

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

Game Branch / HPC Project Number:	14-701
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PROJECT INFORMATION

Project Title: Population Genetic Analysis and Genetic Database for Arizona's Bighorn Sheep

Region and Game Management Unit: Statewide

<p>Local Habitat Partnership Committee (LHPC):</p> <ul style="list-style-type: none"> • N/A 	<p>Was the project presented to the LHPC? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/></p>
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Has this project been submitted in previous years? YES NO
If Yes, was it funded? YES NO → **Funded HPC Project #(s):**

Project Type: Genetic Analysis and Database Creation

Brief Project Summary. This project will inform managers and stakeholders about the genetic status of bighorn sheep in Arizona through estimates of:

- Population level genetic diversity – heterozygosity and allelic diversity
- Population structure, subdivision, and inbreeding coefficients thereof
- Admixture, migration, and relative gene flow among regional populations/sub-populations identified based on population genetic data

This project shall utilize DNA from tissue samples collected by the AZGFD from hunter-harvested bighorn sheep and samples obtained during captures and translocations in previous years.

This project will establish a population genetic database to guide future management actions (such as translocations) and continued research on bighorn sheep in the state of Arizona and beyond.

Big Game Wildlife Species to Benefit: Bighorn Sheep (*Ovis canadensis*)

<p>Implementation Schedule (Month/Day/Year):</p> <p><u>Project Start Date:</u> Phase 1 - January 1, 2015</p> <p><u>Project End Date:</u> Phase 1 - December 31, 2015</p>	<p>Environmental Compliance:</p> <p>NEPA Completed: Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input checked="" type="checkbox"/> Projected Completion Date: _____</p> <p>State Historic Preservation Office - Archaeological Clearance: Yes <input type="checkbox"/> No <input type="checkbox"/> N/A <input checked="" type="checkbox"/> Projected Completion Date: _____</p> <p>Arizona Game and Fish Department EA Checklist: N/A <input checked="" type="checkbox"/> To be Completed by: _____ Projected Completion Date: _____</p>
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PROJECT FUNDING

Special Big Game License Tag Funds Requested:	\$ \$8,500
Cost Share or Matching Funds:	\$ \$2,000
Total Project Costs:	\$ \$10,500

PARTICIPANT INFORMATION

Applicant (please print): John Clemons	Address: 2511 N. Val Vista Dr. Mesa, AZ 85213-1721	E-mail: clemonsjp@aol.com
Telephone: 602-316-2373		Date: 9/1/2014
AGFD Contact and Phone No. (If applicant is not AGFD personnel): Brian Wakeling 602-561-3732, Amber Munig 602-722-9048		
Project has been coordinated with: Brian Wakeling, Anne Justice-Allen, Amber Munig, Esther Rubin		

NEED STATEMENT – PROBLEM ANALYSIS: Founder effects, bottlenecks and genetic drift in small populations of wild animals are problematic because they increase the risk of reduced individual fitness due to inbreeding depression, which can translate to near-term declines in demographic parameters. In the long term, such processes reduce the amount of genetic variation available for populations to adapt to changing local conditions. Maintaining genetic diversity in free ranging herds of bighorn sheep in Arizona is an important component in aiding their long-term survival by reducing the risk of local population extinctions due to inbreeding depression. Assessing genetic diversity will provide an insight into the degree of possible inbreeding and potential genetic bottlenecks occurring in isolated herds of bighorn sheep throughout the state.

PROJECT OBJECTIVES:

1. Review the bighorn sheep translocation history for Arizona and establish if current populations are of first-order, second-order or of mixed ancestry.
2. Extract DNA and analyze Single Nucleotide Polymorphisms (SNPs) from representative samples to determine population structure and genetic diversity (heterozygosity and allelic diversity) among and within populations.
3. Calculate inbreeding coefficients of identified sub-populations to estimate the level of inbreeding.
4. Estimate the relative levels of gene flow (a genetics-based estimate of migration) among the identified sub-populations.
5. Overlay estimates of population genetic connectivity and relatedness among sampled sheep populations.
6. Develop an online database system that can provide raw genetic data for future research and comparisons on population genetic status.
7. Suggest management actions that may include future translocations for improvements in natural connectivity among populations as well as genetic diversity of bighorn sheep in Arizona.

PROJECT DESCRIPTION AND STRATEGIES: For this analysis, we will utilize approximately 200 tissue or blood samples collected from bighorn sheep as a result of hunter harvest, capture and translocation activities conducted previously by AZGFD during 2000-2014.

Laboratory Analysis:

DNA will be extracted from all representative samples and sequenced for the characterization of population and phylogeographic structure for bighorn sheep (e.g. Kijas *et al.* 2009). A high-throughput SNP genotyping platform—the Illumina ovineSNP50 BeadChip (Magee *et al.* 2010) or RAD sequencing will be used for identifying and analyzing SNPs from all samples.

