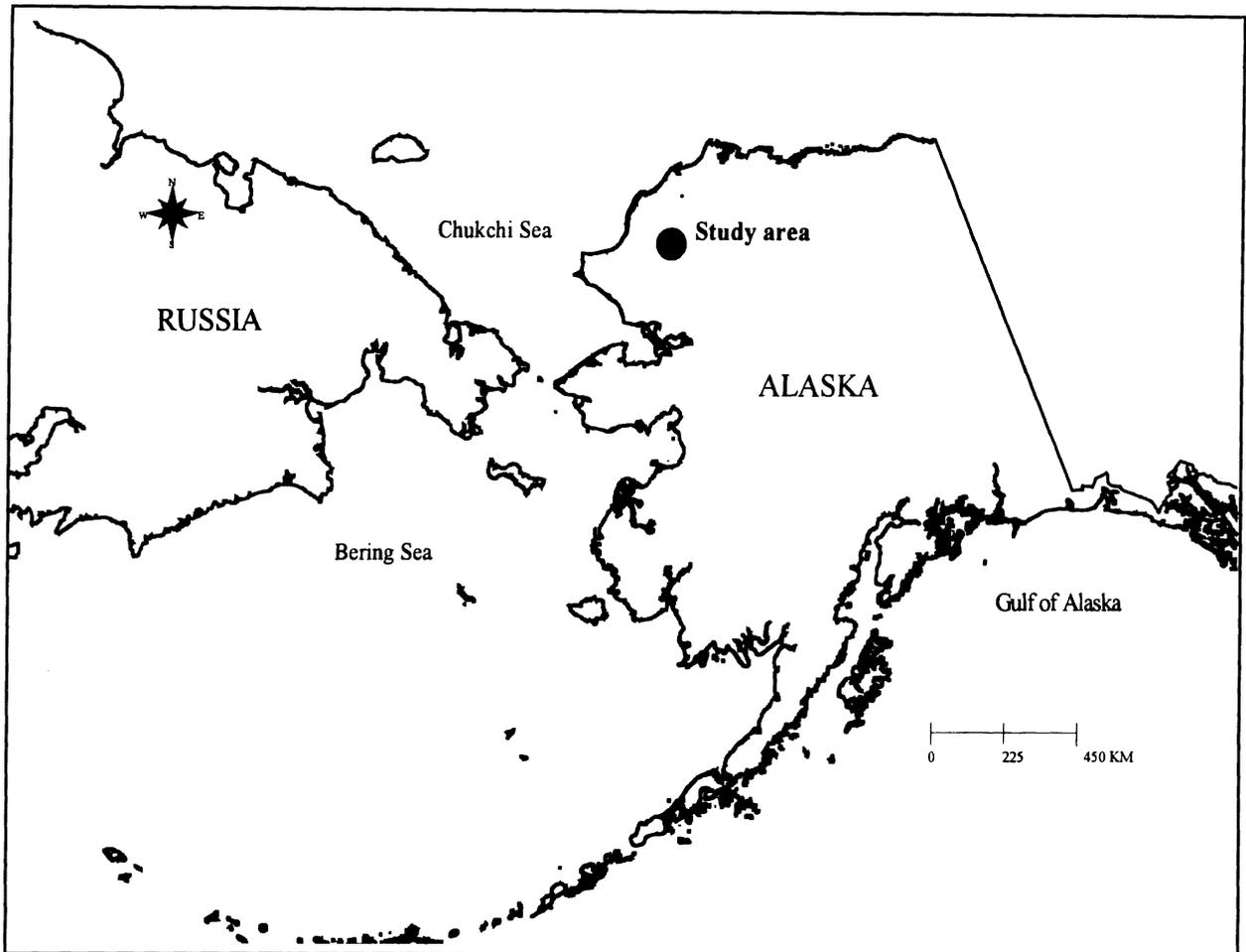


Fig. 1. Study area on the north side of the western Brook Range, Alaska.

dence of local genetic structure (Craighead 1994, Craighead and Vyse 1996).

## RESULTS

The specificity of the primer sets, short length of the PCR products, and high degree of resolution (1 base pair) of the sequencer allowed each individual bear to be identified by a unique combination of alleles and provided evidence for paternity (Craighead et al. 1995). A total of 2,432 alleles were enumerated in the WBR population. The alleles segregated in a Mendelian fashion with no evidence of null alleles (Craighead et al. 1995). A mutation rate of about  $2 \times 10^{-3}$  was estimated from a mismatch between genotypes of a known mother and cub (Craighead et al. 1995) and from the effective number of alleles (Craighead 1994). Despite our relatively small sample size, this estimate is close to independent estimates of

microsatellite mutation rates between  $1 \times 10^{-3}$  and  $2 \times 10^{-4}$  per generation (Amos et al. 1996, Paetkau et al. 1997a). This rate was not high enough to confuse the analysis.

