

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2019 – 2021**

TITLE: Enhancing the Efficacy of Molecular Approaches to Agricultural and Natural Resource Issues in Eastern Oregon by Developing a Regionally Targeted DNA Sequence Library

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COOPERATORS: None

EXECUTIVE SUMMARY:

Many issues facing agricultural producers and natural resource managers involve trophic relationships – in other words, organisms eating one another. The nature of these trophic relationships are diverse (e.g., agricultural pests eating crops; biological control agents feeding on agricultural pests or vectors of human disease; bees provisioning nests with pollen; salmon eating aquatic invertebrates; top predators feeding on fish). Managing these relationships to maximize ecosystem services, such as crop production or fisheries output, requires understanding these complex trophic relationships. In the past, much of our knowledge of who eats whom was based on field observations or collecting the contents of collected and/or ingested material and attempting to visually identify the material. Recently, molecular techniques have been developed that allow scientists to extract DNA from collected food or prey samples that contain multiple species (e.g., pollen balls, gut contents) and to use a process, DNA metabarcoding, to identify the consumed organisms. DNA metabarcoding combines DNA barcoding with advanced sequencing technology. DNA can be extracted from mixed species samples and amplified, and then particular regions of the DNA (barcode regions) that have low variation within a given species, but high variation among species, can be examined. The barcoding regions from the collected, unknown sample are compared with available sequencing data from known species. These known sequences are publicly available in DNA sequence libraries such as GenBank, a NIH genetic sequence database. Although metabarcoding techniques provide much promise as a relatively rapid, inexpensive, and highly specific means of identifying trophic relationships, one challenge is having a robust library with which to compare collected samples. Because the approach is relatively new, data on specific barcoding regions for many species are not available on GenBank. Fortunately, this issue can be addressed by collecting known species that are not represented for barcoding regions of interest, sequencing them, and adding them to existing libraries. The goal of this project was to collect and sequence species that are important in trophic relationships in eastern Oregon but are currently missing sequence data in public databases. The significant accomplishments of this project included conducting all field sampling to collect plant and insect tissue, extracting and amplifying DNA for each species, and

obtaining sequence data for each species. This work contributes to a more comprehensive regionally-specific library that can benefit researchers in solving agricultural and natural resource issues that occur state-wide and beyond.

OBJECTIVES:

The objectives of the project are to:

1. Collect tissue samples from all 125 plant and 40 invertebrate species.
2. Extract the DNA and amplify the barcoded region(s) of interest for each species.
3. Sequence the barcoded regions.
4. Upload the sequences to GenBank and share with other groups, such as the Oregon Biodiversity Genome Project.

PROCEDURES:

To accomplish our first objective, we located each of the plant and animal species on our developed lists and collected tissue from each. Field sampling occurred in Umatilla, Morrow, Union, and Wallowa Counties from April to September 2019.

For our second objective, we took collected samples and extracted DNA from each. We used PCR (polymerase chain reaction) to amplify specific barcode markers with previously validated primers that are designed to target these barcodes. For plants, we used the rbcL and ITS2 gene regions based on previous experiments that have deemed them suitable for identification of plants to the genus and species level. For animals, we used the cytochrome oxidase I (COI) barcode region, which has been identified as a good target for species identification of many invertebrates and has been used to conduct diet analyses in several studies (King et al. 2008). Success of the PCR was confirmed by gel electrophoresis against positive and negative controls. Samples that amplified successfully were cleaned using the Qiagen PCR Cleanup Kit and sent to Oregon State University's Center for Genome Research and Biocomputing to be sequenced.

For our third objective, we sent samples to OSU's Center for Genome Research and Biocomputing and Biopharm for sequencing.

For our fourth objective, we have taken our sequence data and are in the process of preparing it for uploading the data into GenBank and for sharing with other scientists and managers in the region that can use the data to inform their work. This includes sharing our COI data with the Oregon Biodiversity Genome Project, which is a collaboration of OSU, Oregon Department of Fish and Wildlife, The Oregon State Ichthyology Collection, and the USDA Forest Service.

SIGNIFICANT ACCOMPLISHMENTS:

The significant accomplishments of this project included conducting all field sampling to collect plant and insect tissue, extracting and amplifying DNA for each species, and obtaining sequence data for each species.

BENEFITS & IMPACT:

The data generated from this project allows us to make significant progress on several projects in our laboratory, including refining analyses of crop pest vectors, diets of bees, efficacy of beneficial insects, and functional roles of crayfish. This information can be used by growers and natural resource managers in the area to improve decision-making, including altering field margin management to decrease pest loads, enhancing natural control of crop pest, informing plant selections for enhancing native bee habitat, and understanding the functional role of crayfish as ecosystem engineers. At a broader scale, the data will improve other groups' work in the region by providing a more extensive reference library that can improve the quality of inferences made from current and future research.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

We have not received additional funding for this project at this time.

FUTURE FUNDING POSSIBILITIES:

These data will strengthen several proposals we'll be submitting that involve metabarcoding, including applications for funding from programs such as the USDA Agriculture and Food Research Initiative Program and the National Science Foundation.