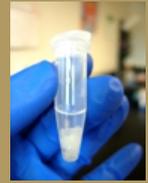


## Developing Genetic Methods to Manage Livestock-Wildlife Interactions



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### Groups Affected:

- Health officials
- Livestock producers and farmers
- U.S. citizens
- Oil and gas development
- Wildlife and natural resource managers

### Major Cooperators:

- Christopher Newport University
- Colorado State University
- Savannah River Ecology Laboratory
- Tejon Ranch Conservancy
- Texas A&M University
- University of Colorado Boulder
- University of Florida
- University of Texas Medical Branch
- U.S. Fish and Wildlife Service
- U.S. Geological Survey
- Utah State University

### National Wildlife Research Center Scientists Develop Genetic Methods for Wildlife Damage Management

Wildlife Services' (WS) National Wildlife Research Center (NWRRC) is the only Federal research organization devoted exclusively to resolving conflicts between people and wildlife through the development of effective, selective, and socially responsible methods, tools, and techniques.

Information about wildlife populations and diseases can be difficult to obtain with traditional sampling techniques. This is especially true for rare or elusive wildlife and emerging diseases. The field of wildlife genetics uses DNA samples collected from tissue, blood, hair, feces, saliva, water and soil to uncover valuable information about animals, their abundance, behavior, movements, and evolution. Genetic analysis includes approaches, such as DNA forensics, species abundance and monitoring, kinship/relatedness, gender determination, and species or individual animal identification.

NWRRC scientists at the Center's wildlife genetics laboratory apply genetic expertise to understand the intercontinental movement of pathogens, monitor endangered and invasive species, detect invasive species using environmental DNA found in water, and understand the movement of animal populations.

### Applying Science & Expertise to Wildlife Challenges

**Simplifying Sample Collection for Environmental DNA.** — Environmental DNA (eDNA) refers to DNA that is shed by an organism into the environment (e.g., water, soil or air). The genetic material could come from shed skin, hair or scales, mucous, urine, and feces. The collection of eDNA in water has been used to detect the presence of invasive species, such as Burmese python and feral swine. Capturing eDNA from water begins by filtering water samples at the collection site or collecting a water sample and concentrating the eDNA it contains using laboratory methods prior to extraction. To preserve DNA in water, samples require cold storage or the addition of a preservative for transportation of filters from the field to the lab. Requiring field personnel to filter and/or manage a continuous cold chain can be expensive, challenging, and time-consuming. Furthermore, freezing and thawing samples prior to analysis reduces DNA viability and thus detection. To eliminate the need for costly cold chain storage and the preservation of water filters, NWRRC researchers tested the effectiveness of adding Longmire's lysis buffer (a solution of 100 mM Tris, 100 mM EDTA, 10 mM NaCl, 0.5 % SDS, 0.2 % sodium azide) to freshwater samples for preservation of eDNA. Results showed that eDNA was effectively preserved in 15 mL water samples treated with Longmire's solution. eDNA detection in samples treated at the highest concentrations of Longmire's solution was similar to samples stored at -80° C at both 28 and 56 days extraction. Medium and low concentrations of Longmire's solution preserved eDNA out to 56 days, but not as well as freezing or the highest concentration of Longmire's solution. NWRRC researchers conclude that Longmire's lysis buffer used at the high concentration treatment level (1:3) is a viable alternative to cold chain storage and can simplify the collection of eDNA in water by eliminating the need for filtering. This allows more time for sample collection which could translate to an increase in the chances of detecting a rare or elusive species.

**Feral Swine.** — Feral swine are one of the most destructive invasive species threatening habitats, native wildlife, agricultural resources, and property in the United States. Feral swine are generalists and thrive in a variety of habitats, eating almost anything they find. To better understand the diversity of plant and animal species feral swine impact, NWRRC geneticists are using high-throughput sequencing technology to analyze the remains of plants and animals found in feral swine feces. Samples from Florida, California, and Texas indicate that feral swine feed upon quail and salamanders, both of which are experiencing major population declines.

Genetics techniques are also being used to evaluate feral swine eradication efforts and help prevent new invasions. In trials with captive feral swine, researchers learned that eDNA can be detected from a single boar wallowing for 15 minutes in a pool of water and that it degrades after 3.5 days. eDNA from multiple wallowing animals takes almost twice as long to degrade. Researchers developed a lab assay that could detect low-quality and low-quantity eDNA found in turbid water, such as that at feral swine

wallowing sites. The tool will help managers determine if feral swine are present in an area and monitor their distribution.

#### **DNA-based Approach for Identifying Sage-Grouse Predators.**

— The greater sage-grouse is a ground-nesting bird at risk of extinction in multiple U.S. states and Canada. Predation events on sage-grouse nests are rarely seen, and it is difficult to identify the responsible predator species from visual nest remains. To help identify common mammalian sage-grouse predators, NWRC geneticists analyzed predator saliva DNA collected from depredated sage-grouse eggshells and bird carcasses in Wyoming. Sage-grouse nests were monitored using radio telemetry of hens and infrared trail cameras. Researchers collected egg remains and/or hen carcasses when a nest failed or a hen was eaten. In 79 percent of the nest and 47 percent of the carcass samples, researchers successfully identified the mammalian predator species from DNA remnants. Eighty-six percent of the detected mammal predators were canids, including coyotes and dogs. Other taxa included rodents, striped skunk and cattle. NWRC researchers acknowledge that nest and adult predator identification is challenging considering the lack of species-specific signs at nests and the difficulty in differentiating predators from scavengers using DNA evidence. The results of this study suggest that the best approach is to use multiple techniques including field surveys, camera monitoring of depredation events, and DNA forensics-based methods.

