

*AN ASSESSMENT OF THE LANDSCAPE GENETIC STRUCTURE OF THE
WESTERN CONTINENTAL GOLDEN EAGLE POPULATION*

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Introduction

Over the past several decades there has been an increasing push by federal and state governments to develop renewable sources of energy including electricity generated at wind, solar, hydro and geothermal facilities. Among these sources, the development of wind energy facilities has increased in the U.S. largely as a result of financial incentives and tax credits established by the Energy Policy Act of 1992, 2005 (GAO 2005) and the American Recovery and Reinvestment Act of 2009 (Public Law 111-5).

In spite of their attractiveness as a clean, renewable energy source, wind energy developments are not ecologically benign. Potential impacts of wind energy facilities on avian species include collision mortality, habitat degradation or loss, and displacement of migration patterns caused by disturbance (Erickson et al. 2002, Drewitt and Langston 2006, Madders and Whitfield 2006, Morrison 2006, Kuvlesky et al. 2007, TWS 2007). For example, Altamont Pass Wind Resource Area, California has high raptor abundance and correspondingly high raptor fatality rates (Smallwood and Thelander 2008).

Impacts of wind energy facilities on endangered, threatened or rare species are of particular conservation concern. One species that has specifically elicited concern regarding wind turbine-related mortalities is the golden eagle (*Aquila chrysaetos*; Lewis et al. 2010). Golden eagles are a 'K-selected' species: long-lived birds that mature late (~ 4 – 5 years of age) and have low reproductive rates (Watson 1997). Consequently, their population growth rates can be severely impacted by increases in adult mortality (Caswell 2001). Due to potential impacts to the western continental golden eagle population, we began a study of this population with the following objectives:

Objectives

1. Develop and maintain a tissue and feather archive housed at the Department of Fish, Wildlife and Conservation Ecology (FWCE), New Mexico State University (NMSU) that will be used to assess the landscape genetic structure of golden eagles using molecular genetic and stable isotope methods (Caut et al. 2006, Rundel et al. 2013).

2. To host a meeting at NMSU to develop a collaborative team that will work together to illuminate the landscape genetic structure of golden eagles.
3. To begin the process of preparing and analyzing golden eagle tissue and feather samples according to the framework decided upon by the collaborative team described under objective 2 above.

Progress to Date

Objective 1: We initiated a collaboration with USFWS biologists, Mr. Brian Millsap and Dr. Robert Murphy, to develop a tissue and feather database and this effort resulted in the first-ever relationship with the National Eagle Repository (NER) and the collection of tissue and feather samples from golden eagle carcasses of known provenance that arrive there. As a result we have acquired a new federal permit authorizing the sharing of samples among all pertinent participants (Permit # MB58285B-0) and which is being used as the legal vehicle for further developing the project. Samples from golden eagles are now stored in the Department of Fish, Wildlife, and Conservation Ecology, New Mexico State University. This database has increased to 853 entries of individual eagles that have either been trapped under other authorized permits or submitted to the NER, this is 79 more entries than at the same time last year. The development of the database and storage of all tissues and feathers over the past year was achieved by two undergraduate students supported by the project (Ms. Diana Meza and Mr. Marcus Montoya).

Objective 2: This grant enabled us to expand the collaborative network and supported a workshop held December 17-18, 2014 at NMSU's Pete Domenici Center. A consortium of scientists, post-docs, private contractors, and students from federal resource agencies and universities participated (Table 1). The workshop was specifically held to determine the best approach to describe the phylogeographic structure of the western continental population of golden eagles. It was decided that: 1) two labs (Drs. DeWoody and Van Den Bussche) would pursue a SNP (single nucleotide polymorphism) analysis, 2) another lab (Dr. Brook Milligan) would pursue the use of a new sequencing platform (MinION) for the landscape genetic analysis,

and 3) that a fourth lab (Dr. David Nelson) would conduct a stable isotope analysis of golden eagle feathers.

Objective 3: One hundred and sixty tissue samples of nestling golden eagles were added to existing samples for a total of 523 samples genotyped in the laboratory of Dr. Andrew DeWoody (Purdue University, West Lafayette, IN) using a panel of 162 SNP loci; these data revealed that there are at least three phylogeographic clusters of golden eagles in the western U.S., including clusters in Alaska, California, and the western states, and has resulted in a publication (Doyle et al. 2016).

