Finding and Retaining Varroa-Resistant Bees

This information about VSH is for all beekeepers, beginners as well as queen producers. Information is presented in 7 parts.

Acknowledgement: This armadillo (née Tatou) is my co-worker in the bee yard (she's not much of a beekeeper).

In 1995 Roger Hoopingarner, Jeff Harris and I discovered varroa sensitive hygiene (the VSH trait). We first called it SMR, which stood for "Suppressed Mite Reproduction" because colonies with this trait had very low mite populations. When we found mites in the brood, they were not producing progeny. It appeared to us that the bees were causing the mites to be non-reproductive. But we didn't understand how that worked.

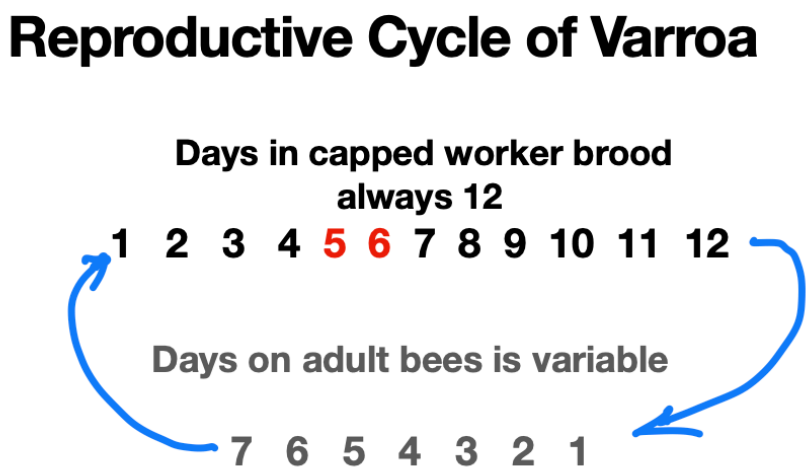
A break came in 2004 in a telephone call from Marla Spivak at the University of Minnesota. We had shared the SMR stock with her. Marla said that she and her graduate student, Abdullah Ibrahim, thought that the mechanism of resistance of SMR was a form of hygienic behavior…the removal of varroa infested brood cells. I was skeptical because we had seen no correlation between our varroa resistance and the freeze-kill test for hygiene.

But we learned that they were right. So we changed the name from SMR (suppressed mite reproduction) to VSH (**varroa sensitive hygiene**). Worker bees destroy brood cells if the cells are infested with a mite. But while we were testing that, we learned a critical detail. If a cell contained a mite that had produced no progeny, that cell was not disturbed. So the bees were not causing the mites to be non-reproductive. It just seemed that way because the bees were selectively removing the cells containing reproductive mites, the mites that had progeny.

Further study revealed that varroa sensitive hygiene is a heritable trait—neither a line nor a breed. It seems to involve only two genes. Certainly, a complex behavior such as VSH involves more than changing the alleles of two genes (alleles are the variable forms of a gene). My guess is that the other genes involved are either non-variable or usually have compatible alleles.

My goal is to enable all beekeepers to have varroa resistant bees while maintaining the genetic diversity of the world's honey bee populations. My approach is to explain how the VSH trait works and how to find and retain it. If all beekeepers —novices included—are able to measure VSH, both bees and beekeepers will benefit. I explain how you can use your cell phone to measure the VSH trait. By measuring varroa resistance, one can know whether or not a queen is producing varroa resistant workers long before there is a varroa crisis.

Part 2. How bees with the VSH trait control mites



The first step is to understand how VSH controls varroa. This figure illustrates the time, in days, of varroa's reproductive cycle in worker brood.

The top line of 1 through 12 represents the number of days that mites spend in worker brood, entering (as the arrow shows) just before the cell is capped. Once a cell is capped, the mite is trapped. We call the mite that enters the cell, the **foundress** or the **foundress female.**

After the cell is capped, and after the host larva has eaten the rest of the brood food and spun her cocoon, the **foundress** lays the first of what will be about 5 eggs, each egg about 30 hours apart.

The first egg laid is a male, so he has time to mature and mate with the females when they become adults. The males cannot live outside the cell, so they and any immature females die when their host bee emerges.

The numbers 1 to 7 (running right to left) represent the estimated time that mites spend on adult bees before they enter or re-enter a brood cell. It's called

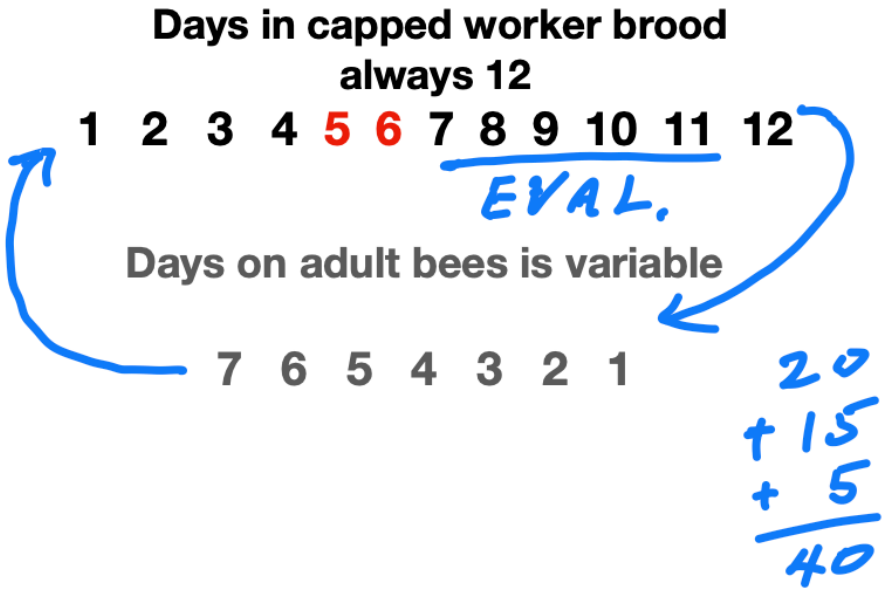
the phoretic phase. We found that the average time spent on adult bees is variable but that 7 days is common. So one complete reproductive cycle of varroa in worker brood is roughly 20 days.

