



The pinTest for hygienic brood

Anna-Carina Schmidt

Imagine a busy honey bee city, teeming with life and laden with honey, where every bee goes about its business. But danger constantly lurks in this seemingly perfect world – among them pathogens, which can only be countered through social immunity. But how do I know if my bees are hygienic and spot and dispose of varroa in the brood? My bee buddy Billy Shepherd and I found out with the pin test.

Social immunity in honey bees is a collective defence system that protects the colony from diseases and parasites by preventing the spread of pathogens. This behaviour is carried out by the worker bees operating in groups to detect, open and remove diseased or damaged brood, and is described as hygienic behaviour (HYG+).

HYG+ is thought to be one of the main factors of genetic resistance in queen selection and breeding. However, while genetics play a crucial role, other factors like stress, hive strength and nutrition also influence hygienic behaviour. A struggling colony, even with a strong genetic predisposition, may lack the resources to mount a strong defence against pathogens.

Isn't that why we give medication, you might ask yourself? Well, in my opinion, beekeeping now and in the future should involve minimally invasive interventions in the bee colony to deal with parasites and diseases. Through targeted selection, we could restore the honey bee's ability to heal itself. This would lead to healthier bee colonies, fewer losses and higher yields.

Genetical resistance in queen breeding

My bee buddy Billy Shepherd and I wanted to find out which of our hives with favourable records have a trait of HYG+ so that we could breed our own strong and local queens. We decided to try the pin-killed brood assay to attempt to identify colonies that had a genetic predisposition to combating pathogens.

The test involves selectively killing individual pupae, triggering the brood-hygienic bees to respond. If a colony demonstrates a swift and thorough removal of the dead brood, it signifies a strong colony, genetically predisposed to hygienic characteristics.

In its original form, the pinTest was first performed in 1986 by John Newton and Harry Ostasiewski Jr with a single needle pin. In 2019, Lutz Eggert, a German queen breeder and lecturer patented the so-called Weimar needle stamp or pinTest, which won the Apimondia bronze prize for innovation in 2022. With 50 pins, it is efficient and ideal to test a large number of colonies. The parts are individually replaceable and made from an alcohol-resistant material.

Our experience with the pinTest

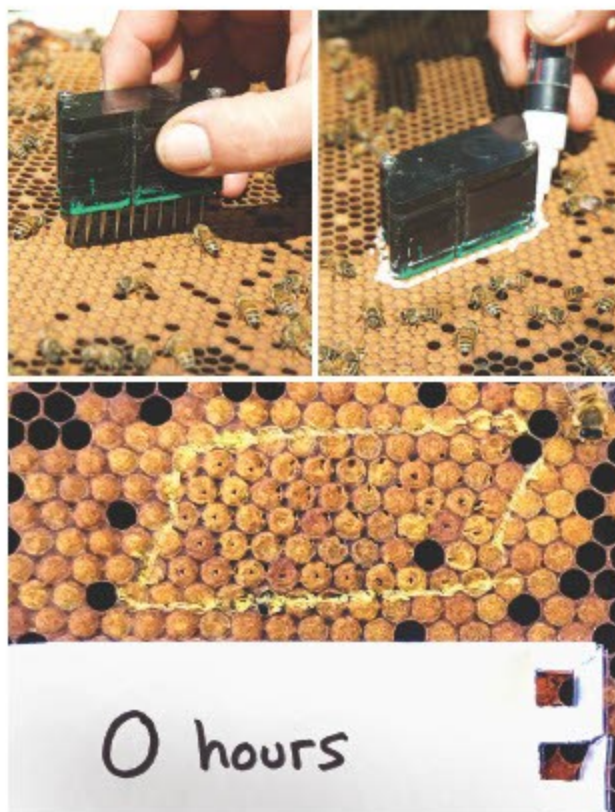
You can test at any time if there is sufficient brood pupae in the pink-purple eye state. Ideally, test all hives in spring, shortly before the start of foraging and again after the honey harvest. But keep in mind that the test cannot be used for high-precision scientific recording as it inevitably includes faulty cells.