More recently we sent the same and additional tissue samples to the lab of Dr. Ron Van Den Bussche (Oklahoma State University, Stillwater, OK) and he is now using a more extensive SNP panel of 30,006 loci which has confirmed the results from Dr. DeWoody's lab that a minimum of 3 phylogeographic clusters of eagles are present in the western continental U.S. However, these results are preliminary and Dr. Van Den Bussche is currently conducting a more thorough analysis of these data.

Three tissue samples were transferred to the lab of Dr. Brook Milligan (NMSU) who is currently using an emerging, portable, handheld sequencing technology developed by Oxford Nanopore Technologies, the MinION sequencer, which is ideally suited to the task of simple and inexpensive acquisition of genetic information for assigning individuals to natal populations. Two important elements to validate the use of the MinION for acquiring genetic information have been accomplished. First, genomic DNA was sequenced from two distinct individuals. This yielded 152,999 and 31,087 reads respectively for the two samples, many of which are greater than 10 kb in length. There are only $2n = 62$ chromosomes in the golden eagle genome, so the available genome sequences are far from complete, but will be compared to a published sequence of the golden eagle genome (Doyle et al. 2014). This work was completed and a second experiment for genotyping individuals based upon MinION data was initiated. This experiment is crucial for transforming the MinION into an inexpensive genotyping device, which is necessary for rapidly assigning golden eagles to natal populations. Unfortunately, the computational issues surrounding the application of this sequencer, and the mechanism it uses, have resulted in equivocal results and we have stopped funding this research. Both the laboratory

and the analytical phases of this project supported two different Ph.D. students in the Department of Biology here at NMSU.

A total of 265 feather samples, including 78 previously sent nestling feather samples, were sent to the lab of Dr. David Nelson (University of Maryland, Frostburg, MD) where stable isotope signatures of carbon, nitrogen, and hydrogen are being generated to begin the development of a stable isotope base map for golden eagles and an analysis of the movements of juvenile, subadult and adult eagles. One of the main issues we have with either the genetic or stable isotope analyses is to obtain greater sample coverage, both in density and extent, to characterize the western U.S. region. This next year (2017) we will begin a sampling effort whereby we will contact and or visit eagle researchers to actively collect nestling eagle samples from on-going projects. We hope to use the genetic and stable isotope data to illuminate the phylogeographic structure of golden eagles in the western continental U.S. and then use this information to assign recovered carcasses to specific regions to understand both the movements of golden eagles and the mortality factors that maybe impacting the western continental population (*sensu* Rundel et al. 2013).

Table 1. Workshop participants (n = 22) that formed a collaborative team to assess the landscape genetic structure of golden eagles in the western continental United States. Note that undergraduate and graduate students, and post-doctoral scholars were involved in the workshop

Name	Affiliation
Ms. Emily Bjerre	Division of Migratory Bird Management, USFWS
Dr. Peter Bloom	Bloom Biological, Inc.
Mr. Charles Britt	Mesa Ecological Services LLC
Dr. James Cain	NM Cooperative Fish & Wildlife Research Unit, USGS
Dr. Michael Collopy	University of Nevada, Reno
Dr. Andrew DeWoody	Dept. of Biological Sciences, Purdue University
Dr. Jacqueline Doyle	Dept. of Biological Sciences, Purdue University (Post-Doc)
Ms. Kristin Engebretsen	New Mexico State University (Technician)
Ms. Megan Judkins	Oklahoma State University (Ph.D. Student)
Dr. Todd Katzner	US Geological Survey
Dr. Carol McIntyre	US National Park Service
Dr. Brook Milligan	Dept. of Biology, New Mexico State University
Mr. Brian Millsap	Division of Migratory Bird Management, USFWS
Mr. Jacob Naranjo	New Mexico State University (Undergraduate Student/Tech)
Dr. David Nelson	University of Maryland – Appalachian Lab
Dr. Barry Noon	Colorado State University
Dr. Gary Roemer	New Mexico State University
Dr. Kristen Ruegg	University of California, Santa Cruz (Post-Doc)
Dr. Sarah Sonsthagen	Alaska Science Center, USGS
Ms. Miranda Butler -Val Verde	New Mexico State University (Undergraduate Student/Tech)
Dr. Ronald Van den Bussche	Oklahoma State University
Mr. Brian Woodbridge	US Fish and Wildlife Service

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