

PATERNITY DETERMINATION WITH DNA FINGERPRINTING IN A GRIZZLY BEAR POPULATION

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Abstract: We extracted DNA from 120 grizzly bears (*Ursus arctos horribilis*) in an arctic population for paternity analysis using DNA fingerprinting. Preliminary results indicate that a combination of several probes and/or enzymes will be necessary to identify sires of offspring with known mothers. Development of genetic profiles will provide estimates of population genetics parameters such as inbreeding coefficients, heterozygosity, and degree of polymorphism to use as a baseline in managing this and other, more endangered, populations. We present these preliminary results in order to inform others of the direction of our research and to facilitate sample collection and lab work in other studies.

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In order to effectively conserve and manage small wildlife populations, it is necessary to determine the degree of genetic variation that exists. A genetic baseline derived from larger, more viable populations is a necessary starting point for comparison. The techniques of DNA fingerprinting with genomic DNA are well suited to the analysis of inter-population genetic variation (Lynch 1992) and paternity analysis (Jeffreys et al. 1985b) and can provide estimates of kinship and inbreeding among individuals, and heterozygosity and degree of polymorphism for the population.

DNA fingerprinting can be defined as the use of detectable DNA probes that hybridize to hypervariable tandem repeat segments (Wyman and White 1980) of DNA. The technique was first developed by Jeffreys lab (Jeffreys et al. 1985a) to describe unique genetic profiles of individuals using human DNA. This technique has been used to develop pedigrees of dogs (*Canis lupis familiaris*), cats (*Felis catus*) (Jeffreys and Morton 1987), and mice (Jeffreys et al. 1987); to demonstrate multiple paternity in house sparrows (*Passer domesticus*) (Burke and Bruford 1987, Wetton et al. 1987); to reveal paternity of snow goose (*Chen caerulescens*) nestlings (Quinn et al. 1987) and old world monkeys (Weiss et al. 1988); and for animal identification, paternity testing, and linkage analysis in horses (*Equus caballus*), dogs, pigs (*Sus scrofa*), chicken (*Gallus gallus*), and fish (Georges et al. 1988).

Paternity, and the development of pedigrees for wild populations, has been used to measure realized reproductive success in red-winged blackbirds (*Agelaius phoeniceus*) (Gibbs et al. 1990), and to analyze kinship in prides of Serengeti lions (*Panthera leo*) (Packer et al. 1991). We have been investigating the use of DNA fingerprints in a viable population of arctic grizzly bears to determine paternity and to use these data for population genetic analysis. The study population is located in the northern foothills of Alaska's Western

Brooks Range (Reynolds 1991).

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METHODS

Whole blood was collected in the field and stored in saline sodium citrate buffer (SSC). Ear tissue samples were removed with a leather punch while attaching ear tags. DNA was isolated from blood using the techniques of Mullenbach et al. (1989) and from tissue using the techniques of Cronin et al. (1991). DNA was re-suspended in sterile distilled water at a concentration of 500 micrograms per milliliter.

Samples of DNA were digested with the restriction endonucleases HinfI and HaeIII. The resulting fragments were separated by electrophoresis in 1.0% agarose gels at 20-25 milliamps for 18-24 hours, and transferred to nylon membranes using the Southern blot technique. Both charged and neutral nylon membranes were used. DNA filters prepared in this way were probed for tandem repeat sequences of genomic DNA with radiolabelled Pv47 and M13 probes, and with alkaline phosphatase-labelled oligonucleotide subunits of Jeffreys 33.15 and 33.6 probes. Labelled membranes were used to expose x-ray film which was then developed to reveal a characteristic banding pattern or DNA fingerprint.

RESULTS AND DISCUSSION

DNA has been extracted from 120 individuals in the study population. This represents over 90% of the bears currently alive as estimated from aerial census (Reynolds 1991, 1992 unpubl. data). Approximately 15

times as much DNA can be extracted per milliliter of tissue sample as from blood. Eight to 10 micrograms of DNA appears to be an optimum amount for electrophoresis. HaeIII digestion reveals more diagnostic bands than does HinfI using Pv47 and M13 probes. The alkaline-phosphatase-labelled oligo probe Jeffreys 33.15 produces the best banding patterns for paternity determination, but does not alone reveal sufficient variation for identification of the sire.