Application

- 1 Choose a frame with a dense brood pattern in the middle-aged brood stage with no empty cells if possible. Lay the frame down.
- 2 Position the Weimar needle stamp so that the needles point at the middle of the cell.
- 3 Gently push the stamp as far as possible and mark the test area and frame with a water-based lacquer pen.
- 4 Place a note (stating hive name or number and time 0–6–24 hrs) next to the marked area and take a picture before putting the frame back in its original position.

Important: To avoid cross-contamination, press the needle stamp into an alcohol-soaked sponge after every use and let the alcohol evaporate for at least three minutes (use a solution with a minimum of 75% alcohol).

- 5 Take a second picture of the same area and the notepad after six hours and a third after 24 hours.



Interpretation

The percentage of the cells is then calculated for each photograph. If a bee colony possesses a high degree of the genetic trait of brood hygiene (recognising, opening and cleaning out), this will have been happening:

After about 30 minutes

the bees close the pierced cells again.

After 2–3 hours

the bees recognise the dead brood and open the cells.

After 6–9 hours

the cells are partially cleared and cleaned.

After 20–24 hours (at the latest)

80–100% of the cells are empty.

Possible outcomes

Nothing is cleared

It is not advisable to breed from this colony.

Little is cleared

HYG+ seems to be present and can be improved by breeding and adding traits via drones.

A lot is cleared

F1 can be bred to stabilise and maintain the properties.

In addition to the genetic factors influencing brood hygiene and the test result, there are other internal and external stress factors such as extreme temperature, the wrong disinfectant or testing at the wrong brood stage.

Our results

After six hours our bees would have closed the pierced cells – ideally, some would have been cleared and cleaned. So we were positively surprised when after 24 hours 70–80% of the cells had been opened and emptied by the bees. As you can see in the image taken by the pinTest inventor Lutz Eggert, bees with a high HYG+ trait can clean out 100%.

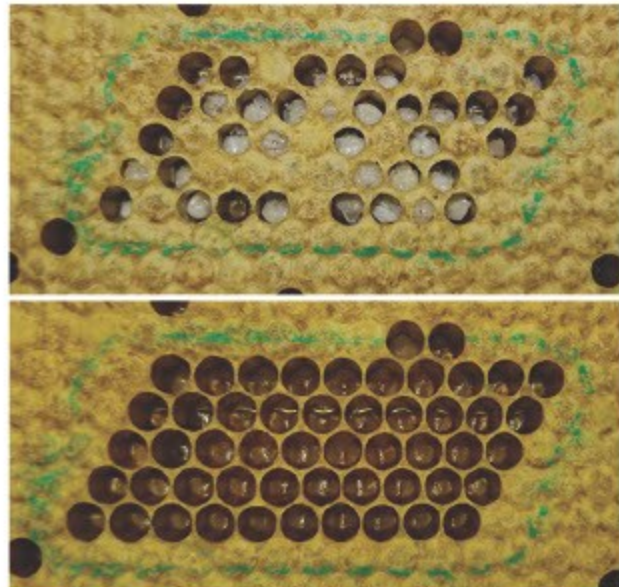
Unfortunately, we could not assess all our preferred hives because of the wet Scottish summer of 2023, but we are looking forward to rerunning the pinTest this year.

Billy and I think that the Weimar needle stamp offers beekeepers a valuable tool for identifying hygienic behaviour by revealing the resilience potential of a queen's lineage. But keep in mind, the needle stamp is not a silver bullet – it is one piece of a larger puzzle. Good hive records are also essential to selection process. By understanding both the genetic influence and environmental factors, beekeepers can nurture resilient colonies and, by providing optimal conditions, can also help them thrive with as little medication as possible.

When a pupa is punctured, a fluid consisting of haemolymph, body fat cells and other organic material emerges. However, the organic material, which may also reach the cell cap via the needle, has no falsifying influence on the result of the pin test.¹



Images showing test in Anna-Carina's hives



Test performed by Lutz Eggert with HYG+ bees after 6 and 24 hours

REFERENCE

¹Gramacho K et al (1999). Influence of body fluid from pin-killed honey bee pupae on hygienic behavior. *Apidologie* 30(5):367–374
<https://doi.org/10.1051/apidoc:19990502>

English information website: pin-test.com/en



For more than two years, Anna-Carina Schmidt has been learning under the mentorship of Billy Shepherd, caring for 10 hives on the Ochil Hills, north of the Forth Valley in Scotland.