

Project Title: Genetic diversity and structure in the Whiskey Mountain bighorn sheep herd

Authors: Sierra Love Stowell, PhD & Holly Ernest, DVM, PhD

Project Type: Research

Affiliate: University of Wyoming with support from Wyoming Game & Fish Department

Location of Project: Whiskey Mountain Herd Unit, northwest Wyoming; University of Wyoming, Laramie, WY, USA

Description of Project:

The Ernest Lab received funding from the Wyoming Wild Sheep Foundation and the Wyoming Governor's Big Game Grant in 2015 and 2016 to conduct a state-wide genetic assessment of Wyoming's bighorn sheep. The goal of that project is to identify genetic groups, describe genetic diversity and population size within groups, and provide a foundation for understanding movement and disease dynamics. To conduct the state-wide assessment, we are working with the Wyoming Game & Fish Department to collect multiple samples from locations across the state. Below we propose a related but currently unfunded project that takes an in-depth look at a single herd.

The Whiskey Mountain herd of bighorn sheep is one of Wyoming's Core Native Herds and has been an important source of animals for the restoration of other herds across Wyoming and the West. Between 1965 and 1990, nearly 400 individuals from Whiskey Basin and Whiskey Mountain were translocated to other states including South Dakota, Utah, New Mexico, Nevada, and Idaho. Between 1949 and 1998, an additional 1,500 individuals from Whiskey were translocated to locations around Wyoming. With an estimated population size of 900-1000 individuals distributed over more than 1,400 square miles and four Wyoming Game & Fish Department (WGFD) hunt areas (Fig. 1), the Whiskey herd is crucial component of bighorn sheep diversity in Wyoming. Hunters and managers have long suspected that the Whiskey herd is actually composed of multiple subherds.

The Whiskey herd was subject to a catastrophic all-age die-off from pneumonia in 1992. Recovery has been slow, with the population remaining below the target size of 1350 individuals since that target was adopted in 2002. The slow rate of recovery has been attributed to continued low recruitment associated with persistent lamb pneumonia. However, the slow recovery could

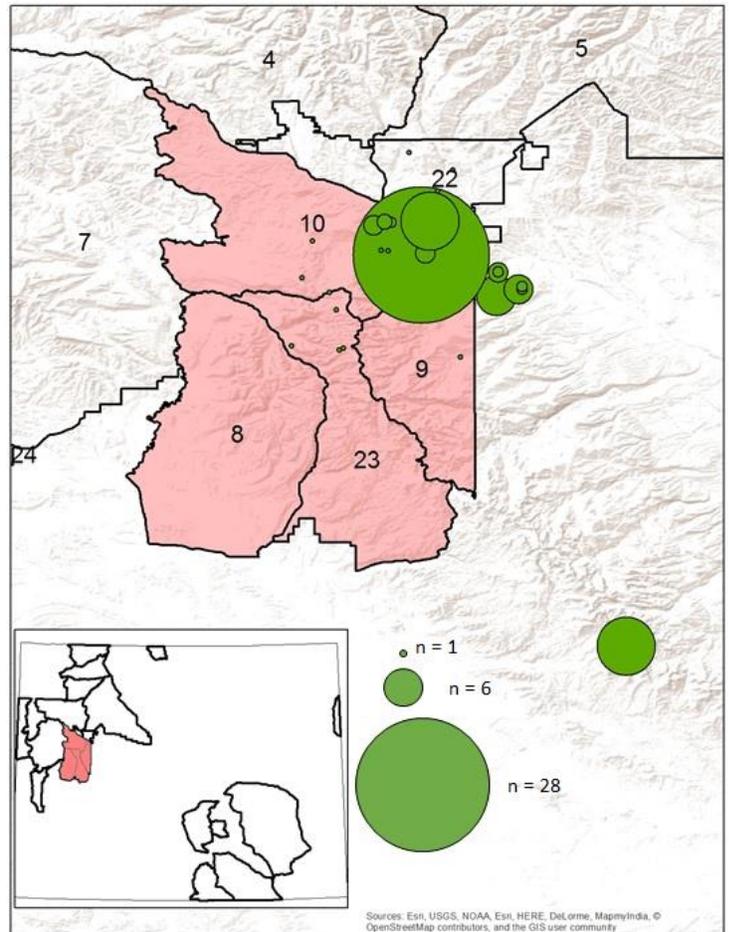


Figure 1 – Map of Whiskey Mountain bighorn sheep boundaries as managed by WGFD with samples in the Ernest Lab in green (scaled to number of samples obtained to date)

also be due to genetic bottleneck: while there may have been more diversity historically, if there was a large die off event and then recovery from a limited source, the current herd will no longer reflect that diversity. Herds that experience such bottlenecks may lack the genetic diversity to overcome diseases and other pressures.

We propose to assess the structure of the Whiskey herd using a genetic approach.

