Measuring Varroa Sensitive Hygiene

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Varroa sensitive hygiene (VSH) is a heritable trait of the honey bee that controls varroa. This trait can be added to any population of bees and may already be present in many others, but only by knowing how to measure the level of VSH can a bee breeder add VSH to an existing bee population or enhance what is already there. The objective here is to describe how VSH works and to explain how to measure it.

Common techniques for evaluating colonies for hygienic behavior or mite infestation do not measure VSH. For example, a freeze-kill test does not measure VSH. And counting mite drop onto the bottom board or counting mites in a sample of adult bees is only for estimating mite populations.

The following is a practical form of selective breeding that is not as precise as the methods that I used when I worked at the bee lab. My goal here is to describe an effective selection technique that strikes a balance between maximum accuracy and minimal effort. This simpler technique has two weaknesses. First, by not equalizing the mite population when setting up test colonies we may be selecting for less virulent varroa as well as for varroa resistant bees. Secondly, we get what we select for. When changing selection methods, one is likely to get a slightly different result.

VSH is Tested in Brood Cells

Varroa sensitive hygiene is expressed only by adult worker bees that are over 10 days old. Therefore, a colony cannot be evaluated until the queen being tested has produced a group of workers that are at least that old. This takes about 7 weeks. During those first 5 weeks the presence of a VSH queen has no effect on the mite population.

Hygienic removal of varroa-infested cells takes place only during a two-day segment of the mite's reproductive cycle and only in worker brood that is 4 - 6 days post capping. Therefore, we measure VSH in worker brood that is aged 7 - 11 days post capping, immediately after the mites have passed through their two days of vulnerability (Figs. 1 and 2).

Critical Components

VSH has two important components:

(1) During those vulnerable two days of the reproductive cycle of the mite, worker bees with the VSH trait will disrupt mite reproduction in a varroa-infested cell ***if*** the foundress female (the adult mite that entered the cell) has produced progeny. Disruption involves removing the bee pupa, thereby causing the death of all mite progeny and an uncertain future for the foundress.

(2) If a foundress mite has **no** progeny, her cell is **not** disturbed, and because of this second component, we can estimate the extent of varroa removal without knowing the initial rate of mite infestation.

We can do this because of a fact about varroa that is unrelated to VSH. In mite-susceptible colonies, about 15% of the mites that enter brood cells will not produce progeny. These non-reproducing mites may be found dead at the bottom of the cell, or if the mite died before the host bee has spun her cocoon, the mite may be at the bottom of the cell on the other side of the cocoon ("entombed" by the bee's cocoon). However, in most cases a non-reproducing foundress is alive but not producing progeny (Figs. 3 & 5). The frequency of non-reproducing mites varies somewhat but is rarely greater than 20% of the mite-infested cells. If a colony expresses little or none of the VSH trait, reproductive mites will all survive, and the percentage of non-reproducing mites will remain below 20%.

On the other hand, bees with the VSH trait remove some or all of the cells with reproductive mites. This removal of reproductive mites increases the relative percentage of non-reproducing mites, so when VSH is present at a significant level, the frequency of non-reproducing mites is greater than 30% of the mite-infested cells. VSH is additive, so bees with more VSH alleles will remove more reproductive mites, and bees with 100% of the VSH alleles will remove all of them.

The term "allele" is unfamiliar to many people. Most are familiar with the word gene which is a unit of inheritance located in a fixed location on a chromosome. Alleles are different forms of a gene. For example, a bee without the VSH trait hasn't lost a gene, the bee simply has a different allele at the site where the VSH allele would otherwise be. Some genes have many alternate forms (or many possible alleles), but they can have only one at a time.

Determining the Level of VSH

I use a sharp forceps to uncap a cell, and if the cell is between 7 and 11 days post capping (Fig. 2), I remove the pupa and check for mites (see Figs. 6 & 7). I examine about 100 cells and record varroa-infested cells as having either reproducing (Fig. 4) or non- reproducing mites. When non-reproducing mites represent 33, 50, or

100% of the infested cells, the workers have 50, 75, or 100% of the VSH trait, respectively. Admittedly, this relationship is a rough estimate, but it guides us in the right direction when selecting for VSH.

The Problem of Having Few Mites in a Sample

If a colony has a high level of the VSH trait, one is not likely to find many mites. If I check 100 cells and find no mites, I continue to 150. If I still have no mites at 150, I stop counting and give the colony a tentative score of 75% VSH. I may resample that colony later if it is a potential breeding source. On the other hand, if you find 5 reproductive mites before you find any nonreproducing mites, stop sampling That colony will not be a VSH breeder.

How many mites is too few? Suppose one only finds 2 infested cells in a sample of 150. There is no need to treat the colony with miticide, but that doesn't provide information about VSH ***unless*** one or more of the cells had a non-reproductive mite. For example, if both of those two mites are non-reproductive, I give it a top score of 100% VSH.

I'm satisfied with infestation data that consist of only 2 non-reproducing mites. And I am okay with finding no infested cells. Examine 100 or 150 cells. If you find no infested cells, score the colony 75% VSH; if you find 1 or 2, score it 50%. If you find any non-reproducing mites, use the percentage guidelines above to determine the level of VSH. If necessary, one can increase the mite population by adding a frame of infested brood to a colony and then evaluate that brood frame exactly 7 days later.

Caveat

I described a method for getting the most information about VSH with minimal effort. Mistakes will be made. However, unlike maintaining pure pedigrees, it is usually simple to re-establish a measurable trait such as VSH.