#### **Monitoring Endangered Mexican Wolves Using Genetics.**

— The Mexican gray wolf (*Canis lupus baileyi*) is the smallest, most genetically distinct and endangered subspecies of gray wolf in North America. Monitoring the population status of Mexican gray wolves in the wild is a critical component of the species' recovery efforts. Contemporary molecular approaches make it possible for biologists to identify individuals by obtaining and amplifying DNA from noninvasively collected samples including feces, hair, or saliva. In collaboration with the U.S. Fish and Wildlife Service and several universities, NWRC researchers and geneticists applied 10 canid microsatellite loci to 235 Mexican gray wolf samples, 48 coyote (*C. latrans*) samples, and 14 domestic dog (*C. lupus familiaris*) samples to identify genes that distinguish the species. Experts then evaluated an approach for using noninvasive fecal DNA genotypes from Mexican gray wolves combined with mark-recapture methods to estimate wolf populations. The genetic methodology proved effective for distinguishing Mexican gray wolf scat from dogs and coyotes and researchers were able to identify 5 of 14 individual wolves known to be members of 3 different packs and 3 previously undetected wolves occurring in the study area. The DNA-based estimates of abundance corresponded with the known number of wolves in the study area. Researchers recommend this approach as an alternative method for the long-term monitoring of recovering Mexican gray wolf populations.

**Endangered Species Translocation.** — Proactive endangered species conservation and management often involve reintroducing animals to historical ranges. This increases the overall number and geographic distribution of populations and lessens the chance that an isolated random event could cause the complete extinction of a species. There are two recognized subspecies of white-tailed deer in the Pacific Northwest, the Columbian white-tailed deer (*Odocoileus virginianus leucurus*) and the Northwest white-tailed deer (*O. v. ochrourus*). The Columbian white-tailed deer is listed as an endangered subspecies with two remaining isolated populations in Washington and Oregon. NWRC geneticists investigated genetic variation, connectivity, and hybridization in the Columbian white-tailed deer populations. They found that Columbian white-tailed deer and Northwest white-tailed deer in the Pacific Northwest originated from a single historic gene pool. This calls into question the two

subspecies' current taxonomic status. Also, the results indicated that the current populations are genetically isolated but may have hybridized with black-tailed deer in the past. Despite the taxonomic ambiguity, the study revealed the presence of some unique genetic variation within each population, which supports ongoing conservation efforts. Such genetic investigations help to guide important endangered species management and translocation decisions.

#### **Selected Publications:**

Hopken, M.W., T.M. Lum, P.M. Meyers, and A.J. Piaggio. 2015. Molecular assessment of translocation and management of an endangered subspecies of white-tailed deer (*Odocoileus virginianus*). *Conservation Genetics* 16:635-647. doi: 10.1007/s10592-014-0689-6.

Hopken, M.W., E.K. Orning, J.K. Young, and A.J. Piaggio. 2016. Molecular forensics in avian conservation: a DNA-based approach for identifying mammalian predators of ground-nesting birds and eggs. *BMC Research Notes* 9:14. doi: 10.1186/s13104-015-1797-1.

Kierepka, E.M., S.D. Unger, D.A. Keiter, J.C. Beasley, O.E. Rhodes Jr., F. L. Cunningham, and A.J. Piaggio. 2016. Identification of robust microsatellite markers for wild pig fecal DNA. *Journal of Wildlife Management* 80:1120-1128. doi: 10.1002/jwmg.21102.

Piaggio, A.J., C.A. Cariappa, D.J. Straughan, M.A. Neubaum, M. Dwire, P.R. Krausman, W.B. Ballard, D.L. Bergman, and S.W. Breck. 2016. A noninvasive method to detect Mexican wolves and estimate abundance. *Wildlife Society Bulletin* 40(2):321-330. doi: 10.1002/wsb.659.

Reed, R.N., M.W. Hopken, D.A. Steen, B.G. Falk, and A.J. Piaggio. 2016. Integrating early detection with DNA barcoding: species identification of a non-native monitor lizard (*Squamata: Varanidae*) carcass in Mississippi, U.S.A. *Management of Biological Invasions* 7(2):193-197. doi: 10.3391/mbi.2016.7.2.07.

#### **Major Research Accomplishments:**

- WS geneticists developed a method using Longmire's lysis buffer for collecting and transferring eDNA in water from the field that eliminates the need for filtering and cold storage.
- WS geneticists used high-throughput sequencing technology to analyze the remains of plants and animals found in feral swine feces to better understand feral swine impacts on biodiversity. Researchers also developed a method to detect low-quality and low-quantity feral swine eDNA found in turbid water, such as that at feral swine wallowing sites. The tool is used to monitor feral swine populations.
- WS geneticists analyzed predator saliva DNA collected from depredated sage-grouse eggshells and bird carcasses to help identify common sage-grouse predators.
- WS geneticists developed an approach that uses wolf fecal samples combined with mark-recapture methods to estimate endangered Mexican wolf populations.
- WS geneticists investigated genetic variation, connectivity, and hybridization in the Columbian white-tailed deer populations. They found that Columbian white-tailed deer and Northwest white-tailed deer in the Pacific Northwest originated from a single historic gene pool.