Such an assessment would be valuable for managers in determining herd boundaries and sustainable harvest rates for individual subherds. This assessment will also allow us to test whether the slow recovery has a genetic component: recovery may be lagging because of a lack of genetic diversity and founder effects resulting from the die-off.

Problem to be Solved:

We will address two main questions:

1) Is there genetic structure within the Whiskey Mountain herd?

For this question, we will identify whether there are genetic clusters within the Whiskey herd. These clusters may be at the scale of subherds, drainages, or families. By identifying clusters, we can further ask, where are those clusters found on the landscape? What is the effective size of each cluster? What is the extent of diversity in each cluster? How closely related are the clusters and are they exchanging genes? Answering these questions will inform how any substructure should be managed. They will also provide the basis for answering basic questions about disease dynamics, as the identity and size of clusters are crucial parameters for determining disease prevalence.

2) How has the die-off affected the genetic structure of the Whiskey Mountain herd?

This question will help us understand whether and how the die-off shaped the genetic substructure of the Whiskey herd. This question suggests two alternative hypotheses: first, the catastrophic all-ages die-off may have affected the entire region equally. If this is the case, then genetic substructure may persist as sub-regions recover with local individuals. Second and alternatively, the catastrophic die-off affected the region differentially. If this is the case, some of the subherds may have been affected more strongly than others so that recovery has occurred from a single or few sources such that substructure no longer persists.

Describe how you propose solving problem:

The first step in assessing population genetic structure in the Whiskey herd is to improve the spatial resolution of our sampling in that area. Wildlife populations are notoriously difficult to census completely, especially when the target species relies on rough terrain and can only be safely captured in cold winter months, such as bighorn sheep. We currently have 82 samples from Whiskey, mainly from the Dubois area as a result of recent captures.

Harvest rates in the Whiskey herd area are approximately 6% of adult males, 1 % of the total population, this amounts to the harvest of 14-16 rams annually. Our collaboration with hunters and WGFD to collect tissue and horn core samples from harvested animals has been reasonably successful: of the 154 bighorn sheep licenses filled across the state in 2015, we received 81 samples. Six of those samples are from the 16 licenses filled in the Whiskey Mountain herd unit. However, six males are not representative of the Whiskey population and will not allow us to accurately describe population structure. Thus, we will rely on blood samples from captures and fecal pellets from the field. We will work with WGFD to continue getting blood and fecal samples.

In 2014, WGFD initiated a collaring and sampling project to monitor the population and assess connections between ewe health and environmental conditions. Of the 89 sheep that were collared in 2014 and 2015, we have whole and/or dried blood samples from 53 of those individuals (Figure 1). Support from Wyoming Wild Sheep Foundation will allow us to sequence DNA from all the collared sheep for which we have samples. In summer 2016, we initiated fecal pellet collection with WGFD and have received 10 samples from Middle Mountain in the Wind Rivers southeast of Dubois and another 13 samples from Circle Ranch along Jakey's Fork (Figure 1). With support from the Wyoming Wild Sheep Foundation, we will continue to collect and analyze fecal samples, as these samples are often the most effective method for sampling a greater number of individuals without the expense and stress of captures. Unlike the hunter samples, fecal samples also allow us to sample a greater number of females, which will give us a better understanding of herd structure than using only the more vagile males. Taken together, the hunter, capture, and fecal samples will allow us to describe genetic structure in the Whiskey herd. We will target a minimum of 30 individuals from each hunt area. To account for potential duplicate individuals in fecal samples, we will target a total of 270 samples from the Whiskey Mountain herd.

The second step in assessing population genetic structure in the Whiskey herd is to employ the same genetic markers that we are using for our state-wide genetic assessment. We are using three complementary genetic approaches: microsatellites, mitochondrial DNA, and genome-wide variation. Each marker type provides information at different evolutionary time scales. Microsatellites are DNA sequences where a motif of a few DNA bases are repeated many times. The number of repeats varies among individuals and is heritable, so these markers can be used to distinguish among individuals and families. Because microsatellites are thought to be rapidly evolving and non-coding, they can be used to estimate population genetic diversity and infer recent demographic events. Microsatellite data have been used in bighorn sheep to identify groups and barriers to gene flow (Buchalski et al. 2015, 2016), among many other applications. Our lab and various others have successfully used DNA from fecal pellets for microsatellite analysis (Buchalski et al. 2015; Luikart et al. 2008).

DNA from the mitochondrion, a cellular organelle involved in metabolism, is inherited only from the mother. It has a slower mutation rate than the microsatellites and can be used to distinguish among high order groups such as populations, subspecies, and species. Mitochondrial DNA data have been used in bighorn sheep to identify subspecies and clarify evolutionary history (Buchalski et al., 2015; Rezaei et al., 2010).

