

**ARIZONA GAME AND FISH DEPARTMENT
HABITAT PARTNERSHIP PROGRAM
HABITAT ENHANCEMENT AND WILDLIFE MANAGEMENT PROPOSAL**

PROJECT INFORMATION

Project Title: Landscape Genetic Assessment of Arizona Desert Bighorn Sheep

Project No. 10-701

Region/GMU: Statewide

HPC: N/A

Project Type: Game Management: Genetic Analyses

Project Description:

This project will partner with wildlife geneticists at University of California, Davis to produce genetic data and analyses for scientifically-informed genetic management of desert bighorn sheep of Arizona. DNA will be analyzed from Arizona bighorn sheep samples (whole blood and/or tissue) that are provided by AZGFD to UC Davis.

AZGFD will coordinate delivery of up to 300 Arizona bighorn sheep samples and at least 30 known Rocky Mountain bighorn sheep samples to UC Davis for comparison of Desert and Rocky Mountain bighorn sheep subspecies/races. Each sample will be DNA-extracted and analyzed for at least thirty microsatellites (nuclear DNA markers) and genetically typed for DNA sequence of the mitochondrial control region (“D loop”). AZGFD will provide data associated with each sample such as capture location and date, sex, age class, etc. The DNA data will be statistically analyzed to determine population structure, genetic diversity, inbreeding, and genetic interchange (gene flow) across the landscape in relation to environmental features (such as potential barriers to gene flow including mountain ranges, roads, urban development, etc.).

Genetic structure of herds, populations and metapopulations will be determined – that is, determination of which groups of bighorns sheep regularly interbreed and landscape patterns of the gene flow among populations. Genetic diversity will be characterized – this is the level of DNA variation within and between populations. Individual bighorn sheep will be assessed for assignment to (and/or fractional membership in) bighorn sheep subspecies (particularly desert vs. Rocky Mountain bighorn sheep races) using multilocus microsatellite genotypes and mitochondrial sequence haplotypes. Levels of admixture (introgression) between Desert and Rocky Mountain bighorn sheep within herds will be computed. Inbreeding indices and average pairwise relatedness among bighorn sheep will be computed. Calculations will be made using DNA data to estimate the numbers of breeding individuals (effective population sizes). Recommendations will be provided for management and conservation of bighorn sheep genetic integrity based on the genetic analyses. AZGFD will provide necessary samples, data and GIS layers (shape files, etc.) detailing topographic and environmental factors (vegetation layers, etc.) that are to be assessed with the DNA data.

Wildlife Species to Benefit: Desert bighorn sheep. Agencies will use information from this study to draft and implement management action plans focused towards the benefit of the highly vulnerable bighorn sheep herds throughout the state.

Possible Funding Partners:

Implementation Schedule:

Beginning: January 2011

Completed: December 2012

NEPA Compliance: (if applicable)

Completed: Yes _____ No _____

Projected Completion Date:

PROJECT FUNDING

SBG Funds Requested: \$28,788.50 per year x two years. \$57,577 total

Cost Share Funds: \$ 0.0 (although there is no specific cost share identified for this portion of the project, it is important to recognize the contribution of the Department in the collection of the samples. Bighorn sheep sample collection kits and personnel time for the past 3-5 years were necessary to obtain this sample collection.)

Total Project Costs: \$28,788.50 per year x two years. \$57,577 total

PARTICIPANT INFORMATION

Applicant: Holly Ernest DVM, PhD Assoc. Prof.
(please print)
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Medicine, University of California Davis, One
Shields Ave, Davis, CA 95616

AGFD Contact and Phone No. Brian Wakeling, Game Branch Chief 623-236-7385
(If applicant is not AGFD personnel)

Coordinated with: Rick Langley; Jon Hanna; Tom McCall; Erin
Butler; Bob Henry; Jim Hinkle; Jim Heffelfinger

Date: 8/30/2010

Applicant's signature: Holly Ernest

Date: 8/30/2010

SEND COMPLETED APPLICATIONS TO:

AZ Game and Fish Department
Attn: Game Branch
5000 W. Carefree Highway
Phoenix, AZ 85086
rgregory@azgfd.gov

WAS PROJECT PRESENTED TO THE LOCAL HPC? YES _____ NO X

HAS PROJECT BEEN SUBMITTED IN PREVIOUS YEARS? No IF SO WAS IT FUNDED?

NEED STATEMENT/PROBLEM ANALYSIS:

Monitoring and a plan for maintaining desert bighorn sheep genetic integrity is vital for their conservation. This study is considered a priority within Arizona as there has been a scarcity of population genetic data for Arizona desert bighorn sheep. The proposed work would provide the genetic data and analyses necessary for assessments of genetic diversity, gene flow among Arizona desert bighorn sheep herds, and levels of interbreeding among Desert and Rocky Mountain bighorn sheep.

Genetic methods have improved greatly in recent years. The data and analyses generated in these proposed projects will be form some of the next vital steps to provide much more accurate assessments of genetic health of bighorn sheep. Microsatellite analysis proposed here will allow accurate population assignment, and genetic diversity assessments. This level of analysis is vital for best practice management and conservation of desert bighorn sheep.

