To uncover what plants honey bees rely on, researchers from The Ohio State University are using the latest DNA sequencing technology and a supercomputer. They spent months collecting pollen from beehives and have developed a multi-locus metabarcoding approach to identify which plants, and what proportions of each, are present in pollen samples.

A single beehive can collect pollen from dozens of different plant species, and this pollen is useful evidence of the hive's foraging behavior and nutrition preferences.

"Knowing the degree to which certain plants are being foraged upon allows us to infer things like the potential for pesticide exposure in a given landscape, the preference of certain plant species over others, and the degree to which certain plant species contribute to the honey bee population."
diet," says graduate student Rodney Richardson. "One of the major interests of our lab is researching honey bee foraging preferences so we can enhance landscapes to sustain robust honey bee populations."

For Richardson and his colleagues, metabarcoding is key to this research. It is a DNA analysis method that enables researchers to identify biological specimens.

Metabarcoding works by comparing short genetic sequence "markers" from unidentified biological specimens to libraries of known reference sequences. It can be used to detect biological contaminants in food and water, characterize animal diets from dung samples, and even test air samples for bacteria and fungal spores. In the case of pollen, it could save researchers countless hours of identifying and counting individual pollen grains under a microscope.

Richardson and his colleagues devised the new metabarcoding method using three specific locations in the genome, or loci, as markers. They found that using multiple loci simultaneously produced the best metabarcoding results for pollen. The entire procedure, including DNA extraction, sequencing, and marker analysis, is described in the November issue of Applications in Plant Sciences.

To develop the new method, the researchers needed a machine powerful enough to process millions of DNA sequences. For this work, the team turned to the Ohio Supercomputer Center.

"As a researcher, you feel like a kid in a candy store," Richardson says. "You can analyze huge datasets in an instant and experiment with the fast-evolving world of open source bioinformatics software as well as the vast amount of publicly available data from previous studies."

In previous metabarcoding experiments, the researchers worked solely
with a marker found in the nuclear genome called ITS2. ITS2 successfully identified plant species present in pollen samples, but it could not produce quantitative measurements of the proportions of each.

While searching for something better, they decided to test two markers from the plastid genome. Pollen was previously thought to rarely contain plastids, but recent studies showed promise for plastid-based barcoding of pollen. Richardson and his colleagues found that the combined data from the two plastid markers, \textit{rbcL} and \textit{matK}, successfully correlated with microscopic measurements of pollen abundance.

The new multi-locus metabarcoding method involves all three markers and could serve as a valuable tool for research on the native bee species that comprise local bee communities.

"With a tool like this, we could more easily assess what plants various bee species are relying on, helping to boost their populations as well as the economic and ecological services they provide to our agricultural and natural landscapes." Richardson says, "While the \textit{honey bee} is seen as our most economically important pollinator, it's only one of several hundred \textit{bee species} in Ohio, the vast majority of which are greatly understudied in terms of their foraging ecology."


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