DNA Fingerprinting of Wolves

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There are proposals to reintroduce *(Canis lupus)* to Yellowstone National Park through translocation of individuals from other populations. Ideally, these wolves should have a genotype similar to that possessed by the subspecies previously found in the Park. This genetic similarity would enhance the probability that genetically based adaptations to the environment of the Park were present, and thus enhance the likelihood that a viable population would be established with minimum animals and effort. Concern has been expressed about preserving the genetic purity of potentially translocated wolves. A genetic test which would permit differentiation between restored pure wolves and clandestinely introduced ones, as well as between pure wolf and wolf x coyote, and wolf x dog hybrid genotypes would be valuable. Furthermore, it is important to understand the power of DNA fingerprinting as a tool for monitoring breeding structure of the restored pack(s) at intervals subsequent to their release in Yellowstone.

This research tests the applicability of DNA technology utilizing hypervariable minisatellite loci ("DNA fingerprinting") in monitoring and controlling the genetic constitution of the Yellowstone wolf pack. DNA fingerprinting was first described by Jeffreys et. al. (1985), and has been proved successful for individual identification, as well as for parental testing in humans and in several species of animals (Burke and Bruford 1987, Helminen et. al. 1988, Morton et. al. 1987, Rabenold et. al. *in press*).

Knowing the original restored wolves' DNA "fingerprints", the National Park Service could then periodically monitor and control the genetic constitution of the Yellowstone wolves, since they could exactly determine each puppy's parents. Action could also be taken to determine which offspring could not have been derived from the park wolves, due to possible hybridization with coyotes or dogs living in or around the Park.

✦ Summary of Research Project Accomplishments to Date

This project, aimed at examining the effectiveness of DNA fingerprinting as a tool for monitoring breeding structure in planned gray wolf restoration populations in Yellowstone, has made logistical and technical progress. Because DNA fingerprints of individual wolves of known genetic relatedness are required, we have contacted 60 private breeders, zoos, and wildlife parks, requesting blood samples from wolves taken at next handling, particularly requesting samples from sets of wolves of known relatedness (siblings, parents and offspring, etc.). We provide a collecting kit including appropiate solutions and containers for blood handling and shipment to all interested parties, as well as import permits for foreign shippers. Five such keepers of wolves have responded

positively, although some have not yet had their next scheduled handling, and others report that they will keep our request in mind when their next handling occurs, but that it will be not for some time. We have, however, obtained 16 individual samples from 3 locations, representing 4 sibships with as many as 4 full siblings each, a father and 2 offspring, and some cousins.

We have extracted high quality DNA from all samples obtained (mean yield = 100 mg) and prepared DNA fingerprints from 2 sibships screening 2 enzymes (HAE III and HINF I) with M13 multilocus probe. Both enzymes produced excellent results, with HINF I showing greater polymorphism.

Wolf blood samples were suspended in PBS (phosphate buffered saline) solution, SDS (sodium dodecyl sulfate), and proteinase K, and incubated at 55°C overnight. This was followed by one phenol, one phenol/chloroform, and one chloroform extraction; and alcohol precipitation. We then made a digestion and hybridization test on 7 samples, using HaeIII and Hinf1 restriction enzymes, with M13 DNA probe. Banding patterns produced by Hinf1 were slightly more polymorphic, although both sets were of excellent quality.

We will produce final gels containing DNA fingerprints for all possible combinations of gray wolf kinships. We will analyze them by making all possible pairwise comparisons between lanes. Only individuals on the same gel will be compared, and estimates of 3 genetic parameters, mean average percent difference (MAPD), frequency of polymorphic loci (P), and average heterozygosity (H) will be derived based on the banding scores of the fingerprints. The proportion of bands shared by individuals of known relatedness can be used to estimate the average allele frequency across the family of loci screened. Allele frequency is the fundamental feature of the system under study that can be used to determine confidence in assignment of parentage through a simple series of calculations (Rabenold et. al., in press, Jeffreys et. al., 1985).

This will allow us to determine the efficiency of DNA fingerprinting in establishing relatedness and testing parentage within gray wolf populations, which in turn might allow us to propose the technique as a tool which could be used to monitor and control the genetic constitution of Yellowstone's wolves.

We are technically prepared to produce the necessary gels, having calibrated conditions on the preliminary set mentioned above. However, slow arrival of samples (often due to long intervals between handlings of animals by their keepers and the simple low availability of this material) impedes our progress. We await samples from the Toronto Zoo and the North Dakota Zoo, and potentially more samples from the Quebec Zoo that would round out a complete nuclear family. We will also be in touch with Ernest Vyse at Montana State University to inquire about providing each other with samples.

For all existing samples and any yet to be received, we will prepare DNA fingerprints for all possible combinations of gray wolf kinships. Through a series of calculations explained in the executive summary, we will estimate the power of DNA fingerprinting in assignment of parentage in gray wolves, based on the similarities of banding patterns of individuals of known degrees of relatedness.

✦ Literature Cited

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