PROJECT OBJECTIVES:

- 1) generate a population genetic (DNA) database for desert bighorn sheep of the Arizona.
- 2) produce assessments of genetic integrity. Genetic integrity includes important population characteristics such as landscape patterns of genetic diversity, levels of inbreeding, population structure, gene flow among populations, and admixture among subspecies (particularly Rocky Mountain vs. desert bighorn sheep).
- 3) provide recommendations toward optimum genetic management of desert bighorn sheep to enable scientifically-informed management for conservation of genetic diversity, population structure, and genetic interchange (gene flow).

PROJECT STRATEGIES:

Summary: UC Davis will extract DNA from samples provided from AZGFD, generate microsatellite and mitochondrial DNA data, then statistically analyze the data using a number of specialized population genetic software packages and ArcGIS. Annual reports will provide both detailed explanations and a plain language summary and interpretation of the findings will be provided so that wildlife biologists and managers can understand the content and implications of the genetic findings.

Details: UC Davis will generate DNA data for up to 300 Arizona desert bighorn sheep samples using the following techniques. DNA will be extracted using Qiagen extraction kits. Fluorescently labeled PCR (polymerase chain reaction) primers (probes) will be used to amplify and detect microsatellite DNA segments and sequence mitochondrial DNA Control Region segments on an ABI 3730 96-capillary electrophoresis DNA analyzer. Geneticists will proof read, annotate, and organize microsatellite DNA data using STRand software and Microsatellite Toolkit. Mitochondrial sequence DNA data will be proof read, annotated, and organized using Sequencher software. The Veterinary Genetics Laboratory (of which the Ernest lab is a unit) has generated this type of data for numerous wildlife species including bighorn sheep for 20 years, and over 100,000 domestic species per year.

Statistical Methods: Summary statistics for population genetics will be calculated using several software packages including Microsatellite Toolkit (observed and expected heterozygosity, mean number of alleles per locus; Park 2001), and GENEPOP (deviations from Hardy–Weinberg equilibrium and linkage equilibrium; Rousset 2007). We will calculate the number of private alleles using CONVERT version 1.31 (Glaubitz, 2004) and number of female/male migrants per generation using software such as MIGRATE. Allelic richness will be calculated (corrected for sample size) in FSTAT (Goudet, 1995) and pairwise population differentiation (F_{ST}) estimates between sampling regions using software such as GENEPOP and ARLEQUIN (Excoffier et al., 2005).

We will assess population structure and test genetic assignment to population and subspecies (including Rocky Mountain vs. Desert) using programs such as STRUCTURE (Pritchard et al. 2000) and GeneClass2 (Cornuet et al., 1999). We will test for evidence of inbreeding using indices including F_{IS} (software such as GENEPOP and ARLEQUIN) and of recent population size reductions in each sampling region with one-tailed Wilcoxon sign-rank tests for heterozygote excess in the program bottleneck version 1.2.02 (Cornuet and Luikart, 1997). Average pairwise relatedness and parentage analyses will be performed using programs such as CERVUS (Kalinowski et al 2007), and ML RELATE. Assessments of genetic findings with landscape variables will be conducted using ArcGIS and programs such as GeneticStudio (Dyer et al. 2010).

PROJECT LOCATION: Laboratory analysis (no field component): Dr. Ernest's lab: Wildlife & Ecology Unit, Veterinary Genetics Laboratory, School of Medicine, University of California Davis, One Shields Ave, Davis, CA 95616

LAND OWNERSHIP AT PROJECT SITE (Please state specifically if PRIVATE PROPERTY and provide landowner's name): N/A: samples have already been collected and archived by AZGFD

IF PRIVATE PROPERTY, IS THERE A STEWARDSHIP AGREEMENT BETWEEN THE LANDOWNER AND THE DEPARTMENT? N/A

HABITAT DESCRIPTION: Sonoran and Mojave Desert (desert bighorn sheep habitat throughout Arizona)

ITEMIZED USE OF FUNDS:

Item	Total cost Over 2 yrs	Cost per year
Salary (including benefits and student expenses):	\$39,516	\$19,758
Lab expenses	\$11,827	\$ 5,914
Travel (UCD researchers to meet with AZDGF)	\$ 1,000	\$ 500
Subtotal	\$52,343	\$26,172
Institutional indirect costs (10%)	\$ 5,234	\$2,617
Total	\$57,577	\$28,789

LIST COOPERATORS AND DESCRIBE POTENTIAL PARTICIPATION:

Coordination to deliver archived bighorn sheep samples to UC Davis: Brian Wakeling, Rick Langley; Jon Hanna; Tom McCall; Erin Butler; Bob Henry; Jim Hinkle; Jim Heffelfinger

PROJECT MONITORING PLAN:

N/A

PROJECT MAINTENANCE:

UC Davis will provide a one-year and final report to AZGFD and partner funding societies to facilitate dissemination of information and adaptive management implementation. Annual reports will provide both detailed explanations and a plain language summary and interpretation of the findings will be provided so that wildlife biologists and managers can understand the content and implications of the genetic findings.

PROJECT COMPLETION REPORT TO BE FILED BY: The completed project report is due two years following receipt of contract for funding and receipt of necessary samples.

WATER DEVELOPMENT PROJECTS (see attached worksheet):

N/A

TREE SHEARING (AGRA-AXE, PUSH) PROJECTS (see attached worksheet):

N/A