April 24th, 2021

FACULTY GRANT PROGRESS REPORT

Dear Faculty Grants Committee,

I'm a fourth-year tenure-track faculty member in the Biology Department. Last year I received $780 in Faculty Grant funding for project "Analysis of reproductive modes of Boechera genus species in south-eastern Pennsylvania". As this past year wet lab research was limited by the COVID pandemic I decided to focus on a similar project "A genetic test for serpentine-specific natural selection in the lyre-leaf rockcress, *Arabidopsis lyrata*, an important element of the State Line Serpentine Barrens of Eastern North America".

Arabidopsis lyrata is a short-lived perennial in the mustard family, growing on rocky soils and often used in studies of plant ecology and evolution. It can grow in disturbed or disadvantaged habitats, such as on nutrient poor or toxic soils. In this project, carried out in collaboration with Dr. Chris Hardy, an MU botanist, we aim to provide insight into adaptation and gene flows between populations of this species. We will carry out a genetic comparison between *A. lyrata* populations growing on more hospitable non-serpentine (granitic and limestone) soils and nutrient poor and high in heavy metals serpentine soils. We will examine selected regions of the genome called microsatellites, which consist of multiple repeats of a short DNA sequence, with the specific number highly variable in the population. We will carry out a comparison of microsatellites between serpentine and non-serpentine populations. This will allow us to determine whether serpentine soil populations are genetically closer to each other and gene flow between them and their non-serpentine neighbors is selected against or whether serpentine populations exchange genetic material with local non-serpentine populations and are likely the result of local adaptation.

We started working on this project during late Fall of 2019. So far, three undergraduate students worked with me on this project – Carter Farmer, Matt Godwin and Brianna Steward. Based on the literature, we selected eight microsatellite loci likely to characterize well the *A. lyrata* populations under study: ICE3, ICE13, ICE14, ATHATPASE, AthS0392, F20D22, ArabpetC10, ArabpetJ17. These loci were selected based on the high heterozygosity (diversity) of microsatellite variants present in *A. lyrata* populations and on the size of the repeating DNA motif (between two base pairs and four). Carter and Brianna isolated DNA from a local *A. lyrata* plant and we used it to optimize the PCR runs for all selected microsatellites. We also visualized and sized the samples using gel electrophoresis. Likewise, the students, with my help, selected three suitable markers from the chloroplast genome to confirm the samples' species identity: TrnL, TrnL-F and MatK. Chloroplasts are plant organelles involved in photosynthesis, which contain genomes separate from those in the nucleus. Students optimized PCR conditions for amplification of the selected chloroplast loci.

During Spring 2020 Dr. Hardy and I, sometimes accompanied by Carter Farmer, visited selected serpentine and non-serpentine locations in Pennsylvania and Maryland and collected plant samples for this study. The following serpentine barrens locations were selected and visited between mid-May and late July 2020: Goat Hill, Chrome, and Nottingham Serpentine Barrens (all three in Lancaster County, PA), Lake Roland Serpentine Restoration Area and Soldier's Delight Serpentine Meadow (both in Baltimore County, MD). Non-serpentine locations include Lock 12, Lancaster County Central Park, Enola Low Grade Trail (all in Lancaster County, PA) and a location off a trail in Centre County, northern PA. From each location one full *A. lyrata* specimen was identified and collected by Dr. Hardy as an herbarium specimen, then leaf samples were taken from 10-14 additional *A. lyrata* individuals in that population. Samples were frozen at -80°C for preservation. GPS coordinates were taken at the plant specimen for each population.

Currently, we are finishing DNA isolation from the collected samples using the standard plant DNA isolation method – the Mini CTAB protocol from UCLA's Jacobsen Lab. When this step is completed, we will be ready to perform the polymerase chain reaction (PCR) amplification of the

selected microsatellite loci for all collected samples. PCR products will be sent to the University of Pennsylvania genomics core facility for sizing. Data for eight microsatellite loci will give us an accurate molecular "fingerprint" of each studied plant and will allow us to compare all (serpentine and non-serpentine) populations under study.

The next steps in this project will be microsatellite data collection, data analysis using statistical approaches and preparation of a manuscript for publication.

This year, despite of COVID, was productive and we made significant progress in the project on *A. lyrata* gene flows between serpentine and non-serpentine populations. We have collected sample plant material and DNA is mostly isolated from samples and ready for further experiments. My students presented at Women in Math, Science and Technology conference at MU, CPUB and Made in Millersville this Spring. One of them was accepted to a graduate program at Northeastern University while the other became a recipient of the MUSE fellowship for this Summer and a BSI grant. Carter Farmer and I plan to spend this Summer running PCR reactions, having the fragments sized ay UPenn, analyzing our results and preparing a manuscript for publication.

Sincerely,

Maja Klosinska