On day 12 when the host bee emerges from the cell, the reproductive phase for that mite comes to an end. The foundress and her adult female progeny emerge from the cell to spend time on adult bees, and the cycle continues. Adult females are the only varroa mites that live outside the brood cell.

Worker bees with the VSH trait disrupt the mite's reproductive cycle at the days in red (4 - 6 days post-capping). The bees detect varroa infested cells and remove or cannibalize the soft bodied bee. As the bee pupa in the cell is being destroyed, the foundress female escapes to an unknown fate. This is how varroa sensitive hygiene controls mite reproduction.

To summarize, worker bees with the VSH trait break the reproductive cycle of varroa at only one place. But that's enough. When measuring varroa sensitive hygiene, we look for evidence of what happened at that vulnerable point by examining older capped brood.

Reproductive Cycle of Varroa



More about varroa's life cycle

After introducing a new queen, how soon can a colony be scored? At least 6 weeks, but to be conservative, I recommend 7 weeks.

Why so long? Because only worker bees express VSH, and it is at least 20 days before a queen produces her first adult worker. So that's 20 days. A few very young workers bees probably would not express VSH, so it's best to wait at least 15 more days so that a large proportion of the workers and all the young workers are progeny of the new queen. So add 15 more days. Then it takes 5 more days for those vulnerable cells to progress into the older cells that we choose to evaluate, those that are 7 - 11 days post-capping. That gives us 40 days as the absolute minimum, starting from the time a queen begins to lay eggs in a colony.

During those 40 days, the mite population will continue to grow. So if you look for mites on adult bees within 40 days after introducing a queen whose

progeny have a high level of VSH, you would conclude that there is no varroa resistance. But after 40 days, the mite population begins to decline, Because mites have a reproductive cycle of about 20 days, it takes about 15 more days or a total of 55 days for the entire mite population to be exposed to that vulnerable segment.

If you evaluate mite populations by sampling mites on adult bees, you need to wait even longer. Two months is not long enough. Remember, when bees disrupt mite reproduction, the foundress is not killed. She is prematurely returned to the phoretic state and therefore adds to the number of mites on adult bees.

How long does a displaced foundress stay on adult bees? Does she re-enter a cell and try again? If so, is she reproductive? Does she catch the first flight out? How long does she live? Does she become weak and therefore vulnerable to being killed by worker bees? I don't have those answers, but my concern is this, if we equate varroa resistance with having fewer mites on adult bees, we may be selecting against VSH.

That's one reason why I never examine adult bees for mites. Another reason is that if mites are not reproducing, a mite population can only decline; and I don't need to know how many are present on the adult bees.

Part 3. Measuring VSH

Varroa sensitive hygiene (VSH) can be added to any population of bees and may be naturally present in many others, but only by measuring the level of VSH can a beekeeper confirm the presence of VSH and then preserve or enhance what is there.

VSH bees do not disturb a cell that contains a non-reproducing mite. Less than 15% of infested cells have non-reproducing mites, so when one finds non-reproducing mites in 40, 65 or 100% of the mite-infested cells, we know that cells with reproducing mites have been destroyed, and the colony has 50, 75, or 100% of the VSH trait, respectively.

Don’t expect to find more than 1 or 2 non-reproducing mites per hundred cells examined. To take advantage of the presence of non-reproducing mites, it’s best to check the brood as soon as possible, 50-80 days after introducing a VSH queen. This is because cells with non-reproducing mites will also disappear from colonies that express VSH.

It is also perfectly 0K to wait 3 months or a year before testing for VSH. The 50 to 80 day range is recommended only because one is more likely to find non-reproducing mites, and finding non-reproducing mites makes one more confidant that the absence of mites is caused by VSH rather than the possibility that the colony had no mites for some other reason. Early testing also provides an early warning when a colony is susceptible to varroa.

