RESEARCH

DNA TESTING: A COMMON WAY TO LEARN ABOUT YOUR BEES AND YOUR HONEY

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This article gives a brief introduction to DNA testing for those who wonder how it works.

Abstract

All living organisms have unique DNA which can be used to identify them, and DNA testing checks whether these unique pieces of DNA are present in samples. It is used in apiculture in a range of ways—examples include identifying mānuka pollen in honey, and checking for a range of diseases or pathogens found in bees, brood or honey.

Importantly, DNA testing is badly affected by cross-contamination, and anyone submitting samples for DNA tests should take care when collecting them to avoid this.

DNA is in all living things, and is a blueprint for life

DNA is found in the cells of all living organisms. It is made up of building blocks called bases, and the order of these bases in DNA forms the blueprint for that organism to develop and grow. The effective regions of DNA are called genes, which are the parts of DNA that are used to produce the proteins that are essential to that organism's life.

DNA testing works because all living things are different at the level of their DNA. The testing looks for genes (or other parts of DNA) which are unique to a particular organism—when the testing finds that unique DNA in a sample, we can confidently say that organism is present. The more copies which are there, the larger the number of organisms that must be there.

Polymerase chain reaction (PCR) is an important way of doing DNA testing

The basis of many DNA tests which are carried out is a technique called PCR. It is a very clever piece of science which allows a laboratory to target genes of interest in a sample and multiply them up to make them easy to detect. It was the invention of an American scientist in the early 1980s, who was awarded a Nobel Prize for it in 1993.

Before a lab can use PCR, it first prepares the sample so that any DNA has been released from the cells which may contain it—animal/insect/plant cells, pollen grains, and bacterial cells or spores are all examples. Then some special reagents are added to the sample, and the PCR process can begin. PCR takes place as repeating cycles of three steps:

1. the sample is heated, causing any DNA in there to separate into single strands (its natural state is to be in a stable double strand)
2. the sample is cooled. Special DNA probes target unique pieces of DNA found only in the organism(s) you have an interest in, then attach to the single-stranded DNA in the sample if DNA from those organisms is present
3. an enzyme called 'polymerase' then causes an extra copy of the piece of DNA that the probe has attached to be created.

So, after one cycle of PCR testing:

- if there are none of the organisms of interest in the sample, no probes will have attached to the DNA, and no copies of the DNA of interest will have been produced
- if there are organisms of interest in the sample, there will be twice as many copies of the target pieces of DNA at the end of that cycle than there were at the start.

The PCR process is then repeated for up to 40–45 cycles, with the number of copies of the target DNA doubling each time. The amount of DNA present at the end of

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the process can then be used to work out whether (1) the organism of interest is in the sample and (2) how many of them there are.

Sometimes you will see a Cq value reported from a DNA test, which is the number of PCR cycles that it took for the amount of target DNA in the sample to reach a threshold defined in that particular test. For example, the MPI mānuka pollen DNA test specifies a Cq value of 36. This means that the amount of mānuka DNA in the sample must meet the minimum threshold set by MPI in the test within 36 PCR cycles.

Cross-contamination is a big problem with PCR testing

PCR testing multiplies the number of copies of the target DNA in a sample hugely during a test. If there is one copy of a target piece of DNA at the start, after 30 cycles there will be over one billion copies of that piece of DNA (2 to the power of 30). For this reason, any cross-contamination between samples can easily produce false results.

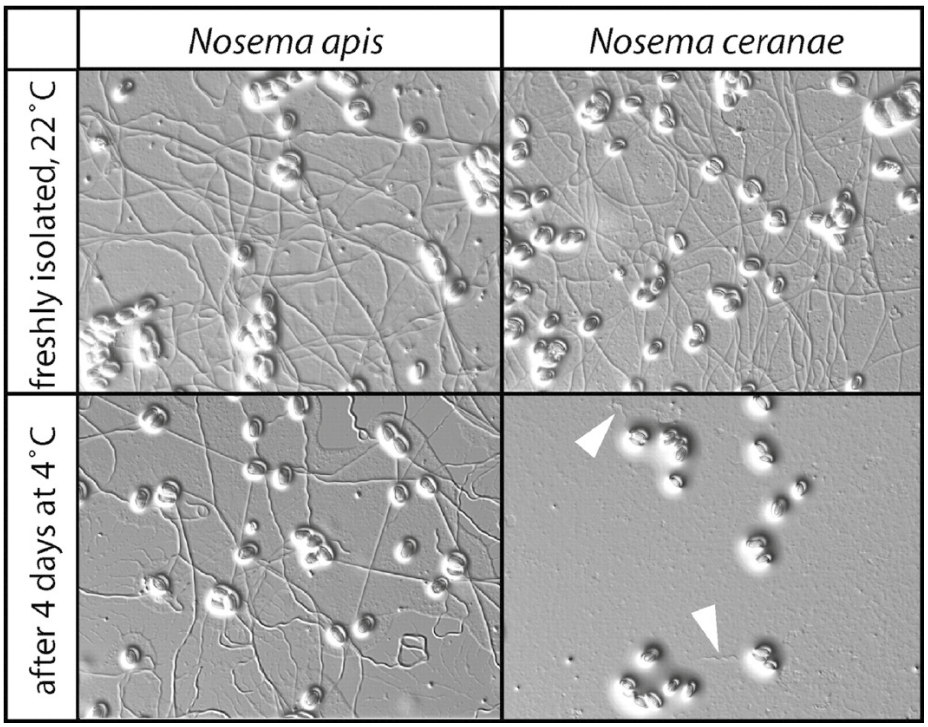
Labs doing DNA testing are very careful to avoid cross-contamination between samples, and keep their work areas clean of DNA. Sample collection is another significant risk of cross-contamination creeping in.

