Determining The *Asclepias* Phylogenetic Tree Through Combined Target Enrichment & Genome Skimming

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Abstract

The milkweed genus *Asclepias* has long served as a model for studies of reproductive biology (Wyatt and Broyles 1994) and plant defenses (Agrawal and Fishbein 2008). Two American clades, the Sonoran Desert and temperate North American, contain up to 50% of the species' diversity and serve as the main focus of this experiment. Resolving evolutionary relationships in these clades is crucial to determining the origins and early diversification of the genus, as well as the milkweed species (Fishbein *et al.* 2011). This project has been ongoing for many years now, collecting and extracting thousands of DNA samples. Through modern genomic sequencing technology and resources, specifically the Hyb-Seq protocol discussed in Weitemeir *et al.* (2014), the goal is to understand the historical origins for plant speciation.

Keywords: *Asclepias*, Genomes, Phylogenetic Tree

Introduction

The milkweed genus *Asclepias* has long served as a model for studies of reproductive biology (Wyatt and Broyles 1994) and plant defenses (Agrawal and Fishbein 2008). Two American clades, the Sonoran Desert and temperate North American, contain up to 50% of the species' diversity and serve as the main focus of this experiment. Resolving evolutionary relationships in these clades is crucial to determining the origins and early diversification of the genus, as well as the milkweed species. (Fishbein *et al.* 2011).

Methods

Because this project has been ongoing over the course of several years now, my personal contributions up to this point have involved steps in sample preparation but not data analysis. My two biggest areas of focus have been in DNA extractions and sample preparation for genomic sequencing. In preparing the samples for sequencing, many steps involved purifying the DNA fragments prior to enzymatic modification.

For the DNA extraction portion, begin by weighing out 100 mg of plant material. Add 800 mL of CLS-VF and 200 mL of PPS to each sample and load the tubes into the Fastprep machine. Run at 6.0 for 40 seconds, cool samples and repeat. After allowing the samples to cool to room temperature after the second run, centrifuge them for 1 minute at 14,000 rcf. Discard the supernatant. Add 500 mL Sews-M to resuspend the solution and centrifuge again, discarding the supernatant afterwards. Do this until you have a dry pellet, allowing excess Sew-M to evaporate off. Add 100 mL TE buffer and lightly vortex, then heat for 5 minutes at 55° C. Place supernatant into a clean, labeled centrifuge tube to store at -20° C.

For the sample purification portion, begin by vortexing Sample Purification Beads (SPB). Add 80 mL of SPB to each 50 mL of fragmented DNA and pipette to mix. Place samples on magnetic stand, allowing SPB to separate from the supernatant. Once a clear distinction can be made between the beads and supernatant, carefully pipette out and discard supernatant without disturbing SPB. Add 300 mL of 80% ethanol to each well, allow SPB to reseparate if necessary, and remove without disturbing SPB. Repeat this step once more. Add 52.5 mL of Resuspension Buffer (RSB) to each well and remove from the magnet. Resuspend the beads by pipetting and transfer the solution to a well plate. Place the plate on a magnet, then proceed to transfer 50 mL from each well of RSB to a new plate without disturbing the suspended beads.

Results

Because of the large scope of this project, there have been no conclusive results with the samples I have worked with thus far. However, I have assisted in the running of a gel, which shows the relative size of the DNA in each sample.