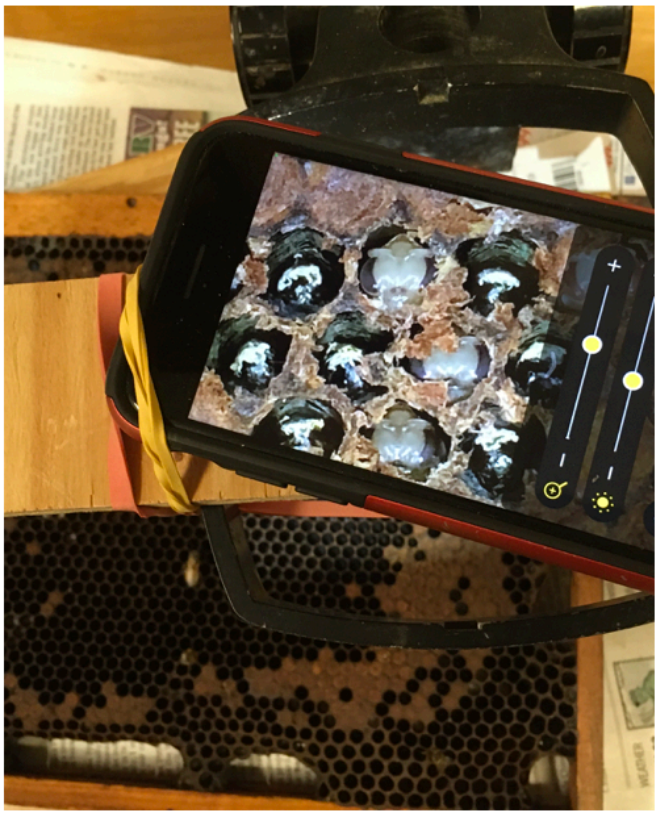
Equipment needed. Probably 90% of our beekeepers already have the equipment needed to measure the VSH. You need a good light, magnification of 3 to 4X, and a focal distance of 4 or 5 inches (10 - 12 cm). You can do that with the magnify utility on your iPhone or iPad or you can use a dissecting scope. If you use a dissecting scope, you may need to add a 0.5 objective. The 0.5 objective decreases the magnification and increases the focal distance, thereby creating the space needed to use your hands between the magnifier and the brood comb that is being examined.

Magnify utility on a cell phone. Cell phones have a built-in, bright light that is in a perfect position next to the lens, with a bonus of having zoom magnification and autofocus. It’s perfect. My experience has been limited to the magnify utility on an iPhone and an iPad, but all iPhones and iPads seem to come with magnify, and I understand that Android phones also have a magnify app available. Older iPhones, such as my 10 year-old 5s can be used but the magnify feature is included within the camera app and not separately.

Set the phone on a horizontal support that leaves it about 6 inches (15 cm) above the tabletop. I used rubber bands and a horizontal bar attached to a heavy support. Any sturdy horizontal support will work. For example, I used two top bars strapped tightly to a stack of heavy books.

The autofocus on the cell phone is good in some ways but it becomes a problem when you lift the comb to focus on the contents at the base of the cell. As you lift the comb, the auto-focus moves up too, focusing on the surface of the comb. To solve that problem, use the focus lock feature. Focus on the surface of the comb where you normally examine a pupa that is removed, then press focus lock and leave it as that. Then, when you lift the comb to focus on the base of the cell, the focus will be at the base of the cell.

An issue with using a cell phone is that constant use of the light drains the phone's battery pretty fast. When evaluating brood, I keep the phone plugged into power. So if you're using it in an area without a power source, know that your work time will be limited.



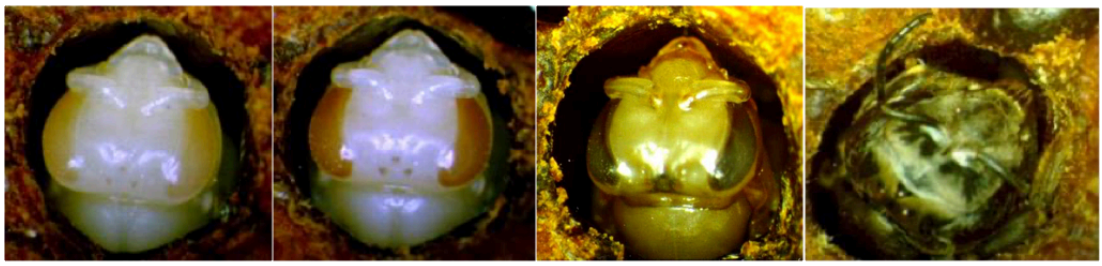
Cell phone setup

Dissecting scope setup.

