Ferns as an Indicator Species of Soil pH, Moisture, and Nitrogen Levels in Northern New Jersey

Madeline Davis and Julia Smolyak, Tenafly High School

Cold Spring Harbor Laboratory DNA Learning Center

Funded by the Thompson Family Foundation

Abstract

Environmental factors, such as soil pH, nitrogen content, and moisture content, can serve as a limiting factor of plant survival and growth. Different species are capable of withstanding different environmental factors. The objective of this Urban Barcoding Project was to identify specific species of vascular plants that can serve as indicators of nitrogen, pH, and moisture levels in soil, while helping Tenafly Nature Center better describe its biodiversity. Samples of soil and ferns from 4 different areas within the Nature Center were collected. The ferns were tested for the species, and the levels of nitrogen, pH, and moisture were tested in the soil. We seeked to determine specific species that thrive in these various environments. Using a novel rapid extraction method, we obtained DNA from 26 fern samples and ran PCR with two primers, rbcL and ITS. Our samples were then sequenced and successfully identified the species of these fern samples.

Introduction & Goals

Thousands of different fern species fill various ecological niches:

- providing food and shelter to small animals,
- filtering toxins, including heavy metals, from the environment (important for the environment in Northern New Jersey due to its close proximity to New York City).

Environmental factors, such as soil pH, nitrogen content, and moisture content, can serve as a limiting factor of plant survival and growth. Different species are capable of withstanding different environmental factors. For example:

- Boston ferns (Nephrolepis exaltata) prefer pH of 5.0 to 5.5
- Dagger ferns (Polystichum acrostichoides) thrive in pH of 5.6 to 7.8.

Tenafly Nature Center, a 350-acre nature preserve in Northern New Jersey, has a list of the species of plants and fungi found in the preserve with names that don't entirely match. This Urban Barcoding Project could assist Tenafly Nature Center in better describing their biodiversity.

Figures 1 and 2: Examples of collected fern samples from Tenafly Nature Center

Materials & Methods

1. Collection

- Collected samples from 4 different locations in Tenafly Nature Center. Samples consisted of a piece of a fern, and the soil surrounding the plant.
- The soil was obtained using a sanitary shovel. All soil collected was 5 inches from the surface.
- Locations:
  - Northern Area: Pfister's Pond & the Lagoon
  - Southern Area: Middle Brook & Haring Rock

Samples were kept frozen until we went to the DNALC laboratory in Harlem.

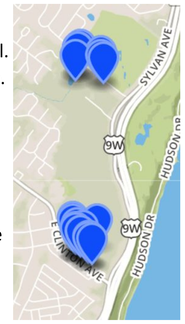

Figure 3: Map of the GPS coordinates of our collected samples from Tenafly Nature Center

2. Soil Analysis

Soil was tested for:

- pH (California Lab pH test kit)
- Nitrogen (California Lab nitrogen testing kit)
- moisture level (mass comparison).

3. Rapid DNA Extraction

- Lysis solution was added to test tubes filled with fern samples , and they were grinded in the solution.
- Punched piece of FTA paper was soaked in the solution.
- Wash buffer was added to a clean tube. The disc was transferred from the lysis solution tube to this r tube, and soaked for 5 seconds. The disc was slid up to the side of the tube to air dry for 2 minutes.
- The disc was transferred to a new tube with 30 µl TE, and was incubated for 15 minutes. We repeated these steps for each one of our samples.
- The samples were mixed with loading dye and put into a gel electrophoresis with rbcl and ITS primers. The gel was run and the samples were compared to ensure proper DNA isolation.

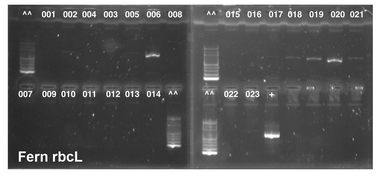

Figure 4: Gel electrophoresis results after using rbcL primer

4. Sequencing & Species Analysis

The samples were sent off to be sequenced, where they were organized and compared to sequences found of the DNAsubway.org website.