DNA testing is already used in apiculture in a range of ways

Some examples of DNA tests used in apiculture in New Zealand include:

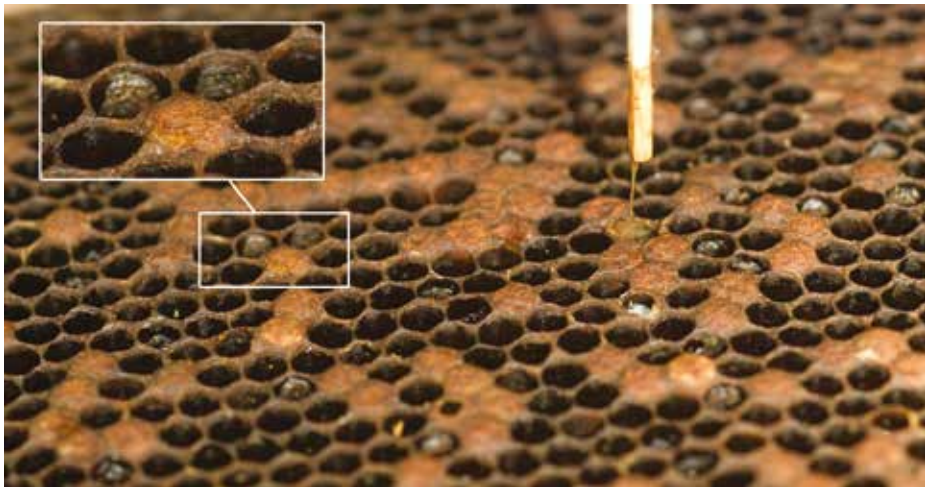
- the mānuka pollen DNA test, which is one of the five attributes specified in MPI's definition of mānuka honey. As its name implies, this test looks for DNA that is uniquely found in mānuka pollen grains to confirm that sufficient mānuka pollen is present in the sample.
- American foulbrood (AFB), which can be tested for in brood, bees, or honey
- other organisms that affect bee health, such as *Nosema ceranae, Nosema apis* and *Lotmaria passim*
- viruses that affect bees.

The technology used in DNA testing continues to advance at an incredible rate. It won't be surprising to see more tests becoming available in future at prices that are affordable for beekeepers and honey processors to use for hive health and product quality purposes.

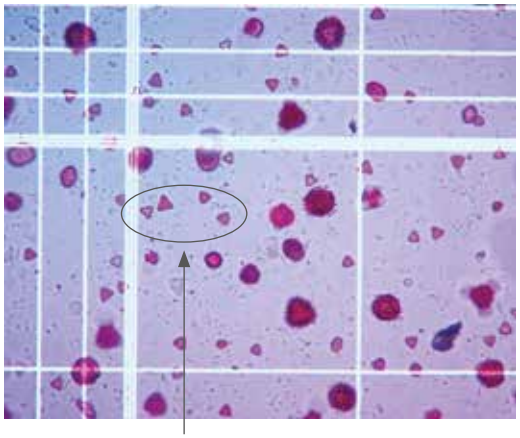


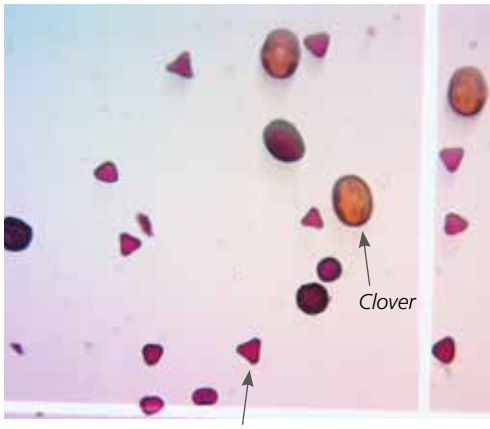
In vitro germination of N. apis *and* N. ceranae *spores. Source: Sebastian Gisder et al.* Appl. Environ. Microbiol. 2010;76:*3032-3038. Copyright © American Society for Microbiology.*

American Foulbrood symptoms: Ropey dead larvae and black scale. Photo: Michael E Wilson, provided courtesy of Bee Informed Partnership, Inc.



Pollen grains recovered from honey and observed under the microscope.





Mānuka/kānuka (can't tell the difference easily, need more sophisticated microscopy analysis or DNA!) Images provided by Analytica Laboratories.