Sampling

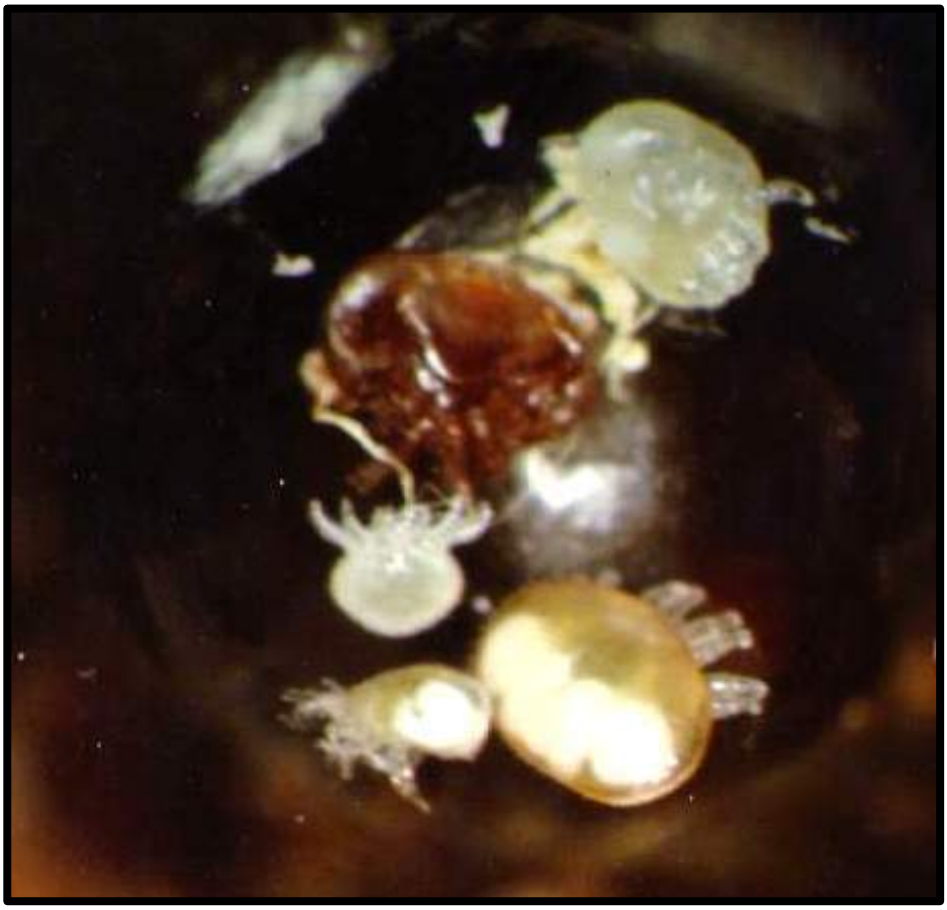
The first step is to collect a comb that has worker pupae, many of which are at least 7 days postcapping (purple-eyed pupae or older). I collect from about 9 colonies at a time which saves me from going back and forth to the apiary. I try to avoid frames with a lot of uncapped larvae because larvae do not survive well when held overnight without worker attendants at room temperature, whereas capped brood and eggs survive reasonably well.

I mentioned that I check only worker cells that are 7 – 11 days post-capping. Below are 4 pupae that I have uncapped.



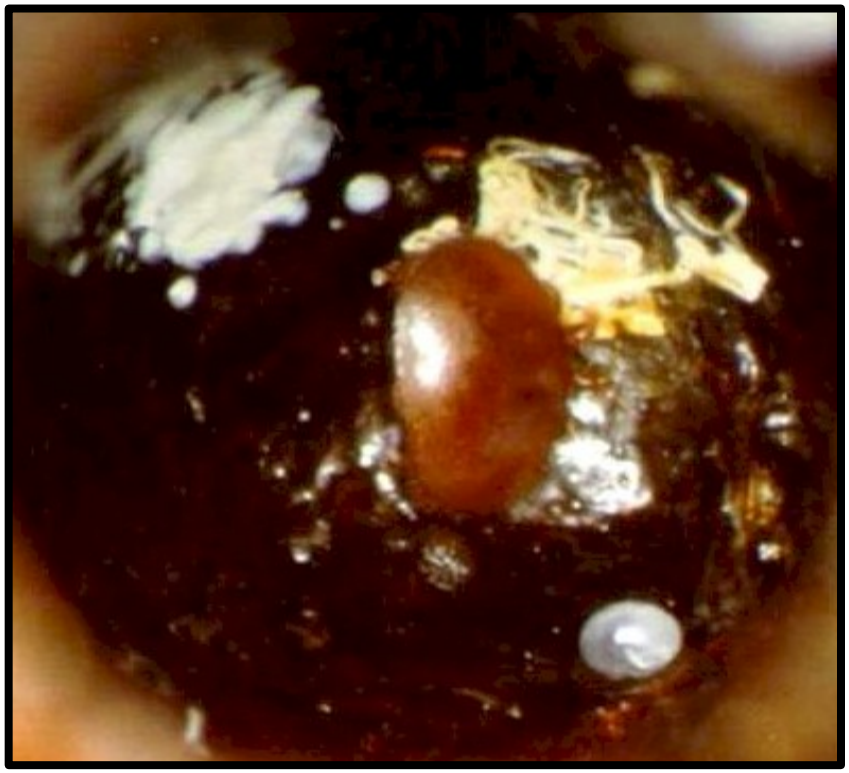
The first one is much too young, the second one is about 6 days post capping, so not quite old enough. If a pupa is too young, leave her and move on. A 7 day pupa is the youngest that we evaluate. It has a white body and dark purple eyes. One can evaluate any pupa that is purple eyed and older. The third example is old enough, 9 or 10 days post-capping. The last one has shed her pupal skin and expanded her wings, so she is an adult. One can evaluate this stage, but I don't.