We examined the frequency of genotypes observed in our population versus those predicted by allele frequencies at each locus (Craighead 1994) and determined using a chi-square test that they exhibited Hardy-Weinberg equilibrium at all loci except locus G10L, where there was an excess of heterozygotes of one genotype, G10L159/G10L161. Eleven bears exhibited this genotype one of which was the mother (1745) of a single offspring (1747). This sole discrepancy is probably due to chance, since a departure from expected frequencies due to population genetic structure should cause the reverse condition (an excess of homozygotes) to be observed. There was no significant difference between observed and expected number of homozygotes, and thus no evi-

dence of population subdivision or genetic structure at this scale.

### Paternity Analysis

We demonstrated that each cub in a litter can be sired independently, and we estimated maximum reproductive success for males. Of 57 offspring with known mothers we established paternity for 36; 21 others were sired by males that had not been sampled in the study area (Craighead 1994, Craighead et al. 1995). The probability of paternal exclusion ranged from  $1 \times 10^{-5}$  (1 offspring: 1702) to  $4 \times 10^{-9}$  (3 offspring: 1708, 1709, 1710; Craighead et al. 1995).

Essentially all adult females living in our study area were sampled. Unmarked males were occasionally observed, and these were captured and sampled whenever possible. Because of the large size of the study area (5,200 km<sup>2</sup>) and the extensive movements of males, our sampling undoubtedly missed several males. An analysis of the paternal alleles (alleles contributed by the sire) of the 21 offspring with unidentified fathers indicated that a minimum of 7 other males were successful breeders (Craighead et al. 1995). Unsampled breeding males probably used the area as a small part of their home range, and the genetic evidence of their successful breeding provides our only clue to their presence and numbers.

In general, a male tends to remain with the same female for her entire estrous period; the same pair can reliably be found in the same location day after day. Occasionally a male is displaced by another male who then remains with the female, and in some instances 2 or more males will remain near the female; tolerating each other's presence. As far as our limited observations and genetic data indicate, males in groups like this are not necessarily related.

In several cases, females were observed breeding with more than 1 known male during their estrous period, yet offspring born the next year had been sired by yet another unsampled male. For example, a female (1440) was seen consorting with a known male (1459) in 1988. In 1989 she gave birth to a single cub (1707). No known males were found to share all 8 paternal alleles with her offspring; in fact, all males differed by 2 alleles or more.

Another female (1454) was seen consorting with a known male (1453) in 1988 and gave birth to 3 cubs in 1989. They were captured with her as 2-year-olds in 1991 (1498, 1499, and 1500). The mother and her 3 offspring were the only bears sampled with the rare allele G10L173. All sampled adult males, including the sampled consorting male, differed by at least 2 paternal alleles.

A third female (1425) was seen consorting with known males in previous years, but she was not observed in 1988. She gave birth to 3 cubs in 1989 (1708, 1709, and 1710). All known adult males differed at 3 or more loci from the offspring.

A fourth female (1097) was seen consorting with 3 known males (1081, 1096, and 1172) in previous years. She was not observed in 1988. In 1989 she had 3 cubs (1480, 1481, and 1482). Fathers were identified for 2 of the offspring (1480, and 1481), but all sampled males differed at 3 or more loci from the third offspring (1482).

These examples provide clear evidence of the presence of unobserved breeding males. In other cases, sires were not accounted for but there was a slight possibility that the true sire could be obscured by mutations.

For example, 1 female (1479) was captured and marked as a 6-year old in 1989; she was 9 years old in 1992. In 1989 and 1990 she was seen consorting with known males (1478, 1405, and 1491). In 1991 she was seen consorting with male 1712. In 1992 she gave birth to a single cub (1758) which was captured with her and sampled. No sampled male matched the cub's genotype at all 8 paternal alleles. However, 1 resident male (1463), differed at only 1 locus. Because of the possibility of mispairing during replication, there is a probability of about  $1 \times 10^{-3}$  (or 1 in 1000; Amos et al. 1996, Paetkau et al. 1997a) that 1463 could be the father. Similarly, other males excluded from paternity at only 1 locus may be possible fathers if no other father has been determined. Among 57 offspring sampled (a total of 456 paternal alleles), there were 5 instances where this was the case; it is possible that in at least 1 of these cases (1 in 1000 alleles) paternity was obscured by mutation. There is no way of knowing for certain, and if this were the case it resulted in an inability to identify the father. More than 1 such omission due to mutation is unlikely in our sample.

