Pollen Shape: Invasive versus Native Plants of Long Island

Brooke Hopkins, Julianna Scianablo, Roisin Pfaff , Dr. Steven Gordon
Garden City High School

Funded by the Thompson Family Foundation

Abstract

In our research we planned to study pollen shapes that we collected. We researched articles including, pollen cell wall patterns form from the articles; Palynology, *Pollen grain surface pattern terminology, and* Pollen power. These articles helped us determine how pollen shape varies in different local species found at the Garden City Bird Sanctuary. In our experiment we barcoded both known plants, determined through the iNaturalist app and unknown plants that we collected. We conducted PCR from the rbcL gene to identify the plant species after sequencing of PCR products. The plant species identified were *Iris anguifuga, Narcissus tazetta*, the red deadnettle (*Lamium purpureum*), which is a common invasive species of the Northeastern United States, was misidentified as Iris, indicating that the wrong lane was sent for sequencing.Forward and reverse sequences yielded similar results but different variants. For example KXG-002 forward was *Narcissus tazetta* and KXG reverse was *Pancratium canariense,* the Canary Sea Daffodil We also used microscopes and computer-based image capture software to examine the pollen shape. We concluded that there was a variety of pollen shapes. The distribution of pollen may also be found to play a role in which insects are attracted to certain plants. In the future, if the non-native species are attracting more insects than it is evident they are invasive and outcompeting the native plants. This may prove beneficial to society as researchers are interested in the dwindling native plant and insect species of Long Island. This research will help determine the impact that non-native insect species are having on native plant population of Long Island. It is of interest if the reason that plants attract more pollinators is due to their pollen shape, especially if non-native plants could have more attractive pollen shapes then native plants. The native foliage of Long Island is important to preserving biodiversity and maintaining food chains.

Introduction

The article, "*Physics explains how pollen gets its stunning diversity of shapes* discusses" different types of pollen. Using computer simulations of the process, scientists reproduced the shapes of lumpy, patterned pollen, which make up roughly 10 percent of the pollen from cataloged flowering plant species *(A. Radja et al.)*.

The independent variables discussed in this article include: developing pollen grains, a material called primexine clumps together into regions with different densities, confined inside a temporary cell wall, that lumpy material creates ripples in the cell membrane, which remain after the original cell wall dissolves. There were no dependent variables described in the article.

The article, "*Pollen Power"* evaluates how plants attract pollinators. They do this using color, and shape. Additionally, plants also use petal patterns to guide insects towards the pollen. "*Pollen Power"* also explains how plants also may use aromas to attract insects. The most important point of this article is the explanation on how plants attract pollinators. We plan on evaluating which pollen shape insects are attracted to.

We will then investigate whether these plants are native or invasive. It is important to understand pollen shape in order to make conclusions about which insects are more attracted to certain types of pollen. The independent variables discussed are color, shape, aroma, and petal shape and the dependent variables are the species of insect attracted to plants.

The article, "*Palynology"* explains the study of fossil pollen extracted from lake sediment, peat bog, or other matrices. This will help us understand how animals play a role in pollen distribution for our research. Palynology is also used to help reconstruct the probable habitats and foods of ancient humans and of wild animals. The independent variable is the type of pollen, and the dependent Variable and the dependent variable is the type of species attracted.

The Article, "*Pollen Grain Surface Pattern Terminology"* explains all of the different kinds of pollen shapes. We need to know these for when we look at the pollen under the microscope. The most important point of this article is the explanation of the different pollen shapes including pilsate, perforated, foveolate, and scabrated pollen; this will be helpful in furthering our understanding of plants and pollen shapes. The independent variables discussed include texture of pollen, shape of pollen and size of pollen and the dependent variable is dispersion of pollen. All these articles explain pollen shape, and different types of pollen. We are trying to figure out if different shapes of pollen will attract different types of pollinators, these articles should help us with our research and experiment. Overall these articles will help us understand the patterns of pollen distribution and pollen shapes for our experiment for the Urban Barcode Project, evaluating plants and their pollinators in Long Island.