When you see that a pupa is old enough, remove her from the cell. Pull the bee out with a forceps and look for mites on the bee and then in the cell. If there's a mite present, and if it's a reproductive mite, you will usually see a fecal patch near the base of the cell at about the 10 o'clock or 2 o'clock location—the top of the comb being away from you.



fertile

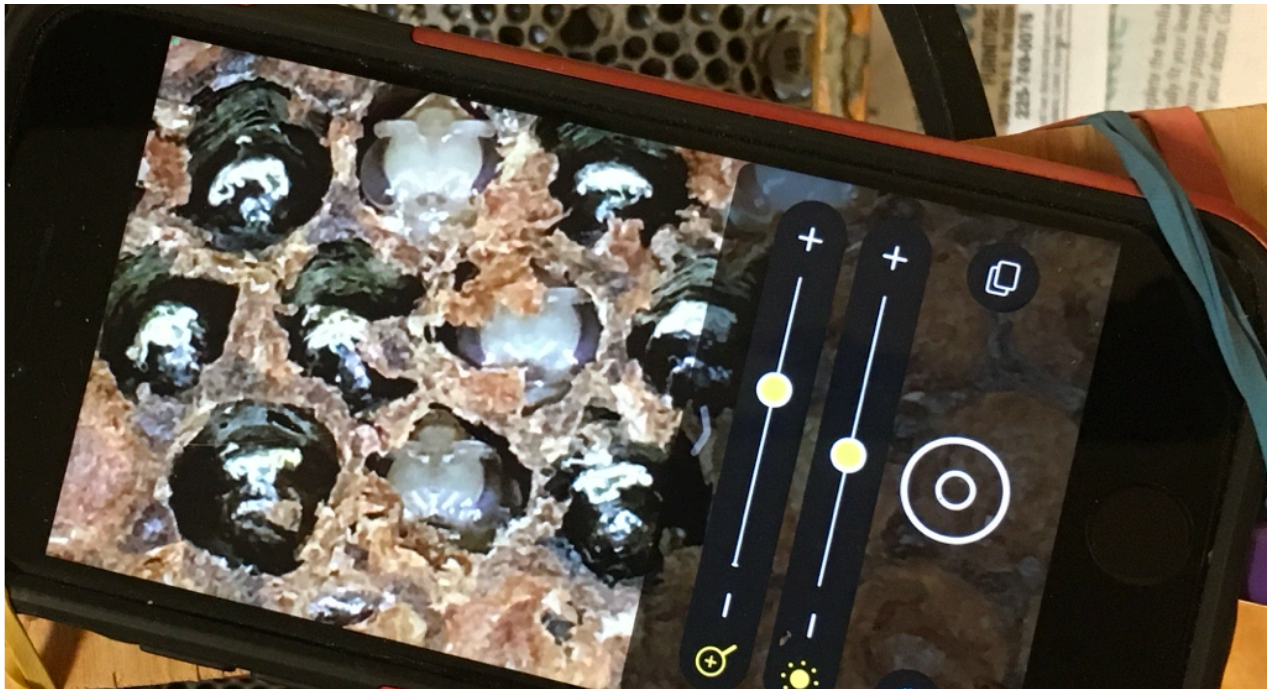
Above is an example of a cell with a reproductive mite. The mite's fecal patch is at the 10 o'clock position. There is always a shed larval skin at the base of the cell, but that has nothing to do with mites. Mites are covering most of it (I'll show the shed larval skin in the next figure). The foundress female is the large brown oval in the center. The progeny (an egg and 3 nymphs) are white, four of them. If the immature mites are immobile, they are not dead. They become immobile while transitioning from protonymph to deutonymph and from deutonymph to adult, perhaps comparable to the immobility of bee pupae.



The situation above is hard to classify. The mite is non-viable because it has an egg only and an egg at this stage does not have time to reach adulthood. Sometimes these non-viable cells have an egg and also a male. So the cell is reproductive, but non-viable. I record it in a third category as non-viable. So when scoring, I don't include it unless the score is borderline, then I count it as non-reproductive because there may have been no progeny a few days earlier when the cell was within the vulnerable period for VSH removal.

Cells with reproducing mites are obvious. Whereas, cells with a non-reproducing mite or a dead mite are easy to miss, especially if there is no fecal patch. A single foundress is hard to spot against a dark cell wall, as are dead foundresses or foundresses "wallpapered" (trapped) between the base of the cell and the cocoon. The cocoon of a worker bee is cellophane-like and very thin. It remains as part of the cell wall.

It takes about 15 minutes to examine 100 cells. If you find 5 cells with reproductive mites before you reach 100, you can quit…that colony does not express VSH.



Cell phone view.

In the previous photo, you saw the contents of two varroa infested cells. Here are cells that are not infested. Note that we have part of the screen taken up with the control panel on the right. Double tap the screen to toggle the control panel in or out. Tap the gear image on the lower left of the control screen and go to settings (activate only 4 settings (brightness, contrast, flashlight, and focus lock).

Ten cells have been uncapped and 7 of them (4 on the left and 3 on the right) have had their pupae removed. The white material at the bottom of the cell is the shed larval skin, not to be mistaken for a mite fecal patch. A mite fecal patch is a pile of white dots found only in varroa-infested cells. The shed larval skin is always present in brood cells that contain a pupa. It looks like a flattened

membrane. None of those cells is mite-infested. The three cells that are still occupied by bee pupae are purple-eyed…just old enough to evaluate.

Fecal patch on the abdomen

If there is no fecal patch on the cell wall, there is usually no mite in that cell. However, infertile mites often place their fecal patch on the bee (commonly on the abdomen of the bee) rather than on the cell wall. Sometimes the mite feces are scattered on the pupae. I don't know what causes the mite to change the placement of feces from the normal cell wall placement to the bee's body. The only time I see the fecal patch on the bee is when a mite does not produce progeny.

