KYHTL at OPaL: Developing Skills in Melissopalynology in Support of Regional Beekeepers

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What is KYHTL?

The Kentucky Honey Testing Laboratory (KYHTL) is a new venture at Bluegrass Community and Technical College that helps honey producers assure the quality of their product and provide certification required for compliance with truth-in labeling guidelines. KYHTL is the first project to utilize melissopalynology in support of DNA metabarcoding to identify pollen in honey samples.
This endeavor aims to bring the US closer to Jones and Bryant's (1980) goal of having a data base of the compositional properties of honey in our 50 states and eventually for individual regions.

KYHTL's Process

Melissopalynology
The study of pollen in honey to identify major nectar contributors to the end product. It has only been used regularly in the United States since the mid-1970's. We use melissopalynology to optically identify pollen through microscopy.

Honey Typing
We use the data collected from melissopalynology to pollen type the honey. These are like fingerprints unique to the region the honey was collected from. By comparing our results with the pollen profile of the region a honey producer claims they collected their honey from, we could either confirm or reject these claims.

DNA Metabarcoding
KYHTL's main lab at Bluegrass Community and Technical College (BCTC) uses DNA metabarcoding to identify the pollen and establish a honey type.

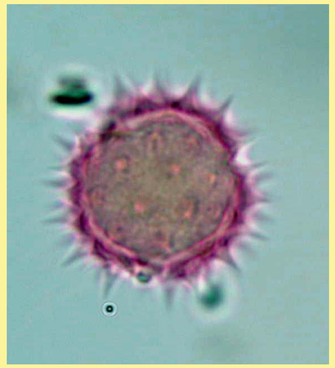
Comparison
During the establishment phase, KYHTL is using a double-blind comparison, meaning neither lab knows where the honey samples originated from nor what pollen it contains. So, after both labs have developed a honey type, we compare the results to ensure accuracy.

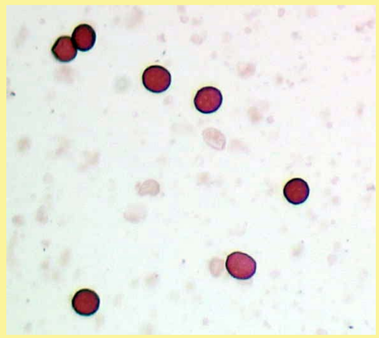
My Process

Learning
For the first couple months of research, I was learning not only how to use the microscope, but also how to optically identify pollen. I started by learning how to identify apertures, which categorize pollen using the types of openings it has, such as pores (holes) and/or colpi (slits). Then, I learned how to identify ornamentation, which further categorizes pollen based on how the outside of the grain is decorated. After that, I was finally able to move on to identifying the family or genus of individual pollen grains.

Counting
After learning to identify pollen grains, I began to count them. Counting quantifies the identified pollen grains (and spores used as markers) in the sample to determine pollen concentration (as recommended by Lieux, 1980) and collecting this data in a spreadsheet. Knowing how much to count in each sample is tricky. In the OPaL lab, we use a rarefaction curve, (Figure 1). When the number of unique grains counted stops increasing relative to the total number of pollen grains counted, we can stop counting as we have captured the sample's diversity and concentration.

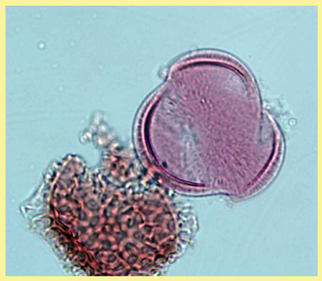
This *Helianthus* pollen grain has echinate ornamentation and tricolporate aperturation. Tricolporate types have three pores and colpi, often occurring together.





This is an example of the microscopic view of a honey sample after processing. This sample is unifloral with the pollen pictured a part of the family Chenopodiaceae

This *Acer* grain is a striate, tricolpate type. Pictured with it is a *Lycopodium* spore, which is used as a control for the counting and typing process.



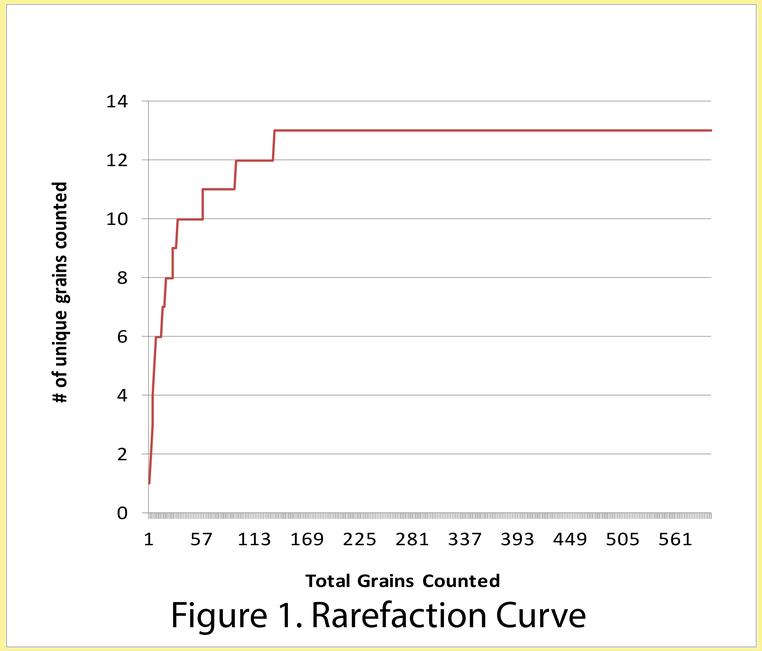


Figure 1. Rarefaction Curve

Typing
Using the data we collected during the counting process, we were able to pollen type the honey. Typing honey entails categorizing it by pollen concentration and diversity. We categorized honey according to Maurizio's (1975) recommendations, with Category I having the lowest concentration (0-20,000 grains per 10 grams) and Category IV having the highest (500,000-1,000,000 grains per 10 grams). Following Louveaux's (1978) recommendations, the pollen present in the samples were classified as predominant, secondary, important minor, and minor pollen types.