Results

Species	Soil pH	Soil Moisture (g)	Soil Nitrogen Content
Parathelypteris noveboracensis	- 6.5 - 6.0	- 2.8 - 2.17	- <0.5 - <0.5
Scutellaria galericulata	- 6.5	- 2.17	- <0.5
Osmundastrum cinnamomeum	- 6.5	- 2.11	- 2.0
Dennstaedtia punctilobula	- 6.5 - 6.0	- 1.99 - 2.14 Average: 2.065	- 0.5 - <0.5

Figure 5: Determined species of fern samples with their corresponding soil factors (pH, moisture, nitrogen content)

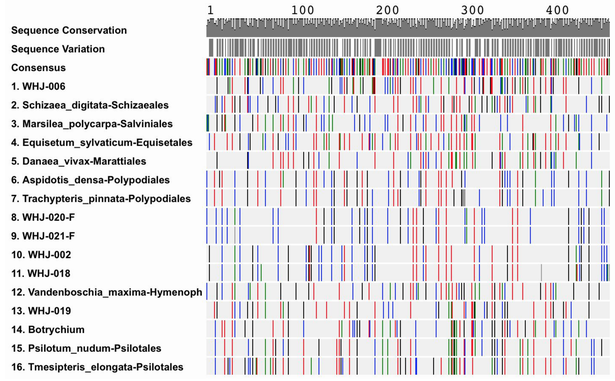

Figure 6: Results of DNA trimming and alignment over our fern samples and the DNA from common fern species from a database

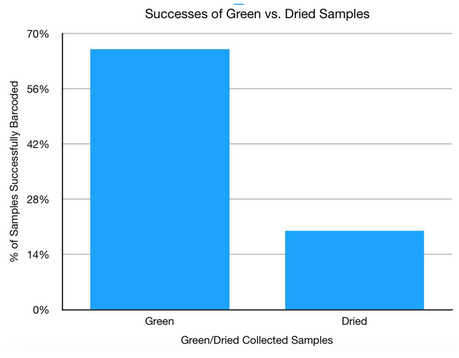

Figure 7: Bar graph showing the relationship with the green vs. dry status of samples and whether they were successfully barcoded

Discussion

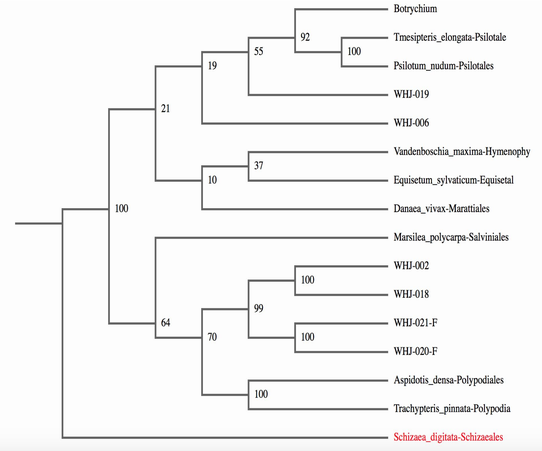

Figure 8: Phylogenetic tree of samples amplified

- After receiving our results, we were able to come to the conclusion that ferns can serve as an indicator of a soil pH of 6.0-6.5.
- The rbcl is a more successful and reliable primer when barcoding small vascular plants.
- In the future we plan to send our findings to the Tenafly Nature Center to correct their lists of biodiversity.

References

1. Fern Archives. (n.d.). Retrieved from http://www.jerseyyards.org/plant-type/fern/
2. Raine, R. (2016, October 07). What Types of Ferns Prefer Acidic Soil? Retrieved from https://homeguides.sfgate.com/types-ferns-prefer-acidic-soil-51940.html
3. Tenafly Nature Center - Home. (n.d.). Retrieved from http://tenaflynaturecenter.org/
4. Vernon, C., Armstrong, G., Davis, J., Wieting, J., Dallimore, K., Worthing, M., & Pearson, T. (2018, June 28). Ecosystem 101: The role of ferns. Retrieved from https://sierraclub.bc.ca/ecosystem-101-the-role-of-ferns/

Acknowledgements

We would like to thank Ms. Rubenchik for her guidance throughout the year. We would also like to thank Dr. Kennedy for assisting us with soil testing. Finally, we would like to thank Dr. Marizzi and Ms. Lee for providing us with the resources necessary to complete this project.