Part 4: Scoring VSH

In selective breeding, we must put a numerical score on potential breeding stock. Although queens and drones are the reproductive castes, adult workers are the only ones who express the VSH trait. This requires us to use indirect methods to score queens and drones.

Below is the scoring method that I use. It scores worker bees on a scale of 0 to 4 with 0 indicating that the workers have none of the VSH alleles and 4 stating that they have all of them. (This may not represent reality, but use it until we find something better).

Percent of infested worker cells with nonreproductive mites (NR)[1]	**If only reproductive mites or no mites are found [2]**	**Score**
80 - 100% nonreproductive)	0/200 (no mites found in 200 cells examined)	4
50 - 80% NR	0/100 or 1/200	3
30 – 50% NR	1 or 2/100	2
20 - 30% NR	3 or 4/100	1
Less than 20% NR	5 or more/100	0

[1] Examine brood cells that are at least 7 days post-capping (the worker bee in the pupal stage should be purple eyed or older).

[2] If you find one or more worker cells with non-reproducing mites, use the first column to calculate your score. In column one, the number of cells examined does not figure into the conversion, but I recommend checking a minimum of 100 cells. However, if you find 5 cells with reproducing mites before you reach 100, there may be no need to continue. If you find no infested cells or none with nonreproducing mites, use the second column. Finding no mites is common in

VSH colonies that have had highly resistant workers for longer than 3 months or if the queen was introduced into a colony that already had a very low mite count.

Part 5: Recording and using your data

Three VSH scores are affixed to every mated queen. These are the queen score (Q) which I list first; the score for the sperm in her spermatheca (D), which I call the drone score; and the worker score (W), the score of the workers (her daughters,.. whether workers or queens). The worker score is the only one that can be measured.

I record those scores in a Q(D)W grouping, with the parentheses symbolizing the sperm in her spermatheca. It is the bee version of mother(father)daughter. If we had perfect heritability and exact measurements, the worker or daughter score would be the midpoint between the Q and D (mother and father) scores.

How does a queen get the scores that are assigned to her for life? The worker score of a colony is the score for any queen that is grafted or superseded from that colony. If one does not have a score for the workers that are her sisters, a queen cannot be scored. Let's assume her sisters scored a 3. Her VSH score would be recorded as 3( ).

A drone score is added when a queen is mated. Because drones are produced from their mother's unfertilized eggs, they have the same score as their mother. Assume we collected drones from a colony that had a queen score of 2. Our queen now carries a 3(2) score. If the drones had come from unscored colonies or if our pretend queen was free-mated, her drone score would be unknown and would be recorded as an asterisk, 3(*). If a colony has an unscored, mated queen, such as a swarm queen, and if the workers in that colony had a score of 2, I would score it as *(*)2.

To get the worker score, a queen must produce progeny for at least 7 weeks. After I measure VSH by uncapping and checking worker cells for mites, I save the data in the format R, NR, NV / N. The R = the number cells with reproductive mites, NR = the number of cells with non-reproductive mites, NV =

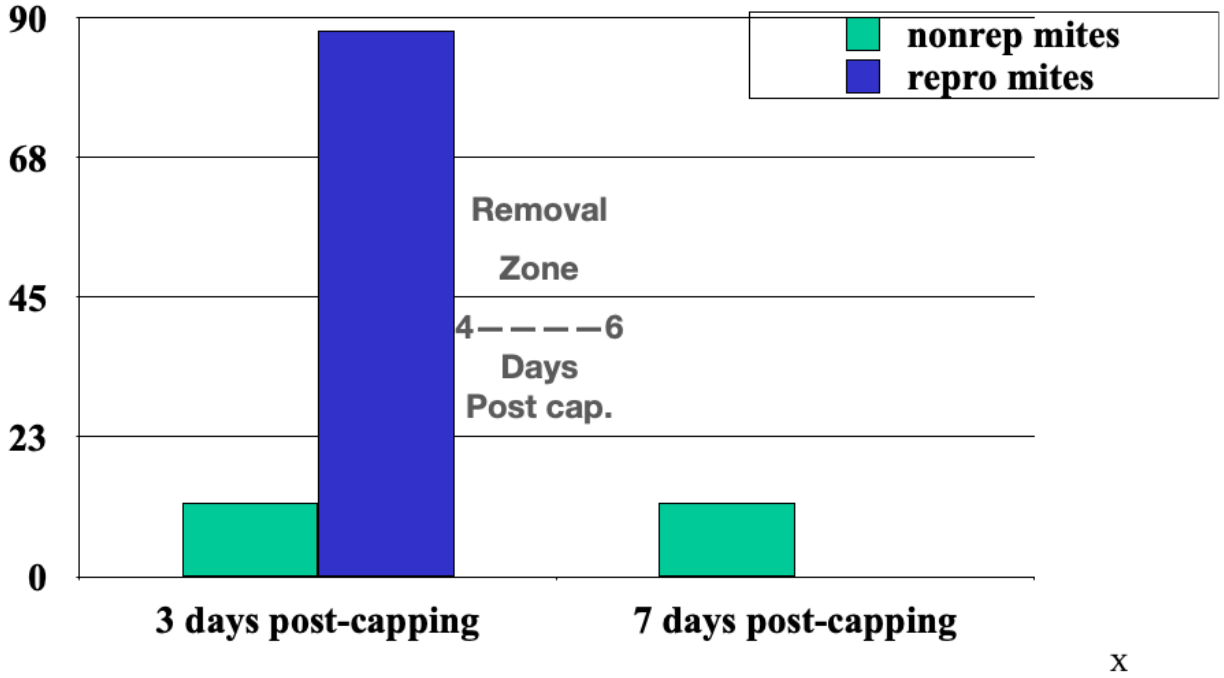
the number of cells with non-viable mites (borderline observations where the foundress has progeny, perhaps a male and/or an egg, but too late to produce a viable female), and N = the number of cells checked. So, if I open 100 cells and find no cells with a reproductive mite, none with a non-reproductive mite, and one that was reproductive but non-viable, I would record a 0,0,1/100. That's a worker score of 3, Now our queen has all her scores. Her 3(2)3 score and her sample tally of 0,0,1/100 are stored on the same line in your spreadsheet as the queen in columns headed by score and sample. Those scores stay with a queen for her lifetime.