Materials & Methods

Methods: Plants are collected from the Garden City Bird Sanctuary. Three different plant species were collected and then inputted into the DNA barcoding database to determine their species. Any plants were preserved in freezers to maintain the pollen shape and DNA. The plants were cut using a scalpel to cut them at the stem from the ground. The next phase is pollen collection from the plants. Then pollen was observed under the microscope. Next DNA barcoding was run on the insects. Based on the results it was determined how the plants native or non-native to Long Island compared.

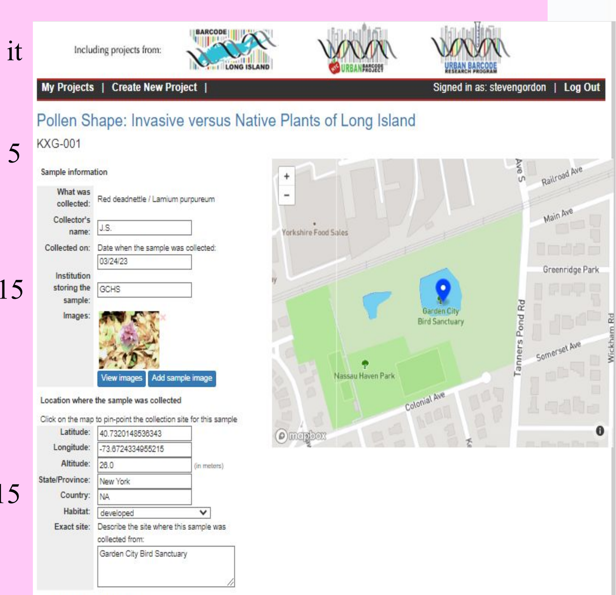
Materials: Dyes, trays, power supplies, electrodes, cables, gel mixtures, 3 plant samples, Scalpel

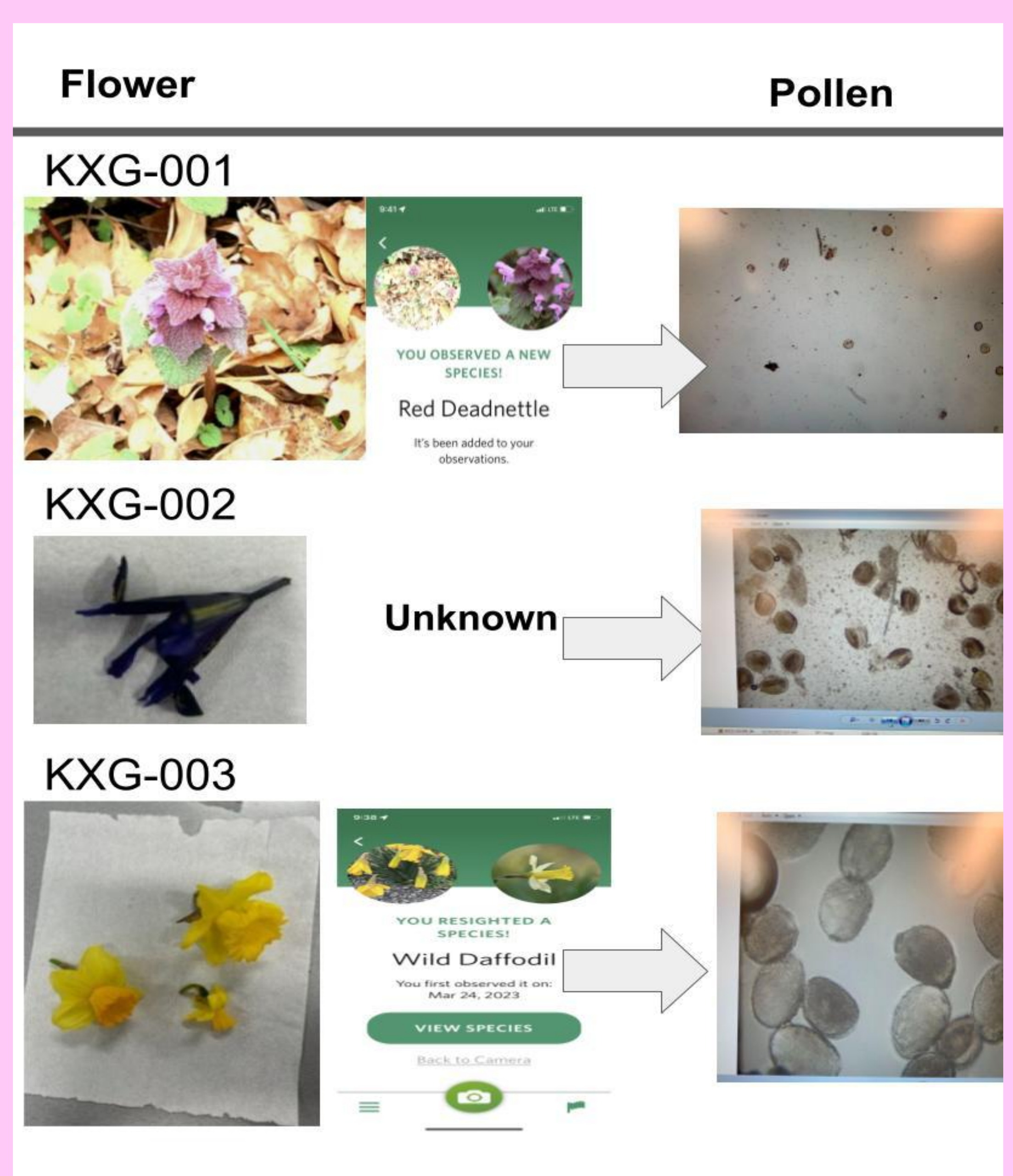
DNA Isolation Methods:

1. Measure 50 microliters of lysis buffer
2. Take sample pour into lysis buffer
3. Take pestle and grind for 2 minutes; after throw out pestle
4. Take filter paper (binds to DNA) and tap paper in lysis solution (so it absorbs DNA)
5. Wait 1 minute and let disc soak up DNA
6. Measure out wash buffer; then transfer paper to wash bufferTap for 5 seconds and soak for two minutes
7. in wash buffer to wash out impurities
8. Let filter paper dry on top of tube
9. Add filter paper to purified water (sit in fridge overnight) *or 15 minutes at room temperature
10. Label tubes (initials #)

PCR methods:

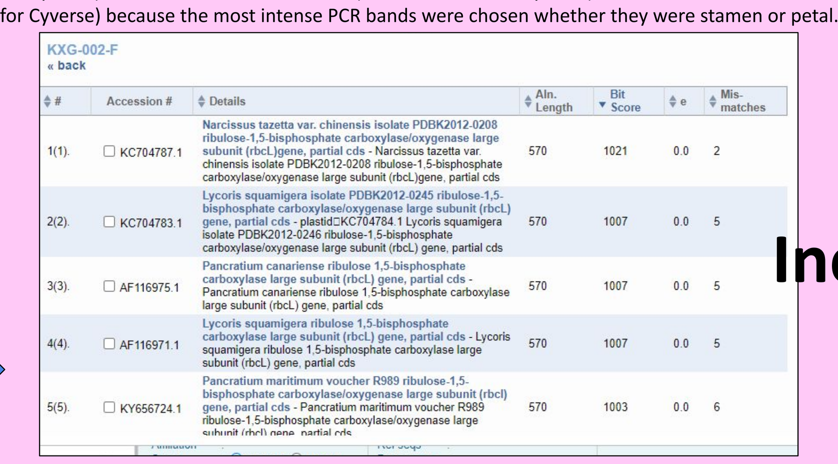
1. FIll tubes with 23 μL primer and 2 μL of DNA
2. 1 minute at 94℃ to melt DNA for 1 cycle
3. For 35 cycles: 94℃ for 15 seconds (denaturation); 54℃ for 15 seconds (annealing); 72℃ for
4. 30 seconds (extending)
5. Set to 4℃ for 59 hours and 59 seconds (cooling)