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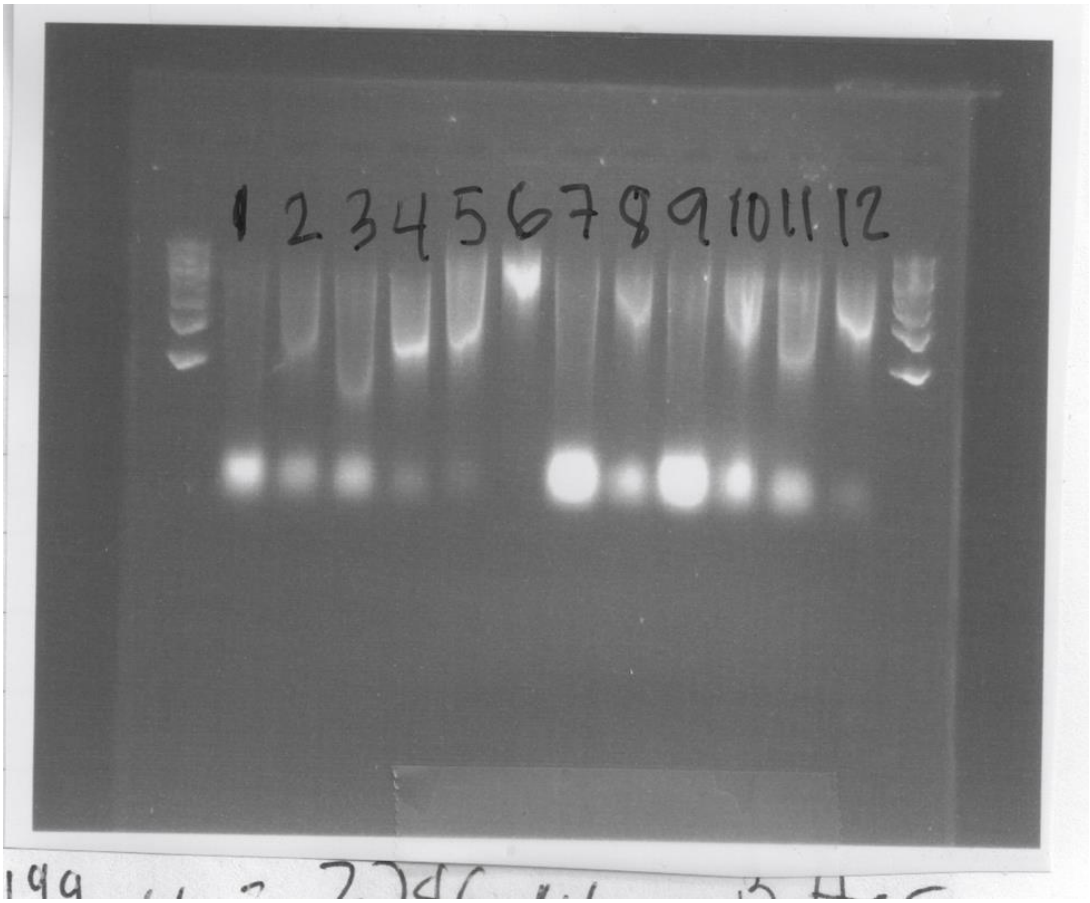

Figure 1 *- This gel shows 12 extractions from 6 samples. Rows 1-2, 7-12 are* A. subulata, *3-4 are* A. nyctaginifolia *and 5-6 are* A. albicans. *This was run after the DNA extraction but before sample purification.*

In Figure 1, a gel was run for 6 different samples, 2 extractions from each. The brighter spots near the top are where the larger fragments of DNA are found, which is what we are able to use for the sample purification and purification process. Further down each well is another bright patch, which is where the smaller DNA and RNA fragments are found. These two components together give us a relatively good idea of the size of the DNA we are looking at and if they are large enough to use in the next step.

Discussion

This research will continue to improve knowledge about phylogenetic relationships and the process of speciation. This knowledge has grown greatly in recent years because of modernized genomic sequencing technology. I am interested in personally continuing to do research and to learn about the different opportunities offered here at OSU as well as what lies beyond my time here. The aspirations of this team are to use the Hyb-Seq protocol, which allows simultaneous data collection for low-copy nuclear genes and high-copy genomic targets for plant systematics, to form a more detailed phylogenetic tree for *Asclepias* and similar species, identifying any tree incongruities and resolving them through the enrichment of nuclear genes and low-coverage sequencing (Weitemier *et al.* 2014).

References

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