Suppose a score is borderline, and you choose to evaluate that queen again at a later date. (Unless I am considering using that queen as a breeder, I wouldn't bother) It doesn't matter if the queen is in a different colony as long as she has been laying eggs in that colony for at least 7 weeks. After evaluating 100 more cells only 1 varroa-infested cell is found, and that cell had a reproductive mite. The data from the second count (1,0,0/100) is combined with the earlier count and we get 1,0,1/200, so the measurement changes, but her worker score remains at 3.

If you're not satisfied with your results, especially if you find no mites and need to know if VSH is the cause, drop a frame of newly capped worker brood from a known varroa-infested colony into the colony that you want to test, and then evaluate the brood on that frame exactly 7 days later.

Using your data comes to this: when selecting a colony to graft from, choose a colony with a high worker score. If you're looking for a drone source, choose a colony with a high queen score, even if the queen is free mated and has unscored workers.

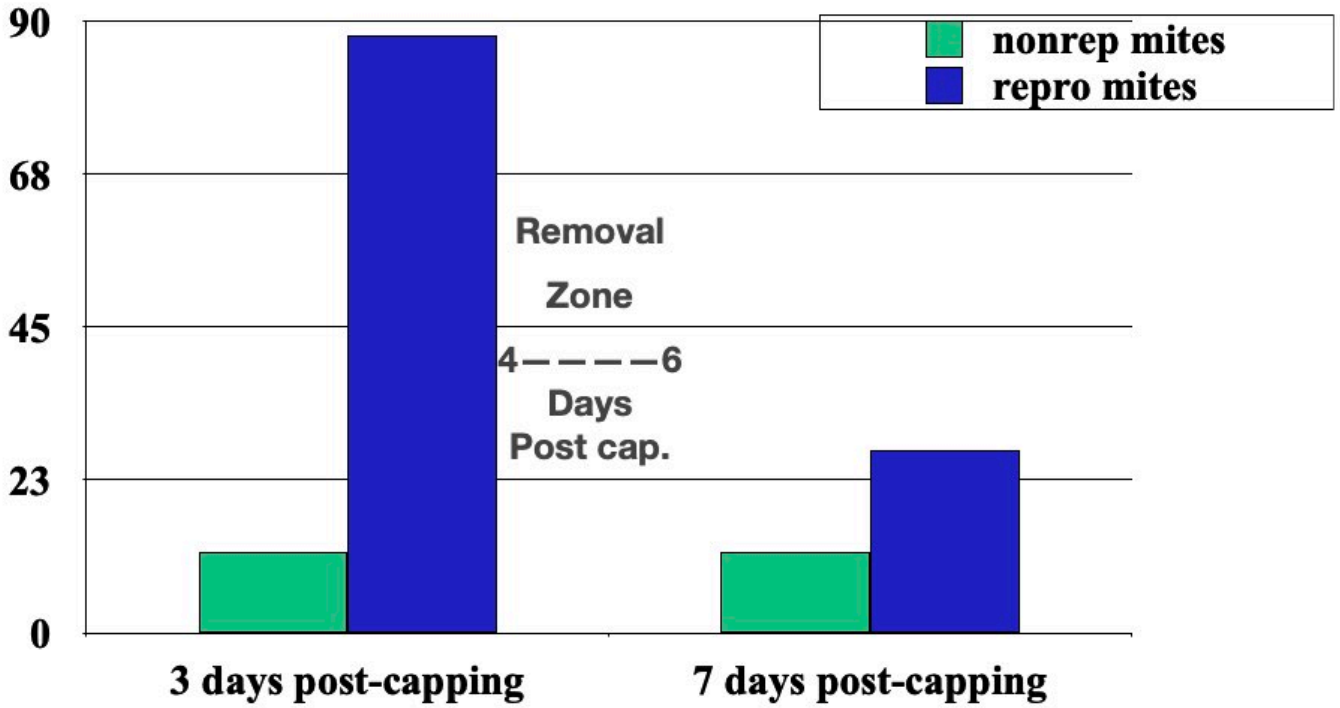
Mite populations before and after VSH bees destroy cells with reproductive mites



What to expect from a colony with a worker score of 4

The columns at the left represent a varroa infestation (in worker brood) before the mite population enters the vulnerable segment (4 - 6) days post capping. Note that in this example, about 12% of the mites that enter a cell did not produce progeny. That's close to average. We don't need to know the initial mite infestation because we know that a non-VSH colony has less than 15% of its worker brood with non-reproducting mites. So when we look at cells 7 days post capping and find that all the varroa infested cells have non-reproducing mites (usually just 1 or 2 cells), we conclude that all of the cells with reproductive mites have been destroyed, and the colony has all of the VSH alleles. That's full expression of VSH or a score of 4.