To date, 30 individuals have been typed using HinfI and Jeffreys 33.15 probe. Results from similar numbers of trials using HaeIII, Pv47, and Jeffreys 33.6 indicate that a combination of several enzyme-probe combinations is necessary for paternity identification. Additional trials are planned using oligo probes of M13, MS1, and CMM101.

Approximately 10 diagnostic bands are revealed in the genome using each probe and enzyme combination (Fig. 1). This is less than the number revealed in humans (Jeffreys et al. 1985b), other primates (Weiss

et al. 1988), dogs, and cats (Jeffreys and Morton, 1987), but is equivalent to other mammal groups including black bear (*Ursus americanus*) (Fain 1992, these proceedings). The use of 2 or more enzyme-probe combinations will provide sufficient data for population genetic analysis. We present these preliminary results in order to inform others of the direction of our research and facilitate sample collection and lab work in other studies.

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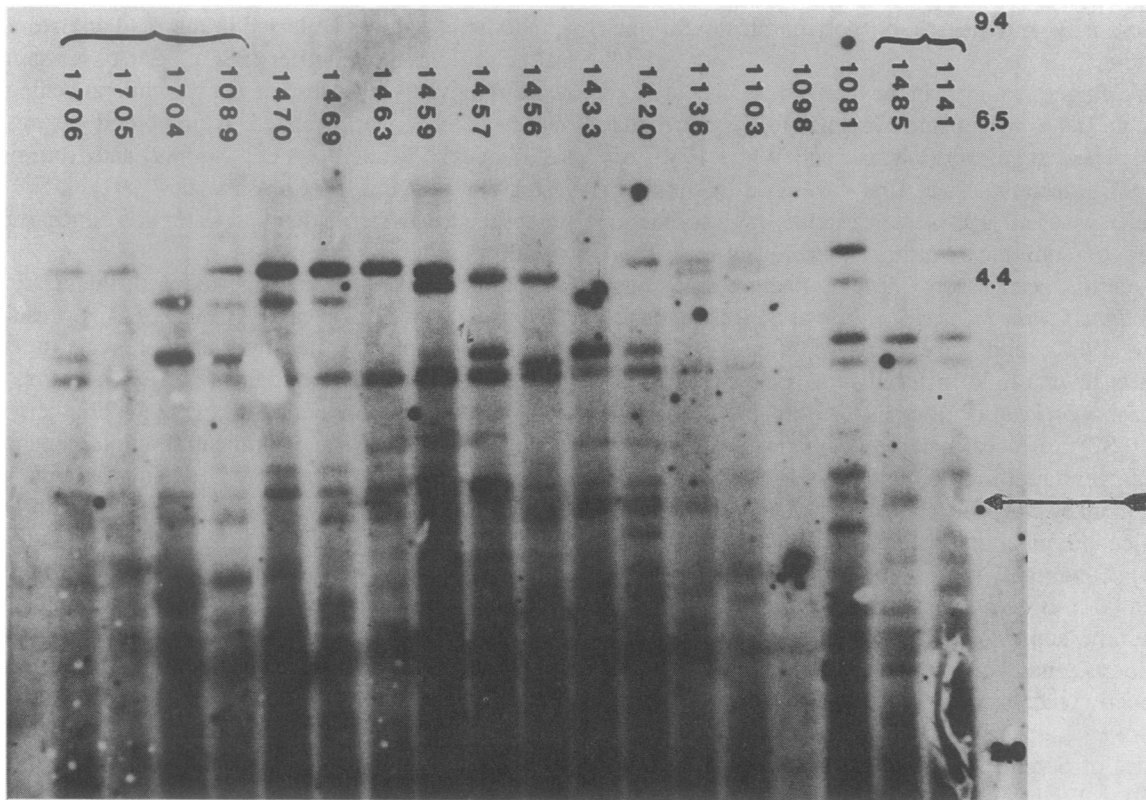


Fig. 1. A DNA fingerprint of 2 family groups and 11 assorted males. Bear numbers are along horizontal axis: fragment size in kilobases is along vertical axis. Bear 1456 was observed breeding with female 1141 the year before 1485 was born. Only 1 band (arrow) can be assumed to be inherited from the father, but this excludes many potential mates. Additional fingerprints using other enzyme-probe combinations are necessary to determine paternity.

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