## DISCUSSION

### Hardy–Weinberg Equilibrium

In a large, idealized, randomly-mating population, the allele frequencies (as reported in Craighead et al. 1995) should predict the frequencies of genotypes, or allele pairs, found throughout the population. Further, if there is random mating with respect to genotype, a gene with  $j$  alleles should exhibit genotype frequencies in Hardy–Weinberg equilibrium (Hartl 1988), where  $p_i$  and  $p_j$  represent the frequencies of the  $i$ th and  $j$ th alleles, respectively, and  $A_i$  and  $A_j$  represent the type of allele found in each possible allele pair, respectively:

$$p_i^2 \text{ for } A_i A_i \text{ homozygotes}$$

$$2p_i p_j \text{ for } A_i A_j \text{ heterozygotes}$$

$$p_j^2 \text{ for } A_j A_j \text{ homozygotes}$$

This model assumes that alleles are selectively neutral, any individual has equal probability for mating with any other individual, and there is negligible migration, insignificant mutation, and non-overlapping generations. However, Hardy–Weinberg frequencies are relatively insensitive to departures from most of these assumptions, so that the frequencies are often valid even if some of these assumptions are not completely met (Hartl 1988). Although the occurrence of genotypes in Hardy–Weinberg proportions does not prove that all these assumptions are valid, a departure from these frequencies can indicate that some assumptions, particularly those of random mating, are not met.

In panmictic populations there are no important barriers to gene flow and any individual has equal probability for mating with any other individual. A population is said to exhibit population or genetic structure if the average genetic constitution (allele frequencies) of individuals from different areas of the total population varies. Each such subpopulation is a reproducing unit (a randomly mating or panmictic group of individuals) and any further subdivision should not reveal additional genetic differences between subgroups (Chakraborty and Leimar 1987). If a population is subdivided genetically the proportion of homozygotes in the total population will be greater than Hardy–Weinberg expectations. Such a deficiency of heterozygotes, known as the Wahlund effect, occurs if the population is sampled over an area large enough to contain subpopulations whose individuals are more closely related to each other than they are to the larger group.

Because female offspring tend to remain near their maternal home range after weaning and in some cases establish an overlapping home range (Reynolds and Hechtel 1984), we expected that over time, genetic structure of the population would develop: adjacent females would be more closely related than distant ones. If males also tended to breed in localized areas, we would expect that homozygotes would accumulate in portions of the study area and that there would be a deficiency of heterozygotes (or Wahlund effect) when the study population is viewed as a panmictic unit. In the western Brooks Range, the absence of a Wahlund effect indicates that the area sampled contained an essentially panmictic population. To capture any significant genetic structure within the sample we would have needed to sample over much larger area such as the entire state of Alaska (Paetkau et al. 1997).

The alleles examined appeared to be selectively neutral. The occurrence of Hardy–Weinberg proportions in the genotypes (or pairs of these alleles) supports the assumption of selective neutrality. It also suggests that: (1) we are sampling from a large effective population; (2) there is little migration from other populations with differing allele frequencies; (3) mutation rates are not high enough to significantly alter allele frequencies; and (4) mating is random with respect to these alleles. Since we did not observe departures from expected Hardy–Weinberg proportions, we infer the following about breeding structure.

Since we know that most females are philopatric and establish lifetime home ranges adjacent to or overlapping with their mother's (Reynolds 1992, Reynolds and Hechtel 1984), the lack of a Wahlund effect in microsatellite genotypes and panmixia in allele frequencies was accomplished through male breeding behavior. Gene flow is primarily mediated by the wide-ranging movements of the male segment of the population, with males being reproductively successful over areas larger than our study area.

Male grizzlies can travel hundreds of kilometers. One adult male resident of our study area for >7 years was later shot near Barrow about 300 kilometers away (H.V. Reynolds, unpubl. data). Another male traveled 163 km to the Arctic Ocean coast and returned (Reynolds and Hechtel 1984). Although it has been known that males leave their natal area after weaning and travel widely, it was not known whether they were able to breed successfully throughout their lifetime home ranges, as this population genetics analysis indicates. This is further corroborated by our paternity analysis.

## MANAGEMENT IMPLICATIONS

This collaborative research combined behavioral observations of family relationships (mother–offspring) with detailed genetic profiles of individuals. The field data allowed us to assess the accuracy of the genetic techniques, assign pedigrees, and infer mechanism of gene flow. Both types of data are necessary to understand the mechanisms of genetics at the population level. We urge that behavioral and ecological research include at least a genetic sampling component. Similarly, we urge that genetic results be interpreted in light of evidence collected in the field.