Most errors come from evaluating and breeding from queens that are multiply mated or free-mated. This is because the VSH trait is expressed at a high level when only some of the bees in a colony express the trait. Therefore, some colonies with free-mated VSH queens score as high as the best VSH breeder queens. But, when *grafting* from such a colony, some of the daughter queens will have a much lower level of the VSH trait than expected. For maximum precision, evaluate progeny of queens that have been inseminated with semen from a single drone.

Once the progeny of a queen has been evaluated there is no need to evaluate her progeny again. Keep the score with the queen if you move her to a different colony.

NOTE: I have described how to estimate VSH at levels equal to or greater than 50% but not at lower levels. This is because low levels of VSH cannot be separated from normal variation found in non-VSH populations.

FIG 1

This bee pupa is about one day too young to evaluate. After uncapping the cell, move on to the next one. White body *with purple eyes* is the youngest stage to evaluate for VSH.

FIG 2

Bee pupae with tan bodies are probably 8 or 9 days postcapping, well within the age to remove and evaluate.

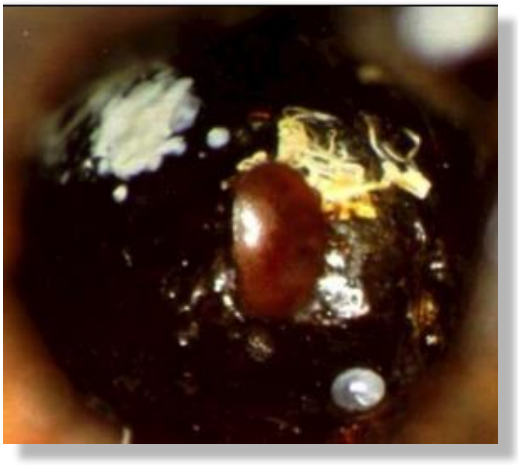

FIG 3

Looking down into a cell that we have uncapped and then removed the pupal bee, we find only a foundress female (brown) and one egg (at 5 o'clock). The material above and to the right of the mite is the bee's shed larval skin. Score this infested cell as non-reproductive because the egg would not have been present 4 – 6 days postcapping.

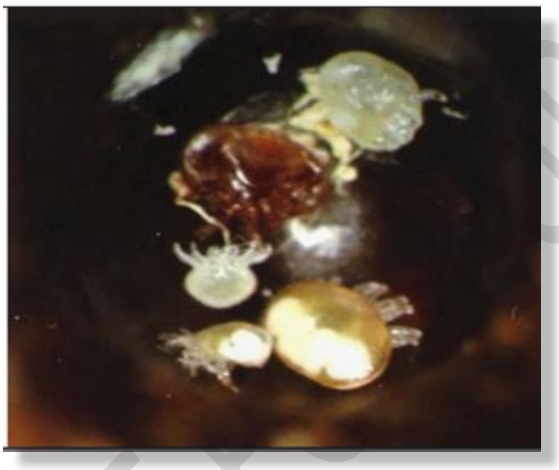

FIG 4

A foundress mite at the bottom of a cell after the host pupa has been removed. The other mites are her progeny. Note the fecal patch on the cell wall and in Fig. 3, both at 11 o'clock .

FIG 5

A non-reproducing mite often leaves her fecal patch on the bee (in this case the bee's abdomen) rather than on the cell wall. No, I don't know why.

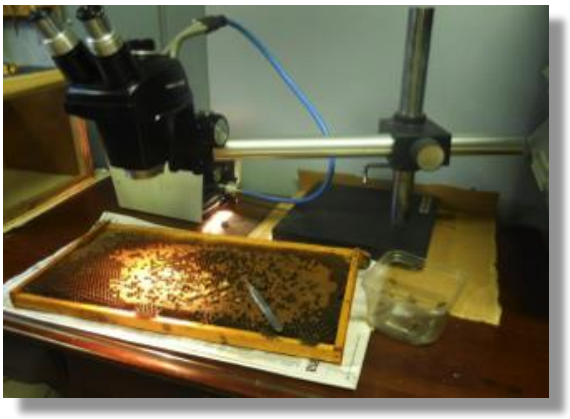

FIG 6

To evaluate brood I collect a comb with worker pupae at least 7 days postcapping (purple-eyed or older). I use 4X magnification and focus a bright light on the bottom of the cell where the mites are usually found, It takes about 20 minutes to examine 100 cells.

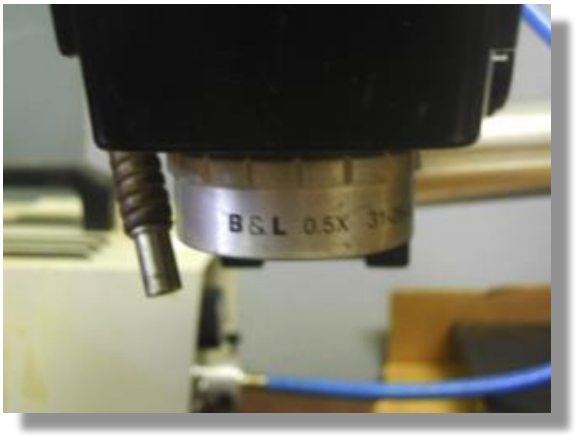

FIG 7

A 0.5 objective reduces the magnification. More importantly, it increases the depth of field and the focal distance, providing more working space between the scope and the comb.