

Proposed genetics monitoring protocol for grizzly bears in the Greater Yellowstone Area

Background

Genetic issues are of concern for the Greater Yellowstone Area (GYA) grizzly bear population because it is an isolated population where genetic declines over time are expected due to inbreeding effects. According to Miller and Waits (2003), recommend that 1-2 effective migrants per generation is an appropriate level of gene flow into the Yellowstone grizzly population. will maintain the current level of divergence between the Northern Continental Divide Ecosystem (NCDE) and GYA bears.

Recent long-distance movements of grizzly bears documented by DNA samples and telemetry have shown that grizzly movements of at least 140 air miles are possible in the northern Rockies. Such movements highlight the need to monitor all Yellowstone bears captured or dead in order to determine if movement into this area has occurred.

Movement of grizzly bears into the GYA from the NCDE can occur naturally via immigration. The Conservation Strategy (USFWS 2007) established a general policy for monitoring the possible migration of new genetic material from the NCDE via genetic monitoring. Genetic samples will be collected from all captured and dead bears in the GYA, and analyzed as part of a cooperative effort between the Interagency Grizzly Bear Study Team and a recognized genetics laboratory. Results will be used to fulfill genetic monitoring requirements for the distinct population segment (DPS) of grizzly bears occupying the GYE as specified in the Final Rule (FR) Removing the Yellowstone Distinct Population Segment (DSP) of Grizzly Bears From the Federal List of Endangered and Threatened Wildlife (Federal Register 72:40 [March 29 2007] pages 14866-14938). The primary task specified in FR is to document natural connectivity, or lack thereof between the GYE-DPS and the NCDE grizzly population using genetic samples from all captured and dead bears. Gene flow between these 2 populations will be detected by using assignment tests which identify the ecosystem from which each individual is most likely to have originated based on its unique genetic signature. This technique also has the ability to identify bears that may be the product of reproduction between GYE and NCDE bears. These tests require that the 16 microsatellite markers used to genotype NCDE bears also be used in the GYE-DPS. If gene flow into the GYE from the NCDE grizzly population is not documented by 2020 then the signatories of the Conservation Strategy U.S. Fish and Wildlife Service in conjunction with their partners will begin to implement -required to- translocation of 1-2 e-grizzly bears every 10 years from other grizzly populations into the GYE-DPS to maintain current levels of genetic diversity. Thus integrity of results is critical and must be of the highest possible standards. Genetic analyses will determine if NCDE-origin genetic materials are found in the GYA confirming migration of genetic material from the NCDE into the GYA.

Methods

Genetic samples will be collected from all bears captured or killed in the GYA. Samples will be stored at -45°C by the IGBST and shipped to Wildlife Genetics International, Inc.

for analysis. Each sample will be genotyped at 20 microsatellite markers and gender determined. Microsatellite markers will include G1A, G1D, G10B, G10C, G10H, G10J, G10L, G10M, G10P, G10U, G10X, MU50, MU59, CXX20, CXX110, MU23, P07, MU51, A06, and CPH9. Sixteen microsatellite markers will be used for the assignment tests because data for the NCDE exists: G1D, G10B, G10P, G10J, G10M, G1A, G10H, MU59, MU50, G10C, MU23, G10L, CXX20, CXX110, G10U, and G10X. Assignment test will be performed using software Genetix (reference) and ~~Structure~~ Structure (referece) to determine if NCDE genes are detected in GYA samples.

Contractor Requirements

The contract laboratory (Wildlife Genetics International, Inc.) will use methods that have been published in a peer-reviewed technical journal. Procedures proposed for the analyses have well-established track records. Peer-reviewed lab-specific error rates associated with those methods are required. Electrophoretic scores will be examined by at least two technicians, at least one of whom has a minimum of two years of experience with 1) microsatellite analysis, 2) working with analysis of small samples of non-invasively collected hair, and 3) experience with or knowledge of *ursid* marker systems. Microsatellite separations shall be accomplished using laser-induced fluorescence denaturing capillary electrophoresis. Laboratory protocols must be strictly adhered to.

To ensure that differences between two samples are not due to genotyping error, samples with similar genotypes will be verified. Allele scores must be calibrated to be consistent with published designations. Extraction and PCR negative and positive controls must be used at all times. Records of these controls shall be available upon request. Initial lab observations, samples, DNA extracts, and capillary electrophoretic results must be readily accessible to the Project Leader at all times during the contract period. All materials related to the project remain the property of the U.S. Geological Survey.

To ensure the integrity of the genotyping data, samples and extracted DNA must be kept in secure facilities. Samples shall be certified by the laboratory as they are received. Accountability is critical and standard procedures at the laboratory must include a chain-of-custody protocol. From the time of receipt of samples by the analytical laboratory, all hair and tissue samples and DNA extractions must be kept in locked rooms when authorized personnel are not present. Computers used to conduct analyses and store results shall be protected from unauthorized access through password protections, stringent firewall and antivirus software and/or having no hard-wire connection to the internet. Data must be backed-up on a regular basis with one copy to remain off premises.

Literature Cited

Miller, C. R., and L. P. Waits. 2003. the history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. Proceedings of the National Academy of Sciences, USA. 100:4334-4339.

U.S. Fish and Wildlife Service. 2007. Final Conservation Strategy for the grizzly bear in the Greater Yellowstone Area. U.S. Fish and Wildlife Service, Missoula, Montana, USA.