Our work revealed no evidence of a Wahlund effect and thus no indication of genetic structure to the population. Other larger Arctic study areas also show no evidence of genetic structure (Paetkau et al. 1997, 1998,

Paetkau and Strobeck 1998). Single large reserves, or groups of connected reserves, should ideally be large enough to contain subdivided populations (genetic structure) and therefore to conserve more genetic diversity within a species (Hedrick 1996, Hedrick and Gilpin 1996). Our findings are important for management concerns since even very large reserves will not capture this range of genetic diversity, which can be considered the genetic component of biodiversity (Cronin 1993).

A conservation reserve can be considered a refuge from extinction: an adequate reserve will buffer a population or species from stochastic and deterministic factors. The Greater Yellowstone Recovery Zone is almost 5 times as large as the WBR study area, and the Greater Yellowstone Ecosystem is about 8 times as large. Even areas this large may not encompass genetic subdivision and thus be adequate for long-term persistence (Craighead et al. 1997). Maintaining habitat connections wherever possible between reserves for male dispersal (gene flow) and demographic rescue should be a management priority.

## LITERATURE CITED

- AMOS, W., S.J. SAWCER, R.W. FEAKES AND D.C. RUBINSTEIN. 1996. Microsatellites show mutational bias and heterozygote instability. *Nature Genetics* 13:390–391.
- BECKMAN, J.S., AND J.L. WEBER. 1992. Survey of human and rat microsatellites. *Genomics* 12:627–631.
- CHAKRABORTY, R. AND O. LEIMAR. 1987. Genetic variation within a subdivided population. Pages 89–120 in N. Ryman and F. Utter, eds. *Population genetics and fishery management*. Univ. Wash. Press, Seattle, Wash.
- CRAIGHEAD, F.L. 1994. Conservation genetics of grizzly bears. Ph.D. Diss. Montana State Univ., Bozeman. 251pp.
- , M. GILPIN, AND E.R. VYSE. 1998. Genetic considerations for carnivore conservation in the Greater Yellowstone Ecosystem. In T. Clark, S. Minta, and P. Karieva, eds. *Greater Yellowstone carnivores*. Univ. Chicago Press, Chicago, Ill.
- , D. PAETKAU, H.V. REYNOLDS, E.R. VYSE, AND C. STROBECK. 1995. Microsatellite analysis of paternity and reproduction in Arctic grizzly bears. *J. Hered.* 86:255–261.
- , AND E.R. VYSE. 1996. Brown/grizzly bear metapopulations. Pages 325–351 in D. McCullough, ed. *Metapopulations and wildlife conservation*. Island Press, Covelo, Calif.
- CRONIN, M.A. 1993. Mitochondrial DNA in wildlife taxonomy and conservation biology: cautionary notes. *Wildl. Soc. Bull.* 21:339–348.
- HARTL, D.L. 1988. *A primer of population genetics*. Second ed. Sinauer Assoc., Sunderland, Mass. 305pp.
- HEDRICK, P.W. 1996. Genetics of metapopulations: aspects of a comprehensive prospective. Pages 29–51 in D. McCullough, ed. *Metapopulations and wildlife conservation management*. Island Press, Washington D.C.
- , AND M. GILPIN. 1996. Metapopulation genetics: effective population size. Pages 161–185 in I. Hanski and M. Gilpin, eds. *Metapopulation dynamics: ecology, genetics, and evolution*. Acad. Press, New York, N.Y.
- LEWONTIN, R.C., AND J. KRAKAUER. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics* 74:175–195.
- PAETKAU, D., W. CALVERT, I. STIRLING, AND C. STROBECK. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecol.* 4:347–354.
- , AND C. STROBECK. 1994. Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecol.* 3:489–495.
- AND ———. 1998. Ecological and genetic studies of bears using microsatellite analysis. *Ursus* 10:299–306.
- , L.P. WAITS, P.L. CLARKSON, L. CRAIGHEAD, AND C. STROBECK. 1997. An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics* 147:1943–1957.
- , ———, ———, ———, E. VYSE, R. WARD, AND C. STROBECK. 1998. Variation in genetic diversity across the range of North American brown bears. *Conserv. Biol.* 12:418–429.
- REYNOLDS, H.V. 1992. Grizzly bear population ecology in the Western Brooks Range, Alaska. *Prog. Rep.*, 1990 and 1991. Alaska Dep. Fish and Game and U.S. Nat. Park Serv., Fairbanks. 64pp.
- , AND J.L. HECHTEL. 1984. Structure, status, reproductive biology, movement, distribution, and habitat utilization of a grizzly bear population. *Fed. Aid in Wildl. Restor. Final Rep. Proj. W-21-1, W-21-2, W-22-1, and W-22-2*. Alaska Dep. Fish and Game, Juneau. 29pp.
- SLATKIN, M. 1982. Testing neutrality in a subdivided population. *Genetics* 100:533–545.