



Notes

from the Lab:

The Latest Bee Science Distilled

by Scott McArt

Most beekeepers probably think they know where their honey comes from. For example, when goldenrod blooms around the Dyce Lab in upstate New York in September, I'm pretty sure that's what my bees are visiting and turning into "goldenrod honey." During apple bloom in May, it's "apple blossom honey." In November and December in New Zealand, it's "manuka honey."

But what do we really know? How much goldenrod nectar is actually in "goldenrod honey?" How much manuka nectar is in "manuka honey?" And are we naïve to think

that bees are only collecting plant nectar to make honey? What about other sources of sugar, such as honeydew from insects? These are the topics for our tenth "Notes from the Lab," where we highlight **"Entomological signatures in honey: an environmental DNA metabarcoding approach can disclose information on plant-sucking insects in agricultural and forest landscapes,"** written by Valerio Utzeri and colleagues and published in the journal *Scientific Reports* [8:9996 (2018)].

The question of where honey comes from is not new. In fact, there's a longstanding field of study on the

topic called melissopalynology (from the Greek words *meli* and *mellisa*, meaning "honey" and "bee," and *palynology*, the "study of pollen"). Melissopalynologists spend lots of time at the microscope looking for pollen grains in honey. Those pollen grains, which are picked up by accident as the bee forages for nectar, provide clues to the honey's origin, since pollens from different plants can look very different.

But melissopalynology is not an exact science. Sometimes pollens from different plant species look similar under the microscope. Also, because bees accidentally pick up different amounts of pollen at different plant species, it's difficult to estimate exactly how much nectar a particular plant species contributes to the honey. And to be honest, it is a little odd to focus on pollen while trying to figure out where the nectar comes from. Why not just look at the honey directly?

Recently, major improvements in DNA sequencing allow us to look directly at honey to infer its origins. In fact, the whole field of palynology is going through a bit of an upheaval as DNA metabarcoding stands to replace traditional palynology approaches. But what new insights can these metabarcoding methods provide? This is the question Utzeri and colleagues were interested in pursuing.

For their study, Utzeri and colleagues purchased 13 different honeys from Italy, France and Eastern Europe. They extracted DNA from the honeys, amplified the DNA by a process called PCR, then sequenced the



Valerio Joe Utzeri, lead author, sampling a honey for DNA metabarcoding analysis.



One of the jars of honey the scientists analyzed.

PCR products (essentially lots of copies of the original DNA) with a new DNA sequencing technology. Then the real work began. Because the new sequencing technology yields lots and lots of data, the authors spent lots and lots of time putting the DNA puzzle together. They assembled the DNA sequences from the honey and checked them against known DNA sequences from plants and animals.

So what did they find? Is honey coming from where beekeepers think it's coming from? To a large degree, yes. For example, "chestnut blossom honey" had 66% of its DNA sequences match with the plant genus that includes chestnut. "Acacia honey" (produced from the *Robinia pseudoacacia* tree, known in the US as black locust) had 41% of its DNA sequences match with acacia. "Apple blossom honey" had 35% of its DNA sequences match with the plant family that includes apple. And "honeydew honey" – honey that bees supposedly collect from the sugary frass (i.e., poop) of insects such as aphids – did indeed have DNA sequences match with aphids and other sap-feeding insects.

But there was also an interesting surprise. It wasn't just the "honeydew honey" that had sap-feeding insect DNA. In fact, all of the honeys had sap-feeding insect DNA, including the 5 "honeydew honeys" and 8 "blossom honeys."

Wait, does that mean lots of honey is actually comprised of insect poop, not plant nectar? That's prob-

ably correct. While it's not possible to say exactly how much insect frass was in the honeys, the quantity of sap-feeding insect DNA was often very high, sometimes even higher in "blossom honeys" compared to "honeydew honeys." In addition, many of the honeys had DNA from a large diversity of sap-feeding insects. For example, "apple blossom honey" contained DNA from 14 sap-feeding insects. The French "wildflower honey" contained DNA from no fewer than 17 sap-feeding insects! These results are really fascinating since they suggest honey bees are not just foraging at flowers when they look for sugar. Indeed, every single honey tested by the authors probably contained sugary sap-feeding insect frass.

What about quantity? We still can't tell exactly how much nectar is coming from particular plants (or animals) with the metabarcoding approach? Unfortunately, that's correct. Just like traditional melissopalynology, metabarcoding still isn't quantitative. And this is a very interesting topic for both fundamental and applied reasons.

For example, I'm sure you're curious to know how much insect poop is in your honey. But on a more practical level, sometimes it's important to know how much nectar is coming from a particular origin, for example in the case of manuka honey. Beekeepers in New Zealand can sell their honey for a much higher price when it's comprised of more nectar from the manuka plant. And this is

major money we're talking about! Last time I was in New Zealand, it was common to see small 500 g jars of "high percentage" manuka honey being sold for over \$200. So how do we know that the honey contains a "high percentage" of manuka? There are some markers used to define manuka honey based on purity, allowing packers to export it as mono or multifloral manuka honey. Unfortunately, there's still a fair amount of uncertainty due to the limitations with metabarcoding, melissopalynology and other chemical methods.

With the rapid improvements in DNA sequencing technology nowadays, this is a topic to keep tabs on if it interests you. There are several labs working to improve metabarcoding methods, and in the process, the quality of information for beekeepers and regulatory agencies. For my part, I'm pretty curious to know whether a large portion of the honey I eat is from insect poop.

Until next time, bee well and do good work,

Scott McArt

REFERENCE:

Utzeri, V. J., G. Schiavo, A. Ribani, S. Tinarelli, F. Bertolini, S. Bovo and L. Fontanesi. 2018. Entomological signatures in honey: an environmental DNA metabarcoding approach can disclose information on plant-sucking insects in agricultural and forest landscapes. *Scientific Reports* 8: 9996. <http://www.nature.com/articles/41598-018-27933-w>

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