All haplotypes and genotypes obtained will be analyzed to derive phylogenies, population structure

and relatedness estimates. Cluster analysis of SNP data will be used to estimate the degree of sheep population substructure. Mantel tests, multiple linear regression models, and coalescent simulations will be used to infer changes in gene flow and diversity of SNP markers in sheep populations (e.g. Epps *et al.* 2010).

Computer programs such as GENALEX, STRUCTURE, and MIGRATE will be used to obtain estimates of heterozygosity, allelic richness, population structure, inbreeding coefficients, migration, and gene flow. Bayesian assignment tests will be performed to assign individuals to populations.

A best estimate of gene flow will then be mapped across the compared populations (and sub-populations), and habitat types. Software such as ArcGIS and CIRCUITSCAPE will be used to contrast genetic data with expert-based habitat-type/quality-based connectivity corridors for bighorn sheep.

Map overlays of genetic data obtained from sheep samples will be used in predictive modeling of habitat connectivity using GIS. All genetic and spatial data will be stored in a reference database for future use by managers in monitoring population genetic status, determining origins of individuals, and understanding colonization/movements of bighorn sheep.

The total project will be split into three phases.

Phase 1- Sample collection, examination of translocation history and population classification and sample preparation, inventory and storage. Extraction of DNA and Polymerase Chain Reaction (PCR). Sample storage at -20C.

Phase 2- DNA sequencing, data analysis and generation of database. Rough-order magnitude cost is \$50,000 for this phase. We are unable to determine accurate pricing for Phases 2 and 3 until the total number of samples to be analyzed and SNP analyses method is defined. Proposals for these phases will be completed in 2015 and 2016.

Phase 3- Continuation of data analysis, database generation and final report. Rough order magnitude cost is \$30,000 for this phase.

References:

Epps CW, Wehausen JD, Palsboll PJ, McCullough DR. 2010. Using Genetic Tools to Track Desert Bighorn Sheep Colonizations. *Journal of Wildlife Management* 74:522–531.

Kijas JW et al., International Sheep Genomics Consortium. 2009. A Genome Wide Survey of SNP Variation Reveals the Genetic Structure of Sheep Breeds. *PLoS ONE* 4:e4668.

Magee DA, Park SDE, Scraggs E, Murphy AM, Doherty ML, Kijas JW, International Sheep Genomics Consortium, MacHugh DE. 2010. Technical note: High fidelity of whole-genome amplified sheep (*Ovis aries*) DNA using a high-density single nucleotide polymorphism array-based genotyping platform. *Journal of Animal Science*. Published Online.

PROJECT LOCATION: Southwest Wildlife Forensics Center, P.O. Box 20803, Mesa AZ in collaboration with the University of Arizona, Conservation Genetics Laboratory and the University of Arizona Genomics Core.

LAND OWNERSHIP AT THE PROJECT SITE(S):

(if the project area is private property, please state specifically and provide the landowner's name)

- N/A

*IF PRIVATE PROPERTY, IS THERE A COOPERATIVE BIG GAME STEWARDSHIP or
LANDOWNER AGREEMENT BETWEEN THE LANDOWNER AND THE DEPARTMENT?*

YES[] NO[] N/A[x]

HABITAT DESCRIPTION: N/A

ITEMIZED USE OF FUNDS:

Special Big Game License Tag Funds

Laboratory equipment (Pipettes, centrifuge, vortexer, etc.) \$3,500
Laboratory supplies and consumables (tubes, gloves and reagents) \$1,500
Extraction/PCR kits \$1,500
Salaries - Research Associate \$3,000
 Lab Technician \$1,000

Total \$ 10,500

Cost Share or Matching Funds (for volunteer labor rates please refer to the worksheet below)

ADBSS matching funds \$2,000

LIST COOPERATORS AND DESCRIBE POTENTIAL PARTICIPATION: Arizona Desert
Bighorn Sheep Society matching funds from other than SBGLT. Other possible grants yet TBD.

**WOULD IMPLEMENTATION OF THIS PROJECT ASSIST IN PROVIDING, MAINTAINING,
OR FACILITATING RECREATIONAL ACCESS?**

YES[] NO[x] N/A[]

PROJECT MONITORING PLAN: Quarterly status updates

PROJECT MAINTENANCE: Possible database maintenance would be required if additional analysis is attempted beyond the scope of this project. The Southwest Wildlife Forensics Center will be responsible for any needed maintenance.

PROJECT COMPLETION REPORT TO BE FILED BY: Melanie Culver, John Clemons and Ashwin Naidu.

WATER DEVELOPMENT PROJECTS (*please use the worksheet below*): N/A

TREE CLEARING/REMOVAL PROJECTS (*please use the worksheet below*): N/A