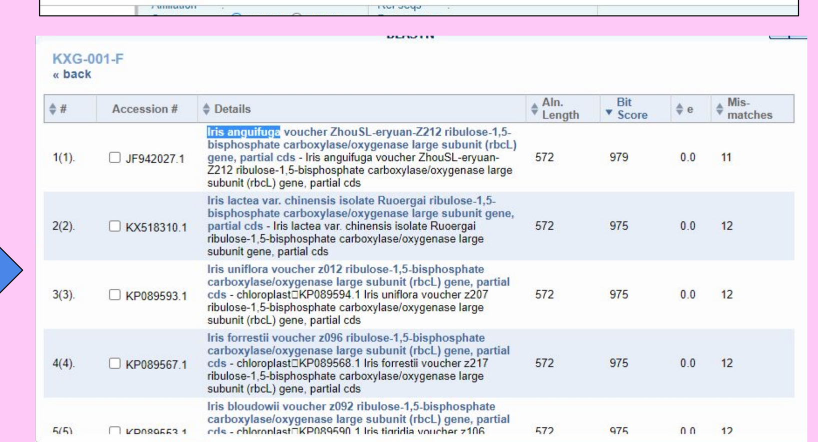


Results

Note: DNA sample codes do not correspond to specimen codes because well 5 was used for KXG-001 (labeled AF-002 for Cyverse), well 3 was used for KXG-002 (labeled AF-001 for Cyverse), well 7 was used for KXG-003(labeled AF-003 for Cyverse) because the most intense PCR bands were chosen whether they were stamen or petal.



Incorrect



Correct

Correct

Discussion

Based on the findings of this experiment the lengths of the DNA displayed in the gel electrophoresis were very similar across all species. This proves that similarly constructed plants thrive in the same area. The slight advantages of some invasive species might make them more suitable to the environment. However, based on these results the plants have very similar molecular characteristics. The differences lay in their pollen shape. For this reason it makes it interesting that pollen shape could possibly impact the success of invasive species. However it is difficult to gather enough information on this in a school setting making a limitation on this research.The red deadnettle is called "a beautiful early bloomer for hungry bees", thus we did find a species in late March that could attract pollinators later in the season as we observed no bees. source:https://www.honeybeesuite.com/honey-bee-forage-red-deadnettle/

Identified Plant based on DNA Barcoding Database	Date Located	Location of Plant	Organismal DNA Identified on Gel Electrophoresis
Daffodil	3/24/2023	GC Bird Sanctuary	Daffodil
Invasive	3/24/2023	GC Bird Sanctuary	Red Deadnettle through iNaturalist tube switched?with Iris?
Unknown	3/24/2023	GC Bird Sanctuary	Iris

References

1. A. Radja *et al*. Pollen cell wall patterns form from modulated phases. *Cell*. Vol. 176, p. 856, February 7, 2019. doi:10.1016/j.cell.2019.01.014.
2. Palynology. (2014). In K. L. Lerner & B. W. Lerner (Eds.), *The gale encyclopedia of science* (5th ed., Vol. 6, p. 3233). Gale.
3. *Pollen grain surface pattern terminology*. (n.d.). Retrieved October 3, 2022, from https://research.fit.edu/media/site-specific/researchfitedu/applied-biogeography/documents/protocols/Quick-Reference-Glossary-with-Illustrations.pdf
4. Shimizu, H. H. (2011). Pollen power. *National Geographic Explorer*, *10*(6), 2.
5. Katifori, E., Alben, S., Cerda, E., Nelson, D. R., & Dumais, J. (2010, April *Foldable structures and the natural design of pollen grains*. PNAS.org. Retrieved October 6, 2022, from https://www.pnas.org/doi/10.1073/pnas.0911223107
6. Hörandl, E. (Ed.). (2021, February 18). *Pollen morphology and variability of polish native species from genus salix L.* National Library of Medicine. Retrieved October 6, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7891718/

Acknowledgements

Thank you Dr.Gordon and Garden City High School for supporting our research and funding our work.Thanks to Cold Spring Harbor laboratories, DNALC NYC, and Arden Feil for providing us with the opportunity to be a part of the Urban Barcode Project and Symposium.