Genome-wide data gives us snapshots across the entire genome, with different regions having different mutation rates as some locations are in genes that code for proteins and other locations are essentially neutral. Genome-wide data is especially interesting because by sequencing a greater number of loci across the entire genome, we increase the probability of identifying genes that are involved with traits of interest, such as disease resistance, migratory behavior, and growth rate (Kardos et al., 2015; Roffler et al., 2016).

Each sample type presents unique challenges for lab processing. While easier to acquire and store, dried blood, horn cores, and fecal pellets are trace DNA samples and require special DNA extraction techniques. Low and fragmented DNA yield mean that traditional DNA markers such as mitochondrial sequences and microsatellite genotypes might be the most reliable. Funding from the Wyoming Wild Sheep Foundation will be used to analyze these sensitive samples.

Agreement between data sets increases our confidence in our inferences, but disagreement between data sets can also be biologically meaningful. For example, because mitochondrial DNA is maternally inherited, it reflects the life history of females. In mammals such as bighorn sheep, ewes tend to be more philopatric (have smaller home ranges that they remain in or return to), while the males tend to more wide-ranging. So, differences in genetic diversity between microsatellite and mitochondrial markers can reflect differences in life history and behavior. For example, if the sexes are differentially affected by pneumonia and other diseases, and a greater proportion of one sex dies than the other, this difference will be reflected in the genetic effective population size of each kind of genetic marker.

In summary, we propose to closely examine the genetic makeup of the Whiskey Mountain bighorn sheep herd. We will use multiple sample types collected with the assistance of hunters and WGFD and multiple genetic marker types to provide a detailed description of herd structure. The state-wide assessment for which we currently have funding includes a few samples from each hunt area in the Whiskey herd. From these few samples, we can differentiate Whiskey from other herds, but not they are not enough to determine herd substructure within Whiskey. From this research, we will provide a written and oral report to WGFD and Wyoming WSF and outreach materials on the Whiskey herd to the National Bighorn Sheep Center in Dubois, WY.

Alignment with Wild Sheep Foundation Conservation Vision 2020 Objectives

Enhance wild sheep populations and their habitats: Whiskey Mountain is a core native herd in Wyoming and provides many opportunities for hunting and a source for translocations across Wyoming and the West. Given the importance of this herd, research is needed to understand the health and structure of this herd. Genetic information is now considered a fundamental and necessary component in wildlife population management and conservation. Genetics provides vital information not provided by other bighorn sheep population health and monitoring tools. Genetic assessment specifically directed at a detailed description of a core native herd has not yet been conducted.

Promote scientific wildlife management: Our research will provide estimates of genetic diversity, population structure, and health in the Whiskey Mountain herd. This work will also allow us to more precisely estimate genetic population size, which is critical for modeling disease prevalence and spread and determining sustainable levels of harvest. Genetic data can be used to estimate population size when populations are difficult to census. We will communicate this work with the Wyoming Department of Game & Fish and other state management agencies, as well as the Wyoming WSF, so that they may better set harvest targets, plan translocation efforts, and monitor populations.

Literature Cited

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Biography

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Are you currently a member: no

Budget

We are requesting \$24,725 in funding from the Wyoming Wild Sheep Foundation to support this project over one year. Budget request includes partial salary support for the postdoctoral researcher and research technician who will complete this project, as well as sequencing and lab materials costs. Because this research is focused specially on the Whiskey Mountain herd and will require detailed sampling in that area, this project is not currently supported under the grant to conduct a state-wide genetic assessment.

Item	Cost to be funded by WY-WSF grant	Cost to be funded by other cooperators
Equipment		All necessary equipment has been purchased by Dr. Ernest using her University of Wyoming startup funds.
Services (no permanent full-time salaries)	\$14,725 (temporary 2 months postdoc, temporary 0.5 month technician), \$7,500 (genomic sequencing)	
Publishing		\$1000
Monitoring	Not applicable	
Supplies (itemize then total)	DNA extraction kits, reagents, enzymes, lab plastics = \$2,500	

Other (specify)	Not applicable	
Total	\$24,725	

Other organizations providing financial aid or support of the project (include any pending amounts applied for):

Wild Sheep Foundation (national), applied August 2016 to study genetics of bighorn sheep translocation in Wyoming

Safari Club International Foundation, applied October 2016 to study relationship of disease and population genetics in Wyoming bighorn sheep

Eastern Wild Sheep Foundation, applied June 2016 for state-wide genetic assessment of Wyoming bighorn sheep

Iowa Chapter of the Foundation of North American Wild Sheep, applied June 2016 for state-wide genetic assessment of Wyoming bighorn sheep

Signature:

Sierra Love Stowell & Holly Ernest

Title: Postdoctoral Research Associate (Love Stowell); Professor & Wyoming Excellence Chair in Disease Ecology (Ernest)

Date: November 1, 2016

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