What to expect from a colony with a worker score of 2



x

The same before and after scenario as in the previous graph, but in this case the worker bees have about half of the VSH alleles, such as when a VSH queen free-mates with drones that do not carry any VSH alleles.

Again, the only measurement that we have is the one taken from older brood. But that is all we need. This is what you can expect if you purchase a free-mated VSH queen. A score of 2 produces an acceptable level of resistance to varroa, enough to control mite populations.

VSH does not affect the population of non-reproducing mites. But as VSH reduces the mite population, the green column of non-reproducing mites declines proportionally. Therefore, it is common to find no cells with nonreproducing mites when measuring a VSH colony more than 3 months after introducing a queen or when measuring any colony with a low mite population.

Part 6: Breeding strategies

Benefits of natural mating and artificial insemination.

The live or let die approach to breeding is nature's way, and I respect nature. But we have the power of the single drone insemination, nature does not. The ability to inseminate a queen with a single drone enables us to evaluate a single gamete and thereby speed-up the selection process.

There is a lot of genetic variation within a colony when a queen is naturally mated with multiple drones. She mates with drones that may come from colonies many kilometers away. A free mated queen ends up having half of the neighborhood represented in her progeny. That may be beneficial for colony fitness, but that will not enable us to detect and isolate a trait that may be universally present allow frequency.

Single drone inseminations produce genetic uniformity within each test colony and therefore reveals genetic differences between colonies. The best way to detect the genetic diversity in a group of colonies is to inseminate a group of queens, each queen with semen from a single drone.

We used that approach in 1995. We set up an experiment with 43 colonies, each with a queen inseminated with a single drone; two months later we identified the VSH trait in 3 of the colonies. If we had relied on natural mating, we'd still be looking.

Free mating is also very powerful, but not in the early stages of selective breeding. The benefit of natural mating is its role in drone selection. When collecting drones for artificial insemination, we choose a colony whose queen has a high score. We take every drone that we can catch so we bypass the benefit of within-colony drone competition that nature uses so effectively.

I estimate that fewer than 1% of the drones successfully mate with a queen, and those that do are probably those which **(1)** are not diseased, **(2)** are not parasitized by varroa during their development, **(3)** have strong and well-functioning bodies, **(4)** come from successful colonies, and probably most importantly **(5)** are competing in all of those challenges with only 1 set of chromosomes.

When successful in mating, a drone transfers identical copies of his winning gamete to a queen bee. Thus, natural mating maintains the quality of our bee populations and may also support varroa resistance *as long as the drone source colonies are <u>not</u> treated to control mites*.

The VSH trait can be added to any bee population. It is not a breed or a line that needs to be maintained in a closed population. So if you discover that your best breeder queen has been superseded, view it as an opportunity to refresh your stock with nature-selected drones. As long as you can measure VSH, you can recover it.

VSH bees do not seem to disrupt varroa reproduction in drone brood. When I check drone brood in VSH colonies, I find reproducing mites. Nevertheless, mite reproduction in drone brood has not been a problem, so I never remove drone brood. I step aside and let nature proceed with drone selection.

Part 7: Where do you fit in?

Checking 100 cells of worker brood enables a beekeeper to predict a colony's varroa status at a very early stage, long before there is a crisis. Whether you're a beginner or a seasoned beekeeper, you will benefit from an ability to measure VSH in your bees. But start slowly, don't stress about checking all of your colonies.

There are different strategies for different situations. If you have the ability to use artificial insemination, you have many options. The VSH trait is probably present in bee populations worldwide, so one option is to find the trait yourself.

The Buckfast breeders in the Netherlands did that, so now they have VSH resistance in a Buckfast bee. A simpler solution is to get a queen that has the VSH trait and take it from there. One is all you need. Remember this is a trait not a line or a breed. Your colonies supply the breeding stock.

If you are a queen producer who does not use artificial insemination, you need to buy a breeder queen. Here's where your ability to measure VSH comes in handy. Measure the progeny of the breeder queen to see if they score at least a 3. If not, you should inform the person who produced her. You may also want to measure and score VSH in some of the colonies produced by your free-mated daughter queens. If you have had VSH breeders in previous years, you may find high levels of VSH in some of those colonies. After about 3 years of breeder queens, you may reach a point where you have an adequate level of VSH in your bees, enabling you to select your own VSH breeders.

If you're a sideliner, a hobby beekeeper or a beginner with one hive, you don't want to lose your bees but neither do you want to expose them, the honey, or your bee equipment to toxic materials. Buy free mated VSH queens. Don't waste your money on breeder queens. After you get those queens established, measure and score their progeny. They should score at least a 1 and hopefully a 2. If you get a zero, you are entitled a replacement or refund. If you get a 2 or better, congratulate the seller. Your ability to measure the quality of your queens makes you a better consumer and improves the industry. When you order, you may want to tell the seller that you measure VSH in your colonies and that you would "be happy to provide feedback if that would be helpful to them."

When consumers know how to measure varroa resistance in their colonies, queen producers will take notice.